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Characterization of ethoxylated fatty alcohols using liquid chromatography with density and refractive index detection

I. Quantitative analysis of pure homologous series by size-exclusion chromatography

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ABSTRACT

Ethoxylated fatty alcohols can be characterized by two-dimensional chromatography with LC under critical conditions as the first and size-exclusion chromatography (SEC) as the second dimension. The dependence of response factors on the chemical composition can be compensated by two different approaches. The results obtained are in good agreement. The SEC calibrations for the individual homologous series showed considerable differences, hence for each fraction the corresponding calibration should be used.

INTRODUCTION

Ethoxylated fatty alcohols (FAE) are in widespread use as non-ionic surfactants for various applications [1–5]. Nevertheless, their analysis is still somewhat problematic, because these products typically consist of several polymeric homologous series of polyoxyethylene with different end-groups, depending on the purity of the fatty alcohol used as the starting material, and also, in some instances, unwanted polyethylene glycols.

A complete characterization of FAE must provide information on the distributions of the chain length of the polyoxyethylene and the carbon number of the alkyl group. This can only be achieved by two-dimensional analytical methods, but the common methods are one-dimensional. Moreover, there are problems with detection, as will be pointed out later.

Lower oligomers can be analysed by GC [6–8] and for higher molecular mass products HPLC in different variations is commonly used [9–18]. Recently, high-temperature capillary GC [19] and supercritical fluid chromatography (SFC) [18] have also been applied to higher molecular mass samples. Despite the high separation ef-

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ficiency of these techniques, quantification of the results is problematic, because the detector response for each oligomer is not predictable.

In the different modes of liquid chromatography, the separation occurs according to different criteria, as follows: size-exclusion chromatography (SEC) [20] separates according to molecular dimensions, *i.e.*, to the number of ethylene oxide units attached to the fatty alcohol; normal phase LC [9,10] also separates according to the number of ethylene oxide units; and reversed-phase LC [11–18] separates according to the alkyl end-groups.

In a special modification of liquid chromatography, called "LC at the critical point of adsorption" or "LC under critical conditions" (LCCC) [21–24], each homologous series elutes in a sharp peak, regardless of how long the polyether chain is. This allows a separation of the individual homologous series. As LCCC has been described in numerous publications [21–24], its principle will be described only briefly. The chromatographic mobility of a molecule is closely related to its distribution coefficient K_d between the stationary and mobile phases, which in turn depends on the change in free energy, ΔF, when the molecule is adsorbed:

$$K_d = \exp\left(-\frac{\Delta F}{kT}\right) \quad (1)$$

According to the second law of thermodynamics, ΔF is composed of the changes in enthalpy and entropy (ΔH and ΔS, respectively):

$$\Delta F = \Delta H + T\Delta S \quad (2)$$

where ΔH depends on the number of adsorbed segments N_a and the interaction energy ε of each segment:

$$\Delta H = -\varepsilon k T N_a \quad (3)$$

In adsorption chromatography, the retention depends on ΔH, and hence on N_a: the larger a molecule is, the later it elutes. In SEC, the opposite is true: the interaction energy here should be close to zero or at least very small, and the elution behaviour should be governed exclusively by the entropic term: smaller molecules will elute later than larger molecules.

If ε for one segment approaches a critical value ε_{cr}, ΔH and $T\Delta S$ will compensate each other, thus leading to $\Delta F = 0$. At this "critical point of adsorption" the corresponding block will become chromatographically invisible, because its chain length will have no effect on the elution volume. Hence the separation will occur only according to the end-groups, or to any groups different from the repeating unit. Applied to FAE this means, that under critical conditions for polyethylene oxide all oligomers of a homologous series (with a given alkyl end-group) will elute within a narrow peak, regardless of their chain length, thus yielding the functionality type distribution (FTD).

If LCCC is performed on a semi-preparative scale, the separated pure homologous series can be characterized using SEC with respect to their molecular mass distribution (MMD). With such a combination of LCCC as the first and SEC as the second dimension, it should be possible to obtain a "map" of these products, with the number of ethylene oxide (EO) units on the x-axis, the length of the alkyl chain on the y-axis and the amount of each oligomer on the z-axis. Even though a qualitative characterization may be fairly easy to achieve, no satisfactory quantification of the chromatograms obtained in the first or second dimension has been described up to now.

In this paper, we present a method that provides quantitative characterization of the individual homologous series in the second dimension (SEC). The quantification of the first dimension (LCCC) will be described in Part II of this series.

EXPERIMENTAL

These investigations were performed using a DDS70 density detection system (Paar, Graz, Austria), which was developed in our group and has been described in full detail in previous papers [25–27]. In SEC measurements it was combined with a SICON LCD 201 refractive index (RI) detector and in LCCC with a Bischoff 8110 RI detector. Each system was and connected to an MS-DOS computer via the serial port. Data acquisition and processing were performed using the software package CHROMA [27], which has been developed for the DDS 70.

SEC measurements were performed in chloroform (HPLC grade, Rathburn) at a constant flow-rate of 1.0 ml/min, which was maintained by a Gynkotek 300C HPLC pump. Samples were injected using a VICI injection valve equipped with a 100-μl loop; the concentration range was 4–8 g/l.

A set of four Phenogel columns, (500/500/1000/1000 Å), 30 cm each, was used for all SEC measurements. The columns were connected to an electrically switched valve (VICI WE-10-C6W) as shown in Fig. 1. When the valve is switched from position A to B before the peaks of interest leave column 4, they are sent back to column 1, and the number of columns through which they have to pass is increased by 2. By switching at appropriate intervals, the separation efficiency of a set of 10–12 columns can be achieved with only four columns. The SEC calibrations were obtained using pure oligomers of EO (from Fluka) and SEC standards from Polymer Laboratories.

In LCCC, two JASCO 880 PU pumps were used, which were equipped with Rheodyne Model 7125 injection valves with a 50- and 500-μl loop, respectively.

Reversed-phase LC was performed with methanol and methanol–water mixtures (from Merck, HPLC grade) on different analytical columns and a semi-preparative column filled with Spherisorb from Phase Separations (ODS-2, 3 μm, 100 × 4.6 mm I.D.; ODS-2, 5 μm, 250 × 4.6 mm I.D.; and ODS-2, 5 μm, 250 × 10 mm I.D.). The flow-rate was 0.5 ml/min in the analytical mea-

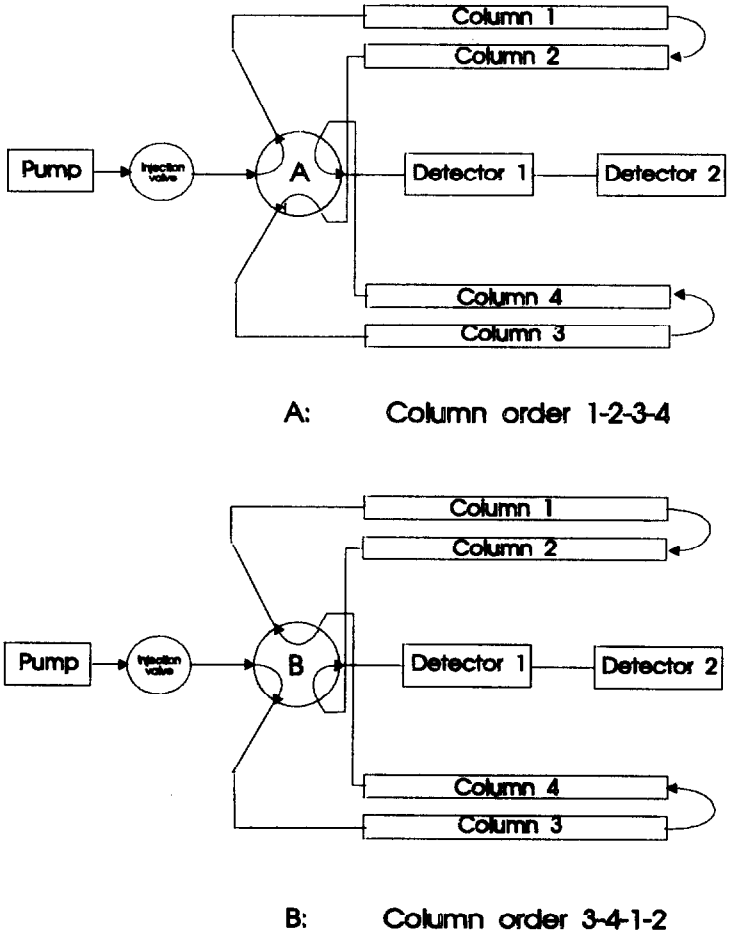

Fig. 1. Configuration used for recycling in SEC measurements.

surements and 2 ml/min in semi-preparative LCCC. An Advantec 2120 fraction collector was used in the semi-preparative separations.

The alkanols and polyoxyethylenes were purchased from Fluka and used as received. Pure homologous series were prepared by anionic ethoxylation [28] of pure 1-alkanols using standard procedures. A monodisperse oligomer octyl-$(EO)_4$ was synthesized by a modified Williamson synthesis [29–31] from 1-octyl bromide and tetraethylene glycol.

Problems in quantitative characterization of FAE

As FAE do not contain any UV-absorbing groups (unlike ethoxylated alkylphenols), different approaches have been described: some workers used derivatization (typically with 3,5-dinitrobenzoyl chloride) and subsequent UV [9] or polarographic detection [15], and others used RI [11–14] or evaporative light-scattering detectors [16]. If one wants to avoid the problems associated with derivatization, there are basically three detectors suitable for this purpose: (1) the evaporative light-scattering detector (ELSD), (2) the differential refractive index (RI) detector and (3) the density detector.

Because of its poor linearity [32], the ELSD is not the instrument of choice, even though it allows the use of gradients. Moreover, it is very likely that the response of this detector will depend on the molecular mass and composition of the sample (because they may determine the size and the transparency of the droplets or particles formed on evaporation of the mobile phase), and the nature of this dependence is not yet clear. RI and density detectors are universal detectors, which can only be used in the isocratic mode. If a separation can be achieved under isocratic conditions, both detectors perform fairly well. One has to take into account, however, that the response factors of universal detectors are closely related to specific properties, such as refractive index increment or apparent specific volume. Hence they will depend on molecular mass (in this instance on the number n of EO units) [33–39].

As has already been shown [11,20,40], this dependence can be compensated using

$$f_i = f_{i,\infty} + \frac{K}{M_i} \tag{4}$$

wherein f_i is the response factor of the oligomer with the molecular mass M_i, $f_{i,\infty}$ is the response factor of a polyether chain with infinite (or at least sufficiently high) molecular mass and K is a

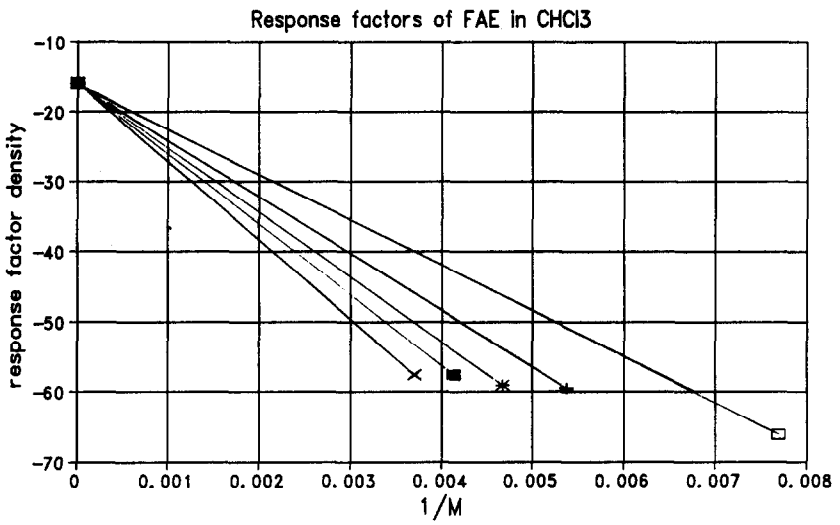

Fig. 2. Response factors (density detection) of homologous series of FAE with different end-groups, as determined by two-point calibration with PEG 3000 and the corresponding alkanols. □ = C_8; + = C_{12}; * = C_{14}; ■ = C_{16}; × = C_{18}.

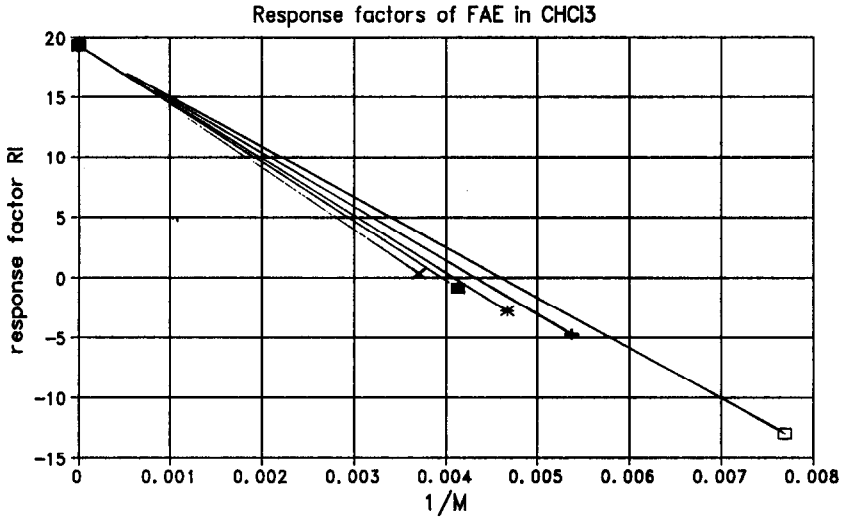

Fig. 3. Response factors (RI detection) of homologous series of FAE with different end-groups, as determined by two-point calibration with PEG 3000 and the corresponding alkanols. Symbols as in Fig. 2.

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.

As no pure oligomers are available for FAE, one can determine K from a two-point calibration using a high-molecular-mass polyoxyethylene and the fatty alcohol (with $n = 0$), or by an iteration procedure, which is provided by CHROMA.

In order to have sufficient amounts of FAE with defined end-groups available, we synthesized several samples by ethoxylation of pure *n*-alkanols and analysed them by analytical LCCC. When necessary, polyethylene glycols were removed by preparative LCCC. As can be seen from Figs. 2 and 3, the response factors of the alcohols vary considerably with their carbon number, hence different values of K are obtained, which are given in Table I.

In the analysis of pure homologous series, this approach yields satisfactory results, provided that the sign of the response factor does not change within the MWD of the sample. As can be seen from Fig. 4, which shows a separation of

TABLE I

RESPONSE FACTORS OF DIFFERENT 1-ALKANOLS IN CHLOROFORM WITH DENSITY AND RI DETECTION AND SLOPES K CALCULATED BY INTERPOLATION AND BY ITERATION (IN CHROMA)

Carbon number of PEG 3000	f_D ($f_{D,\infty} = -16.06$)	Slope for oligomers (density), K_D		f_R ($f_{R,\infty} = 19.287$)	Slope for oligomers (RI), K_R (2-point)
		2-Point	Iteration		
6	−67.07	−5202.7		−22.870	−4300.0
8	−66.11	−6505.9	−6882.9	−13.036	−4202.0
12	59.52	−8082.7	−8431.6	−4.733	−4467.7
14	59.11	−9211.3	−9211.5	−2.765	−4719.1
16	−57.60	−10051.0		−0.904	−4886.2
18	−57.67	−11232.5		0.359	−5110.6

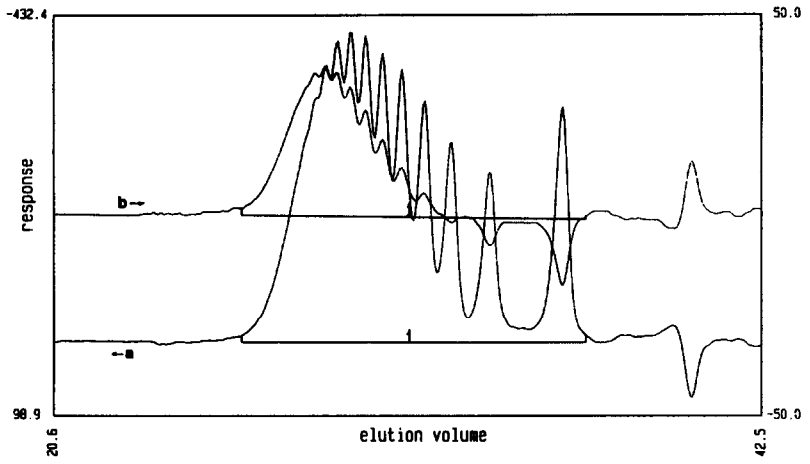

Fig. 4. Chromatogram of an ethoxylated 1-octanol, as obtained with density (a) and RI detection (b).

an ethoxylated 1-octanol by SEC in chloroform, this is fulfilled with density detection, but not with RI detection. In this case, the MWD can only be calculated from the density trace and not from the RI trace. Without compensation of the molecular mass dependence of the response factors an erroneous MWD will be found, because the lower oligomers will be overestimated. Such a compensation can be performed using eqn. 4. The results thus obtained are shown in Figs. 5 and 6.

There is, however, still the problem of the SEC calibration: the calibration line obtained with PEG standards need not necessarily be valid for the monoalkyl ethers [41]. A solution to this problem may be to use dual detection.

Determination of chemical composition using dual detection

As we have shown previously [42,43], the mass fractions m_A and m_B of the monomeric units A and B in a copolymer or a polymer mixture can be determined at any point of the MWD, by SEC with coupled density and RI detectors from the corresponding responses x_D and x_R:

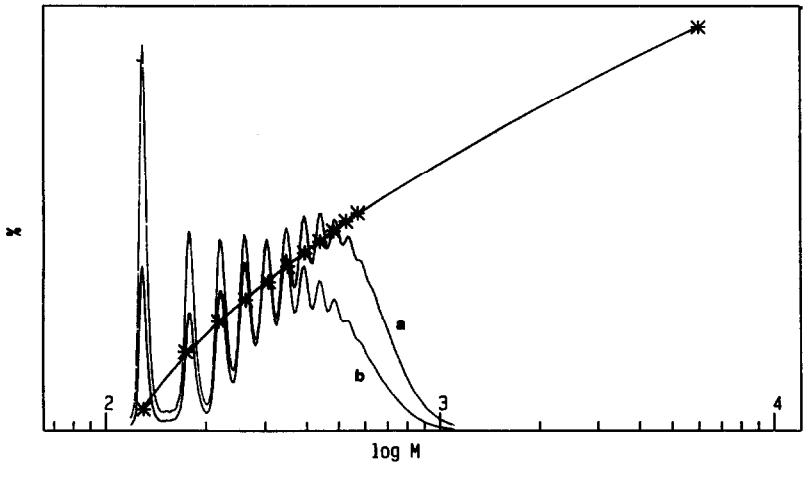

Fig. 5. MWD of the ethoxylated octanol (Fig. 4) from density detection without compensation for molecular mass dependence of response factors. a = Mass distribution; b = number distribution; *——* = calibration.

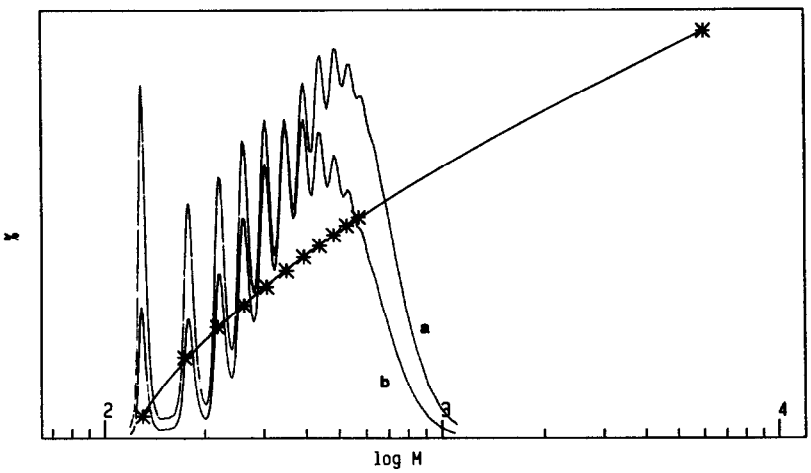

Fig. 6. MWD of the ethoxylated octanol (Fig. 4) from density detection with compensation for molecular mass dependence of response factors using eqn. 4. Curves as in Fig. 5.

$$\frac{1}{m_A} = 1 - \frac{f_{\infty,R,A} \cdot \frac{x_D}{x_R} - f_{\infty,D,A}}{f_{\infty,R,B} \cdot \frac{x_D}{x_R} - f_{\infty,D,B}} \quad (5)$$

If the parameters $f_{\infty,D}$, $f_{\infty,R}$, K_D and K_R for both components (A and B) are known, one can calculate the response factors for any point of the MWD using eqn. 4, which allows the application of these methods also to low-molecular-mass samples.

Once the mass fractions of the components for any point of the peak have been determined, the correct mass m_i eluted within each interval can be calculated using the equations

$$m_i = \frac{x_D}{m_A(f_{D,A} - f_{D,B}) + f_{D,B}} \quad (6)$$

$$m_i = \frac{x_R}{m_A(f_{R,A} - f_{R,B}) + f_{R,B}} \quad (7)$$

In the case of FAE, one may consider the

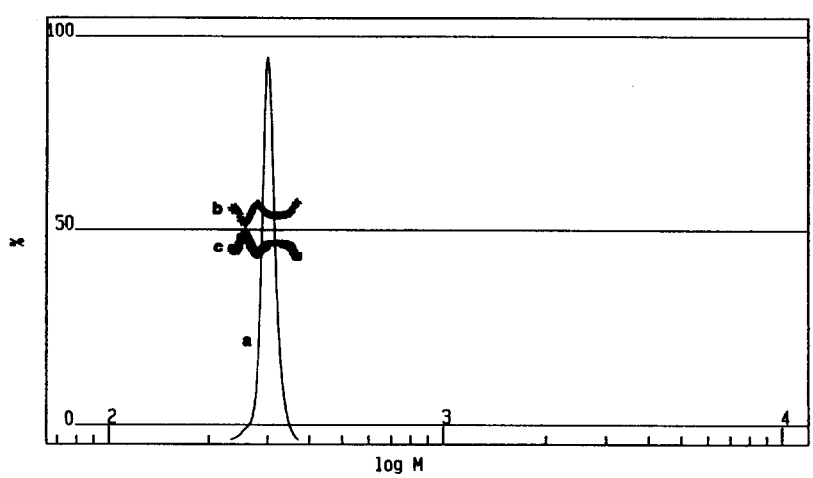

Fig. 7. MWD of octyl-tetraethylene glycol from density detection with compensation for molecular mass dependence of response factors using the mass fraction of the alkanol. a = Mass distribution; b = ethylene oxide; c = octanol.

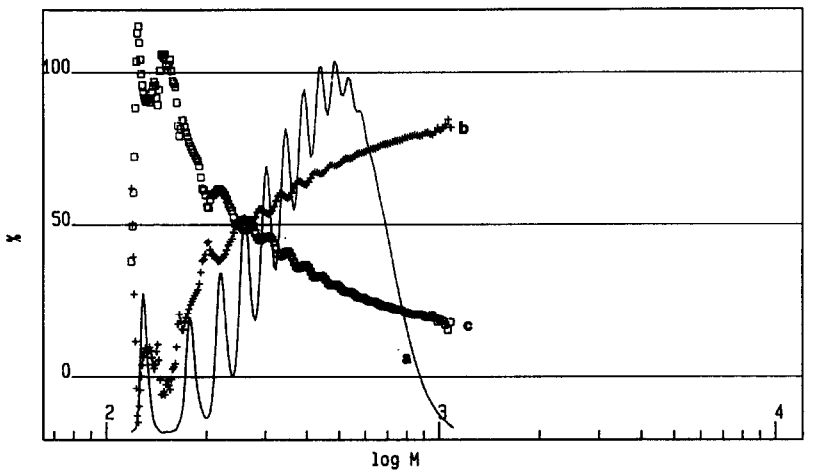

Fig. 8. MWD of the ethoxylated octanol (Fig. 4) from density detection with compensation for molecular mass dependence of response factors using the mass fraction of the alkanol; a, b and c as in Fig. 7.

polyoxyethylene chain as component A, which is inserted as a centre block between the alkyl and the hydroxy groups 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, and only the carbon number of the alkanol has to be accounted for. Using the response factors of high-molecular-mass PEG and the corresponding alkanol, one can calculate the composition and thereby the number of EO units for each fraction.

As a test of this approach, we analysed an octyl-$(EO)_4H$, which has been synthesized by condensation of tetraethylene glycol with 1-octanol, and again the ethoxylated octanol from Figs. 4 and 5. The results obtained are shown in

TABLE II

MOLECULAR MASS AVERAGES OF DIFFERENT OLIGOMERS OF OCTYL-$(EO)_n$ OBTAINED WITH TWO DIFFERENT SEC CALIBRATIONS (PEG AND OCTYL-PEO)

Correction of response factors using slope K or mass fraction of alkanol (m_A).

Sample	Parameter[a]	Calibration					
		PEG			Octyl-PEO		
		No correction	Slope	m_A	No correction	Slope	m_A
R_8EO_4	M_w	299	301	302	300	303	302
	M_n	298	300	300	299	301	301
	M_w/M_n	1.004	1.004	1.003	1.004	1.004	1.004
R_8EO_5	M_w	375	419	413	388	436	428
	M_n	288	340	331	302	352	344
	M_w/M_n	1.301	1.232	1.247	1.285	1.240	1.245
R_8EO_{10}	M_w	613	652	644	649	697	685
	M_n	507	563	533	521	587	572
	M_w/M_n	1.209	1.156	1.164	1.246	1.188	1.198

[a] M_w = Mass-average molecular mass; M_n = number-average molecular mass.

TABLE III

MOLECULAR MASS AVERAGES OF A 1-DODECYL-(EO)$_n$ OBTAINED WITH TWO DIFFERENT SEC CALIBRATIONS (PEG AND DODECYL-PEO)

Correction of response factors using slope K or mass fraction of alkanol (m_A).

Parameter	Calibration					
	PEG			Dodecyl-PEO		
	No correction	Slope	m_A	No correction	Slope	m_A
M_w	587	632	621	611	659	648
M_n	484	539	527	506	559	548
M_w/M_n	1.211	1.172	1.180	1.207	1.178	1.182

Figs. 7 and 8. Obviously, the algorithm works even in cases where the linear interpolation cannot be applied.

As soon as the mass fraction of the polyoxyethylene chain in each peak is known, one can identify each oligomer and use the maxima to establish an SEC calibration for the corresponding homologous series.

For the monodisperse oligomer, the mass fraction of the ethylene oxide chain was found to be $m_A = 53.8\%$, which agrees fairly well with the theoretical value of 57.5%. In the analysis of the ethoxylated tetradecanol, the lowest molecular peak was clearly identified as tetradecanol, and the others as the higher homologues with increasing number of EO units. In Tables II–IV the molecular mass averages for several polyoxyethylene monoalkyl ethers are given, which were obtained with different SEC calibrations (obtained as described above or with commercial PEG standards), and with two different methods of correcting the response factors (using the slope K or the chemical composition from dual detection). As can be seen, the two approaches agree fairly well. It is very important, however, to use the individual SEC calibrations instead of those for PEG.

The separation efficiency of SEC can be enhanced by a recycling technique: when the valve between the columns (Fig. 1) is switched three times during a chromatographic run, the sample has to pass ten column volumes instead of four, and the chromatogram of an octyl-(EO)$_5$ (from Fig. 4) now shows much better resolution (the

TABLE IV

MOLECULAR MASS AVERAGES OF A 1-TETRADECYL-(EO)$_n$ OBTAINED WITH TWO DIFFERENT SEC CALIBRATIONS (PEG AND TETRADECYL-PEO)

Correction of response factors using slope K or mass fraction of alkanol (m_A).

Parameter	Calibration					
	PEG			Tetradecyl-PEO		
	No correction	Slope	m_A	No correction	Slope	m_A
M_w	273	295	288	277	294	290
M_n	246	263	257	257	269	266
M_w/M_n	1.107	1.123	1.122	1.078	1.094	1.092

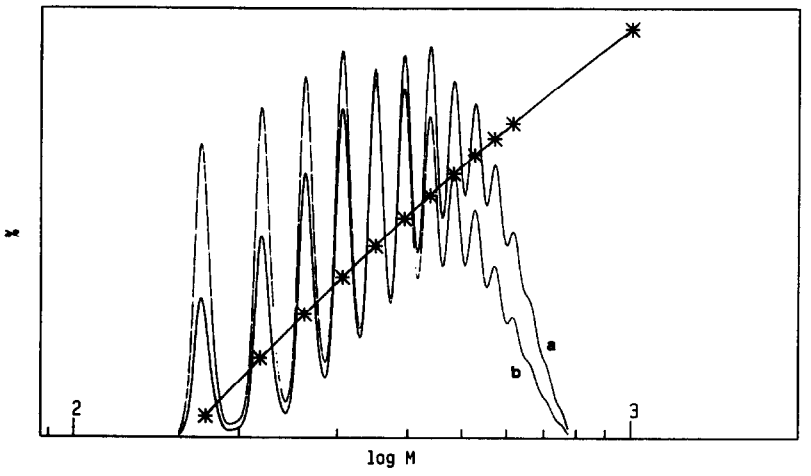

Fig. 9. MWD of the ethoxylated octanol (Fig. 4), as obtained with recycling (three switches of valve, Fig. 1), with density detection. Compensation for molecular mass dependence of response factors using the slopes (eqn. 4). a = Mass distribution; b = number distribution; *——* = calibration.

unreacted alkanol has been cut off in this separation). In Fig. 9, the MWD calculated therefrom using the slope is shown.

CONCLUSIONS

The problem of the quantitative characterization of FAE can be solved by separating the homologous series using CC (on a semi-preparative scale) as the first dimension [44] and analysing the separated homologous series in the second dimension using SEC with dual detection. The quantification of the first dimension will be described in Part II of this series [45].

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