Journal of Chromatography, 673 (1993) 145–156
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 905

Rapid separation of non-ionic surfactants of polyethoxylated octylphenol and determination of ethylene oxide oligomer distribution by C1 column reversed-phase liquid chromatography

Zhendi Wang* and Merv Fingas

Emergencies Science Division, River Road Environmental Technology Centre, Environment Canada, 3439 River Road, Ottawa, K1A 0H3 (Canada)

(First received October 28th, 1992; revised manuscript received January 25th, 1993)

ABSTRACT

A rapid, simple and reproducible reversed-phase high-performance liquid chromatographic (HPLC) method was developed for the separation and characterization of individual oligomers in polyethoxylated octylphenol (PEOP) surfactants with high resolution and sensitivity, using a C1 trimethylsilyl (TMS) column. With this technique, oligomers of PEOP surfactants containing up to 40 ethylene oxide units (molecular mass distributions up to *ca.* 2000) were successfully resolved and identified. The oligomer distribution of the eight PEOP surfactants studied were calculated and depicted graphically. The effects of the mobile phase composition and of the solvents used to prepare PEOP surfactant solutions on the separation of oligomers were investigated in detail.

INTRODUCTION

Polyethoxylate alkylphenols (PEAP) are widely used as non-ionic surfactants in liquid laundry detergents, wetting agents and emulsifiers and in institutional and industrial cleaners [1,2]. They are manufactured by the addition of ethylene oxide (EO) to alkylphenols. The highly branched 4-octyl- and 4-nonylphenols are often the main raw materials used to manufacture polyethoxylated alkylphenols. The distribution of oligomers with varying lengths of the polyethoxy chain is determined mainly by the relative rates of ethoxylation of the ethylene oxide to alkylphenol in the initiation and propagation steps. As a result of their method of synthesis, these surfactants are complex mixtures in which the oligomer index (the number of EO units in each individual oligomer) varies over a considerable range.

Ethoxylated surfactants provide the blender with more versatility than their ionic counterparts. In the latter, the solubilizing power of the sulphate and sulphonate group is fixed, and it is the hydrophobic moiety which must be modified to produce differing hydrophilic–lipophilic balance (HLB) values. The hydrophilic characteristic of the ethoxylate, however, is attributed to the hydration of the ether-link oxygen atoms in the chain via hydrogen bonding, and therefore increases with increasing number of EO units. The distribution of oligomers can affect the chemical and end-use properties of these surfactants. Hence its reliable determination is important for both performance and quality control considerations. Environmental concern is another important reason to know both the

* Corresponding author.

0021-9673/93/$06.00

concentration of surfactants in water and oligomer distribution. Surfactants can have high abundance in wastewater. It has been suggested that the toxicities increase with decreasing length of the polyethoxylate chains [3].

The separation and identification of such complex samples is not trivial, and much research has been carried out in this area. Some classical and non-specific methods including metal ion complexation of the polyethoxylate chain, potentiometric titration and fission techniques [4] have been used to measure the total contents of non-ionic surfactants. These methods fail, however, to differentiate between PEAP and linear alcohol polyethoxylates, which are also widely used as non-ionic surfactants. In addition, they cannot give any information about oligomer distributions and are time consuming to perform.

Various other methods using gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS) with chemical ionization have been carried out in order to identify oligomers of PEAP. Analysis by GC is currently limited to oligomers containing relatively few ethylene oxide units. The formation of volatile derivatives prior to GC analysis is usually required [5,6] because of the lack of volatility of most non-ionic surfactants. High-temperature GC can be extended to the analysis of higher molecular mass components. For example, the high-temperature GC analysis of Triton X-100 has been reported [7]. Stephanou [8,9] reported a method using GC–MS to identify alkylphenols and linear polyethoxylated alcohols in untreated municipal wastewater. Another attempt to determine the EO distribution in complex mixtures of ethoxylated alcohols was the application of probe-distillation chemical ionization mass spectrometry [10].

One of the most suitable techniques for determining non-ionic surfactants and the oligomer distribution of PEAP is high-performance liquid chromatography (HPLC). The characterization of these surfactants has generally been performed with normal-phase columns [11–15]. The initial work on the use of normal-phase LC, with UV detection at 277 nm, to separate some commercial polyethoxylated surfactants was done by Huber *et al.* [16].

Giger and co-workers [17–20] reported on reversed-phase HPLC methods for the simultaneous determination of linear alkylbenzenesulphonates (LAS) and total polyethoxylated alkylphenols in wastewater and other environmental samples. In their methods, all oligomers of PEAP eluted as one or two peaks and the determination of individual oligomers required additional information attainable from normal-phase HPLC. Allen and Rice [21] reported the separation of alkylphenol ethoxylate adducts with up to nine or ten ethylene oxide units by reversed-phase HPLC. To our knowledge, the use of reversed-phase conditions for the separation and identification of individual oligomers of PEAP surfactants with up to 40 EO units (molecular mass distribution up to *ca.* 2000) has not previously been reported.

Escott and Chandler [22] recently reported the application of the LC–thermospray MS for the analysis of mixtures of sulphonated surfactants with in-line UV and on-line MS detection. This technique, as reported, can achieve a high degree of selectivity for the analysis of sulphonated surfactants with the addition of a sufficiently volatile ionic material to the mobile phase.

This paper presents a rapid and simple approach for the separation and identification of polyethoxylated alkylphenols with high resolution and sensitivity, and the determination of the distribution of oligomers with satisfactory reproducibility using reversed-phase HPLC with a C1 TMS (trimethylsilyl) column.

EXPERIMENTAL

Materials

The polyethoxylated octylphenol surfactant samples (Triton is the trade name for many of these surfactants) were purchased from Sigma (St. Louis, MO, USA) and Rohm and Haas (Philadelphia, PA, USA). These octylphenol-based products contained ethoxy oligomers with varying numbers of ethoxy units. PEAP, with an average of <6 EO units, will not dissolve in water (a dispersion forms), but will dissolve in methanol. PEAP, with an average of >7 EO units dissolves in both water and methanol. The sample solutions of the surfactants studied were

prepared in HPLC-grade methanol and deionized water as follows: octylphenol (0.02 μg/μl), Triton X-15 (0.02 μg/μl), Triton X-35 (0.1 μg/μl) and Triton X-45 (0.1 μg/μl) in methanol; Triton X-114 (0.2 μg/μl), Triton X-100 (0.2 μg/μl), Triton X-102 (0.5 μg/μl), Triton X-165 (0.8 μg/μl) and polyethoxylated ($n = 30$) octylphenol (1.0 μg/μl) in water. The standard solution used for testing the reversed-phase HPLC system was *tert.*-octylphenol in methanol.

Chromatography

The method development work was performed on a Shimadzu system, consisting of two LC-600 pumps, an SCL-6B system controller and a variable-wavelength SPD-6AV UV–Vis detector. The chromatograms with peak areas and retention times were recorded on a Shimadzu CR-601 Chromatopac integrator. The attenuation of the integrator was set at 4 or 5 in most measurements.

The chromatographic separation was carried out isocratically in the reversed-phase mode with a 150 mm × 4.6 mm I.D. stainless-steel C1 TMS column (CSC-C1 or Supelcosil LC-1 column, particle packing size 5 μm) purchased from Chromatography Sciences (Montreal, Canada) and Supelco (Bellefonte, PA, USA), respectively.

The mobile phase used was a mixture of deionized water (0.02 g/l of ammonium acetate was added as to improve the baseline stability) and 50–62% of HPLC-grade methanol, depending on the sample to be analysed. The column effluent was monitored at 225 nm (deuterium lamp, flow cell volume 8 μl), which is the approximate wavelength of maximum absorption of polyethoxylate octylphenols obtained from UV spectrophotometric scanning. The flow-rate was maintained at 1.0 ml/min and the column was maintained at ambient temperature (22 ± 1°C). The injection system was a Rheodyne Model 7125 sample injector equipped with a 20-μl sampling loop.

Quantification

Because all oligomers have almost identical molar absorptivities (this will be discussed later), the integrated peak area can be used directly to determine the mole fraction of each oligomer. The following equation shows how the mole fractions are related to peak area:

$$F_i = \frac{A_i}{\sum_{i=0}^{n} A_i} \quad (1)$$

where F is the mole fraction of each oligomer, A represents the peak area of the oligomer and i is the number of EO units in the ethoxylate adducts.

The average EO number may be calculated for each sample using the following equation:

$$\bar{n} = \frac{\sum_{i=0}^{n} A_i n_i}{\sum_{i=0}^{n} A_i} = \sum_{i=0}^{n} n_i F_i \quad (2)$$

where $\bar{n}$ is the average EO number of the sample and n is the number of EO units in each oligomer. After $\bar{n}$ has been obtained, the average molecular mass of each polyethoxylated alkylphenol sample can be determined with the following relationship:

$$\overline{M_r} = (M_r)_R + \bar{n}(M_r)_{EO} \quad (3)$$

where $(M_r)_R$ is the molecular mass of the octylphenol group (t-$C_8H_{17}C_6H_5$–) and $(M_r)_{EO}$ is the molecular mass of an ethylene oxide unit (CH_2CH_2O–).

If the mass fraction (M_i) of an individual oligomer containing n EO units is required, it can be determined using the following equation:

$$M_i\,(\%) = \frac{F_i[(M_r)_R + n(M_r)_{EO}]}{\overline{M_r}} \quad (4)$$

RESULTS AND DISCUSSION

Characterization of surfactant samples

In order for the method to be able to produce quantitative distributions, the following conditions should be met: (1) satisfactory separation of individual components; (2) identification of the ethoxylate peaks; (3) knowledge of the response factors of individual oligomers; and (4) sensitivity and reproducibility of detection.

Various columns and HPLC conditions were

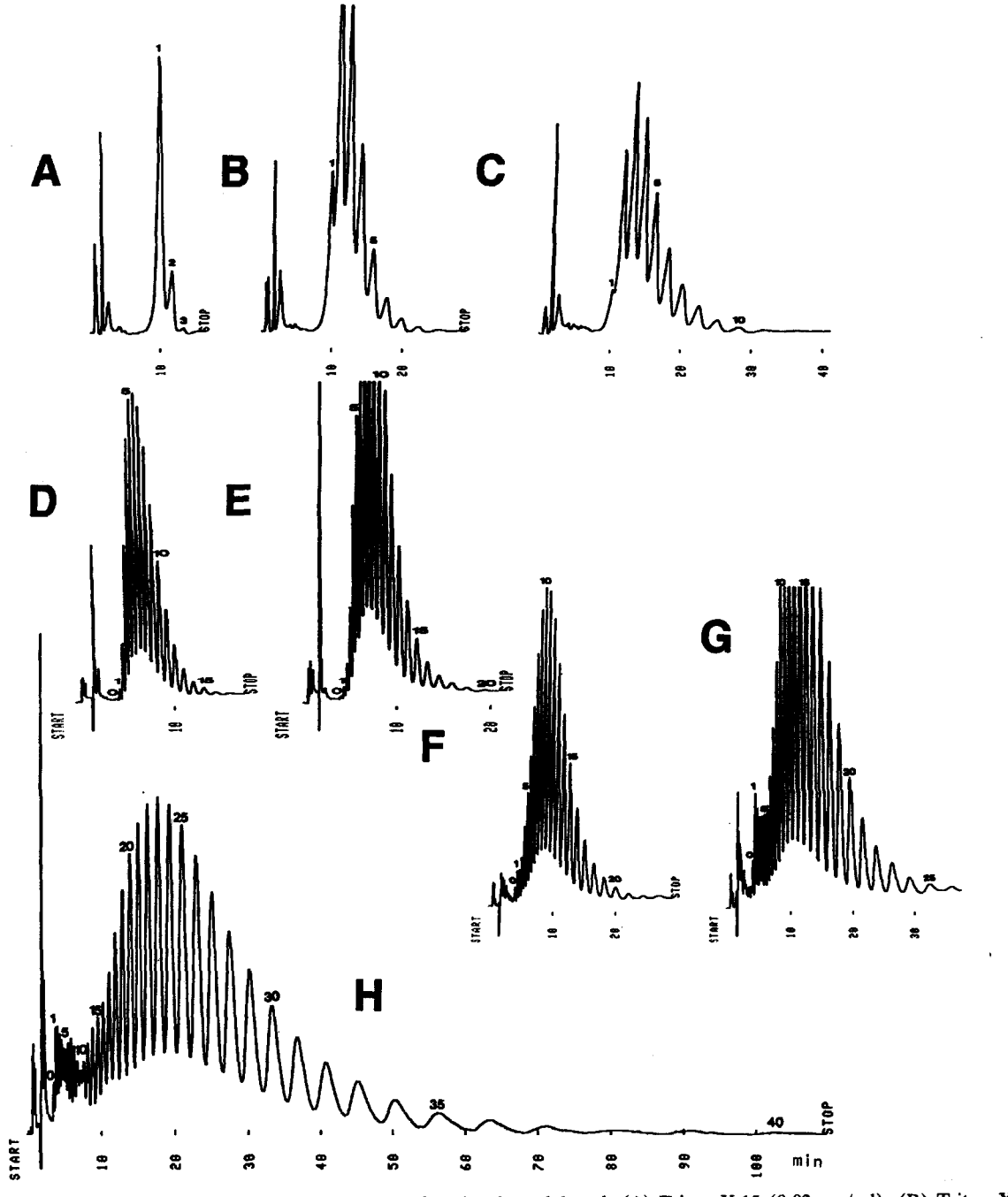

Fig. 1. HPLC of non-ionic surfactants of polyethoxylated octylphenol. (A) Triton X-15 (0.02 mg/ml); (B) Triton X-35 (0.1 mg/ml); (C) Triton X-45 (0.1 mg/ml); (D) Triton X-114 (0.2 mg/ml); (E) Triton X-100 (0.2 mg/ml); (F) Triton X-102 (0.5 mg/ml); (G) Triton X-165 (0.8 mg/ml); (H) POE (30) octylphenol (1.0 mg/ml). Conditions: CSC-C1 TMS column; temperature, 22 ± 1°C; mobile phase, methanol–water [(A)–(C) 53:47; (D)–(H) 60:40]; elution mode, isocratic; flow-rate, 1.0 ml/min; UV detection at 225 nm. The numbers assigned to the individual peaks represent the number of EO units in the oligomers; 0 represents the parent *tert.*-octylphenol. The integrator attenuation was set at 3, 4 or 5 according to the intensities of the peaks.

studied. The Wescan RP/S surfactant column separated ionic sulphate and sulphonate surfactants, but failed to separate polyethoxylated alkylphenols. Both the Supelco LC-1 C1 (TMS) column and CSC Spherisorb C1 (TMS) column separated PEAP oligomers, but better peak shapes and resolution and higher sensitivities were obtained using the latter column under comparable HPLC conditions. Consequently, all work further reported here was carried out using the CSC C1 column.

It has been demonstrated that the alkylphenol oligomers containing different numbers of EO units have nearly identical molar absorptivities at the selected UV detection wavelength because the phenyl ring is the only chromophore in the molecules [23]. Therefore, HPLC with UV detection offers the advantage that the molar response factors for the individual oligomers can be taken as identical, and serve as the basis to determine the EO distribution and average EO mole number directly from the integrated peak areas.

Fig. 1A–H show the chromatograms of eight samples of octylphenol polyethoxylate mixtures with 1–40 EO units. It can be seen that oligomers are well separated. Peak broadening is evident with increase in the number of EO units of oligomers. The small characteristic peak which was eluted prior to ethoxylate adducts and was detected in most of the samples is not part of the surfactant but is attributed to the parent *tert.*-octylphenol (EO = 0) remaining unconverted.

The numbers assigned to the individual peaks in Fig. 1A–H represent the number of EO units in each oligomer. Because of the lack of single-EO-number alkylphenol standards, the identification of these peaks was based on a comparison of the retention times with those of reference materials octylphenol (EO = 0) and Triton X-15 [its major component being EO (1) octylphenol], and on the well accepted assumption that peaks differ from each other by only 1 EO unit [16,23–25].

Reproducibility was measured by repeated analyses of the same sample. If not specifically mentioned, the measurement was carried at least four times ($n = 4$). It should be noted that the luminance of the deuterium lamp decreases as time elapses, which affects the detection sensitivity of the UV detector for the same samples.

TABLE I

RETENTION TIME AND PEAK AREA REPRODUCIBILITY IN ANALYSIS OF TRITON X-114

Peak	Retention time (min)				Peak area (counts)				Average ($\bar{x}$) (counts)	S.D. (counts)	R.S.D. (%)	F (%)[a]
	Run 1	Run 2	Run 3	Run 4	Run 1	Run 2	Run 3	Run 4				
1	4.625	4.625	4.622	4.613	12 121	11 868	11 026	11 028	11 511	568	4.93	0.311
2	4.922	4.987	4.983	4.977	63 715	63 319	63 525	61 452	63 003	1046	1.66	1.705
3	5.377	5.373	5.368	5.362	191 182	175 703	188 503	184 733	185 030	6757	3.65	5.007
4	5.770	5.765	5.758	5.750	355 404	324 687	345 217	338 206	340 879	12 898	3.78	9.224
5	6.100	6.177	6.170	6.167	443 634	411 401	433 946	423 345	428 082	13 867	3.24	11.584
6	6.638	6.633	6.625	6.620	486 022	455 984	475 745	467 963	471 429	12 677	2.69	12.757
7	7.120	7.115	7.108	7.100	499 764	483 654	496 802	491 002	492 806	7104	1.44	13.336
8	7.648	7.642	7.635	7.625	467 690	457 712	463 662	466 086	463 788	4376	0.94	12.550
9	8.225	8.222	8.217	8.205	396 676	387 800	395 056	393 334	393 217	3860	0.98	10.641
10	8.872	8.865	8.863	8.848	302 709	295 058	305 588	300 478	300 958	4455	1.48	8.144
11	9.587	9.577	9.580	9.562	212 793	206 667	221 664	212 711	213 459	6177	2.89	5.776
12	10.385	10.368	10.373	10.355	138 749	136 446	150 785	142 299	142 070	6289	4.43	3.844
13	11.270	11.243	11.258	11.240	84 957	82 894	89 696	86 018	85 891	2849	3.32	2.324
14	12.253	12.225	12.250	12.225	46 468	46 890	50 064	50 481	48 476	2089	4.31	1.312
15	13.380	13.325	13.335	13.325	28 888	28 968	30 396	31 178	29 858	1120	3.75	0.808
16	14.920	14.533	14.467	14.538	17 603	17 200	18 248	16 390	17 360	778	4.48	0.470
17	15.990	15.933	15.962	15.893	7681	7129	7569	8104	7621	401	5.26	0.206
Total area (counts)					3 756 056	3 593 380	3 747 492	3 684 808	3 695 434	65 025	1.76	
Average EO number (n)							7.43					

[a] Mole fraction of oligomers.

TABLE II

COMPARISON OF R.S.D. BY TWO CALCULATION METHODS IN ANALYSIS OF TRITON X-114

Peak	Area percentage				Average ($\bar{x}$) (%)	S.D. (%)	R.S.D. (%)	R.S.D. (%) (by area calc.)
	Run 1	Run 2	Run 3	Run 4				
1	0.323	0.330	0.294	0.299	0.311	0.018	5.79	4.93
2	1.696	1.762	1.695	1.668	1.705	0.040	2.35	1.66
3	5.090	4.890	5.030	5.013	5.007	0.084	1.68	3.65
4	9.462	9.036	9.212	9.178	9.223	0.177	1.92	3.78
5	11.811	11.449	11.580	11.489	11.583	0.162	1.40	3.24
6	12.940	12.690	12.695	12.700	12.756	0.123	0.96	2.69
7	13.306	13.460	13.257	13.325	13.337	0.087	0.65	1.44
8	12.452	12.738	12.373	12.649	12.552	0.169	1.35	0.94
9	10.561	10.792	10.542	10.675	10.642	0.116	1.09	0.98
10	8.059	8.211	8.154	8.155	8.145	0.063	0.77	1.48
11	5.665	5.751	5.915	5.773	5.776	0.104	1.80	2.89
12	3.694	3.797	4.024	3.862	3.844	0.138	3.59	4.43
13	2.262	2.307	2.393	2.334	2.324	0.055	2.37	3.32
14	1.237	1.305	1.336	1.370	1.312	0.057	4.34	4.31
15	0.769	0.806	0.811	0.846	0.808	0.032	3.96	3.75
16	0.469	0.479	0.487	0.445	0.470	0.018	3.83	4.48
17	0.204	0.198	0.202	0.220	0.206	0.010	4.85	5.26
Total (%)	100	100	100	100	100			
Average EO number (n)					7.43			

TABLE III

MOLE FRACTION (F) AND AVERAGE EO NUMBER OF TRITON X-15, X-35 AND X-45

Peak	Triton X-15			Triton X-35			Triton X-45		
	F (%)	S.D. (%)	R.S.D. (%)	F (%)	S.D. (%)	R.S.D. (%)	F (%)	S.D. (%)	R.S.D. (%)
1	80.992	0.092	0.11	11.898	0.242	2.03	3.895	0.091	2.34
2	17.389	0.075	0.43	31.531	0.278	0.88	15.251	0.399	2.62
3	1.595	0.024	1.50	26.879	0.224	0.83	21.872	0.355	1.62
4				15.701	0.189	1.20	19.820	0.240	1.21
5				7.440	0.139	1.87	14.359	0.140	0.97
6				3.678	0.166	4.51	9.674	0.129	1.33
7				1.733	0.093	5.37	6.259	0.226	3.61
8				0.790	0.044	5.57	4.006	0.209	5.22
9				0.361	0.018	4.99	2.698	0.131	4.86
10							1.555	0.065	4.18
11							0.614	0.042	6.84
Total (%)	100			100			100		
Average EO number (n)	1.21			2.99			4.32		

Hence the results vary slightly according to the time at which they were measured.

Determination of oligomer distribution

The peak areas were tabulated once each sample had been run and each peak representing different oligomers had been identified. Table I gives the retention times and the peak areas of the oligomers of Triton X-114 from four repeated analyses. The average area for each peak, standard deviation (S.D.), relative standard deviation (R.S.D.) and mole fraction (F) for each ethylene oxide adduct calculated from the average area are also given. The average EO number for Triton X-114 was determined to be 7.43.

There is another method for determining the distribution of oligomers. The data from each run are expressed as an area percentage (that is, mole fraction), and the average mole fraction for each component is then statistically calculated. These values are identical with those obtained using the first method, but R.S.D.s were general-

TABLE IV

MOLE FRACTION (F) AND AVERAGE EO NUMBER OF PEAP STUDIED

Peak	Triton X-100			Triton X-102			Triton X-165			POE(30) octylphenol		
	F (%)	S.D. (%)	R.S.D. (%)	F (%)	S.D. (%)	R.S.D. (%)	F (%)	S.D. (%)	R.S.D. (%)	F (%)	S.D. (%)	R.S.D. (%)
1	0.138	0.009	6.52	0.445	0.003	0.67	0.683	0.005	0.73	0.312	0.011	3.53
2	0.516	0.028	5.43	0.562	0.002	0.36	0.671	0.004	0.60	0.326	0.017	5.21
3	1.481	0.029	1.96	0.844	0.008	0.95	0.662	0.013	1.96	0.345	0.008	2.32
4	3.473	0.048	1.38	1.535	0.025	1.63	0.705	0.016	2.27	0.364	0.008	2.20
5	5.618	0.048	0.85	2.294	0.030	1.31	0.748	0.017	2.27	0.351	0.006	1.71
6	7.742	0.064	0.83	3.250	0.020	0.62	0.912	0.020	2.19	0.379	0.014	3.69
7	9.978	0.074	0.74	4.753	0.010	0.21	1.309	0.010	0.76	0.405	0.008	1.98
8	11.672	0.087	0.75	6.535	0.039	0.60	2.017	0.012	0.59	0.432	0.014	3.24
9	12.237	0.071	0.58	8.306	0.066	0.79	3.054	0.018	0.59	0.469	0.011	2.35
10	11.690	0.094	0.80	9.785	0.068	0.69	4.412	0.013	0.29	0.486	0.012	2.47
11	10.141	0.045	0.44	10.592	0.074	0.70	5.946	0.024	0.40	0.371	0.019	5.12
12	8.188	0.048	0.59	10.597	0.042	0.40	7.444	0.022	0.30	0.535	0.014	2.62
13	6.076	0.044	0.72	9.833	0.039	0.40	8.742	0.035	0.40	0.610	0.037	6.07
14	4.259	0.049	1.15	8.520	0.060	0.70	9.549	0.076	0.80	0.796	0.008	1.01
15	2.813	0.049	1.74	6.871	0.048	0.70	9.759	0.088	0.90	1.025	0.030	2.93
16	1.819	0.072	3.96	5.189	0.021	0.40	9.356	0.047	0.50	1.274	0.028	2.20
17	1.138	0.069	6.06	3.718	0.026	0.70	8.405	0.059	0.70	1.744	0.035	2.01
18	0.624	0.040	6.41	2.493	0.050	2.01	7.117	0.100	1.41	2.117	0.158	7.46
19	0.292	0.019	6.51	1.635	0.060	3.67	5.696	0.074	1.30	3.096	0.065	2.10
20	0.109	0.008	7.34	1.066	0.029	2.72	4.228	0.093	2.20	3.865	0.083	2.15
21				0.513	0.027	5.26	3.026	0.151	4.99	4.738	0.077	1.63
22				0.261	0.017	6.51	2.062	0.136	6.60	5.541	0.045	0.81
23				0.405	0.012	2.96	1.558	0.072	4.62	6.324	0.100	1.58
24							0.840	0.029	3.45	6.854	0.079	1.15
25							0.491	0.020	4.07	7.219	0.051	0.71
26							0.385	0.021	5.45	7.296	0.015	0.21
27							0.224	0.011	4.91	7.169	0.080	1.12
28										6.765	0.092	1.36
29										6.099	0.056	0.92
30										5.360	0.127	2.37
31										4.425	0.136	3.07
32										3.591	0.081	2.26
33										2.744	0.094	3.43
34										2.176	0.128	5.88
35										1.548	0.085	5.49
36										0.984	0.063	6.40
37										0.792	0.029	3.66
38										0.505	0.013	2.57
39										0.319	0.013	4.08
40										0.240	0.010	4.17
Total (%)	100			100			100			100		
Average EO number (n)		9.42			11.61			14.70			24.91	

ly better. Table II gives the area percentage from each run, the average mole fraction, the S.D. and the R.S.D. obtained by the second method. As a comparison, the corresponding R.S.D. obtained from direct area calculation is also shown in Table II.

Table III lists values of the mole fraction, the S.D., R.S.D. and the average EO number for Triton X-15, X-35 and X-45. Their EO units range from 1 to 11. Similarly, Table IV gives the data for another four longer chain polyethoxylated octylphenol samples [Triton X-100, X-102 and X-165 and EO (30) octylphenol] with EO units ranging from 1 to 40 and average molecular mass ranging from 206 to *ca.* 2000. All statistical data given in Tables III and IV were obtained from four analyses for each sample. The average EO number for these seven surfactants were determined to be 1.21, 2.99, 4.32, 9.42, 11.61, 14.70 and 24.91, respectively.

Figs. 2–4 depict graphically the oligomer distribution determined for samples corresponding to Tables II–IV. The Poisson distribution for the samples with lower molecular mass and the Gaussian distribution for the samples with higher molecular mass are obvious from the shapes of the curves.

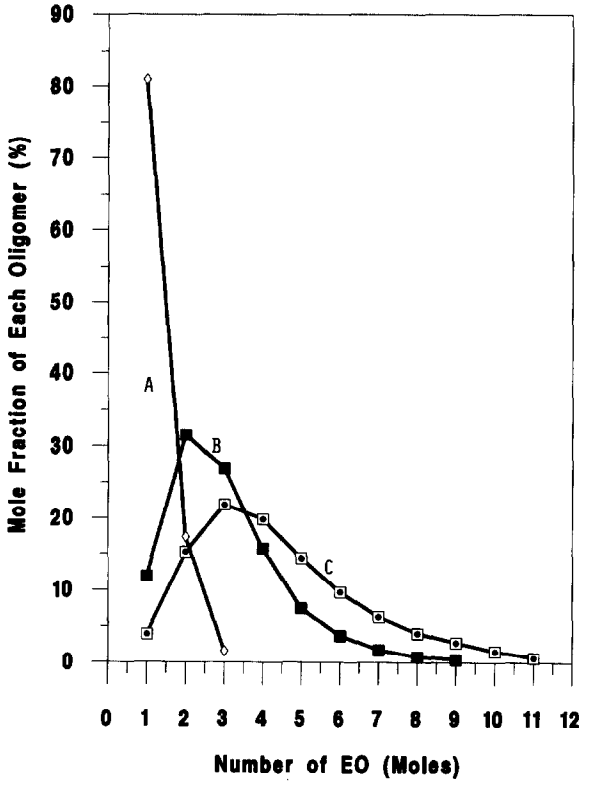

Fig. 2. Oligomer distribution curves for (A) Triton X-15, (B) Triton X-35 and (C) Triton X-45.

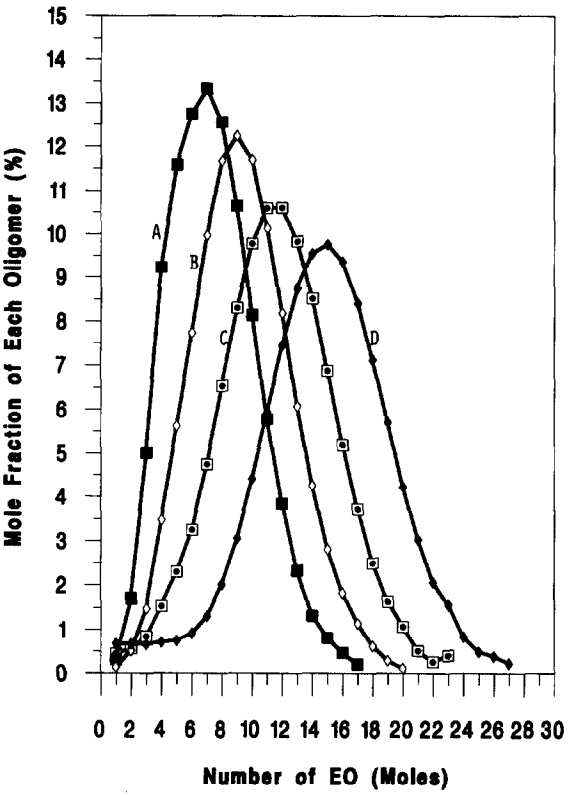

Fig. 3. Oligomer distribution curves for (A) Triton X-114, (B) Triton X-100, (C) Triton X-102 and (D) Triton X-165.

Effect of mobile phase on separation of oligomers

Methanol and acetonitrile were tested as components of the mobile phase. A methanol–water mobile phase produced good separations for all of the surfactant oligomers studied. Acetonitrile–water, however, gave poor separations. Therefore, methanol–water was used as the mobile phase throughout this study.

The experimental results demonstrated that the composition of the mobile phase has a great effect on the separation of ethoxylate adducts. Figs. 5 and 6 show chromatograms of Triton X-114 and X-35 recorded using different mobile

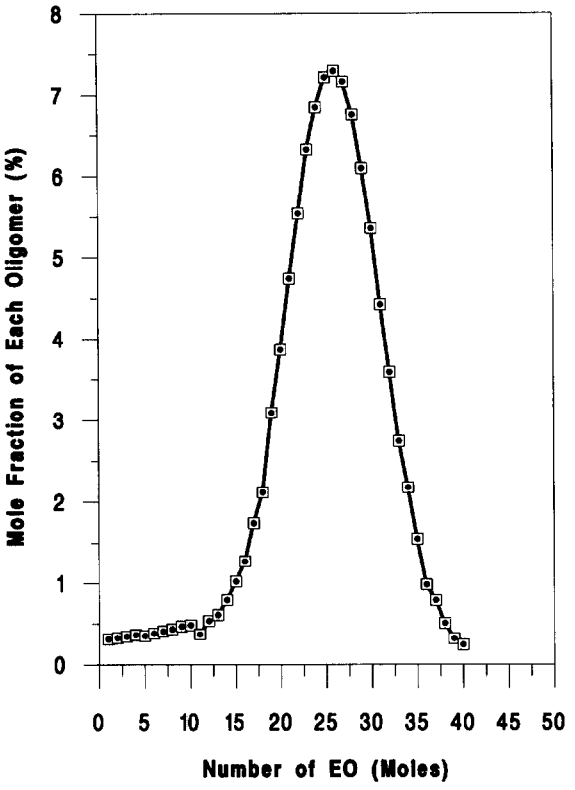

Fig. 4. Oligomer distribution curve for POE (30) octylphenol.

phase compositions. Table V summarizes the retention times for peaks 1, 5, 10 and 15, the total peak numbers identified, the total times needed for complete elution of all components and total integrated peak areas at different mobile phase compositions. The average EO numbers determined for different mobile phase compositions for Triton X-114 are also given in the last column of Table V. When the polarity of the mobile phase was decreased (that is, with a larger proportion of methanol), a shorter elution time was observed. For example, it took 53 min for peak 15 of Triton X-114 to be eluted when the ratio of methanol to water in mobile phase was set at 52:48. It took only 10 min for the same component peak to be eluted when the proportion of methanol in the mobile phase was increased from 52% to 62%. Obviously, the more ethoxylate adducts the sample contains, the more significant is the decrease with time.

It should be noted that for those samples which consisted of shorter chain oligomers and which were dissolved in methanol, poorer separation was observed as the proportion of methanol in mobile phase was increased. No separation between peaks 1 and 2 was observed for Triton X-15, X-35 and X-45 as the methanol proportion increased from 52% to 60%.

In contrast, for the samples which consisted of

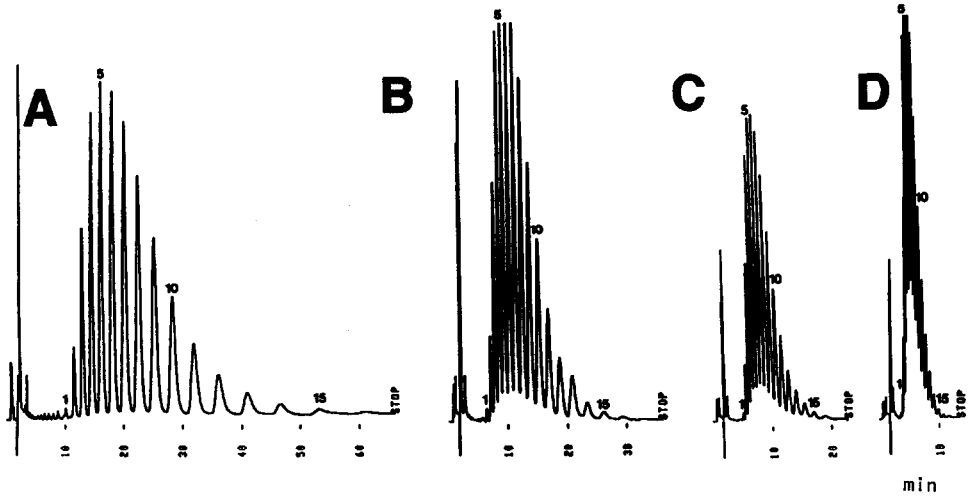

Fig. 5. Chromatograms of Triton X-114 using different methanol–water mobile phase compositions: (A) 53:47; (B) 55:45; (C) 57.3:42.7; (D) 62:38. Other conditions as in Fig. 1.

TABLE V

EFFECT OF MOBILE PHASE COMPOSITION ON THE ANALYSIS OF TRITON X-114

Mobile phase (CH_3OH–H_2O)	N [a]	Retention time (min)				Total peak number	Elution time (min)	Total area counts	Average EO number (n)
		Peak 1	Peak 5	Peak 10	Peak 15				
52:47	4	10.1	16.1	28.3	53.1	16	65	3 666 487	7.37
55:45	2	6.4	9.5	15.3	26.1	17	36	3 592 992	7.44
57.3:42.7	1	5.2	7.3	10.9	17.3	17	23	3 638 398	7.45
60:40	4	4.6	6.2	8.9	13.4	17	17	3 695 434	7.43
62:38	1	4.0	5.1	7.0	10.1	17	13	3 624 514	7.41

[a] N = number of measurements.

longer chain oligomers and which were dissolved in water, such as Triton X-114, X-100, X-102 and X-165, the effect of the composition of the mobile phase on peak resolution was much smaller. When the proportion of methanol in the mobile phase was increased from 52% to 60%, very little resolution was sacrificed, but good separation with much sharper peaks was achieved in a much shorter run time.

Effect of solvent on separation of oligomers

Three solvents, methanol, water and the mobile phase, were used to prepare solutions of PEAP surfactant Triton X-114, X-100, X-102 and X-165. The HLB values of all these surfactants are >12, and therefore they can be easily dissolved in water, methanol and the mobile phase. It is interesting that the sample solutions of the same surfactant, but prepared with different solvents at the same concentration, gave very different chromatograms (the sample volumes injected were all 20 μl). If the sample was an aqueous solution, the oligomers were completely separated. An identical chromatogram was obtained for the sample solution prepared with the mobile phase. However, if the sample was prepared in methanol, the oligomers were very poorly separated, especially for the shorter chain oligomers.

Typical chromatograms of Triton X-114 in methanol solution with methanol–water mobile phases of 60:40 and 55:45 are shown in Fig. 7.

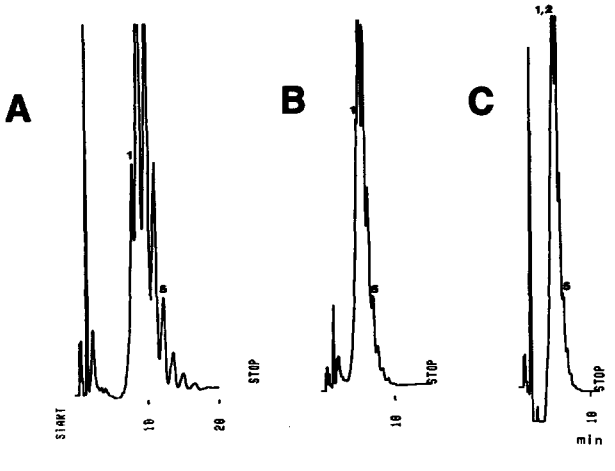

Fig. 6. Chromatograms of Triton X-35 using different methanol–water mobile phase compositions: (A) 55:45; (B) 57.3:42.7; (C) 60:40. Other conditions as in Fig. 1.

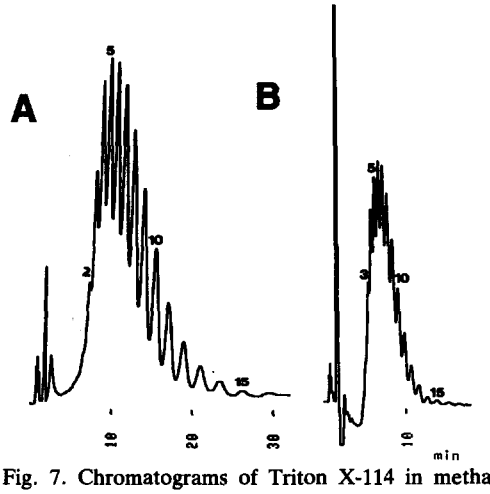

Fig. 7. Chromatograms of Triton X-114 in methanol solutions. (A) Methanol–water (55:45); integrator attenuation 4; (B) methanol–water (60:40); integrator attenuation 5. Other conditions as in Fig. 1.

TABLE VI

EFFECT OF SOLVENT ON SEPARATION OF TRITON X-114 AT VARIOUS MOBILE PHASE COMPOSITIONS

Values given are retention times (min).

Methanol–water mobile phase	Dissolved in	Peak No.																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
60:40	Water[a]	4.75	5.14	5.55	5.96	6.40	6.88	7.38	7.94	8.55	9.22	9.97	10.81	11.73	12.76	13.89	15.14	16.54
	Methanol[b]	–	–	5.51	5.91	6.34	6.82	7.31	7.85	8.48	9.12	9.86	10.69	11.61	12.66	13.75	15.04	16.38
55:45	Water[c]	6.35	7.05	7.78	8.55	9.37	10.31	11.32	12.45	13.72	15.16	16.80	18.65	20.75	23.20	25.99	29.18	32.80
	Mobile phase[d]	6.31	7.00	7.73	8.50	9.33	10.26	11.27	12.40	13.67	15.11	16.78	18.61	20.71	23.12	25.83	28.96	32.57
	Methanol[e]	–	–	7.75	8.52	9.34	10.28	11.28	12.41	13.68	15.12	16.78	18.66	20.75	23.17	26.02	29.17	32.70

[a] Total integrated area = 3 695 434 counts.
[b] Total integrated area = 3 747 324 counts.
[c] Total integrated area = 3 592 992 counts.
[d] Total integrated area = 3 584 481 counts.
[e] Total integrated area = 3 760 714 counts.

Comparison of Figs. 1D and 7 shows the differences in peak shape and component resolution. Table VI gives a detailed comparison of the retention times and total integration peak areas for Triton X-114 samples prepared with three different solvents. Fig. 7 and Table VI show that the retention times and total peak areas are almost identical, regardless of the solvent used. Obviously, it is not a surfactant solubility problem; if it were, the total peak areas would not be the same. Hence the question remains as to the cause of the separation difference between the aqueous and methanol solutions, and why different solvents have such a great effect on the separation of oligomers with identical injection volumes under identical HPLC conditions.

The non-aqueous surfactant solution is a very complex system. A detailed discussion and explanation of the phenomena noted above is beyond the scope of this paper, and requires more experimental work emphasizing physical chemistry studies. However, the general explanation for this phenomenon could possibly be as follows: it is well known that surfactants form micelles if their concentrations are higher than the critical micellar concentration (CMC), and that the CMC of the same surfactant is different in different solvents; hence the microstructure of micelles so formed can also be different [26]. Methanol is less polar than water. The Snyder polarity index of methanol is 5.1, only half that of water, 10.2 [27]. Another factor that should be considered is that the surfactants studied here are not pure compounds, but are a series of oligomers with varying hydrophilic EO chain lengths, and therefore the micelles may consist of varying oligomers. The combination of these factors results in physical chemical properties of surfactants in methanol that are different from those in water, and in different behaviours on the stationary phase of the HPLC column for the surfactants in methanol solution. As a result, the separation of oligomers is incomplete.

CONCLUSIONS

A method employing C1 column reversed-phase liquid chromatography has been developed for the rapid separation of non-ionic surfactants of polyethoxylated alkylphenols and the determination of their ethoxylate oligomer distribution. As a chromatographic technique, it offers several advantages for the analysis of ethoxylated non-ionic surfactants, such as the separation of oligomers, simplicity and economy with respect to time, while still having precision and accuracy. With this technique, complex distributions can be resolved and individual oligomers containing up to 40 or more EO units can be detected, allowing differences in molecular mass and mole fraction to be qualitatively and quantitatively determined. It is possible to apply this technique to the separation and identi-

fication of many other non-ionic surfactant oligomers. More studies are being conducted in this laboratory to apply HPLC and other chromatographic techniques to identify and characterize surfactants in environmental samples.

REFERENCES

1 P.L. Layman, *Chem. Eng. News*, January (1984) 17.
2 P.L. Layman, *Chem. Eng. News*, January (1986) 21.
3 M. Ahel and W. Giger, *Anal. Chem.*, 57 (1985) 1577.
4 J. Cross, *Nonionic Surfactants: Chemical Analysis*, Marcel Dekker, New York, 1987.
5 H.G. Nadeau, D.M. Oaks, Jr., W.A. Nichols and L.P. Carr, *Anal. Chem.*, 36 (1964) 1914.
6 F.J. Ludwig, Sr., *Anal. Chem.*, 40 (1968) 1620.
7 S.R. Lipsky and M.L. Duffy, *LC·GC*, 4 (1986) 898.
8 E. Stephanou, *Chemosphere*, 13 (1984) 43.
9 E. Stephanou, *Int. J. Environ. Anal. Chem.*, 27 (1985) 41.
10 P. Rudewicz and B. Munson, *Anal. Chem.*, 58 (1986) 674.
11 F.P.B. Van der Maeden, M.E.F. Biemond and P.C.G.M. Janssen, *J. Chromatogr.*, 149 (1978) 539.
12 M.S. Holt, E.H. McKerrell, J. Perry and R.J. Watkinson, *J. Chromatogr.*, 362 (1986) 419.
13 J.A. Pilc and P.A. Sermon, *J. Chromatogr.*, 398 (1987) 375.
14 *Separating Homologs and Polymers by HPLC (HPLC Bulletin 796D)*, Supelco, Bellefonte, PA, 1988.
15 M.C. Allen and D.E. Linder, *J. Am. Oil Chem. Soc.*, 58 (1981) 950.
16 J.F.K. Huber, F.F.M. Kolder and J.M. Miller, *Anal. Chem.*, 44 (1972) 105.
17 A. Marcomini and W. Giger, *Anal. Chem.*, 59 (1987) 1709.
18 A. Marcomini, S. Capri and W. Giger, *J. Chromatogr.*, 403 (1987) 243.
19 M. Ahel and W. Giger, *Anal. Chem.*, 57 (1985) 1577.
20 M. Ahel and W. Giger, *Anal. Chem.*, 57 (1985) 2584.
21 C.F. Allen and L.I. Rice, *J. Chromatogr.*, 110 (1975) 151.
22 R.E.A. Escott and D.W. Chandler, *J. Chromatogr. Sci.*, 27 (1989) 134.
23 J. Kelly and H.L. Greenwald, *J. Phys. Chem.*, 62 (1958) 1096.
24 N.T. Crabb and H.E. Persinger, *J. Am. Oil Chem. Soc.*, 45 (1968) 611.
25 S. Hayano, T. Nihongi and T. Asahara, *Tenside*, 5, Nos. 3/4 (1968) 80.
26 K.L. Mittal (Editor), *Solution Chemistry of Surfactants*, Vols. 1 and 2, Plenum Press, New York, 1979.
27 A.F.M. Barton, *CRC Handbook of Solubility Parameters and Other Cohesion Parameters*, CRC Press, Boca Raton, FL, 1983, pp. 198–199.