

Analytical study of polyoxyethylene surfactants of high degree of condensation by normal-phase liquid chromatography on *p*-nitrophenyl-bonded silica

P.L. Desbène*

Laboratoire d'Analyse des Systèmes Organiques Complexes, Université de Rouen, Institut Universitaire de Technologie, 43 rue Saint Germain, 27000 Evreux (France)

B. Desmazieres

Institut Européen de Recherches Multidisciplinaires sur les Peptides, Université de Rouen, B.P. 118, 76134 Mont Saint Aignan Cédex (France)

ABSTRACT

Polyoxyethylene-based non-ionic surfactants resulting from the condensation of ethylene oxide with fatty alcohol cuts have been widely studied by high-performance liquid chromatography. Even though satisfactory analyses are obtained in normal-phase partition liquid chromatography (*e.g.*, using a diol stationary phase) and in reversed-phase partition liquid chromatography (*e.g.*, using C₈ bonded silica) for polyoxyethylenic (POE) surfactants with an average ethylene oxide (EO) number lower than 20 ($\bar{n} < 20$ EO), the analysis of the total distribution of the whole EO oligomers is impossible with more condensed POE surfactants ($\bar{n} > 20$ EO on C₁₆ and C₁₈ alcohols). In order to establish the total distribution of such surfactants, the potential offered by stationary phases with electron-acceptor characteristics used in normal-phase liquid chromatography was studied. The stationary phase selected was a *p*-nitrophenyl-bonded silica with an *n*-propyl spacer. Different mobile phases were tested. The results obtained indicated the possibility of determining the total distribution of polyoxyethylenic oligomers from 1 to more than 80 EO units with baseline resolution using this stationary phase and *n*-heptane–dichloromethane–methanol as the mobile phase, with either UV detection after derivatization of compounds that do not possess chromophoric groups or light-scattering detection, the latter technique having the great advantage of not requiring any derivatization.

INTRODUCTION

Various chromatographic methods have been proposed for the analysis of polyoxyethylenic surfactants. Most studies have been performed by normal-phase high-performance liquid chromatography (HPLC). The literature [1–5] suggests that adsorption chromatography will not give a satisfactory analysis of surfactants that have a wide distribution of polyoxyethylene (POE) units. The only HPLC technique that

appeared to offer promise for the analysis of surfactants in terms of their ethylene oxide (EO) distribution is normal-phase chromatography using amino-bonded silica [6–9], cyano-bonded material [10–13] and more recently diol-bonded packings [14–16]. In particular, we have previously reported the analysis of non-ionic surfactants (KL6, KM11 and KM20) containing EO units of various lengths condensed with long-chain (C₁₆ and C₁₈) aliphatic alcohols via normal-phase chromatography on diol-bonded silica [16,17]. In order to perform the analysis of these surfactants in crude oil phases, a specific and sensitive detector such as the electrochemical

* Corresponding author.

detector was required [18]. As this detection technique is difficult to apply in normal-phase chromatography, we optimized the reversed-phase analysis of these surfactants [19]. Other workers have studied the potential of reversed-phase chromatography [20,21]. Unfortunately, none of these chromatographic methods allows the analysis of polyoxyethylene (POE) surfactants with a high degree of condensation such as KM25 ($\bar{n} = 25$ EO), condensed with a mixture of saturated fatty alcohols (C_{16} and C_{18}). Okada [22,23], using a combination of ion-exchange chromatography and complexation of metallic cations by POE chains, first analysed POE surfactants ($\bar{n} = 9$ EO) condensed with a C_{12} fatty alcohol. Moreover, using a temperature gradient [24], he performed the partial analysis of a non-ionic surfactant having a mean chain length of 25 EO units condensed again with a C_{12} saturated fatty chain. This technique allowed the separation of oligomers up to 41 EO units. However, the resolution of the peaks between $n = 6$ and 14 EO units was limited. Recently, we demonstrated [25], after optimization (nature of the stationary phase, nature and concentration of the complexing cation and its associated counter ion, nature and concentration of the organic co-solvent, pH of the aqueous phase and temperature of the separation), the possibility of analysing the total distribution of the POE surfactants with a high degree of condensation, such as KM25.

However, although this method allows the resolution of compounds with a degree of condensation corresponding to 60 oligomers, it has the disadvantage of a total resolution loss of the first units, *i.e.*, oligomers with a condensation of 1–6 EO units. Under such conditions, and with a view to obtaining a satisfactory resolution both of the first members of the distribution and of the oligomers of high degree of condensation, *i.e.*, more than 60 EO units, we undertook to study the potential offered by normal-phase partition stationary phases with the characteristic of interacting with the solutes by charge transfer. As the polyoxyethylene surfactants we studied are potential electron donors, we chose a stationary phase functioning as an electron acceptor.

The stationary phase selected for this study was a *p*-nitrophenyl-bonded silica with an *n*-propyl spacer.

EXPERIMENTAL

Reagents

KM25 non-ionic surfactant ($\bar{n} = 25$ EO) was of technical grade from Marchon France (Saint Mihiel, France). Cetalox AT non-ionic surfactant ($\bar{n} = 50$ EO) from Witco (Saint Pierre les Elbeuf, France) was also of technical grade. Igepal non-ionic surfactants Co-720 ($\bar{n} = 12$ EO), Co-890 ($\bar{n} = 40$ EO) and Co-990 ($\bar{n} = 100$ EO), obtained by condensation of *p*-nonylphenol with ethylene oxide, were supplied by Aldrich (Strasbourg, France). A surfactant standard with six EO units and a C_{18} moiety was purchased from Nikko Chemicals (Tokyo, Japan). All the solvents used (*n*-heptane, dichloromethane, chloroform, ethyl acetate, tetrahydrofuran, acetonitrile, 2-propanol and methanol) were of LiChrosolv HPLC grade (Merck, Darmstadt, Germany) and were filtered through a 0.45- μ m FH filter (Millipore, Molsheim, France). Analyte compounds that do not possess chromophoric groups were derivatized to esters with 3,5-dinitrobenzoyl chloride according to a previously described method [16].

Instrumentation

Chromatographic separations were performed either on a 250 mm \times 3 mm I.D. column filled with 5- μ m Nucleosil *p*-nitrophenyl-bonded silica (Macherey–Nagel, Düren, Germany) or on a 250 mm \times 4.6 mm I.D. column filled with 5- μ m Ultraspher cyano-bonded silica (Beckman, Fullerton, CA, USA). Analyses were carried on using an HP 1090 low-pressure gradient liquid chromatograph (Hewlett-Packard, Palo Alto, CA, USA) fitted with a Rheodyne Model 7010 injector (5- or 20- μ l loop). The detector was either a PU 4020 UV–Vis spectrophotometer (Pye Unicam, Cambridge, UK) fitted with an 8- μ l cell (operated at 254 nm) or an ACS 950 light-scattering detector (ACS, Manchester, UK) using a 12 l min⁻¹ flow-rate of dry air at 45°C.

RESULTS AND DISCUSSION

As non-ionic surfactants such as KM25 and Cetalox AT do not possess chromophoric groups, we optimized the analytical conditions after derivatizing these compounds to esters via reaction with 3,5-dinitrobenzoyl chloride. As a result of the wide range of polarity of these surfactant oligomers, owing to their high degree of condensation, isocratic analysis could not be considered. Hence only UV detection could be used during the optimization phase, as light-scattering detection, which is compatible with an elution gradient, had never been used to detect surfactants presenting such a high degree of condensation [26].

Different co-solvents were tested, with a view to finding the mobile phase composition giving the best possible resolution in unit time. The choice of co-solvents appeared to be relatively limited, as the mobile phase should present both a high elution strength because of the high polarity of the samples being analysed and a low viscosity in order to keep a high performance of the system.

With respect to these constraints and in order to optimize the system selectivity, we selected (see Table I) solvents belonging to each of the eight solvent groups defined by Snyder [27], with the exceptions of solvents of group 1 (too weak eluent), solvents of group 4 (too strong eluent) and solvents of the group 7 (too high cut-offs and

incompatible with UV detection), *n*-heptane being used as the base solvent.

The solvents of groups 5 and 8, dichloromethane and chloroform, which are too weak eluents, were not used as co-solvents but were used as a third solvent in order to allow miscibility with *n*-heptane of solvents belonging to group 2, *i.e.*, methanol or 2-propanol. The solvents belonging to groups 3 and 6, tetrahydrofuran and ethyl acetate, give insufficient selectivities and are not suitable for resolving the complex mixtures studied.

Hence only solvents belonging to group 2 present suitable eluent strengths and selectivities. However, 2-propanol, previously used as a co-solvent in normal-phase partition chromatography with a diol-bonded silica stationary phase to analyse non-ionic POE surfactants with an intermediate average number of EO groups (*e.g.*, KM11, $\bar{n} = 11$ EO) [16], does not allow in the present instance the satisfactory resolution of oligomers more condensed than 25 EO units, as shown in Fig. 1.

In contrast, methanol, which is a sufficiently strong eluent and shows good selectivity, allows a satisfactory analysis of the 3,5-dinitrobenzoyl ester of KM25. However, owing to the non-miscibility of methanol and *n*-heptane, as previously reported, a third solvent is required. Hence, the total resolution of the KM25 dis-

TABLE I
SOLVENTS TESTED TO OPTIMIZE THE CHROMATOGRAPHIC SYSTEM

Solvent	Solvent group ^a	Viscosity at 20°C (cP)	Miscibility number, <i>M</i>
2-Propanol	2	2.37	15
Methanol	2	0.60	12
Tetrahydrofuran	3	0.45	17
Dichloromethane	5	0.42	
Ethyl acetate	6	0.45	19
Chloroform	8	0.57	19

^a According to Snyder [27].

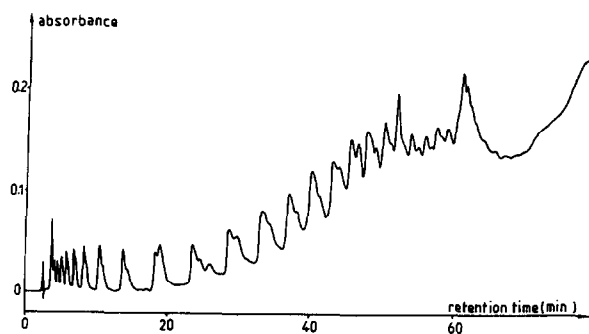


Fig. 1. Analysis of KM25 by normal-phase partition chromatography. Stationary phase, *p*-nitrophenyl bonded silica (250 mm × 3 mm I.D., $d_p = 5 \mu\text{m}$); mobile phase elution gradient from *n*-heptane–dichloromethane–2-propanol at 85:7.5:7.5 to 50:25:25 in 40 min, then isocratic elution at 50:25:25 for 50 min; flow-rate, 0.7 ml/min; temperature, 25°C.

tribution is possible with an elution gradient from the initial mobile phase [*n*-heptane–dichloromethane–methanol (90:5:5)] to the final phase [*n*-heptane–dichloromethane–methanol (65:17.5:17.5)] (see Fig. 2).

This important point having been settled, we attempted to analyse non-ionic POE surfactants with a degree of condensation considerably higher than that of KM25, such as Cetalox AT ($\bar{n} = 50$ OE).

As no pure standard of a POE surfactant with a high degree of condensation is commercially available, we performed, as a first step, the analysis of Cetalox AT in the presence of KM25. The broad EO distribution of the latter, between 1 and 50 EO, allows the precise attribution of the chromatographic peaks corresponding to the oligomers of Cetalox AT. The chromatogram obtained for a mixture of dinitrobenzoyl esters of KM25 (10^4 ppm, w/w) and Cetalox AT (10^4 ppm, w/w) is reported in Fig. 3. The analysis was performed as previously on a *p*-nitrophenyl-bonded silica stationary phase and using methanol as co-solvent in the mobile phase.

The analysis of this complex mixture with a high degree of condensation appears to be relatively satisfactory. It is possible to visualize oligomers between 1 and about 80 EO units. Moreover, the system allows a satisfactory separation of the first 30 oligomers. With more condensed oligomers, the efficiency of the chromatographic system is insufficient with respect to the selectivity obtained. In order to improve the resolution for highly condensed oligomers, we studied the influence of temperature. A tempera-

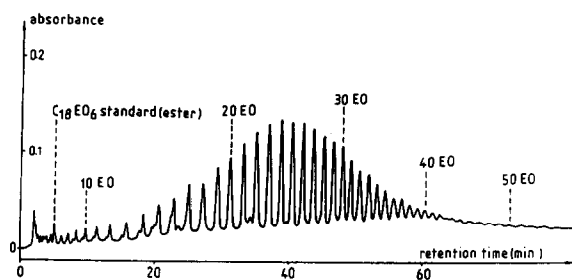


Fig. 2. Analysis of KM25 by normal-phase partition chromatography. Conditions as in Fig. 1, except final mobile phase composition *n*-heptane–dichloromethane–methanol at 65:17.5:17.5 and isocratic elution for 30 min.

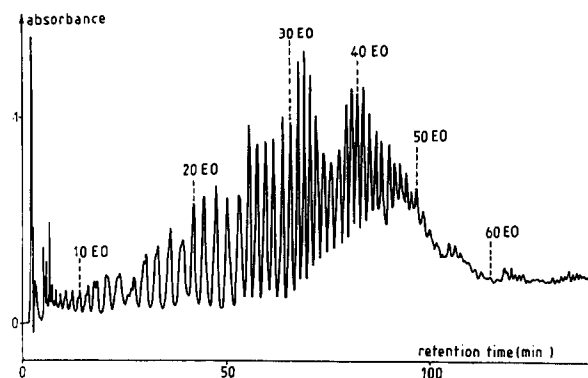


Fig. 3. Analysis of a mixture of non-ionic POE surfactants KM25 (10^4 ppm, w/w) and Cetalox AT (10^4 ppm, w/w) as esters by normal-phase partition chromatography. Conditions as in Fig. 1, except elution gradient from *n*-heptane–dichloromethane–methanol at 90:5:5 to 65:17.5:17.5 in 190 min, then isocratic elution at 65:17.5:17.5 for 30 min; flow-rate, 0.6 ml/min.

ture increase should lead to an improved efficiency of the chromatographic system owing to the decreased viscosity of the mobile phase. The chromatogram obtained at 45°C for a mixture of KM25 and Cetalox AT esters is reported in Fig. 4. The other operating conditions were kept identical with those previously reported.

As evidenced by this chromatogram, it is possible, using these new operating conditions, to analyse correctly a highly condensed non-ionic POE surfactant. The resolution remains constant over the whole distribution of oligomers between 1 and >80 EO units.

Having established the optimum conditions for highly condensed non-ionic surfactants, *viz.*, a *p*-nitrophenyl-bonded silica stationary phase,

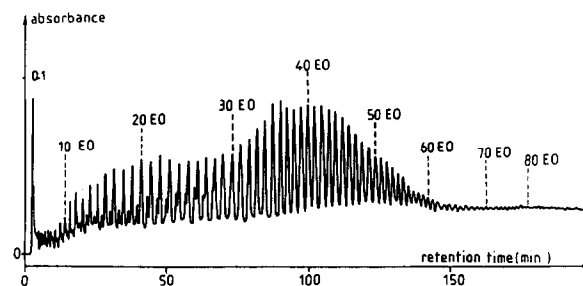


Fig. 4. Analysis of a mixture of non-ionic POE surfactants KM25 (10^4 ppm, w/w) and Cetalox AT (10^4 ppm, w/w) as esters by normal-phase partition chromatography. Conditions as in Fig. 3, except temperature, 45°C.

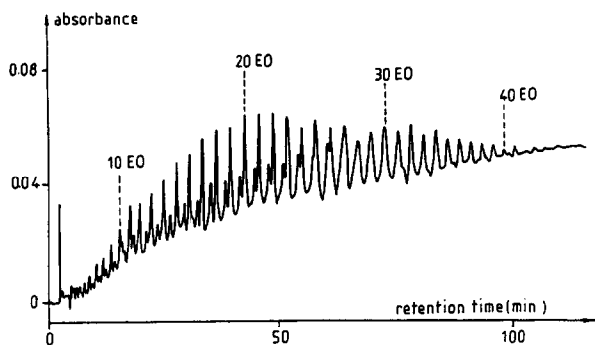


Fig. 5. Analysis of the non-ionic POE surfactant KM25, as the ester, at a 10^4 ppm (w/w) concentration by normal-phase partition chromatography. Conditions as in Fig. 4.

an elution gradient between an initial mobile phase of *n*-heptane–dichloromethane–methanol (90:5:5) and a final composition of *n*-heptane–dichloromethane–methanol (65:17.5:17.5) and a temperature of 45°C, we studied the separation of KM25 and Cetalox AT oligomers. The chromatograms obtained under these conditions are reported in Figs. 5 and 6 and their oligomer distributions as a function of EO number are given in Figs. 7 and 8.

The average numbers of EO units determined for both KM25 and of Cetalox AT are satisfactory, being 23 and 43 EO, respectively, compared with the values given by the manufacturers of 25 and 50 EO, respectively.

These satisfactory results led us to attempt the analysis of even more condensed non-ionic POE surfactants and we decided to study a mixture of Igepal Co-720, Co-890 and Co-990. These non-ionic surfactants result from the condensation of

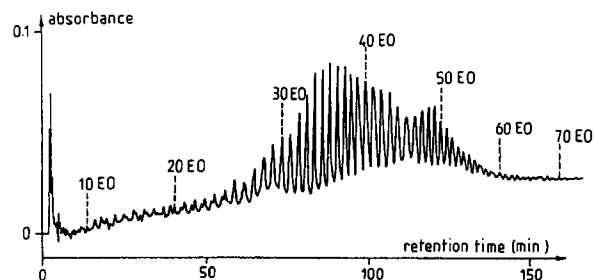


Fig. 6. Analysis of the non-ionic POE surfactant Cetalox AT, as the ester, at a 10^4 ppm (w/w) concentration by normal-phase partition chromatography. Conditions as in Fig. 4.

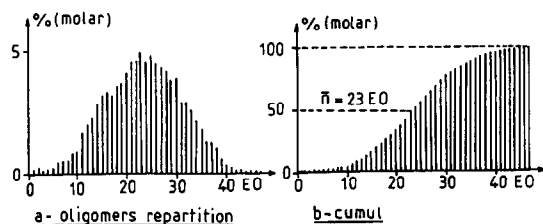


Fig. 7. Distribution histograms obtained from the chromatographic analysis of the non-ionic POE surfactant KM25, as the ester, reported in Fig. 5.

ethylene oxide with *p*-nonylphenol. Their mean EO numbers are $\bar{n} = 12$ EO for Igepal Co-720, 40 EO for Co-890 and 100 EO for Co-990. As they possess an aromatic group, these non-ionic surfactants can be detected without derivatization by UV spectrophotometry, unlike KM25 and Cetalox AT. The chromatogram obtained for the mixture of the three Igepal surfactants under the optimum conditions is reported in Fig. 9a.

Although this chromatographic system appears satisfactory for Igepal Co-720 ($\bar{n} = 12$ EO) and Co-890 ($\bar{n} = 40$ EO), it is insufficient to resolve

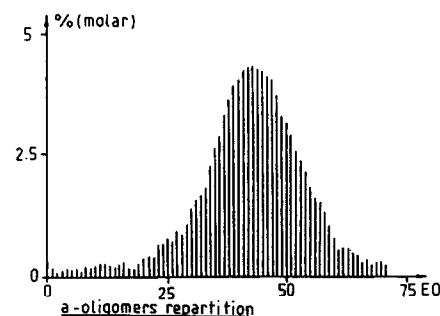
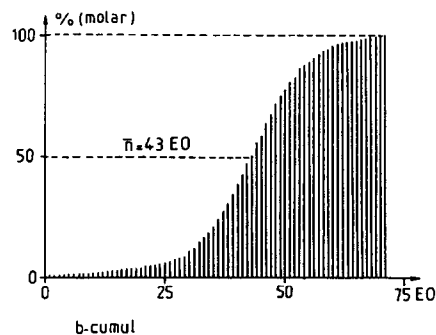


Fig. 8. Distribution histograms obtained from chromatographic analysis of the non-ionic POE surfactant Cetalox AT, as the ester, reported in Fig. 6.

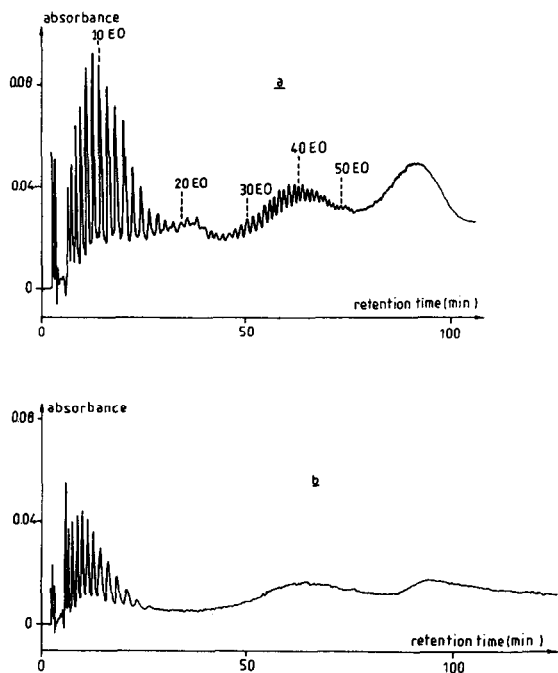


Fig. 9. Analysis of a mixture of the non-ionic POE surfactants Igepal Co 720, Co 890 and Co 990 by normal-phase partition chromatography at 45°C, using an elution gradient from *n*-heptane–dichloromethane–methanol at 90:5:5 to 65:17.5:17.5. Stationary phase: (a) *p*-nitrophenyl-bonded silica; (b) cyano-bonded silica.

the complex mixture constituting Igepal Co-990 ($\bar{n} = 100$ EO). It must be noted that the *p*-nitrophenyl-bonded silica stationary phase is clearly better adapted to the analysis of these complex compounds than the normal-phase partition stationary phases commonly used with non-ionic POE surfactants. For instance, Fig. 9b reports the analysis of this three-surfactant mixture under the same conditions using a cyano-bonded silica stationary phase.

To complete this study, as the procedure of derivatization of KM25 and Cetalox AT can be a drawback for some applications, we replaced UV detection with light-scattering detection, the other conditions remaining unchanged. Light-scattering detection appears relatively universal and is moreover compatible with gradient elution. Fig. 10 shows the chromatogram obtained with this detection technique in the analysis of a mixture of KM25 (5000 ppm, w/w) and Cetalox AT (10^4 ppm, w/w). This chromatogram indi-

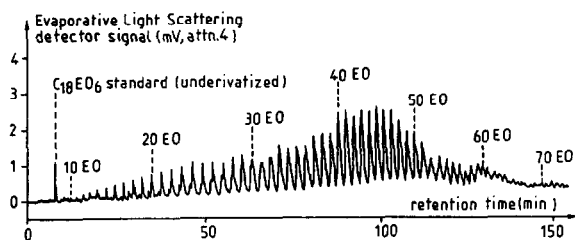


Fig. 10. Analysis of a non-ionic POE surfactant mixture containing KM25 at 5000 ppm (w/w) and Cetalox AT at 10^4 ppm (w/w) by normal-phase partition chromatography on *p*-nitrophenyl-bonded silica at 45°C. Detection system: light scattering. Other conditions as in Fig. 3.

cates that the light-scattering detector is well adapted to the detection of non-ionic POE surfactants whatever their degree of condensation. However, the signal is slightly more noisy than with UV detection.

CONCLUSIONS

A *p*-nitrophenyl-bonded silica stationary phase provides a satisfactory solution to the problem of non-ionic POE surfactant analysis. This phase, used in association with a ternary elution gradient with *n*-heptane–dichloromethane–methanol, allows the analysis, with a satisfactory and nearly constant resolution of the total oligomers of a POE surfactant containing a C_{16} – C_{18} base and from 1 to >80 EO units.

For non-ionic surfactants resulting from the condensation of ethylene oxide with *p*-nonyl phenol (Igepals), the results are less satisfactory as the correct resolution of this complex mixture is obtained only for the first 60 oligomers.

Finally, the evaporative light-scattering detector is well adapted to the detection of these compounds, whatever the degree of condensation.

REFERENCES

- 1 C.F. Allen and L.I. Rice, *J. Chromatogr.*, 110 (1975) 151.
- 2 K. Nakamura and I. Matsumoto, *Nippon Kagaku Kaishi*, 8 (1975) 1342.
- 3 M.C. Allen and D.E. Linder, *J. Am. Oil Chem. Soc.*, 58 (1981) 950.
- 4 J.D. McClure, *J. Am. Oil Chem. Soc.*, 59 (1982) 364.

- 5 A. Aserin, M. Frenkel and N. Garti, *J. Am. Oil Chem. Soc.*, 61 (1984) 805.
- 6 N. Cortesi, E. Moretti and E. Fredli, *Riv. Ital. Sostanze Grasse*, 57 (1980) 141.
- 7 B.F. Bogatzki and L.H. Lippmann, *Acta Polym.*, 34 (1983) 219.
- 8 M. Ahel and W. Giger, *Anal. Chem.*, 57 (1985) 1577.
- 9 M. Ahel and W. Giger, *Anal. Chem.*, 57 (1985) 2584.
- 10 A.C. Hayman and N.A. Parris, paper presented at the 1979 Pittsburgh Conference, paper 24.
- 11 A.M. Rothman, *J. Chromatogr.*, 253 (1982) 283.
- 12 R.E.A. Escott, S.J. Brinkworth and T.A. Steedman, *J. Chromatogr.*, 282 (1983) 655.
- 13 J.A. Pilc and P.A. Sermon, *J. Chromatogr.*, 398 (1987) 375.
- 14 I. Zeeman, J. Silha and M. Bares, *Tenside Deterg.*, 23 (1986) 4.
- 15 I. Zeeman, *J. Chromatogr.*, 363 (1986) 223.
- 16 P.L. Desbène, B. Desmazieres, V. Even, J.J. Basselier and L. Minssieux, *Chromatographia*, 24 (1987) 857.
- 17 P.L. Desbène, B. Desmazieres, V. Even, J.J. Basselier and L. Minssieux, *Chromatographia*, 24 (1987) 588.
- 18 P.L. Desbène, B. Desmazieres, J.J. Basselier and A. Desbène-Monvernay, *J. Chromatogr.*, 465 (1989) 69.
- 19 P.L. Desbène, B. Desmazieres, J.J. Basselier and A. Desbène-Monvernay, *J. Chromatogr.*, 461 (1989) 305.
- 20 A. Marconi and W. Giger, *Anal. Chem.*, 59 (1987) 1709.
- 21 R.E.A. Escott and N. Mortimer, *J. Chromatogr.*, 553 (1991) 423.
- 22 T. Okada, *Anal. Chem.*, 62 (1990) 327.
- 23 T. Okada, *Anal. Chem.*, 62 (1990) 734.
- 24 T. Okada, *Anal. Chem.*, 63 (1991) 1043.
- 25 B. Desmazieres, F. Portet and P.L. Desbène, *Chromatographia*, 36 (1993) 307.
- 26 G.R. Bear, *J. Chromatogr.*, 459 (1988) 91.
- 27 L.R. Snyder, *J. Chromatogr. Sci.*, 16 (1978) 223.