SURFACTANTS & DETERGENTS

HPLC Determination of Ethoxylated Alcohol Surfactants in Wastewater

T.M. Schmitt[*,a], **M.C. Allen**[b], **D.K. Brain**[c], **K.F. Guin**[d], **D.E. Lemmel**[c] and **Q.W. Osburn**[c]
[a]BASF Corporation, Wyandotte, MI, [b]Conoco, Inc., Ponca City, OK, [c]Procter and Gamble Co., Cincinnati, OH and [d]Shell Development Co., Houston, TX

High performance liquid chromatography (HPLC) may be used for analysis of municipal wastewater containing ethoxylated alcohol surfactants. After cleanup of the sample by XAD-2 resin, liquid-liquid extraction, ion exchange and cobalt thiocyanate extraction, the surfactant is derivatized with phenyl isocyanate to permit UV detection and analyzed by both normal phase and reversed-phase HPLC. The alkyl chain length distribution is determined using reversed-phase HPLC, while the ethoxy chain length is determined by normal phase HPLC. The limit of quantification is 0.1 ppm.

Synthetic surfactants are used daily in practically every household in developed countries. The nature of this use results in their chemically intact discharge into municipal sewage treatment facilities, as well as into individual septic tank systems. Thus, systematic investigations into their environmental fate are required to assure that they are ultimately transformed to inert compounds.

Nonionic surfactants make up an important category of general-purpose surfactants, with the alcohol ethoxylates (AE) being the nonionics used most often in domestic laundry and dishwashing detergents. Ethoxylated alcohols have the formula $C_nH_{2n+1}(OC_2H_4)_xOH$, where the alkyl chain is predominantly linear and is in the C_{12} range, and x ranges from 1 to 30 or more.

Many studies have been performed which demonstrate the biodegradibility of AE in laboratory apparatus designed to model real-life conditions. Relatively simple analytical methodology is sufficient for bench scale studies since usually only a single surfactant is examined at a time. However, in order to confirm these studies by following the degradation of AE in a waste treatment plant, it is necessary to use an analytical method which will differentiate AE from other nonionic surfactants (alkylphenol ethoxylates, sorbitan esters, etc.) and from non-surface-active nonionic polymers, such as poly(ethylene glycol). In addition to knowledge of total concentration, information about the alkyl and ethoxy chain length distribution is essential in understanding the changes in AE as it undergoes degradation, and also in assessing the environmental impact of these changes (1).

Analytical methods commonly applied to wastewater analysis respond to broad classes of compounds, yielding a value for total nonionic surfactant. These include three widely used methods: The cobalt thiocyanate (CTAS) spectrophotometric procedure (2), the iodobismuthate (BiAS) titration method (3,4) and the potassium picrate spectrophotometric method (5). The results of these tests are calibrated against some arbitrarily chosen surfactant, and, for example, reported as "cobalt thiocyanate active substance." While these methods provide useful preliminary information, they are sensitive to many positive and negative interferences in complex environmental matrices, and can only be considered quantitative if preliminary steps are added to the procedure to separate the synthetic surfactants from other compounds (6,7). By their nature, these general tests do not distinguish between types of nonionics, nor do they provide information on the distribution of homologs.

Recently, large scale studies of the surfactant material balance at municipal sewage treatment plants have been conducted under the auspices of the Soap and Detergent Association (8) in the USA and jointly by AIS/CESIO (6, 9) in Europe. As a part of such studies, it is important to show how much of the value for total nonionic surfactant by the CTAS or BiAS methods is actually surfactant.

A study was undertaken by the Analytical Subcommittee of the Soap and Detergent Association to develop an AE-specific procedure for analysis of the difficult matrix of municipal wastewater. The result is the comprehensive procedure described here.

EXPERIMENTAL

Apparatus. Several research quality high performance liquid chromatography (HPLC) instruments were used during the course of this study. A typical instrument consisted of two Model 6000A pumps, a U6K injector, a Model 480 variable wavelength UV detector (all Waters Chromatography Division, Millipore Corp., Milford, MA) and a Model 3390 Integrator/Data System (Hewlett-Packard, Palo Alto, CA). All other apparatus can be obtained from ordinary laboratory supply houses.

Reagents. Cobalt nitrate hexahydrate, ammonium thiocyanate, hydrochloric acid, sodium hydroxide, sodium chloride, anhydrous sodium sulfate and phenyl isocyanate were ACS Reagent Grade. Methylene chloride, 1,2-dichloroethane, diethyl ether, methanol, chloroform (containing hydrocarbon stabilizer, but not ethanol), ethyl acetate and acetonitrile were distilled-in-glass (American Burdick & Jackson, Muskegon, MI). Water was purified with a Millipore Milli-Q system. Cobalt thiocyanate solution was prepared by dissolving 15 g $Co(NO_3)_2 \cdot 6H_2O$ and 100 g NH_4SCN in water to make 500 ml. Purified Amberlite XAD-2 resin was obtained from Applied Science Division, Milton Roy Co. (Rochester, NY). Anion exchange resin (AG1-X2, Cl^- form, 50–100 mesh) and cation exchange resin (AG50W-X8, acid form, 50–100 mesh) were obtained from Bio-Rad Laboratories, Richmond, CA.

Standard preparation. The nonionic reference standard solution, 1 mg/ml, was prepared in methylene chloride from a 50:50 blend of Neodol 25-9 and Alfonic 1218-70 (these are an average 9-mole ethoxylate of C_{12}–C_{15} alcohols and an average 7-mole ethoxylate of C_{12}–C_{18} alcohols, commercially available from Shell Chemical Co. [Houston, TX] and Vista Chemical Co. [Ponca City, OK], respectively). The internal standard solution was prepared by weighing 0.250 g each 1-octanol and 1-eicosanol (Aldrich Chemical Co., Milwaukee, WI) into a 100-ml volumetric flask and diluting to volume with 1,2-dichloroethane. An ethylene dichloride solution containing 2.5 mg/ml each of the seven C_8–C_{20} even-numbered primary alcohols (Aldrich) was prepared.

*To whom correspondence should be addressed at: 1419 Biddle Avenue, Wyandotte, MI 48192-3736.

Samples. The samples used in this study were taken from a sewage treatment plant in Enid, OK, as part of a comprehensive program (8). This is a typical activated sludge plant of 8 million gallon/day design which receives 95% of its input from domestic and commercial sources, and 5% of its input from industrial sources. The following paragraphs describe the procedure in detail.

Concentration of surfactants from the sample with XAD-2 resin. A 1.2-cm i.d. glass column was filled to a depth of approximately 18 cm with a methanol slurry of XAD-2 resin, with a plug of glass wool against the top and bottom of the resin bed.New columns were conditioned by following the procedure in the regeneration sequence (below). A volume of sample was chosen to contain at least 1.0 mg nonionic surfactant and was passed through the column at a rate of approximately 1 drop/sec. Sample size was 500 ml for a typical sewage plant influent sample, and about 10 l for an effluent. The resin was then rinsed with 30 ml petroleum ether, applying pressure with a squeeze bulb to force all of the petroleum ether out of the column. This fraction was then discarded.

A 250-ml extraction flask was placed beneath the column and elution was conducted sequentially with 40 ml ethyl ether, 60 ml 1:1 ethyl ether/methanol, and 30 ml methanol. A flow rate of 1 drop/second was maintained. If necessary, a squeeze bulb was used to force the ether through the column. The eluate was evaporated to approximately 5 ml on a steam bath under a stream of nitrogen. Five ml water was added and evaporation was continued to a volume of approximately 5 ml.

Regeneration sequence. The upper glass wool plug was replaced. The column was rinsed sequentially with 40 ml methanolic 0.05 M NaOH, 55 ml conc. HCl/methanol/chloroform (1:5:5), 25 ml methanol, and, using suction to increase the flow rate, 250 ml water.

Isolation of surfactants by liquid-liquid extraction. The solution from the first step was rinsed with 45 ml 5 M NaCl solution into a 250-ml separatory funnel containing 50 ml ethyl acetate. The funnel was shaken vigorously for 1 min and the layers allowed to separate. The aqueous layer was drained into a second separatory funnel, and 50 ml of 5 M NaCl solution added to the first funnel. The funnel was shaken 1 min and the layers allowed to separate, again draining the lower layer into the second funnel. The contents of the first funnel were swirled and the separated aqueous layer (1-2 ml) drained into the second funnel. Fifty ml ethyl acetate was added to the second funnel, which was then shaken for 1 min. The layers were allowed to separate and the lower aqueous layer was discarded. The remaining ethyl acetate layer was decanted into the first separatory funnel, about 30 g of anhydrous sodium sulfate was added, and the funnel was shaken for 10-15 sec. The ethyl acetate extract was filtered through folded, 9-cm, Whatman No. 40 filter paper containing about 30 g anhydrous sodium sulfate, collecting the filtrate in a 150-ml extraction flask. The second separatory funnel was rinsed with 25 ml ethyl acetate and the rinsings decanted into the first separatory funnel, again shaking 10-15 sec and filtering through the Na_2SO_4. This rinse was repeated. Ethyl acetate in the extraction flask was evaporated just to dryness on a steam bath with the aid of a gentle stream of dry nitrogen. The residue was dissolved in 10 ml methanol and saved for ion exchange chromatography.

Ion exchange separation of nonionic surfactants. Preparation of ion exchange columns. A glass wool plug was placed in the bottom of a glass chromatography column, 15 × 300 mm, just above the stopcock. About 20 g of anion exchange resin was slurried in water and transferred to the ion exchange column. To put the resin into the hydroxide form, 50 ml 1 M aqueous sodium hydroxide was passed through the column at a rate of 1-2 drops per second, then the column was rinsed with water until the eluent was free of alkali. Water was displaced by elution with 50 ml of methanol. Air pockets and channels were removed by backflushing. A glass wool plug was placed on top of the anion resin bed. Approximately 16 g of cation exchange resin was slurried in methanol and added to the ion exchange column. The column was then rinsed with 50 ml of methanol prior to use in the following step. (The ion exchange column may be used for up to six samples before discarding the packing material.)

Removal of interfering ionic surfactants by ion exchange chromatography. The methanol solution of the residue from the isolation step was quantitatively transferred to the ion exchange column using two 10-ml methanol rinses. The methanol level was allowed to fall just to the top of the resin bed before adding each successive rinse. The effluent was collected in a 150-ml extraction flask. Methanol elution was continued at a rate of 1-2 ml/min until a total of 125 ml of effluent had been collected. The methanol was evaporated just to dryness on a steam bath with the aid of a gentle stream of nitrogen. (Do not evaporate until ready to run CTAS analysis.)

CTAS analysis. Preparation of calibration curve. Aliquots (1.00, 2.00, 3.00 and 4.00 ml) of the reference standard solution were pipetted into a series of four 150-ml extraction flasks and evaporated just to dryness. These flasks contained 1, 2, 3, and 4 mg surfactant, respectively. The contents of each flask were quickly transferred to individual 125-ml separatory funnels with the aid of 10.0 ml methylene chloride. Cobalt thiocyanate solution (5.0 ml) was added, and the funnel shaken vigorously for 60 sec. After the phases had separated, the methylene chloride layer was drained into a centrifuge tube and spun at approximately 4,000 rpm for 3 min. The absorbance of the centrifugate was measured in a 2-cm cuvette at 620 nm vs CH_2Cl_2. If a haze developed in the cell, the cell was warmed either in the hand or with a heat lamp until the solution was clear. In the case of samples, the solutions, the beaker, the separatory funnel, the centrifuge tube, the transfer pipette, etc., were retained for the recovery of ethoxylates. A calibration curve was prepared by plotting net absorbance vs mg ethoxylate (CTAS). The slope should lie in the range of 0.1-0.3 absorbance units per milligram CTAS.

Analysis of sample. Methylene chloride (10.0 ml) was pipetted into the extraction flask containing the evaporated sample from the ion exchange separation, swirled for a few seconds, then quickly transferred to a 125-ml separatory funnel. Then the preparation of the calibration curve procedure was followed, starting with "5.0 ml cobalt thiocyanate solution. . . ." The number of mg of CTAS in the final residue was determined by comparing its absorbance to the calibration curve. The concentration of CTAS in the original sample was calculated based on the mg found and the starting sample volume.

Recovery of ethoxylates from CTAS solution. For a particular sample or standard we obtained the solutions, the extraction flask, the separatory funnel, the centrifuge tube, the transfer pipette, etc., from the CTAS analysis. After measuring the absorbance, all the methylene chloride-CTAS solution was transferred to the retained flask. Aqueous CTAS solution in the separatory funnel was extracted with two additional 10-ml portions of methylene chloride, adding the extracts to the flask. The miscellaneous glassware used in the CTAS analysis was rinsed with an additional 10 ml of methylene chloride and the rinsings added to the flask. The methylene chloride-CTAS solution was transferred from the flask to a clean 250-ml separatory funnel using two 10-ml portions of methylene chloride to rinse the flask. The methylene chloride phase was passed through 10–15 g anhydrous sodium sulfate into a 150-ml extraction flask. The salt solution was extracted with two additional 10-ml portions of methylene chloride, drying the extracts by passage through the sodium sulfate. The sodium sulfate and filter paper were rinsed with 20 ml methylene chloride and all extracts and rinsings were evaporated on a steam bath using a stream of nitrogen to hasten evaporation.

Derivatization with phenyl isocyanate. To the vial containing the residue from the recovery of the ethoxylates was added 50 μl alcohol internal standard solution (0.25 g each C_8 and C_{20} primary alcohol per 100 ml ethylene dichloride) as well as 10 μl phenyl isocyanate. The walls of the vial were rinsed with 50 μl ethylene dichloride. The vial was swirled to mix, capped loosely, and held at 55 ± 2°C for 45 min in a vacuum oven at 70–100 kPa below atmospheric pressure. The vial contents were dissolved in 250 μl ethylene dichloride. A standard solution was prepared similarly by adding 50 μl of the C_8–C_{20} standard solution (containing all the even-numbered alcohols) to a vial, and derivatizing with 10 μl phenyl isocyanate.

HPLC separation—reversed-phase. LC conditions for separation by alkyl chain length. The column was μBondapak C_{18} (Waters Associates, Milford, MA), 3.9 × 300 mm. The mobile phase varied as a linear gradient, 80:20 methanol/water to 100% methanol in 30 min, at a flow rate of 2.0 ml/min. Detection was by UV absorbance at 235 or 240 nm, 1 AUFS. Injection volumes of 10 μl were used for influent samples, and 20 μl for effluents.

The sample and standard were analyzed according to the above conditions. See Figure 1 for typical chromatograms. The chromatogram of the standard was used for peak identification. The peak areas from the sample chromatogram were used in the calculations.

HPLC separation—normal phase (optional). LC conditions for separation by degree of ethoxylation. A μBondapak NH_2 (Waters Associates) column was used, 3.9 × 300 mm, with a mobile phase of solvent A: 350:150 hexane/ethylene dichloride; solvent B: 185:65 acetonitrile/isopropanol (add 800 μl acetone per liter); linear gradient: 0% B to 35% B in 50 min, at a flow rate of 3.0 ml/min. Detection was by UV absorbance at 235 or 240 nm, 0.1 AUFS. Injection volumes of 10 μl were used for influent samples, and 20 μl for effluents.

Another portion of the derivatized sample was analyzed by the above conditions. See Figure 2 for typical chromatograms. Alternatively, fractions were collected from the reversed-phase analysis, concentrated, and injected as samples for normal phase HPLC. Note that the mobile phase and solvent gradient may have to be modified somewhat to compensate for the characteristics of individual columns.

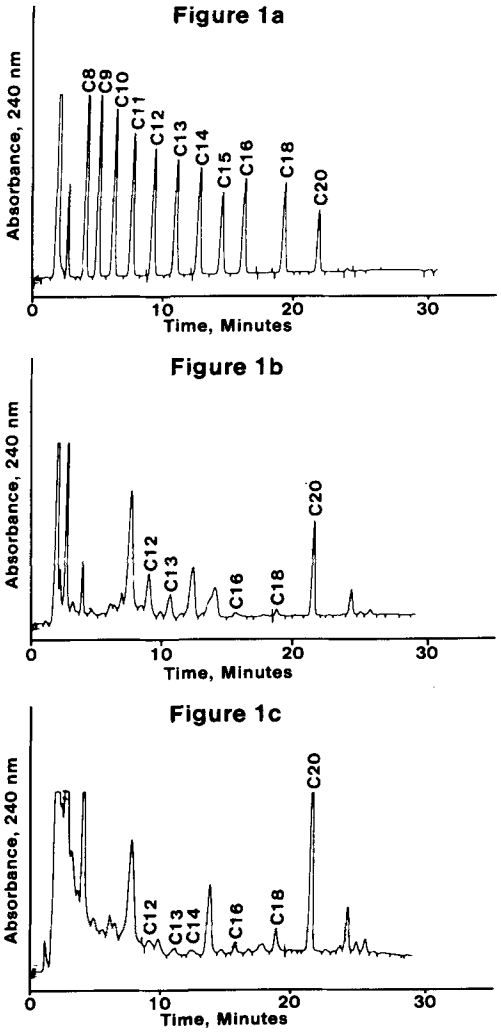


FIG. 1. Separation of AE according to length of alkyl chain, reversed-phase HPLC. (a) Calibration with alcohol standard mixture. (b) Influent wastewater sample. (c) Effluent wastewater sample.

Calculations. The peak areas from the first chromatogram (separation by alkyl chain) were used to calculate the micrograms of AE in the sample, assuming an average degree of ethoxylation of eight (Table 1). Optionally, the peak areas from the second chromatogram (separation by ethoxy number) were used to calculate the average molecular weight of the ethoxy chain. This value was then substituted into the calculation. The total micrograms of AE in the sample were then calculated.

RESULTS

The ruggedness of the procedure was tested in two collaborative studies. The same samples were analyzed in laboratories at different locations, using different types of HPLC apparatus, over a period of several months. The results of the collaborative studies of the method are shown in Tables 2 and 3.

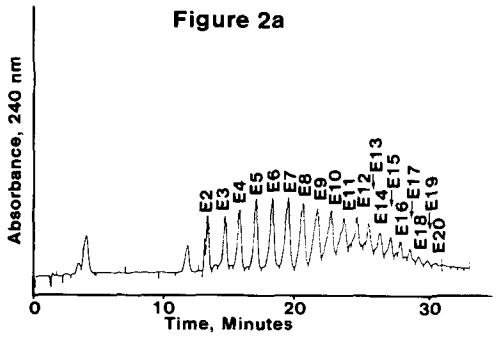
Table 2 shows the results from both an influent and an effluent sample. There is a range of values reported by

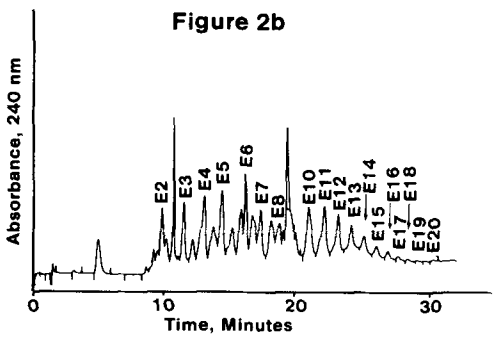
TABLE 1

Example of Calculation (Eight EO Units Assumed)

Alkyl chain length	Area of C_x peak / Area of C_8 alc peak*		FW of alkyl + FW of ethoxy / FW of C_8 alcohol[a]		Total μg C_8 alcohol internal standard		μg AE
12	5.9270 / 20.4652	×	186 + 352 / 130	×	125	=	149.8
12	7.8265 / 20.4652	×	200 + 352 / 130	×	125	=	203.0
14	5.4966 / 20.4652	×	214 + 352 / 130	×	125	=	146.2
15	1.6134 / 20.4652	×	228 + 352 / 130	×	125	=	44.0
16	.6550 / 20.4652	×	242 + 352 / 130	×	125	=	18.3
18	.7776 / 20.4652	×	270 + 352 / 130	×	125	=	22.7
						Total μg AE =	584.0

[a]Alternately, the C_{20} alcohol peak may be used for these calculations, using a MW of 298.





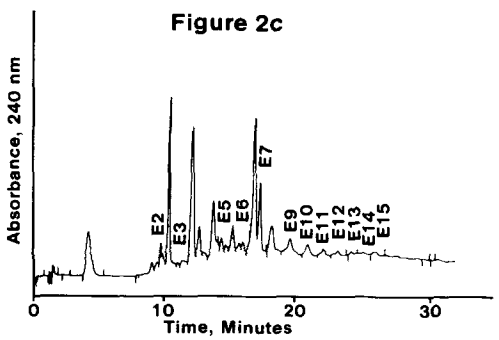


FIG. 2. Separation of AE according to length of ethoxylate chain, normal phase HPLC. (a) Calibration with Neodol 25-9/Alfonic 1218-70 blend. (b) Influent wastewater sample. (c) Effluent wastewater sample.

TABLE 2

Interlaboratory Collaborative Study I

	Analysis of influent stream	Analysis of effluent stream
Laboratory 1	0.67 ± 0.11	0.02 ± 0.01
Laboratory 2	0.92 ± 0.45	0.009 ± 0.001
Laboratory 3	0.71 ± 0.10	<0.01
Laboratory 4	1.31 ± 0.19	0.02 ± 0.01
CTAS value	3.22 ± 0.71	0.32 ± 0.21

All values in mg/l AE.

different laboratories, with the highest value being about twice the lowest. It is interesting to compare the results of HPLC analysis with the standard method for determining nonionic surfactants in water by CTAS. For these samples, AE represented less than a third of the influent CTAS, and only a few percent of the effluent CTAS.

Table 3 shows the results of a second study. In this case also, both influent and effluent samples were analyzed. Collaborators were asked to determine recovery by analyzing the same samples after spiking with 2.0 mg/l AE (for the influent) or 0.5 mg/l (for the effluent). As shown in the table, the agreement between laboratories is much improved if the results are corrected for percent recovery. Recovery of "spikes" ranged from 62-110% for various experiments. By comparison, results of CTAS spiking studies, by an experienced laboratory, showed recoveries of 74-88%, using the same sample preparation methods. The results for the influent are generally more precise than for the effluent because the latter values are near the detection limit of the method.

Table 4 shows the results of repetitive analyses of samples taken from the same points at a sewage treatment plant over a 3-day period. These analyses were performed in a single laboratory by chemists experienced with the method, and show very acceptable precision.

TABLE 3

Interlaboratory Collaborative Study II

	Analysis of influent stream			Analysis of effluent stream		
	Raw value	% Recovery of spike	Corrected value	Raw value	% Recovery of spike	Corrected value
Laboratory A	2.7	65	4.2	0.03	74	0.04
Laboratory B	4.2	110	3.8	0.04	88	0.05
Laboratory C	3.3	72	4.6	0.05	62	0.08
Standard deviation			0.4			0.02
CTAS value	4.7			0.44		

All values in mg/l AE.

TABLE 4

Monitoring AE Concentration at a Sewer Treatment Plant

Sample		Replicate analyses	
Raw influent	Day 1	0.676	0.633
Raw influent	Day 2	0.865	0.814
Raw influent	Day 3	0.912	0.852
Primary influent	Day 1	0.385	0.363
Primary influent	Day 2	0.769	0.719
Primary influent	Day 3	0.607	0.584
S. primary effluent	Day 1	0.499	0.455
S. primary effluent	Day 2	0.578	0.621
S. primary effluent	Day 3	0.296	0.290
N. primary effluent	Day 1	0.252	0.243
N. primary effluent	Day 2	0.307	0.303
N. primary effluent	Day 3	0.242	0.234
S. final effluent	Day 1	0.026	0.023
S. final effluent	Day 2	0.019	0.018
S. final effluent	Day 3	0.014	0.013
N. final effluent	Day 1	0.008	0.008
N. final effluent	Day 2	0.008	0.010
N. final effluent	Day 3	0.011	0.010

All values in mg/l AE.

These and a number of other results from duplicate analyses made at the same time were used to determine the standard deviation (10). The standard deviation based on the duplicates was 4.5%.

Retention time reproducibility in a single laboratory was quite good for the gradient runs using either method, with a standard deviation of less than 1% for all analyses conducted during the plant trial.

The typical value for a distilled water "blank" was 10–20 ppb AE. This reflects possible contamination of glassware, as well as impurities in the mobile phase solvents which cause baseline fluctuations in the chromatogram.

DISCUSSION

This methodology was developed to support a series of studies by the Soap and Detergent Association (SDA). The studies are aimed at obtaining a "material balance" of surfactants in an activated sludge process municipal wastewater treatment plant. The requirements of the method were: (i) differentiating AE surfactants from a similar category of nonionic surfactants, the ethoxylated alkylphenols (APE); (ii) identifying alkyl chain lengths of the AE still intact at various points in the process; and (iii) providing information on the ethoxy chain length of AE at various points in the treatment process.

A number of techniques have been used to characterize AE. Gas chromatography (after derivatization) has been applied to the analysis of AE (11), as has mass spectrometry (12). SFC has also been applied to the separation of AE according to ethoxy distribution, although the method has not yet been demonstrated for analysis of environmental materials (13). Such procedures allow adequate resolution of AE oligomers to distinguish them from other nonionics. HPLC methods, however, are generally more useful for determination of AE because of the low volatility of the higher molecular weight homologues.

HPLC is already an established technique for characterization of ethoxylated surfactants (14). Normal phase gradient elution HPLC will separate ethoxylated alcohols according to their degree of ethoxylation (15–18). (Isocratic elution is sufficient to qualitatively show the molecular weight distribution, but baseline resolution is not obtained [19]). Reversed-phase HPLC, generally also using gradient elution, will give the carbon number distribution of the alkyl chain of AE. A reversed-phase system is also capable of separating according to EO chain length (20), but, by proper choice of the mobile phase, this mechanism can be eliminated (21).

For gradient elution chromatography, it is most convenient to use an ordinary UV absorbance detector. While some AE compounds can be detected directly by low wavelength UV absorbance (15), most require formation of UV-absorbing derivatives, usually with 3,5-dinitrobenzoyl chloride (20) or phenyl isocyanate (16). Detection of underivatized material is also possible using mass spectrometry (18) or a flame ionization detector (17). We chose to prepare the phenyl isocyanate derivatives.

SDA subcommittees have been working with HPLC for the analysis of environmental samples since 1975. Experience had shown that analysis of neat commercial surfactants is straightforward, but that determination of low levels of the same surfactants in environmental samples requires considerable preliminary work-up. Figure 3 gives an outline of the analytical scheme. Several steps are required before HPLC analysis is possible.

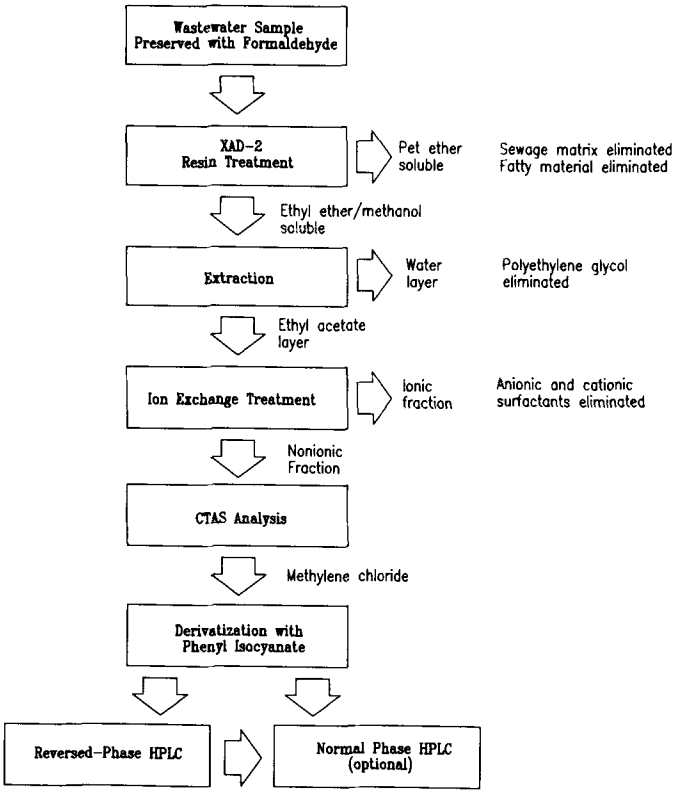


FIG. 3. Outline of analytical method.

Formalin treatment of sewage samples was found to be adequate for preservation at room temperature for at least six months. Samples should be stored in glass, since we observed that some surfactants in dilute solutions adsorb to the surface of plastic containers.

The lipophilic portion of the sample is isolated from the water matrix by passage through Amberlite XAD-2 resin, a non-polar styrene/divinylbenzene polymer. This technique has been shown by other investigators to be effective in concentrating ethoxylated surfactants from an aqueous matrix (22,23). A preliminary petroleum ether wash of the resin removes much of the fatty material. The surfactants are then eluted from the resin with ethyl ether/methanol. This fraction contains (among other impurities) polyethylene glycols, some of them contributed by primary biodegradation of ethoxylated surfactants. While these do not have surface-active properties, they will interfere with CTAS analysis. In previous studies, they were removed by solvent sublation of the surfactants (2,3); in this work, we used liquid-liquid extraction. Passage through a mixed-bed ion exchange column removes anionic and cationic surfactants. CTAS analysis serves the dual purpose of providing a polyether-specific separation step, and also giving an estimate of the concentration of nonionic surfactant to aid in choosing the proper aliquot size for the subsequent HPLC analysis. Alumina clean-up prior to HPLC analysis is occasionally required in order to remove unidentified contaminants which interfere with the liquid chromatography. This step is not required for most environmental samples and the methodology has been omitted from the Experimental section.

The two HPLC separations can be carried out in parallel or in sequence. Greatest selectivity is attained if the reversed-phase separation (i.e., separation by alkyl chain length) is performed first, and the eluate is collected, concentrated and reinjected for the normal phase separation (separation by degree of ethoxylation). The chromatograms in Figure 2b and c were obtained in this manner. If the normal phase chromatography is run on an unpurified sample, non-AE components appear in the chromatograms which interfere with accurate quantification.

Clearly, the clean-up required by this HPLC method is extensive. It should be noted, though, that even the standard CTAS method requires solvent sublation and ion exchange prior to the actual analysis (2). Note also that this is a comprehensive method, designed to cover most environmental samples with a single protocol. Portions of the cleanup procedure may be omitted if the procedure is applied to another matrix.

The method was refined in a series of collaborative studies, the results of two of which are presented in Tables 2 and 3. Certain problems surfaced in the collaborative studies. The major difficulty is that APE surfactants survive all clean-up steps and appear in the HPLC traces. APE gives a large peak in the reversed-phase chromatogram at a retention time corresponding to a C_{11} ethoxylate. This was confirmed by trapping the C_{11} peak and examining it by infrared spectroscopy; the main component was indeed APE. Minor APE interference is also seen with the C_{18} ethoxylate peak. In effluent samples, where measurements are made at or below the nominal quantification limit of the methodology, there is also an interfering peak contributed by the phenyl isocyanate derivatizing reagent, which partially obscures the C_{15} peak. (This is not a problem with influents.) Chromatograms can be obtained at AE concentrations of 0.02 ppm or lower (original sample basis). However, the factors described above result in the chromatogram presenting less usuable information as the concentration drops. For this reason, the method is not suitable for quantitative measurements below about 0.1 ppm.

Similar problems occur in application of the normal phase HPLC method at low levels. Some interference is removed if the peaks from reversed-phase HPLC are collected and reanalyzed. However, the presence of APE "under" the C_{11} and C_{18} peaks will interfere with the determination of the EO chain length if these peaks are collected. For most work, it is preferable to use assumed values for EO chain length. Errors in the values for total AE as a result of making this assumption will be small—a deviation of 3 EO units from the expected eight will only affect the total by 23% for an average C_{14} alkyl chain.

We have found that the same HPLC methodology can be used for APE determination. In this case, the extracts are not derivatized. This permits UV detection to be used to selectively measure APE. The optimum wavelength for APE quantification is 280 nm. Of course, if these values are used to correct for interference in the AE determination, measurements must also be made at 240 nm.

As part of our investigation, we pursued trapping each of the peaks from reversed-phase HPLC individually, and then analyzing by normal phase HPLC. This approach was successful in giving the actual concentration of each homologue. However, we considered the careful technique required, as well as the multiplication of the chromatographic analyses, to be too demanding for a method aimed at semi-routine use. Knowledge of the total mass and

average composition of the AE is sufficient for most purposes.

Ahel and Giger (24) developed a similar procedure for determination of APE in environmental samples. Future work by our committee will include use of this approach to correct the interference of APE with AE determination, as well as to extend the method to determination of AE in sludge and soil.

ACKNOWLEDGMENTS

We thank the following people for their valuable suggestions during the development and writing of the procedure: R. E. Laramy and E. A. Setzkorn, Conoco, Inc.; R. A. Llenado, Procter and Gamble; H. O. Locke, GAF Corporation; D. C. Long, Texise Co.; R. H. Larkin, Rohm and Haas Co.; D. Wharry, Vista Chemical Co.; H. F. Robinson, Lever Brothers Co.; T. Taulli, Monsanto; and K. A. Booman and R. I. Sedlak, Soap and Detergent Association. The following people also participated in the collaborative studies: W. Rorie, Industrial Testing Laboratories and C. T. Desmond, Union Carbide Corporation. This work was performed under the auspices of the Analytical Subcommittee of the Soap and Detergent Association, New York, NY 10016.

REFERENCES

1. Swisher, R.D., *Surfactant Biodegradation*, Marcel Dekker, Inc., New York, 2nd edn., 1986.
2. Boyer, S.L., K.F. Guin, R.M. Kelley, M.L. Mausner, H.F. Robinson, T.M. Schmitt, C.R. Stahl and E.A. Setzkorn, *Environ. Sci. Tech. 11*:1167 (1977).
3. Wickbold, R., *Tenside 9*:173 (1972).
4. Waters, J., J.T. Garrigan, A.M. Paulson, *Water Res. 20*:247 (1986).
5. Favretto, L., B. Stancher and F. Tunis, *Analyst 105*:833 (1980).
6. Brown, D., H. De Henau, J.T. Garrigan, P. Gerike, M. Holt, E. Keck, E. Kunkel, E. Matthijs, J. Waters and R.J. Watkinson, *Tenside 23*:190 (1986).
7. Hellmann, H., *Vom Wasser 64*:29 (1985).
8. Sedlak, R.I., K.A. Booman, *Soap, Cosmet., Chem. Spec. 62*(4):44 (1986).
9. Brown, D., H. De Henau, J.T. Garrigan, P. Gerike, M. Holt, E. Kunkel, E. Matthijs, J. Waters and R.J. Watkinson, *Tenside 24*:14 (1987).
10. Youden, W.J., E.H. Steiner, *Statistical Manual of the Association of Official Analytical Chemists*, AOAC, Arlington, VA, 1975, p. 21.
11. Szymanowski, J., H. Szewczyk and B. Atamanczuk, *Tenside 21*:139 (1984).
12. Stephanou, E., *Chemosphere 13*:43 (1984).
13. Matsumoto, K., S. Tsuge and Y. Hirata, *Shitsuryo Bunseki 35*:15 (1987).
14. Garti, N., B.R. Kaufman and A. Aserin, in *Nonionic Surfactants—Chemical Analysis*, edited by J. Cross, Marcel Dekker, New York, 1987.
15. Aserin, A., M. Frenkel and N. Garti, *J. Am. Oil Chem. Soc. 61*:805 (1984).
16. Allen, M.C. and D.E. Linder, *Ibid. 58*:950 (1981).
17. McClure, J.D., *Ibid. 59*:364 (1982).
18. Levsen, K., W. Wagner-Redeker, K.H. Schaefer and P. Dobberstein, *J. Chromatogr. 323*:135 (1985).
19. Zeman, I., *Ibid. 363*:223 (1986).
20. Nozawa, A., and T. Ohnuma, *Ibid. 187*:261 (1980).
21. Kudoh, M., *Ibid. 291*:327 (1984).
22. Jones, P., and G. Nickless, *Ibid. 156*:87, 99 (1978).
23. Saito, T., and K. Hagiwara, *Fresenius' Z. Anal. Chem. 312*:533 (1982).
24. Ahel, M., and W. Giger, *Anal. Chem. 57*:2584 (1985).

[Received August 8, 1986; accepted December 15, 1989]
[JSD5031]