Analysis of Nonionic Surfactants in Bench-Scale Biotreater Samples[1]

S.T. Dubey*, L. Kravetz and J.P. Salanitro

Shell Development Company, Westhollow Technology Center, Houston, Texas 77251-1380

ABSTRACT: The effluents and activated sludges used in bench-scale biotreater units have been analyzed for nonionic alcohol ethoxylates and their residues. Separate bench-scale units were fed linear alcohol ethoxylates (AE), highly branched and branched nonylphenol ethoxylates. Effluents and sludges were first pretreated by a foam sublation technique to provide a gross separation of surfactants from the environmental matrix. This step was followed by normal-phase high-performance liquid chromatography (HPLC) with either fluorescence detection (FD) or evaporative light-scattering detection (ESLD). The AEs were derivatized with phenylisocyanate and analyzed by normal-phase HPLC coupled with FD. At extremely low surfactant levels, pretreatment of large sample volumes resulted in interferences on derivatization. Hence, a normal-phase HPLC method with ELSD was developed. Although some interferences do appear using ELSD, this method appears to be a more viable alternative to derivatization/FD for very low levels of AE. HPLC with FD and ELSD detection methods are more quantitative and provide information on the polyoxyethylene chain than is possible with traditional methods like cobalt–thiocyanate active substance.

JAOCS 72, 23–30 (1995).

KEY WORDS: Activated sludge, bench-scale biotreater, environmental analysis, evaporative light-scattering detection, fluorescence detection, HPLC, nonionic surfactants.

Surfactants are increasingly used in a variety of household, personal-care and industrial and institutional applications. Upon completion of their cleaning and/or processing functions, these surfactants are discharged into waste treatment plants and ultimately into various environmental compartments. For the end uses mentioned above, it is important to select surfactants which biodegrade in the waste treatment plants to products which are compatible with state and federal discharge regulations. The capability of analyzing for these surfactants throughout the stages of biotreatment has received much attention. For nonionic surfactants, methods such as cobalt thiocyanate active substance (CTAS) (1) or bismuth active substance (BiAS) (2) although simple to use result in interferences from the environmental matrices, show decreased sensitivity to lower polyoxyethylene (POE) chainlength and cannot differentiate between hydrophobes.

One approach to more specific determination of alcohol ethoxylates (AE) in environmental samples is the HBr cleavage–gas chromatography (GC) (3,4) procedure. The alkyl bromide cleavage products are determined by GC. However, HBr–GC provides no significant information about POE ether chainlength.

In recent years, high-performance liquid chromatography (HPLC) has emerged as the most useful technique to separate the distribution of ethylene oxide (EO) adducts comprising the POE chain. When coupled with ultraviolet (UV) (5) or fluorescence (6–8) detection (FD), this approach is useful for analysis of alkyl phenol ethoxylates (APE) in environmental samples.

For AE, derivatization with a chromophore-bearing agent (e.g., phenylisocyanate) has yielded phenylurethanes of the AEs which are detected by UV or fluorescence. However, when applied to low levels of AE in environmental samples, the derivatization step yields interferences from the environmental matrix which can interfere with the determination (9), since large quantities of sample are required for isolation of the surfactant.

More recently, HPLC coupled with evaporative light-scattering detection (ELSD) has been reported for analysis of surfactants in standard solutions (10). In ELSD, eluant from the HPLC column is nebulized and the nonvolatile components determined by light-scattering, thereby eliminating the need for a chromophore. The application of ELSD to environmental samples containing AE is discussed in this paper and compared with the CTAS and HPLC/FD approaches. A comparison of the advantages/disadvantages of CTAS, HPLC/ELSD and FD is given in Table 1.

This work is the analytical extension of a recently published bench-scale continuous activated sludge study (11). In that study, the biodegradation and effluent toxicities of a linear AE, a branched AE and a nonylphenol ethoxylate (NPE) were investigated.

EXPERIMENTAL PROCEDURES

Apparatus. Two research quality HPLC instruments were used. A Varian Vista 5500 (Palo Alto, CA), with a single piston reciprocating pump, was used with the FD. A Varian Star

[1]Presented at the 82nd AOCS Annual Meeting & Expo, May 1991, Chicago, Illinois.

*To whom correspondence should be addressed at Shell Development Co., Westhollow Technology Center, P.O. Box 1380, Houston, TX 77251-1380.

TABLE 1
Comparison of CTAS, HPLC/ELSD and HPLC/FD Methods for Analysis of AE in Aqueous Samples[a]

	Advantages	Disadvantages
CTAS	Does not require specialized instrumentation; easy wet chemistry method.	Most sensitive between EO_6 and EO_{25}; higher limits of detection (100 ppm) than HPLC/ELSD or FD; laborious and tedious; use of methylene chloride—a suspected carcinogen.
HPLC/ELSD	Molecule does not need a chromophore; no need of derivatization, hence, reduces interferences from matrix and time of analysis.	Could not quantitate EO_0[b] and EO_1[b]; higher limits of detection (100 ppb) than FD; very volatile components may escape detection, if detector and HPLC gradient not optimized.
HPLC/FD	Very low limits of detection—100 ppb for AE (Ref. 9); can detect all EOs	Molecule needs chromophore that absorbs in ultraviolet or fluorescence; molecule needs derivatization if no chromophore, which involves an extra analytical step; derivatization can lead to likely interferences from matrix.

[a]CTAS, cobalt thiocyanate active substance; HPLC/ELSD, high-performance liquid chromatography/evaporative light-scattering detector; HPLC/FD, HPLC/fluorescence detector; EO, ethylene oxide; AE, alcohol ethoxylates.
[b]This has changed with newer detectors on the market.

9010, also a single piston pump, was used with the ELSD. The FD, from Perkin-Elmer Corporation (Norwalk, CT), was an LC-250 luminescence detector with a 1.0 mm square flow cell and a 4-μL illuminated volume. The ELSD, a model 750/14 made by Applied Chromatography System, Ltd. (Macclesfield, United Kingdom) was obtained from Polymer Laboratories, Inc. (Amherst, MA). A Pickering Laboratories CHX 650 heater was used for controlling the temperature of the HPLC column. A VG Multichrom System from VG Instruments (Danvers, MA) was used for chromatographic data collection and processing.

Reagents. All reagents used were ACS reagent grade. These include ammonium thiocyanate, cobalt nitrate hexahydrate, hydrochloric acid, sodium bicarbonate, sodium chloride, anhydrous sodium sulfate and phenyl isocyanate. Solvents, 1, 2-dichloroethane (EDC), ethylacetate, isopropanol (IPA), methanol, methylene chloride, hexane and tetrahydrofuran (THF) used for sample preparation and HPLC work were glass-distilled and were Omni Solv® from EM Science (Cherry Hill, NJ). Isooctane was obtained from Burdick and Jackson (Muskegon, MI). Water used was from a Millipore (Milford, MA) Milli-Q system. Anion exchange resin (AG®1-X_4, Cl-form, 50–100 mesh) and cation exchange resin (AG®50W-X_4, acid form, 50–100 mesh) were from Bio-Rad Laboratories (Richmond, CA).

Standards. For NPE analysis, commercial samples of 4-nonylphenol and 4-nonylphenol ethoxylates having average EO content of 1,2 and 9 EO units per mole of nonylphenol were used as standards. For AE analysis, specific C_{12} AE having 1 to 8 EO per mole of alcohol and a commercial linear primary AE with an average of 9 EO units per mole of alcohol were used as standards.

The commercial nonionic surfactants used as standards and substrates in this study are listed with their main structural features and sources in Table 2 and will be referred to in this paper by their acronyms listed in column 2 of the table.

Biotreater samples. Samples preserved in 1–5% (vol) formalin were obtained from bench-scale biotreaters run at 25 and 8°C to simulate summer and winter conditions, respectively (11). Separate units were run for C_{12-15} AE-9(L), C_{13} AE-7(B) and NPE-9(B) and a sodium benzoate control. These biotreating experiments were reported in a previous study (11).

Isolation and CTAS analysis of surfactants from environmental matrices. The following procedure was used to isolate nonionic surfactants from sludges and effluents obtained in the bench-scale biotreater units: (i) Nonionic surfactants were

TABLE 2
Nonionics Tested and Analytical Standards Used

		Alkyl carbon number		
		Distribution		Average
Surfactant	Acronym	range	Average	EO
Linear C_{12-15} AE-9 EO[a]	C_{12-15} AE-9(L)	12–15	13.5	9
Linear C_{12} AE-1 to 8 Specific Ethoxylates[b]	C_{12} AE-(1–8)	12	12	1–8
Branched C_{13} AE-7 EO[c]	C_{13} AE-7(B)	11–15	13.4	7
Branched 4-nonylphenol[d]	NP	8–10	9	0
Branched NPE-1 EO[e]	NPE-1(B)	8–10	9	1
Branched NPE-2 EO[f]	NPE-2(B)	8–10	9	2
Branched NPE-9 EO[g]	NPE-9(B)	8–10	9	9

[a]NEODOL® 25-9 (Shell Chemical Co., Houston, TX); NPE, nonylphenol ethoxylates; other abbreviations as in Table 1.
[b]Imported by American Tokyo Kasei Inc. (Portland, OR).
[c]Made by lab-scale ethoxylation of Exxal 13 alcohol (Exxon Chemical Co., Houston, TX).
[d]Aldrich Chemical Company, Inc. (Milwaukee, WI).
[e]Surfonic N10 (Huntsman Corporation, Austin, TX).
[f]Surfonic N40 (Huntsman).
[g]Igepal CO-630 (Rhône-Poulenc Inc., Laurenceville, GA).

isolated using sublation followed by CTAS determination using a procedure described in the literature (1). The schematic of the sample preparation and analyses are shown in Figure 1. (ii) In addition, samples from the NPE biotreating units were subjected to steam distillation in a modification of a literature procedure (5) in which isooctane was used instead of cyclohexane. This modification permitted determination of short (NPE-0, 1, and 2) POE chains.

Derivatization with phenyl isocyanate. Derivatization was carried out only for AE in order to provide a chromophore for determination by HPLC/FD.

The standards [~0.005 μmoles each of C_{12}AE-(1–8) specific ethoxylates in 200 μL EDC] were transferred to a vial, to which was added 12 μL of a 25% phenyl isocyanate solution (~28 μmoles) in EDC. The vial was swirled, tightly capped and heated in a 60°C oven overnight (~15 h). Excess phenyl isocyanate was removed under a gentle stream of nitrogen at 100°C for 10 min. The residue was taken up in EDC (200 μL), which was then injected onto the HPLC column. The same procedure was followed for derivatizing the surfactants from the residue recovered from the sample preparation procedure.

HPLC analysis. Both for the analysis of NPE and AE, either by FD or ELSD, a normal-phase HPLC procedure was used, as outlined in Tables 3–5.

FD. For the analysis of NPE by FD, the HPLC column and conditions were similar to those used earlier (6) (Table 3), except that a 50 μL injection loop was used. For the analysis of derivatized AE by FD, the conditions are reported in Table 4.

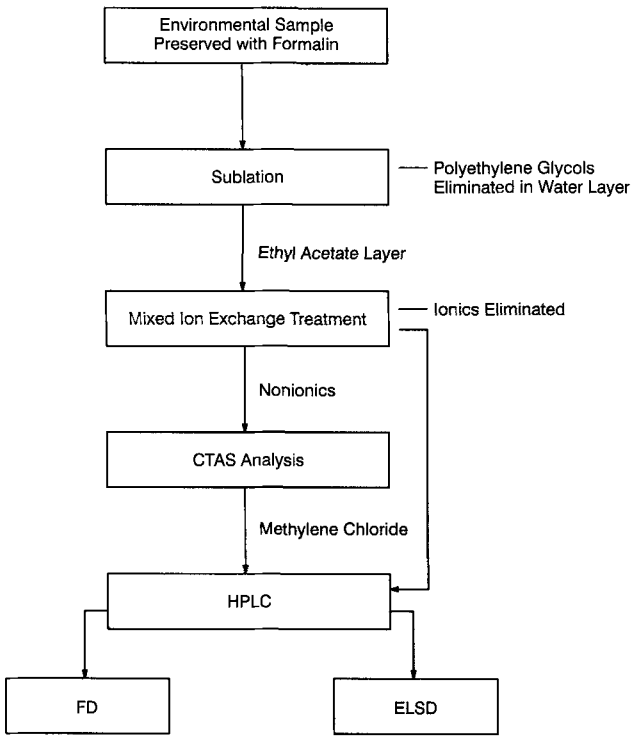

FIG. 1. Schematic of sample preparation analysis. HPLC, high-performance liquid chromatography; FD, fluorescence detection; ELSD, evaporative light-scattering detector; CTAS, cobalt–cyanate active substance.

TABLE 3
HPLC Conditions for Analysis of NPE-9(B) (8) by FD[a]

Column:	Microsorb™ cyanopropyl bonded, 5 micron, 4.6 × 250 mm		
Detector:	Perkin-Elmer[b] LC-240 luminescence detector, Ex = 230 nm, Em = 310 nm		
Solvent A:	20:80 (vol/vol) Tetrahydrofuran/hexane		
Solvent B:	90:10 (vol/vol) Isopropanol/water		
Flow Rate:	1.0 mL/min		
Injection volume:	50 μL		
HPLC gradient:	Time (min)	%A	%B
	0	99	1
	1.40	99	1
	1.50	97	3
	20	58	42
	22	58	42
	25	99	1
	35	99	1

[a]See Table 1 for abbreviations.
[b]Norwalk, CT.

ELSD. Underivatized AE, branched or linear, were separated on a Microsorb® cyanopropyl bonded column, 5 micron 4.6 × 250 mm, from Rainin Instrument Co., Inc. (Woburn, MA) and detected by ELSD. This method of detection has the advantage over UV and FD in that the presence of a chromophore is not required. The detector measures the refracted light from nonvolatile particles retained from the eluant of the HPLC column after it is nebulized and the solvent evaporated. The refracted light is proportional to the concentration of the solute species. The detector conditions and the HPLC gradient used are reported in Table 5.

Calculations. Response factors for NPEs with 0, 1 and 2 EO units were calculated from NP for nonylphenol and NPE-2 standard for nonylphenol with one and two EO groups. Response factors for NPE containing 3 to 18 ethoxylate groups were calculated using commercial NPE-9(B). The reason for

TABLE 4
HPLC Conditions for Analysis of Derivatized C_{12-15} AE-9(L) and C_{13} AE-7(B) by FD[a]

Column:	Zorbax® NH_2, 5 micron, 4.6 × 250 mm			
Detector:	Perkin-Elmer LC-240 luminescence detector Ex = 240 nm, Em = 310 nm			
Solvent A:	Hexane			
Solvent B:	Tetrahydrofuran			
Solvent C:	Isopropanol/water (90:10, vol/vol)			
Flow Rate:	1 mL/min			
Injection volume:	50 μL			
HPLC gradient:	Time (min)	%A	%B	%C
	0	100	0	0
	1.5	100	0	0
	2	80	20	0
	42	10	85	5
	48	10	85	5
	49	80	20	0
	53	80	20	0
	54	100	0	0
	63	100	0	0

[a]See Table 1 for abbreviations; Table 2 for source location.

TABLE 5
HPLC Conditions for Analysis of C_{12-15} AE-9(L) and C_{13} AE-7(B) by ELSD[a]

Column:	Microsorb® CN, 5 micron, 4.6 × 250 mm, 45°C			
Detector:	ACS Model 750/14			
	Temperature	35		
	Time constant	10		
	PMT	5		
	Nitrogen pressure	12 psi		
Solvent A:	Hexane			
Solvent B:	Tetrahydrofuran			
Solvent C:	Isopropanol/water (90:10, vol/vol)			
Flow Rate:	1.5 mL/min			
Injection volume:	100 µL			
HPLC gradient:	Time (min)	%A	%B	%C
	0	100	0	0
	3	100	0	0
	5	80	20	0
	20	52	30	18
	25	40	40	20
	26	80	20	0
	29	80	20	0
	30	100	0	0
	40	100	0	0

[a]See Table 1 for abbreviations.

using separate EO standards for NPE is that the commercial NPE-9(B) has very small amounts of 0, 1 and 2 EO units. Using response factors of lower EOs based on its calibration curve would contribute to large inaccuracies in results. From the HPLC data, areas were used to obtain area/µmole, which was normalized over the ethoxylate distribution and from the µg injected onto the column response factors (area/µg), were calculated for the standard. The sample areas obtained from HPLC were divided by response factors to obtain µg of each ethoxylate, which when divided by the original volume in mL or liters results in values in ppm or ppb.

In the analysis of AE by FD or ELSD, EO distributions in percent-weight of the commercial standards were obtained by an independent GC/HPLC procedure previously reported (11), to calculate response factors at ppm or ppb levels.

RESULTS AND DISCUSSION

CTAS results. The CTAS method is claimed to be most sensitive to surfactants with 6 to 25 EOs (1). Thus it probably underestimates nonionic surfactants having short POE chains. However, the CTAS method does provide a rough estimate of the nonionic surfactant, which is useful in determining the sample size needed for HPLC.

The results of CTAS analyses on both effluent and sludge samples at 25 and 8°C, are shown in Table 6. The relative standard deviation at the highest level analyzed (12 ppm) was ±1.6%, and at 0.3 ppm it was ±12.7%. With the exception of C_{13} AE-7(B) effluent at 25°C, the wet sludges at 8°C show the greatest amount of intact surfactant followed by the effluents at 8°C. Units at 25°C showed a more extensive surfactant biodegradation than those at 8°C, and the linear AE

TABLE 6
Comparison of CTAS Results for NPE-9(B), C_{13} AE-7(B) and C_{12-15} AE-9(L)

	CTAS (ppm)[a]		
	NPE-9(B)	C_{13} AE-7(B)	C_{12-15} AE-9(L)
Effluent[c]	4.1	2.4	0.27
Effluent[d]	0.73	3.6	0.02
Wet sludge[b,c]	11.9	4.9	0.45
Wet sludge[b,d]	2.0	0.18	0.09

[a]After subtraction of control benzoate CTAS values. See Tables 1 and 2 for abbreviations.
[b]Mixed liquor suspended solids.
[c]Influent concentration, 10 ppm; biotreater temperature, 8°C.
[d]Influent concentration, 50 ppm; biotreater temperature, 25°C.

biodegraded to a greater extent than the branched nonionics. In fact, at 25°C, C_{12-15} AE-9(L) does not show a significant CTAS response.

HPLC results: NPE-9(B). The recovery of the sample, preparation and HPLC methods was tested on a commercial NPE-9(B) blend. The normalized EO distribution, before and after sublation and ion-exchange, is shown in Figure 2, indicating that the methods do not change the EO distribution. Spike recoveries of NPE-9(B) in a benzoate control matrix are shown in Table 7.

The HPLC results for NPE-9(B) are given in Table 8. The relative standard deviation was ±1.7%. The NP, NPE-1 and NPE-2 values are based on results from steam distillation experiments. Chromatograms of a standard (1 ppm) NPE-9(B) and those of the effluents containing NPE-9(B) at 25 and 8°C are shown in Figure 3. Chromatograms of sludges are similar to those of effluents and are not shown. NPE surfactants remaining in effluents and sludges at 25°C showed an EO distribution skewed toward the lower EO homologs and confirms other reported observations (5) in environmental samples. Also, the largest increase over the normal EO distribution in the influent NPE-9(B) is the NPE-2 EO. These results are in line with a biodegradation mechanism for NPE in which the longer POE chains are shortened selectively to an average of approximately 2 EO units per molecule (12). In contrast, chromatograms of 8°C sludges and effluents did not show a skewed EO distribution. Note that biodegradation of

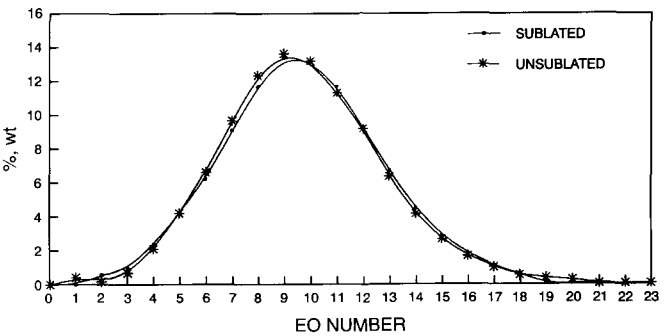

FIG. 2. Comparison of ethylene oxide (EO) distribution of nonylphenol ethoxylate (NPE)-9(B) by fluorescence detection in unsublated and sublated sample.

TABLE 7
Spike Recoveries[a] of NPE-9(B) and AE-9(L) from Matrices Used in Experiments

	Matrix	Spike level (ppb)	Spike recovery (%)	HPLC limit of detection (µg)
NPE-9(B)	Benzoate control	100	98	0.05 (FD)
AE-9(L)	Benzoate control	80	82.5	10 (ELSD)[b]
	Effluent	80	80	

[a]Spike recoveries of AE-7(B) are assumed to be as good as AE-9(L) or better. See Tables 1 and 2 for abbreviations.
[b]Based on 750/14 detector. There are currently more sensitive ELSDs available on the market.

the NPE-9(B) was significantly less at 8°C than at 25°C, and these results are comparable to those reported previously (13). The EO distribution in terms of normalized weight percent is shown in Figures 4 and 5 for 25 and 8°C effluents and sludges, respectively.

The above results also show that CTAS overestimated NPE levels remaining in the 8°C biotreater units and underestimated those values in the 25°C units. The underestimation occurs because at 25°C NPE biodegrades to intermediates with EO distributions skewed to shorter EO content, which responds minimally to CTAS.

C_{13} AE-7(B). Since C_{13} AE-7(B) or C_{12-15} AE-9(L) do not have chromophore groups, the usual method of analysis of such compounds is to carry out a derivatization procedure to introduce a chromophore that can be detected by UV. Although a number of derivatizing agents are available, phenyl isocyanate was chosen because of our previous studies (7). Both for C_{13} AE-7(B) and C_{12-15} AE-9(L), the environmental matrix was not separated completely from the surfactants, and these resulting carry-over impurities tended to derivatize as well, resulting in interferences in the chromatograms. This problem was greater for C_{12-15} AE-9(L) than for C_{13} AE-7(B), due to the larger amount of sample required to be analyzed for C_{12-15} AE-9(L).

To reduce or eliminate derivatizing impurities, the ELSD was used for the analysis of C_{12-15} AE-9(L) and C_{13} AE-7(B) as no chromophore is needed to detect surfactants by this detector. Bear (10) had used ELSD to analyze nonionic ethoxylated surfactants like AE-5(L), AE-7(L) and AE-11(L), although not at trace levels. The principle of detection is that the HPLC column eluant be evaporated by nebulizing with nitrogen gas in a heated tube, and the light scattered by the solute is measured after the solvent is volatilized. If the volatility of the solute is low compared to that of the eluant, the EO distribution of molecules like C_{12-15} AE-9(L) and C_{13} AE-7(B) could be detected. The volatility of the solute is dependent upon the nebulizer gas flow rate and the heated tube temperature. Even under optimized detector conditions it was

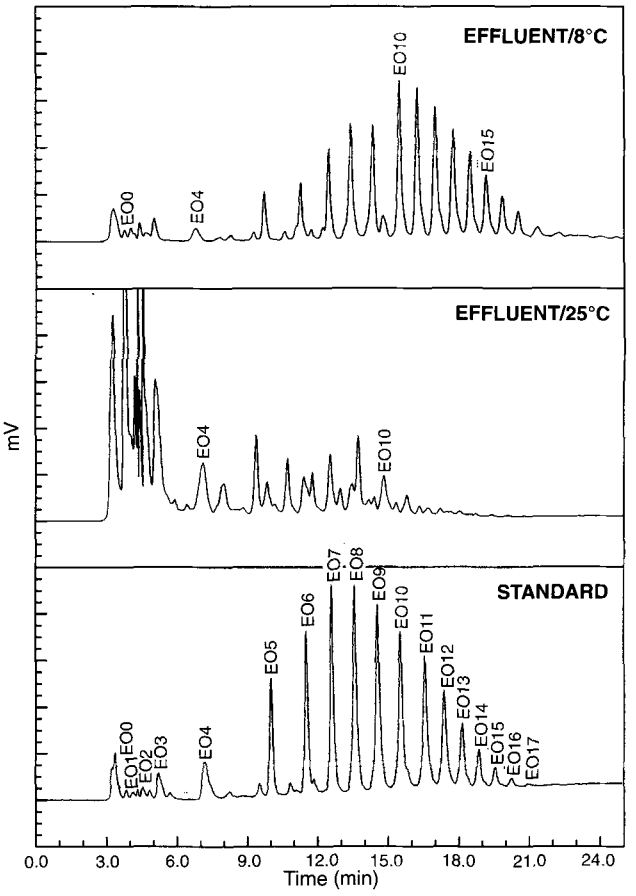

FIG. 3. Normal-phase high-performance liquid chromatography of NPE-9(B) by fluorescence detection. See Figure 2 for abbreviations.

TABLE 8
Summary of Results for NPE-9(B)

	Effluent (ppm, wt/vol)		Sludge (ppm, wt/vol)[a]	
	8°C[b]	25°C[c]	8°C[b]	25°C[c]
EO (0–3)[d]	0.12	2.5	0.41	10.8
EO (4–19)[d]	1.9	0.83	7.7	2.5
Total[d]	2.0	3.4	8.1	13.3
CTAS	4.1	0.73	11.9	2.0

[a]Suspended solids = 4000 mg/L. See Tables 1 and 2 for abbreviations.
[b]Influent: 10 ppm.
[c]Influent: 50 ppm.
[d]By HPLC/ELSD.

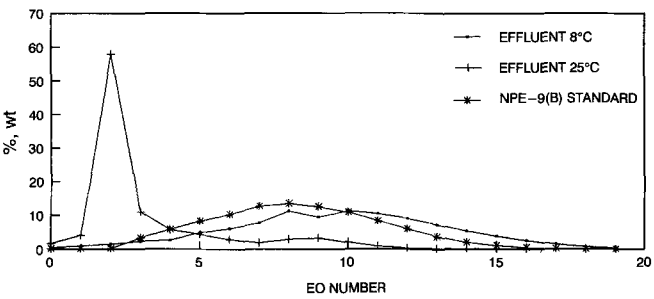

FIG. 4. Ethylene oxide (EO) distribution of NPE-9(B) in effluent by high-performance liquid chromatography/fluorescence detection. See Figure 2 for abbreviation.

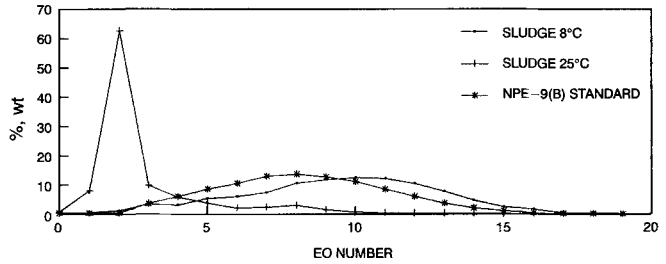

FIG. 5. EO distribution of NPE-9(B) in sludges by high-performance liquid chromatography/fluorescence detection. See Figure 2 for abbreviations.

TABLE 9
Summary of Results for C_{13} AE-7(B)

	Effluent (ppm, wt/vol)		Sludge (ppm, wt/vol)[a]	
	8°C[b]	25°C[c]	8°C[b]	25°C[c]
EO (3–20)[d]	1.8	2.3	3.7	0.5
EO(3–20)[e]	1.6	1.8	3.6	0.3
CTAS	2.4	3.6	4.9	0.2

[a]Suspended solids = 4000 mg/L. See Table 1 for abbreviations.
[b]Influent: 10 ppm.
[c]Influent: 50 ppm.
[d]By HPLC/ELSD.
[e]By derivatization with phenyl isocyanate followed by HPLC/FD.

not possible to detect EO_0 or EO_1 for either C_{12-15} AE-9(L) or C_{13} AE-7(B). EO_2 could be detected at surfactant-levels greater than 20–50 µg injected on column. We were unsuccessful in detecting EO_0, EO_1 and EO_2 at low concentrations in either C_{12-15} AE-9(L) or C_{13} AE-7(B). Also, in the concentration range of 25–150 µg of surfactant standard injected, the response factor for individual EOs was a quadratic rather than a linear function of concentration. Hence, in the analysis of unknown samples, care was taken to stay within the calibration concentration range. ELSD, on the whole, was a better alternative than derivatization/FD, especially if sufficient sample was available.

Chromatograms of C_{13} AE-7(B) standard and its treated effluent at 25 and 8°C by ELSD are shown in Figure 6. HPLC results for C_{13} AE-7(B) are also shown in Table 9. There was some coelution of contaminant species in the area where EO_3 and EO_4 elute, and with the 25°C sludge samples coelution of contaminant species occurred at the same retention time as EO_8 and EO_9. The EO distribution of effluents and sludges at 8 and 25°C are shown in Figures 7 and 8, respectively. Unlike the NPE, the EO distribution for the C_{13} AE-7(B) in the 3–20 EO range does not differ from that of the standard. This suggests that the branched AE biodegraded by a mechanism

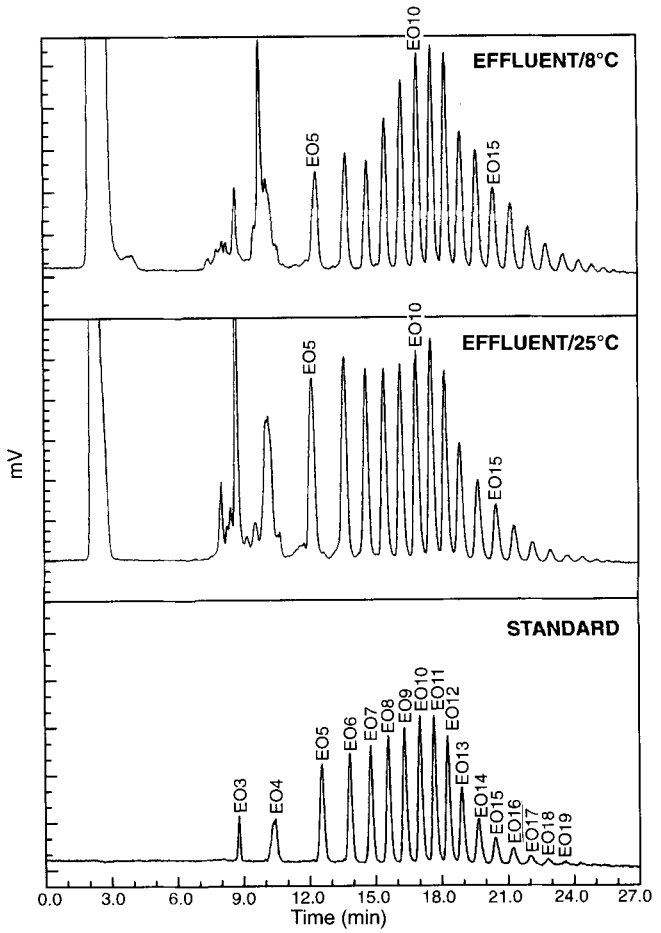

FIG. 6. Normal-phase high-performance liquid chromatography of C_{13} alcohol ethoxylate-7(B) by evaporative light-scattering detection. See Figure 2 for abbreviation.

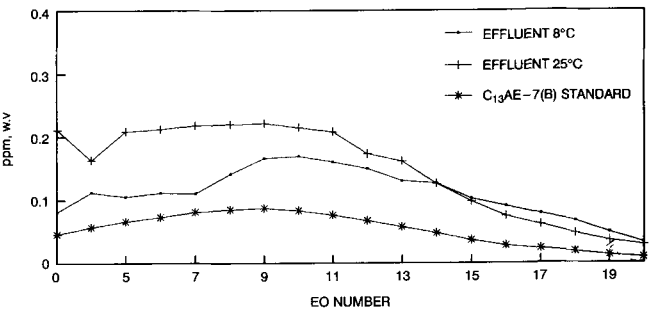

FIG. 7. Ethylene oxide (EO) distribution of C_{13} alcohol ethoxylate-7(B) in effluents by high-performance liquid chromatography/evaporative light-scattering detection. AE, alcohol ethoxylate.

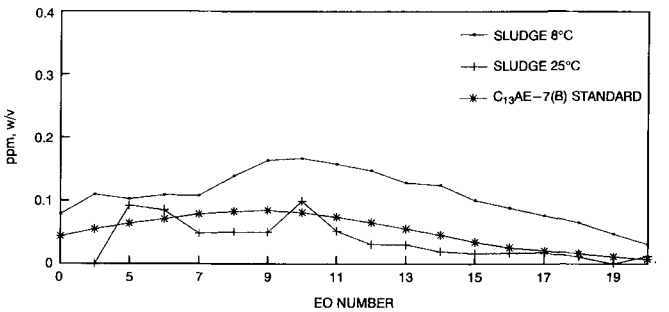

FIG. 8. EO distribution of C_{13} AE-7(B) in sludges by high-performance liquid chromatography/evaporative light-scattering detection. See Figure 7 for abbreviations.

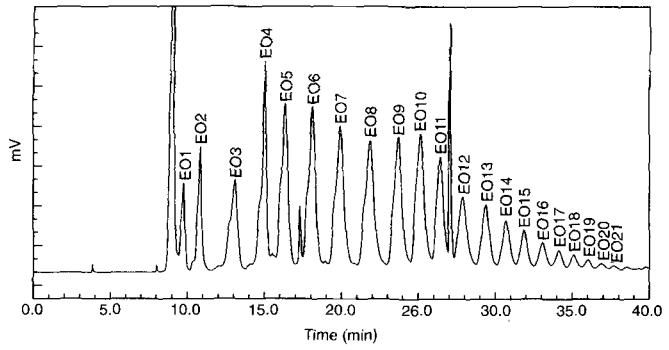

FIG. 9. Normal-phase high-performance liquid chromatography of C_{13} AE-7(B) standard by derivatization/fluorescence detection. See Figure 7 for abbreviations.

in which the POE chain is initially cleaved from the hydrophobe (14). Since the EO distribution for C_{13} AE-7(B) in effluents was similar to that of the intact surfactant, it appears that some species of this surfactant had not yet begun to degrade. It is likely that these species are heavily enriched with quaternary carbon suggesting that the branched AE biodegrades by a mechanism in which the POE chain is initially cleaved from the hydrophobe.

In addition, the higher EOs do not decrease, but maintain their distribution as in the standard. The HPLC results for C_{13} AE-7(B) show the same trend as observed for NPE-9(B) in that the levels of EO_{3-20} are lower than the CTAS value. The results of derivatization/FD for C_{13} AE-7(B) and C_{12-15} AE-9(L) are also included and show results similar to those obtained by ELSD (Figs. 9 and 10).

C_{12-15} AE-9(L). Large amounts of the C_{12-15} AE-9(L) treated effluent were sublated, and the matrix clean-up procedure showed interference peaks both in the ELSD and derivatization/FD chromatograms. The control benzoate samples also had matrix interferences. The C_{12-15} AE-9(L) chromatograms in derivatization/FD showed nondetectable signals over those of the control for up to 500 mL of sublated effluent sample. Increasing the sample size to increase detection limit just increases the interferences. When using the ELSD technique, however, there was some signal above the noise level beyond a retention time of 13 min (corresponding to EO_7) in the chromatogram when sublating 500 mL of sample. Using that part of the chromatogram and a standard at the lowest possible detection level (12.5 µg), the best estimate of intact surfactant in the effluent or sludge at 25 or 8°C was ≤0.1 ppm. The assumption made was that the EO distribution of the surfactant under the interfering peaks is the same as that in the original surfactant in the influent. This assumption is based on reported studies which suggest that linear AE biodegrades by a mechanism in which the hydrophobe is initially separated from the POE chain (12). Spike recoveries of

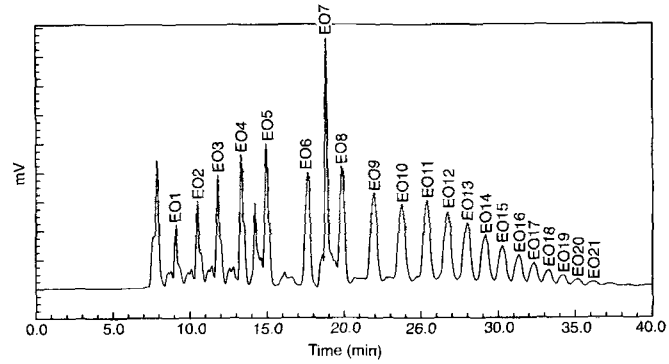

FIG. 10. Normal-phase high-performance liquid chromatography of C_{12-15} AE-9(L) standard by derivatization/fluorescence detection. See Figure 7 for abbreviations.

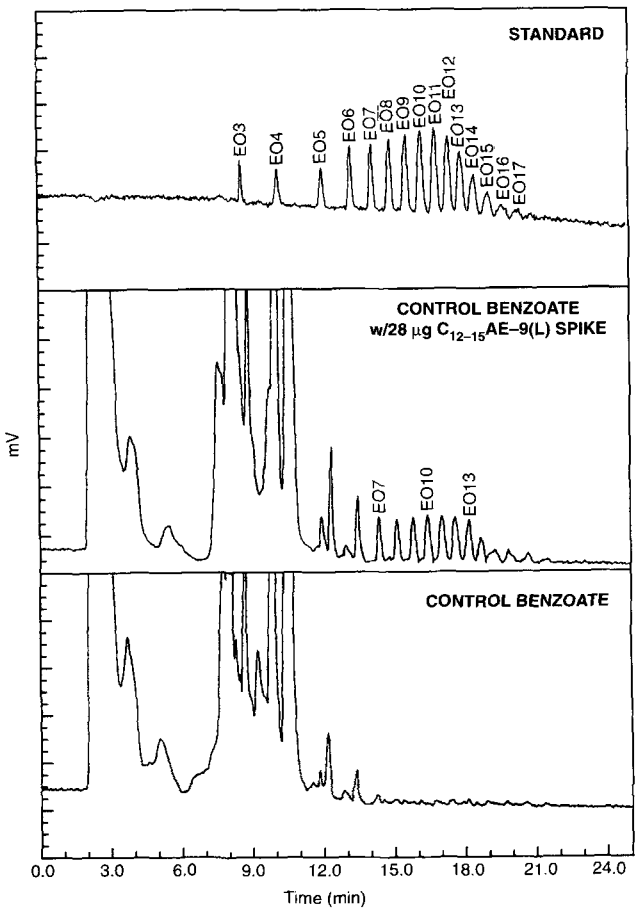

FIG. 11. High-performance liquid chromatography of control benzoate spiked with C_{12-15} AE-9(L) by evaporative light-scattering detection. See Figures 2 and 7 for abbreviations.

TABLE 10
Summary of Results for C_{12-15} AE-9(L)

	Effluent (ppm, wt/vol)		Sludge (ppm, wt/vol)[a]	
	8°C[b]	25°C[c]	8°C[b]	25°C[c]
EO (3–20)[d]	<0.1	<0.1	<0.1	<0.1
CTAS	0.27	<0.02	0.45	0.1

[a]Suspended solids = 4000 mg/L. See Table 1 for abbreviations.
[b]Influent: 10 ppm.
[c]Influent: 50 ppm.
[d]By HPLC/ELSD.

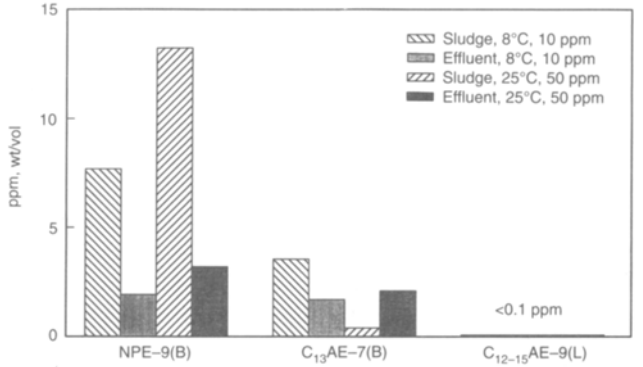

FIG. 12. Comparison of high-performance liquid chromatography results for NPE-9(B), C_{13} AE-7(B) and C_{12-15} AE-9(L). See Figures 2 and 7 for abbreviations.

TABLE 11
Comparison of HPLC Results for NPE-9(B), C_{13} AE-7(B) and C_{12-15} AE-9(L)

	Effluent (ppm, wt/vol)		Sludge (ppm, wt/vol)[a]	
	8°C[b]	25°C[c]	8°C[b]	25°C[c]
NPE-9(B)	2.0	3.4	7.9	13.3
C_{13} AE-7(B)	1.7	2.0	3.6	0.3
C_{12-15} AE-9(L)	<0.1	<0.1	<0.1	<0.1

[a]Suspended solids = 4000 mg/L. See Tables 1 and 2 for abbreviations.
[b]Influent 10 ppm.
[c]Influent 50 ppm.

AE-9(L) are given in Table 7, and chromatograms of the control and spiked control are shown in Figure 11.

The HPLC results for C_{12-15} AE-9(L) show a trend similar to the CTAS values (Table 10) and those reported by Schmitt *et al.* (9) in which effluent streams with 0.32 ppm CTAS produced HPLC results in the range of 0.01–0.02 ppm, i.e., <0.1 ppm. Since HPLC data are more specific than CTAS for nonionics, the above results show CTAS values tend to overestimate nonionic surfactant levels in waste treatment plant effluents.

Comparison of HPLC results for NPE-9(B), C_{13} AE-7(B) and C_{12-15} AE-9(L). HPLC results for the three surfactant types are compared in Table 11 and Figure 12. These data show that biotreater sludge and effluent contain significant levels of branched [C_{13} AE-7(B) and NPE-9(B), but not linear C_{12-15} AE-9(L)] nonionic surfactant isomers. In the case of the branched nonionic surfactants (at 25°C in both sludge and effluent), the distribution of the EOs is skewed toward the lower EOs for NPE-9(B). The EO distribution trend for the C_{13} AE-7(B) was difficult to interpret because of matrix interference and/or inability to detect lower EOs. However, concentration of higher EOs in effluent and sludge at 25°C appears to match that of the intact original surfactant and was unlike that of NPE-9(B). The HPLC results for the C_{12-15} AE-9(L) show the same large decreases in all isomers suggesting that the initial step in linear AE biodegradation is enzymatic scission of hydrophobe from the hydrophile (11) for all the isomers present in the intact surfactant.

ACKNOWLEDGMENTS

We wish to acknowledge the efforts of R.L. Mueller and E.K. Nkomo for carrying out the sample preparation and the HPLC analyses, and L.A. Diaz for setting up and collecting the biodegradation data.

REFERENCES

1. 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).
2. Wickbold, R., *Tenside Detergents 9*:173 (1972).
3. Kaduji, I.I., and J.B. Sted, *Analyst 101*:728 (1976).
4. Wee, V.T., *Advances in the Identification and Analysis of Organic Pollutants in Water,* Vol. 1, Ann Arbor Science Publishers, Inc., 1981, Chapter 30.
5. Ahel, M., and W. Giger, *Anal. Chem. 57*:2584 (1985).
6. Kudoh, M., H. Ozawa, S. Fudano and K. Tsuji, *J. Chromatogr. 287*:337 (1984).
7. Holt, M.S., E.H. McKerrell, J. Perry and R.J. Watkinson, *Ibid. 362*:419 (1986).
8. Kubeck, E., and C.G. Naylor, *J. Am. Oil Chem. Soc. 67*:400 (1990).
9. Schmitt, T., M.C. Allen, D.K. Brain, K.F. Guin, D.E. Lemmel and Q.W. Osburn, *Ibid. 61*:103 (1990).
10. Bear, G.R., *J. Chromatogr. 459*:91 (1988).
11. Kravetz, L., J.P. Salanitro, P.B. Dorn and K.F. Guin, *J. Am. Oil Chem. Soc. 68*:610 (1991).
12. Swisher, R.D., *Surfactant Biodegradation,* Surfactant Science Series, Vol. 18, Marcel Dekker, Inc., New York, 1987.
13. Kravetz, L., H. Chung, K.F. Guin, W.T. Shebs and L.S. Smith, *Tenside Detergents 21*:1 (1984).

[Received April 11, 1994; accepted October 20, 1994]