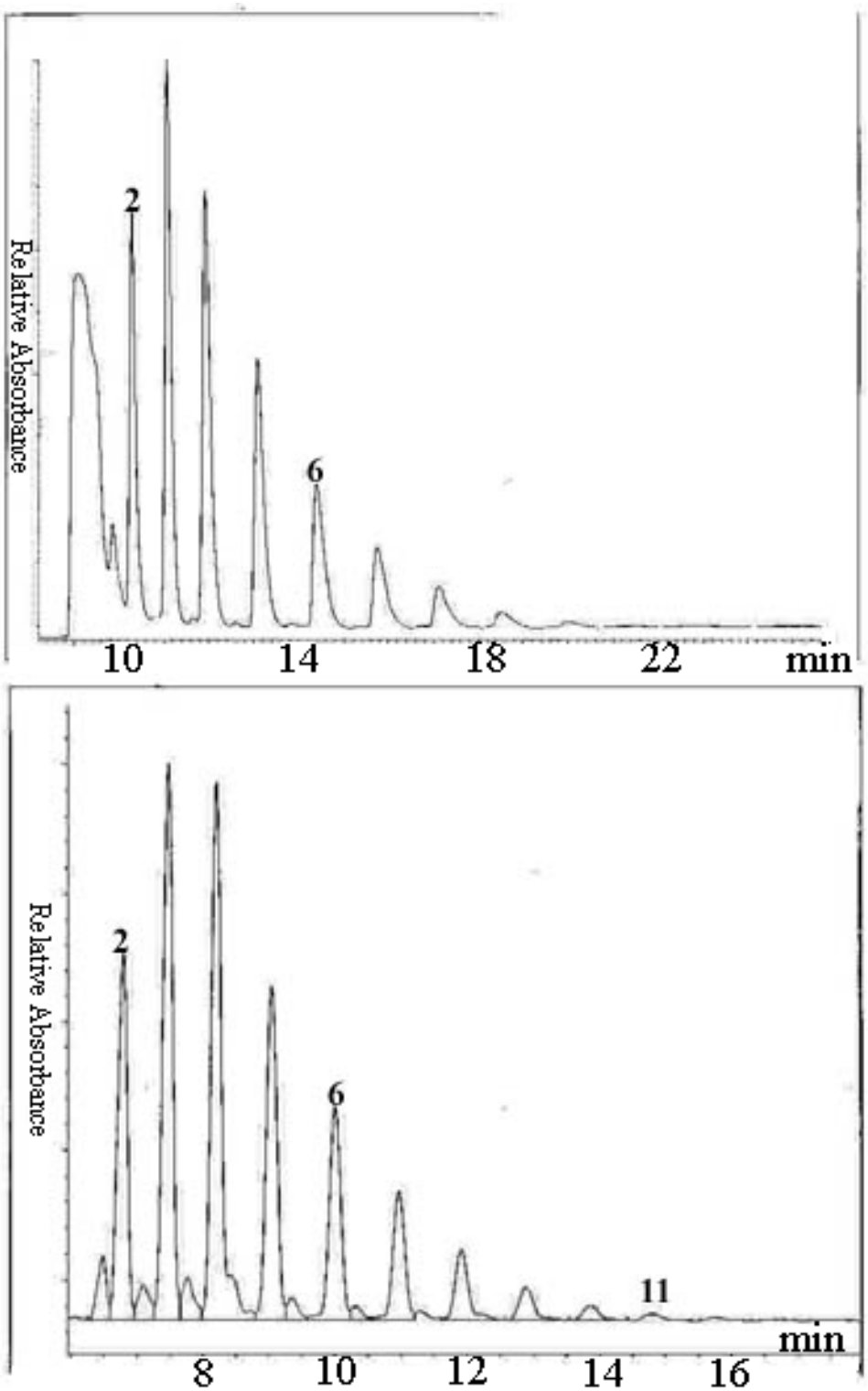

Figure 9. HPLC and SFC chromatograms of POE (5) tert-OP.
Normal phase HPLC-UV (top) with one Supelcosil LC-Diol column and SFC-UV (bottom) with two Supelcosil LC-Diol columns. See Figure 6 for SFC conditions. The peak annotations represent the number of ethoxylate units.

Table II. Chromatographic peak retention times (minutes) of POE (5) tert-octylphenol (OPEOs)

POE (5) tert-octylphenol		
EO Unit	SFC RT	HPLC RT
2	6.79	9.28
3	7.48	10.06
4	8.20	10.92
5	9.05	12.08
6	10.00	13.39
7	10.96	14.75
8	11.91	16.10
9	12.88	17.51
10	13.86	18.96
11	14.80	
12	15.76	

Separation by SFC using two Supelcosil LC-Diol columns and by HPLC using one Supelcosil LC-Diol column.

The effect of stationary phase on separation was sequentially tested using a single diol, amino, and cyano column (Figure 10). Retention of oligomers with longer ethoxylate chains varied with each stationary phase tested. The diol column had the least retention, the amino column had intermediate retention and the cyano column had the greatest retention. It was not possible to elute all of the compounds off the cyano column using the corresponding gradient. In general, a larger methanol modifier concentration was needed to elute longer ethoxylate chain compounds. Because of this we can concluded that APEOs with a longer ethoxylate chain are more polar than ones with shorter chains. Following this reasoning the cyano column must be the most polar stationary phase because it retained the more polar components longer and the diol column was the least polar.

Columns of different stationary phases were coupled in series to test how the arrangement would affect retention of an APEO sample. Two column arrangements were tested, the first consisted of one diol column followed by one cyano column. The second setup contained three columns, a diol column, a cyano column, and an amino column in series (Figure 11). A steeper gradient was needed than previously used to elute all of the compounds because of the presence of the cyano column, as mentioned before. The modifier gradient that was used is described in Figure 11.

One goal of this study was to achieve separation that would allow easy collection of individual oligomers for use as standards. The combined diol/cyano setup rendered shorter retention times than the combined diol/cyano/amino setup and therefore this arrangement was used for preparative fraction collection. In the chromatograms of

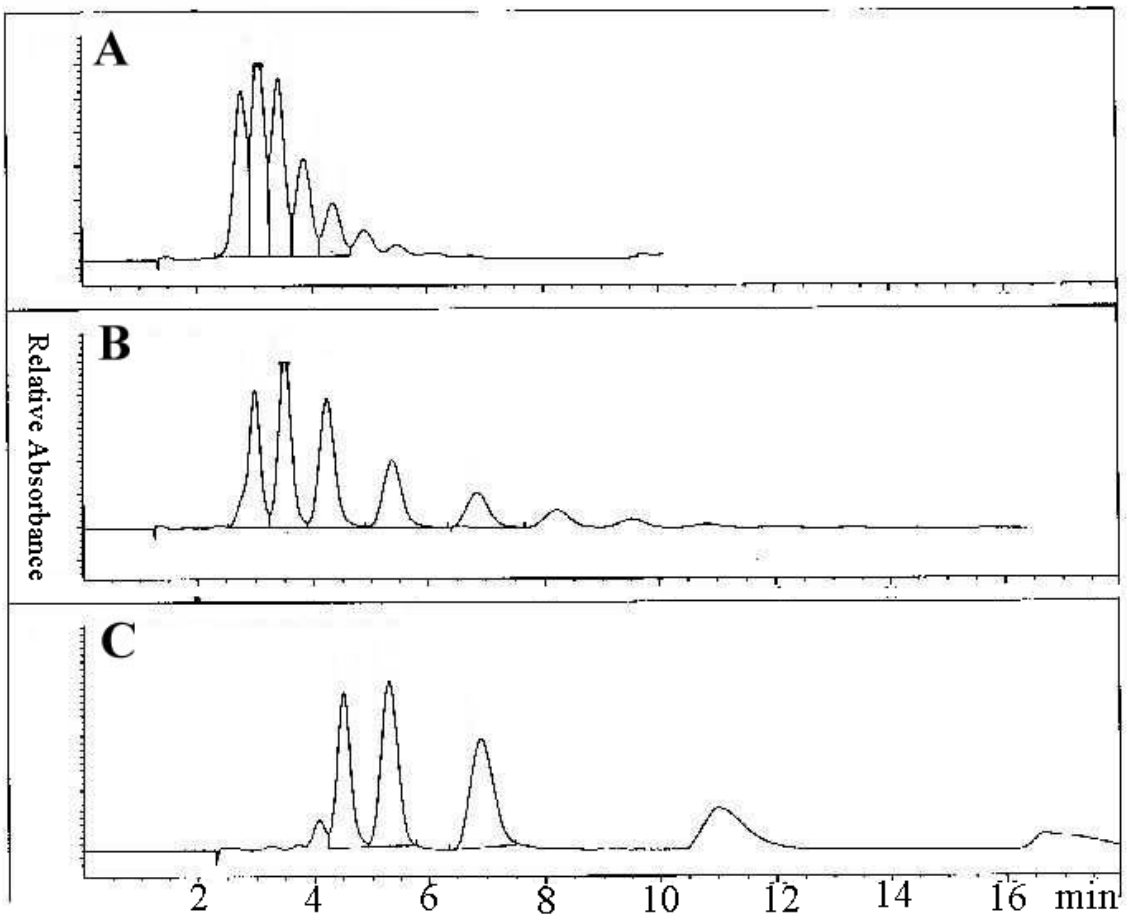

Figure 10. Packed column supercritical fluid chromatograms using single columns of different polar packing material.
A) Supelcosil LC- Diol column B) Spherisorb NH_2 column C) Supelcosil LC-PCN column. The sample used in each chromatogram was POE (4) nonylphenol (2.0 mg/mL). The outlet pressure was maintained at 120 bar and the oven temperature was kept at $60^{O}C$. A linear modifier gradient was used: 10.0% methanol was increased to 26.0% at a rate of 0.6%/min with a 2.0 minute hold then returned to 10.0% in 4.0 minutes followed by a 2.0 minute hold. The mobile phase flow rate was 2.0 mL/min.

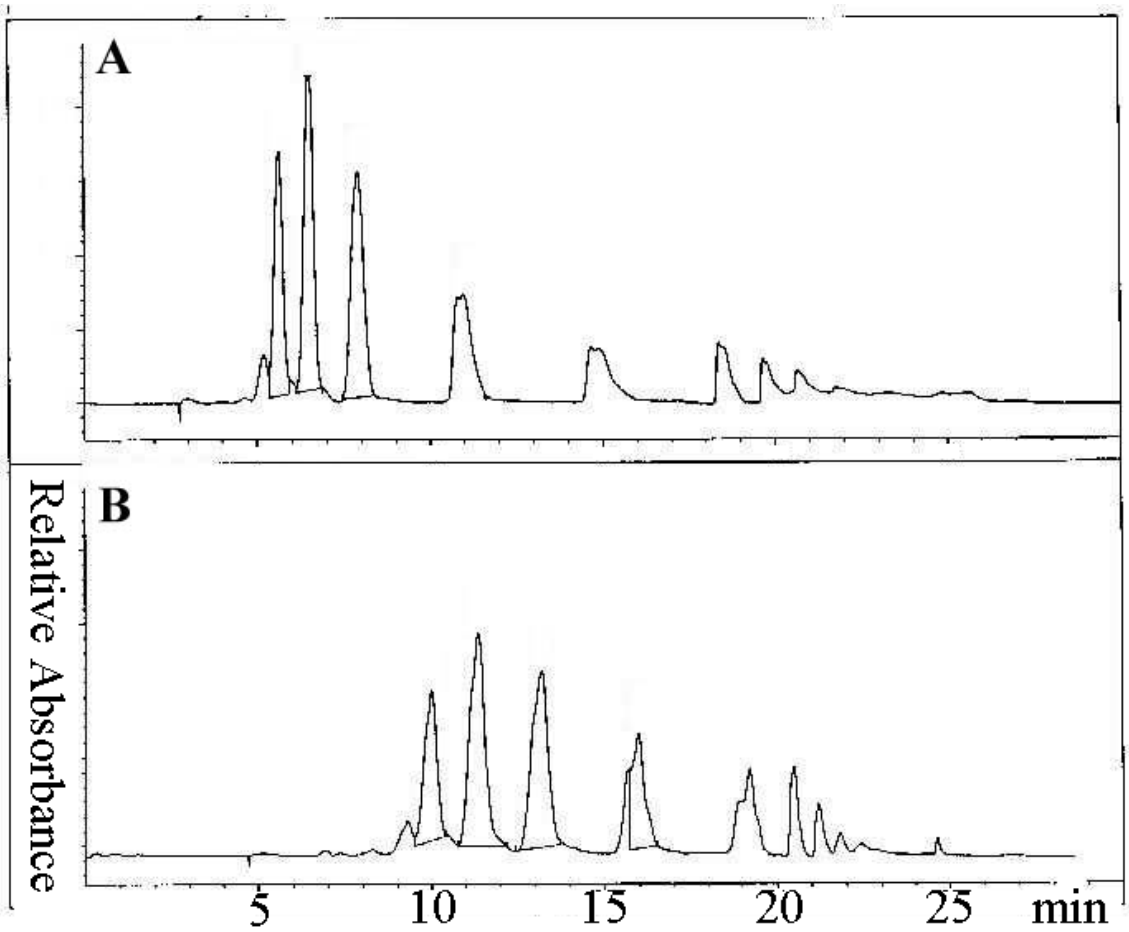

Figure 11. Packed column supercritical fluid chromatograms using stacked columns of different polar stationary phases.
A) one Supelcosil LC-Diol column and one Supelcosil LC-PCN column. B) one Supelcosil LC-Diol column, one Supelcosil LC-PCN column, and one Spherisorb NH_2 column. The sample used in each chromatogram was POE (4) nonylphenol (2.0 mg/mL). The outlet pressure was maintained at 120 bar and the oven temperature was kept at $60^{O}C$. Multiple linear modifier gradients were used: 10.0% methanol was increased to 13.2% by 0.5%/min, the gradient continued to 14.4% at 0.7%/min, 16.6% at 0.8%/min, 20.0% at 1.0%/min, 45.0% at 8.0% hold 5.0 min chromatogram A, 8 min chromatogram B, and returned to 10.0% at 15.0%/min. The mobile phase flow rate was 2.0 mL/min

stacked columns using different stationary phases, peak splitting was observed for later eluting peaks. POE (4) nonylphenol and POE (5) tert-octylphenol were separated and five fractions of each sample were collected. A large volume (75µL) of concentrated sample was injected six to eight times in the collection process. Isolated fractions were re-analyzed both by SFC for purity (Figure 12 and 13) and by flow injection analysis electrospray mass spectrometry for identification. The concentrations used for the semi-preparative work caused the chromatographic peaks to significantly broaden and in some cases combine. Because of this phenomenon it was not possible to collect individual fractions of the two initial oligomers of POE (4) nonylphenol and fractions of the three initial oligomers of POE (5) tert-octylphenol as evidenced by SFC-UV of the early fractions.

Flow injection analysis mass spectrometry was used to identify the components in each fraction. Electrospray ionization mass spectrometry was chosen because it is amenable to high molecular weight analytes and works well with liquid mobile phases. Samples were dissolved in methanol, a compatible solvent for ESI-MS, which made ESI-MS a desirable tool for fraction identification. It was possible to produce sodium adducted molecular ions rather easily. To create optimum response the fractions were first injected and the cone voltage was varied to produce the greatest response for each individual analyte. After MS tuning conditions were perfected the fractions were re-injected into the instrument. A spectrum was created between m/z 200-700 by averaging scans of the injected sample. Figures 14 and 15 show the average mass spectrum of each fraction. The spectra confirm that each chromatographic peak varied by one ethoxylate

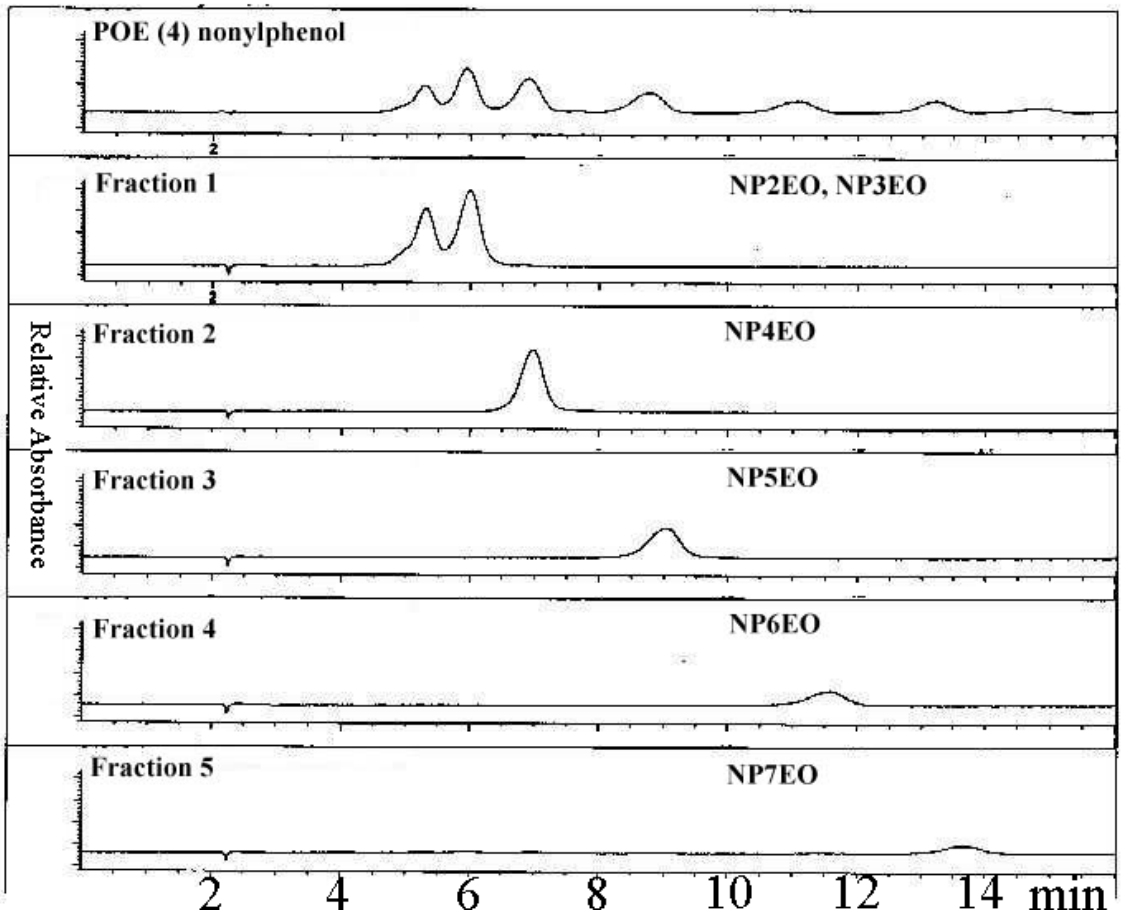

Figure 12. Supercritical fluid chromatograms of collected POE (4) nonylphenol fractions. Separation conducted on one Supelcosil LC-Diol column and one Supelcosil LC-PCN column in series. See Figure 11 chromatogram A for system settings.

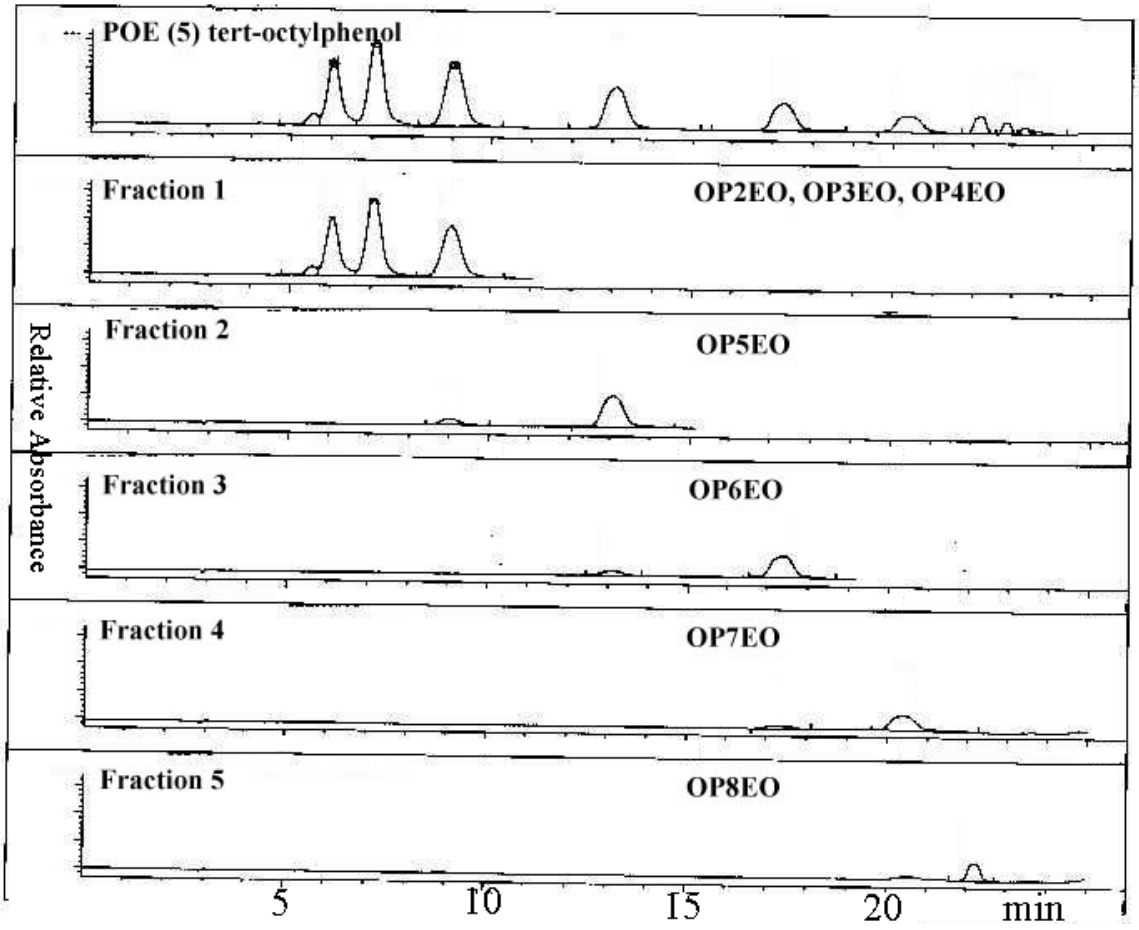

Figure 13. Supercritical fluid chromatograms of collected POE (5) tert-octylphenol fractions. Separation conducted on one Supelcosil LC-Diol column and one Supelcosil LC-PCN column in series. See Figure 11 chromatogram A for system settings.

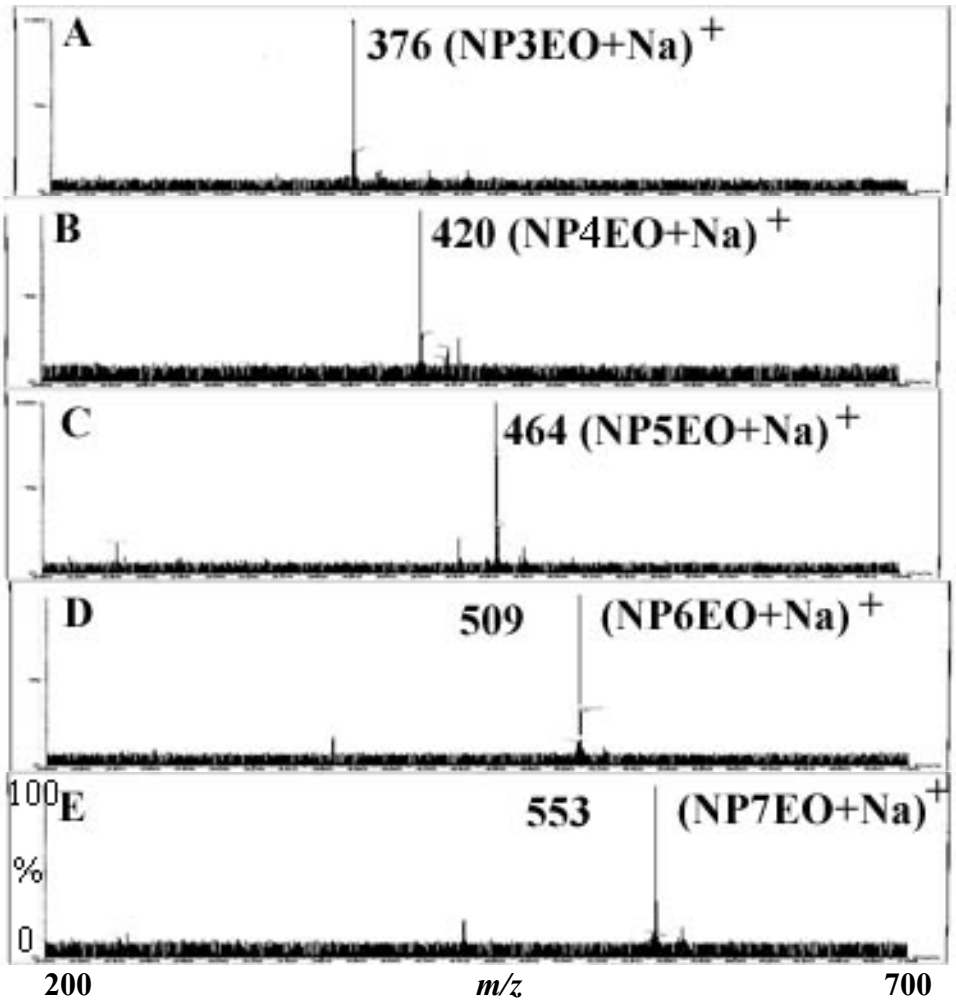

Figure 14. Positive ion FIA-ESI-MS of POE (4) nonylphenol fractions.
Operation in full scan mode. Ions are in the form $[M+Na]^+$, each separated by *m/z* 44, the mass of one ethoxyl unit. A, Fraction 1, cone voltage 59V. B, Fraction 2, cone voltage 53V. C, Fraction 3, cone voltage 62V. D, Fraction 4, cone voltage 65V. E, Fraction 5, cone voltage 67V. Each Spectrum was averaged over the sample injection peak.

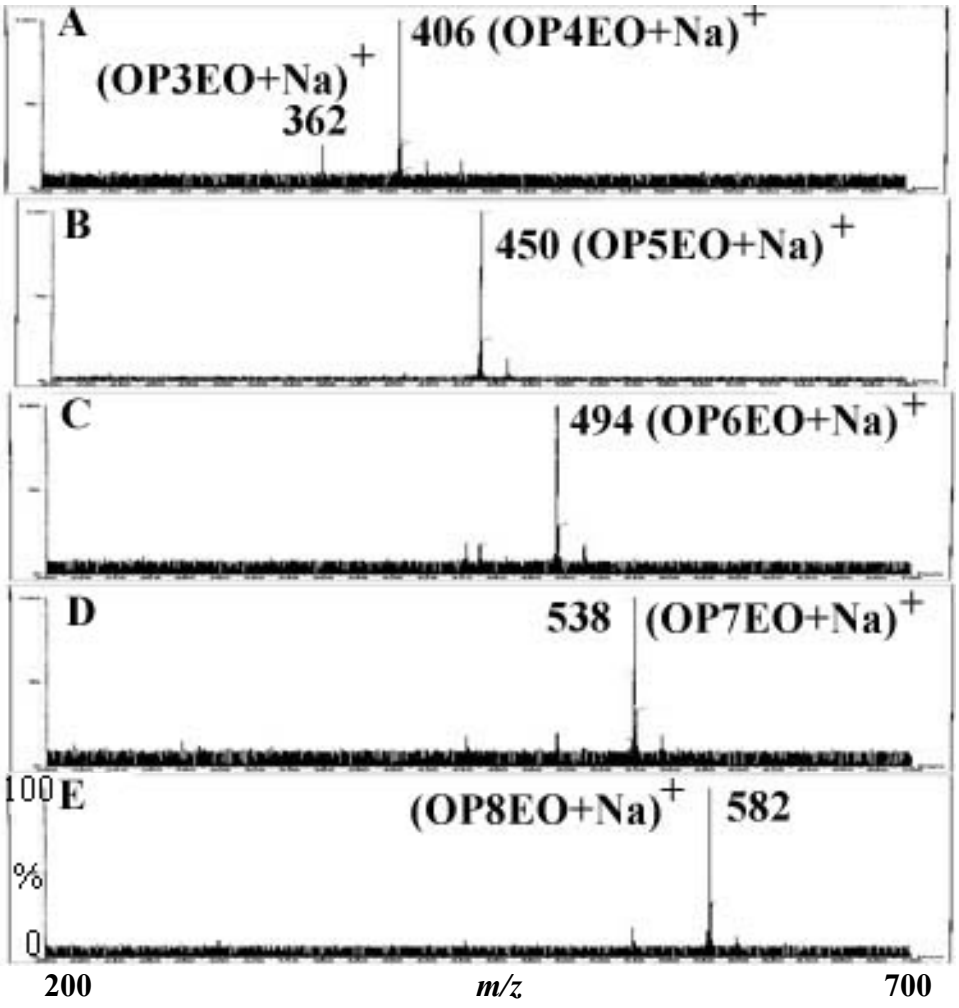

Figure 15. Positive ion FIA-ESI-MS of POE (5) tert-octylphenol fractions. Operation in full scan mode. Ions are in the form $[M+Na]^+$, each separated by *m/z* 44, the mass of one ethoxyl unit. A, Fraction 1, cone voltage 63V. B, Fraction 2, cone voltage 68V. C, Fraction 3, cone voltage 65V. D, Fraction 4, cone voltage 75V. E, Fraction 5, cone voltage 75V. Each spectrum was averaged over the flow injection peak.

unit, a separation of *m/z* 44 amu represents an ethoxylate unit. It was possible to identify NP3EO through NP7EO in basically pure collected fractions of POE (4) nonylphenol and OP5EO through OP8EO in fractions collected from POE (5) tert-octylphenol.

Major ion peaks consisted of $[M + Na]^+$ adduct ions and minor peaks were produced by $[M+K]^+$ adduct ions under positive electrospray conditions. Trace levels of sodium and potassium must be present in the mobile phase used for FIA-ESI-MS because electrolyte was not added to the solutions. According to Okada's research, APEOs have an affinity for alkali metals and have a flexible structure that allows them to form complexes with alkali metals.[37] This explains the ion pairing seen in the mass spectra. Crescenzi and co-workers performed an experiment to see if detector response would decrease due to complexation of oligomers competing for the limited metal pool available. When equivalent amounts of ethoxylated compounds were analyzed by ESI-MS, it was found that detector response increased exponentially from 1 to 6 EO units and then leveled off at 8 EO units (the scope of the study).[38] A decrease in signal was most noticeable for lower ethoxylated oligomers. This can be explained by noting that ethoxylated compounds can form increasingly stable complexes with alkali metal ions as EO unit number increases.[37]

It was important to perform chromatographic separations with absorbance detection on the fractions as well as mass spectrometry analysis, allowing positive identification of sample components since mass spectrometry could not detect all of the compounds present. The first fraction of both POE (4) nonylphenol and POE (5) tert-octylphenol contained more than one compound, as seen in their SFC-UV chromatograms. The sodium ion affinity of the smaller ethoxylate chain compounds was

lower than the larger chain oligomers, and because of this they were not detectable in the mass spectra.

APEOs can be categorized by their average ethoxylate unit value. According to Wang and Fingas, all of the oligomers have almost identical molar absorptivities, which allows integrated chromatographic peak areas to be used directly to determine the mole fraction of each oligomer.[22] Determining percent area of each oligomer peak and then multiplying the percent by the oligomer's assigned EO value was the method used to calculate average molar oligomer values. The EO value of chromatographic peaks was assigned by comparing the elution order and mass spectrometric data discussed above. Summing all of the products of peak area and oligomer value produced an average molar EO value. POE (4) nonylphenol contained nonylphenol predominantly with short ethoxylate chains. NP2EO through NP11EO were observed in its SFC-UV separation. An average ethoxylate unit value of 4.20 was calculated from peak areas. POE (5) tert-octylphenol had a similar distribution as POE (4) nonylphenol, its average ethoxylate unit value was calculated as 4.48 and it contained OP2EO through OP12EO in its SFC-UV separation. Triton N-101 contained a greater range of NPEOs, its calculated average ethoxylate unit was 9.97. NP2EO through NP18EO were observed in its SFC-UV chromatogram. Higher EO peaks were detected in SFC separations which were not detected by HPLC analysis. Wang and Fingas produced similar average EO unit values from their capillary SFC data. Their analysis of Igepal CO-430 (trade name for POE (4) nonylphenol), Triton X-45 (trade name for POE (5) tert-octylphenol) and Triton N-101 produced average EO values of 4.14, 4.50 and 9.52 respectively.[31,32] Chromatographic

data from the SFC-UV separations on two diol columns performed in this study were used to calculate average molar EO values.

4.0 SUMMARY

SFC employing normal phase packed columns and a methanol-modified CO_2 mobile phase produced a similar separation of APEO mixtures compared to HPLC, which employed a similar stationary phase and a hexane/iso-propanol mobile phase. Column length, stationary phase and combining columns with different stationary phases all affected the separation of the APEO mixtures tested. Longer column lengths increased resolution between oligomers. More polar stationary phases retained oligomers with larger ethoxylate units longer. Combination of columns with different stationary phases produced separations combining both of the effects of longer columns and separation ability of each stationary phase. Retention times for SFC separations were notably shorter than normal phase HPLC. One of SFC's advantages is its ability to use longer combined column lengths without elevated back pressure, which occurs in HPLC. Combining multiple columns with different stationary phases seemed to provide the best separation.

An advantage of using packed columns, over the use of capillary columns, is the ability to inject larger amounts of sample and collect eluted fractions. In this study it was possible to isolate and identify individual APEOs. Additionally it was possible to identify the remaining chromatographic peaks due to each peak differing by one ethoxylate unit. This study demonstrated the importance of using both absorbance

detection as well as mass spectrometry. Mass spectrometry alone did not detect all of the components present in initial fractions due to decreased detector response.

Less solvent waste was produced using SFC compared to HPLC. Each SFC separations that used cyano packing as part of its column arrangement used as much as 14.6 mL of methanol. The remaining SFC setups (the study of column length and study of stationary phase) used 12.5 mL of methanol. All separations performed by NP-HPLC used 34.75 mL of hexane and 5.25 mL of iso-propanol, for a combined volume of 40 mL. The HPLC system used almost 275% more organic solvent than the SFC system using a cyano stationary phase and approximately 320% more than the other SFC setups studied, this is not including the volume of solvent needed to initially equilibrate the systems. Reduction of solvent waste is an important step of reducing pollution.

Due to the fact that APEOs are used as industrial cleaners and other processing aids they enter wastewater and end up in sewage treatment plants. Some APEO waste is transferred into the environment and is metabolized into lower ethoxylated alkylphenols which are considered endocrine disrupters.[2] APEOs have been found in fish, river sediment and other environmental samples through analytical techniques.[1,21,27,28,38-42] The results of this study could lead to further use of the method developed for applications in the analysis of environmental samples. Additionally, the separation method could be altered for use in future large-scale separation and collection of individual ethoxylated alkylphenols. Access to standards of individual ethoxylated alkylphenols is important for their quantitative analysis.

CHAPTER 3

Separation of Derivatized Alcohol Ethoxylates and Propoxylates by Low Temperature Packed Column Supercritical Fluid Chromatography using UV Detection

1. INTRODUCTION

The industrial synthesis of alcohol ethoxylates (AEOs) and propoxylates (APOs) yield complex oligomeric mixtures that contain a distribution of either EOs or POs of varying chain length. It is possible to also have various alkyl chain distributions if the starting materials contain fatty alcohols of different chain lengths. Since the molecular size and structure of these surfactants determine their particular properties and hence application, therefore it is necessary that they are well characterized. Alcohol polyethers surfactants may contain oligomeric mixtures that span a wide molecular weight range and be composed of a wide distribution of individual oligomers. The average oligomer size and general oligomer distribution can be determined via chromatographic separation. Many techniques have, therefore, been used such as high temperature gas chromatography (HTGC) coupled with flame ionization detection (FID)[43] or atomic emission detection (AED)[44] for the quantitative characterization of relatively low molecular weight silyl and acetyl derivatized AEO samples. Both reversed-phase[45-48] and normal-phase[47,49] liquid chromatographic (HPLC) separations have been performed on samples containing AEOs. Evaporative light scattering detection (ELSD) has also been used with HPLC separation of non-derivatized AEOs.[45-49] In another study, capillary electrophoresis (CE) was employed[50] for the separation of ionic and nonionic polymers containing ethoxylate chains.

Supercritical fluid chromatography (SFC) is another option for separation of ethoxylated surfactants and has been performed on non-derivatized polyethers with both wall coated open tubular columns[47,51-56] and packed columns[34,58]. As mentioned in previous chapters, the physical properties of supercritical fluids allow high flow rates to be used in SFC and separation of relatively high molecular weight compounds. SFC can perform separation of alcohol polyethers similar to separation performed with alkylphenol ethoxylates due to their similarity in structure. Density programming of pure CO_2 used as the mobile phase has been used to control elution of the oligomeric analytes and can elute higher molecular-weight oligomers than high temperature GC.[43] Flame ionization detection (FID) has been the detector of choice with open tubular SFC for analysis of AEOs.[47,51,53,56] In a few reports SFC has also been combined with chemical ionization[52-54](CI), low energy collisionally induced dissociation[52] (CID), and atmospheric pressure chemical ionization[55] (APCI) mass spectrometry for detection and identification of AEOs.

Unlike alkylphenol ethoxylates, alcohol ethoxylates and propoxylates lack either a strong ultraviolet (UV) (above 205 nm) or a fluorescent active component. Several surfactant derivatization methods have been developed for the addition of an UV chromophore in association with HPLC.[58-63] Naphthyl-isocyanate[61], 1-naphthoyl chloride[62], and 1-anthroylnitrile[63] have each been used to incorporate a fluorescent functional group into surfactants for detection with HPLC. Fatty alcohols in wastewater samples were derivatized by Dunphy and co-workers[64] with 2-fluoro-N-methylpyridinium p-toluenesulfonate for subsequent mass spectrometric detection. Silver and Kalinoski[43] and Asmussen and Stan[44] both used

bis(trimethylsilyl)trifluoroacetamide (BSTFA) for the silylation of hydroxyl-terminated ethoxylated samples wherein GC analysis of the resulting homologous trimethyl silyl (TMS) ethers was performed. Berger and Todd[65] and Rumbelow and co-workers[66] took advantage of the UV transparency of pure CO_2. They formed the TMS derivatives of oligomeric alcohol ethoxylates and propoxylates and separated them by packed column SFC at relatively high temperature and pressure with UV absorbance detection at 191 and 195 nm.

Derivatization reactions, however, can produce by-products that interfere with analyte separation and identification. Katayama and co-workers[67], for example, used an ODS Sep-Pak cartridge to extract the esters formed via derivatization of fatty alcohols with 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole (CDB) in order to achieve the addition of a fluorescent tag. Meisnner and Engelhart[68] developed both an off-line solid phase extraction (SPE) cleanup method and an on-line cleanup method for derivatization of alcohols using carbazole-9-carbonyl chloride or 9-fluorenylmethyl chloroformate. They found that the off-line method produced better results for the analysis of AEO samples.

The separation methods noted above for analysis of alcohol polyethers have strengths and weaknesses. HTGC produces high resolution of low molecular weight oligomers but it is unable to elute the highest molecular weight ones (EO of ~20 or above). HPLC is able to elute high molecular weight non-derivatized oligomers but UV detection is precluded above 210 nm due to UV absorbance by the mobile phase. Tremendous advances have been made in HPLC-ELSD. The ELSD has the advantage of universality for non-volatile species but the UV detector still surpass the ELSD in

dynamic range, operational simplicity, and reliability. SFC separation using pure CO_2 is able to elute high molecular weight TMS-derivatized oligomers but high temperature and high CO_2 pressure are necessary for the best separations.

In the present study, an ethoxylated and a propoxylated alcohol were separated via packed column supercritical fluid chromatography. Acetonitrile-modified CO_2 was used as the mobile phase in an attempt to lower the temperature and CO_2 pressure required for elution compared with SFC separations that used pure CO_2 as the mobile phase. Addition of an UV active chromophore to oligomers was attempted by derivatization of the surfactant samples with a phenyl-containing disilazane-chlorosilane mixture and benzoyl and napthoyl chlorides. SFC, using pure CO_2 as the mobile phase, was used to determine if absorbance of derivatized species was partially due to the polyether chain or if the absorbance was unique to the silylether tag. Electrospray ionization mass spectrometry (ESI-MS) detection was used for making peak assignments in the acetonitrile-modified CO_2 SFC packed column separations. The tacticity of polyoxypropylene (PO) units in the APO sample analyzed in this study was determined by carbon-13 nuclear magnetic resonance spectrometry (^{13}C-NMR).

2. EXPERIMENTAL

2.1 Packed-Column SFC

Three A5000 analytical SFC systems (Mettler-Toledo Autochem Berger Instruments, Inc., Newark, DE), were used in this study. One instrument was located at Virginia Tech's Blacksburg, VA campus, a second at the Technical Innovation Center of

Uniqema in New Castle, DE, and a third at The Procter and Gamble Company's Miami Valley Laboratories location in Cincinnati, OH. SFC-grade carbon dioxide (Air Products and Chemicals, Inc., Allentown, PA) was used as the primary mobile phase for each system.

2.1.1 Pure Carbon Dioxide System

A Deltabond methyl "SFC" packed column (Thermo-Hypersil-Keystone, Bellefonte, PA) was used for separation. The column dimensions were 2 x 250 mm with an average particle size of 5μm and pore size of 300 Å. A 2-μL loop was used for injections. The mobile phase flow rate (liquid) was 0.5 mL/min (calculated 14.4 cm/sec average linear velocity). The oven temperature was 200°C and UV detection was at either 195 nm or 215 nm. CO_2 pressure programming up to a maximum of 370 bar was used for elution.

2.1.2 Acetonitrile-Modified Carbon Dioxide System

Discovery C18 and Discovery RP-AmideC16 stationary phases (SUPELCO, Bellefonte, PA) were used for separation. The column dimensions were 4.6 x 250 mm with an average particle size of 5μm and pore size of 180Å. A 10-μL loop was used for injections. The mobile phase flow rate (liquid) was 2.4 mL/min (calculated 14.4 cm/sec average linear velocity). The oven temperature was 40°C, outlet pressure was held at 120 bar, and absorbance detection was recorded as described above. Modifier programming with acetonitrile up to 25% was used for elution.

2.1.3 SFC-ESI-MS System

Column, mobile phase, and oven conditions were the same as the acetonitrile modified carbon dioxide system. A Hewlett-Packard G1205 column oven (Little Falls, DE) was used to control temperature and UV detection was performed by a Berger Instruments diode array detector. An Isco Model 260D syringe pump (Lincoln, NE) was used to add a make-up flow post UV detector of methanol containing 1 mM ammonium acetate. Make up flow was supplied at a flow rate of 100μL/min. Electrospray ionization mass spectra were obtained with a PerkinElmer Sciex API 365 triple quadrupole mass spectrometer (PerkinElmer, Inc., Toronto, Canada) in the positive ion mode. Turbo gas temperature was 450°C and the (mass-to-charge ratio) *m/z* scan range was 150-1500. The scan stepsize and dwell time were 0.2 *m/z* and 5 ms, respectively.

2.2 Surfactant Samples and Derivatizing Reagents

Alcohol polyether samples were provided by Uniqema (New Castle, DE). A stearyl alcohol polyoxypropylene ether with an average nominal PO length of 15 ($C_{18}PO_{15}$) and a stearyl alcohol polyoxyethylene ether with an average nominal EO length of 10 ($C_{18}EO_{10}$) were analyzed in this study. 1,3-Diphenyl-1,1,3,3-tetramethyldisilazane (DPTMDS), bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), benzoyl chloride, pentafluorobenzoyl chloride, 2-napthoyl chloride, 3,5-dinitrobenzoyl chloride, and pyridine were obtained from Sigma-Aldrich, Inc. (Milwaukee, WI). Phenyldimethylchlorosilane (PDMCS) was purchased from Gelest, Inc. (Tullytown, PA). Sodium hydroxide was obtained from Mallinckrodt, Inc. (Paris, KY). Acetonitrile and dichloromethane (DCM) were obtained

from Burdick & Jackson (Muskegon, MI). ACS grade ammonium chloride was obtained from J.T. Baker (Phillipsburg, NJ).

2.3 Spectroscopy of Derivatized Samples

UV absorbance spectroscopy of derivatized and non-derivatized samples was performed with an Agilent 8453 diode array spectrophotometer (Little Falls, DE). ^{13}C-NMR spectra were collected at 100 MHz on a Varian Unity 400 (Varian, Walnut Creek, CA) spectrometer. A 50 mg sample of $C_{18}PO_{15}$ was dissolved in 1 mL of $CDCl_3$ containing 0.05% tetramethylsilane (Cambridge Isotope Laboratories, Andover, MI). A relaxation delay of 1.0 second between pulses was used with a pulse width of 7.0 μs. Approximately 1500 scans were recorded for the spectra obtained.

3. RESULTS AND DISCUSSION

The goals of this study were to develop a chromatographic method that used mild chromatographic operating conditions and UV detection for analysis of alcohol polyether samples. Previous reported separations of TMS derivatives of alcohol polyethers, which used pure CO_2 as a mobile phase, necessitated the use of high CO_2 pressure (in excess of 300 bar outlet) and relatively high oven temperature (above 150°C). These conditions, which, over the long term, can lead to stationary phase decomposition, approach the maximum operating parameters of commercial analytical SFC instruments. Previous studies of polar and non-polar solutes have shown that use of an organic solvent-modified CO_2 mobile phase can significantly increase column efficiency.[69] Pure CO_2 allows detection of polyether surfactants at 195 nm, unfortunately use of an organic solvent to

modify CO_2 may interfere with UV detection of alcohol polyethers at this wavelength. Therefore, in the work conducted here, silylether derivatives were formed that contained a phenyl moiety, which allowed UV detection outside the absorbance region of liquid solvents commonly used to modify CO_2 for SFC separations.

3.1 Derivatization

Alcohol polyethers were derivatized as their TMS ethers to increase their solubility in pure supercritical CO_2 and reduce undesirable interactions with the stationary phase. BSTFA with 1% TMCS was used for TMS ether formation. The reaction was performed using the derivatizing agent as the solvent. BSTFA is used as a common silylation reagent for GC analysis due to the relatively high volatility of the by-products of the derivatization reaction.[70] Approximately 45 mg of surfactant was dissolved in 1.5 mL of BSTFA-TMCS solution and heated for 60 minutes in an 80°C oven.

All derivatization reactions took place in a 2.0 mL crimp sealed vial. An attempt was made to derivatize surfactant samples for the addition of a functional group capable of absorbing UV light outside the absorbance region of organic solvents. Various esters were formed by reacting alcohol polyether samples with acid chlorides in the presence of pyridine, a basic catalyst and acid scavenger, using hexane or acetonitrile as a solvent. Benzoyl chloride, pentafluorobenzoyl chloride, 2-naphthoyl chloride, and 3,5-dinitrobenzoyl chloride were used for ester formation. The reaction, if performed in hexane, could be stopped by quenching the reaction with 1M sodium hydroxide. The derivatization of alcohol polyether samples to form esterified compounds was successful

but the esterified compounds produced poor chromatographic separation under the conditions used. Broad peaks that had severe tailing were produced by the esterified derivatives. The poor peak shapes may be attributed to poor solubility of the formed esters in the mobile phase or undesirable interactions with the stationary phase compared to silylether derivatives.

Due to the success of TMS ether derivative separations with pure CO_2, it was of interest to form an analogous silylether that contained a phenyl moiety, to use as an UV chromophore. Research into other silylether forming reagents revealed the use of disilazanes for the protection of hydroxyl groups and other protic functional groups. Research has been conducted with hexamethyldisilazane (HMDS) and TMCS for the formation of TMS ethers. HMDS has been designated as both an acid acceptor and the source of TMCS in the derivatization of alcohols.[71] It has been shown in some cases that chlorosilanes are necessary for the derivatization reaction to occur.[72] Following HMDS research, it was decided to evaluate disilazane-chlorosilane reagent mixtures in an effort to form phenyl containing silyl ethers. Diphenyltetramethyl disilazane (DPTMDS) with phenyldimethyl chlorosilane (PDMCS) were used to form phenyldimethyl silylethers (1Ph) from alcohol polyethers. Protic solvents were avoided for the derivatization reaction to discourage the possibility of derivative hydrolysis, therefore, acetonitrile was used as the solvent. Derivatization with DPTMDS alone produced a poor derivatization yield. It was found that PDMCS was necessary in order to catalyze the reaction just as TMCS is used to catalyze silylation reactions with hexamethyldisilazane.[72]

The alcohol disilazane-chlorosilane derivatization reaction is found in Figure 16. The proposed reaction mechanism is as follows: 1) the alcohol attacks the chlorosilane

1Ph: R'= CH_3, R"= Ph

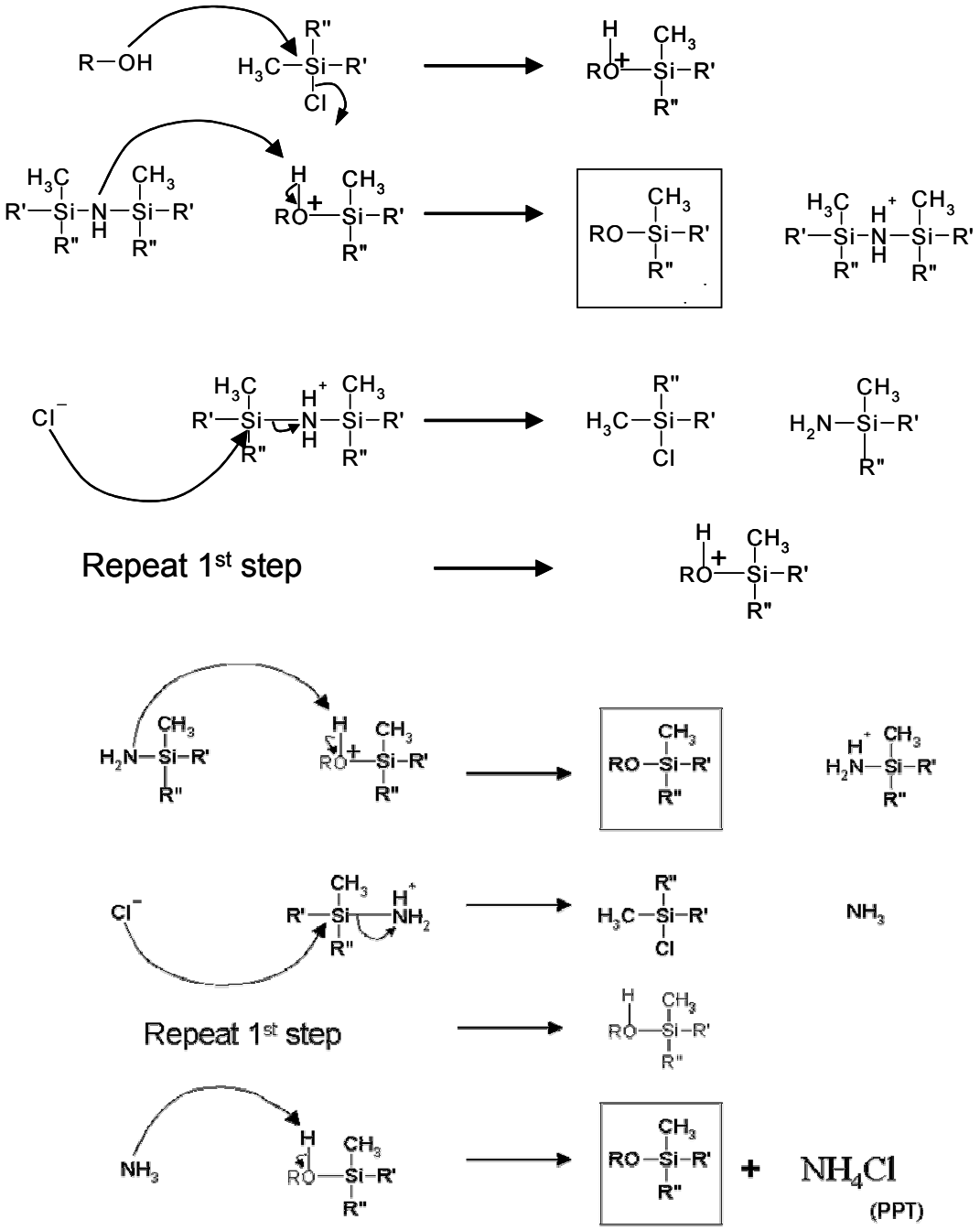

Figure 16. Proposed mechanism for derivatization of primary and secondary alcohols to silylethers using a disilazane-chlorosilane mixture. Acetonitrile solvent

used in the reaction and the disilazane acts as a Lewis base, accepting the proton of the reaction intermediate, thus the silyl ether is formed. 2) Chloride in solution attacks the protonated disilazane and cleaves the Si-N bond, forming the initial chlorosilane and forming an amine $H_2NSi(CH_3)RR'$. 3) The amine performs similarly as the disilazane with the protonated ether intermediate also removing the proton and forming the silylether. 4) Chloride in solution again cleaves the protonated amine to form the original chlorosilane and ammonia. 5) The reaction may take place one more time resulting in ammonium chloride and another equivalent of the silylether.

Previous research that used HMDS-TMCS for TMS ether formation had indicated that a white precipitate was formed during reaction.[71,72] A white precipitate was also produced during reaction of DPTMDS-PDMCS with alcohol polyethers. The precipitate had been presumed to be ammonium chloride. To confirm the identity of the precipitate a reaction blank was performed DPTMDS and PDMCS were added to acetonitrile in the same manner as when derivatizing alcohol polyether samples. The derivatization blank formed a white precipitate instantaneously upon mixture of the reagents. This is most probably due to the presence of water and the formation of disiloxanes. The precipitate was washed several times with acetonitrile. Following the definition of precipitation it was poorly or insoluble in acetonitrile while the disilazanes and chlorosilanes used were soluble in acetonitrile and therefore acetonitrile was a suitable solvent for cleanup of the precipitate. The cleaned-up precipitate was dissolved in deuterated water and analyzed by ^{1}H-NMR. ^{1}H-NMR showed that there were no aromatic protons present in the precipitate. Addition of neat ammonium chloride to the dissolved sample produced an increase in the area of a singlet absorbance with a chemical shift 4.544 ppm, presumably

due to the ammonium protons. The precipitate of the 1Ph reaction was also analyzed by solid IR spectrometry. The precipitate produced an IR absorbance spectrum identical to that of a neat ammonium chloride sample (Figure 17). The IR analysis verified the identity of the compound formed in the 1Ph reaction as ammonium chloride and also corroborated the proposed mechanism of the reaction.

A brief study was performed with $C_{18}PO_{15}$ to determine the optimal reagent concentration for quantitative 1Ph ether formation. The ratio of moles of DPTMDS to moles of $C_{18}PO_{15}$ was varied by adding 50, 100, and 200 μL of DPTMDS to 45 mg samples of $C_{18}PO_{15}$ dissolved in 1350 μL of acetonitrile. A single drop of PDMCS was added to catalyze the reaction. DPTMDS-PDMCS derivatized samples were heated in an 80°C oven for 60 minutes. Derivatized $C_{18}PO_{15}$ samples were then transferred to a separate collection vial with 2.0 mL of DCM. In an effort to quench the reaction the samples were next washed with 1M sodium hydroxide and vortexed. The DCM layer, which contained the analytes of interest, was removed and placed in a separate vial. Sodium sulfate was added to the DCM layer to remove any water that may have been transferred. Dehydrated samples were filtered through a PTFE syringe filter (Millipore Corp., Bedford, MA). The samples were first evaporated to dryness under a stream of nitrogen while being gently heated and then re-dissolved in 1.5 mL of acetonitrile for SFC separation. Table III contains the average molar polyoxypropylene (PO) values calculated from the samples derivatized in the reagent ratio study. Each of the reagent concentrations produced similar PO values and were similar to the value calculated from BSTFA-TMCS derivatized $C_{18}PO_{15}$. Work performed by Berger and Todd[65] and

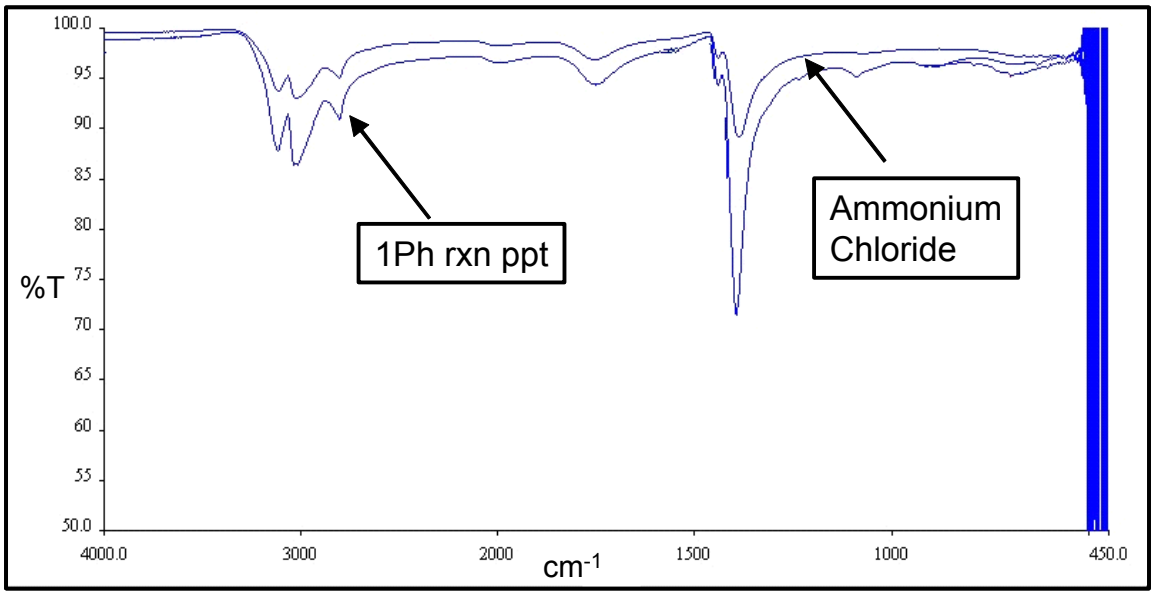

Figure 17. IR spectrum ammonium chloride and 1Ph reaction precipitate.
PerkinElmer Spectrum One FT-IR Spectrometer
Blank = 128 scans; Sample = 64 scans
Resolution = 4cm-1

Table III. DPTMDS to $C_{18}PO_{15}$ reagent molar ratio study

45 mg $C_{18}PO_{15}$						
Volume DPTMDS used	DPTMDS:$C_{18}PO_{15}$	PDMCS:$C_{18}PO_{15}$	Avg PO	SD (n=3)	RSD	Avg. # of Peaks
50uL	4.41	1.59	12.3	0.02	0.12	24.7
100uL	8.90	1.60	12.3	0.01	0.06	27
200uL	18.42	1.66	12.4	0.01	0.1	26
BSTFA w/ 1%TMCS	138.6*		12.2	0.01	0.07	25.3

*=BSTFA:$C_{18}PO_{15}$

Rumbelow and co-workers[66] that used BSTFA-TMCS was the basis for comparison for the current research. The optimal ratio of DPTMDS to surfactant sample was determined by the number of oligomer peaks detected for each derivative. A ratio of DPTMDS to $C_{18}PO_{15}$ as low as 4.4:1 gave similar number of oligomer peaks and average molar oligomer value as the TMS derivative. Final derivatization conditions were: 45 mg of $C_{18}PO_{15}$ with 150 μL of DPTMDS plus one drop of PDMCS dissolved in 1350 μL of acetonitrile. Use of 150 μL of DPTMDS and 45 mg of $C_{18}PO_{15}$ was a molar ratio of approximately 13:1, which would allow surfactant samples of similar molecular weight to be derivatized. As mentioned previously, $C_{18}PO_{15}$ is predominantly terminated with secondary alcohol groups; this derivatization method should therefore work equally as well if not better for primary alcohols, as found in the ethoxylated compounds. A similar method was used for derivatization of $C_{18}EO_{10}$. Samples separated with modified CO_2 were not subjected to liquid-liquid extraction. Initially the liquid-liquid extraction method was employed in an effort to remove residual compounds used in derivatization. The developed methods in this chapter were capable of separating the residual reagents from the derivatized analytes of interest. Because of this, a cleanup method for 1Ph derivatized surfactant samples was forgone. UV spectra of the derivatized samples exhibited an absorption maximum at 215 nm.

3.2 Preliminary Study with Pure Carbon Dioxide

A Deltabond Methyl packed column was used for the SFC separation of both TMS- and 1Ph-derivatized surfactants using pure carbon dioxide. SFC separation with pure CO_2 was initially used as a means to determine if absorbance at 215 nm was unique to 1Ph derivatives. It was necessary to use both high temperature (200 ^{o}C) to provide

high resolution separation and high CO_2 pressure (up to 370 bar outlet) for efficient elution of high molecular weight oligomers. Lower pressure and temperature separations were investigated but were unsatisfactory. Previous research by Berger and Todd[65] and Rumbelow and co-workers[66] used a similar separation scheme for TMS derivatives of ethoxylates and propoxylates. The chromatogram of non-derivatized $C_{18}EO_{10}$ (Figure 18), with detection at both 195 nm and 215 nm, demonstrated the poor solubility and or undesirable column interactions of alcohol polyethers in pure CO_2, even at extreme instrument operating conditions. Minuscule absorbance was detected at 215 nm for non-derivatized surfactants.

Chromatographic separation was improved by the formation of TMS and 1Ph ether derivatives, which exhibited better interaction with the stationary phase (Figure 19). Since the solvent used for TMS-ether formation was the reagent solution itself, it is possible that injection of sample may cap any free silanol groups on the methyl phase, therefore improving the phase or contributing to its longevity. Separations that used pure CO_2 confirmed the absorbance of the 1Ph derivatives was solely due to the phenyl group incorporated into the oligomers. This was done by comparing UV traces of TMS ether derivative separations at 195 and 215 nm. No absorbance was detected at 215 nm for TMS ether derivatives, therefore absorbance at 215 nm of 1Ph ether derivatives was solely due to the added phenyl group. The absorbance of the $C_{18}EO_{10}$ 1Ph ether derivative oligomers at 215 nm was greater than the absorbance of the TMS ether oligomers at 195 nm due to the apparent larger molar absorptivity of the 1Ph ether derivative.

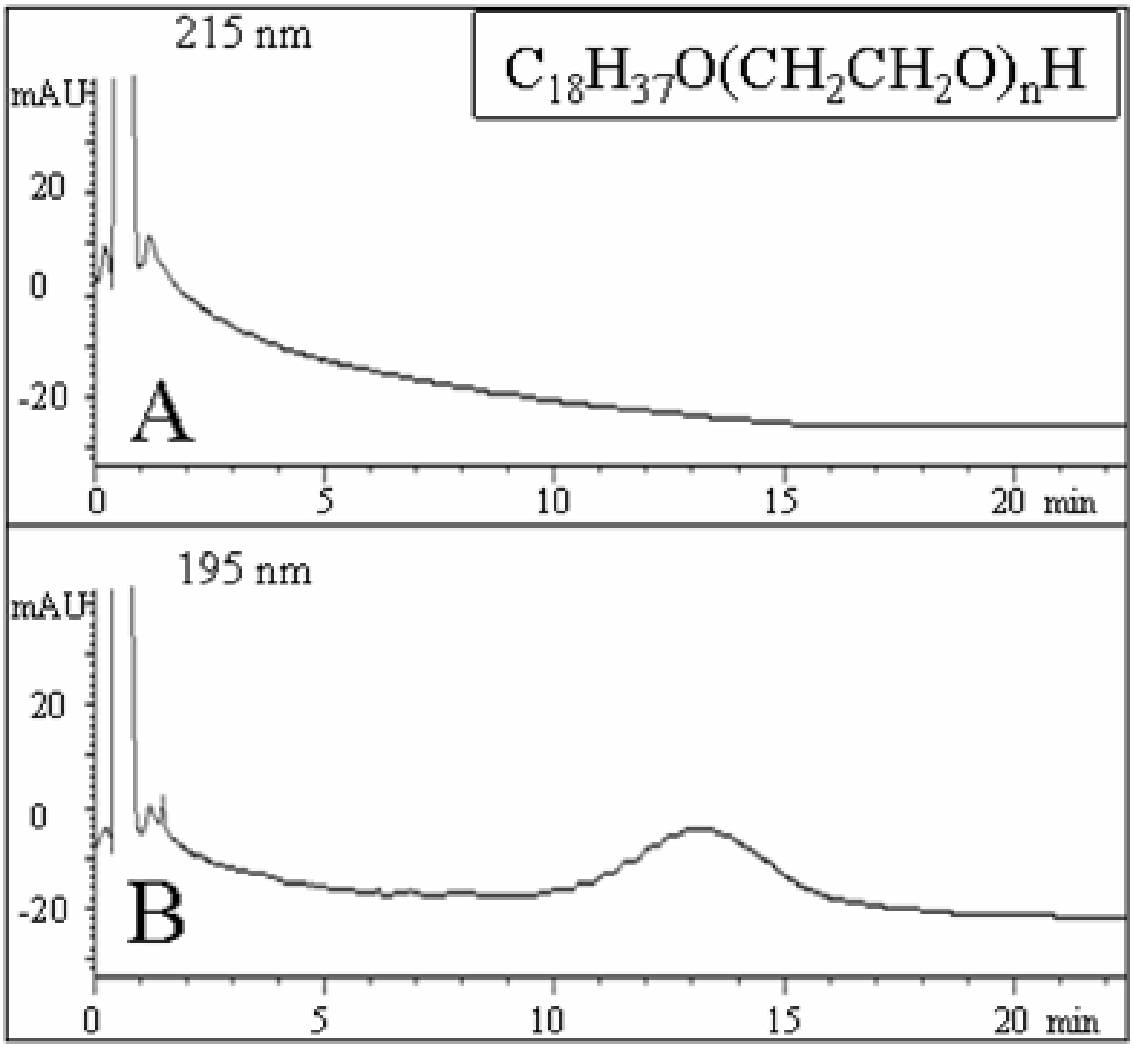

Figure 18. SFC of Non-derivatized $C_{18}EO_{10}$.
Deltabond Methyl column (2.1 x 250 mm), oven temperature 200°C, CO_2 flow rate 0.5 mL/min. A linear pressure gradient was used: 100 bar held for 1 minute, increased to 370 bar at 20 bar/min, hold at 370 bar for 8 minutes. A) Absorbance at 215 nm B) Absorbance at 195 nm

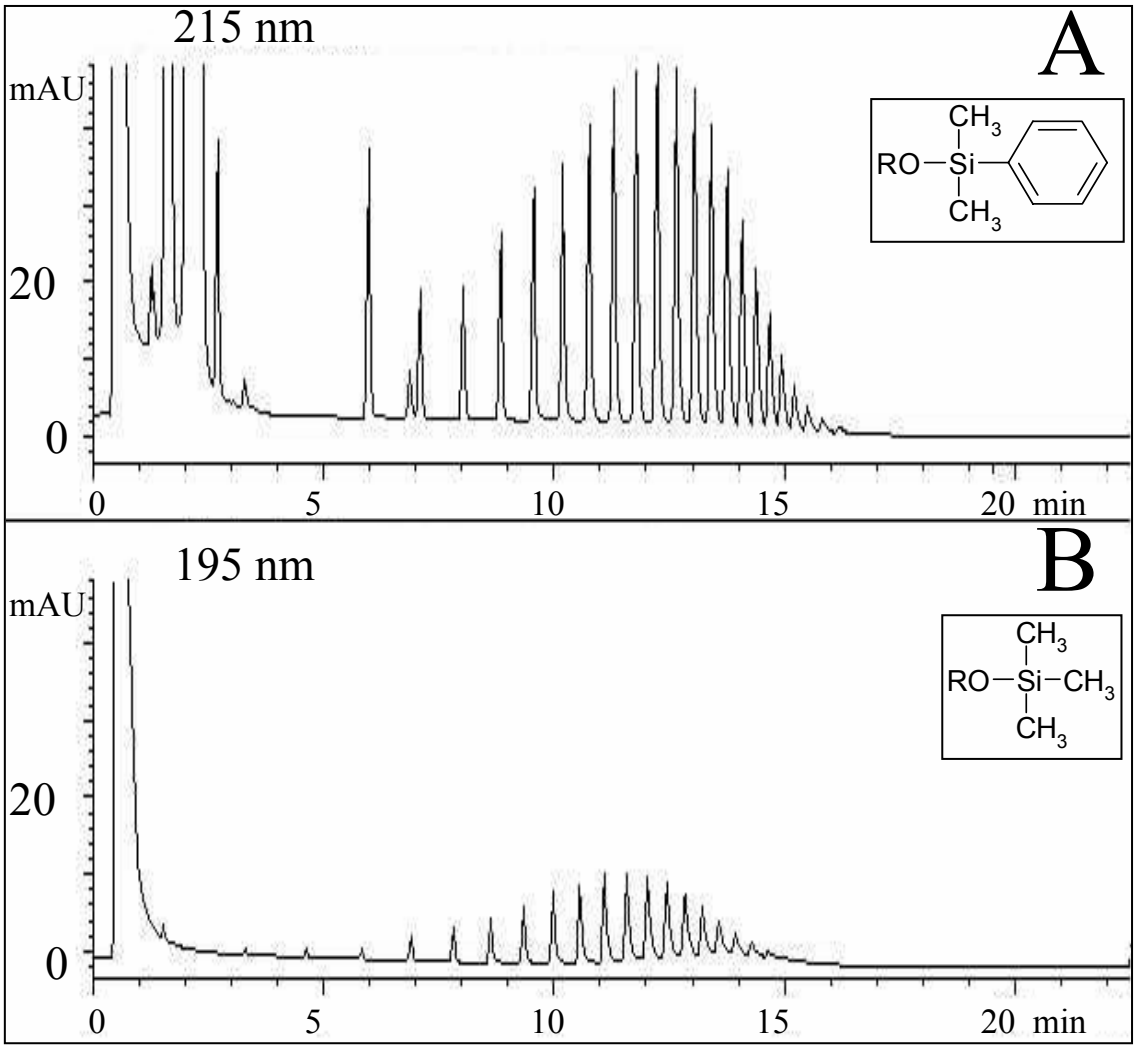

Figure 19. SFC of TMS and 1Ph derivatized $C_{18}EO_{10}$.
A) $C_{18}EO_{10}$ derivatized with DPTMDS and PDMCS, detection at 215 nm B) $C_{18}EO_{10}$ derivatized with BSTFA with 1% TMCS, detection at 195 nm. See Figure 18 for chromatographic conditions

No reference to the use of phenylated disilazanes to derivatize alcohol polyethers for chromatographic purposes has been found in the literature. Traditional uses for DPTMDS have included (a) modification of glass surfaces[73] and (b) the derivatization of PEGs for photometric determination of hydroxyl groups.[74] White and co-workers[75] have used PDMCS for the formation of monosaccharide phenyldimethyl silyl derivatives for analysis by HPLC.

3.3 Acetonitrile Modified Carbon Dioxide

Use of acetonitrile-modified carbon dioxide allowed the use of lower temperature and CO_2 pressure due to its increased solvating strength relative to pure CO_2 at the same temperature and pressure. The lower temperature and pressure conditions also allowed for the use of bonded silica phases with greater alkyl chain length. Several silica-bonded phases were evaluated for separation of the surfactant samples including: bare silica, aminopropyl, cyanopropyl, polyethylene glycol, C_{18}, and amide-embedded alkyl stationary phases. Discovery C_{18} and Discovery RP-AmideC16 provided the most satisfactory separations in preliminary investigations and were thus used as stationary phases with modified carbon dioxide. Both Discovery phases contained the same base silica, but the RP-AmideC16 phase differs in that it contained an amide group embedded in the C_{18} alkyl chain close to the silica surface. Acetonitrile was picked as the modifying solvent due to its low wavelength UV cutoff and its moderate polarity. Each sample was separated individually employing four column configurations: (a) a single Discovery C18 column, (b) a single Discovery RP-AmideC16 column, (c) a Discovery C18 column

followed by a Discovery RP-AmideC16 column, and (d) two RP-AmideC16 columns (Figures 20 and 21). The single C_{18} column yielded good resolution between the initial peaks which where made up of excess derivatizing agent and by-products of the reaction, and the derivatized analytes of interest. The RP-AmideC16 column produced good resolution between the oligomers in both samples, but it did not give a good separation of by-products of the derivatizing agent and the derivatized analytes of interest. A combination of a Discovery C18 column followed by a Discovery RP-AmideC16 column produced both good resolution between the oligomers themselves and between the initial residuals and oligomer peaks. Combination of the alkyl and amide-embedded alkyl stationary phases produced a separation that eliminated the need for sample cleanup. Two RP-AmideC16 columns were tested in series but they gave a similar result to the one obtained with a single column but with longer retention times.

In all of the separations, $C_{18}EO_{10}$ produced chromatograms with more narrow peaks than $C_{18}PO_{15}$ derivatives. The propylene groups of the $C_{18}PO_{15}$ repeat units are branched which creates the probability of different oligomeric combinations. Polymerization of polyoxypropylene can form “tail” to “head”, “head” to "head", and “tail” to “tail” linkages. Along with direction of the methyl group is the possibility of differing chirality between repeat units due to the presence of an asymmetrical tertiary carbon. The $C_{18}PO_{15}$ surfactant studied was commercially produced by base catalysis with potassium hydroxide. It is known that potassium hydroxide catalyzed polymerization of polyoxypropylene forms atactic (random) chirality with predominantly “head” to “tail” propagation.[7,9] ^{13}C-NMR was used to confirm the tacticity of non-derivatized $C_{18}PO_{15}$ sample. Chemical shifts are dependent on sample concentration,

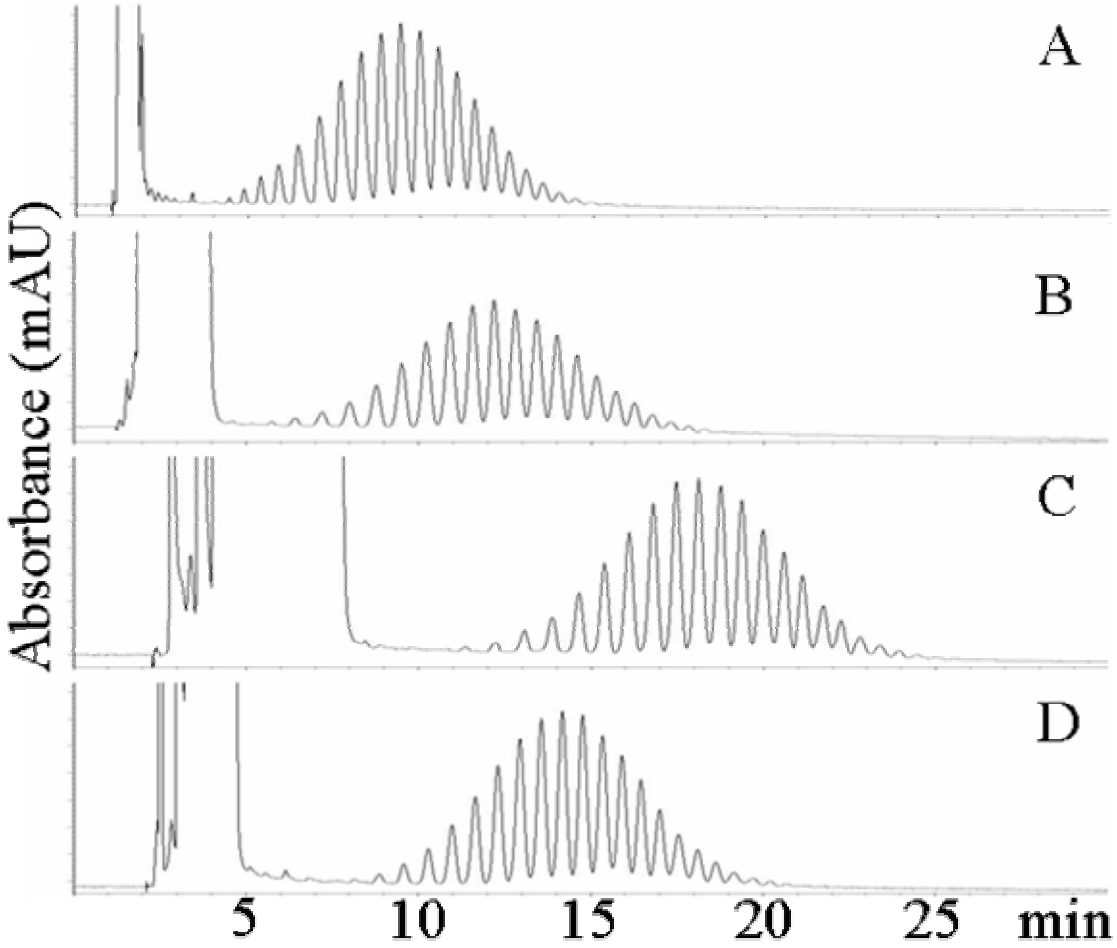

Figure 20. Comparison of an alkyl and an amide-embedded alkyl stationary phase by SFC of 1Ph derivatized $C_{18}PO_{15}$.
Oven temperature 40°C, CO_2 flow rate 2.4 mL/min, outlet pressure 120 bar, detection at 215 nm, modifier acetonitrile. A linear modifier gradient was used: 1% modifier held for 5 minutes increased to 25% at 1%/min, hold at 25% for 5 minutes. A) Discovery C18 column; B) Discovery RP-AmideC16 column; C) Two Discovery RP-AmideC16 columns; D) Discovery C18 column + Discovery RP-AmideC16 column. All columns were 4.6 x 250 mm, 5µm.

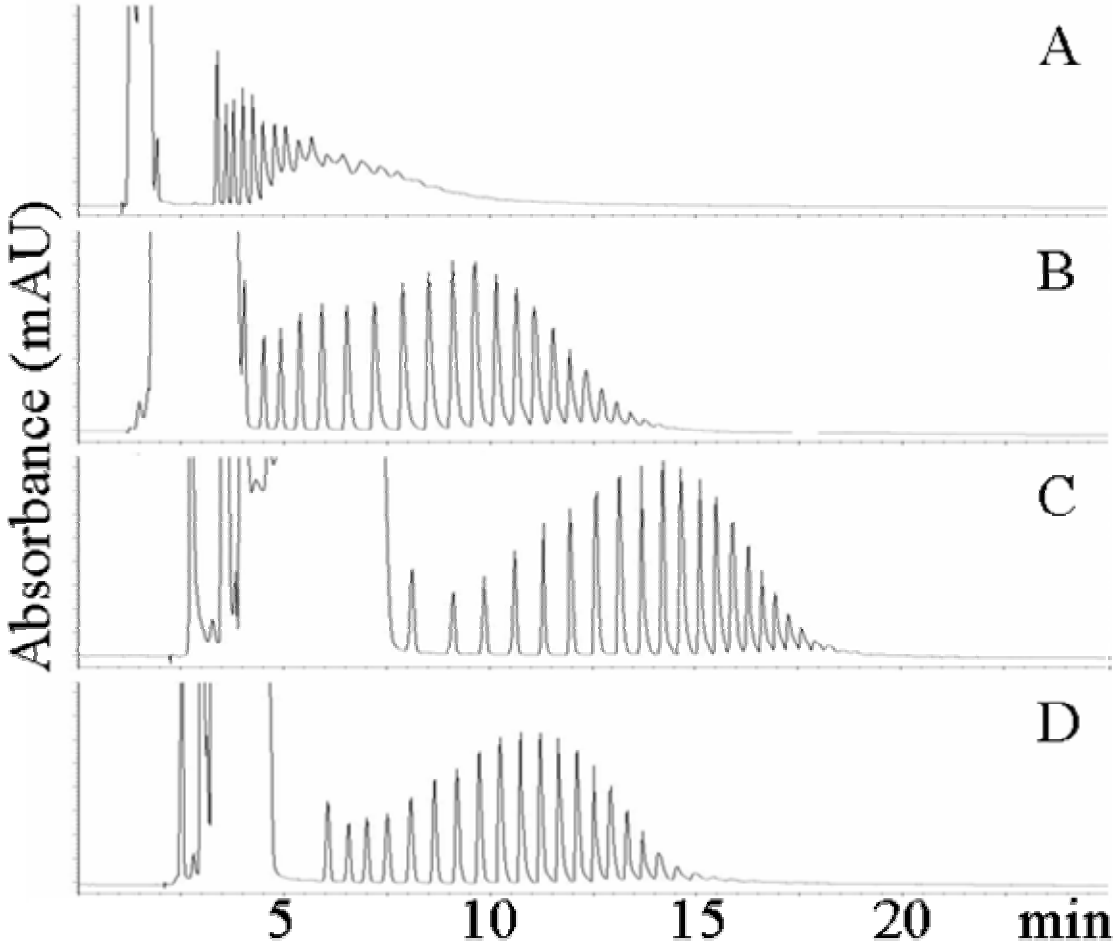

Figure 21. Comparison of an alkyl and an amide-embedded alkyl stationary phase by SFC of 1Ph derivatized $C_{18}EO_{10}$.
Oven temperature 40°C, CO_2 flow rate 2.4 mL/min, outlet pressure 120 bar, detection at 215 nm. A linear modifier gradient was used: 1% modifier held for 5 minutes increased to 20% at 1%/min, hold at 20% for 5 minutes. A) Discovery C18 column; B) Discovery RP-AmideC16 column; C) Two Discovery RP-AmideC16 columns; D) Discovery C18 column + Discovery RP-AmideC16 column. All columns were 4.6 x 250 mm, 5µm.

temperature, and solvent.[8,9] Figure 22 is a ^{13}C-NMR spectra of the $C_{18}PO_{15}$ sample dissolved in $CDCl_3$. Methine carbons present in the PO repeat unit had a chemical shift of approximately 75 ppm and PO methylene carbons had a chemical shift of approximately 73 ppm. The tacticity associated with the polyether units were determined by absorbance due to methine triads and methylene diads, which were assigned from chemical shift values produced by Chilsom and Navarro-Llobet[9] and Schilling and Tonelli[8]. The γ-gauche effect method, which is sensitive to conformation[76], was used by Schilling and Tonelli[8] to calculate relative ^{13}C-NMR chemical shifts as a function of stereo-and regio-sequence for polyoxypropylene. In Figure 22 four resonance peaks at 75.478, 75.323 and 75.279, and 75.074 ppm were due to isotactic-isotactic (ii), mixed isotactic-syndeotactic (is/si), and syndeotactic-syndeotactic (ss) methine triads, respectively. Resonance at 73.328 and 72.929 ppm were due to isotactic (i) and syndiotactic (s) methylene diads, respectively. Since the intensities of resonances of each carbon class were somewhat similar it indicated that no preferred orientation was present, therefore confirming that the polyoxypropylene units were atactic. The greater isomer distribution of $C_{18}PO_{15}$ oligomers, thus, may account for wider chromatographic peaks compared to $C_{18}EO_{10}$, which contains unbranched chain polyoxyethylene groups.

As previously mentioned, the surfactants in this study contained a hydrophobic non-polar region and a hydrophilic slightly polar region. It is possible that the reason the Discovery RP-AmideC16 phase was able to produce better resolution of the oligomers compared to Discovery C18 was because it may have provided more modes of stationary phase-analyte interaction. The Discovery C18 phase separates the surfactant sample via a partition-adsorption mechanism, while the addition of a polar amide group in the

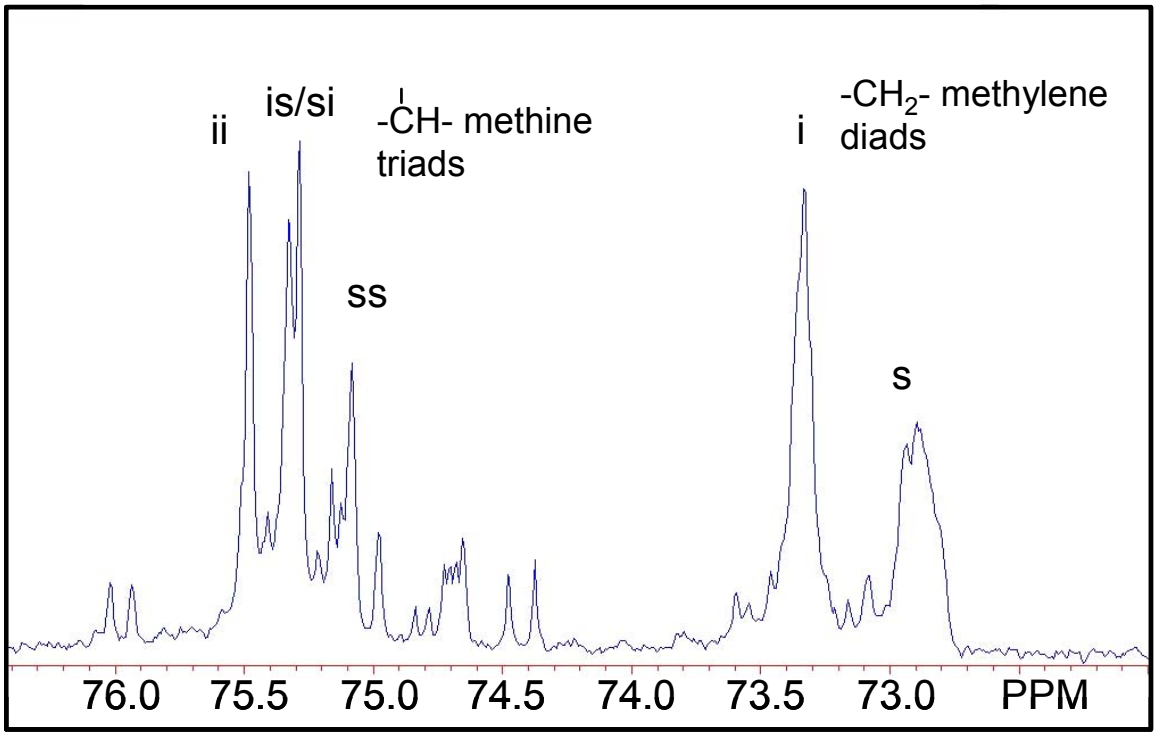

Figure 22. ^{13}C-NMR of non-derivatized $C_{18}PO_{15}$ in $CDCl_3$

Discovery RP-AmideC16 phase makes available hydrogen bonding and dipole-dipole interactions with the polyether region of the surfactant thus producing increased retention and resolution. It is also possible that hydrogen bonding between the adjacent chains of amide groups in the Discovery RP-AmideC16 phase more effectively shields active sites on the base silica. This could account for the improved resolution of $C_{18}EO_{10}$ on Discovery RP-AmideC16 seen in Figure 21 compared to the Discovery C18 phase. The additional carbon atom in polyoxypropylene repeat units of $C_{18}PO_{15}$, compared to the polyoxyethylene repeat units of $C_{18}EO_{10}$, may be responsible for the increased resolution between $C_{18}PO_{15}$ oligomers on the C18 stationary phase. Research on similar phases containing polar embedded groups has been conducted for HPLC (but not SFC) applications. Polar embedded phases have shown improved peak shape for acidic, basic, and zwitterionic analytes[77] compared to conventional C_{18} phases. It is possible that some of the phenomenon associated with polar embedded phases used for HPLC can accrue with SFC applications as well.

3.4 Average Molar Oligomer Values

Work by Wang and Fingas,[22] previously discussed in chapter 2 referring to alkylphenol ethoxylates, demonstrated that oligomers of surfactants containing a phenyl group produce an equal molar UV response and that oligomer repeating unit is not involved in UV detection. They were able to calculate an average molar oligomer value by the summation of the products of oligomer mole fraction (from % peak area) and number of repeating units of each oligomer. Molecular identification of oligomer peaks is, however, necessary for this calculation. The phenyl group added to alcohol polyethers

in this research can be used for similar calculations. SFC-ESI-MS of 1Ph ether derivatives using the acetonitrile-modified CO_2 system was used for peak identification in this study. The SFC-ESI-MS setup was similar to that used by Pinkston and co-workers.[78] The schematic diagram of the SFC-ESI-MS instrument is illustrated in Figure 23. A make-up flow of methanol containing 1 mM ammonium acetate was added to the chromatographic eluent post UV detector to aid in adduct ion formation. Oligomers were subsequently detected as their $[M+NH_4]^+$ adducts. Using the peak identifications it was possible to calculate average molar oligomer values for each analyzed sample. SFC separation of the 1Ph ether derivatives using the tandem stacked Discovery C18-Discovery RP-AmideC16 configuration yielded an average polyoxyethylene (EO) value of 9.7 for $C_{18}EO_{10}$ and an average PO value of 12.6 for $C_{18}PO_{15}$. Deviation from the nominal value is not uncommon and may be due to variation between manufactured surfactant batches.

4. Summary

The goal of this research, the addition of an UV absorbing group to AEO and APO samples, was successfully met. Samples derivatized with DPTMDS-PDMCS produced favorable separations and afforded detection at 215 nm. The ability to detect analytes at 215 nm allowed the use of acetonitrile modified CO_2, which made it possible to separate a wide molecular weight distribution of derivatized oligomeric surfactants using relatively low temperature and CO_2 pressure with UV detection. The use of lower temperature and CO_2 pressure for SFC allows a wide variety of stationary phases to be used for separations compared to conditions needed for pure CO_2 separations. Tandem

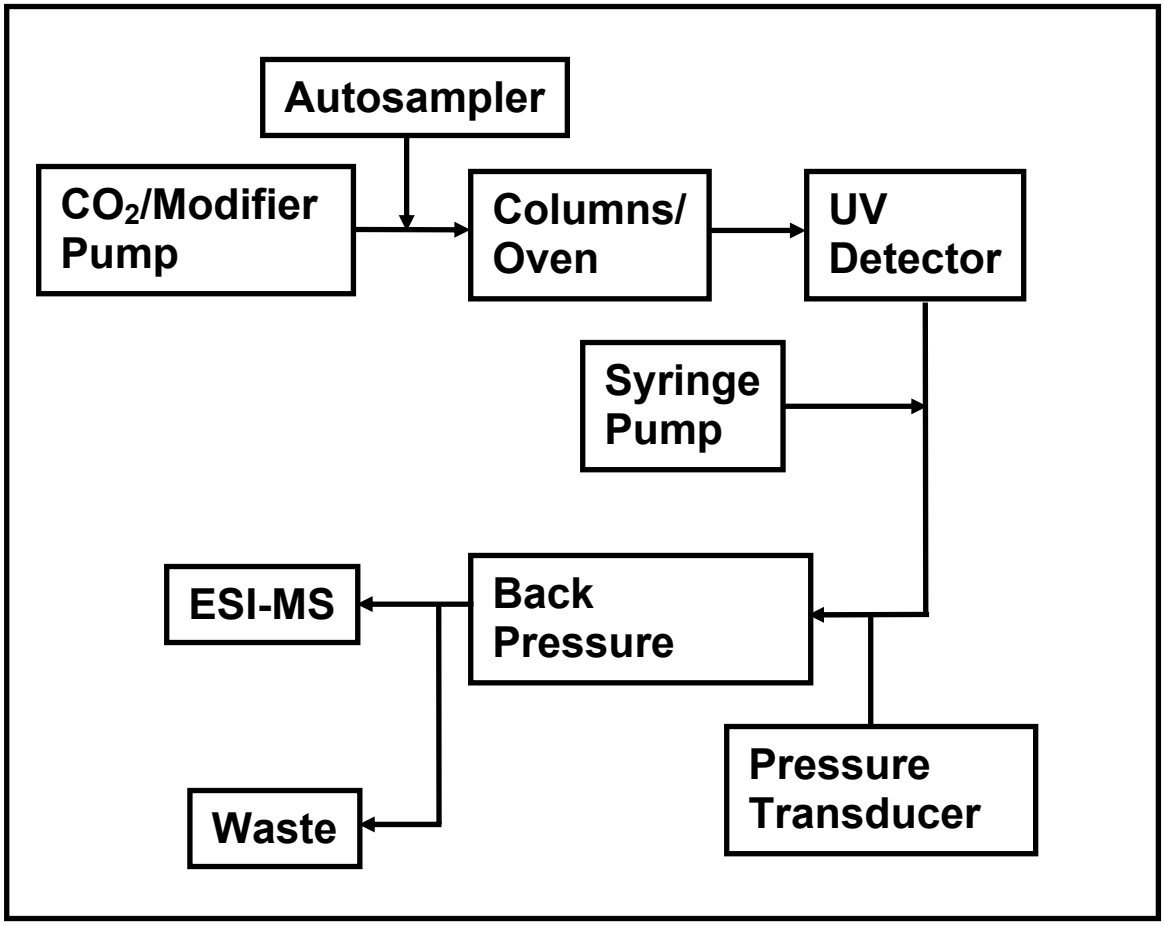

Figure 23. SFC-ESI-MS instrument diagram.

stacking of an ODS stationary phase and a polar-embedded alkyl phase provided enhanced separations. A cleanup step was able to be avoided due to the separation of residual reagents from derivatized species in the chromatograms. When combined with mass spectrometric detection, it was possible to calculate average oligomer values for the samples analyzed. Subsequent chapters investigate increasing chromatographic resolution and sensitivity of detection via evaluation of other derivatives and optimization of stationary/mobile phase conditions.

CHAPTER 4

Determination of Alcohol Polyether Average Molar Oligomer Value/Distribution via Supercritical Fluid Chromatography Coupled with UV and MS Detection

1. INTRODUCTION

Average molar oligomer value and oligomer distribution are important characteristics of alcohol ethoxylates (AEOs) and propoxylates (APOs). The chain length of the hydrophobe and the average molar oligomer value are important factors in assigning commercial uses for manufactured surfactants. AEOs can be classified by their hydrophile-lipophile balance (HLB). The HLB dictates the emulsifying and solubilizing characteristics of non-ionic surfactants.[3] To determine the HLB of alcohol polyethers, it is necessary to know the average molar oligomer value. Nuclear magnetic resonance (NMR) spectrometry and various chromatographic methods have been used most often thus far for the determination of average molar oligomer values.

Proton NMR (^{1}H-NMR) spectroscopy yields data for calculation of average molar oligomer values by integration of (a) the absorbance due to the protons of the repeating ethylene oxide (EO) unit of AEOs and (b) the terminal methyl protons of the alkyl chain.[11-13] A similar calculation can be performed with propylene oxide (PO) groups of APOs. ^{1}H-NMR calculation of average molar oligomer values can, however, be distorted by the presence of polyethylene glycol (PEG) in AEOs.[13] PEG can be produced during the formation of AEOs if water is present during their synthesis.

In Chapter 3 it was demonstrated that an amide-embedded alkyl phase produced a better separation of oligomers than a conventional alkyl phase possibly due to effective

interactions between the analyte and both the alkyl and polar embedded regions of the stationary phase. Low temperature and relatively low-pressure separations, reported in Chapters 2 and 3 as well as published in the literature[57,79], have been made possible by using organic solvent-modified supercritical CO_2 and allows a wide variety of commercially available stationary phases to be used in packed column SFC. Research using polar embedded stationary phases has been historically conducted for HPLC of acidic, basic, and zwitterionic analytes compared to conventional C_{18} phases.[77] Nevertheless, these phases have proven effective in the SFC of derivatized surfactants.

In the current study, a sulfonamide-embedded alkyl phase was investigated for the SFC separation of AEO and APO surfactants. Samples were derivatized with disilazane-chlorosilane mixtures for the formation of phenylated silylethers. A brief study of surfactant concentration on the ability to detect oligomers of low abundance was performed. Acetonitrile and methanol were evaluated as mobile phase modifiers to determine their effect on peak shape. Mass spectrometry, proton nuclear magnetic resonance spectrometry, and ultraviolet absorbance detection were used to determine the average molar oligomer value of surfactant samples.

2. EXPERIMENTAL

2.1 Surfactant Samples and Derivatizing Reagents

$C_{18}PO_{15}$, $C_{18}EO_{10}$, a cetyl alcohol polyoxyethylene ether with a nominal average EO value of 20 ($C_{16}EO_{20}$), and a stearyl alcohol polyoxyethylene ether with a nominal average EO value of 2 ($C_{18}EO_2$) were provided by Uniqema (New Castle, DE). 1,3-

Diphenyl-1,1,3,3-tetramethyldisilazane (DPTMDS) (96% pure) was obtained from Sigma-Aldrich, Inc. (Milwaukee, WI). Phenyldimethylchlorosilane (PDMCS) (98.9% pure) was purchased from Gelest, Inc. (Tullytown, PA). HPLC grade acetonitrile (MeCN) and methanol (MeOH) were obtained from Burdick & Jackson (Muskegon, MI). ACS grade ammonium chloride was obtained from J.T. Baker (Phillipsburg, NJ).

2.2 Packed-Column SFC-UV System

An A5000 analytical SFC system (Mettler-Toledo Autochem Berger Instruments, Newark, DE) was used in this study in conjunction with a Berger automatic liquid sampler (ALS) that contained a 10-μL loop injector and a thermal control module (TCM) used to control column temperature. SFC-grade carbon dioxide (Air Products and Chemicals, Inc., Allentown, PA) was used as the primary mobile phase. Discovery C18, Discovery RP-AmideC16 (SUPELCO, Bellefonte, PA) and Acclaim PA C16 (Dionex, Sunnyvale, CA) packed columns were employed. The dimensions of the Discovery C18 and Discovery RP-AmideC16 columns were 4.6 x 250 mm with an average particle size of 5μm. The dimensions of the two Acclaim PA C16 columns were 4.6 x 150 and 250 mm, respectively, with an average particle size of 5μm. Figure 24 contains the structures of the stationary phases evaluated in this chapter. The mobile phase flow rate (measured in the liquid state at the pump outlet) was 2.4 mL/min. Oven temperature was 40°C, column outlet pressure was held at 120 bar, and UV absorbance was recorded at 215 nm. Modifier programming with acetonitrile and methanol started with a 5-minute hold at 1% modifier to elute excess derivatizing materials. All chromatographic methods then

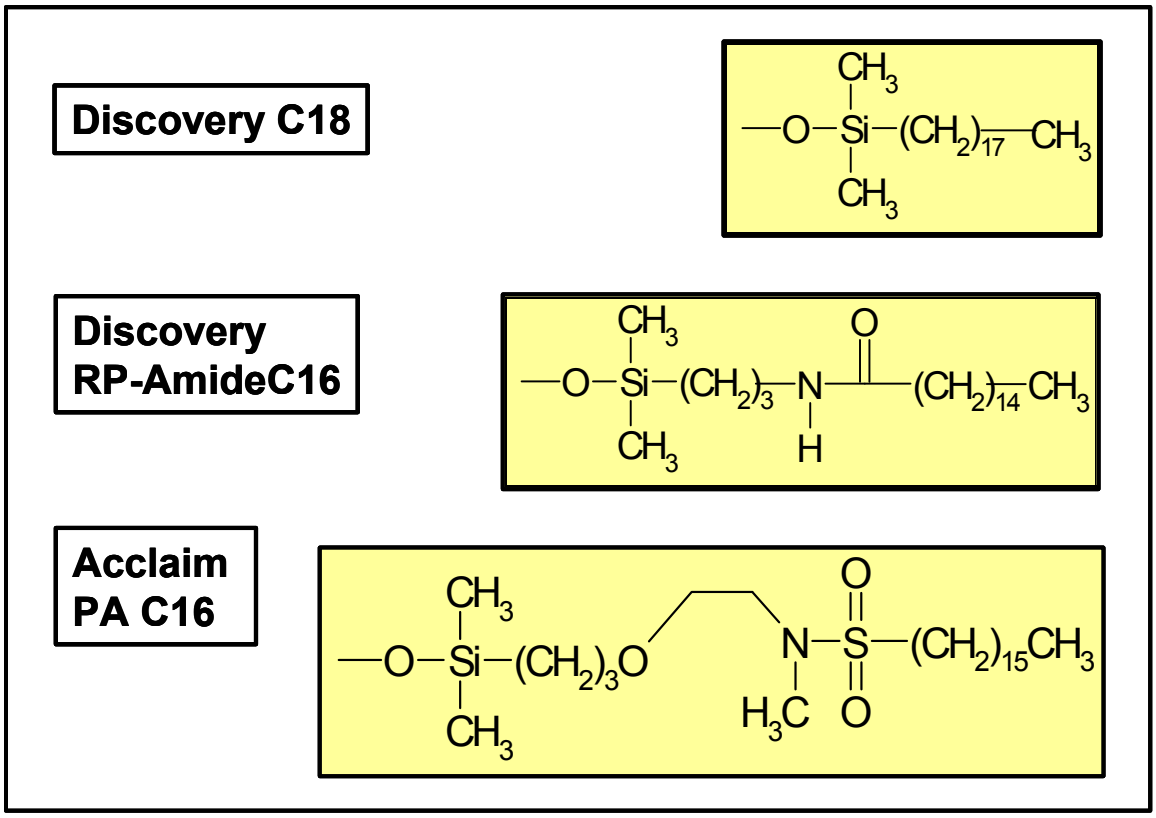

Figure 24. Structures of chromatographic stationary phases.

utilized a linear gradient at 1% modifier per minute to a set concentration depending on the sample composition. A two minute hold at the upper modifier concentration was then followed by a return to 1% modifier at 25%/minute. A 5-minute post-run time was used for system equilibration.

2.3 Packed-Column SFC-ESI-MS System

An A5000 analytical SFC system was also used for SFC-ESI-MS analysis. The column, mobile phase, and oven temperature were the same as described in the packed-column SFC-UV system. A Hewlett-Packard G1205 column oven (Little Falls, DE) was used to control column temperature. An Isco Model 260D syringe pump (Lincoln, NE) delivered 1 mM ammonium acetate (in methanol) make-up flow downstream of the UV detector. Make-up flow was supplied at 200μL/min. A portion of the SFC effluent was diverted to the mass spectrometer via a Valco zero-dead-volume tee (Houston, TX) positioned downstream of the backpressure regulator. The remaining flow was sent to waste. Electrospray ionization mass spectra were obtained using an API 365 mass spectrometer (PerkinElmer Sciex, Boston, MA) in the positive ion mode. Turbo gas temperature was 450°C and the *m/z* range scanned was 150-2000 (step size was 0.2 Da and dwell time was 0.2 ms with a 5.0 ms pause).

2.4 Spectroscopy of Derivatized Samples

To determine an appropriate detection wavelength, UV absorbance spectroscopy was performed with an Agilent 8453 diode array spectrophotometer (Little Falls, DE). IR absorbance spectroscopy was performed either with a PerkinElmer Spectrum One FT-IR

or a 1600 series FT-IR (PerkinElmer, Inc., Shelton, CT). IR spectra were recorded from 450-4000 cm^{-1} with a resolution of 4 cm^{-1}. ^{1}H-NMR spectra were collected at 400 MHz on a Varian Unity 400 (Varian, Walnut Creek, CA) spectrometer. A relaxation delay of 1.0 second between pulses was used with a pulse width of 3.5 μs. Samples were dissolved in $CDCl_3$ at a concentration of approximately 40-90 mg/mL. Both $CDCl_3$ with 0.05% (v/v) tetramethylsilane (Cambridge Isotope Laboratories, Andover, MI) and neat $CDCl_3$ (Isotec, Miamisburg, OH) were used.

3. RESULTS AND DISCUSSION

In the present study, research was conducted to evaluate a sulfonamide-embedded alkyl stationary phase for the supercritical fluid chromatographic analysis of alcohol polyethers. The surfactant samples did not contain strong UV active chromophores above 210 nm and were therefore derivatized forming phenylated silylethers. Once derivatized, it was possible to use UV-absorbance detection combined with mass spectrometric detection for the identification of chromatographic peaks and the calculation of average molar oligomer values. The values obtained from chromatographic data were compared with those calculated via ^{1}H-NMR spectrometry. The effect of CO_2 modifier on oligomeric separation was also investigated.

3.1 Derivatization

Samples were derivatized for formation of phenyldimethyl silylethers, as described in Chapter 3 and reported by Hoffman and co-workers.[79] In Chapter 3 a brief study was performed to determine the optimal ratio between derivatization reagent and

surfactant necessary to adequately yield derivatized species. In this section a further investigation of derivatization was performed to determine the concentration of surfactant necessary to detect the most number of oligomer peaks. The surfactant samples tested included fatty alcohols and contained upwards of 30 oligoether repeating units. The breadth of the oligomeric series was a wide molecular weight span. Each individual oligomer was found at a different concentration in the sample, making a concentration distribution among oligomers. The varying concentration of oligomers allows a portion of the derivatized oligomers to be readably detected by UV detection while oligomers present at low concentration were not discernable from detector noise. As with any sample being analyzed with UV detection, molar concentration and molar absorptivity needed to be taken into account, not just the mass of sample being analyzed. A sample with a high average molecular weight would contain fewer molecules per equal mass than a sample with a lower molecular weight. Assuming the samples contained the same chromophore, at equal mass a low molecular weight sample would produce more intense chromatographic peaks than a high molecular weight sample. Therefore, it is necessary to use higher concentrations of high molecular weight samples to achieve detection of small molar concentration species when using UV detection.

To illustrate this, consider the average molecular weight of $C_{18}EO_2$ is 358 g mol^{-1} which is about half the molecular weight of $C_{18}EO_{10}$ (710 g mol^{-1}) and a third of $C_{18}EO_{20}$ (1150 g mol^{-1}) and $C_{18}PO_{15}$ (1140 g mol^{-1}). In the past experiments, equal masses of sample had been used. Since less moles of sample were being derivatized per equal mass of sample compared to $C_{18}EO_2$ (Table IV) then there should be greater excess of derivatizing compounds in the heavier molecular weight samples. Therefore, a higher

Table IV. Sample molecular weight comparison and molar ratio between surfactant and reagent

				Molar Ratio (Reagent/Sample*100)			
Reagent	M.W.	Mass Used	# Moles	$C_{18}EO_2$	$C_{18}EO_{10}$	$C_{18}EO_{20}$	$C_{18}PO_{15}$
DPTMDS	285.5	0.150 g	5.25E-04	4.2	8.3	13.4	13.3
PDMCS	170.7	0.05 g	2.93E-04	2.3	4.6	7.5	7.4

Sample	M.W.	Mass Used	# Moles	Ratio of moles to $C_{18}EO_2$
$C_{18}EO_2$	358	0.045 g	1.25E-04	1
$C_{18}EO_{10}$	710	0.045 g	6.34E-05	0.5
$C_{18}EO_{20}$	1150	0.045 g	3.91E-05	0.31
$C_{18}PO_{15}$	1140	0.045 g	3.95E-05	0.31

concentration of sample should be able to be derivatized if the sample is of the heavier molecular weight compounds. Twice the concentration of $C_{18}EO_{10}$ and three times the concentration of $C_{18}EO_{20}$ and $C_{18}PO_{15}$ were derivatized compared to the original concentrations used (30 mg/mL).

Data from SFC separations on Acclaim PA C16 with methanol-modified CO_2 (Table V) indicate that each of the higher concentration ethoxylated samples increased the number of oligomers detected by the chromatographic process. Due to the method used to calculate average oligomer value, more detectable peaks increased the average EO values for both $C_{18}EO_{10}$ and $C_{18}EO_{20}$. $C_{18}PO_{15}$ produced a similar number of peaks and an increase in surfactant concentration did not increase the average molar oligomer value. Research in Chapter 5 will further investigate increasing oligomer detection sensitivity through evaluation of a different derivative. The method used for average oligomer value determination weighs each peak by its assessed EO value; therefore, a small chromatographic peak with high EO value has an impact on calculating the average oligomer value of a sample. Depending on the average molecular weight of the sample, 45-135 mg of surfactant was placed in a 2.0 mL vial. The sample was dissolved in 1350 μL of acetonitrile, 150 μL of DPTMDS plus one drop of PDMCS were then added to the vial. The vial was crimp sealed, shaken for 30 seconds, and placed in an 80°C oven for 60 minutes. A white precipitate formed during the derivatization reaction. Samples were allowed to cool and then filtered through a 0.45 μm PTFE syringe filter (National Scientific, Duluth, GA). The precipitate which formed during derivatization was analyzed by IR spectroscopy and identified as ammonium chloride by comparison with the IR spectrum of neat ammonium chloride.

Table V. Surfactant concentration data.

	Concentration	Average Oligomer	Avg. # of oligomers detected
$C_{18}EO_{10}$	30 mg/mL	9.96 EO	29.3
	60 mg/mL	9.99 EO	38.3
$C_{18}EO_{20}$	30 mg/mL	17.89 EO	40
	90 mg/mL	18.34 EO	48.3
$C_{18}PO_{15}$	30 mg/mL	12.55 PO	35
	90 mg/mL	12.36 PO	31.7

1Ph derivatives separated on (2) Acclaim PA C16 columns 4.6 x 150 mm each. Methanol modifier. Values from three injections of a single sample

3.2 Modifier Effect

Methanol and acetonitrile are the most common modifiers of CO_2 for SFC.[69,80-84] Under supercritical conditions, it has been shown that CO_2 and methanol are sorbed by C_{18} and silica stationary phases and subsequently function as part of the stationary phase.[84] Work by Lesellier and co-workers, which used a C_{18} stationary phase, revealed that a larger mobile phase adsorption occurred with methanol modified-CO_2 than acetonitrile modified-CO_2.[84] The adsorption increased stationary phase thickness, which allowed a larger portion of test solutes to enter the bonded-solvated phase. These workers also reported that methanol also made the stationary phase more homogeneous, which in turn created better peak shape. Solute retention has been shown to decrease with the addition of modifier due to increased mobile phase solvent strength .[69] Undesirable interactions with residual silanols are a possible source of band broadening.[69,80] Modifier molecules at a stationary phase surface may thus interact with, and possibly "hide", active silanol sites.[83]

Separations were performed with methanol- and acetonitrile-modified CO_2 on a Discovery C18 column to determine the effect of modifier on oligomer separation. Modifier gradient and other chromatographic conditions were identical for both separations. UV absorbance was recorded at 215 nm, beyond the UV cutoff of both modifying solvents. The first three oligomer peaks of the 1Ph derivatized $C_{18}EO_{10}$ sample separated with acetonitrile-modified CO_2 have asymmetry values of 1.15, 1.21, and 1.44. The corresponding peaks in the methanol-modified CO_2 have asymmetry values of 1.09, 1.10, and 1.14. Peak symmetry values were calculated by SFC Chemstation software revision 3.4 (Mettler-Toledo AutoChem Berger Instruments,

Newark, DE). Peak symmetry is measured by the ratio of peak area prior to the peak apex divided by peak area after the peak apex. A symmetry value of 1.0 would indicate a symmetrical peak. Peak asymmetry is the inverse value of peak symmetry calculated by the software. Peak asymmetry values greater than 1.0 indicate peak tailing and values less than 1.0 indicate peak fronting. SFC of the 1Ph derivatized $C_{18}EO_{10}$ sample revealed that methanol-modified CO_2 produced less peak tailing than separations using acetonitrile-modified CO_2 (Figure 25).

The properties of methanol-modified CO_2 increasing phase thickness and "hiding" residual silanols may explain why better peak shapes were seen using methanol-modified CO_2. As the polyether chain length of each oligomer increased a greater increase in retention time was observed using acetonitrile-modified CO_2 compared to methanol-modified CO_2, indicating acetonitrile-modified CO_2 had weaker solvating strength than methanol-modified CO_2. Retention times of oligomers with shorter EO chain lengths were less influenced by modifier type than those with longer EO chains. Based upon these results, methanol was chosen as the mobile phase modifier for the remainder of this study.

3.3 Stationary Phase

Acclaim PA C16, a sulfonamide-embedded alkyl stationary phase, was evaluated for separation of derivatized surfactants by oligomer number. Previously, 1 Ph derivatized surfactants had been separated on an octadecyl alkyl-bonded phase serially connected to an amide-embedded alkyl phase.[79] The amide-embedded alkyl phase by itself was unable to separate the excess derivatizing material from the oligomeric series and, at the same time, provide good oligomer resolution. Figure 26 contains

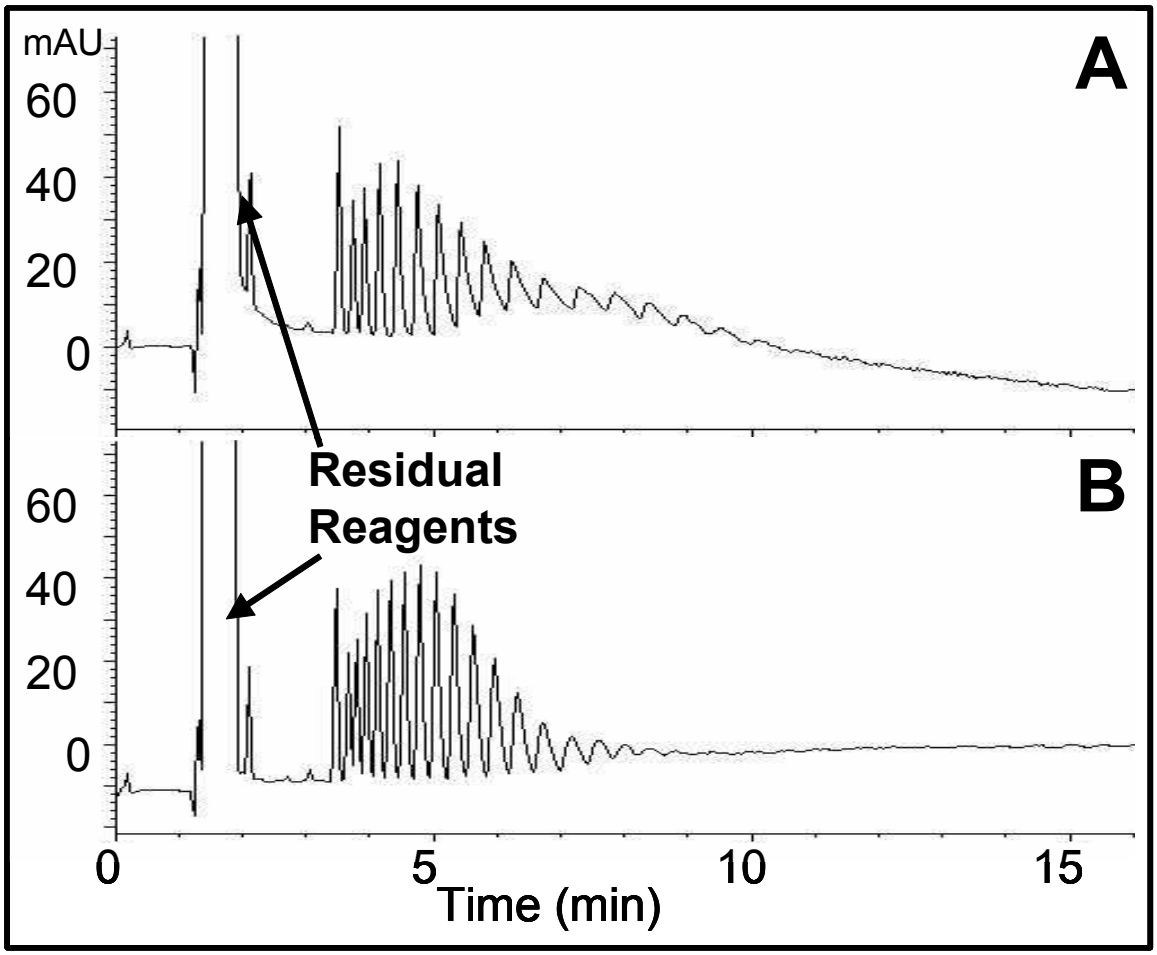

Figure 25. Comparison of acetonitrile and methanol-modified CO_2.
Discovery C_{18}, 4.6 x 250 mm, 5µm. Oven = 40°C, Outlet pressure = 120 bar, Flow rate = 2.4 mL/min, detector absorbance 215 nm, modifier gradient: 1% for 5 min, linear increase 1%/min to 15%, hold 2 minutes, linear decrease to 1% at 25%/min, 5 minute post run. A) Acetonitrile-modified CO_2; B) Methanol-modified CO_2

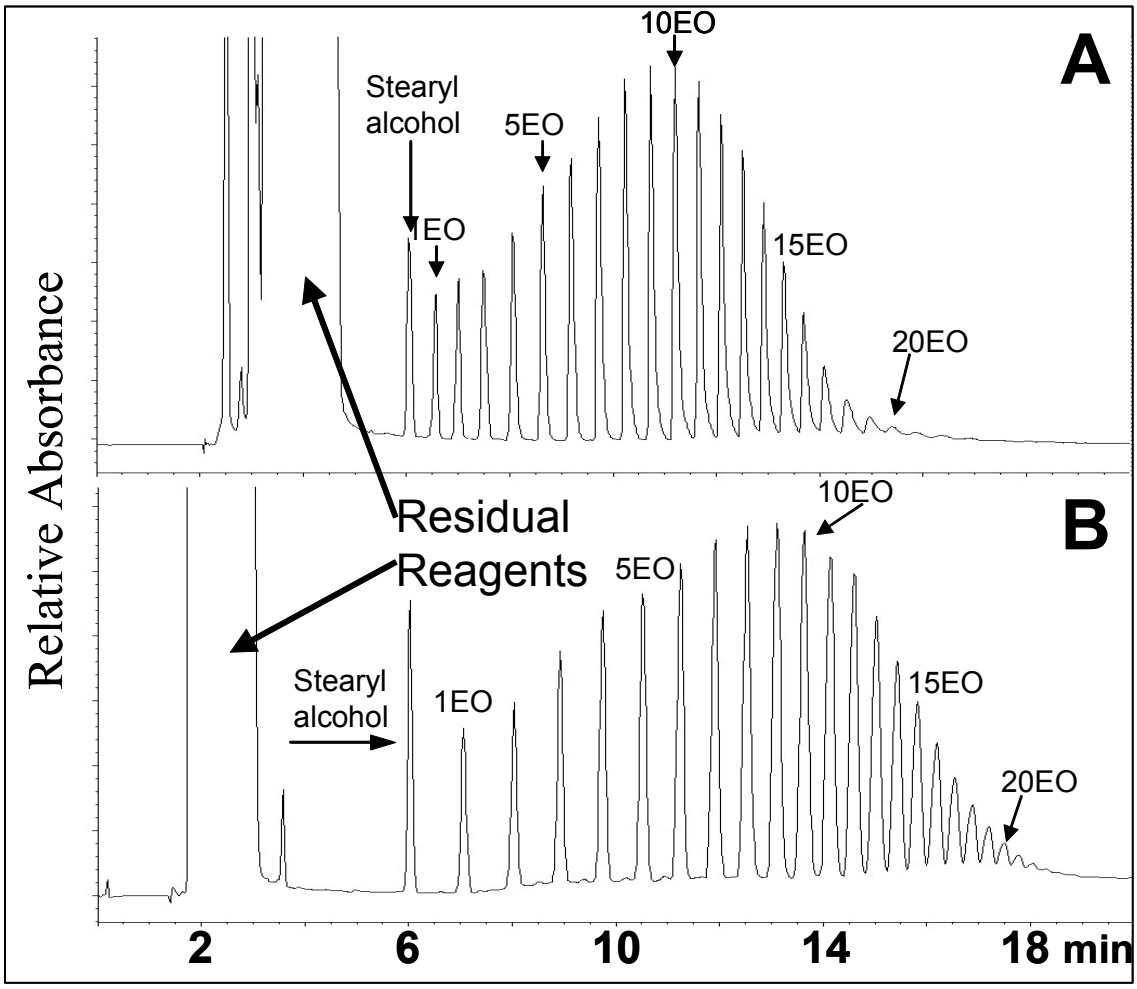

Figure 26. SFC-UV separation of $C_{18}EO_{10}$ 1Ph derivative.
Oven = 40°C, Outlet pressure = 120 bar, Flow rate = 2.4 mL/min, detector absorbance 215 nm, modifier gradient: 1% for 5 min, linear increase 1%/min to 25%, hold 2 minutes, linear decrease to 1% at 25%/min, 5 minute post run. A) Discovery C18 + Discovery RP-AmideC16 (4.6 x 250 mm, 5µm each). Acetonitrile-modified CO_2. B) Acclaim PA C16, 4.6 x 250 mm, 5µm. Methanol-modified CO_2

chromatograms of two 1Ph derivatized $C_{18}EO_{10}$ samples separated on two column setups. One of the chromatograms was produced on a combination of a Discovery C18 column coupled in series with a Discovery RP-AmideC16 column using acetonitrile-modified CO_2 as the mobile phase and the second chromatogram was produced by separation on a Acclaim PA C16 column using methanol-modified CO_2 as the mobile phase. Table VI contains the peak asymmetry and resolution values for each of the derivatized peaks. The Acclaim PA C16 column with methanol-modified CO_2 produced asymmetry values close to 1.0 and deviated with highly ethoxylated oligomers when resolution approached 1.0 (not baseline resolved). The Discovery C18 + Discovery RP-AmideC16 column setup using acetonitrile-modified CO_2 produced chromatographic peaks that had asymmetry values significantly deviating from 1.0 even though resolution was greater between most peaks compared to the separation method using the Acclaim PA C16 stationary phase. The Acclaim PA C16 setup produced less peak tailing for oligomers and better resolution between early eluting surfactant peaks compared to the Discovery C18 + Discovery RP-AmideC16 setup.

The Acclaim PA C16 stationary phase contains 22 carbons in its bonded ligand compared to 19 carbon atoms in the RP-AmideC16 stationary phase. The RP-AmideC16 amide-embedded alkyl phase contains a hydrogen bond donor proton; while the Acclaim PA C16 sulfonamide-embedded phase does not possess hydrogen donor bond capability. Both phases may interact with the hydrophilic polyether region of the AEO and APO samples through dipole-dipole interactions. As mentioned in Chapter 3, the polar embedded functionalities may shield free silanols and thus reduce the possibility of unfavorable interactions with active sites, which may cause peak tailing. The Discovery

Table VI. Peak asymmetry and resolution data for 1Ph derivatized $C_{18}EO_{10}$ on Acclaim PA C16 and Discovery C18 + RP-AmideC16.

	Acclaim PA C16		Discovery C18 + RP-AmideC16		
Peak #	Asymmetry	Resolution	Peak #	Asymmetry	Resolution
1	0.99		1	1.14	
2	1.01	6.01	2	1.09	3.24
3	0.99	5.01	3	1.09	2.88
4	1.01	4.5	4	1.22	2.93
5	0.96	4.03	5	1.43	3.22
6	0.92	3.58	6	1.49	3.43
7	0.91	3.18	7	1.79	3.23
8	0.91	2.78	8	2.04	3.12
9	0.89	2.44	9	2.27	3.08
10	0.9	2.15	10	2.33	3.07
11	0.91	1.9	11	2.5	2.92
12	0.86	1.73	12	2.5	2.84
13	0.85	1.56	13	2.63	2.73
14	0.81	1.44	14	2.7	2.65
15	0.84	1.31	15	2.78	2.54
16	0.83	1.25	16	3.03	2.38
17	0.77	1.17	17	3.13	2.22
18	0.8	1.07	18	2.63	1.89
19	0.73	1.03	19	2.22	1.46
20	0.74	0.95	20	2.27	1.17
21	0.74	0.9	21	2.17	0.95
22	0.75	0.84	22	1.22	0.89
23	0.69	0.87	23	1.45	0.91
24	1.72	1	24	1.09	1.15
25	2.38	1.18	25	0.47	1.09
26	2.13	1.36	26	0.95	0.91

Data from chromatograms in Figure 26

C18 + RP-AmideC16 column setup with acetonitrile-modified CO_2 reduced peak asymmetry to values comparable to the Discovery C18 column with methanol-modified CO_2 as seen in Figure 25. Acteonitrile-modified CO_2 was not studied with the Acclaim PA C16 stationary phase. Methanol-modified CO_2 combined with the Acclaim PA C16 stationary phase produced the best peak asymmetry values observed in all of the studies conducted. This may be attributed to the stationary phase and the mobile phase's ability to shield free silanols. Figure 27 contains chromatograms of two 1Ph derivatized $C_{18}PO_{15}$ samples separated on the coupled C_{18} and amide-embedded alkyl phase and sulfonamide-embedded alkyl phase setups mentioned above. Peak asymmetry data are found in Table VII. Both column arrangements produced chromatographic peaks with asymmetry values close to unity. The uniform peak shape may be due to the presence of a methyl group and greater number of carbon atoms in the polyoxypropylene repeat unit compared to ethyleneoxide repeat units. The PO structure itself may reduce non-ideal interactions with the stationary phase that could lead to peak tailing. Figure 28 contains a chromatogram of the 1Ph derivatized $C_{16}EO_{20}$ separated on the Acclaim PA C16 column. Good oligomer resolution as well as good separation of excess derivatizing agent were observed for each of the samples analyzed.

The sulfonamide embedded group may work in conjunction with methanol for suppression of free silanol interactions. In HPLC, some researchers believe that water forms a strongly adsorbed layer close to the silica surface at the polar groups in polar embedded phases, which effectively shields free silanols.[77] A similar phenomenon may occur in SFC between modifying solvents and polar embedded groups in the stationary phase. The single sulfonamide-embedded column produced separations similar to those

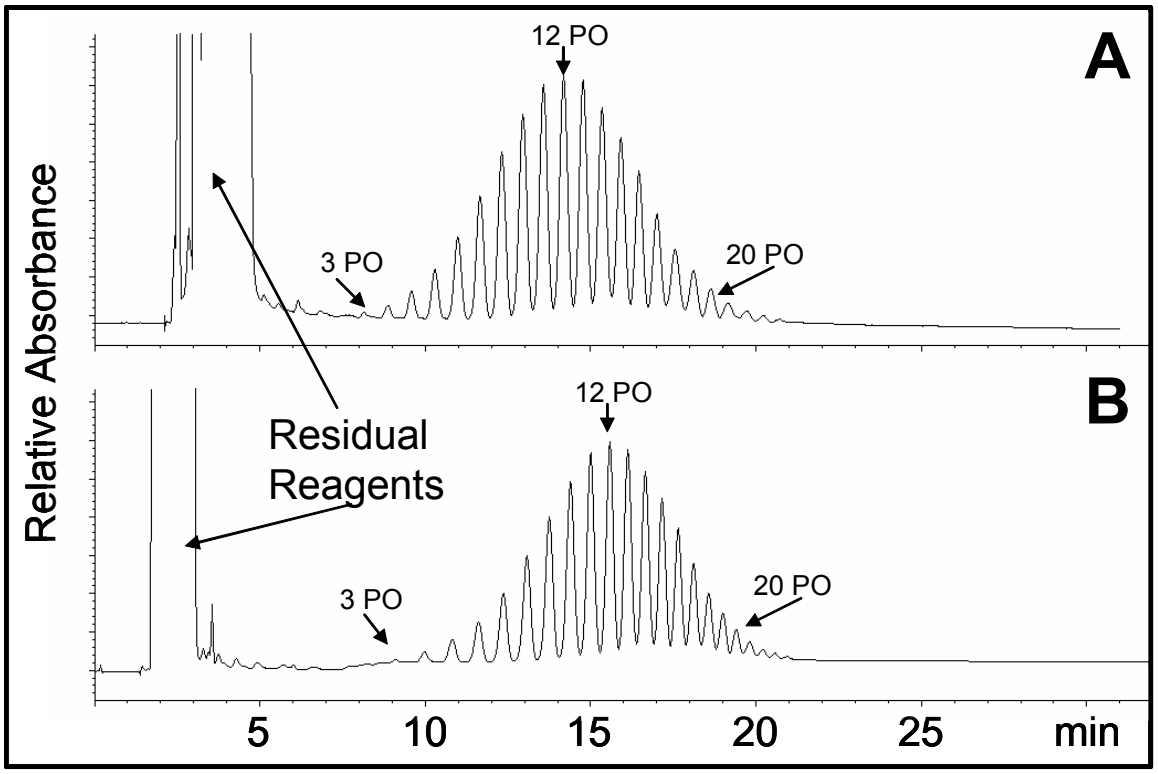

Figure 27. SFC-UV separation of $C_{18}PO_{15}$ 1Ph derivative.
Oven = 40°C, Outlet pressure = 120 bar, Flow rate = 2.4 mL/min, detector absorbance 215 nm, modifier gradient: 1% for 5 min, linear increase 1%/min to 25%, hold 2 minutes, linear decrease to 1% at 25%/min, 5 minute post run. A) Discovery C18 + Discovery RP-AmideC16 (4.6 x 250 mm, 5µm each). Acetonitrile-modified CO_2. B) Acclaim PA C16, 4.6 x 250 mm, 5µm. Methanol-modified CO_2

Table VII. Peak asymmetry data for 1Ph derivatized $C_{18}PO_{15}$ on Acclaim PA C16 and Discovery C18 + RP-AmideC16.

PO#	A	B
3	1.49	0.63
4	1.10	1.03
5	1.09	0.94
6	1.02	0.90
7	0.96	0.91
8	1.05	0.91
9	0.97	0.93
10	1.05	0.96
11	0.97	0.96
12	1.08	0.96
13	1.00	0.99
14	1.14	0.99
15	1.05	1.00
16	1.09	1.00
17	1.09	0.99
18	1.05	0.94
19	0.99	0.96
20	1.10	1.03
21	1.23	0.88
22	1.10	0.91
23	1.64	1.11
24	1.23	0.88
25	2.56	0.90

A) Discovery C18 + Discovery RP-AmideC16

B) Acclaim PA C16

Data from chromatograms in Figure 27

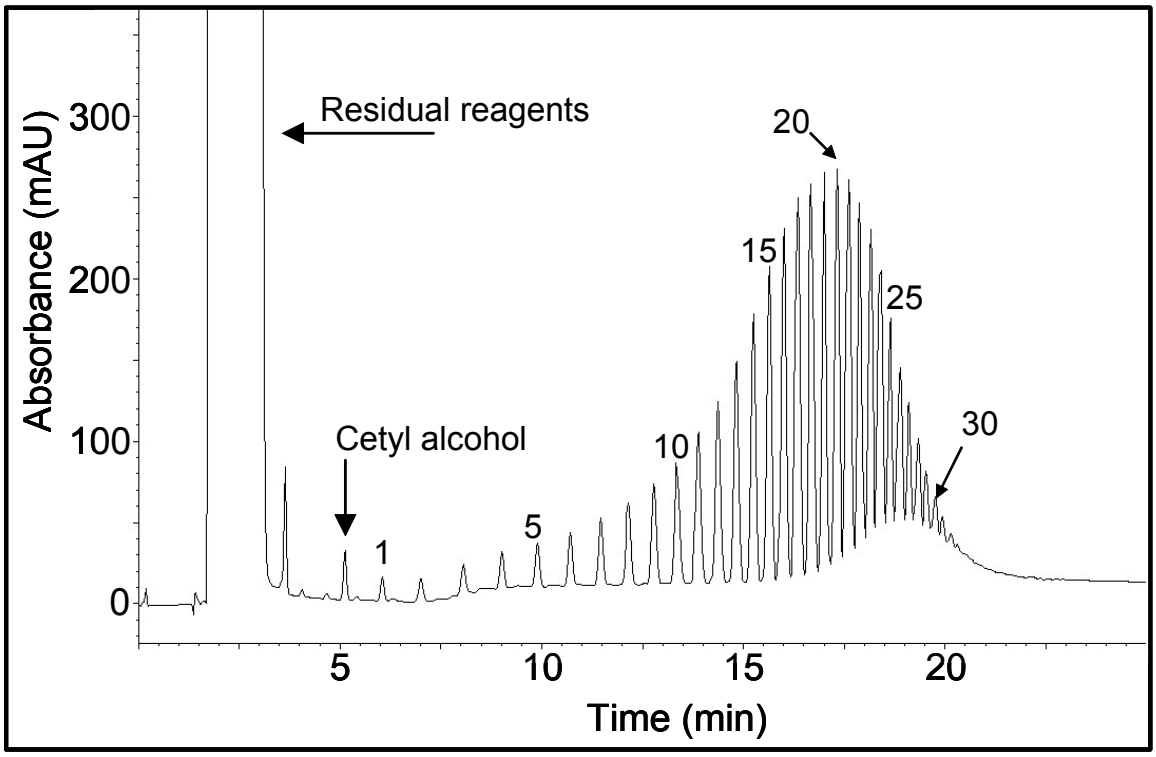

Figure 28. SFC-UV separation of $C_{16}EO_{20}$ 1Ph derivative.
Acclaim PA C16, 4.6 x 250 mm, 5µm. Oven = 40°C, Outlet pressure = 120 bar, Flow rate = 2.4 mL/min, detector absorbance 215 nm, modifier = methanol, modifier gradient: 1% for 5 min, linear increase 1%/min to 25%, hold 2 minutes, linear decrease to 1% at 25%/min, 5 minute post run. Peak annotations denote number of ethoxylate units.

previously only achievable with tandemly stacked columns described above.[79]

3.4 Identification of Oligomers

Oligomeric identification was accomplished through tandem UV-mass spectrometric detection according to a method similar to Pinkston and co-workers.[78] The instrument setup was similar to that discussed in Chapter 3. Electrospray ionization mass spectrometry in the positive ion mode was used. The configuration of the SFC-MS instrument is found in Figure 23 of Chapter 3. A make-up flow of 1 mM ammonium acetate in methanol was added to the effluent, post UV detector, to aid in adduct-ion formation. Oligomers were detected predominately as their $[M+NH_4]^+$ adducts. Mass spectrometry indicated that successively eluted components differed by one oligomer unit. As mentioned in Chapter 2, length of polyoxyethylene repeat unit has been shown to determine ionization efficiency due to association with cations in solution. Mass spectrometric detector response diminished for NH_4^+ adduct ions of oligomers of short ethoxylate chains. In some samples, a peak that preceded the 1EO oligomer was detected in the UV trace, often with larger UV peak area than the 1EO oligomer, but was not detected by ESI-MS as a NH_4^+ adduct ion. An ethoxylated stearyl alcohol with a nominal reported average molar EO value of 2 ($C_{18}EO_2$), which contained predominately short EO oligomers, was analyzed by SFC-ESI-MS to verify the identity of the chromatographic peak eluting before the oligomeric series because it contained the largest concentration of this compound (out of the samples analyzed). Figure 29 contains the UV chromatogram of the $C_{18}EO_2$ 1Ph derivative and extracted ion

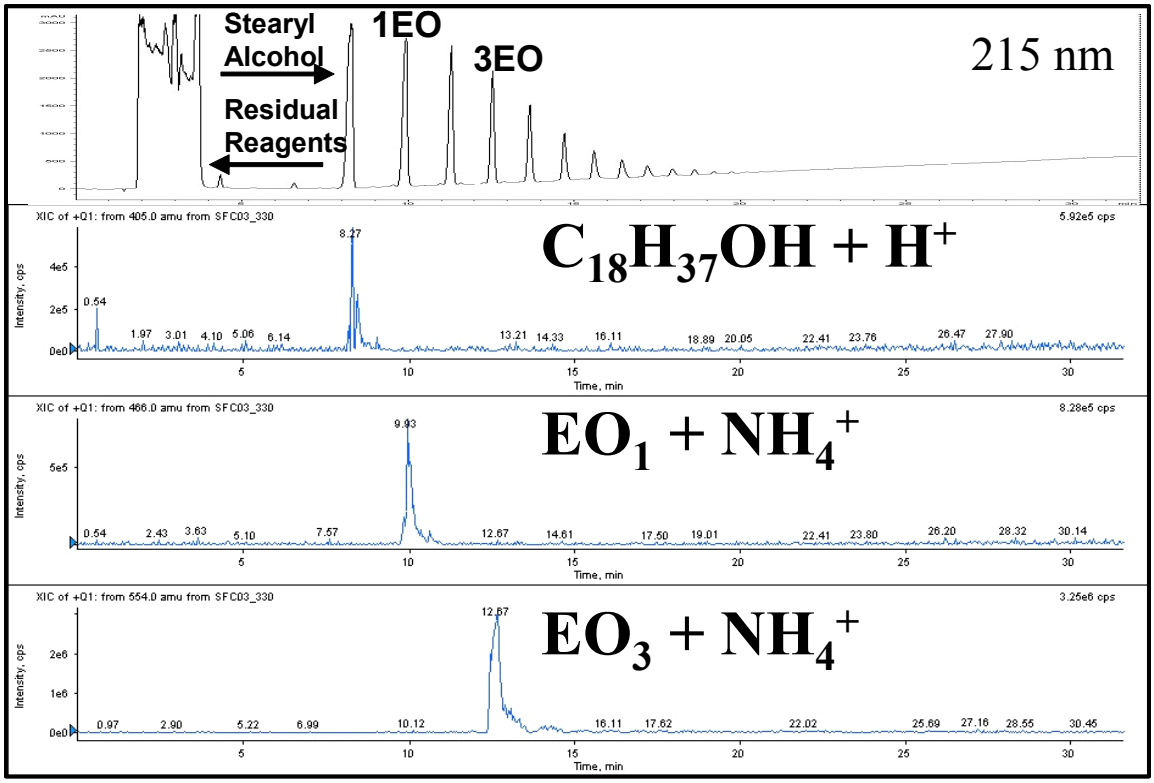

Figure 29. UV and Extracted Ion Chromatograms (XICs) of 1Ph $C_{18}EO_2$.
Acclaim PA C16 (4.6x250 mm); Oven: 40°C; Pressure: 120 bar; Flow rate: 2.4 mL/min; Modifier: MeOH. Gradient: 1% for 5 min, raise 1%/min to 25%, hold 2 minutes

chromatograms of a few oligomers. It was determined that the peak preceding the 1EO oligomer was 1Ph derivatized stearyl alcohol. The $[M + H]^+$ ion was the only adduct ion detected for the 1Ph stearyl alcohol peak. Further discussion of SFC-ESI-MS data interpretation can be found in Chapter 6. Elution order via SFC-ESI-MS data was used to identify oligomers detected by the separate SFC-UV system discussed in this chapter as well.

3.5 Calculation of Average Molar Oligomer Values

3.5.1 ^{1}H-NMR of Non-Derivatized Samples

Calculation of average molar EO values by ^{1}H-NMR followed the method described by Hammond and Kubik.[13] The ^{1}H-NMR spectrum of each sample (64 scans) was acquired three times. The terminal methyl resonance of the alkyl portion of non-derivatized surfactant between 0.84 and 0.91 ppm, relative to TMS, was normalized as three protons. Ethoxylate proton resonances were assigned to the region between 3.55 and 3.76 ppm. Figure 30 is a ^{1}H-NMR spectrum of a non-derivatized $C_{18}EO_{10}$ sample. The absorbance integral due to the polyoxyethylene protons was 42.5 relative to the methyl resonance integral. Dividing the polyoxyethylene integral by 4 (the number of protons in the repeating unit) gave an average molar EO value of 10.6. The average alkyl chain length of surfactant samples can also be determined from ^{1}H-NMR data. The absorbance between 1.22 and 1.35 is due to the methylene repeat unit of the alkyl chain and its integral (normalized to the terminal methyl group) can be divided by 2 (the number of protons in a methylene group) to get the average number of repeat units. The absorbance due to the protons situated on the methylene carbon adjacent (α) to the

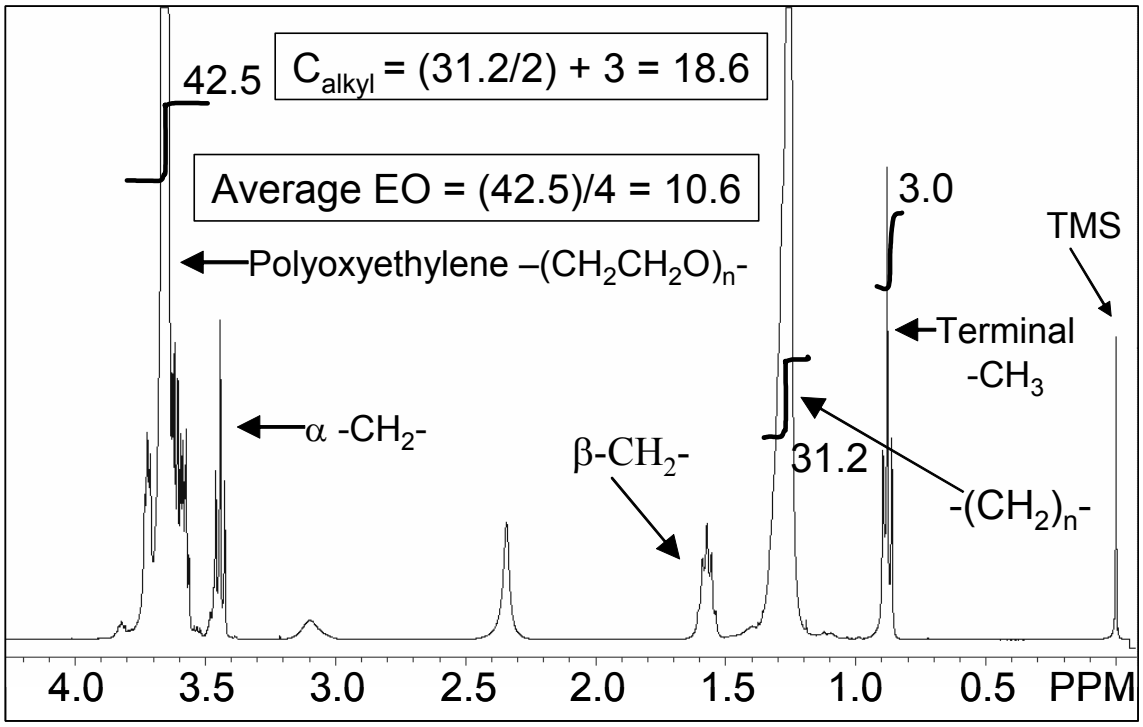

Figure 30. ^{1}H-NMR spectrum of non-derivatized $C_{18}EO_{10}$ in $CDCl_3$.

oxygen of the polyether repeat unit was found between 3.41 and 3.49 ppm and the absorbance due to the protons situated on the methylene carbon twice removed (β) from the oxygen was found between 1.53 and 1.61 ppm. Addition of the average repeat unit carbons, the α and β methylene carbons and the terminal methyl group produced an average alkyl chain length of 18.6 for the $C_{18}EO_{10}$ sample. The ^{1}H-NMR spectra of the non-derivatized $C_{16}EO_{20}$ sample is found in Figure 31. The average molar EO value and average alkyl chain length for the $C_{16}EO_{20}$ sample analyzed was 19.2 and 16.7, respectively, calculated in the same fashion. Discrepancies between nominal values and experimental values may be attributed to variation between manufactured batches of surfactants.

The average PO value of the $C_{18}PO_{15}$ sample was deduced in a similar fashion. The ^{1}H-NMR spectrum of $C_{18}PO_{15}$ is found in Figure 32. The terminal alkyl chain methyl resonance at 0.85 to 0.91 ppm was normalized as three protons. Resonance caused by the polyoxypropylene unit methyl group, with a chemical shift between 1.07 and 1.16 ppm, was integrated relative to the terminal methyl group integral and divided by three (the number of protons in a methyl group) to determine the average PO value. The average alkyl chain length was calculated by dividing the absorbance between 1.22 and 1.35 ppm, due to methylene repeat groups, by two and adding three carbons for the α and β methylene carbons and terminal methyl group. The average molar PO value and average alkyl chain length calculated by ^{1}H-NMR for the $C_{18}PO_{15}$ sample was 13.7 and 18.7 respectively. Average EO and PO values calculated by ^{1}H-NMR were similar to the nominal value assigned by their manufacturer. The RSD of average molar oligomer values calculated from ^{1}H-NMR data was below 1% (Table VIII). The chromatographic

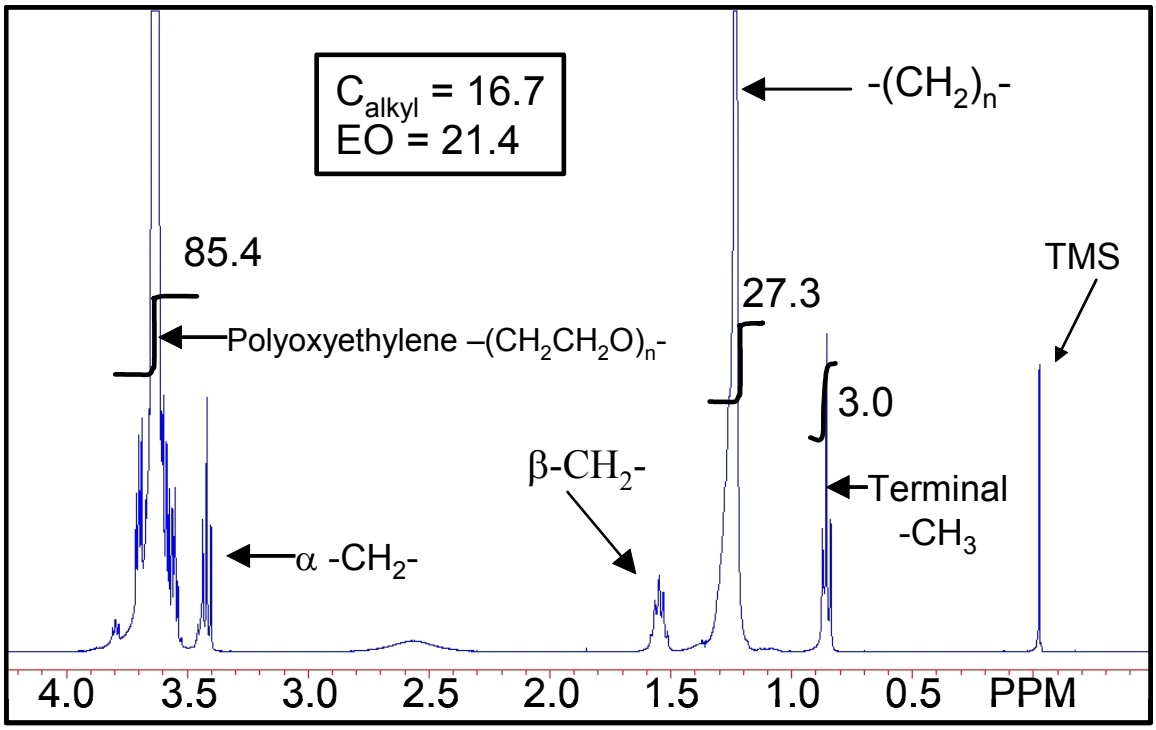

Figure 31. ^{1}H-NMR spectrum of non-derivatized $C_{16}EO_{20}$ in $CDCl_3$.

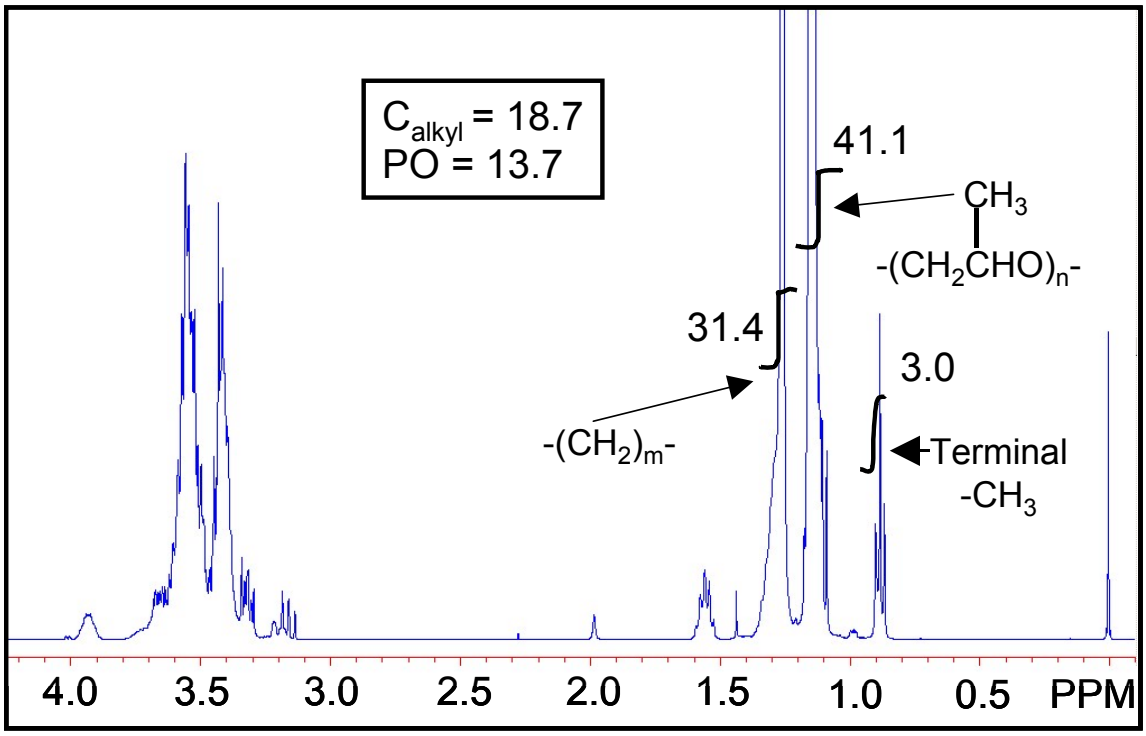

Figure 32. ^{1}H-NMR spectrum of non-derivatized $C_{18}PO_{15}$.

Table VIII. Comparison of average molar oligomer values via ^{1}H-NMR and SFC-UV.

Surfactant	$C_{16}EO_{20}$	$C_{18}EO_{10}$	$C_{18}PO_{15}$
Avg. Mol. Wt.	1122	710	1140
^{1}H-NMR Value[a]	21.4	10.7	13.7
%R.S.D.[a]	0.4	0.3	0.5
SFC-UV Value[b]	19.2	9.6	12.5
# Inj. (# Samples)	15(3)	20(3)	14(3)
% R.S.D.[b]	0.8	0.7	0.2

a = ^{1}H-NMR of three non-derivatized samples
b = SFC-UV of three 1Ph derivatized samples

value should be more informative since samples with different EO or PO distributions, but with the same average molar oligomer value, could appear identical by NMR analysis since only average oligoether values are measured.

3.5.2 SFC-UV of Derivatized Samples

Research in Chapter 3 demonstrated that the absorbance at 215nm of a 1Ph derivative was entirely due to the added functional group and thus, the ether repeating unit is thought to not contribute to the UV signal. Because of this, it was possible to calculate average molar oligomer values by summation of the product of oligomer mole fraction (from percent peak area) and number of repeating units associated with each oligomer peak. SFC-ESI-MS data were employed to assign peak identities in the comparable SFC-UV chromatograms. Combined UV and ESI-MS data thus provided, unlike ^{1}H-NMR, both the average oligomer value and the distribution of oligomers in each surfactant sample. Tables IX, X, and XI contain the detailed peak area information from the 1Ph derivatized $C_{18}EO_{10}$, $C_{18}PO_{15}$, and $C_{16}EO_{20}$ samples, respectively, produced in chromatograms found in Figures 26-28, respectively. The chromatographic data demonstrated the ability of SFC-UV to provide the distribution of an alcohol polyether surfactant. The average molar oligomer value calculated for the 1Ph derivatized $C_{18}EO_{10}$ sample was 9.7 EO units, whereas the NMR method gave a value of 10.6. The average molar oligomer values of the 1Ph derivatized $C_{16}EO_{20}$ and $C_{18}PO_{15}$ samples were calculated in a similar fashion, the calculated values for the individual samples were 19.2 EO and 12.6 PO, respectively. Calculated average molar oligomer values are further

Table IX. SFC-UV peak data for 1Ph derivatized $C_{18}EO_{10}$.

EO#	RT(min)	% Area
1	7.11	2.651
2	8.09	3.170
3	8.99	3.944
4	9.82	4.820
5	10.59	5.303
6	11.31	6.158
7	11.98	7.054
8	12.59	7.897
9	13.16	8.255
10	13.69	8.314
11	14.18	7.975
12	14.64	7.354
13	15.08	6.547
14	15.48	5.488
15	15.86	4.431
16	16.23	3.473
17	16.58	2.569
18	16.91	1.860
19	17.22	1.236
20	17.51	0.780
21	17.79	0.436
22	18.05	0.182
23	18.32	0.061
24	18.56	0.029
25	18.78	0.004
26	18.88	0.003
27	18.99	0.004
Average EO = 9.7		

Data from chromatogram B of 1Ph derivatized $C_{18}EO_{10}$ in Figure 26.

Table X. SFC-UV peak data for 1Ph derivatized $C_{18}PO_{15}$.

PO#	RT (min)	% Area
3	9.27	0.238
4	10.16	0.426
5	11.00	1.065
6	11.80	1.997
7	12.56	3.499
8	13.30	5.433
9	13.98	7.496
10	14.63	9.373
11	15.27	10.633
12	15.86	11.070
13	16.42	10.671
14	16.96	9.661
15	17.47	8.108
16	17.95	6.323
17	18.42	4.772
18	18.88	3.483
19	19.33	2.272
20	19.73	1.432
21	20.12	0.927
22	20.53	0.572
23	20.92	0.278
24	21.28	0.120
25	21.60	0.082
26	21.99	0.031
27	22.29	0.038
Average PO = 12.6		

Data from chromatogram B of 1Ph derivatized $C_{18}PO_{15}$ in Figure 27.

Table XI. SFC-UV peak data for 1Ph derivatized $C_{16}EO_{20}$.

EO#	RT (min)	% Area
1	6.04	0.269
2	7.00	0.333
3	8.05	0.459
4	9.01	0.591
5	9.89	0.606
6	10.70	0.759
7	11.46	0.965
8	12.14	1.262
9	12.77	1.578
10	13.35	1.970
11	13.87	2.430
12	14.36	2.951
13	14.81	3.513
14	15.24	4.122
15	15.63	4.793
16	15.99	5.371
17	16.34	5.900
18	16.68	6.296
19	17.00	6.511
20	17.31	6.611
21	17.60	6.382
22	17.88	6.085
23	18.15	5.544
24	18.39	4.995
25	18.64	4.298
26	18.87	3.598
27	19.11	2.976
28	19.33	2.357
29	19.54	1.845
30	19.75	1.430
31	19.94	1.050
32	20.13	0.749
33	20.30	0.576
34	20.45	0.316
35	20.59	0.267
36	20.77	0.243
Average EO = 19.2		

Data from chromatogram of 1Ph derivatized $C_{16}EO_{20}$ in Figure 28.

discussed in the method reproducibility section. The average molar oligomer value calculated from SFC-UV data may vary from the nominal value or ^{1}H-NMR value due to variation between manufactured surfactant batches or the presence of PEG in the sample.

3.6 Method Reproducibility

Each surfactant sample was derivatized three times and each derivative mixture was injected a minimum of four times. Table VIII includes sample information from the reproducibility study. Separations were performed with the packed column SFC-UV system. Calculated average molar oligomer values for surfactant samples were determined using the method described previously. All 1Ph derivatives gave average molar oligomer values with relative standard deviation (RSD) below 1%. The 1Ph derivative of $C_{16}EO_{20}$ and $C_{18}EO_{10}$ samples produced EO values (19.2 and 9.6 respectively) that were very close to their nominal values (20 and 10 respectively). Both the SFC-UV value (12.5) and the ^{1}H-NMR value (non-derivatized, 13.7) for derivatized $C_{18}PO_{15}$ were below the nominal value (15).

Since "average molar oligomer value" is a relative measure of distribution, it is important to compare peak areas of several chromatograms. As long as all (or a large majority) of the peaks are detectable, and they are equally derivatized, then the correct oligomer value should be obtained. In other words, it would be possible to have chromatograms that vary greatly in total absolute peak area and give equal average molar oligomer values as long as the peak ratios were consistent. The peak area of individual oligomers from the reproducibility study was compared to determine reproducibility of peak area. The peak area of the oligomer containing 4 ethoxylate (4 EO) repeating units of each 1Ph derivatized $C_{18}EO_{10}$ sample was compared. This oligomer was picked

because it was well resolved in each of the chromatograms. The 10 EO peak of $C_{16}EO_{20}$ derivatized samples and the 10 PO peak of $C_{18}PO_{15}$ derivatized samples were also compared for the same reason. The peak areas of the target oligomers were divided by the mass of sample used for each individual derivatizations. This produced an adjusted peak area that was normalized to the mass used for the individual derivatizations, which accounted for slight differences in the mass of sample used. The RSD of peak areas for the 1 Ph derivative of $C_{16}EO_{20}$, $C_{18}EO_{10}$ and $C_{18}PO_{15}$ samples were 2.1, 6.5 and 7.2% respectively. These RSD values indicate that the reproducibility of the derivatization and chromatographic methods were acceptable.

Since derivatized species only absorbed at 215 nm due to the phenyl moiety of the 1Ph derivative, producing an equal molar response, and stearyl (C_{18}) and cetyl (C_{16}) derivatized alcohols were detected in the ethoxylated samples; it was possible to determine the residual alcohol present in analyzed samples. The percent area of the free alcohol peak in relationship to the cumulative area of all derivatized was used to calculate the mole fraction for the free alcohol. The mole fraction of stearyl alcohol in the $C_{18}EO_{10}$ sample was calculated at 3.73 % with an R.S.D. of 1.2 %. The mole fraction of cetyl alcohol in the $C_{16}EO_{20}$ sample was 0.47% with an RSD of 2.6%. The mole fraction of stearyl alcohol and average molar oligomer value for the $C_{18}EO_2$ sample in Figure 27 was 27.6% and 3.2 EO respectively. Table XII contains the oligomer distribution for the 1Ph derivative of the $C_{18}EO_2$ sample. These calculations depend on other possible derivatized species (i.e. PEG) being at concentrations below detection. The developed chromatography and derivatization are therefore also useful for the calculation of residual fatty alcohol present in AEOs. It was not possible to determine free alcohol content of

Table XII. SFC-UV peak data for 1Ph derivatized $C_{18}EO_2$.

EO#	RT (min)	% Area
1	9.93	27.30
2	11.31	22.13
3	12.55	16.53
4	13.66	11.65
5	14.70	7.66
6	15.59	5.09
7	16.43	3.58
8	17.19	2.36
9	17.95	1.38
10	18.62	0.96
11	19.20	0.49
12	19.73	0.34
13	20.19	0.26
14	20.63	0.16
15	21.03	0.13
Average EO = 3.2		

Data from chromatogram of 1Ph derivatized $C_{18}EO_2$ in Figure 29.

the $C_{18}PO_{15}$ sample because the derivatized free alcohol was not detected by SFC-ESI-MS.

4. Summary

In comparison to acetonitrile-modified CO_2, methanol-modified CO_2 provided better peak shape and shorter retention times for 1Ph derivatized alcohol polyethers. The sulfonamide embedded stationary phase used for SFC separations was able to separate excess derivatizing material from the derivatized oligomeric series, as well as provided excellent separation between oligomers. These qualities associated with a single stationary phase is an improvement over a two column configuration wherein an amide embedded phase had to be preceded by an alkyl stationary phase in order to first separate residual derivatizing material from oligomers prior to separation of the oligomeric series. It was found that an increase in the concentration of the 1Ph derivatized AEO samples investigated increased the calculated average molar oligomer value. This may be due to less abundant oligomers being present above the limit of detection. Further investigation into increasing oligomer detection sensitivity will be discussed in Chapter 5. ESI-MS was used for identification of chromatographic peaks, and along with UV detection data allowed average molar oligomer value to be calculated for derivatized surfactant samples. SFC-UV data provided oligomer distribution, mole fraction of initiator alcohol in ethoxylated samples, and average molar oligomer values, which were comparable to nominal values and values obtained by ^{1}H-NMR analysis.

The reproducibility of the developed derivatization and chromatographic methods were evaluated. Low relative standard deviation of average molar oligomer values calculated from SFC separations demonstrate the methods to be reproducible. Thus the

methods are credible for the determination of average molar oligomer value, mole fraction of residual initiator alcohol, and the determination of oligomeric distribution of alcohol polyethers.

CHAPTER 5

Increasing Detection Sensitivity for the Chromatographic Analysis of Alcohol Polyethers

1. Introduction

Since AEOs do not contain functionality capable of absorbing UV light, they are commonly derivatized with UV absorbing groups for detection.[59-61,79,85] Chromatography of derivatized AEOs using UV detection has been useful for both calculation of average oligomer value and oligomer distribution.[25,79,85] Previous chapters have exploited the fact that alkylphenol ethoxylates and alcohol polyethers derivatized to incorporate a phenyl ring provide equal molar response for oligomers. According to Beer's law, UV absorbance is linearly correlated at moderately low concentration. Therefore, oligomers that are present at low concentration, close to the limit of detection, will be difficult to detect. Previously, derivatives formed from AEO and APO samples contained a single phenyl group (1Ph). Calculated average oligomer values were slightly below both the ^{1}H-NMR calculated and reported nominal values. A wide concentration distribution of oligomers was observed from SFC-UV data. Some of the oligomers were at very low concentration with respect to the predominant oligomers in the surfactant samples. It is possible that oligomers present at low concentrations were not detected by UV absorbance because they were below the instrumental limit of detection, which may affect calculated average molar oligomer values.

The intent of the research in this chapter was to further increase the sensitivity of AEOs analyzed by SFC-UV in an effort to observe oligomers present at low

concentrations. This was attempted by evaluating the response of both a methyldiphenyl silylether (2Ph) derivative and comparing it with previous work on a phenyldimethyl silylether (1Ph) derivative. It was anticipated that an increase in the number of phenyl groups associated with derivatized oligomers would increase detection sensitivity. A cursory study of the 2Ph derivative indicated that an amide-embedded alkyl phase could not be used for 2Ph derivative analysis due to co-elution of residual reagents and derivatized oligomers. A SPE cleanup method was unsuccessful as well. Derivatized samples were separated by SFC on a sulfonamide embedded-alkyl stationary phase using methanol-modified CO_2 as the mobile phase. This chromatographic method was shown to separate residual reagents from derivatized species. Electrospray ionization mass spectrometry (ESI-MS) was used for identification of comparable analyte peaks in UV chromatograms.

2. Experimental

2.1 Surfactant samples and derivatizing reagents

$C_{18}EO_2$, $C_{18}EO_{10}$, $C_{16}EO_{20}$, and $C_{18}PO_{15}$ were provided by Uniqema (New Castle, DE). 1,3-Diphenyl-1,1,3,3-tetramethyldisilazane (DPTMDS) (96% pure), 1,3-dimethyl-1,1,3,3-tetraphenyl disilazane (DMTPDS) (97% pure), and chlorodiphenylmethylsilane (MDPCS) (97% pure) were obtained from Sigma-Aldrich, Inc. (Milwaukee, WI). Phenyldimethylchlorosilane (PDMCS) (98.9% pure) was purchased from Gelest, Inc. (Tullytown, PA). Acetonitrile (MeCN) and methanol (MeOH) were obtained from

Burdick & Jackson (Muskegon, MI). ACS grade ammonium chloride was obtained from J.T. Baker (Phillipsburg, NJ).

2.2 Packed-column SFC-UV system

An A5000 analytical SFC system (Mettler-Toledo AutoChem Berger Instruments Newark, DE) was used in this study. The system consisted of an automatic liquid sampler (ALS) with a 10-μL loop used to make injections and a thermal control module (TCM) used to control column temperature. UV data were recorded at 215 nm by a variable wavelength detector. SFC-grade carbon dioxide (Air Products and Chemicals, Inc., Allentown, PA) was used as the primary mobile phase. Acclaim PA C16 (Dionex, Sunnyvale, CA) packed columns were used for SFC separations. The dimensions of the Acclaim PA C16 columns were 4.6 x 150 or 250 mm with an average particle size of 5 μm. The mobile phase flow rate (liquid) was 2.4 mL/min. Oven temperature was 40°C and outlet pressure was held at 120 bar. Mobile phase modifier programming with methanol was used for elution. Each mobile phase method started with a 5-minute hold at 1% modifier to elute excess derivatizing materials. All methods then contained a linear gradient of 1% modifier per minute to a set concentration depending on the sample composition. A two minute hold at the upper modifier concentration was then followed by a return to 1% modifier at 25%/minute. A 5-minute post-run was used for system equilibration.

2.3 SFC-ESI-MS system

An A5000 analytical SFC system was also used for SFC-ESI-MS analysis. Column, mobile phase, and oven temperature were the same as described in the packed-column SFC-UV system. A Hewlett-Packard G1205 column oven (Little Falls, DE) was used to control column temperature and UV detection was performed by a Berger Instruments diode array detector. An Isco Model 260D syringe pump (Lincoln, NE) delivered make-up flow methanol containing 1 mM ammonium acetate downstream of the UV detector. Make up flow was supplied at 200μL/min. The SFC effluent was diverted to the mass spectrometer via a Tee positioned downstream of the backpressure regulator. The remaining flow was sent to waste. Electrospray ionization mass spectra were obtained with an API 365 mass spectrometer (PerkinElmer Sciex, Toronto, Canada) in the positive ion mode. Turbo gas temperature was 450°C and the *m/z* range scanned was 150-2000, step size of 0.2 Da and dwell time was 0.2 ms with a 5.0 ms pause. Figure 21 in Chapter 3 contains a schematic diagram of the SFC-ESI-MS instrument.

2.4 Spectrometry of derivatized samples

UV absorbance spectroscopy of samples, to determine an appropriate detection wavelength, was performed with an Agilent 8453 diode array spectrophotometer (Little Falls, DE). IR absorbance spectroscopy was performed with a PerkinElmer Spectrum One FT-IR (PerkinElmer, Inc., Shelton, CT). IR spectra were recorded from 450-4000 cm^{-1} with a resolution of 4 cm^{-1}.

3. Results and Discussion

Alcohol ethoxylate samples with a wide molar-mass and concentration, by mole fraction, distribution pose an analytical challenge for UV detection due to some components being present close to the limit of detection, while other components represent a substantial portion of the mixture. The goal of this study was to increase UV detection sensitivity of alcohol polyether oligomers. $C_{18}EO_{10}$, $C_{16}EO_{20}$, and $C_{18}PO_{15}$ surfactant samples were derivatized with disilazane-chlorosilane mixtures for formation of silylethers that contained either one or two phenyl groups. SFC-ESI-MS was used to identify the peaks in SFC-UV chromatograms, as well as to identify oligomers present below the limit of UV detection. Data from SFC-UV chromatograms then were used to calculate average molar oligomer values.

3.1 Derivatization

Samples were initially derivatized with an equal concentration of surfactant for both 1Ph and 2Ph derivatives. Then the concentration of surfactant used with 2Ph reagents was decreased in order to determine if 2Ph derivatives might require a lower concentration for achieving adequate sample characterization. The derivatization method used in this study was similar to the one employed in Chapter 3 for the formation of 1Ph derivatives. The proposed reaction mechanism is found in Figure 16 in Chapter 3. The R' and R" groups are both phenyl moieties for the formation of the 2Ph derivative. For the formation of the 1Ph derivative R' is a methyl group and R" is a phenyl group. The method was as follows: approximately 45-90 mg of each sample was placed into a 2.0 mL GC vial. For the formation of 1Ph derivatives, 150 μL of DPTMDS, 1350 μL of acetonitrile, and 31 μL of PDMCS were added to the vial. For the formation of 2Ph

derivatives, 150 mg of DMTPDS, 1350 μL of acetonitrile, and 31 μL of MDPCS were added to the vial. All vials were then capped, mechanically shaken for 30 seconds, and placed in a heating block at 80°C for one hour. After cooling, samples were filtered through a 0.45 μm PTFE syringe filter (Millipore Corp., Bedford, MA). A white precipitate formed during each reaction. The precipitate was washed 5 times with 5 mL of acetonitrile, dried, and analyzed by IR spectroscopy. The precipitates formed during both the 1Ph and 2Ph derivatization reactions were identified as ammonium chloride by comparison of each IR spectrum to the spectrum of a neat sample of ammonium chloride (Figure 33).

3.2 Calculation of Average Molar Oligomer Value

Average molar oligomer values were calculated from UV chromatographic data. SFC combined with mass spectrometry detection (discussed later) provided identification of oligomer peaks in the SFC-UV chromatograms. The sum of the products obtained by multiplying the mole fraction of each oligomer and its assigned EO value produced the average molar oligomer value. Calculation of average molar oligomer value, in this manner, is dependent on the number of oligomers detected. Detection of a greater number of oligomers in theory should increase the calculated average molar oligomer value. In chapter 4 and the literature, the $C_{18}EO_{10}$, $C_{16}EO_{20}$, and $C_{18}PO_{15}$ samples that are examined in the current study were analyzed by ^{1}H-NMR and were found to yield average molar oligomer values of 10.7, 21.4 EO, and 13.7 PO respectively.[85]

3.3 SFC-ESI-MS Analysis

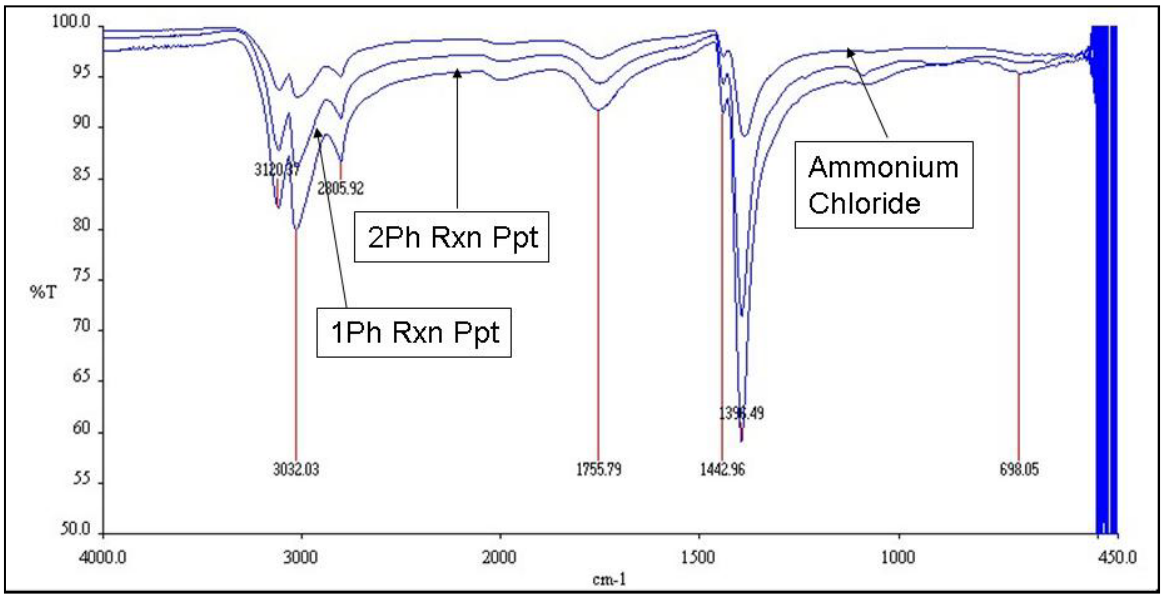

Figure 33. IR spectrum of 1Ph ppt, 2Ph ppt, and ammonium chloride.

Electrospray ionization mass spectrometry (ESI-MS) in the positive ion detection mode was used tandemly with UV detection for identification of oligomers in UV chromatograms. It was necessary to use highly concentrated samples, discussed previously in Chapter 4, due to the large number of oligomers present in the surfactants analyzed. The concentration of oligomers varied greatly and a high concentration of surfactant allowed less abundant oligomers to be detected by UV absorbance. By analyzing highly concentrated samples, it was also possible to observe very low concentration of impurities produced during the alcohol polyether formation process. Although the samples were supposed to be primarily stearyl or cetyl ethoxylated alcohols, derivatized C10, C12, C14, C16, C18 and C20 ethoxylated alcohols were also detected in samples. The concentrations of these impurities were not significant and detection of the impurities was only due to the relatively high concentration of the samples.

Review of contour plots aided in the analysis of SFC-ESI-MS data. A contour plot is similar to a mass spectrometric total ion chromatogram (TIC), but it separates the data into time, mass-to-charge, and detector response in a two-dimensional figure. Retention time is located along the x-axis of a contour plot, mass-to-charge ratio (*m/z*) is found on the y-axis, and relative ion abundance is described perpendicularly out of the plane of the paper. The contour plot of 1Ph derivatized $C_{18}EO_2$ clearly illustrates the homologous series of oligomers (Figure 34). A contour plot can be used to help deconvolute a complicated UV or TIC chromatogram. Figure 35 shows an enlarged region of the contour plot created from SFC-ESI-MS data of the 1Ph derivatized $C_{16}EO_{20}$ sample. A series of peaks can be seen in the contour plots of the analyzed surfactants in

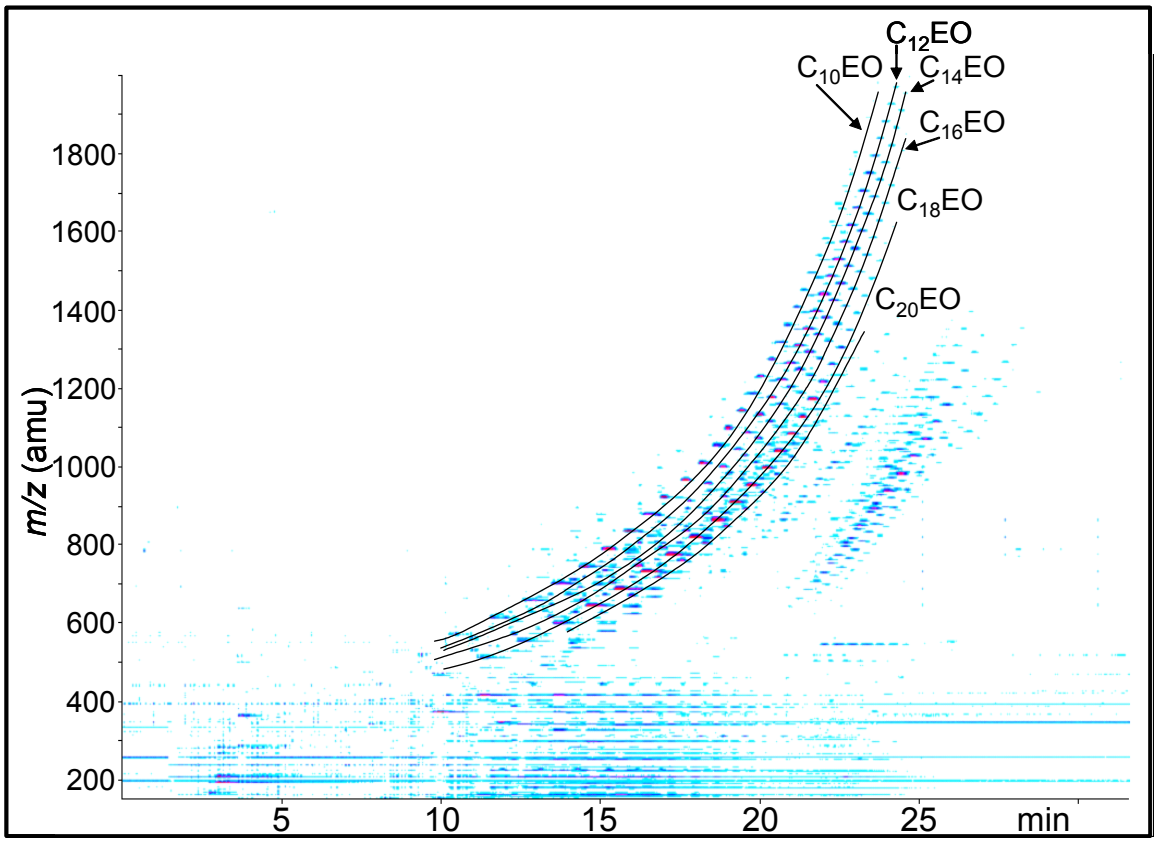

Figure 34. SFC-ESI-MS contour plot of 1Ph derivatived $C_{18}EO_2$.
Acclaim PA C16, (2) 4.6 x 150 mm, 5µm. Oven = 40°C, Outlet pressure = 120 bar, Flow rate = 2.4 mL/min, modifier = methanol, modifier gradient: 1% for 5 min, linear increase 1%/min to 25%, hold 2 minutes, linear decrease to 1% at 25%/min, 5 minute post run. UV and XIC chromatograms in Figure 29.

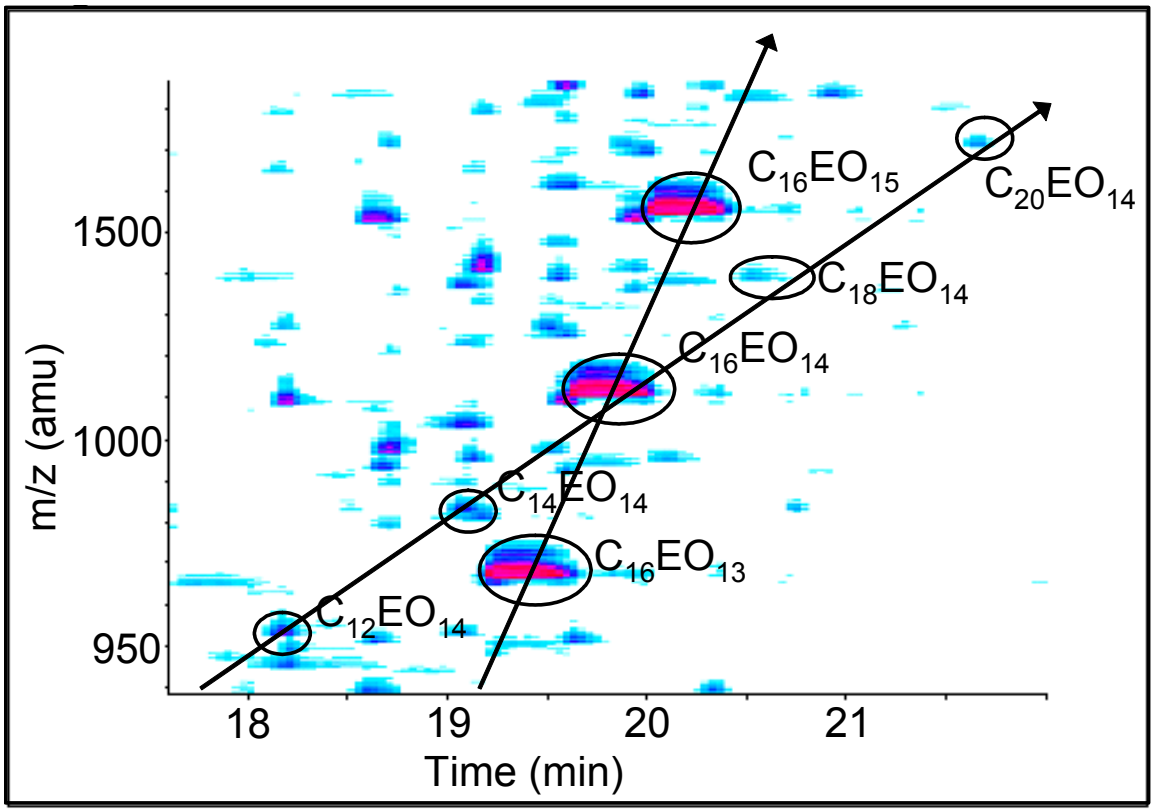

Figure 35. Enlarged SFC-ESI-MS contour plot of 1Ph derivatived $C_{16}EO_{20}$.
Acclaim PA C16, (2) 4.6 x 150 mm, 5µm. Oven = 40°C, Outlet pressure = 120 bar, Flow rate = 2.4 mL/min, modifier = methanol, modifier gradient: 1% for 5 min, linear increase 1%/min to 25%, hold 2 minutes, linear decrease to 1% at 25%/min, 5 minute post run.

which ethoxylate and alkyl chain lengths increase moving diagonally from the lower left corner of the plot to the upper right corner of the plot. It can be seen that the Acclaim PA C16 sulfonamide-embedded alkyl phase efficiently separated homologs with the same degree of ethoxylation as well as oligomers of the same alkyl chain by mass separation. If all of the analytes were at equal concentration then the UV chromatogram would be very complex.

Methanol containing 1 mM ammonium acetate was added post UV detector to aid in detection through formation of ion adducts. Analytes were detected as $[M+H]^+$, $[M+NH_4]^+$, and $[M+Na]^+$ ion adducts, but $[M+NH_4]^+$ ions were primarily used for identification because they appeared to be associated with more oligomers than the other adduct ions. Extracted ion chromatograms were used to identify peaks in UV traces. Figures 36 and 37 contain the extracted ion chromatograms (XICs) and UV absorbance detected chromatograms of the 1Ph and 2Ph derivatives of $C_{18}EO_{10}$, respectively. It should be noted that the mass spectrometric detector response for the first couple of oligomers increased greatly as EO length increased. This may be a function of how well the ethoxylate chains can chealate the ammonium ion. Research by Okada[37] and Crescenzi and co-workers[38] support this notion as each laboratory has demonstrated that ethoxylated compounds increase their ability to form adduct ions as a function of EO chain length. Figures 38 contains XICs and the UV absorbance detected chromatogram of 2Ph derivatized $C_{16}EO_{20}$. ESI-MS detection was useful in discrimination between residual reagents and derivatized oligomers. Multiple charged species could also be seen in the contour plots at the appropriate *m/z* value where z=2. Furthermore, separation between doubly charged oligomers was an *m/z* of 22 amu as compared to singly charged

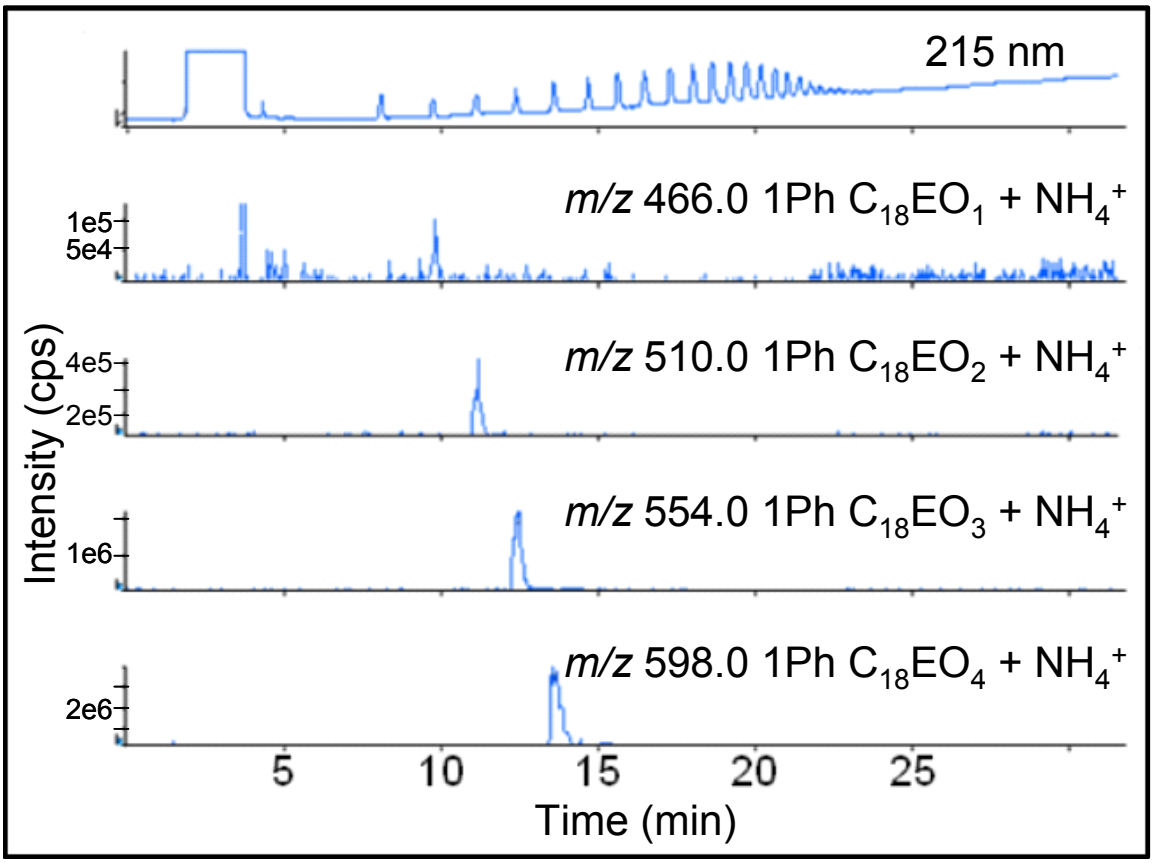

Figure 36. Extracted ion and UV chromatograms of 1Ph derivatized $C_{18}EO_{10}$. Acclaim PA C16, 4.6 x 250 mm. See Figure 35 for conditions. UV detector wavelength 215 nm.

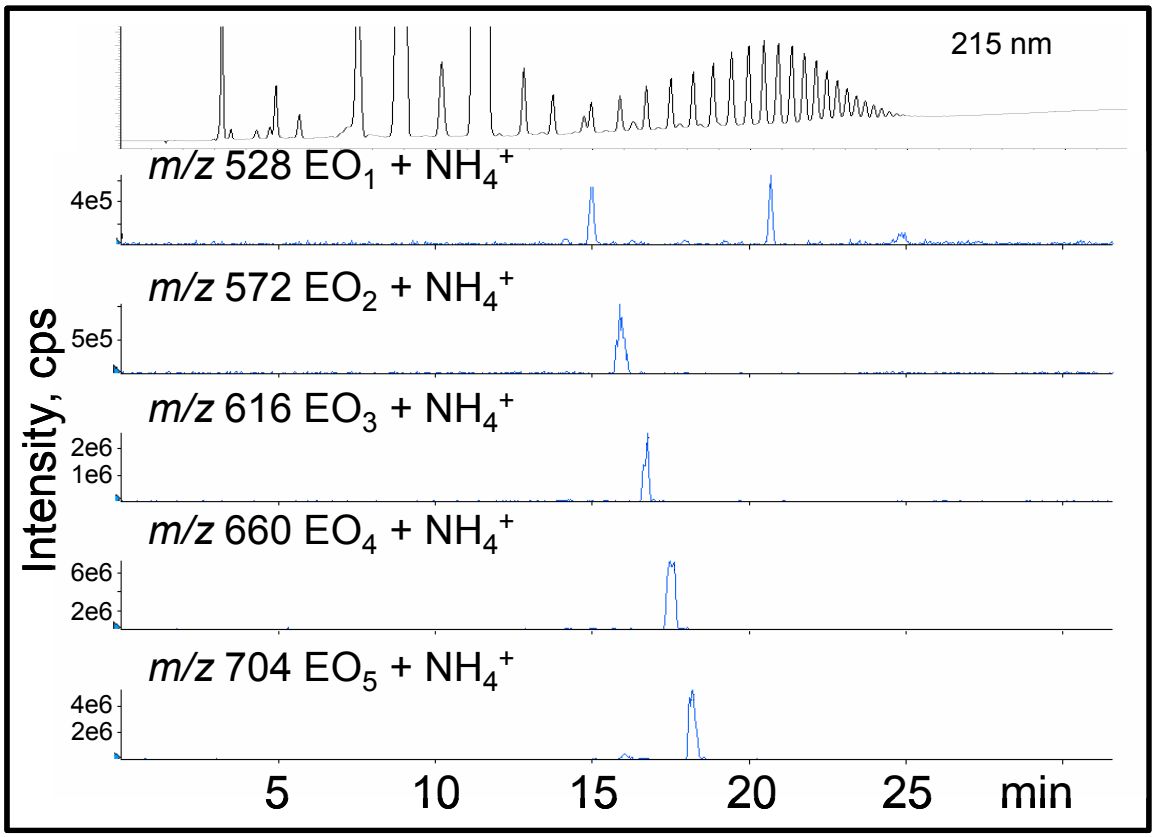

Figure 37. Extracted ion and UV chromatograms of 2Ph derivatized $C_{18}EO_{10}$. Acclaim PA C16, 4.6 x 250 mm. See Figure 35 for conditions. UV detector wavelength 215 nm

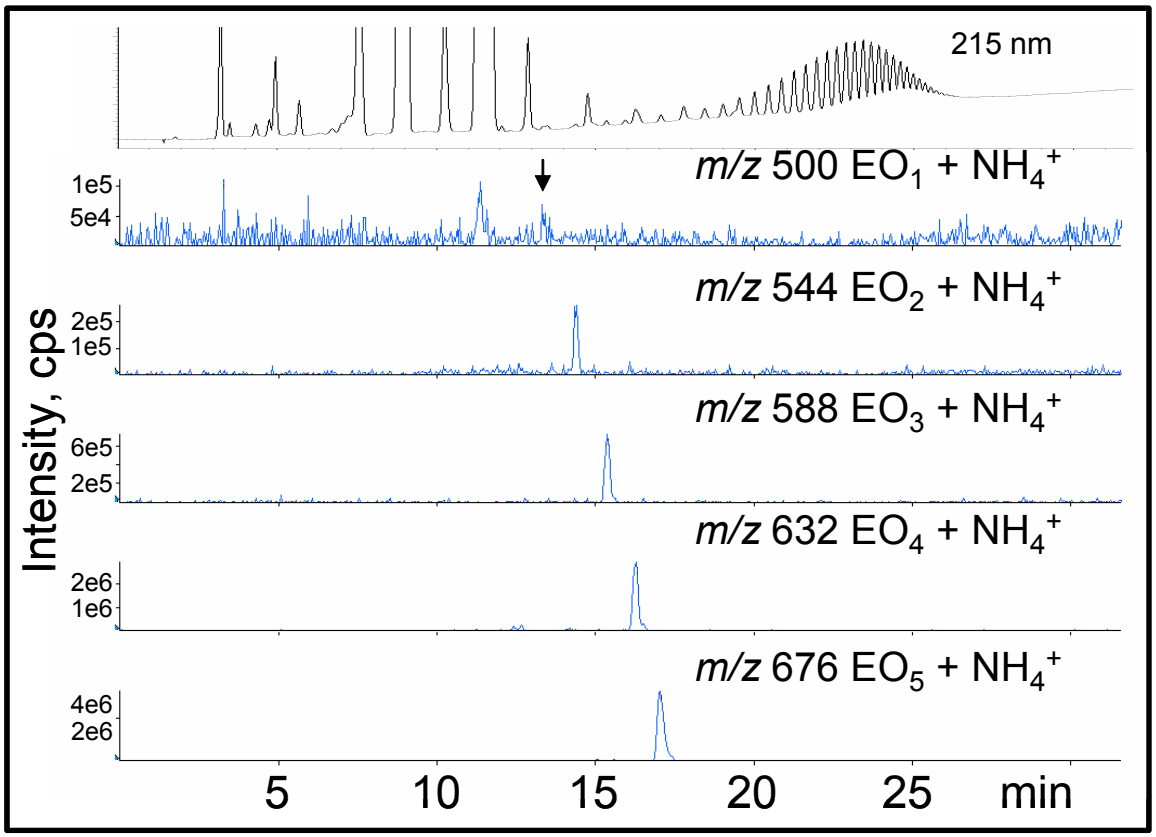

Figure 38. Extracted ion and UV chromatograms of 2Ph derivatized $C_{16}EO_{20}$. Acclaim PA C16, 4.6 x 250 mm. See Figure 1 for conditions. UV detector wavelength 215 nm

oligomers that were separated by an *m/z* of 44 amu. Figure 39 contains a SFC-ESI-MS contour plot of 2Ph derivatized $C_{18}EO_{10}$. The single and doubly charged species are noted in the Figure. By scanning the region between *m/z* 150-2000 it was possible to identify in the 1Ph derivatized $C_{18}EO_{10}$ sample oligomers with up to 35 EO units as their $[M+NH_4]^+$ ion adducts. Oligomers with 30-44 EO units were observed as their $[M+2NH_4]^{2+}$ ion adducts. The mass spectra of the 1Ph $C_{16}EO_{20}$ derivatized sample detected oligomers 2-36 EO as their $[M+NH_4]^+$ ion adducts and oligomers 19-58 EO as their $[M+2NH_4]^{2+}$ ion adducts. The single EO oligomer of the 1Ph derivatized $C_{16}EO_{20}$ sample was only detected as its $[M+Na]^+$ ion adduct. The mass spectra of the 1Ph $C_{18}PO_{15}$ derivatized sample detected oligomers 3-27 PO as their $[M+NH_4]^+$ ion adducts and oligomers 18-40 PO as their $[M+2NH_4]^{2+}$ ion adducts. Singly charged polyoxypropylene oligomers were separated by an *m/z* of 58 amu and doubly charged oligomers were separated by an *m/z* of 29 amu.

3.4 Effect of Derivative

Ideally absorbance is linearly related to concentration and molar absorptivity. Increasing the number of UV active groups per molecule should increase its absorptivity. Therefore, following this reasoning, a derivative that incorporated two phenyl groups was investigated and compared to derivatized AEOs containing a single phenyl group. It should be noted that the commercially available derivatizing reagents were 94-99% pure and therefore contained extraneous compounds. An amide embedded-alkyl stationary phase and a sulfonamide embedded-alkyl phase (Acclaim PA C16) were evaluated for the separation of the derivatized samples. The amide embedded-alkyl phase produced co-elution between the oligomeric series and excess 2Ph derivatizing materials and was

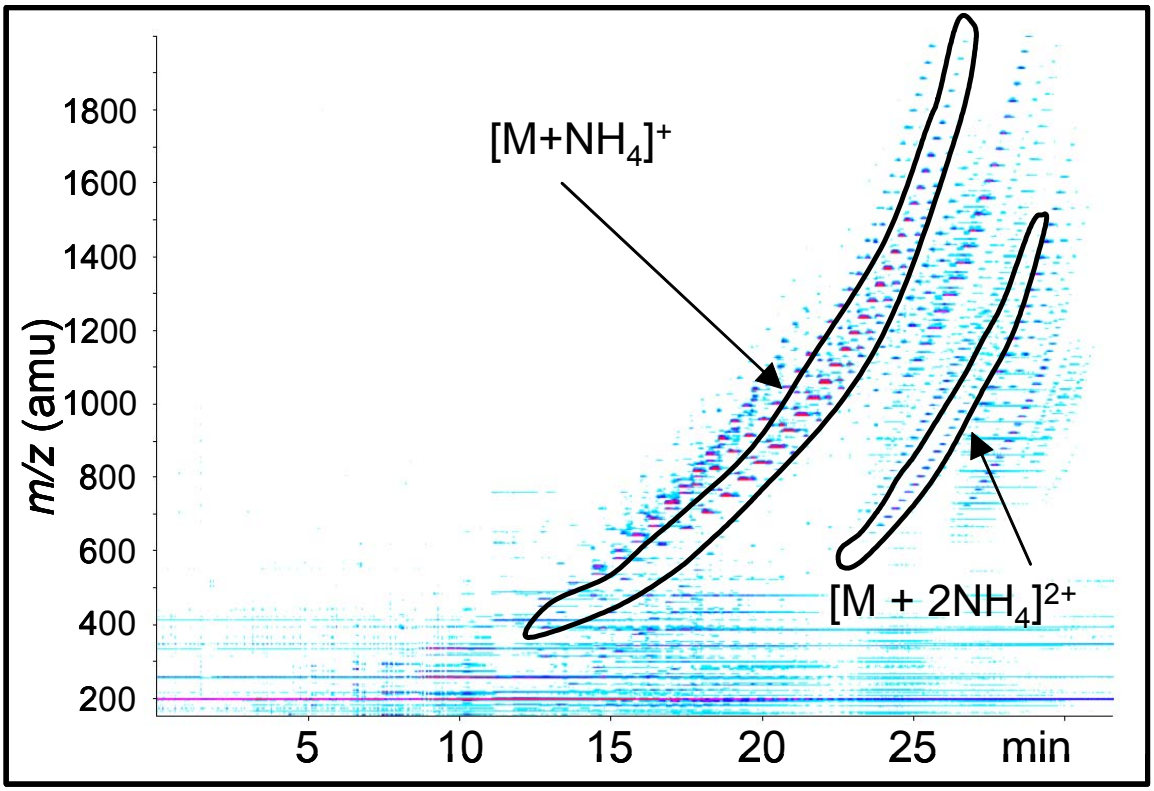

Figure 39. SFC-ESI-MS contour plot of 2Ph derivatized $C_{18}EO_{10}$.

See Figure 37 for UV and extracted ion chromatograms.

therefore not used in this study. Initially an attempt was made to cleanup 2Ph derivatized alcohol polyethers by solid phase extraction. The cleanup methods are described in Appendix A. The SPE methods were successful at removing residual reagents but they also removed the fatty alcohol and oligomers with a short (less than 4 EO) ethoxylate chain. Using the Acclaim PA C16 phase the residual reagents used to form 1Ph derivatives co-eluted in a tight band significantly resolved from the derivatized oligomers. The reagents employed in the 2Ph derivatization, on the other hand, contained compounds that had a greater interaction with the same stationary phase and were longer retained on the column. The excess 2Ph derivatizing reagents were well resolved by Acclaim PA C16 unlike the materials used in the 1Ph derivatization, but also elute prior to the oligomeric series. Since the Acclaim PA C16 stationary phase was capable of eluting residual reagents prior to the oligomeric series investigation into a cleanup step was not further explored. Allowing the analytical column to perform the cleanup "on-line" reduced the possibility of loss of sample that could affect analytical results as well as reduced sample preparation-analysis time. Without MS detection, it would be difficult to differentiate between derivatized oligomers and extraneous peaks in the UV chromatograms. Analysis of reagent blanks confirmed that extraneous peaks were due to impurities in the reagents, not derivatized impurities in the surfactant samples.

A $C_{18}EO_{10}$ sample was derivatized to form 1Ph and 2Ph derivatives employing the same concentration of surfactant (60 mg/mL). Since the concentration of each sample was identical the difference in detection between derivatives should be due to the number of phenyl groups incorporated into the oligomers. An average of 25 oligomer peaks were detected in the chromatograms of the 1Ph derivative, while the chromatograms of the 2Ph

derivative produced an average of 31 oligomers detected by UV absorbance (compared to the 44 oligomer peaks that were detected in the 1Ph $C_{18}EO_{10}$ sample by SFC-ESI-MS). The average molar oligomer values calculated from data for the 1Ph and 2Ph derivatives were 9.6 and 10.1 EO, respectively. A comparison between the two derivatives revealed that the 2Ph derivative produced 246% of the cumulative oligomer peak area of the 1Ph derivative. Figure 40 contains the UV chromatograms of the 1Ph and 2Ph derivatives of the $C_{18}EO_{10}$ sample. It illustrates both the presence of excess derivatizing material and the increased detector response of the 2Ph derivative versus the 1Ph derivative. The calculated molar concentration of individual oligomers ranged from 7.8% to 0.04% (calculated from 2Ph peak area data).

Table XIII shows the average distribution of oligomers of the 2Ph $C_{18}EO_{10}$ sample produced from SFC-UV data of a single injection. The ability to provide both oligomer distribution and average molar oligomer value is an advantage over ^{1}H-NMR, which only provides average molar oligomer information. Oligomer distribution data are useful for surfactant manufacturers and industry employing the surfactant to make sure the correct product is being produced and placed into end user products. Surfactant mixtures with similar average oligomer values that contain different oligomer distribution may perform differently. The increase in molar absorptivity of the 2Ph derivative allowed an average of 7 additional oligomers to be detected. The $C_{18}EO_{10}$ sample was also derivatized to form the 2Ph derivative at approximately 30 mg/mL, half the concentration of the previous samples, to determine if a lower concentration of surfactant could be used for adequate calculation of average molar oligomer values. The less

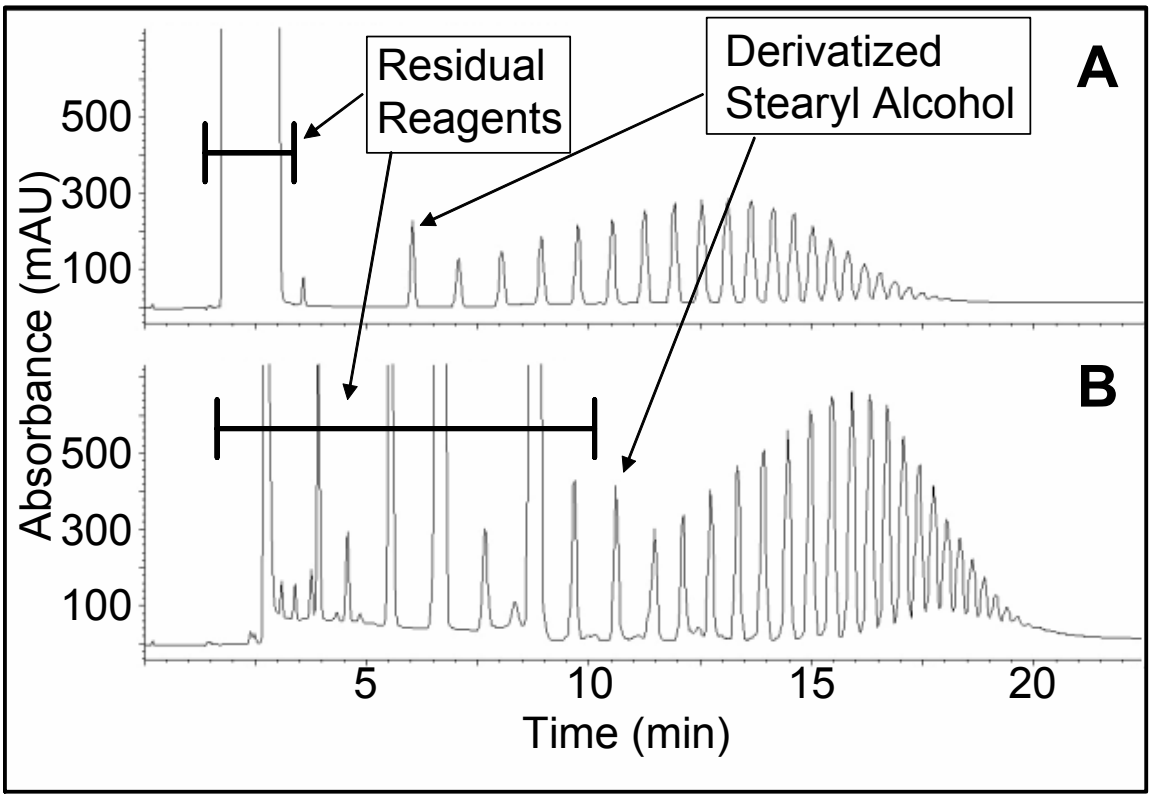

Figure 40. SFC-UV chromatograms of 1Ph and 2Ph derivatized $C_{18}EO_{10}$.
Acclaim PA C16, 4.6 x 250 mm. See Figure 35 for conditions. UV detector wavelength 215 nm. A) 1Ph $C_{18}EO_{10}$ (60 mg/mL) B) 2Ph $C_{18}EO_{10}$ (60 mg/mL)

Table XIII. $C_{18}EO_{10}$ 2Ph Oligomer Distribution.

EO #	% Composition
1	3.05
2	3.22
3	3.78
4	4.47
5	5.30
6	5.94
7	6.81
8	7.43
9	7.64
10	7.80
11	7.58
12	6.91
13	6.16
14	5.26
15	4.36
16	3.51
17	2.75
18	2.11
19	1.66
20	1.19
21	0.87
22	0.61
23	0.43
24	0.29
25	0.19
26	0.18
27	0.16
28	0.12
29	0.11
30	0.08
31	0.04
Average EO = 10.1	

Data from chromatograms in Figure 37.

concentrated sample produced a similar number of detected oligomer peaks and an average molar oligomer value of 10.1 EO.

The ability of the 2Ph derivative to reduce the amount of sample necessary for analysis was also demonstrated with a $C_{16}EO_{20}$ sample. The sample was derivatized at two concentrations: (a) a higher concentration (70 mg/mL) 1Ph derivative and (b) a lower concentration (30 mg/mL) 2Ph derivative. Both derivatives produced approximately 38 detectable oligomer peaks (compared to the 58 peaks detected by SFC- ESI-MS). The calculated average molar EO value for the 1Ph and 2Ph derivatives were 19.2 and 20.1 EO, respectively. Table XIV shows the oligomer distribution of 2Ph $C_{16}EO_{20}$. The extra oligomers that were detected by ESI-MS, not detected in UV, were present at ultra-trace concentration and should have little effect on average molar oligomer calculations. The molar concentration of individual oligomers in the $C_{16}EO_{20}$ sample was between 6.2% and 0.35% (calculated from 2Ph peak area data). Compared to the 2Ph derivatized $C_{18}EO_{10}$ sample, which had an individual oligomer molar concentration range between 7.8% and 0.04%, the 2Ph derivatized $C_{16}EO_{20}$ sample had an oligomer distribution in which its less abundant oligomers were present at a higher concentration than the less abundant oligomers in the $C_{18}EO_{10}$ sample. Due to higher concentrations of individual oligomers in $C_{16}EO_{20}$ the oligomers were most probably not present below the limit of detection and therefore may explain the similar number of oligomers detected in each $C_{16}EO_{20}$ sample regardless of sample concentration or derivative type.

Due to the increased sensitivity of the 2Ph derivative, highly ethoxylated oligomers were more clearly seen than those in 1Ph derivatives for both surfactants. The

Table XIV. $C_{16}EO_{20}$ 2Ph Oligomer Distribution.

EO#	RT (min)	% Area
1	13.48	0.35
2	14.37	0.38
3	15.33	0.44
4	15.93	0.39
5	16.25	1.82
6	17.04	0.59
7	17.76	1.55
8	18.41	0.94
9	18.99	1.22
10	19.50	2.10
11	19.98	1.88
12	20.42	2.32
13	20.83	2.80
14	21.22	3.24
15	21.59	3.88
16	21.93	4.45
17	22.26	5.12
18	22.56	5.50
19	22.85	5.87
20	23.12	6.13
21	23.39	6.26
22	23.64	6.04
23	23.88	5.71
24	24.11	5.26
25	24.34	4.72
26	24.56	4.09
27	24.76	3.50
28	24.96	2.88
29	25.15	2.36
30	25.33	1.87
31	25.51	1.49
32	25.69	1.21
33	25.85	0.91
34	26.02	0.74
35	26.18	0.59
36	26.34	0.55
37	26.57	0.51
38	26.70	0.35
Average EO = 20.1		

Data from chromatograms in Figure 38.

higher sensitivity increased the calculated average molar EO value of the $C_{16}EO_{20}$ sample by 0.9 EO unit bringing the calculated value within 0.1 EO unit of the nominal value. A comparison of the cumulative oligomer peak area of each derivative, divided by the sample's respective concentration, revealed that the 2Ph $C_{16}EO_{20}$ derivative produced 288% of the cumulative oligomer peak area of the 1Ph $C_{16}EO_{20}$ derivatized sample. In each case it would be expected that the 2Ph derivative would produce approximately 200% of the cumulative oligomer peak area of a 1Ph derivative of the same surfactant sample. An increase greater than 200% may be due to co-elution of other derivatized species in the separation of 2Ph derivatives. Regardless, for each surfactant type analyzed the 2Ph derivative was capable of producing higher average molar oligomer values. A noticeable difference between the two types of derivatives was that the 2Ph derivative exhibited a longer retention time compared to the 1Ph derivative.

Figure 41 contains chromatograms of a 1Ph and 2Ph derivatized $C_{18}PO_{15}$ sample. The concentration of surfactant of the 1Ph derivatized sample was approximately 60 mg/mL and the 2Ph derivatized sample was approximately 30 mg/mL. The chromatographic data for both samples produced an average molar oligomer value of 12.8 PO. Table XV contains the oligomer distribution data for both samples. In the UV chromatograms of both of the derivatives approximately 25 oligomers were detected. Even though calculated oligomer values were similar for both derivatives, on a concentration adjusted basis, the 2Ph derivative produced 196 % larger cumulative peak area than the 1Ph derivative. The values calculated in this Chapter are similar to those obtained in Chapter 4 from the 1Ph concentration study. This may indicate the limit of the analysis method. The average molar oligomer value of non-derivatized $C_{18}PO_{15}$ by

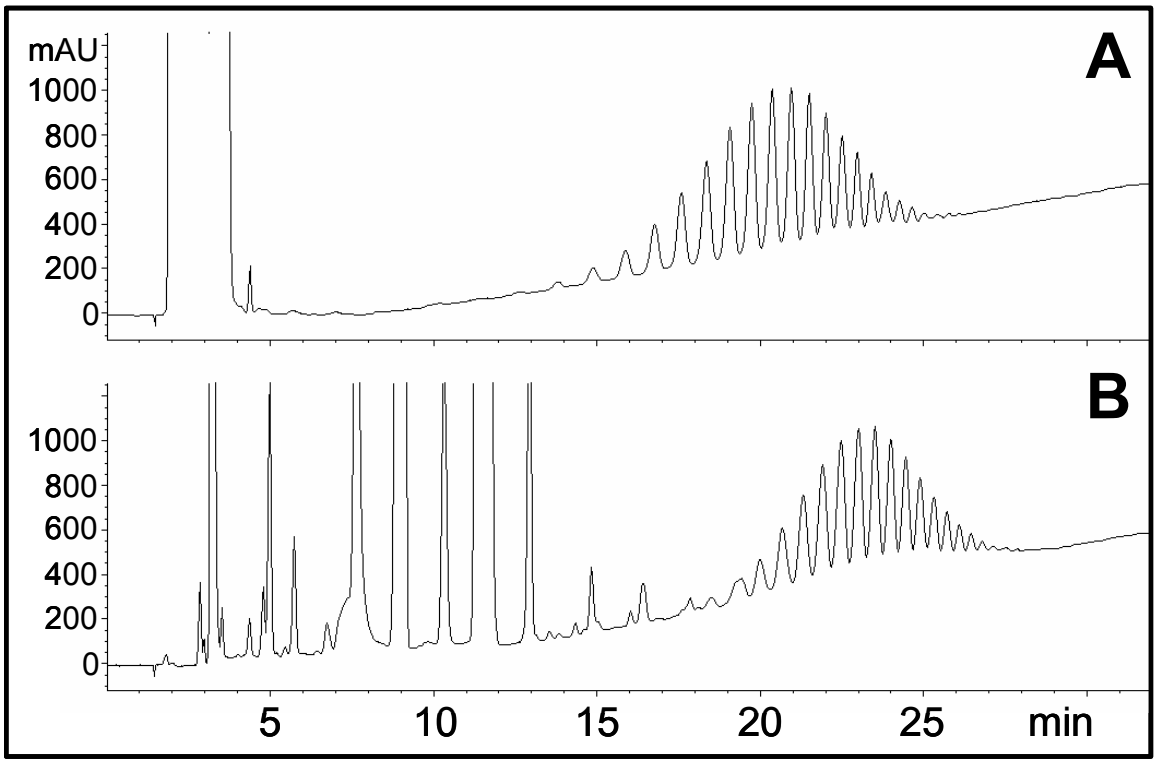

Figure 41. SFC-UV of 1Ph and 2Ph derivatized $C_{18}PO_{15}$.

Acclaim PA C16, 4.6 x 250 mm. See Figure 35 for SFC conditions. UV detector wavelength 215 nm. A) 1Ph $C_{18}PO_{15}$ (~60 mg/mL) B) 2Ph $C_{18}PO_{15}$ (~30 mg/mL)

Table XV. Peak area distribution of 1Ph and 2Ph derivatized $C_{18}PO_{15}$.

	1Ph		2Ph	
PO#	RT (min)	% Area	RT (min)	% Area
3	12.66	0.18		
4	13.83	0.43	17.85	0.39
5	14.89	1.09	18.49	0.77
6	15.86	1.95	19.41	2.74
7	16.77	3.51	19.99	3.46
8	17.58	5.44	20.67	5.72
9	18.35	7.35	21.31	7.62
10	19.07	9.09	21.91	9.37
11	19.74	10.34	22.47	10.51
12	20.36	10.72	23.01	10.62
13	20.95	10.32	23.51	10.10
14	21.49	9.28	23.99	8.92
15	22.01	7.91	24.45	7.48
16	22.49	6.41	24.88	6.04
17	22.96	4.75	25.31	4.62
18	23.40	3.48	25.71	3.37
19	23.83	2.58	26.09	2.40
20	24.25	1.68	26.45	1.84
21	24.64	1.07	26.78	1.17
22	25.00	0.80	27.12	0.92
23	25.41	0.58	27.49	0.71
24	25.77	0.39	27.83	0.52
25	26.08	0.24	28.12	0.31
26	26.50	0.24	28.36	0.23
27	26.75	0.19	28.63	0.16
Avg. PO	12.8 PO		12.8 PO	

Data from chromatograms in Figure 41.

^{1}H-NMR was 13.7 PO, reported in Chapter 4. Inspection of the mass spectrometric data of the 1Ph derivatized $C_{18}PO_{15}$ sample indicates the presence of doubly derivatized (two hydroxyl groups per molecule) polypropylene glycol (PPG). The presence of PPG in the sample may raise the average molar oligomer value calculated by ^{1}H-NMR due to its inability to discriminate between the methyl groups of the repeat unit of an APO or PPG molecule. It should be noted that both chromatography and spectrometry methods produced average molar oligomer values that were below the reported nominal value for the sample. This may be due to the manufacturing process.

3.5 Method reproducibility

Two $C_{18}EO_{20}$ samples were derivatized with DMTPDS-MDPCS to form the 2Ph derivative. Each derivative mixture was chromatographically separated three times. The average molar EO value calculated was 10.1 with an RSD of 0.8%. As mentioned in chapter 4, "average molar oligomer value" is a relative measure of distribution and it was important to compare peak areas of several chromatograms. As long as all (or a large majority) of the peaks were detectable, and they were equally derivatized, then the correct oligomer value should be obtained. In other words, it would be possible to have chromatograms that vary greatly in total peak area but give equal average molar oligomer values as long as the peak ratios were consistent. For this purpose, the peak area of an individual oligomer from the reproducibility study was compared to determine reproducibility of peak area. The peak areas of the 4 EO oligomer of each $C_{18}EO_{10}$ chromatogram were compared. This oligomer was picked because it was well resolved in each of the chromatograms. The peak areas of the 4 EO oligomers were divided by the mass of sample used for each derivatization. This produced an adjusted peak area that

was normalized to the mass used for the individual derivatizations, which accounted for slight differences in the mass of sample used. The RSD of peak areas was 7.6%, which indicated that the derivatization and chromatographic methods were reproducible.

4. SUMMARY

The molar absorptivity of derivatized alcohol ethoxylates was increased in this study by increasing the number of phenyl groups associated with each oligomer. The formation of a 2 Ph derivative was capable of producing more oligomer peaks detected by UV absorbance than the 1Ph derivative for samples of equal concentration. Due to the impurities present in the derivatizing material, interpretation of chromatograms became more complicated for 2Ph derivatives than 1Ph derivatives due to extraneous peaks. Use of reagents of higher purity may alleviate the problems associated with extraneous reagent peaks. The derivative containing two phenyl groups provided higher sensitivity than derivatives containing a single phenyl group and therefore lower concentration of 2Ph derivatives were necessary to provide accurate analysis for distribution of oligomers and average molar oligomer value. The high sensitivity of ESI-MS detection was demonstrated since none of the SFC-UV methods were capable of detecting all of the oligomers by ESI-MS. The derivatization and chromatography methods were capable of producing average molar oligomer values consistent with nominal reported values. Thus, 2Ph derivatization and separation methods described here could be employed for quality control applications of neat alcohol polyethers for the determination of average molar oligomer values and oligomer distributions.

CHAPTER 6

CONCLUSIONS

The analysis of complex oligomeric non-ionic surfactants was conducted in the research. All of the samples analyzed had a wide molecular mass range and a very large mole fraction distribution for individual oligomers. Combining issues of oligomer size and concentration with the lack of a strong UV chromophore associated with alcohol polyethers created a great challenge for both the chromatographic separation and detection of oligomers.

Several techniques were applied to resolve the complex challenges posed by the samples. SFC proved to be useful for the separation of individual oligomers to determine average molar oligomer values as well as oligomer distribution. ^{1}H-NMR also proved to be a useful method for quick determination of average molar oligomer value. ^{13}C-NMR was able to confirm the tacticity of repeat units of propoxylated samples. UV detection coupled with SFC was a rugged technique for calculation of average molar oligomer value. Electrospray ionization mass spectrometry coupled with SFC or liquid flow injection analysis was very useful in the identification of analytes. Without MS detection it would not be possible to confirm analyte identity unless standard compounds were obtained. Mass spectrometry was able to detect some compounds not detected by UV absorbance due to low concentration; while UV detection was able to detect some compounds MS was not able to detect due to poor ionization of analytes. Both detection methods complimented each other. Infrared absorbance spectrometry was also useful in identification of by-products formed during derivatization.

The addition of a chromophore, capable of absorbing UV radiation outside the region in which organic solvents absorb light, was important for detection of alcohol polyethers. Several derivatization schemes were investigated but the formation of silylethers provided the best results for separation and detection of oligomers. Equal molar UV response of oligomers was important for the calculation of oligomer distribution and average molar oligomer values. Research with 1Ph derivatives revealed that a higher concentration of surfactant was necessary for detection of less abundant oligomers in higher average molecular weight samples. The addition of a second phenyl group to the oligomers was capable of increasing detection sensitivity, which allowed lower concentration of surfactant to be used for analysis. In the research conducted, sample was not in limited quantities but may be an issue for other researchers.

The compounds used for derivatizations contained several components as evidenced by blank injections of derivatizing materials. With some stationary phases the components of the reagents co-eluted with derivatized analytes of interest. The use of higher purity reagents or the development of a cleanup method may resolve the issue of extraneous materials present due to derivatization. A cleanup method was investigated but it was not successful. Other cleanup methods may be possible but since a stationary phase was found that separated extraneous chromatographic peaks from those of interest, a cleanup step was not further investigated. The methods developed can ultimately be used for quality control for industrial suppliers and consumers of non-ionic surfactants. The separation and derivatization methods developed make analysis of the different surfactant types easy to perform. Elimination of a cleanup step reduced the possibility of

loss of sample due to handling and increased the productivity of analysis by reducing sample preparation time.

The research conducted was not just method development to solve an industrial problem. The physical chemistry of interactions between analyte, stationary phase, and mobile phase were important elements in this research. As mentioned by Berger,[19] SFC may not be the answer to all separation challenges. HPLC, GC, and other chromatographic techniques have qualities that make them superior in certain aspects of chromatography such as efficiency or ability to analyze rather polar compounds compared to SFC. Due to the characteristics of modified supercritical carbon dioxide, packed column SFC is able to out perform GC in the ability to solvate relatively high molecular weight compounds and HPLC in the ability to operate at higher linear velocity/flow-rate with similar efficiency to HPLC. GC separations were not reported in this thesis, but SFC was shown to perform similar separations as HPLC at reduced analysis time due to higher flow rates.

The importance of interaction between the mobile phase and analytes and the mobile phase and stationary phase were demonstrated by this research. With pure CO_2, non-derivatized alcohol polyethers demonstrated poor solubility in the mobile phase and non-ideal interactions with the stationary phase. Capping the terminal hydroxyl group of the oligomers increased the solubility of the sample in the mobile phase and provided good interaction with the stationary phase. This has been seen in SFC and GC previously.[43,79] It was of interest to reduce the temperature and CO_2 pressure necessary for SFC separations compared to the extreme parameters associated with use of pure CO_2. The addition of an UV chromophore through the capping of the terminal hydroxyl

groups allowed use of organic solvents while being able to use UV detection. The choice of modifier influenced analyte separation by altering the stationary phase and mobile phase. Addition of modifier increased the polarity of the mobile phase, which affected retention time of analytes. Choice of modifier may have also affected the stationary phase structure. The use of methanol as a modifier may have aided in shielding free silanols that produce undesirable interactions between analytes and the stationary phase, causing peak tailing, thus improving peak shape.

The ability to perform separations at relatively mild operating conditions allowed a variety of stationary phases to be evaluated. Interaction between the compounds and the stationary phase are very important for quantitative analysis. This was evidenced by the ability of certain phases to separate derivatized oligomers from residual derivatization reagents and provide good peak symmetry. Polar embedded stationary phases proved to be useful for the analysis of alcohol polyethers. Their possible internal ability to shield free silanols as well as interact with the polyether region of the surfactants analyzed through dipole-dipole or hydrogen bonding interactions produced good resolution between oligomers. Faster analysis coupled with reduced solvent consumption made SFC an exemplary technique for characterization of alcohol polyethers and alkylphenol ethoxylates. Reproducibility studies conducted demonstrated the derivatization and separation methods developed to be robust. The requirements of relatively short analysis time and mild operating conditions for the analysis of non-ionic surfactants were met by the developed methods.

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Appendix A

Evaluation of Sample Clean-up for 2Ph Derivatized Alcohol Polyethers

1.0 Introduction

The reagents used in the disilazane-chlorosilane derivatization were not available at 100% purity; therefore, unwanted compounds were detected in SFC separations. Excess derivatizing compounds were used to insure high derivatization yield. Initial attempts at the separation of 2Ph derivatized alcohol polyethers using the Discovery RP-AmideC16 stationary phase resulted in the co-elution of residual derivatizing material with the derivatized oligomer series. Both acetonitrile- and methanol-modified CO_2 were employed for the separation of 2Ph derivatized $C_{18}EO_{10}$ (Figure 42) and $C_{18}PO_{15}$ (Figure 43). Use of the two modifiers did not change the elution pattern of the residual reagents. Discovery C18 was then evaluated as a stationary phase for SFC separation of the 2Ph derivatives. It was able to elute the residual reagents prior to the oligomeric series but provided poor peak shape as seen with 1Ph derivatives in Chapter 3. A SPE cleanup method was investigated to remove residual reagents. TLC on silica plates with methylene chloride (DCM)/methanol and hexane/iso-propanol mobile phases appeared to be able to separate the derivatizing agents from the derivatized oligomers.

2.0 Experimental

The same chemicals and derivatization methods used in Chapter 5 were used for this research. Acetonitrile (MeCN), hexane, iso-propanol, dichloromethane (DCM) and methanol (MeOH) were obtained from Burdick & Jackson (Muskegon, MI). Thin layer

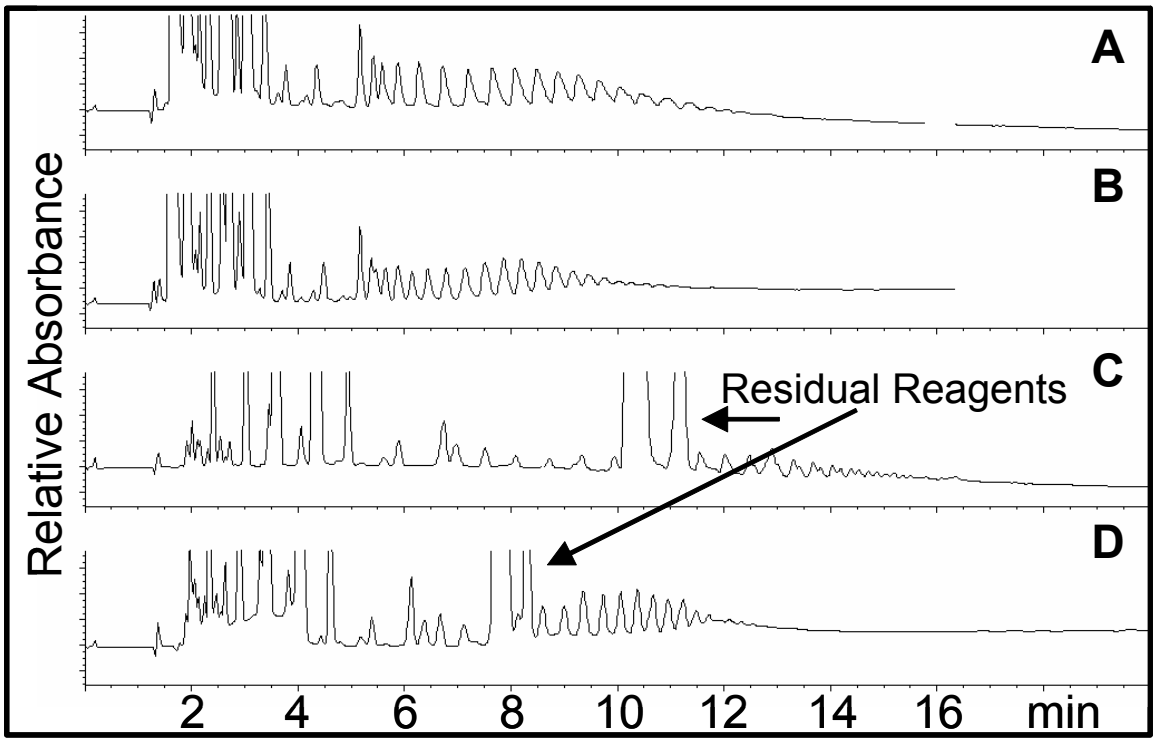

Figure 42. SFC-UV of 2Ph $C_{18}EO_{10}$ on a C18 and Amide-embedded alkyl phase. SFC-UV of 2Ph $C_{18}EO_{10}$ on Discovery C18 (4.6 x 250 mm) with A) MeCN-modified CO_2; B) MeOH-modified CO_2 and on Discovery RP-AmideC16 (4.6 x 250 mm) with C) MeCN-modified CO_2 and D) MeOH-modified CO_2. Oven 40°C, flow rate 2.4 mL/min, absorbance 215 nm, outlet pressure 120 bar.

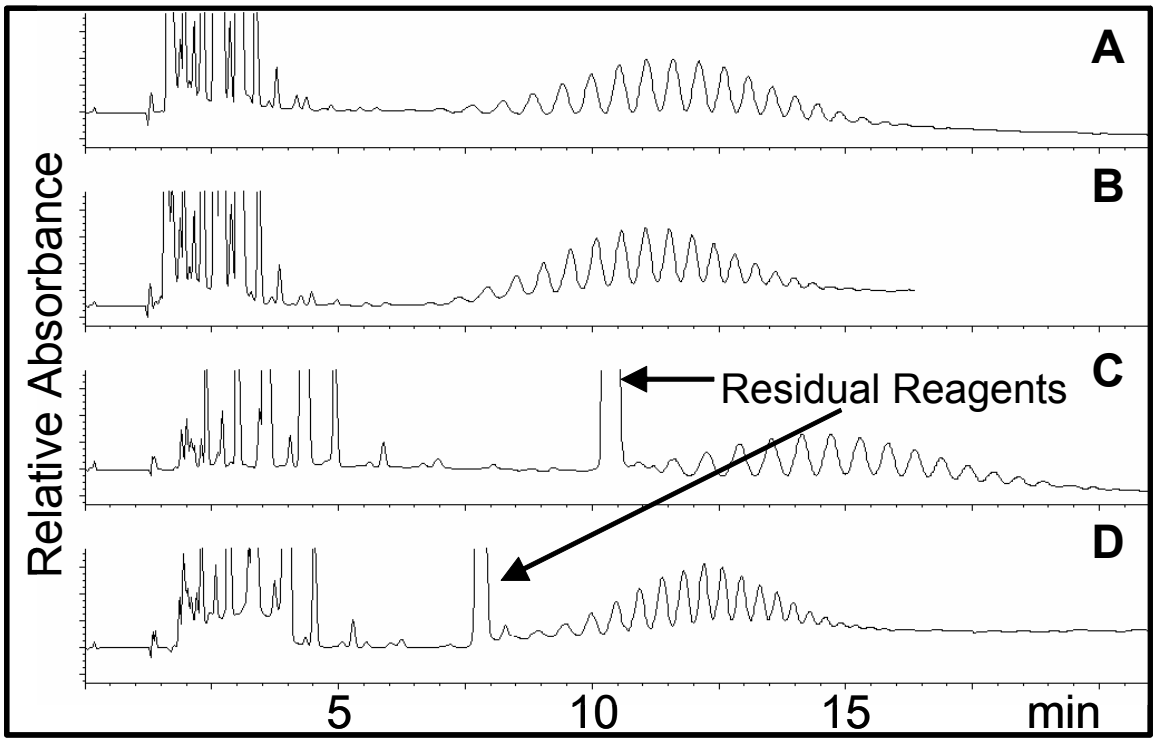

Figure 43. SFC-UV of 2Ph $C_{18}PO_{15}$ on a C18 and Amide-embedded alkyl phase. SFC-UV of 2Ph $C_{18}PO_{15}$ on Discovery C18 (4.6 x 250 mm) with A) MeCN-modified CO_2; B) MeOH-modified CO_2 and on Discovery RP-AmideC16 (4.6 x 250 mm) with C) MeCN-modified CO_2 and D) MeOH-modified CO_2. Oven 40°C, flow rate 2.4 mL/min, absorbance 215 nm, outlet pressure 120 bar.

chromatography (TLC) was performed on aluminum backed silica gel sheets with fluorescent indicator (EM Science, Gibbstown, NJ).

2.1 SPE on silica

Waters silica SEP-PAK cartridges (Milford, MA) were evaluated for clean-up. A syringe was used to pass solvents and samples through the cartridges. Eluted fractions were analyzed by SFC on a Discovery C18 packed column (4.6 x 250 mm, 5µm average particle size) using a Berger Instruments analytical SFC. SFC condtions were: oven temperature 40°C; outlet pressure 120 bar; flow rate 2.4 mL/min, modifier acetonitrile, detection 215 nm. The gradient method was: 1% modifier held for 5 minutes, then raised linearly at 1%/min to 15%, hold at 15 % for 2 minutes then return to 1% at 25%/minute.

Method A: The cartridge was conditioned with 6 mL of DCM. A 2Ph derivatized sample was loaded onto the cartridge. The cartridge was washed with 3 mL of DCM twice and 3 mL of 15/85 (v/v) MeOH/DCM twice. Each wash was collected in a test-tube. Each fraction was analyzed by TLC using DCM as the developing solvent. The fractions were then evaporated under a stream of nitrogen and reconstituted in 1.5 mL of acetonitrile, and then analyzed by SFC.

Method B: The cartridge was conditioned with 6 mL of hexane. Then a derivatized sample which had been evaporated under a stream of nitrogen and reconstituted in hexane was loaded onto the cartridge. The cartridge was then washed with 3 mL of hexane three times and then washed with 3 mL of 15/85 (v/v) MeOH/DCM. Each wash

was collected in a test-tube. The fractions were then evaporated under a stream of nitrogen and reconstituted in 1.5 mL of acetonitrile, thus ready for SFC analysis.

SPE on silica bonded phases

Supelco Discovery CN, NH_2, and Diol 3mL-500 mg SPE tubes (Bellefonte, PA) were evaluated for clean-up. A vacuum chamber was used to pull sample and solvent through the tubes. The tubes were conditioned with 3 mL of hexane twice. The samples were then loaded onto the SPE tubes. The tubes were washed with 3 mL of hexane and then 3 mL of DCM. Each wash was collected in a test-tube, evaporated under a stream of nitrogen, and reconstituted in 1.5 mL of acetonitrile, thus ready for SFC analysis. The same SFC analysis method used for silica SPE clean-up was used for the evaluation of bonded phase SPE clean-up fractions.

3.0 Results and Discussion

Silica TLC plates were used for the evaluation of a cleanup method using methylene chloride (DCM)/methanol or hexane/iso-propanol as the mobile phase. From the results of the TLC separations of derivatizing reagents and derivatized oligomers it appeared that removal of residual reagents was possible. Four stationary phases were used for SPE cleanup of derivatized samples. Residual reagents present in the AEO and APO chromatograms were confirmed by injection of blank reagents. The higher retention of the residual reagents by the Discovery RP-AmideC16 stationary phase may be attributed to hydrogen bonding and dipole-dipole interactions between the embedded amide group and derivatizing reagents. Since 2Ph derivatization reagents were eluted

prior to the derivatized oligomers on the Discovery C18 phase it was used to evaluate fractions obtained during development of the SPE cleanup method.

3.1 SPE Clean-up

Each of the clean-up methods was able to remove excess derivatizing materials or compounds that would interfere with analysis of the oligomeric series. The methods were not successful in retaining all of the derivatized oligomers. Bare silica retained the oligomers better than the bonded phases. When the bonded silica phases were washed with hexane they removed both the compounds that elute before the oligomeric series in SFC and a large portion of the oliogomeric series itself (Figure 44). The oligomeric series was well retained by the Waters silica SEP-PAK using both DCM (Figure 45) and hexane (Figure 46) as wash solvents. In both silica SPE methods the first three to four peaks of the oligomeric series were removed with washes that were intended to only remove extraneous compounds (Figures 45 and 46). Although each method removed oligomeric compounds of interest it seems that DCM was a better solvent for removing unwanted compounds. This method may be useful for derivatized surfactant samples which contain predominantly a high number of ether linkages. Several stationary phases were also evaluated in analytical columns to avoid the need for a cleanup step. In chapter 5 Acclaim PA C16, a sulfonamide-embedded alkyl phase, with methanol-modified CO_2 as the mobile phase was capable of eluting residual 2Ph derivatizing reagents prior to the derivatized oligomeric series. Since a stationary phase was found that was capable of analyzing 2Ph derivatized AEOs and APOs without cleanup further investigation into a cleanup method was not explored.

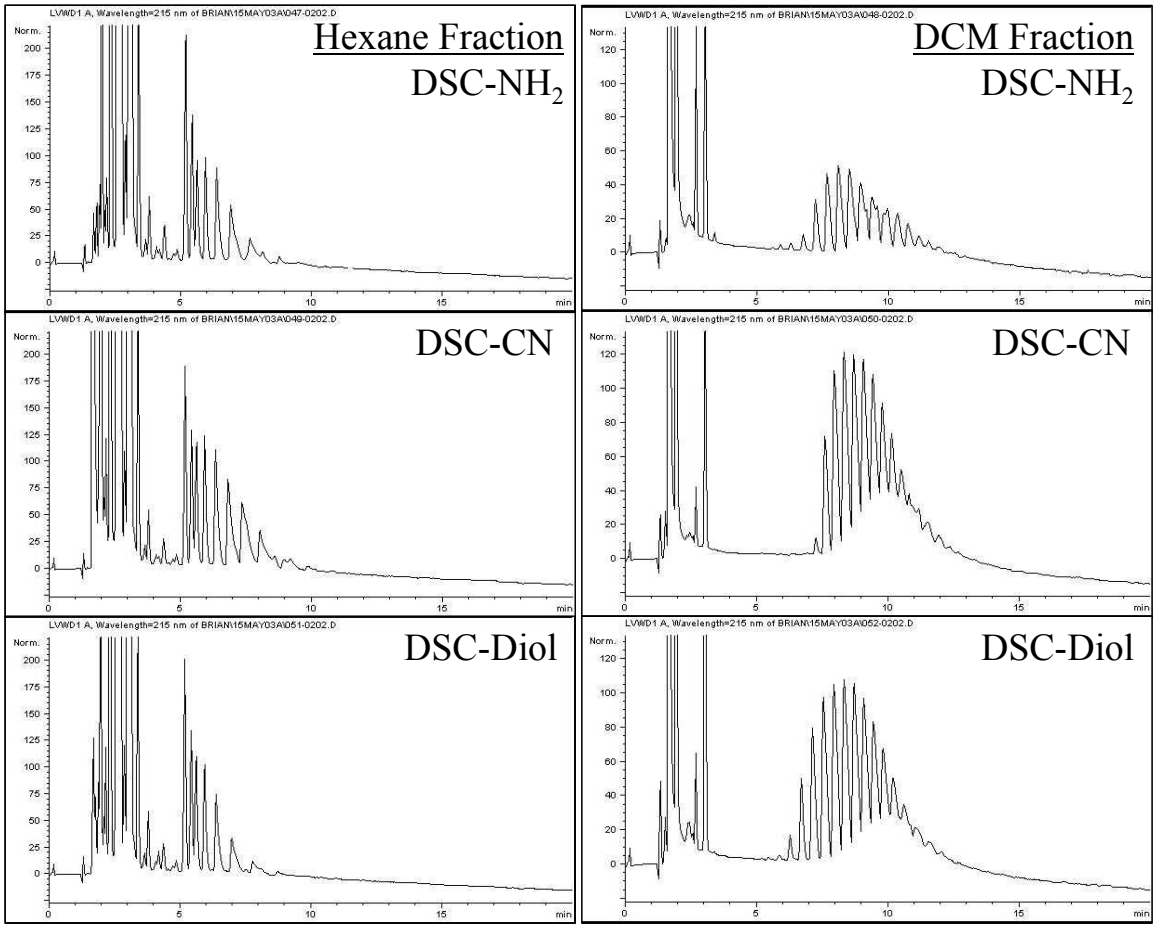

Figure 44. SPE cleanup on bonded silica.
See experimental section for SPE method. SFC conditions: Discovery C18 (4.6 x 250 mm); modifier acetonitrile Oven 40°C, flow rate 2.4 mL/min, absorbance 215 nm, outlet pressure 120 bar. Modifier method: 1% hold 5 min., raise 1%/min to 10 %, hold 2 min., lower 25%/min to 1%. 5 minute post-run.

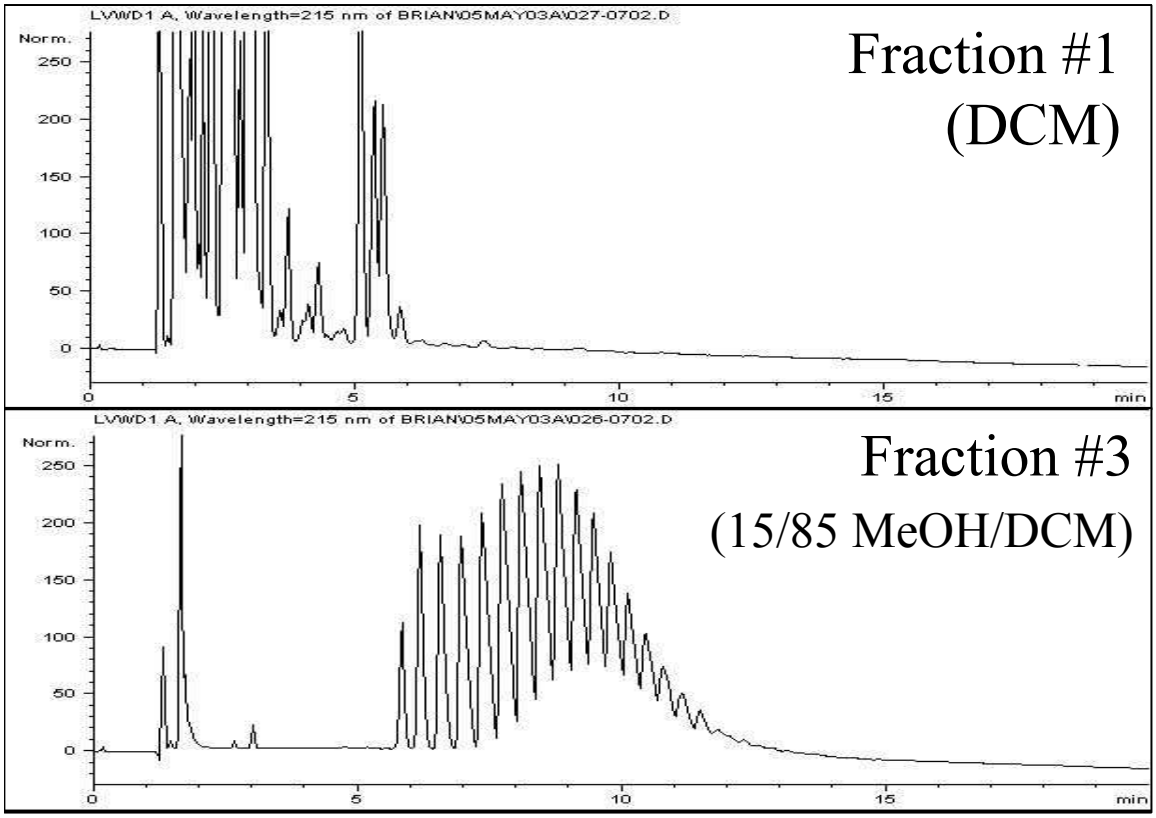

Figure 45. SPE cleanup of 2Ph $C_{18}EO_{10}$ on silica method A.
See experimental section SPE silica method A. SFC conditions: Discovery C18 (4.6 x 250 mm); modifier acetonitrile Oven 40°C, flow rate 2.4 mL/min, absorbance 215 nm, outlet pressure 120 bar. Modifier method: 1% hold 5 min., raise 1%/min to 10 %, hold 2 min., lower 25%/min to 1%. 5 minute post-run.

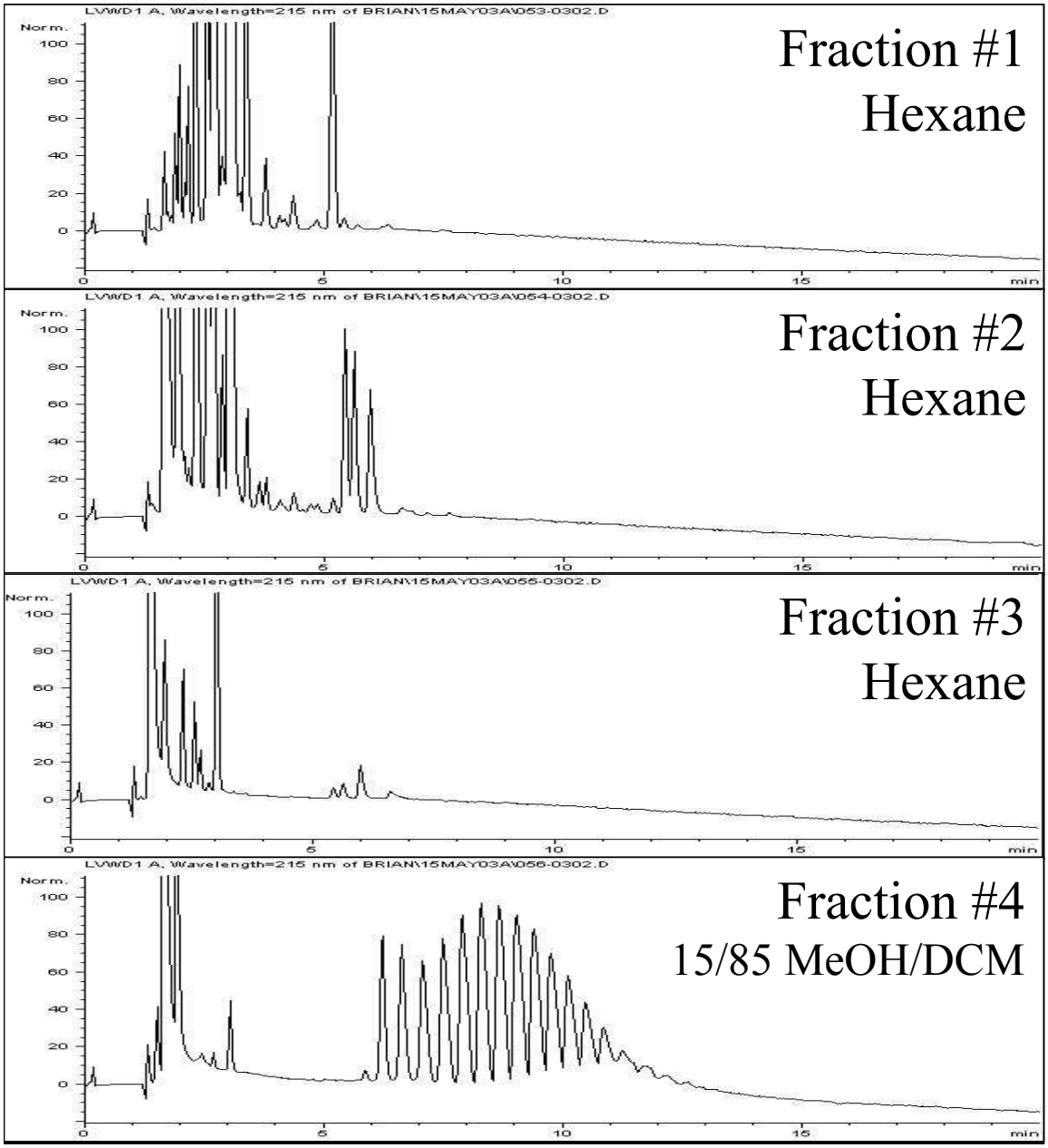

Figure 46. SPE cleanup of 2Ph $C_{18}EO_{10}$ on silica method B.
See experimental section SPE silica method A. SFC conditions: Discovery C18 (4.6 x 250 mm); modifier acetonitrile Oven 40°C, flow rate 2.4 mL/min, absorbance 215 nm, outlet pressure 120 bar. Modifier method: 1% hold 5 min., raise 1%/min to 10 %, hold 2 min., lower 25%/min to 1%. 5 minute post-run.

Appendix B

Separation of Mixed Alkyl Alcohol Polyethers

Mixed alkyl ethoxlyated surfactants were obtained from Uniqema (New Castle, DE). The surfactants contained alkyl chains with a mixture of 13 and 15 carbons ($C_{13/15}EO_n$) and had reported average molar oligomer values between 4 and 20. Several phases were evaluated for separation of the surfactants both by alkyl chain and ethoxylate chain. Figure 48 is the SFC-UV chromatogram of the 1Ph derivative of $C_{13/15}EO_7$. The derivatized sample was separated on an Acclaim PA C16 column with methanol-modified CO_2. It appears that there are two distributions in the sample due to a repeating peak pattern of a larger peak followed by a smaller peak. Upon inspection of the SFC-ESI-MS contour plot (Figure 49) it was evident that isomer separation was occurring. Horizontal bands (same mass) separated into approximately three peaks indicate isomer separation. The sample contains alkyl chains with an odd number of carbons, which indicates that synthetic fatty alcohols were used for production of the ethoxylated alcohol. Due to possible branching in the alkyl chain isomer separation occurs. The contour plot also shows that oligomers of the same homologous series are being separated by the phase but are co-eluting with the other homologous series of oligomers in the manner of $C_{13}EO_{n+1}/C_{15}EO_n$. The alternating set of peaks are therefore due to isomer separation and co-elution between the oligomers of the two homologous series. The same sample was also separated on a Berger Silica column under the same chromatographic conditions. Unlike the Acclaim PA C16 phase, which is a sulfonamide-embedded alkyl phase, that may provide some partition separation interaction, the silica

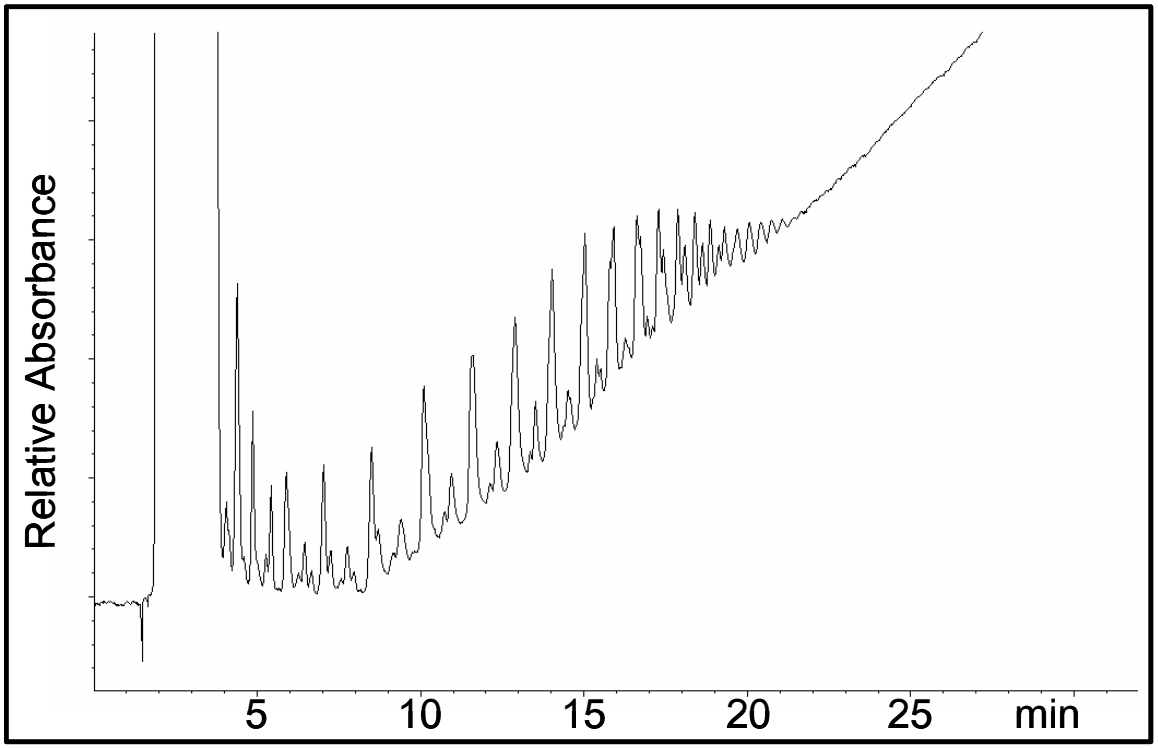

Figure 47. SFC-UV $C_{13/15}EO_7$ on Acclaim PA C16.
Acclaim PA C16, (2) 4.6 x 150 mm, 5µm. Oven = 40°C, Outlet pressure = 120 bar, Flow rate = 2.4 mL/min, modifier = methanol, modifier gradient: 1% for 5 min, linear increase 1%/min to 25%, hold 2 minutes, linear decrease to 1% at 25%/min, 5 minute post run.

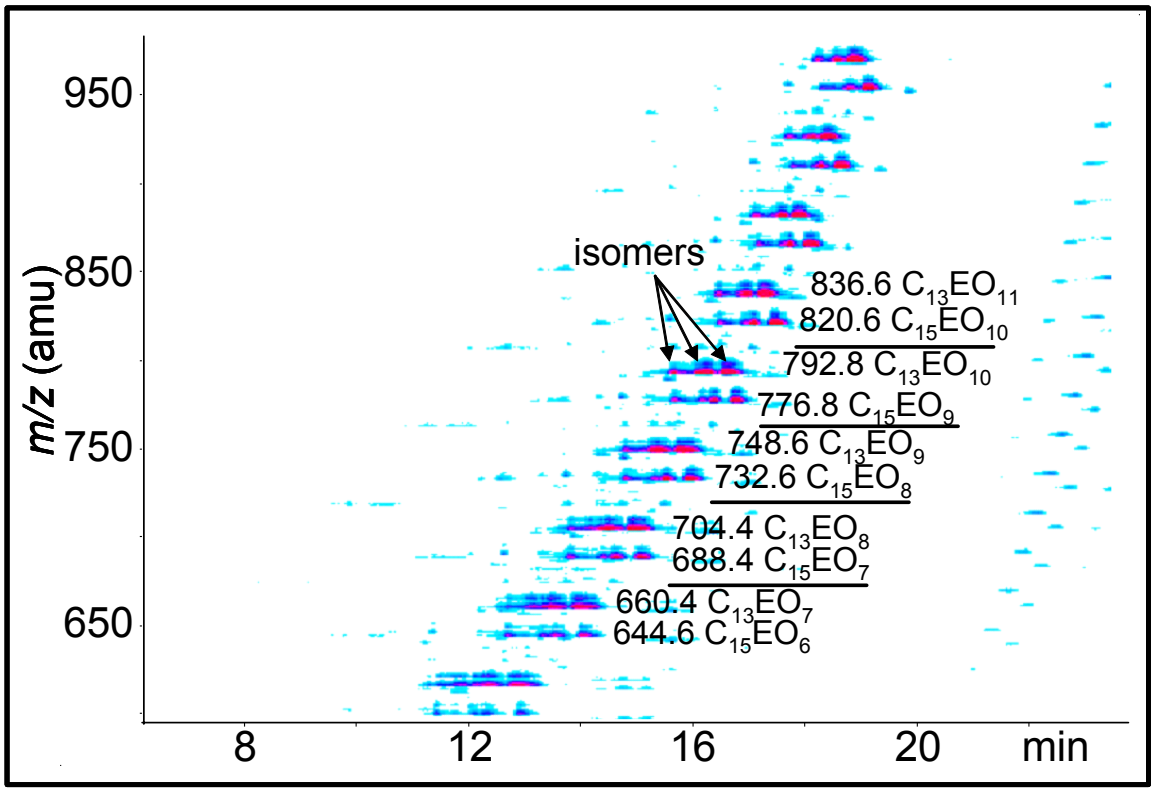

Figure 48. SFC-ESI-MS Contour plot of $C_{13/15}EO_7$ on Acclaim PA C16.
Acclaim PA C16, (2) 4.6 x 150 mm, 5µm. Oven = 40°C, Outlet pressure = 120 bar, Flow rate = 2.4 mL/min, modifier = methanol, modifier gradient: 1% for 5 min, linear increase 1%/min to 25%, hold 2 minutes, linear decrease to 1% at 25%/min, 5 minute post run.

phase could only interact with the analyte through adsorption interactions (i.e. hydrogen bonding and dipole-dipole interactions). The SFC-UV separation of the sample on the silica stationary phase (Figure 50) also exhibited the presence of subsets. Three subsets seem to appear with peak height increasing chronologically in each set. The SFC-ESI-MS contour plot of the sample separated on the silica stationary phase also appears to produce isomer separation (Figure 51). Oligomers with the same number of repeat units from each of the homologous series co-elute. This co-elution may be useful for the calculation of average molar oligomer value of the sample but does not allow characterization of the individual homologous series. Oligomer retention on the silica stationary phase increased with increasing number of repeat units and higher ethoxylated oligomers were not eluted off of the column. Interaction between the highly ethoxylated oligomers and the polar stationary phase must have adsorbed the compounds to the phase. It was not a problem with solubility in the mobile phase since the same oligomers were eluted from the Acclaim PA C16 phase and were detected by ESI-MS. The combination of a column packed with a stationary phase capable of separation only by oligomer repeat unit tandemly coupled to a column containing a polar embedded alkyl phase may be useful for the analysis of mixed alkyl alcohol polyethers. The first phase would have to rapidly separate the sample by oligomer value and the second phase would then perform separation by alkyl group, producing a combined separation by alkyl and oligomer groups. A phase capable of the first dimension was not found and the silica phase was not a possible candidate due to high retention of highly ethoxylated oligomers. This could be a future project based on the work in this thesis.

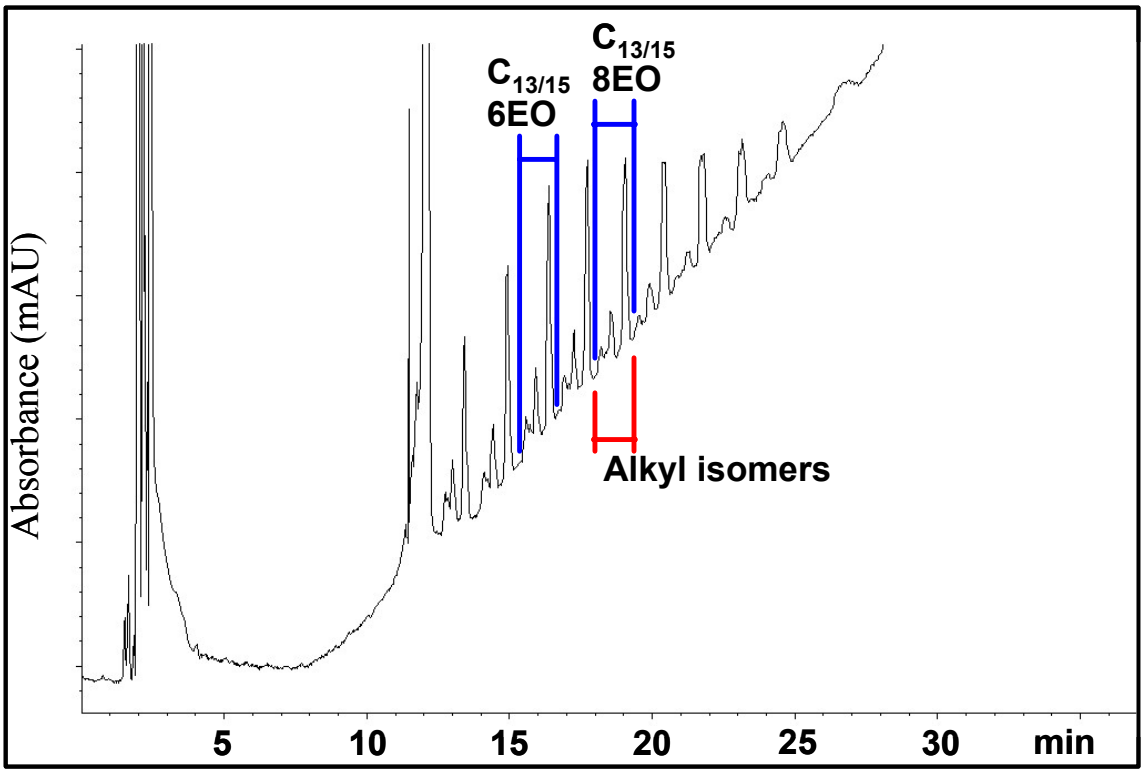

Figure 49. SFC-UV of $C_{13/15}EO_7$ on Berger Silica.
Berger Silica (4.6 x 150 mm, 5µm). Oven = 40°C, Outlet pressure = 120 bar, Flow rate = 2.4 mL/min, modifier = methanol, modifier gradient: 1% for 5 min, linear increase 1%/min to 25%, hold 2 minutes, linear decrease to 1% at 25%/min, 5 minute post run.

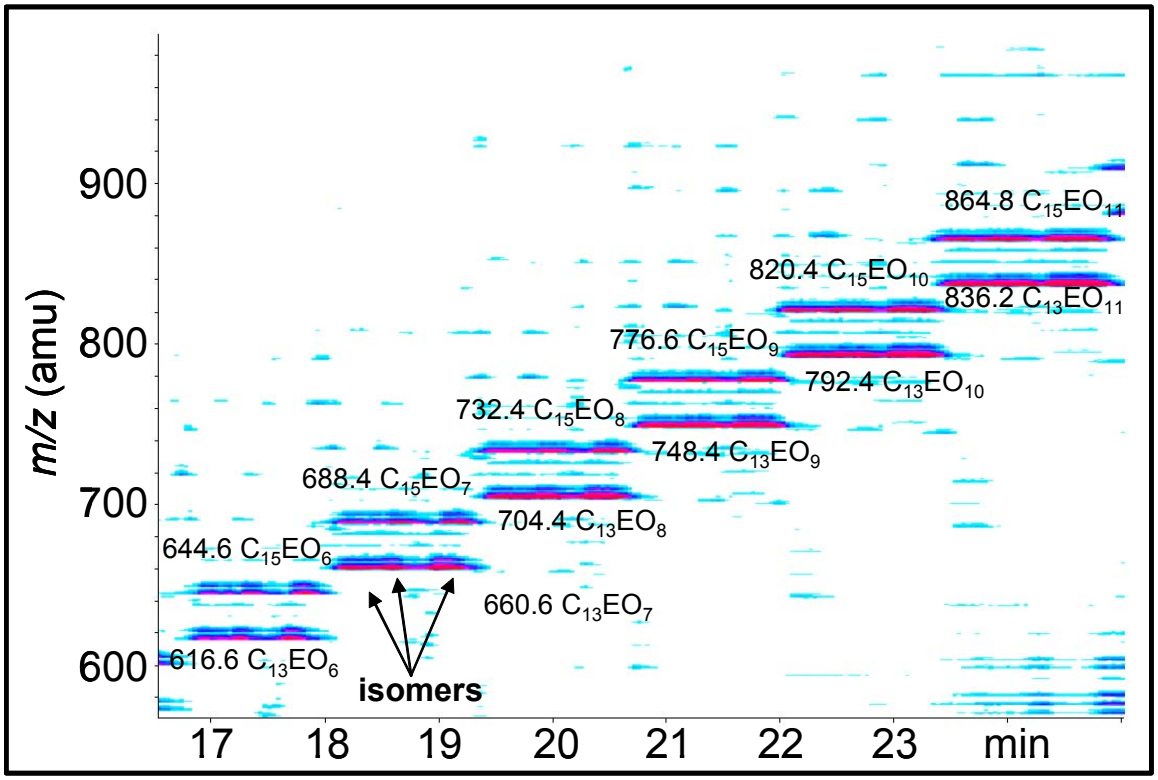

Figure 50. SFC-ESI-MS Contour plot of $C_{13/15}EO_7$ on Berger Silica.
Berger Silica (4.6 x 150 mm, 5µm). Oven = 40°C, Outlet pressure = 120 bar, Flow rate = 2.4 mL/min, modifier = methanol, modifier gradient: 1% for 5 min, linear increase 1%/min to 25%, hold 2 minutes, linear decrease to 1% at 25%/min, 5 minute post run.

Appendix C

Future Work

Methods developed for the analysis of alkylphenol ethoxylates and alcohol polyethers could be applied to development of semi-preparative or preparative collection and purification of monodisperse ethoxylated and propoxylated compounds. Pure standards of individual alkylphenol or alcohol polyethers could be used for quantitation of non-ionic surfactants in environmental samples. Further development of a SFC method for the separation of mixed alkyl chain alcohol polyethers should be investigated.

VITA

Brian Jeffrey Hoffman was born on November 24, 1978 in Philadelphia, PA. He is a member of the Fort Washington Fire Co. No. 1. He graduated from Upper Dublin High School in Fort Washington, PA May 1996. He was a member of the College Park Volunteer Fire Department while attending the University of Maryland at College Park. He received his Bachelor of Science degree in Chemistry from University of Maryland in May of 2000. He began his graduate education at Virginia Polytechnic Institute and State University in September of 2000 and received his Ph.D. in analytical chemistry in May of 2004.