CHAPTER 1

Introduction

Alcohol ethoxylates (AEOs), alcohol propoxylates (APOs), and alkylphenol ethoxylates (APEOs) are non-ionic surfactants. The term surfactant covers surface active compounds characterized by their ability to concentrate at surfaces and to form micelles in solution.[1] AEOs, APOs and APEOs contain two main molecular regions: (1) the oligoether region, a hydrophilic chain, and (2) the alkyl region which is hydrophobic (Figure 1). APEOs are similar in structure to alcohol polyethers, they contain a benzene ring situated between the two regions found in alcohol polyethers.

Since the mid-1940s APEOs have been used commercially for their surfactant ability. They have been used in a wide variety of applications including: industrial process aids, dispensing agents in paper and pulp production, emulsifying agents in latex paints and pesticide formulations, flotation agents, industrial cleaners (metal surfaces, textile processing, and food industry) and household cleaners.[1] Certain APEOs have been determined to be estrogenic in fish, birds, and mammals.[2] Due to the potentially toxic effects of some APEOs and their degradation products, alternative surfactants have been used in their place. Alcohol polyethers have been used in place of APEOs for residential and industrial uses. Household applications of alcohol polyethers include: general home laundry, hand dishwashing, degreasers, carpet cleaners, hard surface cleaners, and automatic dishwashing applications. Industrial applications include use in: manufacturing of pulp and paper, textile and wool scouring, agriculture emulsions and spray adjuvants, and enhanced oil recovery.[3]

Technical synthesis of APEOs start with phenol which is alkylated by

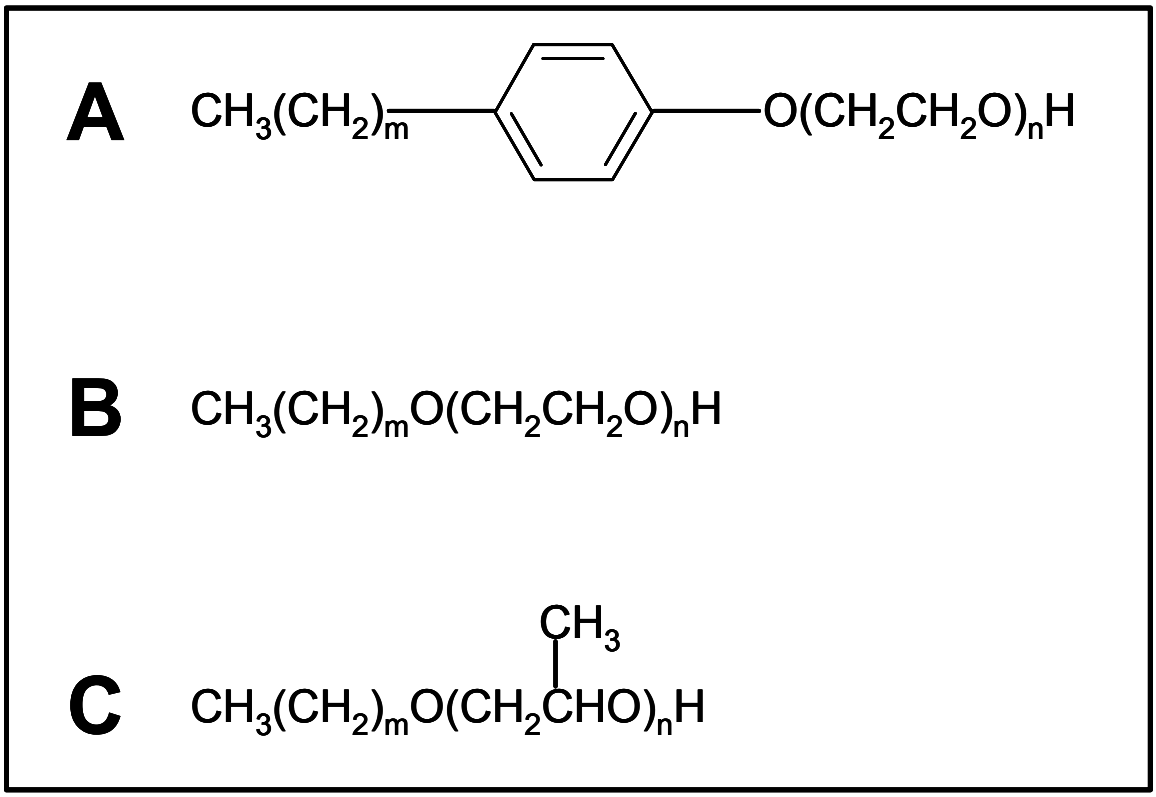

Figure 1. Structure of Selected Non-Ionic Surfactatants
A) alkylphenol ethoxylates B) alcohol ethoxylates C) alcohol propoxylates

trimethylpentene, producing octylphenol (OP) or with nonene isomers which forms nonylphenol (NP) in an acid catalyzed process. Ethoxylation is performed by using KOH/ethanol as a catalyst with a known ratio of ethylene oxide to the alkylphenol.[1] The reaction results in an oligomeric mixture of the alkylphenol containing an ethoxylate chain of varying lengths.

A similar process is used for the formation of alcohol polyethers. Fatty alcohols, used in the formation of alcohol polyethers, are obtained from natural and synthetic sources. Coconut oil and tallow are the most commercially attractive sources of fatty alcohols because alcohols produced from their triglycerides are in the effective detergent range (C_{12}-C_{18}).[3] Early methods used for formation of fatty alcohols involved the direct hydrogenation of triglycerides[3] or esters[4]. These methods required high temperature and pressure. Low pressure methods employ hydrolysis of triglycerides followed by the esterification of the produced fatty acids with the subsequent hydrogenation of esters to alcohols (Figure 2).[3,4] Fatty alcohols obtained from natural sources are almost exclusively linear and contain only even number carbon alkyl chains. Current synthetic methods for production of fatty alcohols include ethylene oligomerization[3,4] as a source of olefin intermediates followed by hydroformulation[3,6] and hydrogenation[3] . Synthetic alcohols can contain either an even or odd number of carbon atoms and can be either 100% linear or 20-25% branched, depending on the method of their formation.[3]

Production of alcohol polyethers is performed by reaction of ethylene oxide or propylene oxide with fatty alcohols. Acidic or basic catalysts can be used for polyether formation. A mixture of adducts result from competition between the epoxide and the

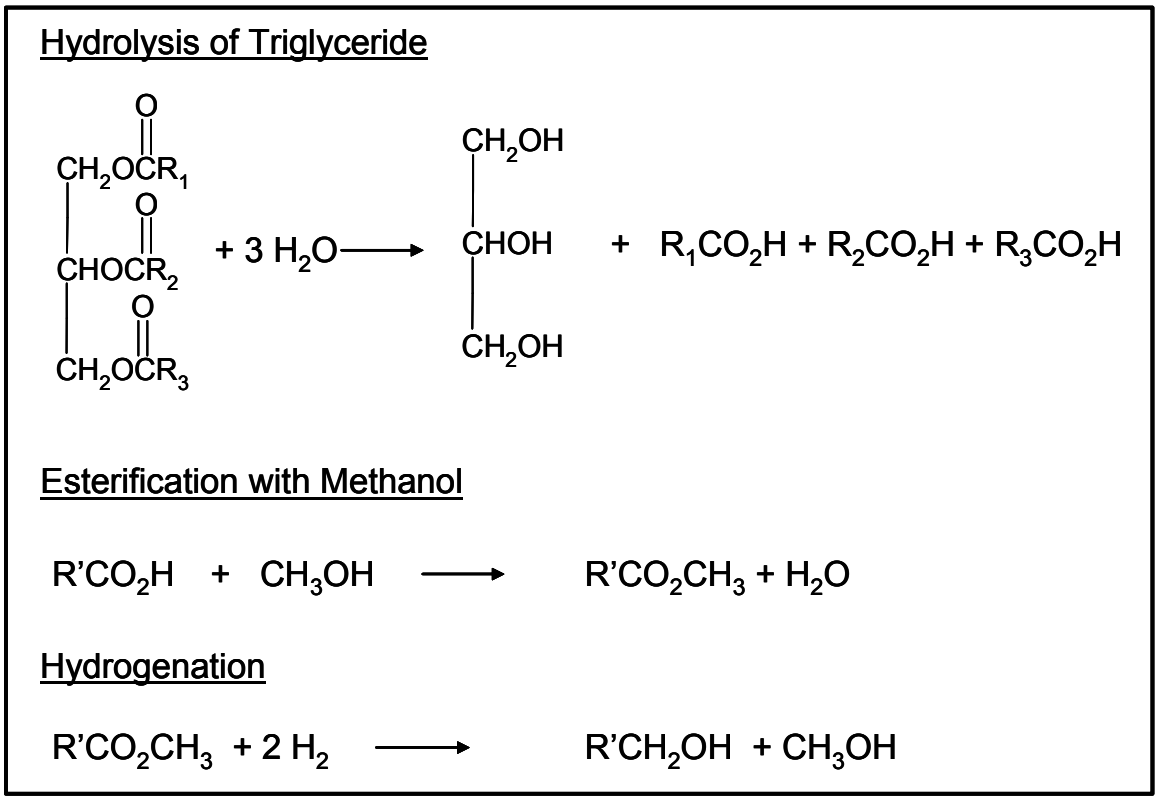

Figure 2. Formation of fatty alcohols from triglycerides.[3]

initiator (fatty alcohol) and formed adducts. The synthesis of these compounds can result in a complex oligomeric mixture of fatty ethers that contain a distribution of either EOs or POs of varying chain length. It is possible to also have various alkyl distributions if the starting materials contain fatty alcohols of different chain lengths. If water is present during the synthesis, it is also possible to produce polyethylene or polypropylene glycol distributions (PEGs or PPGs). Oligomer distribution is dependant on choice of initiator, number of moles of ethylene or propylene oxide reacted per mole of initiator, and choice of catalyst.[3] Basic catalysts produce broad distributions because during chain propagation proton exchange occurs statistically with no preference due to the similarity in acidities of polyoxyethylene alcohols and polyoxyethylene anions having similar structure.[3] Acidic catalysts are rarely commercially used because of production of undesirable side products, such as PEG, dioxane, and 2-methyl-1,3-dioxalane, which must be removed before use.[3] Acidic catalysts associate with ethylene and propylene oxide and reaction is independent of initiator concentration or structure. Oligomerization using acidic catalysts follow a S_N1 reaction mechanism. Basic catalysts associate with the initiator and follow a second order S_N2 nucleophilic substitution reaction mechanism. The equations for ethoxylation of alcohols are found in Figure 3. Similar equations apply for propoxylation of alcohols. Potassium hydroxide is most often used as a basic catalyst for oligomerization.[3]

Propylene oxide, used in the formation of alcohol polyoxypropylene ethers, contains an asymmetric tertiary carbon that may introduce different tacticity in polyoxypropylene oligomer chains. Possibilities of repeat unit propagation include (a) all

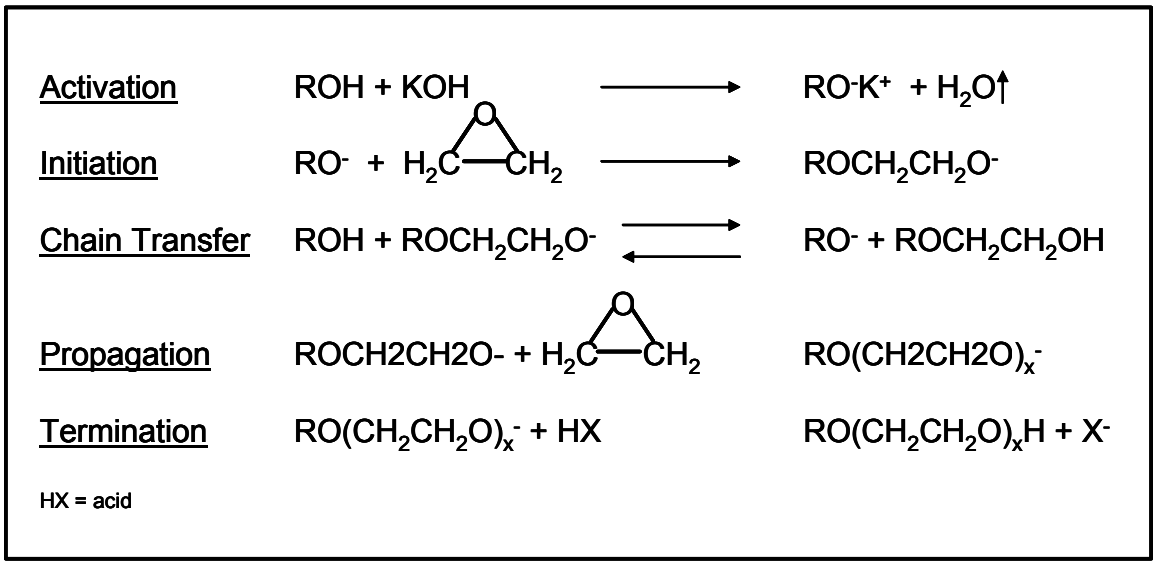

Figure 3. General alcohol ethoxylation equations.[3]

of the repeat units containing the same chirality (isotactic), (b) alternating chirality (syndeotactic), or (c) random chirality (atactic). Isotactic and syndeotactic polyoxypropylene chains form crystalline structures while atactic polyoxypropylene compounds are amorphous.[7] Carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrometry has been used to determine tacticity of polyoxypropylene compounds.[8,9] Along with the issue of repeat unit stereochemistry is the ability for repeat units to propagate in head-to-tail, head-to-head, and tail-to-tail configurations. "Head" refers to the methine carbon and "tail" refers to the methylene carbon of polyoxypropylene repeat units. Use of potassium hydroxide as a catalyst has been shown to produce atactic oligomers that are linked predominantly head-to-tail.[7] Oligomerization with potassium hydroxide predominantly forms propoxylates terminated by secondary hydroxyl groups and small fraction that are terminated by primary hydroxyl groups.[10]

APEOs, AEOs, and APOs have been made commercially available as oligomeric mixtures with specific polyether average values and specific alkane size. The molecular size and structure of surfactants determine their particular applications, therefore it is necessary for them to be well characterized. Several different types of chromatography have been studied previously in efforts to achieve more accurate methods of characterization. Characterization of average molar ethoxylation (EO) and propoxylation (PO) values has been performed by proton nuclear magnetic resonance (^{1}H-NMR)[11-13] and ^{13}C-NMR.[14] NMR is a useful tool for determining average molar oligomer value but it is not capable of revealing oligomer distribution. Average oligomer size and oligomer distribution of surfactants can be determined, however, through chromatographic separation.

Non-ionic surfactants have been analyzed by several chromatographic techniques. The research discussed in this thesis primarily investigated the use of supercritical fluid chromatography (SFC) and various spectrometry techniques for the analysis of oligomeric surfactants. The benefits and weaknesses of different chromatography methods will be discussed in later chapters. SFC is an alternative separation technique for samples that would traditionally be analyzed by gas chromatography (GC) or high performance liquid chromatography (HPLC). The mobile phase used in SFC is a fluid that is above its critical temperature and pressure. CO_2 is most often used as a mobile phase for SFC due to its readily accessible critical conditions, commercial availability, and environmental compatibility. The critical temperature (Tc) and pressure (Pc) of carbon dioxide (CO_2) are 31.0^{o}C and 72.9 bar, respectively.[15]

Supercritical fluids (SFs) have lower intermolecular interactions than normal liquids, this gives SFs advantages over liquids that are used as the mobile phase in HPLC. Supercritical CO_2 exhibits diffusivity similar to gases (gas 0.1-0.4 cm^2/sec ambient, supercritical fluid 0.007 cm^2/sec at Tc/Pc) and densities similar to liquids (liquid 0.6-1.6 g/mL ambient, supercritical fluid 0.2-0.5 g/mL at Tc/Pc).[15] These properties allow SFC to (a) use longer packed column arrangements, due to lower back pressure, (b) operate at higher flow rates, allowing shorter run times and greater sample throughput, and (c) provide higher efficiency separation compared to HPLC per unit time. Work published by Tong and co-workers[16] has illustrated the relationship between mobile phase velocity and column efficiency for GC, LC, and SFC (Figure 4). It is apparent that packed column SFC can operate at higher linear velocity than HPLC; while obtaining similar separation efficiency. CO_2 has a limited solvating range, non-polar through moderately

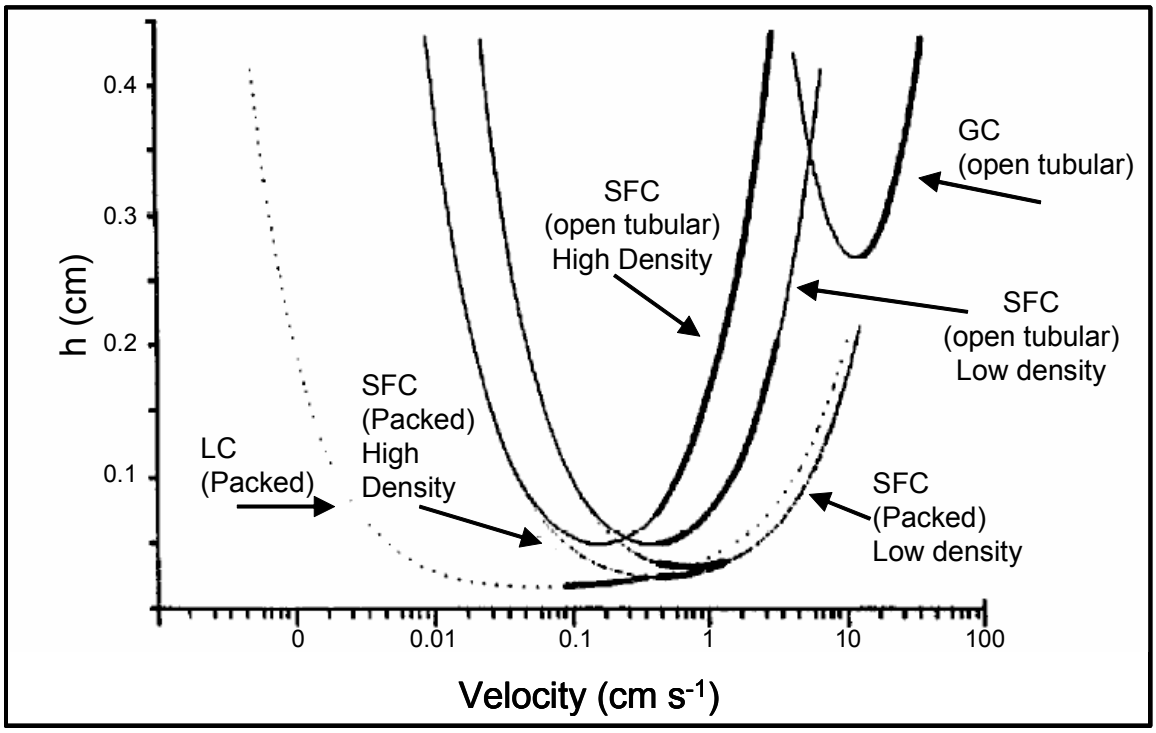

Figure 4. Efficiency as a function of mobile phase velocity[16]
Packed column conditions: 5μm particles. Values calculated from the Knox equation. Open tubular column conditions: 50 μm I.D. (SFC), 300 μm I.D.(GC). Values calculated from the Golay equation. Used with permission from authors of reference 16.

polar compounds can be solvated by pure CO_2. Adjusting temperature and pressure changes CO_2 density, which in turn can be used to control the solvating strength of pure CO_2. Polar organic solvents such as methanol and acetonitrile may be added to CO_2 to adjust the polarity of the mobile phase.[17] Addition of organic solvents to CO_2 also increases the density of the mobile phase as well as deactivates active sites on the surface of bonded silica stationary phases.[18] Detection methods used with GC and HPLC can be utilized with SFC as well. Detection methods include, but are not limited to, ultra-violet absorbance (UV), infrared absorbance (IR), NMR, mass spectrometry (MS), evaporative light scattering (ELSD), and flame ionization detection (FID).[19]

Classical SFC research has been performed with wall coated (0.1 μm-1 μm) open tubular 25-100 μm i.d. (WCOT) capillary columns.[20] Packed columns, with similar construction and dimensions to those used for HPLC applications, can be used for SFC separations as well. Most often bonded porous silica microparticles (approx. 5μm average particle size) are used as a packing material allowing a large variety of commercially available stationary phases to be utilized. Packed columns allow larger sample loading due to greater phase ratio compared to WCOT columns, which provides the ability to perform both analytical and semi-preparative separations. Chemically bonded stationary phases are suited for use with polar modifiers. Modifier addition is also easily controlled due to use of higher flow rates. The 1 cm path length used with 4.6 mm packed columns provides serious sensitivity advantages over the 50-150 μm path length used with capillary columns.[19] Better pressure control devices are available for packed column instruments compared to fixed restrictors used with capillary systems. SFC employing packed columns is also readily coupled with atmospheric pressure

ionization MS detectors associated with HPLC. When pressure is reduced on a supercritical fluid the fluid changes to the gas state, this aids in nebulization for MS ionization sources. A large problem with interfacing HPLC with MS detection is transition of the liquid mobile phase to a gas, use of supercritical fluid mobile phases alleviate this problem. Use of packed column SFC was investigated for analysis of polyether surfactants in the current research due to benefits associated with the method.

The purpose of this research was to develop alternative analytical methods for the analysis of complicated surfactant samples. Classical methods have been shown to have limitations either in chromatographic separation or detection of surfactants. Research focused on the development of SFC techniques that used UV absorbance detection. Currently UV absorbance detectors are economical devices that are rugged and have a wide applicability. Other spectrometric techniques including mass spectrometry and NMR were carried out as well to aid in method development. Requirements for analytical methods were (a) relatively short analysis time and (b) the ability to be performed under relatively mild physical conditions. Chapter 2 deals with the analysis of APEOs by SFC-UV, HPLC-UV and flow injection analysis mass spectrometry (FIA-MS). Tandem stacking of packed columns with different stationary phases was employed to yield separations capable of producing resolution between oligomers sufficient for the collection of individual oligomer fractions. In Chapter 3 the addition of an UV absorbing chromophore to alcohol polyethers is discussed. Initially pure CO_2 was used for elution of trimethylsilyl derivatized surfactant samples. The use of acetonitrile-modified CO_2 was evaluated for the elution of other derivatized surfactants at mild instrument operating conditions. An alkyl bonded phase, an amide-embedded alkyl

phase, and combination of the two phases were investigated for the separation of alcohol polyethers as derivatized esters and silylethers. A sulfonamide-embedded alkyl phase was evaluated in Chapter 4 for separation of alcohol polyethers. Acetonitrile and methanol modified-CO_2 were evaluated for their effect on oligomer separation. SFC-ESI-MS, SFC-UV, and ^{1}H-NMR were used to calculate average molar oligomer values of surfactant samples. Finally in Chapter 5 a silylether derivative containing two phenyl moieties was investigated for increasing oligomer detection sensitivity.

CHAPTER 2

A Study of Ethoxylated Alkylphenols by Packed Column Supercritical Fluid Chromatography

1.0 INTRODUCTION

Separation and identification of the components of an APE mixture can be useful for the determination of their most effective applications. Several different types of chromatography have been studied previously in efforts to achieve better separation conditions. Gas chromatography coupled both with flame ionization detection as well as mass spectrometry has been used in the analysis of APEOs.[21] Isomers of each oligomer tend to be separated into clusters by gas chromatography. Usually it is necessary to derivatize samples containing APEOs for analysis by GC because the compounds are not very volatile. Due to their lower volatility, GC poorly separates higher molecular weight oligomers.

HPLC has been used to separate APEOs of higher mass oligomers. Both reversed phase[22] and normal phase[23-25] chromatographic separations have been performed on solutions containing APEOs. Each oligomer is separated by ethoxylate unit and isomers of each oligomer tend to co-elute. Recently, Gundersen used a graphitic carbon column in her research to separate isomers of individual ethoxylated alkylphenols by HPLC.[26] Ferguson and co-workers used reversed phase HPLC-ESI-MS to analyze APEOs and their metabolites in the aquatic environment.[27] Normal phase HPLC-ESI-MS was used by Shang and co-workers to quantify nonylphenol ethoxylates in marine sediment.[28]

In addition to traditional forms of chromatography, supercritical fluid chromatography has been employed for APE separation. SFC has advantages over both HPLC and GC. SFC can operate at lower temperatures than GC allowing samples that are thermally labile to be analyzed.[29] Supercritical fluids have densities similar to liquids and diffusivities similar to gases. These qualities allow large molecular weight molecules which are not volatile to be separated by SFC similar to HPLC but with shorter retention times due to the physical properties of supercritical fluids. This reduces solvent waste and decreases total analysis time. Due to supercritical carbon dioxide's decreased viscosity, compared to normal liquids, several conventional packed columns can be connected in series without becoming limited by high back pressures, as experienced with HPLC. Berger and Wilson coupled ten 4.6 x 200 mm silica columns for the separation of Brazilian lemon oil.[30] This setup was capable of producing over 200,000 theoretical plates.

Capillary column SFC, using flame ionization detection[31,32], has been used to separate both nonylphenol and octylphenol ethoxylates (NPEO and OPEO). Since sample is destroyed by this method, it is not possible to directly determine analyte identity. Peak identity was surmised by comparing retention times of samples against other APE mixtures that contained a large fraction of a known single oligomer. A disadvantage associated with capillary columns is the inability to inject large sample volumes which precludes semi-preparative fraction collection.

In addition, OPEO mixtures have been separated on packed column SFC using reversed phase[33,34] and normal phase packing material.[35,36] Both Takeuchi and Saito, and Giorgettie and co-workers used C_{18} packed columns to separate octylphenol ethoxylate

samples by SFC. Takeuchi and Saito found that a micro-column (1.0 x 500 mm) had the best separation performance but a semimicro-column (1.7 x 250 mm) produced best results. A conventional column (6.0 x 250 mm) was used in their research as well and was most effective for preparative purposes. Packed column SFC allows larger amounts of sample to be injected into the system for semi-preparative collection of analyte fractions. Giorgettie and co-workers studied mixed mobile phases using the addition of modifier to make their mobile phase more polar. They used pressure programming and modifier addition to produce optimum separations. Highly efficient separations were produced under constant modifier concentration and pressure programming.

The object of this study was to compare the ability of normal phase packed columns to separate APEOs on a SFC system. Individual packed columns as well as stacked packed columns of different stationary phases were used in the SFC experiments. Additional goals of this study were to identify the components that gave rise to the chromatographic peaks in hopes of producing individual ethoxylated alkylphenol standards. Fractions that contain a single ethoxylate compound could later be used as standards for quantifying APEOs in a variety of applications. A comparison of the ability of SFC and HPLC to separate APEOs using normal phase packed columns was studied also.

2.0 Experimental

2.1 Packed column SFC

An A5000 analytical SFC system (Mettler-Toledo Autochem Berger Instruments, Inc., Newark, DE) was used in the SFC analysis. A Berger autosampler with a 10μL

injection loop was used for conventional sample analysis, and a 75μL injection loop was used for injection of semi-preparative samples. SFC-grade carbon dioxide (Air Products and Chemicals, Inc., Allentown, PA) was used with methanol (Burdick & Jackson, Muskegon, MI) as a modifier. The mobile phase flow rate was 2.0 mL/min. The oven temperature was set at 60 ^{o}C and the outlet pressure was kept at 120 bar. Absorbance was read at 225 nm by a diode array detector. The detection wavelength was determined by finding the maximum absorbance of an individual APEO sample by obtaining its UV-VIS spectrum. Supelcosil LC-Diol, Supelcosil LC-CN (Supelco, Bellefonte, PA), and Spherisorb NH_2 (Waters, Millford, MA) columns were used for chromatographic separation of the APEO mixtures. All columns were 4.6 x 250 mm, with a 5 μm average particle size. A diol bonded silica guard column was used.

2.2 Normal phase HPLC

For HPLC analysis, a Hewlett-Packard 1050 series HPLC system (Hewlett-Packard, Little Falls, DE) was used with a variable wavelength detector, reading 225 nm, and an in-line vacuum degasser. Injections were made manually with a Rheodyne injector (Rheodyne, Rohnert Park, CA) equipped with a 20 μL injection loop. Data were collected and chromatograms were processed by MassLynx software (Fisions Instruments, Altricham, England). A Supelcosil LC-Diol column (Supelco, Bellefonte, PA) (4.6 x 250 mm, 5 μm average particle size) was used for chromatographic separation of the APEO mixtures.

2.3 Flow injection analysis mass spectrometer

A Fisions Instruments VG Platform mass spectrometer (Fisions Instruments, Altrichan, England) was used for mass analysis of collected sample fractions. All samples were analyzed under positive electrospray ionization. A syringe pump (Harvard Apparatus, South Natick, MA) supplied an 80:20 methanol:water mobile phase to the probe. Samples were injected by a Rheodyne injector (Rheodyne, Rohnert Park, CA) equipped with a 20 μL injection loop. Nitrogen was used as both the drying gas and sheath gas. Data were collected and analyzed by MassLynx software.

2.4 Alkylphenol Ethoxylate Samples

POE (4) nonylphenol (Chem Service, West Chester, PA) and Triton N-101 (Sigma-Aldrich, Milwaukee, WI) were nonylphenol ethoxylate mixtures. POE (5) tert-octylphenol (Chem Service, West Chester, PA) was an octylphenol ethoxylate mixture. All samples that were analyzed by SFC were dissolved in methanol, while samples analyzed by normal phase HPLC were dissolved in hexane. The Triton N-101 sample used for HPLC was dissolved in 9:1 hexane:acetone to increase solubility. HPLC samples were prepared at approximately 1.0 mg/mL and SFC samples were prepared at approximately 2.0 mg/mL concentrations.

2.5 Semi-preparative SFC

A tee was placed in-line between the column and DAD detector of the SFC system splitting effluent approximately 75% to collection and 25% to the detector. Eluent was diverted using a portion of fused silica capillary tubing. Fractions were collected in pre-weighed 16 mL collection vials. Figure 5 displays a schematic diagram

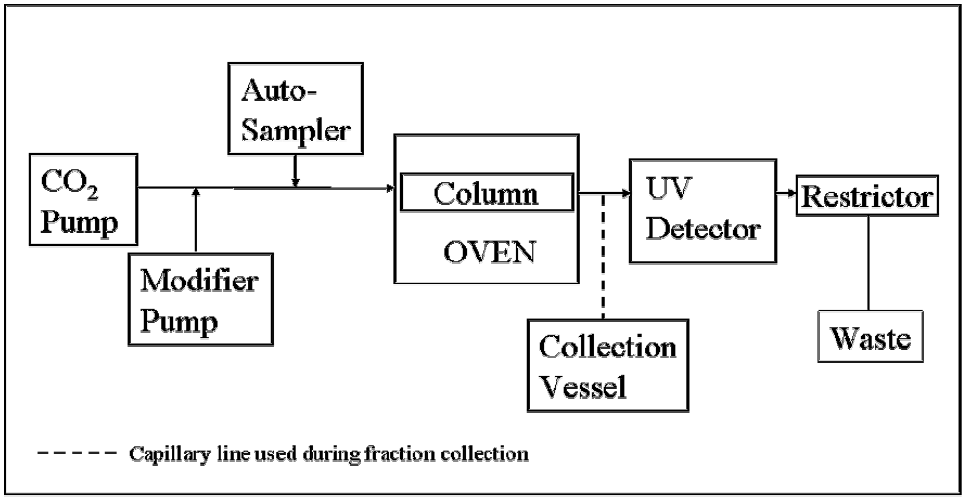

Figure 5. Schematic diagram of SFC system with collection

of the SFC system. Absorbance was monitored and fractions were collected manually between minimum absorbance values. POE (4) nonylphenol and POE (5) tert-octylphenol were separated in this fashion. Fractions were evaporated by N_2 blow-down on a hot plate. The remaining residue was weighed. The fractions were then diluted to 10.0 mL with methanol. Fractions were analyzed by SFC-UV followed by FIA-ESI- MS for purity.

2.6 FIA-ESI-MS method

SFC collected fractions were evaporated by nitrogen blow-down and weighed. Collected fractions were then dissolved in methanol. Optimal mass spectrometer settings were found by injecting each fraction and tuning the instrument. Fractions were then re-injected and mass spectral data were recorded and analyzed. The source temperature was set at 100^{O}C. ESI nebulizing gas flow was set 20 L/h and drying gas flow was 300 L/h. Samples were recorded in full scan mode from m/z 200-700. Cone voltage ranged from 52 to 75 V and the high voltage lens and ESI capillary voltage were kept at 0.88 kV and 3.46 kV respectively.

2.7 HPLC method

Hexane and iso-propanol were used as the mobile phase. A linear gradient was used starting with 100% hexane changing to 70:30 hexane:iso-propanol over 30 minutes. From t= 30 min. to t= 35 min. the mobile phase was returned to 100% hexane and was held for 5 minutes to equilibrate. POE (4) nonylphenol and POE (5) tert-octylphenol were separated in this fashion.

3.0 RESULTS AND DISCUSSION

APEOs are complex mixtures that provide moderate challenges for chromatographic techniques. Research studied how total column length, stationary phase, and column stacking order of different stationary phases affected the SFC separation of ethoxylate units in APEO mixtures. The goal was to find a setup that produced the best separation. To accomplish this all system parameters were kept constant throughout the study other than column setup and modifier gradient. All of the columns used were uniform in size (4.6 x 250 mm, 5 μm average particle size) to verify the effect of column length and packing material. POE (4) nonylphenol was used in all of the diol column studies because of its short elution time.

POE (4) nonylphenol was separated on a combination of one, two, and three packed diol columns connected in series to study the effect of column length (Figure 6). A single diol column poorly separated the sample. Baseline separation was not achieved with a single column. SFC separation on two diol columns increased separation but early eluting peaks were not baseline separated. Using two diol columns, SFC separation was comparable to normal phase HPLC using one diol column. For comparison, POE (4) nonylphenol (Figure 7), Triton N-101 (Figure 8) and POE (5) tert-octylphenol (Figure 9) were separated by SFC on two diol columns and by HPLC on one diol column. The retention time of chromatographic peaks for SFC separation using two diol columns was considerably lower than NP-HPLC separation using one diol column (Table I, NPEO samples. Table II, OPEO sample). Addition of a third diol column to the SFC system generated a better separation but later eluting peaks began to broaden.

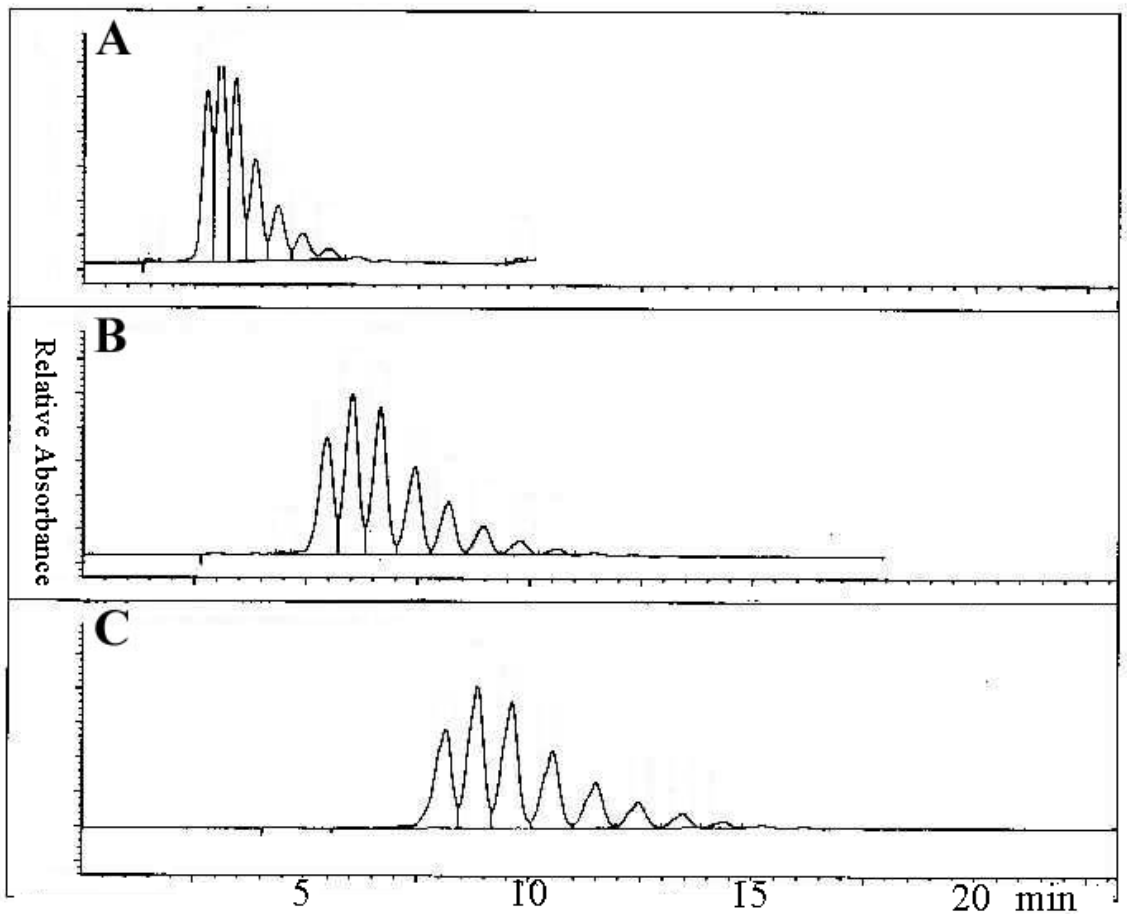

Figure 6. Packed column supercritical fluid chromatograms using stacked diol columns. A, one Supelcosil LC- Diol column. B, two Supelcosil LC-Diol columns. C, three Supelcosil LC-Diol columns. The sample used in each chromatogram was POE (4) nonylphenol (2.0 mg/mL). The outlet pressure was maintained 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.

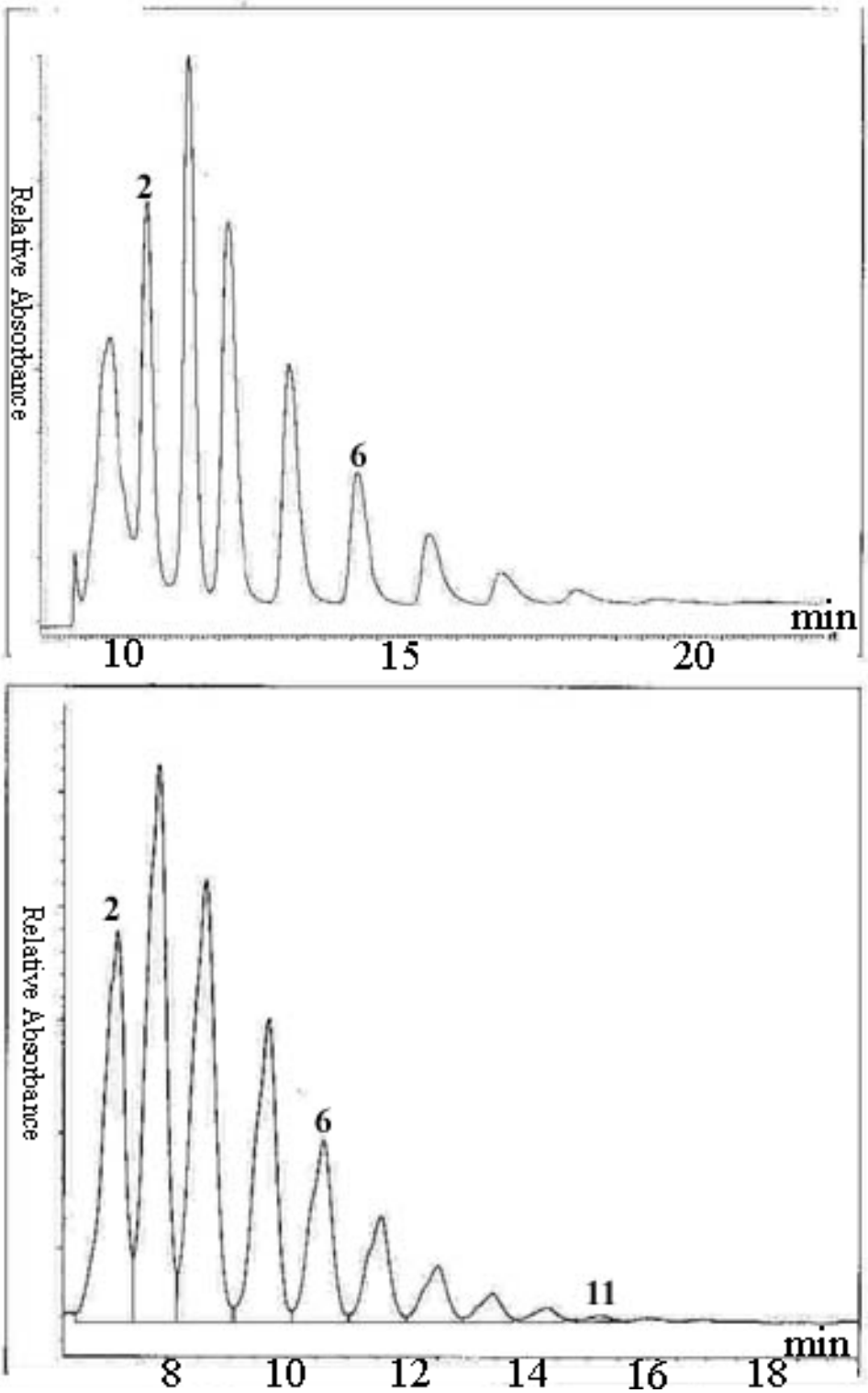

Figure 7. Chromatograms of POE-(4)-NP using normal phase HPLC-UV (top) with one Supelcosil LC-Diol column and SFC-UV (bottom) with two Supelcosil LC-Diol columns. See Figure 6 for SFC conditions. The peak annotations represent the number of ethoxylate units.

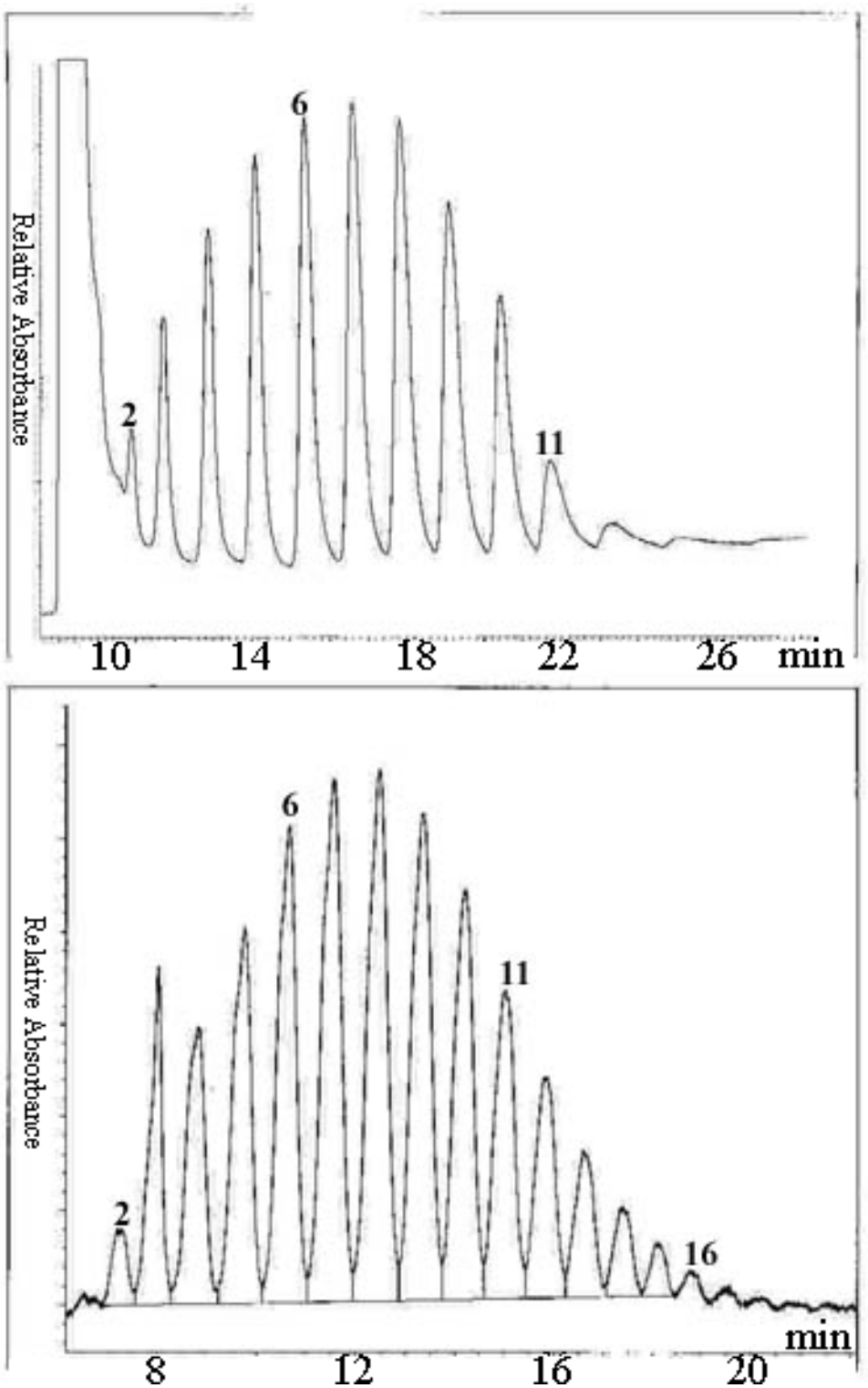

Figure 8. HPLC and SFC chromatograms of Triton N-101.
Normal phase HPLC-UV (top) with one Supelcosil LC-Diol column and SFC-UV (bottom) with two Supelcosil LC-Diol columns. See Figure 6 for SFC conditions. The peak annotations represent the number of ethoxylate units.