Application of 1-alkyl-3-methylimidazolium-based ionic liquids in non-aqueous capillary electrophoresis

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Abstract

In many cases salts, which are liquid at room temperature show a better solubility in organic solvents, and can be used in nonaqueous capillary zone electrophoresis as ionic additives. In this study 1-alkyl-3-methylimidasolium-based ionic liquids were used as additives in separation media to assess the interactions between the analytes and the ionic additive present and to find an influence of the type and concentration of the ionic additive, also the nature of the nonaqueous medium employed. Different organic solvents (acetonitrile and methanol) contribute differently to the conversion of analytes into a charged form. Complexes with either an anionic or a cationic part of the ionic liquid additive were formed. This was the case for electrophoresis separation of Brønsted acids and polyphenolic compounds.
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1. Introduction

In recent years, nonaqueous media have played an important role in electrophoretic separations. A number of research groups have shown that nonaqueous media often compete favourably with aqueous systems in the separation of both charged and uncharged species [1,2]. The nonaqueous capillary electrophoresis (NACE), which is based on the use of the electrolyte solutions prepared from pure organic solvents or their mixtures, offers a number of attractive features such as alteration of selectivity, improved solubility of hydrophobic compounds and reduced electrophoretic currents.

The advantages of nonaqueous solvents in capillary electrophoresis (CE) can be discussed from various viewpoints. Nonaqueous solvents can solubilise a large number of water-insoluble organic compounds 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. Accordingly, changing the organic solvents or varying the proportions of two solvents allows a simple selective manipulation in NACE [3,4].

Organic solvents offer a potential for separation mechanisms based on interactions that cannot take place or are too weak to be measured in aqueous media. Capillary electrophoresis can be used as a tool for a study of interactions taking place in organic solvents.

The organic solvents have been applied in capillary electrophoresis since the early 1980s. However, the wider use them in CE as background electrolyte

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solutions has only recently begun. By changing the solvent and/or the nature of an electrolyte additive allows easy manipulation of the separation selectivity by influencing the effective mobility of the analytes, the mobility of the EOF and the pK values of the analytes.

One of the possibilities for using electrolyte additives, which are easily tuneable by their chemical nature, are room-temperature molten salts or so-called ionic liquids. A class of ionic liquids contains ionic substances with a melting point at or close to room temperature (below 100 °C anyway). Beside their environmentally benign properties, they show a wide range of physicochemical properties, depending on the anionic or cationic part of the salt [5]. The novelty of ionic liquids is expressed in chemical tuneability, 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. For example, the salts with the same cation are miscible in both water and/or organic solvents depending on anionic parts. Because of this they are good candidates for electroosmotic flow modifiers in organic solvents.

The first example of separation by using the ionic liquid as an electrolyte component was demonstrated in Ref. [6] where water-insoluble dyes were separated in acetonitrile with added 1-butyl-3-methyl imidazolium fluoroacetate (3.8 mg/ml). Another application of ionic liquids in CE was presented by Stalcup and co-workers [7] where they analysed the polyphenolic compounds found in grape seed extracts. In this study, different 1,3-dialkylimidazolium ionic liquids are used because of their air and water stability, and miscibility with acetonitrile.

The heteroconjugation between Brønsted acids (phenols, carboxylic acids and alcohols) and small inorganic anions has been explained as a separation mechanism in acetonitrile [8].

$$n\text{RH} + A^- \Leftrightarrow (\text{RH})_n A^-$$
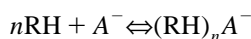
where $n = 1, 2, \ldots$ and RH is a weaker acid than HA. The formation of homo- and heteroconjugates does not occur in protic solvents because the degree of hydrogen bonding in these solvents prevents the association of the dissolved species with one another. In a situation where a nonaqueous electrophoretic buffer contains a deprotonated acid, the resulting anion may form a heteroconjugate with an un-dissociated acidic solute, thus imparting a negative charge to a solute that would otherwise remain uncharged. Accordingly, nonaqueous CE may also be a useful method to study the heteroconjugation of Brønsted acids with anions in solution.

The aim of the present study was to identify a suitable background electrolyte composition based on the use of alkylimidazolium-based ionic liquids in acetonitrile and methanol and to investigate the effect of a background electrolyte on the EOF using different ionic liquids, as well as the actual mobilities of analytes using these background electrolytes.

2. Experimental

CE separations were performed using an ISCO CV4 electropherograph with a UV detector (Isco, Lincoln, NE, USA) 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. The separation was monitored at wavelength 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, AZ, USA) with dimensions 75 cm × 50 μm (effective length 50 cm to 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.

1-Alkyl-3-methyl imidazolium-based ionic liquids with different anionic parts, which are air- and water-stable and miscible with most of CE solvents were used in this study. These were prepared at the Institute of Chemistry at Tallinn Technical University, Estonia, following the procedure described else-

where [9]. The starting materials were obtained from Sigma–Aldrich (Steinheim, Germany).

Background electrolytes: 1-butyl-3-methyl-imidazolium ($[BMIm]^+$)-based ionic liquids with different anionic parts (tetrafluoroborate, $[BF_4]^-$; trifluoroacetate, $[CF_3COO]^-$; heptafluorobutanoate, $[CF_3(CF_2)_2COO]^-$) were prepared as solutions in acetonitrile, and for comparison also sodium acetate CH_3COONa electrolyte in acetonitrile was used.

Polyphenolic compounds: *trans*-resveratrol, catechin, quercetin, gallic acid were obtained from Merck (Darmstadt, Germany).

All solvents were chromatographic grade and were obtained from Sigma–Aldrich.

The separations media were 100% acetonitrile and mixtures of acetonitrile and methanol. All analytes were dissolved separately in acetonitrile (0.5 mg ml^{-1}) for identification purposes and as a mixture for selectivity studies.

3. Results and discussion

3.1. Effect of ionic liquids

Experiments with pure acetonitrile (ACN) showed that the solvent alone was not able to charge the analytes and they remain uncharged. However, there is EOF in pure ACN, but there is no electrophoretic separation of the analytes studied.

A fast and complete separation was estimated for phenolic compounds (1, *p*-ethylphenol; 2, phenol; 3, resorcinol; 4, phloroglycinol dihydrate), which can be seen in Fig. 1 where a background electrolyte was 1-butyl-3-methylimidazolium heptafluorobutanoate (2 mg/ml) in acetonitrile. In this study, all of the separations of phenolic mixtures in ACN-based media were achieved in less than 10 min. The short run times were attributed primarily to the relatively high dielectric constant/viscosity ratio of ACN.

In this case the elution order does not correspond to the charge-to-mass ratio, which is direct indication of a complicated mechanism of charging the analyte. From discussions based on earlier experiments [10] it was proposed that heteroconjugation is responsible for that. The different elution order is expected since the heteroconjugation properties of an analyte are determined primarily by the ability of the analyte to
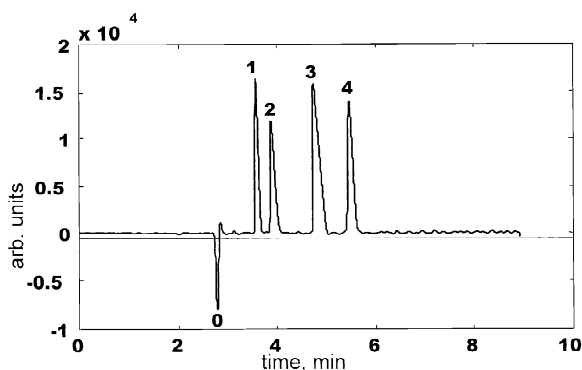

Fig. 1. Separation of selected phenols. Background electrolyte: $[BMIm]^+$ $[CF_3(CF_2)_2COO]^-$ 2 mg/ml in acetonitrile. Analytes: (0) EOF; (1) *p*-ethylphenol; (2) phenol; (3) resorcinol; (4) phloroglycinol dihydrate.

interact with the dissociated background electrolyte components, which, in turn, is linked to the number and position of functional groups on the analyte molecule.

We have explored various 1-butyl-3-methyl imidazolium salts for their efficacy. There is only little change in the selectivity from changing anions, which points on the electrostatic nature of the interaction between the analytes and ionic liquid anion.

3.2. Effect of ionic liquids concentration on mobility

A change of mobility by changing the ionic strength of separation media can point to various conditions for analytes. Miller et al. have studied this problem [11]. By plotting the mobilities of the analytes against the background electrolyte concentration one can estimate the process responsible for separation. In our case the change of mobility when increasing the concentration of ionic liquid confirms the existence of heteroconjugates. This can be seen in Fig. 2 where a change of the mobility of phenol and resorcinol is given for three different ionic liquids. A big difference between $[BMIm]^+$ $[BF_4]^-$ and other ionic liquids shows the difference in size of the conjugates formed or the degree of heteroconjugation by different anions.
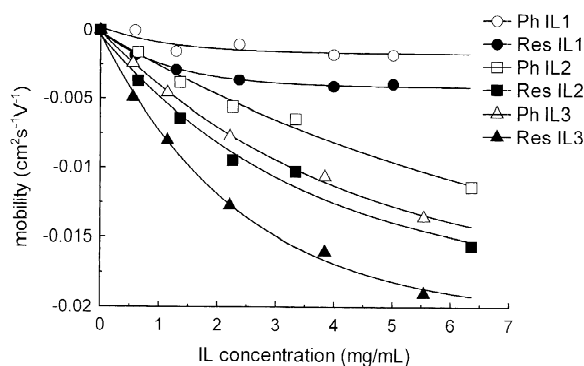
Fig. 2. Separation of phenols (Ph, phenol; Res, resorcinol) with three different ionic liquids (IL1, $[BMIm]^+$ $[BF_4]^-$; IL2, $[BMIm]^+$ $[CF_3(CF_2)_2COO]^-$; IL3, $[BMIm]^+$ $[CF_3COO]^-$) as background electrolytes in acetonitrile.

Fig. 3. EOF in case of two ionic liquids in the acetonitrile–methanol mixture. (A) $[BMIm]^+$ $[CF_3(CF_2)_2COO]^-$; (B) $[BMIm]^+$ $[CF_3COO]^-$.

3.3. Effect of solvent mixtures

The dependence of EOF as a function of the solvent mixture and the salt concentration was studied in case of two ionic liquids (A, 1-butyl-3-methylimidazolium heptafluorobutanoate; B, 1-butyl-3-methylimidazolium trifluoroacetate), and in the ACN–MeOH mixture. The EOF marker was benzene for every separation medium used. As shown in Fig. 3, a large decrease in mobility was observed even if small amounts of methanol were added to an ACN-based background electrolyte. 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. The decreased 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 acetonitrile [12].

The addition of methanol to the background electrolyte with ionic liquids also affects the separation of phenolic compounds very much, and the separation is lost at small concentrations (10%) already as seen in Fig. 4 or even more clearly on pherograms in Fig. 5. This confirms that the formation of heteroconjugates does not occur in protic solvents because the degree of the hydrogen bonding
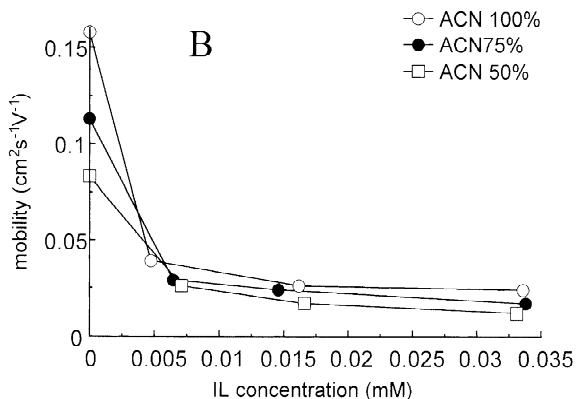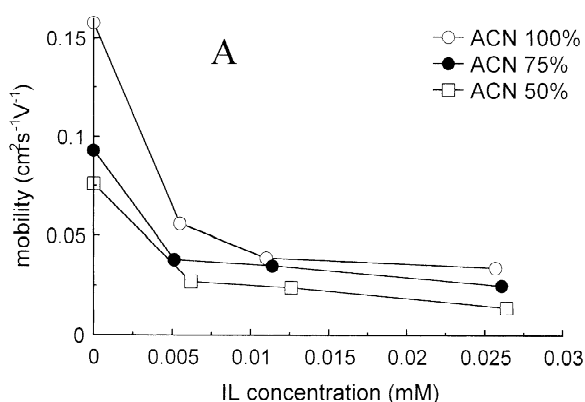
in these solvents prevents the association of the dissolved species with one another. The failure of separation of polyphenols with a simple background electrolyte containing an ionic liquid additive only may be indicative of the same reason. We had to solvate polyphenols in methanol. This small amount of methanol was sufficient to eliminate the conditions for heteroconjugate formation and, thus, the separation of analytes.

We succeeded in achieving good separation of polyphenols when using a buffer system where the analytes were deprotonated and the buffer system
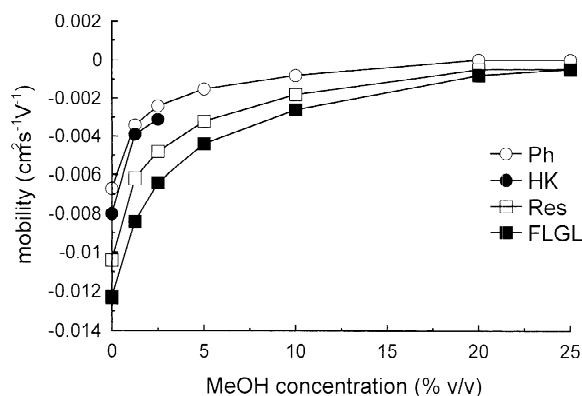
Fig. 4. The mobility of phenols (Ph, phenol; HK, hydrokinone; Res, resorcinol; FLGL, phloroglycinol dihydrate) in the acetonitrile–methanol mixture at ionic liquid $[BMIm]^+$ $[CF_3COO]^-$ concentration of 1.8 mg/ml.
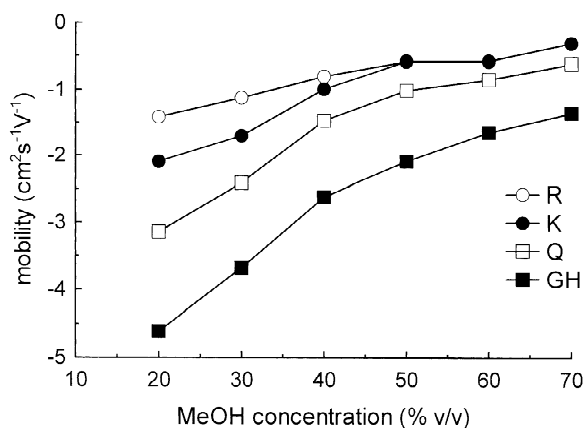

Fig. 6. The mobility of polyphenols (R, *trans*-resveratrol; K, catechin; Q, quercetin; GH, gallic acid) in the acetonitrile–methanol mixture with buffer 50 mM sodium acetate and 1% (v/v) acetic acid.

consisted of sodium acetate and acetic acid. This works well with a high concentration of methanol in acetonitrile despite of a decrease in mobility. Fig. 6 shows a change of the mobility of polyphenols (R, *trans*-resveratrol; K, catechin; Q, quercetin; GH, gallic acid) in an acetonitrile–methanol mixture with

buffer 50 mM of sodium acetate and 1% (v/v) acetic acid.

4. Conclusions

ACN is a good solvent to study the use of alkylimidazolium based salts—ionic liquids—as background electrolyte components. The electrolytes of this type were good to achieve the separation of phenolic compounds, which are soluble in acetonitrile. The ionic liquids studied as components of background electrolytes are not suitable for separation of polyphenolic compounds in acetonitrile.

By changing the composition of a solvent mixture and the salt concentration, it is possible to change the mobilities of the analytes and the separation. The mixture of organic solvents changes the selectivity to large extent. The main mechanism of separation in acetonitrile is heteroconjugation between the salt anion and the analyte molecule.
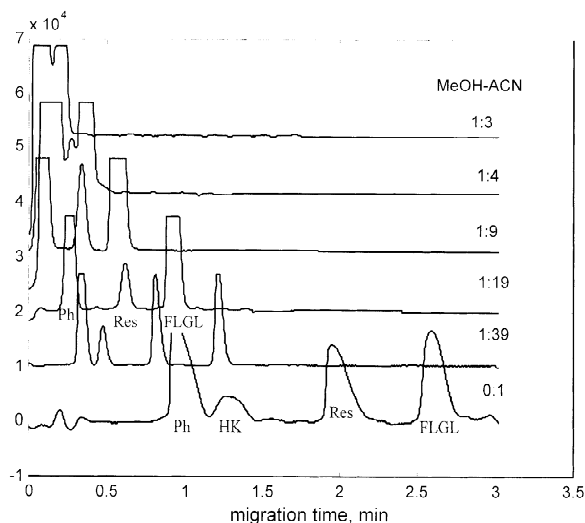

Fig. 5. Influence of added methanol on the migration time and on separation of phenols (Ph, phenol; HK, hydrokinone; Res, resorcinol; FLGL, phloroglycinol dihydrate). Added ionic liquid—1-butyl-3-methyl imidazolium fluoroacetate 3.8 mg/ml.

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References

[1] J.L. Miller, M.G. Khaledi (Eds.), High Performance Electrophoresis, Chemical Analysis Series, Vol. 146, Wiley, New York, 1998, Chapter 15.
[2] F. Wang, M.G. Khaledi, J. Chromatogr. A 875 (2000) 277.
[3] C. Reichardt, Solvent and Solvent Effects in Organic Chemistry, VCH, Weinheim, 1990.
[4] S.P. Porras, M.-L. Riekkola, E. Kenndler, J. Chromatogr. A 924 (2001) 31.
[5] T. Welton, Chem. Rev. 99 (1999) 2071.
[6] M. Vaher, M. Koel, M. Kaljurand, Chromatographia 53 (2001) 302.
[7] E.G. Yanes, S.R. Gratz, M.J. Baldwin, S.E. Robinson, A.M. Stalcup, Anal. Chem. 73 (2001) 3838.
[8] T. Okada, J. Chromatogr. A 771 (1997) 275.
[9] P. Bonhôte, A.-P. Dias, N. Papageorgiou, K. Kalyanasundaram, M. Grätzel, Inorg. Chem. 35 (1996) 1168.
[10] M. Vaher, M. Koel, M. Kaljurand, Electrophoresis 23 (2002) 426.
[11] J.L. Miller, D. Shea, M.G. Khaledi, J. Chromatogr. A 888 (2000) 251.
[12] K. Sarmini, E. Kenndler, J. Chromatogr. A 792 (1997) 3.