# Quantitative trace analysis of surfactant mixtures by reversed-phase high-performance liquid chromatography with refractometric detection

**ARTICLE** *in* JOURNAL OF CHROMATOGRAPHY A · APRIL 1996

Impact Factor: 4.17 · DOI: 10.1016/0021-9673(95)01087-4



**3 AUTHORS**, INCLUDING:

Florence Portet-Koltalo
Université de Rouen
**40** PUBLICATIONS **284** CITATIONS

SEE PROFILE

ELSEVIER

Journal of Chromatography A, 730 (1996) 209–218

JOURNAL OF CHROMATOGRAPHY A

# Quantitative trace analysis of surfactant mixtures by reversed-phase high-performance liquid chromatography with refractometric detection

P.L. Desbène[a,b,*], F.I. Portet[a,b], G.J. Goussot[c]

[a]*LASOC, Université de Rouen, IUT, 43 Rue Saint Germain, 27000 Evreux, France*
[b]*IFRMP, Université de Rouen, 76821 Mont Saint Aignan Cedex, France*
[c]*Laboratoire Janssen, Centre de Recherche, Campus de Maigremont, BP 615, 27106 Val de Reuil Cedex, France*

**Abstract**

Reversed-phase partition liquid chromatography on an octyl column allowed the separation of complex non-ionic poly(ethylene oxide)-type (PEO) surfactant mixtures resulting from the condensation of ethylene oxide with saturated fatty alcohols. As these compounds have no chromophoric group, they were detected by differential refractometry. Accurate quantitation of each oligomer ($C_mE_n$) allowed the main characteristics of each non-ionic surfactant, i.e., the nature and percentage of the different alkyl chains (with $m$ = number of carbons) and the average number of ethylene oxide units ($\bar{n}$) to be obtained in one analysis. Preparative liquid chromatography was used to isolate pure oligomers with a higher degree of ethoxylation ($n = 10$, 11, 12, 14 and more) than the commercially available standards, in order to determine a wide range of refractometric response factors. It appeared that they are constant as a function of alkyl chain length ($C_{10}$–$C_{16}$ range) but that they vary significantly and non-linearly as a function of the degree of ethoxylation, $n$. It was found that neglecting the variation of response factors can result in a distortion of the average ethoxylation number and in an unsatisfactory quantitative analysis. This chromatographic method, involving a quantitative and reproducible trace enrichment procedure with liquid–solid extraction, allowed the analysis of very dilute PEO mixtures in water. The components of complex PEO mixtures in water were determined at concentrations as low as 0.5 mg $l^{-1}$, without any distortion of the distribution, the detection limit being 0.25 $\mu$g $l^{-1}$ for the less abundant oligomers.

*Keywords:* Preparative chromatography; Trace analysis; Surfactants, non-ionic; Poly(ethylene oxides)

## 1. Introduction

Surfactants are usually classified into four main families: anionic, cationic, zwitterionic and non-ionic. Non-ionic surfactants are second to anionic surfactants in world production [1]. Most of them are obtained by condensation of ethylene oxide on compounds possessing a reactive hydrogen, such as alkylphenols or fatty alcohols. The condensation products are complex mixtures of oligomers, i.e., homologous compounds. They can differ in their hydrophobic part and also in their degree of ethylene oxide condensation. Poly(ethylene oxide) units are distributed accord-

* Corresponding author. Address for correspondence: LASOC, Université de Rouen, IUT, 43 Rue Saint Germain, 27000 Evreux, France

0021-9673/96/$15.00 
*SSDI* 0021-9673(95)01087-4

ing to Poisson (or Gaussian) curves [2]. The amphiphilic characteristics of these compounds result in their detergent, emulsifying and foaming properties and they are widely used in various industries (food, cosmetics, detergent, etc.). Their properties are strongly related to their hydrophilic–lipophilic balance (HLB) [3]. Therefore, it is essential to have analytical methods that are sufficiently powerful to allow the determination of both the ethylene oxide part and the nature of the fatty chain(s) in order to characterize these non-ionic surfactants.

High-performance liquid chromatography (HPLC) has been widely used for many years in analyses for non-ionic surfactants [4]. It is preferred to gas chromatography and supercritical fluid chromatography, as the former does not allow the determination of highly ethoxylated surfactants [5] and the latter has been developed only very recently [6].

Two main difficulties appear in the determination of non-ionic surfactants such as polyoxyethylenic surfactants condensed on saturated fatty alcohols (PEO): on the one hand, the resolution of these mixtures, which are often very complex and can contain some dozens of oligomers; and on the other, these compounds do not possess chromophoric groups and their detection by UV–visible absorption spectrometry is impossible (except at very low wavelengths). The chemical derivatization of these compounds allows UV-absorbing groups to be introduced [7], but this supplementary step can result in unsatisfactory quantitative determinations of these surfactants in complex matrices. Other detection techniques make possible the direct detection of these solutes without derivatization, e.g., flame ionization detection [8], low-wavelength UV detection [9], indirect UV detection [10] and evaporative light-scattering detection (ELSD) [11]. None of these techniques could possibly solve the problem of the determination of PEO surfactants contained in detergent formulations, in the aqueous phase, at total concentrations of about 1 ppm, with a view to the subsequent study of their adsorption at liquid–solid interfaces. We have therefore attempted to develop a new strategy for the analysis of complex PEO mixtures.

## 2. Experimental

### 2.1. Equipment

Analyses were performed using the following liquid chromatography system: a Beckman Gold system (Beckman, Fullerton, CA, USA) equipped with a 200-$\mu$l injection loop, a pulse damper from Touzart et Matignon (Vitry sur Seine, France), and an RID-6A differential refractometric detector (Shimadzu, Kyoto, Japan).

$^1$H NMR spectra were recorded using a WP 200 E spectrometer (Brucker, Wissemburg, France) and mass spectra using a Nermag R-10-10-C system equipped with a fast atom bombardment (FAB) source (Delsi-Nermag, Argenteuil, France).

Reversed-phase HPLC analyses were performed using an octyl Ultrasphere column (250 mm $\times$ 4.6 mm I.D., $d_p = 5$ $\mu$m) (Beckman). Preparative chromatography was optimized using a Hyperprep HS silica column (250 $\times$ 4.6 mm I.D., $d_p = 8$ $\mu$m) (Shandon, Eragny, France). The stationary phase used in preparative liquid chromatography was either the same silica or a Merck (Darmstadt, Germany) silica, $d_p = 15$–$40$ $\mu$m. The system used in preparative chromatography was a Prochrom (Champignolles, France) apparatus equipped with 30 cm $\times$ 5 cm I.D. (or 8 cm I.D.) columns and with a Model 202 fraction collector (Gilson Medical Electronics, Villiers le Bel, France).

The liquid–solid extraction cartridges were Sep-Pak $C_{18}$ Plus (Waters, St. Quentin en Yvelines, France).

### 2.2. Reagents

Water was purified and deionized using an Alpha Q system (Millipore, Molsheim, France). The various solvents were used without previous purification: acetonitrile (SDS, Vitry sur Seine, France), methanol and ethyl acetate (Carlo Erba, Rueil-Malmaison, France) of HPLC grade and ethyl acetate and methanol (Carlo Erba) of analytical-reagent grade in the case of preparative liquid chromatography.

The mobile phases used in HPLC were de-

gassed prior to use in USR 3 ultrasonic system (Touzart et Matignon).

### 2.3. Samples

The polyoxyethylenic surfactants (PEO) studied are described according to one of the usual terminologies, i.e., $C_mE_n$, where $m$ = number of carbons in the fatty chain and $n$ = number of ethylene oxide units ($-CH_2-CH_2-O-$) condensed.

We used $C_{10}E_6$, $C_{12}E_6$, $C_{14}E_6$ and $C_{16}E_6$ standards of ≥98% purity from Nikko Chemicals (Tokyo, Japan), and $C_{12}OH$, $C_{12}E_2$, $C_{12}E_4$, $C_{12}E_5$, $C_{12}E_7$, $C_{12}E_8$ and $C_{12}E_9$ standards (puriss. grade) from Fluka (Buchs, Switzerland). All these standards were used without purification. $C_{12}E_{10}$, $C_{12}E_{11}$, $C_{12}E_{12}$ and $C_{12}E_{14}$ standards were obtained from a polydispersed PEO lauryl ether ($C_{12}E_9$), presenting an average degree of ethoxylation $\bar{n} = 9$ EO, furnished by Nikko Chemicals. The surfactants denoted A, B and C in the text were kindly provided by Lever-France (Haubourdin, France). They result from the condensation of ethylene oxide with fatty alcohols which are $C_{13}-C_{15}$ mixtures (A and B) or $C_{10}-C_{12}-C_{14}-C_{16}$ mixtures (C).

### 2.4. Preparative liquid chromatography

The preparation of pure $C_{12}E_n$ oligomers from the polydispersed $C_{12}E_9$ sample (which contains about 24 oligomers from $C_{12}OH$ to $C_{12}E_{23}$) was performed in two steps: first, the isolation of sufficient amounts of fractions enriched in some selected oligomers; and second, the preparation of each of these selected oligomers with high purity.

The first series of oligomers selected contained from $C_{12}E_{10}$ to $C_{12}E_{14}$. The enriched fractions were obtained from five successive fractionations performed by overloading (injection of 1 g of $C_{12}E_9$ in 15 ml of ethyl acetate) a 30 cm × 5 cm I.D. column filled with Shandon 8-μm silica. A step gradient was used and the flow-rate was 40 ml $min^{-1}$. This step gradient was previously optimized using an analytical system with the same stationary phase as mentioned above. We used the following gradient: ethyl acetate–methanol (90:10, v/v) ($V_{eluted}$ = 960 ml); ethyl acetate–methanol (85:15, v/v) ($V_{eluted}$ = 640 ml); ethyl acetate–methanol (80:20, v/v) ($V_{eluted}$ = 640 ml).

The fractionation was performed every 50 ml and only the fractions containing the target oligomers were conserved after identification by analytical HPLC and refractometric detection. This strategy allowed us to isolate four fractions of 300–400 mg of mixtures containing only 5–6 oligomers instead of 24 oligomers in the raw surfactant, i.e., a 60% enrichment in each of the target oligomers. These four enriched fractions were then separately injected on to the preparative column, with elution conditions giving a capacity factor of about 3 for the oligomer selected (see Table 1).

During the last purification, the efficiency of the column was improved (as there was no overload) and the volume of the aliquot fractions was reduced to 20 ml. Under these conditions, it was possible to isolate 150–200 mg of $C_{12}E_{10}$, $C_{12}E_{11}$, $C_{12}E_{12}$ and $C_{12}E_{14}$ pure oligomers. The purity of these different oligomers was found to be in the range 92–99% by HPLC, $^1H$ NMR and FAB-MS.

The enrichment procedure was more tedious in the case of more condensed oligomers (degree of ethoxylation $n \geq 18$ EO), because these oligomers represent about only 1% of the polydispersed surfactant used as raw material. We used a column of larger diameter (8 cm I.D.), filled with a less efficient but cheaper silica ($d_p$ = 15–40 μm).

We injected four times 20 g of the polydispersed $C_{12}E_9$ surfactant and eluted with ethyl

Table 1
Mobile phases used for the final purification of $C_{12}E_n$ standards

| $n$ | Ethyl acetate (%) | Methanol (%) |
|---|---|---|
| 10 | 92 | 8 |
| 11 | 90 | 10 |
| 12 | 88 | 12 |
| 14 | 85 | 15 |
| 18 | 75 | 25 |
| >18 | 70 | 30 |

acetate–methanol (50:50) as the mobile phase at a flow-rate of 80 ml $min^{-1}$. We collected 100-ml fractions at the tail of the distribution, i.e., 1.2 g corresponding to about 10 homologous compounds from $n = 14$ to $n = 23$. A new separation was performed using this whole fraction, which was chromatographed using a 5 cm I.D. column filled with silica of $d_p = 8$ $\mu$m, the mobile phase being ethyl acetate–methanol (70:30) at a flow-rate of 40 ml $min^{-1}$. We collected 60-ml fractions and 600 mg of $C_{12}E_n$ oligomers in the range $n = 17–23$ were isolated.

However, as we required purer standards, we proceeded to a final purification step. The 600-mg fraction of $C_{12}E_n$ ($n = 17–23$) was chromatographed using the same column and a new mobile phase [ethyl acetate–methanol (80:20, v/v] at a flow-rate of 40 ml $min^{-1}$. Fractions of 30 ml were collected and, after analysis, only the fractions corresponding to the mixtures $C_{12}E_{18}$–$C_{12}E_{19}$, $C_{12}E_{20}$–$C_{12}E_{21}$ and $C_{12}E_{22}$–$C_{12}E_{23}$ were retained. Finally, after these three successive preparative chromatographic separations, three mixtures, $C_{12}E_{18}$–$C_{12}E_{19}$, $C_{12}E_{20}$–$C_{12}E_{21}$ and $C_{12}E_{22}$–$C_{12}E_{23}$, enriched at more than 96% were available.

### 2.5. Liquid–solid extraction

The traces of surfactant formulations were concentrated using $C_{18}$-bonded silica cartridges. The formulations diluted in a volume $V_{aq}$ of water were percolated (by vacuum) through the cartridge at a flow-rate of about 10 ml $min^{-1}$. The surfactants, quantitatively adsorbed on the stationary phase, were desorbed with 4 ml of acetonitrile, then 3 ml were diluted with 2 ml of water in order to inject these solutes in a solvent mixture of the same composition as the mobile phase used in chromatographic analyses. Under these conditions, the reconcentration factor ($F$) to consider in quantitative studies is

$$F = (V_{aq}/4) \cdot 0.6$$

## 3. Results and discussion

In spite of the fact that analyses in normal-phase liquid chromatography using cyano- [12], amino- [13], diol- [14,15] and $p$-nitrophenyl-bonded [16] silica and even ion exchangers [17] gave excellent results for the resolution of particularly complex mixtures of non-ionic surfactants, they appeared unsuitable in our case, because the samples are in aqueous solutions. Under these conditions, RP-HPLC was the best choice.

Moreover, the surfactant formulations A, B and C studied resulted from the condensation of ethylene oxide with relatively complex mixtures of fatty alcohols, so it was necessary to characterize them according to their degree of ethoxylation and the nature of their alkyl chains. Systems such as $C_8$ or $C_{18}$ stationary phases, in association with water–acetone [18] or water–methanol [19,20] mobile phases, could not be used because they allow to separate only as a function of hydrophobic chains. In contrast, the combination of $CH_3CN$–$H_2O$ mobile phases with $C_8$ stationary phases allowing the two analyses required, so we adopted such systems [21].

As mentioned above, the surfactants analysed have no chromophoric group and we wanted to avoid any supplementary derivatization step. Therefore, we chose a universal detection method; differential refractometry was preferred to light scattering, which appeared less sensitive in this case. Refractometric detection implies that the analytes are dissolved in a solvent strictly identical with the mobile phase used. Moreover, as reported by Wang and Fingas [22], the same constraint must be met on a chromatographic basis because the separation quality is dependent on the solvent used to dissolve the analytes. As a consequence of the great sensitivity required, we had to minimize the dilution of the samples and this imposed the utilization of a high percentage of water in the mobile phases. Therefore, $C_{18}$-bonded silicas, which are too retentive, could not be used and we performed the analyses on a $C_8$ phase.

As an example, the analysis of surfactant C under the optimized conditions [$CH_3CN$–$H_2O$ (60:40, v/v)] is reported in Fig. 1. As expected, four distributions, corresponding to ethylene oxide condensation on $C_{10}$, $C_{12}$, $C_{14}$ and $C_{16}$ chains, were observed. Peak identification was carried out by spiking the sample with pure

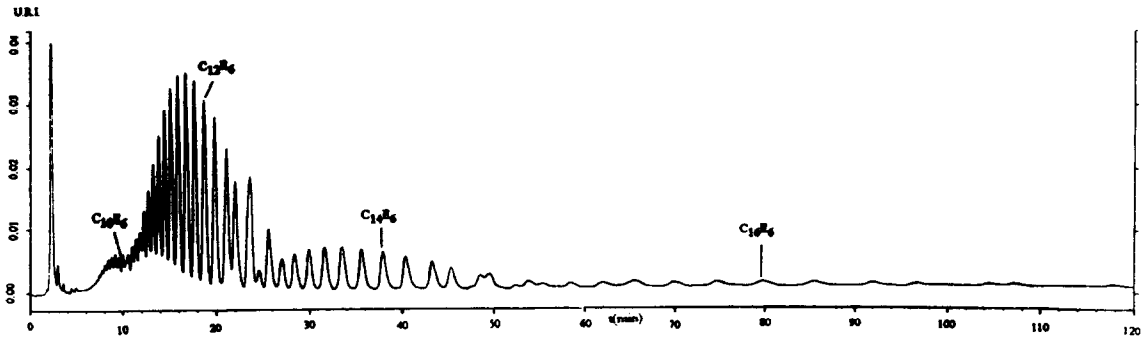


Fig. 1. Analysis of the surfactant C by HPLC. Conditions: $C_8$ column (250 × 4.6 mm, $d_p$ = 5 μm); mobile phase, $CH_3CN$–$H_2O$ (60:40, v/v); flow-rate, 1 ml $min^{-1}$; temperature, 21°C; refractometric detector.

standards differing in their alkyl chains, i.e., $C_{10}E_6$, $C_{12}E_6$, $C_{14}E_6$ and $C_{16}E_6$. Each distribution presents 14–20 peaks corresponding to the different degrees of condensation of ethylene oxide.

In contrast, the chromatograms for surfactants A and B were simpler. Only two distributions of about 20 peaks each were observed, corresponding to $C_{13}$ and $C_{15}$ alkyl chains.

Whatever the sample analysed, the oligomer distributions partly overlap, resulting in a difficult or sometimes impossible direct determination of the retention times of some components. The relationship log $k' = f$ ($m$ carbons), classical in reversed-phase chromatography [19], allowed us to determine without ambiguity the capacity factors $k'$ and therefore the retention times of the different components. Effectively, it is possible to calculate the capacity factor of an oligomer overlapping with other components by proceeding to a linear regression with the values of the capacity factors for the resolved homologous compounds.

The linear regression curves obtained from the chromatograms for surfactants A, B and C, at a constant degree of ethoxylation, as a function of alkyl chains length, are reported in Fig. 2. It was possible to identify in this way all the oligomers of A, B and C, whatever their degree of ethoxylation and the nature of their alkyl chain. The excellent reproducibility is worth noting: the relative standard deviations of retention times, calculated from five independent analyses, are less than 1%.

As this technique appeared reliable and reproducible, it clearly allowed the correct analysis of these surfactants formulations, whatever their complexity, and we could consider, in a second step, the quantitative study of their distributions, both in fatty chains and in ethoxylated chains. However, in an aqueous–organic phase, a coiling phenomenon has been reported in the case of similar surfactants [23–25] and prior to analysis it was necessary to determine eventual modifications of the response factor of the detection

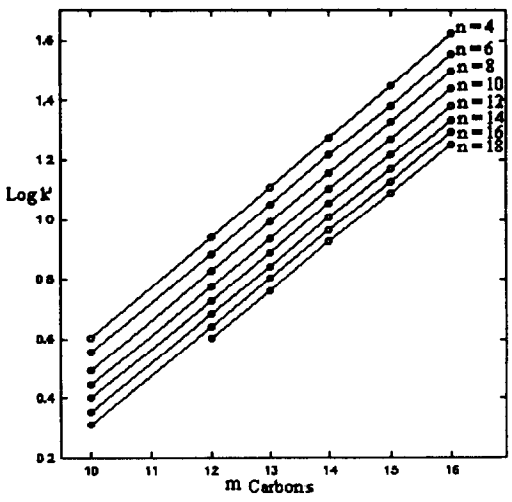


Fig. 2. Log $k'$ = f($m$ carbons) at a constant degree of ethoxylation. Operating conditions as in Fig. 1. $k'$ of $C_{10}$, $C_{12}$, $C_{14}$ and $C_{16}$ fatty chains deduced from HPLC analyses of surfactant C; $k'$ of $C_{13}$ and $C_{15}$ fatty chains deduced from HPLC analyses of surfactants A and B. ● = Experimental values of capacity factors; ○ = capacity factors computed by linear regression.

system, i.e., the differential refractometer, as a function of the structure of the different oligomers analysed.

Using pure standards, characterized by a well defined fatty chain and a precise degree of ethoxylation, either commercially available or obtained by preparative liquid chromatography, we studied the evolution of the refractometric response factors: on the one hand as a function of the nature of the fatty chain, with a constant degree of ethoxylation ($n = 6$ EO); and on the other, as a function of the degree of ethoxylation, with a fatty chain of constant length ($m = 12$ carbons). This systematic study showed that (i) at constant degree of ethoxylation, the response factors in refractometric detection are independent of the nature of the fatty chains in the range studied (from $C_{10}$ to $C_{16}$), and (ii) in contrast, these response factors are strongly dependent on the degree of ethoxylation, as evidenced in Fig. 3.

Fig. 3 shows the evolution of the response factors in refractometry of the different oligomers resulting from the condensation of ethylene oxide with dodecanol. These factors are relative, the response factor of the oligomer $C_{12}E_8$ being the reference. This graph indicates a noticeable difference between the oligomers of low degree of ethoxylation and the highly condensed oligomers. Effectively, from one to eight ethylene oxides, the response factor increase almost linearly as a function of the degree of ethoxylation, then, between 8 and 12 ethylene oxides, the response factors decrease regularly and become quasi-constant after $n = 13$ ethylene oxides.

This behaviour is in good agreement with a recent study by Okada [25], who described the existence of two conformers of poly(ethylene oxide) (PEO) in a water–acetonitrile medium: the first is all-*trans*, linear and very stretched, and its structure is perfectly compatible with the increase in refractometric response factors between 1 and 8 ethylene oxide moieties; the second is *gauche*, helicoidal, and is at the origin of the coiling of the polyoxyethylenic chain at high degrees of ethoxylation. It results in a decrease, then in quasi-constancy of the refractometric response factor above $n = 8$ ethylene oxides. Therefore, our observations could be explained by a transition between the two conformers at around 8–9 ethylene oxides condensed with the dodecanol when we perform analyses in a water–acetonitrile (40:60, v/v) medium.

Being now able to correct satisfactorily the raw data obtained after the analysis of any of our surfactant formulations, we studied in a second step the repartition of such formulations as a function of their fatty chains and of their degree of ethoxylation.

Fig. 4 reports the distributions as a function of these two variables in the case of surfactant B resulting from the condensation of ethylene oxide with a mixture of $C_{13}$–$C_{15}$ fatty alcohols.

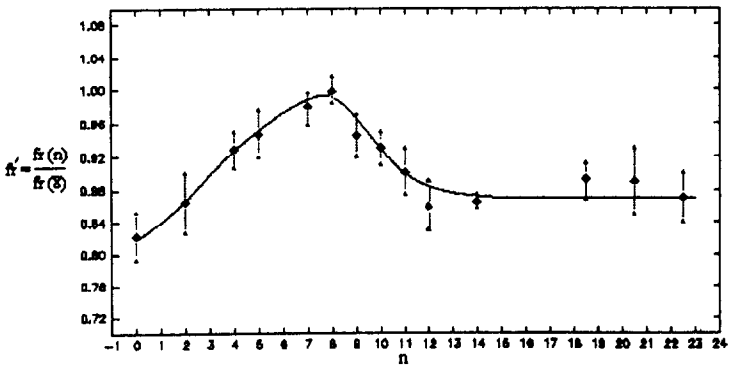


Fig. 3. Evolution of the response factors with refractometric detection as a function of the deggee of condensation of ethylene oxide with dodecanol. ◆ = Average experimental values (values of uncertainties are standard deviations obtained from ten independent analyses); line = graph obtained by regression.

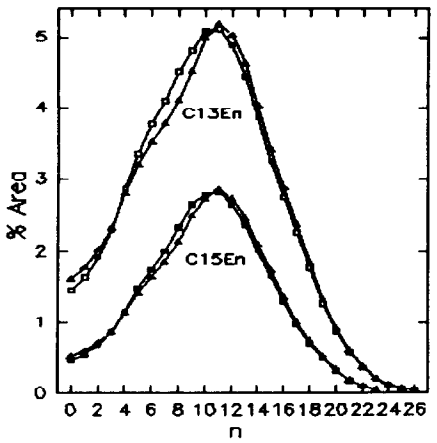


Fig. 4. Repartition of B surfactant oligomers as a function of the degree of ethoxylation and of the fatty chain. Operating conditions similar to those in Fig. 1. □, ■ = Repartition calculated without correction including the variation of the refractometric response factors; △, ▲ = repartition computed after including the variation of the refractometric response factors.

With a view to establishing the improvement in precision obtained by correcting the response factors in refractometry, we also report the distributions obtained when the response factors are assumed to be constant. The correction of response factors results in a greater value of the average degree of ethoxylation ($\bar{n}$). In the present case, a value of $\bar{n} = 9.5$ EO is obtained without correction and a value of $\bar{n} = 10$ EO is obtained using the correct factors; this latter value is in better agreement with the value given by the producer ($\bar{n} = 11$ EO), and it is clearly necessary to use the corrected response factors.

Fig. 5 reports the alkyl chains and ethylene oxide repartitions of the surfactants A and C, obtained after correction of the chromatographic data. These two formulations have similar average degrees of ethoxylation, $\bar{n} = 7.2$ and 6.5, respectively.

These analyses evidence that the surfactant formulations A and B result from the condensation of ethylene oxide on the same fatty alcohols ($C_{13}$–$C_{15}$ mixture). Their surfactant characteristics are different because of their different degrees of ethoxylation. In the case of surfactant B, the degree of ethoxylation is greater than that in surfactant A ($\bar{n} = 10$ and 7.2 EO, respectively).

The differences in the properties can also result from differences in the fatty chains (nature and relative abundances) and it was also important to determine these characteristics. As we demonstrated that the response factors are independent of the nature of the fatty chains, these fundamental data were deduced directly from chromatographic analyses, without correction. Good reproducibility was obtained from 5–6 independent analyses of each of the surfactant formulations analysed (Table 2).

Our objective was to determine with good precision these surfactants in very dilute aqueous solutions with a view to studying their behaviour in the environment. Therefore, we completed

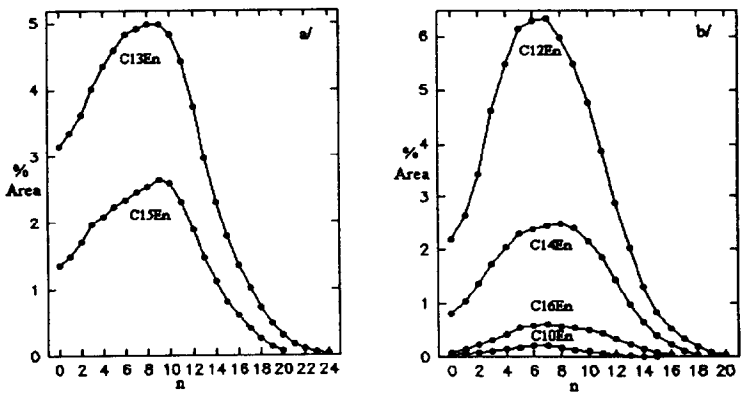


Fig. 5. Repartition of (a) A and (b) C surfactant oligomers, calculated with correction of the refractometric response factors. Operating conditions as in Fig. 1.

Table 2
Characteristics of the surfactant mixtures A, B and C deduced from HPLC analyses with operating conditions as in Fig. 1

| Parameter | Surfactant mixture[a] | | |
|---|---|---|---|
| | A | B | C |
| Relative abundance of fatty chains[a] (%) | $C_{13}$: 67.4 (0.4) | $C_{13}$: 67.6 (0.9) | $C_{10}$: 1.65 (0.15)<br>$C_{12}$: 65.5 (0.8) |
| | $C_{15}$: 32.6 (0.4) | $C_{15}$: 32.4 (0.9) | $C_{14}$: 27.0 (0.4)<br>$C_{16}$: 5.85 (0.20) |
| Average ethoxylation number ($\bar{n}$) | $C_{13}$: 7.2 | $C_{13}$: 10.0 | $C_{10}$: 5.9<br>$C_{12}$: 6.4 |
| | $C_{15}$: 7.3 | $C_{15}$: 10.1 | $C_{14}$: 6.8<br>$C_{16}$: 7.0 |
| Average molar mass (g $mol^{-1}$) | 527 | 650 | 484 |

[a] Values in parentheses are the standard deviations calculated from five independent analyses.

this study by searching for an enrichment technique that would meet this challenge. Effectively, refractometric detection appeared in our case to be more sensitive than the other detection modes usually utilized for these compounds [low-wavelength UV (190–200 nm), indirect UV detection or ELS], but the detection threshold was found to be about 100 ppm for the whole surfactants, i.e., about 50 ppb in the case of the less abundant oligomers. Therefore, we used liquid–solid extraction, which is commonly used for sample concentration [26].

Three experimental parameters were then optimized. (i) The first step was the optimization of the volume of aqueous solution containing the surfactant mixture. The sample volume was percolated through an octadecyl-bonded silica cartridge in order to obtain an important enrichment factor, without prohibitive operating times. (ii) In the second step, the flow-rate was optimized to reach a good compromise between efficiency and operating time. (iii) Finally, the cartridge was rinsed with a definite volume of acetonitrile (solvent used in the HPLC mobile phase). This volume had to be sufficient to desorb quantitatively the surfactant mixture retained on the $C_{18}$ phase and had to be minimized in order to avoid excessive dilution of the sample. After a systematic study, it appeared that a maximum of 1.5 l of sample could be percolated through the $C_{18}$ cartridge at a maximum flow-rate of 10–12 ml $min^{-1}$. Beyond these values, the operating time became prohibitive and important distortions appeared in the oligomer repartitions of the surfactant mixtures.

Finally, we studied the evolution of the recovery yields as a function of the volume of acetonitrile used to desorb the samples (see Table 3). This study showed that the quantitative recovery of the surfactant requires a minimum of 4 ml of acetonitrile, which was adopted as the optimum value. Smaller volumes of acetonitrile resulted in a non-quantitative recovery and larger volumes did not improve the recovery yields of any of the surfactants A, B or C.

Under these conditions, the maximum enrichment factor was 375. However, as mentioned previously, the injection should be performed

Table 3
Evolution of the recovery for the surfactant mixtures A, B and C using a $C_{18}$ cartridge as a function of the acetonitrile volume used for desorption

| Acetonitrile volume (ml) | Recovery (%) |
|---|---|
| 2.0 | 70 |
| 3.0 | 85 |
| 3.5 | 92 |
| 4.0 | 100 |

using a solvent identical with the mobile phase used for the chromatographic analysis, i.e., water–acetonitrile (40:60). Therefore, we used 3 ml of the concentrated organic solution, which was diluted by adding 2 ml of water. Thus, the final maximum enrichment factor was 225. Under these conditions, a practical limit of quantitation of 0.5 mg $l^{-1}$ was obtained for the whole surfactant mixtures, i.e., 0.25 $\mu$g $l^{-1}$ for the less abundant oligomers. Consequently, quantitation of the surfactant mixtures studied (A, B and C) was possible in the concentration range $5 \cdot 10^{-4}$–5 g $\cdot l^{-1}$.

As reported in Table 4, excellent calibration graphs were indeed obtained for the three surfactants in the concentration range 0.1–5 g $l^{-1}$ without enrichment, and this range was considerably broadened towards lower concentrations owing to the enrichment procedure.

To confirm this large quantitation range, we compared the distributions of oligomers of a surfactant mixture at a known concentration with the distribution obtained after concentrating 225-fold a diluted solution of the same surfactant, the initial concentration being chosen to give the same concentration of surfactant after enrichment as in the previous analysis. It is obvious that the enrichment procedure did not cause any distortion in the different distributions, either in alkyl chains ($C_{13}$ and $C_{15}$ in this case) or in the ethoxylated chains (see Fig. 6). In fact, the two chromatograms can almost be superimposed (taking experimental errors into account). Five independent experiments were repeated, confirming the quantitative recovery of the sample, and the relative standard deviations of the quantitation of each oligomers after enrichment were around 5%.

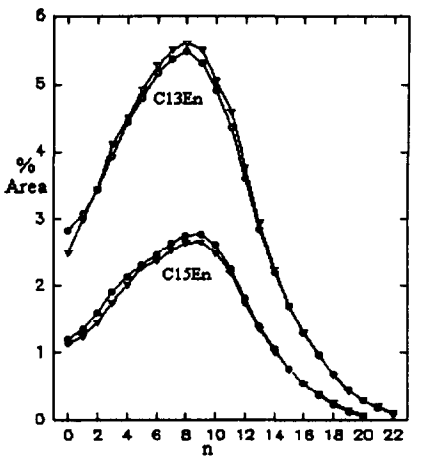


Fig. 6. Comparative study of the oligomer repartitions of the surfactant mixture A as a function of the degree of ethoxylation and of fatty chains, prior to and after enrichment procedure. Conditions as in Fig. 1. ○, ● = Distributions calculated for $C_{13}$ and $C_{15}$ alkyl chains of surfactant A, concentration 1200 mg $l^{-1}$; ▽, ▼ = distributions calculated for $C_{13}$ and $C_{15}$ alkyl chains of the surfactant A, initial concentration 5 mg $l^{-1}$, concentrated 225-fold.

## 4. Conclusion

A method using $C_8$-bonded silica in reversed-phase liquid chromatography has been developed to obtain accurate and reproducible separations of non-ionic surfactant mixtures. This technique allowed us to separate complex distribution of oligomers, with refractometric detection, of all the homologous compounds which differ in their alkyl chains and their degrees of ethoxylation.

The determination of a wide range of response factors allowed us to perform a strict quantitation of all these oligomers, while a simple and reproducible enrichment procedure allowed us to

Table 4
Calibration graphs obtained for the three surfactant mixtures studied

| Surfactant mixture | Slope | Intercept on the ordinate | Correlation coefficient |
|---|---|---|---|
| A | $5.84 \cdot 10^{-2}$ | 0.84 | 0.9999 |
| B | $4.60 \cdot 10^{-2}$ | 0.40 | 0.9998 |
| C | $6.53 \cdot 10^{-2}$ | 4.17 | 0.9995 |

improve considerably the detection threshold under 1 $\mu g\ l^{-1}$.

This high-performance technique should allow us to determine the adsorption isotherms of each oligomer with good precision, with a view to studying their adsorption at liquid–solid interfaces, in a wide range of concentrations. This would allow us to model the behaviour of the complex non-ionic surfactant mixtures used in commercial detergents and to establish their environmental impact.

## Acknowledgements

This work was financially supported by the French Environment Ministry and the French Association of Soaps and Detergents Industries (AISD).

## References

[1] B.F. Greek, Chem. Eng. News, 25 (1991) 36.
[2] A.M. Rothman, J. Chromatogr., 253 (1982) 283.
[3] M.F. Cox, J. Am. Oil Chem. Soc., 66 (1989) 367.
[4] R.A. Llenado and R.A. Jamieson, Anal. Chem., 53 (1981) 174R.
[5] B. Stancher and L. Favretto, J. Chromatogr., 150 (1978) 447.
[6] C.A. Eckert, M.P. Ekart, B.L. Knutson, K.P. Payne, D.L. Tomasko, C.L. Liotta and N.R. Foster, Ind. Eng. Chem. Res., 31 (1992) 1105.
[7] P.L. Desbène, B. Desmazières, V. Even, J.J. Basselier and L. Minssieux, Chromatographia, 24 (1987) 857.
[8] J.D. McClure, J. Am. Oil Chem. Soc., 59 (1982) 364.
[9] R.E.A. Escott and N. Mortimer, J. Chromatogr., 553 (1991) 423.
[10] T. Takeuchi and D. Ishii, J. Chromatogr., 403 (1987) 324.
[11] S. Brossard, M. Lafosse and M. Dreux, J. Chromatogr., 591 (1992) 149.
[12] J.A. Pilc and P.A. Sermon, J. Chromatogr., 398 (1987) 375.
[13] M. Ahel and W. Giger, Anal. Chem., 57 (1985) 2584.
[14] I. Zeman, J. Chromatogr., 383 (1986) 223.
[15] C. Zhou, A. Bahr and G. Swedt, Anal. Chim. Acta, 236 (1990) 273.
[16] P.L. Desbène and B. Desmazières, J. Chromatogr. A, 661 (1994) 207.
[17] B. Desmazières, F. Portet and P.L. Desbène, Chromatographia, 36 (1993) 307.
[18] M. Kudoh, J. Chromatogr., 291 (1984) 327.
[19] N. Nakamura, Y. Morikawa and I. Matsumoto, J. Am. Oil Chem. Soc., 58 (1981) 72.
[20] T. Ban, E. Papp and J. Inczedy, J. Chromatogr., 593 (1992) 227.
[21] G. Barka and P. Hoffman, J. Chromatogr., 389 (1987) 273.
[22] Z. Wang and M. Fingas, J. Chromatogr. A, 673 (1993) 145.
[23] W.R. Melander, A. Nahum and C. Horvath, J. Chromatogr., 147 (1982) 129.
[24] K. Noguchi, Y. Yanagihara, M. Kasai and B. Katayama, J. Chromatogr., 461 (1989) 365.
[25] T. Okada, Anal. Chim. Acta, 281 (1993) 95.
[26] R.W. Frei and K. Zech (Editors), Selective Sample Handling and Detection in HPLC (Journal of Chromatography Library, Vol. 39A), Elsevier, Amsterdam, 1988, p. 5.