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Determination of alcohol polyether average molar oligomer value/distribution via supercritical fluid chromatography coupled with UV and MS detection

Brian J. Hoffman [a], Larry T. Taylor [a,*], Stephen Rumbelow [b], Larry Goff [b], J. David Pinkston [c]

[a] *Department of Chemistry, Virginia Tech, Blacksburg, VA 24061, USA*
[b] *Technical Innovation Center, Uniqema, New Castle, DE 19720, USA*
[c] *Procter & Gamble Pharmaceuticals, Health Care Research Center, Mason, OH 45040, USA*

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Abstract

Supercritical fluid chromatography (SFC) was used for the analysis of ethoxylated and propoxylated surfactants. Samples were derivatized to phenylated silyl ethers with a disilazane–chlorosilane mixture. Addition of a phenyl group to the surfactant allowed UV-absorbance detection of each oligomer. Acetonitrile and methanol were evaluated as mobile phase modifiers. Better peak shape was realized with methanol-modified CO_2 on an octadecyl silica bonded phase than with acetonitrile-modified CO_2. Peak assignments were made via SFC coupled with electrospray ionization–mass spectrometry (ESI–MS) in the positive ion mode. A sulfonamide-embedded alkyl stationary phase was also evaluated for separation of the derivatized samples. SFC–UV and SFC–ESI–MS data were jointly used for calculation of average molar oligomer values which were then compared to values calculated from ^{1}H NMR data of non-derivatized samples. The derivatization or separation method using the sulfonamide embedded phase required no preliminary cleanup and yielded reproducible oligomer values that were consistent with those of the manufacturer's nominal values.

Keywords: Supercritical fluid chromatography; Mobile phase composition; Surfactants; Alcohol ethoxylates; Ethoxylates; Alcohol propoxylates; Propoxylates

1. Introduction

Average molar oligomer value and oligomer distribution are important characteristics of alcohol ethoxylates (AEOs) and alcohol 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 [1]. To determine the HLB of alcohol polyethers, it is necessary to know the average molar oligomer value. Nuclear magnetic resonance (NMR) spectroscopy and various chromatographic methods have been used most often thus far for the determination of average molar oligomer values.

Proton 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 [2–4]. 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 poly(ethylene glycol) (PEG) [4] in AEOs. PEG can be produced during the formation of AEOs if water is present during their synthesis.

Gas chromatography (GC) [5,6], high-performance liquid chromatography (HPLC) [7–11], and supercritical fluid chromatography (SFC) [5,12–18] have all been used for analysis of alcohol polyethers. Often these surfactants are derivatized prior to chromatographic analysis in order to in-

* Corresponding author. Tel.: +1 540 2316680; fax: +1 540 2313255.
E-mail address: itaylor@vt.edu (L.T. Taylor).

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crease volatility of the alcohol polyethers for GC [5,6] and to reduce undesirable stationary phase interactions and/or to improve solubility in the mobile phase in SFC [16–18]. Alcohol polyethers have also been derivatized for introduction of an UV-absorbing [7–9,18] or fluorescent-active [10,11] functional group for HPLC detection.

SFC can provide rapid and efficient separation of relatively high molecular weight surfactants. Increased diffusivity and lower viscosity of supercritical carbon dioxide, compared to traditional HPLC mobile phases, allows SFC to operate at higher flow rates and/or use longer packed columns compared to HPLC [19]. Open tubular [12–14] and packed [15–18] columns have been used for SFC separation of alcohol polyethers. Low temperature and relatively low-pressure separations, recently performed on packed columns, have been made possible by using organic solvent-modified supercritical CO_2 [15,18]. Methanol and acetonitrile are the most common modifiers of CO_2 [20–25] for SFC.

Research by Hoffman et al. have 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 [18]. 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 silyl ethers. 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

A stearyl alcohol polyoxypropylene ether with a nominal average PO length of 15 ($C_{18}PO_{15}$), a stearyl alcohol polyoxyethylene ether with a nominal average EO value of 10 ($C_{18}EO_{10}$), and a cetyl alcohol polyoxyethylene ether with a nominal average EO value of 20 ($C_{16}EO_{20}$) were provided by Uniqema (New Castle, DE, USA). 1,3-Diphenyl-1,1,3,3-tetramethyldisilazane (DPTMDS) (96% pure) was obtained from Sigma–Aldrich (Milwaukee, WI, USA). Phenyldimethylchlorosilane (PDMCS) (98.9% pure) was purchased from Gelest (Tullytown, PA, USA). HPLC-grade acetonitrile (MeCN) and methanol (MeOH) were obtained from Burdick & Jackson (Muskegon, MI, USA). ACS grade ammonium chloride was obtained from J.T. Baker (Phillipsburg, NJ, USA).

Samples were derivatized for formation of phenyl dimethyl silyl ethers, as described earlier by Hoffman et al. [18]. Depending on the average molecular mass of the sample, 45–135 mg of sample was placed in a 2.0 mL vial. The sample was dissolved in 1350 μL of acetonitrile, 150 μL of DPTMDS plus 31 μL of PDMCS were then added to the vial. The vial was capped, shaken for 30 s, and placed in an 80 °C oven for 60 min. A white precipitate (e.g. NH_4Cl) 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, USA).

2.2. Packed-column SFC–UV system

A Berger Analytical A5000 SFC system (Berger Instruments, Newark, DE, USA) 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, Allentown, PA, USA) was used as the primary mobile phase. Discovery C_{18} (Supelco, Bellefonte, PA, USA) and Acclaim PA C_{16} (Dionex, Sunnyvale, CA, USA) packed columns were employed. The dimensions of the Discovery C_{18} column were 250 mm × 4.6 mm with an average particle size of 5 μm. The dimensions of the two Acclaim PA C_{16} columns were 150 mm × 4.6 mm and 250 mm × 4.6 mm, respectively, with an average particle size of 5 μm. 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 min hold at 1% modifier to elute the excess of the derivatizing materials. All chromatographic methods then utilized a linear gradient at 1% modifier per minute to a set concentration depending on the sample composition. A 2 min hold at the upper modifier concentration was then followed by a return to 1% modifier at 25%/min. A 5 min post-run time was used for system equilibration.

2.3. Packed-column SFC–electrospray ionization (ESI)–MS system

A Berger Analytical A5000 SFC system was also used for SFC–ESI–MS analysis. Oligomeric identification was accomplished through tandem UV-mass spectrometric detection according to a method similar to Pinkston et al. [26]. 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, USA) was used to control column temperature. An Isco Model 260D syringe pump (Lincoln, NE, USA) delivered 1 mM ammonium acetate (in methanol) make-up flow downstream of the UV detector to aid in adduct-ion formation. Oligomers were detected as their $[M + NH_4]^+$ adducts. Make-up flow was supplied at 200 μL/min. A portion of the SFC effluent was diverted to the mass spectrometer via a

Valco (Houston, TX, USA) zero-dead-volume tee 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 (Perkin-Elmer Sciex, Boston, MA, USA) in the positive ion mode. Turbo gas temperature was 450 °C and the *m/z* range scanned was 150–1500 (step size was 0.2 U and dwell time was 0.2 ms). Elution order via SFC–ESI–MS data was used to identify oligomers detected by the separate SFC–UV system as well.

2.4. Instrumentation

To determine an appropriate detection wavelength, UV-absorbance spectroscopy was performed with an Agilent (Little Falls, DE, USA) 8453 diode array spectrophotometer. ^{1}H NMR spectra were collected at 400 MHz on a Varian Unity 400 (Varian, Walnut Creek, CA, USA). A relaxation delay of 1.0 s 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 Labs., Andover, MI, USA) and neat C^2HCl_3 (Isotec, Miamisburg, OH, USA) were used.

3. Results and discussion

The present study evaluated 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 silyl ethers. 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.

3.1. Modifier effect

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 [25]. Solute retention has been shown to decrease with the addition of modifier due to increased mobile phase solvent strength [21]. Undesirable interactions with residual silanols are a possible source of band broadening [20,21]. Modifier molecules at a stationary phase surface may thus interact with, and possibly “hide”, active silanol sites [24].

Separations were performed with methanol- and acetonitrile-modified CO_2 on a Discovery C_{18} 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 one Ph 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. SFC of the 1-Ph derivatized $C_{18}EO_{10}$ sample revealed that methanol-modified CO_2 produced less peak tailing than separations using acetonitrile-modified CO_2 (Fig. 1). Acetonitrile is a weak hydrogen bond donor solvent, which may explain why better peak shapes were seen using methanol-modified CO_2. Based upon these results, methanol was chosen as the mobile phase modifier for the remainder of this study.

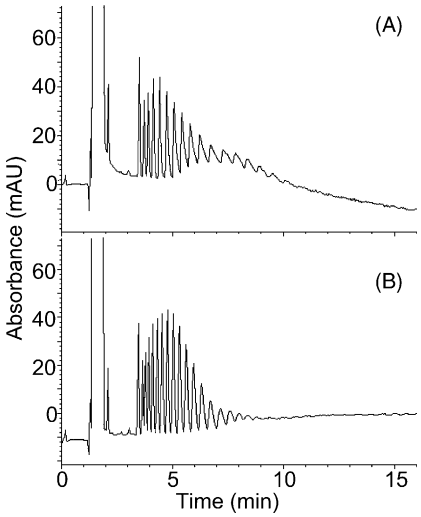

Fig. 1. Comparison of acetonitrile and methanol-modified CO_2. Discovery C18, 250 mm × 4.6 mm, 5 μm. Oven = 40 °C, outlet pressure = 120 bar, flow rate = 2.4 mL/min, detector absorbance 215 nm, modifier gradient: 1% for 5 min, linear increase 1 %/min to 15%, hold 2 min, linear decrease to 1% at 25 %/min, 5 min post run. (A) Acetonitrile-modified CO_2; (B) methanol-modified CO_2.

3.2. Stationary phase

Acclaim PA C_{16}, a sulfonamide-embedded alkyl stationary phase, was evaluated for separation of derivatized surfactant 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 [18]. The amide-embedded alkyl phase by itself was unable to separate the excess of the derivatizing material from the oligomeric series and, at the same time, provide good oligomer resolution. Fig. 2 is a chromatogram of the 1-Ph derivatized $C_{18}EO_{10}$ sample separated by the Acclaim PA C_{16} column using methanol-modified CO_2 as the mobile phase. Chromatographic peaks were identified by SFC–ESI–MS, vide infra. Good oligomer resolution as well as good separation of the excess of the 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.

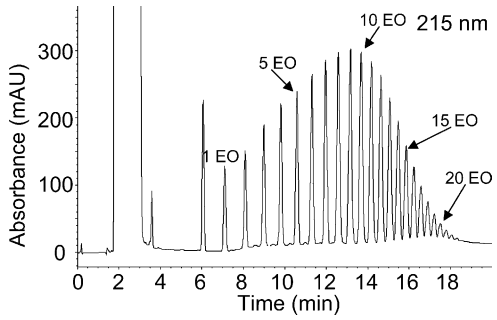

Fig. 2. SFC–UV separation of $C_{18}EO_{10}$ one Ph derivative. Acclaim PA C_{16}, 250 mm × 4.6 mm, 5 μm. Oven = 40 °C, outlet pressure = 120 bar, flow rate = 2.4 mL/min, detector absorbance 215 nm, modifier = methanol, modifier gradient: 1% for 5 min, linear increase 1%/min to 25%, hold 2 min, linear decrease to 1% at 25%/min, 5 min post run.

3.3. Calculation of average molar oligomer values

3.3.1. ^1H NMR of non-derivatized samples

^1H NMR was used to calculate average molar oligomer value for comparison with values calculated by SFC–UV. Calculation of average molar EO values by ^1H NMR followed the method described by Hammond and Kubik [4]. The ^1H NMR spectrum of each sample (64 scans) was acquired three times. Fig. 3 is a ^1H 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 molar EO value for the $C_{16}EO_{20}$ sample analyzed was 19.2, calculated in the same fashion.

The average PO value of the $C_{18}PO_{15}$ sample was deduced in a similar fashion. 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 3 (the number of protons in a methyl group) to determine the average PO value. The average molar PO value calculated by ^1H NMR for the $C_{18}PO_{15}$ sample was 13.7. Average EO and PO values calculated by ^1H NMR were similar to the nominal

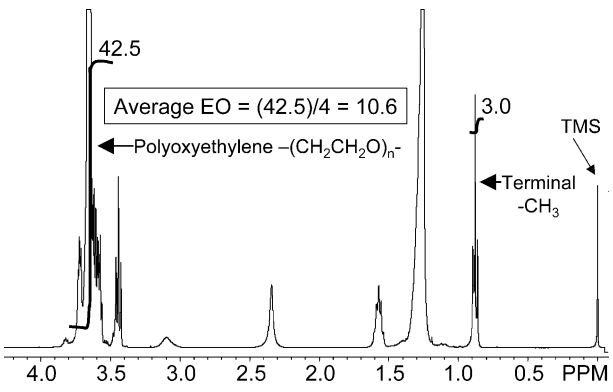

Fig. 3. ^1H NMR spectrum of non-derivatized $C_{18}EO_{10}$.

Table 1
Comparison of average molar oligomer values via ^1H NMR and SFC–UV

Surfactant	$C_{16}EO_{20}$	$C_{18}EO_{10}$	$C_{18}PO_{15}$
Average molecular mass	1122	710	1140
^1H-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
No. of injections (no. of samples)	15(3)	20(3)	14(3)
R.S.D. (%)[b]	0.8	0.7	0.2

[a] ^1H NMR of three non-derivatized samples.
[b] SFC–UV of three one Ph derivatized samples.

value assigned by their manufacturer. The R.S.D. of average molar oligomer values calculated from ^1H NMR data was below 1% (Table 1). The chromatographic 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. Discrepancies between nominal values and experimental values may be attributed to variation between manufactured batches of surfactants.

3.3.2. SFC–UV of derivatized samples

Work by Wang and Fingas [27] has demonstrated that oligomers of surfactants containing an aromatic ring (separated by HPLC) produce equal molar UV responses. Thus, the ether repeating unit is thought to not contribute to the UV signal. They were able 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. This method has been used for calculation of average molar oligomers value in the current research. 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 ^1H NMR, both the average oligomer value and the distribution of oligomers in each surfactant sample. Table 2 contains the detailed peak area information from the 1-Ph $C_{18}EO_{10}$ sample chromatogram found in Fig. 2. The chromatographic data demonstrate the ability of SFC–UV to provide the distribution of an alcohol polyether surfactant. The average molar oligomer value calculated for this sample was 9.7 EO units, whereas the NMR method gave a value of 10.6. The average molar oligomer values of $C_{16}EO_{20}$ and $C_{18}PO_{15}$ were calculated in a similar fashion, results are further discussed in the method reproducibility section. The average molar oligomer value calculated from SFC–UV data may vary from the ^1H NMR value, even though they were the same sample, due to the presence of PEG in the sample.

3.4. Method reproducibility

Each surfactant sample was derivatized three times and each derivative mixture was injected a minimum of four times. Table 1 includes sample information from the reproducibility study. Separations were performed with the

Table 2
SFC–UV peak data for 1-Ph derivatized $C_{18}EO_{10}$ (see chromatogram in Fig. 2)

EO no.	t_R (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.

packed column SFC–UV system. Calculated average molar oligomer values for surfactant samples were determined using the method described previously. All 1-Ph derivatives gave average molar oligomer values with R.S.D.s below 1%. The 1-Ph 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) for derivatized $C_{18}PO_{15}$ and the ^{1}H NMR value (non-derivatized, 13.7) 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 four ethoxylate (4 EO) repeating units of each 1-Ph 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 R.S.D.s 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 R.S.D. values indicate that the reproducibility of the derivatization and chromatographic methods were acceptable.

4. Conclusions

In comparison to acetonitrile-modified CO_2, methanol-modified CO_2 provided better peak shape and shorter retention times. 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. This single column 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 derivatizing material from oligomers prior to separation of the oligomeric series. ESI–MS was used for identification of chromatographic peaks, and along with UV detection data allowed the average molar oligomer value to be calculated for derivatized surfactant samples. Average molar oligomer values 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, we conclude the methods are credible for the determination of average molar oligomer value and the determination of oligomeric distribution of alcohol polyethers.

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