

Electrophoretic mobilities in nonaqueous capillary electrophoresis

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Abstract. In nonaqueous capillary zone electrophoresis separation is based on the interactions between the analytes and the ionic additive present in the separation medium. The choice of the solvents and solvent mixtures can influence the acid–base behaviour and solvation of the analytes and the additives; in addition, it can influence the electroosmotic flow of the separation medium and the intrinsic mobility of the analytes. Large changes in the separation factor and/or resolution, analysis time, and selectivity can be achieved by varying the type and content of the organic solvent in the medium. A simple and reproducible method in the case of which 1-alkyl-3-methylimidazolium based organic salts are used as additives in the separation medium is suggested. Complexes are formed with the anionic part of the ionic liquid additive. This is the case with Brønsted acids and phenolic compounds.

Key words: nonaqueous electrophoresis, ionic liquids, 1-alkyl-3-methylimidazolium salts.

INTRODUCTION

There seems to be no reason to consider water the only useful solvent for capillary electrophoresis (CE). The same laws govern CE separations in aqueous and nonaqueous media; therefore water and the organic solvents can be treated equally. The nonaqueous capillary electrophoresis (NACE), which is based on the use of the electrolyte solutions prepared from pure organic solvents or their mixtures, is extending the applicability of capillary electrophoresis [1, 2]. The advantages of nonaqueous solvents in CE can be discussed from various viewpoints. First of all organic solvents can solubilize organic compounds hardly soluble in water and thus afford a possibility of separating such compounds by CE. Other attractive features of organic solvents are that they greatly differ in physical and chemical properties between themselves and from water and offer large

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changes in the separation factor and/or resolution, analysis time, and selectivity, and they reduce electrophoretic currents. Accordingly, changing the organic solvents or varying the proportions of two solvents allows a simple selective tuning in NACE [3, 4]. Organic solvents have been applied in CE since the early 1980s [5, 6]. However, their wider use in CE as background electrolytes has begun only recently.

Electroosmotic flow (EOF) results from the non-uniform distribution of charge in the vicinity of the capillary wall. Fused silica, the most widely used capillary material in CE, carries dissociable silanol groups on the surface, and the fused silica surface can acquire a positive charge. Dissociation of the silanol groups can take place in solvents without the addition of ionic species if the solvent molecules are able to accept protons. Additionally, the absorption of buffer ions can also contribute to the net charge of the capillary surface. Thus EOF will occur in numerous solvents in CE without the addition of ionic species. The phenomenon of electroosmosis is common to both NACE and aqueous CE. An EOF is present in all organic solvents with high dielectric constants. In aqueous CE, the dependence of electroosmotic mobility on the buffer composition and pH is well known and the EOF is recognized as an important parameter in method development and optimization. However, only a few studies of the EOF of organic solvents without a supporting electrolyte have been performed so far [7].

For efficient and reproducible separation and for charging the analyte a proper modification of the separation medium with ionic species is needed. Room-temperature molten salts, the so-called ionic liquids, are one of the possible electrolyte additives. In addition to their environmentally benign properties, they show a wide range of physicochemical properties, depending on the anionic or cationic part of the salt [8]. The novelty of ionic liquids is expressed in chemical tunability, which means that it is very easy to prepare ionic liquids with the same cationic part but with a different anionic part without significantly changing their physical properties. The chemical properties, in turn, are more dependent on the anionic part. The room-temperature molten salts based on dialkylimidazolium cation are good candidates for EOF modifiers in organic solvents because of their good stability in air and in water, and miscibility with most solvents used in NACE. The first example of separation by using that kind of salts as an electrolyte component was demonstrated in [9] where water insoluble dyes were separated in acetonitrile with added 1-butyl-3-methylimidazolium fluoroacetate (3.8 mg mL^{-1}). Another application of ionic liquids but in aqueous CE was presented by Stalcup et al. [10]; they dynamically modified the surface of the capillary in order to reverse the EOF.

In a situation where a nonaqueous electrophoretic buffer contains a deprotonated acid, the resulting anion may form a heteroconjugate with an undissociated acidic analyte, thus imparting a negative charge to an analyte that would otherwise remain uncharged. The heteroconjugation between Brønsted acids (phenols, carboxylic acids, and alcohols) and small inorganic anions has been explained as a separation mechanism in acetonitrile [11]. In a weak acid (HA) solution the following reaction takes place: $n\text{RH} + \text{A}^- \rightleftharpoons (\text{RH})_n \text{A}^-$, where $n = 1, 2, \dots$ and

RH is a weaker acid than HA. The formation of homo- and heteroconjugates does not occur in protic solvents because the degree of hydrogen bonding in these solvents prevents the association of the dissolved species with one another. Accordingly, NACE may also be a useful method to study the heteroconjugation of Brønsted acids with anions of organic salts in solution [12].

In this study, several 1,3-dialkylimidazolium based salts were used. The aim was to identify a suitable background electrolyte composition based on the use of these salts in different organic solvents and to investigate the effect of a background electrolyte on the EOF, as well as the actual mobilities of analytes using these background electrolytes.

EXPERIMENTAL

CE separations were performed using an ISCO CV4 electropherograph with a UV detector coupled to a personal computer and controlled by in-house written software. All experiments were conducted with an applied voltage of 18 kV with a positive potential at the injector end, which means that the EOF in these nonaqueous electrolyte systems was always cathodic (running from the anode to the cathode). The separation was monitored at 210 nm. All samples were injected using a hydrodynamic injection mode. The duration of an injection was 3 s.

An uncoated capillary (Polymicro Technologies, Phoenix, USA) with dimensions 75 cm \times 50 μ m (effective length 50 cm to the detector) was used throughout the study. Before use, the capillary was rinsed with 1 M sodium hydroxide, water, and methanol, and then with acetonitrile and the separation medium, 10 capillary volumes of each. Between analyses the capillary was washed with a solvent and then with a one-capillary volume of the separation medium. The EOF marker was benzene for every separation medium used.

In this study 1-butyl-3-methylimidazolium (BMIm) and 1-octyl-3-methylimidazolium (OctMIm) based ionic liquids with different anionic parts, which are air- and water-stable and miscible with most CE solvents (hexafluorophosphate – [PF₆]⁻, tetrafluoroborate – [BF₄]⁻, trifluoroacetate – [CF₃COO]⁻, heptafluorobutanoate – [C₃F₇COO]⁻), were used as additives in separation media. These were prepared at the Department of Chemistry at Tallinn Technical University following the procedure described elsewhere [13]. The starting materials were obtained from Aldrich, Germany. All standards, analytes, and solvents were of chromatographic grade and were obtained from Sigma-Aldrich, Germany.

RESULTS AND DISCUSSION

EOF in pure solvents

EOF is a phenomenon generic to capillary electrophoretic separations. Pure solvents have an EOF in the capillary when voltage is applied. It is very characteristic of the solvent and differs between solvents. Only inert solvents

Table 1. Electrophoretic mobility (μ_{eo}) measured in pure solvents at 20°C and some physical parameters taken from the literature [14] for these solvents

Solvent	$\mu_{eo};$ $m^2 V^{-1} s^{-1} 10^{-9}$	ϵ	pK_a	Viscosity, mPs
Acetone	8.9	20.56	32.50	0.33
Acetonitrile	20.5	35.9	35.00	0.34
DMF	3.3	36.71	28.00	0.80
DMSO	1.8	46.45	33.30	1.99
Ethanol	1.9	24.55	18.90	1.08
Methanol	3.2	32.66	17.20	0.55
Water	7.7	78.3	14.00	1.00
Propylene carbonate	4.2	64.92	n.d.	2.53
<i>n</i> -Propanol	0.1	20.45	19.40	1.94

n.d. – no data.

such as chloroform and *n*-hexane do not have EOF. Table 1 presents the EOF values measured in this study at 20°C. The EOF in all these nonaqueous electrolyte systems was directed from the anode to the cathode, which was at the detector end. For the generation of this cathodic EOF the silica surface in solvents must have a net negative charge. The generation of the net charge of the capillary wall is different in amphiprotic solvents, which have the ability to both accept and donate a proton, and dipolar-aprotic solvents, which can accept a proton, but cannot donate one. All this causes different structures of the double layer in amphiprotic and aprotic solvents.

The values of electrophoretic mobility (μ_{eo}) in different solvents varied in a scale as broad as about 2 orders of magnitude. The development of the double layer in aprotic solvents, such as acetonitrile (ACN), is a result of the dissociation of the surface silanol groups. The solvent molecules accept the resulting H^+ ions. In pure aprotic solvents the negative charge of the fused silica surface is solely balanced by cations. If no supporting electrolyte is present in ACN, the negative charge of the fused silica surface is balanced by a low number of cations (protonated ACN molecules), which leads to lower shielding of the negative surface potential [7]. In amphiprotic solvents, such as water, the surface charge is counterbalanced by both cations and anions, the ionic strength is greater, and the double layer is more compact, which results in a slower EOF.

Effect of solvent mixtures

In the present work the mobility of EOF was studied both in the mixtures of MeOH and ACN. At the same time, the viscosities of the given solvents were measured as well. In Table 2 an example of the measured mobilities of EOF in a mixture of ACN and methanol is given together with the respective viscosities; also calculated values of the dielectric constant (ϵ) (based on measured data) and viscosity values taken from the literature [14] are presented.

As shown in Table 2 the electrophoretic mobility of EOF decreases rapidly with the increase of the added MeOH amount. It could be expected because the physical parameters of the mixture (dielectric constant, viscosity), which are important to develop EOF in media, are changing. The decreased viscosity and dielectric constant of the media containing higher amounts of methanol should lead to a decrease of EOF. However, the 150-fold decrease in the EOF mobility cannot be related to the viscosity change of the mixture alone. Mixing of solvents with different proton accepting and/or donating properties affects the interactions of the separation media with the surface of the capillary that are important for the development of EOF in the system. Acetonitrile, which has a stronger proton acceptor ability than donor ability (the latter is negligible), causes stronger deprotonation of the silanol groups than MeOH. Changed EOF mobilities were also observed when the organic solvents were added to an aqueous buffer, with the EOF decreasing more with the addition of methanol than with the addition of ACN [15]. All this means that in the separation media various concurrent processes are taking place, which result in nonlinear dependence of flow mobility change according to mixture composition. This is illustrated in Fig. 1, where curves for three different mixtures of solvents without ionic additives are presented.

Table 2. Electrophoretic mobilities (μ_{eo}) and viscosities in different solvent systems. The average relative span is 0.2%

	ACN 100	ACN:MeOH 75:25	ACN:MeOH 50:50	ACN:MeOH 25:75	MeOH 100
Viscosity, mPs [14]	0.345	0.335	0.370	0.433	0.544
Viscosity, mPs (measured)	0.346	0.333	0.367	0.422	0.524
μ_{eo} , $m^2 V^{-1} s^{-1} 10^{-9}$	89	19.6	14.5	10.4	0.6
ϵ , calculated	35.9	35.1	34.3	33.5	32.7

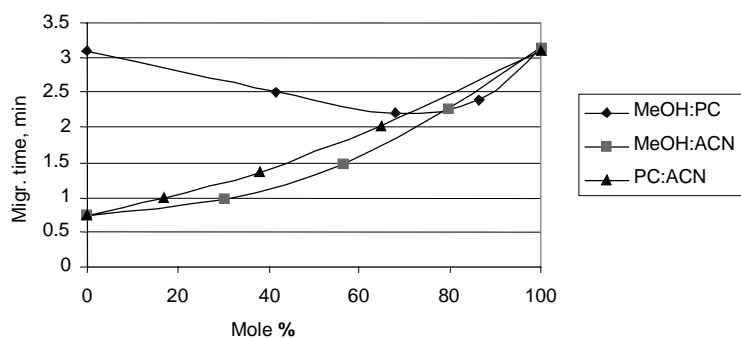


Fig. 1. Electroosmotic flow in binary solvent mixtures.

Effect of salt

The development of EOF in the solvent is in most cases not enough for the electrophoretic separation of analytes in the medium because the analytes remain electrically neutral in a pure solvent. It was clearly seen in experiments with pure ACN that the solvent alone was not able to charge the phenolic compounds and so they remained uncharged. Room-temperature molten salts were chosen as the solvent additives to get electrophoretic separation of analytes. As expected, the adding of salt to a pure solvent changed the EOF of the system. Figure 2 illustrates the changes. Their extent was the greatest in ACN and acetone, and smaller in water and methanol. It must be mentioned that the direction of EOF in all these nonaqueous electrolyte systems remained unchanged and was directed from the anode to the cathode that was on the detector end. We explored various BMIm salts for their efficacy. The salts with the same cation but a different anion did not show great difference. This points to the influence of the salt cation on the formation of EOF in the separation media.

Fast and complete separation was achieved for phenolic compounds in acetonitrile when BMIm heptafluorobutanoate (2 mg mL^{-1}) was added to a background electrolyte. The separations were achieved in less than 10 min. The elution order did not correspond to the charge-to-mass ratio, which is a direct indication of a complicated mechanism of analyte charging. From discussions based on earlier experiments [16] it was proposed that heteroconjugation is responsible for this. A different elution order is expected since the heteroconjugation properties of an analyte are determined primarily by its ability to interact with the dissociated background electrolyte components, which, in turn, is linked to the number and position of functional groups on the analyte molecule (Fig. 3).

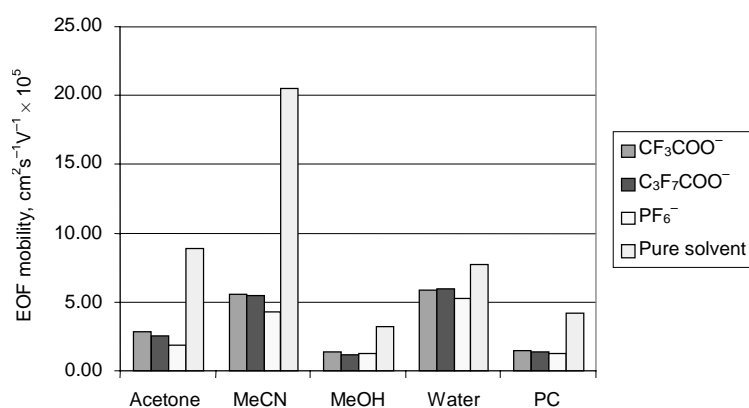


Fig. 2. Influence of added organic salt on the electroosmotic flow of the system. The concentration of added salts (1-butyl-3-methylimidazolium based ionic liquids with different anionic parts: hexafluorophosphate – $[\text{PF}_6]^-$, trifluoroacetate – $[\text{CF}_3\text{COO}]^-$, heptafluorobutanoate – $[\text{C}_3\text{F}_7\text{COO}]^-$) is kept at 5.1 mM.

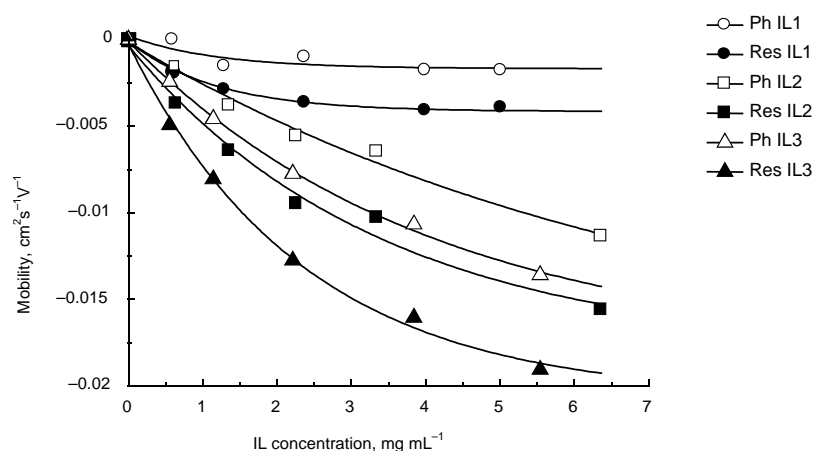


Fig. 3. Separation of phenols (Ph – phenol and Res – resorcinol) with three different ionic liquids (IL1 – [BMIm]⁺ [BF₄]⁻; IL2 – [BMIm]⁺ [C₃F₇COO]⁻; IL3 – [BMIm]⁺ [CF₃COO]⁻) as background electrolytes in acetonitrile.

Effect of the concentration of ionic liquids on mobility

There are only a few systematic studies on the influence of salt concentration on EOF in NACE [17, 18]. The influence of salt concentration on the EOF mobility and on the viscosity of the solvent was studied by the example of ACN. The results are presented in Table 3. The viscosity measurements revealed no major change in the solvent viscosity (however, a slight increase in the viscosity can be observed as expected in this range of salt concentrations). The change in the EOF mobility is quite significant: 2.5-fold over the concentration range tested. As the change in the viscosity is negligible, the main factor responsible for slower EOF mobility is obviously the increase of the ionic strength of the medium. The increase in the ionic strength of the medium results in a decrease in the thickness of the diffuse double layer at the capillary inner wall. As a consequence, the zeta potential of the capillary wall and hence the mobility of EOF are decreasing.

The measurements showed that the size of the salt cation influences the mobility of EOF to a certain extent: when [BMIm]⁺[PF₆]⁻ was used as the elektro-

Table 3. The influence of ionic liquids on the viscosity (η) of the separation medium and the mobility (μ_{eo}) of EOF. The average span of measurements was 0.2%

Concentration of [BF ₄] ⁻ , mM	η , mPs	μ_{eo} , m ² V ⁻¹ s ⁻¹ 10 ⁻⁹
0	0.345	89
2.6	0.350	90
5.7	0.351	72
10.4	0.354	55
22	0.362	35

lyte in ACN, the mobility of EOF was 15% higher than in the case of $[\text{OctMim}]^+[\text{PF}_6]^-$. With the bigger cation the decrease in the thickness of the double layer was not so effective and the decrease in EOF was less compared to salts with a smaller cation at the same concentration.

In the case of solvent mixtures BMIm salts decrease the EOF as illustrated by Fig. 4. Already very small concentrations have a great influence, but starting from a concentration of 0.005 mM of the salt there is no change in the EOF any more. However, in the case of mixtures of different solvents the difference is levelled. As the EOF direction remains unchanged, the shielding of the negative surface potential by salt cations is greater than by methanol, although the ionic strength is greater and the double layer is more compact in the latter case.

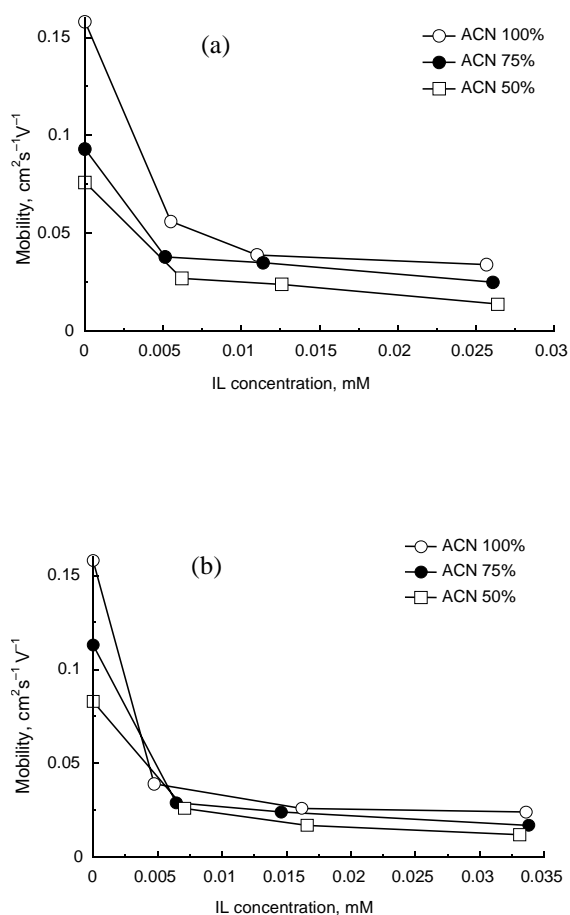


Fig. 4. Electroosmotic flow in the ACN–MeOH mixture in the case of two ionic liquids (IL): (a) – $[\text{BMIm}]^+ [\text{C}_3\text{F}_7\text{COO}]^-$ and (b) – $[\text{BMIm}]^+ [\text{CF}_3\text{COO}]^-$.

CONCLUSIONS

Organic solvents are widening applications of capillary electrophoresis, and alkyylimidazolium based salts – ionic liquids – can be successfully used as background electrolyte components. Electrolytes of this type are good for the separation of phenolic compounds soluble in acetonitrile.

Changing the composition of the solvent mixture and the salt concentration makes it possible to change the electroosmotic flow of the system to a great extent. The main mechanism of separation in acetonitrile is heteroconjugation between the salt anion and the analyte molecule, which enables to separate non-dissociating molecules electrophoretically.

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Elektroforeetilised liikuvused mittevesikeskkonnalises kapillaarelektroforeesis

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Mittevesikeskkonnalisel kapillaarelektroforeesil põhineb lahutumine kas analüüdi dissotsieerumata molekuli ja lahutuskeskkonnas sisalduva ioonse lisandi vahelistel interaktsioonidel või sarnaselt vesikeskkonnaga analüüdi dissotsiatsioonil. Solventide ja nende segude valikuga on võimalik mõjutada happe–aluse tasakaalu ja analüütide solvatatsiooni, mille tulemusel muutub lahutuskeskkonna elektroosmootne voog ja analüütide iseloomulik elektroforeetiline liikuvus. Orgaaniliste lahustite valikust ja nende sisaldusest lahutuskeskkonnas on tingitud suured muutused analüütide lahutuvuses, migratsiooniajas ja lahutamise selektiivsuses.

Meie poolt on välja töötatud lihtne ja reprodutseeritav meetod nõrgalt happeliste analüütide lahutamiseks, kasutades taustelektrolüüdina 1-alküül-3-metüül-imidasooliumkatiooni baasil sünteesitud erinevate anioonidega orgaanilisi soolaid. Lahutamine põhineb nimetatud soolade aniooni ja dissotsieerumata analüüdi molekuli vahel tekkiva negatiivselt laetud kompleksi (heterokonjugaadi) moodustumisel. See fenomen kehtib Brønsted'i hapete (fenoolid, karboksüülhapped ja alkoholid) puhul.