Simon M. Mwongela
Noreen Siminialayi
Kristin A. Fletcher
Isiah M. Warner

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana, USA

Original Paper

A comparison of ionic liquids to molecular organic solvents as additives for chiral separations in micellar electrokinetic chromatography

In this study, we report the effects of adding ionic liquids (ILs), as compared to adding conventional molecular organic solvents (MOSs), to aqueous buffer solutions containing molecular micelles in the separation of chiral analyte mixtures in micellar EKC (MEKC). The molecular micelle used in this study was polysodium oleyl-L-leucylvalinate (poly-L-SOLV). The ILs were 1-alkyl-3-methylimidazolium tetrafluoroborate, where the alkyl group was ethyl, butyl, hexyl, or octyl. These ILs were chosen due to their hydrophobicity, good solvating, and electrolyte properties. Thus, it was expected that these ILs would have favorable interactions with chiral analytes and not adversely affect the background current. Common CE buffers, mixed with a molecular micelle, and an IL or a MOS, were used for these chiral separations. The buffers containing an IL in the concentration range of 0.02 – 0.1 v/v were found to support a reasonable current when an electric field strength of 500 V/cm was applied across the capillary. However, a current break down was observed for the buffers containing more than 60% v/v MOS on application of the above-mentioned electric field. The chiral resolution and selectivity of the analytes were dependent on the concentration and type of IL or MOS used.

Keywords: Chiral separations / Ionic liquids / Micellar electrokinetic chromatography / Molecular micelles / Molecular organic solvents

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1 Introduction

Ionic liquids (ILs) are nonmolecular solvents composed entirely of ions that melt together below 100°C. Typically, ILs consist of nitrogen-containing organic cations and the cation/anion combination can be easily tuned to provide the desired chemical and physical properties. Recently, these solvents have found wide applications in organic, electrochemical, nuclear, inorganic, and analytical chemistry [1]. Their application in analytical chemistry, especially in separating analytes, is merited by their unique properties which include: negligible vapor pressure, high ionic conductivity, good thermal stability, tunable viscosity, and generally good miscibility with water and organic solvents [2 – 4]. ILs have been extensively used in various analytical separation techniques including chromatography and CE as reported in recent reviews [4 – 8].

ILs exhibit dual properties, *i.e.*, they can be used as polar stationary phases to separate polar compounds or as nonpolar stationary phases to separate nonpolar compounds [9, 10]. In addition, the application of ILs can be extended to chiral separations by use of either a chiral IL selector [9 – 12] or by dissolving a chiral selector in an achiral IL [13]. Although the separation of enantiomers has become fairly routine, many chiral selectors cannot distinguish closely related chiral molecules, and as a result, there can be considerable enantiomeric peak overlap. Therefore, additives/modifiers, may be added to the mobile phase in LC or to the BGE in CE in order to achieve enantiomeric separation of these closely related chiral molecules.

Correspondence: Professor Isiah M. Warner, Department of Chemistry, LA State University, Baton Rouge, Louisiana, USA
E-mail: iwarner@lsu.edu
Fax: +1 225-578-3971

Abbreviations: BEE, benzoin ethyl ether; **BME,** benzoin methyl ether; **$BMIMBF_4$,** 1-butyl-3-methylimidazolium tetrafluoroborate; **BNA,** 1,1′-bi-2-naphthyl-2,2′-diamine; **BNP,** (±)-1,1′-bi-2-naphthyl-2,2′-diyl hydrogen phosphate; **BOH,** (±)-1,1′-bi-2-naphthol; **$EMIMBF_4$,** 1-ethyl-3-methylimidazolium tetrafluoroborate; **$HMIMBF_4$,** 1-hexyl-3-methylimidazolium tetrafluoroborate; **ILs,** ionic liquids; **MeOH,** methanol; **MOSs,** molecular organic solvents; **$OMIMBF_4$,** 1-octyl-3-methylimidazolium tetrafluoroborate; **Poly-L-SOLV,** polysodium oleyl-L-leucylvalinate; **1PrOH,** 1-propanol

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CE has become an important tool for the separation of charged analytes. This is due to the enhanced separation efficiency of CE for these analytes. Also, CE, when used in combination with a pseudostationary phase such as micelles or molecular micelles, offers a number of advantages for the separation of both neutral and charged analytes. This pseudostationary approach, referred to as micellar EKC (MEKC), has been evolving with the use of different pseudostationary phases. For example, in our laboratory, the binaphthyl derivatives (±)-1,1′-bi-2-naphthyl-2,2′-diyl hydrogen phosphate (BNP), 1,1′-bi-2-naphthyl-2,2′-diamine (BNA), and (±)-1,1′-bi-2-naphthol (BOH), with an asymmetric plane of chirality, have been separated using different monomeric and polymeric chiral surfactants by MEKC [14, 15]. These compounds are closely related and relatively easy to separate. However, when separating a mixture, the enantiomers of BOH and BNA overlap. To achieve baseline separation of coeluted peaks, one can increase the surfactant (chiral selector) concentration or use BGE modifiers, *i.e.*, molecular organic solvents (MOSs) or ILs.

The main role of MOSs in MEKC is to reduce the retention factor of the highly hydrophobic analytes. In most of the cases, the addition of MOSs leads to a reduction in the EOF, a change in velocity of the pseudostationary phase, and an increase in the size of the elution window, *i.e.*, the time between the elution of a neutral marker and the relatively large, slow moving micellar pseudostationary phase. Besides ILs, other modifiers such as urea and glucose have been applied in MEKC. Urea increases the elution window as well as the solubility of highly hydrophobic solutes in the BGE [16], while glucose, when added to the BGE, enhances resolution [17]. It should be noted that examination of ILs as BGE modifiers is really an examination of the effects of IL component ions since these IL systems are no longer ILs under the conditions examined. It should also be noted that other researchers have explored ILs as additives for separations in HPLC [18 – 24] and in CE [13, 25 – 29].

In a previous report, a newly synthesized molecular micelle, polysodium oleyl-L-leucylvalinate (poly-L-SOLV), was used for the enantiomeric separation of the binaphthyl derivatives described above. In addition, the effect of dissolving ILs in the BGE to resolve the overlapping enantiomer peaks of BOH and BNA was investigated [13]. The present work extends our previous study to include enantiomers with a stereogenic carbon which may be challenging to separate, and compares the effect of ILs to MOSs as BGE modifiers. The ILs (molecular structures shown in Fig. 1) were 1-ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$), 1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$), 1-hexyl-3-methylimidazolium tetrafluoroborate ($HMIMBF_4$), and 1-octyl-3-methylimidazolium tetrafluoroborate ($OMIMBF_4$). The significant properties of these ILs, *i.e.*, good solubility in aqueous solutions, hydrophobicity, and good conductivity, were considered favorable for the separations investigated here. The high solubility of the ILs in aqueous solutions is attributed to the tetrafluoroborate anion. However, as the IL cation alkyl chain length increases, the solubility in aqueous media decreases [2]. The above ILs were shown to assist in the separation of hydrophobic chiral analyte mixtures without adversely affecting the background current. In contrast, the MOSs methanol (MeOH), 1-propanol (1PrOH), and ACN led to longer migration times. High volumes of MOSs in the buffer (above 60% v/v) led to current breakdowns. A comparison was made between the use of 1,3-dialkylimidazolium-based ILs and the above mentioned MOSs.

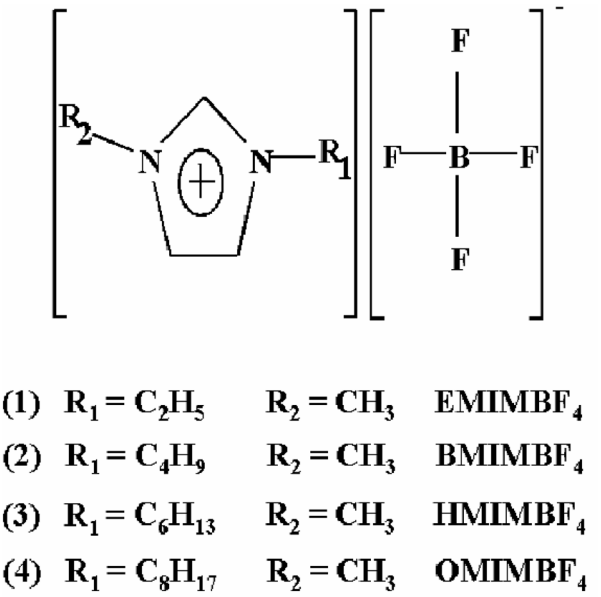

Figure 1. Chemical structures of the ILs used as BGE modifiers in the MEKC separation experiments.

2 Materials and methods

2.1 Reagents

The chiral compounds BNP, BNA, BOH, warfarin, coumachlor, benzoin, benzoin methyl ether (BME), and benzoin ethyl ether (BEE) were purchased from Sigma-Aldrich (Milwaukee, WI). The molecular structures of all the analytes are illustrated in Fig. 2. The oleic acid-*N*-hydroxysuccinimide ester, TRIS, THF, sodium bicarbonate, $EMIMBF_4$, $HMIMBF_4$, and $OMIMBF_4$ were also obtained from Sigma-Aldrich. Sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), and sodium borate (NaB_4O_7) were purchased from Amresco (Solon, OH), Mallinckrodt and Baker (Paris, KY), and Fisher Scientific (Fair Lawn, NJ), respectively. The MOSs MeOH, 1PrOH, and ACN were purchased from Fisher Scientific. $BMIMBF_4$ was

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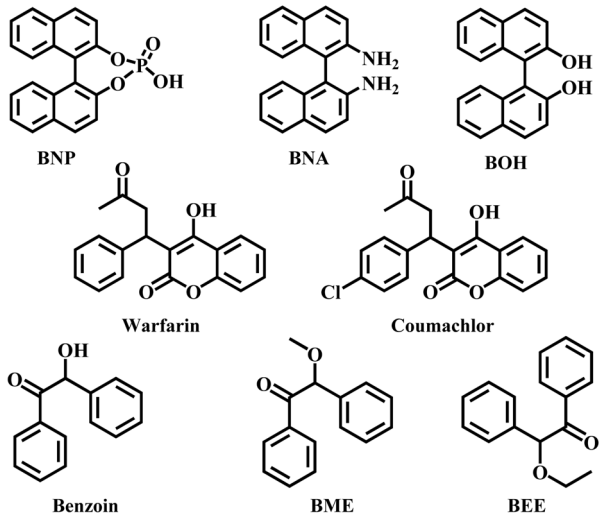

Figure 2. Chemical structures of the chiral analytes used in the current study.

purchased from Chemada Fine Chemicals (Nir Itzhak, Israel). The dipeptide leucine–valine was purchased from Bachem Bioscience (King of Prussia, PA). All the reagents were of analytical reagent grade and used as received.

2.2 Synthesis of poly-L-SOLV

The polymer poly-L-SOLV was synthesized using the modified procedure of Wang and Warner [30, 31]. The CMC of the (L-SOLV) surfactant monomer was determined to be 0.8×10^{-3} M using a surface tensionmeter from CSC Scientific Company (Fairfax, VA). A 6×10^{-3} M aqueous solution of L-SOLV was exposed to a ^{60}Co γ-ray source (70 krad/h) for a total of 7 days for polymerization. After polymerization, the aqueous solution was dialyzed against bulk water using a regenerated cellulose membrane with a 2000 Da molecular weight cutoff. The purified solution was lyophilized under vacuum to obtain the dry product of poly-L-SOLV. Proton NMR (^{1}H NMR) spectroscopy was used to follow the polymerization process. All the polymers used in this study were found to be greater than 99% pure as estimated by elemental analysis (data not shown).

2.3 Preparation of MEKC buffer solutions

The BGE for the separation of the binaphthyl derivatives was 100 mM TRIS mixed with 10 mM sodium borate at pH 10, while the BGE for the separation of warfarin, coumachlor, benzoin, BME, and BEE was 30 mM sodium monophosphate + 20 mM sodium diphosphate at pH 7.2. The pH of the BGE was adjusted before the molecular micelle and modifiers were added. The molecular micelle, in the concentration range of 0.005 – 0.015 g/mL, was added to the BGE and the solution was filtered through a 0.45 μm membrane. IL or organic solvent was then added as a BGE modifier. The resulting solution was used as the running buffer in CE separations in order to assess the effects of various modifiers on separation efficiency and resolution.

2.4 CE

The MEKC experiments were performed by use of a Hewlett-Packard 3D CE instrument (Foster City, CA) equipped with a UV diode array detector. Bare fused-silica capillaries, cut to a length of 60 cm long (52 cm effective length, 50 μm id), were purchased from Polymicro Technologies (Phoenix, AZ). For all the studies, the applied voltage was 30 kV, the detection wavelength was 254 nm, and the temperature of the capillary was maintained at 15°C by the instrument thermostating system. The analytes were prepared in 50:50 methanol/water at concentrations of 0.1 – 0.5 mg/mL depending on the analyte. The samples were introduced into the capillary by hydrodynamic injection at a pressure of 30 mbar for 3 s. Prior to use, each new capillary was conditioned for 60 min with 1 M NaOH followed by a 15 min rinse with triply distilled deionized water. Before each run, the capillary was flushed with the MEKC buffer for 3 min to condition and fill it. When comparing the effect of one IL to another, a new capillary was used in order to eliminate any influence of the previous IL cations. Elution orders were determined by spiking a single pure *S*-(–) enantiomer into the solution of the corresponding racemic analytes. The equations for the calculation of resolution (R_s) and RSD were obtained from references [32, 33]. At least three measurements were made for each experimental condition for which RSD values were calculated. The selectivity (α) is defined as k'_2/k'_1, where k'_1 and k'_2 are the retention factors of the first and second eluting enantiomers [34], respectively. Methanol was used as the neutral (t_0) marker and was measured from the time of injection to the first deviation from the baseline. Dodecanophenone was used as tracer for elution time of the micelle (t_{mc}) at 0.01 g/mL molecular micelle concentration in the buffer containing either the IL or the MOSs.

3 Results and discussion

3.1 Effects of ILs on the separation of binaphthyl derivatives

This group of compounds has a varying degree of hydrophobicity and charge states under the experimental conditions used in this work. Using the molecular micelle, poly-L-SOLV in a 100 mM TRIS + 10 mM sodium borate buffer, the *R*-(+) and *S*-(–) enantiomers of each binaphthyl

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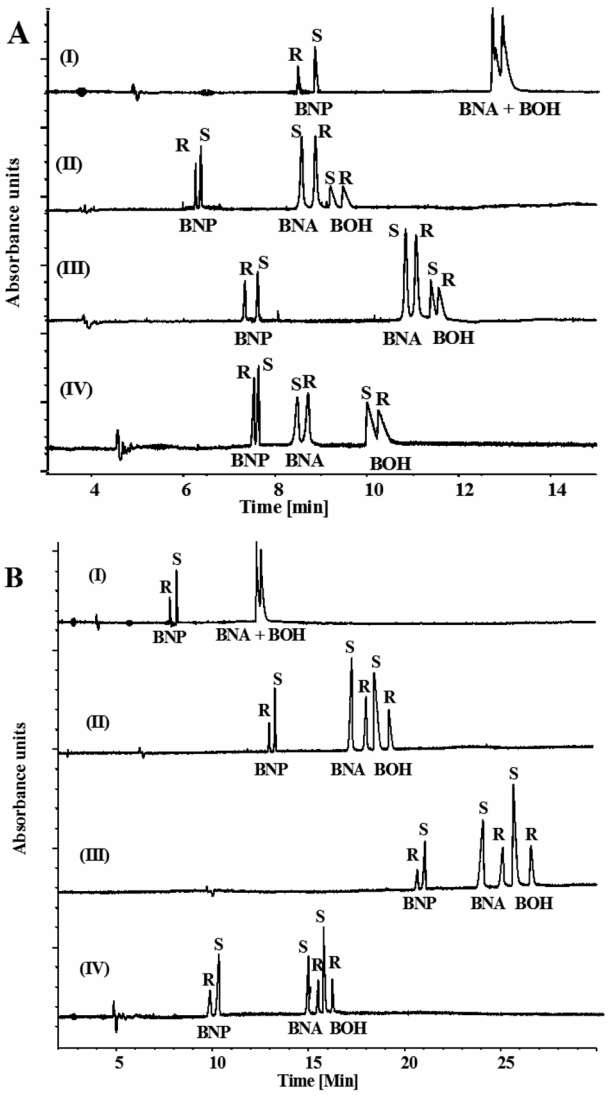

Figure 3. Electropherograms showing the separation of the three binaphthyl derivatives BNP, BNA, and BOH using (**A**) (I) no BGE modifier, (II) 0.06% v/v $EMIMBF_4$, (III) 0.02% v/v $BMIMBF_4$, and (IV) 0.02% v/v $HMIMBF_4$ as modifiers. (**B**) (I) no BGE modifier, (II) 20% MeOH, (III) 20% 1PrOH, and (IV) 10% ACN as modifiers. Conditions: 0.005 g/mL poly-L-SOLV, 100 mM TRIS + 10 mM sodium borate buffer, pH: 10.0, injection size: 30 mbar for 3 s, temp: 15°C, voltage: 30 kV, absorbance detection at 254 nm.

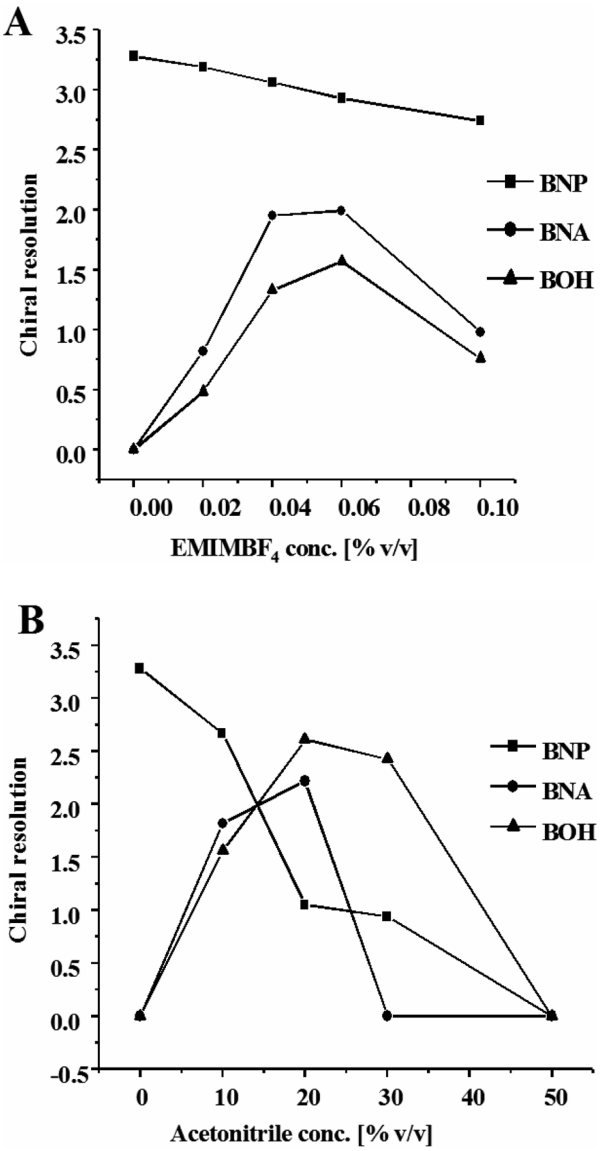

Figure 4. Plots of chiral resolution of BNP (-■-), BNA (-●-), and BOH (-▲-) *vs.* (**A**) $EMIMBF_4$ concentration and (**B**) ACN concentration.

derivative could only be resolved individually, not in a mixture as shown in Fig. 3A, electropherogram I. We investigated the effect of using several different 1,3-dialkylimidazolium tetrafluoroborate ILs as BGE modifiers for the separation of a mixture of enantiomers of the three analytes. A concentration study was done using 0.02 – 0.1% v/v of each IL, and a graph of resolution of each analyte *versus* the IL concentration was generated. A plot of chiral resolution of the three analytes *versus* $EMIMBF_4$ concentration is shown in Fig. 4A and is representative of all ILs except $OMIMBF_4$. A slight decrease in chiral resolution was observed for BNP as the concentration of the IL modifier increased. The chiral and achiral resolution of BNA and BOH was enhanced with an increase in the IL concentration up to an optimum value based on the particular IL used. However, for concentrations greater than optimum, a gradual drop in the chiral resolution was observed. The use of ILs as BGE modifiers led to better selectivity between enantiomers of BNA and BOH, with selectivity increasing as the IL concentration increased from 0.02 to 0.06% v/v. Generally, IL concentrations greater than 0.02% v/v led to a decrease in the chiral resolution of each analyte but an enhancement of selectivity and achiral resolution of the three analytes.

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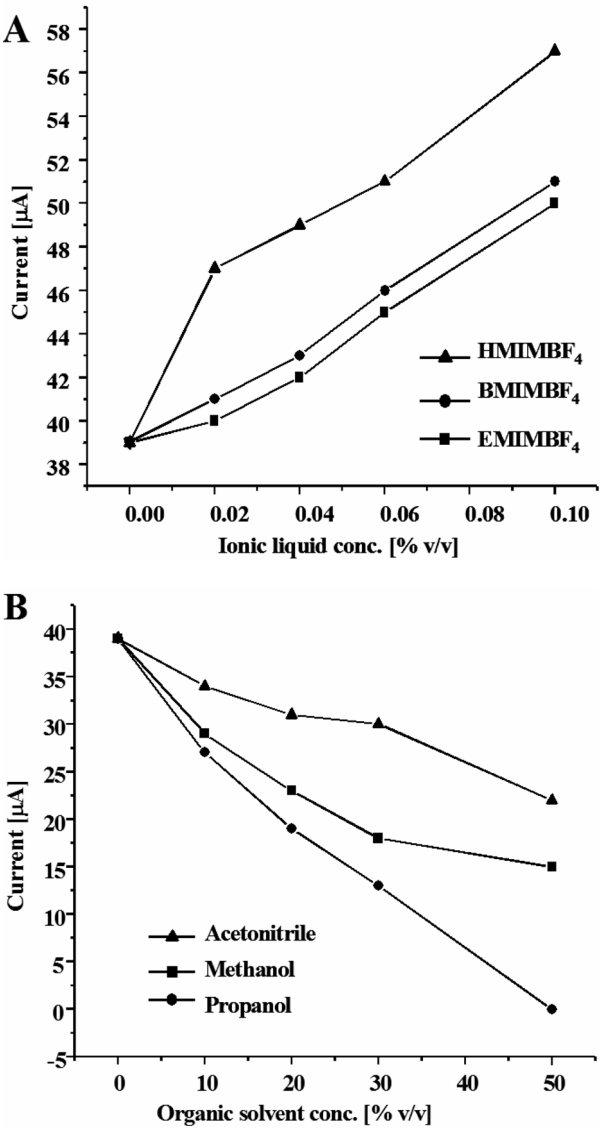

Figure 5. Plots of observed current *vs.* (**A**) concentration of $EMIMBF_4$ (-■-), $BMIMBF_4$ (-●-), $HMIMBF_4$ (-▲-), and (**B**) concentration of MeOH (-■-), 1PrOH (-●-), and ACN (-▲-).

Electropherograms II – IV in Fig. 3A illustrate the optimum separation of a mixture of the binaphthyl derivatives with the addition of optimum concentrations of $EMIMBF_4$, $BMIMBF_4$, and $HMIMBF_4$, respectively. The *R*-(+) enantiomer of BNP eluted faster than the *S*-(–) enantiomer. However, for BNA and BOH the *S*-(–) enantiomer eluted first. This indicates that the *S*-(–) enantiomer of BNA and BOH interacted less with the chiral selector as compared to the *R*-(+) enantiomer of the same compounds.

In addition, Fig. 5A illustrates the enhancement in current as the concentration of IL increased. This enhancement was due to the conductive nature of the ILs. Moreover, a slight increase in the EOF was observed when 0.02 – 0.06% v/v of IL was added (data not shown), but above this concentration a gradual drop in the EOF was observed. This is possibly due to the ability of the imidazolium-based cation to adsorb to the walls of the capillary for sufficiently high IL concentrations. Alternatively, the IL cation may replace the sodium counterion within the electric double layer. If the IL modifies the capillary surface, the EOF and elution time of the analytes should be altered. The abnormality in the tendency of the value of EOF to increase for the lowest IL concentrations added to the BGE is likely due to a slight enhancement in current and the compression of the Helmholtz/mobile layer. A detailed explanation of this phenomenon was presented in our previous study [13]. Of the three ILs presented here, 0.06% v/v $EMIMBF_4$ provided the best resolution as well as the shortest elution time, as shown in electropherogram II, Fig. 3A. In contrast to $EMIMBF_4$, the optimum concentrations for $BMIMBF_4$ and $HMIMBF_4$ were 0.02% v/v for both ILs. The increase in hydrophobicity, viscosity, and conductivity of $BMIMBF_4$ or $HMIMBF_4$ relative to $EMIMBF_4$ could account for the lower concentration required to achieve similar results. It was not clear why $HMIMBF_4$ had a nonlinear increase in current compared to $EMIMBF_4$ and $BMIMBF_4$. Also, we are unable to provide a specific reason why the observed current increases with an increment in alkyl chain length of the imidazolium cation. To the best of our knowledge, the available literature indicates that an increase in the IL concentration in aqueous media leads to a corresponding increase in conductivity up to an optimum concentration after which a decrease in the conductivity is observed [35 – 37]. However, studies are yet to be done that could define the conductivity of ILs in organized media.

The other IL investigated, $OMIMBF_4$, caused peak splitting of BNP. In addition, it resolved the coeluted peaks of BNA and BOH with partial separation of enantiomers of BNA and poor enantiomeric separation of BOH (data not shown). This IL has the highest hydrophobicity and viscosity and reduced water solubility due to its octyl alkyl chain on its cation [2]. Therefore, these properties may have led to unfavorable interactions between the chiral analytes and chiral molecular micelle, thus preventing enantiomeric separation.

3.2 Effects of MOSs on the separation of binaphthyl derivatives

The effect of using MOSs (MeOH, 1PrOH, or ACN) as BGE modifiers in the separation of the three binaphthyl derivatives was also investigated. To determine the optimum concentration of each MOS, a concentration study was performed using 10, 20, 30, and 50% v/v MOS/buffer. The optimum concentration of MOS was found to be 20% for MeOH and 1PrOH and 10% for ACN (Fig. 3B, electrophero-

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Table 1. Select physical properties of the ILs and MOSs used

Compound	Molecular mass (g/mol)	Melting point (°C)	Density (g/mL)	Dielectric constant at 20°C	Water solubility	Reference
$EMIMBF_4$	198	15	1.28 (25°)	Na[a)]	Miscible	[3, 4]
$BMIMBF_4$	226	−81	1.17 (30°)	Na	Miscible	[3, 4]
$HMIMBF_4$	254	Na	Na	Na	Miscible	[4]
$OMIMBF_4$	282	Na	Na	Na	Na	
Methanol	32	−97.6	0.791	33.0	Miscible	[40]
1-Propanol	60	−126.1	0.803	20.8	Miscible	[40]
ACN	41	−44	0.796	36.64	Miscible	[40]

[a)] Not available.

Table 2. Elution times of the first enantiomer (t_{r_1}) and second enantiomer (t_{r_2}) as well as selectivity (α), resolution (R_s), and RSD of the resolution for BNP, BNA, and BOH at the optimum concentration (used in Figs. 3A and B) of each BGE modifier

Modifier concentration v/v	BNP					BNA					BOH				
	$R\,t_{r_1}$	$S\,t_{r_2}$	α	R_s	RSD	$S\,t_{r_1}$	$R\,t_{r_2}$	α	R_s	RSD	$S\,t_{r_1}$	$R\,t_{r_2}$	α	R_s	RSD
$EMIMBF_4$ (0.06%)	6.3	6.5	1.06	3.0	0.03	9.0	9.2	1.04	2.2	0.03	9.6	9.7	1.02	1.2	0.07
$BMIMBF_4$ (0.02%)	7.3	7.6	1.08	3.3	0.02	10.8	11.1	1.03	1.7	0.03	11.5	11.6	1.02	1.1	0.04
$HMIMBF_4$ (0.02%)	6.9	7.0	1.08	1.6	0.08	7.9	8.2	1.07	1.5	0.06	9.7	9.7	1.03	1.1	0.09
MeOH (20%)	13.0	13.3	1.05	3.1	0.03	17.3	18.0	1.07	3.7	0.08	18.4	19.2	1.06	2.7	0.06
1PrOH (20%)	20.5	20.9	1.04	2.0	0.01	23.8	24.8	1.07	3.1	0.08	25.3	26.1	1.06	3.1	0.01
ACN (10%)	9.4	9.6	1.05	2.7	0.02	14.4	14.8	1.05	1.8	0.09	15.1	15.5	1.04	1.2	0.02

grams II–IV). MeOH provided optimal separation of the three analytes in terms of chiral resolution and selectivity. However, the addition of ACN provided the highest peak efficiencies as well as shortest elution time. Figure 4B is a representation of the effect of the MOS concentration on resolution of the three binaphthyl derivatives. Generally, there was a drastic drop in the chiral resolution of BNP. Conversely, both the chiral and achiral resolution of BNA and BOH were slightly enhanced. However, after the optimum MOS concentration was surpassed, the chiral resolution of these two analytes began to decline. It should be noted that the achiral resolution between BNA and BOH was greatly enhanced as the concentration of MeOH, 1PrOH, or ACN was increased.

Increasing the concentration of MOS caused a decline in the observed current (Fig. 5B) leading to an increase in elution time of the three binaphthyl derivatives. The decrease in current is likely due to a reduction in dielectric constant caused by the MOSs [38]. In addition, a current breakdown was observed when buffers containing more than 60% v/v MOSs were used. This is possibly due to a mismatch between the sample zone and separation buffer, which may have led to a high resistance at the interface of the zones when the separation voltage was applied. Therefore, air bubbles were likely to form due to boiling of the MOS by joule heating at the interface, thus influencing or breaking down the separation. A summary of the physical properties of the four ILs included in this study, as well as MeOH, 1PrOH, and ACN, is provided in Table 1. MeOH, with a moderate dielectric constant (≈33 at 20°C) and the capability of undergoing autoprotolysis, is similar to water. It is a neutral amphiprotic solvent in which solvation is favored due to the formation of hydrogen bonds [38]. The 1PrOH is a polar, water-soluble alcohol with medium volatility and a dielectric constant of ≈21 at 20°C. Because of a strong dipole moment, the dielectric constant of ACN (≈37 at 20°C) is higher than that of MeOH and 1PrOH. ACN, a dipolar aprotic solvent with a weak autoprotolysis constant, is also very different from MeOH and 1PrOH. The separation results obtained using these MOSs were consistent with the above-mentioned properties.

The enantiomer elution order of the binaphthyl analytes remained unchanged when the ILs or the MOSs were used as additives. The ILs used led to faster separations without adversely affecting the current. The chiral and achiral resolution, and selectivity of the analytes were dependent on the concentration and type of IL or organic solvent used. Table 2 lists the elution times of the first enantiomer and second enantiomer, t_{r_1} and t_{r_2} respectively, the selectivity (α), as well as chiral resolution (R_s) for BNP, BNA, and BOH at the optimum concentration for each BGE modifier.

3.3 Effects of ILs and MOSs on the separation of coumarin derivatives

Warfarin and coumachlor are both coumarin derivatives. These two structurally related acidic drugs differ only by a chlorine substituent (Fig. 2). $EMIMBF_4$ or

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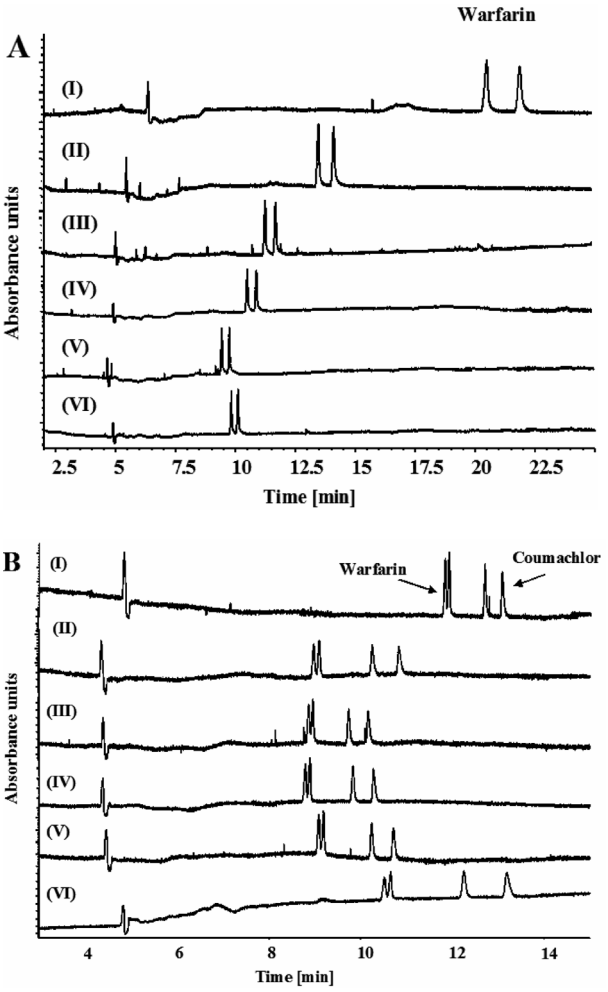

Figure 6. Electropherograms showing the separation of (**A**) warfarin adding $BMIMBF_4$ and (**B**) warfarin and coumachlor adding $BMIMBF_4$ as BGE modifiers at concentrations of (I) no modifier, and (II–VI) 0.02, 0.04, 0.06, 0.1, and 0.2% v/v, respectively. Conditions: 0.01 g/mL poly-L-SOLV, 30 mM NaH_2PO_4 + 20 mM Na_2HPO_4 buffer, pH: 7.2, injection size: 30 mbar for 3 s, temp: 15°C, voltage: 30 kV, absorbance detection at 254 nm.

$BMIMBF_4$ were added to the buffer, and their effect on warfarin and coumachlor separation was investigated. With the exception of elution time, both ILs showed similar results. The addition of $BMIMBF_4$ resulted in shorter elution times. Figure 6A shows the effect of $BMIMBF_4$ concentration on the separation of warfarin. As with the binaphthyl derivatives, increasing the IL concentration enhanced the EOF, led to shorter elution times, and reduced resolution. Despite a reduction in resolution, baseline separation was achieved over the entire IL concentration range studied. The reduced resolution may be due to the increase in EOF, causing the analyte to interact less with the pseudostationary phase. At 0.2% v/v IL, a slight decrease in EOF and increase in elution time of warfarin is observed when compared with the separation obtained using 0.1% v/v IL. As mentioned previously, the decrease in EOF is likely due to modification of the capillary wall by the imidazolium-based cation which would affect column performance.

The separation of a mixture of warfarin and coumachlor was also investigated using $EMIMBF_4$ and $BMIMBF_4$ as BGE modifiers. As mentioned above, warfarin and coumachlor have similar molecular structures, thus it was expected that they would have very similar chiral interactions with the pseudostationary phase and would coelute or elute close to each other. When injected alone, warfarin spends more time in the molecular micelle, and its chiral resolution is enhanced (Fig. 6A). However, when the two analytes were injected in a mixture, the separation indicates that they possibly compete for the same binding sites in the molecular micelle. Coumachlor spends more time in the molecular micelle, thus elutes later, and its enantiomers are better resolved (Fig. 6B (I)). Conversely, warfarin seems to spend less time in the molecular micelle, thus elutes faster and its chiral resolution is highly reduced compared to when it is injected alone as shown by the electropherograms obtained with no modifier in Figs. 6B (I) and 6A (I), respectively.

When $EMIMBF_4$ or $BMIMBF_4$ were used as BGE modifier for the separation of these two coumarin derivatives, results consistent with those observed for the separation of the binaphthyl derivatives were obtained. The EOF was enhanced as the IL concentration increased from 0.02 to 0.06% v/v (Fig. 6B electropherograms (II) to (IV)); however, at a concentration of 0.1% v/v and above the EOF was slightly reduced, leading to longer migration times of the analytes. The achiral resolution of warfarin and coumachlor and the chiral resolution of coumachlor were slightly enhanced when 0.2% v/v $BMIMBF_4$ was added to the BGE (Fig. 6B electropherogram (VI)). Thus, addition of these ILs may have enhanced the interaction of one enantiomer of coumachlor with poly-L-SOLV, the IL may have provided a more hydrophobic environment allowing coumachlor to spend more time in the molecular micelle, or the increase in resolution may have been due to a slight decline in EOF. However, the interactions of the enantiomers with the chiral selector and/or IL, are complex and the cause or mechanism of this interaction is not well understood at this time.

MOSs were also investigated as modifiers in the separation of a mixture of warfarin and coumachlor. All three MOSs, MeOH, 1PrOH, and ACN, led to loss of chiral resolution and selectivity of these analytes. In addition, long migration times and poor baselines were observed (data not shown). The long migration times may be attributed to poor current and poor formation of the electrical double layer. The cause of the poor baselines is unknown. However, it was clear these MOSs did not enhance chiral interactions between the coumarin derivatives investigated and the chiral molecular micelle.

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3.4 Effects of ILs and MOSs on the separation of benzoin derivatives

Additional chiral analytes of benzoin, BME, and BEE were separated. When no modifier was used, baseline separation of the enantiomers of benzoin and BME was obtained, but poor enantiomeric separation was observed for BEE. The ILs and MOSs, at the same concentration range as previously described, were used as modifiers in the separation of these analytes. However, no modifier enhanced the enantiomeric separations of these analytes. In addition, increasing the amount of either IL or organic solvent modifier in the running buffer led to a loss of chiral resolution for BME and BEE (data not shown). The BGE modifiers may have caused unfavorable chiral interactions, thus leading to the results observed.

We are actively investigating the use of ILs as modifiers in MEKC with the goal of understanding the effect of these modifiers in chiral and achiral separations. In addition, other techniques, such as steady state and time-resolved fluorescence are being investigated to gain a better understanding of IL properties and their effect on the behavior of molecular micelles and analytes of interest. At higher IL concentration, the ionic environment may be slightly altered and/or the hydrophobic environment may be enhanced. The latter may cause the analytes to experience stronger interactions with the pseudostationary phase and may lead to a longer elution time, and an enhancement or loss of the chiral and achiral resolution of the chiral analytes. Nevertheless, there are multiple modes of interaction that facilitate chiral separation, *e.g.*, hydrogen bonding, dipole – dipole interactions, steric interactions, and electron-pair π-donor from analyte aromatic rings, as well as van der Waals forces. Some of these interactions are in accord with the three-point rule for chiral recognition described by Dalgliesh [39]. However, total enantioselectivity is strongly dependent on composition, temperature, and pH of the BGE. Thus, it is necessary to optimize the separation medium in order to maximize the interactions necessary for chiral separations.

4 Concluding remarks

Successful separations of individual chiral analytes and mixtures were achieved for binaphthyl and coumarin derivatives using three ILs ($EMIMBF_4$, $BMIMBF_4$, and $HMIMBF_4$) as modifiers in MEKC. The fourth IL ($OMIMBF_4$) did not provide favorable interactions for chiral separation. MOSs were also successfully used as modifiers in the separation of binaphthyl derivatives. The resolution of benzoin, BME, and BEE was not affected by the presence of any of the above modifiers at the same concentration range used for separation of the other chiral analytes, but was reduced at higher concentrations. The elution order of the enantiomers remained unaltered by the presence of any BGE modifier. ILs at the concentrations used led to faster separations without adversely affecting the current, while high volumes of MOSs in the buffer led to current breakdowns. In addition, chiral and achiral resolution and selectivity of the analytes were dependent on the concentration and type of modifier used. However, smaller IL volumes were needed, as compared to MOSs, in order to achieve equivalent chiral resolution and selectivity.

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