Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 329–332
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Separation of nicotinic acid and its structural isomers using 1-ethyl-3-methylimidazolium ionic liquid as a buffer additive by capillary electrophoresis

Michał Piotr Marszałł, Michał Jan Markuszewski, Roman Kaliszan *

Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, ul. Gen. J. Hallera 107, 80-416 Gdańsk, Poland

Received 4 August 2005; received in revised form 7 November 2005; accepted 9 November 2005
Available online 20 December 2005

Abstract

The growing interest in application of ionic liquids (ILs) in analytical chemistry has been observed. The aim of presented investigation was to verify whether ILs would be a suitable modifier of the background electrolyte (BGE) for pharmaceutical analysis of the closely related drug analogues. The study demonstrates the use of 1-ethyl-3-methylimidazolium tetrafluoroborate (1E-3MI-TFB) ionic liquid as modifiers in the separation of nicotinic acid and its structural isomers by capillary electrophoresis. Dependences of the ionic liquid concentration in a BGE on the separation parameters like migration time, resolution factor and width at peak's baseline have been compared. The separation mechanism involves the free imidazolium ions, which can interact with inner surface of the capillary wall. Increased 1E-3MI-TFB concentration to 150 mmol/L caused decrease of migration times of analytes, improve peaks shape and increase of separation performances. At this ionic liquid concentration in a BGE resolution factor between nicotinic and isonicotinic acids increased to 1.86. The proposed CE separation procedure is highly reproducible and can be applied in qualitative and quantitative analysis of carboxylic acids.

Keywords: Nicotinic acid; Ionic liquids; 1-Ethyl-3-methylimidazolium tetrafluoroborate; Capillary electrophoresis

1. Introduction

Ionic liquids (ILs) also known as a room-temperature molten salts are characterized by melting point at or below room temperature. ILs physicochemical properties depend on the anionic or cationic part of the molecule. They are thermally stable, nonvolatile, nonflammable and are very good solvents for most of organic and inorganic compounds. Due to their significant vapor pressure, they are considered environmentally benign [1,2].

Recently, ionic liquids called an attention of analytical chemists working with thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). Ionic liquids of the imidazolium tetrafluoroborate class, added to mobile phases at concentrations of 0.5–1.5% (v/v), blocked silanols and provided excellent thin-layer chromatographic separations of strongly basic drugs, which were otherwise not eluted, even with neat acetonitrile as the mobile phase. The silanol suppressing potency of imidazolium tetrafluoroborates was demonstrated to markedly exceed that of the standard mobile phase additives, like triethylamine, dimethyloctylamine and ammonia [3]. ILs have already been recognized as suitable background electrolyte (BGE) in nonaqueous CE. Using 1-butyl-3-methylimidazolium-based ionic liquids with different anions the analysis of hydrophobic dyes was achieved [4]. The role of the anionic part of the IL salt's on electrophoretic mobility as well as influence of the overall concentration of salts in the buffer system on separation performances have been extensively studied and described [5]. Yanes et al. proposed mechanism of separation of polyphenols that relies on the association of the studied analytes with IL's imidazolium cations either coating the capillary wall or electrophoretically co-migrating in the bulk solution [6]. Similarly in nonaqueous CE when ILs and acetonitrile are BGE constituents' separation is based on the heteroconjugation between the anion salt and the analyte molecule [7]. The reversal of the electroosmotic flow (EOF) caused by covalently bonded 1-alkyl-3-methylimidazolium-based ionic liquid onto the surface of capillary wall was responsible for the separation of the sildenafil citrate and its metabolite in human serum

* Corresponding author. Tel.: +48 583493260; fax: +48 583493262.
E-mail address: roman.kaliszan@amg.gda.pl (R. Kaliszan).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.11.013

[8]. The same class of 1-alkyl-3-methylimidazolium-based IL (1E-3MI-TFB) for the capillary surface coating during separation of the basic proteins was used in CE. Achieved separation for the different basic proteins such as lysozyme, cytochrome *c*, trypsinogen and α-chymotrypsinogen A was characterized by a high efficiency and good repeatability [9]. Qi et al. reported the application of 1-butyl-3-methylimidazolium tetrafluoroborate (1B-3MI-TFB) as running electrolyte with chiral selectors like β-cyclodedextrin (β-CD) to separate the anthraquinones during the purification of natural products [10].

Stepnowski et al. [11] reported simple and selective reversed-phase HPLC method with electrospray ionization (ESI) detection for the analysis of 1-alkyl- and 1-aryl-3-methylimidazolium-based ionic liquids. Also, just recently fast and reproducible capillary electrophoretic method for the separation of the same series of ionic liquid cations was published [12].

There is a need to develop simple and efficient methods for the determination and separation of nicotinic acid and its structural isomers isonicotinic and picolinic acids by means of fast and reproducible analysis. Nicotinic acid and its structural isomer isonicotinic acid are important biological compounds. Nicotinic acid together with derivatives is used for prophylaxis and treatment of pellagra. Isonicotinic acid is a metabolite of isoniazid, still considered to be the primary drug in the chemotherapy of tuberculosis. Sebastiano and co-workers [13] achieved successful separation of nicotinic and picolinic acids with 2 mM quaternarized piperazine [(*N*-methyl-*N*-4-iodobutyl)-*N*9-methylpiperazine] as a capillary wall modifier in CE with tetraborate buffer at pH 9 as a BGE.

The aim of our studies was to verify whether ILs would be suitable BGE modifier for pharmaceutical analysis of the closely related drug analogues. In presented investigation 1-ethyl-3-methylimidazolium tetrafluoroborate (1E-3MI-TFB) ionic liquid was used as an additive in borate buffers to separate nicotinic acid and its structural isomers, isonicotinic and picolinic acids in standard solution. Results of comparative studies of silanol suppressing potency of the 1E-3MI-TFB, 1-methyl-3-hexylimidazolium teterfluoroborate and 1-hexyl-3-heptyloxymethylimidazolium teterfluoroborate in TLC and HPLC were demonstrated in previous work [3]. The Langmuir plots for the three ionic liquids added to acetonitrile eluent as the suppressors of attraction by free silanols of the selected basic drugs on the silica-covered plates shows strong silanol-suppressing potency of analyzed ILs. The strongest one represents 1-methyl-3-hexylimidazolium teterfluoroborate and 1E-3MI-TFB. Due to its commercially availability and demonstrated use in TLC and HPLC we decided to use in presented studies 1E-3MI-TFB as an additive in background electrolyte in CE.

2. Experimental

2.1. Materials

Nicotinic, isonicotinic and picolinic acids were synthesized and kindly donated to us by the Department of Organic Chemistry, Medical University of Gdańsk. 1-ethyl-3-methylimidazolium tetrafluoroborate ionic liquid (1E-3MI-TFB), sodium tetraborate decahydrate and benzyl alcohol were purchased from Fluka (Buchs, Switzerland). Chemical structures of analyzed acids as well as room-temperature imidazolium-based ionic liquid are presented in Fig. 1. Double deionized and distillated water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA). Borate buffer (20 mM) was prepared by dissolving appropriate amounts of sodium tetraborate decahydrate in water to obtain required pH 9.3. Next, 1E-3M-TFB was added to concentration from 10 to 150 mM to the running electrolyte solution (borate buffer solution).

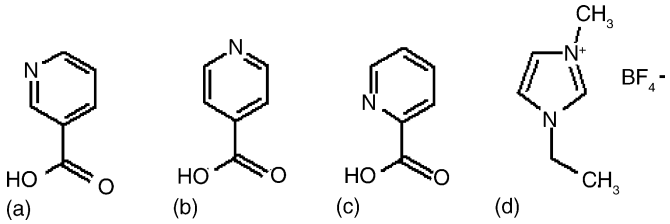

Fig. 1. Chemical structures of nicotinic acid (a), isonicotinic acid (b), picolinic acid (c) and 1-ethyl-3-methylimidazolium tetrafluoroborate ionic liquid (1E-3MI-TFB) (d).

2.2. Apparatus and CE procedure

CE experiments were performed using Quanta 4000E CE System (Waters, Milford, Bedford, MA, USA) equipped with UV detector. A bare fused-silica capillary (Polimicro Technologies, Phoenix, AZ, USA) with 50 μm i.d. and a total length of 46 cm (38.5 cm effective length) was used. New capillaries were first rinsed with 1.0 M NaOH (30 min), then with methanol (30 min) and deionized water (60 min), and finally with the background electrolyte (BGE) (60 min). For conditioning of capillaries, each separation was preceded by a 4 min rinse with BGE. The capillary was air-thermostated (Peltier system) at 20 °C. All injection were done hydrodynamically ($\Delta h = 10$ cm) in 5 s. Applied voltage was either +14 kV or −14 kV depending on concentrations of the 1E-3MI-TFB in the buffer. The UV wavelength for detection was 254 nm. The injected samples were in concentration of 1.0 mg mL^{-1}.

The charge, *q* and radius, *r*, of nicotinic acids were calculated by the molecular modeling program HyperChem for personal computers (HyperCube, Gainesville, FL, USA). The calculations were performed using geometry optimization by the molecular mechanics MM+ force field method [14].

3. Results and discussion

Significant influence on migration times of acidic analytes in capillary electrophoresis could be observed when 1E-3MI-TFB was used as an additive in 20 mM sodium tetraborate buffer. Fig. 2 shows the dependence of the EOF on the concentration of IL's in the BGE when the positive potential at the injector end of the capillary was applied. It can be seen that increasing concentration of 1E-3MI-TFB from 0 to 70 mM results in decrease of EOF. In the concentration above 70 mM of IL in the

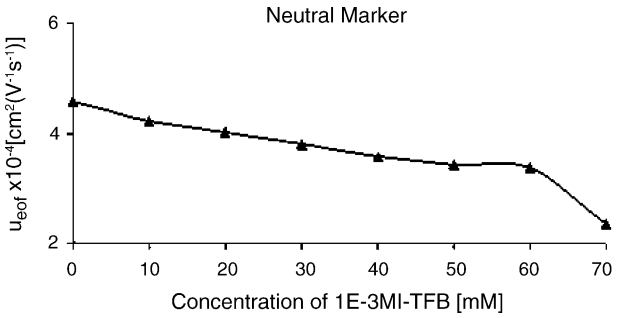

Fig. 2. Effect of concentration of 1-ethyl-3-methylimidazolium tetrafluoroborate on the EOF measured by neutral marker (benzyl alcohol). Electrophoretic condition: applied voltage +14 kV, 20 mM borate buffer, other conditions see Section 2.

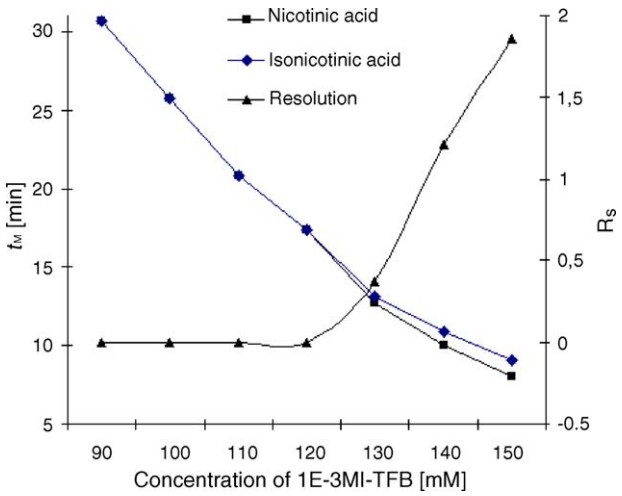

Fig. 3. Influence of concentration (mM) of analyzed ionic liquid on the retention of nicotinic and isonicotinic acids and their resolution. Electrophoretic condition: applied voltage −14 kV, 20 mM borate buffer, other conditions see Section 2.

BGE, the EOF became insignificant. Ionic liquids added to running tetraborate buffer decrease its pH from 9.30 to 8.30. At pH 8.30 the nicotinic acid ($pK_{a1} = 2.07$, $pK_{a2} = 4.73$), isonicotinic acid ($pK_{a1} = 1.70$, $pK_{a2} = 4.89$) and picolinic acid ($pK_{a1} = 1.06$, $pK_{a2} = 5.37$) are negatively charged [15]. Therefore, at a basic pH, the significant influence to the migration of carboxylic acids has electrophoretic mobility, μ_e.

The main goal of the analysis was to demonstrate the applicability of ionic liquid as an additive in BGE for the separation of nicotinic acid from its both isomers, picolinic and isonicotinic acids. In basic pH picolinic acid had been successfully separated even though there was no IL added to the buffer. Increasing concentration of 1E-3MI-TFB in BGE did not effected in separation of nicotinic acid isomers whereas EOF become significantly reduced. 1E-3MI-TFB in concentration of 70 mM in BGE resulted in suppression of free silanol groups on the inner surface of capillary and complete reduction of electroosmotic flow. The EOF became reduced and not reversed because negatively charged silanol groups attracted positively charged 1-ethyl-3-methylimidazolium cations from ionic liquid salt. However, in contrast to standard BGE amine additives used in CE imidazolium cations form uncharged layer on the surface of capillary that did not allow to create the reversal flow of BGE ions under the EOF. This was confirmed by the use of reverse polarity during analysis and applied anodic detection without successful detection of EOF marker. Detection of nicotinic acid derivatives under such defined conditions was possible and with increasing amount of 1E-3MI-TFB, when finally concentration of IL's increased up to 150 mM, the separation of three acids was enabled. Fig. 3 shows that the increasing resolution of neighboring peaks is related to increased concentration of ILs as a BGE modifier. Increasing concentration of added 1E-3MI-TFB in the BGE from 130 to 150 mM resulted in change of separation performances expressed by resolution factor from 0.36 to 1.86.

The separation mechanism of acids can be explained by the presence of imidazolium cations in the BGE. In contradiction to standard surfactants modifiers, they are responsible for formation of single layer on the inner surface of the capillary wall changing its electrophoretic properties (Fig. 4). Simultaneously, due to the ionic interaction between negatively charge silica groups on inner surface of the capillary and IL's cationic molecules, imidazolium moiety became uncharged. Such a created coating on the inner surface of the capillary wall causes suppression of the EOF. At certain concentration of IL's in BGE the total suppression of EOF could be observed. Therefore, negatively charged acids are not retained anymore by electrically neutral single layer of imidazolium ionic. Finally, charged acids' isomers migrate toward the anode and are separated by their different values of μ_e, which can be given by following equation:

$$\mu_e = \frac{q}{6\pi\eta r} \tag{1}$$

where q is the ion charge, η is the solution viscosity and r is ion radius.

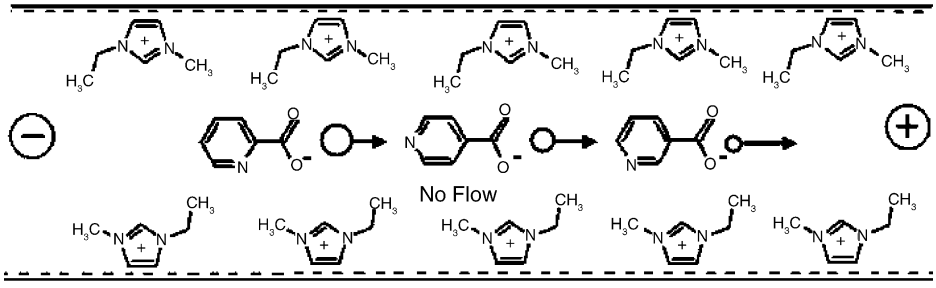

Fig. 4. Proposed mechanism of carboxylic acids' separation using 1E-3MI-TFB. Order of migration according to ratio charge/radius when ⊖→.

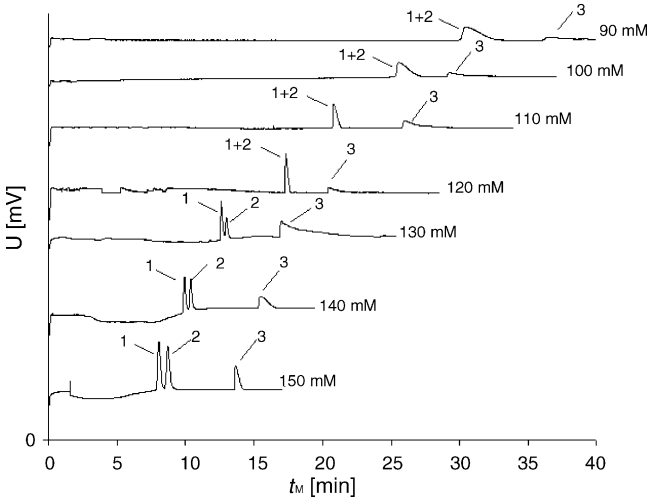

Fig. 5. Electropherograms presenting separation of nicotinic (1), isonicotinic (2) and picolinic acids (3) with increasing concentration of 1-ethyl-3-methylimidazolium tetrafluoroborate ionic liquid added to the BGE. Analyzed acids were in 1.0 mg mL^{-1} concentration. Electrophoretic condition: 20 mM borate buffer, applied voltage −14 kV, other conditions see Section 2.

Fig. 5 presents obtained electropherograms with increasing concentrations of IL's in BGE. Increased 1E-3MI-TFB concentrations caused decrease of migration times of analytes, improve peaks shape and increase of separation performances. According to Eq. (1) the highest mobility has nicotinic acid with largest ratio charge/radius (-2.03×10^{-1}), next isonicotinic acid (-1.60×10^{-1}) and picolinic acid (-1.42×10^{-1}) on the assumption of that η of solution is constant in the current concentration of ionic liquid.

The migration mechanism influence separation and enables analysis of three acid isomers at a single run. It also results in improvement of peak shape and resolution factor (Fig. 3). The peaks widths at base (w_b) of analyzed acids were compared and are presented in Table 1. According to the peak width, the narrowest peaks were obtained in 150 mM concentration of 1E-MI-TFB. With the increase amount of ILs, the improvement of peaks' shape is apparent, especially in the case of picolinic acid (Fig. 5).

The electrophoretic conditions allowing effective separation of isomers of nicotinic acids were used to indicate repeatability of method. The repeatability expressed as relative standard deviation (R.S.D.%) of the migration time, peak area and corrected peak area for all analytes are presented in Table 2. The obtained R.S.D.% values of migration time were in the range from 0.31 to 0.46%. The low values of R.S.D.% for migration time prove proper selection of analytical conditions. The R.S.D.% of peak area and corrected peak area are also in reasonable good ranges from 0.42 to 2.6%.

Table 1
The nicotinic acid and its structural isomers peaks widths (w_b) at base in different concentration of 1E-3MI-TFB added to the BGE

Concentration of 1E-3MI-TFB (mM)	Peak width at base (w_b)		
	Nicotinic acid	Isonicotinic acid	Picolinic acid
90	1.680		1.472
100	1.481		1.414
110	0.827		1.341
120	0.682		1.269
130	0.543	0.712	1.237
140	0.478	0.527	0.919
150	0.341	0.392	0.696

Table 2
Repeatability of nicotinic acid and its structural isomers expressed as relative standard deviation (R.S.D.%, $n = 5$) for migration times, peak area and corrected peak area

	Nicotinic acid	Isonicotinic acid	Picolinic acid
Migration time	0.32	0.35	0.46
Peak area	1.64	1.33	2.60
Corrected peak area	0.61	0.62	0.42

4. Conclusion

In view of the obtained results, the influence of 1E-3MI-TFB ionic liquid is responsible for the simple and efficient separation of nicotinic acid and its structural isomers. Use of 1E-3MI-TFB allows for successful separation of previously unresolved peaks of nicotinic, isonicotinic and picolinic acids. Established method characterizes high repeatability expressed by R.S.D.% of migration times, peak area and corrected peak area. Presented method with application of ILs as a BGE constituent gives unique opportunities for separation of closely related structural isomer in pharmaceutical and biomedical analysis. From the environmental point of view, presently increasing number of commercially available ionic liquids gives opportunity to extend their application in separation methods like CE and HPLC as well as allows to replace the standard environmentally harmful reagents.

References

[1] J.D. Holbrey, K.R. Seddon, Clean Prod. Process 1 (1999) 223–236.
[2] P. Wasserscheid, W. Keim, Angew. Chem. 112 (2000) 3926–3945.
[3] R. Kaliszan, M.P. Marszałł, M.J. Markuszewski, T. Bączek, J. Pernak, J. Chromatogr. A 1030 (2004) 263–271.
[4] M. Vaher, M. Koel, M. Kaljurand, Chromatographia 53 (2001) 302–306.
[5] M. Vaher, M. Koel, M. Kaljurand, Electrophoresis 23 (2002) 426–430.
[6] E.G. Yanes, S.R. Gratz, M.J. Baldwin, S.E. Robinson, A.M. Stalcup, Anal. Chem. 73 (2001) 3838–3844.
[7] M. Vaher, M. Koel, M. Kaljurand, J. Chromatogr. A 979 (2002) 27–32.
[8] W. Qin, S.F.Y. Li, Electrophoresis 23 (2002) 4110–4116.
[9] T.-F. Jiang, Y.-L. Gu, B. Liang, J.-B. Li, Y.-P. Shi, Q.-Y. Ou, Anal. Chim. Acta 479 (2003) 249–254.
[10] S. Qi, S. Cui, X. Chen, Z. Hu, J. Chromatogr. A 1059 (2004) 191–198.
[11] P. Stepnowski, A. Műller, P. Behrend, J. Ranke, J. Hoffmann, B. Jastorff, J. Chromatogr. A 993 (2003) 173–178.
[12] M.J. Markuszewski, P. Stepnowski, M.P. Marszałł, Electrophoresis 25 (2004) 3450–3454.
[13] R. Sebastiano, C. Gelfi, P.G. Righetti, A. Citterio, J. Chromatogr. A 894 (2000) 53–61.
[14] HyperChem Computational Chemistry, Hypercube Inc.: Waterloo, Ontario, Canada, 1996.
[15] A. Albert, E.P. Serjeant, Ionization Constants of Acids and Bases, Wiley, New York, 1962.