

The Design of Stable, Well-Defined Polymer-Magnetite
Nanoparticle Systems for Biomedical Applications

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Dissertation submitted to the Faculty of the Virginia Polytechnic
and State University in partial fulfillment of the requirements for
the degree of

Doctor of Philosophy
In
Chemical Engineering

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August 10, 2009
Blacksburg, Virginia

Keywords: magnetite, poly(ethylene oxide), poly(propylene
oxide), brush extension, steric stabilization, contrast agent

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ABSTRACT

The composition and stability of polymer-magnetite complexes is essential for their use as a treatment for retinal detachment, for drug targeting and delivery, and for use as a MRI contrast agent. This work outlines a general methodology to design well-defined, stable polymer-magnetite complexes. Colloidal modeling was developed and validated to describe polymer brush extension from the magnetite core. This allowed for the observation of deviations from expected behavior as well as the precise control of polymer-particle complex size. Application of the modified Derjaguin-Verwey-Landau-Overbeek (DLVO) theory allowed the determination of the polymer loading and molecular weight necessary to sterically stabilize primary magnetite particles.

Anchoring of polyethers to the magnetite nanoparticle surface was examined using three different types of anchor groups: carboxylic acid, ammonium, and zwitterionic phosphonate. As assessed by dynamic light scattering (DLS), the zwitterionic phosphonate group provided far more robust anchoring than either the carboxylic acid or ammonium anchor groups, which was attributed to an extremely strong interaction between the phosphonate anchor and the magnetite surface. Coverage of the magnetite surface by the anchor group was found to be a critical design variable for the stability of the zwitterionic phosphonate groups, and the use of a tri-zwitterionic phosphonate anchor provided stability in phosphate buffered saline (PBS) for a large range of polymer loadings.

Incorporation of an amphiphilic poly(propylene oxide)-*b*-poly(ethylene oxide) (PPO-*b*-PEO) diblock copolymer attached to the magnetite surface was examined through colloidal modeling and DLS. The relaxivity of the complexes was related to aggregation

behavior observed through DLS. This indicated the presence of a hydrophobic interaction between the PPO layers of neighboring complexes. When this interaction was large enough, the complexes exhibited an increased relaxivity and cellular uptake.

Thus, we have developed a methodology that allows for design of polymer-magnetite complexes with controlled sizes (within 8% of predicted values). Application of this methodology incorporated with modified DLVO theory aids in the design of colloidally stable complexes with minimum polymer loading. Finally, determination of an anchor group stable in the presence of phosphate salts at all magnetite loadings allows for the design of materials with minimum polymer loadings in biological systems.

For my wife and daughter:

Munye and Amelia Miles

*I have fought the good fight, I have finished the race, I have kept the faith.
- 2 Timothy 4:7*

ACKNOWLEDGEMENTS

I have had the privilege of working with **Dr. Richey Davis**, whose open door policy and enthusiasm for the work have made the whole experience worthwhile. I am also indebted to **Dr. Judy Riffle**, who incorporated me as a defacto member of her group, and allowed me to learn (somewhat) the mysterious ways of the polymer chemist. I would also like to thank the members of my committee for their guidance and input: **Dr. William Ducker**, **Dr. David Cox**, and **Dr. John Walz**.

I am appreciative to many colleagues, particularly in Dr. Riffle's group. Without these people and their ability to synthesize new materials, none of this work would have been possible. They include **Nikorn Pothayee**, **Dr. Shane Thompson**, **Christian Reinholz**, **John Boyd**, and **Shannon Ball**. Special thanks to **Dr. Jonathan D. Goff** and **Philip P. Huffstetler** for incorporating me into their group and allowing me to learn with the best. Thanks also to our collaborators **Dr. Robert Woodward** and **Matt Carroll** for their help with the SANS experiments at NIST and their constant questioning, which has made this work significantly better.

Thanks to **Dr. Beth Caba** and **Dr. Akhilesh Garg** for incorporating me into the research group and for introducing me to many laboratory and experimental techniques. Thanks also to my labmates, **Raquel Mejia-Ariza** and **Adam Larkin**, for keeping things interesting.

I would like to thank **Riley Chan** for his ability to fix just about anything and **Angie Flynn**, **Diane Cannaday**, **Chris Moore**, and **Tina Kirk** for assistance with countless matters.

I also thank the following for financial support: NSF Grant Materials World Network for the Study of Macromolecular Ferrofluids (#DMR-0602932), the Macromolecular Interfaces with Life Sciences (MILES) Integrative Graduate Education and Research Traineeship (IGERT) fellowship, the Institute for Critical Technology and Applied Science (ICTAS), and the Eastman Chemical Professional Excellence Travel Award..

Obtaining a Ph.D. is not a singular experience. The support from colleagues and family has been instrumental in the completion of this portion of my life. I wish to thank the colleagues, who became my friends, and in many cases, my family. Thanks to **John and Suzanne Brooks**, **Phil and Tiffany Huffstetler**, **Dr. Jonathan Goff**, **Dr. Ane Turner Johnson**, **John Boyd**, **Michael Heinzer**, **Dean Mastropietro**, and **Adam Larkin**. A special thanks to **Dr. Christopher and Jessica McGrady**: your friendship and council has touched Munye and I from the day we arrived at Virginia Tech. Everything you have received, you deserve.

I am grateful to my family: my parents, **William** and **Kathryn Miles**, my sisters, **Anna Benitez** and **Elizabeth Miles**, and my brother, **Henry Thomas Miles**. We do not always get along, but that is part of what makes getting together so much fun. I love you all.

I thank my Grandfather, **James Sharp**, for the hefalump traps, instilling in me the fear of your club and my love for baseball, and for your service to our country. Thanks to my other grandparents, **Hugh Miles**, **Helen Miles**, and **Doris Sharp**. I thank my extended family: my in-laws, **Tsing** and **Yee Chan**, and my brother-in-laws, **Chi Chan** and **Jose Benitez**.

I would like to thank my daughter, **Amelia Miles**. You have brought meaning to my life that put the stresses of graduate school into proper perspective. You have kept me up at night and woken me up way too early. You have required more time and attention than I could have imagined. I would not trade it for anything. I love you.

Finally, I would like to thank my wife, **Munye Miles**. Your willingness to follow me on this journey and put up with the late nights is what makes you special. While I have tried to help with Amelia, I know that you have experienced the brunt of the responsibility while I have been working. You have accepted that responsibility without complaining and have been instrumental in allowing me to complete my doctorate. I will cherish you always, and I am so blessed to have you in my life. I love you more.

ATTRIBUTION

Dr. Jonathan D. Goff at Virginia Tech synthesized the PDMS, PDMS-magnetite complexes, and PBO-magnetite complexes discussed in section 3.5.1 and sections 8.3.2 – 8.3.5. Dr. Goff also synthesized polyether-magnetite complexes discussed in sections 4.3.6, 5.3.10, 6.3.8, and 7.3.4.

Philip P. Huffstetler at Virginia Tech synthesized the PBO used to make ferrofluid complexes discussed in section 3.5.1. He synthesized the polyethers discussed in sections 4.3.2 – 4.3.4, 5.3.2 – 5.3.9, 6.3.2 – 6.3.6, and 7.3.2. He also synthesized polyether-magnetite complexes discussed in sections 3.5.2, 5.3.10, 6.3.8, and 7.3.4

Dr. Beth Caba at Virginia Tech developed the method by which the radius of gyration was calculated from the size of the particle complex discussed in section 4.4.1 and used extensively in successive chapters.

Dr. Shane Thompson at Virginia Tech synthesized the polyethers discussed in Table 3.3.

Mr. Nikorn Pothayee at Virginia Tech synthesized the polyether-magnetite complexes discussed in Table 3.3.

Mr. John Boyd at Virginia Tech synthesized magnetite used to make complexes discussed in section 4.3.5.

Mr. Christian Reinholz at Virginia Tech synthesized polyether-magnetite complexes discussed in sections 3.5.5, 4.3.6, 5.3.10, and 7.3.4.

Ms. Shannon Ball at Virginia Tech performed TGA measurements for samples discussed in Tables 5.2 and 6.7.

Mr. Jamie Clinton at Virginia Tech performed UV-Vis spectroscopy measurements shown in Figures 9.6 and 9.8 and performed the X-ray photoelectron spectroscopy measurements discussed in section 9.3.

Dr. O. Thompson Mefford at Virginia Tech and Dr. Rob Woodward at the University of Western Australia also helped develop the modified density distribution model as constituted in sections 3.4, 4.4.1, 6.3.10, 7.4.1, and 8.3.8.

Mr. Matthew R.L. Carroll at the University of Western Australia conducted relaxation measurements in section 7.4.4 and aided in the design of the magnetic separation column discussed in section 8.3.6.

Ms. Michelle Gasko at the University of Nebraska-Omaha Medical Center conducted cell culture experiments presented in section 7.4.4.

Dr. Judy S. Riffle at Virginia Tech served as a collaborator and was directly involved in project design and review of publications resulting directly from this work.

Dr. Richey M. Davis at Virginia Tech was my principal advisor throughout my graduate career. He supervised the overall work, including the review of this document.

Table of Contents

1	Introduction	1
1.1	SIGNIFICANCE AND MOTIVATION	1
1.2	SPECIFIC RESEARCH OBJECTIVES	1
1.3	OVERVIEW OF CHAPTERS	3
1.4	REFERENCES.....	7
2	Literature Review	9
2.1	OVERVIEW OF RELEVANT INSTRUMENTATION	9
2.1.1	Light Scattering	9
2.1.1.1	Static Light Scattering	11
2.1.1.2	Dynamic Light Scattering.....	15
2.1.2	Zeta Potential.....	17
2.1.3	Quartz Crystal Microbalance with Dissipation	20
2.1.4	Nuclear Magnetic Resonance	22
2.1.5	Size Exclusion Chromatography	25
2.2	PROPERTIES OF RELEVANT METAL OXIDES	29
2.2.1	Charging of Metal Oxides	29
2.2.2	Iron oxide	32
2.2.2.1	Magnetism	33
2.2.2.2	Superparamagnetism	35
2.2.2.3	Magnetite	36
2.2.2.4	Magnetic Ferrofluids	37
2.2.3	Cerium oxide	38
2.2.3.1	Biological Activity of Cerium Oxide	38
2.2.3.2	Oxygen Vacancies and Cerium Oxide Reactivity.....	39
2.2.4	Interaction of Phosphate with Metal Oxides	41
2.3	POLYMER ADSORPTION ONTO SURFACES.....	44
2.3.1	Mechanism of Polymer Adsorption onto Solid Surfaces.....	44
2.3.1.1	Adsorption of Polymers in Selective and Non-Selective Solvents	45
2.3.1.2	Adsorption of Polyelectrolytes on Charged Surfaces	48
2.3.2	Adsorption of Proteins onto Metal Oxide Surfaces	49
2.3.3	Adsorption of Polymers on Metal Oxide Surfaces	51
2.4	COLLOIDAL STABILIZATION THROUGH POLYMER ADSORPTION	54
2.4.1	Density Distribution Model.....	54
2.4.1.1	Determination of the Radius of the Complex	54
2.4.1.2	Determination of the Radius of Gyration using the Density Distribution Model	58
2.4.2	Extended Derjaguin-Landau-Verwey-Overbeek (DLVO) Theory.....	59
2.4.2.1	Electrostatic Particle-Particle Interactions.....	60
2.4.2.2	Van der Waal's Particle-Particle Interactions.....	61
2.4.2.3	Steric Particle-Particle Interactions.....	63
2.5	MACROMOLECULAR SYNTHESIS.....	65
2.5.1	Condensation vs. Addition Polymerization.....	65

2.5.2	Step Growth Polymerization	66
2.5.3	Chain Growth Polymerization	69
2.5.4	Living Chain Growth Polymerization.....	71
2.5.5	Ring opening polymerizations.....	73
2.6	INTRODUCTION TO MAGNETIC RESONANCE IMAGING	74
2.6.1	Longitudinal (T_1) Relaxation.....	74
2.6.2	Transverse (T_2) Relaxation.....	75
2.6.3	Image Weighting.....	76
2.6.4	MRI T2 Contrast Agents	78
2.7	REFERENCES.....	80
3	Modeling to Predict Solution Sizes and Colloidal Stabilities of the Complexes	87
3.1	ABSTRACT.....	87
3.2	INTRODUCTION.....	88
3.3	EXPERIMENTAL	90
3.3.1	Synthesis of Polymer-Stabilized Magnetite via the Co-Precipitation Method..	90
3.3.2	Synthesis of Polymer-Stabilized Magnetite via the High Temperature Method	90
3.4	MODELING THEORY.....	91
3.5	RESULTS	99
3.5.1	Reproducibility of Particle Size Distributions – Co-precipitation Synthesis Method	99
3.5.2	Reproducibility of Particle Size Distributions – High Temperature Synthesis Method	100
3.5.3	Comparison of Particle Diameters from Image Analysis and X-ray Diffraction	102
3.5.4	Use of the Density Distribution Model to Predict Particle Diameters – Comparison to Dynamic Light Scattering.....	103
3.5.5	Use of $t_{1/2}$ to Predict Complex Stability	104
3.6	CONCLUSIONS	106
3.7	REFERENCES.....	107
4	Synthesis and Colloidal Properties of Polyether-Magnetite Complexes in Water and Phosphate Buffered Saline.....	108
4.1	ABSTRACT.....	108
4.2	INTRODUCTION.....	109
4.3	EXPERIMENTAL	111
4.3.1	Materials.....	111
4.3.2	Synthesis of trivinylsilyl-functional PEO	111
4.3.3	Synthesis of trivinylsilyl-functional PPO-b-PEO (trivinylsilyl-PPO-b-PEO- OH)	112
4.3.4	Functionalization of trivinylsilyl-functional polyethers with ammonium groups.....	113
4.3.5	Magnetite synthesis via reduction of $\text{Fe}(\text{acac})_3$	113
4.3.6	Adsorption of polyether stabilizers onto magnetite nanoparticles	114
4.4	CHARACTERIZATION	114

4.4.1	Modeling to Predict Solution Sizes and Colloidal Stabilities of the Complexes	116
4.5	RESULTS AND DISCUSSION.....	123
4.5.1	Synthesis of polyether-magnetite complexes	123
4.5.2	Relationships among the amphiphilic nature of the polymer dispersion stabilizers, the density of chains on the magnetite surface and the colloidal properties of the complexes.....	126
4.5.3	Influence of material parameters on clustering of the complexes	130
4.5.4	Suitability of three ammonium ions as anchor segments for magnetite in water and PBS	132
4.6	CONCLUSIONS	136
4.7	REFERENCES.....	138
5	Novel Phosphonate-functional Poly(ethylene oxide)-Magnetite Nanoparticles form Stable Colloidal Dispersions in Phosphate Buffered Saline.....	141
5.1	ABSTRACT.....	141
5.2	INTRODUCTION.....	142
5.3	EXPERIMENTAL	143
5.3.1	Materials.....	143
5.3.2	Synthesis of 3-hydroxypropyldimethylvinylsilane (3-HPMVS) and 3-hydroxypropyltrivinylsilane (3-HPTVS)	144
5.3.3	Synthesis of dimethylvinylsilyl-functional PEO-OH (dimethylvinylsilyl-PEO-OH).....	145
5.3.4	Synthesis of trivinylsilyl-functional PEO-OH (trivinylsilyl-PEO-OH).....	146
5.3.5	Functionalization of dimethylvinylsilyl-PEO-OH with carboxylic acid groups.....	146
5.3.6	Functionalization of dimethylvinylsilyl-PEO-OH or trivinylsilyl-PEO-OH with ammonium group(s).....	147
5.3.7	Michael addition of ammonium-PEO-OH or ammonium ₃ -PEO-OH onto diethyl vinyl phosphonate	148
5.3.8	Hydrolysis of diethylphosphonate-PEO-OH (or diethylphosphonate ₃ -PEO-OH) yielding phosphonic acid-PEO-OH (or phosphonic acid ₃ -PEO-OH).	148
5.3.9	Magnetite synthesis via reduction of Fe(acac) ₃	149
5.3.10	Adsorption of functional polyether stabilizers onto magnetite nanoparticles	149
5.3.11	Characterization	150
5.4	RESULTS AND DISCUSSION.....	151
5.4.1	Synthesis of PEO oligomers with different functional endgroups for binding to magnetite	151
5.4.2	Formation of PEO-magnetite complexes via adsorption of the functional PEO oligomers onto the surfaces of magnetite nanoparticle	158
5.4.3	Determination of the efficacies of various anchor groups for magnetite nanoparticles in PBS	160
5.4.4	Investigation of colloidal stabilities of the PEO-magnetite nanoparticle dispersions in water and PBS using DLS.....	161
5.5	CONCLUSIONS	168
5.6	REFERENCES.....	170

6 The Design of Stable, Well-Defined Polyether-Magnetite Complexes In Aqueous Media: Effect of Anchor Group, Molecular Weight, and Magnetite Loading.... 172

6.1	ABSTRACT.....	172
6.2	INTRODUCTION.....	173
6.3	EXPERIMENTAL	174
6.3.1	Materials.....	174
6.3.2	Synthesis of vinylsilyl-PEO-OH oligomers	175
6.3.3	Functionalization of trivinylsilyl-PEO-OH with mercaptoacetic acid.....	176
6.3.4	Functionalization of trivinylsilyl-PEO-OH with 2-mercaptoethylamine hydrochloride.....	176
6.3.5	Michael addition of diethyl vinyl phosphonate to triammonium-PEO-OH	177
6.3.6	De-esterification of tri(diethyl phosphonate)-PEO-OH yielding tri(phosphonic acid)-PEO-OH.....	177
6.3.7	Magnetite synthesis via high temperature reduction of Fe(acac) ₃	178
6.3.8	Adsorption of trifunctional polyether stabilizers onto magnetite nanoparticles	178
6.3.9	Characterization.....	179
6.3.10	Density distribution model to predict sizes of the polyether-magnetite nanoparticle complexes.....	180
6.4	RESULTS AND DISCUSSION.....	184
6.4.1	Synthesis of PEO oligomers with different functional endgroups for binding to magnetite	184
6.4.2	Formation of PEO-magnetite complexes via adsorption of the functional PEO oligomers onto the surfaces of magnetite nanoparticles	185
6.4.3	Colloidal Stability of Polyether-Magnetite Complexes in Deionized Water....	186
6.4.4	Colloidal Stability of Polyether-Magnetite Complexes in Phosphate Buffered Saline.....	195
6.5	CONCLUSIONS	202
6.6	REFERENCES.....	205

7 The Design of Amphiphilic Polyether-Magnetite Complexes for Enhancement of MRI Contrast and Cell Entry 207

7.1	ABSTRACT.....	207
7.2	INTRODUCTION.....	208
7.3	EXPERIMENTAL	209
7.3.1	Materials.....	209
7.3.2	Synthesis of Polyether Stabilizers.....	210
7.3.3	Magnetite synthesis via high temperature reduction of Fe(acac) ₃	210
7.3.4	Adsorption of trifunctional polyether stabilizers onto magnetite nanoparticles	210
7.3.5	Characterization.....	210
7.3.6	Application of a brush model to the polyether-magnetite complexes	212
7.4	RESULTS AND DISCUSSION.....	215
7.4.1	Modeling and Stability in DI Water.....	215
7.4.2	Colloidal Stability of diblock copolymer complexes.....	217

7.4.3	Solvation of the hydrophobic poly(propylene oxide) (PPO) Layer.....	218
7.4.3.1	Small Angle Neutron Scattering to Determine PPO Solvation	219
7.4.3.2	QCM-D to Compare Adsorption of Various Polymer Brushes	224
7.4.4	Relaxation behavior of diblock copolymer complexes.....	228
7.5	CONCLUSIONS	235
7.6	REFERENCES.....	237
8	The Design of Well-Defined Polydimethylsiloxane-Magnetite Complexes.....	239
8.1	ABSTRACT.....	239
8.2	INTRODUCTION	240
8.3	EXPERIMENTAL	241
8.3.1	Materials.....	241
8.3.2	Synthesis of trivinylsilyl-terminated PDMS	242
8.3.3	Functionalization of trivinylsilyl-terminated PDMS with mercaptoacetic acid	243
8.3.4	Magnetite synthesis via co-precipitation of iron chlorides with ammonium hydroxide.....	243
8.3.5	Magnetite synthesis via reduction of Fe (III) acetylacetonate	244
8.3.6	Magnetic separations of PDMS-magnetite nanoparticle fluids.....	245
8.3.7	Characterization.....	246
8.3.8	Density distribution model to predict sizes of the PDMS-magnetite nanoparticle complexes	247
8.4	RESULTS AND DISCUSSION.....	251
8.4.1	Synthesis of PDMS-magnetite complexes	251
8.4.2	Characterization of PDMS-magnetite complexes synthesized through co-precipitation.....	253
8.4.3	Characterization of PDMS-magnetite complexes synthesized through thermal reduction of Fe(acac) ₃	259
8.5	CONCLUSIONS	261
8.6	REFERENCES.....	263
9	Determination of the Catalytic Activity of Cerium Oxide Nanoparticles in Aqueous Media.....	266
9.1	ABSTRACT.....	266
9.2	INTRODUCTION	267
9.3	EXPERIMENTAL	269
9.4	RESULTS AND DISCUSSION.....	274
9.4.1	Characterization of Cerium Oxide Nanoparticles.....	274
9.4.2	pH Effects on Catalytic Activity.....	278
9.4.3	Effect of Phosphate on Catalytic Activity.....	280
9.5	CONCLUSIONS	282
9.6	REFERENCES.....	284
10	Conclusions and Future Work.....	286
10.1	CONCLUSIONS	286
10.2	FUTURE WORK	289
10.2.1	Refinement of the density distribution model	290

10.2.1.1	Incorporation of a Core/Shell Hamaker Constant Calculation	290
10.2.1.2	Incorporation of a Hydrophobic Interaction of PPO Layers	292
10.2.2	Development of Controlled Magnetite Clusters	293
10.2.2.1	Development of Controlled Magnetite Clusters	293
10.2.3	Quantifying the Interaction Between Polymer-Magnetite Complexes and Specific Surfaces.....	298
10.3	REFERENCES.....	302
Appendix – Mathematica Calculations for the Density Distribution Model.....		303

List of Figures

FIGURE 2.1. BASIC CONFIGURATION FOR A LIGHT SCATTERING EXPERIMENT (REDRAWN BY W. MILES). ¹ ...	10
FIGURE 2.2. THE SCATTERING VECTOR OF WATER AT 25 °C AND A WAVELENGTH OF 633 NM VARIES WITH THE SCATTERING ANGLE, ALLOWING PROPERTIES TO BE EXPLORED USING MULTI-ANGLE LIGHT SCATTERING.....	11
FIGURE 2.3. A ZIMM PLOT CONSTRUCTED USING STATIC LIGHT SCATTERING TO EXTRAPOLATE VALUES AT CONCENTRATION AND θ AS THEIR LIMIT APPROACHES ZERO (REDRAWN BY W. MILES). ⁴	14
FIGURE 2.4. AUTOCORRELATION CURVES FOR DIFFERENT PARTICLE SIZES DECAY AT DIFFERENT RATES. ...	16
FIGURE 2.5. A POSITIVELY CHARGED PARTICLE IS SURROUNDED BY AN ELECTRIC DOUBLE LAYER CONSISTING OF THE STERN LAYER AND THE DIFFUSE LAYER. THE ZETA POTENTIAL IS MEASURED AT THE SLIPPING PLANE (REDRAWN BY W. MILES). ^{6,7}	18
FIGURE 2.6. THE DISSIPATION OF THE CRYSTAL OSCILLATIONS IS MUCH SLOWER FOR A RIGID FILM THAN FOR A SOFT FILM (REDRAWN BY W. MILES). ⁹	21
FIGURE 2.7. THE PROTON HIGH AND LOW ENERGY STATES MOVE FARTHER APART AS THE APPLIED MAGNETIC FIELD GETS STRONGER (REDRAWN BY W. MILES). ¹⁴	23
FIGURE 2.8. DIAGRAM OF A TYPICAL NMR SETUP (REDRAWN BY W. MILES). ¹⁶	24
FIGURE 2.9. ILLUSTRATION OF A TYPICAL SEC SET-UP. MULTIPLE COLUMNS ARE SHOWN HERE TO ILLUSTRATE THAT VARIOUS COLUMNS ARE NEEDED DEPENDING UPON THE APPLICATION. ²⁰	26
FIGURE 2.10. THE SURFACE POTENTIAL DECAYS TO THE BULK POTENTIAL LARGE DISTANCES FROM THE SURFACE (REDRAWN BY W. MILES). ⁶	30
FIGURE 2.11. TYPICAL ZETA POTENTIAL VERSUS pH CURVE FOR A METAL OXIDE WITH AN INDIFFERENT ELECTROLYTE ADDED AT INCREASING CONCENTRATIONS. ⁶	32
FIGURE 2.12. THE FOUR MAIN TYPES OF MAGNETISM ARE REPRESENTED HERE BY DEPICTION OF THE SPIN OF THE FREE ELECTRONS, WHICH AFFECT THE MAGNITUDE OF THE MAGNETIZATION VECTOR. ⁴⁵	33
FIGURE 2.13. STRUCTURAL REPRESENTATION OF PROTONATION/DEPROTONATION STEPS AND THE APPROPRIATE pK_a VALUES FOR THE ORTHOPHOSPHATE SPECIES (REDRAWN BY W. MILES). ⁸⁶	42
FIGURE 2.14. THE ADSORPTION ISOTHERM REACHES A PLATEAU AS THE POLYMER CONCENTRATION IS INCREASED. TRANSITIONS FROM HIGH TO LOW POLYMER CONCENTRATION DOES NOT REDUCE THE POLYMER ADSORBED ONTO THE SURFACE (DOTTED LINE) (REDRAWN BY W. MILES). ⁹²	44
FIGURE 2.15. POLYMERS ADSORB TO SOLID SURFACES IN TAILS, LOOPS, AND TRAINS (REDRAWN BY W. MILES). ⁹²	45
FIGURE 2.16. THE SAME DIBLOCK COPOLYMER ADSORBS TO A SOLID SURFACE DIFFERENTLY DEPENDING ON THE TYPE OF SOLVENT BEING USED FOR DISPERSION (REDRAWN BY W. MILES). ⁹²	46
FIGURE 2.17. FOR NON-SELECTIVE SOLVENTS, THE MOLECULAR WEIGHT OF EACH BLOCK DETERMINES HOW THE DIBLOCK POLYMER WILL ADSORB (REDRAWN BY W. MILES). ⁹²	47
FIGURE 2.18. MODELING OF STAR-SHAPED POLYMERS AS CONCENTRIC SHELLS WITH CONSTANT BLOB SIZE FROM A CENTRAL POINT (REDRAWN BY W. MILES). ¹⁰⁷	54
FIGURE 2.19. THE POLYMER VOLUME FRACTION IS CONSTANT AT THE CENTER BUT THEN DECREASES AS THE BLOB SIZE INCREASES (REDRAWN BY W. MILES). ¹⁰⁷	55
FIGURE 2.20. VAGBERG ET AL. TREATED AMPHOPHILIC DIBLOCK COPOLYMERS AS A MICELLE WITH A SOLID NON-SOLVATED CORE AND A HYDROPHILIC STAR-SHAPED POLYMER BRUSH. ¹⁰⁶	55
FIGURE 2.21. INCORPORATION OF A NON-SOLVATED SHELL TO REPRESENT A HYDROPHOBIC BLOCK WAS INTRODUCED BY ZHANG, ET AL. ¹¹⁰	56
FIGURE 2.22. ADDITION POLYMERIZATION OF ETHYLENE TO POLYETHYLENE AND METHYL METHACRYLATE TO POLY(METHYL METHACRYLATE) (REDRAWN BY W. MILES). ¹³⁰	66
FIGURE 2.23. SCHEMATIC REPRESENTATION OF STEP GROWTH POLYMERIZATION MECHANISM (REDRAWN BY W. MILES). ¹³⁰	67
FIGURE 2.24. HIGH MOLECULAR WEIGHTS REQUIRES HIGH PERCENT CONVERSION FOR STEP GROWTH POLYMERIZATION (REDRAWN BY W. MILES). ¹³⁰	68
FIGURE 2.25. FOR CHAIN GROWTH POLYMERIZATIONS, MOLECULAR WEIGHT IS IDENTICAL AT ALL PERCENT CONVERSIONS, MAKING IT IDEAL FOR LOW CONVERSION APPLICATIONS (REDRAWN BY W. MILES). ¹³⁰	69
FIGURE 2.26. INITIATION, PROPAGATION, AND TERMINATION IN A CHAIN GROWTH POLYMERIZATION (REDRAWN BY W. MILES). ¹³⁰	70

FIGURE 2.27. FOR LIVING POLYMERIZATIONS, MOLECULAR WEIGHT VARIES LINEARLY WITH CONVERSION (REDRAWN BY W. MILES). ¹³⁰	72
FIGURE 2.28. RESTORATION OF LONGITUDINAL MAGNETIZATION VECTOR FOLLOWING RF PULSE (REDRAWN BY W. MILES). ¹⁹	75
FIGURE 2.29. COMBINED LONGITUDINAL (T_1) AND TRANSVERSE (T_2) RELAXATION (REDRAWN BY W. MILES). ¹⁹	76
FIGURE 2.30. BY ADJUSTING THE REPETITION TIME AND THE T_1 RELAXATION, CONTRAST ON A T_1 -WEIGHTED IMAGE CAN BE ACHIEVED (REDRAWN BY W. MILES). ¹⁹	77
FIGURE 2.31. BY ADJUSTING THE REPETITION TIME AND THE T_2 RELAXATION, CONTRAST CAN BE ACHIEVED (REDRAWN BY W. MILES). ¹⁹	78
FIGURE 3.1. MODELING OF STAR-SHAPED POLYMERS AS CONCENTRIC SHELLS WITH CONSTANT BLOB SIZE FROM A CENTRAL POINT (REDRAWN BY W. MILES). ²	88
FIGURE 3.2. THE POLYMER VOLUME FRACTION IS CONSTANT AT THE CENTER BUT THEN DECREASES AS THE BLOB SIZE INCREASES (REDRAWN BY W. MILES). ²	89
FIGURE 3.3. VAGBERG ET AL. TREATED AMPHOPHILIC DIBLOCK COPOLYMERS AS A MICELLE WITH A SOLID NON-SOLVATED CORE AND A HYDROPHILIC STAR-SHAPED POLYMER BRUSH. ¹	89
FIGURE 3.4. PROBABILITY HISTOGRAMS OF COMPLEXES WERE GENERATED BY MODIFYING THE ORIGINAL IMAGE (A) TO A CONTRAST-ENHANCED IMAGE (B) USING FOVEA PRO 4.0 SOFTWARE. THE IMAGES FOR A COMPLEX WITH A TRIAMMONIUM-5.6kPPO-B-7.2kPEO-OH COPOLYMER ARE SHOWN.	92
FIGURE 3.5. SMALL DIFFERENCES ARE OBSERVED IN THE PARTICLE HISTOGRAMS FOR COMPLETELY DIFFERENT BATCHES OF THE CO-PRECIPIRATION MAGNETITE SYNTHESIS.	100
FIGURE 3.6. SMALL DIFFERENCES ARE OBSERVED IN THE PARTICLE HISTOGRAMS FOR COMPLETELY DIFFERENT BATCHES OF THE HIGH-TEMPERATURE MAGNETITE SYNTHESIS.	101
FIGURE 3.7. POWDER XRD PATTERN OF HIGH TEMPERATURE MAGNETITE SYNTHESIS.	103
FIGURE 3.8. THE POTENTIAL ENERGY DIAGRAM FOR THE PHOSPHONATE-TERMINATED 2k PEO PREDICTS FLOCCULATION AT 30% POLYMER LOADING, BUT NOT AT 50% OR 70% POLYMER LOADING.	105
FIGURE 4.1. PROBABILITY HISTOGRAMS OF COMPLEXES WERE GENERATED BY MODIFYING THE ORIGINAL IMAGE (A) TO A CONTRAST-ENHANCED IMAGE (B) USING FOVEA PRO 4.0 SOFTWARE. THE IMAGES FOR A COMPLEX WITH A TRIAMMONIUM-5.6kPPO-B-7.2kPEO-OH COPOLYMER ARE SHOWN.	117
FIGURE 4.2. PROBABILITY HISTOGRAMS FOR A TRIAMMONIUM-5.6kPPO-B-7.2kPEO-OH COMPLEX (GRAY) AND A TRIAMMONIUM-7.2kPEO-OH COMPLEX (BLACK) INDICATE THAT THE MAGNETITE CORE SIZE DISTRIBUTION IS REPRODUCIBLE.	118
FIGURE 4.3. SYNTHESIS OF A HETEROBIFUNCTIONAL TRIVINYLSILYL-PPO-B-PEO-OH COPOLYMER.	124
FIGURE 4.4. ^1H NMR ILLUSTRATES THE DISAPPEARANCE OF VINYL GROUPS (1) ON A 7.2kPEO-OH HOMOPOLYMER AFTER ADDITION OF CYSTEAMINE ACROSS THE VINYLSILANE GROUPS.	125
FIGURE 4.5. POTENTIAL ENERGY CURVES FOR MAGNETITE-COPOLYMER COMPLEXES WITH TRIAMMONIUM-7.2kPEO-OH COMPLEXES (A) AND TRIAMMONIUM-5.6kPPO-B-7.2kPEO-OH (B)	129
FIGURE 4.6. INTENSITY WEIGHTED DIAMETERS FOR THE MAGNETITE-COPOLYMER COMPLEXES OBTAINED FROM DLS VS. THE CALCULATED $\text{LN}(T_{1/2})$: 5.6kPPO-B-7.2kPEO-OH (DIAMONDS), 5.6kPPO-B-3.8kPEO-OH (TRIANGLES), 3.3kPPO-B-4.8kPEO-OH (SQUARES), AND 3.3kPPO-B-2.6kPEO-OH (EMPTY CIRCLES)	131
FIGURE 4.7. DLS SHOWS THAT THE COMPLEXES WITH THE TRIAMMONIUM-5.6kPPO-B-7.2kPEO-OH HAVE LONG-TERM STABILITY IN DI WATER (A) BUT NOT IN PBS (B). PERCENTAGES SHOWN ARE % POLYMER IN THE COMPLEXES.	132
FIGURE 4.8. THE ZETA POTENTIAL OF MAGNETITE WITHOUT POLYMER (A) AND MAGNETITE COATED WITH POLYMER (B, TRIAMMONIUM-7.2kPEO-OH) CHANGES AND FOLLOWS THE SAME TREND WHEN MEASURED IN PBS COMPARED TO DI WATER. THE LOWEST pK_a OF PHOSPHORIC ACID IS ~ 2.2 , WHICH CORRESPONDS TO THE ISOELECTRIC POINT OF BOTH SYSTEMS.	134
FIGURE 5.1. ^1H NMR OF A $2,900 \text{ g mol}^{-1}$ DIMETHYL VINYLSILYL-PEO-OH OLIGOMER	153
FIGURE 5.2. GPC CHROMATOGRAM OF A DIMETHYL VINYLSILYL-PEO-OH OLIGOMER SHOWING A M_n OF $7,900 \text{ g mol}^{-1}$ AND A MOLECULAR WEIGHT DISTRIBUTION OF 1.05	153
FIGURE 5.3. ENE-THIOL ADDITION OF MERCAPTOACETIC ACID TO A $2,900 \text{ g mol}^{-1}$ POLYETHER YIELDING A CARBOXYLIC ACID-FUNCTIONALIZED PEO (HOOC-PEO-OH)	154
FIGURE 5.4. ENE-THIOL ADDITION OF MERCAPTOETHYLAMINE HYDROCHLORIDE TO A $2,900 \text{ g mol}^{-1}$ DIMETHYL VINYLSILYL-PEO-OH TO PRODUCE AMMONIUM-PEO-OH	155
FIGURE 5.5. ^1H NMR OF A $2,900 \text{ g mol}^{-1}$ DIETHYLPHOSPHONATE-PEO-OH	157

FIGURE 5.6. ^1H NMR OF A $2,900\text{ G MOL}^{-1}$ ZWITTERIONIC PHOSPHONATE-PEO-OH	158
FIGURE 5.7. DEPICTION OF A TRI-ZWITTERIONIC PHOSPHONATE-PEO-OH BINDING TO THE SURFACE OF A MAGNETITE NANOPARTICLE	158
FIGURE 5.8. TEM OF THE MAGNETITE NANOPARTICLES (A) AND A HISTOGRAM OF THE PARTICLE SIZE DISTRIBUTION (B).	160
FIGURE 5.9. INTENSITY WEIGHTED DIAMETERS FROM DLS OF THE $2,900$ (OPEN CIRCLES) AND $8,300\text{ G MOL}^{-1}$ (BLACK DIAMONDS) AMMONIUM-PEO-MAGNETITE COMPLEXES IN DI WATER (A) AND PBS (B) OVER 24 HOURS.....	161
FIGURE 5.10. DLS INTENSITY WEIGHTED DIAMETERS OF THE $2,900$ (OPEN CIRCLES) AND $8,300\text{ G MOL}^{-1}$ (BLACK DIAMONDS) OF CARBOXYLATE-PEO-MAGNETITE COMPLEXES IN DI WATER (A) AND PBS (B) OVER 24 HOURS. A MAGNIFIED PLOT OF THE $2,900\text{ G MOL}^{-1}$ CARBOXYLATE-PEO-MAGNETITE COMPLEX IN PBS IS SHOWN IN (C).....	163
FIGURE 5.11. ZETA POTENTIALS INDICATE THE CHARGE CHARACTERISTICS OF MAGNETITE NANOPARTICLE SURFACES IN DI WATER VERSUS PBS.	164
FIGURE 5.12. DLS INTENSITY WEIGHTED DIAMETERS OF THE $2,900$ (OPEN CIRCLES) AND $8,300\text{ G MOL}^{-1}$ (BLACK DIAMONDS) MONO-ZWITTERIONIC PHOSPHONATE-PEO-MAGNETITE COMPLEXES IN DI WATER (A) AND PBS (B) OVER 24 HOURS	165
FIGURE 5.13. DLS INTENSITY WEIGHTED DIAMETERS OF (A) THE $2,900\text{ G MOL}^{-1}$ MONO-ZWITTERIONIC PHOSPHONATE-PEO-MAGNETITE COMPLEX IN DI WATER (BLACK DIAMONDS) AND 0.17 M NaCl (OPEN CIRCLES), AND (B) THE $8,300\text{ G MOL}^{-1}$ MONO-ZWITTERIONIC PHOSPHONATE-PEO-MAGNETITE COMPLEX IN DI WATER (BLACK DIAMONDS) AND 0.17 M NaCl (OPEN CIRCLES).....	166
FIGURE 5.14. INTENSITY WEIGHTED DIAMETERS OF THE $3,400$ (OPEN CIRCLES) AND $9,100\text{ G MOL}^{-1}$ (BLACK DIAMONDS) TRI-ZWITTERIONIC PHOSPHONATE-PEO-MAGNETITE COMPLEXES IN DI WATER (A) AND PBS (B) OVER 24 HOURS	167
FIGURE 6.1. A REPRESENTATIVE TEM IMAGE OF POLYETHER-MAGNETITE COMPLEXES INDICATE A WELL- DEFINED PARTICLE DISTRIBUTION.	186
FIGURE 6.2. ALL MONOAMMONIUM- AND MONOCARBOXYLIC ACID-ANCHORED COMPLEXES WERE STABLE FOR 24 HOURS IN DI WATER (APPROXIMATELY 30% MAGNETITE LOADING FOR EACH).....	187
FIGURE 6.3. INTENSITY WEIGHTED DIAMETERS (D_i) OF THE $2,900\text{ G MOL}^{-1}$ MONO-PHOSPHONATE-PEO-OH COMPLEXES IN DI WATER (A) AND $0.17\text{ M SODIUM CHLORIDE}$ (B).....	187
FIGURE 6.4. AGGREGATION OF THE MONOPHOSPHONATE-ANCHORED $8,300\text{ G MOL}^{-1}$ PEO COMPLEXES OCCURRED IN DI WATER (A). THIS AGGREGATION WAS HINDERED WITH THE ADDITION OF 0.17 M SODIUM CHLORIDE (B).....	188
FIGURE 6.5. PLOTS OF THE STERIC POTENTIAL AS A FUNCTION OF SURFACE TO SURFACE SEPARATION FOR THE $8,300\text{ G MOL}^{-1}$ COMPLEX WITH 32% AND 47% MAGNETITE LOADINGS.....	190
FIGURE 6.6. STERIC POTENTIALS FOR THE $2,900\text{ G MOL}^{-1}$ COMPLEXES WITH 37 AND 48% MAGNETITE LOADINGS.	191
FIGURE 6.7. INTENSITY WEIGHTED DIAMETERS (D_i) OF (A) $3,400\text{ G MOL}^{-1}$ AND (B) $9,100\text{ G MOL}^{-1}$ PEO COMPLEXES WITH TRIPHOSPHONATE ANCHORS IN DI WATER OVER 24 HRS.....	192
FIGURE 6.8. VISUALIZATION OF THE AREA OCCUPIED BY THE PHOSPHONATE ANCHOR GROUP IN CONTACT WITH THE MAGNETITE SURFACE.	193
FIGURE 6.9. ZETA POTENTIAL MEASUREMENTS INDICATE THAT PHOSPHATE IS A POTENTIAL DETERMINING ION FOR MAGNETITE.	196
FIGURE 6.10. AGGREGATION FOR THE COMPLEXES MADE WITH TRICARBOXYLATE ANCHOR GROUPS (A) INDICATES TWO DISTINCT DESORPTION MECHANISMS COMPARED TO THE AMMONIUM ANCHOR GROUPS (B).	199
FIGURE 7.1. CARTOON DEPICTION OF A MAGNETITE CORE, NON-SOLVATED PPO LAYER, AND AN EXTENDED PEO BRUSH IN CONCENTRIC BLOBS EXTENDING OUT FROM THE PPO SURFACE.....	212
FIGURE 7.2. DLS OF THE TRIAMMONIUM $3.3\text{kPPO-B-2.6kPEO-OH}$ COMPLEX (A) AND THE TRIPHOSPHONATE 3kPPO-B-3kPEO-OH COMPLEX OVER TIME IN PBS AT 25°C	218
FIGURE 7.3. THE DIFFERENCE IN SCATTERING IN 100% H_2O BETWEEN A MAGNETITE COMPLEX WITH AN ATTACHED NON-SOLVATED, DEUTERATED PPO LAYER AND A COMPLEX WITH A NONSOLVATED, HYDROGENATED PPO LAYER.	221
FIGURE 7.4. SANS DATA FOR MAGNETITE COMPLEXES STABILIZED WITH THE $3.1\text{kDPPO-B-3.4kPEO-OH}$ (LEFT) AND $3.3\text{kPPO-B-2.6kPEO-OH}$ (RIGHT) BLOCK COPOLYMERS AT THE INDICATED $\text{H}_2\text{O}/\text{D}_2\text{O}$ RATIOS.....	222

FIGURE 7.5. DLS MEASUREMENTS OF MAGNETITE COMPLEXES MADE WITH HYDROGENATED DIBLOCKS WITH TRIAMMONIUM AND TRIPHOSPHONATE ANCHORS AT VARIOUS WT% POLYMER LOADINGS IN 100% H ₂ O.	224
FIGURE 7.6. ADSORPTION OF THE TRIAMMONIUM-TERMINATED 5kPEO-OH HOMOPOLYMER AND 3.3kPPO-B-2.6kPEO-OH DIBLOCK COPOLYMER FROM DI WATER AT 25°C ONTO AN Fe ₃ O ₄ -COATED QUARTZ CRYSTAL. THE LEVEL OF THE ADSORPTION OF THE DIBLOCK IS ABOUT TWICE THAT OF THE HOMOPOLYMER.	225
FIGURE 7.7. ADSORPTION OF THE TRIAMMONIUM-TERMINATED 5kPEO-OH HOMOPOLYMER AND 5.6kPPO-B-3.8kPEO-OH DIBLOCK COPOLYMER FROM DI WATER AT 25°C ONTO AN Fe ₃ O ₄ -COATED QUARTZ CRYSTAL. THE DISSIPATION FACTOR OF BOTH POLYMERS IS NEARLY THE SAME.	226
FIGURE 7.8. ADSORPTION OF THE TRIAMMONIUM-TERMINATED 5.6kPPO-B-3.8kPEO-OH DIBLOCK COPOLYMER FROM DI WATER AT 25°C ONTO AN Fe ₃ O ₄ -COATED QUARTZ CRYSTAL. THE DISSIPATION FACTOR IS MUCH HIGHER THAN THE DISSIPATION FOR THE POLYMERS SHOWN IN FIGURE 7.7.	227
FIGURE 7.9. MODIFIED DLVO POTENTIAL ENERGY DIAGRAMS FOR POLYMER-STABILIZED MAGNETITE PARTICLES AND CLUSTERS. IN THE LATTER, THE NUMBER OF MAGNETITE PARTICLES PER CLUSTER, N, IS VARIED. THE RANGE OF THE MAGNETIC POTENTIAL INCREASES (RIGHT) BUT THE RANGE OF THE VAN DER WAAL'S POTENTIAL DOES NOT (LEFT) AS N INCREASES.	229
FIGURE 7.10. A SECONDARY POTENTIAL ENERGY MINIMUM CAN BE INDUCED BY A MAGNETIC FIELD WHEN THE CLUSTER SIZE GETS LARGE ENOUGH (ASSUMES 10kPEO WITH A SURFACE COVERAGE OF 1 CHAIN NM ⁻²).	230
FIGURE 7.11. THE LOCAL MAGNETIC FIELD BECOMES EXTREMELY INHOMOGENEOUS EVEN FOR SMALL MAGNETIC CLUSTERS.	231
FIGURE 7.12. TRI-ZWITTERIONIC PHOSPHONATE-ANCHORED 3.4kPEO-OH COMPLEXES IN DI WATER AT 25°C – MODIFIED DLVO ENERGY DIAGRAM AS A FUNCTION OF WT% POLYMER LOADING (A) AND RELAXIVITIES AND DLS INTENSITY WEIGHTED HYDRODYNAMIC DIAMETERS (B).	232
FIGURE 7.13. RELAXATION BEHAVIOR FOR TRIAMMONIUM 3.3kPPO-B-4.8kPEO-OH (A) AND 3.3kPPO-B-2.6kPEO-OH (B) COMPLEXES.	232
FIGURE 7.14. RELAXATION DATA VS. MAGNETITE LOADING FOR THE TRI-ZWITTERIONIC PHOSPHONATE 3kPPO-B-5kPEO-OH COMPLEXES.	234
FIGURE 7.15. MEASURE OF R123 ACCUMULATION OF 51% MAGNETITE 3.3kPPO-B-4.8kPEO-OH (A) AND 48% MAGNETITE 3.3kPPO-B-2.6kPEO-OH (B) COMPLEXES IN MCF7/ADR CELLS.	235
FIGURE 8.1. ¹ H NMR CONFIRMS THE ADDITION OF MERCAPTOACETIC ACID ACROSS VINYL-SILANE GROUPS ON A 3,100 G MOL ⁻¹ TRIVINYLSILYL-TERMINATED PDMS. THE UPPER SPECTRUM IS TRIVINYLSILYL-TERMINATED PDMS; LOWER SPECTRUM IS THE TRICARBONYLATE-FUNCTIONAL PDMS	252
FIGURE 8.2. TEM IMAGES OF A 5,100 G MOL ⁻¹ PDMS-MAGNETITE COMPLEX BEFORE MAGNETIC SEPARATION (A) AND FOLLOWING FIVE MAGNETIC SEPARATIONS (B).	253
FIGURE 8.3. THE NUMBER WEIGHTED DIAMETER DISTRIBUTIONS OF THE 3,050, 5,100, 6,900, AND 10,200 G MOL ⁻¹ PDMS-MAGNETITE COMPLEXES IN CHLOROFORM AT 25 °C DISPLAY THE EFFECT OF MAGNETIC SEPARATION FOR A MAGNETIC FIELD ABOVE THE SATURATION MAGNETIZATION AT 10 MG ML ⁻¹ .	254
FIGURE 8.4. IMAGE ANALYSIS SHOWS NO SIGNIFICANT DIFFERENCE BETWEEN THE PARTICLE SIZE HISTOGRAMS FOR THE STARTING MATERIAL (3.4 ± 2.0 NM), FIRST MAGNETIC SEPARATION (3.2 ± 1.8 NM), AND FIFTH MAGNETIC SEPARATION (3.1 ± 1.6 NM) OF THE 6,900 G MOL ⁻¹ PDMS-MAGNETITE COMPLEX.	256
FIGURE 8.5. FOR EACH MOLECULAR WEIGHT, APPROXIMATELY 80 PERCENT OF THE MATERIAL IS REMOVED AFTER FIVE SEPARATIONS.	257
FIGURE 8.6. POTENTIAL ENERGY DIAGRAMS OF THE 3,050 (A), 5,100 (B), 6,900 (C), AND 10,200 G MOL ⁻¹ (D) PDMS-MAGNETITE COMPLEXES (COMPOSITIONS SHOWN IN TABLE 8.2) SHOW DEEPER SECONDARY MINIMUM ENERGY WELLS FOR THE SMALLER POLYMER BRUSH WITH AN APPLIED MAGNETIC FIELD ABOVE THEIR SATURATION MAGNETIZATION.	258
FIGURE 8.7. A REPRESENTATIVE TEM IMAGE (A) SHOWS A WELL-DEFINED MAGNETITE CORE WHILE PARTICLE HISTOGRAMS OF REPEATED MAGNETITE SYNTHESSES (B) SHOW A HIGH DEGREE OF REPRODUCIBILITY FOR THREE DIFFERENT MAGNETITE BATCHES.	259
FIGURE 9.1. CONCENTRATION OF HYDROGEN PEROXIDE BASED ON ABSORBANCE MEASUREMENTS AT 400 NM IS LINEAR FOR THE POTASSIUM TITANIUM OXALATE ASSAY FOR THE HYDROGEN PEROXIDE CONCENTRATIONS USED IN THIS STUDY.	272

FIGURE 9.2. TEM IMAGES OF THE NM (A), N130 (B), AND N160 (C) CERIUM OXIDE NANOPARTICLE SHOW SIGNIFICANT DIFFERENCES IN PARTICLE SIZE AND SHAPE. THE (111) CRYSTAL FACES CORRESPOND TO THE TRIANGULAR-SHAPED PARTICLE SURFACES.	275
FIGURE 9.3. XRD OF THE NM (A) AND N160 (B) SAMPLES SHOW SIGNIFICANT DIFFERENCES IN THE RELATIVE ABUNDANCE OF THE (111) CRYSTALLOGRAPHIC FACE.	276
FIGURE 9.4. CATALYTIC ACTIVITY OF THE THREE CERIUM OXIDE NANOPARTICLE SAMPLES WAS MEASURED BY ANALYZING THE DECOMPOSITION OF HYDROGEN PEROXIDE IN THE PRESENCE OF THE MATERIAL (1 MG mL ⁻¹) AT A pH OF ~ 7.5.	276
FIGURE 9.5. CHARACTERIZATION OF THE CATALYTIC ACTIVITY OF THE CERIUM OXIDE NANOPARTICLES SHOWS A NORMALIZED REACTION RATE CONSTANT OF $0.0143 \pm 0.003 \text{ M}^{-2}\text{MIN}^{-1}$ FOR THE N130 CERIA AT A pH OF ~ 7.5.	277
FIGURE 9.6. DIFFERENCES WERE OBSERVED IN THE UV-VIS SPECTRA FOR THE NM VS. THE N130 CERIA SAMPLES INDICATING A HIGHER CONCENTRATION OF Ce^{3+} FOR THE NM SAMPLE.	277
FIGURE 9.7. THE ISOELECTRIC POINTS OF THE CERIUM OXIDE NANOPARTICLES USED IN THIS STUDY ALL ARE IN LINE WITH LITERATURE VALUES.	279
FIGURE 9.8. CATALYTIC ACTIVITY OF CERIUM OXIDE NANOPARTICLE INCREASES AS pH IS INCREASED (A) AND SEEMS TO BE ASSOCIATED WITH A CHANGE IN THE UV-VIS ABSORBANCE SPECTRA (B) (UNITS OF k' ARE $\text{M}^{-2}\text{MIN}^{-1}$).	280
FIGURE 9.9. ZETA POTENTIAL CONFIRMS THAT PHOSPHATE IS A POTENTIAL DETERMINING ION FOR THE NM (A) AND N130 (B) CERIUM OXIDE SAMPLES.	281
FIGURE 9.10. THE ADDITION OF SODIUM PHOSPHATE SIGNIFICANTLY REDUCES THE CATALYTIC ACTIVITY OF BOTH THE NM (A, $[\text{CeO}_2] = 6.75 \text{ MG mL}^{-1}$) AND N130 (B, $[\text{CeO}_2] = 1 \text{ MG mL}^{-1}$) SAMPLES. HOWEVER, THE NM SAMPLE SHOWS SIGNIFICANTLY MORE REDUCTION IN ACTIVITY (pH ~ 7.5).	281
FIGURE 9.11. THE pH DEPENDENCE OF REACTION RATE IS STILL PRESENT EVEN IN THE PRESENCE OF 0.05 M SODIUM PHOSPHATE FOR THE N130 SAMPLE.	282
FIGURE 10.1. AT THE SAME SURFACE TO SURFACE SEPARATION, A LARGE PPO SHELL MAY INCREASE THE RANGE OF THE HAMAKER CONSTANT.	290
FIGURE 10.2. A CORE/SHELL HAMAKER MODEL HAS A LONGER RANGE THAN A CORE ONLY HAMAKER MODEL.	291
FIGURE 10.3. THE INTENSITY WEIGHTED DIAMETER INCREASES AS POLYMER LOADING DECREASES. HOWEVER, DEVIATION IS SEEN FROM THE EXPONENTIAL FIT IN CERTAIN CASES, WHICH INDICATES INCREASED CLUSTERING.	294
FIGURE 10.4. MAGNETITE CLUSTERS FORMED THROUGH CIJ MIXING.	295
FIGURE 10.5. STABILIZATION OF MAGNETITE NANOPARTICLES VIA A MALEIMIDE FUNCTIONALIZED PEO.	296
FIGURE 10.6. FUNCTIONALIZATION OF THE MALEIMIDE GROUP WITH EITHER CYSTEAMINE HYDROCHLORIDE OR MERCAPTOACETIC ACID ALLOWS CONTROL OF THE COMPLEX SURFACE CHARGE.	296
FIGURE 10.7. CROSS-LINKING CAN BE ACHIEVED THROUGH ADDITION OF DCC OR HOBT.	297
FIGURE 10.8. CROSS-LINKING OF THE MAGNETITE COMPLEXES CAN BE ACHIEVED THROUGH THE ADDITION OF 1,2-ETHANEDITHIOL.	298
FIGURE 10.9. QCM-D DATA COMPARING ADSORPTION OF MAGNETITE COMPLEXES CONTAINING 30% MAGNETITE LOADING WITH 3kPPO-B-5kPEO-OH AND 3kPPO-B-3kPEO-OH STABILIZERS ON OTS-FUNCTIONALIZED SILICA.	300
FIGURE 10.10. NO SIGNIFICANT DIFFERENCES IN ADSORPTION ON AN OTS-FUNCTIONALIZED SILICA SURFACE WERE OBSERVED FOR A MAGNETITE COMPLEX STABILIZED WITH A 3kPPO-B-5kPEO-OH DIBLOCK COPOLYMER AT APPROXIMATELY 30, 40, AND 50% MAGNETITE LOADING.	300

List of Tables

TABLE 2.1. CHEMICAL SHIFTS OF SOME TYPICAL PROTONS FOR ^1H NMR (REDRAWN BY W. MILES). ¹⁶	25
TABLE 2.2. CHEMICAL COMPOSITION AND UNIT CELL CONFIGURATION AFFECTS PHYSICAL PROPERTIES (SUCH AS DENSITY) OF IRON OXIDES. ⁴⁶	32
TABLE 2.3. DIFFERENT CURIE TEMPERATURES, MAGNETIC STRUCTURES, SATURATION MAGNETIZATIONS, AND ANISOTROPY CONSTANTS FOR VARIOUS IRON OXIDES. ⁴⁵	36
TABLE 2.4. CALCULATION OF THE CRITICAL PARTICLE RADIUS FOR SUPERPARAMAGNETIC BEHAVIOR AT ROOM TEMPERATURE AT THE EXTREMES OF THE RANGE OF THE ANISOTROPY CONSTANT.	37
TABLE 2.5. CHARGE COMPLICATES ADSORPTION BOTH WHEN THE SURFACE IS CHARGED AND THE POLYMER IS CHARGED. ⁹²	48
TABLE 2.6. ONLY SIX-MEMBER RINGS ARE NOT THERMODYNAMICALLY FAVORED TO PARTICIPATE IN RING-OPENING POLYMERIZATIONS AT 25 °C. ¹³⁰	73
TABLE 2.7. RELAXATION TIMES FOR DIFFERENT TISSUES IN A 1.5 T MAGNETIC FIELD. ¹⁹	78
TABLE 3.1. THE CALCULATED NUMBER AVERAGE MEAN DIAMETERS ARE ALL WITHIN THE STANDARD DEVIATION OF THE MEASUREMENT FOR THE CO-PRECIPIATION SYNTHESIS.	100
TABLE 3.2. THE CALCULATED NUMBER AVERAGE MEAN DIAMETERS ARE ALL WITHIN THE STANDARD DEVIATION OF THE MEASUREMENT FOR THE HIGH-TEMPERATURE SYNTHESIS.	101
TABLE 3.3. DENSITY DISTRIBUTION PREDICTIONS ARE WITHIN AN AVERAGE OF 7% FOR DIFFERENT MOLECULAR WEIGHT STABILIZERS, MAGNETITE SYNTHESSES, AND ANCHOR GROUPS.	104
TABLE 3.4. AS THE POLYMER LOADING IS REDUCED, DEVIATION OF DLS FROM THE MODEL ALSO INCREASES.	104
TABLE 4.1. POLYETHER MOLECULAR WEIGHTS	125
TABLE 4.2. A DIVERGENCE IN THE EXPERIMENTAL (MEASURED OVER 24 HOURS) AND PREDICTED DIAMETERS OF THE COMPLEXES ALLOWS FOR ESTIMATING THE LOWER LIMIT OF POLYMER LOADING WHERE APPRECIABLE FLOCCULATION OCCURS. THIS PHENOMENON IS MORE PRONOUNCED WHEN COMPARING EXPERIMENTAL AND PREDICTED VOLUME AVERAGE DIAMETERS.	127
TABLE 4.3. AN AVERAGE DEVIATION OF 8% WAS OBSERVED BETWEEN EXPERIMENTAL (MEASURED IMMEDIATELY AFTER DISPERSION BY SONICATION FOLLOWED BY FILTRATION) AND PREDICTED NUMBER AVERAGE DIAMETERS FOR COMPLEXES THAT WERE PREDICTED TO BE STABLE BY DLVO THEORY.	130
TABLE 4.4. DIALYSIS EXPERIMENTS SHOW THAT THESE POLYMER STABILIZERS DESORB APPRECIABLY FROM THE MAGNETITE SURFACE IN PBS OVER 24 HOURS (AFTER DISPERSION BY SONICATION AND FILTRATION). NO DESORPTION WAS OBSERVED AFTER DIALYSIS AGAINST DI WATER.	133
TABLE 4.5. THE CHANGE IN DIAMETER OVER TIME IN PBS INDICATES THAT THE PPO LAYER OF THE DIBLOCK COMPLEXES HINDERS POLYMER DESORPTION FROM THE MAGNETITE SURFACE.	136
TABLE 5.1. MOLECULAR WEIGHTS OF DIMETHYLVINYLSILYL-PEO-OH AND TRIVINYLSILYL-PEO-OH OLIGOMERS	152
TABLE 5.2. COMPOSITIONS OF THE PEO-MAGNETITE NANOPARTICLE COMPLEXES	159
TABLE 6.1. MOLECULAR WEIGHTS OF DIMETHYLVINYLSILYL- AND TRIVINYLSILYL-PEO-OH OLIGOMERS	185
TABLE 6.2. PERCENT SURFACE COVERAGE CALCULATED BASED ON THE SURFACE AREA OCCUPIED BY THE PHOSPHONATE ANCHOR GROUP.	194
TABLE 6.3. RATE OF AGGREGATION OVER 24 HOURS FOR COMPLEXES MADE WITH INDICATED ANCHOR GROUPS AT 25°C IN PBS.	197
TABLE 6.4. FOR MONO-ANCHOR COMPLEXES, COMPARISON OF PREDICTED NUMBER AVERAGE DIAMETERS TO EXPERIMENTAL DIAMETERS MEASURED BY DLS INDICATES A SPECIFIC INTERACTION FOR THE CARBOXYLIC ACID-FUNCTIONALIZED PEO COMPLEXES.	198
TABLE 6.5. SURFACE COVERAGE CALCULATIONS FOR THE AMMONIUM ANCHOR GROUP EXPLAIN STABILITY BEHAVIOR IN PBS.	200
TABLE 6.6. TRIPHOSPHONATE-ANCHORED COMPLEXES SHOW NO APPRECIABLE AGGREGATION IN PBS OVER 24 HOURS.	201
TABLE 6.7. POLYMER DESORPTION CAN BE QUANTIFIED THROUGH DIALYSIS AGAINST PBS.	202
TABLE 7.1. ^1H NMR AND GPC DATA FOR THE AMPHIPHILIC DIBLOCK COPOLYMERS.	215
TABLE 7.2. COMPARISON OF DLS SIZE MEASUREMENTS MADE IN DI WATER AT 25°C TO MODEL PREDICTIONS FOR THE TRIAMMONIUM COMPLEXES.	216

TABLE 7.3. COMPARISON OF DLS SIZE MEASUREMENTS MADE IN DI WATER AT 25°C TO MODEL PREDICTIONS FOR THE TRI-ZWITTERIONIC PHOSPHONATE ANCHORED COMPLEXES.	217
TABLE 7.4. NEUTRON SCATTERING LENGTH DENSITIES FOR RELEVANT MATERIALS. ³⁶	220
TABLE 7.5. DLS OF SANS SAMPLES SHOWS SIMILAR RESULTS TO OTHER DIBLOCK COPOLYMER COMPLEXES.	223
TABLE 8.1. TRIVINYLSILYL-TERMINATED PDMS MOLECULAR WEIGHTS AND DISTRIBUTIONS	252
TABLE 8.2. COMPARISON OF THE MODEL PREDICTIONS OF HYDRODYNAMIC DIAMETER WITH EXPERIMENTAL MEASUREMENTS INDICATE THAT FIVE MAGNETIC SEPARATIONS ARE NECESSARY TO OBTAIN A WELL-DEFINED PARTICLE SIZE DISTRIBUTION.	255
TABLE 8.3. COMPARISON OF THE NUMBER AVERAGE DIAMETERS OBTAINED FROM DLS WITH THOSE PREDICTED BY THE MODEL INDICATE A WELL-DEFINED PARTICLE SIZE DISTRIBUTION.	260
TABLE 9.1. CHARACTERIZATION OF CERIUM OXIDE NANOPARTICLE SIZE AND CATALYTIC ACTIVITY.	274
TABLE 10.1. THERE APPEARS TO BE A CORRELATION BETWEEN THE RATIO OF BRUSH EXTENSION TO THE Σ PARAMETER OF THE EXPRESSION PROPOSED BY LIKOS.	299

1 Introduction

1.1 Significance and Motivation

The motivation for this research is to create biologically viable polymer-nanoparticle complexes for use in retinal detachment therapy, for specific targeting of cells, and as an MRI contrast agent.¹⁻⁴ Controlling the size, dispersion, and interaction of the complexes with proteins and other biological molecules is essential to understanding their biological viability. This control is obtained by adding a steric stabilizing layer which is large enough to counteract the large van der Waal's forces typical of metal and metal oxide nanoparticles.⁵ By modeling the complex interactions using extended Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, predicted sizes in solution could be compared to experimental values. In addition, the viability of different groups used to anchor the polymer to the metal oxide particles was explored in media containing phosphate salts because the steric repulsion provided by the polymer is useful only if the polymer remains on the surface of the particle. Thus, measurement of the binding behavior of these anchor groups was necessary in aqueous media mimicking conditions encountered in biological systems. Prior to this work, there was no way to use a model to systematically design polymer-particle complexes to obtain controlled complex sizes, as well as to explain aggregation phenomena. Additionally, methods have been developed to characterize the efficacy of functional anchor groups in the presence of phosphate salts.

1.2 Specific Research Objectives

The overall objective of this research was to develop a model to aid in the design of polymer-particle complexes that would be appropriate for various *in vivo* applications. In order to accomplish this general objective, several specific research objectives were necessary:

1. Apply the modified density distribution model to magnetite-polymer complexes to obtain an understanding about the colloidal stability of these materials *in vitro* and to validate the model.

Understanding colloidal stability of polymer-magnetite complexes is paramount to the design of materials for biomedical applications.⁶⁻⁸ Previous work has focused on the application of a density distribution model by Vagberg, et al.⁹ to describe polymer brushes extending into solution from either a hydrophobic polymer core, a magnetite core, or a magnetite core surrounded by a non-solvated hydrophobic shell.⁸⁻¹³ However, understanding how to validate this model and under what conditions the model breaks down is of significant interest. Understanding when to apply the model allows for comparison of complexes with different molecular weights and to detect deviation from expected behavior.

2. Examine the stability of various anchor groups used to attach polymers to the magnetite surface in physiological media.

Stability of polymer-magnetite complexes in suspension is important for their use in biological systems.^{6, 8} Stability of these types of materials has been examined in saline solutions, but there is very little information regarding stability in solutions containing phosphate salts.¹⁴⁻¹⁶ Because there is a drive to use these complexes in biological systems, testing their stabilities in physiological analogs is critical to determining their efficacy. Additionally, there is a moral obligation to ensure that minimal cell and animal tests are performed by designing well-defined, stable systems. This objective is aimed at synthesizing anchor groups that are stable in water, saline, and the presence of phosphate salts.

3. Design well-defined polymer-stabilized single-particle systems.

Much of the current biomedical research utilizes commercially available materials with some degree of aggregation in solution.¹⁷⁻²⁰ There is no current metric to determine whether a suspension consists of individual single particles or clusters. While light scattering can be used to see large-scale aggregation, small amounts of aggregation can go undetected. Thus, the development of a methodology to determine when a system consists of single particles in suspension and the application of this methodology to aid in separations or new synthetic routes is critical.

4. Examine the effect of the addition of a PPO block to give the polymer-magnetite complexes amphiphilic character.

Pluronic® block copolymers have shown an ability to enter cells and to interact with cell membranes.^{18, 19} The critical variable appears to be the ratio of hydrophilic character to hydrophobic character.²¹⁻²³ There is a need to develop nanomaterials that interact with cell membranes much like these Plurionics®. Thus, the incorporation of an amphiphilic diblock copolymer adsorbed onto a magnetite nanoparticle may aid in cell entry. How the addition of this copolymer affects variables such as colloidal stability, MRI contrast, and cellular uptake needs to be examined in a controlled fashion.

5. Examine the catalytic activity of cerium oxide nanoparticles.

Lifespans of neural cells and *Drosophila* exposed to paraquat have been extended after exposure to commercially available cerium oxide nanoparticles. Evidence suggests that this biological activity is related to the oxidative state of cerium oxide.²⁴⁻²⁸ Previous studies have indicated a preference of the more reactive oxidative state to certain crystal surfaces.^{29, 30} Comparison of samples that do and do not exhibit biological activity is critical to understanding the mechanism by which these materials work.

1.3 Overview of Chapters

Chapter 2 is a literature review providing an overview of relevant topics including basic theoretical background and a review of current research. The six primary topics covered in the review are: (1) introductory information on the main experimental techniques used, (2) relevant properties of metal oxides, (3) polymer adsorption onto solid surfaces, (4) colloidal stabilization through polymer adsorption, (5) macromolecular synthesis techniques, and (6) an introduction to magnetic resonance imaging.

In **Chapter 3**, the density distribution model developed by Vagberg et al.⁹ is modified to incorporate a magnetite core and a hydrophobic shell surrounding the magnetite core for a hydrophobic block of an amphiphilic diblock copolymer. The concentric blob model that Vagberg describes was used to explain the behavior of hydrophilic brushes extending out from the surface of the magnetite core. A probability averaging method described by

Mefford et al.^{10, 11} was used to obtain a core probability size distribution and was incorporated into the model. Finally, based on work by Russel³¹, the stability ratio of the solution and the half-life for doublet formation was added to the model to allow for comparison between different molecular weight polymer stabilizers. Confirmation of the method for determining the particle core size distribution was also established.

In **Chapter 4**, the study of triammonium anchor groups on the adsorption of polyethers onto magnetite nanoparticles was studied. The polymers in this study were triammonium-PEO-OH homopolymers and triammonium-poly(propylene oxide)-*b*-PEO-OH diblock copolymers (PPO-*b*-PEO-OH). This work examines the effectiveness of the triammonium anchor group to stabilize polyether-magnetite complexes in both DI water and phosphate buffered saline (PBS). The change in behavior in the presence of phosphate salts with the addition of PPO blocks was explored, and the viability (or non-viability) of these complexes in PBS was explained. Through the use of dynamic light scattering (DLS) and extensive modeling as described in Chapter 3, important design parameters for future diblocks were elucidated.

Chapter 5 describes the synthesis and characterization of three different anchor groups—carboxylate, ammonium, and a phosphonate zwitterion—used to anchor PEO to the magnetite surface. The functionalization of the polyether with each anchor group is discussed in detail. The stability of these anchor groups and varying the polymer molecular weight was explored through measuring the size over time of each complex in both DI water and PBS. Reasoning for the stability of each complex and differences between anchor groups is given.

Chapter 6 builds on the work in **Chapter 5**, only this time the efficacy of six combinations of anchor group chemistry and architecture were explored: a monocarboxylate, a tricarboxylate, a monoammonium, a triammonium, a monophosphonate zwitterion, and a triphosphonate zwitterion. The stability of each of these complexes was again measured over time in both DI water and PBS. A polymer brush model was implemented to explain differences in behavior for each complex.

Magnetite surface coverage by the anchor groups was calculated to determine its effect on stability. An additional attractive force between the monophosphonate zwitterion-magnetite complexes was also examined.

Chapter 7 explores the stability of the triphosphonate zwitterion anchor group for complexes containing an amphiphilic diblock copolymer. Effects of molecular weight and polymer loading on stability in PBS were examined. Comparisons of the DLS data for triammonium complexes with similar molecular weights were made. Magnetic relaxivity data and cellular uptake data were presented for the triammonium complexes and the polymer brush model was used to describe some of the results.

Chapter 8 describes the synthesis and characterization of poly(dimethylsiloxane) (PDMS)-magnetite complexes using two different magnetite synthetic routes (coprecipitation of iron chloride salts and the high temperature reduction of iron acetylacetonate). Four different PDMS molecular weights (3,000; 5,000; 7,000; and 10,000 g mol⁻¹) were adsorbed to the magnetite surface. The extended density distribution polymer brush model was applied to both systems and it was determined whether the polymer-magnetite system showed aggregation. Magnetic separation columns were employed to remove aggregates. The quality of the magnetite synthetic routes was compared based on number of separations needed and the comparison of the model to experimental DLS data.

Chapter 9 describes the measurement of the catalytic activity of commercially available-supplied cerium oxide nanoparticles. The catalytic activity measurements were done by measuring the decomposition of hydrogen peroxide in the presence of different cerium oxide particles. X-ray diffraction, DLS, BET surface area, XPS, and TEM were employed to determine differences between the samples. The preferred crystallographic orientation of the materials was determined and compared to their catalytic activity. The activities were then measured in the presence of phosphate salts and compared.

Chapter 10 describes the conclusions drawn from the preceding chapters and the future direction of this research. Overall conclusions are presented as well as conclusions for each individual chapter. Future work topics presented include refinement of the extended density distribution polymer brush model, synthesis of polymer-magnetite clusters for increased relaxivity, and quantifying the interaction between polymer brushes and surfaces, specifically hydrophobic surfaces and lipid bilayers.

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2 Literature Review

This literature review has two main objectives. First, the background relevant to the research put forth in later chapters is explained. Second, relevant current research—as well as past research on which the current work is based—is presented.

This review addresses six main topics. These are: 1) Relevant instrumentation to the work presented in later chapters, 2) Relevant properties of metal oxides, particularly those of magnetite and cerium oxide, 3) Polymer adsorption onto surfaces, 4) Colloidal stabilization through polymer adsorption, 5) Macromolecular synthesis techniques, and 6) Introduction to magnetic resonance imaging.

2.1 Overview of Relevant Instrumentation

The purpose of this section is to highlight relevant instrumentation used in this document to analyze and characterize samples. It is placed first in the literature review because much of the review deals with research where these techniques were employed to prove something specific and relevant to the research proposal. Also, fundamental understanding of the techniques is essential to understanding the proposal and the results contained herein. Relevant instrumentation techniques reviewed in this section are light scattering, zeta potential, quartz crystal microbalance with dissipation, nuclear magnetic resonance, and size exclusion chromatography.

2.1.1 Light Scattering

Light scattering is a non-destructive technique for probing various characteristics of materials, such as weight-average molecular weight, radius of gyration, and particle size.¹⁻⁴ Essentially, light scattering is the adsorption and re-emission of electromagnetic radiation. A sample is introduced to an oscillating electromagnetic field. This field interacts with the charges of bound atoms or molecules and the charges begin to oscillate. Based on Maxwell's relations, an accelerated charge gives off secondary electromagnetic radiation in all directions, and this secondary radiation is called scattered light.¹

Light scattering occurs due to heterogeneities in a system. If a system is completely homogeneous, there is no net scattering due to destructive interference. For dispersed

particle systems, the heterogeneity in the system is caused by differences in the optical properties of the suspended particles and the solvent.¹ Thus, materials with differences in refractive index induce light scattering that can be “seen” by a photodetector. Figure 2.1 shows the typical configuration for a light scattering experiment.

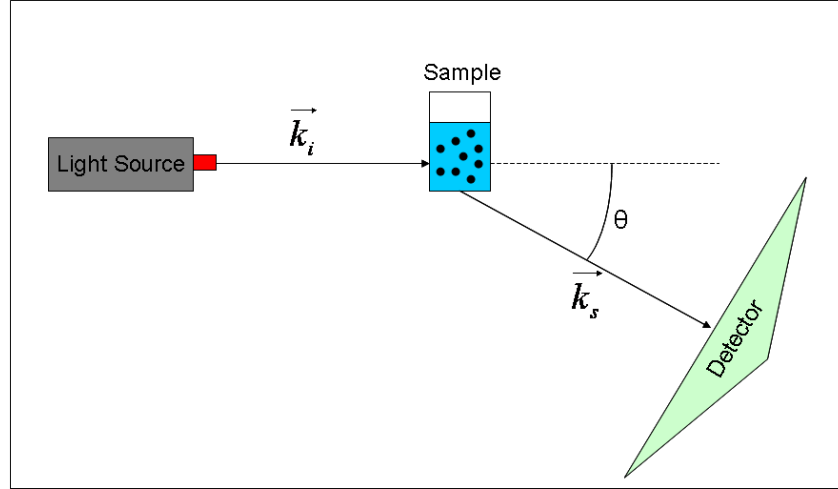


Figure 2.1. Basic configuration for a light scattering experiment (redrawn by W. Miles).¹

In this configuration, a monochromatic light source (typically a laser) is introduced to the sample. The incident beam is described by the incident wave vector $\vec{k}_i$, which has a magnitude described by equation 2.1.¹

$$|\vec{k}_i| = \frac{2\pi n_1}{\lambda_o} = \frac{m_1 \omega_o}{c} \quad (2-1)$$

Here, m_1 is the refractive index of the solvent, ω_o is the circular frequency of the incident light in a vacuum, c is the velocity of light in a vacuum, and λ_o is the wavelength of the incident light. The scattered wave vector, denoted by $\vec{k}_s$, describes the light scattered at an angle θ with respect to the incident light. These two wave vectors can be related to the scattering vector, $\vec{q}$, through equations 2.2 and 2.3.^{1,4}

$$\vec{q} = \vec{k}_i - \vec{k}_s \quad (2-2)$$

$$|\vec{q}| = \frac{4\pi n_1}{\lambda_o} \sin\left(\frac{\theta}{2}\right) \quad (2-3)$$

Thus, the magnitude of the scattering vector is angle dependent. Figure 2.2 shows how the scattering vector for water (RI = 1.331) varies with the scattering angle at a wavelength of 633 nm (He-Ne laser).⁴

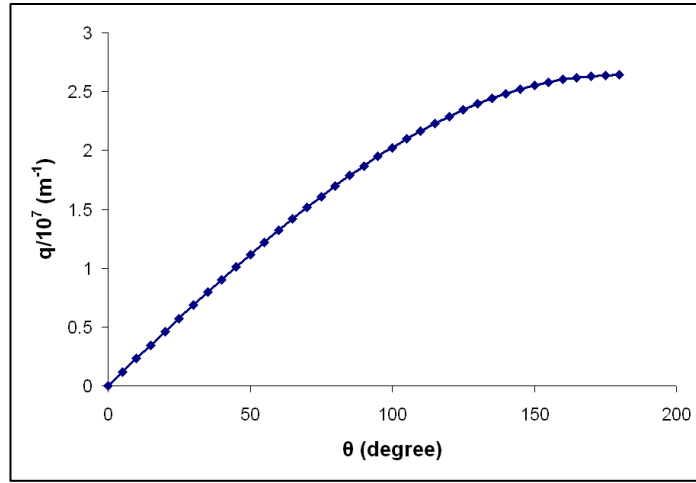


Figure 2.2. The scattering vector of water at 25 °C and a wavelength of 633 nm varies with the scattering angle, allowing properties to be explored using multi-angle light scattering.

The magnitude of q^{-1} is typically between 10-1000 nm. Light scattering gives useful information if the inhomogeneities of a system are of the same order of magnitude as the magnitude of the inverse scattering vector.¹ Light scattering can be further divided up into static and dynamic measurements. The following sections explain each in more detail, including advantages and limitations of each.

2.1.1.1 Static Light Scattering

Static light scattering (SLS) experiments measure time-averaged scattering of a system. When the incident and scattered radiation have the same frequency, this is called Rayleigh scattering. This occurs for small particles and polymers and so is useful for things such as determining the molecular weight, radius of gyration, and second virial coefficient of a polymer.^{1, 2, 4}

The Rayleigh scattering intensity measured by the detector in a vacuum is described in equation 2.4.⁴

$$\frac{I}{I_o} = \frac{\pi^2 \alpha^2 \sin^2 \theta'}{\lambda^4 \epsilon_o^2 r^2} \quad (2-4)$$

Here I_o is the incident beam intensity, α is the sample polarizability, θ' is the vertical scattering angle, λ is the incident beam wavelength, ϵ_o is the electric permittivity of a vacuum (8.854×10^{-12} F/m), and r is the distance of the detector from the point of scattering.

Of course, light scattering experiments occur in a solvent rather than a vacuum, and so the wavelength of the beam and the electric permittivity are affected by the refractive index of the medium, as shown in equation 2.5.⁴

$$\frac{I}{I_o} = \frac{\pi^2 \alpha_{ex}^2 \sin^2 \theta'}{(\lambda/n)^4 (\epsilon_o n^2)^2 r^2} = \frac{\pi^2 \alpha_{ex}^2 \sin^2 \theta'}{\lambda^4 \epsilon_o^2 r^2} \quad (2-5)$$

Here α_{ex} is the excess polarizability between the solvent and the suspended solute. For static light scattering experiments, the scattering of the solvent has to be considered. For I_s denoting the intensity of the solvent and I denoting the intensity of the sample, the excess scattering $I_{ex} = I - I_s$. Thus the excess scattering is the scattering due to the scattering entity in the sample.

Recall that the scattering arises from the difference in refractive index of the suspension and the solvent. This difference is concentration dependent and can be represented by the specific refractive index increment (also called the differential refractive index), dn/dc , shown in equation 2.6.⁴

$$\frac{dn}{dc} \approx \frac{(n_{suspension} - n_{solvent})}{v_{sp}} \quad (2-6)$$

Here, $n_{suspension}$ and $n_{solvent}$ are the refractive indices of the suspension and solvent, respectively, and v_{sp} is the specific volume of the suspension in solution.

The excess scattering of a suspension in solution can be described by equation 2.7.⁴

$$\frac{I_{ex}(k)}{I_o} = \frac{4\pi n^2}{\lambda^4 r^2} \left(\frac{dn}{dc} \right)^2 \Psi_{cc}(k) \quad (2-7)$$

Here, r is the distance of the scattering object from the detector and $\Psi_{cc}(k)$ is the Faurier transform of the autocorrelation function, which is the mathematical function that describes concentration variations in a sample as described in equation 2.8.⁴

$$\Psi_{cc}(k) = \frac{cV}{N_A} \left(1 + \frac{k^2 R_g^2}{3} \right)^{-1} \left[\frac{1}{M} + 2A_2c + \dots \right]^{-1} \quad (2-8)$$

Thus, the excess scattering can be described as shown in Equation 2.9.⁴

$$\frac{I_{ex}(k)}{I_o} = \frac{4\pi n^2}{\lambda^4 r^2} \left(\frac{dn}{dc} \right)^2 \frac{cV}{N_A} \left(1 + \frac{k^2 R_g^2}{3} \right)^{-1} \left[\frac{1}{M} + 2A_2c + \dots \right]^{-1} \quad (2-9)$$

The ratio I_{ex}/I_o can be related to the Rayleigh ratio, $R_\theta V/r^2$, such that equation 9 can be rewritten as shown in equation 2.10.²

$$\frac{Kc}{R_\theta} = \frac{1}{P(\theta)} \left(\frac{1}{M} + 2A_2c + \dots \right) \quad (2-10)$$

Here, $K = (1/N_A)[2\pi n/\lambda^2(dn/dc)]^2$ and $P(\theta)$ is the structure factor for scattering that arises when there are multiple centers that contribute to the overall scattering. When $P(\theta) = 0$, the equation reduces to the Rayleigh equation. However, there are three main limitations to Rayleigh scattering. First, the scattering centers must be isotropic, dielectric, and nonadsorbing. Second, the scatterers must not have a refractive index that is too large because there is a simplification in the polarizability term where $(n^2-1)/(n^2+2)$ is estimated as $(n-1)$. Finally, particles must be small compared to the wavelength of incident light (diameter $< \lambda/20$). This is because in Rayleigh scattering, it is assumed that the electromagnetic wave that drives the oscillating dipole is the same throughout the particle. When the particle size increases to that of the wavelength of the incident beam,

the particle is no longer in the same phase of the wave at all times and the scattering is affected.²

The Debye equation, as shown in equation 2.11, attempts to address some of these complications with the Rayleigh equation. The Debye equation is angle dependent and is only valid for small values of $\theta/2$.²

$$\frac{Kc}{R_\theta} = \left(1 + \frac{16\pi^2 R_g^2}{3\lambda^2} \sin^2\left(\frac{\theta}{2}\right) \right) \left(\frac{1}{M} + 2A_2c + \dots \right) \quad (2-11)$$

Two things are readily apparent from equation 2.11. First, as the limit of concentration approaches zero, Kc/R_θ varies proportionally with $\sin^2(\theta/2)$. Second, as the limit of both concentration and θ approach zero, Kc/R_θ varies proportionally with $1/M$. Of course, measurements can not be made at zero concentration or zero angle, so the data has to be extrapolated in the form of a Zimm plot, as shown in Figure 2.3.^{2, 4, 5} For this measurement, the Rayleigh ratio is measured at varying angle and concentration. The intercept of both concentration and angle extrapolated to zero is equal to $1/M_w$. Furthermore, the slopes of each line give information about the radius of gyration and second virial coefficient.

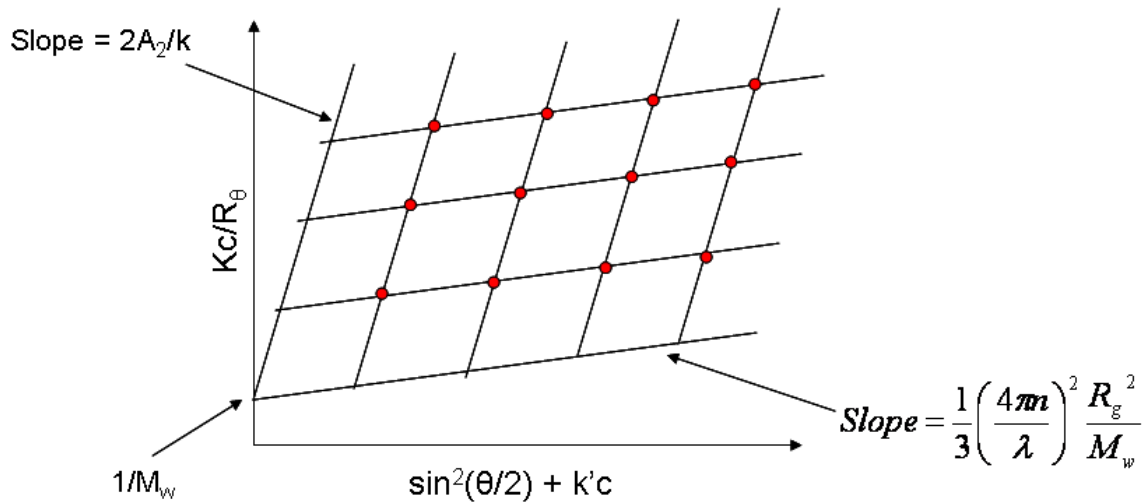


Figure 2.3. A Zimm plot constructed using static light scattering to extrapolate values at concentration and θ as their limit approaches zero (redrawn by W. Miles).⁴

Thus, we can determine the radius of gyration, second virial coefficient, and weight average molecular weight of a polymer sample through static light scattering.

2.1.1.2 Dynamic Light Scattering

Dynamic light scattering (DLS)—also called photon correlation spectroscopy (PCS) and quasi-elastic light scattering (QELS)—allows for measurement of particle sizes down to 0.6 nm and up to 6 μm . It is ideal for many applications because of the sensitivity of the measurement to aggregation and because multiple peaks can be resolved for multimodal systems.^{1,3}

DLS measures temporal scattering variations of a sample. The physics of the scattering are the same as for static light scattering, but the way in which the measurement is made is altered. For DLS, the intensity is measured at two times, $I(t)$ and $I(t + \tau)$, where τ is the delay time. Essentially, what is being measured is how the scattering changes as a function of time. These changes in scattering intensity with time can be described by the autocorrelation function, shown in equation 2.12.^{3,4}

$$G(\tau) = \int_0^{\infty} I(t)I(t + \tau)dt \quad (2-12)$$

What this illustrates is that the intensity is always continuous, and that the fluctuations about the mean intensity are due to movement of the particles from one position at time t to a position very close to the original position at time $t + \tau$.

So why do these particles move constantly in solution to generate the variations in scattering intensity with time? As mentioned in section 2.1.1.1, even when a solution is completely homogeneous, there are continuous density fluctuations at the molecular scale which impart a pressure gradient in the fluid.² This pressure gradient imparts a force on the particles suspended in the solution and causes them to move. This movement is called Brownian motion and is a completely statistically random movement. The magnitude of the movement is dependent upon the system temperature and the particle size. As the temperature is increased, the density fluctuations increase and so the force imparted on the particle increases also. As particle sizes get larger, there is more

resistance (drag) to the force imparted from the density fluctuations, leading to less total movement of the particle system.^{2, 4}

The velocity at which the particle moves through solution is called the diffusion coefficient. This is the parameter measured by DLS and can be related to the particle size. The diffusion coefficient can be measured by examining the decay of the autocorrelation function with time. The autocorrelation function can be represented by correlation curves for each material, where decay to baseline occurs quicker for a faster diffusing material as shown in Figure 2.4.³

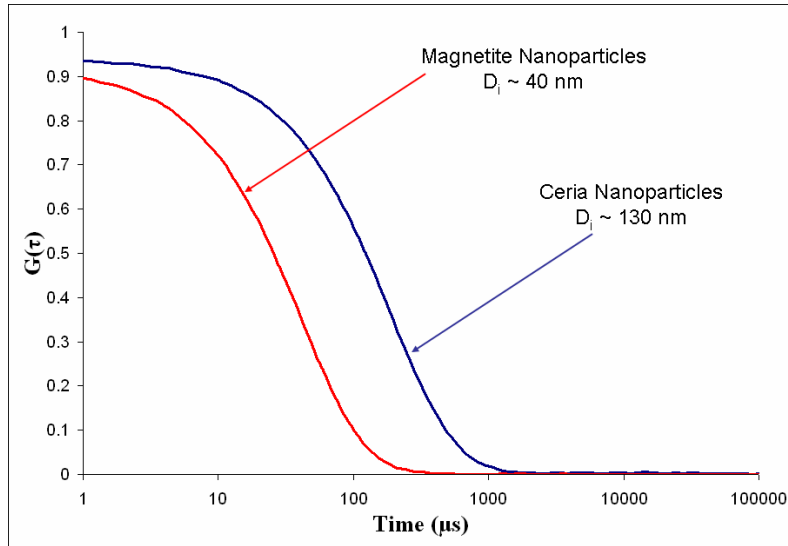


Figure 2.4. Autocorrelation curves for different particle sizes decay at different rates.

To relate the autocorrelation curve (and hence the diffusion coefficient) to the particle size, the autocorrelation function can be related to various parameters by equation 2.13, when the sample is relatively monodisperse.³

$$G(\tau) = \int_0^{\infty} I(t)I(t+\tau)dt = B + A^{-2q^2D\tau} \quad (2-13)$$

Here, B is a coefficient related to the baseline of the autocorrelation function, A is the amplitude of the autocorrelation function, q is the scattering vector, D is the diffusion

coefficient, and τ is the delay time. Thus at a fixed scattering angle, all variables but D are specified and D can be calculated.

The diffusion coefficient, D , can then be related to the particle radius through the Nernst-Einstein equation as shown in equation 2.14.⁴

$$D = \frac{k_B T}{\xi} \quad (2-14)$$

This equation is related to the calculation of a constant velocity (v) of a particle in a viscous medium, which requires a constant force of $F = v\zeta$, where ζ is the coefficient of friction. For a system of spherical particles, $\zeta = 6\pi\eta_s R_H$ and so the Nernst-Einstein equation reduces to the Stokes-Einstein equation as shown in equation 2.15.²⁻⁴

$$R_H = \frac{k_B T}{6\pi\eta_s D} \quad (2-15)$$

Here, R_H is the hydrodynamic radius of the particle and η_s is the viscosity of the medium.

2.1.2 Zeta Potential

Zeta potential is a useful tool to describe repulsive electrostatic interactions in a colloidal system. For typical colloids, zeta potentials greater than 30 mV or less than -30 mV provide large enough electrostatic forces for the suspension to be deemed “stable.”⁶ Of course, the exact stability threshold varies based on the material in suspension and other factors, such as particle size. In addition, stability can be defined many different ways depending upon the time-frame being discussed. Regardless, this definition works well as a general definition for most colloids.

To define the zeta potential, the environment of a charged particle in solution must also be discussed. A potential difference occurs at any interface due to excess electrons in one or both phases aligning nonuniformly at that interface.⁶ The origin of surface charges and the effect of pH will be discussed in detail in later sections.

At the colloid-water interface, a charged particle is surrounded by an electric double layer consisting of two components. First, an inner layer—called the Stern layer—where oppositely charged ions are strongly bound to the particle surface. Second, an outer layer—called the diffuse region—where ions of both charges are weakly bound to the surface. A boundary exists within the diffuse layer where the particle and ions act as one unit, and so as the particles move, the electric double layer also moves. This boundary is called the slip plane and the potential at the slip plane is called the zeta potential. This phenomenon is illustrated in Figure 2.5.^{6,7}

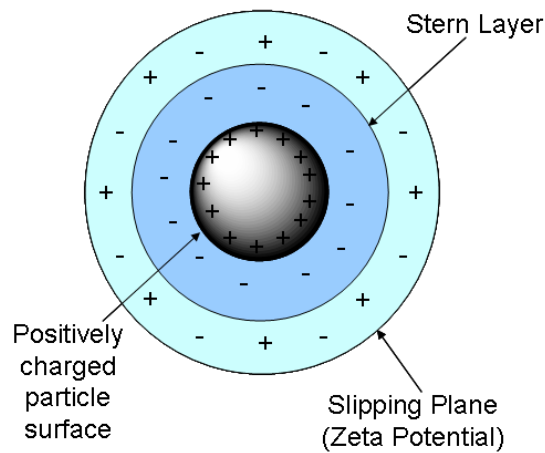


Figure 2.5. A positively charged particle is surrounded by an electric double layer consisting of the stern layer and the diffuse layer. The zeta potential is measured at the slipping plane (redrawn by W. Miles).^{6,7}

Measurement of the zeta potential is possible because of electrophoresis, which is the movement of a charged particle due to an applied electric field. When the electric field is introduced, the charged particles move toward the electrode of opposite charge. This movement is opposed by viscous forces of the medium, and when these two forces reach equilibrium, a constant velocity is achieved. This velocity in a unit electric field is called the electrophoretic mobility. This electrophoretic mobility can be related to the zeta potential through the Henry equation as shown below.^{6,7}

$$U_E = \frac{2\varepsilon\zeta f(\kappa a)}{3\eta} \quad (2-16)$$

Here, U_E is the electrophoretic mobility, ϵ is the dielectric constant of the solvent, ξ is the zeta potential, η is the solvent viscosity, and $f(\kappa a)$ is Henry's function, where κ is the inverse Debye length and a is the particle radius.

κ^{-1} is the Debye length, which is a measure of how far out the electric double layer extends and is given by equation 2.17.^{2, 6, 7}

$$\kappa^{-1} = \left[\frac{\epsilon_o \epsilon_r k_B T}{2000 e^2 I N} \right]^{0.5} \quad (2-17)$$

Here, ϵ_o is the permittivity of free space, ϵ_r is the dielectric constant of the medium, k_B is Boltzman's constant, T is temperature, e is the charge of an electron, I is the solution ionic strength, and N is Avogadro's number. The ionic strength is defined as $I = 0.5 \sum C_i Z_i^2$ where C_i is the concentration of an ionic species and Z_i is the valence of that ion.

κa is the ratio of the particle radius to the thickness of the electric double layer and varies from two extreme limits. The first limit is when the particles are relatively large ($> 0.2 \mu m$) and the electric double layer is small ($I > 10^{-3} M$) and is called the Smoluchowski limit. At this limit, $f(\kappa a)$ is 1.5. The other limit is called the Hückel limit and is for small particles with large electric double layers. At this limit, $f(\kappa a)$ is 1.0. For many systems, including the ones explored in this work, neither the Smoluchowski or Hückel limits correctly describe the system.^{2, 6} In those cases, the zeta potential has to be corrected from the Smoluchowski limit as shown in equation 2.18.

$$\xi_{corrected} = \frac{\xi_{measured}}{\frac{2}{3} f(\kappa a)} \quad (2-18)$$

This requires a calculation of $f(\kappa a)$, which can be done using equations 2.19 and 2.20.⁶

$$for \kappa a < 1 \quad f(\kappa a) = 1 + \frac{(\kappa a)^2}{16} - \frac{5(\kappa a)^3}{48} - \frac{(\kappa a)^4}{96} - \left[\frac{(\kappa a)^4}{8} - \frac{(\kappa a)^6}{96} \right] e^{\kappa a} \int_{\infty}^{\kappa a} \frac{e^{-t}}{t} dt \quad (2-19)$$

$$\text{for } \kappa a > 1 \quad f(\kappa a) = \frac{3}{2} - \frac{9}{2\kappa a} + \frac{75}{2\kappa^2 a^2} - \frac{330}{\kappa^3 a^3} \quad (2-20)$$

Thus, for water at 298K, the Debye length can be written as $0.304/I^{0.5}$ and $f(\kappa a) = 1.0$. For phosphate buffered saline at 298K with an ionic strength of 0.14 M, $f(\kappa a) = 1.2$.

2.1.3 Quartz Crystal Microbalance with Dissipation

The quartz crystal microbalance (QCM) is essentially an ultrasensitive mass balance and is very similar to surface plasmon resonance (SPR) spectroscopy. Unlike SPR, QCM technology allows for determination of mass adsorbed on surfaces that are not just metal, which is important for many applications. In addition, QCM is extremely sensitive to mass adsorption ($< 1 \text{ ng/cm}^2$) due to the ability to measure changes in frequency accurately.⁸

QCM is based on the oscillation of a quartz crystal, which is a piezoelectric material. A material is piezoelectric if it produces mechanical work due to an applied electric field. In the case of QCM, an external driving oscillator circuit induces the quartz crystal to oscillate at its resonant frequency, f . The basic principle is that any mass added to the crystal dampens the oscillatory behavior and there is a negative change in frequency, Δf . Conversely, if mass is removed from the crystal, this increases the oscillatory frequency and there is a positive change in Δf .⁸ Sauerbrey found that this change in frequency was linearly related to the change in mass, Δm . This relationship is described by the Sauerbrey equation, shown in equation 2.21.⁸⁻¹⁰

$$\Delta m = \frac{-C}{n} \Delta f \quad (2-21)$$

Here, C is a constant that depends on the thickness and specific properties of the quartz and n is the harmonic being examined. Harmonics are a natural resonance of a system that is an integer multiplier of the resonance frequency (usually 5 or 10 MHz).⁹

The Sauerbrey equation is only valid for rigid materials and does include other key assumptions. First, it is assumed that the mass added is distributed evenly on the crystal surface. Second, it is assumed that the mass adsorbed is much smaller than the mass of

the crystal itself ($\Delta f/f \ll 1$). Finally, it is assumed that the mass is rigidly attached and that there is no slip or deformation due to the oscillation of the crystal.^{8, 10} It is this third assumption where the Sauerbrey equation fails to describe the behavior of softer materials. Softer materials begin to exhibit viscoelastic behavior which causes deformation with the crystal oscillation. Thus, there is a need to further characterize the relationship between mass adsorbed and frequency for softer materials.

Traditional QCM operates with a continuous current applied to the quartz crystal. However, by interrupting this current and measuring the decay in the oscillation, information can be gained about the adsorbed material's rigidity or softness. This decay is called the energy dissipation, and is denoted D .^{8, 9} Figure 2.6 shows two quartz crystals, one with a rigid substrate and one with a soft substrate.

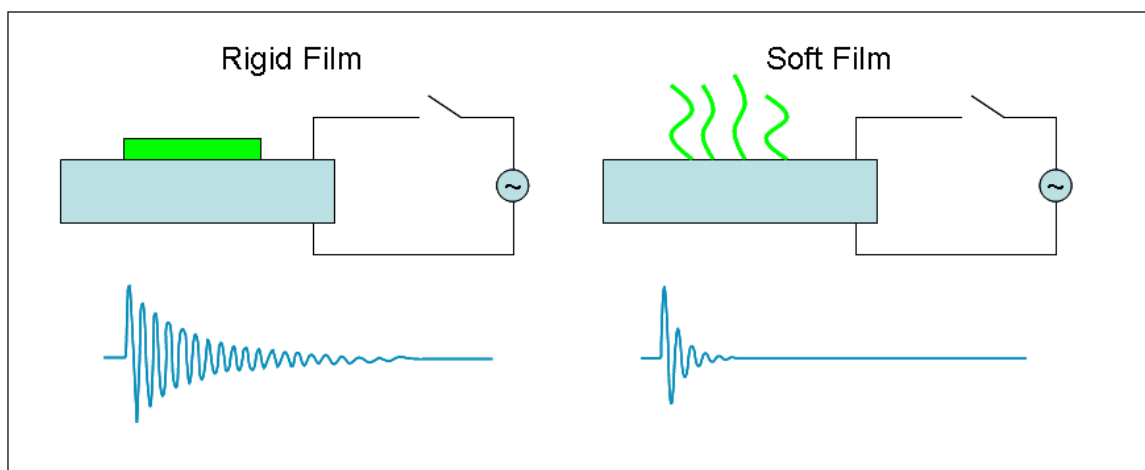


Figure 2.6. The dissipation of the crystal oscillations is much slower for a rigid film than for a soft film (redrawn by W. Miles).⁹

For rigid films, the dissipation—the decay of the crystal oscillation following removal of the current—is relatively slow. This is because there is limited resistance to the oscillation from the adsorbed film. For softer films, there is resistance to the oscillation from the adsorbed film. Thus, the change in dissipation for a soft film is significantly larger than the change in dissipation for a rigid film when they are adsorbed on the same surface. In addition, if the two samples in Figure 2.6 had identical masses, traditional

QCM would treat them as identical materials, while QCM-D would show large differences in the structure (and perhaps mechanism) of adsorption.

Not only does dissipation data provide information about the rigidity of a film, but it also provides other benefits. First, the validity of the Sauerbrey model can be confirmed based on the change in dissipation. Because the Sauerbrey equation only works for rigid films, large changes in dissipation indicate that it may not appropriately describe the data. Second, viscoelastic modeling of the frequency and dissipation data is possible. Typically for soft films the Sauerbrey equation underestimates the film thickness. Thus, by using a viscoelastic model—such as the Voigt model—more accurate measurements of film thicknesses and mass loadings can be achieved. Third, dissipation allows for examination of hydration or swelling of a film by observation of a softening of a film when swelling occurs. Finally, structural changes on a surface may be observable.⁹ This can be particularly important for adsorption of materials where the kinetics of adsorption determine how a material adsorbs (i.e. whether it spreads out over the entire surface or extends out into solution at various concentrations).

QCM-D has many different applications. Dolatshahi-Pirouz et al. studied protein adsorption on platinum surfaces.¹¹ Liu and Zhang studied the oscillating swelling and collapsing behavior of polyelectrolyte brushes forced by chemical oscillation.¹² Reimhult et al. studied blocking agents and how to protect gold and polystyrene surfaces for eventual use in biosensors that need reductions in non-specific binding.¹³ Essentially, any application dealing with a flat-plate geometry has at least some use for QCM-D.

2.1.4 Nuclear Magnetic Resonance

NMR is a rapid, nondestructive method to probe the molecular structure of various molecules. NMR focuses on absorbance properties of nuclei, which is different from other spectroscopic techniques such as UV-Vis or IR spectroscopy that focus on absorbance due to properties of electrons. NMR is fairly analogous to MRI, which will be discussed in much more detail later on.¹⁴⁻¹⁶

Nuclei with an odd number of protons and neutrons have their own magnetic moment due to the spins of the protons. When a magnetic field is applied to the proton, the proton spin either aligns with the magnetic field or against it. More proton spins align parallel than antiparallel, and so there is a net magnetic moment in the direction of the applied magnetic field. The proton spins that are aligned parallel with the magnetic field are relatively low energy (denoted $-1/2$) and the proton spins that are aligned antiparallel to the magnetic field are relatively high energy (denoted $+1/2$).¹⁴⁻¹⁹ Figure 2.7 shows that as the applied magnetic field is increased, the energy gap between the high and low energy states gets larger.¹⁴

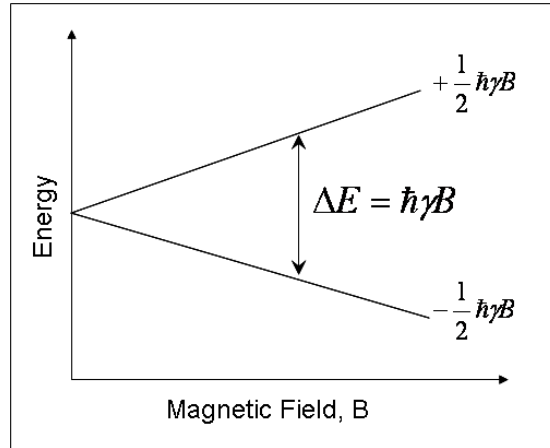


Figure 2.7. The proton high and low energy states move farther apart as the applied magnetic field gets stronger (redrawn by W. Miles).¹⁴

The energy gap is calculated from Planck's equation, and is illustrated in equation 2.22.

$$\Delta E = \hbar \gamma B \quad (2-22)$$

Here, ΔE is the amount of energy between the low and high energy states, $\hbar$ is Planck's constant, γ is the gyromagnetic ratio ($2.674 \times 10^4 \text{ G}^{-1}\text{S}^{-1}$), and B is the incident magnetic field strength. In the magnetic field, the number of protons aligned parallel and antiparallel to the magnetic field reaches an equilibrium.

The protons are then exposed to radio frequency energy, which is adsorbed and drives some of the parallel aligned protons into an antiparallel configuration. When the radio

frequency is removed, the system moved back to the original equilibrium, but the higher energy state protons must release a photon equal to the energy gap to return to the lower energy state. This photon can be collected and measured.¹⁴⁻¹⁶

Figure 2.8 shows a typical NMR configuration where the main components are the sample tube, the permanent magnets, a field sweep generator, an RF source, an RF receiver, and a recorder/integrator. The field sweep generator is a set of coils used to alter the magnetic field over a very small range.¹⁶

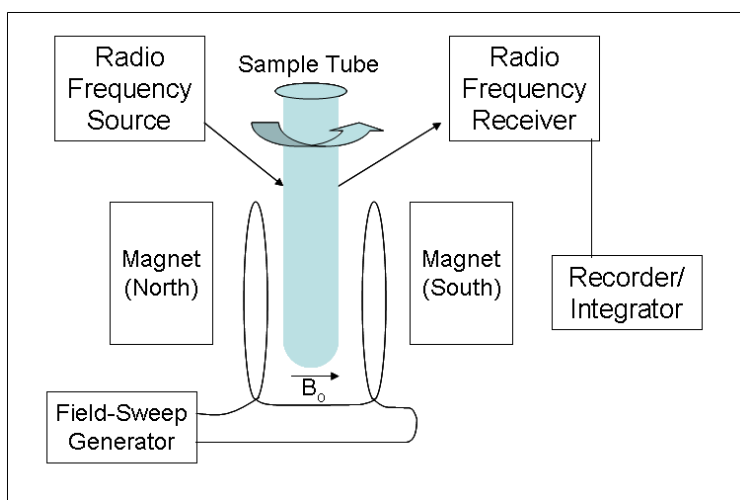


Figure 2.8. Diagram of a typical NMR setup (redrawn by W. Miles).¹⁶

Not all protons adsorb RF radiation in the same manner. Differences, caused by the magnetic fields of surrounding protons, tend to “shield” the protons from the magnetic field, especially electrons in covalent bonds. The shielding effect is proportional to the electron density around the proton. The chemical shift is the magnetic field strength at which a given proton adsorbs energy compared to an arbitrary reference. This can be expressed in ppm, as shown in equation 2.23.¹⁶

$$ppm = \frac{RF_s - RF_{Ref}}{RF_{Ref}} \times 10^6 \quad (2-23)$$

Here, RF_s is the resonance frequency of the sample and RF_{Ref} is the resonance frequency of the reference, both in Hz. Table 2.1 illustrates chemical shifts for common types of protons for ^1H NMR.

Table 2.1. Chemical shifts of some typical protons for ^1H NMR (redrawn by W. Miles).¹⁶

Type of Proton	Chemical Shift (ppm)
Primary	0.9
Secondary	1.3
Tertiary	1.5
Vinyllic	4.6 - 6.0
Acetylenic	2.0 - 3.0
Aromatic	6.0 - 8.5
Iodo (primary)	2.0 - 4.0
Bromo (primary)	2.5 - 4.0
Chloro (primary)	3.0 - 4.0
Fluoro (primary)	4.0 - 4.5
Alcohol	3.4 - 4.0
Hydroxyl	1.0 - 5.6
Alkoxyl	3.3 - 4.0
Carbonyl	2.0 - 2.7
Aldehydic	9.0 - 10.0
Carboxylic	10.5 - 12.0

High ppm values are related to lower electron densities (deshielding) while low ppm values are related to higher electron densities, relative to the arbitrary standard.

2.1.5 Size Exclusion Chromatography

Size exclusion chromatography (SEC)—also known as gel permeation chromatography—is a method used in chemical synthesis to identify a species' molecular weight, composition, microstructure, and molecular architecture. It relies on the separation of polymers based on size. Unlike other chromatography techniques, the SEC column does not adsorb substances differently to cause separation, but instead consists of a porous network. This pore network is well-defined, and while smaller molecular weight species get trapped in this network, larger molecular weight species pass through. Thus, large molecular weight species are eluted first, and the smallest molecular weight species are eluted last. In this way, SEC allows for a molecular weight distribution to be obtained, unlike other methods such as intrinsic viscosity (M_v) or nuclear magnetic resonance (NMR, M_n).^{20, 21}

Figure 2.9 illustrates an example of a typical SEC set-up. The sample is placed in the autoinjector, and then the solvent is pumped from the reservoir (pure water in the case of aqueous SEC or THF for organic SEC) and passed through a degasser to remove any moisture adsorbed from the atmosphere.

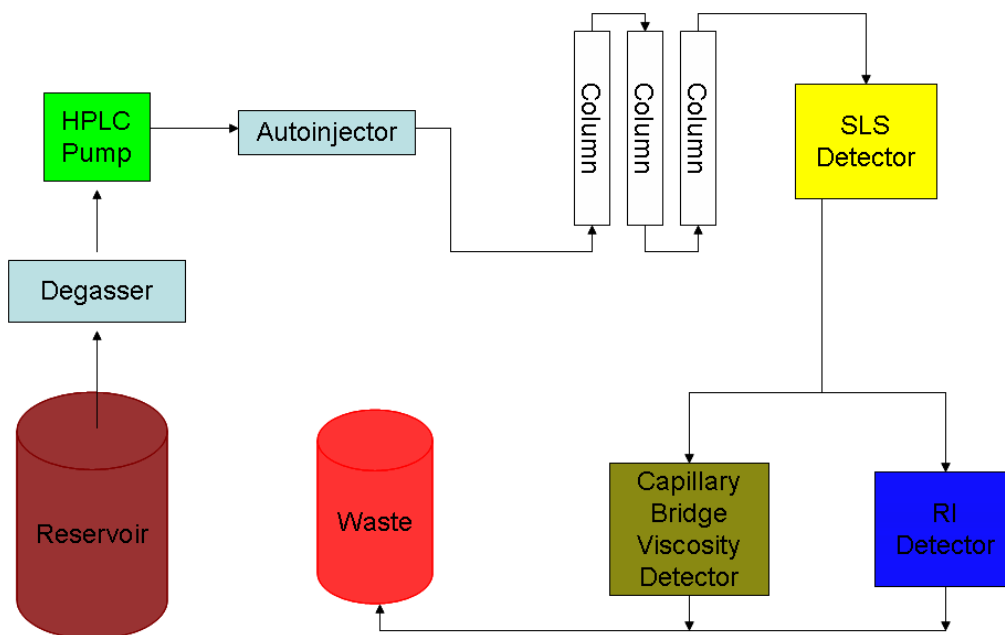


Figure 2.9. Illustration of a typical SEC set-up. Multiple columns are shown here to illustrate that various columns are needed depending upon the application.²⁰

This is done by a high performance liquid chromatography (HPLC) pump. From there, the solvent is pumped through the autoinjector and the sample is injected. The solvent and sample are then pumped through the chromatography columns. Here, three columns are shown to illustrate that various pore networks are needed depending upon the size of the molecular weight distribution. Following separation in the columns, the solvent and sample are pumped to a static light scattering (SLS) detector, where the scattered light of the sample is measured at multiple angles. Finally, the sample is divided and pumped into both a refractive index (RI) detector and a capillary bridge viscosity detector and then pumped to waste. For each run, blank solvent and then a molecular weight standard are run through the system for comparison to the sample of interest and to ensure that the system is operating as it should.

By changing the size of the porous network within a column, one can change the molecular weight distributions that can be measured. There is always a trade-off between resolution and the breadth of a distribution that one can measure. The detectors that are used are normally used in tandem, where one detector is sensitive to molar mass fluctuations, while another detector is sensitive to fluctuations in concentration.²¹

As mentioned previously, static light scattering (SLS) is a technique that uses the time average scattering of light at a specific angle to calculate the weight average molecular weight and the radius of gyration of a species. It does this through the generation of a dipole in the species due to the oscillating electromagnetic radiation that is emitted from the light source, typically a laser. This dipole generates its own electromagnetic radiation, which is what we call light scattering. A photodetector can be placed at various angles to measure the intensity of this scattering.²²

The capillary bridge viscosity detector measures the specific viscosity of the solution, which can be used to obtain the species molecular weight. The viscosity detector has multiple capillaries (two for this example). When both capillaries are filled with only solvent, then there is no pressure drop that can be measured across the “bridge.” However, when some of the polymer is contained within the solution, it enters the first capillary before it enters the second capillary. Thus, there is a viscosity difference between the capillaries and a pressure drop can be measured and related to the viscosity of the fluid at a given time.

The refractive index (RI) detector is used to measure the concentration of the species at a given time. Prior to any SEC measurements, one wants to measure the differential index of refraction, dn/dC , which measures the differential amount that the RI changes with a differential change in concentration. Thus, this means that by measuring the RI of the species after it passes through the columns, it is possible to know the concentration of the species.²¹

All three of these detectors give a specific piece of information that can be used to determine something about the molecular weight, composition, microstructure, or architecture. For example, while a linear and highly-branched polymer might both have the same molecular weight, both the radius of gyration and the viscosity of the solution would be different at a given concentration for each sample. For this case, by using multiple detectors, the polymer architecture can be probed in a way that a single detector cannot.

2.2 Properties of Relevant Metal Oxides

Metal oxides are abundant in nature and are prevalent in industrial products and processes. Sometime, as in the case of rust (iron oxide), the metal oxide is an undesirable product and knowledge of its composition allows for the prevention of material failures.^{23, 24} Other times, metal oxides are directly used in common household products, such as titanium dioxide (TiO_2) in paint²⁵ or zinc oxide (ZnO) in sunscreens.²⁶⁻²⁹

As research into nanomaterials has become more prevalent, the use of metal oxides in biological systems has also increased.³⁰⁻³² Cytotoxicity has become a concern, and studies on the effects of nanoparticles exposure have been conducted.³³⁻³⁵ Iron oxide nanoparticles have been used as contrast agents for the enhancement of magnetic resonance imaging (MRI) and cerium oxide nanoparticles have been used to treat oxidative stress in neural cells.³⁶⁻⁴¹ These two oxides, in particular, show promise for use in biological systems due to their unique properties (magnetism and oxidative stress treatment). Additionally, generating alloys of the two materials might provide a better material than either on its own.

The purpose of this section is to detail both fundamental properties universal to nearly all metal oxides and then specifically describe properties unique to both magnetite and cerium oxide. The charging behavior, adsorption of proteins, adsorption of polymers, and interaction of phosphates with metal oxides are discussed generally. The magnetic properties of iron oxides, particularly magnetite, and the oxygen vacancy formation of cerium oxide are discussed in detail.

2.2.1 Charging of Metal Oxides

When a system consists of two phases, excess ions in one or both phases causes the ions to align nonuniformly at the interface.⁶ This also holds true for metal oxide-water interfaces where the particle has a charge and is surrounded by an electric double layer as described in section 2.1.2. This generates a situation where a positively charged particle attracts negatively charged ions, whose concentration gradually decreases to the bulk ion

concentration with increasing distance from the particle surface. This is illustrated in Figure 2.10, where the surface charge is plotted versus distance from the surface.

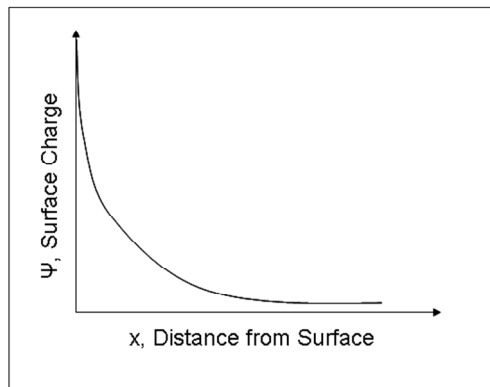
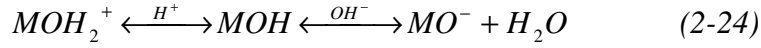


Figure 2.10. The surface potential decays to the bulk potential large distances from the surface (redrawn by W. Miles).⁶

As mentioned in section 2.1.2, how far out the surface charge extends is dictated by the Debye length and in turn, the ionic strength. Ionic species in solution that control the extension of this double layer but do not participate in specific interactions with the surface are called indifferent electrolytes. However, some ionic species do interact with the surface and control the surface charge. These ionic species are called potential determining ions. Whether an ionic species is an indifferent electrolyte or a potential determining ion is dependent upon the material that is being examined.^{6, 42-44}

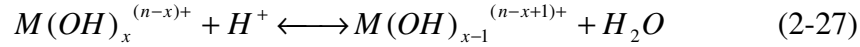
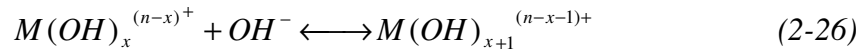
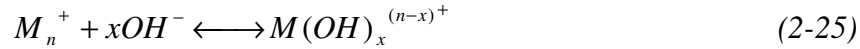
Testing to see whether an ionic species is a potential determining ion is fairly straightforward. The point of zero charge (PZC) is the pH at which the surface charge is zero. Similarly, the isoelectric point (IEP) is the point at which the zeta potential is zero. Both definitions are essentially the same except for the point at which the charge is measured, as the PZC is at the surface while the IEP is at the slipping plane of the electric double layer.⁶

The surface charges of metal oxides are affected greatly by changes in pH. A simplistic view of how the surface charge of the metal oxide changes with pH is depicted in equation 2.24.



Thus, in very acidic conditions the surface charge is positive and in basic conditions the surface charge is negative. In this way, the H^+ and OH^- ions can be seen as potential determining ions.

However, this definition does not really encompass what is occurring on the surface. From equation 2.24, it appears that at the IEP the metal oxide surface only consists of hydroxyl groups. A more complicated view of how surface charge varies on metal oxides with pH changes is provided in equations 2.25-2.27.⁶



Here, we can see that at no point is there a neutral surface species. Instead, there are varying degrees of positive or negative charge. When the net number of positive charges equals the net number of negative charges, then the system is at the PZC or IEP. Figure 2.11 shows a typical zeta potential versus pH curve for a metal oxide where the ionic species added to solution is an indifferent electrolyte.

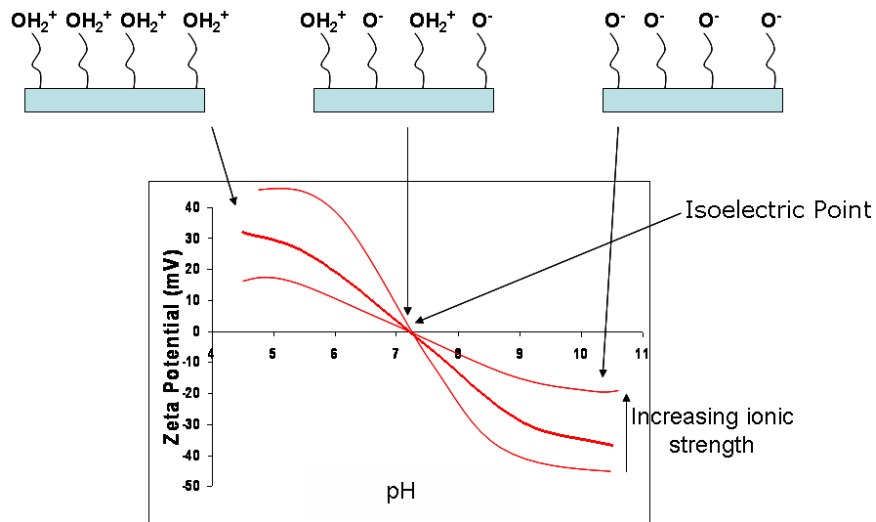


Figure 2.11. Typical zeta potential versus pH curve for a metal oxide with an indifferent electrolyte added at increasing concentrations.⁶

As illustrated in Figure 2.11, an indifferent electrolyte does not alter the IEP, although it does decrease the magnitude of the zeta potential due to the decrease in the Debye length. Thus, if an ionic species is added where the IEP changes as a function of ionic strength, then that ionic species is a potential determining ion.⁶

2.2.2 Iron oxide

Iron oxides are materials commonly found in nature and have many uses. There are approximately 16 iron oxides, hydroxides, and oxide hydroxides, and each exhibit different properties and behavior based upon their chemical composition and crystal structure.^{45, 46} Table 2.2 shows the unit cell configuration of three iron oxides and how that configuration affects their densities.

Table 2.2. Chemical composition and unit cell configuration affects physical properties (such as density) of iron oxides.⁴⁶

Oxide	Chemical Formula	Unit Cell Configuration	Density (g-cm ⁻³)
Magnetite	Fe ₃ O ₄	Cubic or tetragonal	5.18
Maghemite	γ-Fe ₂ O ₃	Cubic or tetragonal	4.87
Hematite	α-Fe ₂ O ₃	Trigonal	5.26

The unit cell configuration of most iron oxides is primarily octahedron, with the O or OH ions arranging in either a hexagonal close packed (hcp) or cubic close packed (ccp) configuration. Those arranged as hcp are designated the α -phase and those arranged as ccp are designated the γ -phase. Typically, the α -phase, such as in goethite or hematite, is more stable than the γ -phase, such as maghemite.⁴⁶ Magnetite and maghemite are both fairly resistant to oxidation and retain their magnetic properties over time, which makes them suitable for many applications, including biological applications.^{41, 47, 48} However, magnetite has a higher saturation magnetization than maghemite, and so research has recently shifted in that direction.⁴⁵

2.2.2.1 Magnetism

There are four main types of magnetism that will be discussed herein: ferromagnetism, antiferromagnetism, ferrimagnetism, and paramagnetism. These are all interrelated and arise from the ordering (or randomness) of the spins of unpaired electrons randomly oriented on different atoms.^{45, 46} Figure 2.12 illustrates the different types of magnetism and how the orientation of the electron spin dictates the magnitude of the magnetic moment.

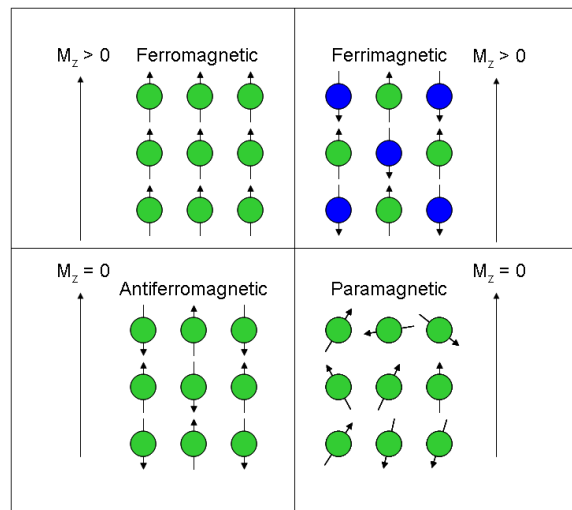


Figure 2.12. The four main types of magnetism are represented here by depiction of the spin of the free electrons, which affect the magnitude of the magnetization vector.⁴⁵

Ferromagnetism is when all the free electrons spin in the same direction (parallel), producing a net magnetic moment in that direction. Thus, for materials that are

ferromagnetic—such as iron—the magnetic moment is permanent and the material exhibits permanent magnetic properties.

Antiferromagnetism is when the free electrons spin in opposite directions (antiparallel) but have the same magnitude. The spins of each electron cancel each other out and so there is no net magnetic moment. This means that these materials do not exhibit magnetic behavior without an applied magnetic field.

Ferrimagnetism is very similar to antiferromagnetism in that the free electrons spin antiparallel to each other. However, they do not have the same magnitude, and so there is a magnetic moment produced. This means that these materials do exhibit permanent magnetic properties without an applied magnetic field.

Paramagnetism is when the free electrons spin with a random orientation. This produces no net magnetic moment and so these materials do not exhibit magnetic properties without an applied magnetic field. However, when a magnetic field is applied the spins align and the material exhibits magnetic characteristics. The strength of this magnetic field is related to the magnetic susceptibility by the following equation.

$$M = \chi H \quad (2-28)$$

Here, M is the magnetization, χ is the magnetic susceptibility, and H is the magnitude of the applied magnetic field. For paramagnetic materials, the magnetic susceptibility varies according to the Curie-Weiss law, described by the following equation.

$$\chi = \frac{C_M}{T - T_c} \quad (2-29)$$

Here, C_M is the Curie constant, T_c is the Curie temperature, and T is the temperature of the system. The Curie constant and Curie temperature are parameters that vary based on the material. As equation 2.29 illustrates, the magnetic susceptibility—and thus the magnetization—decreases as the system temperature approaches the Curie temperature. This is because there is a balance between thermal energy needed to randomize the

electron spins in paramagnetic materials. Below the Curie temperature, the thermal energy is not large enough to randomize the spins and the material exhibits either ferromagnetic, antiferromagnetic, or ferrimagnetic behavior.⁴⁵

Ferromagnetic, antiferromagnetic, and ferrimagnetic behavior is based on domains, where particles between 50-500 nm consist of one domain. As stated before, these domains will have either parallel or antiparallel spins and the magnetic moment generated is dependent on the type of spin. However, when a magnetic field is applied, the spins begin to align. When the magnetic field is large enough, the spins of all the domains align parallel to each other. The magnitude of this magnetic field is called the saturation magnetization, which plays a role in the maximum force that an external magnetic field can exert on a material.⁴⁵

2.2.2.2 Superparamagnetism

When the size of the particles becomes much smaller than a typical domain size, instead of domains that align in either parallel or antiparallel configurations, magnetic anisotropy results. Essentially, electron spins are more easily aligned along preferred crystallographic planes. This is called superparamagnetism and materials that exhibit this behavior have the advantage of exhibiting strong paramagnetic properties and have large magnetic susceptibilities.⁴⁵ For MRI applications, this is important because materials with large magnetic susceptibilities distort local magnetic fields, which is important for relaxivity as will be discussed later.

In addition, while superparamagnetic materials exhibit paramagnetic behavior, they are not restricted to that behavior only above the Curie temperature. The superparamagnetic behavior can be quenched, but at a much lower temperature known as the blocking temperature, shown in equation 2.30.

$$T_B = \frac{V \cdot K_{eff}}{25k} \quad (2-30)$$

Here, V is the volume of the particle, K_{eff} is the anisotropy constant, k is Boltzman's constant, and T_B is the blocking temperature. Table 2.3 shows the Curie temperature, saturation magnetization, and magnetic susceptibility of some iron oxides.

Table 2.3. Different Curie temperatures, magnetic structures, saturation magnetizations, and anisotropy constants for various iron oxides.⁴⁵

Oxide	Curie Temperature (°C)	Magnetic Structure	Saturation Magnetization at 27 °C (A-m ² kg ⁻¹)	Anisotropy Constant, K_{eff} (J-m ⁻³)
Magnetite	577	Ferrimagnetic	92 - 100	$10^4 - 10^5$
Maghemite	547 - 713	Ferrimagnetic	60 - 80	10^5
Hematite	683	Weakly Ferromagnetic	0.3	$1 \times 10^4 - 6 \times 10^4$

As noted earlier and shown in Table 2.3, the saturation magnetization of magnetite is significantly higher than maghemite. This means that for the same magnetic field, more force will be exerted on a magnetite particle than a maghemite particle of a certain size.

Additionally, superparamagnetic materials show little to no magnetic hysteresis, whereas paramagnetic materials will show magnetic hystereses when comparing magnetization versus demagnetization processes for a magnetization vs. applied magnetic field curve.⁴⁵

2.2.2.3 Magnetite

Magnetite has an inverse spinel structure and consists of 33% tetrahedral coordinated and 67% octahedral coordinated interstices that are completely filled with Fe^{3+} in the tetrahedral interstices and equal amounts Fe^{2+} and Fe^{3+} in the octahedral interstices.^{45, 46} The difference in these is the cation in these interstices causes two magnetic lattices which are antiparallel. Because they are not the same magnitude, there is a net magnetic moment, and magnetite is considered ferrimagnetic.^{45, 46} When the particles get small enough, magnetite exhibits superparamagnetic behavior. Based on equation 2.30 and the range of anisotropy constants, the particle radius at which magnetite exhibits superparamagnetic behavior can be calculated, as shown in Table 2.4.

Table 2.4. Calculation of the critical particle radius for superparamagnetic behavior at room temperature at the extremes of the range of the anisotropy constant.

	$K_{\text{eff}} = 10^4 \text{ J m}^{-3}$	$K_{\text{eff}} = 10^5 \text{ J m}^{-3}$
r (nm)	T_B (°C)	T_B (°C)
0	-273	-273
3	-270	-240
6	-247	-11
9	-185	612
12	-63	1824
15	137	3823
18	435	6805
19	559	8052
21	851	10967

As illustrated in red, the critical radius is between 6-9 nm for $K_{\text{eff}} = 10^5$ and between 15-18 nm for $K_{\text{eff}} = 10^4$ (shown in blue). This is similar to values in the literature which observe superparamagnetic behavior at room temperature at a radius of approximately 12 nm.⁴⁹

2.2.2.4 Magnetic Ferrofluids

Magnetic ferrofluids are colloidal suspensions of magnetic nanoparticles suspended in an appropriate carrier fluid.⁵⁰⁻⁵³ They are used for many different applications, both industrial and biological. Industrial uses of ferrofluids include applications in sealing, damping and heat transfer.⁵⁴ Biological applications include cellular therapy, cellular targeting, tissue repair, drug delivery, magnetic resonance imaging (MRI), and hyperthermia.⁵⁵⁻⁶⁵

Synthesis of ferrofluid particles can be achieved through various methods, including wet grinding, coprecipitation, microemulsion, decomposition of organometallic compounds, inverse microemulsion techniques, and reduction of metal salts in aqueous solution.⁵² The particles must then either be coated with a surfactant to provide steric stability or have their surface modified to produce a repulsive electrostatic force to prevent aggregation.⁵⁰⁻⁵² The physical properties of the ferrofluid—such as viscosity, surface tension, temperature stability, oxidative stability, and vapor pressure—can be tailored by varying the carrier fluid in which the particles are suspended.⁵² Common carrier fluids

include organic solvents, inorganic solvents (water), hydrocarbons, synthetic esters, polyglycols, polyphenyl ethers, and many others.^{50-52, 54}

Our interest in ferrofluids is their application to biological systems, particularly as a treatment for retinal detachment.^{66, 67} However, a complication that arises with the traditional ferrofluid configuration (particles suspended in a carrier fluid) is the occurrence of phase separation, which can reduce the efficacy of the ferrofluid in biological systems.⁶⁸ Additionally, from both a biological systems perspective—as well as a magnetic characterization perspective—we would like to have a well-defined particle size distribution and be able to quantify that distribution.⁶⁹

2.2.3 Cerium oxide

2.2.3.1 Biological Activity of Cerium Oxide

Nanoparticulate cerium oxide has been shown to have profound biological effects in cell culture and animal experiments.^{38, 70} Additionally, these same effects have not been seen in experiments with micron-sized ceria. Nanosize ceria has extended the lifespan of mixed brain cell cultures by six fold, and the lives of *Drosophila* significantly. During the cell cultures' extended lifespan, calcium signaling was preserved. The fruit flies were fed 10 nM ceria suspended in saline in their normal commercial fly food and showed resistance to paraquat, a common component of herbicides.³⁹

In addition to increased lifespan, free radical damage due to UV-light, H_2O_2 , and excitotoxicity was mitigated by the ceria nanoparticles. This seems to be convincing evidence that the ceria nanoparticles are acting as free radical scavengers. This illustrates another interesting feature of the ceria particles used in these studies. They seem to have a regenerative nature, where the Ce^{3+} ion reacts with the free radical, and through some series of reactions or rearrangements, regains its activity. Conversely, the CeO_2 could be acting as a catalyst, lowering the activation energy for the decomposition of H_2O_2 compared to the bulk.

Evidence in the literature seems to suggest that nanoceria has far different characteristics than microceria. This may be due to increased oxygen defects seen throughout the crystal structure.⁷¹⁻⁷⁴ Oxygen defects are crystal defects caused by oxidation of the crystal lattice without a change in stoichiometry.⁷⁵ Nanoceria has also shown to increase cell survival rates in normal human breast tissue, but not in tumor-affected human breast tissue⁷⁶. It is believed that the ceria nanoparticles act as free-radical scavengers in the cell, and the ability of the nanoparticle to infiltrate the cell and then to scavenge free radicals is the crux of the problem. In addition, nanoceria has also been used as a semiconducting material in photovoltaic cells.⁷⁷

Cerium oxide has been used in many industrial applications, ranging from catalysis to chemical mechanical polishing (CMP). It is a pale yellow-white powder that is slightly hygroscopic. It exists in two forms, cerium(IV) oxide, denoted CeO_2 , and cerium(III) oxide, denoted Ce_2O_3 . This oxidation state change leads to many of the interesting properties of ceria.

The appearance of ceria is very sensitive to stoichiometry. As mentioned above, pure ceria has a pale yellow-white color, while a blue color has been observed in non-stoichiometric ceria samples, most probably due to the Ce^{4+} to Ce^{3+} transitions. These large deviations from stoichiometry can occur at low oxygen partial pressures (CeO_{2-x} , where x can be 0.3), but at normal ambient conditions, x is usually zero. The oxygen defects of the crystal lattice are controlled by the stoichiometry, and thus, for micron-sized ceria under ambient conditions, these defects can only be altered by doping.⁷⁸

2.2.3.2 Oxygen Vacancies and Cerium Oxide Reactivity

Oxygen vacancies are a kind of surface defect inside a crystal structure. Surface defects can be produced on oxide surfaces when modifying or cleaning the surface or from impurities in the crystal lattice. These defects can be either structural, which are generally caused by the surface thermodynamically favoring a certain geometrical configuration, or stoichiometrically, which leads to oxygen and/or cation vacancies. Vacancies have been shown to be active sites for chemical reactions, and thus, there is great interest in their ability to enhance the catalysis of certain reactions.⁷⁹

These defects formed due to nonstoichiometry are formed because of redox reactions that take place with a compound without a change in crystal structure. Thus, thermodynamic equilibrium dictates that these defects are formed to balance out this change. When these defects are completely saturated, an additional phase will separate (i.e. FeO oxidizing to Fe_3O_4 or reducing to Fe metal). This phenomenon seems to be exaggerated in multi-valent ions.⁷⁵ Ceria is known to participate in various redox reactions, and is a multi-valent ion, so it is not surprising that there would be nonstoichiometric defects formed on its surface.

Because ceria is already used abundantly in catalysis, the surface defects, in particular the oxygen vacancies, have been studied extensively. Yang, et al. found that vacancy formation was favored in the surface layer and subsurface layer of cerium oxide depending upon the surface conditions. The surface layer was favored for vacancy formation for the (110) ceria surface, while the subsurface layer was favored for vacancy formation for the (110) ceria surface. In addition, the (111) surface had relatively small surface relaxation when compared to the (110) and (100) surfaces, and the surfaces with higher surface relaxations were less stable and more susceptible to vacancy formation. Even though there were differences in occurrence of vacancies at each surface, the surface was much more susceptible to vacancy formation than the bulk. Yang also found an oscillatory behavior for the vacancy formation energy on the subsurfaces of the ceria (111) surface.⁸⁰

Conesa found very similar results to Yang, in that he found that the stability of the ceria surfaces (stability meaning resistance to vacancy formation) again went in the order of the (111), (211), (110), and (100) surfaces. While anion vacancy (oxygen vacancy in this case) formation was less favorable for the (111) surface, anion vacancy pairs were formed much easier on the (111) surface, and did not form on the (110) and (100) surfaces at all.⁸¹ The vacancy formation energy for each of these ceria surfaces has been researched by other groups as well, and seems to be fairly well established.^{82, 83} This indicates that by differing the ratio of crystal surfaces, one could alter the reactivity or selectivity of cerium oxide nanoparticles.

Mamontov, et al. compared pure ceria nanoparticles with zirconia-doped ceria. They found that the ceria-zirconia vacancies had large oxygen deficiency and that this required the formation of Ce(III) ions to keep the system electronically neutral. The pure ceria nanoparticles did not show this behavior, probably due to the interstitial anions in the lattice providing charge compensation. In addition, EPR studies of the pure ceria versus the ceria-zirconia systems showed no active oxygen species for the pure ceria, but some active oxygen species for the ceria-zirconia system.⁸⁴ This indicates that vacancies formed by doping may produce free radicals, while vacancies of pure ceria would tend to be reactive towards free radicals.

Removal of excess oxygen from the ceria lattice leads to localization of the left over electrons on the empty f-states. This causes ceria to change from the Ce(IV) valence state to the Ce(III) valence state, which is far more reactive. Esch et al. found that the electrons left behind with the removal of oxygen from the crystal lattice localize around the cerium ions. They postulate that oxygen vacancy formation is in clusters, such that the Ce(III) valence state is exposed exclusively.⁸⁵ This ceria valence state is much more reactive than the Ce(IV) valence state, and may help to explain the reactivity of the ceria nanoparticles, since as mentioned earlier, smaller ceria particles seem to have a higher ratio of Ce(III) to Ce(IV) valence states.

2.2.4 Interaction of Phosphate with Metal Oxides

Phosphate is a nutrient that is required for all living tissue. It contains 2% phosphorous and is needed for many different processes including photosynthesis for aquatic plants, biological waste treatment, and industrial water softening. There are four main types of phosphates: orthophosphates, polyphosphates, metaphosphates, and organic phosphates. Figure 2.13 shows the structural representation of the orthophosphates, as well as their pK_a values.⁸⁶

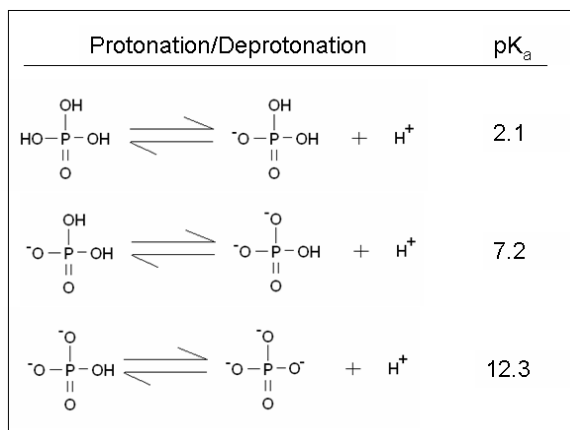


Figure 2.13. Structural representation of protonation/deprotonation steps and the appropriate pK_a values for the orthophosphate species (redrawn by W. Miles).⁸⁶

This is important because after phosphate adsorbs to the surface of a material, the charge of the phosphate will dictate the charge of the complex. In this case, phosphate would be a potential determining ion.⁶

Phosphate has been found to adsorb strongly with various oxides, including iron oxide and magnetite.⁸⁷⁻⁹¹ Daou et al. found that phosphate adsorbs heavily to magnetite, but that it doesn't affect the magnetization of the magnetite or the chemical structure. They found that the phosphonation occurred specifically at octahedral sites (Fe³⁺)⁸⁹ Bocher et al. found that when performing coprecipitation of ferrous sulphate heptahydrate and ferric sulphate pentahydrate with calcium carbonate, addition of phosphate promoted the product to be all [Fe^{II}₄Fe^{III}₂(OH)₁₂]²⁺•[CO₃, nH₂O]²⁻, also known as green rust one [GR1(CO₃²⁻)]. However, when the coprecipitation was done without phosphate, the product was GR1(CO₃²⁻), magnetite, and ferrous hydroxide. This was because the phosphate species appeared to preferentially adsorb to a specific type of coordination of OH groups in the surface.⁸⁷ Thus, it appears that phosphate adsorbs preferentially to specific sites on iron oxides, meaning that design of iron oxides that adsorb or do not adsorb phosphate may be possible.

The adsorption of phosphate to various iron oxides can be seen as an advantage or disadvantage, depending upon the application. Borch et al. found that phosphate adsorption to iron oxides in subsurface environments inhibits the reactivity of the iron

oxide linearly with phosphate concentration.⁸⁸ Thus, prevention of this adsorption would allow the iron oxide to react with contaminants in the water, but perhaps also react with possible nutrients. Eskandarpour et al. designed nanoaggregates to remove phosphate from wastewater.⁹⁰ Here the adsorption is positive and necessary for the application. White et al. designed phosphate anchor groups to attach polymers to iron oxide nanoparticles.⁹¹ Here, the adsorption was used in the design of a specific anchor group, taking advantage of the adsorption to overcome the entropy loss due to the chains organizing on the surface.

2.3 Polymer Adsorption onto Surfaces

2.3.1 Mechanism of Polymer Adsorption onto Solid Surfaces

Polymer adsorption onto solid surfaces occurs when the thermodynamic loss of entropy due to the increased arrangement of the polymer chains is overcome by the force of attraction of the polymer chains to the surface. In addition, there are kinetic phenomena that determine whether or not a polymer adsorbs to the solid surface. First, the polymer must diffuse to the solid surface. Second, the polymer must stick to the surface, which is mainly determined by the thermodynamic forces described above. Finally, the polymer must undergo conformation changes to minimize the free energy.⁹²

Typical polymer adsorption of a homopolymer that is attracted to a solid surface is illustrated in Figure 2.14.

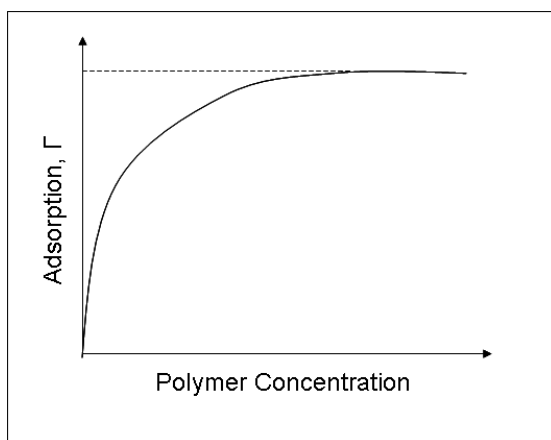


Figure 2.14. The adsorption isotherm reaches a plateau as the polymer concentration is increased. Transitions from high to low polymer concentration does not reduce the polymer adsorbed onto the surface (dotted line) (redrawn by W. Miles).⁹²

The polymer adsorbs differently in different solvents. In good solvents ($\chi < 1/2$), the surface adsorption is proportional to molecular weight but the plateau is independent of molecular weight at high molecular weight. In theta solvents, the surface adsorption increases without bound (i.e. no plateau). Obviously then, adsorbed amounts are higher in theta solvents than in good solvents.

A polymer can have a positive or negative adsorption, the determination of which depends on the net interaction of the polymer with the surface compared to the entropy loss due to organization of the polymer on the surface. Small molecules can affect this energy balance and cause desorption, particularly if the small molecules attach more strongly than the polymer. Polymers adsorb to solid surfaces in tails, loops, and trains. Figure 2.15 illustrates the difference between these three adsorption steps.⁹²

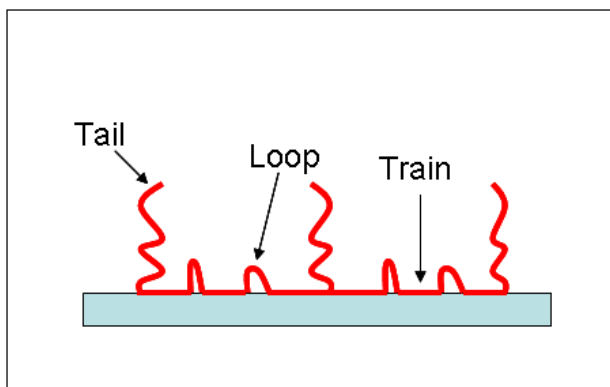


Figure 2.15. Polymers adsorb to solid surfaces in tails, loops, and trains (redrawn by W. Miles).⁹²

As the name suggests, tails are polymer chains that extend out from the surface into solution, loops are polymer chains that have both ends connected to the solid surface but otherwise are disconnected from the surface, and trains are polymer chains that are completely in contact with the surface. For systems of multiple polymers, higher molecular weight species adsorb preferentially. Thus, polymers with large PDIs affect adsorption as the larger molecular weight polymers lose out to the smaller molecular weight polymers due to competitive adsorption.⁹²

2.3.1.1 Adsorption of Polymers in Selective and Non-Selective Solvents

When dealing with adsorption of block copolymers, it is important to specify what type of solvent the copolymer is in. A non-selective solvent is one where both blocks are soluble in the solvent. Typically one block is preferentially adsorbed onto the surface, and this is called the anchor block. The other block forms a brush of tails extending out from the surface in the solvent, which is called the buoy. For steric stabilization of

colloids, a block copolymer has the advantage that the anchor block can keep the polymer on the particle surface while the buoy block provides steric stabilization.⁹²

A selective solvent is a solvent where one block of the copolymer is insoluble in the solvent. This leads to formation of micelles in the solvent and affects the way in which the polymer adsorbs onto the surface. Figure 2.16 shows the difference in the adsorption behavior for the same diblock copolymer in both a non-selective and selective solvent.

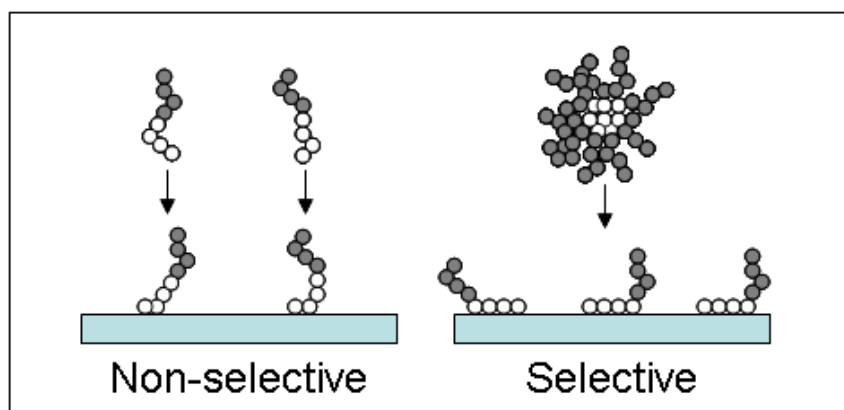


Figure 2.16. The same diblock copolymer adsorbs to a solid surface differently depending on the type of solvent being used for dispersion (redrawn by W. Miles).⁹²

The most striking difference is that in the selective solvent, the insoluble block attaches almost completely to the surface, while there is significant swelling in the anchor block during non-selective adsorption. Additionally, the diblock copolymer is completely dispersed before adsorption in the non-selective solvent while the copolymer has formed micelles in the selective solvent. Thus, above the critical micelle concentration (CMC), the adsorption of the diblock copolymer can be significantly slowed because of the energy needed to disrupt the micelle structure.

For non-selective solvents, the ratio of molecular weights of the blocks affects how the polymer adsorbs to the surface. For cases where the anchor block is short and the buoy block is long, there is significant steric repulsion between the buoy blocks. Thus, the buoy block molecular weight determines the number of chains adsorbed onto the surface and the anchor layer is undersaturated. This is called the buoy dominated regime and is shown in Figure 2.17.

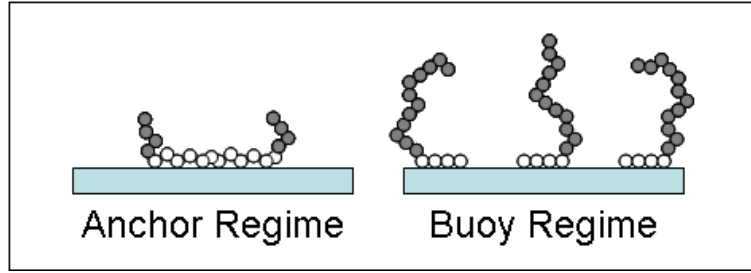


Figure 2.17. For non-selective solvents, the molecular weight of each block determines how the diblock polymer will adsorb (redrawn by W. Miles).⁹²

Also shown in Figure 2.17 is the case where the anchor block molecular weight is large and the buoy block molecular weight is small. In this case, the overlap (and hence steric repulsion) of the buoy block is minimal and so adsorption is limited by adsorption of the anchor layer. This is called the anchor dominated regime. The ratio of the molecular weights in the anchor and buoy dominated regimes can be described by the asymmetry ratio, β_n , as shown in equation 2.31.⁹²

$$\beta_n = \left(\frac{N_B}{N_A} \right)^{6/5} = \left(\frac{1 - v_A}{v_A} \right)^{6/5} \quad (2-31)$$

Here, the total number of segments, N , can be described as the sum of the number of anchor segments, N_A , and the number of buoy segments, N_B . The fraction of anchor segments is then $v_A = N_A/N$. The point at which the asymmetry ratio is equal to the number of anchor segments, N_A , is where the system transitions from the anchor dominated regime to the buoy dominated regime. Each regime can then be described by estimating the surface chain density, σ , as shown in equations 2.32 and 2.33.⁹²

$$\text{Buoy regime: } \sigma \propto \frac{1}{N_A} = \frac{1}{N v_A} \quad (2-32)$$

$$\text{Anchor regime: } \sigma \propto \frac{N_B}{N_A^{1/3}} = \frac{1 - v_A}{v_A^{1/3}} N^{2/3} \quad (2-33)$$

There are no anchor or buoy dominated regimes for selective solvents, as the anchor layer is not thermodynamically stable with both the solvent and the surface. Thus, the anchor group adsorbs strongly to the surface. Marques, Joanny, and Leibler described the anchor layer as forming a melt on the surface and called this the Van der Waal's brush regime.^{93, 94} The buoy segments remain solvated and extend out like brushes into the solvent. The asymmetry ratio and surface chain density for selective solvents can be described as shown in equations 2.34 and 2.35.⁹²

$$\beta_n = \left(\frac{N_B^{6/5}}{N_A} \right) = \left(\frac{(1-v_A)^{6/5}}{v_A} \right) \quad (2-34)$$

$$\sigma \propto N_A^{-1/2} N_B^{-1/4} = N^{-3/4} v_A^{-1/2} (1-v_A)^{-1/4} \quad (2-35)$$

It should be noted that the values for the asymmetry ratio and surface chain densities are equilibrium estimates. It is important to note that the kinetics of adsorption of a diblock copolymer in a selective solvent will change based on concentration, especially at the critical micelle concentration (CMC).

2.3.1.2 Adsorption of Polyelectrolytes on Charged Surfaces

The introduction of charge complicates adsorption significantly. Table 2.5 shows the different cases where charge plays a role in polymer adsorption to a surface.

Table 2.5. Charge complicates adsorption both when the surface is charged and the polymer is charged.⁹²

Polymer	Surface	Main Factors	Salt Effect
Uncharged	Uncharged	Ion and segment competition	Negligible Effect
Charged	Uncharged	Segment-segment repulsion	Increases adsorption
Both with Same Charge		Segment-segment and segment-surface repulsion	Increases adsorption
Both with Opposite Charge		Pure electrostatic attraction	Decreases Adsorption
		Both electrostatic and surface affinity attraction	Can Increase or Decrease Adsorption

As shown in Table 2.5, there are four main categories of charge to consider. First is when both the polymer and surface are uncharged. In this case, adsorption occurs based

on mechanisms that have been discussed in earlier sections. As such, salt has very little effect other than to possibly change how the polymer and solvent interact.

The second case is when the polymer is charged and the surface is not. In this case, packing of the polymer on the surface is directly impacted not only by steric considerations, but also by electrostatic repulsion of the charged groups in the polymer. Thus, as salt is added to the solution and the electrostatic charges are screened, adsorption increases.

The third case is when both the polymer and surface have the same sign charge, whether it be positive or negative. In this case, there is electrostatic repulsion between the polymer segments just as in case 2. However, in this case there is also electrostatic repulsion between the surface and the polymer. Again, by screening out these electrostatic effects with salt, adsorption can be increased.

The final case is when the polymer and the surface have opposite charge signs. In this case, there is an electrostatic attraction between the surface and the polymer. In fact, this electrostatic attraction can be large enough to hold the polymer to the surface absent any specific affinity for the surface due to the polymer. In this case, adding salt screens out this attractive force and reduces the amount of polymer adsorbed. In the case where the polymer does have specific affinity for the surface, salt can either increase or decrease the amount of polymer adsorbed. This is determined by the balance of the electrostatic repulsion between the polymer segments, the electrostatic attraction between the polymer and the surface, and the specific affinity of the polymer to the surface.⁹²

2.3.2 Adsorption of Proteins onto Metal Oxide Surfaces

Other than for stability, it may be unclear as to why metal oxides must have adsorbed polymers on the surface to be effective biological agents. The following sections details interactions between metal oxides and proteins, many of which can be mitigated with the application of a steric stabilizing polymer layer. There is relatively sparse literature detailing protein adsorption on magnetite or ceria, but there is a wealth of literature on protein adsorption on other metal oxides, which is what is presented here.

It has been shown that adsorption of different proteins will occur at titanium dioxide surfaces.⁹⁵⁻⁹⁷ As mentioned before, the point of zero charge, or isoelectric point, is the point at which there are an equal number of both positive and negative charges. Giacomelli, et al. have shown a distinct decrease in the isoelectric point (IEP) of titanium dioxide when exposed to Bovine Serum Albumin (BSA) from the IEP of TiO_2 (6.5) to that of BSA(4.7).⁹⁸ This indicates that the entire surface of the titanium dioxide nanoparticles is saturated in the protein and thus, the mobility of the particles is dependent upon only the charge of the protein. The amount of adsorbed protein was determined through UV-Vis spectroscopy following centrifugation. The protein adsorption was highly pH dependent, but there was adsorption at all pH levels, with the maximum adsorption coinciding with the IEP of BSA. In addition, there are monomeric, dimeric, and polymeric species in BSA, and there was a difference in adsorption of each of these components at all pH levels, with the exception of the exact same amount being adsorbed for each at the IEP. In addition, sodium chloride concentration did not seem to affect adsorption.⁹⁸

Olivia, et al. saw many of the same characteristics when exposing titanium dioxide to Human Serum Albumin (HSA). Again, the IEP shifted from that of the titanium dioxide down to that of the HSA, indicating complete coverage. The explanation for the adsorption occurring at all pH levels is that the macromolecular protein—in this case HSA—has both positive and negative charges on it, as does the titanium dioxide. Thus, no matter the pH—which correlates with the surface charge of the titanium dioxide—there is an opposite charge on the protein where adsorption can occur.⁹⁹

These charging characteristics also indicate why the maximum adsorption occurs at or right above the HSA isoelectric point, where the charge on the titanium dioxide would be largely positive, while the charge on the HSA would be neutral or negative. At a pH of approximately 6.5 (the IEP of TiO_2), this is no longer the case, as the titanium dioxide now takes on a net negative charge as does the HSA.

The adsorption of proteins on metal oxide surfaces is not limited to just titanium dioxide. Topoglidis, et al. observed the adsorption of cytochrome c and hemoglobin on tin oxide⁹⁶ and Diniz, et al. looked at the adsorption of concanavalin A on oxidized platinum.¹⁰⁰ Fukuzaki, et al. observed the adsorption behavior of BSA on silicon dioxide, titanium dioxide, zirconium oxide, and aluminum oxide as functions of pH. Adsorption trends for BSA were similar for the titania, zirconia, and alumina, but not for the silica. They concluded that the surface charge density of the metal oxides was a determining factor in the adsorption of BSA molecules. They also concluded that the amount adsorbed corresponded to different orientations of the BSA molecule at different metal oxide surface charges.¹⁰¹

Scotchford, et al. produced metal oxide based surfaces using photolithographic techniques that consisted of titanium oxide, aluminum oxide, vanadium oxide and niobium oxide, which had isoelectric point ranges of 3.5-6.7, 8.8-9.5, 1.5-2.3, and 3.4-3.8, respectively. They found that vanadium oxide and aluminum oxide had the largest effect on the morphology of the cells and that they did not facilitate cell growth nearly as much as the other surfaces. They postulated that one reason for this phenomena in aluminum oxide is that the isoelectric point dictates that at biological pH (7.0-7.4), there is a net positive charge on the alumina surface.¹⁰² It may also be that the vanadium oxide dictates changes in cellular morphology because of the strength of its surface charge. The surface potential for vanadium oxide would be very large at biological pH because the IEP is 5-6 pH units away. Thus, the potential felt by the cells would be greater for vanadium oxide than for titanium oxide or niobium oxide.

2.3.3 Adsorption of Polymers on Metal Oxide Surfaces

It appears that generally, most protein structures—particularly amphiphilic ones—adsorb to metal oxide surfaces, and that this generally holds true for most metal oxides due to the similarities in their charging behavior and surface chemistry. The next logical step is to control the adsorption of certain proteins through the adsorption of polymers selective to certain proteins. In addition, adsorption of polymers that are not selective towards any proteins might give insight into various biological transport mechanisms as well as preventing undesired adsorption.

Indeed, adsorption of protein-selective polymers has already been performed on some metal oxides. Ruiz-Taylor, et al. coated silicon oxide, titanium dioxide, and niobium oxide with poly(L-lysine)-grafted-poly(ethylene glycol) copolymers (PLL-*g*-PEO). This was done because PEO reduces the adsorption of many proteins in the blood, and the copolymer adsorbed readily to any negatively charged surface at biological pH. They showed that these coated polymers, with active sites built into the PEG, adsorbed streptavidin, while preventing other proteins from adsorbing, even in complex protein mixtures.¹⁰³

But the coating of various metal oxides is not always as trouble free as described above. Indeed, the introduction of particle shapes and sizes introduces a variable not observed by Ruiz-Taylor, et al., as they adsorbed their copolymer onto a flat metal oxide substrate. While the surfaces should react the same way, the introduction of particles in solution may introduce other hurdles to surface coating. This was observed by Berret, et al., where they were able to coat ceria and iron oxide nanoparticles with poly(trimethylammonium ethylacrylate)-*b*-poly(acrylamide) or (PTEA(5k)-*b*-AM(30K)). They found that after coating, the 7 nm particles had formed stable clusters of around 70 nm.¹⁰⁴ It is unclear what affect clustering would have on the biological activity of various nanoparticles, but particle size is most likely an important parameter. Thus, it is important to be able to coat each nanoparticle individually to make transport and reactivity comparisons.

Harris, et al. found that anionic triblock copolymers, consisting of two poly(ethylene) oxide tail blocks and polyurethane center blocks could be chemisorbed to magnetite nanoparticles about 10 nm in size via the carboxylic acid groups. They do note however that because the polyurethane center blocks are not water soluble, the magnetite particles did not react with the triblock copolymer unless dichloromethane was introduced into the system.¹⁰⁵ Thus, it appears that the chemisorption process may be far more involved than the physisorption process described by Ruiz-Taylor, where cationic polymer was physisorbed onto an anionic surface. In the case of magnetite, the IEP is around 6.8, and

thus, at biological pH is an anionic surface, so reaction with the anionic carboxylic acids may have been hindered by repulsive interactions.¹⁰⁵

2.4 Colloidal Stabilization through Polymer Adsorption

2.4.1 Density Distribution Model

2.4.1.1 Determination of the Radius of the Complex

The density distribution (DD) model is a tool used to describe star polymers and particle-polymer complexes that behave like star polymers. The need for a model arises from the desire for an understanding of the polymer behavior in solution to be able to predict the steric force imparted by the polymers when they overlap. The density distribution model was developed by Vagberg et al.¹⁰⁶ based on the ideas of Daoud and Cotton that proposed modeling of star polymers as concentric shells emanating from a central point with the same number of blobs, as shown in Figure 2.18.¹⁰⁷

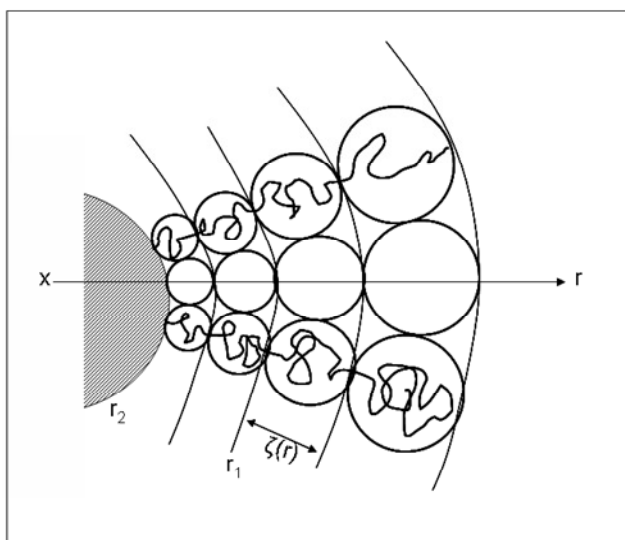


Figure 2.18. Modeling of star-shaped polymers as concentric shells with constant blob size from a central point (redrawn by W. Miles).¹⁰⁷

The effect of this model is that there is a constant density (or volume fraction) of polymer at the center, and then that volume fraction decreases for each successive blob, as shown in Figure 2.19. The radii r_1 and r_2 in Figure 2.18 and Figure 2.19 correspond to distances from the core surface to where the concentric shell of blobs begins.

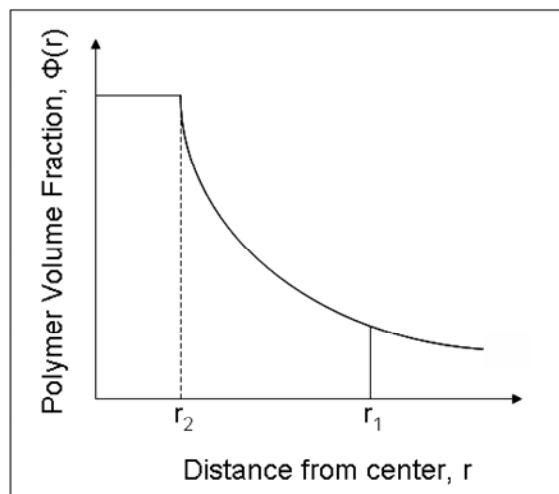


Figure 2.19. The polymer volume fraction is constant at the center but then decreases as the blob size increases (redrawn by W. Miles).¹⁰⁷

Vagberg et al. further developed this model to describe the dimensions of amphiphilic diblock copolymers that form micelles.¹⁰⁶ The hydrophobic blocks were treated as a solid core, while the hydrophilic blocks were treated as concentric shells with an equal number of blobs, as shown in Figure 2.20.

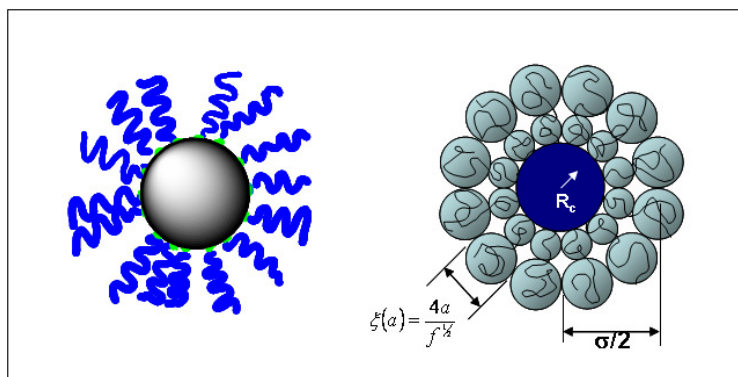


Figure 2.20. Vagberg et al. treated amphiphilic diblock copolymers as a micelle with a solid non-solvated core and a hydrophilic star-shaped polymer brush.¹⁰⁶

The DD model predicts micelle radius, R_m as shown in equation 2.36.

$$R_m = \left[Na_s^{1/\nu} \frac{8f^{(1-\nu)/2\nu}}{3\nu 4^{1/\nu}} + R_c^{1/\nu} \right]^\nu \quad (2-36)$$

Here, ν is the Flory exponent, R_c is the radius of the core, N is the number of Kuhn segments, and f is the number of hydrophilic chains. The blob diameter can be described as shown in equation 2.37.

$$\xi(a) = \frac{4a}{f^{1/2}} \quad (2-37)$$

Here, a is the particle radius and f is again the number of hydrophilic chains emanating from the core.¹⁰⁶

The DD model was further expanded upon by Mefford et al. by treating a particle-polymer system consisting of magnetite and poly(dimethylsiloxane) (PDMS) similarly to the system of Vagberg.^{108, 109} In these studies, the magnetite particle core was quantified with TEM and then fit with a probability distribution. Then, the PDMS chains were treated with the blob model to describe their behavior in both good and theta solvents. Further expansion of the model was done by Zhang, et al., where a magnetite-polymer complex consisted of the magnetite core particle and a pentablock copolymer consisting of a urethane center block and two tails, each consisting of a hydrophobic poly(propylene oxide) (PPO) block and a hydrophilic poly(ethylene oxide) (PEO) block.¹¹⁰

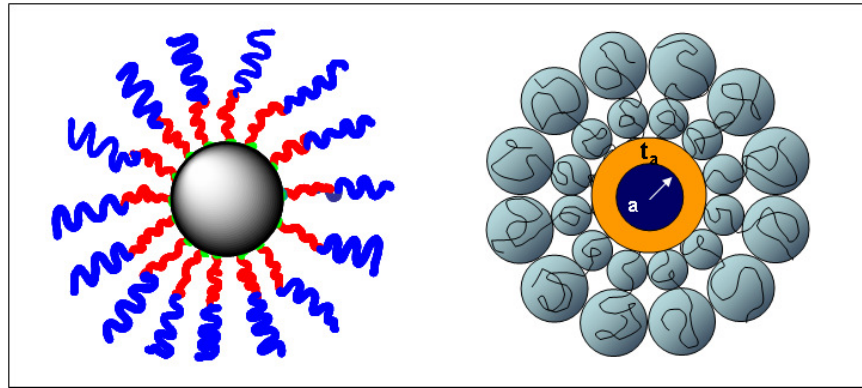


Figure 2.21. Incorporation of a non-solvated shell to represent a hydrophobic block was introduced by Zhang, et al.¹¹⁰

The DD model was altered to include a particle core, but then also a non-solvated shell to represent the hydrophobic PPO blocks followed by the concentric shell of blobs to

describe the hydrophilic PEO blocks. This representation is shown in Figure 2.21, where t_a is the thickness of the anchor layer. This anchor layer thickness can be calculated as shown in equation 2.38.

$$t_a = \left(\left(\frac{\rho_{mag} a^3 (1 - W_{mag})}{W_{mag} MW_{PPO} \rho_{PPO} MW_{Total}} \right) + a^3 \right)^{1/3} - a \quad (2-38)$$

The number of chains per particle is a function of the radius as shown in equation 2.39 owing to the probability distribution fit of Mefford et al.^{108, 109}

$$f(a) = 4\pi a^2 \alpha \quad (2-39)$$

Thus, the modified DD model predicts a complex radius, $R_m(a)$, as shown in equation 2.40.

$$R_m(a) = \left(\frac{8N_k f(a)^{\frac{1-\nu}{2\nu}} L_k^{1/\nu}}{4^{1/\nu} 3\nu} + (a + t_a)^{1/\nu} \right)^\nu \quad (2-40)$$

Note that the complex radius is a function of the particle radius, a , and that this also is due to the fitting of the core radius with a probability distribution.

Other studies have used the DD model with various levels of success. Farinha et al. studied polystyrene-b-poly(ethylene oxide) (PS-b-PEO) micelles in both water and cyclopentane.¹¹¹ They found that the DD model predicted the micelle size well when the ratio of thickness to core radius was less than 0.72. The model did slightly overpredict the brush thickness when compared to DLS, but this was attributed to polydispersity in the molecular weights of the blocks and also to loops and trains formed during adsorption due to interaction of the PEO with the PS in solution. Harada et al. studied the size of micelles made with a mixture of poly(ethylene glycol)-b-poly(α - β -aspartic acid) (PEO-b-P(Asp)) and PEG-b-poly(L-lysine) (PEG-b-P(Lys)).¹¹² Again, the micelle size was compared to that obtained from DLS and again, the predicted size was larger than the measured size. Conversely, both Johnsson et al. and FitzGerald et al. found that the predicted size was slightly less than the experimental size in their studies.^{113, 114} However, these studies did not use DLS for comparison. Johnsson studied PEG

derivatized lipids in water where the phospholipid core had a negative charge. The micelle size was estimated by using the measured aggregation number and the core volume occupied. FitzGerald studied polyoxyethylene surfactants (nonionic Gemini surfactants) and measured the size of the micelles by SANS at varying temperature. Thus, the method of determining the micelle size may be just as important as the model to which it is being compared.

2.4.1.2 Determination of the Radius of Gyration using the Density Distribution Model

As noted earlier, the purpose of using the density distribution model to describe the behavior of star polymers is so that the radius of gyration (R_g) can be calculated. The radius of gyration is a common measure of chain dimension, and R_g^2 is the second moment about the chain's center of mass.⁴ The R_g can be measured using static light scattering or neutron scattering or can be estimated as shown in equation 2.41.

$$R_g = \sqrt{\frac{I_{total}}{m_{total}}} \quad (2-41)$$

Here I_{total} is the total moment of inertia of the complex, and m_{total} is the total mass of the complex. The total moment of inertia can be calculated by deconvoluting each segment—the particle core and the solvated PEO corona—into its own moment of inertia because of the additive nature of the term as shown in equation 2.42.^{109, 115}

$$I_{total} = I_{core} + I_{corona} \quad (2-42)$$

The core moment of inertia can be described using the equation for the moment of inertia for a sphere with a density ρ_{core} and a distance r from the center.

$$I_{core} = \int_0^{R_c} r^2 \rho_{core} 4\pi r^2 dr = \frac{4\pi R_c^5 \rho_{core}}{5} \quad (2-43)$$

The corona density is described by the DD model as shown in equation 2.44.¹⁰⁶

$$\rho(r) = m_s A \left(\frac{r}{a_s} \right)^{\frac{1}{v}} r^{-3} \quad R_c < r < R_m \quad (2-44)$$

Here, m_s is the mass of the corona chains and A is the proportionality constant described by Vagbert et al. and described as shown in equation 2.45.

$$A = \frac{(3)4^{\frac{1}{\nu}} f^{\frac{3\nu-1}{2\nu}}}{32} \quad (2-45)$$

Thus, the moment of inertia of the corona chains can be described as shown in equation 2.46.

$$I_{corona} = \int_{R_c}^{R_m} r^2 m_s A \left(\frac{r}{a_s} \right)^{\frac{1}{\nu}} r^{-3} 4\pi r^2 dr = \frac{4\pi A m_s}{a_s^{\frac{1}{\nu}} \left(2 + \frac{1}{\nu} \right)} \left(R_m^{(2+\frac{1}{\nu})} - (R_c)^{(2+\frac{1}{\nu})} \right) \quad (2-46)$$

The total mass of the complex can be also be described additively, as shown in equation 2.47.

$$m_{total} = m_{core} + m_{corona} \quad (2-47)$$

These values can be determined through thermogravimetric analysis for particle-polymer complexes where the particle does not degrade at the degradation temperatures of the polymer.

The radius of gyration can then be used to calculate the stability of the colloidal suspension in various solvents.^{109, 115} Caba et al. measured R_g s for polymer micelles with small angle neutron scattering (SANS) and the calculated R_g s were within 7% of experimental values.^{109, 115} An explanation of the stability calculations can be found in the following section.

2.4.2 Extended Derjaguin-Landau-Verwey-Overbeek (DLVO) Theory

Colloidal stability can be predicted from a model for the total potential energy V_{Total} as described by DLVO theory and shown in equation 2.48.²

$$V_{Total} = V_{vdW} + V_M + V_{ES} + V_S \quad (2-48)$$

V_{vdW} is the attractive potential due to van der Waal's interactions, V_M is the attractive potential produced by an applied magnetic field, V_{ES} is the repulsive potential due to

electrostatic interactions, and V_s is the repulsive potential due to steric repulsion of the polymer brushes.

Typically a potential energy maximum of > 10 kT is usually necessary for long-term colloidal stability although this is material specific. This specification is for dilute solutions and as the solution becomes more concentrated, the calculations of particle-particle interactions no longer apply.¹¹⁶

2.4.2.1 Electrostatic Particle-Particle Interactions

An electrostatic repulsive potential results because of the overlap of the diffuse layers around the two particles when they come into close proximity. At low potentials after application of the Poisson-Boltzman distribution, the surface charge density can be described as $\sigma_o = \epsilon_o \epsilon \kappa \psi_o$, where ϵ is the dielectric constant of the solvent, ϵ_o is the permittivity of free space, κ is the inverse Debye length, and ψ_o is the surface potential.¹¹⁷ If the double layer is compressed—which occurs when the particles come together (i.e. κ increases), either the surface charge density increases, the surface potential decreases, or both occur. While it is believed that both of these phenomena occur, it is only important when the radii of the particles interacting are not identical. However, if the radii are assumed to be identical and the particle is assumed to be spherical, the electrostatic potential can be calculated as shown in equation 2.49.¹¹⁷

$$V_{ES} = 2\pi a \epsilon \epsilon_o \psi_o^2 \ln(1 + e^{-\kappa(r-2a)}) \quad (2-49)$$

Here, r is the center to center separation, and a is the particle radius. For a small electric double layer overlap (i.e. $\exp(-\kappa(r-2a)) \ll 1$), the electrostatic potential can be calculated as shown in equation 2.50.

$$V_{ES} = 2\pi \epsilon \epsilon_o \psi_o^2 e^{-\kappa(r-2a)} \quad (2-50)$$

Both equations 2.49 and 2.50 assume the Debye-Hückel approximation, which essentially limits their use to low surface potential systems ($ze\psi_o/2kT \ll 1$).¹¹⁷

Another method for calculating the electrostatic potential was derived by Rerrink and Overbeek and does not assume the Debye-Hückel approximation.¹¹⁸ However, it does assume small electric double layer overlap. For particles of the same size, the electrostatic potential can be described by equations 2.51 and 2.52.

$$V_{ES} = \frac{32\pi\epsilon\epsilon_0 a k^2 T^2 \gamma^2}{e^2 z^2} \quad (2-51)$$

$$\gamma = \frac{e^{\left[\frac{ze\psi_0}{2kT}\right]} - 1}{e^{\left[\frac{ze\psi_0}{2kT}\right]} + 1} \quad (2-52)$$

If the Debye-Hückel approximation is applied to this system, equation 2.51 reduces to equation 2.50. Thus, for materials with high potential (> 25 mV) it is best to use equation 2.51 while equation 2.50 is suitable for low potentials. For systems with large electric double layers, equation 2.49 is more appropriate.

2.4.2.2 Van der Waal's Particle-Particle Interactions

Van der Waal's (VdW) forces act between all atoms and molecules. They are also called dispersion forces, London forces, charge-fluctuation forces, electrodynamic forces, and induced-dipole-induced-dipole forces. While they may not be the strongest force, VdW forces are always present, meaning that they always make a contribution to the overall stability of a system.⁴³

VdW forces arise even in non-polar molecules because while the time-average dipole moment of that non-polar molecule may be zero, there is a transient dipole. This transient dipole is caused by the instantaneous positioning of the electrons about the nucleus, and it generates an electric field, which in turn polarizes a nearby neutral molecule. This induces a dipole in the neighboring molecule and generates an attractive force between the molecules.⁴³

For two spherical particles with the same radius, the VdW pair potential can be calculated as shown in equation 2.53.

$$V_{vdW} = -\frac{1}{6}A_{eff} \left(\frac{2a^2}{r^2 - 4a^2} + \frac{2a^2}{r^2} + \ln \left(\frac{r^2 - 4a^2}{r^2} \right) \right) \quad (2-53)$$

Here, a is the particle radius, r is the center to center separation of the particles, and A_{eff} is the effective retarded Hamaker constant. The Hamaker constant is a coefficient in the VdW pair potential interaction that takes into account specific material parameters that affect the interaction. Equation 2.54 shows the equation for the Hamaker constant.⁴⁴

$$A_{eff} = A_{v=0} + A_{v>0} = \frac{3}{4}kT \left(\frac{\bar{\varepsilon}(0) - \varepsilon(0)}{\bar{\varepsilon}(0) + \varepsilon(0)} \right)^2 + \frac{3\hbar\omega}{16\sqrt{2}} \frac{(\bar{n}_o^2 - n_o^2)^2}{(\bar{n}_o^2 + n_o^2)^{3/2}} F(H) \quad (2-54)$$

In this equation, k is Boltzman's constant, T is the temperature, $\varepsilon(0)$ and $\bar{\varepsilon}(0)$ are the dielectric constants for the medium (water) and the substrate, n_o and $\bar{n}_o$ are the refractive indices (in the visible range) of the medium and substrate, $2\pi\hbar$ is Planck's constant, and ω is the frequency of the dominant relaxation in the UV. $F(H)$ accounts for retardation effects and is shown in equation 2.55.⁴⁴

$$F(H) = \frac{4\sqrt{2}}{\pi} \int_0^\infty \frac{(1+2Hx)e^{-2Hx}}{(1+2x^2)^2} dx \quad (2-55)$$

H is given by equation 2.56.

$$H = n_o (\bar{n}_o^2 + n_o^2)^{1/2} \frac{\hbar\omega}{c} \quad (2-56)$$

Here, h is the surface to surface separation of the particles and c is the speed of light in a vacuum. At the limiting forms of F , where H approaches zero or infinity, the Hamaker constants reduce to the forms shown in equations 2.57 and 2.58.⁴⁴

$$\text{For } H \rightarrow 0 \text{ (non-retarded): } A_{eff}(0) = \frac{3}{4}kT \left(\frac{\bar{\varepsilon}(0) - \varepsilon(0)}{\bar{\varepsilon}(0) + \varepsilon(0)} \right)^2 + \frac{3\hbar\omega}{16\sqrt{2}} \frac{(\bar{n}_o^2 - n_o^2)^2}{(\bar{n}_o^2 + n_o^2)^{3/2}} \quad (2-57)$$

$$\text{For } H \rightarrow \infty \text{ (fully retarded): } A_{eff}(h) = \frac{3}{4}kT \left(\frac{\bar{\varepsilon}(0) - \varepsilon(0)}{\bar{\varepsilon}(0) + \varepsilon(0)} \right)^2 + \frac{3}{4\pi} \frac{\hbar c}{n_o h} \left(\frac{\bar{n}_o^2 - n_o^2}{\bar{n}_o^2 + n_o^2} \right)^2 \quad (2-58)$$

It should be noted that the Derjaguin approximation (approximating a sphere as many small flat surfaces) breaks down at small particle sizes. The VdW potential expressions do make this assumption and so it is expected to introduce error as particle sizes get smaller.⁴⁴ It also should be noted that this calculation is only for hard spherical particles of one material. For systems with adsorbed polymers, it neglects the contributions of the anchor layer thickness and adsorbed polymer brush. Typically Hamaker constants for polymers are significantly lower than for metal oxides and so the magnetite contribution should be greater than the polymer contribution to the overall Hamaker constant.

2.4.2.3 Steric Particle-Particle Interactions

When two polymer-coated surfaces are brought into proximity to each other, the collision causes the polymer surfaces to compress. This confines the polymer chains to a limited number of configurations and decreases the entropy of the system. This leads to a repulsive osmotic potential known as steric or overlap repulsion.^{43, 117}

Additionally, when the segments overlap, this leads to an increase in the local segment density in the overlapping region. Thus, the interaction of the chains with each other and with the solvent plays a role in whether the overall steric potential is attractive or repulsive. The free energy of mixing can be estimated using Flory-Krigbaum theory, and is shown in equation 2.59.¹¹⁹

$$\delta(\Delta G_{mix}) \approx \frac{kT}{V_1} [(\chi - 1)\phi_2 + (1/2 - \chi)\phi_2^2] \quad (2-59)$$

Here, χ is the Flory segment-segment interaction parameter, ϕ_2 is the fraction of segments within a differential volume δV , and V_1 is the volume of a solvent molecule.

Thus, there are two components to the steric potential: the mixing component based on free energy, which can be attractive or repulsive and the osmotic pressure-driven component which is always repulsive.¹¹⁹ Good solvent conditions always generate a repulsive potential for terminally anchored chains.⁴⁴ Thus, by using a polymer in a system with a good solvent, adsorption of the polymer to a particle surface can increase the stability of the system, as the repulsive steric potential dominates the other pair

potentials and repels the particles apart to maintain colloidal stability. This rationale has been used extensively in the literature to stabilize colloidal systems.^{105, 120-124}

The radius of curvature plays a central role in the repulsive steric potential of an adsorbed polymer on a particle. As the particle gets smaller, the radius of curvature increases, and the space in which the polymer has to extend is larger than for flat-plate geometries. Utilizing the blob model put forth by Daoud and Cotton¹⁰⁷, Likos et al. derived an expression for the particle-particle repulsive steric interaction potential for star-shaped polymers with a densely adsorbed brush layer, which is shown in equation 2.60.^{125, 126}

$$V_s = \frac{5}{18} kT f^{3/2} \left\{ \begin{array}{ll} -\ln\left(\frac{r}{\sigma}\right) + \frac{1}{1 + \frac{\sqrt{f}}{2}}; & r \leq \sigma \\ \frac{1}{1 + \frac{\sqrt{f}}{2}} \left(\frac{\sigma}{r}\right) \exp\left(-\frac{\sqrt{f}}{2\sigma}(r - \sigma)\right); & r > \sigma \end{array} \right\} \quad (2-60)$$

Here, f is the number of chains per particle and $\sigma/2$ is the distance from the center of the core to the center of the outermost blob layer.¹²⁵⁻¹²⁷ For this expression, large quantities of simulations estimate $\sigma = 1.3R_g$, the radius of gyration of the ensemble.¹²⁶ For $\sigma < r$, the logarithmic expression is derived from the pair interaction potential derived by Witten and Pincus, shown in equation 2.61.¹²⁸

$$V(r) = v\theta(f)T \ln\left(\frac{R_g}{r}\right) \quad (2-61)$$

Here, v is the Flory exponent, $\theta(f)$ can be approximated as $f^{3/2}$, and r is the core radius. The logarithmic form ($r > \sigma$) takes over when the outmost blobs fully overlap ($\sigma/2$).^{125, 126}

For $\sigma > r$, the term denotes a long-term interaction that takes the Yukawa form (such as that used to describe a screened Coulomb potential¹²⁹) with the amplitude set such that the functions smoothly interpolate at $r = \sigma$.^{125, 126} At the limit of infinite chains ($f \rightarrow \infty$), the Yukawa decay function returns to the description of the potential between two hard spheres.^{125, 126}

2.5 Macromolecular Synthesis

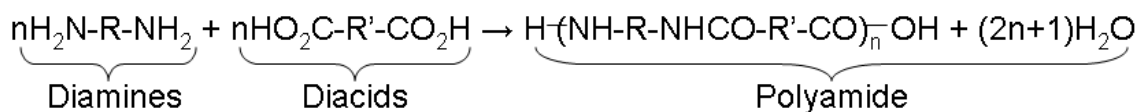
To understand the rationale for using the synthesis techniques employed in this research, a basic understanding of macromolecular synthesis is necessary. Typically, macromolecules or polymers are classified in one of two ways. The first is a composition or structure-based classification, and polymers are denoted either condensation or addition polymers. The second classification is a mechanism-based nomenclature where the polymers are named based on their mechanism of synthesis: step or chain polymerization.^{130, 131} Further discussion of the differences between these classifications follows.

2.5.1 Condensation vs. Addition Polymerization

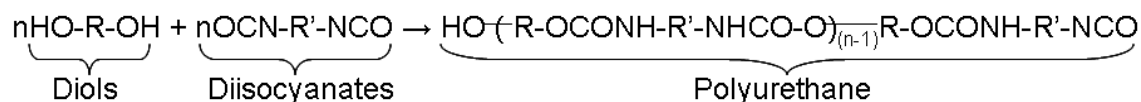
Condensation and addition polymers were originally classified by Carothers, where the composition of the polymer dictated the classification. A polymer must meet one of three requirements to be classified as a condensation polymer.

- The synthesis involves elimination of small molecules (such as water)
- The polymer contains functional groups in the polymer backbone
- The polymer repeat unit lacks atoms present in the monomer (possibly a hypothetical monomer)

Any other polymerizations that do not meet one of these conditions is classified as an addition polymerization. An example of a condensation polymer is the formation of polyamides through the reaction of diamines with diacids.¹³⁰



Here, the elimination of a small molecule is obvious as the water is a byproduct of this reaction. However, for the reaction of diols with diisocyanates to make polyurethanes, this is not the case, as there is no small molecule expelled as a byproduct.¹³⁰



However, the polyurethane does have the –O-CO-NH- functional group linkage and so it fits under the definition as given above.

Addition polymers do not contain functional groups in the polymer backbone (although they can contain them as pendant groups), do not lose small molecules during the synthesis, and do not lose atoms that are in the original monomer. Thus, addition polymers consists of mainly entities that have completely carbon-based backbones. Good examples of this are polyethylene and poly(methyl methacrylate) (PMMA), which are synthesized from ethylene and methyl methacrylate, respectively, shown in Figure 2.22.

Polymer	Monomer	Repeat Unit
Polyethylene	$\text{CH}_2=\text{CH}_2$	$-\text{CH}_2-\text{CH}_2-$
Poly(methyl methacrylate)	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_2=\text{C} \\ \\ \text{CO}_2\text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ -\text{CH}_2-\text{C} \\ \\ \text{CO}_2\text{CH}_3 \end{array}$

Figure 2.22. Addition polymerization of ethylene to polyethylene and methyl methacrylate to poly(methyl methacrylate) (redrawn by W. Miles).¹³⁰

As shown, the backbones of both polymers are carbon-based, and functional groups, while present in the PMMA, are attached as pendant groups to the main polymer backbone.

2.5.2 Step Growth Polymerization

The mechanism by which a polymer is synthesized allows for prediction of polymer molecular weight and the breadth of the molecular weight distribution. Thus, classifying polymers by their synthetic mechanism immediately gives information about the polymer that is not present when a polymer is classified as an addition or condensation polymer. It is for this reason that further discussion of polymers will classify them by their mechanism.

Step growth polymerization is the mechanism by which the polymer grows in steps, so that at any time, there is high, medium, and low molecular weight polymer as well as monomer. Figure 2.23 shows a schematic of step growth polymerization.

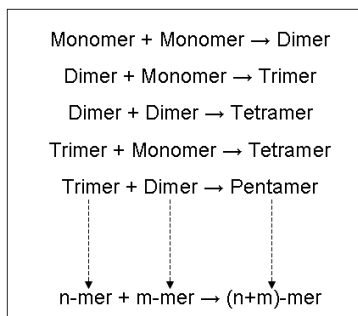


Figure 2.23. Schematic representation of step growth polymerization mechanism (redrawn by W. Miles).¹³⁰

As Figure 2.23 shows, any molecule in the reaction is free to react with any other molecule (i.e. a monomer can react with a dimer, trimer, or tetramer). For this reason, high conversion is needed for high molecular weight polymer.

The polydispersity index (PDI) can be measured by taking the weight average degree of polymerization (X_w) divided by the number average degree of polymerization (X_n). The closer the PDI is to unity, the smaller the molecular weight distribution. For step growth polymerization, the PDI can be defined as shown in equation 2.62.¹³⁰

$$\frac{X_w}{X_n} = 1 + p \quad (2-62)$$

Here, p is the extent or fraction of conversion. This means that as conversion of monomer to polymer is increased, the breadth of the molecular weight distribution also increases. This is undesirable because well-defined polymers are ideal for model systems.

Additionally, for self-catalyzed step-growth polymerizations, the degree of polymerization is related to time as shown in equation 2.63.

$$X_n^2 = 1 + 2[M]_0^2 kt \quad (2-63)$$

Here, $[M]_0$ is the initial concentration of monomer, k is the reaction rate constant, and t is time. Since degree of polymerization (and consequently, polymer molecular weight) are proportional to $t^{0.5}$, long times are needed for high molecular weights. Figure 2.24 shows how molecular weight changes with percent conversion for step growth polymerizations.

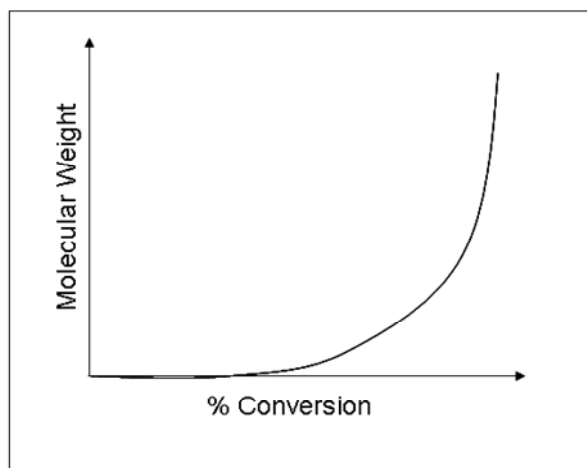


Figure 2.24. High molecular weights requires high percent conversion for step growth polymerization (redrawn by W. Miles).¹³⁰

Thus, step growth polymers have the disadvantage of taking a long time to achieve high molecular weights, and as the time (and conversion) increases, the breadth of the molecular weight distribution increases.¹³⁰ Advantages include that scale-up is typically easy and that contamination is not as large of a concern as in other types of polymerizations.

Step growth polymerizations are still used extensively both in research and development and in industry. Zia et al. investigated the synthesis of chitin-based polyurethane elastomers via step growth polymerization in hopes of making a biodegradable elastomer for biomedical applications.¹³² Rojas et al. used a step growth acyclic diene metathesis (ADMET) polymerization as a model system for polyethylene systems. The idea being that making long-chain branched polyethylene is difficult and so using the ADMET

polymerization can provide model systems to describe properties such as rheological behavior that increases processing efficiency.¹³³ Step growth polymerizations are also used in the manufacture of Kevlar and nylon, two well-known and profitable materials.¹³⁴

2.5.3 Chain Growth Polymerization

Chain polymerizations proceed through a mechanism where a reactive center is generated and then passed on—or propagated—to the next molecule through the addition of monomer. Only monomer reacts with the reactive center, and so unlike step growth polymerizations, in chain growth polymerizations at any given time there is only monomer, growing chains, and high molecular weight polymer. This is illustrated by Figure 2.25, which shows how molecular weight varies with percent conversion for chain growth polymerizations.

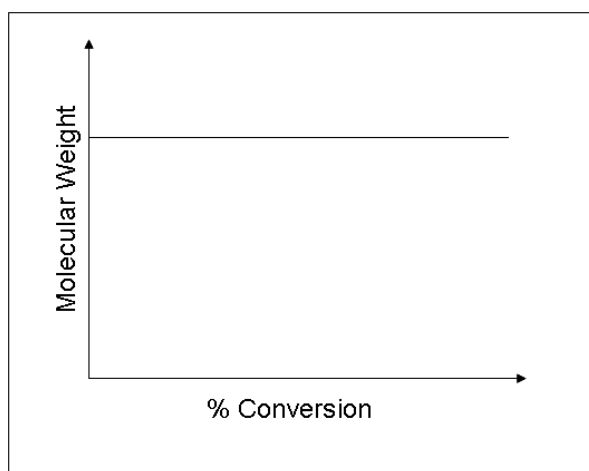


Figure 2.25. For chain growth polymerizations, molecular weight is identical at all percent conversions, making it ideal for low conversion applications (redrawn by W. Miles).¹³⁰

Chain growth polymerizations have three main steps: initiation, propagation, and termination. Figure 2.26 shows the three steps for chain growth polymerization for a typical polymerization.

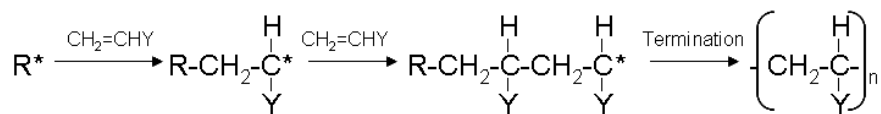
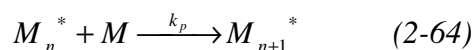


Figure 2.26. Initiation, propagation, and termination in a chain growth polymerization (redrawn by W. Miles).¹³⁰

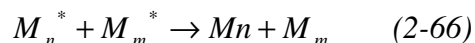
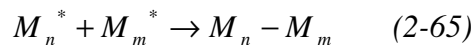
In the initiation step, the initiator is activated to produce an active center (R^*). This active center is typically a free radical, a cationic group, or an anionic group. What type of active group is used is dependent upon the specific reaction. Each reaction typically will proceed thermodynamically, but if the reaction is kinetically slow, then the reaction does not appreciably progress. Also, side reactions, chain transfer reactions, and polymer bite-back are considerations when choosing the type of active center.

The monomer then interacts with the active center, and attaches to the initiator, forming a new active center. This step is called the propagation step and can be represented as shown in equation 2.64.¹³⁰



Here, M_n is the growing chain with an active center, M is the monomer, M_{n+1} is the new growing chain with an active center, and k_p is the kinetic rate of propagation.

Termination can occur through two different mechanisms: combination (coupling) or disproportionation. Termination by combination is when two active centers combine to form one polymer chain. Termination by disproportionation is when one chain generates a double bond and discards the active center. The termination mechanisms can be described as shown in equations 2.65 and 2.66.



Following termination, the polymer is deemed “dead” because cessation of chain growth has occurred.¹³⁰

Advantages of chain growth polymerizations are that they are relatively fast compared to step growth polymerizations. In addition, high molecular weight polymers can be obtained at low conversion. The PDI of chain growth polymerizations can be described as shown in equation 2.67.

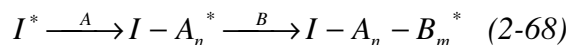
$$\frac{X_w}{X_n} = \frac{2+p}{2} \quad (2-67)$$

Again, the PDI increases as the extent of conversion increases. However, at very low conversion, the molecular weight distribution is relatively small, meaning that well-defined polymers can be synthesized. However, at very high conversions, the molecular weight distributions can get high ($PDI = 2-5$) because the molecular weight is proportional to $[M]/[I]^{0.5}$ where $[M]$ is the concentration of monomer and $[I]$ is the concentration of initiator. Since the initiator concentration typically decreases faster than the concentration of monomer, the molecular weight distribution widens as this ratio does not remain the same. The average molecular weight is still the same, but the breadth of the distribution has increased.¹³⁰

Many common plastics are made via chain growth polymerization. Polyethylene is used in toys, bottles, and plastic bags. Poly(vinyl chloride) (PVC) is used in pipes and flooring. Poly(tetrafluoroethylene) (Teflon) is used for non-stick surfaces, liners, and insulation. Poly(methyl methacrylate) is also known as Plexiglas and has many uses including windshields, skylights, solar panels and more.¹³⁴

2.5.4 Living Chain Growth Polymerization

Typically, chain growth polymerizations proceed with chain-breaking reactions, such as bimolecular termination or chain transfer. This limits the lifetime of the propagating centers and increases the breadth of the molecular weight distribution as conversion is increased. Living polymerizations are chain growth polymerizations that have no chain breaking reactions. This has the advantage of high conversion with good molecular weight control. Also, because the active center must be terminated by adding a material, addition of another monomer allows for growth of different blocks while still retaining a narrow PDI. An example of this is shown in equation 2.68.



Living polymerizations (LP) are often found in biological syntheses often. Typically, synthetic living polymerizations involve either a cationic or anionic active center (CLP or ALP). CLP processes are sometimes difficult to achieve because there is a built-in termination reaction that has to be worked around. No such problem exists with ALP, and they are relatively easy to carry out with a well-purified system.¹³⁰

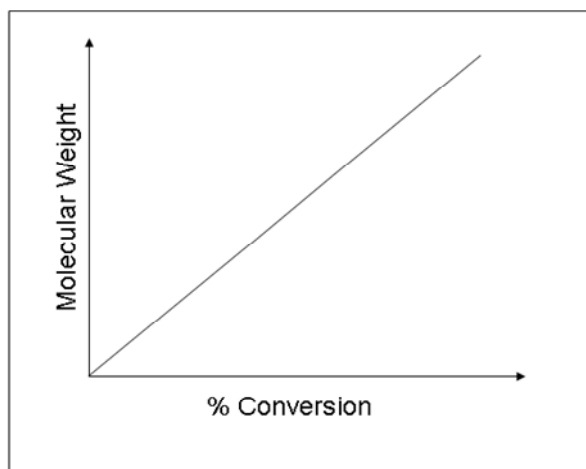


Figure 2.27. For living polymerizations, molecular weight varies linearly with conversion (redrawn by W. Miles).¹³⁰

The molecular weight of a living polymerization varies linearly with conversion, as shown Figure 2.27. As mentioned, the molecular weight distribution for living polymerizations is much narrower than either step growth or chain growth polymerizations. This is illustrated in equation 2-69.

$$\frac{X_w}{X_n} = 1 + \frac{1}{X_n} \quad (2-69)$$

Thus, as the molecular weight increases, the PDI gets significantly smaller. At large conversion for step and chain growth polymerizations, the PDI can be significantly larger than 2. For living polymerizations, the PDI can be as low as 1.01.¹³⁰

2.5.5 Ring opening polymerizations

Ring opening polymerizations, as the name implies, involves the polymerization of a molecule based on the opening of a cyclic ring. This is especially important for this work because polymerization of poly(ethylene oxide) and poly(propylene oxide) can be performed through ring opening polymerizations. Ring opening polymerizations are typically chain growth polymerizations. Many are living polymerizations, which means that good molecular weight control at high conversion is attainable.

Ring opening polymerizations are determined by the thermodynamics of the ring, and as Table 2.6 illustrates, are favorable for all but six-member ring structures due to issues associated with ring strain. The “lc” subscript denotes a conversion of liquid monomer to crystalline polymer.

Table 2.6. Only six-member rings are not thermodynamically favored to participate in ring-opening polymerizations at 25 °C.¹³⁰

Number of Carbons in Ring	ΔH_{lc} (kJ/mol)	ΔS_{lc} (J/mol·K)	ΔG_{lc} (kJ/mol)
3	-113.0	-69.1	-92.5
4	-105.1	-55.3	-90.0
5	-21.2	-42.7	-9.2
6	2.9	-10.5	5.9
7	-21.8	-15.9	-16.3
8	-34.8	-3.3	-34.3

Table 2.6 gives valuable information about how to proceed with ring-opening polymerizations. For three and four-member rings, the free energy of polymerization is virtually identical, but is significantly lower for five-member rings. Additionally, the free energy of formation for linear polymerizations is significantly more favorable than any of the ring opening polymerizations. Thus, these ring opening polymerizations may proceed significantly slower than typical chain growth polymerizations and a coordination catalyst may be needed to increase the rate of polymerization.¹³⁰ However, heterogeneities in the coordination catalyst can increase the PDI.

2.6 Introduction to Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is an important diagnostic tool for medical applications. MRI relies on contrast between different types of tissues to allow for determination of disease. Enhancement of this contrast for particular types of tissues could allow for earlier determination of diseases, such as cancer. In addition, more precise imaging could reduce the invasiveness of surgical procedures and allow for better monitoring of cancer in remission.¹⁷⁻¹⁹

To understand how MRI contrast agents work, one must first understand the fundamentals of relaxivity and how they relate to the intensity of an MRI signal. MRI works through the manipulation of protons contained within a material and their spin, as well as their magnetic moment. The protons are exposed to a magnetic field, B_0 , which is aligned in the z-direction. The proton spins reach an equilibrium alignment, with the majority of the spins aligned with the magnetic field while some are aligned against it. The percentage aligned with the magnetic field is directly related to the strength of the magnetic field. Thus, a stronger magnetic field produces a larger magnetic moment in the z-direction (M_z), which is called the longitudinal magnetization vector. These protons have an angular momentum, ω , and are rotating about the z-axis.^{14, 15, 17-19}

2.6.1 Longitudinal (T_1) Relaxation

Following this initial equilibrium alignment, a strong radio frequency (RF) pulse is introduced in the xy-plane, exciting the protons and aligning the spins in that plane, which produces a magnetic moment (M_{xy}), called transverse magnetization. In the intermediate time between pulses, the spins realign with the magnetic field in the z-direction (B_0). As the spins realign in the z-direction, they release the excess energy from absorption of the RF pulse to the surrounding environment (lattice), which is why this is sometimes called spin-lattice relaxation. The time that it takes for the spins to completely realign in this direction is called the longitudinal, or T_1 , relaxation, illustrated in Figure 2.28.^{18, 19}

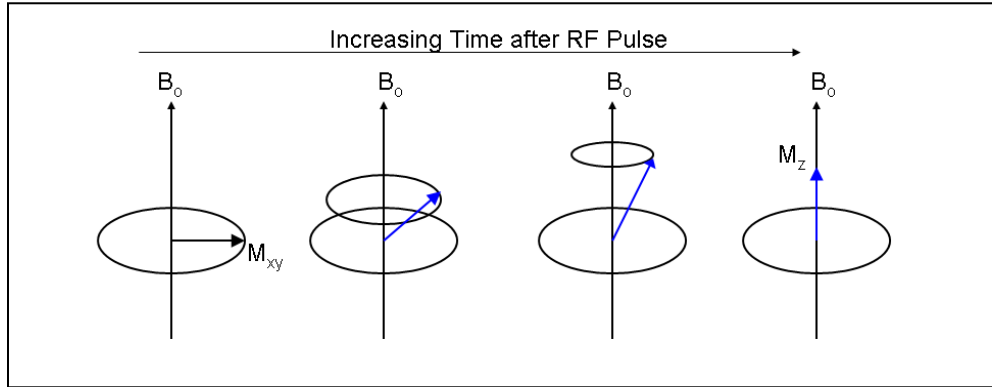


Figure 2.28. Restoration of longitudinal magnetization vector following RF pulse (redrawn by W. Miles).¹⁹

T1 relaxation restores M_z as a function of time, which can be described by equation 2.70.

$$M_z(t) = M_z[1 - e^{-t/T_1}] \quad (2-70)$$

M_z is the initial magnetic moment, t is time measured after the RF pulse, and T_1 is the longitudinal relaxation time. Two things are readily apparent from equation 2.70. First, the recovery of M_z depends on both the T_1 relaxation time and the time over which the RF pulse is introduced (i.e. 63% recovery if $t = T_1$). Second, the magnitude of the initial M_z vector is included as a coefficient, meaning that if a new RF pulse is introduced before complete recovery, then the recovery of the next phase will be slower, decreasing image contrast.¹⁸

2.6.2 Transverse (T_2) Relaxation

Recall that the proton spins also have angular momentum. Each spin has a random angular momentum about the z-axis before the RF pulse. However, this randomness of the angular momentum is disrupted by the RF pulse, and the proton spins move in synch, creating what is called phase coherence. However, due to factors that will be discussed shortly, phase coherence is lost as some spins rotate at different angular momentums than others. This process is called dephasing, and occurs until the angular momentums of the spins are again in the equilibrium random configuration about the z-axis. The time that it takes for the dephasing to occur is called the transverse (T_2) relaxation.¹⁸ Figure 2.29

illustrates both the T_1 and T_2 relaxation, which occur simultaneously following the RF pulse.

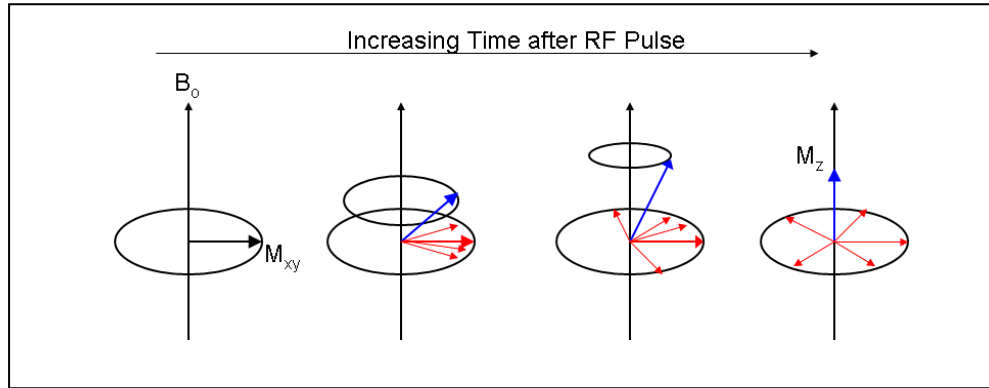


Figure 2.29. Combined longitudinal (T_1) and transverse (T_2) relaxation (redrawn by W. Miles).¹⁹

The dissipation of the transverse magnetization vector (M_{xy}) can be described by equation 2.71.

$$M_{xy}(t) = M_z[e^{-t/T_2}] \quad (2-71)$$

The inverse exponential relationship applies as compared to $M_z(t)$ and the recovery time is again dependent upon the initial magnitude of M_z . For T_1 relaxation, it is apparent that the presence of B_0 generates the impetus for realignment of the protons. However, the reason for T_2 relaxation is a little less obvious. T_2 relaxation occurs because of local inhomogeneities of the magnetic field. The protons are interacting with each other and each have their own magnetic moment. Thus, when they interact, they affect each other's path and cause dephasing, which is why T_2 relaxation is sometimes referred to as spin-spin relaxation. Inhomogeneities of the external magnetic field (B_0) as well as differences in tissues or the introduction of materials with their own local magnetic fields can also cause dephasing, which is called free induction decay (FID).^{18, 19}

2.6.3 Image Weighting

The significance of the difference between T_1 and T_2 relaxation is because the image contrast can be modified depending upon the weighting given to each component. The image can be weighted by use of one of three parameters shared by all biological tissues:

proton density, T_1 relaxation time, and T_2 relaxation time. Proton density is controlled simply by the number of available excitable protons in a sample. The T_1 and T_2 weightings are controlled by adjusting the repetition time (TR), which is the time between RF pulses. Figure 2.30 illustrates how the T_1 time can be controlled by adjusting the repetition time.

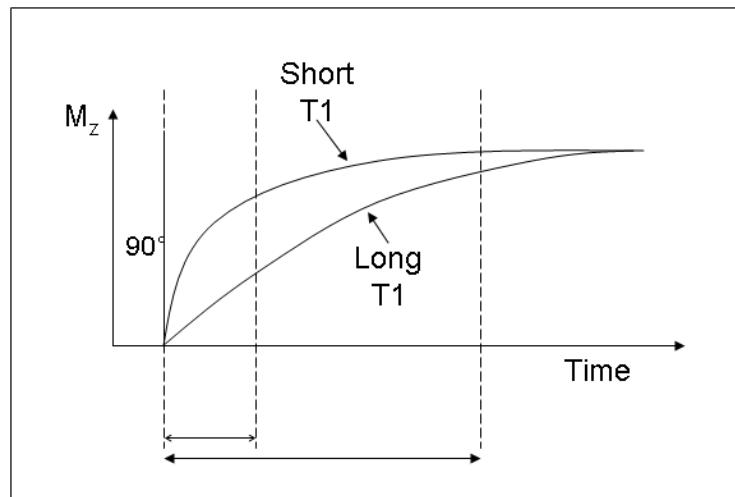


Figure 2.30. By adjusting the repetition time and the T_1 relaxation, contrast on a T_1 -weighted image can be achieved (redrawn by W. Miles).¹⁹

For tissues with significantly different T_1 relaxation times, there is contrast at small TR while at long TR the signals approach each other and show significantly less contrast. In this case, the short TR time is a more heavily weighted T_1 image than the long TR. In addition, if one were looking at an MR image of two tissues with the T_1 relaxations shown in Figure 2.30 at the shorter TR, the tissue with the shorter T_1 relaxation would be brighter than the tissue with the longer T_1 relaxation.

The same concept is illustrated in Figure 2.31 for T_2 relaxation, and again the contrast is controlled by the TR. At short TR, there is little to no contrast between the two tissues shown. However, at long TR, there is significant contrast between the two samples.

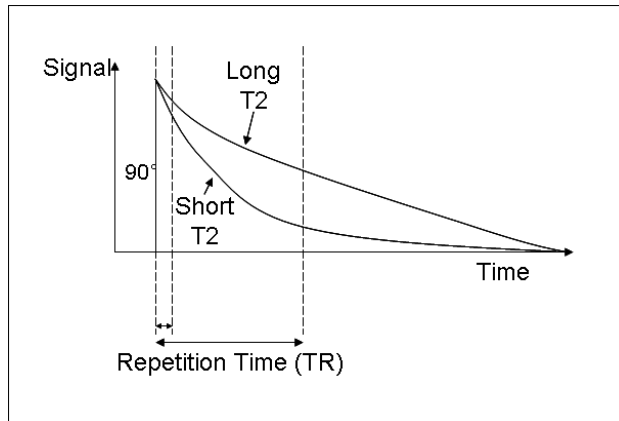


Figure 2.31. By adjusting the repetition time and the T₂ relaxation, contrast can be achieved (redrawn by W. Miles).¹⁹

Thus, at long TR an image is said to be T₂ weighted, and the sample with a short T₂ relaxation will appear dark while a sample with a long T₂ will appear bright.¹⁹

2.6.4 MRI T₂ Contrast Agents

As mentioned above, inhomogeneities in a magnetic field cause dephasing of the angular momentums of protons and so increase the T₂ relaxation.¹⁹ This inhomogeneity can be caused by inherent properties of the tissues themselves. Table 2.7 shows the T₁ and T₂ relaxation times in a 1.5 T magnetic field for different types of tissue.

Table 2.7. Relaxation times for different tissues in a 1.5 T magnetic field.¹⁹

Tissue	T ₁ (msec)	T ₂ (msec)
Cerebral Spinal Fluid	> 4000	> 2000
White Matter	780	90
Gray Matter	920	100
Meningioma	400	80
Metastasis	1800	85
Fat	260	80

As Table 2.7 shows, meningioma (a type of brain cancer) has a T₂ relaxation time fairly close to that of white matter and gray matter. This indicates that contrast between this type of cancer and the normal brain would be relatively weak on a T₂-weighted image. There is significantly more contrast in T₁ relaxation times, and so a T₁-weighted image would be warranted. However, by lowering the T₂ relaxation of either the healthy tissue or the cancer tissue, better contrast can be obtained with a T₂-weighted image.

Typically to increase contrast, contrast agents are used to either change the proton density of a tissue or to change the local magnetic field of a tissue.¹⁹ In the case of T₂ relaxation agents, the latter method is used to decrease the T₂ relaxation. Typically, the measure of relaxivity is used to join the relaxation and the concentration of the contrast agent as shown in equation 2.72 where [M] is the concentration of the contrast agent.¹³⁵

$$R_1 = \frac{1}{T_1[M]} \quad \text{and} \quad R_2 = \frac{1}{T_2[M]} \quad (2-72)$$

There are distinct advantages to using T₂ contrast agents. Typically a stabilizer is needed to both keep the contrast agent colloidally stable and to avoid cytotoxicity concerns. However, this stabilizer separates the actual contrast agent material from the protons that are being affected. Indeed, stabilizers have been found to decrease the relaxivity for various materials.¹³⁶ In addition, this distance affects the R₁ and R₂ relaxivities differently. For R₁ relaxivity, the proximity of the proton to the contrast agent surface is important while it is less important for R₂ relaxivity.¹³⁷ Thus, stabilization of T₂ contrast agents has less of an effect on the relaxivity and so different strategies for stabilization and targeting can be used without concern of completely canceling out the increased relaxivity.

Superparamagnetic iron oxide nanoparticles (SPION) have been studied intensely as T₂ contrast agents.¹³⁸⁻¹⁴⁴ These SPIONs have been used for many different biological systems, including examining the liver, spleen, brain and many others.^{141, 143-145} The R₂ relaxivity of these materials is influenced by the strength of the magnetic field, the particle size, and the magnetic property of the particle.^{139, 145} Exploring the effect of clustering on the relaxivity has been of interest, but there are competing results on each front. Roch et al. found that as the size of the particle increased, the R₂ relaxivity increased but the R₁ relaxivity decreased.¹⁴⁶ Plaza et al. and Tanimoto et al. found that as the size of the particle increased, the R₂ relaxivity decreased.^{145, 147} Each system consists of considerably different particle sizes and even different fundamental materials, meaning that considerably more research is needed to truly determine the effect of each particle characteristic on R₂ relaxivity.

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3 Modeling to Predict Solution Sizes and Colloidal Stabilities of the Complexes

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This chapter has not been submitted for peer-reviewed publication.

Dr. Jonathan Goff synthesized the PDMS, PDMS-magnetite complexes and PBO-magnetite complexes discussed in section 3.5.1. The phosphonate-anchored complexes in section 3.5.5 were synthesized by Dr. Goff and Christian Reinholz. Philip Huffstetler synthesized the polyether-magnetite complexes in discussed in section 3.5.2 and the PBO used to make the PBO-magnetite complexes. Dr. Shane Thompson synthesized the polyethers discussed in Table 3.3.

3.1 Abstract

The ability to predict colloidal stability is important for many applications. When adsorbing polymers to the surface of a colloid, the ability to predict the steric component added by the polymer is essential to design parameters such as polymer molecular weight and polymer loading. Previous work has used a density distribution model to predict the polymer density as a function of distance from a particle core. This chapter extends that treatment to include a Weibull probability distribution to describe the particle core size distribution. This analysis is performed on multiple core particle synthesis methods and the results from the distribution are compared to results obtained via X-ray diffraction. Additionally, a non-solvated hydrophobic shell is assumed for amphiphilic diblock copolymers and the thickness of the polymer layer is also calculated using the model. The density distribution model is then used to calculate the intensity, volume, and number average of the polymer-magnetite complex. Finally, parameters such as the stability ratio and half-life for doublet formation are estimated using extended DLVO

theory and a model for steric interaction between extended polymer brushes is used to predict when flocculation should occur for a variety of complexes.

3.2 Introduction

The density distribution (DD) model is a tool used to describe star polymers and particle-polymer complexes that behave like star polymers. The need for a model arises from the need for an understanding of the polymer behavior in solution to be able to predict the steric force imparted by the polymers when they overlap. The density distribution model was developed by Vagberg et al.¹ based on the ideas of Daoud and Cotton that proposed modeling of star polymers as concentric shells emanating from a central point with the same number of blobs, as shown in Figure 3.1.²

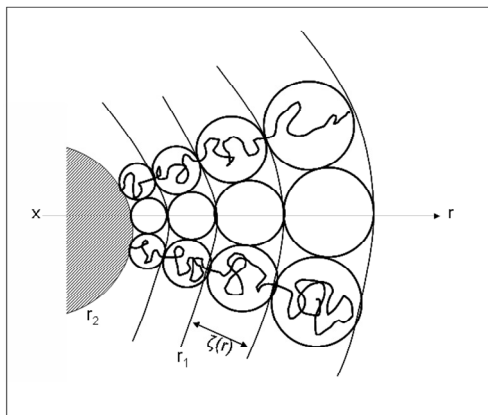


Figure 3.1. Modeling of star-shaped polymers as concentric shells with constant blob size from a central point (redrawn by W. Miles).²

The effect of this model is that effectively, there is a constant density (or volume fraction) of polymer at the center, and then that volume fraction decreases for each successive blob, as shown in Figure 3.2. The radii r_1 and r_2 in Figure 3.1 and Figure 3.2 correspond to distances from where the blobs begin.

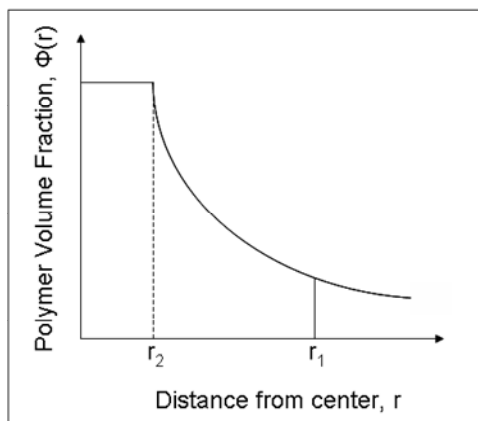


Figure 3.2. The polymer volume fraction is constant at the center but then decreases as the blob size increases (redrawn by W. Miles).²

Vagberg et al. further developed this model to describe the dimensions of amphiphilic diblock copolymers that form micelles.¹ The hydrophobic blocks were treated as a solid core, while the hydrophilic blocks were treated as concentric shells with an equal number of blobs, as shown in Figure 3.3.

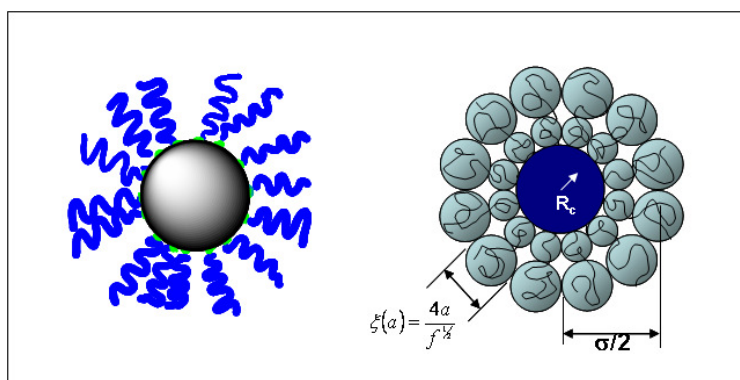


Figure 3.3. Vagberg et al. treated amphiphilic diblock copolymers as a micelle with a solid non-solvated core and a hydrophilic star-shaped polymer brush.¹

Mefford et al. expanded the density distribution model to incorporate a Weibull probability distribution to describe the core particle size.^{3, 4} Zhang et al. incorporated a non-solvated shell surrounding the particle core to model the adsorption of an amphiphilic polymer to the core surface.^{5, 6}

This chapter expands on the work of Mefford et al. and Zhang et al. to incorporate the Weibull distribution into the calculation of the radius of gyration, and thus the steric stability of the dispersion. Additionally, the reproducibility of the image analysis for each synthesis is explored and compared to results obtained from X-ray diffraction (XRD). Finally, the stability ratios and half-lives for doublet formation ($t_{1/2}$) are calculated based on the probability distribution, and compared to the results from dynamic light scattering (DLS).

3.3 Experimental

3.3.1 Synthesis of Polymer-Stabilized Magnetite via the Co-Precipitation Method

The synthesis of magnetite via a co-precipitation method was achieved following the synthesis published by Mefford et al.^{3,4} In this case, 2.32 g of ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and 0.86 g of ferrous chloride tetrahydrate ($\text{FeCl}_2 \cdot \text{H}_2\text{O}$) were each dissolved in 10 mL of deionized (DI) water and deoxygenated via N_2 purge for an hour. This was then placed in a 3-prong roundbottom flask and mixed with a high-speed homogenizer while still under N_2 purge. A deoxygenated (also with N_2 purge) aqueous solution of 50% v/v ammonium hydroxide was rapidly added to the iron chloride mixture, forming magnetite.

An appropriate mass of tricarboylsilyl-functional poly(dimethylsiloxane) (PDMS) or poly(butylene oxide) (PBO) was then dissolved in approximately 5 mL of chloroform, which was added to the mixture. The pH was adjusted with hydrochloric acid to a pH of approximately 6, and the solution was allowed to stir for an hour to allow the polymer to interfacially bind to the magnetite nanoparticles. The homogenizer was then stopped and the water and chloroform were separated and the resulting polymer-magnetite complex could be collected from the chloroform.

3.3.2 Synthesis of Polymer-Stabilized Magnetite via the High Temperature Method

Magnetite nanoparticles were synthesized using a reduction method adapted from Pinna *et al.*⁷ Fe(III) acetylacetonate (2.14 g, 8.4 mmol) and benzyl alcohol (45 mL, 0.43 mol) were added to a 250-mL, three-necked roundbottom flask equipped with a water

condenser and overhead mechanical stirrer. N₂ was passed through the solution for 1 h. While stirring under N₂, the solution was heated to 100 °C for 4 h, and the temperature was then increased to 205 °C at a rate of ~25 °C h⁻¹. Following 24 h at 205 °C, the reaction was cooled to room temperature, then the magnetite particles were collected with a magnet and the benzyl alcohol was decanted. The magnetite nanoparticles were washed 3X with acetone, then were dispersed in chloroform (20 mL) containing oleic acid (0.3 g). The solvent was removed under vacuum at room temperature, and the oleic acid-stabilized magnetite nanoparticles were washed 3X with acetone. The particles were dried under vacuum for 24 h at 25 °C. The composition of the particles obtained from thermogravimetric analysis (TGA) showed 5% organic residue to 95% magnetite.

A representative method for preparing a targeted composition of 70:30 wt:wt polyether:magnetite complex is provided. Oleic acid-stabilized magnetite nanoparticles (50 mg) prepared as described above were dispersed in chloroform (10 mL) and added to a 50-mL roundbottom flask. A polyether with a functional anchor group (100 mg) was dissolved in chloroform (10 mL) and added to the dispersion. The reaction mixture was sonicated in a VWR 75T sonicator for 16 h under N₂, and then the nanoparticles were precipitated in hexanes (300 mL). A magnet was utilized to collect the magnetite nanoparticles and free oleic acid was decanted with the supernatant. The complexes were dried under N₂ at 25 °C for 24 h. The complexes were then dispersed in aqueous media using a Tekmar TM-200 probe-tip sonicator at medium power for 20-30 s.

3.4 Modeling Theory

The magnetite particle core radii were determined through analyses of TEM images and by XRD. Figure 3.4 shows an original and the contrast-enhanced TEM image used for image analysis.

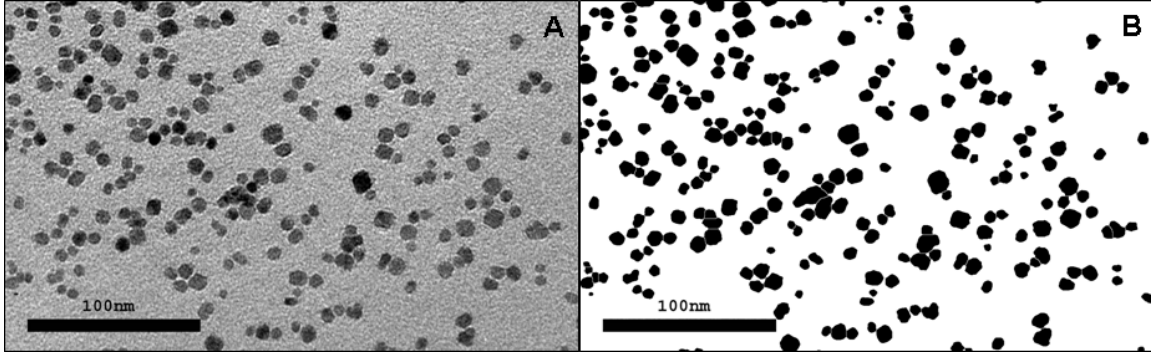


Figure 3.4. Probability histograms of complexes were generated by modifying the original image (A) to a contrast-enhanced image (B) using Fovea Pro 4.0 software. The images for a complex with a triammonium-5.6kPPO-b-7.2kPEO-OH copolymer are shown.

At least 2000 particles per sample were analyzed and each image was scrutinized manually to correct any software errors. The equivalent radii were graphed versus the probability of their occurrence and this histogram was fit with a Weibull probability distribution as shown in equation 3-1.

$$P(a) = \frac{c}{b} \left(\frac{a}{b} \right)^{c-1} \exp \left[- \left(\frac{a}{b} \right)^c \right] \quad (3-1)$$

Here, c is the Weibull scale parameter, b is the Weibull shape parameter, and a is the particle radius. The Weibull distribution has the advantage that it is flexible. If $b = 1$, then $P(a)$ reduces to an exponential distribution. If $b = 3.4$, $P(a)$ reduces to a Gaussian distribution.

Hydrodynamic radii (R_m) of the magnetite-polymer complexes were calculated by the method of Mefford, et al.^{3, 4} TEM image analysis data was first fitted with a Weibull probability distribution to calculate an average specific surface area of the magnetite particles. Combining average surface area with the average polymer loading per mass of the complex (from TGA) leads to an average number of chains per magnetite surface area, α , as shown in Equation 3-2.³

$$\alpha = \frac{(1 - W_{mag}) N_{Av} \rho_{mag} \int_0^{\infty} a^3 P(a) da}{3 MW_{Total} W_{mag} \int_0^{\infty} a^2 P(a) da} \quad (3-2)$$

Here, ρ_{mag} is the density of magnetite (5.21 g cm⁻³),⁸ W_{mag} is the weight fraction of the complex that is magnetite, and MW_{Total} is the polymer number average molecular weight. The integral must be taken at this point to ensure self-consistency of the model to bulk calculations. This is distinctly different from previous work where the integral was taken after the division of the radii (i.e. integrating $aP(a)da$ instead of $a^3P(a)da/a^2P(a)da$).^{3, 4} Also, the average must be taken here because the measurement of the polymer mass via TGA is inherently an average measurement of the entire distribution. The assumption is made that each magnetite particle has the same ratio of magnetite to polymer, and so the average of the distribution must be taken at this juncture.

Using a modification of Vagberg's density distribution model,⁹ we calculate the number of chains per particle, $f(a)$, and the complex radius $R_m(a)$ —the radius of the particle plus its attached brush—as

$$f(a) = 4\pi a^2 \alpha \quad (3-3)$$

$$R_m(a) = \left(\frac{8N_k f(a)^{\frac{1-\nu}{2\nu}} L_k^{1/\nu}}{4^{1/\nu} 3\nu} + a^{1/\nu} \right)^\nu \quad (3-4)$$

where N_k is the number of statistical segments in the polymer brush chain, ν is the Flory exponent of the brush in the solvent, L_k is the length of a statistical segment, and a is the particle radius.

For complexes with an amphiphilic diblock copolymer, the hydrophobic layer was assumed to be a nonsolvated, hydrophobic shell surrounding the magnetite core and the brush was modeled as concentric blobs extending out from the surface of the PPO layer.

To account for the hydrophobic layer, the model was adapted to include a new effective core size, which is the radius of the magnetite core obtained from the Weibull distribution plus the thickness of the hydrophobic anchor layer (t_a) (equation 3-5).

$$t_a = \left(\left(\frac{\rho_{mag} a^3 (1 - W_{mag})}{W_{mag} MW_{anchor} \rho_{anchor} MW_{Total}} \right) + a^3 \right)^{1/3} - a \quad (3-5)$$

Here, ρ_{anchor} is the density of the hydrophobic anchor layer, MW_{anchor} is the anchor layer number average molecular weight, and MW_{Total} is the total polymer number average molecular weight. Thus, the predicted hydrodynamic radius of the complex as a function of magnetite particle radius can be described by equation 3-6.

$$R_m(a) = \left(\frac{8N_k f(a)^{\frac{1-\nu}{2\nu}}}{4^{1/\nu} 3\nu} L_k^{1/\nu} + (a + t_a)^{1/\nu} \right)^\nu \quad (3-6)$$

This function along with the Weibull distribution function for the magnetite particle radius is used to determine radial distribution averages for comparison with DLS experimental data. Each average weights particles of various diameters differently and thus gives an estimate of the homogeneity of the particle systems. The number average diameter, D_n , of the magnetite complex can be calculated as

$$D_n = 2 \int_0^\infty R_m(a) P(a) da \quad (3-7)$$

The volume average diameter, D_v , of the magnetite complex can be calculated as

$$D_v = 2 \frac{\int_0^{\infty} R_m(a)^4 P(a) da}{\int_0^{\infty} R_m(a)^3 P(a) da} \quad (3-8)$$

Using a method by Mefford, et al. and assuming the particles are in the Rayleigh scattering regime,³ the intensity average diameter, D_i , for the magnetite complex was calculated as

$$D_i = 2 \frac{\int_0^{\infty} R_m(a)^6 P(a) da}{\int_0^{\infty} R_m(a)^5 P(a) da} \quad (3-9)$$

As equations 3-7 through 3-9 indicate, the intensity average diameter weights larger particles significantly more than the volume average diameter, which, in turn, weights larger particles more than the number average diameter. Thus, if a system has small amounts of clustered nanoparticles, this would affect the intensity average more so than the number average. This is important when considering which weighted average from the model to compare to the DLS results. If the model matches the measurements obtained via DLS closely, the model can then be used with DLVO calculations to predict stabilities of the complexes in solution.

Colloidal stability can be predicted from a model for the total potential energy V_{Total} as described by DLVO theory and shown in equation 3-10.¹⁰

$$V_{Total} = V_{vdW} + V_M + V_{ES} + V_S \quad (3-10)$$

V_{vdW} is the attractive potential due to van der Waal's interactions, V_M is the attractive potential produced by an applied magnetic field, V_{ES} is the repulsive potential due to electrostatic interactions, and V_S is the repulsive potential due to steric repulsion of the polymer brushes. The magnetic potential energy term was neglected because for the

stability calculations there is no magnetic field being applied to the system. The electrostatic potential can be calculated as

$$V_{ES} = 2\pi\epsilon\epsilon_o\psi_o^2 \ln(1 + e^{-\kappa(r-2a)}) \quad (3-11)$$

where ϵ is the dielectric constant of the solvent, ϵ_o is the permittivity of free space, ψ_o is the surface potential, κ is the inverse Debye length, r is the center to center separation, and a is the particle radius.¹¹ The van der Waal's potential can be calculated as

$$V_{vdW} = -\frac{1}{6}A_{eff}\left(\frac{2a^2}{r^2 - 4a^2} + \frac{2a^2}{r^2} + \ln\left(\frac{r^2 - 4a^2}{r^2}\right)\right) \quad (3-12)$$

A_{eff} is the effective retarded Hamaker constant calculated from equation 3-13.

$$A_{eff} = A_{v=0} + A_{v>0} = \frac{3}{4}kT\left(\frac{\bar{\epsilon}(0) - \epsilon(0)}{\bar{\epsilon}(0) + \epsilon(0)}\right)^2 + \frac{3\hbar\omega}{16\sqrt{2}}\frac{\left(\bar{n}_o^2 - n_o^2\right)^2}{\left(\bar{n}_o^2 + n_o^2\right)^{3/2}}F(H) \quad (3-13)$$

In this equation, k is Boltzmann's constant, T is the temperature, $\epsilon(0)(8.0 \times 10^1)^{12}$ and $\bar{\epsilon}(0)(2.0 \times 10^4)^{13}$ are the dielectric constants for the medium (water) and the substrate (magnetite), $n_o(1.33)^{12}$ and $\bar{n}_o(1.97)^{12}$ are the refractive indices (in the visible range) of the medium and substrate, $2\pi\hbar$ is Planck's constant, and $\omega(1.88 \times 10^{16} \text{ rad/sec})^{12}$ is the frequency of the dominant relaxation in the UV. $F(H)$ accounts for retardation effects and is unity at the nonretarded limit.¹² For this treatment, the Hamaker constant was calculated for magnetite only, and was 9.0×10^{-20} J in the nonretarded limit. The Hamaker constant for magnetite has been reported elsewhere as 16.4×10^{-20} J.¹⁴ Our calculation neglects the contributions of the anchor layer thickness and adsorbed polymer brush. Typically Hamaker constants for polymers are significantly lower than for metal oxides and so the magnetite contribution should be greater than the polymer contribution to the overall Hamaker constant. Further refinement of the model will incorporate a core/shell estimation of the Hamaker constant.

For the case of a densely adsorbed brush layer, the steric contribution to the interaction potential can be described by the expression ¹⁵

$$V_s = \frac{5}{18} kT f(a)^{3/2} \left\{ \begin{array}{ll} -\ln \left(\frac{r}{\sigma} + \frac{1}{1 + \frac{\sqrt{f(a)}}{2}} \right); & r \leq \sigma \\ \frac{1}{1 + \frac{\sqrt{f(a)}}{2}} \left(\frac{\sigma}{r} \right) \exp \left(-\frac{\sqrt{f(a)}}{2\sigma} (r - \sigma) \right); & r > \sigma \end{array} \right\} \quad (3-14)$$

Here, $f(a)$ is the number of chains per particle (equation 3-3) and $\sigma/2$ is the distance from the center of the core to the center of the outermost blob layer.^{11, 15} For this expression, σ is defined as $1.3R_g$, the radius of gyration of the ensemble.¹¹ The inner, logarithmic term in the equation which describes close approaches was derived originally by Witten and Pincus¹⁶ to describe the entropic repulsion when chains from adjacent star-like objects overlap. The outer, exponential term ($r > \sigma$) is based on a Yukawa-type decay (such as that used to describe a screened Coulomb potential¹⁷) with the decay length set to the diameter of the largest blobs and has been extensively tested using machine simulations.¹⁵

The radius of gyration, R_g , can be approximated using the previously described model and the Weibull particle radius distribution fit by calculating the moment of inertia of the complex as described by Mefford et al.^{3, 4} The moment of inertia of a mass, m , rotated about an axis at a distance x is given by¹⁸

$$I = x^2 dm \quad (3-15)$$

Extension of this to the moment of inertia of each component of the complex can be calculated as

$$I_{Total} = I_{Mag} + I_{PPO} + I_{PEO} = \int_0^a \rho_{mag} 4\pi a^4 da + \int_a^{a+t_a} \rho_{PPO} 4\pi a^4 da + \int_{a+t_a}^{R_m(a)} m_s A \left(\frac{a}{a_s} \right)^{\frac{1}{\nu}} 4\pi a da \quad (3-16)$$

where each component (the core, the anchor, and the brush) is separated into its individual component, and then added together.

The total mass of the complex can be calculated as

$$M_{Total} = M_{Mag} + M_{PPO} + M_{PEO} = \frac{4}{3} \pi a^3 \rho_{mag} + \frac{M_{mag} (1 - W_{mag})}{W_{mag}} \quad (3-17)$$

and the radius of gyration is defined as

$$R_g = \int_0^\infty \sqrt{\frac{I_{Total}}{M_{Total}}} P(a) da \quad (3.18)$$

Note that the radius of gyration is now a function of the particle size distribution. This allows us to essentially compare the pair-pair potentials of each mode of the size distribution.

To test this method, the calculated R_g s were compared to measured values of R_g for polymer micelles from small angle neutron scattering (SANS) with agreement within 7% of experimental values.¹⁹ This allows for calculation of the stability ratio and the half-life for doublet formation using equations 3-19 and 3-20, respectively.

$$W = 2\bar{a} \int_{2\bar{a}}^\infty \frac{e^{\frac{V_{Total}}{kT}}}{r^2} dr \quad (3-19)$$

$$t_{1/2} = \frac{\bar{a}^3 \pi \mu W}{\phi k T} \quad (3-20)$$

Here, μ is the solvent viscosity, Φ is the volume fraction of particles in solution, and $\bar{a}$ is the average particle radius.^{10, 20} The classical expression for W does not account for polydispersity and only concerns pair interactions for particles of like sizes. We account for the size distribution by computing $\bar{a}$ (4.0 nm) from the Weibull distribution (equation 3-2). The half-life for doublet formation is the time required for half of the particles to flocculate in the form of doublets. This parameter serves as a basis of comparison for complexes with different polymer molecular weights.

3.5 Results

3.5.1 Reproducibility of Particle Size Distributions – Co-precipitation Synthesis Method

The reproducibility of the co-precipitation method was tested to determine whether a particle size distribution needs to be obtained every time a new batch of magnetite is made, or whether the same distribution can be used to model many different complexes. Figure 3.5 illustrates five different complexes, each made with a completely separate batch of magnetite. Some of the batches have undergone magnetic separation (“pass” refers to the number of times the solution is passed through the separation column), which is addressed further in Chapter 8. These complexes were stabilized with poly(butylene oxide) (PBO) and polydimethylsiloxane (PDMS), which are hydrophobic materials that are very stable in organic solvents, such as chloroform or tetrahydrofuran (THF). Synthesis of these materials was carried out using living polymerizations to ensure narrow molecular weight distributions (< 1.1).²¹

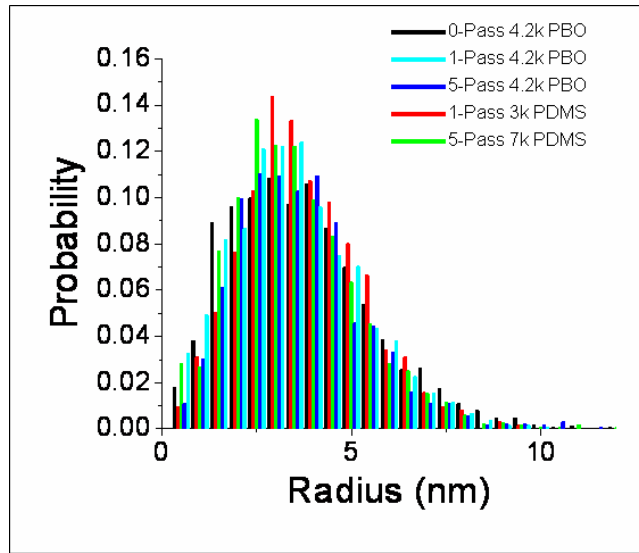


Figure 3.5. Small differences are observed in the particle histograms for completely different batches of the co-precipitation magnetite synthesis.

Not only does each distribution look similar, but the calculated number average means are all well within each standard deviation as shown in Table 3.1.

Table 3.1. The calculated number average mean diameters are all within the standard deviation of the measurement for the co-precipitation synthesis.

Sample	Mean Diameter (nm)	Standard Deviation (nm)
0-Pass 4.2k PBO	7.0	3.8
1-Pass 4.2k PBO	6.4	3.4
5-Pass 4.2k PBO	6.9	4.3
1-Pass 3k PDMS	7.5	3.1
5-Pass 7k PDMS	6.2	2.9

Thus, for each co-precipitation synthesis, an aggregate of each of the five distributions can be used to estimate the distribution of a new synthesis. This yields a Weibull distribution with scale parameter $c = 2.32$ and shape parameter $b = 3.93$ with a sum of squared errors of 8.8×10^{-4} .

3.5.2 Reproducibility of Particle Size Distributions – High Temperature Synthesis Method

The reproducibility of the high temperature magnetite synthesis method was tested to determine whether a particle size distribution needs to be obtained every time a new batch of magnetite is made, or whether the same distribution can be used to model many

different complexes. Figure 3.6 illustrates five different complexes, each made with a completely separate batch of magnetite.

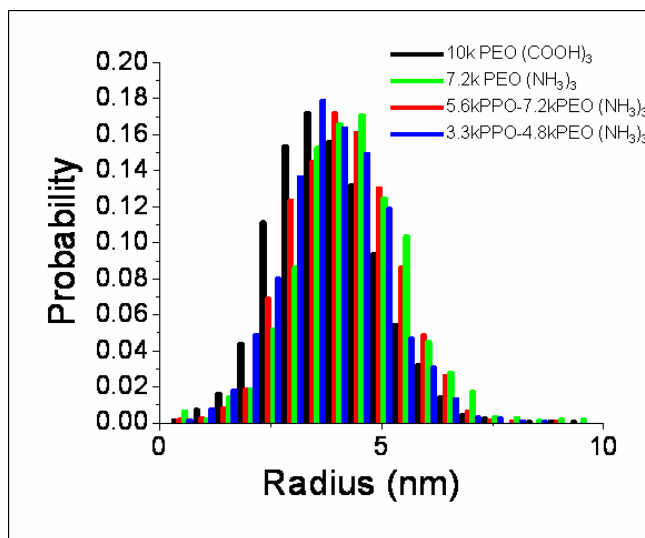


Figure 3.6. Small differences are observed in the particle histograms for completely different batches of the high-temperature magnetite synthesis.

There is a range of molecular weights and anchor groups used in this analysis, and each time, the distributions look remarkably reproducible. The calculated number average means are also all well within each standard deviation as shown in Table 3.2. The denotation $(\text{COOH})_3$ or $(\text{NH}_3)_3$ indicates whether the polyether was anchored to the magnetite with a tricarboxylate anchor group or a triammonium anchor group. The polyethers used were either poly(ethylene oxide) homopolymers (PEO) or poly(ethylene oxide)-*b*-poly(propylene oxide) amphiphilic diblock copolymers (PPO-*b*-PEO).

Table 3.2. The calculated number average mean diameters are all within the standard deviation of the measurement for the high-temperature synthesis.

Sample	Mean Diameter (nm)	Standard Deviation (nm)
10k PEO $(\text{COOH})_3$	7.7	2.5
7.2k PEO $(\text{NH}_3)_3$	8.1	2.9
5.6kPPO-7.2kPEO $(\text{NH}_3)_3$	7.8	2.2
3.3kPPO-4.8kPEO $(\text{NH}_3)_3$	7.7	2.3

Thus, for each high temperature synthesis, an aggregate of each of the four distributions can be used to estimate the distribution of a new synthesis. This yields a Weibull distribution with scale parameter $c = 4.09$ and shape parameter $b = 4.32$ with a sum of squared errors of 8.3×10^{-4} .

3.5.3 Comparison of Particle Diameters from Image Analysis and X-ray Diffraction

The particle diameters calculated from image analysis are equivalent diameters using the assumption that the particles are spherical. X-ray diffraction (XRD) was performed on the particles to confirm the magnetite composition and to determine the average particle diameter. Powder XRD patterns were obtained using a Scintag XDS-2000 diffractometer with a Ni-filtered Cu-K $_{\alpha}$ ($\lambda = 0.154$ nm) radiation source. The patterns were obtained at a scan rate of $1.0 \text{ } 2\theta \text{ s}^{-1}$ and were scanned from 10 to 90° . Particle diameters were obtained using the Scherrer formula, which allows for estimation of a spherical particle diameter as a function of the width of the diffraction curves (equation 3.21).

$$d_{XRD} = \frac{0.9\lambda}{\beta \cos \theta} \quad (3.21)$$

Here, λ is the wavelength of radiation, β is the peak width at half height in radians, and θ is the angle of reflection.²² Figure 3.7 shows the XRD peak of the high temperature magnetite synthesis.

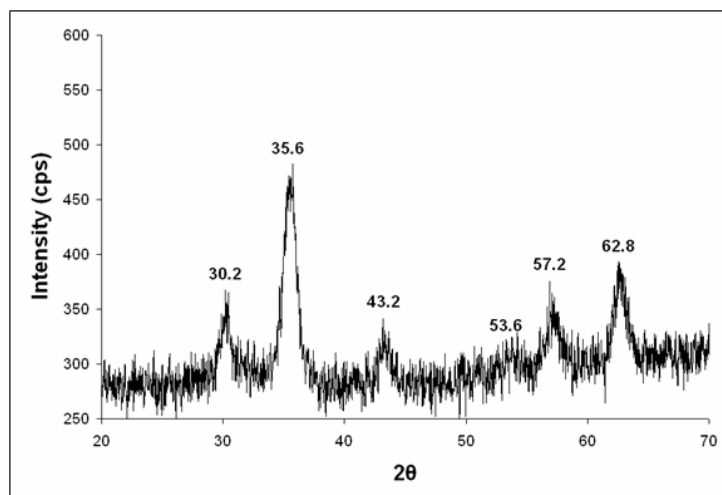


Figure 3.7. Powder XRD pattern of high temperature magnetite synthesis.

The diameters based on five different XRD peaks (30.2°, 35.6°, 43.2°, 57.2°, and 62.8°) were averaged, giving a diameter of 8.2 ± 1.2 nm, which compares favorably to the diameters of 7.8 ± 2.2 nm (5.6kPPO-*b*-7.2kPEO-OH) and 8.1 ± 2.9 nm (7.2kPEO-OH) obtained from the images in Figure 3.4. Thus, the validity of assuming a spherical particle is supported by the consistency of data derived from the multiple methods.

3.5.4 Use of the Density Distribution Model to Predict Particle Diameters – Comparison to Dynamic Light Scattering

Now that we have a model and the proper definition of the particle core distribution, the diameter of the polymer-magnetite complexes can be calculated. Table 3.3 shows the predicted number average diameters from the density distribution model and the experimental DLS number average diameters for complexes made using the magnetite from the high temperature synthesis.

The number average diameter is the appropriate diameter for comparison because it is less susceptible to small amounts of flocculation. As Table 3.3 illustrates, for polymer-magnetite complexes with significantly different molecular weights, different magnetite syntheses, and different anchor groups, the model predicts the number average diameter within 7%.

Table 3.3. Density distribution predictions are within an average of 7% for different molecular weight stabilizers, magnetite syntheses, and anchor groups.

Polymer	% Polymer	DLS D_n (nm)	Model D_n (nm)	% Deviation
Urethane Pentablock (3kPEO-1kPPO-Urethane-1kPPO-3kPEO)	65	27.6	24.4	12%
10k PEO (COOH) ₃	72	34.2	40.5	18%
7.2k PEO (NH ₃) ₃	65	37.2	37.6	1%
5.6kPPO- <i>b</i> -7.2kPEO (NH ₃) ₃	68	34.9	34.9	0%
3kPEO- <i>g</i> -0.6PEI ($m = 1$)	61	23.6	26.8	14%
2kPEO (R-PO ₃)	66	24.7	24.7	0%
1-Pass 4.2k PBO	69	26.7	26.3	1%
5-Pass 5k PDMS	71	20.1	22.7	13%

3.5.5 Use of $t_{1/2}$ to Predict Complex Stability

The following is just an example of the model and its use for predicting stability. A far more detailed analysis is provided in the following chapters.

At some point, the polymer loading gets so low that the magnetite will begin to flocculate. Interestingly, we have found that the particles do not flocculate continuously until falling out of solutions, but rather they flocculate into a cluster size that can be predicted by DLVO theory. Table 3.4 illustrates this more clearly for a 2k PEO (molecular weight = 2,000 g mol⁻¹) terminated with a phosphonate group. Details about the synthesis of this polymer are given in Chapter 5.

Table 3.4. As the polymer loading is reduced, deviation of DLS from the model also increases.

Complex	Polymer Loading (%)	Chains/nm ²	Model D_n (nm)	DLS D_n (nm)	Deviation (%)
2kPEO Phosphonate	32	1.0	19.4	29.3	34%
2kPEO Phosphonate	63	3.5	24.1	24.7	2%
2kPEO Phosphonate	66	4.1	24.7	26.0	5%
2kPEO Phosphonate	30	0.9	19.1	27.4	30%
2kPEO Phosphonate	50	2.1	22.0	24.3	9%
2kPEO Phosphonate	70	4.9	25.5	24.8	3%

Two separate batches of each material were synthesized with small variations in how the polymer was functionalized with the phosphonate. As illustrated, the deviation of the

DLS from the model is rather low above 50% polymer loading. However, when the loading is reduced below 32%, significant deviation occurs. We believe this is due to clustering of the individual complexes.

From the R_g calculations described above, extended DLVO theory can be used to plot the total system potential energy vs. the center to center separation of the particles. Figure 3.8 illustrates this potential energy diagram for the phosphonate-terminated 2k PEO.

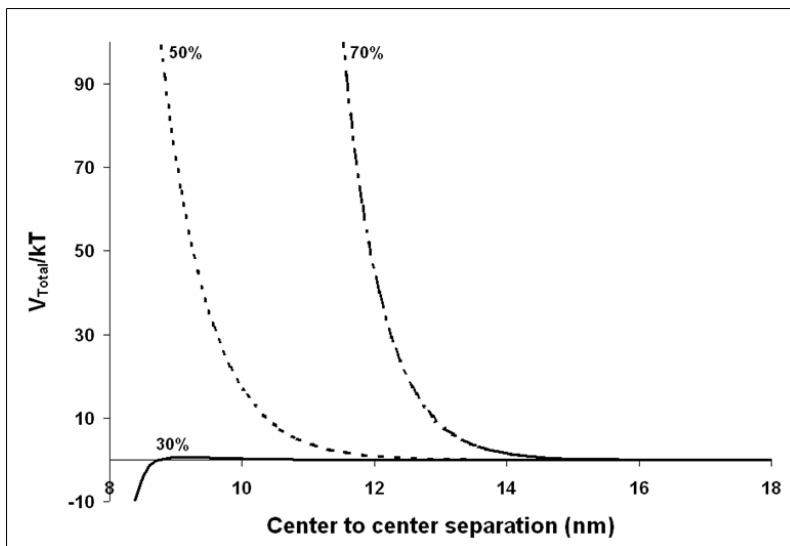


Figure 3.8. The potential energy diagram for the phosphonate-terminated 2k PEO predicts flocculation at 30% polymer loading, but not at 50% or 70% polymer loading.

The potential energy diagram predicts that at 30% polymer loading there should be flocculation, but not at 50% or 70% loading. However, flocculation is not just based on thermodynamics, but also the diffusion kinetics of the particles in suspension. Thus, we must account for the concentration of the complex by way of a volume fraction (assumed to be 2.0×10^{-5} , which approximates a concentration of approximately 0.5 mg mL^{-1}). At that concentration, the $t_{1/2}$ values calculated from the model are 5.0×10^{221} seconds, 1.3×10^{228} seconds, and 3.7×10^{-4} for complexes with 70%, 50%, and 30% polymer loadings, respectively. Even the 32% polymer loading has a $t_{1/2}$ of 7.0×10^{-3} seconds. The model predicts that the flocculation point is at approximately 35% polymer loading, which is supported by the DLS data.

3.6 Conclusions

The density distribution model was adapted to combine a Weibull probability distribution fit of the core particle radius and a non-solvated hydrophobic shell surrounding the core for amphiphilic stabilizers. Each parameter was then calculated by integrating over the entire distribution at the appropriate point, allowing for the incorporation of the distribution into key calculations, such as the radius of gyration of the polymer complex.

The image analysis technique using TEM was shown to be reproducible for two different magnetite syntheses, and average Weibull shape and scale parameters were found for each distribution. X-ray diffraction was performed on one synthetic route, and using the Scherrer equation, the average particle diameter was calculated and compared to the image analysis technique. Both techniques were well within their standard deviations and compared quite well. This is important because both techniques assume a spherical particle, and so arriving at the same answer from two different techniques lends credence to treating the particles as spherical entities.

The extended density distribution model number average diameter predictions were compared with those obtained by DLS for eight different complexes, with varying polymer stabilizers, magnetite synthetic routes, and anchor groups. The average deviation of the model from the DLS data was 7%.

Additionally, a phosphonate-terminated 2k PEO was complexed with magnetite at varying polymer loadings. Deviation from the model at low polymer loadings was observed, and is thought to be due to the combining of the magnetite complexes into stable clusters. The model predicted stability at 50% and 70% polymers loadings, but not at 30% polymer loading, which is exactly where flocculation was seen experimentally. Thus, it appears that this extended density distribution model does a good job of both predicting the complex size, but also of predicting stability limits. This has far-reaching impact in colloidal systems, particularly for biological applications.

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4 Synthesis and Colloidal Properties of Polyether-Magnetite Complexes in Water and Phosphate Buffered Saline

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This chapter is in print: Langmuir (2009), 25, 803-813

Dr. Jonathan Goff and Christian Reinholz synthesized the polyether-magnetite complexes discussed in section 4.3.6. Philip Huffstetler synthesized the polyethers discussed in sections 4.3.2, 4.3.3, and 4.3.4. John Boyd synthesized the magnetite discussed in section 4.3.5. Dr. Beth Caba helped develop the modeling procedure discussed in section 4.4.1.

4.1 Abstract

Biocompatible magnetic nanoparticles show great promise for many biotechnological applications. This paper addresses the synthesis and characterization of magnetite nanoparticles coated with poly(ethylene oxide) (PEO) homopolymers and amphiphilic poly(propylene oxide-*b*-ethylene oxide) (PPO-*b*-PEO) copolymers that were anchored through ammonium ions. Predictions and experimental measurements of the colloidal properties of these nanoparticles in water and phosphate buffered saline (PBS) as functions of the polymer block lengths and polymer loading are reported. The complexes were found to exist as primary particles at high polymer compositions and most formed small clusters with equilibrium sizes as the polymer loading was reduced. Through implementation of a polymer brush model, the size distributions from DLS were compared to those from the model. For complexes that did not cluster, the experimental sizes matched the model well. For complexes that clustered, equilibrium diameters were predicted accurately through an empirical fit derived from DLS data and the half-life for

doublet formation calculated using the modified Derjaguin-Landau-Verwey-Overbeek (DLVO) theory. Deviation from this empirical fit provided insight into possible additional interparticle hydrophobic interactions for select complexes for which the DLVO theory could not account. While the polymers remained bound to the nanoparticles in water, most of them desorbed slowly in PBS. Desorption was slowed significantly at high polymer chain densities and with hydrophobic PPO anchor blocks. By tailoring the PPO block length and the number of polymer chains on the surface, flocculation of the magnetite complexes in PBS was avoided. This allows for in vitro experiments where appreciable flocculation or sedimentation will not take place within the specified timescale requirements of an experiment.

4.2 Introduction

The properties of magnetite nanoparticles make them ideal for biomedical applications including cell separations, treatment of detached retinas and use as contrast-enhancing agents for MRI.¹⁻⁶ Their low cytotoxicities make them promising candidates for in vivo applications.^{2, 7} In addition, the magnetite surface is amenable to adsorption of biocompatible macromolecules. Thus, dispersants and targeting or imaging agents can be bound to these nanoparticles.^{2, 7-12}

At room temperature, magnetite nanoparticles below approximately 23 nm in diameter exhibit superparamagnetic behavior, and their colloidal properties can be influenced by interparticle magnetic attractive forces when they are placed in an external magnetic field.^{7, 13-17} When a magnetic field is applied, the magnetic dipoles align in the field direction and the particles begin to self-assemble in the absence of repulsive interactions.^{18, 19} Attaching soluble nonionic polymer chains to form a brush on a surface generally increases colloidal stability and inhibits aggregation due to steric repulsion. This arises from a combination of enthalpic and entropic effects resulting from the confinement of soluble tail chains in the gap between two particles. Steric stabilization occurs when the steric forces keep the particles separated by a distance that prevents aggregation due to attractive forces. With sufficiently thick brushes, the interaction potentials can be entirely repulsive, resulting in a very stable dispersion.²⁰ In addition, by

choosing the correct stabilizer molecular weight and chain density on the magnetite surface, we show herein that small controlled clusters of these complexes can form, while still maintaining sufficient steric forces to disperse the clusters. This can occur even in the absence of an applied field.

Various steric stabilizers for magnetite have included polyvinylpyrrolidone, dextran, polydimethylsiloxane and PEO.^{2, 3, 5, 13, 14, 21} Adsorption of these polymers onto the surface of magnetite is enhanced by introducing specific functional anchor groups.^{2, 3, 5, 22} In aqueous media, amphiphilic block copolymers with controlled hydrophobic and hydrophilic blocks can also contribute to polymer adsorption and better colloidal stability as reported herein. Previous work in our laboratories has utilized poly(propylene oxide-*b*-ethylene oxide) (PPO-*b*-PEO) block copolymers functionalized with carboxylates on the hydrophobic end to adsorb onto the magnetite surface, but the lack of stability of those complexes in physiological media is a concern.^{2, 3, 5, 22, 23}

This paper describes magnetite nanoparticles that have been sterically stabilized with homo- and diblock copolyethers having three alkylammonium groups on one end to promote adsorption onto the magnetite surface. The three main thrusts include 1) relationships of stabilizer chain densities and hydrophobic interactions to colloidal stabilities of the complexes, 2) influences of material parameters such as polymer composition and loading on forming controlled clusters of the complexes, and 3) the suitability of alkylammonium ions as anchor groups for the magnetite nanoparticles in physiological media, particularly in solutions that contain phosphate salts. Additionally, by observing deviations of experimental data from the model, aggregation phenomena are observed that would otherwise go unseen. We believe that developing an understanding of these three areas will enable the design of magnetic complexes wherein sizes, clustering, and interactions with physiological media and with cells can be controlled through judicious choices of molecular parameters.

4.3 Experimental

4.3.1 Materials

Tetrahydrofuran (THF, EMD Chemicals, 99.5%) was refluxed over sodium with benzophenone until the solution reached a deep purple, and fractionally distilled just prior to use. Azobisisobutyronitrile (AIBN), benzyl alcohol (>98%), ethylene oxide (EO, 99.5+%), cysteamine hydrochloride, potassium (98%), hexanes (HPLC grade), hexamethylphosphoramide (HMPA, 99%), propylene oxide (PO, >99%), oleic acid, NaOH (1 N, aq), sodium iodide (>98%), iron(III) acetylacetonate (Fe(acac)₃) and 1.6 M vinyl magnesium chloride were purchased from Aldrich and used as received. 3-Chloropropyltrichlorosilane (Gelest) was used as received. Dichloromethane and nitric acid (0.1 N) (Fisher Scientific) were used as received. Naphthalene (Aldrich, 99%) was sublimed prior to use. Glacial acetic acid (Aldrich) was diluted with THF to yield a 2.5 M solution. N,N-Dimethylformamide (DMF, EMD Chemicals) was dried over CaH₂, fractionally distilled under vacuum and stored under nitrogen at 25 °C. A double-metal catalyst, Impact™ (Zn₃[Co(CN)₆]₂), was kindly donated by Bayer and was diluted with distilled THF to yield a 4.1 mg mL⁻¹ dispersion. Dulbecco's PBS (10X, Aldrich) without calcium and magnesium was diluted to a concentration of 1X.

4.3.2 Synthesis of trivinylsilyl-functional PEO

A characteristic procedure for the synthesis of a trivinylsilyl-initiated PEO is provided. A 7.2k M_n PEO was initiated with 3-hydroxypropyltrivinylsilane (3-HPTVS). 3-HPTVS was prepared according to a procedure developed by Vadala et al.^{23, 24} A 300-mL high-pressure Series 4561 Parr reactor was utilized for the polymerizations. EO (20.4 g, 0.463 mol) was distilled from a lecture bottle into the pressure reactor cooled with an isopropanol-dry ice bath. THF (40 mL) was added to the reactor via syringe. An initiator solution consisting of 3-HPTVS (0.704 mL, 6.80 mmol), THF (10 mL) and potassium naphthalene (0.91 mL of a 0.94 M solution, 0.85 mmol) was prepared in a separate flame-dried, 100-mL roundbottom flask. The initiator solution was added to the stirring reaction mixture via syringe. The cooling bath was removed, and the reaction mixture was allowed to reach room temperature and maintained for 24 h. The polymerization was terminated by adding acetic acid (2.72 mL of a 2.0 M solution in THF, 6.80 mmol)

to the pressure reactor via syringe. The pressure reactor was purged with N₂ for 1 h, then opened and its contents were transferred to a 250-mL roundbottom flask. The solvent was removed under vacuum at room temperature, and the product was dissolved in 200 mL of dichloromethane. The product was washed twice with deionized (DI) water (2X 100 mL). The solution was concentrated under vacuum at room temperature and precipitated in cold diethyl ether.

4.3.3 Synthesis of trivinylsilyl-functional PPO-b-PEO (trivinylsilyl-PPO-b-PEO-OH)

A representative procedure for a 5.6k PPO-*b*-7.2k PEO (M_n) trivinylsilyl-functional copolymer is provided. PO (10 g, 0.175 mol) was added to a 300-mL pressure reactor via syringe, followed by 5 mL of distilled THF. An initiator solution consisting of 3-HPTVS (0.3 g, 1.8 mmol), THF (5 mL), and Impact™ catalyst (0.25 mL, 100 ppm based on PO) was prepared in a flame-dried, 100-mL roundbottom flask. The initiator dispersion was agitated rapidly for 10 min, then introduced into the pressure reactor, and the reactor was charged with N₂ to a pressure of 30 psi. The polymerization was conducted at 90 °C until a cessation in pressure drop was observed (~3 h). After cooling to room temperature and purging with nitrogen, the reactor was opened and the reaction mixture was transferred to a 250-mL roundbottom flask. The solvent was removed under vacuum, yielding trivinylsilyl-functional PPO.

EO (20.4 g, 0.463 mol) was distilled from a lecture bottle into a 300-mL pressure reactor cooled with an isopropanol-dry ice bath. THF (10 mL) was added to the reactor via syringe. In a 50-mL roundbottom flask, the hydroxy terminus of the trivinylsilyl-functional PPO (5 g, 0.9 mmol) was reacted with potassium naphthalene (0.91 mL of a 0.94 M solution, 0.85 mmol) in THF (5 mL) to form a PPO alkoxide macroinitiator. The macroinitiator solution was added to the pressure reactor via syringe. The polymerization and polymer isolation conditions were identical to those for the PEO homopolymer described above.

4.3.4 Functionalization of trivinylsilyl-functional polyethers with ammonium groups

Polyethers with three ammonium groups on one terminus were obtained via the ene-thiol addition of cysteamine hydrochloride across the vinylsilane groups. In a characteristic procedure, trivinylsilyl-PPO-*b*-PEO-OH (2 g, 0.16 mmol), cysteamine hydrochloride (80 mg, 0.7 mmol), and AIBN (26 mg, 0.15 mmol) were dissolved in deoxygenated DMF (6 mL) in a roundbottom flask. The reaction was conducted at 80 °C for 24 h with stirring, then the reaction mixture was cooled to room temperature. DI water (100 mL) was added to the flask, and the mixture was transferred to a separatory funnel. Dichloromethane (200 mL) was added to the separatory funnel to extract the alkylammonium-functionalized polyether from the water layer. The dichloromethane layer was washed with a 1 N solution of sodium bicarbonate (3X), followed by three washes with DI water. The dichloromethane was removed under vacuum, and the triammonium-PPO-*b*-PEO-OH copolymer was dried at 50 °C under vacuum for 12 h. ¹H NMR confirmed the expected chemical structure.

4.3.5 Magnetite synthesis via reduction of Fe(acac)₃

Magnetite nanoparticles were synthesized using a reduction method adapted from Pinna et al.²⁵ Fe(III) acetylacetonate (2.14 g, 8.4 mmol) and benzyl alcohol (45 mL, 0.43 mol) were added to a 250-mL, three-necked roundbottom flask equipped with a water condenser and overhead mechanical stirrer. N₂ was passed through the solution for 1 h. While stirring under N₂, the solution was heated to 100 °C for 4 h, then the temperature was increased to 205 °C at a rate of ~25 °C h⁻¹. Following 24 h at 205 °C, the reaction was cooled to room temperature, then the magnetite particles were collected with a magnet and the benzyl alcohol was decanted. The magnetite nanoparticles were washed 3X with acetone, then were dispersed in chloroform (20 mL) containing oleic acid (0.3 g). The solvent was removed under vacuum at room temperature, and the oleic acid-stabilized magnetite nanoparticles were washed 3X with acetone. The particles were dried under vacuum for 24 h at 25 °C. The composition of the particles obtained from thermogravimetric analysis (TGA) showed 5% organic residue to 95% magnetite.

4.3.6 Adsorption of polyether stabilizers onto magnetite nanoparticles

A representative method for preparing a targeted composition of 70:30 wt:wt polyether:magnetite complex is provided. Oleic acid-stabilized magnetite nanoparticles (50 mg) prepared as described above were dispersed in chloroform (10 mL) and added to a 50-mL roundbottom flask. A triammonium-functional polyether (100 mg) was dissolved in chloroform (10 mL) and added to the dispersion. The pH of each solution and the resulting mixture were approximately neutral. The reaction mixture was sonicated in a VWR 75T sonicator for 16 h under N₂, and then the nanoparticles were precipitated in hexanes (300 mL). A magnet was utilized to collect the magnetite nanoparticles and free oleic acid was decanted with the supernatant. While it is clear from NMR of the supernatant that some of the oleic acid is displaced, it should be noted that removal of the small composition of oleic acid may not be quantitative. The complexes were dried under N₂ at 25 °C for 24 h. TGA was used to determine the polymer loading for each complex. The complexes were then dispersed in aqueous media using a Tekmar TM-200 probe-tip sonicator at medium power for 20-30 s. The polyether:magnetite complexes exhibited superparamagnetic behavior as measured by Superconducting Quantum Interference Device (SQUID) magnetometer.

4.4 Characterization

Spectral analyses of compounds were performed using a Varian Unity 400 NMR and a Varian Inova 400 NMR. An Alliance Waters 2690 Separations Module with a Viscotek T60A dual viscosity detector and laser refractometer equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel column set was used for gel permeation chromatography (GPC) analyses. GPC data were collected in chloroform at 30 °C. Data were analyzed utilizing a Universal calibration to obtain absolute molecular weights.

TGA was carried out on the PEO-stabilized and PPO-*b*-PEO-stabilized magnetite nanoparticles using a TA Instruments TGA Q500. Each sample was first held at 110 °C for 10 min to drive off any excess moisture. The sample was then equilibrated at 100 °C and the temperature was ramped at 10 °C min⁻¹ to a maximum of 600 °C in a nitrogen atmosphere. The mass remaining was recorded throughout the experiment.

TEM was conducted with a Philips EM-420 field-emission-gun transmission electron microscope. The microscope was equipped with a 2000 x 3000 pixel digital imaging system. Dry samples of the magnetite complexes were dispersed in water and cast onto amorphous carbon-coated copper grids for analysis. Great care was taken to ensure that both eucentric height and focus were set consistently from one sample to another to reduce uncertainty in the digital image analyses. Images were acquired at a magnification of 96 kx, corresponding to a resolution of 3.7 pixels nm⁻¹. This magnification gave both sufficient resolution and contrast for digital image analysis, and provided a large enough field of view to obtain adequate numbers of particles. Particle distribution analyses were performed using Reindeer Graphics' Fovea Pro 4 plug-in for Adobe Photoshop 7.0.

DLS measurements were conducted with a Malvern Zetasizer NanoZS particle analyzer (Malvern Instruments Ltd, Malvern, UK) at a wavelength of 633 nm from a 4.0 mW, solid-state He-Ne laser at a scattering angle of 173° and at 25 ± 0.1 °C. Intensity, volume and number average diameters were calculated with the Zetasizer Nano 4.2 software utilizing an algorithm, based upon Mie theory, that transforms time-varying intensities to particle diameters.²⁶ Zeta potential measurements were also performed with the Zetasizer ZS at 25 ± 0.1 °C to measure the particle velocity imparted due to an applied electric field which can be related to the surface charge.²⁷

For DLS analysis, each complex was dispersed in DI water as described above. The solution was then diluted to approximately 0.05 mg mL⁻¹ and filtered through a Whatman Anotop 100-nm alumina filter directly into a polystyrene cuvette. This corresponds to a volume fraction of 1.3×10^{-5} to 2.2×10^{-5} depending on the polymer loading. In experiments where PBS was added, 0.1 mL 10X PBS was added to 0.9 mL of the 0.05 mg mL⁻¹ complex solution and the new solution was mixed and then filtered through a 100-nm alumina filter into a clean polystyrene cuvette. Each sample was analyzed directly following filtration and measured either 3 times in a 5-min period or every 30 minutes over 24 hours depending on the experiment.

Zeta potential measurements were taken by dispersing the sonicated complexes in either 25 mL of DI water or 1X PBS at a concentration of $\sim 0.05\text{-}0.1 \text{ mg mL}^{-1}$. The pH was adjusted up to $\sim 7.5\text{-}8.5$ using 0.1 N sodium hydroxide. The zeta potential was measured as the pH was decreased in small increments using 0.1 N nitric acid. Both nitric acid and sodium hydroxide were determined to be indifferent electrolytes and so they did not greatly affect the surface charge density of the magnetite.

Powder X-Ray Diffraction (XRD) patterns were obtained using a Scintag XDS-2000 diffractometer with a Ni-filtered Cu-K α ($\lambda = 0.154 \text{ nm}$) radiation source. The patterns were obtained at a scan rate of $1.0 \text{ } 2\theta \text{ s}^{-1}$ and were scanned from 10 to 90° . Particle diameters were obtained using the Scherrer formula, which allows for estimation of particle diameter as a function of the width of the diffraction curves (equation 4-1).

$$d_{XRD} = \frac{0.9\lambda}{\beta \cos \theta} \quad (4-1)$$

Here, λ is the wavelength of radiation, β is the peak width at half height in radians, and θ is the angle of reflection.²⁸

4.4.1 Modeling to Predict Solution Sizes and Colloidal Stabilities of the Complexes

The magnetite particle core radii were determined through analyses of TEM images and by XRD. Figure 4.1 shows an original and the contrast-enhanced TEM image used for image analysis. At least 2000 particles per sample were analyzed and each image was scrutinized manually to correct any software errors. The equivalent radii were graphed versus the probability of their occurrence and this histogram was fit with a Weibull probability distribution as shown in equation 4-2.

$$P(a) = \frac{c}{b} \left(\frac{a}{b} \right)^{c-1} \exp \left[- \left(\frac{a}{b} \right)^c \right] \quad (4-2)$$

Here, c is the Weibull scale parameter, b is the Weibull shape parameter, and a is the particle radius.

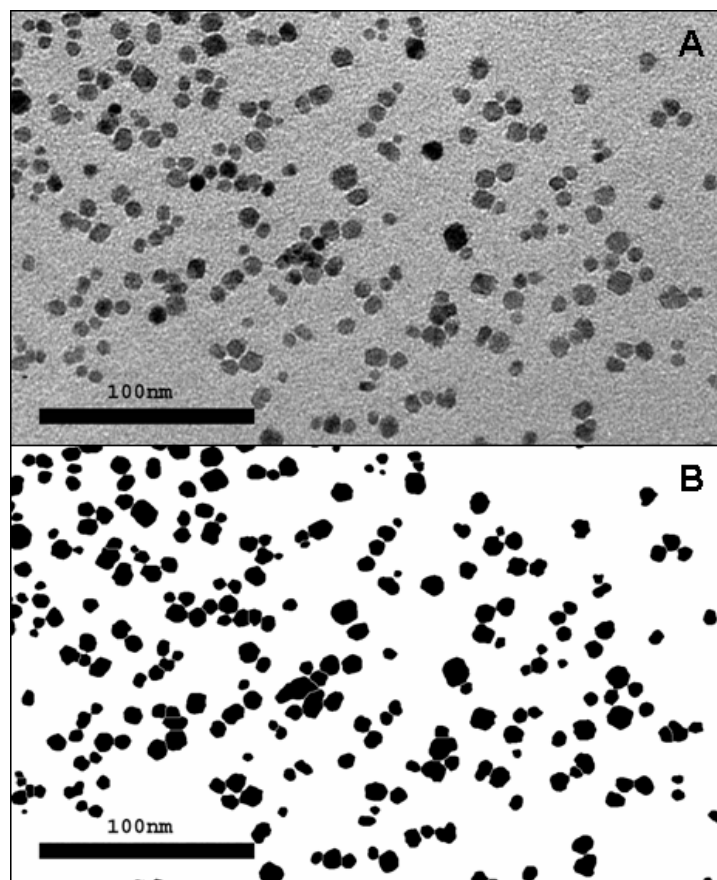


Figure 4.1. Probability histograms of complexes were generated by modifying the original image (A) to a contrast-enhanced image (B) using Fovea Pro 4.0 software. The images for a complex with a triammonium-5.6kPPO-*b*-7.2kPEO-OH copolymer are shown.

Figure 4.2 shows two histograms of particle radii versus probability for a triammonium-7.2kPEO-magnetite complex and a triammonium-5.6kPPO-*b*-7.2kPEO-OH diblock complex. Each complex was prepared with a different batch of magnetite, and the variability between the histograms and their respective probability distributions is well within standard error. The particle radii reported herein are equivalent radii assuming the particles were spherical. XRD was performed on the particles to confirm the magnetite composition and to determine the average particle diameter using the Scherrer equation, which also assumes a spherical particle. The diameters based on five different XRD peaks were averaged, giving a diameter of 8.2 ± 1.2 nm, which compares favorably to the diameters of 7.8 ± 2.2 nm (5.6kPPO-*b*-7.2kPEO-OH) and 8.1 ± 2.9 nm (7.2kPEO-OH)

obtained from the images in Figure 4.2. Thus, the validity of assuming a spherical particle was supported by the consistency of data derived from the multiple methods.

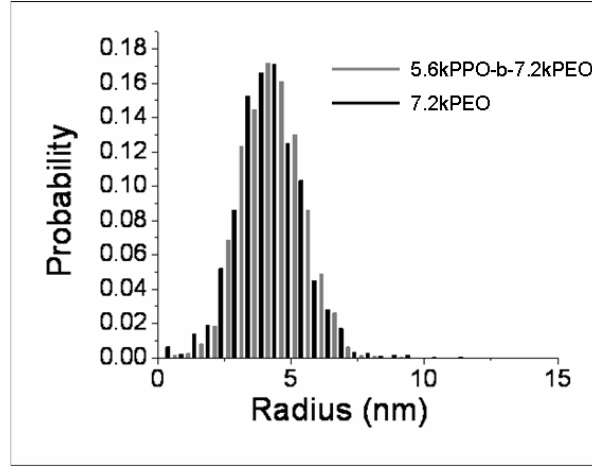


Figure 4.2. Probability histograms for a triammonium-5.6kPPO-b-7.2kPEO-OH complex (gray) and a triammonium-7.2kPEO-OH complex (black) indicate that the magnetite core size distribution is reproducible.

Hydrodynamic radii (R_m) of the magnetite-polymer complexes were calculated by the method of Mefford, et al.^{29, 30} TEM image analysis data was first fitted with a Weibull probability distribution to calculate an average specific surface area of the magnetite particles. Combining average surface area with the average polymer loading per mass of the complex (from TGA) leads to an average number of chains per magnetite surface area, α , as shown in Equation 4-3.²⁹

$$\alpha = \frac{(1 - W_{mag})N_{Av}\rho_{mag}}{MW_{Total}W_{mag}} \int_0^{\infty} \left(\frac{3}{a}\right) P(a) da \quad (4-3)$$

Here, ρ_{mag} is the density of magnetite (5.21 g cm^{-3}),³¹ W_{mag} is the weight fraction of the complex that is magnetite, and MW_{Total} is the polymer number average molecular weight. Using a modification of Vagberg's density distribution model,³² we calculated the number of chains per particle, $f(a)$, and the complex radius $R_m(a)$ —the radius of the particle plus its attached brush—as

$$f(a) = 4\pi a^2 \alpha \quad (4-4)$$

$$R_m(a) = \left(\frac{8N_k f(a)^{\frac{1-\nu}{2\nu}}}{4^{1/\nu} 3\nu} L_k^{1/\nu} + a^{1/\nu} \right)^\nu \quad (4-5)$$

where N_k is the number of statistical segments in the PEO chain (63), ν is the Flory exponent of PEO in water at 25 °C (0.583)³³, L_k is the length of a statistical segment (0.6 nm), and a is the particle radius. For complexes with the PPO-*b*-PEO diblock copolymers, the PPO layer was assumed to be a non-solvated, hydrophobic shell surrounding the magnetite core and the PEO was modeled as concentric blobs extending out from the surface of the PPO layer. To account for the PPO layer, the model was adapted to include a new effective core size, which is the radius of the magnetite core obtained from the Weibull distribution plus the thickness of the hydrophobic anchor layer (t_a) (equation 4-6).

$$t_a = \left(\left(\frac{\rho_{mag} a^3 (1 - W_{mag})}{W_{mag} MW_{PPO} \rho_{PPO} MW_{Total}} \right) + a^3 \right)^{1/3} - a \quad (4-6)$$

Here, ρ_{PPO} is the density of the PPO anchor layer (1.04 g cm⁻³),³¹ MW_{PPO} is the anchor layer number average molecular weight, and MW_{Total} is the total polymer number average molecular weight. Thus, the predicted hydrodynamic radius of the complex as a function of magnetite particle radius can be described by equation 4-7.

$$R_m(a) = \left(\frac{8N_k f(a)^{\frac{1-\nu}{2\nu}}}{4^{1/\nu} 3\nu} L_k^{1/\nu} + (a + t_a)^{1/\nu} \right)^\nu \quad (4-7)$$

This function along with the Weibull distribution function for the magnetite particle radius is used to determine radial distribution averages for comparison with DLS experimental data. Each average weights particles of various diameters differently and thus gives an estimate of the homogeneity of the particle systems. The number average diameter, D_n , of the magnetite complex can be calculated as

$$D_n = 2 \int_0^\infty R_m(a) P(a) da \quad (4-8)$$

The volume average diameter, D_v , of the magnetite complex can be calculated as

$$D_v = 2 \frac{\int_0^{\infty} R_m(a)^4 P(a) da}{\int_0^{\infty} R_m(a)^3 P(a) da} \quad (4-9)$$

Using a method by Mefford, et al. and assuming the particles are in the Rayleigh scattering regime,²⁹ the intensity average diameter, D_i , for the magnetite complex was calculated as

$$D_i = 2 \frac{\int_0^{\infty} R_m(a)^6 P(a) da}{\int_0^{\infty} R_m(a)^5 P(a) da} \quad (4-10)$$

As equations 4-8 through 4-10 indicate, the intensity average diameter weights larger particles significantly more than the volume average diameter, which in turn, weights larger particles more than the number average diameter. Thus, if a system has small amounts of clustered nanoparticles, this would affect the intensity average more so than the number average. This is important when considering which weighted average from the model to compare to the DLS results. If the model matches the measurements obtained via DLS closely, the model can then be used with DLVO calculations to predict stabilities of the complexes in solution.

Colloidal stability can be predicted from a model for the total potential energy V_{Total} as described by DLVO theory and shown in equation 4-11.²⁰

$$V_{\text{Total}} = V_{\text{vdW}} + V_M + V_{\text{ES}} + V_S \quad (4-11)$$

V_{vdW} is the attractive potential due to van der Waal's interactions, V_M is the attractive potential produced by an applied magnetic field, V_{ES} is the repulsive potential due to electrostatic interactions, and V_S is the repulsive potential due to steric repulsion of the polymer brushes. The magnetic potential energy term was neglected because for the

stability calculations there is no magnetic field being applied to the system. The electrostatic potential can be calculated as

$$V_{ES} = 2\pi a \epsilon \epsilon_o \psi_o^2 \ln(1 + e^{-\kappa(r-2a)}) \quad (4-12)$$

where ϵ is the dielectric constant of the solvent, ϵ_o is the permittivity of free space, ψ_o is the surface potential, κ is the inverse Debye length, r is the center-to-center separation, and a is the particle radius.³⁴ The van der Waal's potential can be calculated as

$$V_{vdW} = -\frac{1}{6} A_{eff} \left(\frac{2a^2}{r^2 - 4a^2} + \frac{2a^2}{r^2} + \ln \left(\frac{r^2 - 4a^2}{r^2} \right) \right) \quad (4-13)$$

A_{eff} is the effective retarded Hamaker constant calculated from equation 4-14.

$$A_{eff} = A_{v=0} + A_{v>0} = \frac{3}{4} kT \left(\frac{\bar{\epsilon}(0) - \epsilon(0)}{\bar{\epsilon}(0) + \epsilon(0)} \right)^2 + \frac{3\hbar\omega}{16\sqrt{2}} \frac{(\bar{n}_o^2 - n_o^2)^2}{(\bar{n}_o^2 + n_o^2)^{3/2}} F(H) \quad (4-14)$$

In this equation, k is Boltzmann's constant, T is the temperature, $\epsilon(0)$ (8.0×10^1)³⁵ and $\bar{\epsilon}(0)$ (2.0×10^4)³⁶ are the dielectric constants for the medium (water) and the substrate (magnetite), n_o (1.33)³⁵ and $\bar{n}_o$ (1.97)³⁵ are the refractive indices (in the visible range) of the medium and substrate, $2\pi\hbar$ is Planck's constant, and ω (1.88×10^{16} rad/sec)³⁵ is the frequency of the dominant relaxation in the UV. $F(H)$ accounts for retardation effects and is unity at the nonretarded limit.³⁵ For this treatment, the Hamaker constant was calculated for magnetite only, and was 9.0×10^{-20} J in the nonretarded limit. The Hamaker constant for magnetite has been reported elsewhere as 16.4×10^{-20} J.³⁷ Our calculation neglects the contributions of the anchor layer thickness and adsorbed polymer brush. Typically Hamaker constants for polymers are significantly lower than for metal oxides and so the magnetite contribution should be greater than the polymer contribution to the overall Hamaker constant. Further refinement of the model will incorporate a core/shell estimation of the Hamaker constant.

For the case of a densely adsorbed brush layer, the steric contribution to the interaction potential can be described by the expression³⁸

$$V_s = \frac{5}{18} kT f(a)^{3/2} \left\{ \begin{array}{ll} -\ln\left(\frac{r}{\sigma}\right) + \frac{1}{1 + \frac{\sqrt{f(a)}}{2}}; & r \leq \sigma \\ \frac{1}{1 + \frac{\sqrt{f(a)}}{2}} \left(\frac{\sigma}{r}\right) \exp\left(-\frac{\sqrt{f(a)}}{2\sigma}(r - \sigma)\right); & r > \sigma \end{array} \right\} \quad (4-15)$$

Here, $f(a)$ is the number of chains per particle (equation 4-4) and $\sigma/2$ is the distance from the center of the core to the center of the outermost blob layer.^{34, 38} For this expression, σ is defined as $1.3R_g$, the radius of gyration of the ensemble.³⁴ The inner, logarithmic term in the equation which describes close approaches was derived originally by Witten and Pincus³⁹ to describe the entropic repulsion when chains from adjacent star-like objects overlap. The outer, exponential term ($r > \sigma$) is based on a Yukawa-type decay (such as that used to describe a screened Coulomb potential⁴⁰) with the decay length set to the diameter of the largest blobs and has been extensively tested using machine simulations.³⁸

The radius of gyration, R_g , can be approximated using the previously described model and the Weibull particle radius distribution fit by calculating the moment of inertia of the complex as described by Mefford et al.^{29, 30} The moment of inertia of a mass, m , rotated about an axis at a distance x is given by⁴¹

$$I = x^2 dm \quad (4-16)$$

Extension of this to the moment of inertia of each component of the complex can be calculated as

$$I_{Total} = I_{Mag} + I_{PPO} + I_{PEO} = \int_0^a \rho_{mag} 4\pi a^4 da + \int_a^{a+t_a} \rho_{PPO} 4\pi a^4 da + \int_{a+t_a}^{R_m(a)} m_s A \left(\frac{a}{a_s}\right)^{\frac{1}{v}} 4\pi a da \quad (4-17)$$

The total mass of the complex can be calculated as

$$M_{Total} = M_{Mag} + M_{PPO} + M_{PEO} = \frac{4}{3} \pi a^3 \rho_{mag} + \frac{M_{mag}(1 - W_{mag})}{W_{mag}} \quad (4-18)$$

and the radius of gyration is defined as

$$R_g = \int_0^\infty \sqrt{\frac{I_{Total}}{M_{Total}}} P(a) da \quad (4-19)$$

To test this method, the calculated R_g s were compared to measured values of R_g for polymer micelles from small angle neutron scattering (SANS) with agreement within 7% of experimental values.⁴² This allows for calculation of the stability ratio and the half-life for doublet formation using equations 4-20 and 4-21, respectively.

$$W = 2\bar{a} \int_{\bar{a}}^\infty \frac{e^{-\frac{V_{Total}}{kT}}}{r^2} dr \quad (4-20)$$

$$t_{1/2} = \frac{\bar{a}^{-3} \pi \mu W}{\phi kT} \quad (4-21)$$

Here, μ is the solvent viscosity, Φ is the volume fraction of particles in solution, and $\bar{a}$ is the average particle radius.^{20, 43} The classical expression for W does not account for polydispersity and only concerns pair interactions for particles of like sizes. We account for the size distribution by computing $\bar{a}$ (4.0 nm) from the Weibull distribution (equation 4-2). The half-life for doublet formation is the time required for half of the particles to flocculate in the form of doublets. This parameter serves as a basis of comparison for complexes with different polymer molecular weights.

4.5 Results and Discussion

4.5.1 Synthesis of polyether-magnetite complexes

The synthesis of a trivinylsilyl-PPO-*b*-PEO-OH is shown in Figure 4.3. A $Zn_3[Co(CN)_6]_2$ coordination catalyst (ImpactTM from Bayer) was used for preparing the PPO blocks to avoid side reactions associated with base catalyzed ring-opening polymerizations of PO. It is well-known that significant amounts of undesired allyl-functional PPO form when hydroxide or alkoxide bases are utilized.⁴⁴ ¹H NMR of the trivinylsilyl-PPO-OH synthesized with the ImpactTM catalyst revealed well-defined polymers without allyl endgroups. Trivinylsilyl-PPO-OH was then utilized as a macroinitiator for base catalyzed polymerization of EO, resulting in an amphiphilic,

heterobifunctional trivinylsilyl-PPO-*b*-PEO-OH diblock copolymer. 3-HPTVS was also utilized as the initiator for base catalyzed polymerization of EO to form trivinylsilyl-PEO-OH.

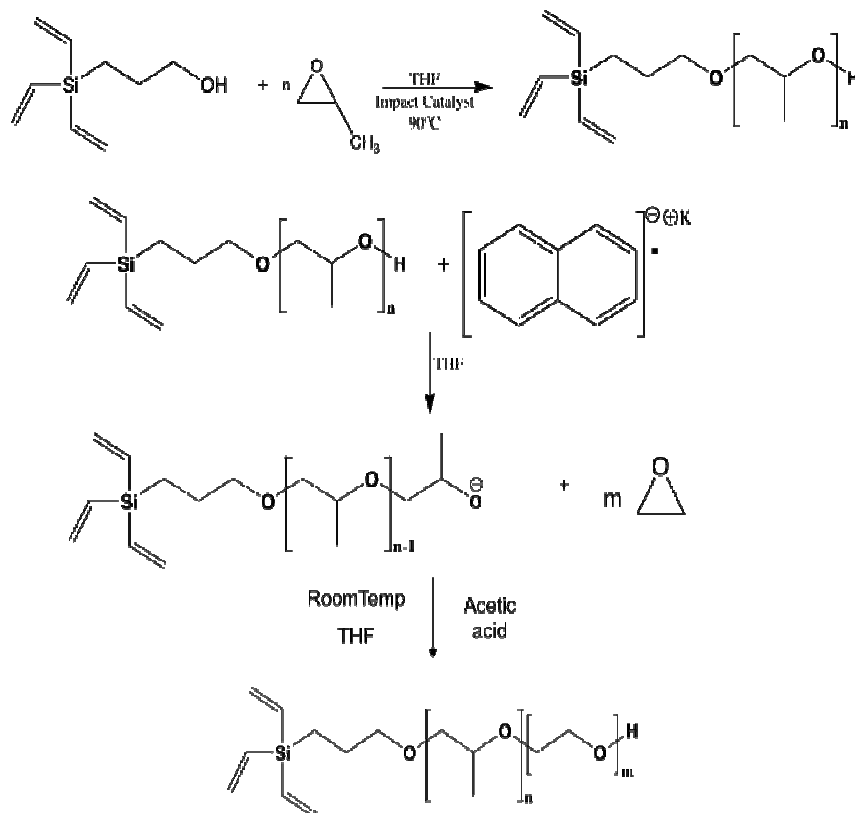


Figure 4.3. Synthesis of a heterobifunctional trivinylsilyl-PPO-*b*-PEO-OH copolymer.

Molecular weights and block lengths were analyzed by NMR and GPC with good agreement among the targeted values and both methods of analysis (Table 4.1). Molecular weight distributions of the PPO blocks were somewhat broader than for the PEO blocks. This arises from the heterogeneous nature of the coordination catalyst, and we are currently exploring synthetic avenues for narrowing those distributions. As expected, the PEO homopolymers that were synthesized by conventional anionic polymerizations had very narrow molecular weight distributions.

Table 4.1. Polyether molecular weights

Polymer	M _n (g mol ⁻¹)			Molecular Weight Distribution
	¹ H NMR of PPO	¹ H NMR of PEO	GPC	
7.2kPEO-OH	N/A	7,200	7,800	1.1
5.6kPPO- <i>b</i> -3.8kPEO-OH	5,600	3,800	10,100	1.3
5.6kPPO- <i>b</i> -7.2kPEO-OH	5,600	7,200	13,100	1.2
3.3kPPO- <i>b</i> -2.6kPEO-OH	3,300	2,600	5,400	1.3
3.3kPPO- <i>b</i> -4.8kPEO-OH	3,300	4,800	7,900	1.3

* PDI = Polydispersity index

Ene-thiol additions of cysteamine hydrochloride on the trivinylsilyl-functional polyethers were performed to introduce ammonium functionality on the vinylsilyl termini. The reaction mixtures were deoxygenated prior to heating to afford complete conversion of the vinyl groups. After thiol addition, the polymers were washed with aqueous base and DI water to remove DMF and excess cysteamine, resulting in triammonium-functional polyethers. Figure 4.4 shows a representative ¹H NMR spectrum that confirms the addition of cysteamine hydrochloride across the vinyl groups of a 7.2k M_n trivinylsilyl-PEO-OH homopolymer.

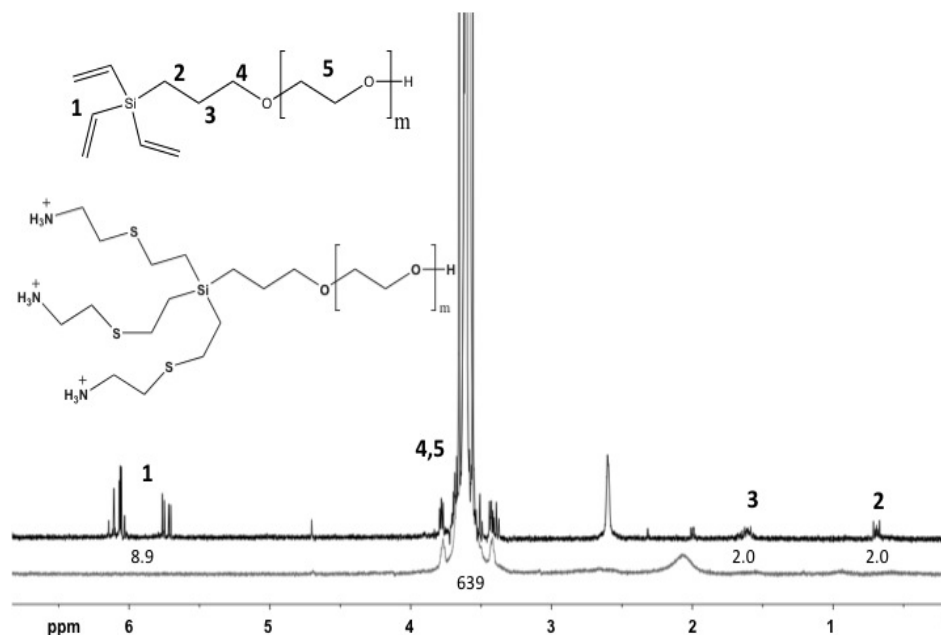


Figure 4.4. ¹H NMR illustrates the disappearance of vinyl groups (1) on a 7.2kPEO-OH homopolymer after addition of cysteamine across the vinylsilane groups.

Triammonium-functional diblock copolymers with varied PPO and PEO block lengths as well as a triammonium-PEO-OH homopolymer were utilized to prepare the magnetite-copolymer complexes for this study.

4.5.2 Relationships among the amphiphilic nature of the polymer dispersion stabilizers, the density of chains on the magnetite surface and the colloidal properties of the complexes

One objective of this work has been to generate small clusters of magnetite-copolymer complexes that remain stable in dispersion. Clusters are desirable because larger particles may provide larger relaxivities and increase contrast enhancement in MRI imaging.⁴⁵ To understand colloidal stability in physiological media, it was first helpful to examine the properties of these materials in water. Diameters of the complexes in water were measured via DLS every half-hour for 24 hours and the mean of the final 10 measurements were compared to those predicted by the model (Table 4.2). At high polymer compositions, the diameters of complexes with the PEO homopolymer and the first two diblocks described in Table 4.2 matched the model closely, indicating that these were dispersed as single particles. However, the DLS volume average diameters for complexes with those polymers became significantly higher than those predicted by the model as the polymer loading was reduced (suggesting clustering). For complexes with the triammonium-3.3kPPO-*b*-2.6kPEO-OH complexes where the block lengths were relatively short and the hydrophobic PPO was the dominant copolymer block, the volume averages were larger than the predicted values at all of the polymer loadings (33, 49 and 66% polymer). Thus, controlled clusters had formed with all of the complexes prepared with this particular copolymer. Surprisingly, these particles flocculated slightly until they reached some equilibrium diameter and then the sizes were stable (measured for >24 hours). This may be attributable to some mobility of the polymer chains on the surface or that the PPO layer of one particle generates an attractive hydrophobic force with the PPO layer of another particle. While the present model cannot account for the slight clustering, comparison of the DLS data to the model can identify when the flocculation takes place.

Table 4.2. A divergence in the experimental (measured over 24 hours) and predicted diameters of the complexes allows for estimating the lower limit of polymer loading where appreciable flocculation occurs. This phenomenon is more pronounced when comparing experimental and predicted volume average diameters.

		Dynamic Light Scattering		Distribution Model	
Complex	Polymer Loading (%)	Volume average diameter (nm)	Number average diameter (nm)	Volume average diameter (nm)	Number average diameter (nm)
7.2kPEO-OH	39	43.2	33.8	33.7	31.7
7.2kPEO-OH	45	38.5	30.7	35.2	33.2
7.2kPEO-OH	65	45.5	37.2	41.1	38.8
5.6kPPO- <i>b</i> -7.2kPEO-OH	33	46.9	34.7	28.1	26.0
5.6kPPO- <i>b</i> -7.2kPEO-OH	51	39.3	31.0	32.8	30.4
5.6kPPO- <i>b</i> -7.2kPEO-OH	68	42.2	34.9	38.4	35.5
5.6kPPO- <i>b</i> -3.8kPEO-OH	31	45.1	35.6	21.9	19.9
5.6kPPO- <i>b</i> -3.8kPEO-OH	49	39.1	29.5	25.8	23.3
5.6kPPO- <i>b</i> -3.8kPEO-OH	67	34.0	27.4	30.7	27.7
3.3kPPO- <i>b</i> -2.6kPEO-OH	33	51.5	36.9	20.9	18.8
3.3kPPO- <i>b</i> -2.6kPEO-OH	49	39.0	29.8	24.1	21.7
3.3kPPO- <i>b</i> -2.6kPEO-OH	66	37.6	30.6	28.4	25.5

Potential energy curves were generated for the complexes. Measured zeta potentials of the particles ranged from a minimum of -6 mV to a maximum of +6 mV. This indicated that surface charge contributed very little electrostatic stabilization and that steric stabilization was the dominating factor at close ranges (10^{-2} kT (electrostatic) vs. 10^2 kT (steric) at surface-to-surface separation distances <5 nm for the 5.6kPPO-*b*-3.8kPEO-OH complex with 70% polymer loading). Figure 4.5 shows potential energy curves for complexes with the triammonium-7.2kPEO-OH and triammonium-5.6kPPO-*b*-7.2kPEO-OH. Typically a potential energy maximum of >10 kT is sufficient to prevent flocculation.⁴⁶ As illustrated in Figure 4.5(B), the maximum for the diblock complex at 30% polymer loading is approximately 2 kT and is approximately 28 kT for the case of 50% polymer loading. At a volume fraction of 2.0×10^{-5} , these loadings correspond to $t_{1/2}$ values of 7.5×10^{-4} and 6.1×10^{10} seconds, respectively. Thus, one would expect clustering to occur for the particles with 30% polymer, but not for those with 50% polymer. This is consistent with the data in Table 4.2, which shows clustering to an equilibrium diameter at 33% ($t_{1/2} = 1.8 \times 10^{-3}$ sec) but not at 51% polymer loading ($t_{1/2} = 1.2 \times 10^{12}$ sec) as indicated by the number average diameters. The instability exhibited

with low polymer loadings is even more evident in the data for the triammonium-5.6kPPO-*b*-3.8kPEO-OH complex, which is reasonable since it has a much shorter hydrophilic block. The potential energy curves predict clustering for the case of 31% polymer loading ($t_{1/2} = 3.0 \times 10^{-4}$ sec) and this is confirmed by DLS. For the complex with 49% polymer ($t_{1/2} = 1.6 \times 10^8$ sec), clusters with equilibrium diameters were observed via DLS. Although the $t_{1/2}$ seems high for clustering to occur in this case, some clustering is not altogether unexpected because the $t_{1/2}$ value is the measure of when half of the particles have formed doublets. Thus, when right at the transition from single particles to clusters (for example, 20% doublets instead of 50%), partial clustering would be observable via DLS even with the slightly elevated $t_{1/2}$. In addition, these $t_{1/2}$ calculations are extremely sensitive to polymer loading at the point of clustering and any slight error in the TGA measurement might cause inconsistencies between experimental and calculated sizes.

By contrast, the potential energy curves for the complexes coated with the triammonium-7.2kPEO-OH predict stability against clustering at 50% ($t_{1/2} = 8.0 \times 10^{114}$ sec) and 70% ($t_{1/2} = 7.0 \times 10^{657}$ sec) polymer loadings, but not at 30% ($t_{1/2} = 1.8 \times 10^1$ sec). These predictions are also consistent with the DLS results, as the volume and number average diameters do not significantly deviate from the predicted values until a small deviation is observed at 39% polymer (Table 4.2). The overall resistance to clustering for the homopolymer complexes compared to the 5.6kPPO-*b*-7.2kPEO-OH and 5.6kPPO-*b*-3.8kPEO-OH diblock complexes is most likely due to an increased number of chains per particle for similar polymer loadings. Thus, it appears that the DLVO theory can reasonably predict the colloidal stabilities of these complexes. By contrast, for the complexes with the 3.3kPPO-*b*-2.6kPEO-OH copolymer, the experimental volume and number average diameters deviate from the predicted values for all polymer loadings while DLVO predicts resistance to clustering at high polymer concentrations ($t_{1/2} = 4.8 \times 10^{225}$ and 2.0×10^{24} seconds for 70% and 50% polymer, respectively). This may be due to an additional hydrophobic interaction between the PPO blocks on separate complexes due to the relatively high composition of PPO combined with the short PEO block. In addition, we note that assumptions inherent in the calculation of the Hamaker constant

may cause some inaccuracies in the DLVO predictions, especially since the Derjaguin approximation is not expected to fully apply for these small particles with highly curved brushes. However, these data indicate that experimental diameters can be compared to those predicted by the density distribution model when the calculated potential energy maximum is greater than about 10kT.

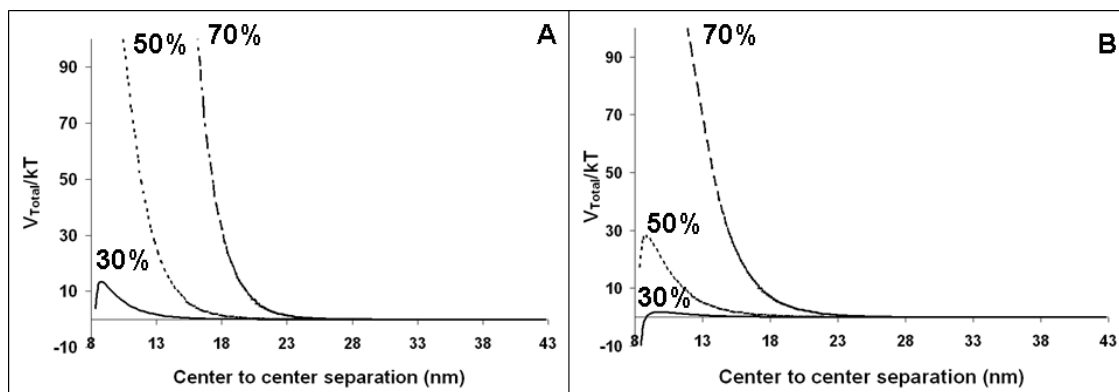


Figure 4.5. Potential energy curves for magnetite-copolymer complexes with triammonium-7.2kPEO-OH complexes (A) and triammonium-5.6kPPO-b-7.2kPEO-OH (B)

Number average diameters from DLS (measured immediately after the complexes were suspended in DI water and sonicated) and the model for complexes with the triammonium-7.2kPEO-OH, 5.6kPPO-*b*-7.2kPEO-OH and 5.6kPPO-*b*-3.8kPEO-OH are compared in Table 4.3. The potential energy curves for each of these complexes show maxima >10kT and so clustering should be minimal. For these complexes, there is a maximum deviation of 23% (6.9 nm) and an average deviation of 8% (2.7 nm). This error is typical for DLS measurements in this size range. Thus, the density distribution model, with no adjustable parameters, can predict the number average diameters of complexes to within an average of 8% for non-clustered complexes. The model also provides calculations of DLVO potential energy curves and half-lives for doublet formation that predict the polymer loading at which complexes will begin to exhibit clusters as the polymer composition is reduced. These correlate well with complex sizes measured by DLS. Deviation in this prediction occurs with the 3.3kPPO-*b*-2.6kPEO-OH complexes, but this may be due to an additional attractive hydrophobic force or dispersion force not accounted for at present in the model.

4.5.3 Influence of material parameters on clustering of the complexes

While the radii of the complexes predicted from the model do not account for clusters, the model can be used to predict aggregation based on the stability ratios and half-lives for doublet formation ($t_{1/2}$). The sizes of these clusters were stable over a 24-hour measurement time and they did not settle, so they could be easily compared. The $t_{1/2}$ values were calculated for each of these complexes, and $\ln(t_{1/2})$ was plotted versus the intensity average diameters measured by DLS (24 hours after the particles were dispersed by sonication and then filtered) (Figure 4.6). Because the intensity average diameter is weighted heavily towards larger particles, it is ideal for probing cluster formation. An exponential function was fit to the data from the 5.6kPPO-*b*-7.2kPEO-OH, 5.6kPPO-*b*-3.8kPEO-OH and 3.3kPPO-*b*-4.8kPEO-OH complexes. This fit indicates that the clustering behavior of these complexes can be accurately described by the $t_{1/2}$ values. This comparison is unique in the colloid literature to our knowledge and allows for predicting the cluster diameter based on the $t_{1/2}$ calculation. More importantly, deviations from the predicted diameter can be used to better understand interactions between specific complexes. Additionally, at higher polymer loadings, the number average diameters of each of these complexes matched those predicted by the model as described earlier. This indicates that by increasing the polymer loading, the complexes form discrete particles and clustering is eliminated.

Table 4.3. An average deviation of 8% was observed between experimental (measured immediately after dispersion by sonication followed by filtration) and predicted number average diameters for complexes that were predicted to be stable by DLVO theory.

Complex	Polymer Loading (%)	Chains/nm ²	Model D _n (nm)	DLS D _n (nm)	Deviation (%)
7.2kPEO-OH	65	1.08	38.8	37.2	4%
7.2kPEO-OH	48	0.55	33.9	32.4	5%
7.2kPEO-OH	45	0.48	33.2	31.0	7%
7.2kPEO-OH	44	0.47	32.9	35.9	8%
7.2kPEO-OH	39	0.37	31.6	33.9	7%
7.2kPEO-OH	30	0.25	29.4	28.7	2%
5.6kPPO- <i>b</i> -7.2kPEO-OH	74	0.62	37.9	30.4	25%
5.6kPPO- <i>b</i> -7.2kPEO-OH	68	0.46	35.5	31.6	12%
5.6kPPO- <i>b</i> -7.2kPEO-OH	52	0.24	30.6	31.0	1%
5.6kPPO- <i>b</i> -7.2kPEO-OH	52	0.24	30.6	27.6	11%
5.6kPPO- <i>b</i> -3.8kPEO-OH	67	0.46	35.1	31.3	12%

By contrast, for the complexes with the more hydrophobic 3.3kPPO-*b*-2.6kPEO-OH copolymer that had the smaller block lengths, the exponential function only fit the experimental data at polymer loadings up to ~60%. The experimental diameters were significantly larger than the predicted values for loadings above 60% polymer. The DLS measurements (Table 4.2) show that complexes with this polymer formed clusters at all polymer loadings whereas the potential energy curves and $t_{1/2}$ calculations indicated that clusters should not form above 55% polymer loading. For these complexes, increasing the polymer loading does not eliminate clustering at all. This suggests that clustering in those complexes at high polymer compositions may be due to a different mechanism that the current DLVO calculations do not account for. Since this deviation occurs in the complexes with very high compositions of copolymer, we hypothesize that the clustering in those materials may be largely attributable to the properties of the polymer. It may be that the hydrophobic PPO blocks on one nanoparticle are not sufficiently shielded from neighboring nanoparticles by the short PEO blocks, and that PPO segments on multiple particles are associating through hydrophobic interactions. This is supported both by the deviation of the cluster diameters from the exponential fit, and the deviation of the experimental sizes from those predicted by the DLVO calculations (Table 4.2).

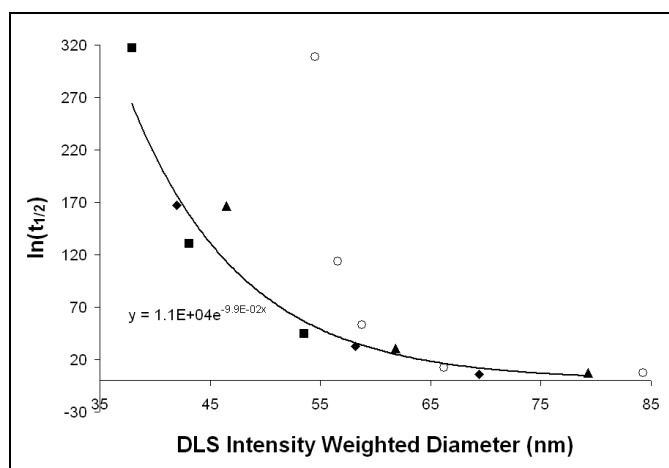


Figure 4.6. Intensity weighted diameters for the magnetite-copolymer complexes obtained from DLS vs. the calculated $\ln(t_{1/2})$: 5.6kPPO-*b*-7.2kPEO-OH (diamonds), 5.6kPPO-*b*-3.8kPEO-OH (triangles), 3.3kPPO-*b*-4.8kPEO-OH (squares), and 3.3kPPO-*b*-2.6kPEO-OH (empty circles)

The accuracy of the exponential fit indicates that the $t_{1/2}$ is a useful tool for comparing complexes with different molecular weight polymers. This, along with the potential energy curves derived with the extended DLVO theory, provides evidence that the steric potential in equation 4-16 is valid for these systems. Moreover, the deviation from the exponential fit for the 3.3kPPO-*b*-2.6kPEO-OH copolymer provides insight into additional interactions that may be at play. The predictions of $t_{1/2}$ and the associated cluster diameter of a particle can aid in the design of copolymers required to obtain resistance to or formation of clusters. Similarly, such an approach can likely be extended to other colloidal systems.

4.5.4 Suitability of three ammonium ions as anchor segments for magnetite in water and PBS

Intensity-average diameters of the magnetite-polymer complexes over time in DI water and PBS were examined with DLS to establish the conditions where these polymers had suitable binding affinities to be considered good dispersion stabilizers. Figure 4.7 shows colloidal sizes over time for magnetite coated with triammonium-5.6kPPO-*b*-7.2kPEO-OH. While the sizes were constant in water over 24 hours, some aggregation was observed with time in PBS, particularly at the lower polymer compositions. This behavior is attributed to some desorption of the polymers from the magnetite surface in PBS.

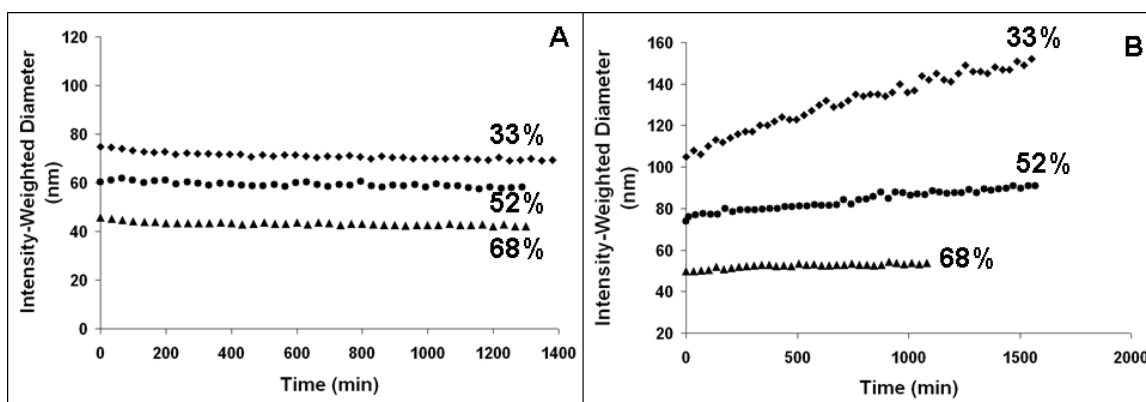


Figure 4.7. DLS shows that the complexes with the triammonium-5.6kPPO-*b*-7.2kPEO-OH have long-term stability in DI water (A) but not in PBS (B). Percentages shown are % polymer in the complexes.

To understand the effects of PBS, the magnetite-copolymer complexes were dialyzed against water for 48 hours to remove any unbound polymer, against PBS for 24 hours to expose the complexes to phosphate, and then against water for another 24 hours to remove any unbound polymer that may have been displaced by the phosphate (as well as to remove phosphate salts from the liquid phase). Compositions of the complexes were measured by TGA after the water dialyses to examine whether any polymer had desorbed from the surface (Table 4.4).

Table 4.4. Dialysis experiments show that these polymer stabilizers desorb appreciably from the magnetite surface in PBS over 24 hours (after dispersion by sonication and filtration). No desorption was observed after dialysis against DI water.

Complex	Dialysis against water (% polymer)	Chains/nm ²	Dialysis against PBS (% polymer)	Chains/nm ²
7.2kPEO-OH	50	0.58	39	0.37
7.2kPEO-OH	71	1.45	60	0.87
5.6kPPO- <i>b</i> -3.8kPEO-OH	64	0.40	44	0.18
5.6kPPO- <i>b</i> -7.2kPEO-OH	64	0.38	46	0.19
5.6kPPO- <i>b</i> -7.2kPEO-OH	52	0.23	28	0.08
3.3kPPO- <i>b</i> -2.6kPEO-OH	62	0.64	51	0.41

Each complex lost a significant amount of polymer after exposure to PBS for 24 hours. Thus, these experiments indicated that the cause of the size increases over time in PBS (as observed in Figure 4.7) versus constant sizes in water was primarily due to polymer desorption in PBS (and not caused by screening of electrostatic charge due to the ionic strength of PBS). This was confirmed by measuring sizes of the complexes in DI water with sodium chloride added at the same ionic strength as PBS. No increase in size over time was observed.

Figure 4.8 shows zeta potentials measured while varying the pH for an oleic acid-coated magnetite (Figure 4.8A) and a magnetite-7.2kPEO-OH complex (Figure 4.8B) in water and PBS. Note how the charge of both systems becomes markedly more negative in PBS, only passing through the zero net charge barrier at a pH of ~2.0-2.5. This pH corresponds with the lowest pK_a of phosphoric acid. A similar change in charging behavior occurs for the other complexes.

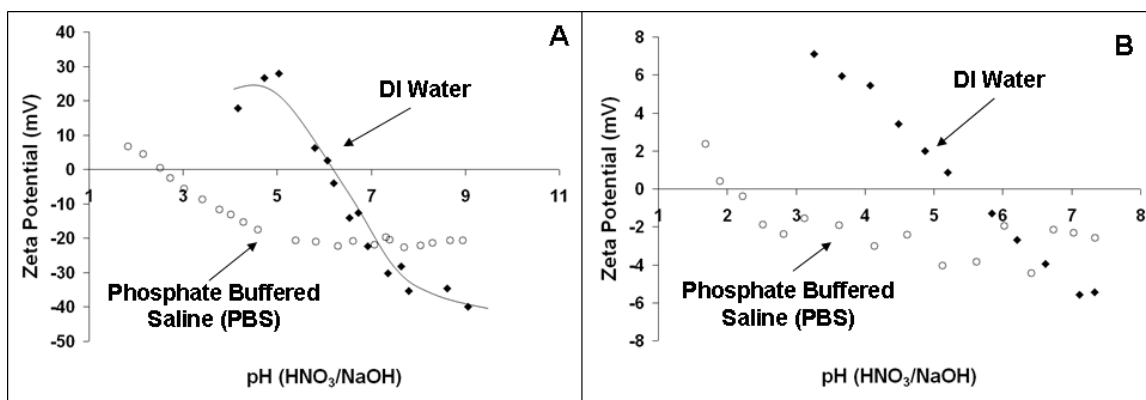


Figure 4.8. The zeta potential of magnetite without polymer (A) and magnetite coated with polymer (B, triammonium-7.2kPEO-OH) changes and follows the same trend when measured in PBS compared to DI water. The lowest pK_a of phosphoric acid is ~ 2.2 , which corresponds to the isoelectric point of both systems.

This, along with the dialysis data, indicates that the phosphate adsorbs relatively strongly onto the magnetite surface, even when a polymer brush is present. For all the complexes examined, phosphate adsorption affects anchoring of the ammonium groups on the magnetite surface and causes some desorption of the polymer. In most cases, this desorption is sufficient to induce flocculation until the particles are large enough to form visible sediments within a few days. This indicates a limitation of the triammonium anchor group for a steric stabilizer when the complexes are to be used in physiological media.

It was of interest to understand how the rates of polymer desorption in PBS were influenced by the nature of the copolymer coatings. In particular, we wondered whether a relatively hydrophobic PPO sheath layer could hinder desorption significantly. The rates of size increase in PBS were approximately constant so the slopes of the lines were compared (e.g., in Figure 4.7). Table 4.5 summarizes the rates of increase for the complexes and predicts their cluster diameters at 24 hours. These data suggest that a PPO layer does slow the rate of desorption. When comparing the magnetite-7.2kPEO-OH complex with 45% polymer, the 5.6kPPO-*b*-7.2kPEO-OH complex with 68% polymer, and the 5.6kPPO-*b*-3.8kPEO-OH complex with 67% polymer—cases in which the chain densities are essentially equal ranging from 0.46-0.48 chains nm⁻²—it is apparent that the PPO layer plays a large role in increasing stability against flocculation.

Notice that the 7.2kPEO-OH homopolymer complex, which is the most resistant to clustering in water, is the least stable in PBS. An order of magnitude more chains nm^{-2} was required for the 7.2kPEO-OH complex to have the same slope as complexes prepared with the 5.6kPPO-*b*-7.2kPEO-OH copolymer (1.08 chains nm^{-2} vs. 0.11 chains nm^{-2} , respectively).

In addition, the sizes of the complexes with the 5.6kPPO-*b*-7.2kPEO-OH are more stable than with the 5.6kPPO-*b*-3.8kPEO-OH, indicating that the longer PEO brush (at similar numbers of chains nm^{-2}) provides better stabilization in PBS. For the 5.6kPPO-*b*-3.8kPEO-OH with 31% polymer, the flocculation rate is essentially equal to that of the 7.2kPEO-OH complex with 39% polymer. This is a much larger rate than for the 5.6kPPO-*b*-7.2kPEO-OH complex with 33% polymer. This can be attributed to two competing factors. First, the hydrophobic PPO block slows desorption of the polymer from the surface, but does not prevent it. Second, the colloidal stability depends directly on the number of chains nm^{-2} . For the 5.6kPPO-*b*-3.8kPEO-OH complex with 31% polymer, the combination of the small hydrophilic block along with the large hydrophobic block and the relatively few chains on the particle surface contribute to the faster flocculation rate. For large PPO blocks, there is a limit to the total number of chains nm^{-2} due to the size of the hydrophobic PPO. Thus, even though the data indicates that the PPO block somewhat protects the complexes from flocculation in PBS, larger PPO blocks result in fewer chains that can be adsorbed onto the magnetite surface.

Thus, our hypothesis is that reducing the PPO molecular weight might allow for better colloidal stability in PBS because the protective PPO layer would still be present, but this time with a higher number of polymer chains on the surface. Additionally, the deviation of the DLS data from the model and the exponential clustering fit indicate that hydrophobic forces are strong for the 3.3kPPO-*b*-2.6kPEO-OH complexes. This is illustrated in Table 4.5, where the rate of flocculation for a 3.3kPPO-*b*-2.6kPEO-OH complex with 48% polymer was zero. Thus, by reducing the molecular weight of the PPO block, colloidal stability in PBS can be obtained for greater than 24 hours.

Table 4.5. The change in diameter over time in PBS indicates that the PPO layer of the diblock complexes hinders polymer desorption from the magnetite surface.

Complex	Polymer Loading (%)	Chains/nm ²	Slope in PBS (nm/hr)	Diameter Increase over 24 hours (nm)
7.2kPEO-OH	39	0.37	28.8	691
7.2kPEO-OH	45	0.48	21.6	518
7.2kPEO-OH	65	1.08	2.0	49
5.6kPPO- <i>b</i> -7.2kPEO-OH	33	0.11	1.7	42
5.6kPPO- <i>b</i> -7.2kPEO-OH	51	0.23	0.6	14
5.6kPPO- <i>b</i> -7.2kPEO-OH	68	0.46	0.2	4
5.6kPPO- <i>b</i> -3.8kPEO-OH	31	0.10	29.3	703
5.6kPPO- <i>b</i> -3.8kPEO-OH	49	0.22	1.0	23
5.6kPPO- <i>b</i> -3.8kPEO-OH	67	0.46	0.5	12
3.3kPPO- <i>b</i> -2.6kPEO-OH	52	0.42	0.0	0

4.6 Conclusions

A heterobifunctional triammonium-PEO-OH homopolymer and triammonium-PPO-*b*-PEO-OH diblock copolymers were synthesized and adsorbed onto magnetite nanoparticles to study their effects on colloidal stability in water and in PBS. A model for the copolymer-magnetite complexes was employed which incorporated the magnetite core surrounded by a hydrophobic PPO layer (treated as a non-solvated shell around the magnetite core) and a hydrophilic PEO layer extending into solution.²⁹ A polymer brush model was combined with the magnetite core size distribution to predict complex size distributions that could be measured by DLS. The model, in concert with a modified DLVO theory, was used to predict the polymer loadings that were needed to prevent clustering. For systems that the DLVO theory predicted to be single particles, the average deviation of the model from the DLS measurements for number average diameters was only 2.7 nm (8%). By comparing the number average and volume average diameters to the predicted values, the point of complex clustering could be experimentally identified. For cases where clustering occurred, the small clusters reached an equilibrium diameter and did not continue to grow. In water, the magnetite-PEO complexes were more resistant to clustering than their diblock counterparts at similar polymer loadings because the relatively hydrophobic PPO block restricts the total number of PEO chains that can be attached to the magnetite nanoparticle surface.

Half-lives for doublet formation ($t_{1/2}$) were calculated for the diblock complexes from the DLVO theory and were correlated with measured intensity average diameters (after 24 hours) in water by fitting the diameters and their $\ln(t_{1/2})$ values with an exponential function. For the complexes with the 3.3kPPO-*b*-2.6kPEO diblocks, the size deviations from the predictions were significantly higher at high polymer loadings, suggesting that copolymer-magnetite complexes with relatively long PPO blocks and short PEO blocks may exhibit additional hydrophobic interactions between the PPO blocks on separate complexes not yet accounted for in the DLVO theory. These hydrophobic interactions could be a key to designing small clusters of nanoparticles that have colloidal stability in aqueous media.

Stability in physiological media was examined through zeta potential measurements, dialysis, and long-term DLS measurements. While the sizes of the complexes were all constant in water for 24 hours, most of them showed a continual increase in diameter over time in PBS, indicating most of the polymers studied were partially displaced by phosphate salts in PBS. However, there was significantly less polymer desorption in PBS for the complexes having the amphiphilic diblocks relative to complexes with the triammonium-7.2kPEO-OH. Moreover, high levels of PEO chains nm^{-2} are essential for colloidal stability in PBS as illustrated by the magnetite-triammonium-3.3kPPO-*b*-2.6kPEO-OH complex ($0.42 \text{ chains nm}^{-2}$) that did not increase in size over time in water or PBS. For biological studies in physiological media, incorporation of a hydrophobic block to shield the magnetite surface from the phosphate salts and the use of lower molecular weight PPO blocks to increase chain density may be essential for use of the triammonium anchor group. Current research in our laboratories is also exploring alternative anchoring groups for magnetite and the relative stabilities of those materials in PBS.

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5 Novel Phosphonate-functional Poly(ethylene oxide)-Magnetite Nanoparticles form Stable Colloidal Dispersions in Phosphate Buffered Saline

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This chapter is currently under review (Chemistry of Materials)

Philip Huffstetler synthesized the polyethers discussed in sections 5.3.2 – 5.3.9 as well as some of the polyether-magnetite complexes discussed in section 5.3.10. Dr. Jonathan Goff and Christian Reinholz synthesized the polyether-magnetite complexes discussed in section 5.3.10. Shannon Ball performed TGA measurements as shown in Table 5.2.

5.1 Abstract

Many studies have concerned the colloidal stability of magnetite nanoparticles coated with poly(ethylene oxide) (PEO), but their long-term stability when such complexes are exposed to physiological media is still not well understood. This paper describes effects of different functional anchor groups, including one carboxylate, one ammonium, one phosphonate zwitterion, and three phosphonate zwitterions, on the structural stabilities of PEO-magnetite nanoparticle complexes in water and phosphate buffered saline (PBS). While PEO oligomers bound to the magnetite through any of these functional groups remain stably bound in water, only the complexes with polymers bound through the zwitterionic phosphonates were stable in PBS. The stabilities of the PEO-magnetite complexes with these phosphonate zwitterions in PBS allowed for investigating colloidal properties of their dispersions as functions of the number of phosphonates per chain and the chain densities and molecular weights. In contrast to all of the other complexes studied, PEO-magnetite nanoparticles bound through three phosphonate zwitterions on

one end of the PEO exhibited no significant change in size for over 24 hours even when they were dispersed in PBS. The colloidal properties of all of these dispersions are discussed in light of their compositions and structures.

5.2 Introduction

Magnetite nanoparticles coated with biocompatible macromolecules have been of great interest due to a wide range of potential biomedical applications including drug delivery, treatment of detached retinas, cell separations and contrast enhancement agents for MRI.¹⁻⁶ PEO has been one of the most widely used coatings for magnetite nanoparticles due to its low cytotoxicity, ability to mask foreign substrates from the immune system, and FDA approval for *in vivo* applications.^{1, 7, 8} Despite the prevalence of PEO as a coating for magnetite, there is a need to improve upon the binding efficacy of the polymer to the magnetite surface so that the materials will remain intact in physiological media.⁹⁻¹¹

Functional anchor groups on polymers can aid in their adsorption to the surface of magnetite. Polymeric magnetite dispersion stabilizers containing carboxylate and alkylammonium anchor groups have been previously reported.^{8, 11-17} Over the past few decades, significant effort has been devoted to the surface modification of magnetite with PEO-containing polymers to improve their biocompatibility, resist protein adsorption and increase their circulation time within the body.^{1, 7, 18, 19} Previous work by others has shown the capacity to coat magnetite nanoparticles with polymers containing pendant carboxylate groups, as well as with carboxylate, alkylammonium, or methoxysilane termini.^{8, 20-27} Investigations of the colloidal stabilities of magnetite and maghemite nanoparticles having organic coatings have been conducted in water and in saline (e.g., sodium chloride).^{12-16, 20} Stabilities of these complexes in water and saline were adequate, but the issue of their stabilities in media simulating physiological conditions that contain phosphate salts has not been adequately addressed.

This paper describes the synthesis of PEO oligomers containing functional anchor groups at one end (one carboxylate, one ammonium, one phosphonate zwitterion, three

phosphonate zwitterions) and their adsorption onto magnetite nanoparticles. Relationships between the chemical nature of the anchor group(s) and the capacity to remain firmly bound to the magnetite, as well as the colloidal dispersion stabilities of the PEO-magnetite complexes, were studied in water and in PBS. Additionally, the influence of PEO molecular weight on colloidal properties of the PEO-magnetite complexes with similar polymer loadings has been investigated.

5.3 Experimental

5.3.1 Materials

Azobisisobutyronitrile (AIBN), benzyl alcohol (>98%), diethyl ether, diethyl vinyl phosphonate (97%), ethylene oxide (EO, 99.5+%), hexanes (HPLC grade), iron (III) acetylacetonate ($\text{Fe}(\text{acac})_3$), mercaptoacetic acid (97+%), oleic acid (90%, technical grade), sodium iodide (99%), triethylamine (TEA, 99.5%), and 1.0 M vinylmagnesium bromide in THF were purchased from Aldrich and used as received. Tetrahydrofuran (THF, Optima Grade, EMD Science, 99.5%) was refluxed over sodium metal with benzophenone until the solution reached a deep purple, fractionally distilled, and deoxygenated just prior to use. Glacial acetic acid (EMD Science) was diluted with THF yielding a 2.0 M acetic acid solution. Naphthalene (Aldrich) was sublimed prior to use. Bromotrimethylsilane (TMS-Bromide, 97%) and mecaptoethylamine hydrochloride were purchased from Alfa Aesar and used as received. Ethanol (Decon Laboratories Inc.) was used as received. Methanol (HPLC grade), chloroform (HPLC grade), *N,N*-dimethylformamide (DMF, Optima Grade), dichloromethane (HPLC grade), sodium bicarbonate, ammonium chloride, sodium chloride, and acetone (HPLC grade) were purchased from Fisher Scientific and used as received. 3-Chloropropyldimethylchlorosilane and 3-chloropropyltrichlorosilane were purchased from Gelest and used as received. Dialysis tubing (25,000 g mol^{-1} MWCO and 1,000 g mol^{-1} MWCO) was obtained from Spectra/Por. Phosphate buffered saline 10X (PBS) was obtained from Lonza and diluted to appropriate concentrations.

5.3.2 Synthesis of 3-hydroxypropyldimethylvinylsilane (3-HPMVS) and 3-hydroxypropyltrivinylsilane (3-HPTVS)

3-HPMVS and 3-HPTVS were prepared utilizing a modified procedure originally developed by Vadala et al.^{11, 17} The procedure described herein that was utilized to prepare 3-HPMVS can be applied to the synthesis of 3-HPTVS with appropriate substitution of the trivinylsilane as the starting reagent. 3-Chloropropyldimethylchlorosilane (10.0 g, 0.06 mol) was syringed into a clean, flame-dried, two-neck, round-bottom flask equipped with a stir bar under a N₂ purge. The reaction flask was placed in an ice bath and cooled to 0 °C. A 1.0 M solution of vinylmagnesium bromide (64.0 mL, 0.064 mol) in THF was slowly added to the flask over 30 min. The flask was allowed to warm to room temperature, and the mixture was stirred for 24 h. The reaction mixture was diluted with dichloromethane (100 mL), transferred to a separatory funnel and washed with a saturated aqueous ammonium chloride solution (150 mL), then the organic layer was further washed with aqueous sodium chloride (3 X 150 mL). Magnesium sulfate was added to the organic layer to remove any residual water, followed by vacuum filtration. Dichloromethane was removed under vacuum and the product was distilled at 100 °C, 0.8 Torr, yielding 3-chloropropyldimethylvinylsilane (8.91 g, 0.55 mol, 94% yield) (3-CPMVS). ¹H NMR was used to confirm the quantitative addition of vinyl groups.

3-CPMVS (8.91 g, 0.55 mol) was placed in a 250-mL round-bottom flask equipped with a stir bar and condenser. In a separate round-bottom flask, sodium iodide (16.4 g, 0.11 mol) was dissolved in acetone (60 mL) and the solution was syringed into the flask. The mixture was heated at 56 °C for 24 h. Acetone was removed under vacuum and the product was dissolved in dichloromethane (100 mL) and vacuum filtered to remove the salt by-products. Dichloromethane was removed under vacuum and the product was distilled at 100 °C, 0.8 Torr, yielding 3-iodopropyldimethylvinylsilane (3-IPMVS, 13.4 g, 0.05 mol). ¹H NMR confirmed the expected structure.

3-IPMVS (13.4 g, 0.05 mol) was placed in a 250-mL round-bottom flask equipped with a stir bar and condenser. DMF (20 mL) was added to the reaction flask followed by sodium bicarbonate (8.8 g, 0.10 mol) and DI water (5 mL). The mixture was heated to

100 °C for 24 h and conversion of the alkyl iodide to an alcohol was monitored via ^1H NMR. The reaction mixture was transferred to a separatory funnel and washed 3X with DI water to remove the excess sodium bicarbonate and DMF. The product was fractionally distilled at 90 °C, 0.8 Torr, yielding 3-hydroxypropyldimethylvinylsilane (3-HPMVS, 7.4 g, 0.048 mol, 95% yield). ^1H NMR confirmed the expected chemical structure.

5.3.3 Synthesis of dimethylvinylsilyl-functional PEO-OH (dimethylvinylsilyl-PEO-OH)

A representative procedure for synthesizing a dimethylvinylsilyl-PEO-OH is provided. An 8,300 g mol⁻¹ M_n PEO oligomer was initiated with 3-HPMVS. A 300-mL, high-pressure Series 4561 Parr reactor was utilized for the polymerizations. EO (10.0 g, 0.23 mol) was distilled from a lecture bottle into the pressure reactor cooled with an isopropanol-dry ice bath. THF (5 mL) was added to the reactor via syringe. A potassium naphthalide solution was prepared by charging naphthalene (14.1 g, 0.11 mol) into a 250-mL, flame-dried, round-bottom flask equipped with a glass stir bar. Dry THF (100 mL) was syringed into the flask to dissolve the naphthalene. Potassium metal (3.96 g, 0.10 mol) was added to the solution followed by a N_2 purge for 30 min. The solution was stirred overnight and titrated with 1 N HCl to determine the molarity of the potassium naphthalide solution, which was shown to be 0.95 M. An initiator solution consisting of 3-HPMVS (0.19 g, 1.29 mmol), THF (5 mL) and potassium naphthalide (1.26 mL of a 0.95 M solution in THF, 1.20 mmol) was prepared in a separate flame-dried, 100-mL, round-bottom flask. The initiator solution was added to the stirring reaction mixture via syringe. The cooling bath was removed, and the reaction mixture was allowed to reach room temperature and maintained for 24 h. The polymerization was terminated by adding acetic acid (0.66 mL of a 2.5 M solution in THF, 1.65 mmol) to the pressure reactor via syringe. The pressure reactor was purged with N_2 for 1 h, then opened and its contents were transferred to a 250-mL round-bottom flask. The solvent was removed under vacuum at room temperature, and the product was dissolved in 200 mL of dichloromethane. The product was washed twice with DI water (2 X 100 mL). The solution was concentrated under vacuum at room temperature and precipitated in cold diethyl ether.

5.3.4 Synthesis of trivinylsilyl-functional PEO-OH (trivinylsilyl-PEO-OH)

An exemplary procedure for synthesizing a trivinylsilyl-PEO-OH oligomer is provided. A $9,100 \text{ g mol}^{-1}$ M_n PEO oligomer was initiated with 3-HPTVS in a 300-mL, high-pressure Series 4561 Parr reactor. EO (10.0 g, 0.23 mol) was distilled from a lecture bottle into the pressure reactor cooled with an isopropanol-dry ice bath (-30°C), followed by addition of THF (5 mL) via syringe. A potassium naphthalide solution was prepared by charging naphthalene (14.1 g, 0.11 mol) into a 250-mL, flame-dried, round-bottom flask equipped with a glass stir bar. Distilled THF (100 mL) was syringed into the flask to dissolve the naphthalene. Potassium metal (3.96 g, 0.10 mol) was added to the solution followed by a N_2 purge for 30 min. The solution was stirred overnight and titrated with 1 N HCl to determine the molarity of the potassium naphthalide solution, which was shown to be 0.97 M. An initiator solution consisting of 3-HPTVS (0.19 g, 1.12 mmol), THF (5 mL) and potassium naphthalide (1.03 mL of a 0.97 M solution in THF, 1.0 mmol) was prepared in a separate flame-dried, 100-mL, round-bottom flask. The initiator solution was added to the stirring pressure reactor mixture via syringe. The dry-ice/isopropanol bath was removed, and the reaction mixture was allowed to reach room temperature and maintained for 24 h. The polymerization was terminated by adding acetic acid (0.66 mL of a 2.5 M solution in THF, 1.65 mmol) to the pressure reactor via syringe. The pressure reactor was purged with N_2 for 1 h, then opened and its contents were transferred to a 250-mL round-bottom flask. The solvent was removed under vacuum at room temperature, and the product was dissolved in 200 mL of dichloromethane and washed twice with DI water (2 X 100 mL). The solution was concentrated under vacuum at room temperature and precipitated in cold diethyl ether.

5.3.5 Functionalization of dimethylvinylsilyl-PEO-OH with carboxylic acid groups

An exemplary procedure for adding a carboxylic acid group via ene-thiol free radical chemistry across the vinylsilyl endgroup is provided for an $8,300 \text{ g mol}^{-1}$ dimethylvinylsilyl-PEO-OH. Dimethylvinylsilyl-PEO-OH (1.0 g, 0.12 mmol) was charged to a 100-mL round-bottom flask equipped with a stir bar and dissolved in 2 mL of deoxygenated toluene. Mercaptoacetic acid (37.0 mg, 0.36 mmol) was syringed into

the reaction flask followed by the addition of AIBN (9.2 mg, 0.06 mmol) dissolved in 0.5 mL of toluene. The mixture was deoxygenated for 10 min by sparging with N₂, then reacted at 80 °C for 24 h. The reaction mixture was dissolved in 200 mL of dichloromethane, then transferred to a separatory funnel and washed with DI water 3X to remove the excess mercaptoacetic acid. The dichloromethane was removed via rotary evaporation and the resulting polymer was precipitated into cold diethyl ether. The polymer was dried at room temperature under vacuum for 24 h yielding 0.94 g of carboxylic acid-functionalized PEO.

5.3.6 Functionalization of dimethylvinylsilyl-PEO-OH or trivinylsilyl-PEO-OH with ammonium group(s)

Heterobifunctional polyethers with a terminal ammonium group (ammonium-PEO-OH) were obtained via ene-thiol addition of mercaptoethylamine hydrochloride across the vinylsilane. In a characteristic procedure, an 8,300 g mol⁻¹ dimethylvinylsilyl-PEO-OH (2 g, 0.24 mmol), mercaptoethylamine hydrochloride (45.2 mg, 0.4 mmol), and AIBN (20 mg, 0.12 mmol) were dissolved in deoxygenated DMF (5 mL) in a 100-mL round-bottom flask equipped with a stir bar. The reaction was conducted at 70 °C for 24 h with stirring, then the reaction mixture was cooled to room temperature. DI water (100 mL) was added to the flask, and the mixture was transferred to a separatory funnel. Dichloromethane (200 mL) was added to the separatory funnel to extract the alkylammonium-functionalized polyether from the water layer. The dichloromethane layer was washed with a 1 N solution of sodium bicarbonate (3X), followed by 3 washes with DI water. The dichloromethane solution was concentrated under vacuum, and the ammonium-PEO-OH oligomer was precipitated into cold diethyl ether and dried at 25 °C under vacuum for 12 h yielding 1.9 g of product (95% yield).

A PEO oligomer with three ammonium groups on one end (ammonium₃-PEO-OH) was prepared by a similar procedure using trivinylsilyl-PEO-OH as the starting substrate, and the same molar ratios of reagents to equivalents of vinyl groups as described above.

5.3.7 Michael addition of ammonium-PEO-OH or ammonium₃-PEO-OH onto diethyl vinyl phosphonate

A characteristic procedure for adding a phosphonate group to an 8,300 g mol⁻¹ ammonium-PEO-OH is provided. An ammonium-PEO-OH oligomer (1.0 g, 0.12 mmol) was charged to a clean, flame-dried, 100-mL, round-bottom flask equipped with a stir bar, and dissolved in ethanol (9 mL). TEA (0.20 mL, 0.14 mmol) was added to the reaction, followed by diethyl vinyl phosphonate (0.26 mL, 0.14 mmol). The reaction was carried out at 70 °C for 24 h. The reaction mixture was diluted with DI water to obtain a 75:25 water:ethanol composition and placed in a 1,000 g mol⁻¹ MWCO cellulose acetate dialysis bag and dialyzed against 4 L of DI water for 24 h to remove excess diethyl vinyl phosphonate. The contents of the dialysis bag were transferred to a 100-mL round-bottom flask and lyophilized, yielding 0.91 g of diethyl phosphonate-functionalized PEO.

A PEO oligomer with three phosphonate groups on one end (phosphonate₃-PEO-OH) was prepared by a similar procedure using ammonium₃-PEO-OH as the starting substrate, and the same molar ratios of reagents to equivalents of ammonium groups as described above.

5.3.8 Hydrolysis of diethylphosphonate-PEO-OH (or diethylphosphonate₃-PEO-OH) yielding phosphonic acid-PEO-OH (or phosphonic acid₃-PEO-OH)

Phosphonic acid-PEO-OH was prepared from diethyl phosphonate-PEO-OH using a hydrolysis procedure adapted from Caplan et al.²⁸ In a representative procedure, an 8,300 g mol⁻¹ diethyl phosphonate-PEO-OH (0.80 g, 0.10 mmol) was charged to a clean, 100-mL round-bottom flask equipped with a stir bar and dissolved in 5 mL of dichloromethane. Trimethylsilyl bromide (0.032 mL, 0.24 mmol) was syringed into the reaction flask and stirred at room temperature for 24 h. Methanol (0.01 mL, 0.24 mmol) was added and stirred for 2 h to cleave the trimethylsilyl groups. Dichloromethane (50 mL) was added, and the mixture was washed 3X with DI water (100 mL each) in a separatory funnel. The dichloromethane layer was concentrated, then the oligomer was precipitated by pouring the mixture into cold diethyl ether. The polymer was vacuum-dried at 25 °C for 24 h yielding 0.77 g of phosphonic acid-PEO-OH.

A PEO oligomer with three phosphonate groups on one end (phosphonate₃-PEO-OH) was prepared by a similar procedure using diethylphosphonate₃-PEO-OH as the starting substrate, and the same molar ratios of reagents to equivalents of phosphonate groups as described above.

5.3.9 Magnetite synthesis via reduction of Fe(acac)₃

Magnetite nanoparticles were synthesized using a reduction method adapted from Pinna et al.²⁹ Fe (III) acetylacetonate (2.14 g, 8.4 mmol) and benzyl alcohol (45 mL, 0.43 mol) were charged to a 250-mL, three-neck, round-bottom flask equipped with a water condenser and placed in a Belmont metal bath with an overhead stirrer with both thermostatic (+/- 1 °C) and revolution per minute control. The solution was sparged with N₂ for 1 h. While stirring under N₂, the solution was heated at 100 °C for 4 h, then the temperature was increased to 205 °C at a rate of ~25 °C h⁻¹. Following 24 h at 205 °C, the reaction was cooled to room temperature, then the magnetite particles were collected with a magnet and the benzyl alcohol was decanted. The magnetite nanoparticles were washed 3X with acetone (100 mL each), then were dispersed in chloroform (20 mL) containing oleic acid (0.3 g). The solvent was removed under vacuum at room temperature, and the oleic acid-stabilized magnetite nanoparticles were washed 3X with acetone (100 mL each). The particles were dried under vacuum for 24 h at 25 °C. The composition of the particles obtained from thermogravimetric analysis (TGA) was 5% organic residue to 95% magnetite.

5.3.10 Adsorption of functional polyether stabilizers onto magnetite nanoparticles

A representative method for preparing a targeted composition of 70:30 wt:wt polyether:magnetite complex is provided. Oleic acid-stabilized magnetite nanoparticles (50.0 mg) prepared as described above were dispersed in chloroform (10 mL) and charged to a 50-mL round-bottom flask. A functional PEO (117.0 mg) was dissolved in chloroform (10 mL) and added to the dispersion. The pH of each solution and the resulting mixture were approximately neutral. The reaction mixture was sonicated in a VWR 75T sonicator for 16 h under N₂, and then the nanoparticles were precipitated in hexanes (300 mL). A magnet was utilized to collect the magnetite nanoparticles and free oleic acid was decanted with the supernatant. The complexes were dispersed in DI water

(20 mL) using sonication for 30-60 s. The complexes were dialyzed against DI water (4 L) for 24 h using 25,000 g mol⁻¹ MWCO dialysis bags.

5.3.11 Characterization

¹H NMR spectral analyses of compounds were performed using a Varian Unity 400 NMR or a Varian Inova 400 NMR operating at 399.97 MHz.

An Alliance Waters 2690 Separations Module with a Viscotek T60A dual viscosity detector and laser refractometer equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel column set was used for gel permeation chromatography (GPC) analyses. GPC data were collected in chloroform at 30 °C. Data were analyzed utilizing a Universal calibration made with polystyrene standards to obtain absolute molecular weights.

TGA was used to determine the polymer loading for each complex. TGA measurements were carried out on the PEO-magnetite nanoparticles using a TA Instruments TGA Q500 to determine the fraction of each complex that was comprised of polymer. Freeze-dried samples were first held at 110 °C for 10 min to drive off any excess moisture. The samples (10-15 mg) were then equilibrated at 100 °C and the temperature was ramped at 10 °C min⁻¹ to a maximum of 700 °C in a nitrogen atmosphere. The mass remaining was recorded throughout the experiment. The mass remaining at 700 °C was taken as the fraction of magnetite in the complexes. The experiments were conducted in triplicate.

DLS measurements were conducted with a Malvern Zetasizer NanoZS particle analyzer (Malvern Instruments Ltd, Malvern, UK) at a wavelength of 633 nm from a 4.0 mW, solid-state He-Ne laser at a scattering angle of 173° and at 25 ± 0.1 °C. Intensity, volume and number average diameters were calculated with the Zetasizer Nano 4.2 software utilizing an algorithm, based upon Mie theory, that transforms time-varying intensities to particle diameters.³⁰ For DLS analysis, the dialyzed complexes dispersed in DI water were diluted to ~0.05 mg mL⁻¹ and filtered through a Whatman Anotop, 100-nm, alumina filter directly into a polystyrene cuvette. This corresponds to a volume fraction of 1.3 × 10⁻⁵ to 2.2 × 10⁻⁵ depending on the polymer loading on the magnetite. In experiments where PBS was added, 0.1 mL of 10X PBS was mixed with 0.9 mL of the 0.05 mg mL⁻¹

complex solution, then the solution was filtered through a 100-nm alumina filter into a clean polystyrene cuvette. Each sample was analyzed immediately following filtration and re-measured every 30 min over 24 h.

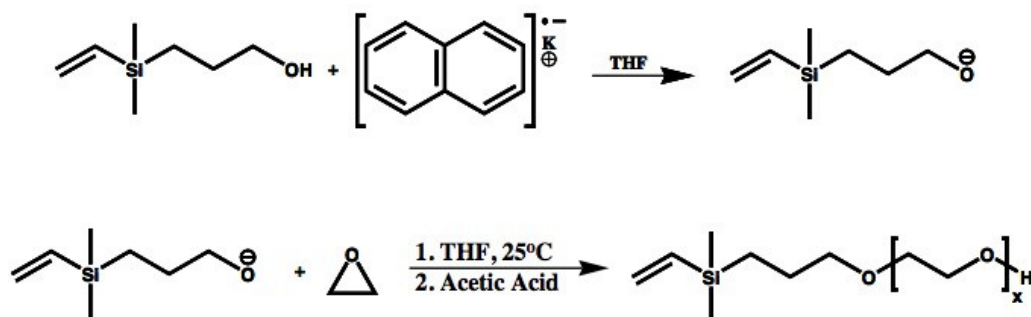
A 7T MPMS SQUID magnetometer (Quantum Design) was used to determine magnetic properties. Hysteresis loops were generated for the magnetite nanoparticles at 300K and 5K. Fe concentration of the magnetite was determined chemically by acid digestion followed by ICP-AES analysis. These concentrations were in good agreement with TGA values.

5.4 Results and Discussion

One primary issue regarding the structures of polymer-magnetite nanostructures for biomedical applications is the colloidal stability of dispersions of such complexes in physiological media.^{9, 10, 21} The aim of this study has been to compare the binding efficacies of PEO oligomers with different functional anchoring groups to magnetite, particularly in the presence of phosphate salts found in physiological media. PEO oligomers with one terminal carboxylate, ammonium or a phosphonate zwitterion, and with three phosphonate zwitterions (all on one end of the PEO) were adsorbed onto magnetite nanoparticles, and the stabilities of the complexes were compared. Knowledge of which binding groups remained stably bound to the magnetite in water and in PBS then allowed us to examine the colloidal properties of the stable nanoparticles in dispersions in light of the molecular weights and chain densities of the bound oligomers on the magnetite surfaces.

5.4.1 Synthesis of PEO oligomers with different functional endgroups for binding to magnetite

3-HPMVS was used as a versatile initiator for living anionic polymerizations of EO to produce heterobifunctional oligomers that could be post-functionalized with the different chemical groups to adsorb onto the magnetite nanoparticles (Scheme 5.1).^{17, 25}



Scheme 5.1. Synthesis of dimethylvinylsilyl-PEO-OH via living anionic polymerization

This approach allowed for utilizing the same oligomer for comparing dispersion properties with the only difference being the functional anchor groups. A variation of this approach using 3-HPTVS as the initiator was also investigated to prepare phosphonic acid₃-PEO-OH oligomers, so that the dispersion properties could be compared with nanoparticles having different surface densities of functional binding groups. Potassium naphthalide was reacted with 3-HPMVS or 3-HPTVS to form an alkoxide for initiating EO, and the number of moles of the alcohol initiator relative to EO controlled the molecular weight. A small deficiency of the naphthalide relative to alcohol ensured that only the alkoxide (and not residual naphthalide) initiated the chains, and the remaining alcohol chains transferred with the growing PEO chains throughout the reaction. It is noteworthy that these polymerizations were terminated with acetic acid prior to opening the reaction vessel to avoid any unwanted oxidative side reactions.

Dimethylvinylsilyl-PEO-OH oligomers with targeted molecular weights of approximately 3,000 and 8,000 g mol⁻¹ were prepared and the materials were characterized using ¹H NMR and GPC (Table 5.1).

Table 5.1. Molecular weights of dimethylvinylsilyl-PEO-OH and trivinylsilyl-PEO-OH oligomers

Targeted Molecular Weight	M _n (g mol ⁻¹)		PDI [*]
	¹ H NMR	GPC	
Monovinylsilyl-3,000 g mol ⁻¹	2,900	3,100	1.09
Monovinylsilyl-8,000 g mol ⁻¹	8,300	7,900	1.05
Trivinylsilyl-3,000 g mol ⁻¹	3,400	3,400	1.04
Trivinylsilyl-9,000 g mol ⁻¹	9,100	9,500	1.09

*PDI = Polydispersity Index

Figure 5.1 shows a representative NMR spectrum of a dimethylvinylsilyl-PEO-OH oligomer.

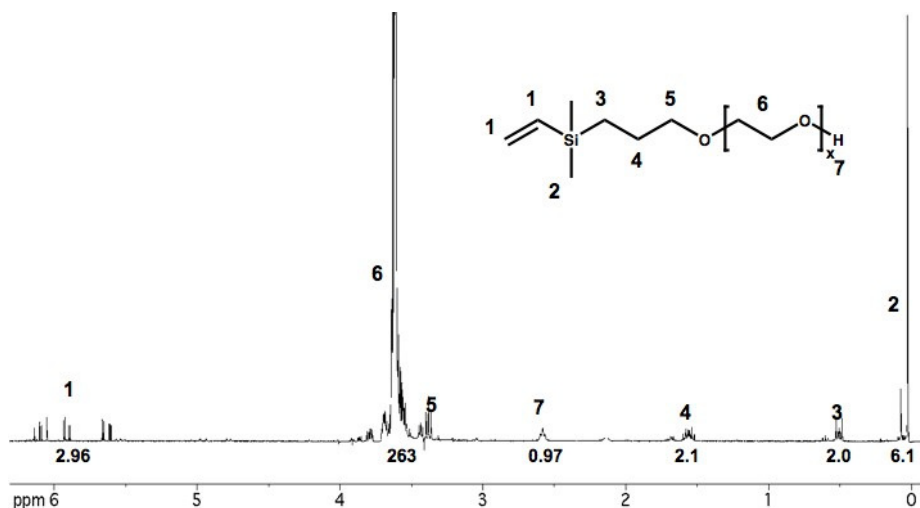


Figure 5.1. ^1H NMR of a $2,900 \text{ g mol}^{-1}$ dimethylvinylsilyl-PEO-OH oligomer

Number average molecular weight was determined by comparing the integral ratios of the resonances corresponding to the methylene groups in the initiator (labeled 3 and 4) to the repeat unit of ethylene oxide labeled 6.

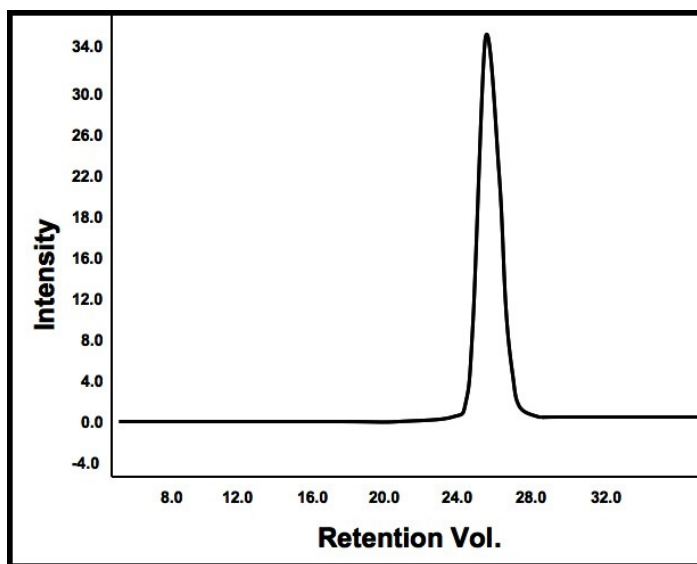
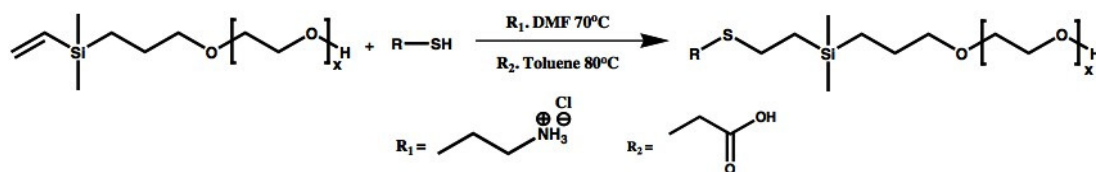


Figure 5.2. GPC chromatogram of a dimethylvinylsilyl-PEO-OH oligomer showing a M_n of $7,900 \text{ g mol}^{-1}$ and a molecular weight distribution of 1.05

GPC of the dimethylvinylsilyl-functional polyethers revealed symmetric monomodal peaks with molecular weight distributions of less than 1.1, which are indicative of living anionic polymerizations (Figure 5.2). Good molecular weight agreement was found between both methods of analysis.

Ene-thiol additions were utilized to introduce carboxylate and ammonium endgroups onto the polyethers (Scheme 5.2).



Scheme 5.2. Ene-thiol additions of 2-mercaptoethylamine hydrochloride (R_1) and mercaptoacetic acid (R_2) to dimethylvinylsilyl-PEO-OH

The vinylsilyl functionality is unusual among vinyl groups in that it does not polymerize readily by free radical reactions, and thus it is seemingly an ideal substrate for ene-thiol functionalization reactions because polymerization does not compete. Nevertheless, excesses of the thiol relative to vinylsilane were utilized for these reactions to ensure quantitative addition.

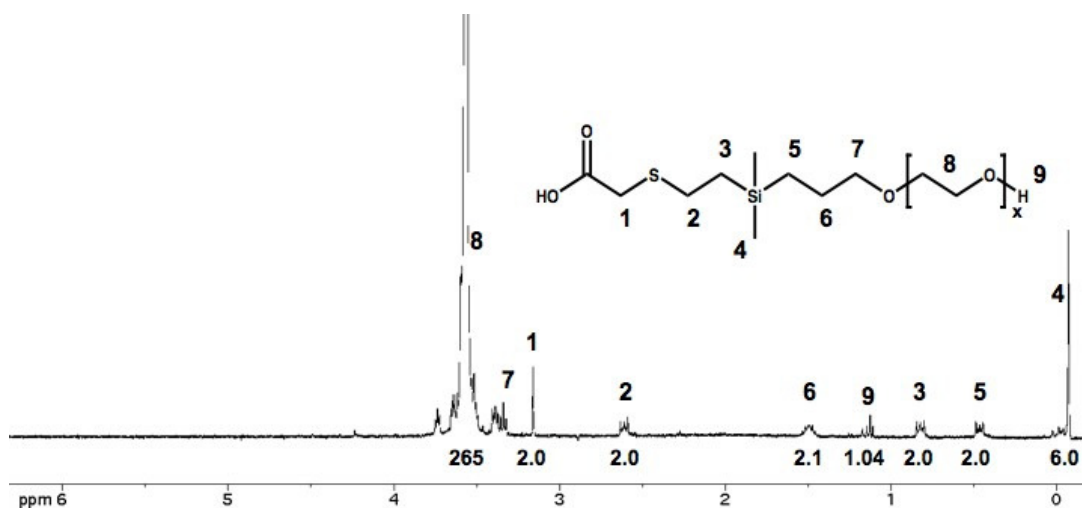


Figure 5.3. Ene-thiol addition of mercaptoacetic acid to a $2,900 \text{ g mol}^{-1}$ polyether yielding a carboxylic acid-functionalized PEO (HOOC-PEO-OH)

Addition of mercaptoacetic acid to dimethylvinylsilyl-PEO-OH was monitored by ^1H NMR. Complete disappearance of the vinyl group at ~ 6 ppm indicated stoichiometric conversion to a carboxylic acid-PEO-OH (Figure 5.3).

Additionally, appearances of the methylene peaks labeled 1-3 in the spectrum indicated addition of mercaptoacetic acid. Comparison of the resonance integrals corresponding to the methylene peaks of the thiol (1-3) to the methylene peak labeled 5 confirmed quantitative addition across the vinylsilane endgroups.

^1H NMR was also utilized to monitor ene-thiol addition of 2-mercaptoethylamine hydrochloride onto the vinylsilane endgroups of dimethylvinylsilyl-PEO-OH and trivinylsilyl-PEO-OH by observing the complete disappearance of the vinyl groups and appearances of methylene peaks labeled 1-4 in the spectra (Figure 5.4). Comparison of integrations confirmed quantitative functionalization with ammonium groups to form ammonium-PEO-OH or ammonium₃-PEO-OH.

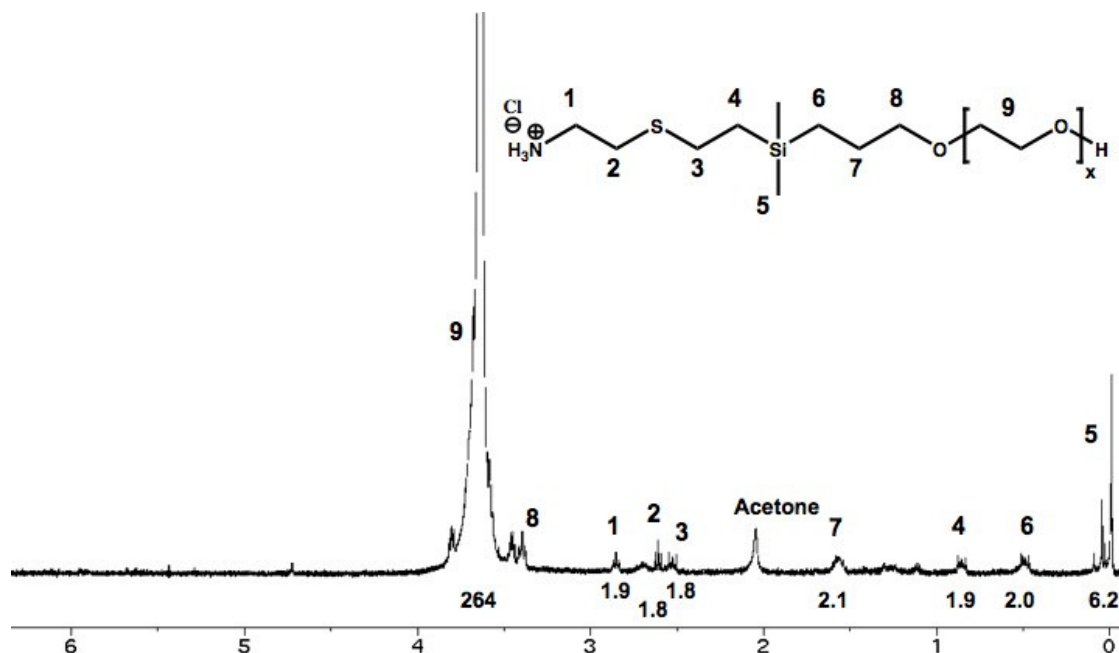
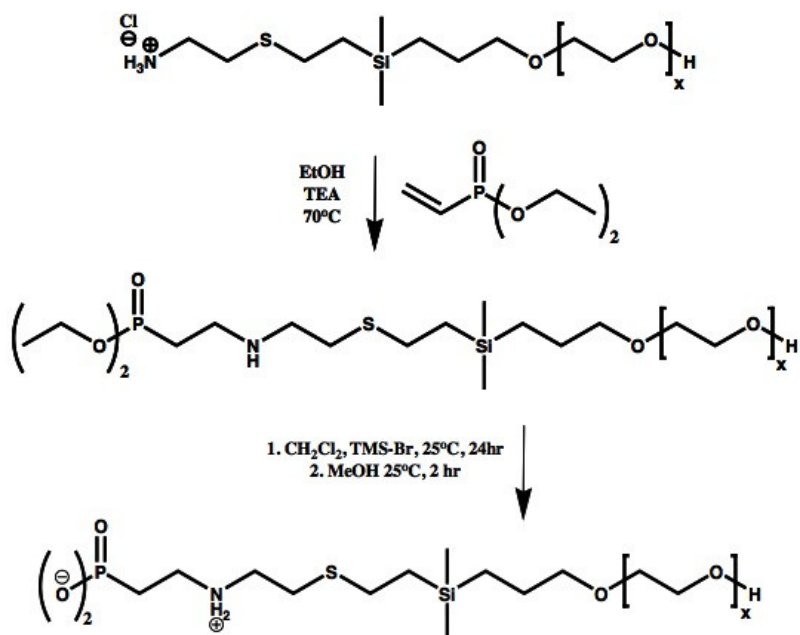


Figure 5.4. Ene-thiol addition of mercaptoethylamine hydrochloride to a 2,900 g mol⁻¹ dimethylvinylsilyl-PEO-OH to produce ammonium-PEO-OH

The ammonium-functional PEO oligomers were used as precursors to form the zwitterionic phosphonate-functional polyethers. The ammonium endgroups were first reacted with triethylamine to afford the corresponding free amines. A slight molar excess of triethylamine was added to maintain basic reaction conditions, aiding in nucleophilic addition to diethyl vinyl phosphonate. Diethyl vinyl phosphonate was added to the amine group(s) via Michael addition, yielding diethylphosphonate-PEO-OH (Scheme 5.3) or diethylphosphonate₃-PEO-OH utilizing a procedure adapted from the literature.³¹



Scheme 5.3. Michael addition of amine-PEO-OH to diethyl vinyl phosphonate and de-esterification to remove the ethyl groups yielding zwitterionic phosphonate-PEO-OH

Figure 5.5 shows the ^1H NMR of a representative diethylphosphonate-PEO-OH oligomer.

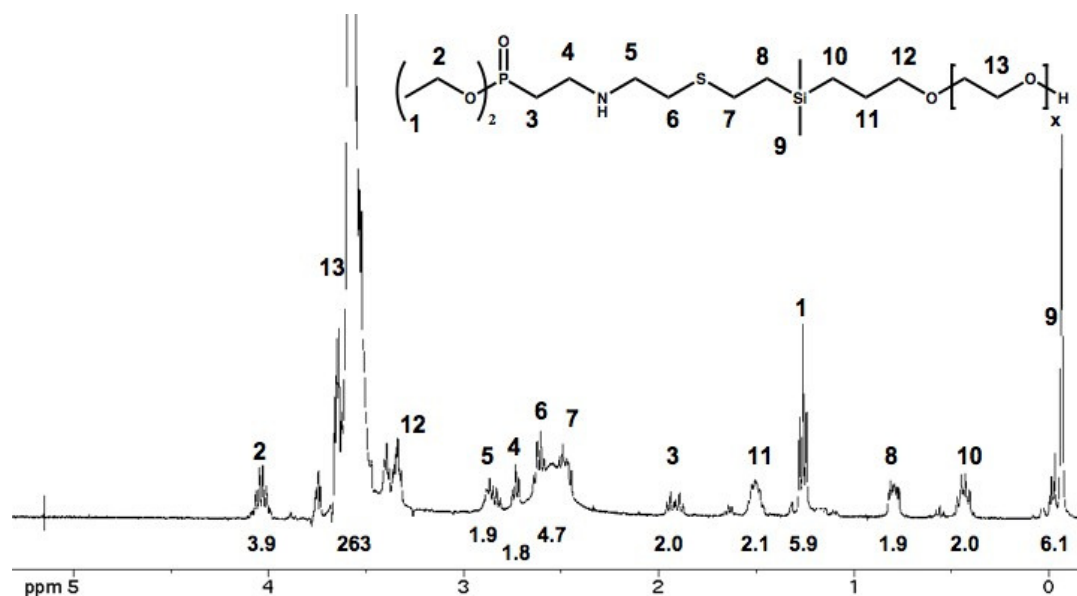


Figure 5.5. ^1H NMR of a $2,900\text{ g mol}^{-1}$ diethylphosphonate-PEO-OH

Two methylene peaks corresponding to the converted vinyl group of the phosphonate reactant are observed as peaks 3 and 4 in the spectrum of the product. Quantitative addition to diethyl vinyl phosphonate was determined by comparing the integrations of peaks 1-4 to the integration of the methylene peak labeled 10.

For binding to magnetite, the ethyl phosphonate groups were cleaved using bromotrimethylsilane (TMS-Br).²⁸ Reaction with TMS-Br yielded an intermediate bis-trimethylsilyl ester with an alkyl halide by-product. In the methanolysis step of the reaction, the alcohol cleaved the silyl ester, yielding phosphonic acid (Scheme 5.3). A zwitterionic endgroup was afforded in the phosphonate-PEO-OH product (or in the corresponding phosphonate₃-PEO-OH) at a pH less than 10, due to the negative charge of the phosphonate and the protonated secondary amine. ^1H NMR spectra confirmed that deprotection of the diethylphosphonate-PEO-OH oligomers was complete (Figure 5.6).

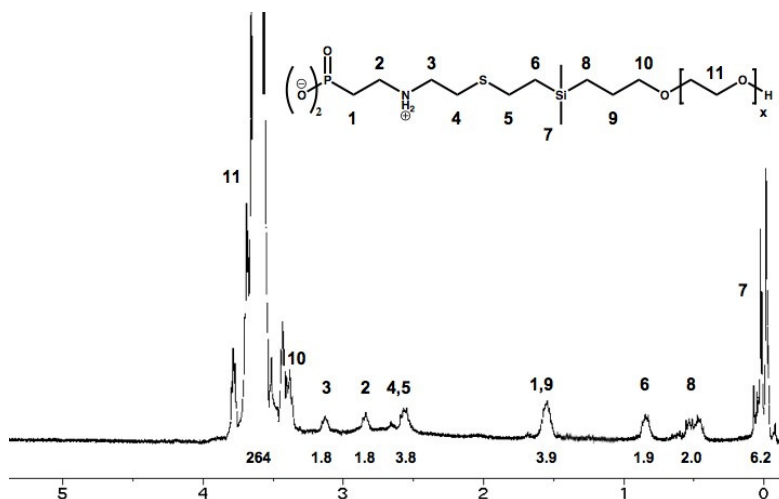


Figure 5.6. ^1H NMR of a $2,900 \text{ g mol}^{-1}$ zwitterionic phosphonate-PEO-OH

In addition to the disappearance of the ethyl resonances in the spectrum, there was an upfield shift ($\sim 1.6 \text{ ppm}$) of the methylene group adjacent to the phosphorus atom.

5.4.2 Formation of PEO-magnetite complexes via adsorption of the functional PEO oligomers onto the surfaces of magnetite nanoparticle

PEO-magnetite complexes with similar polymer loadings were formed with the two sets of different molecular weight PEO oligomers, then the complexes were dialyzed against water to remove any unbound PEO (Figure 5.7).

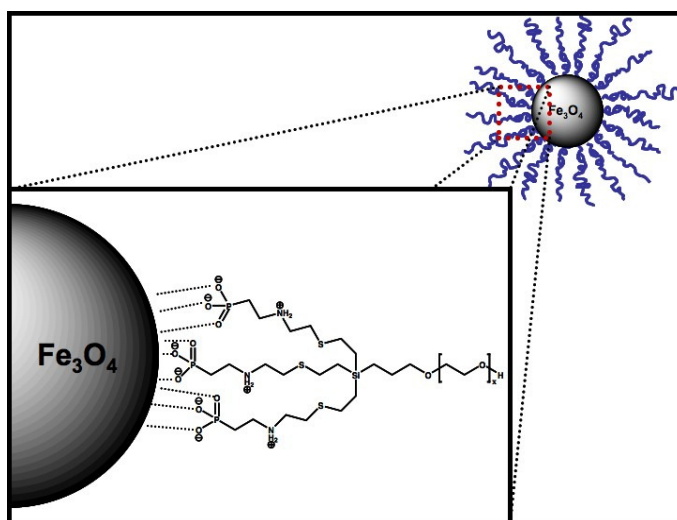


Figure 5.7. Depiction of a tri-zwitterionic phosphonate-PEO-OH binding to the surface of a magnetite nanoparticle

Table 5.2 shows the polymer loadings in the complexes determined by TGA after dialysis and the number weighted average diameters in water as measured by dynamic light scattering (DLS).

Table 5.2. Compositions of the PEO-magnetite nanoparticle complexes

PEO Molecular Weight (g mol ⁻¹)	Anchor Group(s)	Initial Polymer Loading after Dialysis against H ₂ O (Wt %)	D _n (nm)	Polymer Loading after Dialysis against PBS (Wt %)
2,900	Mono-Carboxylate	64.7 ± 0.9	19.9	40.7 ± 1.4
8,300	Mono-Carboxylate	68.4 ± 1.6	24.5	45.3 ± 1.4
2,900	Mono-Ammonium	66.8 ± 0.8	26.8	41.0 ± 0.7
8,300	Mono-Ammonium	77.1 ± 0.9	31.7	42.6 ± 0.7
2,900	Mono-Zwitterionic Phosphonate	63.3 ± 0.4	26.2	65.1 ± 1.4
8,300	Mono-Zwitterionic Phosphonate	70.2 ± 0.7	39.6	67.7 ± 0.7
3,400	Tri-Zwitterionic Phosphonate	69.0 ± 0.6	27.6	68.8 ± 0.4
9,100	Tri-Zwitterionic Phosphonate	70.5 ± 0.8	34.2	69.2 ± 0.8

The compositions of the PEO-magnetite complexes were in close agreement with the targeted 30 wt% magnetite:70 wt% polyether composition. The PEO-magnetite complexes with the ammonium and zwitterionic phosphonate anchor groups had approximately the same number weighted average diameters, while the complexes with the carboxylate anchor groups were significantly smaller. The size of the magnetite nanoparticle core was measured via TEM with an average diameter of 8.1 ± 2.9 nm (Figure 5.8).

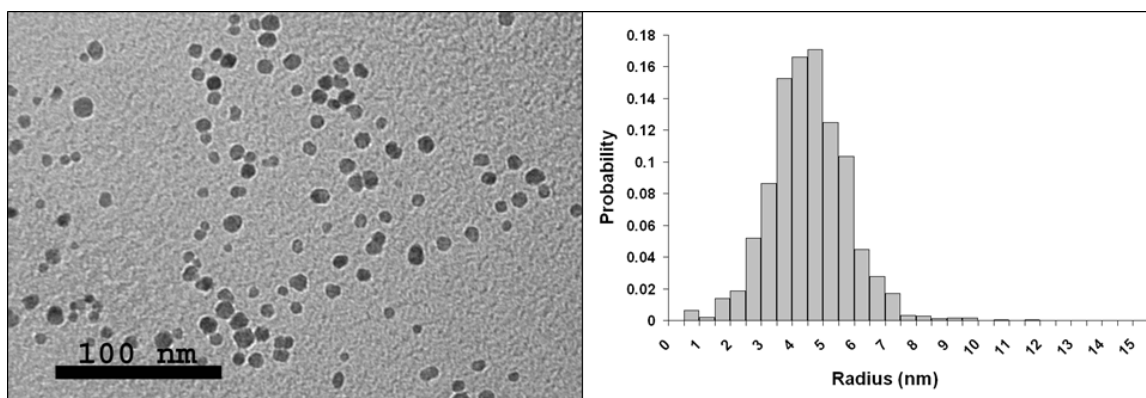


Figure 5.8. TEM of the magnetite nanoparticles (A) and a histogram of the particle size distribution (B).

The magnetic properties of the magnetite nanoparticles were characterized via superconducting quantum interference device analyses. Hysteresis loops revealed superparamagnetic behavior and a saturation magnetization of 80 emu g^{-1} of magnetite.

5.4.3 Determination of the efficacies of various anchor groups for magnetite nanoparticles in PBS

Binding of the polymers to the magnetite surfaces in PBS were studied to compare the stabilities of these complexes in a medium simulating physiological conditions. Dispersions of the complexes were dialyzed against PBS for 24 hours to measure any polymer desorption that might be caused by displacement by salts in the medium. The complexes were subsequently dialyzed against water for an additional 24 hours to remove any desorbed polymer and salts. TGA was used to determine the polymer loadings of the complexes before and after this process to investigate anchor group efficacies in the presence of phosphate salts in the medium (Table 5.2). Desorption of the polymer from the magnetite surface in PBS was indicated by a decrease in polymer loading. Large decreases in polymer loadings ($\sim 25 \text{ wt\%}$) were observed for the complexes bound through carboxylate or ammonium anchor groups. In addition, significant sedimentation of these complexes was observed during the PBS dialysis step, further substantiating instability in PBS. In contrast, no loss in polymer loading was observed for the complexes containing mono-zwitterionic phosphonate anchor groups or tri-zwitterionic phosphonate anchor groups after PBS dialysis. These complexes also did not exhibit sedimentation in the PBS during the 24 hours. Based on this study, it was reasoned that

PEO-magnetite nanoparticles with the zwitterionic phosphonate anchor groups would likely have superior stability in physiological media in comparison to ammonium or carboxylate groups.

5.4.4 Investigation of colloidal stabilities of the PEO-magnetite nanoparticle dispersions in water and PBS using DLS

Colloidal stabilities of the PEO-magnetite complexes against flocculation in DI water and PBS were examined using DLS by measuring the nanoparticle diameters every 30 minutes for 24 hours. Figure 5.9 shows the diameters of the complexes containing the PEO oligomers with the mono-ammonium anchor group.

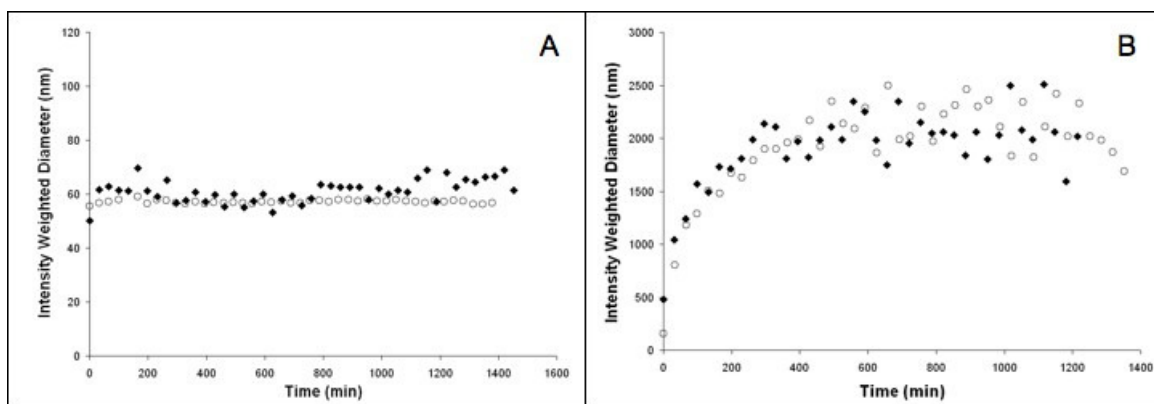


Figure 5.9. Intensity weighted diameters from DLS of the 2,900 (open circles) and 8,300 g mol⁻¹ (black diamonds) ammonium-PEO-magnetite complexes in DI water (A) and PBS (B) over 24 hours

Intensity weighted diameters are reported due to their sensitivity to the presence of aggregates (since they scale with radii to the 6th power), so that any agglomeration with time is magnified. Consistent with the desorption data, sizes of the ammonium-PEO-magnetite complexes did not change significantly over 24 hours in water, while large increases in the diameters were observed for the complexes in PBS. Moreover, sediment was visually observed for the ammonium-PEO-magnetite complexes in PBS. Based on the calculation for the terminal velocity of a sphere (eq. 1) in solution, a magnetite complex should settle to the bottom of the DLS cuvette (traverse a distance of 1 cm) when an aggregate diameter of ~400-700 nm is reached.¹⁹

$$U = \frac{2}{9} \frac{r^2 g (\rho_{mag} - \rho_{solvent})}{\mu_{solvent}} \quad (5-1)$$

Here, U is the terminal velocity of the particle, g is the gravitational constant, r is the particle radius, ρ_{mag} is the density of the magnetite complex (2.28 g cm^{-3}), $\rho_{solvent}$ is the density of the solvent, and $\mu_{solvent}$ is the viscosity of the solvent at 25°C . Thus, after ~200 minutes in PBS the ammonium-PEO-magnetite complexes were likely on the verge of sedimentation and the sizes measured by DLS do not represent complexes that were at equilibrium in terms of size.

Magnetic characterization data shows that the magnetite nanoparticles display superparamagnetic behavior. The maximum potential energy of magnetic dipoles aligned with an applied magnetic field can be estimated by equation 5-2.³²

$$V_M = \frac{8\pi\mu_o a^3 M^2}{9\left(\frac{h}{a} + 2\right)^3} \quad (5-2)$$

Here, a is the particle radius, M is the magnetization, μ_o is the magnetite permeability in vacuum ($1.26 \times 10^{-6} \text{ Tm/A}$), and h is the surface-to-surface separation of two particles.(reference) With the application of the Earth's magnetic field (0.3 – 0.6 Gauss), any dipole-dipole interaction is significantly smaller than the van der Waal's interactions of the actual particle ($V_{vdW}/kT = 0.4$, $h = 5 \text{ nm}$) , even at particle sizes of 1000 nm ($V_M/kT = 2 \times 10^{-7}$, $h = 5 \text{ nm}$) where the particles are settling out of suspension. Thus, van der Waal's forces would dominate and cause flocculation before any magnetic dipole-dipole interaction would become significant.

Figure 5.10 compares the diameters of the complexes containing the carboxylate anchor groups in DI water (A) and PBS (B and C).

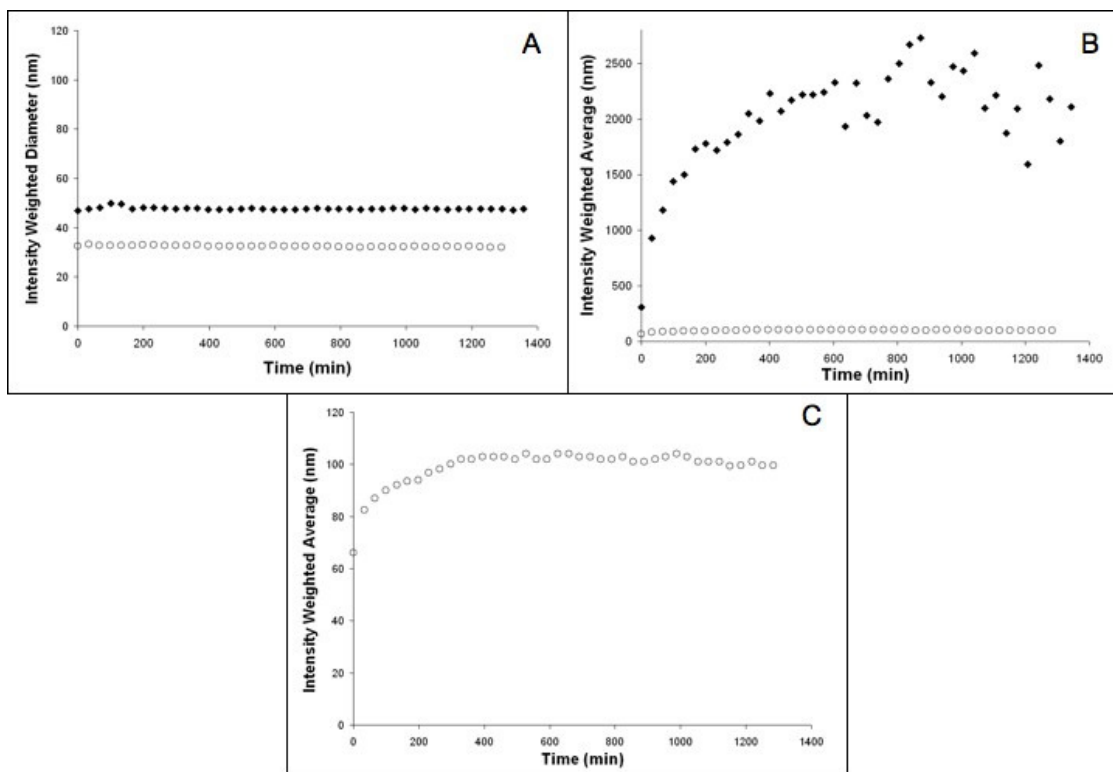


Figure 5.10. DLS intensity weighted diameters of the 2,900 (open circles) and 8,300 g mol^{-1} (black diamonds) of carboxylate-PEO-magnetite complexes in DI water (A) and PBS (B) over 24 hours. A magnified plot of the 2,900 g mol^{-1} carboxylate-PEO-magnetite complex in PBS is shown in (C).

As expected, these complexes did not increase in intensity average diameter in DI water over time. However, flocculation was observed for the 8,300 g mol^{-1} carboxylate-PEO-magnetite complex in PBS over time, and the complexes with the 2,900 g mol^{-1} PEO aggregated to ~ 100 nm in intensity weighted diameter with an equilibrium diameter reached after ~ 5 hours. Thus, even with polymer desorption, the PEO molecular weight affects the behavior of these complexes in PBS. A possible explanation for this difference is the number of polymer chains anchored to the magnetite nanoparticle surfaces in the two cases. Each complex has approximately the same polymer loading (~ 65 wt %), resulting in more total chains for the 2,900 g mol^{-1} complexes (~ 3.0 chains nm^{-2}) relative to the 8,300 g mol^{-1} complexes (~ 1.1 chains nm^{-2}). DLS data presented in Table 5.2 also shows that the complexes with carboxylate anchor groups have significantly smaller number weighted average diameters than their ammonium counterparts. The smaller diameters of the complexes with the carboxylate anchor

groups reflect a higher local density of polymer near the magnetite surface. As reflected in Figures 1.7 and 1.8, the complexes with carboxylate anchor groups also flocculate less rapidly in PBS relative to the complexes with ammonium anchors. This can be partially attributed to the higher polymer density.

The relative colloidal stability of dispersions of these complexes with ammonium or carboxylate endgroups in DI water versus PBS is consistent with the absence of polymer desorption in water and significant desorption in PBS. Based on previous work, it appears that phosphate salts from the PBS can displace these polymer anchor groups.⁹ This reduces steric repulsion and promotes flocculation of the nanoparticles due to pair-pair van der Waal's interactions. Figure 5.11 illustrates adsorption of phosphate salts on the magnetite surface.

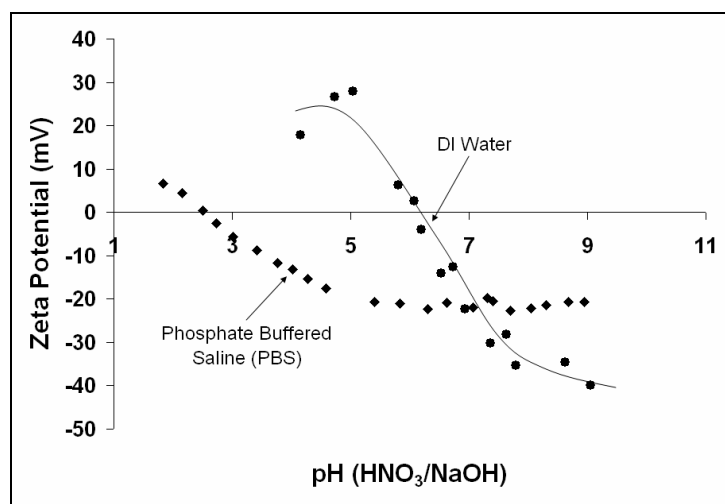


Figure 5.11. Zeta potentials indicate the charge characteristics of magnetite nanoparticle surfaces in DI water versus PBS.

In PBS, the magnetite nanoparticles cross the line of zero charge at ~pH 2.5, and this closely corresponds to the lowest pK_a of phosphonic acid. This suggests that phosphate salts from the PBS adsorb on the magnetite surfaces.

Figure 5.12 shows intensity-weighted diameters of complexes bound through the mono-zwitterionic phosphonate anchor groups in DI water (A) and PBS (B), where polymer

desorption does not occur in either medium.

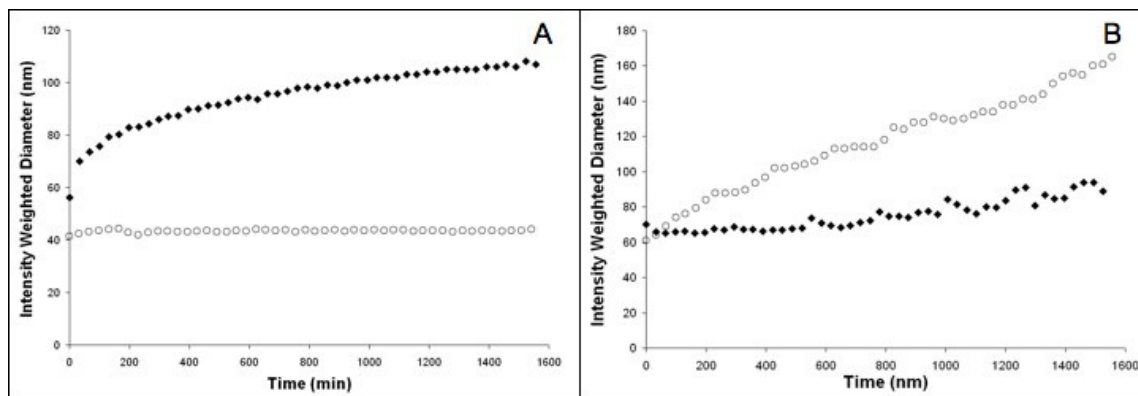


Figure 5.12. DLS intensity weighted diameters of the 2,900 (open circles) and 8,300 g mol⁻¹ (black diamonds) mono-zwitterionic phosphonate-PEO-magnetite complexes in DI water (A) and PBS (B) over 24 hours

To our surprise, while the sizes of some of these nanoparticles remained constant over time, some of them increased in size over time in both media, even without polymer desorption. The hydrodynamic sizes in PBS, however, remained significantly smaller than the analogous materials containing the carboxylate or ammonium anchors, and this result was deemed promising. While the dialysis data in Table 5.2 indicates that the polymer does not desorb, the aggregation behavior observed in PBS does seem to indicate some desorption from the magnetite surface, particularly since there is no aggregation in sodium chloride. This indicates that there may be small-scale desorption of the polymer accompanied by an attraction between phosphonate anchors of one complex with the exposed magnetite of neighboring complex. This will be explored further in Chapter 6.

Figure 5.13 compares intensity-weighted diameters of the complexes containing the PEO with one phosphonate zwitterion anchor group over time in DI water and a 0.17 M NaCl solution (same ionic strength as the PBS).

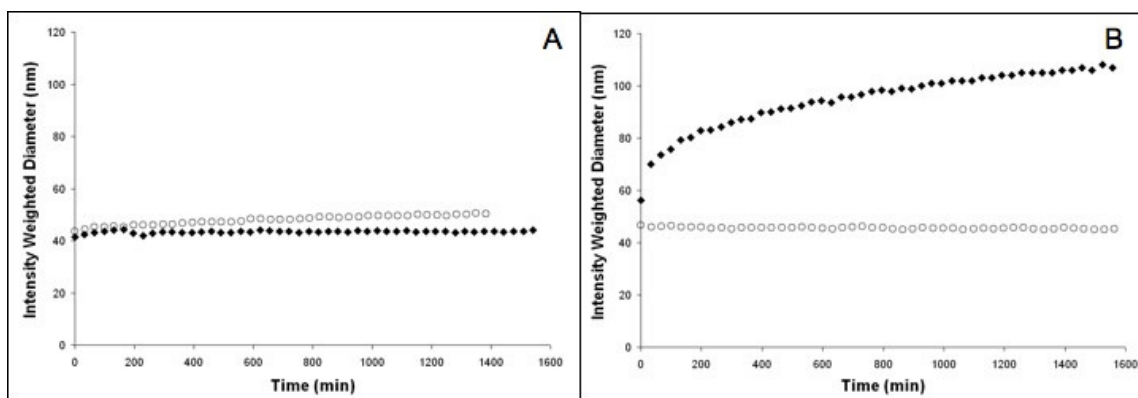


Figure 5.13. DLS intensity weighted diameters of (A) the 2,900 g mol⁻¹ mono-zwitterionic phosphonate-PEO-magnetite complex in DI water (black diamonds) and 0.17 M NaCl (open circles), and (B) the 8,300 g mol⁻¹ mono-zwitterionic phosphonate-PEO-magnetite complex in DI water (black diamonds) and 0.17 M NaCl (open circles)

No difference in size was observed for the complex with the 2,900 g mol⁻¹ PEO in the salt solution, and better stability was observed for the 8,300 g mol⁻¹ complex in the salt solution. This indicates that it is not the ionic strength of the PBS that induces flocculation in these cases. The slight aggregation of the complex with the 8,300 g mol⁻¹ PEO in DI water appears to be due to attractive electrostatic forces—whose origin at this time is unknown—that are screened when the complex is in 0.17 M NaCl. The reduction in Debye length with sodium chloride appears to reduce the electrostatic attraction. Additionally, the stability of the 2,900 g mol⁻¹ complex in DI water indicates that a denser polymer brush layer also prevents aggregation.

Based on the data discussed above, PEO-magnetite complexes containing one zwitterionic phosphonate anchor group showed significant improvement over the other anchor groups, but long-term colloidal stability was still not achieved. To address this issue, three phosphonate zwitterions, all on one end of the PEO oligomers, were investigated as anchor groups to probe any effect of cooperative binding and/or of each chain having a larger "footprint" on the magnetite surface. Figure 5.14 shows the intensity-weighted diameters of PEO-magnetite complexes bound through tri-zwitterionic phosphonates in (A) DI water and (B) PBS.

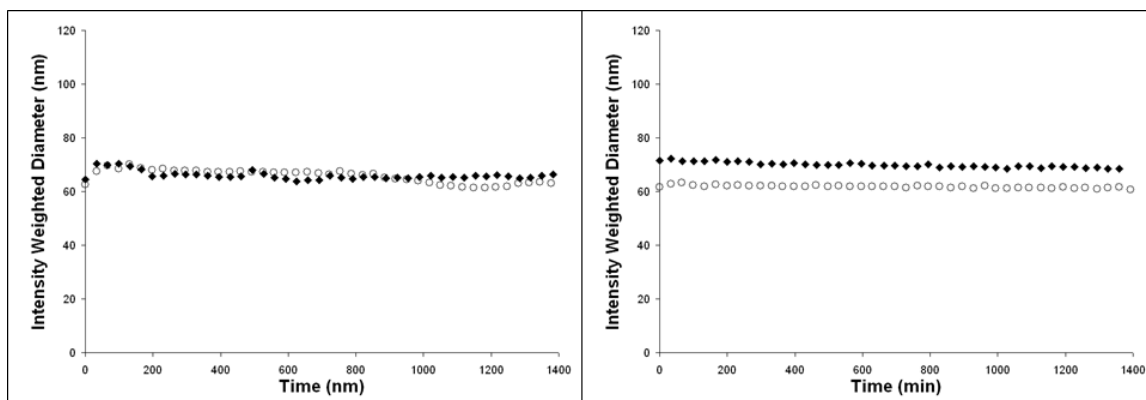


Figure 5.14. Intensity weighted diameters of the 3,400 (open circles) and 9,100 g mol⁻¹ (black diamonds) tri-zwitterionic phosphonate-PEO-magnetite complexes in DI water (A) and PBS (B) over 24 hours

Long-term colloidal size stability was observed for the 3,400 g mol⁻¹ and 9,100 g mol⁻¹ tri-zwitterionic phosphonate PEO-magnetite complexes in both media. Thus, at similar polymer loadings, three phosphonate anchor groups on the polymer chain end yielded complexes that did not undergo polymer desorption AND they did not aggregate over 24 hours in PBS.

We hypothesize that the size stability of the zwitterionic phosphonate₃-PEO-magnetite complexes in PBS can be primarily attributed to high magnetite nanoparticle surface coverage. McDermott et al. performed quartz crystal microbalance experiments on octadecylphosphonate adsorbed onto SiO₂ in THF (a good solvent) and determined the footprint of the phosphonate group to be 18.5 Å.²⁶ Assuming this applies to our system, for a magnetite sphere with a radius of 4.0 nm there is 145 nm² of available surface area, or enough space for 734 zwitterionic phosphonate groups to adsorb. We can calculate the total number of chains per particle for each complex and then calculate how much of the available surface area is taken up by each chain depending on whether there is one or three zwitterionic phosphonate anchor groups. The 2,900 and 8,300 g mol⁻¹ mono-zwitterionic phosphonate complexes and the 3,400 and 9,100 g mol⁻¹ tri-zwitterionic phosphonate complexes have 83%, 36%, >100%, and >100% surface coverage, respectively. Thus, the complex with the most available magnetite surface area also shows aggregation in DI water (Figure 5.11A). At constant polymer loading, decreasing

the PEO molecular weight increases the total number of chains and anchor groups and eliminates this aggregation in DI water. Moreover, by increasing the number of anchor groups three-fold, the predicted surface coverage increases substantially, as do the stabilities of the complexes in both DI water and PBS. This shows that by increasing the total number of anchor groups, either through reduction of molecular weight (more chains nm^{-2}) or by increasing the number of anchor groups per chain (tri-zwitterionic phosphonate anchor group), the colloidal stability of these materials can be controlled in PBS.

We also suspect that the aggregation observed in DI water for complexes with the $8,300 \text{ g mol}^{-1}$ mono-zwitterionic phosphonate anchor group was likely due to an interaction between the magnetite of one complex and the anchor groups of another complex. Were this aggregation due to an interaction between anchor groups of different complexes, we would expect to see more aggregation with an increase in the number of anchor groups. However, we see no aggregation of the tri-zwitterionic phosphonate complexes in DI water, indicating that the critical variable is not an interaction between anchor groups, but rather the availability of the magnetite surface.

5.5 Conclusions

It is clear that the phosphonate zwitterion endgroups remain stably bound to the magnetite in either water or PBS, while the carboxylate and ammonium endgroups allow the polymers to desorb in PBS. The colloidal instabilities of the carboxylate and ammonium complexes are attributed to polymer desorption from the surface of the magnetite nanoparticles in the presence of phosphate salts.⁹

The fact that PEO-magnetite complexes with phosphonate zwitterions remained stably bound in PBS allowed us to consider colloidal size stabilities in light of the density of chains, molecular weights of the bound chains and the predicted surface coverage (i.e., the possible footprint of a trifunctional vs. monofunctional zwitterionic phosphonate chain). The improved colloidal size stability of complexes with the tri-zwitterionic phosphonate anchor groups is likely due to an increase in surface coverage associated with the multiple anchor groups. For a given polymer loading, the availability of the

magnetite surface can also be reduced by shortening the polymer molecular weight, which increases the total number of adsorbed chains.

TGA data confirmed that neither of the novel mono-zwitterionic phosphonate or the tri-zwitterionic phosphonate-anchored polyethers desorbed from the magnetite surface upon exposure to PBS. Coupled with no measurable aggregation of the tri-zwitterionic phosphonate polyether-magnetite complexes in PBS, these seem to be the best-suited complexes for applications that occur in physiological conditions.

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6 The Design of Stable, Well-Defined Polyether-Magnetite Complexes In Aqueous Media: Effect of Anchor Group, Molecular Weight, and Magnetite Loading

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This chapter will be submitted to Nanoletters

Philip Huffstetler synthesized the polyethers discussed in sections 6.3.2 – 6.3.6 and the polyether-magnetite complexes discussed in section 6.3.8. Dr. Jonathan Goff aided in the synthesis of the polyether-magnetite complexes discussed in section 6.3.8. Shannon Ball performed TGA measurements as shown in Table 6.7.

6.1 Abstract

The colloidal stability of polymer-stabilized nanoparticles in physiological media is critical to using nanomaterials for therapeutics. The presence of electrolytes—particularly phosphate—in physiological media presents complications to colloidal stability not found with particles suspended in deionized water. As such, this research looks at three different types of anchor groups—consisting of a carboxylate group, an ammonium group, and a zwitterionic phosphonate group—for the attachment of poly(ethylene oxide) (PEO) to magnetite nanoparticles. Three critical variables were examined: the number of anchor groups, the PEO molecular weight, and the magnetite loading of each complex. Stability of each of these complexes was examined in water and phosphate buffered saline (PBS) which allowed for observation of differences between the two aqueous media. This also allowed conclusions to be drawn about reasons for differences in the stabilities of different complexes. We found that the zwitterionic phosphonate anchor group is considerably more effective than either the carboxylate or ammonium anchor groups in phosphate buffered saline. This is due to the strong

interaction between the phosphonate group and the magnetite, as evidenced by the magnetite's affinity for phosphate through zeta potential measurements, and the aggregation of monophosphonate-anchored complexes in DI water. Because of this aggregation in DI water, we found that a triphosphonate anchor group is necessary for colloidal stability of greater than 24 hours in PBS. Additionally, this was true for both short and long PEO brushes and at varying magnetite loadings, which should allow for the design of materials with very specific functionality.

6.2 Introduction

Research into magnetite nanoparticles has been of great interest due to a wide range of potential biomedical applications including drug delivery, treatment of detached retinas, cell separations and contrast enhancement agents for MRI.¹⁻⁶ Biocompatible macromolecules, such as poly(ethylene oxide) (PEO) has been one of the most widely used coatings for magnetite nanoparticles because it is FDA approved for in vivo applications and its ability to prevent protein adsorption and subsequent phagocytosis.^{1, 7, 8} Despite the prevalence of PEO as a coating for various nanomaterials, the binding efficiency of the PEO to the surface must be improved to ensure colloidal stability of the materials being sterically stabilized by the polymer brush.⁹⁻¹¹

Functional anchor groups on polymers can aid in their adsorption to the surface of magnetite. However, these same anchor groups can be susceptible to desorption with the addition of phosphate salts commonly found in biological systems. Polymeric magnetite dispersion stabilizers containing carboxylate, alkylammonium, and phosphonate zwitterion anchor groups have been previously reported.^{8, 12-14} Significant effort has been devoted to the surface adsorption of PEO-containing polymer onto the magnetite surface to improve biocompatibility, and increase circulation time.^{1, 7, 15, 16} Previous work by others has shown the adsorption of polymers to magnetite nanoparticles using pendant carboxylate groups, as well as with carboxylate, alkylammonium, or methoxysilane termini.^{8, 17-23} Much of this work has examined stability in saline solutions, but the robustness of these anchor groups in solutions containing phosphate salts remains a concern.²⁴⁻²⁶ Previously, fundamental understanding of the effects of anchor group,

molecular weight, and polymer loading on colloidal stability has been difficult to deconvolve. This has made designing stable, effective complexes a challenge with significant guesswork. However, development of models to describe both the complex size and stability have recently been developed which can be applied to these systems.^{4,}

11, 27

This paper describes the colloidal stability of PEO-magnetite complexes where the PEO oligomers contain functional anchor groups at one end (one and three carboxylate(s), one and three ammonium(s), one and three phosphonate zwitterion(s)) to aid adsorption onto the magnetite. Relationships between the type of anchor group, number of anchor groups, polymer molecular weight, and magnetite loading on the capacity of the polymer to remain firmly bound to the magnetite, and thus remain colloidally stable, were studied in water and in PBS. Through comparisons of stability in these two media, reasons for their stability (or instability) can be explored. Previous work has shown that stability in PBS can be achieved with specific tailoring of polymer molecular weight and magnetite loading.^{11, 14} However, through the development of the triphosphonate zwitterion anchor group, we have developed PEO-magnetite complexes that are stable for over 24 hours in PBS regardless of molecular weight or magnetite loading. This allows the design of materials with a polymer stabilizer for a particular application, rather than to ensure stability in a given aqueous media.

6.3 Experimental

6.3.1 Materials

Azobisisobutyronitrile (AIBN), benzyl alcohol (>98%), diethyl ether, diethyl vinyl phosphonate (97%), ethylene oxide (EO, 99.5+%), hexanes (HPLC grade), iron (III) acetylacetonate ($\text{Fe}(\text{acac})_3$), mercaptoacetic acid (97+%), oleic acid (90%, technical grade), sodium iodide (99%), triethylamine (TEA, 99.5%), and 1.0 M vinylmagnesium bromide in THF were purchased from Aldrich and used as received. Tetrahydrofuran (THF, Optima Grade, EMD Science, 99.5%) was refluxed over sodium metal with benzophenone until the solution reached a deep purple, fractionally distilled, and deoxygenated just prior to use. Glacial acetic acid (EMD Science) was diluted with THF

yielding a 2.0 M acetic acid solution. Naphthalene (Aldrich) was sublimed prior to use. Bromotrimethylsilane (TMS-Bromide, 97%) and mecaptoethylamine hydrochloride were purchased from Alfa Aesar and used as received. Ethanol (Decon Laboratories Inc.) was used as received. Methanol (HPLC grade), chloroform (HPLC grade), N,N'-dimethylformamide (DMF, Optima Grade), dichloromethane (HPLC grade), sodium bicarbonate, ammonium chloride, sodium chloride, and acetone (HPLC grade) were purchased from Fisher Scientific and used as received. 3-Chloropropyldimethylchlorosilane and 3-chloropropyltrichlorosilane was purchased from Gelest and used as received. Dialysis tubing (25,000 g mol⁻¹ MWCO and 1,000 g mol⁻¹ MWCO) was obtained from Spectra/Por. Phosphate buffered saline 10X (PBS) was obtained from Lonza and diluted to appropriate concentrations.

6.3.2 Synthesis of vinylsilyl-PEO-OH oligomers

A characteristic procedure for the synthesis of a 9,100 g mol⁻¹ trivinylsilyl-PEO-OH is provided. Further polymerizations of heterobifunctional PEO's involving 3-HPMVS can be found in Goff *et.al*¹⁴. EO (10.0 g, 0.23 mol) was distilled from a lecture bottle into a 300-mL, high-pressure Series 4561 Parr reactor cooled with an isopropanol-dry ice bath (-30 °C), followed by addition of THF (5 mL) via syringe. A potassium naphthalide solution was prepared by charging naphthalene (14.1 g, 0.11 mol) into a 250-mL, flame-dried, round-bottom flask equipped with a glass stir bar. Distilled THF (100 mL) was syringed into the flask dissolving the naphthalene. Potassium metal (3.96 g, 0.10 mol) was added to the solution followed by a N₂ purge for 30 min. The solution was stirred overnight and titrated with 1 N HCl to determine the molarity of the solution, which was shown to be 0.97 M. In a separate flame-dried 100 mL roundbottom flask, 3-HPTVS (0.19 g, 1.12 mmol) and THF (5 mL) were added via syringe followed by the addition of a potassium naphthalide solution (1.03 mL, 1.0 mmol) creating an alkoxide initiator solution. The initiator solution was added to the stirring pressure reactor mixture via syringe. The dry-ice/isopropanol bath was removed, and the reaction mixture was allowed to reach room temperature and maintained for 24 h. The polymerization was terminated by adding an acetic acid solution (0.66 mL 1.65 mmol) to the pressure reactor via syringe. The pressure reactor was purged with N₂ for 1 h, then opened and its contents were transferred to a 250-mL round-bottom flask. The solvent was removed

under vacuum at room temperature, and the product was dissolved in 200 mL of dichloromethane and washed twice with DI water (2 X 100 mL). The solution was concentrated under vacuum at room temperature and precipitated in cold diethyl ether yielding 9.6 g of trivinylsilyl-PEO-OH.

6.3.3 Functionalization of trivinylsilyl-PEO-OH with mercaptoacetic acid

An exemplary procedure for addition of carboxylic acid groups via ene-thiol free radical chemistry across the vinylsilyl endgroups is provided for a $9,100 \text{ g mol}^{-1}$ trivinylsilyl-PEO-OH. Trivinylsilyl-PEO-OH (1.0 g, 0.11 mmol) was charged to a 100-mL roundbottom flask equipped with a stir bar and dissolved in 2 mL of deoxygenated toluene. Mercaptoacetic acid (45.5 mg, 0.49 mmol) was syringed into the reaction flask followed by the addition of AIBN (10.0 mg, 0.06 mmol) dissolved in 0.5 mL of toluene. The mixture was deoxygenated for 10 min by sparging with N_2 , then heated at 80°C for 24 h. The reaction mixture was dissolved in 200 mL of dichloromethane, then transferred to a separatory funnel and washed with DI water 3X to remove the excess mercaptoacetic acid. Dichloromethane was removed via roto-evaporation and the resulting polymer was precipitated into cold diethyl ether. The polymer was dried at room temperature under vacuum for 24 h yielding 0.97 g of tricarboxylic acid-functionalized PEO.

6.3.4 Functionalization of trivinylsilyl-PEO-OH with 2-mercaptoethylamine hydrochloride

Heterobifunctional polyethers with three-ammonium groups (triammonium-PEO-OH) were obtained via the ene-thiol addition of 2-mercaptoethylamine hydrochloride across the vinylsilane groups. A characteristic procedure for a $9,100 \text{ g mol}^{-1}$ trivinylsilyl-PEO-OH is provided. Trivinylsilyl-PEO-OH (2 g, 0.22 mmol), mercaptoethylamine hydrochloride (46.1 mg, 0.4 mmol), and AIBN (21 mg, 0.12 mmol) were dissolved in deoxygenated DMF (5 mL) in a 100-mL roundbottom flask equipped with a stir bar. The reaction was heated at 80°C for 24 h, then the reaction mixture was cooled to room temperature. DI water (100 mL) was added to the flask, and the mixture was transferred to a separatory funnel. Dichloromethane (200 mL) was added to the separatory funnel to extract the triammonium-functionalized polyether from the aqueous layer. The dichloromethane layer was washed with a 1 N solution of sodium bicarbonate (3X), followed by 3 washes with DI water. The dichloromethane solution was concentrated

under vacuum, and the triammonium-PEO-OH oligomer was precipitated into cold diethyl ether and dried at 25 °C under vacuum for 12 h yielding 1.91 g of product (95% yield).

6.3.5 Michael addition of diethyl vinyl phosphonate to triammonium-PEO-OH

A characteristic procedure for addition of diethyl phosphonate groups to a 9,100 g mol⁻¹ triammonium-PEO-OH is provided. Triammonium-PEO-OH (1.0 g, 0.11 mmol) was charged to a clean, flame-dried, 100-mL roundbottom flask equipped with a stir bar and dissolved in ethanol (5 mL). TEA (0.20 mL, 0.14 mmol) was added to the reaction, followed by addition of diethyl vinyl phosphonate (0.26 mL, 0.14 mmol). The reaction was carried out at 70°C for 24 h. The reaction mixture was diluted with DI water to obtain a 75:25 water:ethanol composition and placed in a 1,000 g mol⁻¹ MWCO cellulose acetate dialysis bag and dialyzed for 24 h to remove excess diethyl vinyl phosphonate. The contents of the dialysis bag were transferred to a 100-mL roundbottom flask and lyophilized, yielding 0.94 g of tri(diethyl phosphonate)-functionalized PEO.

6.3.6 De-esterification of tri(diethyl phosphonate)-PEO-OH yielding tri(phosphonic acid)-PEO-OH

Tri(phosphonic acid)-PEO-OH was prepared from tri(diethyl phosphonate)-PEO-OH using a hydrolysis procedure adapted from Caplan et al.²⁸ In a representative procedure, a 9,100 g mol⁻¹ tri(diethyl phosphonate)-PEO-OH (0.90 g, 0.10 mmol) was charged to a clean, 100-mL roundbottom flask equipped with a stir bar and dissolved with 5 mL of dichloromethane. Trimethylsilyl bromide (0.082 mL, 0.62 mmol) was syringed into the reaction flask and stirred at room temperature for 24 h. Methanol (0.03 mL, 0.62 mmol) was added and stirred for 2 h to cleave the trimethylsilyl groups. Dichloromethane (50 mL) was added to the mixture and washed 3X with DI water (100 mL each) in a separatory funnel. The organic layer was concentrated and precipitated by pouring the mixture into cold diethyl ether. The polymer was dried at 25 °C under vacuum for 24 h yielding 0.81 g of tri(phosphonic acid)-PEO-OH.

6.3.7 Magnetite synthesis via high temperature reduction of Fe(acac)₃

Magnetite nanoparticles were synthesized using a reduction method adapted from Pinna et al. Iron(III) acetylacetonate (4.28 g, 16.8 mmol) and benzyl alcohol (90 mL, 0.86 mol) were charged to a 250-mL, three-necked roundbottom flask equipped with a water condenser and placed in a Belmont metal bath with an overhead stirrer with both thermostatic ($\pm 1^\circ\text{C}$) and revolution per minute control. The solution was sparged with N₂ for 1 h to remove any oxygen present in the system. While stirring under N₂, the solution was heated at 100°C for 4 h, then the temperature was increased to 205°C at a rate of $\sim 25^\circ\text{C h}^{-1}$. After 24 h at 205 °C, the reaction was cooled to room temperature and the magnetite nanoparticles were collected with a magnet and the benzyl alcohol was decanted. The magnetite nanoparticles were washed 3X with acetone (100 mL each), followed by dispersion in chloroform (40 mL) containing oleic acid (0.6 g). The solvent was removed under vacuum at room temperature and the oleic acid-stabilized magnetite nanoparticles were washed 3X with acetone (100 mL). The particles were dried under vacuum for 24 h at 25°C and the composition of the particles obtained from thermogravimetric analysis (TGA) was 5% organic residue to 95% magnetite.

6.3.8 Adsorption of trifunctional polyether stabilizers onto magnetite nanoparticles

A representative method for preparing a targeted composition of 70:30 wt:wt polyether:magnetite complex is provided. Oleic acid-stabilized magnetite nanoparticles (50.0 mg) prepared as described above were dispersed in chloroform (10 mL) and charged to a 50-mL roundbottom flask. A trifunctional polyether (117.0 mg) was dissolved in chloroform (10 mL) and added to the dispersion. The pH of each solution and the resulting mixture were approximately neutral. The reaction mixture was sonicated in a VWR 75T sonicator for 16 h under N₂ followed by precipitation into hexanes (300 mL). A magnet was utilized to collect the magnetite nanoparticles and the displaced oleic acid was decanted with the hexanes. The complexes were dried under nitrogen for 30 min and dispersed in DI water (50 mL) using sonication for 30-60 s. The complexes were dialyzed against DI water (4 L) for 24 h using 25,000 g mol⁻¹ MWCO dialysis bags.

6.3.9 Characterization

^1H NMR spectral analyses of compounds were performed using a Varian Inova 400 NMR or a Varian Unity 400 NMR operating at 399.97 MHz.

Gel permeation chromatography (GPC) was performed using an Alliance Waters 2690 Separations Module with a Viscotek T60A dual viscosity detector and laser refractometer equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel column set. GPC data were collected in chloroform at 30 °C. Data were analyzed utilizing a Universal calibration made with polystyrene standards to obtain absolute molecular weights.

Thermogravimetric analysis (TGA) was used to determine the polymer loading for each complex. TGA measurements were carried out on the PEO-magnetite nanoparticles using a TA Instruments TGA Q500 to determine the fraction of each complex that was comprised of polymer. Each sample was first held at 120°C for 10 min to drive off any excess moisture. The sample (10-15 mg) was then equilibrated at 100°C and the temperature was ramped at 10°C min⁻¹ to a maximum of 700°C in a nitrogen atmosphere. The mass remaining was recorded throughout the experiment. The mass remaining at 700°C was used to calculate the fraction of magnetite in the complexes. The experiments were conducted in triplicate.

Dynamic Light Scattering (DLS) measurements were conducted with a Malvern Zetasizer NanoZS particle analyzer (Malvern Instruments Ltd, Malvern, UK) at a wavelength of 633 nm from a 4.0 mW, solid-state He-Ne laser at a scattering angle of 173° and at 25 ± 0.1 °C. Number average diameters were calculated with the Zetasizer Nano 4.2 software utilizing an algorithm, based upon Mie theory, that transforms time-varying intensities to particle diameters.²⁹ For DLS analysis, the polymer-magnetite complexes were received dispersed in DI water and were diluted to ~0.05 mg mL⁻¹ and filtered through a Whatman Anotop, 100-nm, alumina filter directly into a polystyrene cuvette. This corresponds to a volume fraction of 1.3×10^{-5} to 2.2×10^{-5} depending on the polymer loading on the magnetite. In experiments where the complex was exposed to PBS, 0.15 mL of 10X PBS was mixed with 1.35 mL of the 0.05 mg mL⁻¹ complex solution, then the solution was

filtered through either a 100 or 1000-nm filter into a clean polystyrene cuvette. Each sample was analyzed immediately following filtration and re-measured every 30 min over 24 h.

Transmission electron microscopy (TEM) was conducted using a Philips EM-420 field-emission-gun transmission electron microscope. Samples of the polymer-magnetite complexes were suspended in water with probe sonication and cast onto amorphous carbon-coated copper grids. Images were acquired at a magnification of 96 kx at a voltage of 100 kV, corresponding to a resolution of 3.7 pixels nm⁻¹. At least 2000 particles taken from five separate images were used for image analysis. Particle distribution analyses were performed using Reindeer Graphics' Fovea Pro 4 plug-in for Adobe Photoshop 7.0.

A 7T MPMS Squid magnetometer (Quantum Design) was used to determine magnetic properties. Hysteresis loops were generated for the magnetite nanoparticles at 300K and 5K. Fe concentration of the magnetite was determined chemically by acid digestion followed by ICP-AES analysis. These concentrations were in good agreement with TGA values.

6.3.10 Density distribution model to predict sizes of the polyether-magnetite nanoparticle complexes

Modeling of the polyether-magnetite complexes to predict their sizes was performed using methods developed by Mefford, Zhang, and Miles^{3, 4, 17, 30}. The particles were imaged with TEM and image analysis was performed on the magnetite cores to obtain a particle size distribution. This distribution was then fit with a Weibull probability distribution ($P(a)$) as shown in Equation 6-1 where a is the particle radius and b and c are the Weibull shape and scale parameters, respectively.

$$P(a) = \frac{c}{b} \left(\frac{a}{b} \right)^{c-1} \exp \left[- \left(\frac{a}{b} \right)^c \right] \quad (6-1)$$

The average surface area of the magnetite could then be calculated and by combining the average surface area with the average polymer loading per mass of complex (from TGA), the average number of chains per magnetite surface area, α , could be determined.

$$\alpha = \frac{(1 - W_{mag}) N_{Av} \rho_{mag} \int_0^{\infty} a^3 P(a) da}{3 M_n W_{mag} \int_0^{\infty} a^2 P(a) da} \quad (6-2)$$

Here, ρ_{mag} is the density of magnetite (5.21 g cm⁻³),³¹ W_{mag} is the weight fraction of the complex that is magnetite (from TGA), N_{Av} is Avogadro's number, and M_n is the PEO number average molecular weight. Through application of a modified version of the Vagberg density distribution model,^{3, 4, 30, 32} the average number of chains and the radius of the PEO-magnetite complexes was calculated as

$$f(a) = 4\pi a^2 \alpha \quad (6-3)$$

$$R_m(a) = \left(\frac{8 N_k f(a)^{\frac{1-\nu}{2\nu}} L_k^{1/\nu}}{4^{1/\nu} 3\nu} + a^{1/\nu} \right)^\nu \quad (6-4)$$

where N_k is the number of Kuhn segments, L_k is the Kuhn length (0.6 nm)³¹, ν is the Flory exponent (0.583 for PEO in water at 25 °C),³³ and $f(a)$ is the number of chains per particle.

Comparison to DLS measurements were made by calculating the number average diameter D_n from the combination of the Weibull probability size distribution fit and the density distribution model.

$$D_n = 2 \int_0^{\infty} R_m(a) P(a) da \quad (6-5)$$

The focus was on the number weighted average, both from a modeling and a light scattering perspective because the number weighted average is the least sensitive to

aggregation. Compared to the intensity weighted average size, significantly more aggregation must occur before the number average will change significantly. Thus, comparison of a calculated number average to an experimental average from light scattering allows determination of considerable aggregation.

The brush model can also be used to calculate the pair-pair potential for polymer-particle complexes of the same size as previously described by our group.^{4, 30} The calculation of these potentials is described in equations 6-6 through 6-9.

$$V_{Total} = V_{vdW} + V_M + V_{ES} + V_S \quad (6-6)$$

$$V_{ES} = 2\pi\epsilon\epsilon_o\psi_o^2 \ln(1 + e^{-\kappa(r-2a)}) \quad (6-7)$$

$$V_{vdW} = -\frac{1}{6} A_{eff} \left(\frac{2a^2}{r^2 - 4a^2} + \frac{2a^2}{r^2} + \ln \left(\frac{r^2 - 4a^2}{r^2} \right) \right) \quad (6-8)$$

$$V_S = \frac{5}{18} kTf(a)^{3/2} \left\{ \begin{array}{ll} -\ln \left(\frac{r}{\sigma} + \frac{1}{1 + \frac{\sqrt{f(a)}}{2}} \right); & r \leq \sigma \\ \frac{1}{1 + \frac{\sqrt{f(a)}}{2}} \left(\frac{\sigma}{r} \right) \exp \left(-\frac{\sqrt{f(a)}}{2\sigma} (r - \sigma) \right); & r > \sigma \end{array} \right\} \quad (6-9)$$

V_{Total} is the total potential energy, V_{vdW} is the attractive potential due to van der Waal's interactions, V_M is the attractive potential produced by an applied magnetic field, V_{ES} is the repulsive potential due to electrostatic interactions, and V_S is the repulsive potential due to steric repulsion of the polymer brushes. All calculations and measurements are performed without an applied magnetic field, and so V_M is negligible.¹⁴ Here, ϵ is the dielectric constant of the solvent, ϵ_o is the permittivity of free space, ψ_o is the surface potential, κ is the inverse Debye length, r is the center-to-center separation, and a is the

particle radius, h is the surface-to-surface separation of two particles³⁴, r is the center-to-center separation distance of two particles, A_{eff} is the effective retarded Hamaker constant calculated according to previous work^{4, 11}

For the case of a densely adsorbed brush layer, the steric contribution to the interaction potential may be described by equation 12, where r is the center-to-center separation, $f(a)$ is the number of chains per particle, and $\sigma/2$ is the distance from the center of the core to the center of the outermost blob layer, and σ is defined as $1.3R_g$, the radius of gyration of the ensemble.^{35, 36} Thus, determination of the onset of a sharp increase in the steric potential is determined by the R_g , which is calculated as done previously.^{4, 11} By summing these potential energy terms (van der Waals, electrostatic, and steric) in equation 6-6, a pair-pair potential energy diagram can be constructed to determine the effect of a magnetic field on the separation of these materials as a function of molecular weight.

Finally, the calculation of V_{Total} allows for the determination of the stability ratio and the half-life for doublet formation using equations 6-10 and 6-11, respectively, where μ is the solvent viscosity, Φ is the volume fraction of particles in solution, and $\bar{a}$ is the average particle radius.^{37, 38}

$$W = 2\bar{a} \int_{\frac{\sigma}{2}}^{\infty} \frac{e^{\frac{V_{\text{Total}}}{kT}}}{r^2} dr \quad (6-10)$$

$$t_{1/2} = \frac{\bar{a}^{-3} \pi \mu W}{\phi k T} \quad (6-11)$$

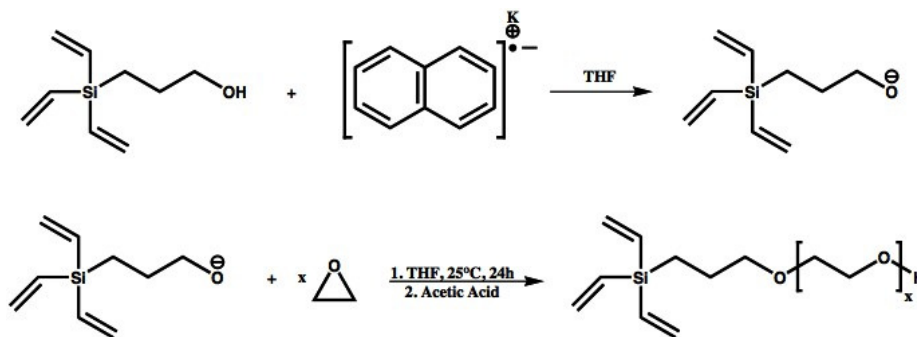
The classical expression for W does not account for polydispersity and only concerns pair interactions for particles of like sizes. We account for the size distribution by computing $\bar{a}$ (4.0 nm) from the Weibull distribution (equation 6-1). The half-life for doublet formation provides a basis to compare complexes with different molecular weights and polymer loadings.

6.4 Results and Discussion

The aim of this study was to compare the stability of different PEO-magnetite complexes by varying the type of functional anchor used, the number of functional anchors used, the polymer molecular weight, and the magnetite loading of each complex. This was done in both DI water and PBS, which while not a perfect biological analog, does contain phosphate salts commonly found in physiological media while still allowing the use of light scattering as a characterization tool.

6.4.1 Synthesis of PEO oligomers with different functional endgroups for binding to magnetite

3-HPMVS and 3-HPTVS were used as versatile initiators for the living anionic polymerization of EO yielding heterobifunctional oligomers useful for post-functionalization with specific chemical groups for the adsorption onto magnetite nanoparticles. A characteristic synthesis of trivinylsilyl-PEO-OH is shown in Scheme 1. This approach allowed for utilization of the same oligomer for comparing dispersion properties while only changing the type and number of functional anchor groups. Functionalization of these oligomers with carboxylic acid, ammonium, and zwitterionic phosphonates have previously been reported by Goff *et al.* Potassium naphthalide was reacted with 3-HPMVS or 3-HPTVS to form an alkoxide for initiating EO, and the number of moles of the alcohol initiator relative to EO controlled the molecular weight. A slight deficiency of naphthalide relative to alcohol ensured the alkoxide and not residual naphthalide initiated the polymer chains, while the residual alcohols transferred with the propagating PEO chains. These anionic ring-opening polymerizations were terminated with acetic acid in order to avoid any unwanted oxidative side reactions.



Scheme 6-1. Synthesis of trivinylsilyl-PEO-OH via anionic ring-opening polymerization

Both Dimethylvinylsilyl-PEO-OH and trivinylsilyl-PEO-OH oligomers with molecular weights ranging from 3,000 g mol⁻¹ to 9,000 g mol⁻¹ were prepared and characterized utilizing GPC and ¹H NMR as shown in Table 6.1.

Table 6.1. Molecular weights of dimethylvinylsilyl- and trivinylsilyl-PEO-OH oligomers

Targeted Molecular Weight	M _n (g mol ⁻¹)		MWD
	¹ H NMR	GPC	
Monovinylsilyl-3,000 g mol ⁻¹	2,900	3,100	1.09
Monovinylsilyl-8,000 g mol ⁻¹	8,300	7,900	1.05
Trivinylsilyl-3,000 g mol ⁻¹	3,400	3,400	1.04
Trivinylsilyl-9,000 g mol ⁻¹	9,100	9,500	1.09

As previously reported, ene-thiol free-radical additions were used to introduce both carboxylic acid and ammonium functionality for both the dimethylvinylsilyl-PEO-OH and trivinylsilyl-PEO-OH oligomers.¹⁴ The vinylsilyl group was used because it does not readily polymerize via free-radical chemistries. Addition of mercaptoacetic acid and 2-mercaptoethylamine hydrochloride were monitored via ¹H NMR through the disappearance of the vinyls between 5.8 and 6.2 ppm as previously reported.¹⁴

The ammonium and triammonium-functionalized PEO oligomers were utilized as precursors towards the formation of zwitterionic phosphonate PEO-OH. The ammonium polyethers were neutralized by addition of triethylamine affording free amines followed by the introduction of diethyl vinyl phosphonate. Diethyl vinyl phosphonate was added to the amine group(s) through the Michael addition. The ethyl groups of both diethyl phosphonate-PEO-OH and tri(diethyl phosphonate)-PEO-OH oligomers were cleaved via addition of bromotrimethylsilane (TMS-Br) followed by methanolysis yielding phosphonic acid functionality.

6.4.2 Formation of PEO-magnetite complexes via adsorption of the functional PEO oligomers onto the surfaces of magnetite nanoparticles

PEO-magnetite complexes were formed with the four sets of varying molecular weight

PEO oligomers, after which the complexes were dialyzed against water to remove any unbound PEO. The compositions of the PEO-magnetite complexes were in close agreement with the targeted magnetite:polyether composition as measured by TGA.

Figure 6.1 shows a representative TEM image of the polyether-magnetite complex, which indicates a well-defined particle size distribution of $7.7 \text{ nm} \pm 2.3 \text{ nm}$.

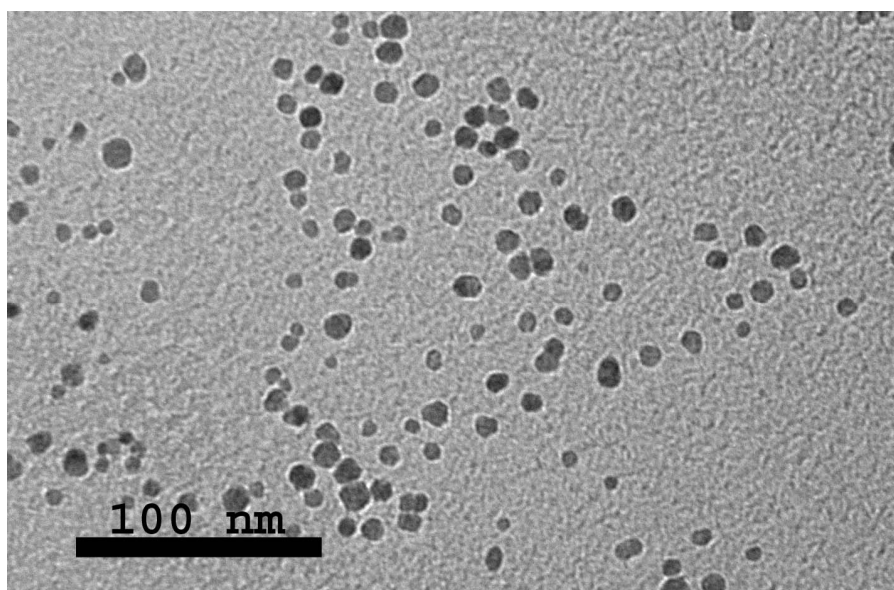


Figure 6.1. A representative TEM image of polyether-magnetite complexes indicate a well-defined particle distribution.

The magnetic properties of the magnetite nanoparticles were characterized via superconducting quantum interference device analyses. Hysteresis loops revealed superparamagnetic behavior and a saturation magnetization of 80 emu g^{-1} for the magnetite nanoparticles. This compares with reported saturated magnetization of 82 emu g^{-1} for bulk magnetite.³⁹

6.4.3 Colloidal Stability of Polyether-Magnetite Complexes in Deionized Water

Before examination of the stability of these materials under physiological conditions, characterization of their colloidal stability in deionized (DI) water was needed to understand the complexities associated with varying molecular weights and polymer

loadings. Figure 6.2 shows DLS over time for two different PEO molecular weights for the monoammonium and monocarboxylic acid anchor groups with approximately 30% magnetite loading.

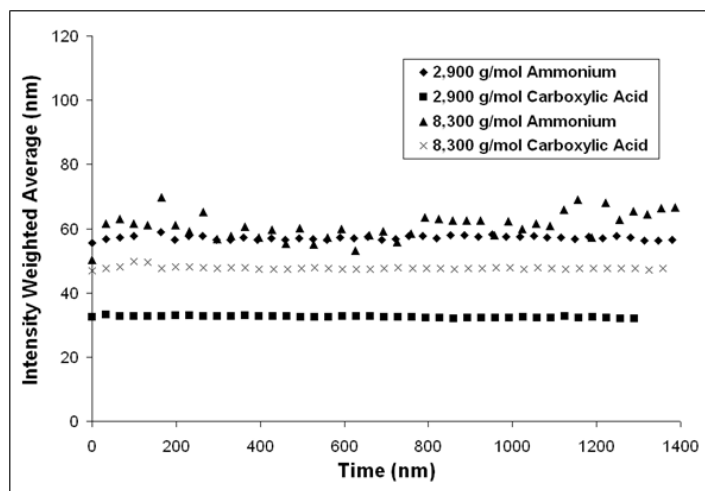


Figure 6.2. All monoammonium- and monocarboxylic acid-anchored complexes were stable for 24 hours in DI water (approximately 30% magnetite loading for each).

Similar stabilities were observed for the triammonium and tricarboxylic acid anchor groups for two similar PEO molecular weights. However, the stability of the monophosphonate anchor groups in DI water was considerably different, as shown in Figure 6.3.

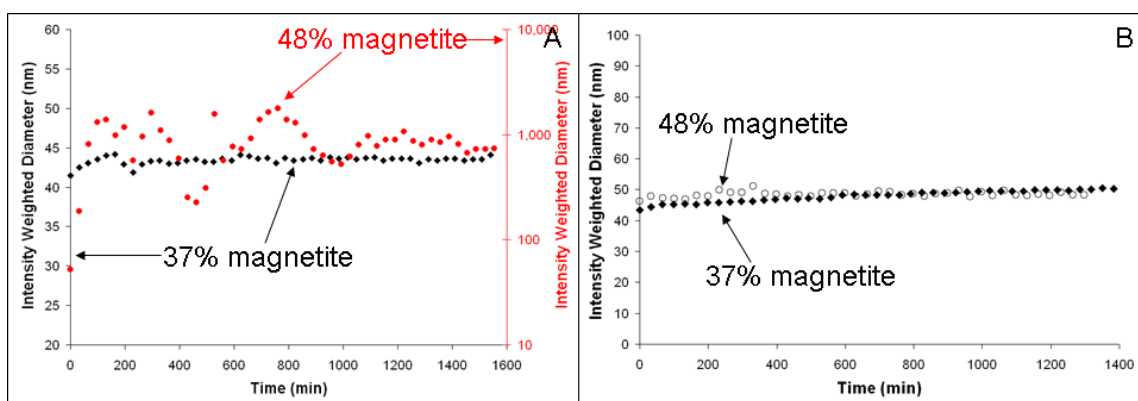


Figure 6.3. Intensity weighted diameters (D_I) of the $2,900 \text{ g mol}^{-1}$ Mono-phosphonate-PEO-OH complexes in DI water (A) and 0.17 M sodium chloride (B).

There was no appreciable aggregation for the monophosphonate-anchored $2,900 \text{ g mol}^{-1}$ complex with 37 wt % magnetite loading and the measured particle size was very close to that predicted for a dispersed primary particle using the Density Distribution model. However, there was significant aggregation of the complex once the magnetite loading was increased to 48 wt % (A). This behavior is very different than the ammonium and carboxylic acid anchor groups. These results indicate that the phosphonate anchor group may contribute to the onset of this aggregation. Through addition of sodium chloride (0.17 M), the aggregation was eliminated, showing that the aggregation appears to be due to electrostatic interactions (Figure 6.3B).

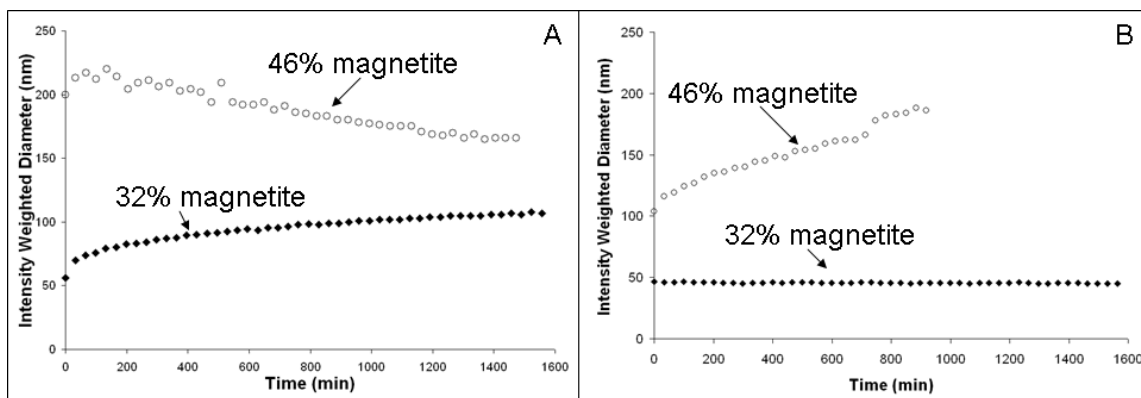


Figure 6.4. Aggregation of the monophosphonate-anchored $8,300 \text{ g mol}^{-1}$ PEO complexes occurred in DI water (A). This aggregation was hindered with the addition of 0.17 M sodium chloride (B).

Similar behavior was observed for the monophosphonate-anchored $8,300 \text{ g mol}^{-1}$ PEO complexes, as shown in Figure 6.4. Aggregation was observed in DI water for both the 32 wt % and 46 wt % magnetite loadings (A). In contrast to the $2,900 \text{ g mol}^{-1}$ complexes, the 46% magnetite loading for the $8,300 \text{ g mol}^{-1}$ complex could not be completely dispersed in the water, as indicated by its initial size of approximately 200 nm. This indicates the presence of a secondary potential energy minimum deep enough to prevent reduction of the complex to its primary size, even with extensive sonication. There was no appreciable growth of the complex size over time, but much of the sample was captured in the filter prior to measurement and there was visible sedimentation upon

completion of the experiment. For the 32% magnetite loading, the particle size is initially very close to that of a dispersed primary particle but then increases with time, indicating flocculation into a more shallow secondary minimum than was the case for the 46% loading.

In Figure 6.4(B), addition of 0.17 M sodium chloride eliminated all aggregation for the complex with 32 wt % magnetite loading. The aggregation could not be completely eliminated for the complex with 46 wt % magnetite loading. However, sonication of the complex did reduce the particle size below that obtainable in water, which confirms that the flocculation observed in DI water is due to a secondary minimum and that the minimum can be reduced with the addition of salt. This indicates an electrostatic interaction and because this is not observed with any of the other anchor groups, it must be due to the zwitterionic phosphonate group.

The length scale at which this electrostatic interaction is significant can be estimated using extended DLVO theory. The Debye length for each complex should be approximately 30 nm (assuming an ionic strength of 10^{-4} M) and the electrostatic potential decays exponentially from the charged surface. Measurement of the zeta potential to quantify this electrostatic potential is not useful because currently there is no model to account for the zwitterionic nature of the anchor group, it is an average measurement which would always results in a negative potential, and it would not be a representative measurement due to the shifting of the shear plane due to the polymer brush. However, it is useful to compare the length scale of the steric repulsive potential with that of electrostatic interactions. Figure 6.5 shows the steric potentials as a function of surface to surface separation for two $8,300 \text{ g mol}^{-1}$ complexes with 32% and 46% magnetite loadings.

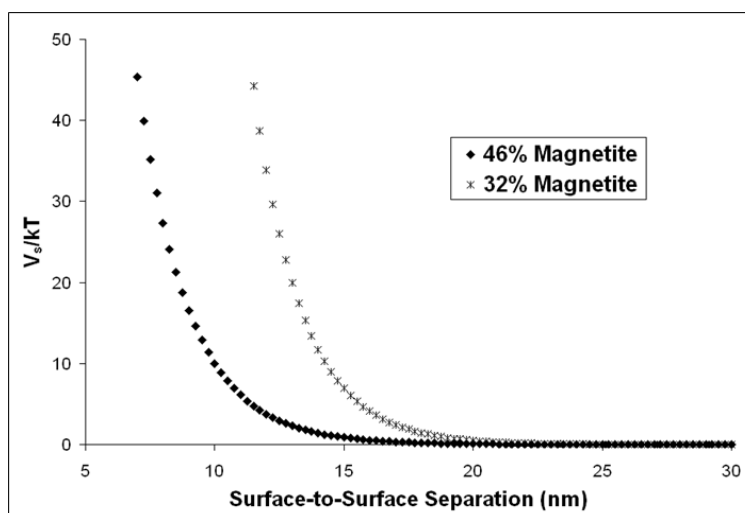


Figure 6.5. Plots of the steric potential as a function of surface to surface separation for the $8,300 \text{ g mol}^{-1}$ complex with 32% and 47% magnetite loadings.

The 32% magnetite loading provides the longer-range steric repulsion, beginning to dominate ($> 10kT$) at a separation distance of 14 nm compared to 10 nm for the complex with 46% magnetite loading. However, a large enough secondary potential energy minimum is created by the electrostatic interaction caused by the phosphonate anchor group to prevent the sample with 46% magnetite from being resuspended as a single particle system. In this case, the electrostatic interaction must increase significantly (enough to produce the large secondary minimum) between 10-14 nm to explain the observed behavior.

Extended DLVO theory also explains the behavior observed for the $2,900 \text{ g mol}^{-1}$ complex with 48% magnetite loading (Figure 6.3A). If the onset of strong electrostatic interaction is indeed between 10-14 nm, then significantly more flocculation would be expected for smaller molecular weight stabilizers due to smaller repulsive steric potentials (Figure 6.6).

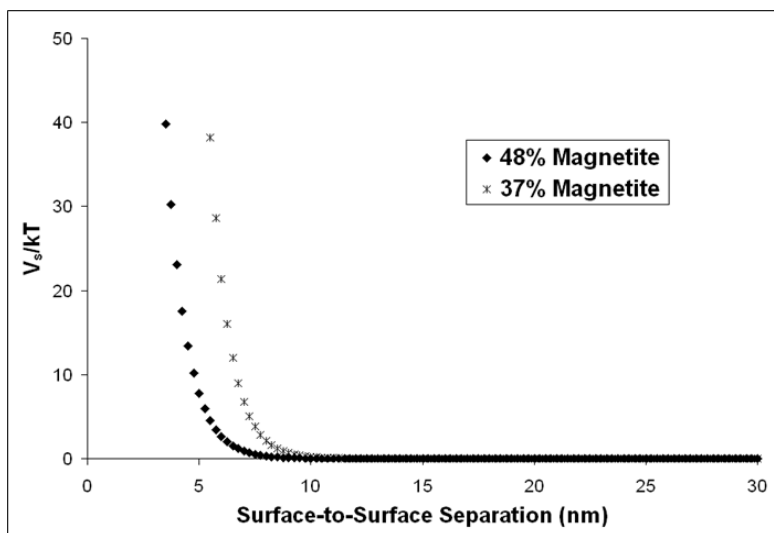


Figure 6.6. Steric potentials for the $2,900 \text{ g mol}^{-1}$ complexes with 37 and 48% magnetite loadings.

However, if this were a complete explanation then both complexes should flocculate in water and not just the complex with 48% magnetite loading. The only real difference between the complexes is that the lower magnetite loading has a denser polymer brush. It is possible that this polymer brush is dense enough to appreciably screen out any electrostatic interaction and prevent flocculation in DI water. This increased stability may also indicate that the electrostatic interaction is between the phosphonate anchor group of one complex and the magnetite of a neighboring complex.

The nature of the electrostatic interaction was examined through the use of triphosphonate anchor groups, which provided more magnetite surface coverage without increasing the polymer chain density compared to the monophosphonate counterparts. Also, an increase in the number of anchor groups would increase the electrostatic interaction were it due to an interaction between anchor groups of neighboring complexes. Stability of these complexes for two different triphosphonate-anchored PEO molecular weights ($3,400 \text{ g mol}^{-1}$ (A) and $9,100 \text{ g mol}^{-1}$ (B)) are shown in Figure 6.7.

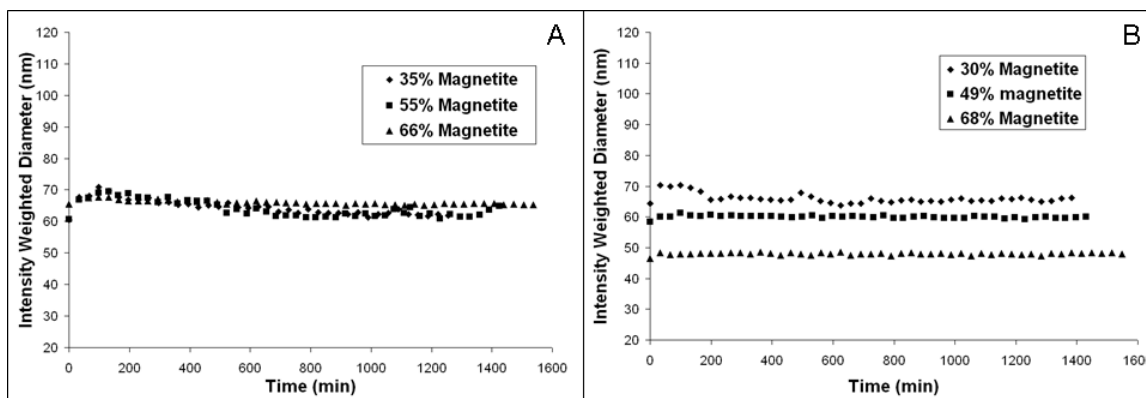


Figure 6.7. Intensity weighted diameters (D_I) of (A) 3,400 g mol⁻¹ and (B) 9,100 g mol⁻¹ PEO complexes with triphosphonate anchors in DI water over 24 hrs.

No aggregation was observed for any of the triphosphonate-anchored 3,400 or 9,100 g mol⁻¹ PEO complexes in DI water, regardless of the magnetite loading of the complex. Addition of two more anchor groups per chain eliminated the aggregation observed for the monophosphonate-anchored complexes. This suggested that the aggregation was not due to attraction between anchor groups of different complexes but rather due to attraction of magnetite of one complex to an anchor group of another complex. This required an estimate of the fraction of the magnetite particle surface covered by the anchor groups.

To determine the magnetite surface coverage, a method was needed to quantify the surface area occupied by a particular anchor group. The program ChemBio3D Ultra 11.0 was used to determine the average bond length for a specific anchor group (1.7 Å for a P-O bond in a phosphonate anchor group and 1.1 Å for an ammonium anchor group). This value was then used as the radius of a sphere representing the anchor group located at the magnetite nanoparticle surface as shown in Figure 6.8. This method neglects the volume occupied by the oxygen molecule, and further work should be done to incorporate this volume and examine whether the cross-sectional area would be a better way to measure the anchor group footprint.

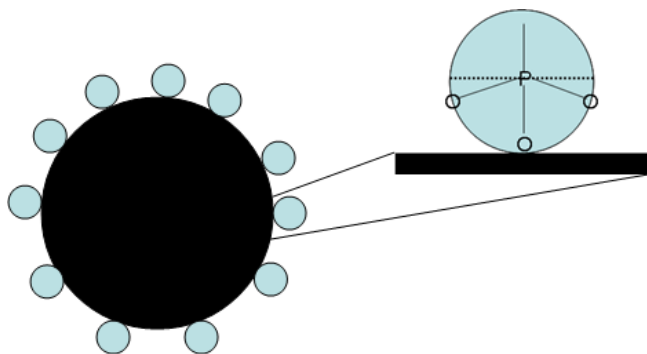


Figure 6.8. Visualization of the area occupied by the phosphonate anchor group in contact with the magnetite surface.

Assuming only the lower half of the sphere can contact the magnetite surface, the surface area occupied by a particular anchor group was taken to be the surface area of the hemisphere. For a monophosphonate, this model yields a molecular footprint of $18.2 \text{ \AA}^2$. This approximation compares well with the literature, where McDermott *et al.* used QCM-D to calculate the molecular footprint of a phosphonate group attached to an alkyl chain to be $18.5 \text{ \AA}^2$.⁴⁰ The model yields a much smaller molecular footprint for the monoammonium group of $7.6 \text{ \AA}^2$.

Table 6.2 illustrates the surface coverage based on a surface area of $122 \text{ m}^2 \text{ g}^{-1}$ for the magnetite particle distribution and a molecular footprint of $18.2 \text{ \AA}^2$. The number of available anchors was determined through the calculation of polymer chains using equations 6-1 through 6-3 and then multiplied by three for the triphosphonate-anchored complexes.

Table 6.2. Percent surface coverage calculated based on the surface area occupied by the phosphonate anchor group.

Anchor Group	Molecular Weight (g/mol)	% Magnetite	Number of Anchors	% Surface Coverage	Stable in DI Water?
Monophosphonate	2,900	37	648	96%	Yes
	2,900	48	381	57%	No
	2,900	67	163	24%	No
	8,300	32	283	42%	No
	8,300	46	133	20%	No
	8,300	71	57	8%	No
Triphosphonate	3,400	31	2166	322%	Yes
	3,400	55	975	145%	Yes
	3,400	66	417	62%	Yes
	9,100	30	849	126%	Yes
	9,100	49	363	54%	Yes
	9,100	68	156	23%	Yes

For the monophosphonate-anchored complexes, the only sample that exhibited 24-hour stability in DI water was the 2,900 g mol⁻¹ complex with 37% magnetite loading. This sample had 96% surface coverage according to the calculation while the next closest complex had a surface coverage of 57%. Thus, it appears that the stability observed in Figure 6.3A of the 2,900 g mol⁻¹ complex with 37% magnetite loading in DI water was due to a decrease in the magnitude of the electrostatic interaction because very little magnetite was available to neighboring anchor groups. Also, none of the 8,300 g mol⁻¹ complexes have complete coverage and both show some flocculation in DI water (Figure 6.4A). The relative amounts of flocculation observed have already been explained using extended DLVO theory. Stability of these materials in DI water is quite complex, with the existence of an interaction potential due to the attraction of the phosphonate anchor group to the magnetite surface coupled with a repulsive steric interaction potential that stabilizes the complexes.

As illustrated in Table 6.2, the complexes made with triphosphonate anchors show no aggregation over 24 hours in DI water. They also show much higher surface coverages, including complexes where the estimated area occupied by the anchor groups exceeds the surface area of the magnetite. However, at higher magnetite loadings, i.e. the 9,100 g mol⁻¹ complex with 68% magnetite loading, the surface coverage becomes relatively

small but no aggregation occurs. This phenomenon may be due to interactions between phosphonate anchors that would cause them to spread out over the magnetite surface and thus increase the footprint of the triphosphonate anchor. Thus, for the complexes made with triphosphonate anchors, the effective % surface coverage of magnetite available for interaction with other complexes might be significantly smaller than that calculated in Table 6.2. This may account for the increased stability of these complexes, even at high magnetite loadings.

6.4.4 Colloidal Stability of Polyether-Magnetite Complexes in Phosphate Buffered Saline

Previous work has shown that colloidal stability in DI water is no guarantee that these polyether-magnetite complexes will be stable in more complex solutions, such as phosphate buffered saline (PBS). Desorption of these polyethers with the introduction of phosphate salts into solution has been a significant barrier to the development of these materials.^{11, 14} Through the incorporation of an amphiphilic diblock copolymer with finely-tuned polymer molecular weights and magnetite loadings, this desorption could be hindered.¹¹ Additionally, recent development of the phosphonate anchor group has allowed for increased stability of these materials.¹⁴ However, previous work has also indicated that a reduction in polymer loading can significantly decrease the stability of a complex in PBS.¹¹ The ability to tailor a polymer-magnetite complex size may be critical to possible biological targeting, and the size of a complex is directly related to its polymer loading.^{11, 27} Thus, it is critical to determine the efficacy of a particular anchor group over a range of magnetite loadings.

Figure 6.9 shows zeta potential measurements made of the magnetite nanoparticles at different pH in two different buffers: PBS and sodium phosphate.

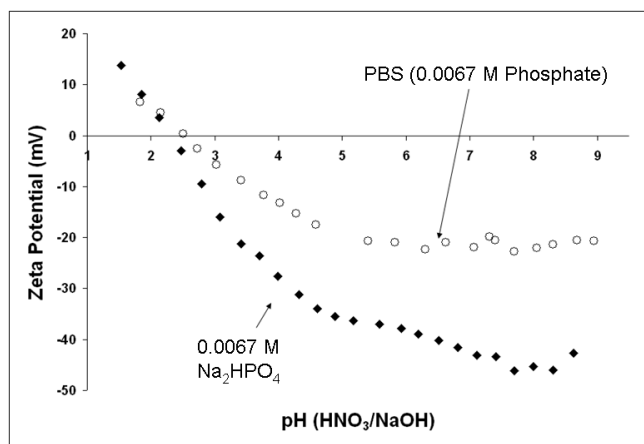


Figure 6.9. Zeta potential measurements indicate that phosphate is a potential determining ion for magnetite.

The molarity of the sodium phosphate was adjusted to the same molarity of phosphate in PBS and the pH was adjusted with indifferent electrolytes. The isoelectric point of the magnetite in water was measured at 6.1. A shift was noted in the charging behavior of the magnetite with the addition of either PBS or sodium phosphate. The isoelectric point for these solutions is around 2.5, which is near the lowest pK_a of phosphate and it appears that phosphate is a potential determining ion that adsorbs strongly to the magnetite surface. This change in charging behavior is observed even after a polymer stabilizer is added to the magnetite surface, and desorption of the polymer is due to competitive adsorption between the phosphate in solution and the polyether anchor block.

One way to measure the stability of these materials in PBS is to measure the rate of aggregation over time with DLS. This allows the comparison across different types of anchor groups and different magnetite loadings. A comparison of anchor group stability in PBS is shown in Table 6.3.

Table 6.3. Rate of aggregation over 24 hours for complexes made with indicated anchor groups at 25°C in PBS.

Anchor Group	Molecular Weight (g/mol)	% Magnetite	Chains/nm ²	Aggregation Rate in PBS (nm/hr)
Monocarboxylate	2,900	35	3.0	18.5
Tricarboxylate	3,400	30	3.4	17.7
Monocarboxylate	8,300	32	1.3	1604
Tricarboxylate	9,100	34	1.0	36.4
Monoammonium	2,900	33	3.3	999
Triammonium	3,400	31	3.2	227
Monammonium	8,300	21	2.3	3598
Triammonium	9,100	36	1.0	2.1
Monophosphonate	2,900	38	2.8	3.5
Triphosphonate	3,400	31	3.2	0.0
Monophosphonate	8,300	32	1.3	1.1
Triphosphonate	9,100	30	1.3	0.0

Three observations are immediately apparent from Table 6.3. First, three anchor groups provide much better stability than one anchor group. This indicates a competitive adsorption of the phosphate salts can trigger anchor group desorption. Hence, the removal of more anchor groups should require more time, slowing the aggregation process. Second, the stability of the complexes made with carboxylate anchor groups was much better than those made with ammonium groups for one anchor group, but, for complexes made with three anchor groups, stability depended on PEO molecular weight. Finally, the phosphonate anchor groups are better than either the ammonium or carboxylate anchor groups, regardless of whether one or three anchor groups were used. The increased stability of the monocarboxylate anchor groups appears to be due to a specific interaction between the carboxylic acid and the PEO. This behavior was inferred by comparing the predicted number average diameters from the density distribution model to DLS measurements as shown in Table 6.4.

Table 6.4. For mono-anchor complexes, comparison of predicted number average diameters to experimental diameters measured by DLS indicates a specific interaction for the carboxylic acid-functionalized PEO complexes.

Anchor Group	Molecular Weight (g/mol)	Magnetite Loading (%)	DLS D _n (nm)	Model D _n (nm)	% Difference
Carboxylate	2,900	35	20	29	43%
Ammonium	2,900	33	27	29	8%
Phosphonate	2,900	38	26	28	6%
Carboxylate	8,300	32	25	42	71%
Ammonium	8,300	23	44	46	4%
Phosphonate	8,300	32	40*	43	8%

*Initial measurement used due to flocculation in DI water

Previous studies have shown agreement between the density distribution model and DLS measurements to within 8% for both polyether-magnetite and polydimethylsiloxane-magnetite complexes.^{11, 27} This level of agreement was observed in the present work for both the monoammonium and monophosphonate anchor groups. However, for the monocarboxylate anchor groups, the measured diameter was significantly smaller than the predicted model diameter. It appears that something about the carboxylate anchor group reduces the extension of the PEO brush away from the magnetite surface for both long and short PEO molecular weights. Table 6.3 indicates that shorter, denser brushes provide better stability against desorption of polymer from the magnetite surface. The interaction between the carboxylate anchor group and the PEO provides a denser brush than other anchor groups. This may be a significant reason for the better stability in PBS for the carboxylate anchor group compared to the ammonium group for the same molecular weight.

The aggregation behavior for complexes made with the tricarboxylate and triammonium anchor groups are very different and show a striking dependence on anchor type and PEO molecular weight as shown in Figure 6.10.

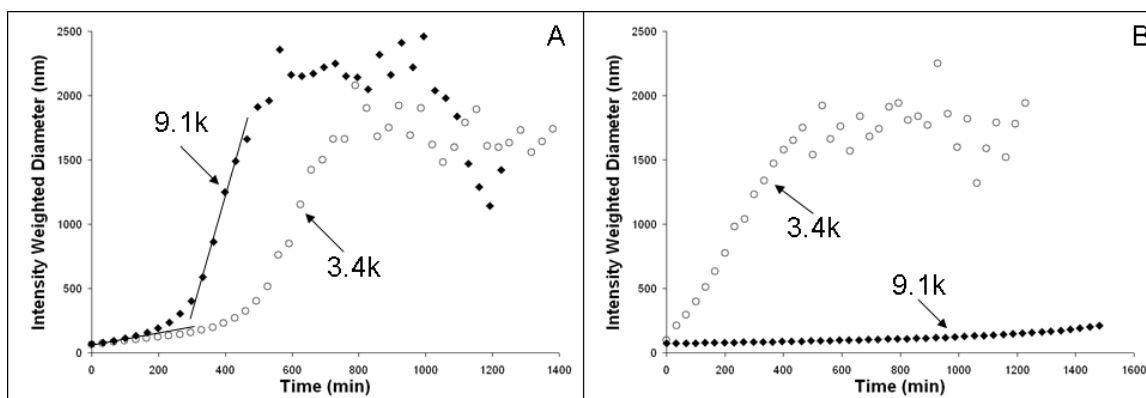


Figure 6.10. Aggregation for the complexes made with tricarboxylate anchor groups (A) indicates two distinct desorption mechanisms compared to the ammonium anchor groups (B).

For the tricarboxylate anchor groups, there are two distinct desorption steps whereas, for the triammonium anchor groups, there is one distinct slope. This provides further evidence of an interaction between the carboxylic acid group and the PEO brush. The three anchor groups initially provide significantly better resistance to desorption. However, after some polymer is removed, there is acceleration in aggregation rate, possibly due to an increased desorption rate caused by the anchor groups attached to a PEO chain interacting with a neighboring PEO chain and aiding in its desorption from the magnetite surface.

Another interesting aspect of Figure 6.10 was the relative stability of the triammonium-anchored complexes. For all of the other complexes, shorter and denser polymer brushes provide better colloidal stability in PBS. The reasoning was that when both complexes are at a magnetite loading of 30 wt %, there are more total chains of a smaller molecular weight species and thus, diffusion of phosphate to the surface is hindered. For the triammonium-anchored complexes, the 9,100 g mol⁻¹ complex exhibits far greater stability than its 3,400 g mol⁻¹ counterpart. Again, this anomalous behavior appears to be due to magnetite surface coverage by the anchor groups. In this case, the 3,400 g mol⁻¹ triammonium complex with 31% magnetite loading has a surface coverage above 100%, as illustrated in Table 6.5.

Table 6.5. Surface coverage calculations for the ammonium anchor group explain stability behavior in PBS.

Anchor Group	Molecular Weight (g/mol)	% Magnetite	Number of Anchors	% Surface Coverage	Slope in PBS (nm/hr)
Monoammonium	2,900	33	773	48%	999
	8,300	23	445	28%	3598
Triammonium	3,400	31	2166	135%	227
	3,400	48	1056	66%	47
	9,100	36	648	40%	2.1

The ammonium anchor is significantly smaller than the phosphonate anchor ($7.6 \text{ \AA}^2$ vs. $18.2 \text{ \AA}^2$), meaning there is significantly less surface coverage for these anchors by comparison. However, there are more anchors than available magnetite surface area for the $3,400 \text{ g mol}^{-1}$ triammonium complex. This leads to free anchor groups that are not attached to the magnetite surface, which weakens the interaction between the polymer chain and the magnetite surface compared to the $9,100 \text{ g mol}^{-1}$ complexes. We would then expect to observe a decrease in aggregation rate in PBS as the magnetite loading is increased for the $3,400 \text{ g mol}^{-1}$ complex. This behavior was indeed observed as the rate of aggregation in PBS was reduced from 227 to 47 nm h^{-1} when the magnetite loading was increased from 31 wt % to 48 wt %. This is the opposite to the behavior observed for triammonium-anchored complexes with a $7,200 \text{ g mol}^{-1}$ stabilizer reported previously.¹¹ This confirms that the surface coverage is an important design parameter and also validates the method being used to at least qualitatively calculate the surface coverage for these anchor groups. To our knowledge, this correlation of particle stability with surface coverage by anchor groups has not been previously reported.

From the results for complexes with mono-anchor groups shown in Table 6.3, the complexes made with the zwitterionic phosphonate anchors exhibited less aggregation in PBS than did those made with ammonium or carboxylate chemistries. In order to overcome this problem, a tri-zwitterionic phosphonate was examined at various polymer loadings and molecular weights as shown in Table 6.6.

Table 6.6. Triphosphonate-anchored complexes show no appreciable aggregation in PBS over 24 hours.

Anchor Group	Molecular Weight (g/mol)	% Magnetite	Chains/nm²	Slope in PBS (nm/hr)
Triphosphonate	3,400	35	2.7	0.0
Triphosphonate	3,400	36	2.6	0.0
Triphosphonate	3,400	42	2.0	0.0
Triphosphonate	3,400	45	1.8	0.0
Triphosphonate	3,400	55	1.2	0.0
Triphosphonate	3,400	56	1.1	0.0
Triphosphonate	3,400	66	0.6	0.0
Triphosphonate	9,100	30	1.3	0.0
Triphosphonate	9,100	49	0.5	0.0
Triphosphonate	9,100	68	0.2	0.0

No appreciable aggregation was observed for any of the tri-zwitterionic phosphonate-anchored complexes over 24 hours in PBS at various polymer loadings. The stability of these complexes is further supported by dialysis experiments, where each sample was suspended in DI water, dialyzed against water for 24 hours, against PBS for 24 hours, and then against DI water again for 48 hours and the polymer desorption was measured using TGA.¹⁴

The amount of polymer desorption that occurs after the PBS dialysis is shown in Table 6.7. Significant desorption was observed for the monocarboxylate, tricarboxylate, monoammonium, and triammonium anchor groups after exposure to PBS. However, significant desorption was not observed for either the monophosphonate or triphosphonate anchor groups. Thus it appears that the aggregation measured over time by DLS combined with the dialysis data in Table 6.7 are effective ways to characterize the colloidal stability of the anchor groups used to attach the PEO to the magnetite nanoparticle.

Table 6.7. Polymer desorption can be quantified through dialysis against PBS.

PEO Molecular Weight (g mol ⁻¹)	Anchor Group	Initial Polymer Loading after Dialysis against H ₂ O (Wt %)	Polymer Loading after Dialysis against PBS (Wt %)
2,900	Monocarboxylate	64.7 ± 0.9	40.7 ± 1.4
8,300	Monocarboxylate	68.4 ± 1.6	45.3 ± 1.4
2,900	Monoammonium	66.8 ± 0.8	41.0 ± 0.7
8,300	Monoammonium	77.1 ± 0.9	42.6 ± 0.7
2,900	Monophosphonate	63.3 ± 0.4	65.1 ± 1.4
8,300	Monophosphonate	70.2 ± 0.7	67.7 ± 0.7
3,400	Tricarboxylate	70.3 ± 0.7	25.0 ± 1.2
9,100	Tricarboxylate	66.2 ± 0.7	27.6 ± 1.4
3,400	Triammonium	69.3 ± 0.8	44.0 ± 0.8
9,100	Triammonium	64.2 ± 0.3	47.4 ± 0.8
3,400	Triphosphonate	69.0 ± 0.6	68.8 ± 0.4
3,400	Triphosphonate	34.5 ± 0.9	35.4 ± 0.5
3,400	Triphosphonate	57.6 ± 0.4	57.9 ± 0.3
3,400	Triphosphonate	55.0 ± 0.9	54.7 ± 0.8
3,400	Triphosphonate	44.0 ± 0.6	44.8 ± 0.5
3,400	Triphosphonate	45.3 ± 0.8	44.7 ± 0.1
9,100	Triphosphonate	32.3 ± 0.7	32.8 ± 0.7
9,100	Triphosphonate	70.5 ± 0.8	69.2 ± 0.8

6.5 Conclusions

Colloidal stability in DI water and PBS is a necessary first step towards the design of polyether-magnetite complexes for biomedical applications. As such, designing anchor groups that effectively attach the polyethers to the magnetite surface is an essential part of obtaining colloidal stability in various aqueous media. Additionally, having a strong interaction between the anchor group and the magnetite allows for a reduction in the polymer loading necessary for sterically stabilizing the complex, which may allow for the maximization of the magnetite's magnetic properties for imaging or other applications.

Both mono- and tri-functional polyethers were synthesized with anchor groups containing carboxylic acid, ammonium, and zwitterionic phosphonate functionalities. These polyethers were attached to magnetite nanoparticles through these anchor groups and the stabilities of the polyether-magnetite complexes were measured in both DI water and

PBS. All the complexes were stable in DI water for greater than 24 hours except for the monophosphonate-anchored complexes. These showed considerable aggregation, particularly as the magnetite loading was increased, indicating a specific interparticle interaction between the phosphonate anchor group of one complex and the magnetite of another. This aggregation was eliminated with the use of a triphosphonate anchor group, indicating that the critical parameter dictating aggregation is the magnetite surface that is occupied by the anchor group.

Both the carboxylate and ammonium anchor groups showed considerable aggregation in PBS due to polymer desorption. The amount of desorbed polymer was determined through TGA measurements made before and after dialysis of the complex against PBS. Aggregation of the complexes was significantly reduced with the use of tri-functional anchors rather than mono-functional anchors. The phosphonate anchor groups showed significantly less aggregation than their carboxylate and ammonium counterparts, and the aggregation could be completely eliminated through use of a triphosphonate anchor group, regardless of the magnetite loading. This is a significant advance from previous work which requires specific control of both molecular weight and magnetite loading to inhibit flocculation. Additionally, the triphosphonate complexes showed no significant polymer loss after dialysis against PBS, indicating that these complexes are indeed stable in PBS. The increased stability of these complexes is attributable to the large interaction energy between the magnetite and phosphonate, which is observed in the adsorption of phosphate to the magnetite surface through zeta potential measurements, and the aggregation of monophosphonate-anchored complexes due to an attraction between the phosphonate groups of one complex with magnetite of another.

Calculations of surface coverage were carried out using a simple method to estimate the size of an individual anchor group. Values obtained from this method were in good agreement with literature values for a phosphonate group. The surface coverage calculations were validated qualitatively through measurement of stability of monophosphonate-anchored complexes in DI water. Extrapolation of this method to ammonium groups was confirmed through stability measurements made in PBS. To our

knowledge, this is a novel approach to exploring colloidal stability and the effect of anchor groups on that stability and might be applied to other polymer-particle systems.

Polyether-magnetite complexes have been synthesized and characterized showing long-term (24 hour) stability in both DI water and PBS. Additionally, these complexes are made with a well-defined magnetite particle size distribution and a brush structure that is well-described by the density distribution model, allowing for fairly precise (within 8%) control of the average particle size. This should allow for the rapid development of biocompatible polyether-magnetite nanoparticles with properties specifically tailored for a desired application.

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7 The Design of Amphiphilic Polyether-Magnetite Complexes for Enhancement of MRI Contrast and Cell Entry

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Portions of this work will be published following additional relaxation experimentation

Philip Huffstetler synthesized the polyethers discussed in sections 7.3.2 and the polyether-magnetite complexes discussed in section 7.3.4. Dr. Jonathan Goff and Christian Reinholz aided in the synthesis of the polyether-magnetite complexes discussed in section 7.3.4. Matthew Carroll and Dr. Robert Woodward provided relaxation measurements for the polyether-magnetite complexes as discussed in section 7.4.4.

7.1 Abstract

Magnetite particles have many uses in biological systems including as contrast agents for MRI, drug delivery, hyperthermia treatments, and many more. Amphiphilic polyethers have shown promise as agents for drug delivery because of their ability to traverse cell membranes. This study is an effort to combine the advantages of both systems by adsorbing an amphiphilic stabilizer—consisting of poly(ethylene oxide)-b-poly(propylene oxide) diblock copolymers with varying molecular weights—to magnetite nanoparticles. The aim is to understand how to determine the optimum molecular weights for maximum relaxivity while still being able to infiltrate the cell. These results provide preliminary data to suggest that a hydrophobic interaction between the amphiphilic polyethers is present and that it may be the reason for increases in cellular

uptake and relaxivity. Modeling of these complexes using a polymer brush model should provide a tool to allow for further understanding of these mechanisms.

7.2 Introduction

Superparamagnetic iron oxide nanoparticles (SPION) coated with biocompatible macromolecules have been of great interest due to a wide range of potential biomedical applications including drug delivery, treatment of detached retinas, cell separations and contrast enhancement agents for MRI, where they have been studied intensely as T₂ contrast agents.¹⁻¹³ These SPIONs have been used for different tissues, including examining the liver, spleen, brain and many others.^{10, 12-14} However, designing the complexes for controlled cellular uptake has continued to be a major hurdle.

PEO has been one of the most widely used coatings for magnetite nanoparticles due to its low cytotoxicity, ability to mask foreign substrates from the immune system, and FDA approval for *in vivo* applications.^{1, 15, 16} Amphiphilic block copolymers (particularly Pluronics®) have shown potential for applications in drug delivery for multi-drug resistant cancer cells.¹⁷⁻¹⁹ Through careful selection of the molecular weights of the blocks, the hydrophobic to hydrophilic ratio can be controlled, as can cellular uptake.¹⁷⁻¹⁹ Small Angle X-Ray Scattering (SAXS) has been used to probe the interaction between a lipid bilayer mimicking a cell membrane and a Pluronic® triblock copolymer. These studies indicated that by carefully selecting the molecular weight of the hydrophobic copolymer (poly(propylene oxide) (PPO)), insertion of the Pluronic® into the cell membrane can be achieved.²⁰

The approach of this work has been to combine the two systems, using magnetite to enhance contrast and an amphiphilic diblock copolymer to facilitate cell uptake. Poly(propylene oxide)-*b*-poly(ethylene oxide) (PPO-*b*-PEO) diblock copolymers were synthesized using an anionic living polymerization, allowing for precise control of molecular weight and the molecular weight distribution. The effect of polymer molecular weight and magnetite loading was explored by comparing the sizes of the complexes measured by dynamic light scattering (DLS) to calculated sizes for dispersed primary particles from a polymer brush model developed by Vagberg, et al.²¹ and further

adapted to polymer-particle systems by Mefford, Miles, Huffstetler, and Goff et al.^{3, 4, 22-25} The difference between the DLS measurements and the model provide a measure of the extent of aggregation of the particles which helps to explain significant relaxation measurements and cellular uptake measurements. This provides a tool to both design materials and to elucidate the mechanism by which increases in relaxivity and cellular uptake are achieved.

7.3 Experimental

7.3.1 Materials

Azobisisobutyronitrile (AIBN), benzyl alcohol (>98%), diethyl ether, diethyl vinyl phosphonate (97%), ethylene oxide (EO, 99.5+%), hexanes (HPLC grade), iron (III) acetylacetonate ($\text{Fe}(\text{acac})_3$), mercaptoacetic acid (97+%), oleic acid (90%, technical grade), sodium iodide (99%), triethylamine (TEA, 99.5%), and 1.0 M vinylmagnesium bromide in THF were purchased from Aldrich and used as received. Tetrahydrofuran (THF, Optima Grade, EMD Science, 99.5%) was refluxed over sodium metal with benzophenone until the solution reached a deep purple, fractionally distilled, and deoxygenated just prior to use. Glacial acetic acid (EMD Science) was diluted with THF yielding a 2.0 M acetic acid solution. Naphthalene (Aldrich) was sublimed prior to use. Bromotrimethylsilane (TMS-Bromide, 97%) and mercaptoethylamine hydrochloride were purchased from Alfa Aesar and used as received. Ethanol (Decon Laboratories Inc.) was used as received. Methanol (HPLC grade), chloroform (HPLC grade), N,N'-dimethylformamide (DMF, Optima Grade), dichloromethane (HPLC grade), sodium bicarbonate, ammonium chloride, sodium chloride, and acetone (HPLC grade) were purchased from Fisher Scientific and used as received. 3-Chloropropyldimethylchlorosilane and 3-chloropropyltrichlorosilane was purchased from Gelest and used as received. Dialysis tubing (25,000 g mol⁻¹ MWCO and 1,000 g mol⁻¹ MWCO) was obtained from Spectra/Por. Phosphate buffered saline 10X (PBS) was obtained from Lonza and diluted to appropriate concentrations.

7.3.2 Synthesis of Polyether Stabilizers

Details of the synthesis of the PPO-b-PEO-OH diblock copolymer stabilizers can be found in the synthesis section of Chapter 4. The Michael addition reaction used to functionalize triammonium-functionalized PPO-b-PEO-OH diblock copolymers with tri-zwitterionic phosphonate functionality was described in Chapter 5, sections 5.3.7 and 5.3.8. As discussed in Chapter 4, the triammonium anchors resist flocculation in phosphate buffered saline (PBS) for specific PPO and PEO molecular weights and magnetite loadings. However, molecular weight and polymer loading was not important for stability in PBS for the tri-zwitterionic phosphonate-anchored homopolymer complexes. Thus, it is important to determine whether this is also the case for the tri-zwitterionic phosphonate-anchored diblock complexes.

7.3.3 Magnetite synthesis via high temperature reduction of $\text{Fe}(\text{acac})_3$

Details of the magnetite nanoparticle synthesis can be found in Chapters 3, 4, 5, 6, and 8. The magnetite was made via the reduction of iron acetylacetonate, which yields a roughly spherical, narrow particle size distribution of 7.8 ± 2.2 nm. Note that variability between magnetite batches was examined in Chapter 3 and the average particle size was within 0.4 nm for each batch.

7.3.4 Adsorption of trifunctional polyether stabilizers onto magnetite nanoparticles

Details of the adsorption of trifunctional polyether stabilizers onto magnetite nanoparticles can be found in Chapters 3, 4, 5, 6, and 8. This was done in chloroform at room temperature through overnight sonication.

7.3.5 Characterization

^1H NMR spectral analyses of compounds were performed using a Varian Inova 400 NMR or a Varian Unity 400 NMR operating at 399.97 MHz.

Gel permeation chromatography (GPC) was performed using an Alliance Waters 2690 Separations Module with a Viscotek T60A dual viscosity detector and laser refractometer equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel column set. GPC data

were collected in chloroform at 30 °C. Data were analyzed utilizing a Universal calibration made with polystyrene standards to obtain absolute molecular weights.

TGA was used to determine the polymer loading for each complex. TGA measurements were carried out on the PEO-magnetite nanoparticles using a TA Instruments TGA Q500 to determine the fraction of each complex that was comprised of polymer. Each sample was first held at 120 °C for 10 min to drive off any excess moisture. The sample (10-15 mg) was then equilibrated at 100 °C and the temperature was ramped at 10 °C min⁻¹ to a maximum of 700 °C in a nitrogen atmosphere. The mass remaining was recorded throughout the experiment. The mass remaining at 700 °C was taken as the fraction of magnetite in the complexes. The experiments were conducted in triplicate.

DLS measurements were conducted with a Malvern Zetasizer NanoZS particle analyzer (Malvern Instruments Ltd, Malvern, UK) at a wavelength of 633 nm from a 4.0 mW, solid-state He-Ne laser at a scattering angle of 173° and at 25 ± 0.1 °C. Number average diameters (D_n) were calculated with the Zetasizer Nano 4.2 software utilizing an algorithm, based upon Mie theory, that transforms time-varying intensities to particle diameters.²⁶ For DLS analysis, the polymer-magnetite complexes were received dispersed in DI water and were diluted to ~0.05 mg mL⁻¹ and filtered through a Whatman Anotop, 100-nm, alumina filter directly into a polystyrene cuvette. This corresponds to a volume fraction of 1.3×10^{-5} to 2.2×10^{-5} depending on the polymer loading on the magnetite. In experiments where the complex was exposed to PBS, 0.15 mL of 10X PBS was mixed with 1.35 mL of the 0.05 mg mL⁻¹ complex solution, then the solution was filtered through either a 100 or 1000-nm filter into a clean polystyrene cuvette. Each sample was analyzed immediately following filtration and re-measured every 30 min over 24 h.

Relaxometry measurements were performed using a Bruker mq-60 minispec NMR Analyzer. Samples were diluted to specific concentrations between 0.01 and 0.0001 wt%. Each sample (0.4 mL) was transferred to a 7.5-mm glass NMR tube and sealed with parafilm. The sample tubes were placed in an aluminum block enclosed in a water bath at

37.5 °C for 30 min prior to relaxometry measurements. A Carl-Purcell-Meiboom-Gill (CPMG) spin echo sequence of 1000 echoes (echo spacing, TE, of 2 ms), and a repetition time of 15 s, was used to measure T_2 .

7.3.6 Application of a brush model to the polyether-magnetite complexes

Details concerning the calculations behind the polymer brush model can be found in Chapters 3, 4, 6, and 8. The incorporation of a non-solvated PPO shell surrounding the magnetite core was implemented to describe the brush behavior as shown in Figure 7.1.

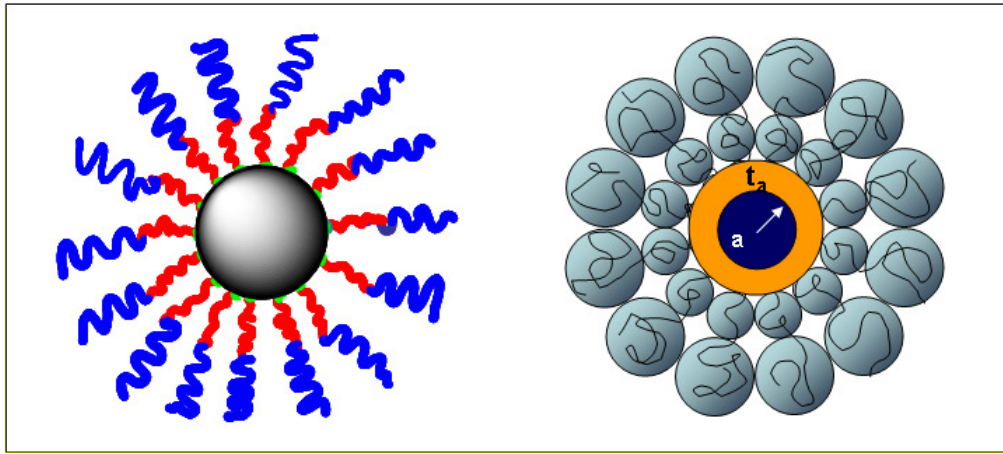


Figure 7.1. Cartoon depiction of a magnetite core, non-solvated PPO layer, and an extended PEO brush in concentric blobs extending out from the PPO surface.

The equivalent radii were graphed versus the probability of their occurrence and this histogram was fit with a Weibull probability distribution as shown in equation 7-1.

$$P(a) = \frac{c}{b} \left(\frac{a}{b} \right)^{c-1} \exp \left[- \left(\frac{a}{b} \right)^c \right] \quad (7-1)$$

Here, c is the Weibull scale parameter, b is the Weibull shape parameter, and a is the particle radius. The Weibull distribution has the advantage that it is flexible. If $b = 1$, then $P(a)$ reduces to an exponential distribution. If $b = 3.4$, $P(a)$ reduces to a Gaussian distribution.

Hydrodynamic radii (R_m) of the magnetite-polymer complexes were calculated by the method of Mefford, et al.^{3, 4} TEM image analysis data was first fitted with a Weibull probability distribution to calculate an average specific surface area of the magnetite particles. Combining average surface area with the average polymer loading per mass of the complex (from TGA) leads to an average number of chains per magnetite surface area, α , as shown in Equation 7-2.³

$$\alpha = \frac{(1 - W_{mag}) N_{Av} \rho_{mag} \int_0^{\infty} a^3 P(a) da}{3 MW_{Total} W_{mag} \int_0^{\infty} a^2 P(a) da} \quad (7-2)$$

Here, ρ_{mag} is the density of magnetite (5.21 g cm^{-3}),²⁷ W_{mag} is the weight fraction of the complex that is magnetite, and MW_{Total} is the polymer number average molecular weight. The integral must be taken at this point to sum up the effect of the particle size distribution. This is because the measurement of the polymer mass via TGA is inherently an average measurement of the entire distribution. The assumption is made that each magnetite particle has the same ratio of magnetite to polymer, and so the average of the distribution must be taken at this juncture.

Using a modification of Vagberg's density distribution model,²¹ we calculate the number of chains per particle, $f(a)$, and the complex radius $R_m(a)$ —the radius of the particle plus its attached brush—as

$$f(a) = 4\pi a^2 \alpha \quad (7-3)$$

$$R_m(a) = \left(\frac{8 N_k f(a)^{\frac{1-\nu}{2\nu}}}{4^{1/\nu} 3\nu} L_k^{1/\nu} + a^{1/\nu} \right)^\nu \quad (7-4)$$

where N_k is the number of statistical segments in the polymer brush chain, ν is the Flory exponent of the brush in the solvent, L_k is the length of a statistical segment, and a is the particle radius.

For complexes with an amphiphilic diblock copolymer, the hydrophobic layer was assumed to be a nonsolvated, hydrophobic shell surrounding the magnetite core and the brush was modeled as concentric blobs extending out from the surface of the PPO layer. To account for the hydrophobic layer, the model was adapted to include a new effective core size, which is the radius of the magnetite core obtained from the Weibull distribution plus the thickness of the hydrophobic anchor layer (t_a) (equation 7-5).

$$t_a = \left(\left(\frac{\rho_{mag} a^3 (1 - W_{mag})}{W_{mag} MW_{anchor} \rho_{anchor} MW_{Total}} \right) + a^3 \right)^{1/3} - a \quad (7-5)$$

Here, ρ_{anchor} is the density of the hydrophobic anchor layer, MW_{anchor} is the anchor layer number average molecular weight, and MW_{Total} is the total polymer number average molecular weight. Thus, the predicted hydrodynamic radius of the complex as a function of magnetite particle radius can be described by equation 7-6.

$$R_m(a) = \left(\frac{8N_k f(a)^{\frac{1-\nu}{2\nu}}}{4^{1/\nu} 3\nu} L_k^{1/\nu} + (a + t_a)^{1/\nu} \right)^\nu \quad (7-6)$$

This function along with the Weibull distribution function for the magnetite particle radius is used to determine radial distribution averages for comparison with DLS experimental data. Each average weights particles of various diameters differently and thus gives an estimate of the homogeneity of the particle systems. The number average diameter, D_n , of the magnetite complex can be calculated as

$$D_n = 2 \int_0^\infty R_m(a) P(a) da \quad (7-7)$$

The volume average diameter, D_v , of the magnetite complex can be calculated as

$$D_v = 2 \frac{\int_0^{\infty} R_m(a)^4 P(a) da}{\int_0^{\infty} R_m(a)^3 P(a) da} \quad (7-8)$$

Using a method by Mefford, et al. and assuming the particles are in the Rayleigh scattering regime,³ the intensity average diameter, D_i , for the magnetite complex was calculated as

$$D_i = 2 \frac{\int_0^{\infty} R_m(a)^6 P(a) da}{\int_0^{\infty} R_m(a)^5 P(a) da} \quad (7-9)$$

7.4 Results and Discussion

7.4.1 Modeling and Stability in DI Water

Polyether-magnetite complexes were synthesized with varying molecular weights and both tri-zwitterionic phosphonate and triammonium functional anchor groups. ^1H NMR and GPC data for the diblock copolymers are shown in Table 7.1.

Table 7.1. ^1H NMR and GPC data for the amphiphilic diblock copolymers.

Polymer	M_n (g mol ⁻¹)			Molecular Weight Distribution
	^1H NMR of PPO	^1H NMR of PEO	GPC	
3.3kPPO- <i>b</i> -2.6kPEO-OH, Triammonium	3,300	2,600	5,400	1.3
3.3kPPO- <i>b</i> -4.8kPEO-OH, Triammonium	3,300	4,800	7,900	1.3
3kPPO-3kPEO-OH, Triphosphonate	3,000	2,900	6,100	1.1
3kPPO-5kPEO-OH, Triphosphonate	3,000	5,000	8,300	1.1

Molecular weights were targeted so that the effect of each anchor group could also be compared. For the magnetite complexes stabilized with polymers with triammonium anchors, referred to hereafter as the triammonium complexes, hydrodynamic diameters measured by dynamic light scattering (DLS) were compared to model predictions from the polymer brush model (Table 7.2).

Table 7.2. Comparison of DLS size measurements made in DI water at 25°C to model predictions for the triammonium complexes.

Anchor Group	PPO Molecular Weight (g/mol)	PEO Molecular Weight (g/mol)	Magnetite Loading (%)	DLS D _n (nm)	Model D _n (nm)	% Difference
Triammonium	3,300	2,600	35	30.6	25.2	18%
Triammonium	3,300	2,600	34	31.4	25.5	19%
Triammonium	3,300	2,600	39	31.7	24.2	24%
Triammonium	3,300	2,600	41	27.5	23.4	15%
Triammonium	3,300	2,600	48	26.8	22.3	17%
Triammonium	3,300	2,600	50	28.4	21.9	23%
Triammonium	3,300	4,800	32	23.5	31.9	36%
Triammonium	3,300	4,800	41	26.4	29.3	11%
Triammonium	3,300	4,800	51	31.1	26.9	14%

For the 3.3kPPO-*b*-4.8kPEO-OH complexes, the DLS particle size increased with increasing magnetite loading. Aggregation appears to be occurring for the 3.3kPPO-*b*-4.8kPEO-OH complex as the magnetite loading is increased due to a reduction in the steric repulsion. However, for the 3.3kPPO-*b*-2.6kPEO-OH complexes, the particle size was essentially constant over all magnetite loadings. This may indicate an interaction between PPO layers of neighboring complexes that prevents complete dispersion of the complex with an increased polymer loading. While there is significant deviation between the model and the DLS measurements for both complexes, the deviation was less for the 3kPPO-*b*-4.8kPEO-OH complexes for the two highest magnetite loadings. Similar trends were observed for the tri-zwitterionic phosphonate-anchored complexes (Table 7.3). For the 3kPPO-*b*-5kPEO-OH complexes, the values of D_n increased with the magnetite loading while no such trend was observed for the 3kPPO-*b*-3kPEO-OH complexes. At low magnetite loadings - 30-40% for the 3kPPO-*b*-3kPEO-OH complexes and 30% for the 3kPPO-*b*-5kPEO-OH complexes - these complexes match the brush model considerably better than did their ammonium counterparts. The model significantly underpredicts values of D_n in Table 1.2 for the triammonium complexes at low magnetite loadings. Since the model assumes that the PPO layer is nonsolvated, it may be that the PPO layer in the triammonium complexes is more solvated compared to the PPO layer in the triphosphonate complexes. This hypothesis is consistent with the results of small angle neutron scattering (SANS) experiments discussed below. It is worth noting that results from Chapters 5 and 6 show that the zwitterionic phosphonate anchor group introduces an interparticle electrostatic attraction while the triammonium anchor group

does not. It is possible that triammonium anchor group also affects the solvation of the PPO layer in the diblock complexes in some way. The issue of solvation will be addressed in a later section.

Table 7.3. Comparison of DLS size measurements made in DI water at 25°C to model predictions for the tri-zwitterionic phosphonate anchored complexes.

Anchor Group	PPO Molecular Weight (g/mol)	PEO Molecular Weight (g/mol)	Magnetite Loading (%)*	DLS D _n (nm)	Model D _n (nm)	% Difference
Triphosphonate	3,000	2,900	30	27.6	28.0	1%
Triphosphonate	3,000	2,900	30	27.8	28.0	1%
Triphosphonate	3,000	2,900	40	26.2	25.3	3%
Triphosphonate	3,000	2,900	40	26.6	25.3	5%
Triphosphonate	3,000	2,900	50	27.2	23.1	15%
Triphosphonate	3,000	2,900	50	27.1	23.1	15%
Triphosphonate	3,000	5,000	36	35.3	31.4	11%
Triphosphonate	3,000	5,000	37	36.2	31.1	14%
Triphosphonate	3,000	5,000	41	40.3	30.0	26%
Triphosphonate	3,000	5,000	43	37.7	29.4	22%
Triphosphonate	3,000	5,000	47	42.3	28.4	33%
Triphosphonate	3,000	5,000	53	42.9	27.0	37%

*TGA measurements pending for the 3kPPO-*b*-3kPEO-OH complexes (values shown are targeted values)

Again, the size of the 3kPPO-*b*-3kPEO-OH complexes does not change with magnetite loading. It seems highly unlikely that the sizes would be constant for both the ammonium and tri-zwitterionic phosphonate complexes with the smaller PEO molecular weight just due to a reduction in steric repulsion. Indeed, for the 3kPPO-*b*-5kPEO-OH complexes, a significant increase in size is noted with an increase in magnetite loading. It may be that the PPO layers for the 3kPPO-*b*-3kPEO-OH complexes are interacting, generating a consistent particle size.

7.4.2 Colloidal Stability of diblock copolymer complexes

The use of these complexes in biological systems requires that they be stable when exposed to biological environments, particularly those with phosphate salts. Complexes made with the diblocks triammonium 3.3kPPO-*b*-2.6kPEO-OH and triphosphonate 3kPPO-*b*-3kPEO-OH (from Tables 1.2 and 1.3) show no increase in size over 24 hours in DI water. The molecular weights compared here are roughly the same. However, in PBS,

the triammonium anchored complexes showed time-dependent aggregation as the magnetite loading was increased (Figure 7.2A).

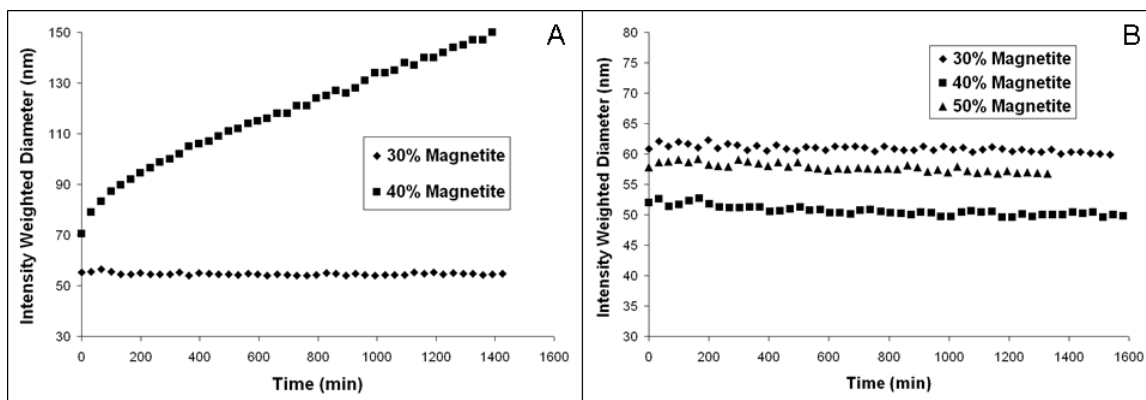


Figure 7.2. DLS of the triammonium 3.3kPPO-b-2.6kPEO-OH complex (A) and the triphosphonate 3kPPO-b-3kPEO-OH complex over time in PBS at 25°C.

By contrast, no aggregation was observed for the triphosphonate complexes, even with a reduction in polymer loading. The differences in size for the triphosphonate complexes are not very significant considering the intensity weighting. The number average diameters of these three complexes are roughly the same.

Slight aggregation can be measured by examining the rate at which the size increases over time. The triphosphonate-anchored 3kPPO-5kPEO did show small amounts of aggregation in PBS (between 0.2 – 0.7 nm h⁻¹). However, because this aggregation was also seen in 0.17 M sodium chloride, we believe this aggregation may be due to an attractive force between the phosphonate anchor groups of one complex with the magnetite of another complex, as discussed in detail in Chapter 6.

7.4.3 Solvation of the hydrophobic poly(propylene oxide) (PPO) Layer

As described in Chapters 3, 4, and 8, the existing extension of the density distribution model can predict the primary particle size of various polymer-magnetite complexes to within 8% over a wide range of magnetite loadings for PEO and PDMS homopolymers. Additionally, the calculated half-life for doublet formation ($t_{1/2}$) can be used to predict, in some cases, particle aggregation and thus correlations between the particle size and magnetite loading can be made. This predictive tool makes it possible to compare

complexes with different polymer molecular weights, loadings, and even different types of polymers.

For studies involving a hydrophobic polymer block, the modeling strategy has been to incorporate the PPO layer as a non-solvated shell while the PEO chains extend out into solution.^{28, 29} Typically, hydrophobic layers have been treated as essentially melts completely covering the surface.³⁰ However, a growing body of evidence indicates that this may not be the case, particularly at elevated temperatures or in mixed solvents.³¹⁻³³ SANS is a powerful technique for probing the solvation of polymer layers attached to colloidal particles.

7.4.3.1 Small Angle Neutron Scattering to Determine PPO Solvation

Neutron scattering is analogous to light scattering, especially in the calculation of structure factors and scattering vectors. While light scattering relies on interactions in the electron cloud, neutron scattering relies on interactions between the neutrons and the atomic nucleus. These nuclear interactions are more short-range than interactions with electrons. This means neutron scattering has the main advantage of an increased penetration depth due to the small interaction range. However, this also means that neutrons are only weakly scattered. Weak scattering combined with the fact that neutron beams typically have much lower flux compared to electron beams means that neutron scattering is a signal-limited technique. This means that no one instrument can be used to study all neutron scattering and so a large array of instruments have been developed to study neutron scattering.^{34, 35}

Small angle neutron scattering (SANS) is one of these techniques and is used to probe molecular structures at the nanoscale. The angles in SANS typically vary from 0.2°-20° which corresponds to a scattering vector (q) range of 0.001 Å⁻¹ to 0.45 Å⁻¹ and d-spacings of 14 Å – 6,300 Å. A principal advantage of SANS is the ability to use deuterium-labeled samples to increase contrast.³⁵

Neutron scattering is based on the scattering length densities of the material, which is the ratio of the scattering length per molecule and the molecular volume of the material.³⁵

Table 7.4 shows scattering length densities for some relevant materials for this study.³⁶

Table 7.4. Neutron scattering length densities for relevant materials.³⁶

Material	SLD ($\times 10^{-6} \text{ \AA}^{-2}$)
Magnetite	6.97
H ₂ O	-5.58
D ₂ O	6.38
DPPO-PEO	5.06
PPO-PEO	1.51

Water and deuterated water are on opposite ends of the scattering length density spectrum. Also, the deuterated and non-deuterated polymers also show significant differences. Thus, through mixing the solvents in different ratios, different scattering patterns can be obtained.

For this study, magnetite complexes were made with two different diblock copolymers, each with triammonium anchor blocks: a 3.3kPPO-*b*-2.6kPEO-OH block copolymer and a 3.1kDPPO-*b*-3.4kPEO-OH block copolymer, where again DPPO is used to describe a deuterated PPO block. The details of the synthesis and characterization of the 3.3kPPO-*b*-2.6kPEO-OH block copolymer were given in Chapter 4, and the synthesis of the deuterated diblock copolymer follows the same general procedure using a deuterated PO monomer. Due to differences in the scattering length density of the polymers, different scattering patterns should emerge. Figure 7.3 depicts the scattering that would be expected from each polymer coating in 100 % water.

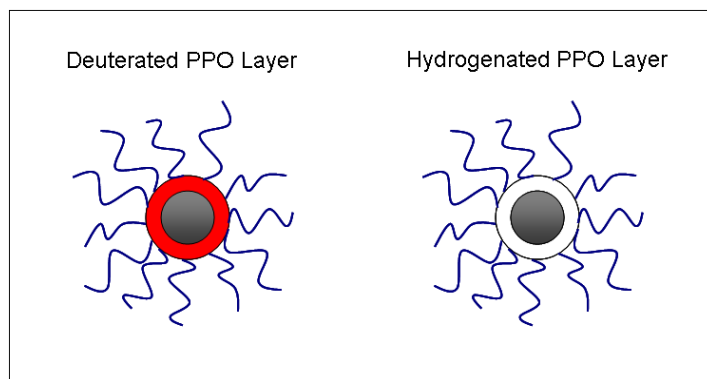


Figure 7.3. The difference in scattering in 100% H₂O between a magnetite complex with an attached non-solvated, deuterated PPO layer and a complex with a nonsolvated, hydrogenated PPO layer.

Because the deuterated polymer has such a high scattering length density compared to the hydrogenated polymer, there is much more contrast between the solvent (water) and the deuterated polymer. Also, there is a high level of scattering from the magnetite. Thus, the scattered intensity for the hydrogenated polymer should be significantly less than for the deuterated polymer. In this case, the PEO layer is so solvated that the effective scattering length density is very close to the solvent. By mixing H₂O and D₂O, the scattering intensities of each component change based on the match between the component scattering length density and the solvent scattering length density. This is analogous to light scattering in that no scattering occurs if the refractive index of the solvent matches that of the material.

SANS was performed at the National Institute for Standards and Technology in Gaithersburg, Maryland using the neutron guide 7 (NG-7), a 30 m SANS instrument that measures artifacts ranging from 1-500 nm. The measurements were carried out at a polymer-magnetite complex concentration of approximately 10 mg mL⁻¹ while varying the D₂O:H₂O ratio from 0 to 100 in 20% increments. Measurements were taken at three scattering distances (1, 4, and 13 meters) and a composite figure was assembled using IGOR Pro 5 software.

Figure 7.4 shows the SANS results for both diblock copolymer-magnetite complexes. The target composition for each complex was 35% magnetite - TGA determined the deuterated complex was 35% and the hydrogenated complex was 37% magnetite.

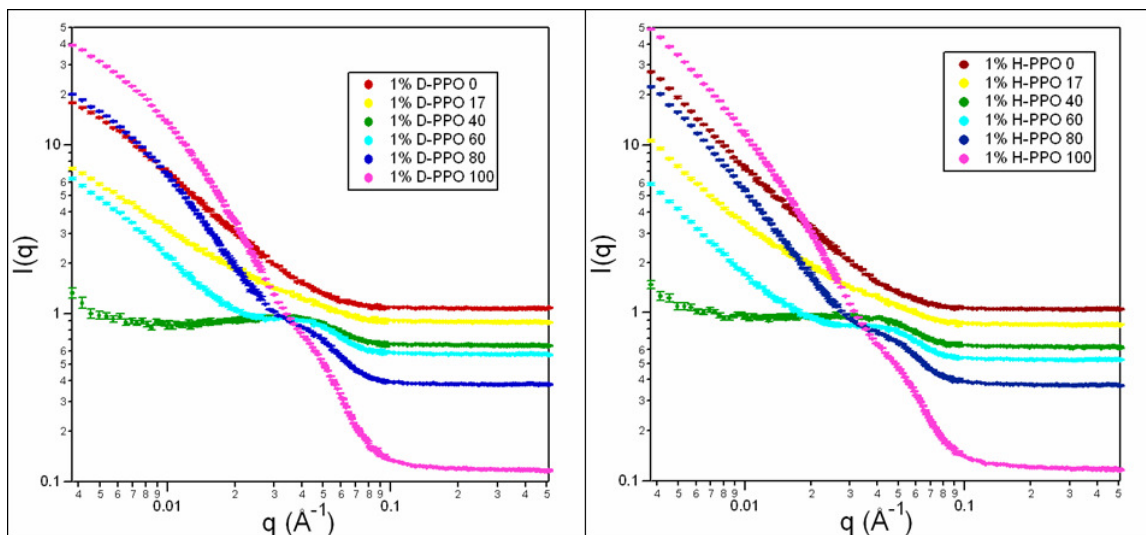


Figure 7.4. SANS data for magnetite complexes stabilized with the 3.1kDPPO-b-3.4kPEO-OH (left) and 3.3kPPO-b-2.6kPEO-OH (right) block copolymers at the indicated H₂O/D₂O ratios.

The most striking result from Figure 7.4 is that there are practically no significant differences between the scattering patterns. This indicates that the only significant scattering taking place is from the magnetite nanoparticles. The only way that both the hydrogenated and deuterated PPO could exhibit the same scattering pattern is if they are heavily solvated. The solvation of the PPO layers would cause the average scattering length density of the PPO layer to approach that of the solvent. That appears to have happened as the scattering patterns from each complex are virtually identical.

The most significant difference in the scattering patterns in Figure 7.4 occurs at low values of the scattering vector q , where, for some of the H₂O/D₂O ratios, the slopes of the complexes made with the deuterated diblock are less than those of the complex made with the hydrogenated diblock. At low q values, the scattering intensity $I(q)$ typically levels off at values corresponding to the size of the largest aggregate in solution. The lower slopes of the $I(q)$ vs q plots for the complexes made with the deuterated diblock

suggest that they are less aggregated than those made with the hydrogenated diblock. This would be consistent with the trend discussed in Chapter 4, where for the same molecular weight PPO block, an increase in the PEO molecular weight decreased the equilibrium cluster size.

To probe the aggregation effect further, the hydrodynamic diameters of the complexes were measured using dynamic light scattering at 0.05 mg/mL after filtration with a 100 nm Whatman Anotop aluminum oxide filter, and these results are shown in Table 7.5.

Table 7.5. DLS of SANS samples shows similar results to other diblock copolymer complexes.

Sample	% Magnetite	Solvent	D _i (nm)	D _v (nm)	D _n (nm)
3.1kDPPO- <i>b</i> -3.4kPEO-OH	35	H ₂ O	64.6	39.6	30.7
3.1kDPPO- <i>b</i> -3.4kPEO-OH	35	D ₂ O	49.3	32.5	24.7
3.3kPPO- <i>b</i> -2.6kPEO-OH	37	H ₂ O	51.8	34.6	26.0
3.3kPPO- <i>b</i> -2.6kPEO-OH	37	D ₂ O	48.2	32.4	25.0

As illustrated in Table 7.5, the value of D_n of the complex made with the deuterated diblock is essentially the same as the size of the hydrogenated diblock, which is the same result as shown in Tables 7.2 and 7.3. This latter comparison provides a useful consistency check. There is a slightly larger size for the deuterated polymer in H₂O, but the number average diameter is not much larger, indicating that the flocculation observed is rather minimal. Thus, it appears that the differences observed in the slopes of the SANS data are either not a function of the aggregation behavior of the samples or that the SANS is more sensitive to aggregation than the DLS.

To probe the solvation of the PPO block further, the sizes of selected complexes made with fully hydrogenated diblocks described in Tables 7.2 and 7.3 were measured by DLS as a function of temperature. Since PPO becomes more hydrophobic (less solvated by water) as the temperature is increased³⁷, it was hypothesized that a change in the solvation of the PPO layer could affect the particle size as the temperature is increased (Figure 7.5).

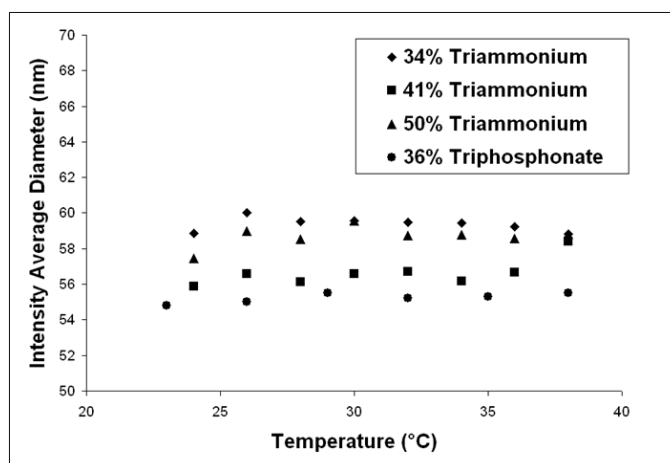


Figure 7.5. DLS measurements of magnetite complexes made with hydrogenated diblocks with triammonium and triphosphonate anchors at various wt% polymer loadings in 100% H₂O.

No change in diameter was observed for temperatures ranging from 22–38 °C. While this is not a definitive test of PPO solvation, it does indicate that whatever changes occur in the degree of solvation of the PPO layer are not sufficient to appreciably alter the complex diameter.

7.4.3.2 QCM-D to Compare Adsorption of Various Polymer Brushes

In order to probe further the issue of the solvation of the PPO block, the adsorption of PPO-PEO diblocks was compared to that of PEO homopolymers that were adsorbed onto magnetite-coated crystals in a quartz crystal microbalance with dissipation monitoring (QCM-D). As mentioned in Chapter 2, QCM-D is advantageous over QCM in that the dissipation of the film can be measured with the former, but not with the latter. The dissipation factor is determined by the damping effect of the film on the quartz crystal that slows the oscillation after the voltage is cut off. In essence, the dissipation factor is the energy dissipated per oscillation per the total energy stored in the system. Thus, direct comparison of the dissipation of two different materials does not require any viscoelastic modeling. This dissipation can be related to the rigidity (in terms of the viscoelasticity and elastic modulus) of the adsorbed polymer film.^{38, 39} It was hypothesized that, if a PPO-*b*-PEO-OH diblock copolymer is completely solvated, that

should be observable using QCM-D technology as a similar change in dissipation to a PEO-OH homopolymer with a similar molecular weight to that of the total diblock. This was done using a Q-Sense E-4 system in conjunction with an Ismatec IPC centrifugal pump flowing the polymer solution through titanium flow cells containing iron oxide coated quartz crystals at $25 \pm 0.1^\circ \text{C}$.

This experiment compared the adsorption of a triammonium-terminated 5kPEO-OH homopolymer (D.P. ~ 113) and a triammonium-terminated 3.3kPPO-*b*-2.6kPEO-OH diblock copolymer (D.P. ~ 116) by adsorption of the polymer from solutions flowed past the crystals in the E-4 flow cell. The 5kPEO-OH homopolymer was synthesized as described in Chapter 4, section 4.3.2 and had a molecular weight distribution of approximately 1.1. Figure 7.6 shows the adsorption characteristics of each polymer on a quartz crystal sputter coated with Fe_3O_4 and cleaned with oxygen plasma. Each polymer was introduced to the QCM crystal in the E-4 flow cell at a concentration of $30 \mu\text{g mL}^{-1}$ and a flow rate of $500 \mu\text{L min}^{-1}$.

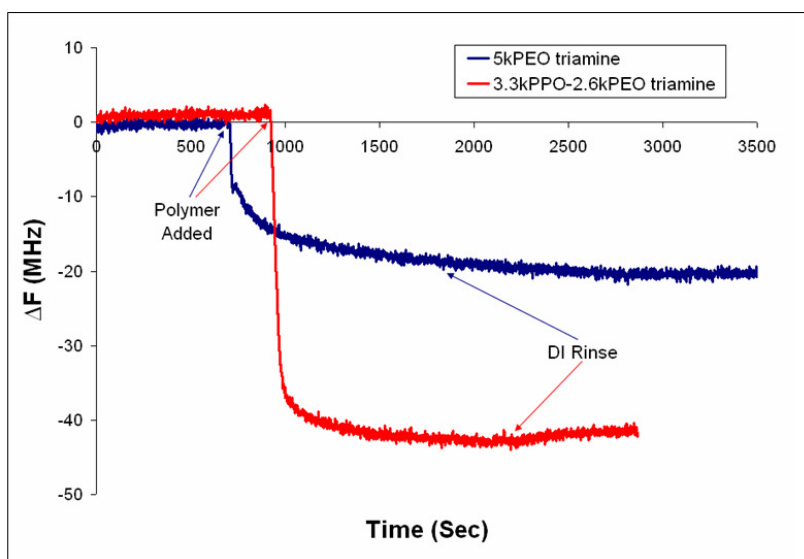


Figure 7.6. Adsorption of the triammonium-terminated 5kPEO-OH homopolymer and 3.3kPPO-*b*-2.6kPEO-OH diblock copolymer from DI water at 25°C onto an Fe_3O_4 -coated quartz crystal. The level of the adsorption of the diblock is about twice that of the homopolymer.

Note that far more diblock copolymer adsorbed to the iron oxide surface than the homopolymer (0.25 and 0.14 chains nm^{-2} and 2.5 and 1.2 mg m^{-2} , respectively) and that the adsorption kinetics are significantly quicker for the diblock also. This is most likely

due to the affinity of the PPO layer to the iron oxide surface compared to the thermodynamics of staying in solution. The higher adsorbed amount for the diblock means that there should be more steric repulsion between polymer chains for the diblock than for the homopolymer, very likely producing a more rigid film and a smaller change in the dissipation factor. However, because the thickness was not independently measured, this may not be the case. Additionally, a non-solvated layer at the iron oxide surface should also generate a more rigid film and a smaller change in dissipation factor. However, Figure 7.7 shows that the dissipation factors for both the 5kPEO-OH homopolymer and 3.3kPPO-*b*-2.6kPEO-OH diblock copolymer are virtually the same.

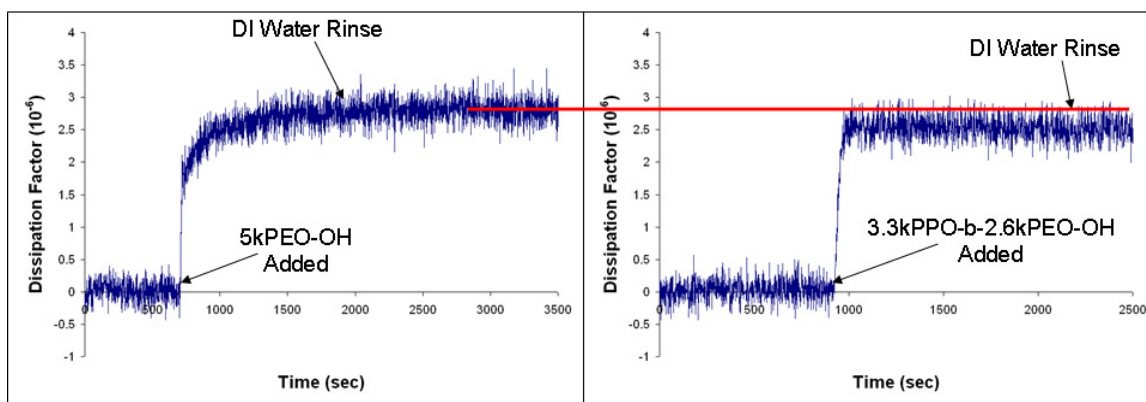


Figure 7.7. Adsorption of the triammonium-terminated 5kPEO-OH homopolymer and 5.6kPPO-*b*-3.8kPEO-OH diblock copolymer from DI water at 25°C onto an Fe₃O₄-coated quartz crystal. The dissipation factor of both polymers is nearly the same.

This is consistent with the hypothesis that the PPO layer is at least somewhat solvated. Otherwise, the dissipation factor would be considerably less with such a small hydrophilic block extending out from a non-solvated layer. Each polymer has approximately the same degree of polymerization, and so there should be a difference in the rigidity of the film were the PPO layer not somewhat solvated.

To probe the effect of diblock composition, the change in frequency and dissipation for a 5.6kPPO-*b*-3.8kPEO-OH diblock copolymer (D.P. ~ 183) was also measured (Figure 7.8).

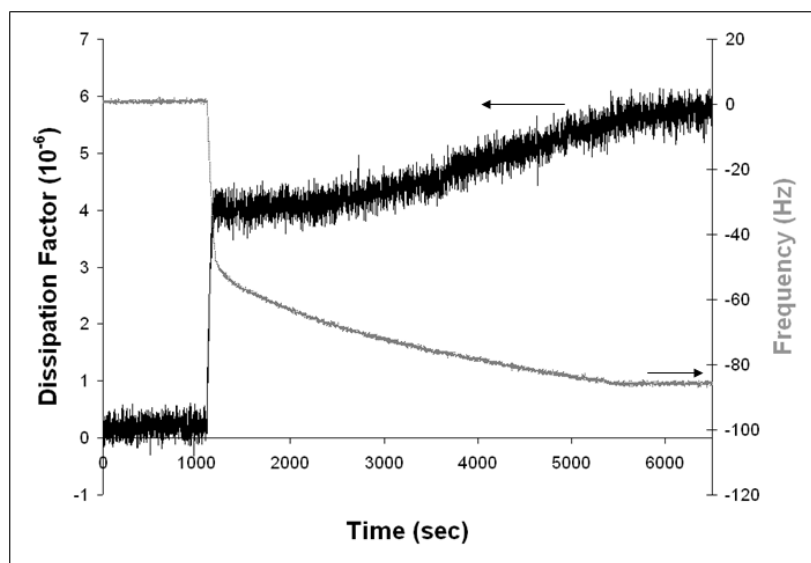


Figure 7.8. Adsorption of the triammonium-terminated 5.6kPPO-*b*-3.8kPEO-OH diblock copolymer from DI water at 25°C onto an Fe₃O₄-coated quartz crystal. The dissipation factor is much higher than the dissipation for the polymers shown in Figure 7.7.

Note that the dissipation factor is more than twice that for the polymers shown in Figure 7.7, indicating a much softer film. Note also the adsorbed amount is about twice that of the 5.6kPPO-*b*-3.8kPEO-OH diblock copolymer. Since the PEO block is fairly short (DP = 86), it appears that the PPO block is also solvated for this polymer. While the QCM-D is sensitive enough to detect differences in the dissipation, it should be noted that these measurements do not confirm solvation of the layer, but merely point towards that possibility. Use of a technique such as neutron reflectivity would likely be a more definitive measure of the solvation of the PPO layer on a flat surface. Additionally, these polymers were adsorbed onto the iron oxide crystals from water while the polyethers are adsorbed onto the magnetite nanoparticles in chloroform. This most likely affects the brush density, as there may be significant electrostatic interactions between the charged anchor groups in water that would not be significant in a low dielectric constant medium such as chloroform. This is illustrated by the typical magnetite nanoparticle, which for 30% magnetite loading has approximately 10 mg of polymer per square meter, compared to 2.5 mg m⁻² and 1.2 mg m⁻² for the 3.3kPPO-*b*-2.6kPEO-OH complex and 5kPEO-OH complex, respectively.

7.4.4 Relaxation behavior of diblock copolymer complexes

A major advantage of magnetite clusters as opposed to larger particles of magnetite is that the clusters should retain their superparamagnetic behavior, but be affected much more strongly by an applied magnetic field. Thus, the cluster can be manipulated in ways that a 10 or 20 nm superparamagnetic particle cannot, but does not exhibit ferromagnetic behavior below the Curie temperature.

A magnetic component can be incorporated into the extended DLVO theory as an attractive term and is calculated as shown in equation 7-10.

$$V_M = \frac{8\pi\mu_o a^3 M^2}{9\left(\frac{h}{a} + 2\right)^3} \quad (7-10)$$

Here, a is the particle radius, M is the magnetization (saturation value of 345,000 A/m), μ_o is the magnetite permeability in vacuum ($1.26\text{e-}6$ Tm/A), and h is the surface to surface separation of two particles.^{40, 41} Equations 7-11 and 7-12 adjust the magnetic potential for systems of clusters where there is space between the particles where x_{mag} is the mass fraction of magnetite, x_{in} is the mass fraction of interstitial space, and ρ_{in} is the density of the interstitial space.

$$V_M = -\frac{8\pi\mu_o (x_{\text{mag}} a)^3 M^2}{9(h/(x_{\text{mag}} a) + 2)^3} \quad (7-11)$$

$$\rho_{\text{cluster}} = x_{\text{mag}} \rho_{\text{mag}} + x_{\text{in}} \rho_{\text{in}} \quad (7-12)$$

For this particular treatment, the cluster was assumed to be hexagonal close packed and 74% of the interior cluster volume, with the other 26% attributed to the polymer. Using the methodology for the modified DLVO theory presented in equations 3-10 through 3-14 from Chapter 3, Figure 7.9 illustrates how the range of the van der Waal's potential and magnetic potential change as the number of particles in the cluster increase as the number of magnetite particles in the cluster, n , increases.

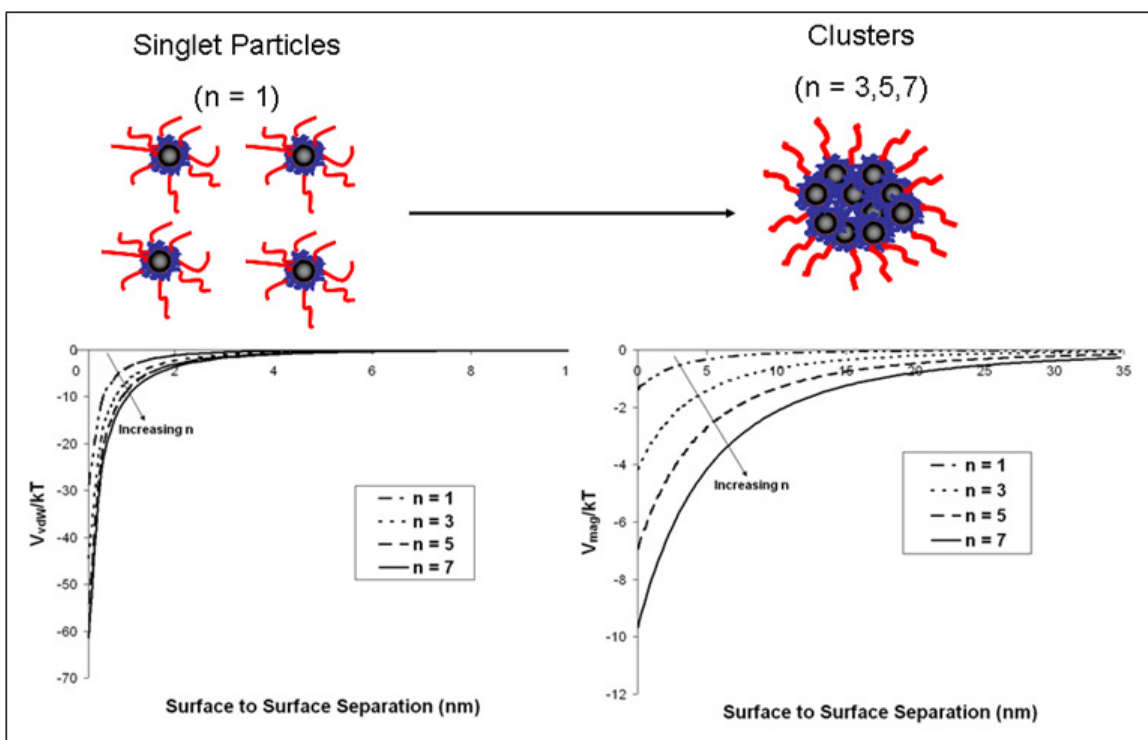


Figure 7.9. Modified DLVO potential energy diagrams for polymer-stabilized magnetite particles and clusters. In the latter, the number of magnetite particles per cluster, n , is varied. The range of the magnetic potential increases (right) but the range of the van der Waal's potential does not (left) as n increases.

While the assumption of hexagonal close packing may overestimate the volume fraction of magnetite in a cluster, the principle remains that the *range* of the magnetic force *increases* while the range of the van der Waal's force does not. This means that formation of a magnetite cluster of a particular size allows for tuning of the colloidal stability with an external field, as shown in Figure 7.10. A secondary potential energy minimum can be induced when a magnetic field is applied. However, this potential energy diagram also indicates that when the magnetic field is removed, the particles should redisperse in solution due to Brownian motion.

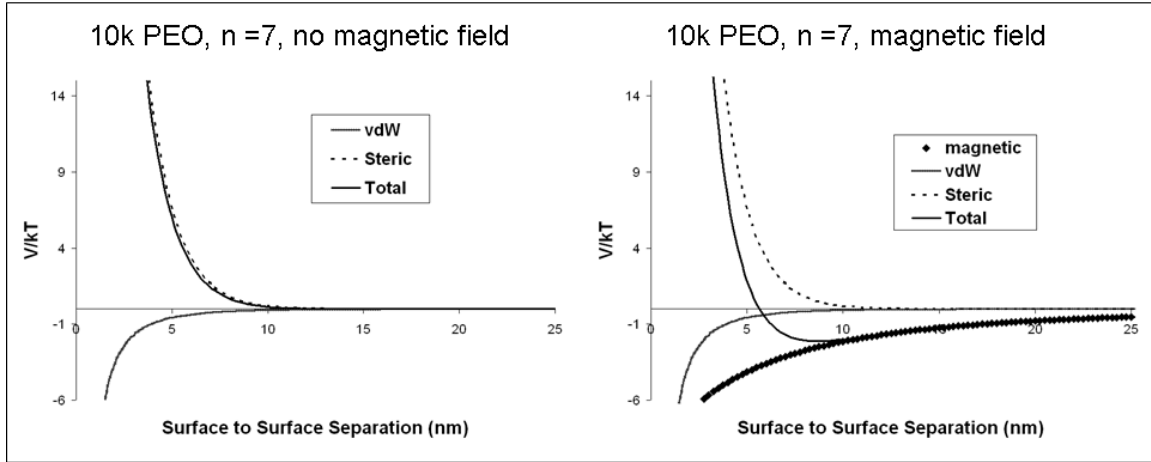


Figure 7.10. A secondary potential energy minimum can be induced by a magnetic field when the cluster size gets large enough (assumes 10kPEO with a surface coverage of 1 chain nm⁻²).

Additionally, the T_2 relaxation can be affected by aggregation of the magnetic particles. Roch et al. studied the effect of aggregation on the T_2 relaxation and found that the relaxation rate increases with the radius as shown in equation 7-13.

$$\frac{1}{T_2} = \frac{16f_a \Delta\omega R_a^2}{45D} \quad (7-13)$$

Here, f_a is the volume fraction occupied by the clusters, $\Delta\omega$ is the difference in angular frequency between the local field and the bulk, R_a is the cluster radius, and D is the diffusion coefficient of water.⁴²

However, when the size of the magnetic sphere gets too large and the translational diffusion time is longer than a diffusion time denoted $\tau_L = (1.49/\Delta\omega)x^{1/2}(1.52 + f_a x)^{5/3}$, the relaxation rate then decreases with the particle radius as shown in equation 7-14, where $x = \Delta\omega(TE/2)$ and TE is the repetition time of the 90° radio frequency pulse.⁴²

$$\frac{1}{T_2} = \frac{1.8f_a x^{1/3}(1.52 + f_a x)^{5/3} D}{R_a^2} \quad (7-14)$$

T_2 relaxation can also be increased through inhomogeneities in a magnetic field.^{43, 44} Figure 7.11 illustrates how a magnetite particle can significantly alter the local magnetic field and how even just a small cluster ensures that the magnetic field is extremely inhomogeneous, speeding up dephasing of the protons' angular momentum.

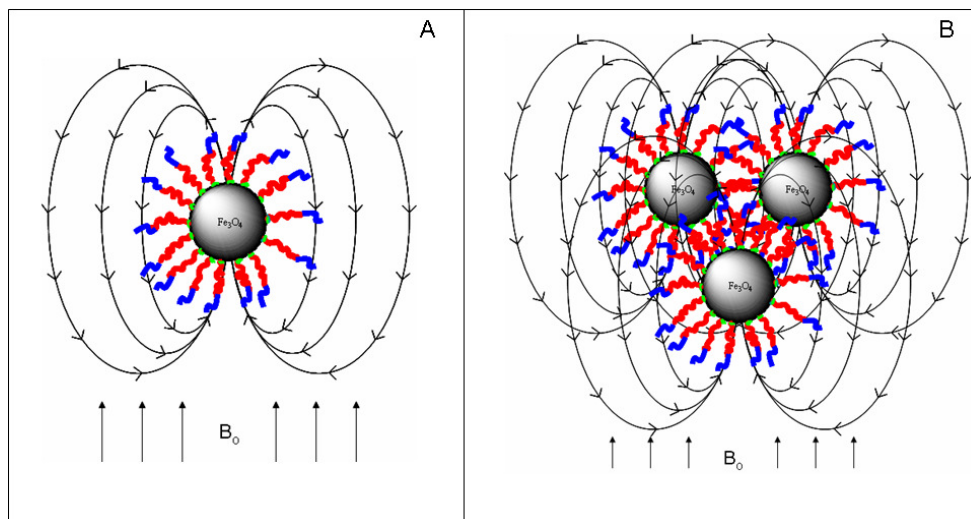


Figure 7.11. The local magnetic field becomes extremely inhomogeneous even for small magnetic clusters.

At this point, only the relaxation behavior of the ammonium diblock complexes has been measured. However, it is worth looking at the colloidal stability and size of a homopolymer complex first, this one with tri-zwitterionic phosphonate anchors (Figure 7.12).

Figure 7.12A shows potential energy diagrams for each of the three polymer loadings and Figure 7.12B shows the relaxation data in conjunction with DLS measurements. Typical commercial relaxation agents exhibit R_2 relaxivities far below those shown by these complexes (107 , 53 , and $72 \text{ (s-mM Fe)}^{-1}$ for ferrumoxides, ferrumoxtran, and ferumoxsil, respectively).¹¹

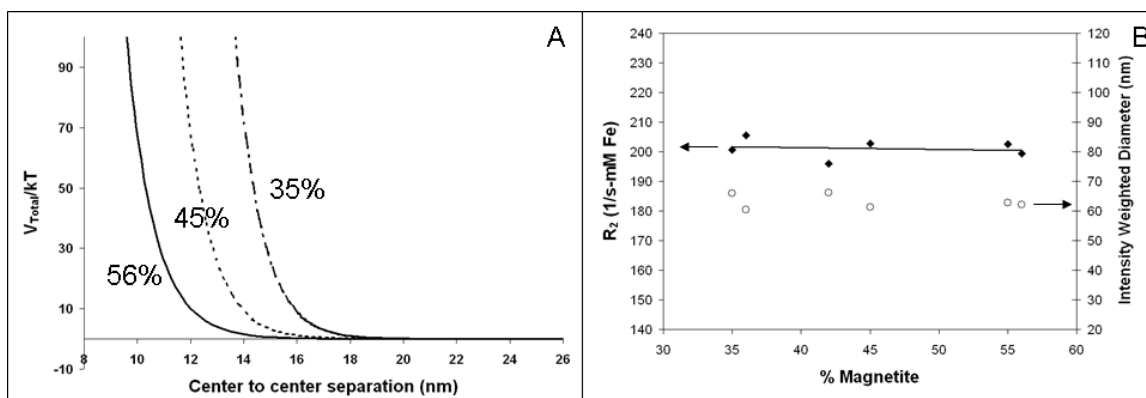


Figure 7.12. Tri-zwitterionic phosphonate-anchored 3.4kPEO-OH complexes in DI water at 25°C – modified DLVO energy diagram as a function of wt% polymer loading (A) and relaxivities and DLS intensity weighted hydrodynamic diameters (B).

The potential energy diagram predicts that none of the materials should aggregate at all, and this is observed with very little change in diameter with reduction in polymer loading. The DLS measurements are in good agreement with the model and indicate that the complexes are primary particles. Additionally, there is almost no change in relaxivity of the materials (note that this measurement is per mM iron) with a reduction in polymer loading. This is not the case for the diblock complexes, whose relaxation behavior is illustrated in Figure 7.13.

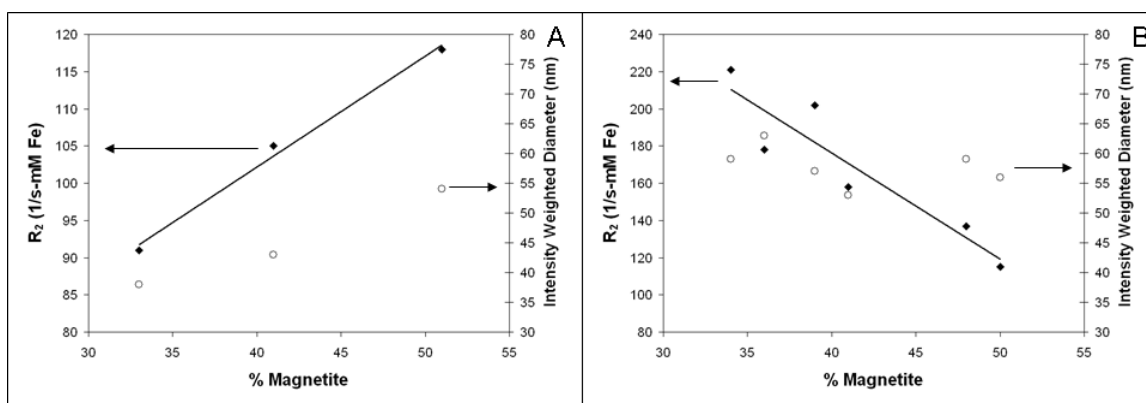


Figure 7.13. Relaxation behavior for triammonium 3.3kPPO-*b*-4.8kPEO-OH (A) and 3.3kPPO-*b*-2.6kPEO-OH (B) complexes.

Opposite trends are observed here - the 3.3kPPO-*b*-4.8kPEO-OH complex shows an increase in relaxation with a decrease in polymer loading while the 3.3kPPO-*b*-2.6kPEO-

OH complex shows an increase in relaxation with an *increase* in polymer loading. Again, the relaxivity of 220 is more than twice that reported in the literature for commercially available materials.¹¹ From the light scattering data for the 3.3kPPO-*b*-4.8kPEO-OH complexes, it appears that reducing the polymer loading causes an increase in particle size, i.e. clustering occurs, and most likely the increase in relaxivity. There also appears to be a slight increase in size with an increase in polymer loading for the 3.3kPPO-*b*-2.6kPEO-OH complex. Thus, it appears that aggregation is a primary cause of the observed increases in relaxivity, but that the aggregation must occur due to two mechanisms. The first mechanism (for the 3.3kPPO-*b*-4.8kPEO-OH complex, i.e. the complex with the longer PEO brush) is the same as presented in chapter 4 where a reduction in polymer loading reduces the steric repulsion, thus causing aggregation. The second mechanism (for the 3.3kPPO-*b*-2.6kPEO-OH complex) must be due to the polymer because the size stays relatively constant with an increase in polymer loading, meaning that the complex is not becoming more dispersed with an increase in steric repulsion. In this case, it is hypothesized that a hydrophobic interaction between the PPO layers of neighboring magnetite particles causes aggregation thus causing the relaxivity to increase. Additionally, none of these DLS measurements were done in the presence of an applied magnetic field. It is distinctly possible that the behavior of both complexes is significantly different in a magnetic field, particularly for complexes that are already aggregating. Future work will need to utilize light scattering that is coupled with the ability to apply a magnetic field of significant strength.

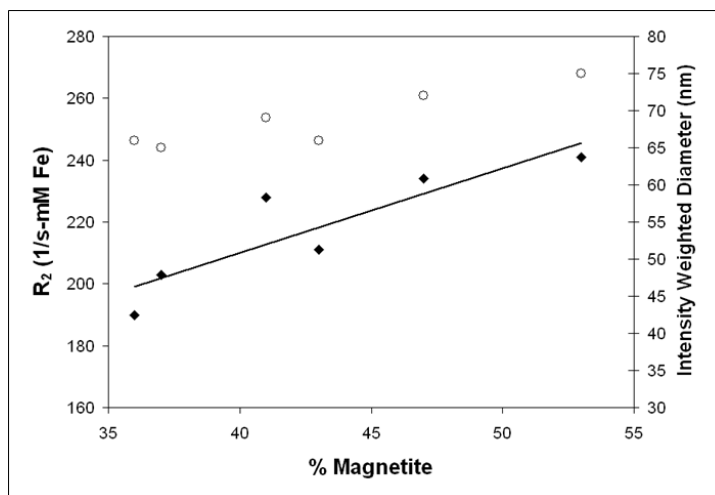


Figure 7.14. Relaxation data vs. magnetite loading for the tri-zwitterionic phosphonate 3kPPO-*b*-5kPEO-OH complexes.

The same phenomenon as observed for the triammonium 3.3kPPO-*b*-4.8kPEO-OH complex was also observed for the tri-zwitterionic phosphonate 3kPPO-*b*-5kPEO-OH as shown in Figure 7.14. Aggregation of the complexes is observed with an increase in magnetite loading as is an increase in relaxivity. However, these complexes have significantly larger relaxivities than their ammonium counterparts, which may be attributable to the strong interaction of phosphonate groups between complexes as discussed in Chapter 6.

The presence of a hydrophobic interaction for the triammonium 3.3kPPO-*b*-2.6kPEO-OH complex is further bolstered by cell culture experiments measuring rhodamine R123 accumulation of multi-drug resistant cancer cells (MCF7/ADR) after exposure to the magnetite complexes. This technique has been used to determine cellular uptake due to exposure of these same cells to Pluronics®.^{17, 18} In these experiments, the hydrophobic to hydrophilic ratio affects the cellular uptake, as has been seen with Pluronics®.

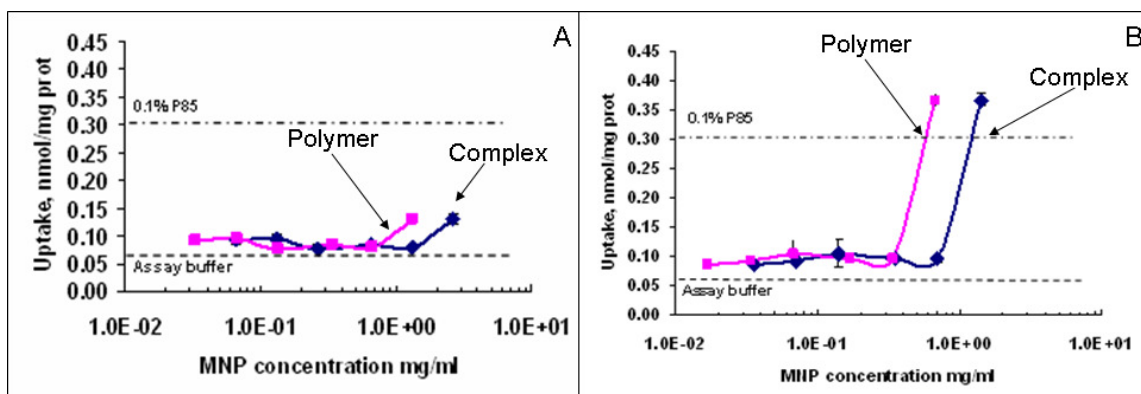


Figure 7.15. Measure of R123 accumulation of 51% magnetite 3.3kPPO-*b*-4.8kPEO-OH (A) and 48% magnetite 3.3kPPO-*b*-2.6kPEO-OH (B) complexes in MCF7/ADR cells.

While it is not clear whether this increased cellular uptake is due to hydrophobic interactions between the complex and the cell membrane, clustering of the complexes, or possibly both, it does indicate that the hydrophobic to hydrophilic ratio of the diblock copolymer is critical to uptake.

7.5 Conclusions

Amphiphilic diblock copolymer-stabilized magnetite nanoparticle complexes were synthesized and characterized for two different molecular weights and two different anchor groups. Both the triammonium and triphosphonate-anchored complexes exhibited the same trends in size with changes in magnetite loading. However, the brush model fit the triphosphonate data considerably better. It is hypothesized that the PPO layer in these complexes is less solvated than that in the triammonium complexes but this has not been proven yet.

However, both SANS and QCM-D data indicate a degree of solvation of the PPO layer that is fairly unexpected. This solvation is in stark contrast with an assumption in the brush model that the PPO layer is a non-solvated shell surrounding the magnetite core. The differences in size between anchor groups may be due to their affect on PPO solvation. There is no increase in size with a change in temperature which means that the degree of solvation is not so large to appreciably affect the mean polymer-particle distribution appreciably (i.e. the change is less than 1-2 nm).

Varying the block copolymer composition leads to considerably different behavior in both relaxivity measurements and in cell uptake experiments. For both experiments, the presence of a smaller hydrophilic PEO brush - while keeping the PPO molecular weight constant – led to what is likely an attractive hydrophobic interaction between complexes that, in the case of relaxivity, leads to enhanced relaxivity due to cluster formation. Further characterization of this hydrophobic interaction will be critical to control cell uptake and contrast enhancement.

Both the triammonium and triphosphonate-anchored complexes were stable in DI water. However, as the polymer loading was reduced for the 3.3kPPO-b-2.6kPEO-OH triammonium complex, flocculation was observed in phosphate buffered saline (PBS). This behavior was not observed for the 3kPPO-b-3kPEO-OH triphosphonate complex. This is in agreement with comparisons in Chapter 5 and 6 of the anchor groups.

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8 The Design of Well-Defined Polydimethylsiloxane-Magnetite Complexes

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This chapter has been accepted for publication in Polymer

Dr. Jonathan Goff synthesized the PDMS discussed in sections 8.3.2 and 8.3.3. Dr. Goff also synthesized the PDMS-magnetite complexes discussed in sections 8.3.4 and 8.3.5. Philip Huffstetler aided in the synthesis of the PDMS discussed in sections 8.3.2 and 8.3.3.

8.1 Abstract

Magnetic nanoparticles have numerous applications, particularly in biological systems for drug delivery, cell targeting, and as MRI contrast agents. However, much of the current research on these materials is focused on a top-down approach focusing on biological efficacy of commercial materials and other aggregated materials. Rapid improvement in biomaterials would be greatly enhanced by starting with a well-defined system with a controlled, narrow particle size distribution. To that end, this paper addresses the synthesis and characterization of magnetite nanoparticles coated with tricarboxylate-functional polydimethylsiloxane (PDMS) oligomers of varying molecular weight. Two methods—co-precipitation of iron chlorides and high temperature reduction of iron(III) acetylacetonate—were used to synthesize the magnetite nanoparticles and compared. Through implementation of a polymer brush model, it was determined that the co-precipitation synthesis method required multiple magnetic separations and significant material loss to produce a well-defined particle distribution. Conversely, the high temperature synthesis method showed a well-defined particle distribution without any magnetic separation. Through adjustment of critical design parameters such as polymer

loading and molecular weight, the diameters of the complexes were predicted to within seven percent of experimental values. Thus, we now have a tool that allows for the design of sterically stabilized single-particle complexes with a specifically tailored particle size. This bottom-up approach should enable the development of biomaterials with more precise control of functionality.

8.2 Introduction

Magnetite nanoparticles have many uses in biomaterials due to their resistance to oxidation and their low cytotoxicities.^{1, 2} Some of these biological applications include uses in cellular therapy, cellular targeting, tissue repair, drug delivery, magnetic resonance imaging (MRI), and hyperthermia.³⁻¹⁴ Biocompatible macromolecules can be adsorbed on the magnetite surface, which allows specific functionality to be built into the polymer-magnetite complexes, such as targeting and imaging agents.^{1, 2, 15-19}

Synthesis of magnetic particles can be achieved through various methods, including wet grinding, co-precipitation, microemulsion and inverse microemulsion methods, thermolysis of organometallic compounds, and reduction of metal salts in aqueous solution.²⁰ The particles must then either be coated with a surfactant to provide steric stability or have their surface modified to produce a repulsive electrostatic force to prevent aggregation.²⁰⁻²² One approach has been to attach soluble non-ionic polymers to a magnetite surface to form a polymer brush. The steric repulsion provided by both the enthalpic and entropic energies arising from confinement of the chains upon the approach of two particles significantly increases colloidal stability.²³ Various polymer stabilizers have been used in conjunction with magnetite, including poly(vinyl pyrrolidone), dextran, polydimethylsiloxane, poly(ethylene oxide), and poly(propylene oxide-*b*-ethylene oxide) diblock copolymers.^{2, 14, 24-29} Through adjustment of polymer loading and molecular weight, extension of the brush can be controlled to produce a stable dispersion.³⁰

Much of the research currently focused on nanoparticle dispersions for biological applications uses an approach that focuses on efficacy of commercial materials or particle

systems that show some degree of aggregation in solution.³¹⁻³⁷ Emerging biomedical applications will require precise control of the size and breadth of the nanoparticle distribution both for determination of biodistribution after administration as well as dose control. Additionally, to our knowledge, no general methodology exists to design polymer-nanoparticle complexes with control of particle sizes within a few nanometers.

Therefore, the focus of our work has been to develop a methodology to allow for the precise design of polymer-nanoparticle complexes. Magnetite nanoparticles stabilized by various molecular weight PDMS stabilizers were used in this study. Two methods were used to synthesize the polymer-magnetite complexes and the materials were compared. The first method, co-precipitation of iron chloride salts with base, required extensive magnetic separation due to aggregation. Through application of a polymer brush model adapted from Vagberg et al.³⁸ and comparison to dynamic light scattering (DLS) measurements, we have been able to determine when separation is necessary and how much material must be removed to achieve a well-defined narrow size distribution.

Due to significant material loss during the magnetic separation steps, a different synthetic route, thermal reduction of organometallic compounds, was also utilized to prepare complexes. The brush model was also applied to this system and comparison to DLS measurements confirmed that no magnetic separation was necessary for this system. This brush model has previously been successfully applied to polyether-magnetite complexes.³⁰ Thus, it appears that the application of this polymer brush model is applicable to significantly different polymer-nanoparticle systems. Additionally, we now have a methodology to determine whether a system is comprised of sterically-stabilized single particles in solution and to predict the size of the complex. This will allow for design of various polymer-nanoparticle complexes for biomedical applications.

8.3 Experimental

8.3.1 Materials

Hexamethylcyclotrisiloxane (D₃, Gelest, Inc., 98%) was dried over calcium hydride prior to use. Cyclohexane (Fischer Scientific, HPLC grade) was stirred with concentrated

sulfuric acid for 48 h, washed with deionized water until neutral and dried over magnesium sulfate. The cyclohexane was then stirred over calcium hydride, fractionally distilled under vacuum, stored over sodium in a nitrogen atmosphere, and distilled just prior to use. Tetrahydrofuran (THF, EMD Chemicals, 99.5%) was refluxed over sodium with benzophenone until the solution reached a deep purple, and fractionally distilled just prior to use. Toluene (Burdick and Jackson, 99.9%) was used as received. 2,2'-Azobisisobutyronitrile (AIBN, 98%), n-butyllithium (2.5 M solution in hexanes) and mecaptoacetic acid (97%) were purchased from Aldrich and used as received. Trivinylchlorosilane (Gelest Inc., 95%) and trimethylchlorosilane (Gelest Inc., 99%) were used as received. Ammonium hydroxide (VWR International) was diluted with Millipore water to yield a 50/50 v/v solution and deoxygenated with nitrogen just prior to use. Iron (III) chloride hexahydrate ($\geq 98\%$) and iron (II) chloride tetrahydrate (99%) were obtained from Sigma-Aldrich and were ground into fine powders and stored under nitrogen prior to use. Iron (III) acetylacetonate ($\text{Fe}(\text{acac})_3$) was purchased from Aldrich and used as received. Hydrochloric acid (EMD, 12.1 M) was added to deionized (DI) water to yield a 3.0 M solution. DI water was deoxygenated just prior to use. Dichloromethane was obtained from EMD Chemicals and used as received. Iron granules (Alfa Aesar, 1-2 mm, 99.98%) were washed repeatedly with a variety of solvents to remove any coating on the surface and subsequently dried overnight in a vacuum oven at 40 °C. The iron granules (6 g) were then placed into a 3-mL syringe packed with glass wool to obtain magnetic separation columns. NdFeB doughnut-shaped magnets were purchased from Engineered Concepts and had an outer diameter of 2.54 cm, an inner diameter of 1.26 cm and were 0.65 cm thick. Magnetic separation columns were prepared by magnetizing the iron-granule-packed syringe with the doughnut-shaped magnets.

8.3.2 Synthesis of trivinylsilyl-terminated PDMS

The synthesis of a targeted $3,000 \text{ g mol}^{-1}$ trivinylsilyl-terminated PDMS is provided. Other molecular weights were prepared in an analogous manner with appropriate ratios of initiator to monomer to control chain length. D_3 (26.5 g, 0.119 mol) was sublimed into a flame-dried round-bottom flask containing a magnetic stir bar and purged with nitrogen. Cyclohexane (26 mL) was added to the flask via syringe and the D_3 monomer was dissolved at room temperature. n-Butyllithium (2.5 M, 3.18 mL, 0.00795 mol) was

added to the reaction flask via syringe and the solution was stirred for 1 h, followed by the addition of THF (10 mL) to the solution as a reaction promoter. The living anionic polymerization was monitored using ^1H NMR, and at ~90% conversion of monomer (~18 h), the polymer was terminated with an excess of trivinylchlorosilane (1.72 mL, 0.0119 mol). The solution was stirred overnight and then concentrated under vacuum at 40 °C. The product was dissolved in 200 mL of dichloromethane, washed three times with DI water (100 mL each), concentrated under vacuum and precipitated into methanol (300 mL). The recovered trivinylsilyl-terminated PDMS oligomer was dried under vacuum at 80 °C overnight. ^1H NMR confirmed the expected chemical structure.

8.3.3 Functionalization of trivinylsilyl-terminated PDMS with mercaptoacetic acid

A representative procedure for functionalizing the PDMS oligomers on one end only is provided below. Other molecular weight oligomers were functionalized in similar reactions. A PDMS oligomer with three carboxylic acid groups on one terminus was prepared via a thiol-ene addition of mercaptoacetic acid across the vinylsilane endgroups. A 3,100 g mol⁻¹ trivinylsilyl-terminated PDMS (10 g, 0.01 mol vinyl) was added to a flame-dried round-bottom flask containing a magnetic stir bar, and dissolved in deoxygenated toluene (10 mL). The reaction mixture was sparged with nitrogen for 2 h to remove oxygen, and then AIBN (2.5 x 10⁻³ g, 1.7 x 10⁻⁴ mol) and mercaptoacetic acid (1.11 mL, 0.016 mol) were added into the flask. The reaction mixture was sparged with nitrogen for 0.5 h and then heated at 80 °C with stirring for 3 h. ^1H NMR was used to observe the quantitative disappearance of the vinyl proton peaks (~6 ppm), indicating completion of the thiol-ene functionalization reaction. The reaction mixture was concentrated under vacuum at 60 °C, and the product was dissolved in methanol (10 mL). DI water was added dropwise to the solution until a white solid precipitate formed, which was collected via vacuum filtration. This precipitation was repeated 3X, and the recovered polymer was dried under vacuum overnight at 80 °C.

8.3.4 Magnetite synthesis via co-precipitation of iron chlorides with ammonium hydroxide

Synthesis of magnetite nanoparticles and subsequent adsorption of a representative carboxylate-functional PDMS dispersion stabilizer onto the nanoparticle surfaces was

achieved via the following procedure. The experimental conditions describe a method to obtain a PDMS stabilizer-magnetite complex comprised of ~30 wt% magnetite and ~70 wt% PDMS as the dispersion stabilizer. Magnetite nanoparticles were prepared using a chemical co-precipitation of iron salts. Iron (III) chloride hexahydrate (3.50 g, 0.013 mol) and iron (II) chloride tetrahydrate (1.28 g, 0.0064 mol) were weighed into separate round-bottom flasks, and each was dissolved in 20 mL of deoxygenated water. The two iron salt solutions were then added to a 500-mL, three-necked, round-bottom flask fitted with an Ultra-Turrax T25 Digital Homogenizer, a pH electrode and a nitrogen purge. The iron salts solution was stirred at 13,000 rpm with the homogenizer and the ammonium hydroxide solution (~20 mL) was added via syringe until the rapidly stirring solution turned black and reached a pH of 9-10. The PDMS dispersion stabilizer (3.5 g) was dissolved in dichloromethane (60 mL), and this solution was added to the basic magnetite dispersion and stirred for 30 min. Aqueous HCl (3.0 M) was then slowly added until a slightly acidic pH was obtained (~12 mL was required to reach pH 5-6). The heterogeneous dispersion was stirred for 1 h, then transferred to a separatory funnel and allowed to separate for 24 h. The dichloromethane layer containing the PDMS-magnetite complex was collected. The dichloromethane layer containing the PDMS-magnetite complex was dried with magnesium sulfate, vacuum-filtered, and concentrated under vacuum. The recovered PDMS-magnetite nanoparticle fluid was washed 3X with methanol (15 mL each) and dried under vacuum overnight at 80 °C. TGA was used to determine the composition of the PDMS-magnetite nanoparticle fluid.

8.3.5 Magnetite synthesis via reduction of Fe (III) acetylacetonate

Magnetite nanoparticles were synthesized using a reduction method adapted from Pinna et al.³⁹ Fe (III) acetylacetonate (2.14 g, 8.4 mmol) and benzyl alcohol (45 mL, 0.43 mol) were added to a 250-mL, three-necked round-bottom flask equipped with a water condenser and overhead mechanical stirrer. N₂ was passed through the solution for 1 h. While stirring under N₂, the solution was heated to 100 °C for 4 h, then the temperature was increased to 205 °C at a rate of ~25 °C h⁻¹. Following 24 h at 205 °C, the reaction was cooled to room temperature, then the magnetite particles were collected with a magnet and the benzyl alcohol was decanted. The magnetite nanoparticles were washed 3X with acetone, then were dispersed in chloroform (20 mL) containing oleic acid (0.3

g). The solvent was removed under vacuum at room temperature, and the oleic acid-stabilized magnetite nanoparticles were washed 3X with acetone. The particles were dried under vacuum for 24 h at 25 °C. The composition of the particles obtained from thermogravimetric analysis (TGA) showed 5% organic residue to 95% magnetite.

A representative method for preparing a targeted composition of 70:30 wt:wt of a PDMS:magnetite complex is provided. Oleic acid-stabilized magnetite nanoparticles (25 mg) prepared as described above were dispersed in chloroform (10 mL) and added to a 50-mL round-bottom flask. A carboxyl-functional PDMS (58 mg) was dissolved in chloroform (10 mL) and added to the dispersion. The reaction mixture was sonicated in a VWR 75T sonicator for 16 h under N₂. The chloroform was removed via evaporation, and the resulting ferrofluid was washed 3X with methanol (15 mL each) to remove excess polymer. A magnet was utilized to collect the magnetite nanoparticles and free PDMS was decanted with the supernatant. The complexes were dried under N₂ at 25 °C and TGA was used to determine the composition of the PDMS-magnetite nanoparticle fluid.

8.3.6 Magnetic separations of PDMS-magnetite nanoparticle fluids

Mefford et al. described the use of magnetic separation techniques to remove aggregates from a 3,000 g mol⁻¹ PDMS-magnetite complex for magnetite made via the co-precipitation method.⁴⁰ In this study, removal of the slightly-aggregated complexes from the total sample was accomplished through repeated magnetic separations in the same fashion. The separation columns were comprised of iron granules tightly packed into 3-mL syringes. The syringes were 6.60 cm in length with an inner diameter of 1.00 cm. The length of the iron granules in the separation columns was approximately 2.45 cm with a diameter of 1.00 cm. Two doughnut-shaped magnets were placed around the circumferences of the iron granule-packed syringes to form the magnetic separation columns, and a Hall probe was used to measure the magnetic field generated by the separation column (~0.1 T). Great care was taken at each step of the column formation to ensure reproducibility.

Two concentrations of the PDMS-magnetite nanoparticle fluids in chloroform (1 mg mL^{-1} and 10 mg mL^{-1}) were investigated in the magnetic separation studies. The concentrated and dilute dispersions of the PDMS-magnetite complexes were passed through five magnetic separation columns. Aliquots of the PDMS-magnetite complexes were collected after the first and fifth magnetic separations and dried under vacuum at 80°C overnight. After each magnetic separation of a PDMS-magnetite complex, the columns were washed with chloroform ($\sim 20 \text{ mL}$) in the absence of a magnetic field to recover the material removed from the bulk sample. The initial material, first separation, fifth separation and column-extracted materials were characterized by DLS, TEM, and TGA.

8.3.7 Characterization

Spectral analyses of compounds were performed using a Varian Unity 400 NMR and a Varian Inova 400 NMR. An Alliance Waters 2690 Separations Module with a Viscotek T60A dual viscosity detector and laser refractometer equipped with a Waters HR 0.5 + HR 2 + HR 3 + HR 4 styragel column set was used for gel permeation chromatography (GPC) analyses. GPC data were collected in chloroform at 30°C . Data were analyzed utilizing a Universal calibration to obtain absolute molecular weights.

TGA was carried out on the tricarboxylate-functional PDMS and PDMS-stabilized magnetite nanoparticles using a TA Instruments TGA Q500. Samples were first held at 110°C for 10 min to remove any residual solvent. Samples were then equilibrated at 30°C and the temperature was ramped at $10^\circ\text{C min}^{-1}$ to 700°C in a nitrogen atmosphere. Char yields were recorded at the maximum temperature. TGA was used to determine the composition of the PDMS-magnetite nanoparticle fluids.

TEM was conducted using a Philips EM-420 field-emission-gun transmission electron microscope. Samples of the polymer-magnetite complexes were dispersed in water through probe sonication and analyzed after being cast onto amorphous carbon-coated copper grids. The eucentric height and focus were set consistently for each sample. The microscope was equipped with a 2000×3000 pixel digital imaging system, and images were acquired at a magnification of 96 kx at a voltage of 100 kV, corresponding to a

resolution of 3.7 pixels nm⁻¹. At least 2000 particles taken from five separate images were used for image analysis. Particle distribution analyses were performed using Reindeer Graphics' Fovea Pro 4 plug-in for Adobe Photoshop 7.0.

A Malvern Zetasizer NanoZS particle analyzer (Malvern Instruments Ltd, Malvern, UK) was used to conduct dynamic light scattering (DLS) experiments of the complexes in chloroform at 25 °C. A 4.0 mW, solid-state He-Ne laser at a wavelength of 633 nm was the incident light source. The NanoZS measures at a scattering angle of 173°, which reduces the effects of multiple scattering and contaminants such as dust. Malvern's Zetasizer Nano 4.2 software was used to calculate intensity, volume and number average diameters utilizing an algorithm that transforms time-varying intensities to particle diameters.⁴¹

Powder X-Ray Diffraction (XRD) patterns were obtained using a Scintag XDS-2000 diffractometer with a Ni-filtered Cu-K α ($\lambda = 0.154$ nm) radiation source. The patterns were obtained at a scan rate of 1.0 2 θ s⁻¹ and were scanned from 10 to 90°. Particle diameters were obtained using the Scherrer formula, which allows for estimation of particle diameter as a function of the width of the diffraction curves.⁴²

8.3.8 Density distribution model to predict sizes of the PDMS-magnetite nanoparticle complexes

Modeling of the PDMS-magnetite complexes to predict their sizes in various solutions was based on methods developed by Mefford, Zhang, and Miles^{29, 30, 40, 43} TEM was used to image the particles and image analysis was performed on the magnetite cores (only the magnetite component of the complexes is visible by TEM). The size distribution of the magnetite was fitted with a Weibull probability distribution as shown in Equation 8-1.

$$P(a) = \frac{c}{b} \left(\frac{a}{b} \right)^{c-1} \exp \left[- \left(\frac{a}{b} \right)^c \right] \quad (8-1)$$

Here, a is the particle radius and b and c are the Weibull shape and scale parameters, respectively.

The average surface area of the magnetite was calculated from the particle size distribution derived from TEM. Combining the average surface area with the average polymer loading per mass of complex (from TGA) gives an average number of chains per magnetite surface area, α , as shown in Equation 8-2.

$$\alpha = \frac{(1 - W_{mag}) N_{Av} \rho_{mag} \int_0^{\infty} a^3 P(a) da}{3 M_n W_{mag} \int_0^{\infty} a^2 P(a) da} \quad (8-2)$$

Here, ρ_{mag} is the density of magnetite (5.21 g cm⁻³),⁴⁴ W_{mag} is the weight fraction of the complex that is magnetite, N_{Av} is Avogadro's number, and M_n is the PDMS number average molecular weight. Equation 8-2 is slightly different than the one previously reported for reasons of self-consistency, but the difference in the number average diameter resulting from this change is less than three percent.^{30, 40, 43} Through application of a modified version of the Vagberg density distribution model,^{30, 38, 40, 43} the average radius of the PDMS-magnetite complexes was calculated as

$$R_m(a) = \left(\frac{8 N_k f(a)^{\frac{1-\nu}{2\nu}} L_k^{1/\nu}}{4^{1/\nu} 3\nu} + a^{1/\nu} \right)^\nu \quad (8-3)$$

where N_k is the number of Kuhn segments, L_k is the Kuhn length (0.81 nm)⁴⁴, ν is the Flory exponent (0.6 for a good solvent), and $f(a)$ is the number of chains per particle, which were calculated from Equation 8-4.

$$f(a) = 4\pi a^2 \alpha \quad (8-4)$$

All of the measurements were performed in chloroform, which is a good solvent for PDMS.

Different modes of the complex distribution were calculated through a Weibull probability distribution fit (Equations 8-5, 8-6, and 8-7) where D_n , D_v , and D_i are the number, volume, and intensity average diameters, respectively.

$$D_n = 2 \int_0^{\infty} R_m(a) P(a) da \quad (8-5)$$

$$D_v = 2 \frac{\int_0^{\infty} R_m(a)^4 P(a) da}{\int_0^{\infty} R_m(a)^3 P(a) da} \quad (8-6)$$

$$D_i = 2 \frac{\int_0^{\infty} R_m(a)^6 P(a) da}{\int_0^{\infty} R_m(a)^5 P(a) da} \quad (8-7)$$

For our purposes, the focus will be on the number weighted average, both from a modeling and a light scattering perspective. The reason is that of these distributions, the number weighted average is the least susceptible to aggregation. Large amounts of aggregation must be present before the number average will change significantly. Thus, comparison of a calculated number average to an experimental average from light scattering allows determination of considerable aggregation.

The brush model can also be used to calculate the pair-pair potential for polymer-particle complexes of the same size as previously described by our group.^{30, 43} The calculation of these potentials is described in equation 8-8.

$$V_{Total} = V_{vdW} + V_M + V_{ES} + V_S \quad (8-8)$$

V_{vdW} is the attractive potential due to van der Waal's interactions, V_M is the attractive potential produced by an applied magnetic field, V_{ES} is the repulsive potential due to electrostatic interactions, and V_S is the repulsive potential due to steric repulsion of the polymer brushes. The electrostatic potential can be calculated as

$$V_{ES} = 2\pi a \epsilon \epsilon_o \psi_o^2 \ln(1 + e^{-\kappa(r-2a)}) \quad (8-9)$$

where ϵ is the dielectric constant of the solvent, ϵ_o is the permittivity of free space, ψ_o is the surface potential, κ is the inverse Debye length, r is the center-to-center separation, and a is the particle radius.⁴⁵ However, chloroform has a significantly lower dielectric constant compared to water (4 vs. 80 at room temperature, respectively). Assuming an

ionic strength comparable to DI water (1×10^{-4}), the repulsive electrostatic interaction is treated as negligible due to the extremely small Debye length. The magnetic potential can be calculated as

$$V_M = \frac{8\pi\mu_o a^3 M^2}{9\left(\frac{h}{a} + 2\right)^3} \quad (8-10)$$

where a is the particle radius, M is the magnetization ($345,000 \text{ A m}^{-1}$), μ_o is the magnetite permeability in vacuum ($1.26 \times 10^{-6} \text{ Tm A}^{-1}$), and h is the surface-to-surface separation of two particles.⁴⁶ The van der Waal's potential can be calculated as

$$V_{vdW} = -\frac{1}{6} A_{eff} \left(\frac{2a^2}{r^2 - 4a^2} + \frac{2a^2}{r^2} + \ln \left(\frac{r^2 - 4a^2}{r^2} \right) \right) \quad (8-11)$$

where r is the center-to-center separation distance of two particles and a is the particle radius. A_{eff} is the effective retarded Hamaker constant calculated according to previous work.^{30, 43}

For the case of a densely adsorbed brush layer, the steric contribution to the interaction potential may be described by the Likos expression.⁴⁷

$$V_s = \frac{5}{18} kT f(a)^{3/2} \left\{ \begin{array}{ll} -\ln \left(\frac{r}{\sigma} + \frac{1}{1 + \frac{\sqrt{f(a)}}{2}} \right); & r \leq \sigma \\ \frac{1}{1 + \frac{\sqrt{f(a)}}{2}} \left(\frac{\sigma}{r} \right) \exp \left(-\frac{\sqrt{f(a)}}{2\sigma} (r - \sigma) \right); & r > \sigma \end{array} \right\} \quad (8-12)$$

Here, r is the center-to-center separation, $f(a)$ is the number of chains per particle, and $\sigma/2$ is the distance from the center of the core to the center of the outermost blob layer.^{45,}

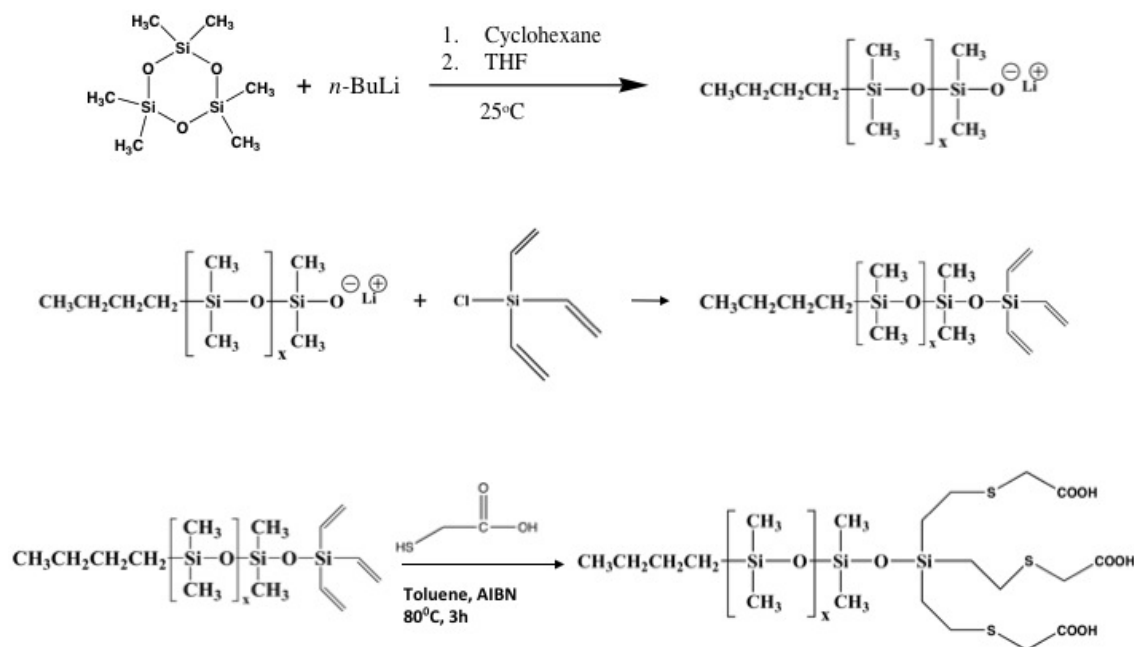
⁴⁷ For this expression, σ is defined as $1.3R_g$, the radius of gyration of the ensemble.⁴⁵ Thus, determination of the onset of a sharp increase in the steric potential is determined by the R_g , which is calculated as done previously.^{30, 43} By taking these potential energy terms in aggregate (van der Waals, magnetic, electrostatic, and steric), a pair-pair

potential energy diagram can be constructed to determine the effect of a magnetic field on the separation of these materials as a function of molecular weight.

8.4 Results and Discussion

8.4.1 Synthesis of PDMS-magnetite complexes

The synthetic procedure for obtaining a tricarboxylate-functional PDMS oligomer has been previously reported (Scheme 8.1).^{24, 40} Great care was taken to purify all solvents and reagents for the D₃ polymerizations to prevent premature termination of the growing PDMS chains. The living anionic polymerization of D₃ yielded polymers with good control over molecular weights and narrow molecular weight distributions, as confirmed by NMR and GPC (Table 8.1). A series of trivinylsilyl-terminated PDMS oligomers were synthesized with targeted molecular weights ranging from 3,000 to 10,000 g mole⁻¹. The molecular weights obtained from GPC and NMR were in close agreement with the targeted values.



Scheme 8.1. Synthesis of tricarboxylate-functional PDMS oligomers for stabilizing fluids and dispersions of magnetite nanoparticles

Table 8.1. Trivinylsilyl-terminated PDMS molecular weights and distributions

Targeted Trivinyl- PDMS MW	M _n (g mol ⁻¹)		PDI
	¹ H NMR	GPC	
3,000	3,050	3,150	1.08
5,000	5,100	4,830	1.09
7,000	6,900	6,910	1.05
10,000	10,200	9,850	1.07

Tricarboxylate-functional PDMS magnetite dispersion stabilizers were synthesized via the ene-thiol addition of mercaptoacetic acid to the trivinylsilyl-terminated PDMS series. Complete conversion of the vinyl groups was promoted by thoroughly deoxygenating the reaction mixtures before the additions. Excess mercaptoacetic acid was removed from the polymer during isolation by precipitating the polymer into a methanol/water mixture, yielding well-defined tricarboxylate-functional PDMS oligomers. The addition of mercaptoacetic acid across the vinylsilane groups was confirmed by ¹H NMR (Figure 8.1).

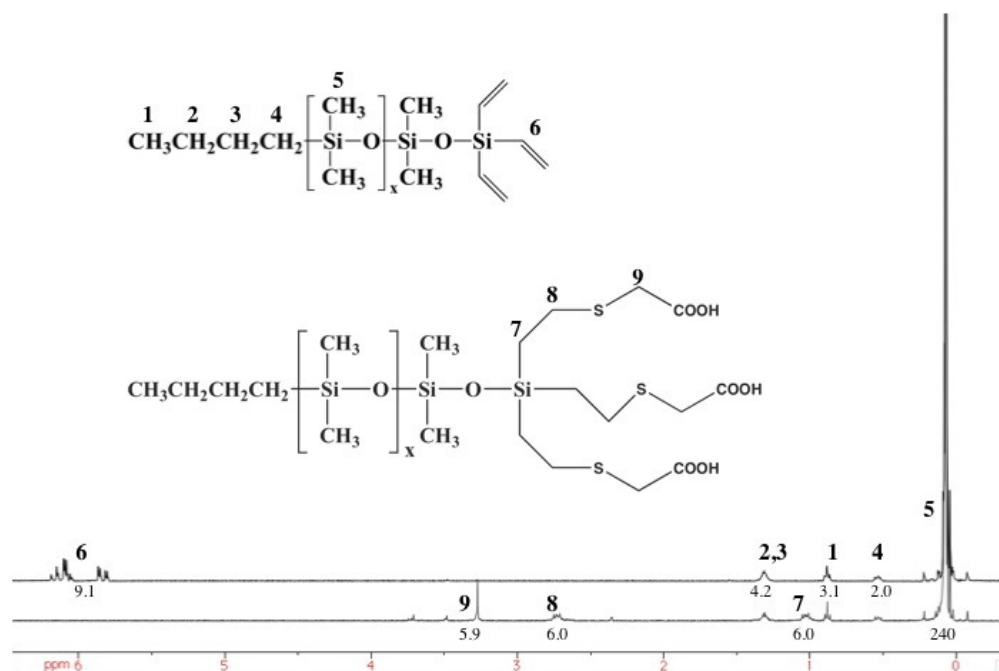


Figure 8.1. ¹H NMR confirms the addition of mercaptoacetic acid across vinylsilane groups on a 3,100 g mol⁻¹ trivinylsilyl-terminated PDMS. The upper spectrum is trivinylsilyl-terminated PDMS; lower spectrum is the tricarboxylate-functional PDMS

Using these polymers, PDMS-magnetite complexes were formed with compositions close to those targeted (approximately 30 wt% magnetite and 70 wt% PDMS coating) as determined by TGA.

8.4.2 Characterization of PDMS-magnetite complexes synthesized through co-precipitation

Due to aggregation in the system, magnetic separation was employed to remove aggregates and obtain a more defined and narrow particle size distribution. Figure 8.2 shows representative images of the $5,100 \text{ g mol}^{-1}$ PDMS-magnetite complex without separation and following five magnetic separations. There are clearly fewer aggregates in the magnetically separated image than in the starting material. However, determining the degree of aggregation in either case is difficult just from images alone.

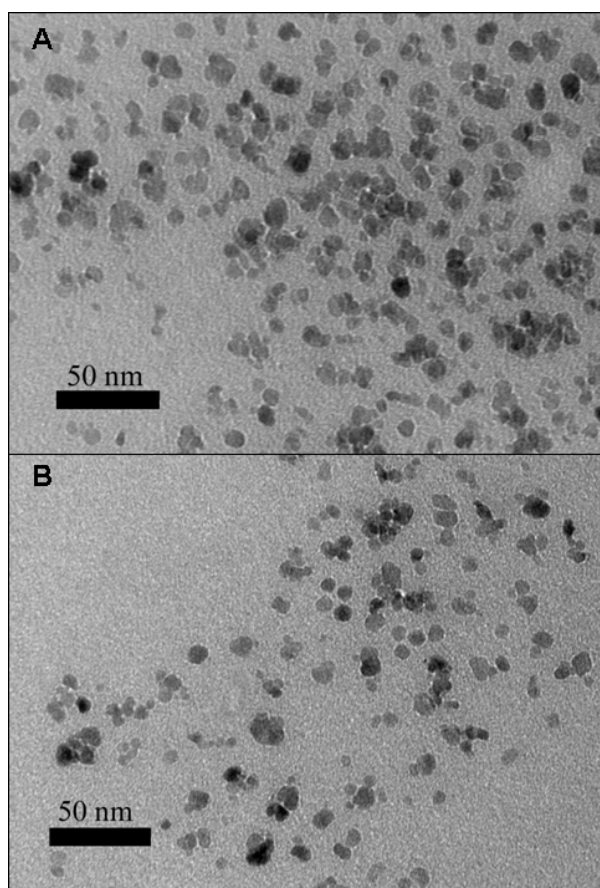


Figure 8.2. TEM images of a $5,100 \text{ g mol}^{-1}$ PDMS-magnetite complex before magnetic separation (A) and following five magnetic separations (B).

DLS was then used to characterize the extent of aggregation of these materials in chloroform (a good solvent for PDMS).

Figure 8.3 shows the number weighted diameter distribution from DLS of the four PDMS-magnetite complexes with differing molecular weight stabilizers during various stages of magnetic separation at a concentration of 10 mg mL^{-1} . Each of these distributions is an average of three consecutive measurements. Passing the PDMS-magnetite complexes through the separation columns removes the larger particles. Each distribution decreases both in width and overall diameter with increasing separations, indicating that multiple separations are necessary to obtain a well-defined distribution of polymer-stabilized single magnetite nanoparticles.

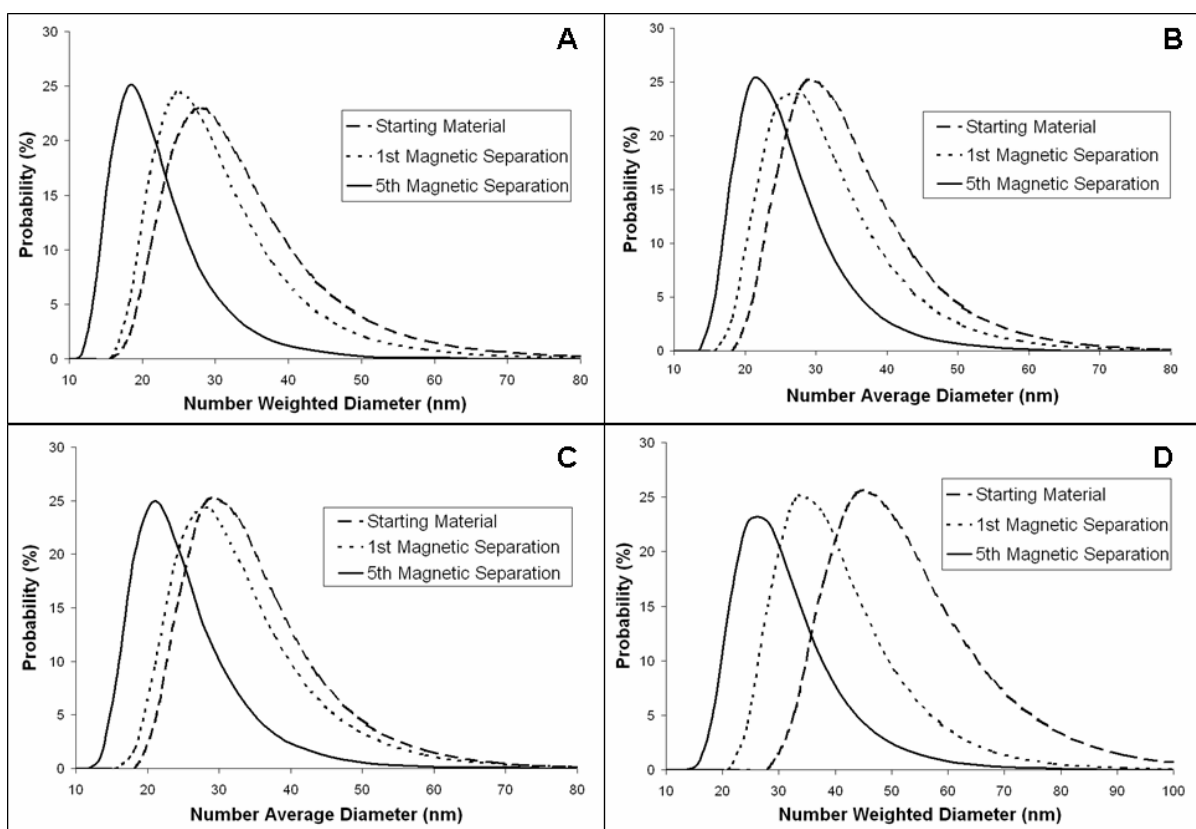


Figure 8.3. The number weighted diameter distributions of the 3,050, 5,100, 6,900, and $10,200 \text{ g mol}^{-1}$ PDMS-magnetite complexes in chloroform at 25°C display the effect of magnetic separation for a magnetic field above the saturation magnetization at 10 mg mL^{-1} .

This is also observed in Table 8.2, which shows the number average diameters predicted by the model compared to those obtained via DLS for the starting material and the material after both one and five magnetic separations for all four PDMS molecular weights. Note that these solutions were separated at a concentration of 1 mg mL^{-1} , both to assure dilute solution conditions and to prevent saturation of the magnetic separation column.

Table 8.2. Comparison of the model predictions of hydrodynamic diameter with experimental measurements indicate that five magnetic separations are necessary to obtain a well-defined particle size distribution.

PDMS-Magnetite Complex	% Magnetite	Starting Material	1 st Separation	5 th Separation	Model
		DLS D_n (nm)*	DLS D_n (nm)	DLS D_n (nm)	D_n (nm)
3,050 g mol^{-1}	35	29.6	27.0	19.7	18.2
5,100 g mol^{-1}	29	31.0	29.7	20.1	22.7
6,900 g mol^{-1}	24	30.1	30.0	21.8	26.5
10,200 g mol^{-1}	26	48.4	36.5	27.6	30.0

* D_n = Number Average Diameter

Note that the model comes into better agreement with the DLS data with an increased number of magnetic separations. This again indicates that multiple magnetic separations are necessary in order to obtain a well-defined system. This treatment does assume a constant number of chains per surface area for each magnetite particle in the distribution, irrespective of the particle size. The increased agreement between the model and the sizes measured by DLS with subsequent magnetic separations indicates that this assumption is reasonable as long as the core particle size is not affected significantly by the magnetic separation (i.e. if only aggregates are removed and not single particles).

This was confirmed through image analysis, where the size distributions of the magnetite core for the starting material, first magnetic separation, and fifth magnetic separation for the $6,900 \text{ g mol}^{-1}$ PDMS-magnetite complexes were compared as shown in Figure 8.4.

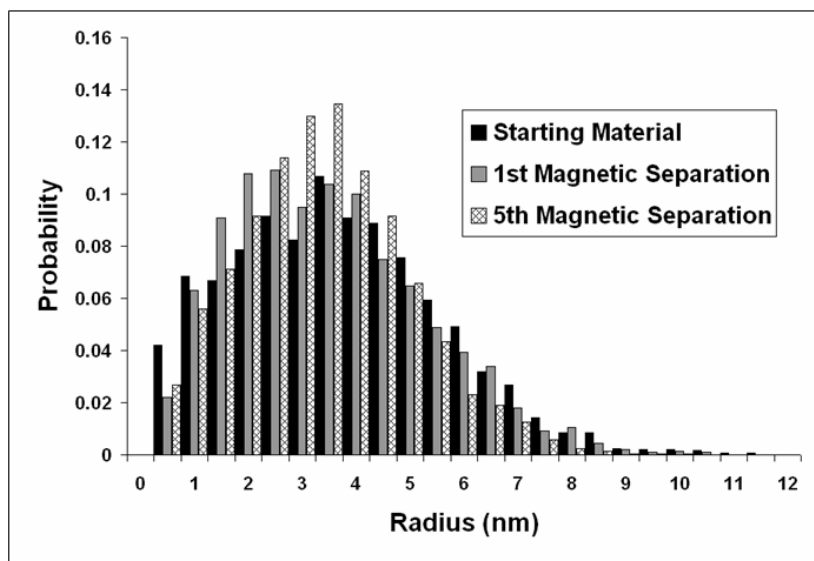


Figure 8.4. Image analysis shows no significant difference between the particle size histograms for the starting material (3.4 ± 2.0 nm), first magnetic separation (3.2 ± 1.8 nm), and fifth magnetic separation (3.1 ± 1.6 nm) of the $6,900 \text{ g mol}^{-1}$ PDMS-magnetite complex.

This method of image analysis divides any obvious aggregates into its components, thus eliminating the complexities of determining which particles are aggregated and which are not. This allows determination of the nominal magnetite core size which does not appreciably change in the magnetic separation process. The only parameter that changes is the degree of aggregation of the system, which is reflected in the DLS measurements.

The primary issue with this separation technique is that approximately 80 percent of the material (by mass) is lost during the series of five separations at a concentration of 1 mg mL^{-1} (Figure 8.5). Figure 8.3 and Table 8.2 illustrate the need for five magnetic separations to obtain a well-defined particle system, and so a significant loss of material is inevitable regardless of molecular weight when using this method. Also, the core particle size does not change, which means that 80 percent of the material is in the form of aggregates, indicating that the synthetic procedure for making the complexes is not ideal.

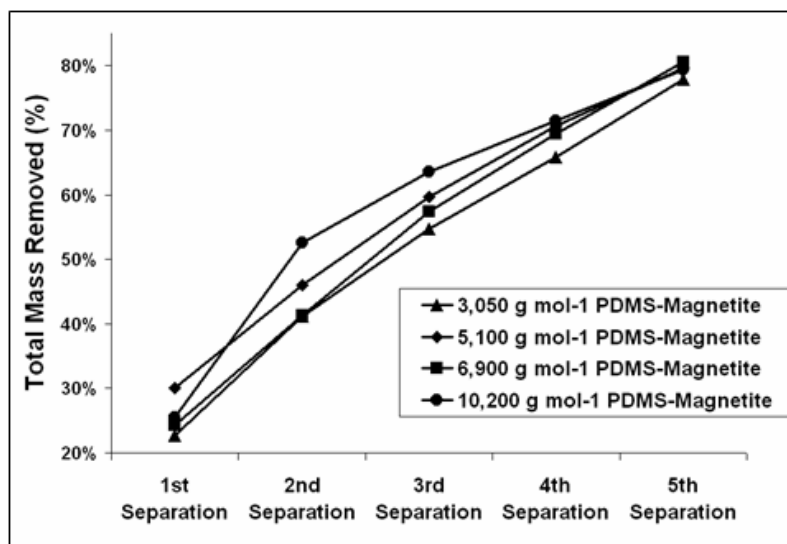


Figure 8.5. For each molecular weight, approximately 80 percent of the material is removed after five separations.

Application of extended DLVO theory also confirms that the single core particles should not be appreciably separated through the magnetic fractionation step irrespective of polymer molecular weight or magnetic field strength. Examination of the pair-pair potentials for the different polymer brushes at a magnetic field strength above the saturation magnetization of the nanoparticles predicts that the majority of the material removed should be magnetite aggregates. Figure 8.6 shows these potential energy diagrams generated for all four molecular weights examined in this study using extended DLVO theory with the added magnetic pair-pair potential as described previously.

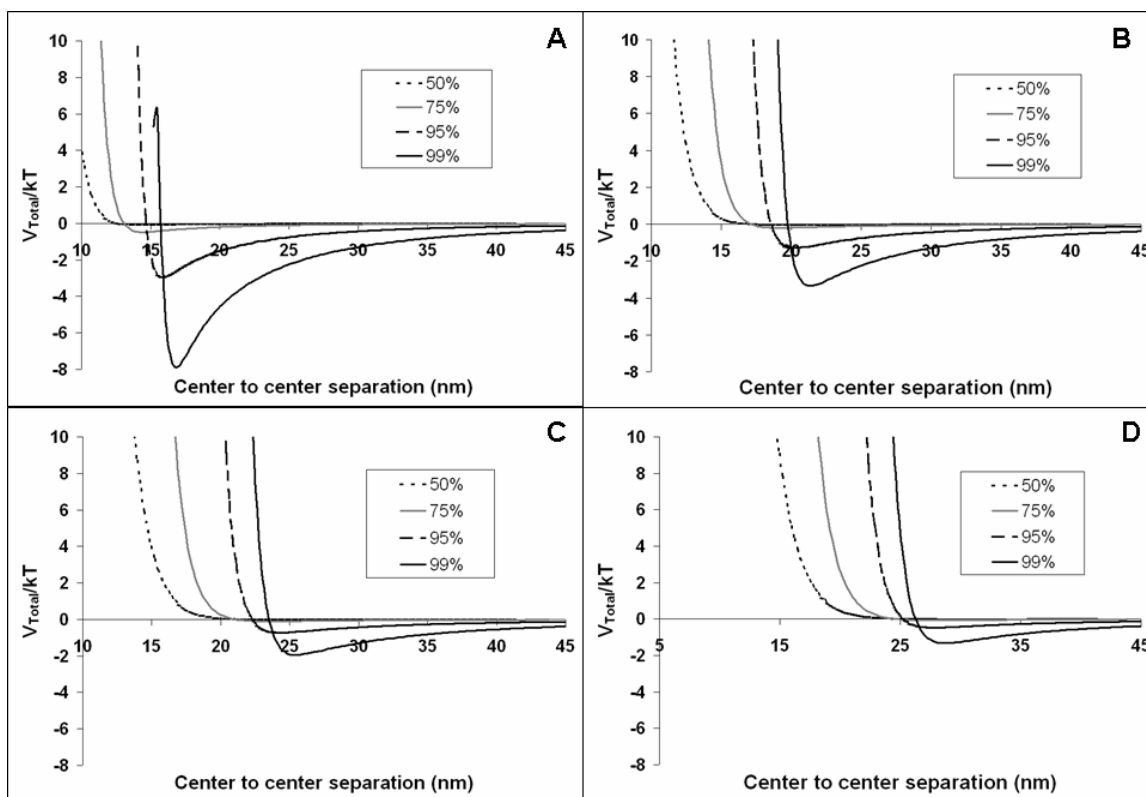


Figure 8.6. Potential energy diagrams of the 3,050 (A), 5,100 (B), 6,900 (C), and 10,200 g mol⁻¹ (D) PDMS-magnetite complexes (compositions shown in Table 8.2) show deeper secondary minimum energy wells for the smaller polymer brush with an applied magnetic field above their saturation magnetization.

The potential energy diagrams were calculated for the 50th (4.1 nm), 75th (4.9 nm), 95th (6.1 nm), and 99th (6.9 nm) percentile of the core particle size distribution as determined by image analysis. A more negative secondary potential energy well indicates a propensity for aggregation of two complexes interacting with each other. As illustrated in Figure 8.6, only the 99th percentile of the particle size distribution is susceptible to magnetically induced flocculation. Of the four complexes examined, only the 3,050 g mol⁻¹ PDMS-magnetite complex might exhibit appreciable flocculation. However, Figure 8.5 indicates no appreciable difference in the percent mass lost during magnetic separation. The secondary potential energy wells for all molecular weights are very similar for the 95th percentile and are identical for the 50th and 75th percentiles of the particle size distribution regardless of molecular weight. Thus, the separation profiles of each molecular weight should be mostly uniform, and the number of single particles removed from the system should be negligible, as observed by image analysis.

8.4.3 Characterization of PDMS-magnetite complexes synthesized through thermal reduction of $\text{Fe}(\text{acac})_3$

Because of the issues outlined in the previous section, a more uniform magnetite size distribution was desired. This was achieved through the elevated temperature reduction of iron (III) acetylacetonate adapted from Pinna et al.³⁹ Figure 8.7 shows a representative TEM image of the $3,050 \text{ g mol}^{-1}$ PDMS-magnetite complex as well as particle histograms for three different magnetite syntheses obtained through image analysis, illustrating the remarkable reproducibility of the particle size distributions ($7.8 \pm 2.2 \text{ nm}$, $7.0 \pm 2.3 \text{ nm}$, and $7.7 \pm 2.3 \text{ nm}$ for batches 1, 2, and 3, respectively).

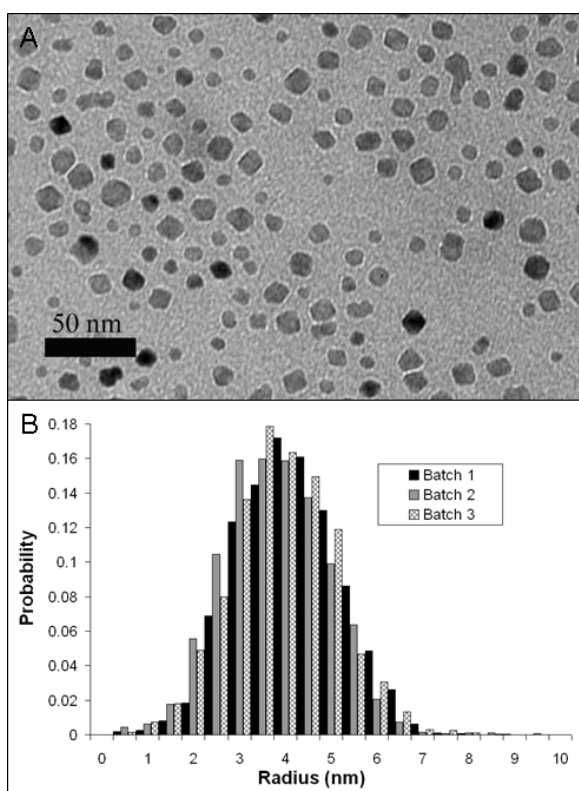


Figure 8.7. A representative TEM image (A) shows a well-defined magnetite core while particle histograms of repeated magnetite syntheses (B) show a high degree of reproducibility for three different magnetite batches.

Additionally, XRD was performed on another magnetite batch prior to coating with oleic acid, and through application of the Scherrer equation ($D_{\text{XRD}} = 0.9\lambda/\beta\cos(\theta)$, where λ is the wavelength of radiation, β is the peak width at half-height in radians, and θ is the angle of reflection),⁴² a diameter of $8.2 \pm 1.2 \text{ nm}$ was obtained from five different XRD

peaks. This compares favorably to the average obtained via image analysis. Because both methods require the assumption that the particle is spherical, the consistency of the data from both image analysis and XRD supports the validity of this assumption.

Polymer-magnetite complexes were prepared with all four PDMS molecular weights. Different polymer loadings for the 5,100 and 6,900 g mol⁻¹ PDMS-magnetite complexes were used to ensure that the model was correctly predicting the PDMS-brush extension. Just as before, all DLS measurements were made in chloroform, a good solvent for PDMS. Table 8.3 shows a comparison of the number average diameters obtained from DLS with those predicted by the model for the PDMS-magnetite complexes.

Table 8.3. Comparison of the number average diameters obtained from DLS with those predicted by the model indicate a well-defined particle size distribution.

PDMS-Magnetite Complex	% Magnetite	DLS D _n (nm)	Model D _n (nm)
3,050 g mol ⁻¹	44	18.9	17.1
5,100 g mol ⁻¹	21	23.7	24.5
	42	21.6	20.6
6,900 g mol ⁻¹	38	23.7	23.5
	47	20.4	22.0
10,200 g mol ⁻¹	41	23.2	26.4

As illustrated in Table 8.3, there is remarkable agreement between the DLS measurements and the model predictions for this system. The largest deviation between the predicted and experimental values is 3.2 nm, and the average deviation is seven percent, which is on par with the error associated with DLS measurements and previous comparisons to the model made with polyether-stabilized complexes.³⁰ Additionally, this suggests that the PDMS brush extension is predicted by the model accurately, as the comparison of the two PDMS-magnetite complexes with different magnetite to polymer compositions illustrates. In both cases, an increase in the magnetite weight fraction decreases the complex size.

The accuracy of the model compared to the DLS measurements is a strong indication that the high-temperature magnetite synthetic route provides a well-defined particle size distribution. The reproducibility of different batches means that this synthetic method can be used repeatedly to obtain the same magnetite distribution, allowing for design and control of various polymer-particle properties. Most importantly, the high-temperature synthetic route requires no magnetic fractionation, which eliminates material loss associated with the co-precipitation.

8.5 Conclusions

Sterically stabilized complexes comprised of PDMS and magnetite nanoparticles were synthesized using two different magnetite synthetic methods and four different PDMS molecular weights. Through comparison of a polymer brush model with DLS measurements, it was determined that the co-precipitation synthetic method resulted in aggregates. Multiple magnetic separations were necessary to obtain a well-defined narrow particle distribution and while this did produce the desired distribution, nearly 80 percent of the total starting material was lost in the separation process. Image analysis and the application of extended DLVO theory with an added magnetic pair-pair potential indicated that aggregates were the main component being removed through the magnetic separation process, and varying the molecular weight of the PDMS brush did not appreciably affect the separation profiles.

A thermolysis method that involved reduction of iron (III) acetylacetonate was implemented to produce a particle size distribution consisting of primarily single particles to eliminate the magnetic separation step. The reproducibility of this method was confirmed through analysis of TEM images for multiple magnetite batches and through X-ray diffraction. The polymer brush model was applied to these complexes and predicted the PDMS-magnetite complex sizes within seven percent of those measured by DLS for both varying molecular weight and polymer loading. This indicates that the model accurately describes how the PDMS brush extends out from the magnetite nanoparticle surface. Previous work in our group has examined the use of this model to predict the sizes of polyether-magnetite complexes with similar agreement.³⁰ The model includes no adjustable parameters, and predicts the sizes of significantly different types

of polymer-magnetite complexes based on published values of the Flory exponent for solvent quality, the statistical polymer segment length, and the statistical number of segments per polymer chain.

Most importantly, through application and validation of this brush model, we now have a readily available methodology for the design of sterically-stabilized single-particle complexes with sizes tailored to within at least ten percent of a desired value. Additionally, the model provides a tool for determining the state of aggregation of a system in solution and whether further separation techniques are necessary to remove unwanted components. This knowledge will allow for the design of polymer-nanoparticle systems for biomedical applications with specific functionalities and the ability to determine the critical design parameters for variables such as cellular uptake and efficacy of delivery.

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9 Determination of the Catalytic Activity of Cerium Oxide Nanoparticles in Aqueous Media

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As constituted, this chapter will not be published in a peer-reviewed journal

Jamie Clinton performed UV-Vis spectroscopy measurements shown in Figures 9.6 and 9.8 and performed the X-ray photoelectron spectroscopy measurements discussed in section 9.3.

9.1 Abstract

The catalytic activity of commercially available cerium oxide nanoparticles was explored by measuring the catalytic degradation of hydrogen peroxide in aqueous solutions. One of the cerium oxide samples showed significant biological activity while the other two did not. The oxidation state of each sample was measured through X-Ray Photoelectron Spectroscopy and did not show a significant change in Ce^{3+} concentration between samples. However, Transmission Electron Microscopy images indicate that while the specific surface areas of the samples are similar, the crystal structures of these surfaces are quite different based on powder X-ray diffraction measurements. The reaction rates were measured and then normalized to the specific surface area of each sample, and Cerium oxide samples manufactured by different synthetic methods showed markedly different catalytic activities based on reaction rates normalized to the specific surface area of each sample. Modifications of the reaction pH were made, and an increase in catalytic activity was observed, as well as changes in the UV-Vis absorbance spectra consistent with an increase in Ce^{3+} . Zeta potential measurements indicated that phosphate was a

potential determining ion and was strongly adsorbing to the cerium oxide nanoparticle surface. Kinetic measurements made in the presence of phosphate confirmed this, as the catalytic activity of the materials was significantly lowered. However, the biologically active nanoparticle system was hindered far less than the others, suggesting that the adsorption of salts onto cerium oxide nanoparticle systems may play a key role in their biological activity.

9.2 Introduction

Cerium oxide (CeO_2 , ceria) nanoparticles (NPs) are used in many different catalytic applications, including the decomposition of hydrocarbon waste products, such as carbon monoxide.¹ This is believed to occur through the abstraction of oxygen from the surface and the reduction of the oxidation state of CeO_2 from the Ce^{4+} to the Ce^{3+} state. This oxygen combines with the carbon monoxide to form carbon dioxide through the reaction $\text{CO(g)} + (1/2)\text{O}_2\text{(g)} \rightarrow \text{CO}_2\text{(g)}$. Ceria nanoparticles have also recently been shown to exhibit profound biological effects in cell cultures, extending the lifespan of neuron cells by up to six-fold while preserving neuronal function as measured by intracellular free calcium signaling.²⁻⁴ In addition to increased lifespan, free radical damage due to UV-light and H_2O_2 was mitigated by the ceria nanoparticles. In *Drosophila* (fruit flies), ceria extended both maximum and average lifespan and protected flies from free radical challenges with paraquat.^{2, 5} There is strong evidence that ceria NPs scavenge free radicals by a catalytic process and that this catalytic activity is related to the ratio of the Ce^{3+} to the Ce^{4+} oxidation state.⁶

A number of prior studies have established that both the stoichiometry and the lattice strain of ceria vary as a function of particle size.⁷⁻¹⁰ It is postulated that the coordination number of cerium to O^{2-} changes from eight to seven when a reducing agent is introduced. Because the ionic radius of Ce^{3+} ions is larger than the ionic radius of Ce^{4+} ions (1.034 Å vs. 0.92 Å), the Ce-O bond length changes, distorting the crystal lattice.⁷ For a large particle, this has little effect, as the increased lattice strain can be relieved through lattice expansion. However, for nanoparticles, this may not be the case, as the increased lattice strain can have a significant effect due to the large surface areas associated with extremely small particle sizes. An increase in Ce^{3+} ion has been observed

with decreasing particle size through X-ray Photoelectron Spectroscopy (XPS) and X-ray Adsorption Near Edge Spectroscopy (XANES), as well as with Raman spectroscopy.⁷⁻¹⁰

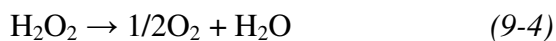
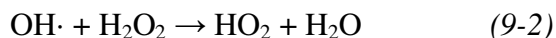
Formation of oxygen vacancies and removal of oxygen from the ceria lattice leads to localization of the unbound electrons on the empty f-states of the neighboring Ce atom. This causes a change from the Ce^{4+} valence state to the Ce^{3+} valence state, which is electron deficient and susceptible to oxidation.¹¹ Esch et al. postulated that oxygen vacancy formation at the surface is in clusters, such that the Ce^{3+} valence state is exposed exclusively. This valence state is much more reactive than the Ce^{4+} valence state, and may help to explain the reactivity of the ceria NP, since smaller ceria particles seem to have a higher ratio of Ce^{3+} to Ce^{4+} valence states.¹¹

Density functional theory has indicated that the crystal structure of the ceria plays a role in the thermodynamics of oxygen vacancy formation. Yang, et al. found that vacancy formation was favored in the surface layer and subsurface layer of cerium oxide depending upon the surface conditions.¹² Vacancy formation was favored on the surface layer for the (110) ceria surface, while vacancy formation was favored for the subsurface layer for the (111) ceria surface. In addition, the (111) surface exhibited relatively little surface relaxation - the rearrangement of surface or near-surface atoms to reduce the surface free energy - when compared to the (110) and (100) surfaces, and the surfaces with higher surface relaxations were less stable and more susceptible to vacancy formation. Even though there were differences in occurrence of vacancies at each surface, the surface was much more susceptible to vacancy formation than the bulk.

Conesa found very similar results to Yang, in that he found that the stability of the ceria surfaces against vacancy formation again went in increasing order of the surfaces as follows: (111), (211), (110), and (100). While oxygen vacancy formation was less favorable for the (111) surface, oxygen vacancy pairs were formed much easier on the (111) surface, and did not form on the (110) and (100) surfaces at all.¹³ The vacancy formation energy for each of these ceria surfaces has been researched by other groups as well.^{1, 14} Thus, the expectation would be that fewer oxygen vacancies would be formed

on a (111) surface, which means a smaller Ce^{3+} concentration. Because the Ce^{3+} is extremely reactive, surfaces with higher levels of oxygen vacancies should show larger catalytic activities.

In this study, we examine a model reaction - the decomposition of hydrogen peroxide - in the presence of cerium oxide. The catalytic mechanism by which the cerium oxide acts is unclear, but it has been suggested that it may act to lower the activation energy for the cleavage of the O-O bond as shown in equation 9-1.



Following the oxygen bond cleavage, the radical combines with the peroxide molecule to form water and dioxyhydrogen (equation 9-2), which in turn dissociates to form water and oxygen (equation 9-3). The overall stoichiometry of the reaction is given in equation 9-4. However, previous studies have indicated that different crystal surface planes of cerium oxide may support different reaction pathways.¹⁵ The experiments were designed to compare cerium oxide samples materials that show high levels of cellular activity with those that do not with the intention of determining critical variables that determine the catalytic activity and, possibly, cellular activity.

9.3 Experimental

Cerium oxide nanoparticles were obtained from Nanophase Inc. (Romeoville, IL) (denoted N130 and N160) and Nanoscale Materials, Inc. (Manhattan, KS) (denoted NM). The N130 and N160 samples were synthesized via a physical vapor synthesis method where metal is vaporized, exposed to a reactant, and then condensed into nanoparticles and the N130 sample showed biological activity as described previously.^{2-6, 16} The NM sample was synthesized using a proprietary process designed to generate large specific surface areas.¹⁷ Hydrogen peroxide (35%), potassium titanium oxalate (> 90%), and sulfuric acid (10N) were used as received from Fisher Scientific. Nanopure water with a

resistivity greater than 17 MΩ-cm was obtained from a Barnstead ROpure ST reverse osmosis/tank system.

Surface areas of the cerium oxide nanoparticles were measured by performing Brunauer-Emmitt-Teller (BET) 6-point scans using a Micromeritics ASAP 2000 nitrogen adsorption instrument. Adsorption/desorption isotherms were also performed on each material to determine the pore size distribution and average pore radius.

Powder X-Ray Diffraction (XRD) patterns were obtained using a Scintag XDS-2000 diffractometer with a Ni-filtered Cu-Kα ($\lambda = 0.1541$ nm) radiation source. The patterns were obtained at a scan rate of $1.0\ 2\theta\text{s}^{-1}$ and were scanned from 10° to 90° . Particle diameters were obtained using the Scherrer equation, as shown in equation 9-5.

$$d_{XRD} = \frac{K\lambda}{\beta \cos \theta} \quad (9-5)$$

The constant K is estimated as 0.9, λ is the wavelength of radiation, β is the peak width at half height in radians, and θ is the angle of reflection.²¹

X-Ray Photoemission Spectroscopy (XPS) measurements were performed using a Perkin-Elmer 5400. A solution of ceria ($\sim 40\ \text{mg mL}^{-1}$), suspended in DI water, was added in equal proportions to an aqueous solution of 20 % poly(vinyl alcohol) (PVA) with average molecular weight of $13,000\text{--}23,000\ \text{g mol}^{-1}$ (Aldrich Chemical Company). The large concentration of ceria was necessary to resolve the 3d Ce spectral peaks from the signal associated with PVA in the XPS spectrum. Droplets of the PVA-ceria solution were then placed on a glass microscope slide using a Pasteur pipette. Sufficient solution was used to fully cover the slide. The slide was placed in a convection oven at $70\ ^\circ\text{C}$ for two hours to remove the water and create a uniform thin film of PVA-ceria. The PVA was used as a dispersant to prevent agglomeration of the ceria nanoparticles while the sample dried. The spectral region of interest was the ten peaks associated with the 3d core levels of cerium, six of which are associated with Ce^{4+} and four of which are associated with Ce^{3+} .

TEM was conducted with a Philips EM-420 field-emission-gun transmission electron microscope. Suspensions (in DI water) of the ceria particles were diluted to approximately 0.1 mg mL^{-1} , probe sonicated at medium power for one minute, and cast onto amorphous carbon-coated copper grids for analysis. The microscope was equipped with a digital camera, such that images acquired at a magnification of 96 kx corresponded to a resolution of $3.7 \text{ pixels nm}^{-1}$.

Dynamic light scattering (DLS) was used to obtain the intensity-weighted particle hydrodynamic diameter in aqueous suspensions. A Malvern Zetasizer NanoZS compact scattering spectrometer (Malvern Instruments Ltd, Malvern, UK) at a wavelength of 633 nm with a 4.0 mW, solid-state He-Ne laser was used at a scattering angle of 173° . Zeta potential measurements were also taken with the Malvern Zetasizer ZS, utilizing Laser Doppler Velocimetry to measure the fluid flow around the particles as an electric field is applied. This is done by utilizing that particles moving through the solution due to an applied electric field scatter light at an intensity that is related to the particle velocity.

The chemical decomposition of hydrogen peroxide in the presence of the cerium oxide nanoparticles was studied typically in 10 mL solutions with approximately 4 mM hydrogen peroxide and cerium oxide typically at a concentration of 1 mg mL^{-1} . Reactions were conducted in 12 mL polypropylene vials at atmospheric pressure. The temperature was regulated to within $\pm 1^\circ\text{C}$ with a Therm-O-Watch L6-1000SS temperature control device (Instruments for Research and Industry) wired to a hot plate while the solution was kept well-mixed via a magnetic stirrer bar. The pH of the solution was adjusted from 3.5 to 8.5 using 0.3 M nitric acid and 0.1 M sodium hydroxide, both obtained from Fisher Scientific.

Reaction rates (based on equation 9-4) were measured by monitoring the hydrogen peroxide concentration as a function of time. This was done by removing one milliliter aliquots from the reaction vessel at predetermined times during the reaction and filtering them with a 20 nm Whatman Anotop aluminum oxide filter to remove the cerium oxide particles. To ensure that the ceria particles were completely removed, the absorbance at

400 nm was measured for DI water, a 1 mg mL^{-1} solution of CeO_2 in DI water, and then that same solution after filtration. In every case, the DI water and the filtered solution had the same absorbance at 400 nm to within ± 0.005 absorbance units, indicating that the ceria was completely removed and did not substantially affect the measurement of the hydrogen peroxide concentration. The filtered sample was then divided into three samples, each of them tested using a potassium titanium oxalate assay for hydrogen peroxide as described by Sellers.¹⁸ The assay consisted of a mixture of 10 N sulfuric acid (25 mL), water (100 mL), and potassium titanium oxalate (0.885 g). The solution to be tested (0.43 mL) was mixed with 1 mL of the clear assay solution, which turned yellow upon exposure to H_2O_2 . The absorbance was measured at 400 nm and was related back to the H_2O_2 concentration in solution. Figure 9.1 shows that this method is linear over the concentration ranges of hydrogen peroxide used in this study. The average of the three measurements taken was used to determine the hydrogen peroxide concentration at specific times.

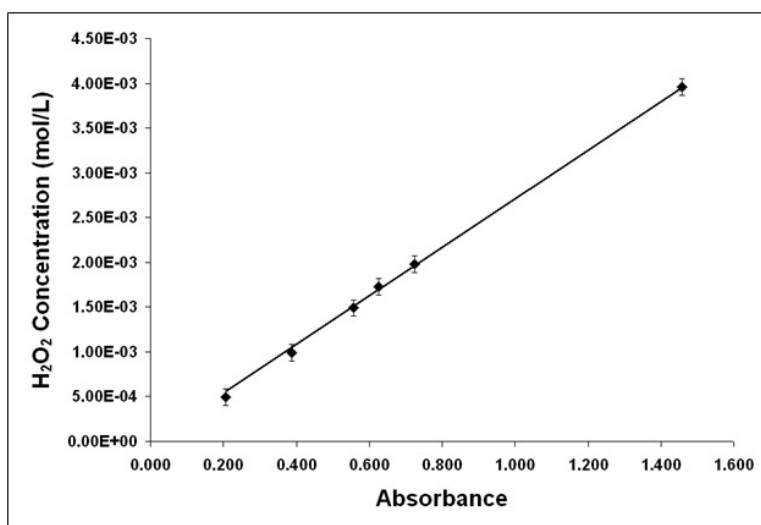


Figure 9.1. Concentration of hydrogen peroxide based on absorbance measurements at 400 nm is linear for the potassium titanium oxalate assay for the hydrogen peroxide concentrations used in this study.

Absorbance measurements were performed using a Shimadzu UV-3101PC spectrophotometer. Methacrylate cuvettes that exhibit negligible absorption above 260

nm were used. Samples were diluted to approximately 0.06 mg/mL—which minimized scattering—and then an absorption scan was performed from 260 nm to 560 nm.

The hydrogen peroxide concentration versus time data was fit with the following first-order approximation, which has been observed by others doing similar experiments¹⁹:

$$\ln\left(\frac{[H_2O_2]}{[H_2O_2]_o}\right) = -kt \quad (9-6)$$

where k is the experimental reaction rate constant and t is time. Experiments were run typically in triplicate and the calculated reaction rate constants had a typical standard deviation of approximately 15 % after normalization for surface area described below, which is relatively small compared to the differences in reaction rates that were observed. This standard deviation was calculated by first performing linear regression on each experimental run to determine the slope and intercept. These slopes were then treated as an experimental data set, and the average and standard deviation of the data set was calculated via equations 9-7 and 9-8, where m is the measured slope.²⁰

$$\bar{m} = \sum_{i=1}^N \frac{m_i}{N} \quad (9-7)$$

$$\sigma_m = \sqrt{\frac{1}{N}(m_i - \bar{m})^2} \quad (9-8)$$

Once these measurements were taken, the reaction rate constants obtained as described above were normalized for each surface area to obtain a reaction rate constant k' (units of $\text{m}^{-2}\text{min}^{-1}$) that was directly related to the reactivity of the cerium oxide surface as shown in equation 9-9:

$$k' = -\left(\frac{1}{A_s m t}\right) \ln\left(\frac{[H_2O_2]}{[H_2O_2]_o}\right) \quad (9-9)$$

where A_s is the specific surface area of the cerium oxide, and m is the mass of cerium oxide in solution.

9.4 Results and Discussion

9.4.1 Characterization of Cerium Oxide Nanoparticles

The purpose of this study was to determine the factors that control the catalytic activity of cerium oxide nanoparticles for the decomposition of hydrogen peroxide. Table 9.1 summarizes some the principal characteristics of the cerium oxide particles relevant to their catalytic activity as indicated by the values of the reaction rate constant normalized for surface area, k' . These include the intensity weighted hydrodynamic diameter from DLS, the BET specific surface area, the diameter of the particles based upon BET surface area, and the diameter of the particles based upon XRD.

Table 9.1. Characterization of cerium oxide nanoparticle size and catalytic activity.

Sample	BET Specific Surface Area (m ² /g)	Diameter based on BET (nm)	Diameter based on XRD (nm)	DLS Diameter (nm)	Average Pore Diameter (nm)	k' (m ⁻² min ⁻¹) Normalized for Surface Area
N130	88.6	9.7	NM	130	8.8	0.0143 ± 0.003
N160	24.6	34.8	37.9	160	NM	0.0120 ± 0.002
NM	86.0	10.0	4.2	245	8.5	0.0374 ± 0.007

The diameter from BET is calculated from the classical model for a spherical particle, where $SSA = 6/\rho D$, where SSA is the specific surface area, ρ is the density of the material, and D is the diameter of the particle. The diameter from XRD is calculated using the Scherrer equation as described earlier. Because the particle diameters from the BET and XRD are in rough agreement, it appears that making the assumption that the particles are spherical and relatively non-porous is a good one. In addition, because the average pore diameters calculated from adsorption/desorption isotherms indicate that the pore diameters are roughly the diameter of the particles, these pores are likely the space between individual particles that aggregate when the particles are dried. Note that the N130 and NM samples have almost identical specific surface areas, even though the reaction rate constant for the NM sample is almost three-times greater than that for N130. Also, the N160 sample, while it has a different specific surface area, has a reaction rate constant that similar to that of the N130 sample when normalized for surface area.

Figure 9.2 shows TEM images at 96kx magnification of ceria for the NM (A), N130 (B), and N160 (C).

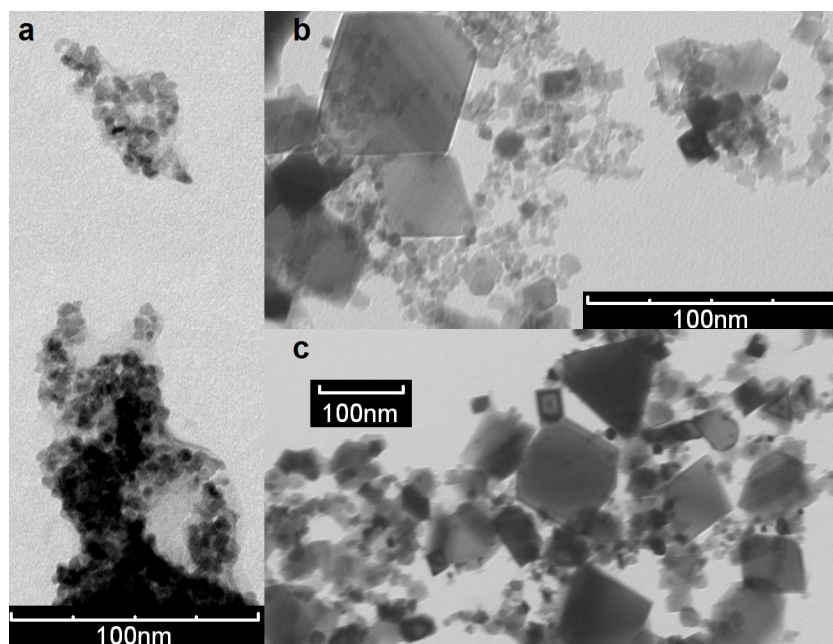


Figure 9.2. TEM images of the NM (A), N130 (B), and N160 (C) cerium oxide nanoparticle show significant differences in particle size and shape. The (111) crystal faces correspond to the triangular-shaped particle surfaces.

Considerable aggregation was found in all three samples, which is in rough agreement with the DLS measurements, listed in Table 9.1. There is a broad distribution for both size and shape in the N130 and N160 samples. Note that the crystallographic nature of each material is quite different, and that there appears to be a larger proportion of (111) crystal faces exposed in the N130 and N160 samples, which correspond to the triangle-shaped particle surfaces. This was examined closer using XRD and a method described by Chen et al.²², where the area of the peak characteristic of the (111) crystal plane was compared with the area of the peak characteristic of the (200) plane shown in Figure 9.3. Due to sample limitations, the N130 sample was not analyzed with XRD. The ratios of the areas of the characteristic peaks ($A_{(111)}/A_{(200)}$) were used to quantitate the relative abundance of the (111) crystallographic face for the two samples. The N160 sample had a significantly higher ratio (11) compared to the NM sample (5).

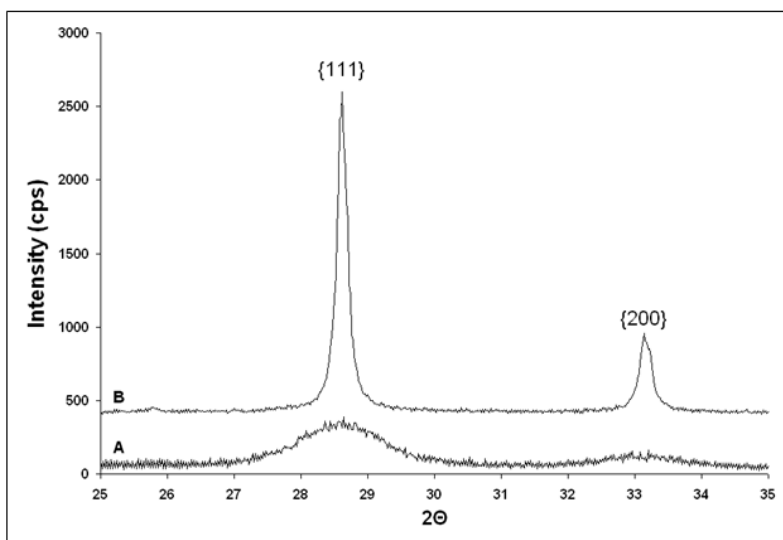


Figure 9.3. XRD of the NM (A) and N160 (B) samples show significant differences in the relative abundance of the (111) crystallographic face.

Thus, the observation that the NM CeO_2 catalyzes the peroxide decomposition at a much higher rate than either the N130 or N160 samples, as documented in Table 9.1 and shown in more detail in Figure 9.4 ($[\text{CeO}_2] = 1 \text{ mg mL}^{-1}$) is consistent with the hypothesis that the (111) crystal surface is less catalytically active.

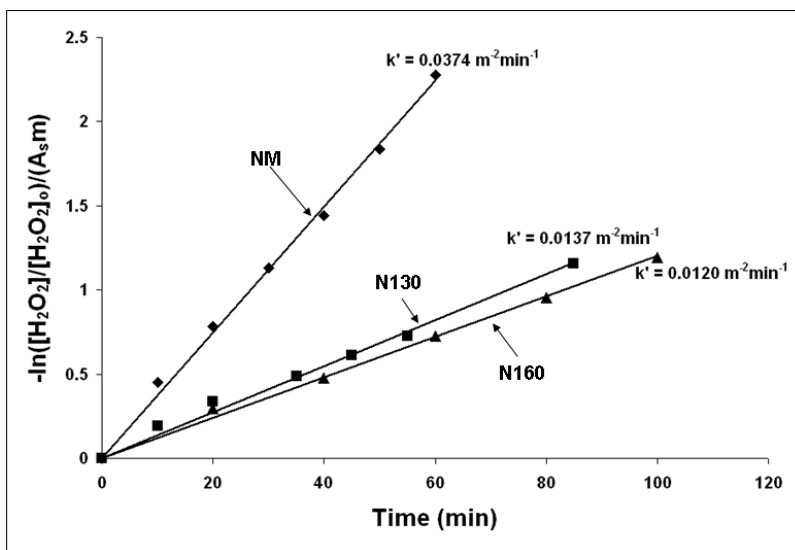


Figure 9.4. Catalytic activity of the three cerium oxide nanoparticle samples was measured by analyzing the decomposition of hydrogen peroxide in the presence of the material (1 mg mL^{-1}) at a pH of ~ 7.5 .

Figure 9.5 illustrates the reproducibility for the experiments used to determine catalytic activity.

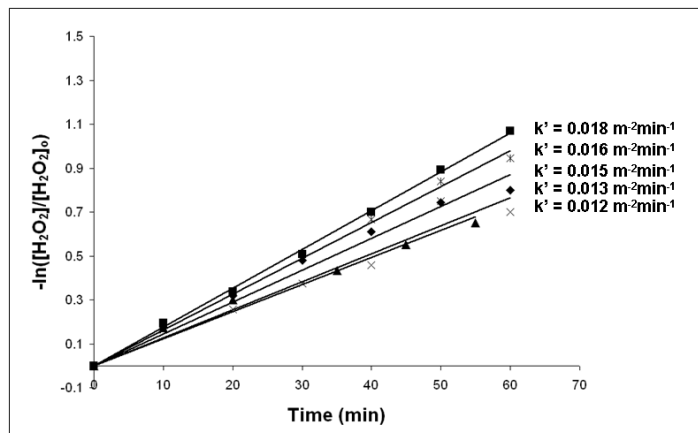


Figure 9.5. Characterization of the catalytic activity of the cerium oxide nanoparticles shows a normalized reaction rate constant of $0.0143 \pm 0.003 \text{ m}^{-2}\text{min}^{-1}$ for the N130 ceria at a pH of ~ 7.5 .

The variability between measurements is most likely due to variations in temperature between reaction runs ($\pm 1^\circ\text{C}$). However, as seen in Table 1.1, the variability is much less than the difference seen between the N130 and N160 samples when compared to the NM sample.

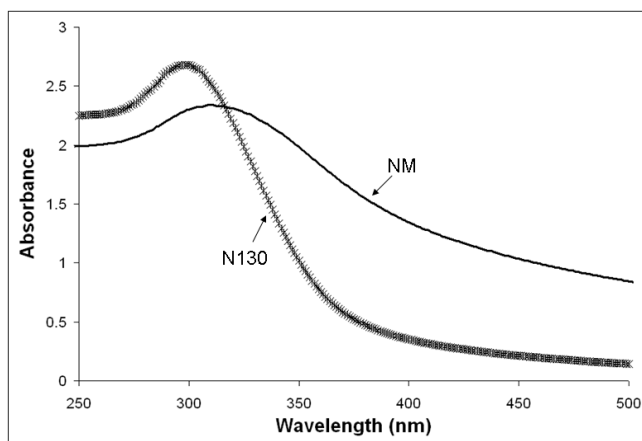


Figure 9.6. Differences were observed in the UV-Vis spectra for the NM vs. the N130 ceria samples indicating a higher concentration of Ce^{3+} for the NM sample.

To gain further insight into the relation between the relative abundance of Ce^{+3} , sample structure, and catalytic activity, the UV-Vis spectra for the NM and N130 samples were

measured as shown in Figure 9.6. Using the N130 trace as a reference, there appears to be a higher concentration of Ce^{3+} in the NM sample as indicated by the slight redshift of the peak at 310 nm, which may be attributable to the Ce^{4+} oxidation state, and the broadening of the shoulder around 400 nm, which may be attributable to Ce^{3+} through the $\text{Ce}5_d - \text{Ce}4_f$ transition.²³ The samples were also examined using XPS, and Ce^{3+} concentrations of 25%, 34%, and 33% were found for the N130, N160, and NM samples, respectively. While these do not agree with the kinetic rate constant data, there is significant curve fitting needed to determine these concentrations, which introduces significant error into the measurement. Additionally, sample preparation can play a large role in the Ce^{3+} concentration measured with XPS.

Because both the NM and N130 samples have essentially the same specific surface area and do not exhibit markedly different average pore sizes, we conclude that the NM CeO_2 has a higher activity per surface area than the N130 CeO_2 . We hypothesize that this increased reactivity is due to an increased thermodynamic driving force for oxygen vacancy formation on different types of crystallographic surfaces of CeO_2 , where an increased prevalence of the (111) surface may lead to decreased catalytic activity. In addition, even though the N130 and N160 samples have considerably different size characteristics, their catalytic activities are similar. Thus, it appears as though in this case that the difference in particle size is not the critical variable, but rather the preferred crystallographic orientation.

9.4.2 pH Effects on Catalytic Activity

Charging of metal oxides is a common phenomenon which can affect colloidal stability.²⁴ Metal oxide particles are amphoteric and thus there is a pH - the isoelectric point (IEP) or point of zero charge - at which the particles exhibit a net zero surface charge. Below the IEP, the ceria exhibits a net positive charge, while at pH above the IEP, it exhibits a net negative charge. Such particles exhibit greater colloidal electrostatic stability at pH values both above and below the IEP. It was initially anticipated that the catalytic activity of the nanoparticles might increase as the pH was adjusted to farther away from the isoelectric point, whether higher or lower. This is because aggregation is expected at the IEP due to a significant decrease in electrostatic repulsion. As the sample pH is adjusted

away from the IEP, better colloidal stability is expected which should ensure that the maximum available surface area is in contact with the hydrogen peroxide. However, the results below show that this was not the case.

Figure 9.7 shows how the zeta potential varies with pH including the isoelectric points for all three ceria samples. The pH was adjusted with indifferent electrolytes (NaOH and HNO_3)²⁵ to determine the IEP, and the IEP of all samples fell within the range of 6.4-7.3, which is well within the range found in the literature (5.5-11.2).²⁵⁻³¹ Thus, one would expect very similar colloidal stabilities for each material at the same pH values.

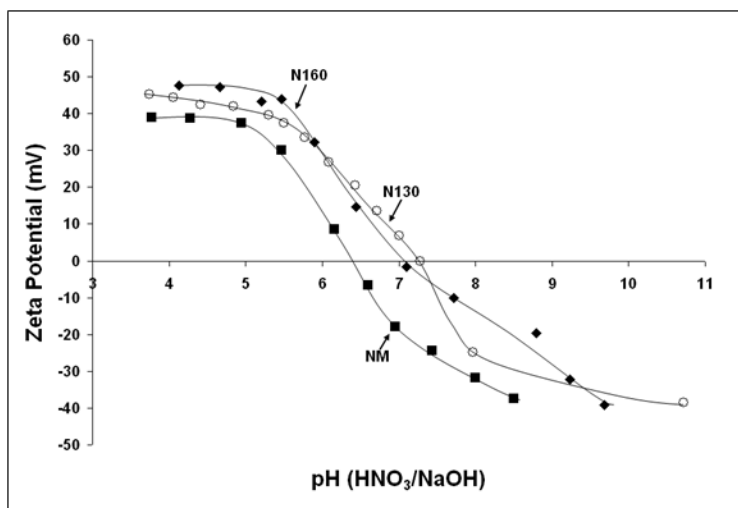


Figure 9.7. The isoelectric points of the cerium oxide nanoparticles used in this study all are in line with literature values.

Figure 9.8 shows that there is a distinct increase in the decomposition rate of hydrogen peroxide catalyzed by the N160 sample with an increase in pH ($[\text{CeO}_2] = 1 \text{ mg mL}^{-1}$). This type of behavior was seen in all three ceria samples, indicating a similar change in each surface. As Figure 9.8 also illustrates, UV-Vis absorbance measurements confirm qualitatively that the Ce^{3+} oxidation state concentration increases as the pH increases for sample N160. This trend was seen for all three samples examined, and we believe that the increase in reaction rate may be due to an increase in Ce^{3+} surface concentration—and thus an increase in oxygen vacancies—on the ceria surface as the pH is adjusted.

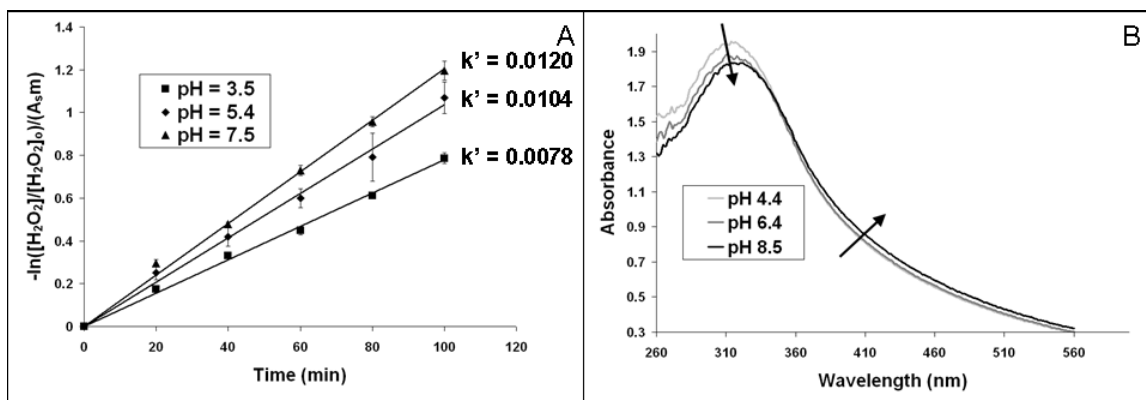


Figure 9.8. Catalytic activity of cerium oxide nanoparticle increases as pH is increased (A) and seems to be associated with a change in the UV-Vis absorbance spectra (B) (units of k' are $m^{-2}min^{-1}$).

Since catalytic activity increased monotonically with pH starting at values well below the IEP, the increased catalytic activity is not due to enhanced particle electrostatic stability. Prior work has indicated that the ceria oxidation state might act as a Lewis acid-base pair, with higher concentrations of Ce^{3+} at lower pH and higher concentrations of Ce^{4+} at higher pH.³² However, the Ce^{3+} oxidation state should be more active due to the formation of oxygen vacancies, which are electron deficient and extremely reactive. Thus, our measurements of reaction rate constants and UV-Vis absorption are at odds with treating the oxidation state as a Lewis acid-base pair because the reaction rate constant increases with increasing pH, indicating an increase in the Ce^{3+} oxidation state at higher pH.

9.4.3 Effect of Phosphate on Catalytic Activity

While the previous data is valuable for comparison, all the experiments were performed in water which is very different from a physiological liquid phase. The first step to simulating a physiological fluid is to add electrolytes and so catalytic activity was measured in the presence of salts common to physiological systems. Previous work has shown phosphate to be a potential determining ion for iron oxide³³ and so experiments in phosphate were performed to determine if this was true for cerium oxide as well.

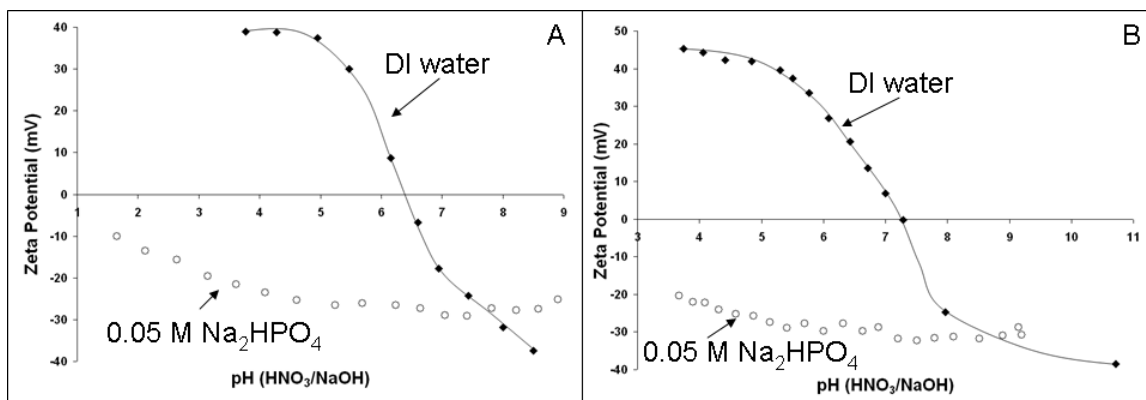


Figure 9.9. Zeta potential confirms that phosphate is a potential determining ion for the NM (A) and N130 (B) cerium oxide samples.

Figure 9.9 shows zeta potential measurements run for the NM (A) and N130 (B) samples in the presence of 0.05 M sodium phosphate. A similar curve was obtained for the N160 sample. The concentration of sodium phosphate was selected to mimic the concentration of the phosphate ion in phosphate buffered saline.

Clearly there is a change in the charging characteristics for both of the ceria samples shown. This is similar charging behavior as seen previously with iron oxide³³ and so it appears that phosphate is a potential determining ion for cerium oxide as well.

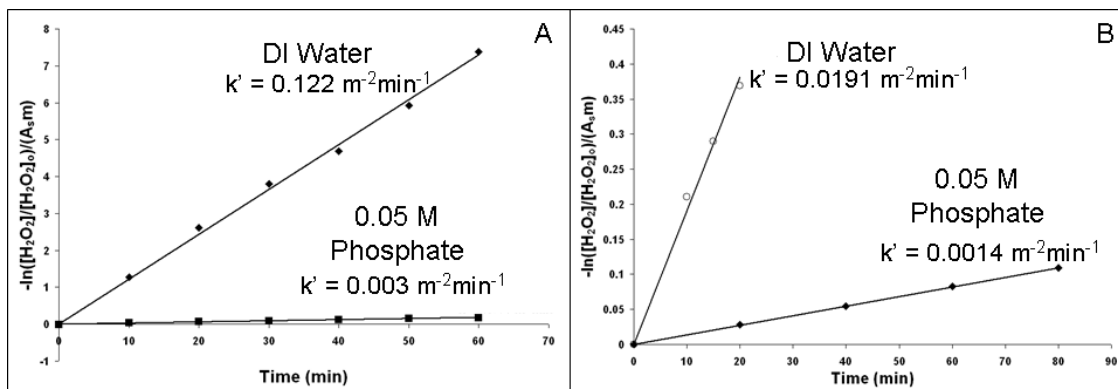


Figure 9.10. The addition of sodium phosphate significantly reduces the catalytic activity of both the NM (A, $[\text{CeO}_2] = 6.75 \text{ mg mL}^{-1}$) and N130 (B, $[\text{CeO}_2] = 1 \text{ mg mL}^{-1}$) samples. However, the NM sample shows significantly more reduction in activity (pH ~ 7.5).

Figure 9.10 illustrates the effect of 0.05 M sodium phosphate on the reactivity of the NM sample (A) and the N130 sample (B) at a pH of approximately 7.5. While there is a

decrease in the catalytic activity of both samples, the decrease is 41 times less for the NM sample while it is only 16 times less for the N130 sample. Additionally, the concentration of the NM sample had to be raised to 6.75 mg mL^{-1} to be able to measure a reaction rate over 60 minutes once phosphate was added.

Figure 9.11 illustrates that while there is significant adsorption of phosphate with the addition of sodium phosphate, lowering the pH still lowers the reaction rate for the N130 sample. Thus, it appears that the surface phenomenon causing the pH dependence may still be important even with phosphate adsorption.

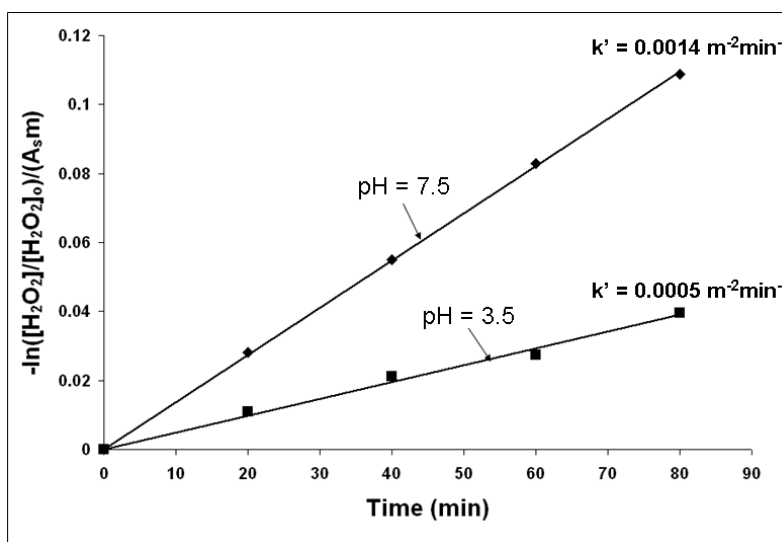


Figure 9.11. The pH dependence of reaction rate is still present even in the presence of 0.05 M sodium phosphate for the N130 sample.

The larger decrease in catalytic activity for the NM sample may be a possible reason for its lack of biological activity. Whether this is due to the difference in preferred crystallographic orientation or not is unclear. However, the fact that there is still a pH dependence on catalytic activity indicates that the ceria surface is still accessible, even after the adsorption of phosphate.

9.5 Conclusions

The catalytic activity of three different cerium oxide nanoparticle dispersions was examined with changes in pH and the addition of sodium phosphate. The samples were

found to have different preferred crystallographic orientations, which seemed to be at least partially responsible for differences observed in the catalytic decomposition of hydrogen peroxide. The calculated kinetic rate constant for the NM ceria nanoparticles was almost a factor of three greater than that of the N130 and N160 ceria nanoparticles. The NM and N130 samples had roughly the same surface area and average pore diameter, and had similar diameters calculated from BET and XRD, from which we can infer that the difference in catalytic activity is not due to differences in surface availability. However, literature suggests that the (111) surface is less reactive than other surfaces. The N160 sample has a preferred crystallographic orientation with significantly more (111) surface than the NM sample. Thus, it is possible that the difference in catalytic activity between samples is due to the difference in preferred orientation. Further evidence of this is that a distinct increase in reaction rate was observed for all samples that were examined when the pH was increased, which indicates that different colloidal stability of the particles is not responsible for their different reaction rates. The oxidation state of the cerium oxide was examined through UV-Vis absorbance spectroscopy, and it was found that the concentration of the Ce^{3+} oxidation state was higher at higher pH, which is in agreement with a higher catalytic activity.

Sodium phosphate was introduced to each ceria system to mimic certain components of biological systems. A significant reduction in catalytic activity was seen for all the systems. However, the NM sample (41 times lower) was significantly more affected than the N130 sample (16 times lower), which is the sample that shows biological activity in cell cultures and drosophila mobility studies. This data illustrates the complexities of looking at catalytic activity of materials in biological systems. While this study has focused on just one particular electrolyte (phosphate) added to the system, biological systems are comprised of many different electrolytes and proteins, some of which may have a specific adsorption to the material surface that affects catalytic activity. These results suggest that the biological activity of these materials is a complex phenomenon and can be dramatically affected by interactions between the cerium oxide surface and the liquid phase environment to which it is exposed.

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10 Conclusions and Future Work

The work contained within this thesis is necessarily focused on colloidal stability of polymer-nanoparticle complexes. The knowledge gained from this work is intended to aid in the design of new materials quickly and efficiently and to allow for interpretations of data that normally would be difficult to understand. To that end, results obtained for relaxivity and biological efficacy of these materials have been interpreted from a colloidal perspective in an attempt to explain complicated phenomena. The first half of this chapter presents these conclusions in sequence. The second half of this chapter outlines work that at this time is not complete, but shows considerable promise for its continuation.

10.1 Conclusions

As nanomaterials become more inclusive in therapeutic medicine, an understanding of toxicity, biodistribution, and dose will be critical to rapid development of efficacious treatments. Well-defined systems will be necessary to generate high-throughput screening of materials for both cytotoxicity and biological efficiency and viability. With that in mind, the main goals of this work have been to develop tools to aid in the design of well-defined polymer-nanoparticle systems and to use this model to observe deviations from expected behavior. This has allowed the development of effective anchor groups for stabilization of magnetite nanoparticles as well as the ability to design polymer-magnetite nanoparticle complexes to a diameter within 10% of a desired value.

In **Chapter 3**, additions and improvements to the density distribution model were presented. These additions included the calculation of the half-life for doublet formation and a non-solvated hydrophobic shell surrounding the magnetite particle to mimic the behavior of an amphiphilic diblock copolymer. Reproducibility of the magnetite nanoparticle synthetic methods was examined, and the particle size distributions were quantified using image analysis and built into the model using a Weibull probability distribution. Model calculations compared favorably to measurements made with DLS for various complexes with differing anchor groups, molecular weights, and polymer stabilizers. Additionally, the polymer loading at which the onset of aggregation occurred

was determined and was correctly predicted by the model for a poly(ethylene oxide) (PEO) brush.

Heterbiofunctional PEO and PPO-*b*-PEO polymers were synthesized with ammonium functionality for adsorption to the magnetite surface in **Chapter 4**. The stability of these materials in both DI water and phosphate buffered saline (PBS) was explored. The ammonium anchor groups were found to be ineffective stabilizers due to desorption of the polymer upon exposure to PBS. This was due to phosphate being a potential determining ion for magnetite and significantly changing the surface characteristics of the magnetite. Flocculation due to PBS exposure was measured using dynamic light scattering and was quantified for different polymer loadings and molecular weights. Complexes containing the amphiphilic diblock copolymer were less susceptible to flocculation in PBS, and careful control of the polymer loading and polymer molecular weights reduced the flocculation to essentially zero. The density distribution model was again applied to these complexes, with an average deviation of the model from the DLS measurements of 8%. The half-life for doublet formation was again a useful tool for comparing different molecular weights and for predicting the polymer loading where the onset of aggregation for both the homopolymer and diblock copolymer complexes began.

Chapters 5 and 6 address the issue of anchoring the polymer to the magnetite surface. Three different anchor group chemistries were explored, resulting in PEOs with carboxylic acid, ammonium, or zwitterionic phosphonate functionality. Both monofunctional and trifunctional PEOs were synthesized. For the monofunctional anchor groups, both the carboxylate and ammonium-anchored complexes were stable in DI water but not in PBS. The monophosphonate-anchored complexes showed far greater stability in PBS but were not stable in DI water, particularly at lower polymer loadings. For the trifunctional anchor groups, again the carboxylate and ammonium-anchored complexes were stable in DI water but not in PBS. However, the triphosphonate-anchored complexes were stable in both DI water and in PBS. Additionally, the P-C bond in the phosphonate is far more stable than the P-O bond in a phosphate group, which should aid in long-term storage and viability. Two main conclusions could be

drawn from this work: 1) the aggregation of the monophosphonate complexes was caused by the attraction of the anchor group to magnetite of another complex and 2) the triphosphonate anchors are the most-likely candidate for success *in vivo*. Additionally, the instability observed in the carboxylate and ammonium-anchored complexes in PBS gives clues as to the accessibility of the magnetite surface to small molecules. The degree of surface coverage was calculated and related back to colloidal stability of the complexes. This may be important for the design of delivery vehicles where access to the surface must be carefully controlled.

Chapter 7 consists of an analysis of PPO-b-PEO-OH block copolymer-magnetite complexes with varying molecular weight and both a triammonium anchor and a triphosphonate anchor. Dynamic light scattering measurements were compared to the density distribution model. While the comparisons do not deviate by a large percentage (?? %), they do deviate more for the diblock complexes than for the homopolymer complexes discussed in the previous chapters. Small Angle Neutron Scattering (SANS) and Quartz Crystal Microbalance with Dissipation (QCM-D) data both seem to indicate a significant amount of solvation of the PPO layer rather than the assumed non-solvated shell. However, there was also no noticeable change in diameter when the complexes were heated from 20°C to 40° C. Current relaxation data indicates that for homopolymer complexes that appear stable, no change is observed in relaxation. However, for the triammonium complexes, significant (and opposite) trends exist depending upon the molecular weight of the polymer stabilizer. Thus, it appears as though the hydrophobic force detailed in Chapter 4 as a reason for deviation from model predictions plays a significant role. This is evidenced by an increase in diameter (and relaxivity) with reduced polymer loading for a 3.3kPPO-b-4.8kPEO-OH complex and an increase in diameter (and relaxivity) for a 3.3kPPO-b-2.6kPEO-OH complex with increased polymer loading. Additionally, the smaller diblock shows increased cellular uptake. This indicates that the hydrophobic to hydrophilic ratio is both a critical variable and extremely sensitive to small variations in polymer molecular weight.

Chapter 8 compared two different magnetite synthetic methods—coprecipitation of iron chlorides with ammonia and the high temperature reduction of iron(acac)—stabilized with polydimethylsiloxane (PDMS) of varying molecular weight. The use of the density distribution model when compared to DLS measurements allowed for the determination that the coprecipitation synthesis leads to the formation of polymer-stabilized aggregates rather than single particles. When these aggregates were removed with a magnetic separation column, the model and DLS measurements matched well. For the high temperature magnetite synthetic route, there was agreement between the model and DLS without any magnetic separation. Thus, the model was validated as an effective tool to determine the degree of aggregation of a system and to determine how much separation was needed to ensure the system consists of a single particle with a polymer stabilizing brush layer.

A body of work largely unrelated to the rest of this thesis is presented in **Chapter 9**. In this chapter, the catalytic activity of different commercially purchased cerium oxide nanoparticles was examined. Catalytic activities were found to be very different for materials with similar pore structures and specific surface areas by measuring the decomposition of hydrogen peroxide in the presence of the nanoparticles. However, the preferred crystallographic orientation for these materials was different. The (111) ceria surface is significantly less reactive according to density functional theory, and the materials with a more preferred orientation towards the (111) crystal plane showed a lower catalytic activity than the other nanoparticle system. Upon addition of phosphate to the reaction system, the catalytic activities of the samples were all reduced. However, the sample with the more preferred orientation towards the (111) crystal plane showed significantly less reduction than the other nanoparticle system. Additionally, this sample also shows significant biological activity and we postulate that the difference in catalytic activities upon the addition of phosphate salt may play a role in why it shows biological activity compared to the other ceria nanoparticle system.

10.2 Future Work

The three main sections presented here are: 1) Refinement of the existing extended density distribution model for polymer-particle design, 2) Rationale for and development

of controlled magnetite clusters, and 3) Quantifying the interaction between polymer brushes and specific surfaces.

10.2.1 Refinement of the density distribution model

10.2.1.1 Incorporation of a Core/Shell Hamaker Constant Calculation

Currently, the extended density distribution model only incorporates the Hamaker constant of the magnetite when calculating the Van der Waal's potential. This assumption was made because typically polymers have a significantly lower Hamaker constant than metal oxides and so an adsorbed organic layer could reduce the effective Hamaker constant of the complex compared to that of the bare magnetite particle. However, even when a non-solvated shell is assumed, at high polymer loadings the thickness of the PPO anchor layer approaches the size of the particle radius. As Figure 10.1 shows, at the same surface-to-surface separation of two particles, the main change is not the magnitude of the Hamaker constant, but the range of the Van der Waal's force.

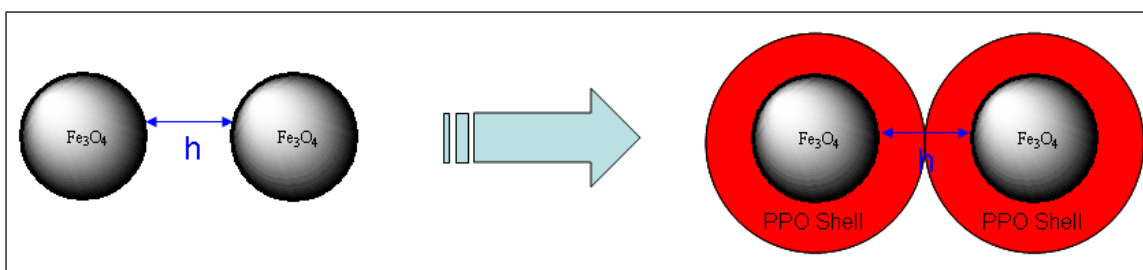


Figure 10.1. At the same surface to surface separation, a large PPO shell may increase the range of the Hamaker constant.

Croll, Prieve, and others have described the calculation of the Hamaker constant particles with adsorbed polymer layers.¹⁻⁵ Changes in van der Waal's potentials for polystyrene particles with a Pluronic brush have also been explored previously by Bevan.^{6, 7} Figure 6.8 illustrates the differences between the traditional core only model used in the DLVO calculations previously versus that of a core/shell model for a magnetite system stabilized with a 3.3kPPO-*b*-2.6kPEO-OH diblock copolymer with a 70% polymer loading.³

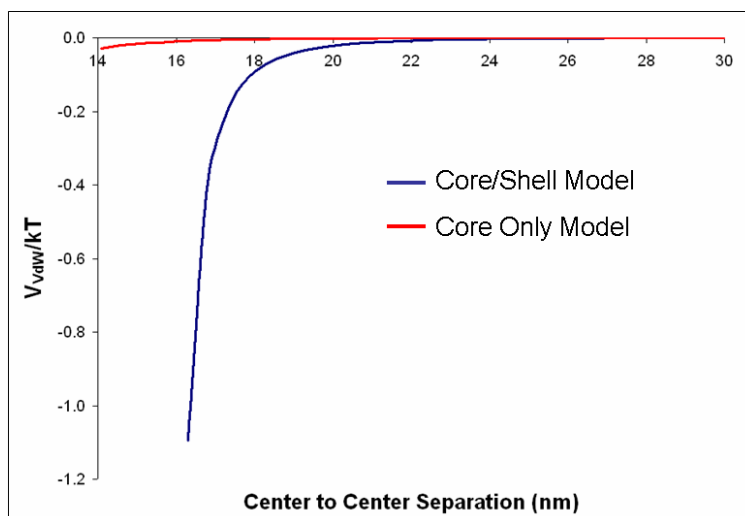


Figure 10.2. A core/shell Hamaker model has a longer range than a core only Hamaker model.

The contribution of the PEO is assumed to be negligible in this calculation. However, for this particular system, a non-solvated PPO layer is approximately 3.9 nm, while the magnetite radius is only 4.0 nm. Thus, the PPO plays a significant role in the Van der Waal's attractive potential. Also, the range of this potential is obviously affected by this polymer layer. However, it also appears that while the range of this attraction has increased, it is not significant enough to form a secondary minimum in the total potential energy diagram. Also, a major limitation of this treatment is that it assumes a non-solvated shell, which may not be appropriate for this interaction as discussed in Chapter 7. Further work should include the use of the same polyether stabilizers on a second particle system. The particle system should have a well-defined particle size distribution, a Hamaker constant significantly different than magnetite, and usability with the current anchor groups discussed in Chapters 5 and 6. This would allow for the decoupling of the contribution of the polymer layer compared to the core particle. Gold would most likely be suitable for this type of experiment, as it has a markedly different Hamaker constant than magnetite, and well-defined particle suspensions of varying size are commercially available. Additionally, gold typically exhibits negative charge and would be amenable to adsorption of the ammonium anchor groups as currently constituted.

10.2.1.2 Incorporation of a Hydrophobic Interaction of PPO Layers

Unique behavior for hydrophobic materials has been observed in water, where the attraction between the hydrophobic materials is stronger in water than in another medium. This force of attraction has been measured between hydrophobic surfaces in water and was found to decay exponentially in the first 0 – 10 nm, a fairly large-range force.^{8,9} Israelachvili proposed the relationship shown in equation 10-1.

$$\Delta G \approx -20\sigma \text{ kJ/mol} \quad (10-1)$$

Here, ΔG is the hydrophobic pair potential and σ is the diameter of small hydrophobic solute molecules.^{8,9} Thus, as the hydrophobic molecules increase in diameter, the free energy for interaction between the hydrophobic molecules decreases. Additionally, for the polyether-magnetite complexes, the force may have an even longer range due to the solvation of the PPO layer as indicated by SANS and QCM-D.

10.2.1.2.1 Modeling of Hydrophobic Interaction

Incorporating this hydrophobic interaction into the density distribution model presents a unique challenge. Typically, the model has treated the PPO layer as a non-solvated surface. As such, the hydrophobic interaction can be treated as an independent component added into the extended DLVO theory as an attractive term in the overall potential energy term as shown in equation 10-2

$$V_{Total} = V_{vdW} + V_M + V_{ES} + V_S + V_{PPO} \quad (10-2)$$

Israelachvili has proposed that for two hydrophobic surfaces in water, the interaction potential between them can be calculated using equation 10-3.⁸

$$V_{PPO} = -2\gamma_i e^{-D/\lambda_o} \quad (10-3)$$

Here, γ_i is the interfacial energy between the PPO and water, D is the particle separation distance (surface-to-surface separation), and λ_o is the decay length. However, this potential energy expression is based on a flat plate geometry (units of energy/area) and so

should be converted to a sphere-sphere interaction form using the Deryaguin approximation. This approximation, however, is not rigorous for nanoparticles with radii comparable to the surface-to-surface separation where the interaction is significant.

10.2.2 Development of Controlled Magnetite Clusters

10.2.2.1 Development of Controlled Magnetite Clusters

Magnetic clusters exhibit unique properties that can be utilized for many different applications. Whenever clustering is proposed, the cluster size distribution must be considered. A distribution that is too broad can cause issues with biocompatibility as well as the deterioration or loss of the unique properties that are desired. As such, unique methods are needed in order to ensure that the clustering method used produces the desired cluster size and distribution. Three main methods of clustering will be discussed in this section. First, clustering through the use of interparticle interactions and their prediction with DLVO theory will be discussed. Second, clustering particles through the use of a confined impingement jet mixer will be discussed. Finally, a mechanism for the formation of chemically cross-linked clusters will be proposed.

10.2.2.1.1 Clustering by Interparticle Interactions

The calculation of interparticle interactions allows one to understand the stability or instability of a system. In Chapter 5, the density distribution model was used to estimate the magnetite-polymer complex size and the complex radius of gyration. That, in turn, was used to predict the steric forces associated with polymer brush repulsion and the half-life for doublet formation ($t_{1/2}$). Even though the model includes the many assumptions put forth earlier in this chapter, there is definitely a correlation between the $t_{1/2}$ and the clustering of the complexes. Figure 10.3 illustrates how the $t_{1/2}$ is related to the particle diameter.

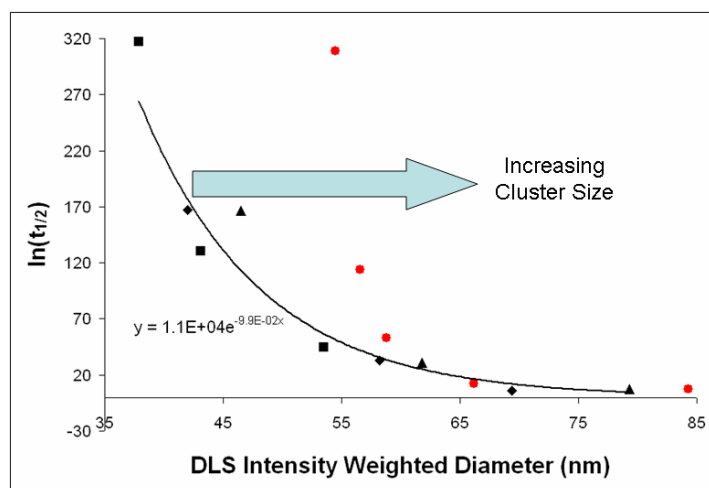


Figure 10.3. The intensity weighted diameter increases as polymer loading decreases. However, deviation is seen from the exponential fit in certain cases, which indicates increased clustering.

Deviation from the exponential fit indicates clustering that is due to a force other than those being calculated in the density distribution model. The complexes that deviate from this model are also the ones that show the increased relaxivity and the increased cellular uptake in Chapter 7. We believe that this increase in clustering is due to the hydrophobic attraction force discussed in Chapter 7. Hence, a refined model to include this parameter is important to be able to increase these cluster sizes.

However, there is a major limitation to this type of clustering. When the hydrophobic attraction becomes too strong, the clusters will continuously aggregate into a primary potential energy minimum, rendering them useless. Also, each magnetite particle is coated with both PPO and PEO blocks, meaning that the total volume fraction of magnetite is significantly less than if the magnetite particles were just packed together without any polymer.

10.2.2.1.2 Clustering by Confined Impingement Jet Mixing

To increase the volume fraction of magnetite in the cluster and also to introduce higher levels of control over the particle size, a technique called confined impingement jet (CIJ) mixing can be used. This technique consists of high velocity streams mixing under turbulent flow conditions.¹⁰⁻¹³ Figure 10.4 shows a schematic of how this technique works for the formation of magnetite clusters.

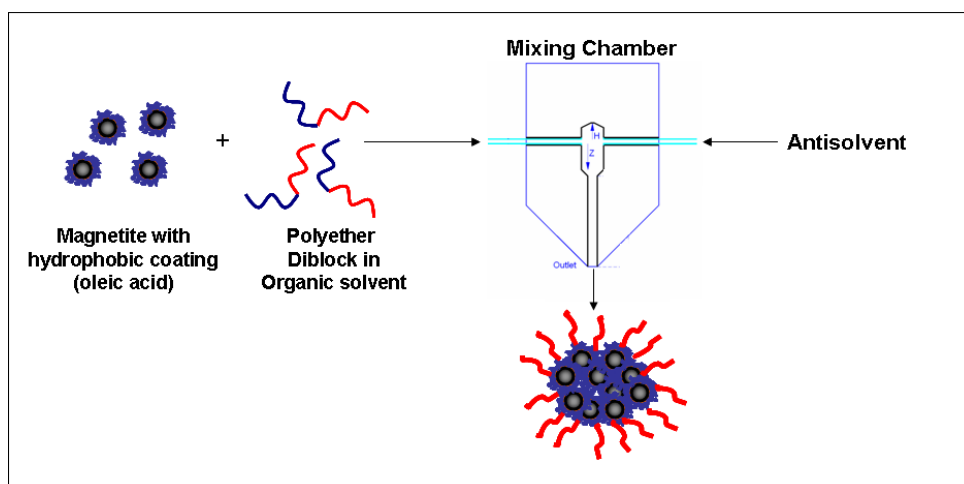


Figure 10.4. Magnetite clusters formed through CIJ mixing.

The basic idea is to have an amphiphilic diblock copolymer and hydrophobic magnetite particles suspended in an organic solvent. The introduction of an antisolvent (in this case water) should induce an attractive hydrophobic force between the magnetite nanoparticles. It also should induce an attractive hydrophobic force from the hydrophobic portion of the diblock copolymer. Based on the mixing parameters used and the molecular weights of each block of the diblock, control of the cluster size should be achievable.

10.2.2.1.3 Clustering by Chemical Cross-linking

Finally, there are two methods to make magnetite clusters from a chemical perspective. The first method involves generating an electrostatic interaction between particles and then crosslinking them together. The other method involves using a dithiol to crosslink the particles together directly.

Both methods involve the use of a triammonium- or phosphate-terminated PEO with a maleimide group on the other end. This polymer can then be adsorbed onto the magnetite nanoparticle through a ligand exchange in chloroform with sonication as illustrated in Figure 10.5.

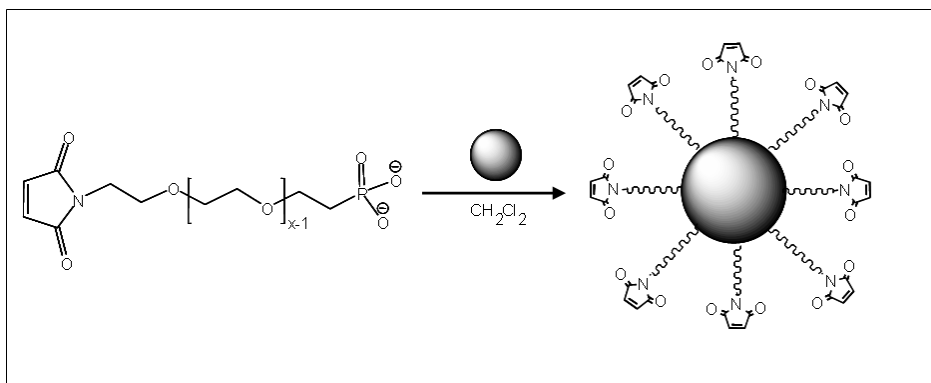


Figure 10.5. Stabilization of magnetite nanoparticles via a maleimide functionalized PEO.

The advantage to the maleimide is that it can be reacted with thiols, unlike the hydroxyl groups that typically used as polymer endgroups. With that in mind, there are two ways to modify the stabilized complexes to generate controlled complexes.

10.2.2.1.3.1 Electrostatic Cross-linking

Through reaction of the maleimide groups with either cysteamine hydrochloride or mercaptoacetic acid, charged groups can be placed on the end of each complex as illustrated in Figure 10.6.

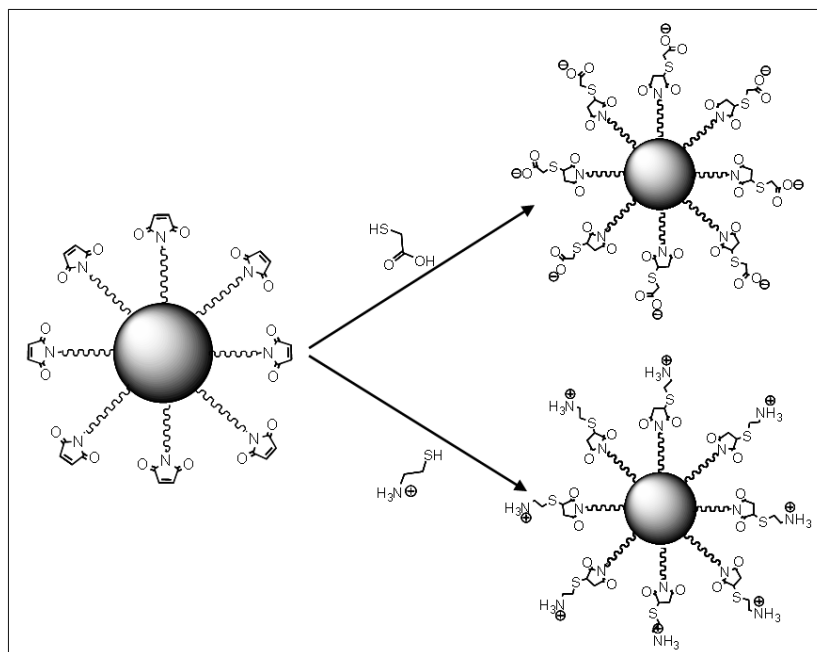


Figure 10.6. Functionalization of the maleimide group with either cysteamine hydrochloride or mercaptoacetic acid allows control of the complex surface charge.

Now, these oppositely charged complexes will attract each other. Through mixing of each in different ratios (12 mol negatively charged to 1 mol positively charged), a complex that consists of a positively charged core surrounded by negatively charged complexes can be designed as shown in Figure 10.7.

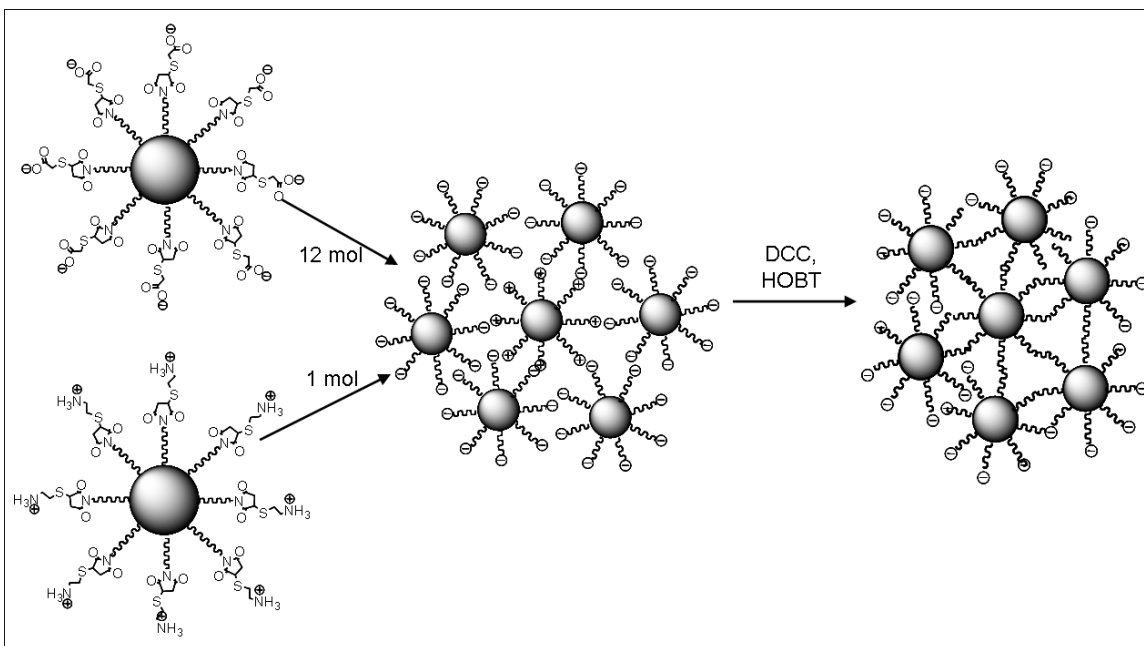


Figure 10.7. Cross-linking can be achieved through addition of DCC or HOBT.

Now an amide linkage can be made through the addition of dicyclohexylcarbodiimide (DCC) or 1-hydroxybenzotriazole hydrate (HOBT). This leaves a magnetite cluster cross-linked at all the previous points of electrostatic interaction, but highly negatively charged on the surface of the cluster.

10.2.2.1.3.2 Direct Cross-linking

Having a charged cluster may not be advantageous for many reasons, including possible interactions with proteins or cell membranes. Additionally, the proper mixture of each component is necessary in the previous reactions to obtain the proper cluster size, and a finite size is achievable without using a step-growth mechanism. Thus, instead of reacting the maleimide PEO-stabilized magnetite with cysteamine hydrochloride or mercaptoacetic acid, a disulfide linkage can be formed through the addition of 1,2-ethanedithiol as shown in Figure 10.8.

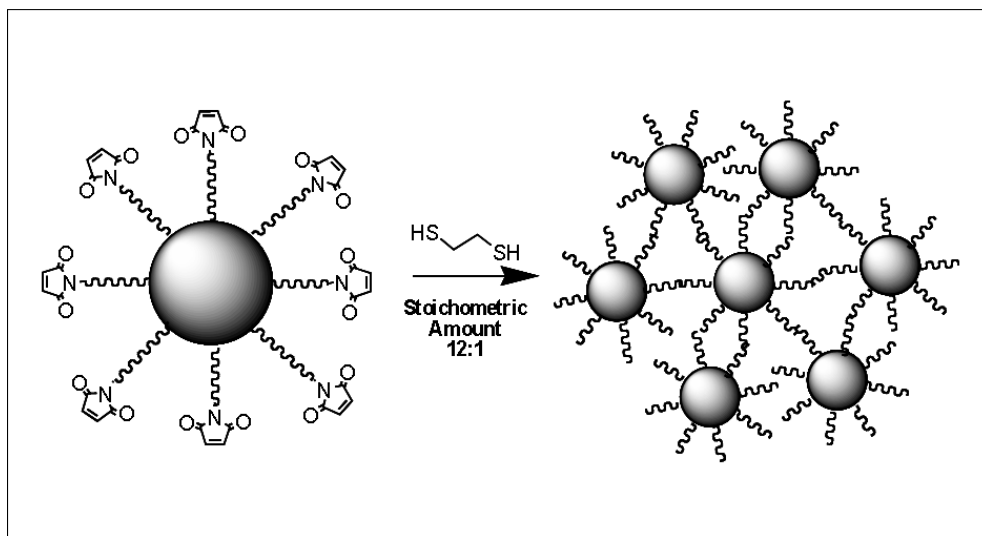


Figure 10.8. Cross-linking of the magnetite complexes can be achieved through the addition of 1,2-ethanedithiol.

By keeping the stoichiometric ratio of complex to dithiol at 12:1, the magnetite clusters remain just lightly cross-linked with maleimide groups extending out into solution. Thus, there still remains the option of reacting those maleimides with either cryeamine hydrochloride or mercaptoacetic acid to give the cluster an electrostatic charge.

10.2.3 Quantifying the Interaction Between Polymer-Magnetite Complexes and Specific Surfaces

Because surface coverage appears to be an extremely important parameter, future work should attempt to examine the effect of surface coverage on the adsorption of various molecular weight proteins onto a magnetite surface. Confirmation of the surface coverage calculations can be obtained through the use of QCM-D to determine the anchor group footprint as illustrated by McDermott et al.¹⁴ Additionally, a more in-depth examination of the density distribution model yields a correlation between the brush extension for each complex (β) and the parameter $\sigma/2$, which denotes the length scale from the center of the magnetite particle to the center of the outermost blob of the model as shown in Table 10.1. This $\sigma/2$ parameter is used in the Likos expression to predict the separation distance where the onset of a sharp increase in steric repulsion occurs.^{15, 16}

Table 10.1. There appears to be a correlation between the ratio of brush extension to the σ parameter of the expression proposed by Likos.

Complex	% Magnetite	$\sigma/2$ (nm)	PEO Brush Extension (β) (nm)	$\beta - \sigma/2$
3.3kPPO-2.6kPEO	34	6.3	5.2	-1.1
	44	5.3	4.8	-0.5
	51	4.8	4.6	-0.2
3.3kPPO-4.8kPEO	32	6.5	8.9	2.4
	41	5.6	8.3	2.7
	51	4.8	7.6	2.8
5.6kPPO-7.2kPEO	30	7.0	10.8	3.7
	40	6.0	9.9	3.9
	50	5.1	9.1	4.0
5.6kPPO-3.8kPEO	30	7.0	6.3	-0.7
	40	5.9	5.8	-0.1
	50	5.0	5.4	0.4

By subtracting $\sigma/2$ from β , a clear delineation appears between complexes. The complexes that show negative values are the ones that show deviation from the exponential fit shown in Figure 4.6. The hypothesis has been that the deviation was due to a hydrophobic interaction of the PPO layers of neighboring complexes, and this certainly seems to suggest that may be the case. Yamakawa has proposed an interpenetration function that shows promise for accurately calculating penetration of these brushes.^{17, 18} Comparison of that penetration depth with the calculated PEO brush extension may allow for design of materials with PPO anchor blocks of a certain molecular weight to ensure they specifically interact.

Experimentally, quantifying this interaction is not a trivial matter. illustrates differences observed in quartz crystal microbalance with dissipation monitoring (QCM-D, E-4 system from Q-Sense) experiment involving the adsorption of magnetite complexes containing 30% magnetite loading and either a 3kPPO-*b*-5kPEO-OH or 3kPPO-*b*-3kPEO-OH stabilizer on an octadecyltrichlorosilane (OTS)-functionalized silica-coated quartz crystal prepared as described in the literature.^{19, 20}

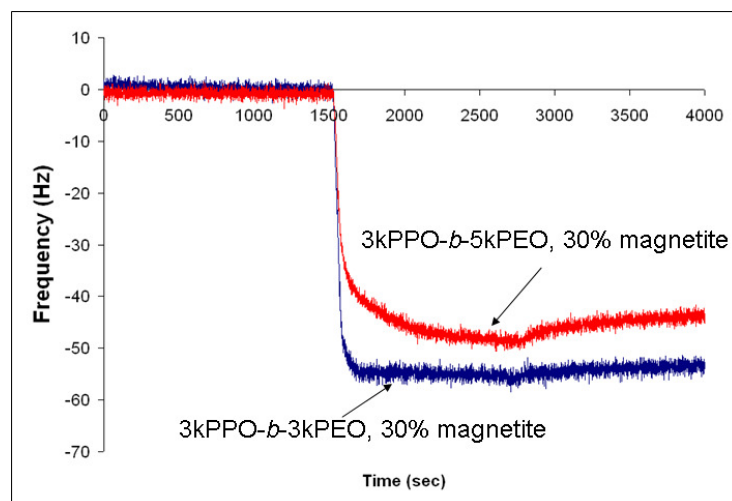


Figure 10.9. QCM-D data comparing adsorption of magnetite complexes containing 30% magnetite loading with 3kPPO-*b*-5kPEO-OH and 3kPPO-*b*-3kPEO-OH stabilizers on OTS-functionalized silica.

Both complexes were adsorbed at a concentration of 0.1 mg mL⁻¹ and clearly there is a difference in the kinetics of adsorption between the two complexes as the 3kPPO-*b*-3kPEO-OH complex adsorbs considerably quicker. Additionally, the desorption following a DI rinse (at approximately 2700 s) is significantly less for the complex with the shorter PEO brush. Initially, it also appears that the magnitude of this complex adsorbed to the surface is also larger.

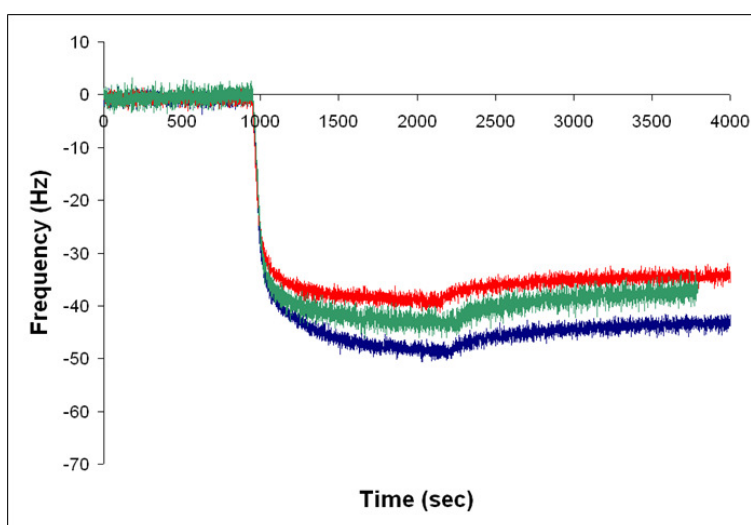


Figure 10.10. No significant differences in adsorption on an OTS-functionalized silica surface were observed for a magnetite complex stabilized with a 3kPPO-*b*-5kPEO-OH diblock copolymer at approximately 30, 40, and 50% magnetite loading.

However, as illustrated in Figure 10.10, repeated measurements of the 3kPPO-b-5kPEO-OH complex show a frequency spread of around 9 Hz while the difference in Figure 10.9 is only 11 Hz. This may indicate that the best way to determine the interaction between complexes and this surface are through measurements of the kinetics and not overall adsorption.

This experiment is also complicated by the presence of PEO. When the same experiment is performed on a magnetite-3.4kPEO-OH, a larger frequency change is observed than for either of the diblock complexes (73 Hz). This indicates that there may be a specific interaction between the PEO chains and the OTS-functionalized surface. A significant (10-15°) change in contact angle is observed on the hydrophobized silica for water containing just PEO (no magnetite) compared to water alone again indicating a specific interaction between the PEO and OTS-functionalized surface.

However, were a technique developed that allowed comparison of these different complexes, it would allow high-throughput evaluation of multiple complexes. As such, magnetite containing a blend of PPO and PEO homopolymers anchored to the surface could be implemented to carefully tailor the hydrophobic to hydrophilic ratio. Quick evaluation of these materials would be possible with this QCM-D method. Thus, continued work with these systems should explore a surface that does not show a specific interaction with PEO. Additionally, progression to a surface that behaves more like a cell membrane would also be advantageous. As such, the next step should probably include the use of phospholipid bilayers to mimic a cell membrane. Comparison of that data with the hydrophobized silica surface should allow for a clear picture as to what variables are important for interaction with different surfaces.

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Appendix – Mathematica Calculations for the Density Distribution Model

```
(*This sheet calculates the intensity, volume,
and number average diameters as well as half-life to doublet flocculation of
polymer/magnetite complexes using a Weibull distribution from TEM and image analysis*)

NoTailblocks := 1 (*Number of tailblocks*)
MWanchor := 3300 (*MW of anchorblock*)
MWTail := 2600 (*MW of tailblock*)
PctMag := 0.51 (*Percent Magnetite via TGA measurement*)

(*c & b are parameters for the Weibull
distribution. P is the Weibull probability function*)
c := 3.59
b := 4.48
P := ((c/b) * (r/(b))^(c-1)) * (e^(-1*(r/(b))^c))
(*A is the Average Surface Area of the magnetite cores*)
A := 4*Pi*r^2
Aavg :=  $\int_0^{\infty} A * P \, dr$ 
(*V is the Average Volume of the magnetite cores*)
V := (4/3)*Pi*r^3
Vavg :=  $\int_0^{\infty} V * P \, dr$ 
rho := 5.17*10^-21 (*density of magnetite*)
ApM := (Aavg/Vavg) * (1/rho) (*Surface Area of particles per unit mass*)
Na := 6.02*10^23 (*Avagadro's Number*)
SAMag := ApM*PctMag (*Total Surface area of magnetite per mass complex*)
MWTTotal := MWTail*NoTailblocks + MWanchor
Ntailblock := MWTail/44.1
Nanchor := MWanchor/58.1
xtailblock := Ntailblock/(Ntailblock + Nanchor)
NoChains := (1 - PctMag) * (Na/MWTTotal) * xtailblock
(*NoChains is the Number of PEO chains per mass of complex*)
"CpA2 is the average number of chains per magnetite surface area"
CpA = NoChains/SAMag

Cinf := 4.1 (*Chain stiffness parameter*)
Lk := 0.6(*length of statistical segment (Kuhn length)*)
Rhoanchor := 1.04*10^-21
Mcore := rho*V
Manchor := Mcore (1 - PctMag) / PctMag * (MWanchor/MWTTotal)
Vanchor := (((rho*4*Pi/3) * (r)^3 * (1 - PctMag)) / PctMag * MWanchor) / (Rhoanchor * MWTTotal)
tanchor := ((3*Vanchor/(4*Pi) + (r)^3)^(1/3) - r
mo := 14.7 (*Molecular weight per bond (g/mol)*)
Nk := MWTail/(mo*Cinf) (*Number of statistical segments in tailblock chain*)
f := 4*Pi*r^2*CpA (*functionality, or number of chains per particle*)
v := 0.583(*Flory Exponent*)
Rm := (8*Nk*Lk^(1/v) * f^((1-v)/(2*v))) / (3*v*4^(1/v) + (r + tanchor)^(1/v))^v
(*Rm is the radius of the complex*)
D1 := NIntegrate[P*Rm^4, {r, 0, 499}]
D2 := NIntegrate[P*Rm^3, {r, 0, 499}]
```



```

I1 := NIntegrate[P*Rm^6, {r, 0, 499}]
(*I1 is the first term in the intensity calculation*)
I2 := NIntegrate[P*Rm^5, {r, 0, 499}]
(*I2 is the second term in the intensity calculation*)
"Intensity-weighted diameter"
Di = 2 * (I1 / I2)
"Volume-weighted diameter"
Dv = 2 * (D1 / D2)
"Number-weighted diameter"
Dn = NIntegrate[2 * Rm * P, {r, 0, 499}]

Mshell := (Mcore / PctMag) * (1 - PctMag) * (NoTailblocks * MWTail) / MWTTotal
"Mass of corona"
Mshell2 =  $\int_0^{\infty}$  Mshell * P dr
(*Mass of corona chains*)
Mtotal := Mshell + Mcore + Manchor (*total mass of complex*)
Icore := 4 * Pi * r^5 * rho / 5 (*moment of inertia of core*)
Ianchor := 4 * Pi * Rhoanchor * ((r + tanchor)^5 - r^5) (*moment of inertia of anchor*)
ms := MWTail / (6.02 * 10^23 * Nk) (*mass of a PEO segment*)
As := 3 * 4^(1/v) * f^((3*v - 1) / (2*v)) / 32 / pi (*constant of proportionality*)
as := 0.6 (*size of monomers in corona*)
Icorona := 4 * Pi * As * ms * (Rm^(2 + (1/v)) - (r + tanchor)^(2 + (1/v))) / (as^(1/v) * (2 + (1/v)))
(*moment of inertia of corona*)
Itotal := Icore + Ianchor + Icorona (*total moment of inertia of complex*)
"Average radius of gyration of the complex"
Rg = NIntegrate[ $\sqrt{Itotal / Mtotal}$  * P, {r, 0, 499}]
"Average anchor layer thickness"
ta = NIntegrate[tanchor * P, {r, 0, 499}]
"Average radius of the complex"
Rm2 = NIntegrate[Rm * P, {r, 0, 499}]
"Sigma of complex"
sigma = 1.3 * Rg
"Average of the total number of chains per particle"
ftot = NIntegrate[f * P, {r, 0, 499}]
"Average particle core radius"
Rc2 = NIntegrate[r * P, {r, 0, 499}]
"Volume Fraction of PEO (solvation w.r.t. water)"
Rhoshell := 1.04 * 10^-21
VolFractPEO = (Mshell2 / Rhoshell) / ((4/3) * pi * ((Dn/2)^3 - (ta + Rc2)^3))
s := 1.3 * Rg * .000000001
eps := 80
epso := 8.85 * 10^(-12)
Sio := -1 * 10^(-3)
i := 0.00001
kappa := 1 / (9.61 * 10^-8)
k := 1.38 * 10^-23
T := 298
c := 3 * 10^8
plank := 6.63 * 10^-34
eo := 2 * 10^4
w := 1.88 * 10^16
no2 := 1.78

```

```

no22 := 3.88
M := 4.5 * 10^5
uo := .00000126
VolFract := 0.01
miu := .001
Rc3 := Rc2 * .000000001
Rm3 := Rm2 * .000000001

PhiES := 2 * Pi * Rc3 * eps * epso * (Sio)^2 * e^(-(kappa) * (r - 2 * Rc3))
PhiS := If[r < s, ((5 / 18) * ftot^(3 / 2)) * (-Log[r / (s)] + 1 / (1 + sqrt(ftot) / 2)),
  ((5 / 18) * ftot^1.5 * 1 / (1 + sqrt(ftot) / 2)) * (s / r) * e^(-sqrt(ftot) / (2 * s) * (r - s))]
PhiM := -8 * Pi * uo * Rc3^3 * M^2 / (9 * ((r - 2 * Rc3) / Rc3) + 2)^3
H := (no2)^0.5 * (no22 + no2)^0.5 * (r - 2 * Rc3) * w / c
F := (1 + (Pi * H / (4 * sqrt(2))))^(3 / 2)^( -2 / 3)
Aeff := (3 / 4) * k * T * ((eo - eps) / (eo + eps))^2 * e^(-2 * (r - 2 * Rc3) * kappa) +
  3 * plank * w / (2 * Pi * 16 * sqrt(2)) * F * (no22 - no2)^2 / (no22 + no2)^(3 / 2)
Phivdw := -(1 / 6) * Aeff * (2 * Rc3^2 / (r^2 - 4 * Rc3^2) +
  2 * Rc3^2 / (r^2) + Log[(r^2 - 4 * Rc3^2) / r^2])
Phitotal := (PhiES + PhiS * k * T + PhiM + Phivdw)
(*Plot[PhiS, {r, 8.08*10^-9, 40*10^-9}]
Plot[Phivdw / (k * T), {r, 8.08*10^-9, 40*10^-9}]
Plot[PhiES / (k * T), {r, 8.08*10^-9, 40*10^-9}]
Plot[PhiM / (k * T), {r, 8.08*10^-9, 40*10^-9}]
Plot[Phitotal / (k * T), {r, 8.08*10^-9, 40*10^-9}]*)
"Stability ratio of particles"
W = 2 * Rm3 * NIntegrate[e^(Phitotal / (k * T)) / r^2, {r, 2 * Rc3, 1000}]
"Time to flocculation (seconds)"
tp = Pi * miu * Rm3^3 * W / (VolFract * k * T)
"Estimated DLS Intensity Weighted Diameter of Cluster (nm)"
DLS = Log[(1 / (Log[tp] + 20))^9.52381] + 92.8825

```