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Separation of nonylphenol ethoxylates and nonylphenol by non-aqueous capillary electrophoresis ☆

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Abstract

Capillary electrophoresis based on non-aqueous solvent background electrolytes was employed, with single and multiple wavelength UV detection, to evaluate discrimination among oligomer components of mixtures of non-ionic, long chain nonylphenol ethoxylates (NP*n*EO, with *n* = number of ethoxy units) and their lipophilic degradation products. The tested organic solvents included acetonitrile, methanol, ethanol, 1- and 2-propanol, 1-butanol and tetrahydrofurane in the presence of sodium acetate. A rational variation of composition of background electrolyte solvent mixtures allowed to modify the mobility of electroosmotic flow and the type and degree of interactions between the ionic additive (sodium acetate) and the components of the analyte mixtures. The physicochemical properties of the solvents, such as dielectric constant, viscosity and electron donor–acceptor ability regarding the additive, were considered to improve the resolution of lipophilic compounds with less than three ethoxy groups and the discrimination attainable for longer chain oligomers. The studied methodologies also allowed discerning between surfactants of similar (nominal) ethoxy chain lengths. This was demonstrated by the different peak distribution patterns observed for NP*n*EO compounds with *n* = 7.5 and 10, respectively.

Keywords: Non-aqueous capillary electrophoresis; Nonylphenol ethoxylates; Lipophilic metabolites; Cationic complexes; Heteroconjugation

1. Introduction

Alkylphenol ethoxylates (AP*n*EO) are non-ionic surfactants in which the hydrophilic part of the molecule is constituted by a polyethoxylated chain with varying ethoxylation degrees (number of ethoxy units *n* between 1 and 40). The hydrophobic region is provided by alkylphenol groups, with the branched hydrocarbon chains most commonly containing eight (octylphenol ethoxylates, OP*n*EO) or nine (nonylphenol ethoxylates, NP*n*EO) carbon atoms. Most of AP*n*EO are introduced into the environment as aqueous solutions through industrial and domestic effluents and discharged to surface waters either directly or after waste treatment in sewage treatment plants [1,2]. It is known that bio-elimination of AP*n*EO is incomplete, leading to persistent, strongly lipophilic degradation products like alkylphenol diethoxylate (AP2EO), alkylphenol monoethoxylate (AP1EO) and the alkylphenols nonylphenol (NP) and octylphenol (OP). These compounds, particularly alkylphenols, have been shown to accumulate in aquatic organisms. They are more toxic than the parent compounds and are considered the origin of a number of estrogenic response effects, even at low concentrations, on a variety of living organisms [3–5].

The availability of methods for the analytical determination of AP*n*EO and their metabolites, including selective procedures able to provide information on about individual homologue distributions, are relevant both in product characterization and for environmental safety controls. A review on determination of surfactants by capillary electrophoresis (CE) by Heinig and Vogt [6] addressed the separation of AP*n*EO oligomers by employing phosphate or borate buffers containing the anionic surfactant

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sodium dodecyl sulfate (SDS) and polar organic solvents like acetonitrile (ACN). This operation mode, based on solvophobic associations, was demonstrated to be useful for the separation of these amphiphilic non-ionic substances which, due to the high partition coefficients in the micellar phase, are impossible to resolve in micellar electrokinetic capillary chromatography [7,8]. The absence of aggregation and of adsorption of surfactant molecules onto capillary walls in polar non-aqueous media have been exploited for the CE separation of ionic detergents [9]. Non-aqueous solvent media may also be particularly useful for the electrophoretic separation of amphiphilic compounds like non-ionic surfactants of the AP*n*EO type. However, suitable separation strategies for these neutral substances are yet to be developed [10].

As discussed by Bowser et al. [11], the adequate choice of organic solvents offers the possibility of CE separations based on interactions that cannot occur or that can hardly be detected in an aqueous medium. For instance, non-dissociated Brønsted acids (phenols, carboxylic acids and alcohols) have been separated in ACN by use of their heteroassociation with small anions like Cl^-, ClO_4^- and CH_3COO^- (Ac^-). Due to the low electron acceptor (or hydrogen-bond formation) ability of that aprotic solvent, the added anions experience very weak solvation and interact preferably with stronger hydrogen donors, like the Brønsted acids, leading to the formation of heteroconjugated anions [9,12–14]. On the other hand, Okada [15,16] has shown that poly(oxyethylenes) (POE) are able to form coordination shells at the optimum distance required by some cations, frequently trapping them in a helix structure. The occurrence of this type of complexation would not be favored in a solvent like water, which has a strong solvation ability for hard cations. In that instance, the polyethers are unable to replace the water molecules existing in the solvation shell of the cation. Capillary electrophoresis separation of a variety of POE has been carried out in methanol (MeOH) by Okada [17], based on complexation of the uncharged analytes with cations such as Na^+, K^+ and NH_4^+, allowing migration of the neutral molecules to the cathode.

To our knowledge, no methods specifically aimed to the analytical determination of AP*n*EO by non-aqueous capillary electrophoresis (NACE) have been reported to date in the literature. The present work explores the use of various solvents and mixtures of solvents as well as the effect of different injection media, electrolyte concentrations and two detection modes on the CE separation and identification of NP*n*EO and their lipophilic metabolites. Analyte interactions with NaAc in non-aqueous media are exploited. Based on complexation with Na^+, separation of NP*n*EO with 3 or more ethoxy groups was investigated, while heteroconjugation with Ac^- was considered for the separation of NP2EO, NP1EO and NP.

2. Experimental

2.1. Chemicals

The chemical formulae of the analytes considered in this work are shown in Table 1. All chemicals were used as received from the suppliers. Sodium acetate ($NaAc{\cdot}3H_2O$) was SigmaUltra quality (Sigma-Aldrich Co., USA). Acetic acid (glacial) was electronic grade reagent (J.T. Baker, NJ, USA). All the organic solvents used were of HPLC grade (Merck, Darmstadt, Germany or similar). Purified water (18 MΩ cm) was obtained from a Simplicity model water purification unit (Millipore, SP, Brazil).

Table 1
Chemical formulae of the surfactants and lipophilic metabolites studied

Compound	Formula
Nonylphenol ethoxylates (NP*n*EO)	$H_{19}C_9$–Ph–O–$(CH_2$–CH_2–$O)_n$–H, $n = 1$–40
Nonylphenol diethoxylate (NP2EO)	$H_{19}C_9$–Ph–O–CH_2–CH_2–O–CH_2–CH_2–OH
Nonylphenol monoethoxylate (NP1EO)	$H_{19}C_9$–Ph–O–CH_2–CH_2–OH
Nonylphenol (NP)	$H_{19}C_9$–Ph–OH

Technical NP (nominal composition: 85% 4-NP/10% 2-NP, Fluka, Buchs, Switzerland), 4-NP1EO and 4-NP2EO (Promochem, Wesel, Germany) were used as analyte standards for the estrogenic metabolites. A sample of NP*n*EO with an average number of four ethylene oxide units (NP4EO) obtained from a manufacturer and consisting of a mixture of oligomers with 1–10 ethoxy groups, was subjected to separation by HPLC in order to isolate oligomers required as secondary standards for CE peak identification (Section 2.2). A stock solution 1.2 g l^{-1} of NP4EO in cyclohexane/2-propanol (90 + 10) was the starting material. Commercially available brands of NP10EO and NP7.5EO (NP*n*EO with average numbers of 10 and 7.5 ethoxy units respectively, the latter usually known as PONPE 7.5) were employed.

2.2. Apparatus

Experiments were carried out with two different CE instruments: (i) a SpectraPHORESIS equipment (ThermoSeparation Products, CA, USA) consisting of an ULTRA separation module, an air-driven capillary cooling system, an UV3000 scanning UV/Visible detector, a VIAL SERVER autosampler, a SN4000 interface and a PC1000 software for data acquisition and processing and (ii) a P/ACE MDQ model system (Beckman Instruments, CA, USA) equipped with a diode array detector (DAD), a liquid-driven capillary cooling unit and a data handling P/ACE System MDQ software. Fused-silica capillaries were obtained from Alltech (IL, USA) and MicroSolv Technology Corporation (NJ, USA). Dimensions were: 75 μm inner diameter, 39.3 cm total length, 33.5 cm effective length for the ThermoSeparation instrument and 75 μm inner diameter, 57 cm total length and 50 cm effective length for the Beckman instrument. Capillary, sample and background electrolyte (BGE) temperatures were maintained at 15 °C during all the experiments to avoid significant evaporation and local boiling or bubble generation with the highly volatile solvents investigated. Running potential was kept at 15 kV and sample introduction was carried out in the hydrodynamic mode at the anodic end of the capillary at 0.8 psi for 2.5 s

(ThermoSeparation instrument) and 0.5 psi for 5.0 s (Beckman Instrument).

A commercial high performance liquid chromatography system consisting of a SpectraSERIES P200 binary pump and a SpectraSERIES UV100 UV/visible detector (ThermoSeparation Products, USA), equipped with a 5-μm particle aminosilica column of 250 mm × 4.6 mm (Pinnacle II, Restek, USA), was used for the separation of oligomers ($n = 4$ through 7) from the NP4EO sample. Elution of individual compounds was carried out by binary gradient. The two mobile phases used were A: hexanes (95% *n*-hexane/5% branched hexanes) and B: 2-propanol. The program followed (at 50 °C temperature) was: 10 min isocratic hold with 96% A–4% B, and a linear gradient through 50% A–50% B in 32 min. Flow rate was 1.0 ml min^{-1}. Fractions were collected by means of a three-way manual switching valve (Rheodyne 7030RV, USA) from inyections of 50 μl NP4EO stock solution. The solvent was then evaporated to dryness and the residues suitably dissolved in ACN for CE injection.

Ultraviolet molecular absorption spectra were obtained with a HP-8453 UV–vis diode-array spectrophotometer (Hewlett-Packard, CA, USA).

2.3. Procedures

Standard solutions were prepared daily by diluting, in the appropriate media, stock solutions 1 g l^{-1} of NP, NP1EO and NP2EO and 10 g l^{-1} of NP10EO and PONPE 7.5 in acetonitrile or methanol. Stock solutions were stored at 4 °C.

Background electrolyte solutions were prepared daily by dissolving an appropriate amount of $NaAc{\cdot}3H_2O$ in the corresponding solvent or mixture of solvents. With the exception of the solvent composition ACN/MeOH 80:20 (in volume), glacial acetic acid was added at a final concentration of 2% (v/v) to allow complete dissolution of the salt in the organic media.

At the beginning of daily operation or when changing the BGE, capillaries were flushed with water (1 min), 0.1 M NaOH (1 min), water (1 min), the corresponding BGE solvent (1 min) and the running electrolyte (2 min). Then the capillary was conditioned by maintaining the running voltage until stable current and baseline were achieved (approximately 20 min). A 2-min washing step with BGE was performed before each run, and a 1-min wash step with the corresponding solvent was added at the end of the runs. All CE solutions were filtered through 0.45 μm PVDF or Nylon membrane filters before use. Acetone (1% v/v in ACN) was used as the electroosmotic flow (EOF) marker.

Electroosmotic mobilities (μ_{eo}) were calculated from:

$$\mu_{eo} = \frac{Ll}{t_{eof}V} \quad (1)$$

where t_{eof} is the migration time for the neutral marker (in s), V is the separation voltage (in V), L is the capillary total length and l is the capillary length to the detection window (in cm).

Electrophoretic mobilities (μ_{ep}) were calculated from:

$$\mu_{ep} = \left(\frac{Ll}{t_m V}\right) - \mu_{eo} \quad (2)$$

where t_m is the migration time for the cationic complex (in s), V is the separation voltage (in V), L is the capillary total length and l is the capillary length to the detection window (in cm).

Identification of electrophoretic peaks for compounds with less than three ethylene oxide units was made directly with standard spikes of NP, 4-NP1EO and 4-NP2EO. For identification of components with $n > 3$, spikes from individual oligomers were obtained by HPLC separation, as described above.

3. Results and discussion

3.1. Ultraviolet spectrometric detection

Ultraviolet (UV) absorption spectra of both long and short chain NP10EO oligomers, dissolved in a BGE consisting of 12.5 mM NaAc in ACN/MeOH/HAc 88:10:2 (in volume), were measured. Simultaneous detection at 195, 200 and 205 nm was performed with the ThermoSeparation instrument and at 196 and 200 nm with the Beckman equipment, because peak intensities at higher wavelengths proved to be insufficient for quantitative measurements. The best S/N were obtained at 195 and 196 nm with each apparatus, respectively. Multiple wavelength recordings using the DAD on the 190–300 nm range were also performed with the Beckman instrument. The characteristic absorption spectra of the analytes, with relative maxima around 200, 230 and 277 nm discriminated them from other signals, allowing positive identification of their presence in poorly resolved zones of the electropherograms.

3.2. Background electrolyte solvent effects

3.2.1. Electroosmotic flow response

The physicochemical properties of the solvents included in the present study are listed in Table 2. Employing NP10EO as analyte, we tested the electrophoretic and electroosmotic responses for BGE compositions consisting of 12.5 mM NaAc/2% (v/v) HAc dissolved in ACN, MeOH, ethanol (EtOH), 1-propanol (1-PrOH) and tetrahydrofurane (THF), respectively. The ThermoSeparation instrument was employed for these experiments. Fig. 1 shows electropherograms recorded by injecting 660 mg l^{-1} of NP10EO in, respectively, ACN, MeOH, EtOH and 1-PrOH. With ACN and MeOH electrolytes analysis times were considerably reduced (less than 8 min). In the case of EtOH, separation of higher ethoxylates seems to improve but a t_{eof} of 57.9 min resulted unacceptable for practical analysis. A better separation of higher oligomers was observed when working with 1-PrOH, but the acetone EOF marker was not detected even for an 80-min run. Neither the EOF marker nor the analytes were detected in THF even after 100 min of electrophoresis run time.

The physical parameters that influence EOF are the dielectric constant (ε_r) and the viscosity (η) of the medium, and the electrokinetic or zeta potential (ζ). These parameters are related to the electroosmotic mobility (μ_{eo}) by the Von Smoluchowski equation [18]:

$$\mu_{eo} = -\frac{\varepsilon_0 \varepsilon_r \zeta}{\eta} \quad (3)$$

Table 2
Physicochemical properties of the organic solvents investigated and water

Solvent	η (mPa s)[a]	ε_r[a]	pK_a[b]	t_{boil} (°C)	DN (kcal mol^{-1})	AN
Water	1.19	82.0	14	100	33	54.8
Methanol	0.62	34.3	17.2	64.7	30	41.3
Ethanol	1.29	26.3	18.9	78.3	32	37.1
1-Propanol	2.42	22.1	19.43	97.2	n.d.a.	n.d.a.
2-Propanol	2.83	21.0	20.8	82.3	36	33.5
1-Butanol	3.25	18.7	21.56	117.7	30	36.8
Acetonitrile	0.40	38.0	>33.3	82	14.1	19.3
Tetrahydrofurane	0.51	7.7	–	66	20	8

η = viscosity coefficient, ε_r = dielectric constant, pK_a = negative logarithm of autoprotolisis constant, t_{boil} = boiling point, DN = solvent donicity, AN = solvent acceptor number (DN and AN as defined by Gutmann, reference [27]). η and ε_r values from reference [26]. pK_a and t_{boil} values from reference [9]. DN and AN values from references [11], [27] and [28]. n.d.a.: no data available.
[a] Values at 15 °C.
[b] Values at 25 °C.

where ε_0 is the permittivity of vacuum. Zeta potential can be described as [19]:

$$\zeta = \sigma_0(\varepsilon_0\varepsilon_r K)^{-1} \quad (4)$$

where σ_0 is the (negative) charge density on the wall surface and K refers to the thickness of the double layer. By introducing the parameters that determine the zeta potential, μ_{eo} results:

$$\mu_{eo} = -\left(\frac{\varepsilon_r^{1/2}}{\eta}\right)\sigma_0 I^{-1/2}\left(\frac{\varepsilon_0 RT}{2000F^2}\right)^{1/2} \quad (5)$$

where I is the ionic strength of the electrolyte solution, R and F are the ideal gas and Faraday constants, respectively, and T is the absolute temperature.

A consideration of Eq. (5) allows a qualitative estimation of the influence of different variables on μ_{eo}. At a constant temperature, departures from a straight, zero intercept line in a μ_{eo} versus $\varepsilon_r^{1/2}/\eta$ plot for varying solvents and mixtures of solvents will evidence the dependence of the term $\sigma_0 I^{-1/2}$ in Eq. (5) on the variation of the physicochemical properties (i.e. dielectric constant and donor–acceptor abilities) of the media studied [20,21]. Fig. 2 presents plots obtained from measurements of μ_{eo} for pure and mixed solvent BGE's (containing in all cases 12.5 mM NaAc/2% HAc). Experimental points correspond to ACN, MeOH and EtOH (lower curve) and the solvents included in Table 2, all of them 10% v/v in ACN (upper points). For the latter, approximate values of viscosity and dielectric constant of the mixtures were estimated from the individual properties of each component, assuming a variation proportional to the mixture composition [21]. Although the responses are different for each set of points (pure solvents or otherwise), a steady increase of μ_{eo} with $\varepsilon_r^{1/2}/\eta$ is observed. The departure from a linear response for the pure solvents may reflect the stronger influence of the lowering of ionic strength expected for an aprotic medium such as acetonitrile.

Electroosmotic mobility values are significantly higher with the mixed solvents tested, but very similar to that measured with pure acetonitrile. We attempted to explain this behavior by examining the response of pure methanol when compared with a 10% (v/v) solution of 2-propanol in ACN (both BGE's having similar $\varepsilon_r^{1/2}/\eta$ ratios). Ion pairing of the added electrolyte would normally be expected to increase electroosmotic flow due

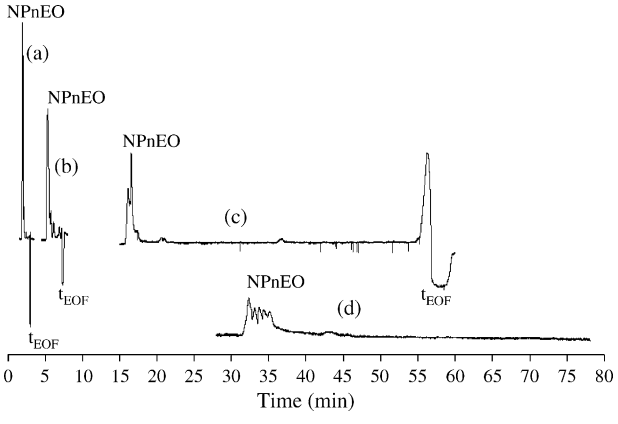

Fig. 1. Electropherograms of 660 mg l^{-1} NP10EO in the corresponding BGE solvent. BGE's: 12.5 mM NaAc (2% HAc) in (a) 100% ACN, (b) 100% MeOH, (c) 100% EtOH, (d) 100% 1-PrOH. Instrumental conditions: V = 15 kV, T = 15 °C, detection wavelength = 195 nm, injection time (0.8 psi) = 2.5 s. The zones ascribed to migrating analytes (NPnEO) and electroosmotic flow (EOF) are indicated for each BGE. Expanded versions of electropherograms (a) and (b) are shown in Fig. 3(a) and (d), respectively.

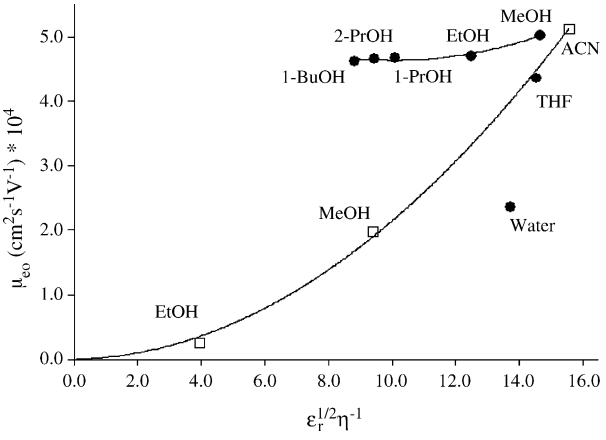

Fig. 2. Electroosmotic mobilities for different solvents and mixtures of solvents as a function of $\varepsilon_r^{1/2}/\eta$. BGE: 12.5 mM NaAc (2% HAc) in: 100% ACN, MeOH and EtOH (squares), and 10% each of MeOH, EtOH, 1-PrOH, 2-PrOH, 1-BuOH, THF and water in ACN (filled circles). Instrumental conditions: same as Fig. 1.

to a decrease in ionic strength. Evidence of the formation of ion pairs has been discussed by Porras and Kenndler [22], who reported that ion pairing seems to be operative for simple 1:1 salts dissolved in methanol, ethanol, ACN and other solvents. In our case, dielectric constants are similar (ε_r MeOH = 34.3, ε_r 2-PrOH/ACN = 36.2), but formation of ion-pairs that reduces ionic strength may be favored in the presence of 90% ACN, possibly due to the poor donor-acceptor properties of this aprotic solvent. Therefore, μ_{eo} should be significantly higher for 10% solvents in ACN when compared with protic solvents like the alcohol compounds tested.

By contrast, the behavior of pure and 10% THF cannot be explained by considering only ionic strength effects. As previously mentioned, the EOF marker was not detected after 100 min of electrophoresis in THF. The dielectric constant of THF is comparatively low and its donor-acceptor properties are unfavorable for electrolyte dissociation, pointing to an enhanced ion pair formation (i.e. lower ionic strength). However, the electroosmotic mobility decreases, suggesting that a significant depletion in the effective negative charge on the capillary inner surface (σ_0) could be the cause of this behavior. A decrease of the autoprotolysis constant of silanol groups upon the addition of a solvent with such a low dielectric constant would decrease their degree of dissociation and concurrently the ζ potential at the silica surface [20,22]. This effect cannot be compensated by the mentioned expected decrease in ionic strength. Moreover, sodium ions arising from the sodium salt contained in the non-dissociating BGE might tend to be extensively adsorbed on the negatively charged wall [21,23]. Further, the absence of EOF marker signal after 100 min of electrophoresis in the case of the pure THF solvent could be ascribed to a reversed (anodic) electroosmotic flow produced by a charge inversion at capillary walls, as that already reported by Cottet et al. [24] in THF-MeOH mixtures under strongly acidic conditions. The behavior observed suggests that separation of higher n oligomers could be performed with good resolution in the THF medium under reversal of polarity conditions. Although we did not perform experiments to demonstrate the feasibility of this assertion, electroosmotic flow reversal for CE analysis of derivatized fatty alcohol ethoxylates has been recently proposed by Desbène el al. [25].

Water (10% v/v in ACN) was included in this series of experiments only for comparison purposes. Its behavior regarding the noticeable lowering of μ_{eo} is in agreement with a higher electrolyte ionic strength produced by the suppressed ion pair formation, which should reduce the double-layer thickness on the capillary walls.

3.2.2. Oligomer resolution

Resolutions of oligomers with $n > 3$ obtained under the tested experimental conditions are depicted in Fig. 3 for several compositions of BGE (pure and mixed solvents).

Positive signals for standards with $n < 3$ (i.e. NP2EO, NP1EO and NP) were not observed (both as individual or mixed peaks), suggesting that those lipophilic compounds migrated at the velocity of the electroosmotic flow. The peak for a compound containing three ethoxy groups, migrating as a cation (i.e. faster than the EOF marker), was barely discernible.

The migration characteristics of the non-ionic compounds may be attributed to formation of charged species by complexation with Na^+ present in the BGE milieu. Consequently, electrophoretic mobility of complex cationic species should be dependant not only on charge and molecular size but also on the degree of complexation. Recorded electropherograms in Fig. 3 show that, in spite of their larger molecular size, long chain NP*n*EO oligomers migrate faster than those having shorter ethoxylated chains. As it is known that formation constants of POE-cation complexes systematically increase with chain lengths [12], the degree of complexation seems to constitute a primary factor in governing migration. In Fig. 4, we have plotted electrophoretic mobility vs. the number of ethoxy groups in the oligomer chain. Each curve corresponds to the different BGE compositions for some of the solvents (or mixtures of solvents) studied. With 100% ACN the breaking point (i.e. the higher number of ethoxy groups in the compound that can be clearly discriminated) occurs for $n = 5$, with good selectivity for shorter chains. Similarly, good selectivity is observed with 10% methanol in ACN and the compound with $n = 6$ can still be discriminated. A poorer separation degree resulted from 100% methanol, but the observation of the individual compounds with $n = 6$ ethoxy groups is still allowed. On the other hand, when 10% water in ACN is employed as BGE solvent, peaks with n up to about 11 can be distinguished, although with very small differences in electrophoretic mobilities.

In general terms, μ_{ep} values are lower both in the presence of methanol (pure or 10% in ACN) and of water (10% in ACN) than in pure ACN. Ethereal oxygen atoms involved in coordination are expected to be arranged as part of the coordination shell of the cation [12]. Because that shell is formerly occupied by solvent molecules, the formation of coordination bonds will compete with solvation. Addition of water to the ACN electrolyte enhances the separation for the longest NP*n*EO chains, while resolution worsens as n decreases. Water, and in lesser extent methanol, should be unfavorable solvents for polyether-cation complexation because of their high solvation properties regarding hard cations, so the mentioned effects may be rather related to the lowering of the complexation constants.

3.3. Behavior of lipophilic NPnEO metabolites

As mentioned in Section 2.3 the presence of HAc in the ACN electrolyte allowed the dissolution of NaAc. Because this acid behaves as a very weak electrolyte in ACN (pK_a: 22.3 [13]), it is not expected to bring enough H^+ concentration to compete with Na^+. This was further verified by running a sample containing 660 mg l^{-1} of NP10EO in ACN with a BGE consisting of 2% (v/v) HAc in ACN (i.e. without NaAc). Under the experimental conditions employed no circulation of current was detected and no analyte signals were observed.

In the BGE containing NaAc and HAc, the ability of the acid to form hydrogen bonds with the conjugate base is expected to hinder heteroconjugation in NP1EO, NP2EO and NP through hydroxyl–acetate interactions. With the purpose of investigating the separation of these lipophilic molecules, dissolution of

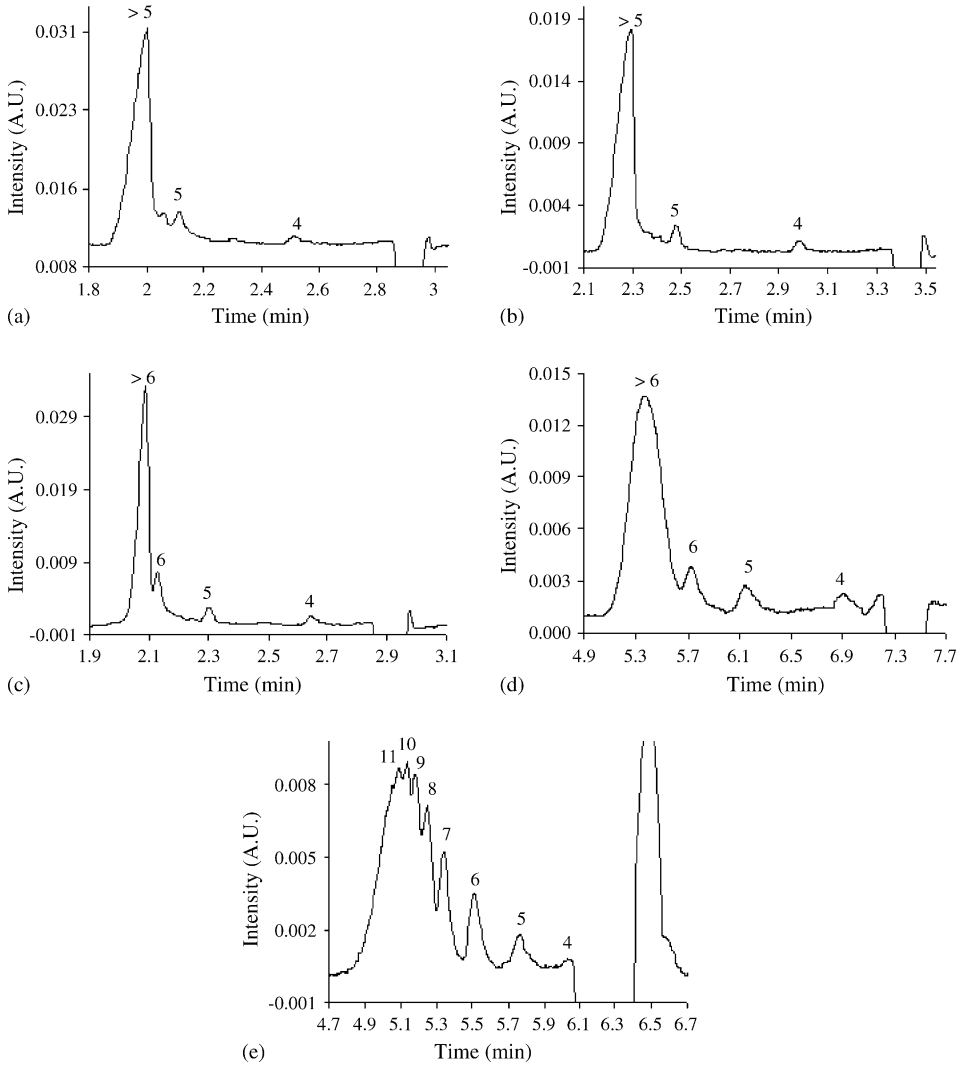

Fig. 3. Electropherograms of 660 mg l^{-1} NP10EO in the corresponding BGE solvents. BGE: 12.5 mM NaAc (2% HAc) in (a) 100% ACN, (b) 10% BuOH in ACN, (c) 10% MeOH in ACN, (d) 100% MeOH, (e) 10% water in ACN. The labels above the peaks indicate the number (*n*) of ethoxy groups in the corresponding NP*n*EO oligomer. Instrumental conditions: same as Fig. 1.

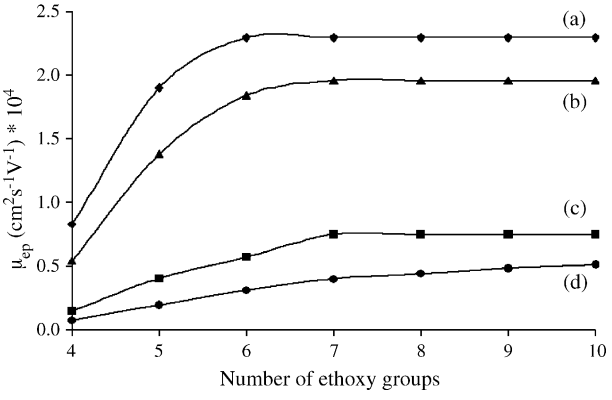

Fig. 4. Electrophoretic mobilities of NP*n*EO as a function of the number (*n*) of ethoxy groups for different BGE solvents. BGE: 12.5 mM NaAc (2% HAc) in (a) 100% ACN, (b) 10% MeOH in ACN, (c) 100% MeOH, (d) 10% water in ACN. Sample: 660 mg l^{-1} NP10EO in the corresponding BGE solvent. Instrumental conditions: same as Fig. 1.

the salt in pure ACN was performed without addition of HAc by adding 20% MeOH instead. It is worth mentioning that dissolution of sodium acetate in that medium actually derives from heteroconjugation between the acetate ion and the methanol solvent [11].

Fig. 5 depicts results obtained with diode array detection (DAD) by employing the Beckman instrument. Besides the conventional electropherogram (signal at a fixed wavelength), a bidimensional absorbance-wavelength image was recorded. Positive identification of NP is supported by the characteristic absorption spectrum in the bidimensional plot. The formation of an heteroconjugated, negatively charged ion of the (undissociated) phenolic compound in absence of HAc is suggested by its separation from the electroosmotic peak. Discrimination of the nonylphenyl ethers NP1EO and NP2EO from the electroosmotic negative signal was not possible under the conditions of Fig. 5. This result could be ascribed to a less favorable heteroconjugation of acetate ions with analyte terminal alcoholic groups caused by the presence of 20% methanol.

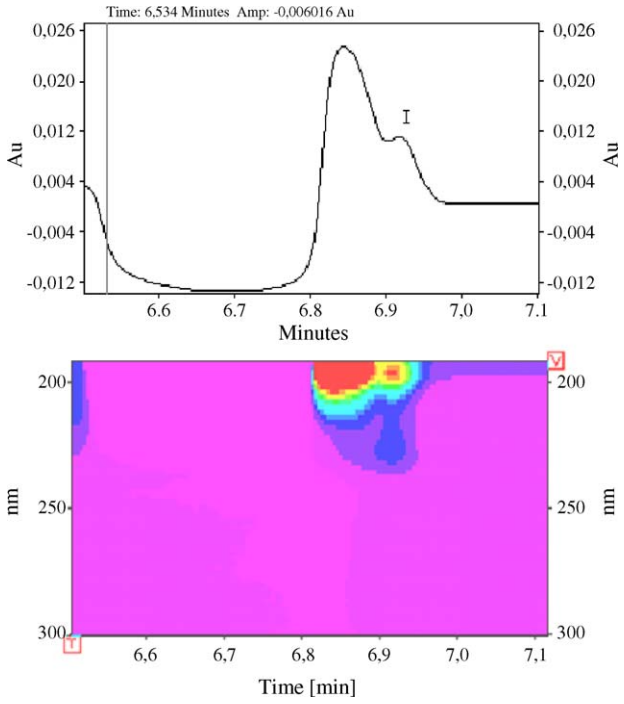

Fig. 5. Single and multiple wavelength electropherogram of 10 mg l^{-1} NP in ACN. BGE: 12.5 mM NaAc in ACN/MeOH (80:20). Peak label I indicates NP. Instrumental conditions: $V = 15$ kV, $T = 15$ °C, single wavelength detection = 196 nm, injection time (0.5 psi) = 5 s.

3.4. Influence of sample diluent on selectivity

We have noticed that the diluent employed in sample preparation affects significantly the selectivity that can be achieved. For instance, it was found that when NP10EO is dissolved in 1-propanol, resolution of higher-*n* oligomers is improved when compared with the sample in pure methanol or ACN. However, the discrimination of lipophilic compounds in the same run could be impaired due to the existence of unidentified signals possibly arising from the solvent in the zone of the electropherogram corresponding to uncharged compounds. Consequently, further experiments were carried out with the running electrolyte (12.5 mM NaAc in ACN/MeOH 80:20 in volume) as sample introduction medium. This procedure allowed the recording of suitable electropherograms both in the lower-*n* oligomer and the lipophilic oligomer zones, without substantial changes in the resolution for higher NP*n*EO species. Fig. 6(a), prepared from experiments carried out with DAD detection, shows the separation achieved for a 660 mg l^{-1} sample of commercial NP10EO. Position of peaks can be correlated with the UV absorption spectra depicted as bidimensional plots. The presence of low concentrations of lipophilic NP2EO and/or NP1EO (migration time around 8.3 min) in the surfactant tested is apparent. In addition, a low intensity peak possibly due to NP3EO, migrating as a positively charged complex, is observed. On the other hand, NP migrating as an heteroconjugate anion, is fairly resolved from the BGE structure (Fig. 6(b)). Absence of signal overlapping is supported by the spectrum recorded with the DAD.

3.5. Effect of electrolyte concentration

In ideal ionic solutions (no ion-pairing present), an increase in electrolyte concentration reduces the ζ potential due to an increase in ionic strength and causes a reduction in electroosmotic flow. According to Eq. (5), for the same operating conditions a plot of electroosmotic mobility versus $C^{-1/2}$, where C is a completely dissociated 1:1 electrolyte concentration in mol l^{-1}, should be linear. In Fig. 7, we have plotted μ_{eo} versus $C^{-1/2}$ for NaAc. A departure from a straight (dotted) line is observed at concentrations higher than 6.25 mM with the ACN/MeOH (80:20) solvent. Since a negative curvature (i.e. a

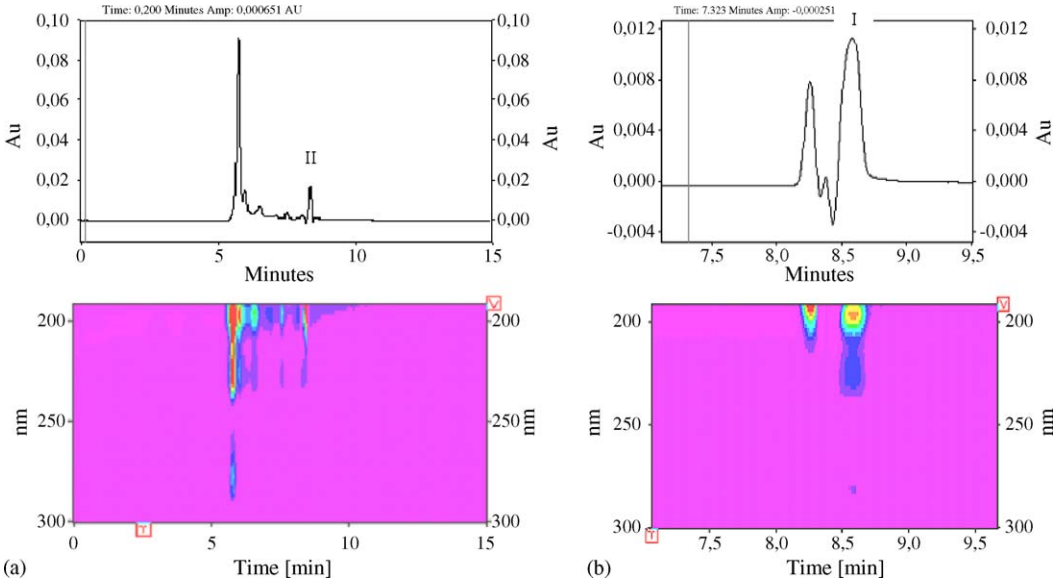

Fig. 6. Single and multiple wavelength electropherograms of (a) 660 mg l^{-1} NP10EO in BGE and (b) NP 20 mg l^{-1} in BGE. BGE: 12.5 mM NaAc in ACN/MeOH (80:20). Peak label II indicates NP1EO + NP2EO. Peak label I: same as Fig. 5. Instrumental conditions: same as Fig. 5.

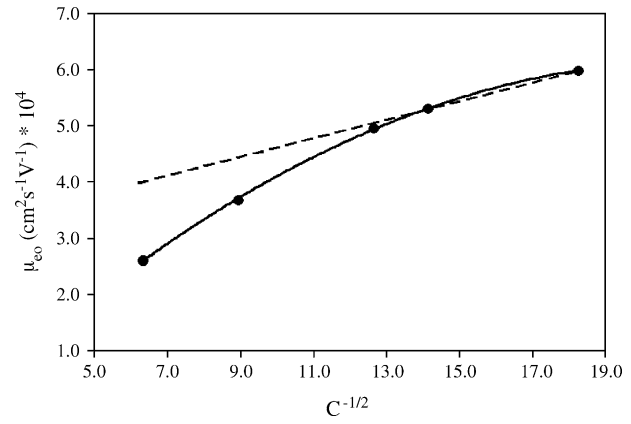

Fig. 7. Electroosmotic mobilities for varying molar concentrations of NaAc in an ACN/MeOH (80:20) BGE. Instrumental conditions: same as Fig. 5.

lowering of μ_{eo}) is opposite to that expected for a higher degree of ion-pair formation at higher salt concentrations, the effect could be attributed to stronger interactions between the silica surface and the electrolyte cation (Na^+) as the concentration of the salt increases, thus causing a decrease of σ_0 in Eq. (5) [23].

Regarding NP10EO oligomer resolution, and when only the effect of analyte–additive interaction is considered, an increase in the additive concentration would be required to compensate for a decrease in the complexation constant with decreasing n [11]. This effect, added to longer electrophoresis times for higher NaAc concentrations, has apparently led to the best discrimination both on the electropherogram zones corresponding to lipophilic compounds and to the lower-n oligomers for the highest NaAc concentration tested (a 25 mM BGE), the separation of NPnEO with six or more ethoxy units remaining virtually unchanged.

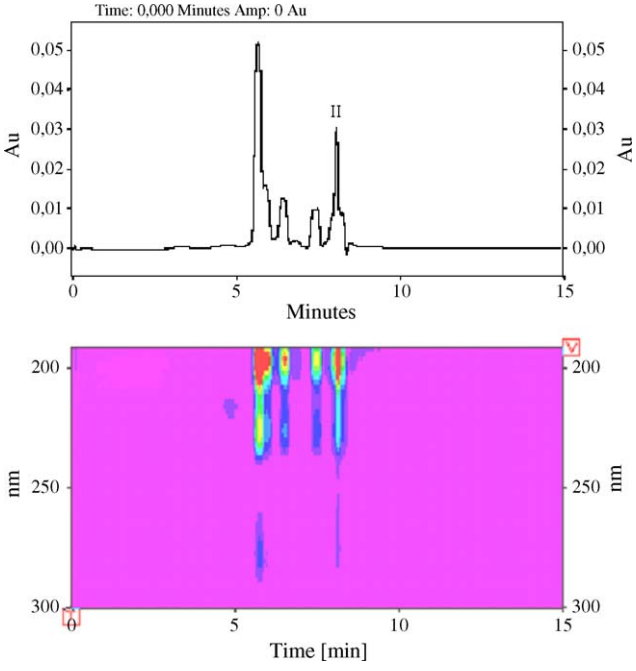

Fig. 8. Electropherogram of 550 mg l^{-1} PONPE 7.5 in BGE. BGE: 12.5 mM NaAc in ACN/MeOH (80:20). Peak label II: same as Fig. 6. Instrumental conditions: same as Fig. 5.

3.6. Discrimination of surfactants

Discrimination among surfactants of similar (nominal) ethoxy chain lengths is one of the potential applications of the studied methodologies. Because the features of the electropherograms obtained with 80% ACN-20% MeOH and including sodium acetate as electrolyte additive should differ according to the characteristics of the raw formulations, the procedure could be useful for fingerprinting of commercial surfactants [6]. To assess the potentiality of the method to discriminate between two commercially available products (NP10EO and NP7.5EO), we recorded electropherograms for a 550 mg l^{-1} PONPE 7.5 solution. The electropherogram obtained, presented in Fig. 8, differed markedly from the characteristic pattern of the NP10EO previously investigated (shown in Fig. 6(a)), indicating that the developed method may be suitable for differentiation of NPnEO commercial products. Signals for higher oligomer ($n > 6$) peaks decreased significantly, with a concomitant increase of the remaining peaks, evidencing the presence of a larger amount of lipophilic NPnEO.

4. Conclusions

The approach described, based on chemical control of the velocity of electroosmotic flow and the migration behavior of species, suggests ways to improve the selectivity in capillary electrophoretic separations when organic solvent BGE's are employed. By varying the composition of the solvent it has been possible to rationally shift the EOF velocity and also to promote the interactions between ionic additives and the analytes. Separations of oligomers of long ethoxy chains were found to be dependant on the presence of sodium acetate, a result that may be attributable to formation of positively charged species arising from complexation of the ethoxy groups with Na^+ ions. It was also observed that the viscosity and dielectric constants of the solvents or solvent mixtures, as well as their donor–aceptor properties regarding ionic additives, may influence electrophoretic and electroosmotic mobilities. Although baseline or better resolution was obtained for compounds with a number (n) of ethoxy groups between 3 and 6, discrimination of oligomers with n up to 11 could be attained with ACN containing 10% water. Discrimination of lipophilic oligomers with $n < 3$ was achieved by employing a mixture of 80% ACN–20% MeOH containing NaAc in concentrations 12.5 or 25 mM. Separation of NP may be ascribed to heteroconjugation with Ac^- ions. The improvement in selectivity for both mono and diethoxylated oligomers arises principally from prior dilution of the sample with the BGE and from the use of extended wavelength recording of absorption spectra with diode array detector. Finally, it is worth mentioning that some practical problems that plagued aqueous based CE operation, such as the strip off of the capillary cladding for BGE compositions required for NPnEO analysis (i.e. with a relatively high concentration of SDS plus an organic solvent), were not observed in NACE.

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