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Specific background electrolytes for nonaqueous capillary electrophoresis

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Abstract

The use of organic solvents or mixture of solvents in capillary electrophoresis is gaining wider attention. The electroosmotic flow mobility of eight organic solvents (acetonitrile, acetone, dimethylformamide, dimetylsulphoxide, propylene carbonate, methanol, ethanol, *n*-propanol) and of mixtures of several solvents (methanol–acetonitrile, methanol–propylene carbonate, acetonitrile–propylene carbonate) has been studied. The influence of 1,3-alkylimidazolium salts in different solvents on the separation of different analytes has been investigated. Some of these salts have shown usefulness for matrix-assisted laser desorption ionization matrices and off-line analysis of electrophoresis fractions. It also appears that nonaqueous capillary electrophoresis with 1,3-alkylimidazolium salts as background electrolytes is suitable for separation small inorganic ions.

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1. Introduction

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 nonaqueous solvents in CE are providing possibilities to fulfill several gaps from using only aqueous solutions. First of all organic solvents can solubilize compounds hardly soluble in water and thus afford a possibility of separating such compounds by capillary electrophoresis (CE). Other attractive features of organic solvents are that their physical and chemical properties can offer large changes in separation factor/resolution, analysis time and selectivity, and reduced electrophoretic currents. Accordingly, changing the organic solvents or varying the proportions of two solvents allows a simple selective tuning of separation in NACE [3,4].

The first attempts to apply the organic solvents in capillary electrophoresis were performed in the very beginning of CE in early 1980s [5,6]. However, the wider use of those in CE as background electrolyte solutions has only recently begun [7].

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 negative 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. This means that electroosmotic flow is occurring in solvents without the addition of ionic species. The phenomenon of electroosmosis is common to both NACE and aqueous CE. 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 on the electroosmotic flow in organic solvents without supporting electrolyte were performed, and possibilities for changing EOF recognized [8].

Selection of appropriate background electrolyte for organic solvent of interest is important for a successful analysis. The electrolyte should be dissolving in the solvent, as well as not be UV light absorbing (in many cases). Different quaternary ammonium salts have been used for this purpose [9]. New trend is to use of alkylimidazolium salts as

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background electrolyte modifiers; most of them are air and water-stable room-temperature molten salts, or so called ionic liquids. They show a wide range of physicochemical properties, depending of an anionic or cationic part of the salt [10] and miscibility with most of solvents used in NACE. The novelty of these salts is expressed in chemical tunability which means that it is very easy to prepare salts with the same cationic part but with a different anionic part without significantly changing their physical properties.

The first example of using that kind of salts as an electrolyte component was demonstrated by authors in [11], where non-soluble in water dyes were separated in acetonitrile with added 1-butyl-3-methyl imidazolium fluoroacetate ($3.8 \, mg \, ml^{-1}$). On the same time the application of the same type of salt but in aqueous CE was presented by Stalcup et al. [12], they dynamically modified the surface of the capillary to achieve reversed electroosmotic flow for separation of phenolic compounds. Another example from the very close field of separation methods can be brought. The effect of imidazolium tetrafluoroborates to suppress deleterious influence of free silanols on liquid chromatographic separations was demonstrated [13], it markedly exceeded that of the standard mobile phase additives on decreasing band tailing, reducing band broadening, and improving resolution. Saturation of adsorption has been observed at the imidazolium tetrafluoroborate concentration of ca. 0.5% (v/v).

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has become one of the most commonly used analytical methods, and not only for big biomolecules but also low-molecular-mass compounds of biological interest. The inhomogeneous distribution of analytes within sample spots is the most serious problem in the use of MALDI-MS. Coupling of MALDI-MS to capillary electrophoresis is intensive object to study [14]. NACE can here provide advantage over the water based systems. Some of alkylammonium and alkylimidazolium salts have been proved to be useful as MALDI matrixes [15], which raise the interest of employing them as electrolyte modifier in nonaqueous capillary electrophoresis, especially in coupling with MALDI-MS. These special liquid salts are combining the properties of ionic liquids with the ability to perform the ionisation and desorption processes necessary for MALDI-MS. These useful properties are very low vapour-pressure and stability of matrix under high-vacuum conditions, and ease of achieving sample homogenity [16].

Besides organic ions also small ions, such as inorganic cations are targets to study in nonaqueous CE [17,18].

2. Experimental

2.1. Sample preparation

The 1-butyl-3-methyl imidazolium (BMIm) based salts with different anionic parts, which are air- and water-stable

and miscible with most of CE solvents (trifluoroacetate: $[CF_3COO]^-$, heptafluorobutanoate: $[CF_3(CF_2)_2COO]^-$) were used in this study as additives in separation media. Also some alkylammonium (triethylammonium and butylammonium) and alkylimidazolium salts of α-cyano-4-hydroxycinnamic acid were used in this study. These were prepared at the Institute of Chemistry at Tallinn University of Technology following the procedure described elsewhere [15,19]. The starting materials were obtained from Aldrich, Germany.

Phenolic compounds: resorcinole, phloroglycinol, chlorogenic acid, caffeic acid, gallic acid were obtained from Merck, Darmstadt, Germany. All analytes were dissolved separately in acetonitrile ($0.5 \, mg \, ml^{-1}$) for identification purposes and as a mixture for selectivity studies.

All solvents were chromatographic grade and were obtained from Sigma–Aldrich, Germany. The investigated mineral waters (Värska and Vichy) were obtained from local grocery store.

2.2. Instrumentation

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 20 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, 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. Benzene was used as the EOF marker.

The MALDI time-of-flight (TOF) MS spectra were obtained on Voyager-DE PRO Workstation (Applied Biosystems, USA) system.

3. Results and discussion

3.1. EOF in pure solvents and solvent mixtures

EOF is a phenomenon generic to capillary electrophoretic separations, and there is an electroosmotic flow in solvents in the silica capillary when the voltage is applied. Except in non-electrolytic solvents (such as diethylether, chloroform, _n_-hexane, cyclohexane) which have low dielectric constants. In this study the behaviour was studied in different
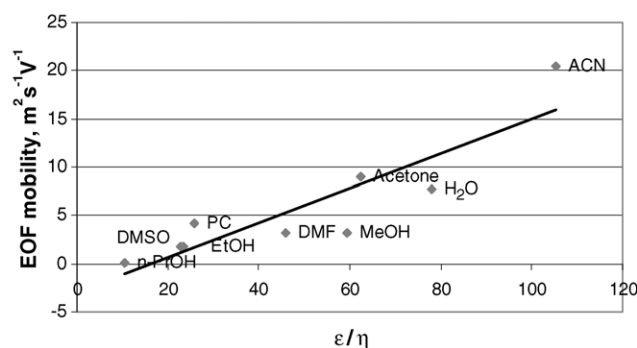
Fig. 1. Correlation of EOF generated in pure solvents [acetonitrile (ACN), acetone, propylene carbonate (PC), methanol (MeOH), ethanol (EtOH), *n*-propanol (*n*-PrOH), dimethylformamide (DMF), dimethyl sulphoxide (DMSO)] with relation ε/η of this solvent (mobilities of EOF are measured at temperature 20 °C and voltage 20 kV).
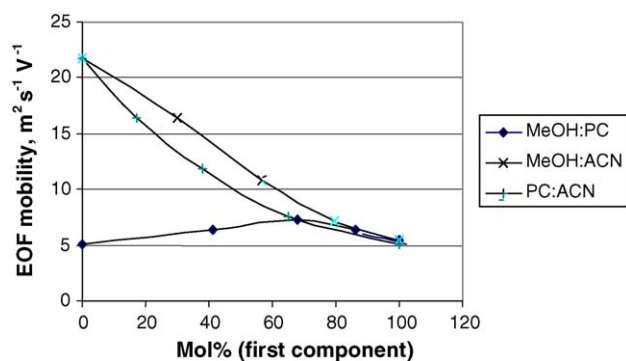

Fig. 2. Change of EOF mobility with changes in content of binary solvents in case of three different binary mixtures (methanol–propylene carbonate; methanol–acetonitrile, and propylene carbonate–acetonitrile).

organic solvents. The electroosmotic flow mobility, μ_{eo}, varied in a large scale in different solvents (from *n*-PrOH to ACN) as can be seen on Fig. 1. The EOF in all these non-aqueous electrolyte systems was directed from the anode to the cathode. For generation of this cathodic EOF the silica surface in the used solvents must have a net negative charge.

It is well-known from the theory of capillary electrophoresis that electrophoretic mobility is proportional to relation ε/η and is a function of zeta potential of the surface in the solvent, which is determined by the surface charge on the capillary wall [20–22]. The relationship between EOF in pure solvent and ε/η value for the corresponding solvent is presented on Fig. 1. According to [8] the discrepancy from straight linear relationship can be explained with changes in zeta potential because of more specific interactions of solvents with silica surface in the column. However, there can be other factors responsible for changes of zeta potential. Also the polarity in the separation media is probably different for different solvents, and this influences on the thickness of double layer on the column wall.

Miscellaneous solvents have different physical properties (dielectric constant, viscosity) thus by mixing various solvents one can change the EOF. Some physical properties of solvents that were used in this study are presented in Table 1. However, the most important are chemical properties of the mixture components. Mixing of solvents with different proton accepting/donating properties affects the interactions in the separation media and on the surface of capillary which are important for develop of electroosmotic flow in the system.

Consequently dependence of EOF mobility from the content of binary mixture is not linear, as shown on Fig. 2. The μ_{eo} of mixtures of different relations methanol–propylene carbonate were not much changed, while the relation ε/η is rather similar for both solvents. In the mixtures with ACN, which have the highest ζ-potential, μ_{eo} decreased approximately four times when going from ACN to methanol or propylene carbonate.

The similar non-linear change of EOF mobilities were 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 [23]. The similar trends are followed in different buffered acetonitrile–methanol mixtures [24,25]. Therefore, in the separation media and on the surface of column there are taking place different concurrent processes which result in nonlinear dependence in change of flow mobility according to mixture composition.

3.2. Effect of salt

Presence of electroosmotic flow in a solvent is not enough in most cases for the electrophoretic separation of analytes because the analytes remain electrically neutral in pure solvent and migrate together with EOF peak. It was clearly seen in experiments with pure acetonitrile that the solvent alone was not able to charge the phenolic compounds and they remain uncharged. Therefore, addition of background electrolyte is needed.

The family of 1,3-alkylimidazolium salts is promising candidate for use as background electrolyte modifiers.

Table 1
Parameters of the investigated solvents

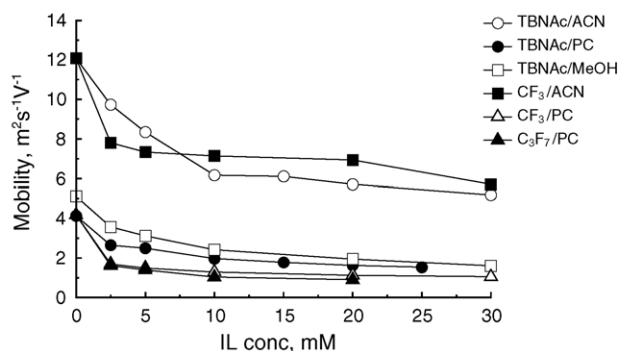| Solvent	EOF mobility[a], μ_{eo} (m^2 V^{-1} 10^{-9})	Dielectric constant, ε	Viscosity[a], η (mP s)	Density, d (g/cm^3)
ACN	21.7	35.90	0.35	0.786
MeOH	5.47	32.66	0.52	0.796
PC	5.17	64.92	1.61	1.189

Data from [2].

[a] Experimental data.

Fig. 3. Change of EOF mobility with changes of electrolyte concentration in acetonitrile (ACN) and propylene carbonate (PC). Added salts were tetrabutylammonium acetate (TBNAc), 1-butyl-3-methylimidazolium fluoroacetate (CF$_3$), and 1-butyl-3-methylimidazolium heptafluorobutanoate (C$_3$F$_7$).
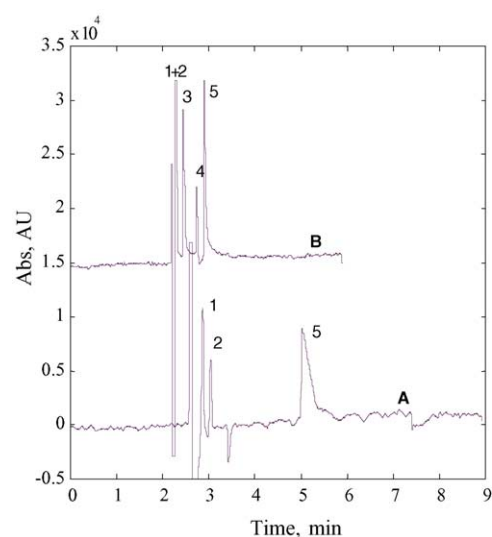

Fig. 4. Electrophrograms on separation of (1) resorcinol, (2) phloroglycinol, (3) chlorogenic acid, (4) caffeic acid, (5) gallic acid. Electrolytes were: (A) triethylammonium α-cyano-4-hydroxycinnamate (8 mM) in pure acetonitrile; (B) butylammonium α-cyano-4-hydroxycinnamate (5 mM) in pure acetonitrile.

Selection of the proper salt gives an additional opportunity to alter the selectivity of a separation approach.

On Fig. 3 is presented the change of electroosmotic mobilities in different electrolyte systems. As expected the adding of salt to pure solvent changes the EOF of the system, and the change is most dramatic in acetonitrile, less in propylene carbonate and methanol. It is known that a presence of the salt in separation media modifies dynamically the double layer on the capillary wall [26]. The BMIm based salts with the same cation but different anion ([CF$_3$COO]$^-$ and [CF$_3$(CF$_2$)$_2$COO]$^-$) do not show big difference which points to the most influence of the salt cation to the formation of EOF in the separation media and modification of surface of column. Interestingly, in the case of background electrolyte additives with other organic cations (imidazolium and ammonium) there is no big difference in EOF mobility. It is demonstrated on Fig. 3 for acetonitrile and propylene carbonate, where the effect of dynamic modification of the capillary surface is seen from the decrease of EOF. Salts used here change EOF remarkably already at low concentration. It must be noted, however, that they are not able to change the sign of the surface charge of capillary wall and the direction of EOF. The EOF was remained unchanged in all studied nonaqueous electrolyte systems, directed from the anode to the cathode. For imidazolium salts the effect is greater than for tetraalkylammonium salts, except in case of acetonitrile solutions.

The similar influence of alkylimidazolium salts to EOF mobility was detected by the authors both in pure acetonitrile [27] and in ACN–MeOH mixtures [28].

In case of NACE there are possibilities for other separation mechanism than charging of the analyte by dissociation. From discussions based on earlier experiments [27] it was proposed that heteroconjugation is responsible for that. To use organic solvents such as acetonitrile and propylene carbonate for the separation of neutral analytes (Brønstedt acids) has been based to form negatively charged heteroconjugate between the analytes and electrolyte anion [29]. The different elution order is expected since the heteroconjugation properties of an analyte are determined primarily by the ability of the analyte 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.

Alkylimidazolium salts have some advantage in use for that purpose because they have good solubilities in many organic solvents used in NACE. It allows separation of analytes by using relatively simple electrolyte system by just adding proper salt to pure solvent.

In this study above mentioned electrolytes (pure solvent and alkylammonium salt or alkylimidazolium salt of α-cyano-4-hydroxycinnamic acid used as MALDI matrix) were used in several applications. At first attempt was made to get separation of phenolic compounds. On the Fig. 4 two sample electropherograms of separation of phenolic compounds are presented. In this case both, the heteroconjugation for resorcinol and phloroglycinol, and the dissociation for other analytes are the possible separation mechanisms according to discussion in [28]. It appears that alkylammonium salts were giving better separation in these conditions than alkylimidazolium salts (the results not shown). In case of A (Fig. 4) better separation take place for uncharged resorcinol and phloroglycinol. Using mixtures of solvents and/or by varying the concentration of salts variation in selectivity of separation can be achieved.

On Fig. 5 the mass spectrum of chlorogenic acid previously separated by NACE is presented as an example. As can be seen from the figure, a MALDI matrix used for determination of phenolic compounds is suitable as background electrolyte for NACE.
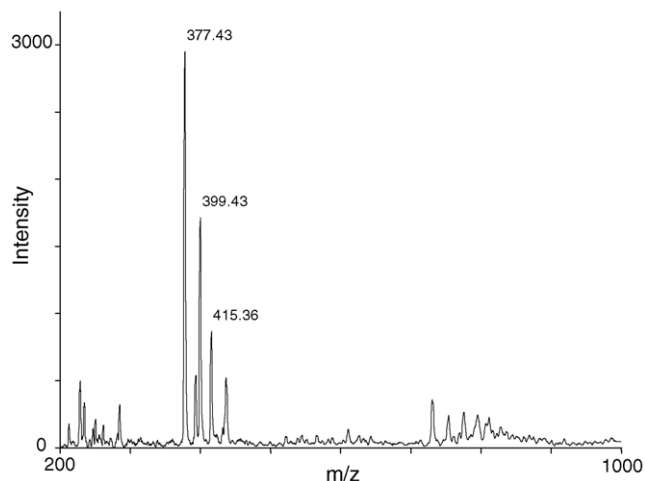
Fig. 5. Positive ion mode MALDI mass spectrum of chlorogenic acid, matrix was α-cyano-4-hydroxycinnamate. The main peaks are $[M+H]^+$ 377.43; $[M+Na]^+$ 399.43; $[M+K]^+$ 415.36.

An attempt was also made to separate small cations using the same type of electrolytes in nonaqueous media. Here again the selection of solvent is crucial. It has been shown that by choosing methanol as a solvent, alkylimidazolium salts can be used for the separation of metal cation. In this study the separation of Na^+, K^+, Ca^{2+} and Mg^{2+} ions was achieved by using 20 mM 1-butyl-3-methylimidazolium heptafluorobutanoate ($[CF_3(CF_2)_2COO]^-$) in methanol with indirect UV detection in mineral water (Fig. 6). Under described conditions alkali and alkaline earth metal cations are well separated without using the complexing agents. The separation mechanism needs further investigation.
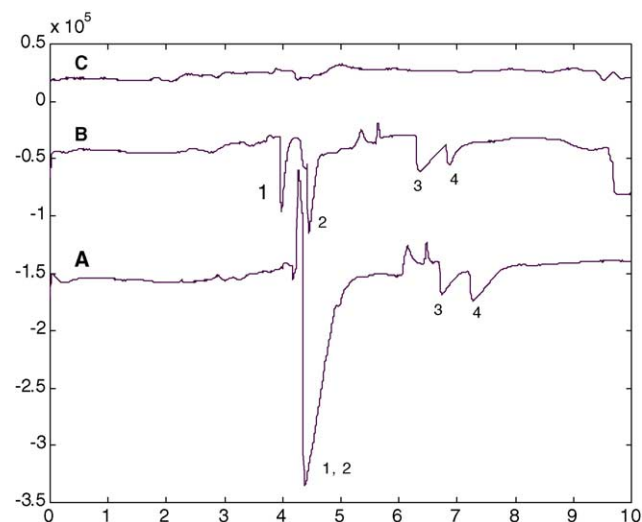

Fig. 6. Separation of Estonian mineral water Värska and Vichy. Background electrolyte: 20 mM 1-butyl-3-methyl imidazolium heptafluorobutanoate in methanol. Indirect UV detection at 210 nm. Applied voltage 20 kV. (A) Mineral water Värska, (B) mineral water Vichy, (C) deionized water. (1) K^+, (2) Na^+, (3) Mg^{2+}, (4) Ca^{2+}.

4. Conclusion

Alkylimidazolium and alkylammonium based salts are well soluble in organic solvents and can be successfully used as background electrolyte components in nonaqueous capillary electrophoresis. By changing the composition of a solvent mixture and the salt concentration it is possible to influence the electroosmotic flow of the nonaqueous electrophoretic system in great extent. The possible mechanism of separation in organic solvent is heteroconjugation between the background electrolyte anion and analyte, giving the possibility to electrophoretically separate non-dissociating analyte molecules in aprotic solvents.

The electrolytes of this type enabled to achieve the separation of phenolic compounds, which are soluble in acetonitrile.

5. Acknowledgment

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