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Liquid chromatography of polyglycerol fatty esters and fatty ethers on porous graphitic carbon and octadecyl silica by using evaporative light scattering detection and mass spectrometry

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Abstract

A liquid chromatographic method has been developed for the analysis of polyglycerol fatty esters and fatty ethers which are non-ionic surfactants. Two methods were compared using either octadecyl silica or porous graphitic carbon. The octadecyl silica system with a hydroorganic mobile phase enables to compare the hydrophobic behavior of the compounds. The porous graphitic carbon enables the separation of the diastereoisomers. Detection of these non-UV-absorbing compounds was achieved by evaporative light scattering detection. Identification of isomers was performed by mass spectrometry. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Porous graphitic carbon; Polyglycerol fatty esters; Polyglycerol fatty ethers; Surfactants

1. Introduction

Polyglycerol fatty esters are industrial surfactants which are constituted of complex mixtures due to: (i) the alkyl chain length, (ii) the degree of polymerization, (iii) the linear, branched or cyclic structure of the oligoglycerol moiety. Moreover some compounds can appear under various diastereoisomeric forms due to stereogenic carbons. Therefore correlations between structure and performances cannot be easily determined. Consequently a European project

[1] was set up to provide the synthesis on the laboratory scale and the structure determination of target compounds in order to evaluate their physico-chemical properties and their environmental impact.

In a previous work [2] we have shown the use of porous graphitic carbon in industrial diglycerol LC analysis using evaporative light scattering detection (ELSD) for these non-UV-absorbing compounds. The aim of this work is to propose an analytical method by liquid chromatography (LC) and mass spectrometry (MS) of various polyglycerol lauryl esters and ethers in order to assess their purity and the isomeric ratio as complementary study to other information given by NMR [3]. To achieve this difficult work, porous graphitic carbon was tested as

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suitable chromatographic support to increase the selectivity with regard to octadecyl bonded silica phase and various mobile phases were investigated using ELSD as universal detection method.

2. Experimental

2.1. Material

The LC apparatus consisted of a Varian (Palo Alto, CA, USA) Model 9010 pump, a Rheodyne (Cotati, CA, USA) Model 7125 injector valve with a 20 μl loop, a Shimadzu (Kyoto, Japan) CR-5A integrator and an ELSD apparatus Model Sedex 55 (Touzart et Matignon, France) which was set as follows: the drift tube temperature was 45°C and nebulizer gas pressure was 2 bar. The columns used were: Hypercarb-S (100 mm \times 4.6 mm I.D., particle size 5 μm) and Hypercarb-S (150 mm \times 4.6 mm I.D., particle size 7 μm) packed with porous graphitic carbon (PGC) from Hypersil (Runcorn, UK) and Alltima C₁₈ (250 mm \times 4.6 mm I.D., particle size 5 μm) from Alltech (State College, PA, USA).

As the influence of the temperature on the retention was weak (approximately 0.05–0.08 min °C⁻¹), all the analyses were carried out at room temperature.

For k values, hold-time t_0 was measured from the elution of NaNO₃ with methanol as the mobile phase.

Mass spectrometry was realized using a Perkin-Elmer Sciex (Forster City, CA, USA) API 300 apparatus triple quadrupole with ionspray source (IS). The flow-rate of the nebulizer gas in ionspray was 0.26 l min⁻¹. MS was operated in positive ion mode. LC–MS coupling was made using a Perkin-Elmer series 200 pump with methanol–dichloromethane as mobile phase at a flow-rate 1 ml min⁻¹ and a split ratio 1/20 into the IS interface. This mobile phase produces a spray slightly less stable than hydro-organic mobile phase involving noise at the peak apex. Moreover, the LC–MS apparatus with extra-column connections was less efficient than the LC–ELSD apparatus. However, the most abundant ions of each peak were sufficiently characteristic for a good determination by operating with selective ion monitoring LC–MS.

2.2. Reagents

The synthesis of solutes was carried out by Cassel and Debaig and was described elsewhere [3]. Fig. 1 displays the various compounds studied in this work with their coding: PG2, PG3 and PG4 represents diglycerol, triglycerol and tetraglycerol, respectively, while ES and ET represents either lauryl ester or lauryl ether bonding. The symbols “ss” or “ps” specify that the coupling of glycerol units is respectively made between two secondary hydroxyl groups or between a primary hydroxyl group and a secondary hydroxyl group. The symbol “lin” signifies that the oligoglycerol moiety is linear in PG3 or PG4 after a coupling between two primary hydroxyl groups. The letters C, T, I indicate that the lauryl chain is bonded in a central, terminal or internal position of the oligoglycerol moiety. Pentaerythritol lauryl ester (PE/ES) was synthesized in order to compare its activity with that of polyglycerol esters.

LC solvents were HPLC grade and purchased from J.T. Baker (Noisy le Sec, France) and water was produced from an Elgastat UHQ II system (Elga, Anthony, France).

3. Results and discussion

3.1. Polyglycerol ester response in ELSD

ELSD is a universal detection method for LC which has been largely used for carbohydrate and polyol analysis [4]. We have previously shown its use for the analysis of polyglycerols in isocratic elution [2] and of a mixture of polyethylene glycols and polyethoxylated alcohols in gradient elution on Hypercarb [5]. The curve of the ELSD response versus the mass (or concentration) in double logarithmic coordinates is linear with a slope b and an intercept $\log a$ where the b values are generally comprised between 1 and 1.6.

The ELSD response of various polyglycerol esters was tested with methanol as eluent without column and the corresponding values of b and $\log a$ are given in Table 1. The average of the slope b is 1.10 and relative standard deviation (RSD) of the slope is 2.16% while that of the intercept is 4.08%. Thus, the RSD of the response of various polyglycerol esters at

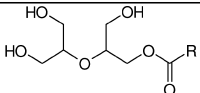
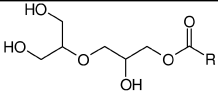
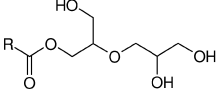
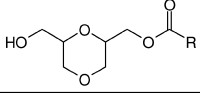
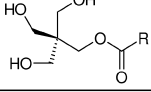
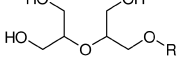
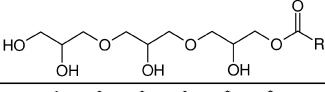
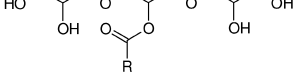
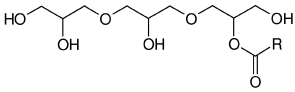
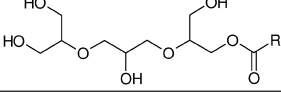
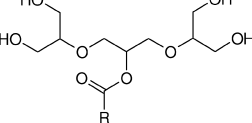
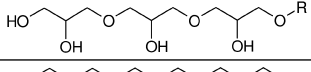
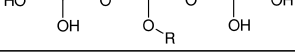
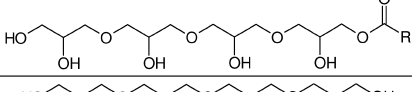
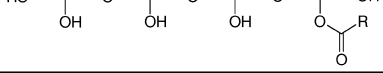
PG2 / ES / ss	
PG2 / ES / ps 1	
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PE / ES	
PG2 / ET / ss	
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PG3 / ES / lin / C	
PG3 / ES / lin / I	
PG3 / ES / sp ps / T	
PG3 / ES / sp ps / C	
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Fig. 1. Description and coding of the various polyglycerol lauryl esters and ethers.

Table 1
ELSD response with methanol as mobile phase according to log
 $A = b \log C + \log a$

Compound	Slope <i>b</i>	Intercept
PG2/ES/ss	1.065	2.616
PG2/ES/ps	1.083	2.574
PG3/ES/sp-ps/C	1.118	2.507
PG2/ET/ss	1.121	2.355
PE/ES	1.100	2.575
Average	1.097	2.525
RSD %	2.159	4.077

C from 200 mg l⁻¹ to 5000 mg l⁻¹.

a given concentration is approximately 15%. Therefore, in this work we consider that the response of the diastereoisomers of a given structure will be similar in an isocratic elution thus simplifying the quantitative determination.

On the other hand, the response value becomes different when the eluent composition changes: thus it is approximately two times larger when dichloromethane–methanol (80:20) replaces pure methanol and it is approximately 10 times smaller when water–methanol (80:20) replaces pure methanol. Thus, care will be taken for a comparison of the response of various compounds in gradient elution.

3.2. Reversed phase liquid chromatography

We have already [5] shown that LC using a suitable octadecyl bonded phase with acetonitrile–water as eluent enables to separate polyethoxylated alcohols in the pure reversed-phase mode. The surfactant interacts by its apolar moiety with the alkyl chain of the support whereas its polar ethylene oxide moiety remains in the polar mobile phase. This mechanism is strongly linked to the nature of the octadecyl-bonded phase. Thus, when silanol groups have been end-capped, the retention of oligomer decreases as the ethylene oxide number decreases. On the other hand, when the silanol groups are accessible, interaction of the polar moiety of the solute involves an increase of the retention when the ethylene oxide number increases.

The Alltima C₁₈ support has a high carbon content (16%) where residual silanols are masked by polymeric bonding and endcapping [6]. This packing affords high retention of weak polarity compounds.

Retention of polyethoxylated alcohols C₁₂EO₄, C₁₂EO₆ and C₁₂EO₉ on this packing with acetonitrile–water mixture as mobile phase confirms a pure reversed-phase mode since the retention decreases as the ethylene oxide (EO) number increases.

Polyglycerol fatty esters were analyzed on this packing with methanol–water and acetonitrile–water mixtures as mobile phases. As expected in reversed-phase liquid chromatography, the methanol–water mixture involves a higher retention (*k* values comprised between 11 and 20) than acetonitrile–water mixture (*k* between 1 and 4) with the same water content (20%) as shown in Fig. 2a. Moreover, the selectivity of PG2s (as that of PG3s) is very weak with the methanol–water eluent and the retention of the fatty ether (PG2/ET/ss) is higher than that of the corresponding ester (PG2/ES/ss).

With acetonitrile–water Fig. 2b shows the following results: (i) the retention follows the reversed-phase mode, i.e. the PGs having five hydroxyl groups (PG4/ES/lin/I and PG4/ES/lin/T) are eluted before PG3s (PG3/ES/sp-ps/C, PG3/ES/lin/C, PG3/ES/sp-ps/T, PG3/ES/lin/T) having four hydroxyl groups, while PG2s (PG2/ES/ps1, PG2/ES/ps2, PG2/ES/ss) and PE/ES having only three hydroxyl groups are more retained; PG2 fatty ether has the highest retention; (ii) the variation of the retention in function of the acetonitrile content is similar for all compounds; (iii) the selectivity between compounds is better than that obtained with methanol–water eluent; (iv) the retention of PG terminal ester is higher than that of central or internal; (v) only the retention of PG3/ES/sp-ps/T does not vary like the other ones.

Fig. 3 shows that the retention of polyglycerol fatty esters is much lower than that of polyethoxylated alcohols (C₁₂EO₄, C₁₂EO₆, C₁₂EO₉) on octadecyl phase in gradient elution. This chromatographic behavior is in agreement with their critical micelle concentration (CMC) [7].

However reversed-phase liquid chromatography (RPLC) does not enable a sufficient selectivity for a complete separation of isomers and diastereoisomers. We have therefore used PGC support (Hypercarb-S) which had successfully been tested for polyethoxylated alcohols [5] and for alkylglycosides [8]. Moreover a selective interaction was observed for the separation of isomeric diglycerols [2].

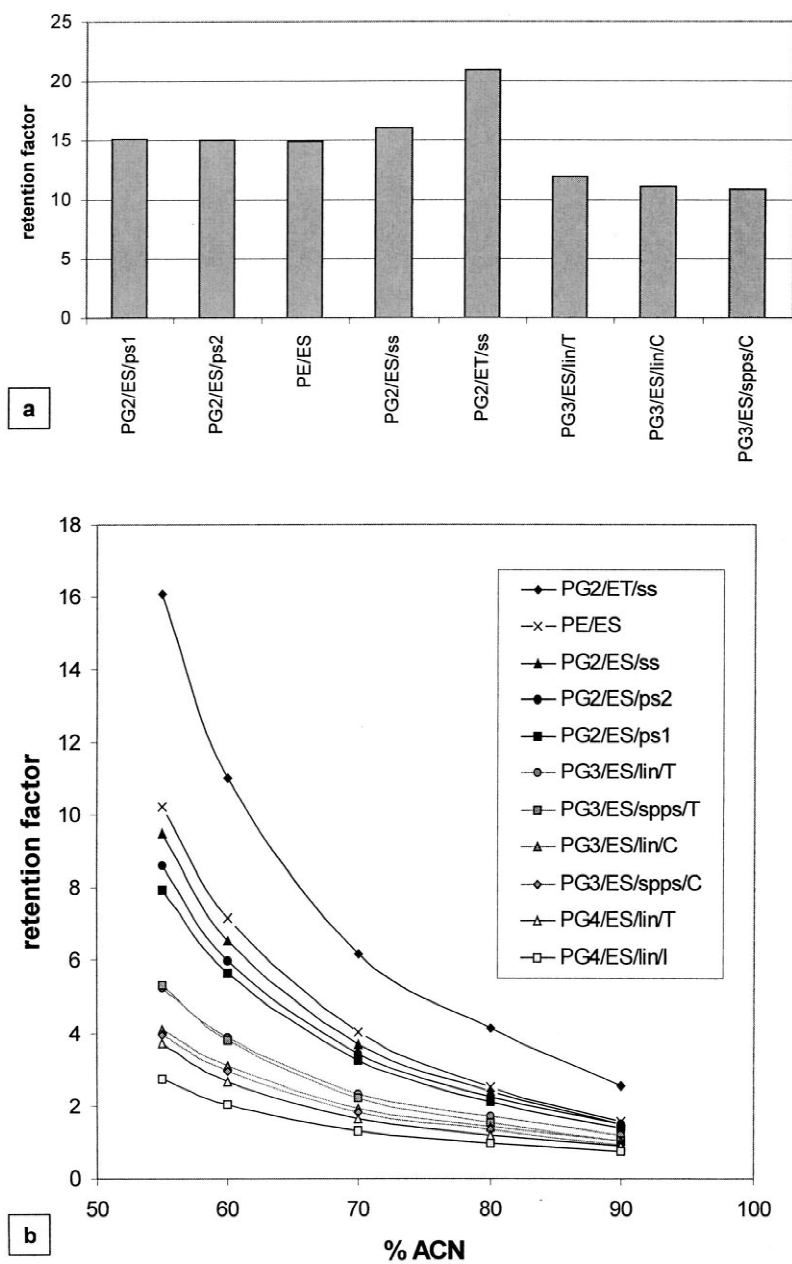


Fig. 2. Retention factor k of polyglycerol esters and ethers. Column: Alltima C_{18} , 250 mm \times 4.6 mm I.D., 5 μ m. (a) methanol–water (80:20) mobile phase; (b) acetonitrile–water mobile phase. ELSD. Concentration 250 mg l^{-1} .

3.3. Porous graphitic carbon LC

Porous graphitic carbon (PGC) possesses a rigid, planar surface in addition to high electronic and

charge transfer interactions. We have previously noted a particular behavior of this support on polyethoxylated alcohols where the retention increases as a function of hydrocarbonaceous chain length as in

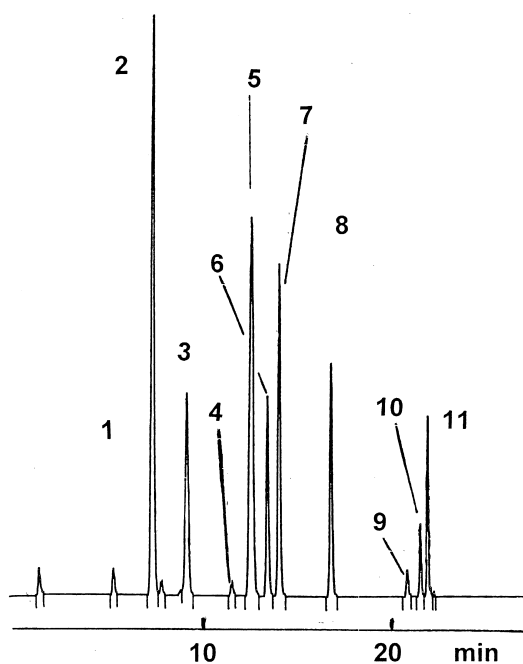


Fig. 3. Chromatogram of polyglycerol esters, ethers and $C_{12}EO_4$, $C_{12}EO_6$ and $C_{12}EO_9$ on Alltima C_{18} , 250 mm \times 4.6 mm I.D., 5 μ m, with acetonitrile (ACN)–water mobile phase in gradient elution: 55% ACN during 8 min, then from 55% ACN to 90% ACN in 10 min, then from 90% ACN to 100% ACN in 1 min, then 100% ACN. Flow-rate: 1.2 ml min $^{-1}$. ELSD. Concentration 250 mg l $^{-1}$. Elution order: PG4/ES/lin/I (1), PG4/ES/lin/T, PG3/ES/lin/C, PG3/ES/sp-ps/C (2), PG3/ES/lin/T, PG3/ES/sp-ps/T (3), PG2/ES/ps1 (4), PG2/ES/ps2 (5), PG2/ES/ss (6), PE/ES (7), PG2/ET/ss (8), $C_{12}EO_9$ (9), $C_{12}EO_6$ (10), $C_{12}EO_4$ (11).

pure reversed-phase (RP)LC and of ethylene oxide number as in normal-phase LC.

Polyglycerol fatty ester analysis was firstly performed on PGC with acetonitrile–water as eluent for a direct comparison with the results obtained on octadecyl bonded silica. The retention decreases while the acetonitrile content increases as in the pure reversed-phase mode. Such a behavior has also been observed with hydroxylated compounds such as oligoglycerols [2] or carbohydrates [9].

Fig. 4 compares the retention of those compounds on Alltima C_{18} and PGC with the same mobile phase (acetonitrile–water, 90:10) and with a similar retention factor for PG2/ET/ss.

With the same mobile phase (acetonitrile–water, 90:10), the retention on PGC is much higher than on

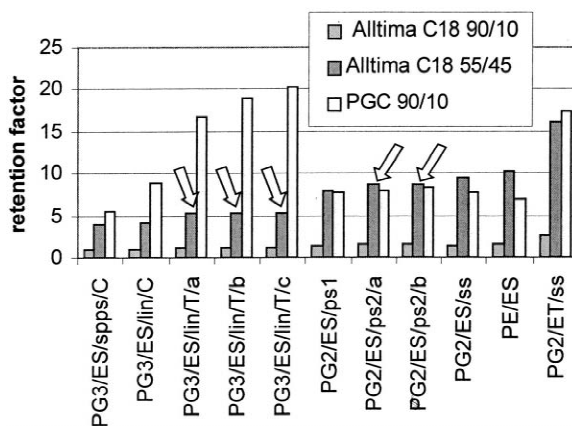


Fig. 4. Comparison of retention on Alltima C_{18} and PGC with acetonitrile–water mobile phase. Dark grey: Alltima C_{18} , 250 mm \times 4.6 mm I.D., 5 μ m, acetonitrile–water (55:45), light grey: Alltima C_{18} , acetonitrile–water (90:10), white: Hypercarb-S, 100 mm \times 4.6 mm I.D., 5 μ m, acetonitrile–water (90:10). ELSD. Concentration 250 mg l $^{-1}$.

the octadecyl silica phase. Moreover, for a similar retention of PG2/ET/ss, PGC affords a different selectivity due to a high interaction of the polar moiety of the solute with the stationary phase as observed for polyethoxylated alcohols [5] and alkylglycosides [8]. On the opposite of the reversed-phase mode, the interactions of PG esters are enhanced on PGC due to the electronic interactions of a higher number of oxygen and more especially when the oligoglycerol moiety is linear (PG3/ES/lin/T). On the other hand, the interaction is lowered when the oxygen are only present in hydroxyl groups as for PE/ES.

The major interest of PGC consists in the separation of diastereoisomers (PG3/ES/lin/T/a, b and c). A separation of diastereoisomers PG2/ES/ps2/a and b appears also but with a weaker resolution. An increase of the water content increases the retention but the resolution remains always low. Only a decrease of the temperature enables enhancement of the selectivity, but the efficiency is lowered and the retention time is increased.

Therefore, we have tested methanol which has been previously noted more suitable than acetonitrile to separate two alkylglycosides which differ in their polar sugar moiety [8]. However, the elution power of methanol was too weak to elute the polyglycerol fatty esters such as PG3s and PG4s under satisfac-

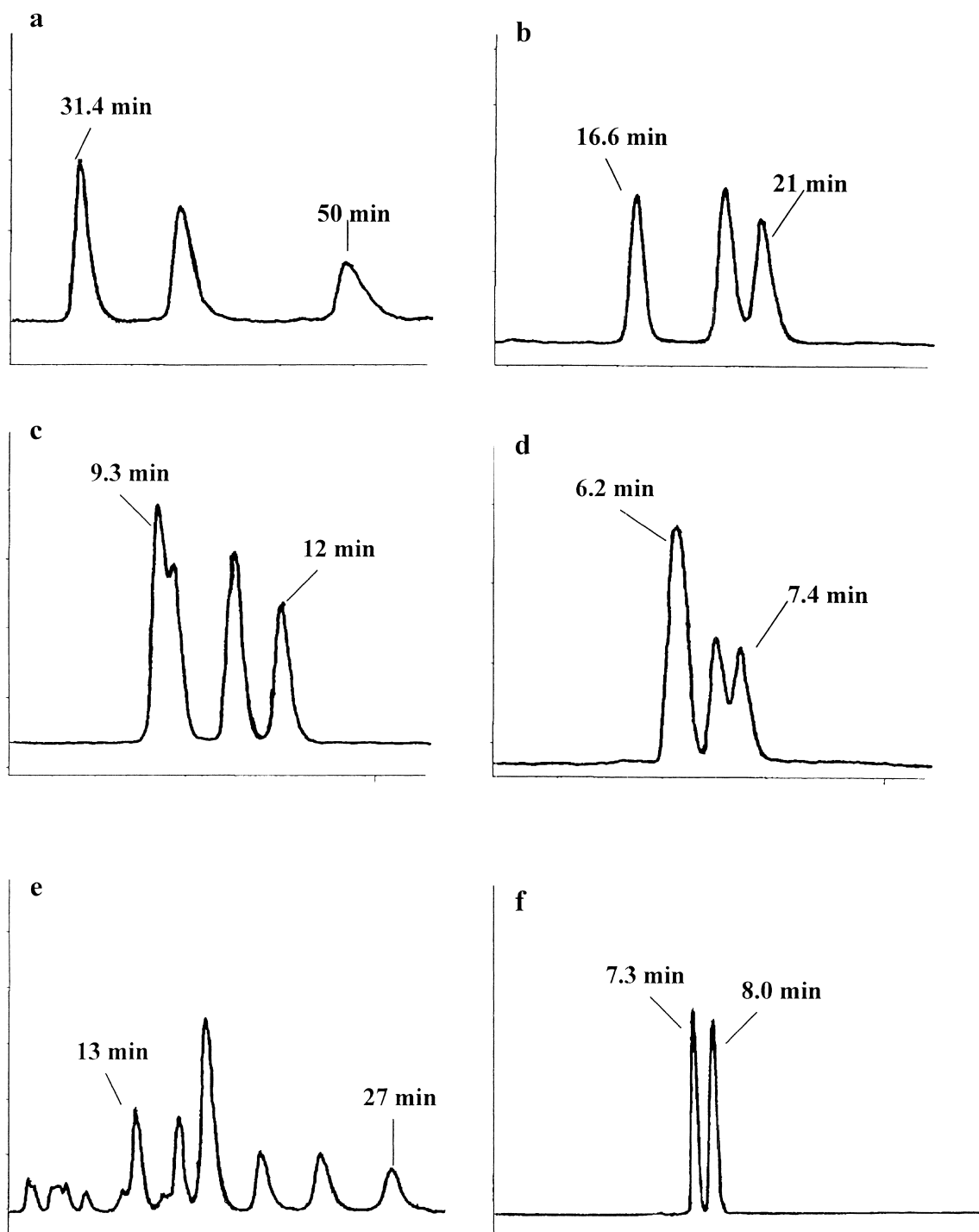


Fig. 5. Separation of diastereoisomeric lauryl esters and lauryl ethers. Column: Hypercarb-S 150 mm \times 4.6 mm I.D., 7 μ m. Flow-rate: 1 ml min^{-1} . (a) PG3/ET/lin/T, methanol–dichloromethane (80:20); (b) PG3/ES/lin/T, methanol–dichloromethane (80:20); (c) PG3/ES/lin/I, methanol–dichloromethane (80:20); (d) PG4/ES/lin/I, methanol–dichloromethane (70:30); (e) PG4/ES/lin/T, methanol–dichloromethane (70:30); (f) PG2/ES/cy, methanol–dichloromethane (70:30). ELSD. Concentration 250 mg l^{-1} .

tory conditions. Therefore the hydroorganic mixture is replaced by a mixture of organic solvents as in lipid analysis by non aqueous reversed-phase mode on octadecyl bonded silica. Thus methanol replaces water as solvent having a weak elution strength and various organic modifiers such as chloroform, dichloromethane, methyl *tert.*-butyl ether, acetone, ethyl acetate were tested in order to increase elution strength.

The retention of these surfactants decreases as the content φ of the less polar solvent in the binary mixture increases according to a linear relationship $\log k = a - m\varphi$ observed in the reversed-phase mode [10].

Moreover the elution strength of this modifier has been evaluated on the retention of PG3/ES/lin/T diastereoisomers and shows the following order:

acetonitrile < acetone \approx ethyl acetate
 < methyl *tert.*-butyl ether
 < dichloromethane < chloroform

Methanol–dichloromethane mixtures were the best ones to separate the whole of groups of isomers as shown in Fig. 5. This mixture was also required for extracting polyethoxylated surfactants from graphitic carbon black cartridges [11].

Under these conditions, the retention of PG terminal is approximately the double of that of the central isomer and the retention of PG linear is approximately the double of that having a branched moiety. Retention of the ether is higher than that of polyglycerol esters as in the reversed-phase mode on Alltima C₁₈. Moreover, the retention is strongly influenced by the stereochemistry of polar moiety because the various diastereoisomers of each polyglycerol fatty ester are well separated.

The couple PG2/ps2 a and b remains poorly separated with the methanol–dichloromethane mobile phase as with acetonitrile–water. A better selectivity was obtained with methanol–acetone and an efficient separation (Fig. 6) is obtained in a suitable retention time with the mixture (95:5) by applying the linear relationship $\log k = a - m\varphi$. A similar result was also obtained with methanol–ethyl acetate. In contrast, these mixtures with acetone or ethyl acetate do not enable a similar enhancement of the

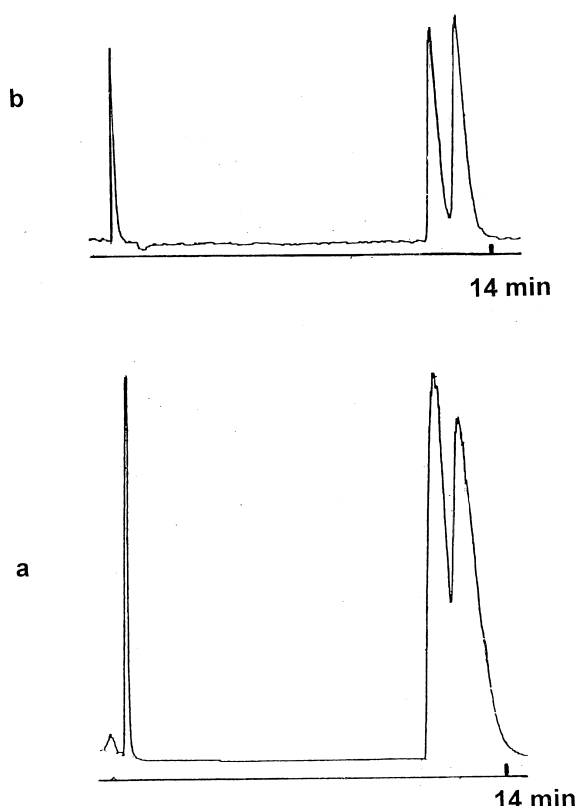


Fig. 6. Separation of PG2/ES/ps2 diastereoisomers. Column: Hypercarb-S 100 mm \times 4.6 mm I.D., 5 μ m. (a) Methanol–dichloromethane (98:2). (b) Methanol–acetone (95:5) flow-rate: 2 ml min^{-1} . ELSD. Concentration 250 mg l^{-1} .

separation of the other mixtures of polyglycerol fatty esters.

As previously done on octadecyl phase in reversed-phase mode, it is possible on PGC using methanol–dichloromethane (80:20) to compare the retention of the diastereoisomers PG3/ET/lin/T and diastereoisomers PG3/ES/lin/T with that of C₁₂EO₆ polyethoxylated alcohol having the same oxygen number (i.e. 7). The retention factor of diastereoisomers PG3/ES/lin/T is comprised between 10 and 13 and that of diastereoisomers PG3/ET/lin/T between 19.6 and 31.6 while that of C₁₂EO₆ is 36 [5]; that comparison illustrates the decrease of the interaction of polar moiety when the oxygen are in a hydroxyl group.

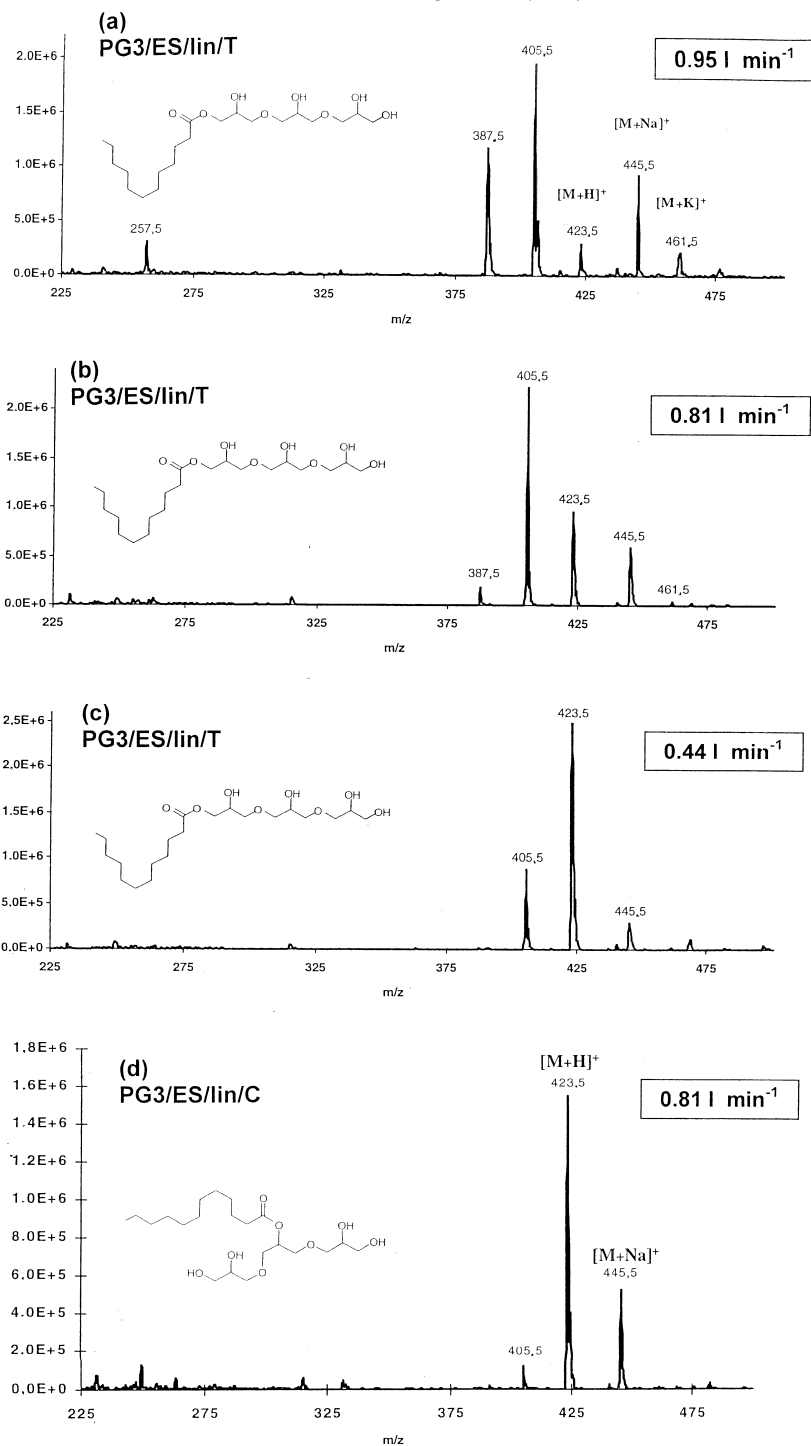


Fig. 7. Influence of curtain gas flow-rate on mass spectrum of polyglycerol lauryl esters. (a) PG3/ES/lin/T, 0.95 l min⁻¹; (b) PG3/ES/lin/T, 0.81 l min⁻¹; (c) PG3/ES/lin/T, 0.44 l min⁻¹. (d) PG3/ES/lin/C, 0.81 l min⁻¹. MS conditions: Ion spray tension +5.7 kV, Orifice voltage 20 V and focusing ring voltage 200 V infusion at 5 μ l min⁻¹ in methanol–water–acetic acid (95:5:0.5). Concentration 300 mg l⁻¹.

3.4. Mass spectrometry

Structure determination of each synthesized compound was performed by NMR spectrometry and MS [3]. Electrospray MS was previously [2,12] used to analyze polyglycerols. Therefore polyglycerol fatty ester MS was achieved with the same method. If the synthesized polyglycerol is a mixture of diastereoisomers, they are considered as having a same spectrum. MS of the mixture was carried out in the ionspray mode under 3 kV between capillary and counter-electrode. Behind the counter-electrode a flow of nitrogen washes away the non-ionic molecules and the residual solvent. In this interface, ions collide with the curtain gas and this collision induces a dissociation which depends on the gas flow-rate as shown in Fig. 7. PG3/lin/T, which is a mixture of diastereoisomers, was studied in MS at various curtain gas flow-rates. At high flow-rates (0.81 and 0.95 l min⁻¹), water loss is enhanced involving a fragmentation of the MH⁺ ion ($m/z=423$) of PG3/lin/T (Fig. 7a and b) whereas it is very low for the PG3/ES/lin/C (Fig. 7d). In the same manner, at low flow-rate (0.44 l min⁻¹) the water loss is higher for PG3/ES/lin/I than for PG3/ES/lin/T. Thus water loss is weaker when the coupling of fatty chain is made in central position of the oligoglycerol moiety. This fragmentation mode is simple and suitable to obtain nice fingerprints characteristic of each isomeric polyglycerol ester.

Another fingerprint can be obtained by MS–MS. Fig. 8 shows the distribution of daughter ions of MH⁺ ($m/z=423$) as a function of the collision energy. The two polyglycerol esters PG3/ES/lin/T (Fig. 8a) and PG3/ES/lin/I present similar distribution curves with two major peaks at $m/z=405$ and 387 corresponding respectively to MH⁺ minus a loss of H₂O (405) and 2H₂O (387). The difference appears with the m/z 131 ion which is slightly major than the m/z 257 ion in PG3/ES/lin/I. On the other hand, the curves of PG3/ES/lin/C (Fig. 8b) are different showing a large peak at m/z 331 corresponding to MH⁺ minus a loss of H₂O and (CH₂–CHOH–CH₂–O). The curves of the other central PG3/ES/sp-ps/C present two important signals at m/z 331 and 257 with a characteristic ion 183 (C₁₁H₂₃CO⁺) at 20 eV (Fig. 8c). Thus, with MS–MS each ester can be characterized.

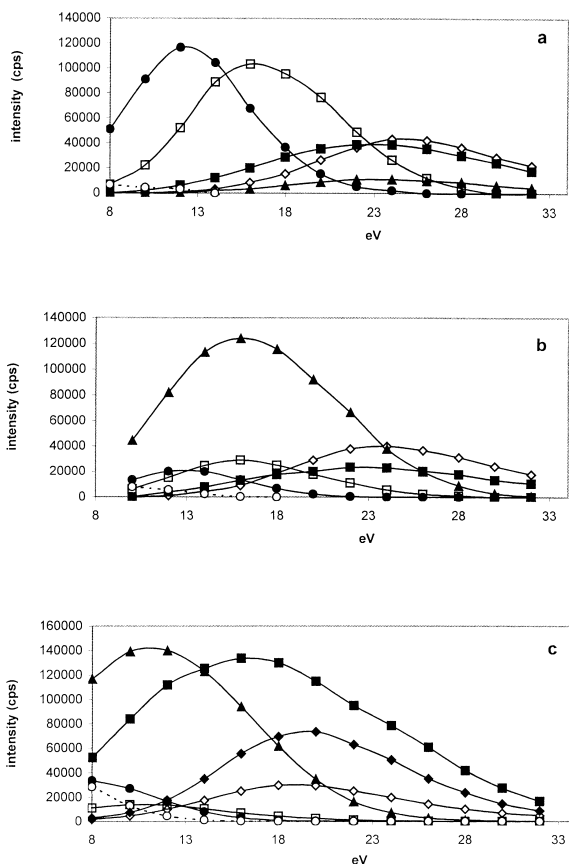


Fig. 8. MS–MS fragmentation as a function of the collision energy. (a) PG3/ES/lin/T, (●) $m/z=405$, (□) $m/z=387$; (b) PG3/ES/lin/C, (▲) $m/z=331$; (c) PG3/ES/sp-ps/C, (▲) $m/z=331$, (■) $m/z=257$, (♦) $m/z=183$. Dotted line (○), $m/z=423$ MH⁺. MS voltages see Fig. 7. Concentration 300 mg l⁻¹.

LC analysis enables an easy quantitative determination of each diastereoisomer because of the mass response of ELSD. In order to verify the identity of each peak, LC–MS of PG3/lin/T using methanol–dichloromethane as eluent was performed. The same peaks (MH⁺, MNa⁺ and MH⁺–H₂O) with a higher signal at MNa⁺ and with the same ratios were obtained for each diastereoisomer. That confirms our previous affirmation that the diastereoisomers have the same spectrum. Moreover, LC–MS allows to determine the nature of the by-products which appeared in the degradation of the PG3/ES/lin/T methanolic solution after several days; in fact the retention times and the proportions of the peaks

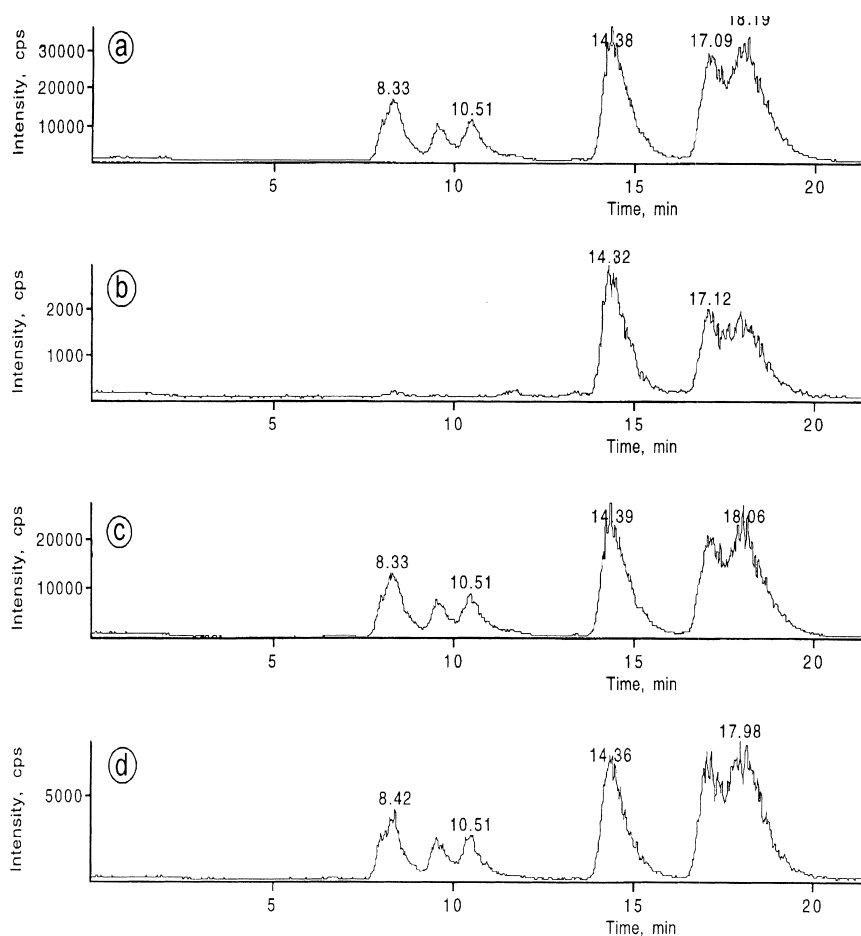


Fig. 9. LC–MS chromatogram of PG3/ES/lin/T solution after degradation. Column: Hypercarb-S, 100 mm×4.6 mm I.D., 5 μ m. Mobile phase: methanol–dichloromethane (70:30), flow-rate: 1 ml min⁻¹, split ratio 1/20. Curtain gas flow: 0.81 l min⁻¹. MS voltages see Fig. 7. Concentration 500 mg l⁻¹. (a) Total ion current with $m/z=405.5$, 423.5 and 445.5. (b) Selective ion monitoring, m/z 423.5. (c) Selective ion monitoring, $m/z=445.5$. (d) Selective ion monitoring, m/z 405.5.

(MH⁺, MNa⁺ and MH⁺–H₂O) enables to identify these by-products as being PG3/ES/lin/I (Fig. 9).

4. Conclusion

LC using an octadecyl bonded phase and coupled to ELSD is a suitable method to control the purity of polyglycerol esters. But porous graphitic carbon appears to be required for diastereoisomer analysis. Mass spectrometry with an adapted setting of curtain gas or by MS–MS enables to characterize the analytes.

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