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Migration of (hydroxy)carboxylic acids in coelectroosmotic capillary electrophoresis Influence of the electrolyte composition

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Abstract

This work focused on the way several electrolyte components could affect the electroosmotic flow and the capillary electrophoretic migration of aliphatic or aromatic (hydroxy)carboxylic acids. The effects exerted by the electroosmotic flow modifier, hexadecyltrimethylammonium bromide, the addition of metal salt to the electrolyte and the absorbance provider (chromophore) used for indirect detection were investigated. A retention of the organic acids was demonstrated. Its magnitude was shown to depend on the amount of cationic surfactant adsorbed onto the capillary walls. The addition of sodium nitrate led to a remobilization of all the acids except glycolic acid. Moreover, the presence of the chromophore was shown to influence mainly the migration of the glycolic acid.

Keywords: Background electrolyte composition; Hydroxycarboxylic acids; Carboxylic acids

1. Introduction

The application of an electrical field when using capillary electrophoresis induces a flow of the bulk solution towards the cathode called electroosmotic flow (EOF). Control of the EOF is of major importance for the optimization of separations. Different strategies have been adopted to control the EOF including changes in the physicochemical buffer properties, modifications of the capillary surface or application of an external radial voltage to the capillary [1–3]. Concerning the first point, use of organic solvent [4], manipulation of ionic strength [5] or pH [6] have been proposed. For the second approach, either dynamic [7–10] or permanent [8,10,11] wall coating has been reported.

Among the compounds mentioned for dynamic coatings, the use of cationic surfactants is of great interest in the design of anion separation. Their ability to reverse the electroosmotic flow allows anions to migrate in the same direction as the electroosmotic flow, resulting in faster analysis. The understanding of the mechanism by which flow reversal is achieved originates from the work of Fuerstenau and coworkers [12,13] who first postulated a surfactant adsorption at a negatively charged surface through the formation of hemimicelles, pairs of surfactant ions which result from the attraction of the hydrophobic surfactant tails. Recently, Lucy and Underhill [14] have also described the role of surfactant in the achievement of flow reversal in

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capillary electrophoresis and have demonstrated the existence of two mechanisms by which a cationic surfactant can adsorb onto the walls of a fused-silica capillary. They have shown that the formation of the surfactant bilayer could occur via the adsorption of individual surfactant ions or through the direct adsorption of hemimicelles, hence leading to double or a single transition adsorption isotherms, respectively which differ from each other around the zero electroosmotic mobility region.

A few articles have focused on the variables affecting flow reversal in capillary electrophoresis, as for example the concentration of the cationic surfactant [14–18], the type of surfactant [14,16–18], pH [14,16,17,19], the electrolyte concentration [16], the addition of metal salt [17] or the electrolyte type [14,16].

As already mentioned, capillary electrophoretic separation of anions often requires the addition of a cationic surfactant to the electrolyte. For example, the use of alkylammonium salts such as hexadecyltrimethylammonium bromide [18,20,21] or tetradecyltrimethylammonium bromide [15,20,22–24] has been well documented. Cationic surfactants have often been added to the electrolyte at a concentration lower than their critical micellar concentration (CMC) in water. However a few studies performed in micellar electrokinetic chromatography with a surfactant concentration greater than the CMC have been reported. In the first case, the influence of the surfactant concentration in the achievement of the separation of inorganic and organic anions has been widely studied [18,20,22,23,25,26]. It has been shown that variations of the surfactant concentration lead to changes in the migration times of the anions, generally related to the EOF variations. However, reversal in the migration orders of anions or modifications of separation selectivity have also been observed. Possible explanations of these effects have been supposed to involve either ion pairing between anions and the cationic surfactant [18,22,26] or hydrophobic interactions between hydrophobic anions and the surfactant [22]. The possibility for the adsorbed cationic surfactant to behave like an anion exchanger has also been proposed [20].

The purpose of this work is to contribute to a better understanding of the way the electrolyte composition can affect the electroosmotic flow and the migration of anions. Hence, the effects of the concentration of the cationic surfactant hexadecyltrimethylammonium bromide, the addition of metal salt and the presence of a chromophore (absorbance provider) on both magnitude of the electroosmotic mobility and migration of six (hydroxy)carboxylate anions have been investigated.

2. Experimental

2.1. Reagents

All reagents were of analytical grade and were used without further purification. Hexadecyltrimethylammonium bromide (CTAB), nicotinic acid (NICO), creatinine (CREA), hydroxyacetic acid (glycolic acid, GLYCO), 3,4-dihydroxybenzoic acid (protocatechuic acid, PROTO) and 3,4,5-trihydroxybenzoic acid (gallic acid, GAL) were from Acros (Noisy Le Grand, France). 2,3-Dihydroxypropanoic acid (glyceric acid, GLYCE) and 2,3,4-trihydroxybutanoic acid (threonic acid, THREO) were used as their calcium salts and were products of Aldrich (Saint Quentin, France). Benzoic acid and cadmium nitrate, tetrahydrate were from Prolabo (Fontenay-sous-Bois, France). Sodium nitrate was purchased from Fluka (Saint Quentin, France).

Nicotinate and creatinine have previously proved to be good candidates as chromophore and buffer agent, respectively, in the separation and detection of (hydroxy)carboxylate anions [27,28].

2.2. Instrumentation

Experiments were performed on a Beckman P/ACE 2000 capillary electrophoresis instrument (Roissy Charles de Gaulle, France) equipped with a UV detection system. A fused-silica capillary (Thermo Finnigan, Les Ulis, France) with a total length of 37 cm and an I.D. of 75 μm was used. A detection window was created at 30 cm from the capillary inlet by removing the polyimide coating. Indirect UV detection was performed at a single wavelength of 254 nm while direct detection was carried out without nicotinate at 254 or 214 nm depending on the absorption properties of the organic acid. Samples were hydrodynamically injected at the

cathodic side for 2 s, which corresponds to an injected volume of ca. 1.4 nl. Experiments were carried out at a constant voltage of 20 kV and a constant temperature of 30 °C.

The determination of the critical micellar concentration of CTAB was carried out using the same capillary electrophoresis instrument, with a greater voltage of 30 kV and a shorter capillary length of 27 cm in order to amplify the variations of the current. The capillary was kept at a constant temperature of 30 °C to avoid Joule heating that could affect the CMC value.

2.3. Procedure

The composition of the electrolytes used to perform the experiments are as follows:

(i) 20 m*M* CREA, 4 m*M* NICO and increasing concentrations of CTAB ranging from 0.1 to 0.5 m*M*, pH 5.5, for the study of the influence of the surfactant concentration,

(ii) 20 m*M* CREA, 4 m*M* NICO, 0.1 m*M* CTAB at pH 5.5 or 0.5 m*M* CTAB at pH 6.2 and increasing concentrations of metal salt, either $Cd(NO_3)_2$ or $NaNO_3$, for the study of the effect of the addition of metal salt,

(iii) 20 m*M* CREA, 20 m*M* $NaNO_3$, 0.5 m*M* CTAB without NICO or with 4 m*M* NICO, pH 5.5, for the study of the influence of the chromophore.

The electrolytes were prepared fresh daily by dissolving appropriate amounts of reagents in water purified with a Milli-Q system (Millipore, Saint Quentin, France), filtered before use through a 0.45-μm filter (Millipore) and degassed.

pH was adjusted, when necessary, with NaOH or HNO_3 solutions depending whether the electrolyte contains nicotinate. In this work, it was assumed, considering the acidity constants (Table 1), that the monovalent anions were the only chemical forms of the (hydroxy)carboxylic acids in solution either at pH 5.5 or 6.2.

Stock solutions of individual (hydroxy)carboxylic acids were prepared at 5 m*M* with the exception of benzoic acid (1 m*M*). Samples containing either a single organic acid or a mixture of organic acids at 0.2 or 0.4 m*M* were made by dilution of these stock solutions.

Prior to the first use, the capillary was rinsed with methanol (30 min), then with 1 *M* NaOH (30 min) and water (30 min) and finally with the electrolyte (at least 30 min). Between two consecutive runs, the capillary has been rinsed for 2 min with the appropriate electrolyte, except when varying the concentration of CTAB where the capillary was rinsed with 1 *M* NaOH (30 min) and water (30 min) and equilibrated with the electrolyte (180 min).

2.4. Calculations

Electroosmotic mobility, m_{eo}, was determined by the measurement of the migration time of water, t_{eo}, according to the following expression:

$$m_{eo} = \frac{L_t L_d}{V t_{eo}} \quad (1)$$

where L_t is the total capillary length, L_d is the capillary length from the injection inlet to the detector and V is the applied voltage.

Similarly, the observed mobility of a given organic anion, m_{obs}, was calculated from the migration

Table 1
Acidity constants of the organic acids from the literature [29]

Acidity constant	GLYCO	GLYCE	THREO	BENZO	PROTO	GAL
pK_{a_1}	3.83[a]	3.54[b]	n.a.	4.20[a]	4.32[c]	4.26[d]
pK_{a_2}					8.83[c]	8.70[d]
pK_{a_3}					11.70[c]	11.45[d]

n.a., not available.
[a] $I=0$, $T=25$ °C.
[b] $I=2.0$, $T=25$ °C.
[c] $I=0.2$, $T=25$ °C.
[d] $I=0.1$, $T=20$ °C, where I stands for ionic strength and T for temperature.

time of this anion, t, according to the following relation:

$$m_{obs} = \frac{L_t L_d}{Vt} \quad (2)$$

The electroosmotic and observed mobilities were directed towards the anode and were considered as positive values. The mobilities reported in this work represent the means of at least two consecutive measurements.

3. Results and discussion

3.1. Effect of hexadecyltrimethylammonium bromide concentration

3.1.1. Effect on the electroosmotic mobility

The effect of CTAB concentration on the magnitude of the electroosmotic flow was examined. Fig. 1 shows the variations of the electroosmotic mobility with increasing CTAB concentrations ranging from 0.1 to 0.5 m*M*. A reversal of the electroosmotic flow was obtained even for the lowest CTAB concentrations. The electroosmotic mobility increases when varying the CTAB concentration from 0.1 to ca. 0.2 m*M* while it remains constant for higher CTAB concentrations. The increase in the electroosmotic mobility is probably the result of an increase in the positive charge of the capillary walls which might be induced by a growing adsorption of the surfactant. The existence of a plateau from a CTAB concentration of 0.2 m*M* suggests that the charge of the capillary surface remains unchanged even in the presence of increasing concentrations of CTAB, which allows to assume that no further cationic surfactant adsorbs onto the capillary walls. In order to check whether this plateau might be attributed, as shown by other studies [14,30], to the formation of micelles, the determination of the CMC of CTAB was performed in the electrolyte used for capillary electrophoretic measurements reported in Fig. 1. For that purpose, the procedure proposed by Cifuentes et al. [31] was used. This approach is based on the measurement of the current, i, generated by the electrolyte containing increasing amounts of CTAB under the application of a constant voltage. As the conductivity of the surfactant depends on its aggregation state (monomer or micelle), the plot of the electrical current values versus the surfactant concentration must fit into two straight lines, the abscissa of their intersection point corresponding to the CMC of the surfactant. Fig. 2 shows the values of the current as a function of the surfactant concentration ranging from 0.05 to 1.20 m*M*. As expected, two fitting lines with different slopes were found, with an intersection point around 0.6 m*M* (for comparison, the CMC values of CTAB in different background electrolytes are listed in Table 2). Even if the CMC cannot be determined accurately from Fig. 2 because of a data dispersion due to relatively low current variations, it appears that the CMC of CTAB is much greater than 0.2 m*M*. Consequently, the existence of the plateau in Fig. 1 cannot be

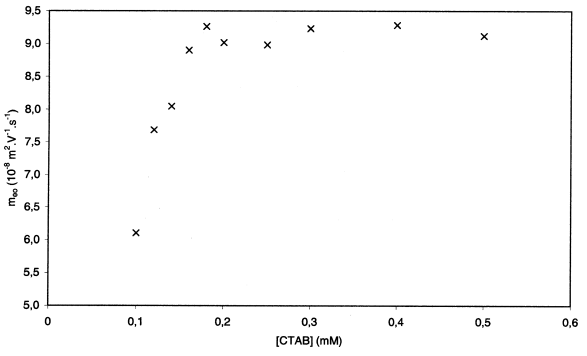

Fig. 1. Effect of CTAB concentration on the electroosmotic mobility measured in an electrolyte containing 20 m*M* CREA and 4 m*M* NICO, pH 5.5.

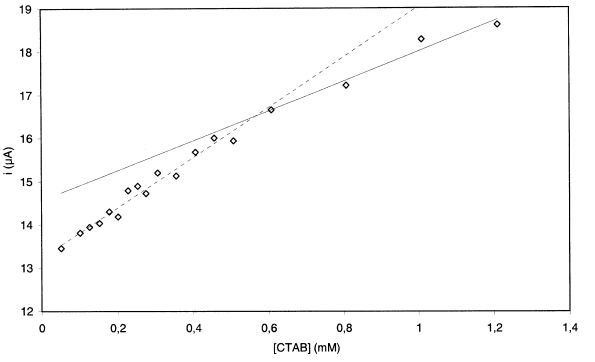

Fig. 2. Effect of CTAB concentration on the electrical current measured in an electrolyte containing 20 m*M* CREA and 4 m*M* NICO, pH 5.5.

Table 2
Critical micellar concentrations (CMC) of hexadecyltrimethylammonium bromide, in different buffers, from the literature

Buffer	CMC (m*M*)
Water	0.93[a]
10 m*M* H_3BO_3, 10 m*M* Na_2BO_3, pH 9.0	0.75[b]
16 m*M* HCl, 16 m*M* Tris, pH 7.0	0.40[b]
10 m*M* CH_3COOH, 10 m*M* $NaCH_3COO$, pH 4.6	0.37[b]
10 m*M* K_2HPO_4, *I* adjusted to 50 m*M* with KCl, pH 9.0	0.15[c]
10 m*M* K_2HPO_4, *I* adjusted to 50 m*M* with KCl, pH 3.5	0.17[c]

[a] $T=25$ °C [31].
[b] $T=22.5$ °C [17].
[c] T not determined. pH adjusted with H_3PO_4 or KOH [14].

correlated with the formation of micelles and might simply result from the saturation of the capillary walls with the surfactant.

3.1.2. Effect on the migration of the carboxylate anions

The effect of CTAB concentration on the migration of the organic anions was also investigated. Fig. 3 shows the influence of CTAB concentration on the differences between observed and electroosmotic mobilities, $m_{obs}-m_{eo}$, of the aliphatic anions. The mobility difference of all these anions follows the same trend, i.e. it decreases as the CTAB concentration increases from 0.1 to 0.2 m*M* and remains constant with a further increase in the CTAB concentration. The occurrence of an interaction in solution between the monomeric surfactant and the aliphatic anions seems to be highly improbable as it would lead, in the absence of micelles, to a decrease in the mobility differences on the whole range of

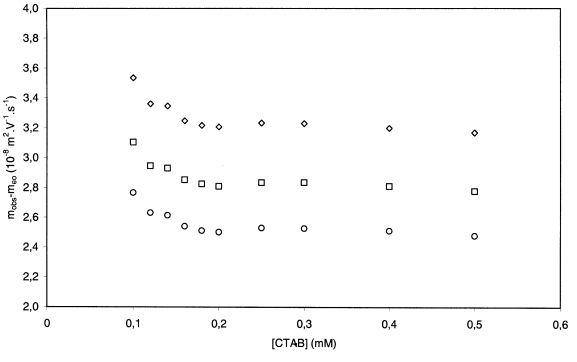

Fig. 3. Effect of CTAB concentration on the mobility differences of glycolate (◇), glycerate (□) and threonate (○), measured in an electrolyte containing 20 m*M* CREA and 4 m*M* NICO, pH 5.5.

CTAB concentrations and not only between 0 and 0.2 m*M*. The profile of the mobility differences observed in Fig. 3 is consistent with the quantity of CTAB adsorbed onto the capillary walls. It might then result from a possible retention of the aliphatic anions through interactions with the adsorbed cationic surfactant. Because of this retention, only a fraction of the aliphatic anion is present in the mobile phase. Its observed mobility can thus be expressed using the following equation:

$$m_{obs} = x_A(m_{ep,A} + m_{eo}) \quad (3)$$

where x_A represents the fraction of unretained anion and $m_{ep,A}$ the electrophoretic mobility of the anion in the absence of retention.

The following relation can be deduced from Eq. (3) for the observed mobility of an anion subject to retention:

$$m_{obs} = \frac{m_{ep,A} + m_{eo}}{1 + k'} \quad (4)$$

where k' represents the capacity factor which corresponds to the ratio of quantities of organic anion in the stationary (capillary wall) and mobile (electrolyte) phases, as described in chromatography.

As a consequence, the difference between the observed and the electroosmotic mobilities, $m_{obs}-m_{eo}$, which is a constant without any retention, depends, in the presence of retention, on the capacity factor and on the electroosmotic mobility, two parameters which vary for CTAB concentrations up to 0.2 m*M*.

The same experiment was performed for aromatic anions. A similar trend was observed for CTAB

concentrations ranging from 0.1 to 0.2 m*M*, i.e. a decrease in the mobility differences which was however more pronounced than the one obtained for aliphatic anions and was attributed to the retention of these anions. Nevertheless, no conclusion could have been drawn from the results obtained for CTAB concentrations greater than 0.2 m*M* because of detection problems that arose from these CTAB concentrations and for which no explanation was found at that time.

3.2. Effect of metal salt concentration

3.2.1. Effect on the electroosmotic mobility

The influence of metal salt on the electroosmotic mobility was studied for two CTAB concentrations: 0.1 and 0.5 m*M*. This corresponds, according to the results presented above, to a partial and a total coverage of the capillary surface, respectively. The variations of the electroosmotic mobility were studied as a function of the metal salt $Cd(NO_3)_2$. The electroosmotic mobilities plotted versus the nitrate concentration are shown in Figs. 4 and 5 for CTAB concentrations of 0.1 and 0.5 m*M*, respectively. The influence of metal salt concentration on the electroosmotic flow depends strongly on the CTAB concentration. For 0.1 m*M* of CTAB in the electrolyte, the electroosmotic mobility first increases for concentrations of $Cd(NO_3)_2$ ranging from 0 to ca. 3 m*M* and then decreases as the concentration of $Cd(NO_3)_2$ further increases. With a CTAB concentration of 0.5 m*M*, an increase in $Cd(NO_3)_2$ concentration leads to a decrease in the reversed electroosmotic flow, similarly to what is observed with metal salt on normal electroosmotic flow [32–34]. This decrease in the reversed flow was also observed on the one hand by Tavares et al. [16] with increasing concentrations of either chromate as its potassium salt or 3,5-dinitrobenzoate in the presence of 0.115 m*M* CTAB in the electrolyte and on the other hand by Dworschak and Pyell [17] with the addition of metal salts such as potassium chloride or calcium chloride in an electrolyte containing 40 m*M* tetradecyltrimethylammonium bromide. In the first case, this decrease was assumed to result, as for normal electroosmotic flow, from the simultaneous effect of a compression of the double layer thickness at the interface solution–surface and an adsorption of anions onto the capillary walls through interactions with hemimicelles. The explanation proposed in the second case involved a competitive adsorption between the cationic surfactant and the metal cation. In our work, an experiment performed with $NaNO_3$ as metal salt gave the same variations of the electroosmotic mobility as those observed with $Cd(NO_3)_2$ (Fig. 5). Hence, the nature of the metal cation seems to have no influence on the electroosmotic mobility, which allows to suppose that the decrease in the electroosmotic mobility with increasing metal salt concentrations involves the nitrate anions. This was reinforced by another experiment carried out by varying the cadmium concentration while keeping

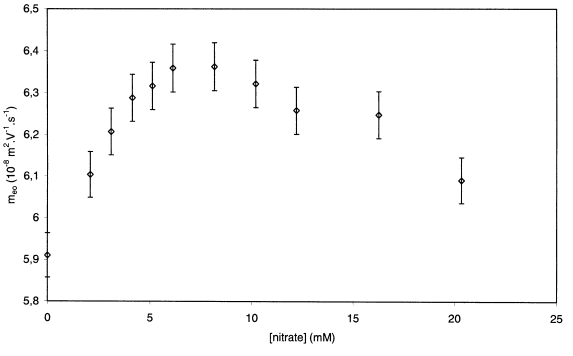

Fig. 4. Effect of $Cd(NO_3)_2$ concentration on the electroosmotic mobility measured in an electrolyte containing 0.1 m*M* CTAB, 20 m*M* CREA and 4 m*M* NICO, pH 6.2.

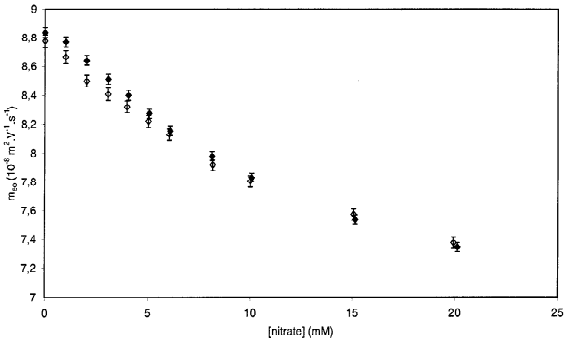

Fig. 5. Effect of $Cd(NO_3)_2$ (◇) or $NaNO_3$ (♦) concentration on the electroosmotic mobility measured in an electrolyte containing 0.5 m*M* CTAB, 20 m*M* CREA and 4 m*M* NICO, pH 6.2.

the nitrate concentration at a constant value of 20 m*M* by means of appropriate proportions of $NaNO_3$ and $Cd(NO_3)_2$. Maintaining constant the nitrate concentration led to a constant electroosmotic mobility (results not shown). Consequently, the hypothesis proposed by Tavares et al. which would involve an adsorption of the nitrate ions is likely to occur.

The behaviour of the electroosmotic mobility in the presence of 0.1 m*M* CTAB in the electrolyte suggests the occurrence of at least two phenomena. The increase in the electroosmotic mobility means that the positive charge of the capillary surface becomes more important, which could be explained by a possible adsorption of the metal cation Cd^{2+}. This adsorption requires the existence of dissociated silanol groups at the capillary surface, which is consistent with a partial coverage of the capillary with the surfactant and favours the hypothesis that the cationic surfactant adsorbs onto the capillary walls through the single transition adsorption isotherm depicted by Lucy and Underhill [14]. Concerning the decrease in the electroosmotic mobility from a $Cd(NO_3)_2$ of 3 m*M*, it could be induced by a predominant effect of the nitrate ions as observed when using CTAB 0.5 m*M*.

3.2.2. Effect on the migration of the carboxylate anions

The influence of the metal salt concentration on the migration of the organic anions was also investigated with the aim of checking whether the adsorbed cationic surfactant acts like an anion exchanger, as suggested by the results of the study of the influence of the CTAB concentration (Section 3.1.2). For that purpose, sodium nitrate was chosen rather than cadmium nitrate as the sodium cation is supposed to have a negligible interaction with these organic anions.

The differences between the observed and electroosmotic mobilities, $m_{obs} - m_{eo}$, of the organic anions as a function of the sodium nitrate concentration are shown in Fig. 6 for a CTAB concentration of 0.5 m*M* in the electrolyte. It is noticeable that benzoate was not detected in the absence of sodium nitrate and in the presence of sodium nitrate concentrations of 15 and 20 m*M*.

For all the organic anions with the exception of glycolate, the mobility differences increase with increasing sodium nitrate concentrations in the electrolyte, while the mobility differences related to glycolate remain constant. The increase in the mobility differences might be due to a decrease in

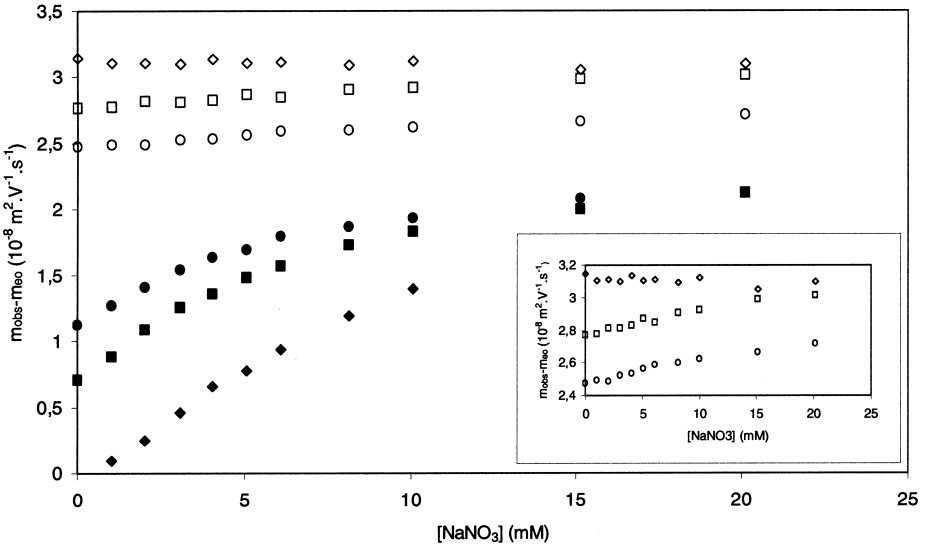

Fig. 6. Effect of sodium nitrate concentration on the mobility differences of the glycolic (◇), glyceric (□), threonic (○), gallic (●), protocatechuic (■) and benzoic (♦) anions, measured in an electrolyte containing 0.5 m*M* CTAB, 20 m*M* CREA and 4 m*M* NICO, pH 6.2.

the retention of the organic anions resulting from a competitive adsorption between the organic anions and the nitrate ions. These results show that the adsorbed cationic surfactant seems to actually behave like an anion exchanger and that the retention involves electrostatic interactions.

The main difference between aliphatic and aromatic anions is the magnitude of the mobility variations induced by the addition of sodium nitrate. Indeed, the mobility variations are much greater for the aromatic anions, which allows to suppose that additional hydrophobic interactions might be involved in the retention of these aromatic anions.

For glycolate, assuming that the mobility difference is equal to the electrophoretic mobility of the free anion is inconsistent with the existence of retention of this anion shown in Section 3.1.2. It can however be assumed that the retention of glycolate is not affected by the presence of nitrate ions.

Separation efficiencies calculated for different sodium nitrate concentrations are summarized in Table 3. Protocatechuic and gallic acids give increasing efficiencies with growing sodium nitrate concentrations, which supports the hypothesis of a decreasing retention of these acids in the presence of sodium nitrate. The efficiencies relative to the aliphatic acids show little variations that cannot, however, be correlated with any changes in the magnitude of retention with the addition of sodium nitrate.

The former results were compared to those obtained with a CTAB concentration of 0.1 m*M*. Table 4 gives the differences between the observed and electroosmotic mobilities of the organic anions in the absence and in the presence of sodium nitrate (5 and 10 m*M*).

Table 3
Separation efficiencies[a] (10^5 plates m^{-1}) in the absence of $NaNO_3$ and in the presence of $NaNO_3$ concentrations of 10 and 20 m*M*

	$NaNO_3$ concentration (m*M*)		
	0	10	20
GLYCO	0.77	0.59	1.26
GLYCE	2.43	1.36	1.70
THREO	3.48	3.44	3.53
PROTO	0.28	0.85	1.56
GAL	0.21	1.20	1.39

Electrolyte, 0.5 m*M* CTAB, 20 m*M* CREA and 4 m*M* NICO, pH 6.2.

[a] Separation efficiencies were not calculated for benzoic acid since it was not detected in the absence and in the presence of 20 m*M* of $NaNO_3$.

Table 4
Mobility differences, $m_{obs} - m_{eo}$, of the organic anions (10^{-8} m^2 V^{-1} s^{-1}) as a function of the $NaNO_3$ concentration, measured in an electrolyte containing 0.1 m*M* CTAB, 20 m*M* CREA and 4 m*M* NICO, pH 5.5

	$NaNO_3$ concentration (m*M*)		
	0	5	10
GLYCO	3.53±0.01	3.43±0.02	3.25±0.03
GLYCE	3.11±0.01	3.12±0.01	3.12±0.01
THREO	2.78±0.01	2.76±0.01	2.77±0.02
BENZO	2.19±0.01	2.23±0.01	2.37±0.01
PROTO	2.01±0.01	2.06±0.01	2.13±0.01
GAL	2.03±0.01	2.05±0.01	2.09±0.02

For all the anions except glycolate, the variations of the mobility differences are consistent with a smaller retention of these anions which might result not only in a lower quantity of adsorbed cationic surfactant but also in possible electrostatic repulsion between the organic anions and the dissociated silanol groups.

These results highlight once again the unusual behaviour of the glycolate anion. The decrease in its mobility differences shows that its retention is favoured in the presence of sodium nitrate, which could be due to a possible screening of the negative charges at the capillary wall by the sodium cations. However it seems to be clear that the retention of glycolate involves the cationic surfactant as, in the absence of sodium nitrate, the mobility difference obtained for a CTAB concentration of 0.5 m*M* ($3.15\pm0.01\times10^{-8}$ m^2 V^{-1} s^{-1}) is lower than the value obtained for a CTAB concentration of 0.1 m*M* ($3.53\pm0.01\times10^{-8}$ m^2 V^{-1} s^{-1}).

3.3. Influence of the chromophore

To further elucidate the unusual behaviour of glycolate, the study of the influence of another variable, the nicotinate concentration, on the migration of the organic anions was carried out. Table 5 compares the mobility differences of four organic anions obtained with and without nicotinate in the electrolyte. In the absence of nicotinate, the anions

Table 5
Mobility differences, $m_{obs} - m_{eo}$, of the organic anions (10^{-8} m^2 V^{-1} s^{-1}) as a function of the nicotinate concentration, measured in an electrolyte containing 0.5 m*M* CTAB, 20 m*M* CREA and 20 m*M* $NaNO_3$, pH 5.5

	Nicotinate concentration (m*M*)	
	0	4
GLYCO	3.72±0.01	3.10±0.02
GLYCE	3.15±0.02	3.02±0.01
THREO	2.75±0.02	2.72±0.01
PROTO	2.05±0.01	2.13±0.01

were directly detected at 214 nm for GLYCO, GLYCE and THREO and at 254 nm for PROTO. The addition of nicotinate in the electrolyte decreases the mobility differences of glycolate while the values are closer or similar for the other organic anions, which would suggest that the predominant mechanism for the retention of glycolate involves the nicotinate anion.

In this work, it was demonstrated that the migration of the (hydroxy)carboxylate anions combined both electrophoretic and chromatographic phenomena. The retention of these anions was shown to involve electrostatic interactions with the cationic surfactant adsorbed at the capillary walls and possible hydrophobic interactions which seem to be important for the aromatic anions. Another mechanism involving the electroosmotic flow modifier and the chromophore appeared to be predominant for the retention of glycolate. Further experiments will be necessary for a better understanding of this mechanism.

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