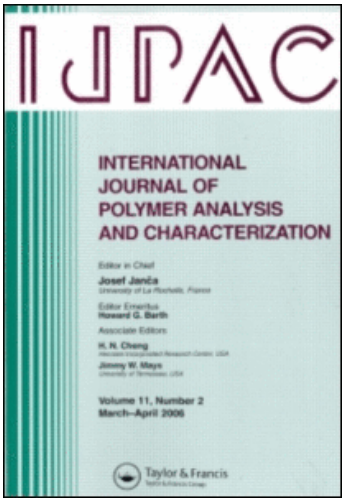

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Mapping of Polyethers Using Two-Dimensional Liquid Chromatography With Coupled Density and RI Detection

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Mapping of Polyethers Using Two-Dimensional Liquid Chromatography With Coupled Density and RI Detection

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Two-dimensional liquid chromatography is a useful method for the characterization of nonionic surfactants, such as ethoxylated fatty alcohols with respect to the distributions of molecular weight, chemical composition, and functionality. In the first dimension, liquid chromatography under critical conditions is used to separate according to functionality. The fractions thus obtained, which consist of pure homologous series, are analyzed by size exclusion chromatography. From these data, a three-dimensional map is established. With a combination of a density detector and a refractive index detector in both dimensions, the correct altitudes in such a map are obtained. By this technique, the contribution of preferential solvation in each peak can also be determined.

KEY WORDS Nonionic surfactants, liquid chromatography, molecular weight measurements, size exclusion chromatography, density detector

INTRODUCTION

Ethoxylated fatty alcohols (FAE) typically consist of different homologous series depending on the purity of the fatty alcohol used as the starting material. Low-molecular-weight samples can also contain unreacted fatty alcohol, and high-molecular-weight samples often contain polyethylene glycols. This means that a complete characterization of FAE requires information on both the distributions of the chain length of the polyoxyethylene and the carbon number of the alkyl group, as well as the functionality type distribution (FTD). This can be achieved using two-dimensional liquid chromatography (LC), which involves a separation of the homologous series using LC under critical conditions (LCCC) [1–4] on a semi-preparative scale as the first dimension, and the analysis of the separated homologous series by size exclusion chromatography (SEC) as the second dimension, which yields the molecular weight distribution (MWD) for each fraction. From the data thus obtained, it should be possible to characterize a surfactant in the form of a three-dimensional map.

This approach is, however, not easy; even if a good separation is achieved, the quantification of the results is often unsatisfactory because of detection problems. As the indi-

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vidual oligomers have a different chemical composition, they will be seen by the detector with a different sensitivity. Moreover, preferential solvation [5–9] of amphiphilic molecules can cause additional complications.

Detection in Liquid Chromatography of FAE

Since FAE products do not contain any UV-absorbing groups (unlike ethoxylated alkyl phenols), different approaches have been described in the literature. Some authors have used derivatization (typically with 3,5-dinitrobenzoyl chloride) and subsequent UV [10] or polarographic detection [11]. Others used an evaporative light scattering detector (ELSD) [12] or a differential refractive index (RI) detector [13–17]. The density detector [18,19], which has been developed in our group, can also be applied to the analysis of FAE, as we have shown previously [20,21].

It is clear that derivatization is not an appropriate method in two-dimensional chromatography. The ELSD is not the instrument of choice because of its poor linearity. Moreover, the effect of operating conditions, molecular weight and composition of the sample on the response of this detector is not yet clear. RI and density detectors are bulk property detectors, which can only be used in the isocratic mode. If a separation can be achieved under isocratic conditions, both instruments will work well. One has to take into account that the response factors of bulk property detectors are closely related to specific properties, such as refractive index increment or apparent specific volume, which will be dependent on molecular weight [22–28]. As has already been shown [27–31], this dependence can be compensated using

$$f_i = f_{i,\infty} + \frac{K}{M_i} \tag{1}$$

where f_i is the response factor of an oligomer with the molecular weight M_i and $f_{i,\infty}$ is the response factor of a polyether chain with infinite (or, at least sufficiently high) molecular weight, and K is a constant representing the influence of the end groups. In a plot of f_i vs. $1/M_i$, K is the slope of the regression line.

For a given polymer homologous series of FAE, increasing molecular weight means also increasing content of ethylene oxide units and decreasing alkanol content. Hence, a different approach for quantification should be used so that the chemical composition at any point of the chromatogram can also be obtained from dual detection.

Determination of Chemical Composition Using Dual Detection

As we have shown previously [20,31–33], the weight fractions w_A and w_B of the units A and B in a polymer can be determined at any point of the peak from the signals x_D and x_R of coupled density (D) and RI (R) detectors and the corresponding response factors:

$$\frac{1}{w_A} = 1 - \frac{f_{R,A} \cdot \dfrac{x_D}{x_R} - f_{D,A}}{f_{R,B} \cdot \dfrac{x_D}{x_R} - f_{D,B}} \tag{2}$$

In the low-molecular-weight range, there are basically two different situations:

- In the case of polymer mixtures or copolymers, the response factors in Equation (2) have to be calculated for each molecular weight using Equation (1) from $f_{\infty,D}$, $f_{\infty,R}$ K_D, and K_R.
- In the case of FAE, one may consider the polyoxyethylene chain as component A, which is inserted as a center block between the alkyl and the hydroxy group of component B (the fatty alcohol). In this case, no correction for the end groups is required because the PEO-chain has no end groups, only the carbon number of the alkanol has to be accounted.

Once the weight fractions of the components w_A and w_B of a fraction have been determined, one may calculate the corresponding response factor for each detector

$$f_i = w_A \cdot f_A + w_B \cdot f_B = w_A \cdot (f_A - f_B) + f_B \tag{3}$$

from which the correct mass m_i eluted within the fraction can be calculated:

$$m_i = \frac{x_i}{f_i} \tag{4}$$

It is evident that Equations (1–4) can only be applied to pure homologous series (with the same end groups), as they are obtained from LCCC.

Provided that the separation in the first dimension is good, the molecular weight distributions of the individual homologous series can be determined with high accuracy by SEC as the second dimension [20]. The quantification of the first dimension is, however, much more difficult [21]: in LCCC, each peak contains an unknown amount of polymer of an unknown composition (i.e., with given end groups, but an unknown number of EO-units). Moreover, each peak can also contain an unknown contribution from preferential solvation. Hence, there are three unknown variables for each peak, namely

- The amount m_P of the polymer in the fraction.
- The composition w_A of the fraction (which determines the average response factor f_{av}).
- The amount m_s of preferentially adsorbed solvent.

If preferential solvation can be neglected, the amount and composition of the fraction can be determined from dual detection (density and RI), as is the case in SEC, where single mobile phases are used. LCCC is, however, typically performed in mixed mobile phases, because critical conditions can seldom be found in one-component mobile phases.

As we have shown previously [34], the extent of preferential solvation of the repeating units and the end groups can be considerably different. For FAE, the amount of solvent preferentially adsorbed by each oligomer depends on both the relative length of the hydrophobic alkyl group and the hydrophilic polyether chain. Hence, considerable errors may arise in the determination of the mass of the fractions.

The determination of all three parameters (m_P, w_A, and m_S) requires additional information, which could be provided by a third detector, if the response to the components (A and B) and to the solvent were sufficiently defined. If, however, the composition of the fraction is known, one may determine the amount of fraction and

preferentially adsorbed solvent with only two detectors. This information can be obtained by analyzing each fraction by SEC with dual detection in the second dimension. The principle of this method has already been described in full detail in a previous communication [21].

Quantification in Two-Dimensional LC With Dual Detection

In a solution of a polymer in a mixed solvent, the composition of the latter inside the coils will be different from outside because of different affinity of the polymer towards the components of the solvent. If such a solution is injected onto a chromatographic column, the zone of "dialyzed solvent" will be separated from the sample peak, the area of which will contain a contribution from preferential solvation. The response factors thus obtained can therefore be regarded as apparent ones. The true response factors will be found when the sample is injected (on) directly into the detector(s), by bypassing the column.

In column measurements, the area X of a peak eluting in the first dimension can be calculated using:

$$X = m_P \cdot (w_A \cdot f_A + w_B \cdot f_B) + m_S \cdot f_S \tag{5}$$

where mp is the polymer mass containing weight fraction w_A and w_B of components A and B, m_s is the mass of preferentially adsorbed solvent, and f_A, f_B, and f_s are the corresponding (true) response factors

Once the response factor f_A and f_B and the mass fractions w_A and w_B of the components of the polyer are known, one may calculate its average response factor f_{av} using Equation (3). (In the case of FAE, which consist of the end groups R- and -OH and a PEO-chain without end groups, f_A is the response factor of the fatty alcohol ROH, and f_B the response factor of high molecular PEO.) Hence one may calculate the mass of polymer in the fraction using

$$m_P = \frac{X_D \cdot f_{S,R} - X_R \cdot f_{S,D}}{f_{av,D} \cdot f_{S,R} - f_{av,R} \cdot f_{S,D}} \tag{6}$$

where the indices D and R denote the peak areas and response factors obtained from density and RI detection, respectively.

The amount of preferentially adsorbed solvent can be determined using

$$m_S = \frac{X_D \cdot f_{av,R} - X_R \cdot f_{av,D}}{f_{av,R} \cdot f_{S,D} - f_{av,D} \cdot f_{S,R}} \tag{7}$$

EXPERIMENTAL

These investigations were performed using the density detection system DDS70 (commercially available from Chromtech, Graz, Austria), which has been developed in our group. This instrument has been described in full detail in previous communications [18,19]. In SEC, as well as in LCCC, a Sicon LCD 201 RI detector (Bio-Trade, Vienna, Austria) was used in series with the density detector.

Each chromatographic system was connected to a MS-DOS computer via the serial port. Data acquisition and processing was performed using the software package Chroma (Chromtech, Graz, Austria), which has been developed for the DDS 70.

SEC measurements were performed in chloroform (ChromAr HPLC grade, Mallinckrodt, USA) at a constant flow rate of 1.0 mL/min, which was maintained by a Gynkotek 300C HPLC pump (Gynkotek, Munich, Germany). Samples were injected using a Vici injection valve (Valco Europa, Schenkon, Switzerland) equipped with a 100-μL loop, the concentration range was 4–8 g/L. A column set of four Phenogel columns (2 × 500 Å + 2 × 100 Å) 30cm each (Phenomenex, Torrance, CA, USA) was used. SEC calibrations were obtained using pure oligomers of EO (Fluka, Buchs, Switzerland) and SEC standards from Polymer Laboratories (Church Stretton, Shropshire, UK).

For LCCC, a Jasco 880 PU pump was used (Japan Spectrosopic Co., Ltd., Tokyo, Japan), which was operated at a flow rate of 2 mL/min. LCCC was performed in methanol and methanol-water 90:10 (w/w) (LiChroSolv HPLC grade, Merck, Darmstadt, Germany) on a semipreparative column filled with Spherisorb ODS2 (5 μm, 10 × 250 mm) (Phase Separations Ltd., Deeside, Clywd, UK). For the column measurements, a Valco injection valve (Valco Europa) equipped with a 500-μL loop was used, and the fractions were collected by means of an Advantec 2120 fraction collector (Toyo Roshi International Inc., Dublin, CA, USA). For bypass measurements, we used, as well with the manual injector, an autosampler (Spark SpH 125 Fix, Spark Holland, Emmen, Netherlands), which was equipped with a 50-μL sample loop. From the comparison of both series of measurements, the injected volume of the autosampler was found to be 53 μL (assuming the larger loop to be exactly 500 μL). In order to avoid adsorption effects in the syringe, samples were transferred to the larger loop (on the manual injector) via a Teflon capillary directly from the flask by displacement with air. By this procedure, the reproducibility of peak areas was dramatically improved (typically about ± 0.5%).

The alkanols, polyoxyethylenes, and FAE (Brij) samples were purchased from Fluka and used without further purification.

RESULTS AND DISCUSSION

As the first step, the response factors of water, polyethylene glycols, alkanols, and—as far as they were available—FAE oligomers had to be determined. Figure 1 shows a plot of the peak areas of water from density and RI detection, as obtained from bypass measurements, versus the sample size. From the slopes of the regression lines, the true response factors were determined. Figure 2 shows a plot of the true response factors of oligoethylene glycols as a function of 1/M. Values of $f_{\infty,D}$, $f_{\infty,R}$, K_D, and K_R were determined from the intercepts and slopes of the regression lines.

In Figures 3 and 4 the corresponding plots for the alkanols and the available pure FAE oligomers are shown. As can be seen, the individual oligomers fall perfectly on the lines obtained from a two-point calibration using sufficiently high molecular PEG and the corresponding alkanol, which indicates, that this simple approach works quite well.

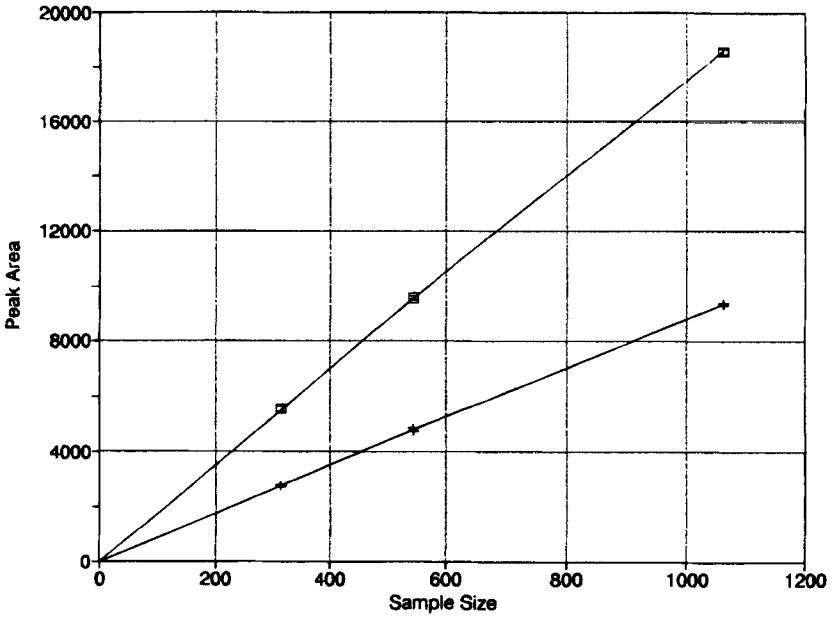

FIGURE 1 Peak areas of water from density (❑) and RI (+) detection as a function of sample size (in μg), as obtained from bypass measurements with an autosampler.

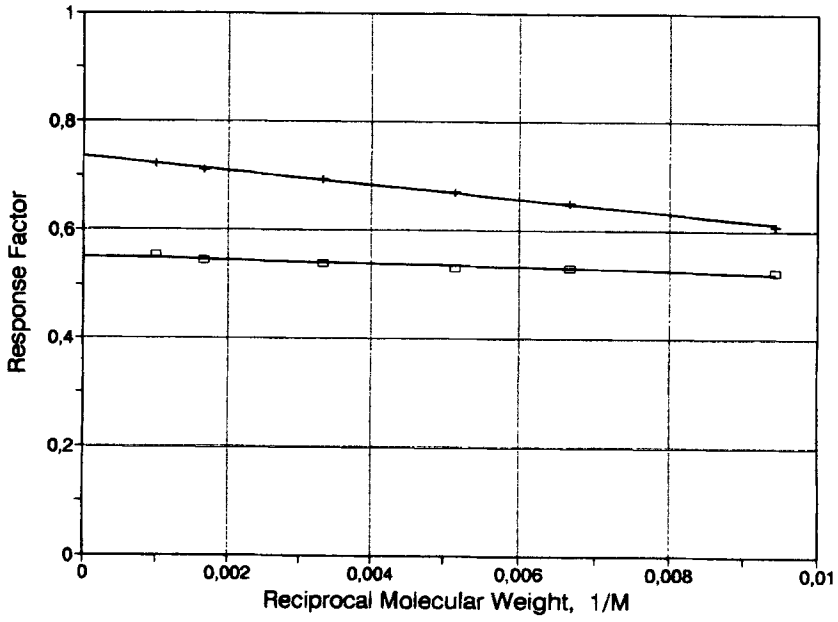

FIGURE 2 True response factors of oligoethylene glycols from density (❑) and RI (+) detection as a function of reciprocal molecular weight (from bypass measurements with autosampler).

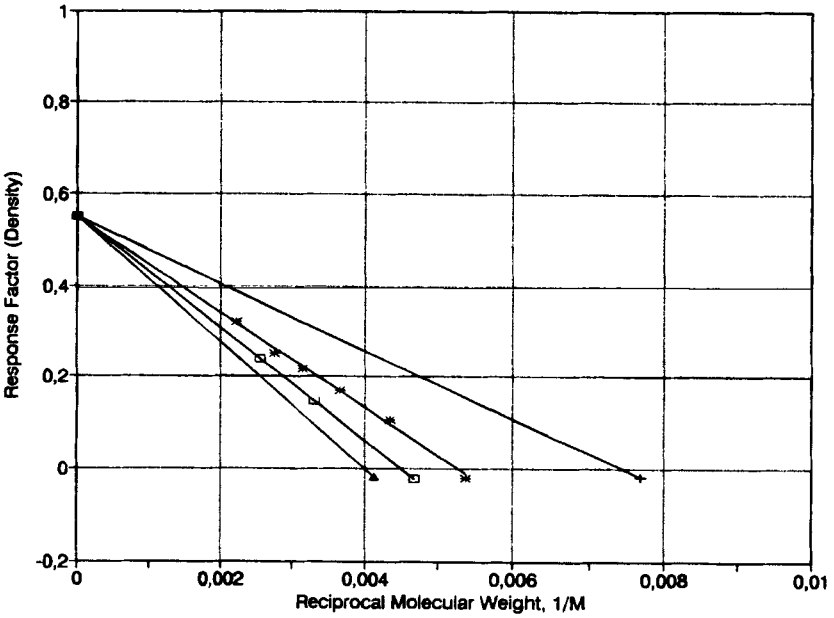

FIGURE 3 True response factors of alkanols and FAE-oligomers from density detection as a function of reciprocal molecular weight (from bypass measurements with autosampler). +: C8; *: C12; ❑: C14; ▲: C16

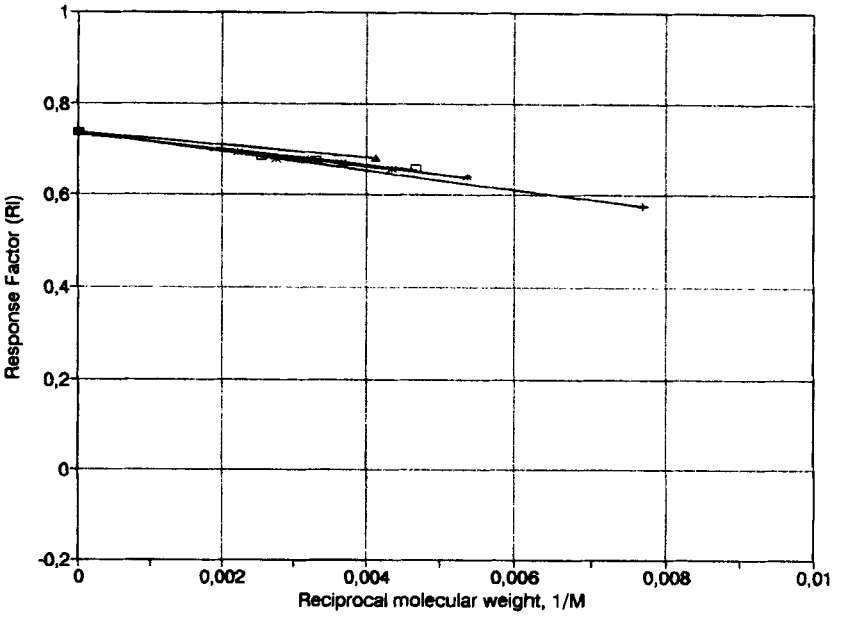

FIGURE 4 True response factors of alkanols and FAE-oligomers from RI detection as a function of reciprocal molecular weight (from bypass measurements with autosampler). +: C8; *: C12; ❑: C14; ▲: C16

The next step was the evaluation of the accuracy of the method, which was done by multiple injection of the alkanols in LCCC and comparison of the mass m_P calculated using Equation (7) with the sample size, which agreed well. From the same measurements, the dependence of preferential solvation on the molecular weight (or the carbon number) of the alkanol could be studied. The results are given in Table I. While the lower alcohols preferentially adsorb water, the higher alcohols adsorb methanol, which appears to be reasonable because of the increase of hydrophobicity with carbon number. As can be seen from Figure 5, the mass percentage of water in alkanol within a peak increases almost linearily with 1/M, as would be expected.

TABLE I

Recovery of alkanols in LCCC with dual detection and extent of preferential solvation

Carbon number	m_P/sample size Equation (6)	m_P/sample size (density alone)	m_P/sample size (RI alone)	m_P/sample size Equation (7)
4	104.8%			3.5%
6	98.4%	100.0%	100.0%	1.3%
8	101.8%	93.7%	96.5%	0.4%
12	99.0%	98.6%	103.2%	–0.3%
14	100.8%	89.0%	96.3%	–0.5%

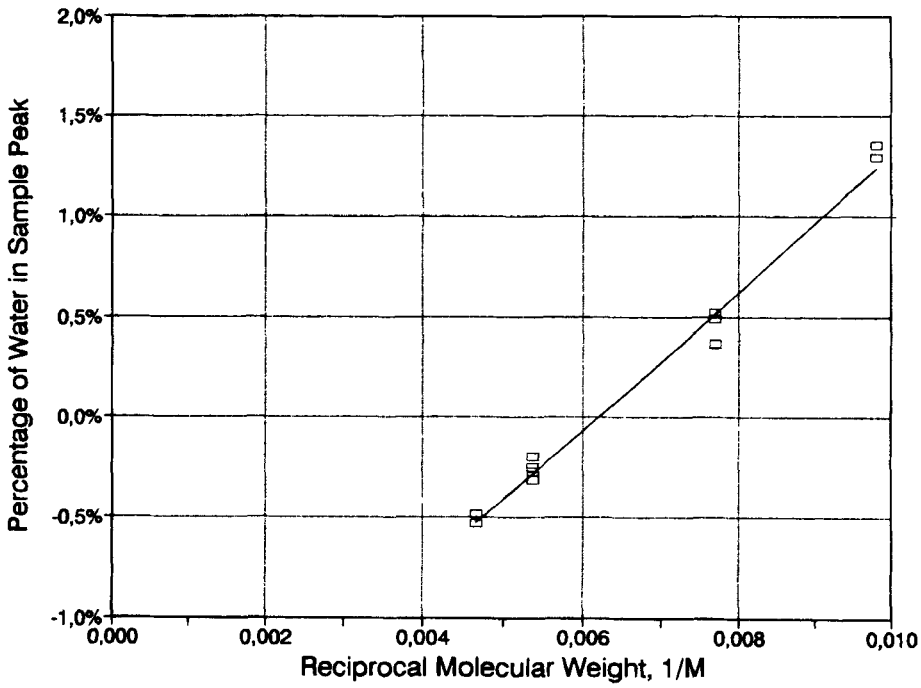

FIGURE 5 Percentage of mass of solvent (from preferential solvation) in sample within a peak for 1-alkanols in methanol-water 90:10 (w/w)

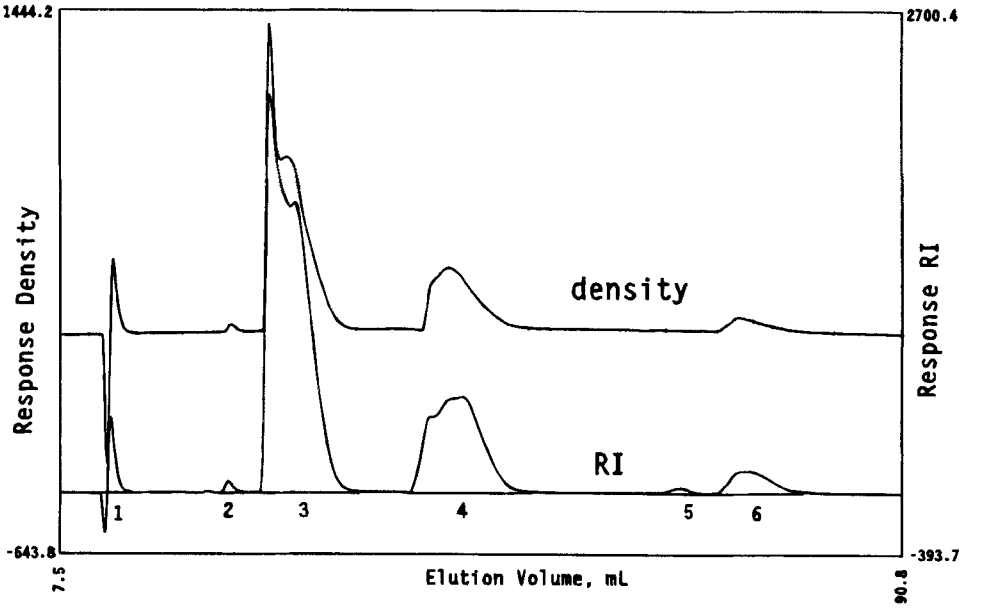

FIGURE 6 LCCC of Brij 30 with density and RI detection (peak 1: PEG + solvent; peak 2: unknown; peak 3: C12-fraction; peak 4: C14-fraction); peaks 5 and 6: C16-fraction).

In the third step, commercial ethoxylated fatty alcohols with a different MWD (Brij 30 and Brij 35), were analyzed by two-dimensional liquid chromatography. In figure 6 the chromatogram of Brij 30 (from LCCC, first dimension) is shown. The individual fractions were collected, the solvent evaporated, and the dried fractions analyzed by SEC. In Figure 7 the chromatogram of the C12-fraction thus obtained is shown. As can be seen clearly, the response factors for the RI detection depend strongly on the number of EO-units (Please note that the peaks of the lower oligomers have the opposite sign in RI detection).

Figure 8 shows the MWD and the composition calculated using Equation (2). From the average composition of each fraction, the average response factors in the first dimension (LCCC) were calculated using Equation (3), and therefrom the amount of each LCCC fraction using Equation (7). The same procedure was the applied to Brij 35.

As can be seen from Table II, the recovery for all fractions was very good for both samples, which indicates that the algorithm works well. When the apparent response factor were used, quite similar values for the C12- and C14-fractions were found, but for the PEG-fraction, which elutes at the solvent peak, no mass could be determined.

With the correct amounts of each fractions, the maps of Brij 30 and Brij 35 were calculated, as is shown in Figures 9–11. Since the samples contain discrete homologous series, the representation in Figures 10 and 11 should be preferred to the three-dimensional surface shown in Figure 9, which suggests the existence of data points, were there can be none.

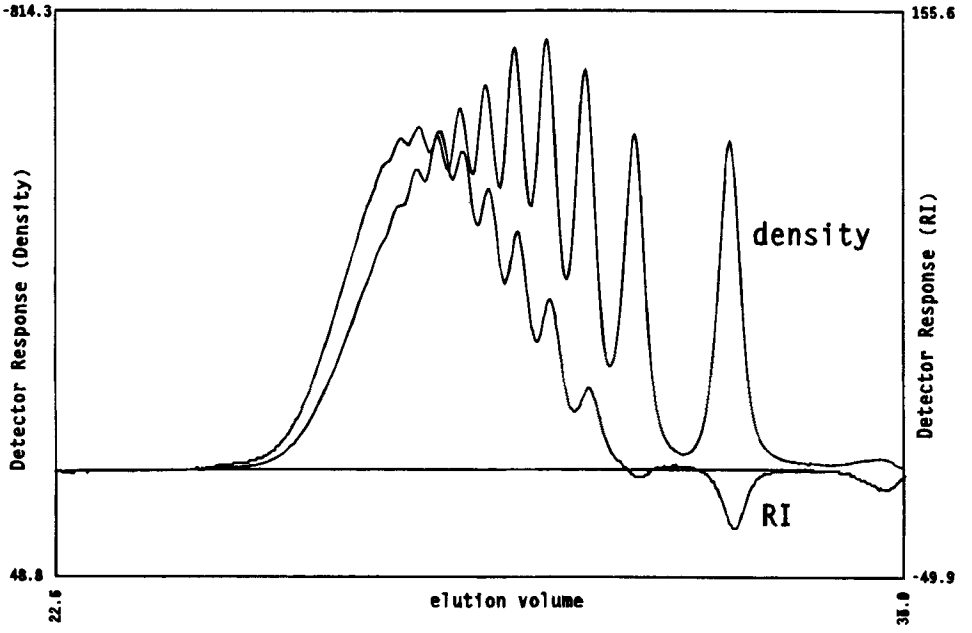

FIGURE 7 SEC of C12-fraction of Brij 30, as obtained with density and RI detection.

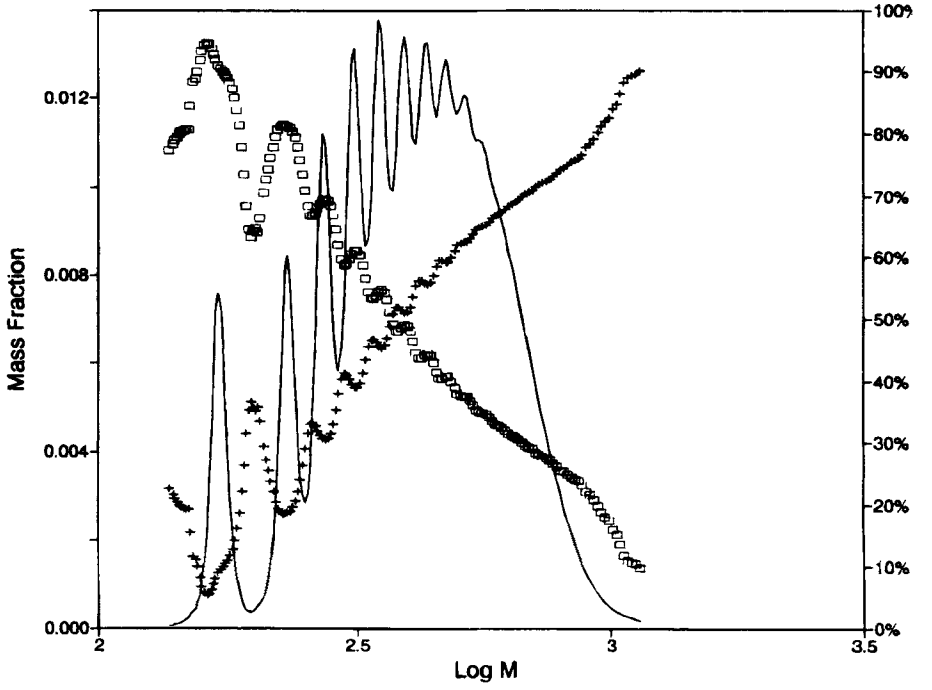

FIGURE 8 MWD and chemical composition of C12-fraction of Brij 30 (from Figure 7). _____: mass distribution; +: weight fraction of ethylene oxide; ❑: weight fraction of dodecanol.

TABLE II

Recovery of fatty alcohol ethoxylates from two-dimensional liquid chromatography with coupled density and RI detection.

	Brj 30			Brij 35		
	Eqn.(6)	density	RI	Eqn.(6)	density	RI
PEG	2.8%			1.9%		
C12	69.8%	70.3%	70.0%	66.9%	70.7%	68.2%
C14	24.5%	27.9%	24.7%	18.8%	20.1%	19.2%
C16	5.0%			8.1%		
Sum	102.0%			95.7%		

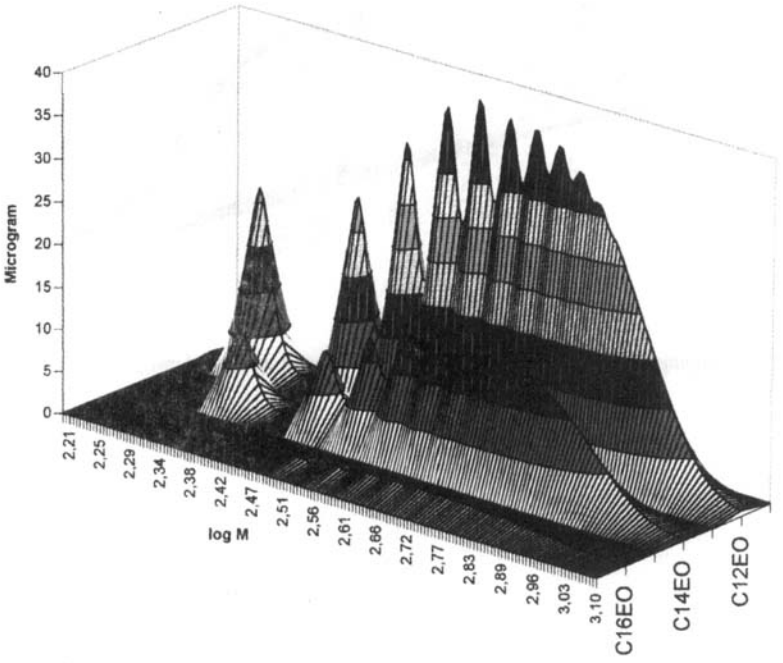

FIGURE 9 Three-dimensional map of Brij 30 from two-dimensional LC with coupled density and RI detection in both dimensions.

CONCLUSIONS

Two-dimensional liquid chromatography with coupled density and RI detection in both dimensions yields quantitatively correct maps of ethoxylated fatty alcohols, which could not be obtained by other techniques. The method also is an excellent approach for studying preferential solvation of amphiphilic substances in mixed mobile phases.

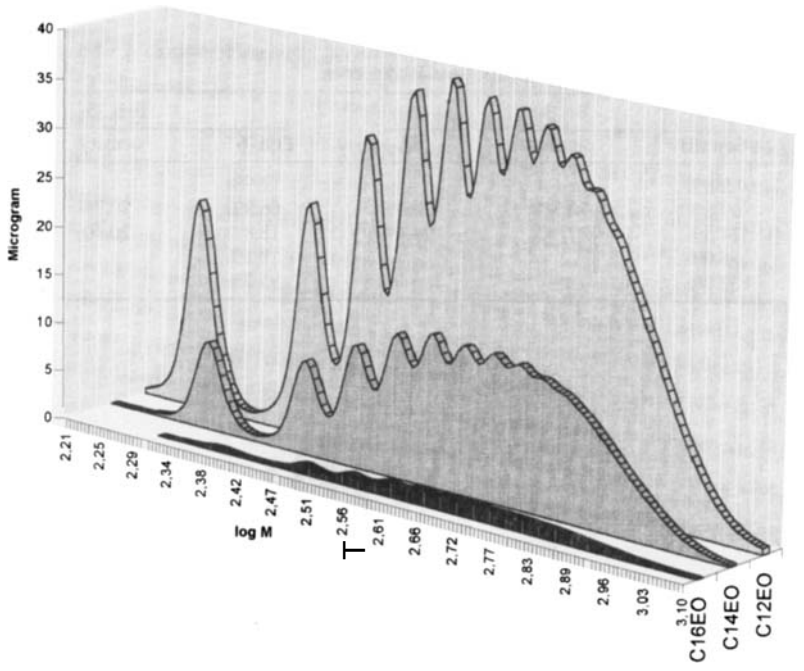

FIGURE 10 Mapping of Brij 30 from two-dimensional LC with coupled density and RI detection in both dimensions.

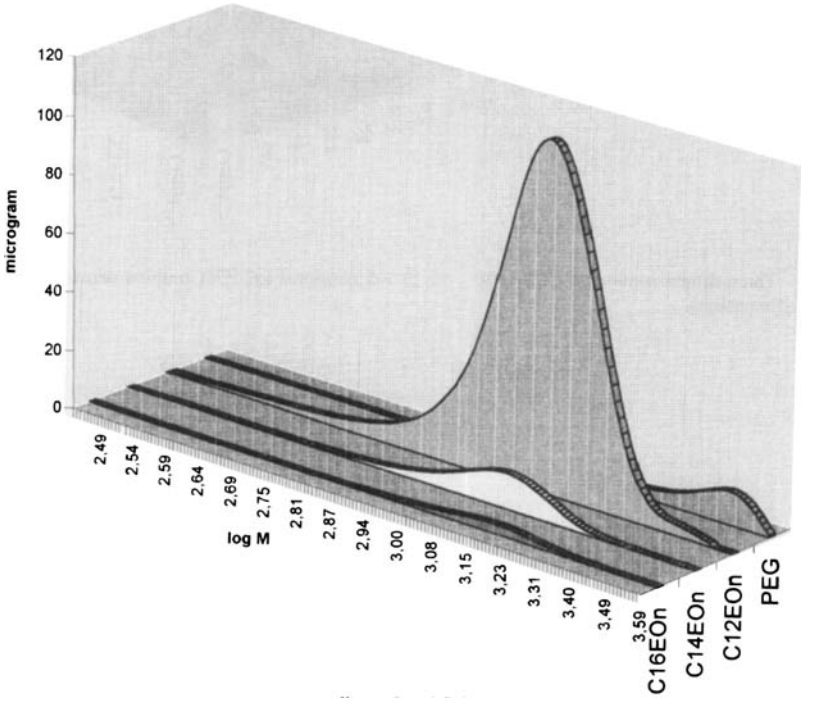

FIGURE 11 Mapping of Brij 35 from two-dimensional LC with coupled density and RI detection in both dimensions.

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