A Study of Polyethoxylated Alkylphenols by Packed Column Supercritical Fluid Chromatography

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Abstract

Alkylphenol polyethoxylates (APEs) are a widely used group of nonionic surfactants in commercial production. Characterization of the composition of APE mixtures can be exploited for the determination of their most effective uses. In this study sample mixtures contain nonylphenol polyethoxylates and octylphenol polyethoxylates. The separation of individual alkylphenols by ethoxylate units is performed by supercritical fluid chromatography (SFC)-UV as well as normal-phase high-performance liquid chromatographic (HPLC)-UV employing packed columns. The stationary phase and column length are varied in the SFC setup to produce the most favorable separation conditions. Additionally, combinations of packed columns of different stationary phases are tested. The combination of a diol and a cyano column is found to produce optimal results. An advantage of using packed columns instead of capillary columns is the ability to inject large amounts of sample and thus collect eluted fractions. In this regard, fractions from SFC runs are collected and analyzed by flow injection analysis–electrospray ionization–mass spectroscopy in order to positively identify the composition of the fractions. In comparing the separation of APE mixtures by SFC and HPLC, it is found that SFC provides shorter retention times with similar resolution. In addition, less solvent waste is produced using SFC.

Introduction

Alkylphenol polyethoxylates (APEs) are referred to as nonionic surfactants. Since the mid 1940s, APEs have been used commercially for their surfactant ability. The term surfactant includes surface-active compounds characterized by their ability to concentrate at surfaces and form micelles in solution (1). 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). These compounds are commercially available as oligomeric mixtures with varying ethoxylate chain lengths as well as varying alkyl sizes. Certain APEs have been determined to be estrogenic in fish, birds, and mammals (2).

APEs contain two main molecular regions: the polyethoxylate (POE) chain (EO) is polar and thus hydrophilic and the alkylphenol is the hydrophobic area. The hydrophilic nature of the EO is attributed to the hydration of the ether-linked oxygen atoms (3). A technical synthesis of APEs start with phenol, which is alkylated by trimethylpentane and thus produces octylphenol (OP), or by 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 EO chain of varying lengths.

The 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 (GC) coupled with flame ionization detection as well as mass spectrometry (MS) has been used in the analysis of APEs (4). Isomers of each oligomer tend to be separated into clusters by GC. Usually, it is necessary to derivatize samples containing APEs for analysis by GC, because the compounds are not very volatile. GC poorly separates higher molecular-weight oligomers because of their lower volatility.

High-performance liquid chromatography (HPLC) has been used to separate APEs of higher mass oligomers. Both reversed-phase (3) and normal-phase (5–7) chromatographic separations have been performed on solutions containing APEs. Each oligomer is separated by an ethoxylate unit, and isomers of each oligomer tend to coelute. Recently, Gundersen used a graphitic carbon column in research to separate isomers of individual ethoxylated alkylphenols by HPLC (8). Ferguson et al. used reversed-phase HPLC–electrospray ionization (ESI)–MS to analyze APEs and their metabolites in aquatic environments (9). Normal-phase HPLC–ESI–MS was used by Shang et al. to quantitate NPEOs in marine sediment (10).

In addition to traditional forms of chromatography, supercritical fluid chromatography (SFC) 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. Supercritical fluids have densities similar to liquids and diffusivities similar to gases. These qualities allow large molecular-weight molecules that are not volatile to be

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separated by SFC similar to HPLC but with shorter retention times because of the physical properties of supercritical fluids. This reduces solvent waste and decreases the total analysis time. Capillary-column SFC using flame ionization detection (11,12) has been used to separate both NPEO and OPEO. Because a sample is generally destroyed by this method, it is not possible to directly determine analyte identity. Peak identity can be surmised by comparing retention times of samples with other APE mixtures that contain a large fraction of a known single oligomer. A disadvantage associated with capillary columns is the inability to inject large sample volumes, which precludes semipreparative fraction collection.

In addition, OPEO mixtures have been separated on packed-column SFC using reversed-phase (13,14) and normal-phase (15,16) packing material. Both Takeuchi and Saito and Giorgettie et al. used C18 packed columns to separate OPEO samples by SFC. Takeuchi and Saito found that a microcolumn (1.0 × 500 mm) had the best separation performance, but a semimicrocolumn (1.7 × 250 mm) produced the best results. A conventional column (6.0 × 250 mm) was used in their research for preparative purposes. Packed-column SFC allows larger amounts of sample to be injected into the system for the semipreparative collection of analyte fractions. Giorgettie et al. studied mixed mobile phases using the addition of a modifier in order to make their mobile phase more polar. They used pressure programming and a modifier additon 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 APEs on an 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 quantitating APEs in a variety of applications. A comparison of the ability of SFC and HPLC to separate APEs using normal-phase packed columns was also studied.

Experimental

Packed-column SFC

A Berger (Newark, DE) SFC system was used in the SFC analysis. A Berger autosampler with a 10-µL injection loop was used for conventional sample analysis, and a 75-µL injection loop was used for the injection of semipreparative 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°C, and the outlet pressure was kept at 120 atm. Absorbance was read at 225 nm by a diode-array detector. The detection wavelength was determined by finding the maximum absorbance of an individual APE 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 the chromatographic separation of the APE mixtures. All columns measured 4.6 × 250 mm with a 5-µm particle size. A diol bonded silica guard column was used.

Normal-phase HPLC

For HPLC analysis, a Hewlett-Packard (Little Falls, DE) 1050 Series HPLC system was used with a variable wavelength detector (reading 225 nm) and an inline vacuum degasser. Injections were made manually with a Rheodyne (Rohnert Park, CA) injector equipped with a 20-µL injection loop. Data were collected and chromatograms were processed by MassLynx software (Fisions Instruments, Altricham, U.K.). A Supelcosil LC-Diol column (4.6 × 250 mm, 5 µm) was used for the chromatographic separation of the APE mixtures.

Flow injection analysis–MS

A Fisions Instruments VG Platform MS was used for the mass analysis of collected sample fractions. All samples were analyzed under positive ESI. 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 equipped with a 20-µL injection loop. Nitrogen was used as both the drying and sheath gas. Data were collected and analyzed by MassLynx software.

Alkylphenol samples

POE-(4)-NP (ChemService, West Chester, PA) and Triton N-101 (Sigma-Aldrich, Milwaukee, WI) were used as NPEO mixtures. POE-(5)-*tert*-OP (ChemService) was used as an OPEO mixture. All of the samples that were analyzed by SFC were dissolved in methanol, and samples analyzed by normal-phase HPLC were dissolved in hexane. The Triton N-101 sample that was used for HPLC was dissolved in 9:1 hexane–acetone in order 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.

Semipreparative SFC

A tee was placed inline between the column and diode-array detector of the SFC system, splitting effluent approximately 75% to the collection and 25% to the detector. Eluent was diverted using a portion of fused-silica capillary tubing. Fractions were collected in preweighed 16-mL collection vials. Absorbance was monitored, and fractions were collected manually between minimum absorbance values. POE-(4)-NP and POE-(5)-*tert*-OP were separated in this fashion. Fractions were evaporated by nitrogen 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 flow injection analysis (FIA)–ESI–MS for purity.

FIA–ESI–MS method

SFC-collected fractions were evaporated by nitrogen blow-down and weighed. Collected fractions were then dissolved in methanol. Optimal MS settings were found by injecting each fraction and tuning the instrument. Fractions were then reinjected, and mass-spectral data were recorded and analyzed. The source temperature was set at 100°C. ESI nebulizing gas flow was set at

20 L/h, and the drying gas flow was 300 L/h. Samples were recorded in full-scan mode from *m/z* 200 to 700. The cone voltage ranged from 52 to 75 V, and the high voltage lens and ESI capillary voltage were kept at 0.88 and 3.46 kV, respectively.

HPLC method

Hexane and isopropanol were used as the mobile phase. A linear gradient was used starting with 100% hexane and then changing to 70:30 hexane–isopropanol over 30 min. From t = 30 to 35 min, the mobile phase was returned to 100% hexane and held for 5 min in order to equilibrate. POE-(4)-NP and POE-(5)-*tert*-OP were separated in this fashion.

Results and Discussion

APEs are complex mixtures that provide moderate challenges for chromatographic techniques. Our research studied how the total column length, stationary phase, and column stacking order of different stationary phases affect the SFC separation of ethoxylate units in APE mixtures. Our goal was to find a setup that produced the best separation. In order to accomplish this we kept all system parameters constant throughout the study other than column setup and modifier gradient. All of the columns used were uniform in size (4.6 × 250 mm, 5 µm) in order to allow us to verify the effect of column length and packing material. POE-(4)-NP was used in all of the diol column studies because of its short elution time.

POE-(4)-NP was separated on a combination of one-, two-, and three-packed diol columns connected in series to study the effect of column length (Figure 1). 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 with normal-phase HPLC using one diol column. For comparison, POE-(4)-NP, POE-(5)-*tert*-OP, and Triton N-101 were separated by SFC on two diol columns and HPLC on one diol column (Figures 2–4). The retention time of the chromatographic peaks for SFC separation using two diol columns was considerably lower than normal-phase HPLC separation using one diol column (Tables I and II shows data for the NPEO sample and Table III shows data for the OPEO sample). The addition of a third diol column to the SFC system generated a better separation, but later-eluting peaks began to broaden.

The effect of the stationary phase on separation was sequentially tested using a single diol, amino, and cyano column (Figure 5). The retention of oligomers with longer ethoxylated units 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 com-

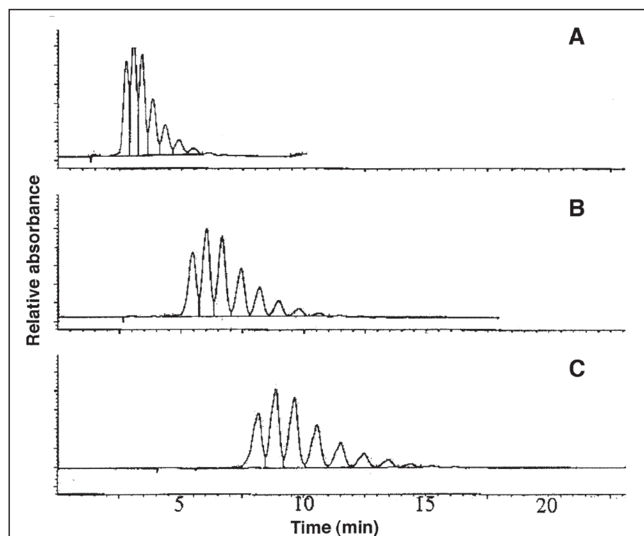

Figure 1. Packed-column supercritical fluid chromatograms using stacked diol columns: (A) one Supelcosil LC-Diol column, (B) two Supelcosil LC-Diol columns, and (C) three Supelcosil LC-Diol columns. The sample used in each chromatogram was POE-(4)-NP (2.0 mg/mL). A linear modifier gradient was used by the following program: 10.0% methanol was increased to 26.0% at a rate of 0.6%/min with a 2.0-min hold and then returned to 10.0% in 4.0 min followed by a 2.0-min hold.

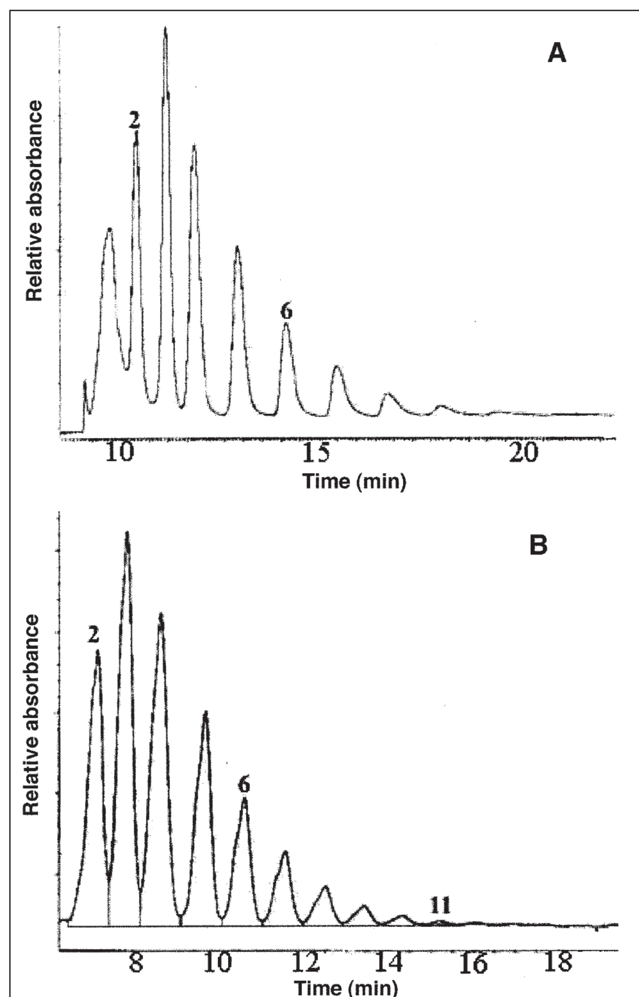

Figure 2. Chromatograms of POE-(4)-NP using (A) normal-phase HPLC-UV with one Supelcosil LC-Diol column and (B) SFC-UV with two Supelcosil LC-Diol columns. The peak annotations represent the number of ethoxylate units.

pounds. Because of this, we can conclude that APEs with a longer ethoxylate chain are more polar than those 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 is the least polar.

Columns with different stationary phases were coupled in series to test how the arrangement would affect the retention of an APE 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 6). A steeper gradient was needed than previously used in order to elute all of the compounds because of the presence of the cyano column (as previously mentioned). The modifier gradient that was used is described in Figure 6.

One of our goals in this study was to achieve separation that would allow us to easily collect individual oligomers for use as standards. The combined diol–cyano setup rendered shorter retention times than the combined diol–cyano–amino setup; therefore, this arrangement was used for preparative fraction collection. In the chromatograms of stacked columns using different stationary phases, peak splitting was observed for later-eluting peaks. POE-(4)-NP and POE-(5)-*tert*-OP were separated, and five fractions of each sample were collected. A large volume (75 µL) of concentrated sample was injected six to eight times in the collection process. Isolated fractions were reanalyzed both by SFC for purity (Figures 7 and 8) and FIA–ESI–MS for identification. The concentrations used for the semipreparative work caused the chromatographic peaks to significantly broaden and in some cases combine. Because of this phenomenon we were not able to collect individual fractions of the two initial oligomers of POE-(4)-NP and fractions of the three initial oligomers of POE-(5)-*tert*-OP as evidenced by the SFC-UV of the early fractions.

FIA–MS was used to identify the components in each fraction. ESI–MS 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. In order to create an optimum response, the fractions were first injected and the cone voltage varied in order to produce the greatest response for each individual analyte. After MS tuning conditions were perfected, the fractions were reinjected into the instrument. A spectrum was created between

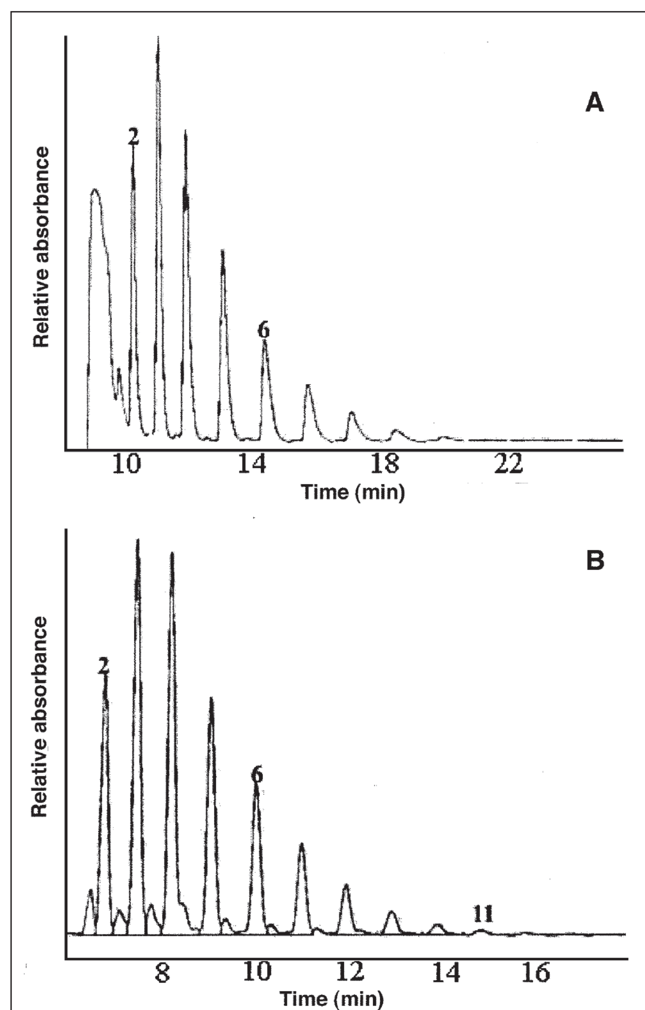

Figure 3. Chromatograms of POE-(5)-*tert*-OP using (A) normal-phase HPLC-UV with one Supelcosil LC-Diol column and (B) SFC-UV with two Supelcosil LC-Diol columns. The peak annotations represent the number of ethoxylate units.

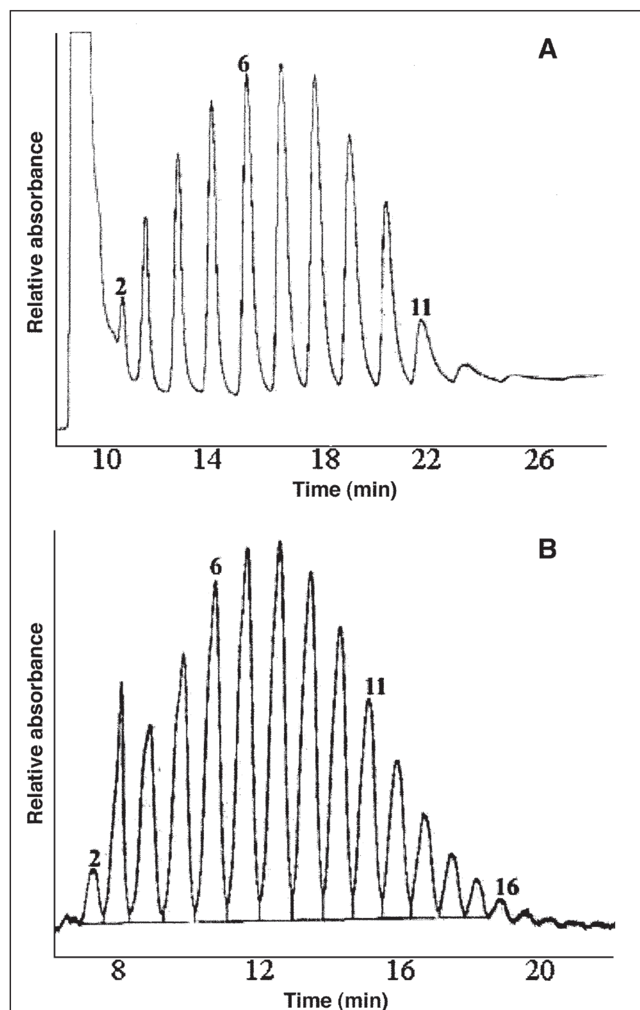

Figure 4. Chromatograms of Triton N-101 using (A) normal-phase HPLC-UV with one Supelcosil LC-Diol column and (B) SFC-UV with two Supelcosil LC-Diol columns. The peak annotations represent the number of ethoxylate units.

m/z 200 and 700 by averaging scans of the injected sample. Figures 9 and 10 show the average mass spectrum of each fraction. The spectra confirm that each chromatographic peak varied by one ethoxylated unit (a separation of *m/z* 44 represents an ethoxylate unit). It was possible to identify NP3EO through NP7EO in basically pure collected fractions of POE-(4)-NP and OP5EO through OP8EO in fractions collected from POE-(5)-*tert*-OP.

Major ion peaks consisted of Na^+ adduct ions, and minor peaks were produced by K^+ adduct ions under positive electrospray conditions. Trace levels of sodium and potassium must be present in the mobile phase that was used for FIA–ESI–MS because electrolyte was not added to the solutions. According to Okada's research (17), APEs have an affinity for alkali metals and have a flexible structure that allows them to form complexes with alkali metals. This explains the ion pairing seen in the mass spectra. Crescenzi et al. performed an experiment to see if the detector response would decrease because of the 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 the detector response increased exponentially from 1 to 6 EO units and then leveled off at 8 EO units (the scope of the study) (18). 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 the EO unit number increases (17).

Table I. Chromatographic Peak Retention Times of POE-(4)-NP (NPEO) Separated by SFC Using Two Supelcosil LC-Diol Columns and HPLC Using One Supelcosil LC-Diol Column

EO unit	SFC RT*	HPLC RT
2	7.18	9.14
3	7.86	9.93
4	8.64	10.66
5	9.68	11.82
6	10.61	13.08
7	11.56	14.46
8	12.49	15.83
9	13.43	17.28
10	14.37	18.74
11	15.16	

* RT, retention time.

Table II. Chromatographic Peak Retention Times of Triton N-101 (NPEOs) Separated by SFC Using Two Supelcosil LC-Diol Columns and HPLC Using One Supelcosil LC-Diol Column

EO unit	SFC RT*	HPLC RT
2	7.29	9.88
3	8.02	10.68
4	8.83	11.84
5	9.75	13.08
6	10.66	14.33
7	11.57	15.55
8	12.48	16.80
9	13.36	18.06
10	14.20	19.39
11	15.03	20.68
12	15.84	22.29
13	16.61	24.11
14	17.37	
15	18.10	
16	18.81	
17	19.58	
18	20.10	

* RT, retention time.

Table III. Chromatographic Peak Retention Times of POE-(5)-*tert*-OP (OPEOs) Separated by SFC Using Two Supelcosil LC-Diol Columns and HPLC Using One Supelcosil LC-Diol Column

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	

* RT, retention time.

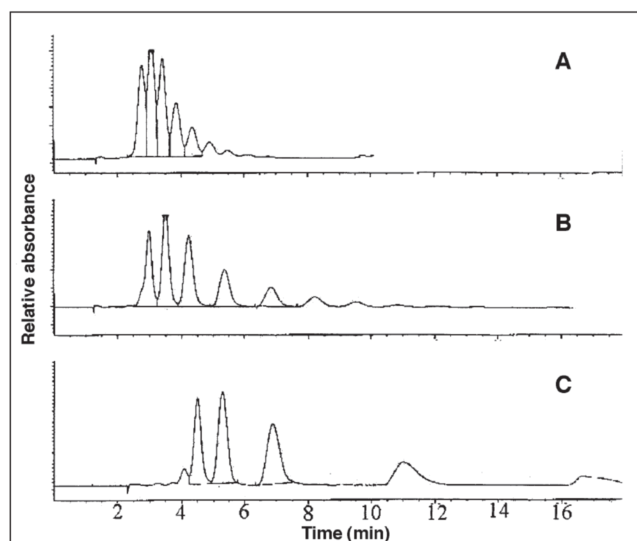

Figure 5. Packed-column supercritical fluid chromatograms using single columns of different polar packing material: (A) Supelcosil LC-Diol column, (B) Spherisorb NH_2 column, and (C) Supelcosil LC-PCN column. The sample used in each chromatogram was POE-(4)-NP (2.0 mg/mL). A linear modifier gradient was used by the following program: 10.0% methanol was increased to 26.0% at a rate of 0.6%/min with a 2.0-min hold and then returned to 10.0% in 4.0 min followed by a 2.0-min hold.

It was important to perform chromatographic separations with absorbance detection on the fractions as well as MS analysis, thus allowing us to positively identify sample components because MS could not detect all of the compounds present. The first fraction of both POE-(4)-NP and POE-(5)-*tert*-OP contained more than one compound (as seen in their SFC-UV chromatograms). The sodium ion affinity of the smaller ethoxylate chain compounds is lower than the larger chain oligomers, and because of this they were not detectable in the mass spectra.

APEs can be categorized by their average ethoxylate unit value. According to Wang and Fingas (3), all of the oligomers have almost identical molar absorptivity, which allows integrated chromatographic peak areas to be used directly to determine the mole fraction of each oligomer. POE-(4)-NP contained NP predominantly with short ethoxylate chains. NP2EO through NP11EO were observed in its SFC-UV separation. An average ethoxylate

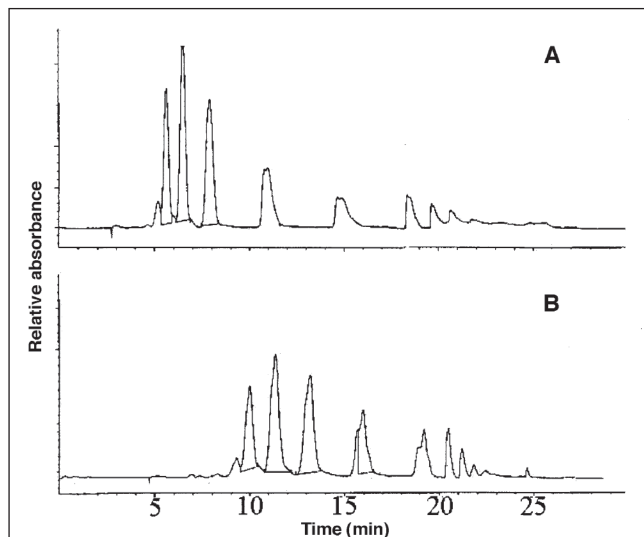

Figure 6. Packed-column supercritical fluid chromatograms using stacked columns of different polar stationary phases: (A) one Supelcosil LC-Diol column and one Supelcosil LC-PCN column and (B) one Supelcosil LC-Diol column, one Supelcosil LC-PCN column, and one Spherisorb NH_2 column. The sample used in each chromatogram was POE-(4)-NP (2.0 mg/mL). Multiple linear modifier gradients were used by the following program: 10.0% methanol was increased to 13.2% by 0.5%/min and then continued to 14.4% at 0.7%/min, 16.6% at 0.8%/min, 20.0% at 1.0%/min, 40.0% at 8.0%/min (held for 5.0 min), and then returned to 10.0% at 15.0%/min.

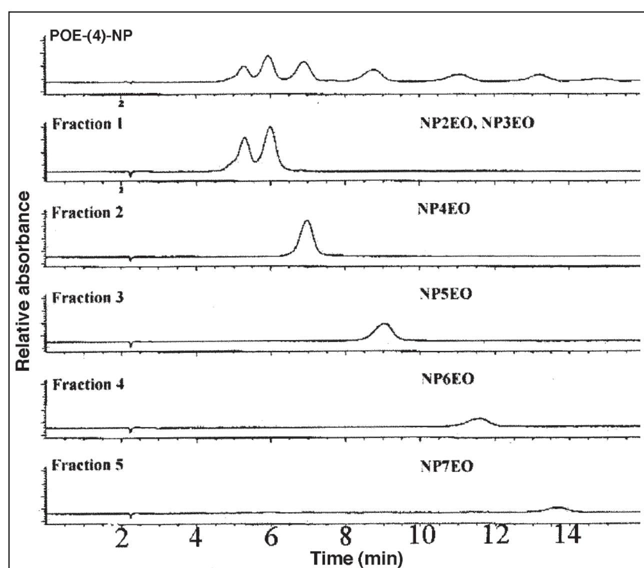

Figure 7. Supercritical fluid chromatograms of collected POE-(4)-NP fractions. Separation was conducted on one Supercosil LC-Diol column and one Supelcosil LC-PCN column in series (the system settings were the same as Figure 3).

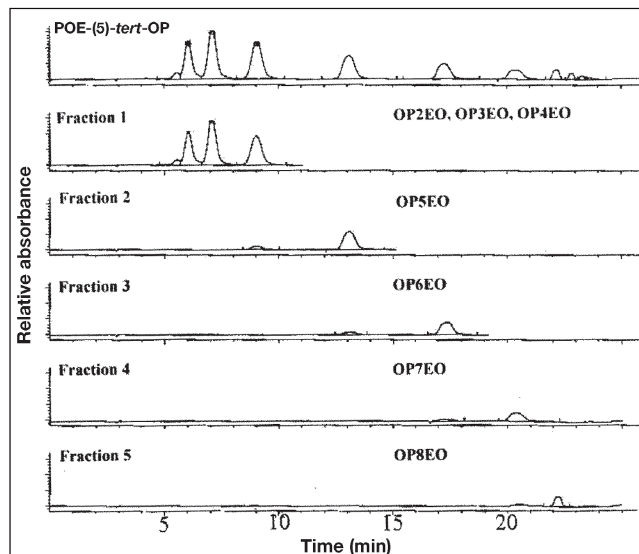

Figure 8. Supercritical fluid chromatograms of collected POE-(5)-*tert*-OP fractions. Separation was conducted on one Supelcosil LC-Diol column and one Supelcosil LC-PCN column in series (the system settings were the same as Figure 3).

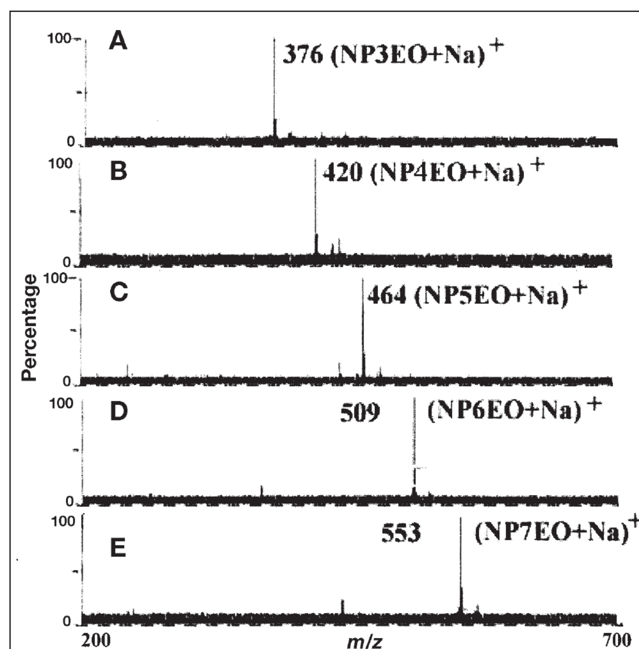

Figure 9. Positive-ion FIA–ESI–MS of POE-(4)-NP fractions operated in full-scan mode. Ions were in the form of $(M+Na)^+$ and each were separated by *m/z* 44 (the mass of one ethoxyl unit): (A) fraction 1, cone voltage of 59 V; (B) fraction 2, cone voltage of 53 V; (C) fraction 3, cone voltage of 62 V; (D) fraction 4, cone voltage of 65 V; and (E) fraction 5, cone voltage of 67 V. Each spectrum was averaged over the sample injection peak.

unit value of 4.20 was calculated from peak areas. POE-(5)-*tert*-OP had a similar distribution as POE-(4)-NP. 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 CO430 (trade name for POE-(4)-NP), Triton X-45 (trade name for POE-(5)-*tert*-OP), and Triton N-101 produced average EO values of 4.14, 4.50, and 9.52, respectively (11,12). We used the chromatographic data from the SFC-UV separations on two diol columns to calculate our average EO values.

Conclusion

Normal-phase packed-column SFC produced a similar separation of APE mixtures compared with normal-phase HPLC. Column length, stationary phase, and column combinations with different stationary phases all affected the separation of the APE mixtures tested. Longer column lengths increased the separation of oligomers. More-polar stationary phases retained oligomers with larger ethoxylate units for a longer time. A combination of columns with different stationary phases produced separations combining both the effects of longer columns and the 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. It is possible to isolate and identify individual APEs. Additionally, it is possible to identify the remaining chromatographic peaks because of each peak differing by one ethoxylate unit. Our study demonstrated the importance of using both absorbance detection as well as MS. MS alone did not show all the components of our initial fractions because of the decreased detector response.

Less solvent waste was produced using SFC compared with HPLC. Each SFC separation that used cyano packing as part of its column arrangement used 6.7 mL of methanol. The remaining SFC setups (the studies of column length and stationary phase) used 11.8 mL of methanol. All separations performed by normal-phase HPLC used 34.75 mL of hexane and 5.25 mL isopropanol for a combined volume of 40 mL. The HPLC system used almost 600% more solvent than the SFC system using a cyano stationary phase and over 330% more than the other SFC setups studied (this is not including the volume of solvent needed to initially equilibrate the systems). The reduction of solvent waste is an important step of reducing pollution.

Because of the fact that APEs are used as industrial cleaners and other processing aids, they enter wastewater and end up in sewage treatment plants. Some APE waste is transferred into the environment and metabolized into lower ethoxylated alkylphenols, which are considered endocrine disrupters (2). APEs have been found in fish, river sediment, and other environmental samples through analytical techniques (1,4,9,10,18–22). The results of our study could lead to the further use of the method developed for applications in the analysis of environmental samples. Additionally, our method could be altered for use in a future large-scale separation and collection of individual ethoxylated alkylphenols. Access to standards of individual ethoxylated alkylphenols is important for their quantitative analysis.

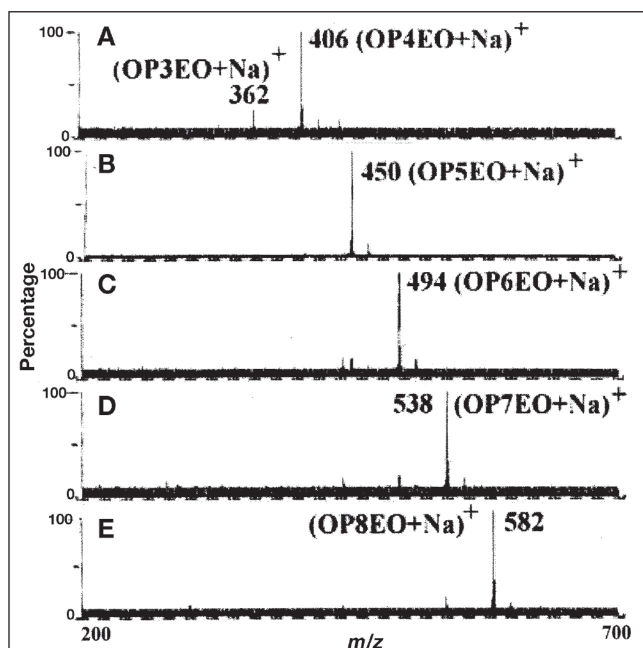

Figure 10. Positive-ion FIA–ESI–MS of POE-(5)-*tert*-OP fractions operated in full-scan mode. Ions were in the form of $(M+Na)^+$ and each were separated by *m/z* 44 (the mass of one ethoxyl unit): (A) fraction 1, cone voltage of 63 V; (B) fraction 2, cone voltage of 68 V; (C) fraction 3, cone voltage of 65 V; (D) fraction 4, cone voltage of 75 V; and (E) fraction 5, cone voltage of 75 V. Each spectrum was averaged over the flow injection peak.

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References

1. B. Thiele, K. Gunther, and M.J. Schwunger. Alkylphenol ethoxylates: trace analysis and environmental behavior. *Chem. Rev.* **97:** 3247–72 (1997).
2. R. White, S. Jobling, S.A. Hoare, J.P. Sumpter, and M.G. Parker. Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* **135:** 175–82 (1994).

3. Z. Wang and M. Fingas. Rapid separation of nonionic surfactants of polyethoxylated octylphenol and determination of ethylene oxide oligomer distribution by C1 column reversed-phase liquid chromatography. *J. Chromatogr.* **673:** 145–56 (1993).
4. C. Whalberg, L. Renberg, and U. Wideqvist. Determination of nonylphenol and nonylphenol ethoxylates as their pentafluorobenzoates in water, sewage sludge, and biota. *Chemosphere* **20:** 179–95 (1990).
5. R.E.A. Escott, S.J. Brinkworth, and T.A. Steedman. The determination of ethoxylate oligomer distribution of nonionic and anionic surfactants by high-performance liquid chromatography. *J. Chromatogr.* **282:** 655–61 (1983).
6. I. Zeman. Applications of bonded diol phases for separation of ethoxylated surfactants by high-performance liquid chromatography. *J. Chromatogr.* **363:** 233–30 (1986).
7. A.M. Rothman. High-performance liquid chromatographic method for determining ethoxymer distribution of alkylphenoxy polyoxyethylene surfactants. *J. Chromatogr.* **253:** 283–88 (1982).
8. J.L. Gundersen. Separation of isomers of nonylphenol and select nonylphenol polyethoxylates by high-performance liquid chromatography on a graphitic column. *J. Chromatogr. A* **914:** 161–66 (2001).
9. P.L. Ferguson, C.R. Iden, and B.J. Brownawell. Analysis of alkylphenol ethoxylate metabolites in the aquatic environment using liquid chromatography–electrospray mass spectrometry. *Anal. Chem.* **72:** 4322–30 (2000).
10. D.Y. Shang, M.G. Ikonomou, and R.W. MacDonald. Quantitative determination of nonylphenol polyethoxylate surfactants in marine sediment using normal-phase liquid chromatography–electrospray mass spectrometry. *J. Chromatogr. A* **849:** 467–82 (1999).
11. Z. Wang and M. Fingas. Quantitative analysis of polyethoxylated octylphenol by capillary supercritical fluid chromatography. *J. Chromatogr.* **641:** 125–36 (1993).
12. Z. Wang and M. Fingas. Analysis of polyethoxylated nonylphenols by supercritical fluid chromatography and high-performance liquid chromatography. *J. Chromatogr. Sci.* **31:** 509–18 (1993).
13. A. Giorgettie, N. Pericles, H.M. Widmer, K. Anton, and P. Datwyler. Mixed mobile phases and pressure programming in packed column supercritical fluid chromatography: a unified approach. *J. Chromatogr. Sci.* **27:** 318–24 (1989).
14. M. Takeuchi and T. Saito. Combination of semi micro and micro packed column supercritical fluid chromatography with some other instruments for qualitative analysis. *J. High Resolut. Chromatogr.* **14:** 347–51 (1991).
15. T.A. Dean and C.F. Poole. Solventless injection for packed column supercritical fluid chromatography. *J. High Resolut. Chromatogr.* **12:** 773–78 (1989).
16. E.S. Francis, M.L. Lee, and B.E. Richter. Modifier addition in microcolumn supercritical fluid chromatography with a high pressure pulsed valve. *J. Microcolumn Sep.* **6:** 449–57 (1994).
17. T. Okada. Efficient evaluation of poly(oxyethylene) complex formation with alkali-metal cations. *Macromolecules* **23:** 4216–19 (1990).
18. C. Crescenzi, A. Di Corcia, R. Sampri, and A. Marcomini. Determination of nonionic polyethoxylate surfactants in environmental waters by liquid chromatography/electrospray mass spectrometry. *Anal. Chem.* **67:** 1797–1804 (1995).
19. T.L. Keith, S.A. Snyder, C.G. Naylor, C.A. Staples, C. Summer, K. Kannan, and J.P. Giesy. Identification and quantification of nonylphenol ethoxylates and nonylphenol in fish tissues from Michigan. *Environ. Sci. Technol.* **35:** 10–13 (2001).
20. H.B. Lee, T.E. Peart, D.T. Bennie, and R.J. Maguire. Determination of nonylphenol and their carboxylic acid metabolites in sewage treatment plant sludge by supercritical fluid carbon dioxide extraction. *J. Chromatogr. A* **785:** 385–94 (1997).
21. T.L. Potter, K. Simmons, J. Wu, M. Sanchez-Olvera, P. Kostecki, and E. Calabrese. Static Die-away of a nonylphenol ethoxylate surfactant in estuarine water samples. *Environ. Sci. Technol.* **33:** 113–18 (1999).
22. M. Petrovic and D. Barcelo. Determination of anionic and nonionic surfactants, their degradation products, and endocrine-disrupting compounds in sewage by liquid chromatography/mass spectrometry. *Anal. Chem.* **72:** 4560–67 (2000).

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