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Analysis of neutral surfactants by non-aqueous capillary electrophoresis using an electroosmotic flow reversal

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Abstract

The separation of KM 20, that is in fact a mixture of non-ionic surfactants, was carried out by non-aqueous capillary electrophoresis. This complex mixture resulting from the condensation of ethylene oxide with fatty alcohols does not have chromophoric moieties. So, we analysed it after derivatization by means of 3,5-dinitrobenzoyl chloride. The proposed approach is based both on the formation of complexes with alkaline or ammonium cations in methanol and on the utilisation of a positively charged capillary. From a comparative study on the capillary treatment procedure, we used hexadimethrine bromide as electroosmotic flow reverser in order to obtain both repeatable analyses and good resolutions of the largest KM 20 oligomers. Then, among the five cations used to form complexes with KM 20, we pointed out that ammonium cation led to the best resolutions. Moreover, we evidenced that the counter-ion of this cation had a great influence on resolution because it modified the magnitude of electroosmotic flow. Ion pair formation that is more or less strong between ammonium and its counter-ion was involved in this variation of electroosmotic flow. So, we calculated the association constants for various ammonium salts in methanol. Then, using ammonium chloride as background electrolyte, we optimised the concentration of this salt, in methanol, in order to reach the optimal separation of KM 20 oligomers. Thus, a baseline separation was obtained by using 6×10^{-2} mol/L NH_4Cl as running electrolyte. In these conditions, we separated, in about 30 min, more than 30 oligomers of KM 20. The distribution of these oligomers that was determined from the optimal separation, appeared consistent with that obtained from HPLC analyses. Indeed, we determined that the mean ethoxylation number was equal to 18 while its real value is equal to 20.

Keywords: Non-aqueous capillary electrophoresis; Electroosmotic flow reversal; Neutral surfactants; Hexadimethrine coating; Dissociation constants

1. Introduction

Non-ionic surfactants are widely used in detergent industry because of their amphiphilic properties that enable to form micelles and to decrease the surface tension of water. Among them, polyethylene oxides (POEs) are of a great interest for environment because of the low toxicity of their degradation products. These compounds are constituted of homologues with a statistical distribution of ethylene oxide (OE). Their general formula is C_mOE_n, where m ranges from 11 to 18 and n from 2 to about 80. The characterisation of these complex mixtures, based on the utilisation of high performance separation techniques, was carried out essentially by liquid chromatography (HPLC). By using non-polar column, the separation is based essentially on their alkyl chain lengths [1–3], while polar phases lead to a separation based on OE number [4–6]. Moreover, we can note that, under particular chromatographic conditions, the separation can be performed as a function of double distribution, i.e. alkyl length and OE number [7]. Because POE do not have chromophoric moieties, they have been essentially analysed after derivatization either by means of 3,5-dinitrobenzoyl chloride [5] or with phenyl [8] or naphthyl isocyanate [9]. The analysis of underivatized POEs has been also performed using universal detectors such as differential refractometer, evaporative light scattering detector or mass spectrometer [10]. In other respects, the utilisation of capillary zone electrophoresis (CZE) appears difficult because POE does not have ionic or ionizable group [11]. Contrariwise, the utilisation of electrolytes

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containing high sodium dodecylsulfate (SDS) concentrations and high acetonitrile percentages that prevent micelle formation enables the seperation of homologues as a function of their hydrophobicity [12]. By using phthalic anhydride as derivatization agent, Bullock [13], Wallingford [14], Heinig et al. [15] and more recently Oudhoff [16], conferred on POEs both a charge for CZE analysis and a chromophoric moiety for UV detection. Okada [17,18] carried out another approach in order to give a charge to POE. This one was based on the ability of POEs to form positively charged complexes with some alkali and alkaline earth metals in non-aqueous medium. Nevertheless, the obtained resolution for the homologues having more than 16 OE units appeared unsatisfactory using a bare fused silica capillary for separation. So, in this work, we aimed to resolve this analytical difficulty. Indeed, we undertook, in non-aqueous capillary electrophoresis (NACE), the analysis of KM 20 ($m = 16$ and $m = 18$; $\bar{n} = 20$) derivatized with 3,5-dinitrobenzoyl chloride (KM 20-DNB) by using ammonium or alkaline cations to form complexes with this POE. The analysis was carried out by using a reversal of electroosmotic flow that was obtained by coating the capillary with hexadimethrine. In such conditions, the electroosmotic flow (EOF) migrated towards the anode and the homologues with the lower positive charge, i.e. the compounds having the shorter ethoxylated chains, migrated faster than the other compounds towards the anode where was performed the UV detection. The observed migration order was the opposite of the one previously reported by Okada [17] but this seems to be favourable to the separation of the homologues having the higher OE units. Besides, the use of hexadimethrine as EOF reverser enabled us to reduce the adsorption of positively charged compounds on the inner capillary wall. This should improve the analysis repeatability. The constants of complex formation being previously determined in methanol [17], we used this solvent in our study.

2. Experimental

2.1. Chemicals

Ammonium acetate (98%) and 3,5-dinitrobenzoyl chloride (99%) were provided by Acros (Acros France, Noisy-le-Grand, France). KM 20 (technical grade) was purchased from Marchon (Marchon, Saint Mihiel, France). Methanol (RS-HPLC grade) came from Carlo Erba (Carlo Erba France, Val de Reuil, France). Hexaethylene glycol monohexadecyl ether (99%), used as standard, was purchased from Fluka Chemie (Sigma–Aldrich–Fluka France, L'Isle d'Abeau Chesne, France). Lithium chloride, sodium chloride, potassium chloride, tetramethylammonium chloride and ammonium chloride, used as background electrolytes, were of analytical purity and were provided by Aldrich France. Hexadimethrine bromide, used as electroosmotic flow reverser, and sodium hydroxide came also from Aldrich France. All solutions were prepared by using the 18 MΩ cm water produced by means of an Alpha Q Millipore system (Millipore, Bedford, MA, USA). KM 20 and the standard used for peak identification were derivatized with 3,5-dinitrobenzoyl chloride and using magnesium as a catalyst, in dry benzene, following a previously described method [5].

2.2. Apparatus

All analyses were carried out by using a P/ACE 2100 system (Beckman-Coulter, Fullerton, CA, USA). The UV detection was performed at 214 nm. P/ACE software version 2.64 (Beckman-Coulter) was used to control the capillary electrophoresis and for the data acquisition. Samples were injected for 5 s in hydrodynamic mode (injection pressure: 0.5 psi, i.e. 3447 Pa). The fused silica capillaries [57 cm (50 cm effective length) × 50 μm i.d.] were obtained from Thermo Electron (Thermo Electron France, Les Ulis, France) and eCap Amine capillaries [57 cm (50 cm effective length) × 50 μm i.d.] were purchased from Beckman-Coulter. The regeneration solution used for eCap Amine capillary came also from Beckman-Coulter. The injections were carried out at the cathode. Before use, the solutions were systematically degassed for 15 min by sonication performed with a Branson 2510 apparatus (Bransonic Ultrasonic Cleaner, Danbury, CT, USA).

2.3. Regeneration process for eCap Amine capillaries

With regard to eCap Amine capillaries, regeneration was carried out, after each analysis, using a commercial regenerating solution. The rinsing procedure provided by Beckman-Coulter consists in flushing the capillary consecutively with the commercial regenerating solution for 3 min, then with the running electrolyte for 5 min.

2.4. Dynamic coating procedure of fused silica capillaries

First, new fused silica capillaries were activated using the following rinsing procedure: 0.5 mol/L NaOH (30 min) then water (5 min). After this activation step, the capillaries were flushed with 0.2% (w/v) hexadimethrine bromide (methanolic solution) for 15 min. Then, the electrophoretic separation was carried out maintaining, in the running electrolyte, hexadimethrine bromide at a 0.02% (w/v) concentration. At last, before each analysis the capillary was flushed with the running electrolyte containing 0.02% (w/v) hexadimethrine bromide.

2.5. Static coating procedure of fused silica capillaries

First, new fused silica capillaries were activated using the procedure described earlier. After this activation of the silica surface, the capillaries were flushed consecutively with methanol for 5 min, 0.2% (w/v) hexadimethrine bromide (methanolic solution) for 45 min, then with methanol for

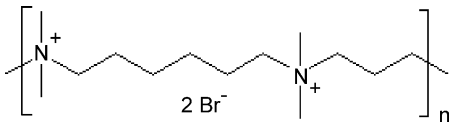

Fig. 1. Structure of hexadimethrine bromide used as electroosmotic flow reverser.

5 min. For the purpose of obtaining a satisfactory repeatability, the capillary was flushed, after each analysis, for 3 min with 0.2% (w/v) hexadimethrine bromide in methanol.

3. Results and discussion

3.1. NACE analysis of KM 20

3.1.1. Comparison of three different approaches for the EOF reversal

Three approaches were studied in order to obtain a reproducible reversed electroosmotic flow. The first was based on the utilisation of the eCap Amine capillary. Concerning the two other approaches, we coated a fused silica capillary with hexadimethrine bromide whose structure is described in Fig. 1. We used this polymeric cationic surfactant with the static or the dynamic mode that are described in the experimental part.

The potentialities of the three approaches were studied by using a methanolic solution of 5×10^{-2} mol/L NH_4Cl as running electrolyte. We injected a KM 20-DNB solution (1 g/L) for 5 s. The applied voltage equalled −20 kV. The electroosmotic flow was measured from the migration time corresponding to the non-complexed oligomers of KM 20-DNB. We can note that these non-complexed oligomers co-migrated with the excess of alcohol (hexadecyl and octadecyl alcohols) resulting from KM 20 synthesis. For each approach, the analysis of the test sample was consecutively repeated seven times then we calculated: the electroosmotic flow (μ_{eo}), the efficiency on $C_{16}E_{18}$-DNB peak (N) and the resolution on $C_{16}E_{18}$-DNB/$C_{16}E_{19}$-DNB pair (R_s). The corresponding mean values ($\bar{X}$) and the related variation coefficients (CV) are gathered in Table 1.

We can note that the same analyses were carried out by using an untreated silica capillary but the repeatability was very poor because of EOF instability. The latter may be due to the adsorption of cations on the inner capillary walls.

This comparative study pointed out the advantages and the drawbacks of each approach. Thus, in order to obtain a good repeatability, the utilisation of eCap Amine capillaries required before each analysis to flush the capillary with the commercial regeneration solution, and then, with the running electrolyte. The absolute value of the electroosmosis mobility obtained with this capillary was lower than those generated with fused silica capillaries coated, statically or dynamically, with hexadimethrine bromide (HDMB). This could be due to a lower surface charge density. At last, this capillary led to very satisfactory resolutions although efficiency was lower than the one obtained with hexadimethrine-coated capillaries.

The dynamic coating of fused silica capillaries led to a higher electroosmotic flow ($|\mu_{eo}| = 1.72 \times 10^{-8}$ m^2 V^{-1} s^{-1}). Although, these capillaries gave good efficiencies (N = 85,000 for $C_{16}E_{18}$-DNB), the resolution was poor.

Lastly, the reversed EOF obtained following the static mode required regeneration between two analyses. The latter was carried out flushing for 3 min with HDMB in methanolic solution. Contrary to eCap Amine capillaries, the capillaries coated following the static mode exhibited a high electroosmotic flow that enabled us to reach very good efficiencies and so good resolutions.

Taking into account the previous results, we chose the static mode to carry on the experimentation. Indeed, for a low cost, such a capillary enabled to obtain very good efficiencies (N = 115,000) and relatively high electroosmotic flows ($|\mu_{eo}| = 1.6 \times 10^{-8}$ m^2 V^{-1} s^{-1} or so).

3.1.2. Optimisation of the resolution

The capillary coating mode being chosen, we determined the background electrolyte that led to the best separation of KM 20-DNB in NACE. First, we studied the nature of the cation used to form complexes with the solutes. Before studying the influence of counter-ion on separation, chloride was systematically used as counter-ion. Using a methanolic solution containing 10^{-2} mol/L of the studied salts, we obtained the electropherograms reported in Fig. 2.

These analyses evidenced that $N^+(CH_3)_4$ and Li^+ did no lead to the complexation of KM 20-DNB because no peak corresponding to positively charged compounds was observed after electroomosis. These results were consistent with those obtained by Okada [17]. Indeed, this author proved that complexation of $(C_2H_5)_3NH^+$ by POEs did

Table 1
Comparative study of the three procedures used to reverse the electroosmotic flow: evaluation of the electroosmotic mobility ($|\mu_{eo}|$), efficiency[a] (N) and resolution[b] (R_s)

	eCap Amine		Dynamic treatment		Static treatment	
	$\bar{X}$	RSD (%)	$\bar{X}$	RSD (%)	$\bar{X}$	RSD (%)
$\|\mu_{eo}\|$ (m^2 V^{-1} s^{-1})	1.45×10^{-8}	0.6	1.72×10^{-8}	0.2	1.63×10^{-8}	0.5
N[a]	70000	11	85000	13	115000	12
R_s[b]	1.9	7	1.3	10	1.9	10

[a] Calculated on the peak corresponding to $C_{16}E_{18}$-DNB oligomer.
[b] Calculated on the $C_{16}E_{18}$-DNB/$C_{16}E_{19}$-DNB pair.

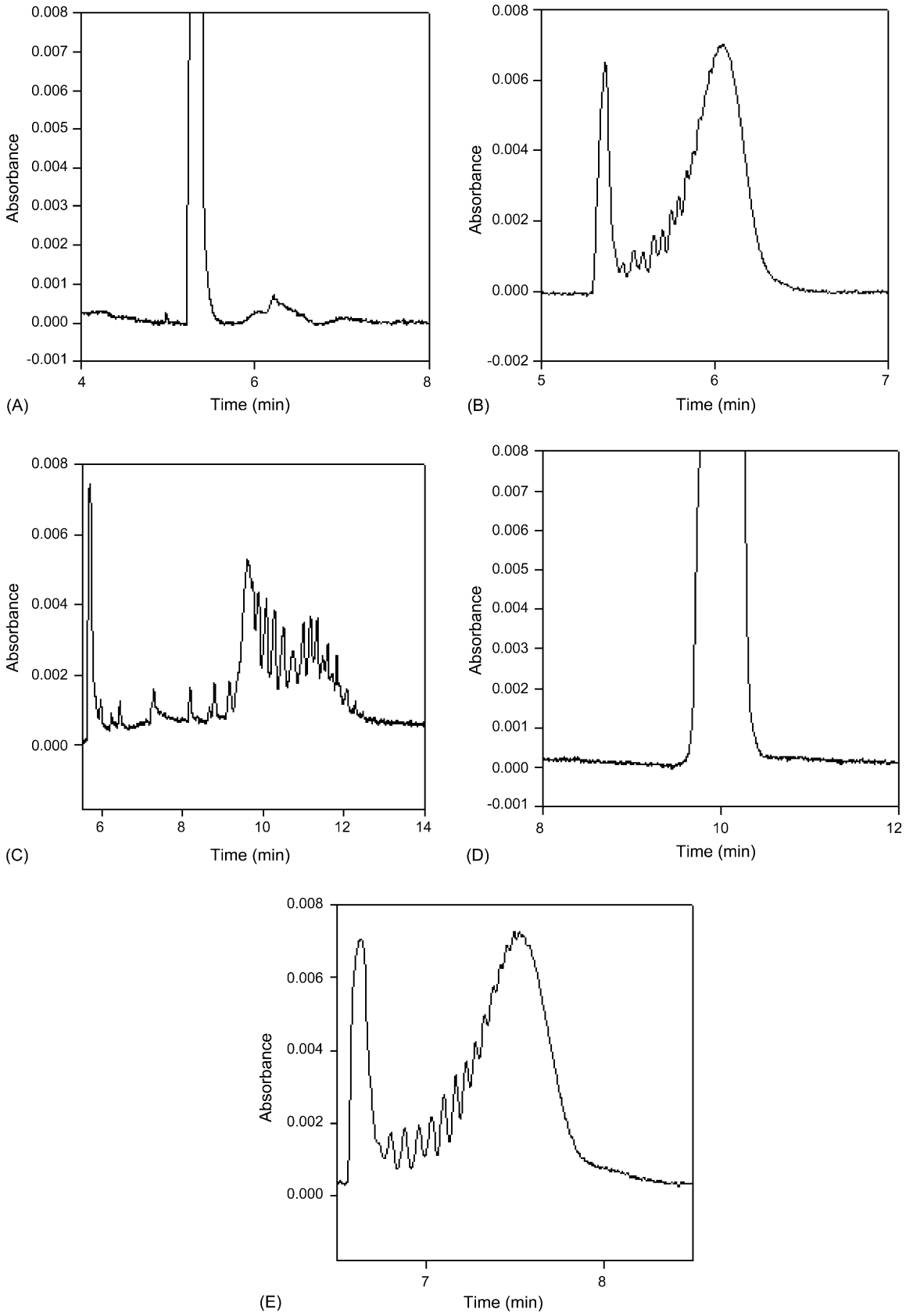

Fig. 2. Electropherograms corresponding to the choice of complexing cation for the analysis of KM 20-DNB in NACE. Operating conditions: fused silica capillary [57 cm (50 cm effective length) × 50 μm i.d.] coated with HDMB following the static procedure; electrolyte: 10^{-2} mol/L of LiCl (A), NaCl (B), KCl (C), $(CH_3)_4NCl$ (D), NH_4Cl (E) in methanol; hydrodynamic injection of 1 g/L KM 20-DNB for 5 s; applied voltage: −30 kV; temperature: 23 °C; anodic detection at 214 nm.

not occur and it was very weak with $(CH_3)_2NH_2^+$. With regard to lithium cation, Ono et al. [19] demonstrated that its complexation, in methanol, was unfavourable because of its high degree of solvation. Contrariwise, we observed, with Na^+ and K^+ cations, several peaks migrating after the electroosmotic flow that evidenced the formation of KM 20-DNB complexes. The peaks distribution appeared particularly regular for ammonium cation. Consequently, we chose this cation to study, on the one hand, the influence of cation concentration on the separation of KM 20-DNB and, on the other hand, the influence of counter-ion.

Concerning the ammonium salt concentration, it modified both electroomosis, via ionic strength, and complexation between oligomers of KM 20-DNB and ammonium cation. We observed that the number of detected peaks increased and resolution was improved when salt concentration increased. There was an optimal salt concentration for which the resolution per time unit was maximal but this concentration varied as a function of ammonium counter-ion. Above this optimal concentration, the number of visualised oligomers decreased and resolution became poor.

Various ammonium salts (NH_4F, NH_4Cl, NH_4Br, NH_4I, CH_3COONH_4, NH_4SCN), that are soluble in methanol up to 10^{-1} mol/L, were studied. The electropherograms corresponding to the optimal concentration of the most representative salts are presented in Fig. 3.

For NH_4I, that absorbs in the UV range, the detection sensitivity was poor (Fig. 2A). Consequently, we were not able to study the oligomers distribution by using NH_4I as background electrolyte although the complexation occurred. With respect to the electropherogram obtained with NH_4SCN that absorbs in the UV range to a lesser degree, about 15 oligomers were observed but resolution appeared unsatisfactory for the less abundant oligomers, i.e. those having the higher OE numbers (Fig. 2B). Contrariwise, for NH_4Cl (Fig. 2C) and NH_4Br (not shown), we observed a baseline separation of all oligomers while ammonium acetate (Fig. 2D) and ammonium fluoride (not shown) led to a satisfactory resolution only for the oligomers with the lower numbers of OE unit.

So, this comparative study allowed us to evidence that the choice of counter-ion is critical with regard to the separation of KM 20 oligomers.

In an attempt to understand why the resolution is dependent on the nature of ammonium counter-ion, we studied successively the parameters involved in the following equation of resolution in capillary zone electrophoresis:

$$R_s = \frac{1}{4}\frac{\Delta\mu_e}{\overline{\mu} + \mu_{eo}}\sqrt{N} \quad (1)$$

where $\Delta\mu_e$ is the difference of electrophoretic mobilities defined as: $\Delta\mu_e = (\mu_{n+1} - \mu_n)$ [where μ_n and μ_{n+1} are the electrophoretic mobilities of two consecutive oligomers ($C_{16}E_n$ and $C_{16}E_{n+1}$) ($m^2\,V^{-1}\,s^{-1}$)]; N is efficiency; $\overline{\mu}$ is the mean electrophoretic mobility calculated from the equation $\overline{\mu} = (\mu_{n+1} + \mu_n)/2$ ($m^2\,V^{-1}\,s^{-1}$); and μ_{eo} is the electroosmotic mobility ($m^2\,V^{-1}\,s^{-1}$).

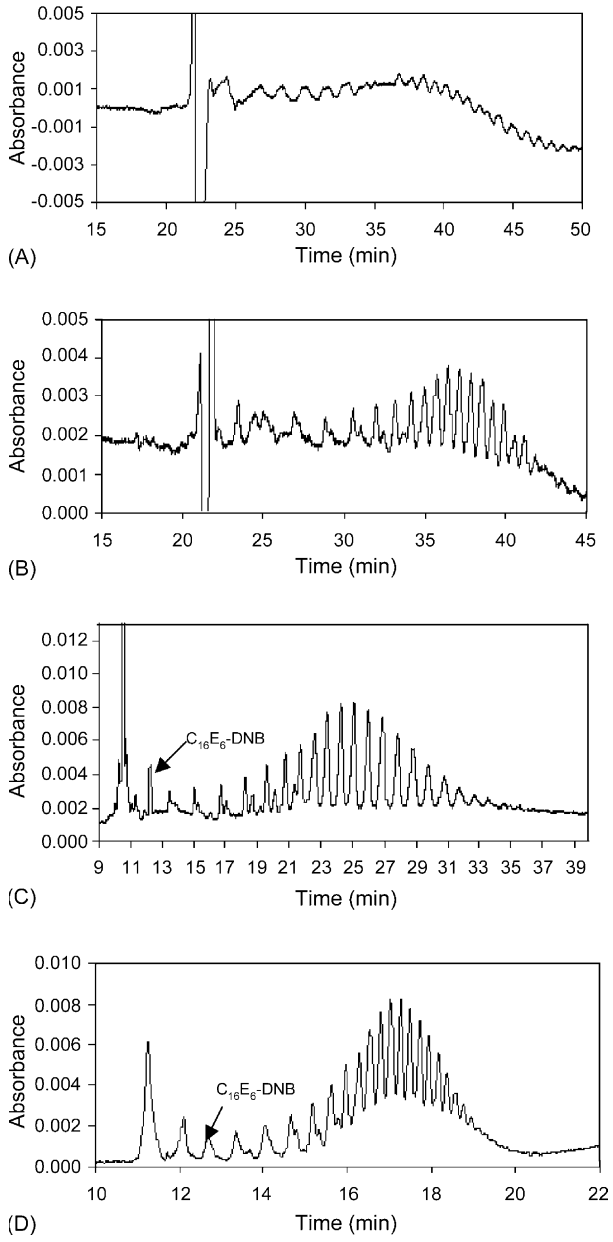

Fig. 3. Electropherograms corresponding to the choice of running electrolyte for the analysis of KM 20-DNB in NACE. Operating conditions: fused silica capillary [57 cm (50 cm effective length) × 50 μm i.d.] coated with HDMB following the static procedure; electrolyte: 2×10^{-2} mol/L NH_4I (A), 4×10^{-2} mol/L NH_4SCN (B), 6×10^{-2} mol/L NH_4Cl (C), 6×10^{-2} mol/L CH_3COONH_4 (D) in methanol; hydrodynamic injection of 1 g/L KM 20-DNB for 5 s; applied voltage: −15 kV; temperature: 23 °C; anodic detection at 214 nm.

From this equation, it clearly appears that a reversal of the electroosmotic flow should increase the resolution if $-\mu_{eo2} < 2\overline{\mu} + \mu_{eo}$. As regards the optimisation of the resolution, we can note that the applied voltage was maintained constant and equalled −15 kV for studying a broad range of background electrolyte concentrations (10^{-2} to 10^{-1} mol/L range) without problem of Joule heating. In such conditions, we restricted our study to the following

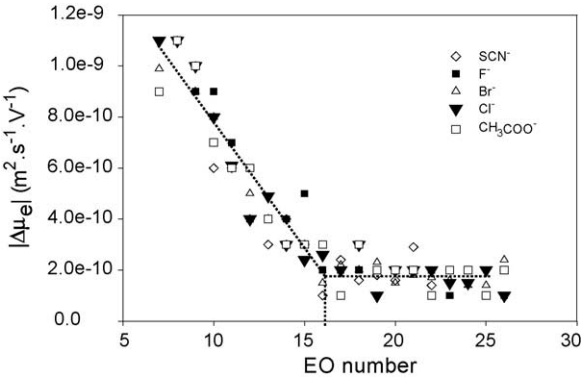

Fig. 4. Evolution of $|\Delta\mu_e|$ as a function of the OE number of KM 20-DNB oligomers for the various studied counter-ions. The OE number reported in this figure corresponds to the mean ethoxylation degree considering two neighbouring oligomers. Operating conditions: separation capillary coated with HDMB following the static procedure; applied voltage: −15 kV; the concentrations of ammonium salts are equal to 4×10^{-2} mol/L; other operating conditions as in Fig. 3.

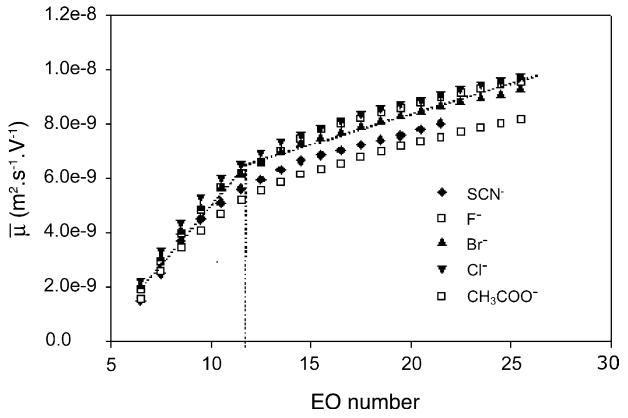

Fig. 5. Evolution of mean electrophoretic mobility of KM 20-DNB oligomers as a function of their ethoxylation degree with regard to various ammonium salts. Operating conditions as in Fig. 4.

three factors: the selectivity via $\Delta\mu_e$, the electrophoretic mobility via $\bar{\mu}$ and the electroosmotic flow (μ_{eo}).

3.1.2.1. Difference of electrophoretic mobilities. The evolution of $|\Delta\mu_e|$ as a function of KM 20-DNB oligomers is reported in Fig. 4 for the different studied counter-ions.

As shown in Fig. 4, the evolution of $|\Delta\mu_e|$ depends on ethoxylated chain lengths. Indeed, for $n < 16$, $|\Delta\mu_e|$ decreases when n increases whereas, for $n \geq 16$, this parameter remains constant when OE number varies from 16 to 27. These two different evolutions could be attributed to the evolution of conformation related to the complexes formed between KM 20-DNB oligomers and NH_4^+. In fact, the POE having the lower ethoxylation degree should preferentially have a zig-zag conformation while the helix conformation should be favoured when the OE unit increases. These changes of conformation have been reported several times in literature in presence of monovalent cation [20] and even in absence of cation [21,22]. Moreover, as illustrated by Fig. 4, $|\Delta\mu_e|$ appears almost constant as a function of studied counter-ion. Consequently, the observed difference of resolution as a function of running electrolyte cannot be due to a change of $|\Delta\mu_e|$. Therefore, in a second time, we wanted to know if this evolution of resolution originates from the electrophoretic mobilities of KM 20-DNB oligomers.

3.1.2.2. Electrophoretic mobility. For each ammonium counter-ion, we studied the evolution of the mean electrophoretic mobility of KM 20-DNB oligomers as a function of OE number. As presented in Fig. 5, $\bar{\mu}$ increases with ethoxylation degree. Besides, we observe two segments with different slopes.

Indeed, the slope is lower for $n > 12$ than the one obtained for a degree of ethoxylation inferior to 12. Thus, the break observed on the plot $\bar{\mu} = f(n)$ could again be attributed to a change of complex conformation. By referring to Fig. 5, the latter may occur at about $n = 12$. This value is inferior to that previously obtained from the plot $|\Delta\mu_e| = f(n)$ (see Fig. 4). This difference can originate from the inaccuracy linked to the experimental determination. However, if the electrophoretic mobility of the studied oligomers does not depend on the nature of ammonium salt, in contrast, it varies as a function of the ammonium salt concentration within the running electrolyte. Thus, for a given oligomer, the electrophoretic mobility increases, in absolute value, with ammonium salt concentration. Such behaviour is consistent with Eq. (2) mentioned below:

$$\mu = \frac{K[\mathrm{M}]}{1 + K[\mathrm{M}]}(\mu_{comp} + \mu_{eo}) \quad (2)$$

where μ is the oligomer electrophoretic mobility ($m^2\,V^{-1}\,s^{-1}$), K is the complex formation constant, [M] is the complexed cation concentration in the running electrolyte (mol/L), μ_{comp} is the complex electrophoretic mobility ($m^2\,V^{-1}\,s^{-1}$), and μ_{eo} is the electroosmotic mobility ($m^2\,V^{-1}\,s^{-1}$).

This equation is defined on the basis of a fast exchange between complexed and non-complexed species and for an electroosmotic flow migrating in opposite direction of complexes.

Moreover, like for $|\Delta\mu_e|$, the evolution of mean electrophoretic mobilities does not show significant difference as a function of ammonium counter-ion. So, whatever the background electrolyte, this mean mobility has no influence on the resolution of KM 20.

Accordingly, the modification of resolution should originate from electroosmotic flow that should increase the electrophoretic path length.

3.1.2.3. Electroosmotic mobility. The evolution of electroosmotic flow as a function of ammonium salt concentration is plotted in Fig. 6.

Thus, if the nature of the counter-ion associated with ammonium has nearly no influence on $|\Delta\mu_e|$ and on the mean electrophoretic mobility, we observe that it modified

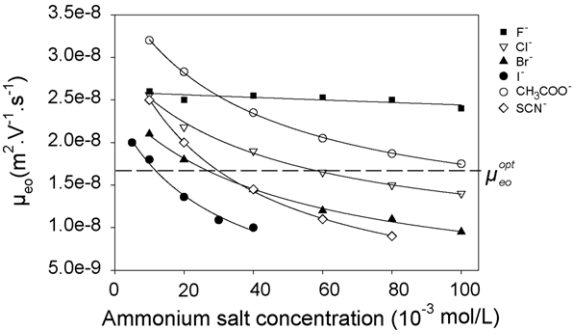

Fig. 6. Evolution of electroosmotic flow as a function of salt concentration for various ammonium salts. Operating conditions as in Fig. 3.

significantly the electroosmotic flow. So, we can make several comments about this evolution. First, taking into account the double layer theory, electroosmosis decreases when the ionic strength increases. Nevertheless, this decrease is more or less important as a function of the studied counter-ion. As an example, electroosmotic flow markedly decreases when iodide is used while it is almost constant in the case of fluoride anion. We will try to rationalize such results below (see Section 3.2).

On the whole, the separation of KM 20-DNB oligomers showed an optimum when the EOF was equal to 1.7×10^{-8} m^2 V^{-1} s^{-1}, whatever the ammonium salt used. For example, such an EOF was obtained by using 6×10^{-2} M NH_4Cl in methanol as running electrolyte. The analysis of KM 20-DNB carried out in these conditions is reported in Fig. 7.

The oligomers identification was carried out by injecting $C_{16}E_6$ derivatized by 3,5-dinitrobenzoyl chloride. As an example, the main oligomers were marked in Fig. 7. As is shown by this figure, we can easily visualise all the oligomers between $C_{16}E_5$ and $C_{16}E_{30}$ or even $C_{16}E_{33}$. Moreover, the baseline resolution allowed carrying out quantitative studies. The various oligomers bearing the same chromophoric moiety, we can reasonably assume that they have the same response co-

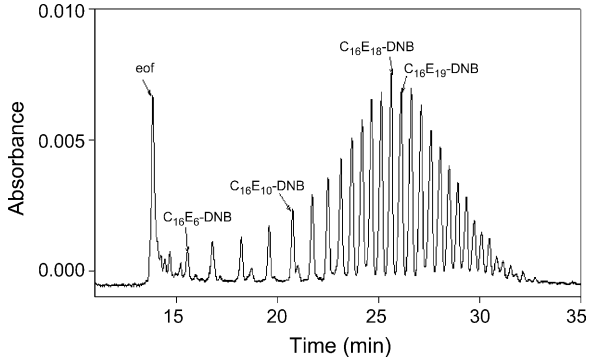

Fig. 7. NACE analysis of KM 20-DNB using the optimal conditions. Operating conditions: fused silica capillary [57 cm (50 cm effective length) × 50 μm i.d.] coated with HDMB following the static procedure; electrolyte: 6×10^{-2} mol/L NH_4Cl in methanol; hydrodynamic injection of 1 g/L KM 20-DNB for 5 s; temperature: 23 °C; applied voltage: −15 kV; anodic detection at 214 nm.

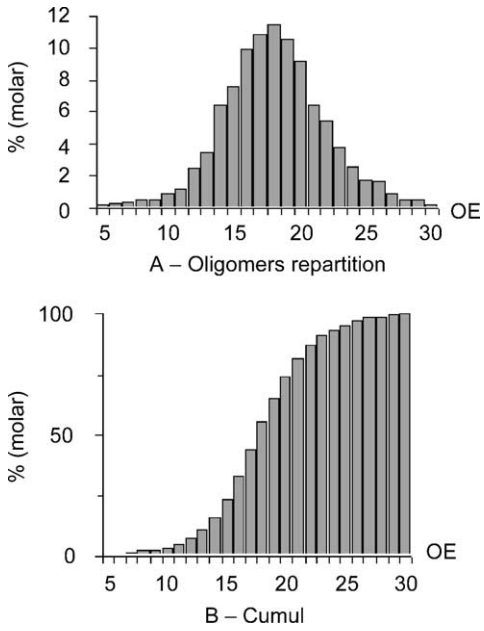

Fig. 8. Distribution histograms of KM 20-DNB obtained from the NACE analysis reported in Fig. 7.

efficient. Consequently, it is possible to characterise the distribution of KM 20 oligomers from the peak areas corrected by the migration times. Fig. 8A and B give, respectively, the distribution and cumulative distribution histograms for KM 20-DNB as deduced from quantitative NACE analysis.

The distribution determined by NACE is consistent with the one obtained previously from HPLC analyses [5]. Nevertheless, it is slightly narrower than the latter because it is truncated not only for low ethoxylation degrees ($n \leq 4$) but also for high OE unit numbers (the last visualised oligomers correspond to $n = 34$ or so). Consequently, we determined that the mean ethoxylation number is equal to 18 while its real value is equal to 20, as evaluated by HPLC. In our opinion, this results from two different reasons. First, it is well known that alkaline or alkaline earth cations can form complex with polyoxyethylenic chains if the latter have five OE units as a minimum [17]. So, the oligomers with less than five OE units migrated with electroosmotic flow because they have no charge. Consequently, we used them as electroosmosis markers. Besides, the oligomers possessing the highest ethoxylation degrees formed complexes with ammonium cation but they were not detected as mentioned earlier. This may originate from the detection sensitivity of the used technique.

3.2. Electroosmotic flow and ion pair formation

We demonstrated that the electroosmosis mobility is a crucial parameter with respect to the separation of KM 20-DNB oligomers. Besides, as reported in Fig. 6, the nature of the ammonium salt led to different EOF magnitude. Indeed, for a given ammonium salt concentration, the intensity of electroosmosis varies significantly with the nature of the considered counter-ion. In the studied concentration range,

Table 2
Molar ionic conductivity at infinite dilution (Λ_0) of the studied ammonium salts in methanolic solutions and binding constants (K_b) of related ion pairs (values deduced by plotting $F(z)/\Lambda$ as a function of $C\Lambda/F(z)$)

Anion	R^2	$\Lambda_0 \times 10^{-4}$ ($m^2\,S\,mol^{-1}$)	$\Lambda_0^{H_2O} \times 10^{-4}$ ($m^2\,S\,mol^{-1}$)	K_b
CH_3COO^-	0.96	113	114.4	37
F^-	0.97	109	128.9	22
Cl^-	0.96	135	149.8	20
Br^-	0.91	133	151.6	14
I^-	0.95	133	150.3	6
SCN^-	0.92	141	139.5	15

R is the correlation coefficient.

the electroosmotic flow rises by varying the ammonium counter-ion from iodide to fluoride:

$$I^- < Br^- \approx SCN^- < Cl^- < CH_3COO^- < F^-$$

Ion pair formation that is more or less strong between ammonium and its counter-ion may be involved in this variation of electroosmotic flow. To validate this hypothesis, we calculated the binding constants of theses ion pairs, K_b, by using the following equation [23]:

$$\frac{F(z)}{\Lambda} = \frac{1}{\Lambda_0} + \frac{K_b}{\Lambda_0^2}\frac{C\Lambda}{F(z)} \quad (3)$$

with

$$F(z) = \frac{4}{3}\cos^2\left[\frac{1}{3}\cos^{-1}\left(-3^{3/2}\frac{z}{2}\right)\right]$$

$$z = \left(\frac{A + B\Lambda_0}{\Lambda_0^{3/2}}\right)(C\Lambda)^{1/2}$$

$$A = \frac{82}{\eta(\varepsilon T)^{1/2}}$$

$$B = 0.82 \times 10^6(\varepsilon T)^{-3/2}$$

where η and ε are the viscosity and the dielectric constant of the solvent, respectively, and T is the temperature of analysis.

Thus, A and B being able to be evaluated for methanol at 25 °C, by plotting $F(z)/\Lambda$ as a function of $C\Lambda/F(z)$, we obtain a linear relationship for which:

(i) the origin ordinate equals $(1/\Lambda_0)$, and
(ii) the slope corresponds to K_b/Λ_0^2.

So, Λ_0 and K_b can be calculated taking into account the origin ordinate and the slope of this straight line. As reported in Table 2, the correlation coefficients ranged from 0.91 to 0.97, so they were relatively satisfactory. In this table, for comparison, we also reported the molar ionic conductivities at infinite dilution measured in water ($\Lambda_0^{H_2O}$) that correspond to the same salts [24]. As reported in this table, the molar ionic conductivity at infinite dilution of ammonium haloids is lower of about $18 \times 10^{-4}\,m^2\,S\,mol^{-1}$ in methanol than those measured in water.

Consequently, ammonium cation may be more solvated by methanol than by water. Moreover, this difference of solvation was evidenced previously by Porras et al. [25] for alkaline cations. Unlike ammonium haloids, ammonium thiocyanate and ammonium acetate have about the same molar ionic conductivity at infinite dilution in methanol than in water. For the purpose of explicating this result, we can note that SCN^- and CH_3COO^- have a more complicated geometry and a delocalised charge unlike haloids that possess a spherical symmetry.

On the whole, the binding constants of ion pairs gathered in Table 2 range from 6 to 37. These values are coherent with those, ranging from 10 to 40, reported by Porras et al. [25] for nitrate/alkaline metal ion pairs. Thus, the magnitude of electroosmotic flow depends on the ability of ammonium salts to form strong ion pairs in methanol. Among the studied background electrolytes, NH_4F and CH_3COONH_4 have the higher binding constants and led, for a given concentration, to the thicker double layer on the capillary surface. So they gave the higher electroosmotic flows. On the contrary, in the same experimental conditions, ammonium iodide, that owns the lower binding constant, led to a thinner double layer. Indeed, a more efficient electro-neutralisation occurred in the internal layer because of a higher concentration of free anions in the electrolyte. Lastly, for NH_4Br and NH_4SCN, the same magnitude of electroosmotic flow was reached because these salts have about the same ability to form ion pairs in methanol.

We must now explain the particular behaviour of electroosmotic flow with respect to ammonium fluoride. Indeed, unlike the other ammonium salts, the electroosmosis is almost constant when NH_4F concentration increases. This evidences probably that fluoride ions have a low ability to neutralise the positive charges of the capillary surface. Such behaviour is consistent with the very low elution strength of fluoride ions in exchange ion chromatography linked to its lack of interaction with the positive charges of the stationary phase [26].

To sum up the previous results, we pointed out that the difference of electrophoretic mobilities, $|\Delta\mu_e|$, was almost independent of the nature of ammonium salt and we evidenced that electrophoretic mobility of oligomers (μ) depended solely upon the salt concentrations. So, according to Eq. (1) and whatever the considered ammonium salt, there must exist one salt concentration that gives the optimal electroosmotic flow leading to $R_s = 1.5$.

We showed that only two running electrolytes, 6×10^{-2} mol/L NH_4Cl and 3×10^{-2} mol/L NH_4Br, allowed to obtain good resolution for KM 20-DNB ($R_s > 1.5$ for $C_{16}E_{18}/C_{16}E_{19}$ pair, see Fig. 3 for NH_4Cl). By referring to Fig. 6, these electrolytes led to the same electroosmotic flow that equalled $1.7 \times 10^{-8}\,m^2\,V^{-1}\,s^{-1}$. This value, noted μ_{eo}^{opt} in Fig. 6, was previously retained as optimum for electroosmotic flow with regard to the separation of KM 20-DNB. It is now easy to understand why the resolution was poor using NH_4F and CH_3COONH_4. Indeed, whatever

their concentration in the running electrolyte, these two salts did not enough slow down electroosmosis to reach the optimal value. On the contrary, we might perform a good resolution of KM 20 oligomers with ammonium iodide if the problems of detection were resolved.

4. Conclusion

In an attempt to improve the resolution of KM 20-DNB, that is a complex mixture of oligomers, an electroosmotic flow reversal was performed in pure organic medium. A comparative study was carried out to determine the best procedure to maintain constant the reversed electroosmotic flow. Thus, a static treatment of the capillary with hexadimethrine bromide appeared to lead to a satisfactory repeatability. In a first approach for optimising the resolution, we selected ammonium cation to form positively charged complexes with KM 20-DNB. Then, studying the influence of ammonium counter-ion in NACE, we evidenced that the nature of this anion has an influence on the resolution of KM 20, essentially by modifying the electroosmosis magnitude. We pointed out a good correlation between the distribution histograms of KM 20 oligomers established from NACE analyses and the one previously determined from HPLC analyses. This validates our approach carried out in NACE.

In such a context, we want to optimise mass detection of KM 20 in order to analyse this complex mixture without any derivatization. Consequently, we want to perform KM 20 analysis using NACE–electrospray ionization MS coupling.

References

[1] M. Ahel, W. Giger, Anal. Chem. 57 (1985) 2584.
[2] P.L. Desbène, B. Desmazières, J.J. Basselier, A. Desbène-Monvernay, J. Chromatogr. 461 (1989) 305.
[3] K. Lemr, M. Zanette, A. Marcomini, J. Chromatogr. A 686 (1994) 219.
[4] I. Zeeman, J. Chromatogr. 363 (1986) 223.
[5] P.L. Desbène, B. Desmazières, V. Even, J.J. Basselier, L. Minssieux, Chromatographia 24 (1987) 857.
[6] E. Forgács, T. Cserháti, J. Chromatogr. A 661 (1994) 239.
[7] P.L. Desbène, F.I. Portet, G.J. Goussot, J. Chromatogr. A 730 (1996) 209.
[8] M.C. Allen, D.E. Linder, J. Am. Oil Chem. Soc. 58 (1981) 950.
[9] A. Marcomini, M. Zanette, J. Chromatogr. A 733 (1996) 193.
[10] K. Rissler, J. Chromatogr. A 742 (1996) 1.
[11] K. Heinig, C. Vogt, Electrophoresis 20 (1999) 3311.
[12] K. Heinig, C. Vogt, G. Werner, J. Chromatogr. A 745 (1996) 281.
[13] J. Bullock, J. Chromatogr. 645 (1993) 169.
[14] R.A. Wallingford, Anal. Chem. 68 (1996) 2541.
[15] K. Heinig, C. Vogt, G. Werner, Anal. Chem. 70 (1998) 1885.
[16] K.A. Oudhoff, J. Chromatogr. A 985 (2003) 479.
[17] T. Okada, J. Chromatogr. A 695 (1995) 309.
[18] T. Okada, J. Chromatogr. A 804 (1998) 17.
[19] K. Ono, H. Konami, K. Murakami, J. Phys. Chem. 20 (1979) 2665.
[20] T. Okada, Anal. Chim. Acta 281 (1993) 95.
[21] F.E. Bailey Jr., T.V. Koleske, in: M.J. Shick (Ed.), Non-Ionic Surfactants, Marcel Dekker, New York, 1967 (Chapter 23).
[22] P.L. Desbène, F.I. Portet, G.J. Goussot, J. Chromatogr. A 730 (1996) 209.
[23] J. Barthel, R. Watcher, M. Knerr, Electrochim. Acta 16 (1970) 723.
[24] D.R. Lide (Ed.), Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 1993, p. 5.
[25] S.P. Porras, M.L. Riekkola, E. Kenndler, Electrophoresis 23 (2002) 367.
[26] P. Schoenmaker, Handbook of High Performance Liquid Chromatography, Chromatographic Sciences Series, Marcel Dekker, New York, 1998.