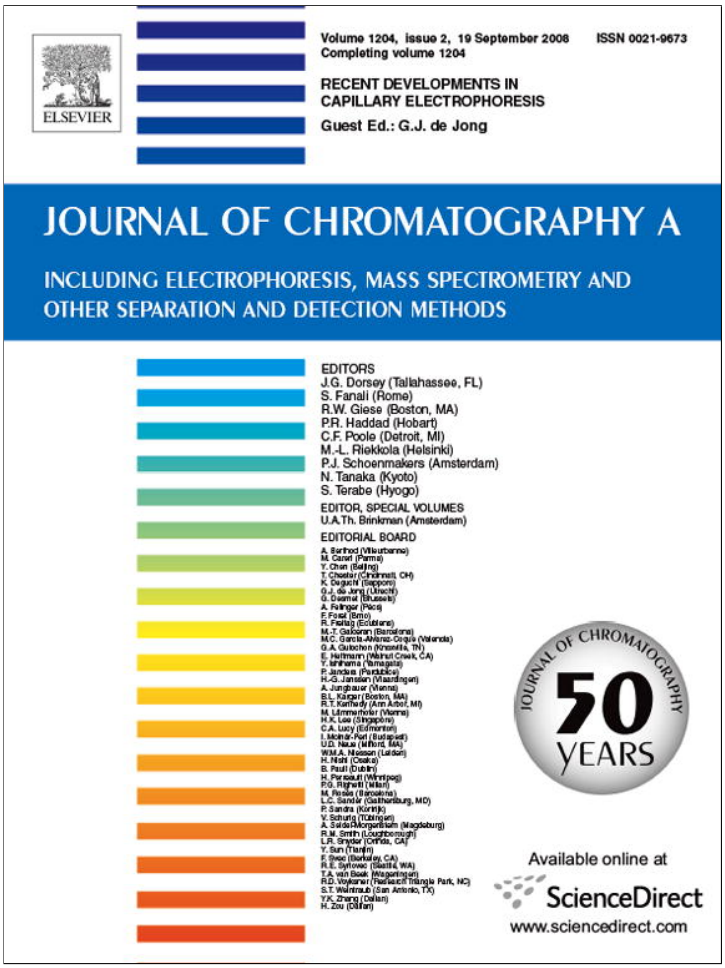

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Chiral ionic liquids for enantioseparation of pharmaceutical products by capillary electrophoresis

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ABSTRACT

A chiral ionic liquid (IL), *S*-[3-(chloro-2-hydroxypropyl)trimethylammonium] [bis((trifluoromethyl) sulfonyl)amide] (*S*-[CHTA]$^+$[Tf_2N]$^-$), which can be easily and readily synthesized in a one-step process from commercially available reagents, can be successfully used both as co-electrolyte and as a chiral selector for CE. A variety of pharmaceutical products including atenolol, propranolol, warfarin, indoprofen, ketoprofen, ibuprofen and flurbiprofen, can be successfully and baseline separated with the use of this IL as electrolyte. Interestingly, while *S*-[CHTA]$^+$[Tf_2N]$^-$ can also serve as a chiral selector, enantioseparation cannot be successfully achieved with *S*-[CHTA]$^+$[Tf_2N]$^-$ as the only chiral selector. In the case of ibuprofen, a second chiral selector, namely a chiral anion (sodium cholate), is needed for the chiral separation. For furbiprofen, in addition to *S*-[CHTA]$^+$[Tf_2N]$^-$ and sodium cholate, a third and neutral chiral selector, 1-*S*-octyl-β-D-thioglucopyranoside (OTG), is also needed. Due to the fact that the chirality of this chiral IL resides on the cation (i.e., -[CHTA]$^+$), and that needed additional chiral selector(s) are either chiral anion (i.e., cholate) or chiral neutral compound (OTG), the results obtained seem to suggest that additional chiral selector(s) are needed to provide the three-point interactions needed for chiral separations.

1. Introduction

Enantiomeric separation is an important subject in science and technology. The popularity stems from the fact that very often, only one form of enantiomers is chemically and/or biologically active. The other or others can reverse or otherwise limit the effect of the desired enantiomer [1–5]. It is thus hardly surprising that pharmaceutical industry needs effective chiral separation methods. High-performance liquid chromatography (HPLC) and gas chromatography (GC) are the two most widely used methods for chiral separations [1–5]. The recent advances in the capillary electrophoresis have provided an alternative means for chiral separations, especially for cases where samples are available in limited amounts, and when short analysis time is needed [6–18]. However, the number of chiral selectors which is known to be effective in CE is considerably less than the chiral stationary phases available in GC and HPLC [1–18]. As a consequence, search for new chiral selectors becomes an important issue in CE separation.

A variety of compounds including cyclodextrins (CDs), crown ethers, proteins, polysaccharides, macrocyclic antibiotics, chiral micelles and metal–chiral ligand complexes can be effectively used as chiral selectors for CE [6–18]. Unfortunately, when used by themselves (i.e., without any additives), these chiral selectors may not provide adequate selectivity and resolution. In some cases, more than one chiral selectors are needed for chiral separation. For example, as a sole chiral selector CD cannot provide chiral separation by CE for some amino acids but when used in combination with other chiral selector such as 1-*S*-octyl-β-thioglucopyranoside or 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate, it provided baseline separation for these compounds [15,16]. Considerable efforts have, therefore, been made in the last few years to find novel chiral selectors which can offer high selectivity and resolution for enantioseparation by CE. Chiral ionic liquids offer an attractive solution for this problem because in addition to being chiral, they are ionic liquid at room temperature, and hence, can also serve as electrolyte for CE.

Ionic liquids (ILs) are a group of organic salts that are liquid at room temperature [19–23]. They have unique chemical and physical properties, including the fact that they are air and moisture stable, have a high solubility power, and have virtually no vapor pressure [19–23]. Because of these properties and also because some ILs have either non-toxicity or relatively lower toxicity than volatile organic compounds that are traditionally used as industrial solvents, they can serve as a "green" recyclable alternative

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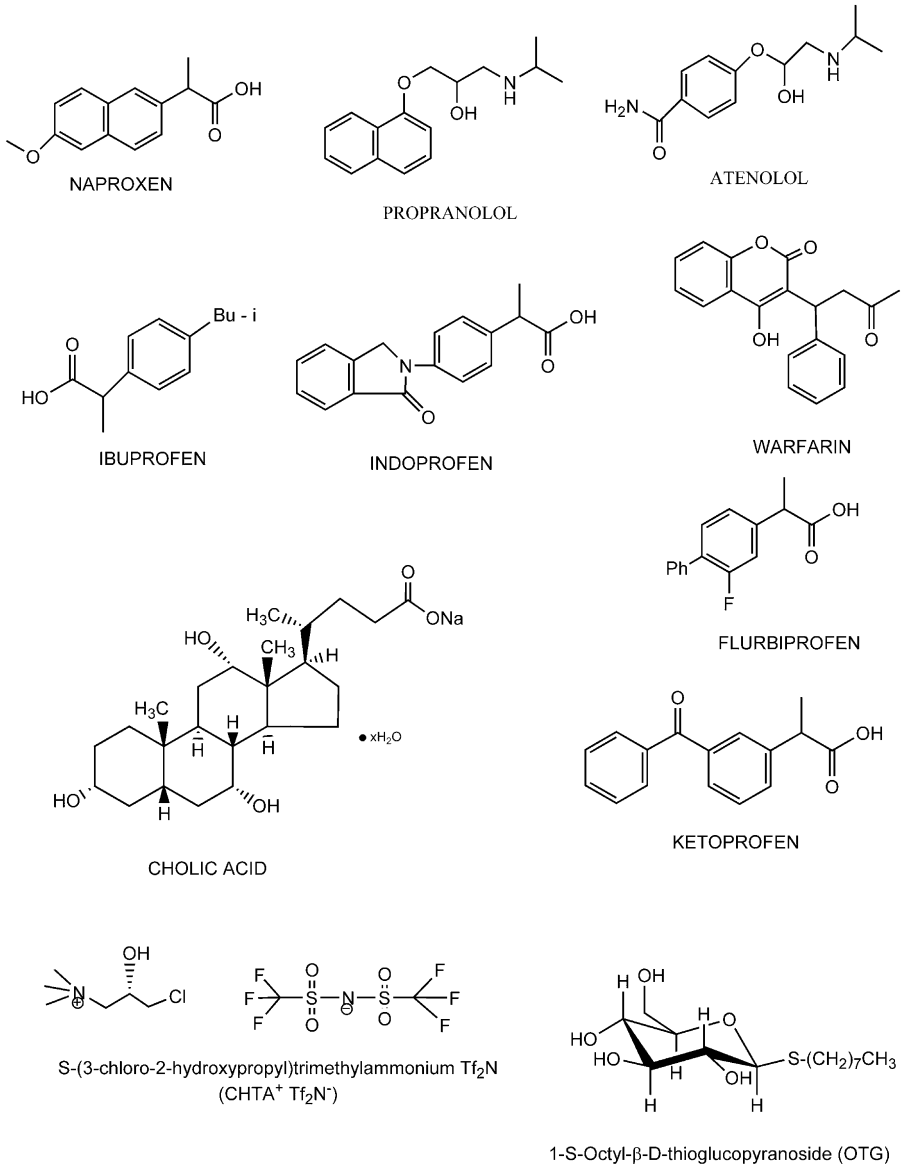

Fig. 1. Structure of compounds used in this study.

solvents [19–23]. The ILs have, in fact, been successfully used in many applications, including replacing traditional organic solvents in (1) organic and inorganic syntheses, (2) solvent extractions, (3) liquid–liquid extractions, (4) electrochemical reactions and (5) as a medium to enhance the sensitivity of thermal lens measurements [19–27]. Advances in ILs have made synthesis of chiral ILs a subject of intense study in recent years. The popularity stems from the fact that it is possible to use chiral ILs as chiral solvents for optical resolutions, for asymmetric induction in synthesis and as chiral stationary phase in chromatography [19,20]. It may, therefore, be possible to use chiral IL as chiral selector for CE. Because of their ionic nature, when used in CE, the chiral IL also provide additional advantage, namely, they can also serve as electrolyte as well. Unfortunately, chiral ILs are not commercially available. Only a few chiral ILs have been synthesized, and the synthesis of reported chiral ILs required rather expensive reagents and elaborated synthetic schemes [19,20]. Because of these limitations, in spite of potential of chiral ILs and extensive efforts made by various groups, to date, the study and applications of chiral ILs either as electrolyte and/or chiral selector in CE have been severely hindered.

We have demonstrated recently that both enantiomeric forms of a novel chiral ionic liquid, *R*- and *S*-(3-chloro-2-hydroxypropyl) trimethylammonium Tf_2N^- ((*R*)- and *S*-$[CHTA]^+[Tf_2N]^-$) can be readily synthesized in enantiomerically pure form by a simple ion exchange reaction from corresponding (*R*)- and (*S*)-chloro-2-hydroxypropyl)trimethylammonium chloride salts which are commercially available [25,26]. (*R*)- and *S*-$[CHTA]^+[Tf_2N]^-$ are liquid at room temperature (glass transition temperature of −58.4 °C) and are thermally stable up to at least 400 °C. More importantly, near-infrared and ^{19}F NMR studies shows that that optically active *R*- and *S*-$CHTA^+$ Tf_2N^- ionic liquid do exhibit relatively strong enantiomeric recognition and that their chiral recognition is relatively stronger than those for other reported chiral ILs [19,20,25].

The information presented is, indeed, provocative and clearly demonstrate that it is possible to exploit the ionic nature, the high solubility power and the enantiomeric recognition ability of this chiral *S*-$[CHTA]^+[Tf_2N]^-$ ionic liquid for enantioseparation by CE.

Such considerations prompted us to initiate this study to explore the use of this chiral IL as both electrolyte and chiral selector for CE. Preliminary results are reported in this communication.

2. Experimental

2.1. Chemicals

Cholic acid, sodium salt hydrate, 98%, tris(hydroxymethyl) aminomethane, 99 + % (*S*)-(−)-(3-chloro-2-hydroxypropyl)trimethylammonium chloride ($CHTA^+Cl^-$), 99% *N*-lithiotrifluoromethane ($Li^+Tf_2N^-$), 99.95%, atenolol, (*S*)-naproxene, warfarin, (*R*)- and (*S*)- and (*RS*)-flurbiprofen, (*RS*)- and (*S*)-ibuprofen, indoprofen, (*S*)- and (*RS*)-ketoprofen, were obtained from Sigma–Aldrich (Milwaukee, WI, USA). (*R*)-Naproxene was a product of Fluka (Buchs, Switzerland), 1-*S*-octyl-β-thioglucopyranoside (OTG) was from Pierce (Rockford, IL, USA), propranolol and (*R*)- and (*S*)-ibuprofen were from ICN Biomedicals (Aurora, OH, USA) and Biomol Research Labs (Plymouth Meeting, PA, USA), respectively.

S-[3-Chloro-2-hydroxypropyl trimethylammonium] [bis((trifluoromethyl)sulfonyl)amide] (*S*-$[CHTA]^+[Tf_2N]^-$) was prepared using the same procedure as reported in our previous papers [25,26]. Essentially, 1.88 g (10 mmol) of (*S*)-(−)-(3-chloro-2-hydroxypropyl) trimethylammonium chloride and 2.87 g (10 mmol) of *N*-lithiotrifluoromethane sulfonimide were each dissolved in 10 mL of distilled water. Two solutions were then mixed together and stirred for 2.5 h at room temperature. The mixture formed two layers. Top layer was removed; the remaining layer was washed with distilled water several times. After drying under vacuum at 60 °C for 10–12 h, the product was characterized by ^1H and ^{13}C NMR, IR, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC).

2.2. Methods

All capillary electrophoresis measurements were performed using the same instrument as in our previous studies [15,16], i.e., the ISCO Model 3850 electropherograph equipped with a UV detector (Isco, Lincoln, NE, USA) and coupled to a personal computer. Data were retrieved and analyzed by an in-house written software. An untreated, bare fused silica capillary (MicroSolv Technology, Eatontown, NJ, USA) with dimensions of 50 cm × 50 μm (effective length 37 cm to detector) was used throughout the study. Before use, the capillary was rinsed with 0.5 M sodium hydroxide, distilled water and separation medium (each for 10–15 min). Between analyses the capillary was washed with distilled water and separation medium. Study was conducted at applied voltage in the range from 10 to 30 kV with a positive potential at the injector end. All samples were injected using vacuum injection mode (5 s injection time) and were monitored at 214 nm. *N*,*N*-Dimethylformamide was used as neutral marker to determine electroosmotic mobility. Sample solutions were prepared by dissolving ca. 0.5 mg of each analyte in 1 mL of separation buffer.

3. Results and discussion

It is expected, based on its ionic structure, that the chiral IL may be used as electrolyte for CE. This possibility was investigated by using a 50 mM aqueous solution of *S*-$[CHTA]^+[Tf_2N]^-$ as the buffer solution which has pH 6.25 for separation of eight pharmaceutical compounds shown in Fig. 1. No separation was found with this buffer (i.e., all compounds were eluted as a single broad peak). Interestingly, as shown in Fig. 2A, when 30 mM of an anionic surfactant, cholic acid (see Fig. 1 for structure),

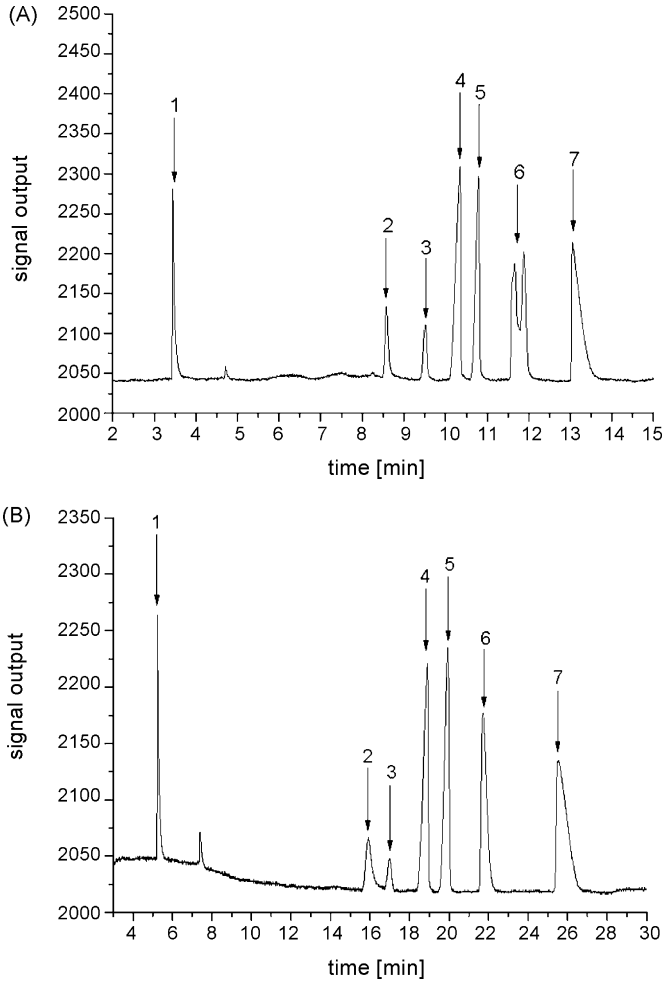

Fig. 2. Electropherograms of a sample containing a mixture of seven compounds. Bare fused-silica capillary 50 cm (effective length, 37 cm) × 50 μm I.D. electrolyte: 20 mM *S*-$[CHTA]^+[Tf_2N]^-$, 30 mM sodium cholate; applied voltage: (1) Atenolol, (2) propranolol, (3) warfarin, (4) indoprofen, (5) ketoprofen, (6) ibuprofen and (7) flurbiprofen. (A) 25 kV and with *RS*-ibuprofen; (B) 18 kV and with *S*-ibuprofen.

was added into an aqueous solution of 20 mM *S*-$[CHTA]^+[Tf_2N]^-$, and the mixture was used as a buffer, baseline separation of the mixture of seven compounds including atenolol, propranolol, warfarin, indoprofen, ketoprofen, ibuprofen and flurbiprofen was achieved (naproxen was not included in this mixture as it was found that with this buffer, the elution time of naproxen is almost the same as that of ibuprofen). Of particular interest is band no. 6 which corresponds to *RS*-ibuprofen. As illustrated, this band contains two equal peaks. Since a racemic mixture of ibuprofen was used in the mixture, the result seems to indicate that this buffer is capable of promoting chiral separation of ibuprofen. To confirm this possibility, the CE experiment was repeated with the same buffer and the same seven compounds except *RS*-ibuprofen was replaced with optically active *S*-ibuprofen. The electropherogram obtained (Fig. 2B) is the same as that obtained previously (Fig. 2A) except in this case, band #6 which corresponds to *S*-ibuprofen has only a single peak. Electropherogram (not shown) similar to that shown in Fig. 2B was obtained when the other enantiomer of ibuprofen, i.e., *R*-ibuprofen was used). These results clearly indicate that a buffer containing 20 mM of *S*-$[CHTA]^+[Tf_2N]^-$ and 30 mM of sodium cholate can successfully serve not only as electrolyte but also as chiral selector for enantioseparation of ibuprofen.

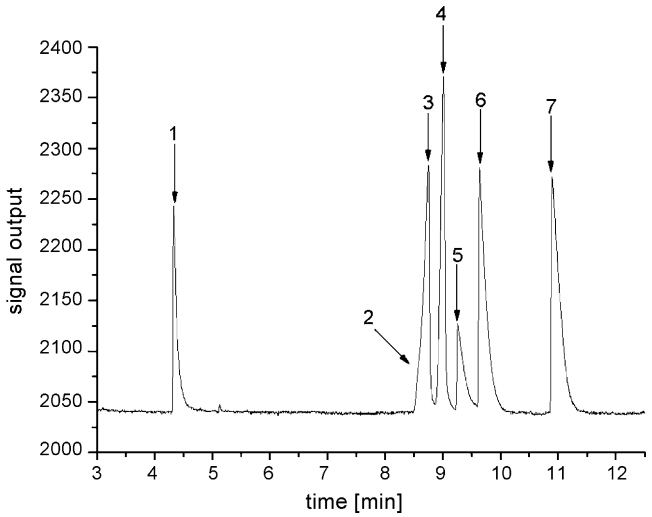

Fig. 3. Electropherograms of a sample containing a mixture of seven compounds. Bare fused-silica capillary 50 cm (effective length, 37 cm) × 50 μm I.D. electrolyte: 50 mM sodium cholate; applied voltage: 20 kV. (1) Atenolol, (2) indoprofen, (3) ketoprofen, (4) warfarin, (5) propanol, (6) ibuprofen and (7) flurbiprofen.

Additional information can be obtained by comparing electropherograms shown in Fig. 2A and B with the electropherogram shown in Fig. 3. This electropherogram was obtained for the same seven compounds but with the buffer contains only 50 mM sodium cholate. As illustrated, separation efficiencies by buffer containing only sodium cholate are relatively inferior to those obtained with buffer containing both sodium cholate and chiral IL, *S*-CHTA]$^+$[Tf_2N]$^-$. Specifically, it was not possible to separate indoprofen from ketoprofen with sodium cholate buffer whereas they were baseline separated by the [SC + *S*-CHTA]$^+$[Tf_2N]$^-$] buffer. More important is the fact that even though sodium cholate is chiral and can serve as electrolyte, when used alone, it cannot provide any chiral separation ability at all.

The results presented seem to suggest that interactions between only chiral cholate anion and *RS*-ibuprofen do not provide sufficient chiral discrimination (toward *R*- and *S*-ibuprofen) for enantioseparation. Rather, cooperative interactions of both chiral cation (*S*-[CHTA]$^+$) and chiral anion (cholate) with the analyte (ibuprofen) are needed to achieve chiral separation. The results are, in fact, in agreement with the mechanism of chiral separations which states that three-point interactions are needed for enantioseparation [2–4,7]. Efficiency of the chiral separation, in terms of resolution (R_s) and selectivity (α) was also found to be strongly dependent on concentrations of *S*-[CHTA]$^+$[Tf_2N]$^-$ and sodium cholate. Shown in Fig. 4 is plot of resolution (R_s) as a function of concentration of *S*-[CHTA]$^+$[Tf_2N]$^-$ and sodium cholate, respectively. It is interesting to observe that no separation was achieved with sodium cholate concentration less than 30 mM. At 30 mM sodium cholate, both R_s and α

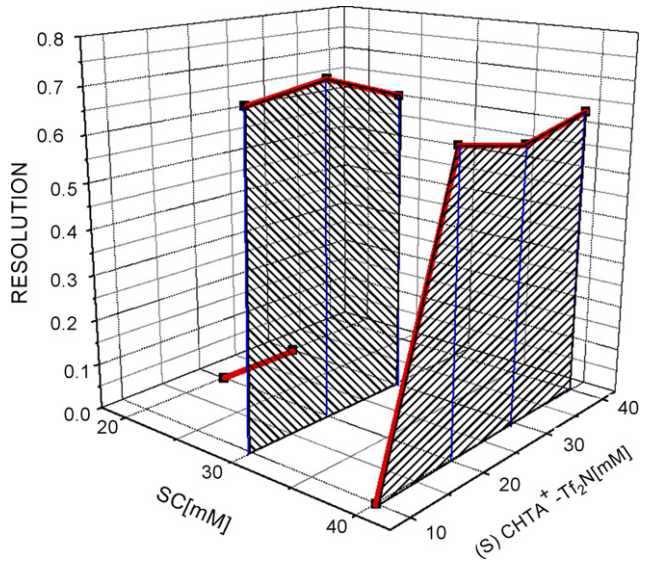

Fig. 4. Plots of concentration of sodium cholate (SC) and *S*-[CHTA]$^+$[Tf_2N]$^-$ vs. resolution (R_s) factor for ibuprofen. See text for experimental conditions.

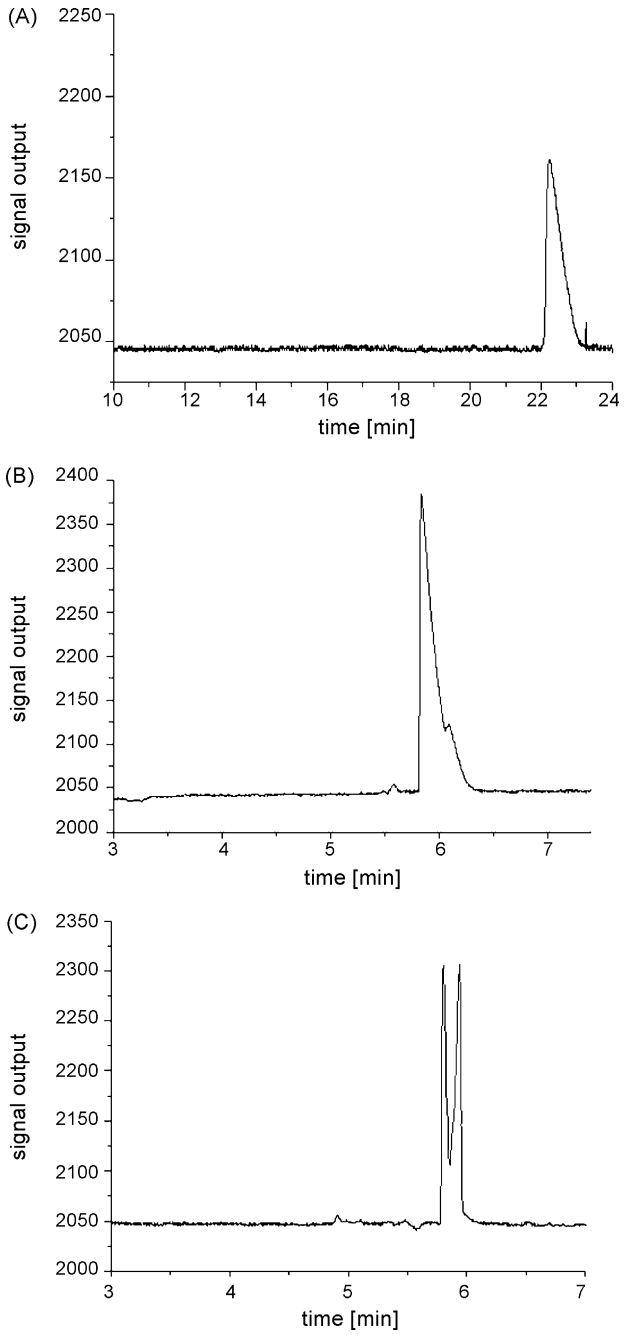

Fig. 5. Electropherograms of a sample of (*RS*)-flurbiprofen. Bare fused-silica capillary 50 cm (effective length, 37 cm) × 50 μm I.D. electrolyte: 50 mM sodium cholate (A); 20 mM *S*-[CHTA]$^+$[Tf_2N]$^-$, 30 mM sodium cholate (B); and 20 mM *S*-[CHTA]$^+$[Tf_2N]$^-$, 30 mM sodium cholate and 10 mM OTG (C). Applied voltage: 18 kV for (A), 30 kV for (B) and (C).

values (not shown) remain constant over the 10–30 mM concentration range of *S*-$[CHTA]^+[Tf_2N]^-$. Further increasing concentration of sodium cholate (to 40 mM) does not lead to any improvement in resolution and selectivity of the separation. Rather it shifts the optimal resolution and selectivity to relatively higher concentration of *S*-$[CHTA]^+[Tf_2N]^-$ (from 10–30 mM range to 20–40 mM range).

Interestingly, even though a buffer containing both *S*-$[CHTA]^+[Tf_2N]^-$ and sodium cholate can effectively serve as chiral selector for ibuprofen, it cannot chirally separate other six compounds in the mixture regardless of their relative concentrations. The best result found using this buffer for compounds other than ibuprofen is that found for *RS*-flurbiprofen which is shown in Fig. 5B. For comparison, electropherogram obtained with buffer contains only one chiral selector (50 mM of sodium cholate) is also provided in Fig. 5A. As shown, buffer with two chiral selectors (B) provides relatively better chiral separation efficiency than that by the buffer with only one chiral selector (A) as the peak obtained with the former has a broad shoulder compared to the peak obtained with the latter. Adding a third and neutral chiral compound 1-*S*-octyl-β-D-thioglucopyranoside (OTG) to the [*S*-$[CHTA]^+[Tf_2N]^-$–sodium cholate] buffer leads to substantial improvement in the enantioseparation (compare Fig. 5A–C which show electropherograms of flurbiprofen with a buffer contained three chiral selectors: 20 mM [*S*-$[CHTA]^+[Tf_2N]^-$ + 30 mM sodium cholate + 10 mM OTG). Interestingly, adding a neutral chiral OTG as the third chiral selector provides not only no improvement but rather somewhat worsen resolution of the separation for ibuprofen (see, for example electropherograms shown in Fig. 6A (no OTG) with (B) (with 10 mM OTG)). A variety of reasons may account for the need of a third neutral chiral selector for flurbiprofen (as compared to ibuprofen) but the most likely one is probably due to the fact that a third and neutral chiral selector which has a glucose ring is needed for chiral interactions with the extra phenyl ring (compared to ibuprofen) in flurbiprofen (see, their structures in Fig. 1).

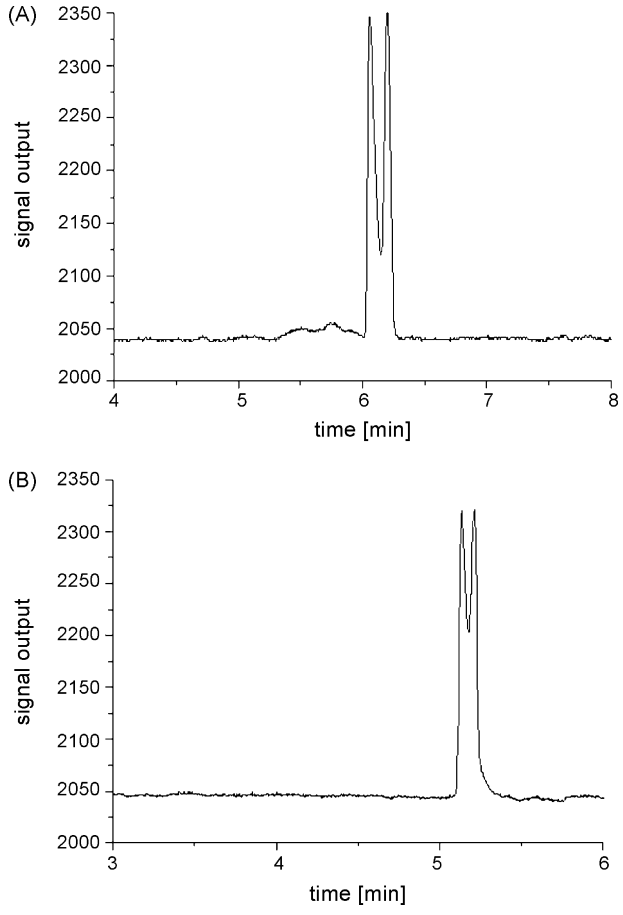

Fig. 6. Electropherograms of a sample of (*RS*)-ibuprofen. Bare fused-silica capillary 50 cm (effective length, 25 cm) × 50 μm I.D. electrolyte: 20 mM *S*-$[CHTA]^+[Tf_2N]^-$, 30 mM sodium cholate; applied voltage: 25 kV. (A) Buffer contains no OTG; (B) buffer contains 10 mM OTG.

4. Conclusions

In summary, we have demonstrated that a novel and chiral IL, *S*-$[CHTA]^+[Tf_2N]^-$, which can be easily and readily synthesized in one-step process from commercially available reagents, can successfully serve both as electrolyte and also as a chiral selector for CE. A variety of pharmaceutical products including atenolol, propranol, warfarin, indoprofen, ketoprofen, ibuprofen and flurbiprofen, can be successfully and baseline separated with the use of this IL as electrolyte. Interestingly, while *S*-$[CHTA]^+[Tf_2N]^-$ can also serve as a chiral selector, enantioseparation cannot be successfully achieved with *S*-$[CHTA]^+[Tf_2N]^-$ as the only chiral selector. In the case of ibuprofen, a second chiral selector, namely a chiral anion (sodium cholate), is needed for the chiral separation. For furbiprofen, in addition to *S*-$[CHTA]^+[Tf_2N]^-$ and sodium cholate, a third and neutral chiral selector, OTG, is also needed. Due to the fact the chirality of this chiral IL resides on the cation (i.e., -$[CHTA]^+$), and that needed additional chiral selector(s) are either chiral anion (i.e., cholate) or chiral neutral compound (OTG), the results obtained seem to suggest that additional chiral selector(s) are needed because the chiral IL cation ($[CHTA]^+$) can probably provide either one or two of three-point interactions needed for chiral separations. The results also seem to suggest that it is entirely possible to use only a chiral IL as a sole chiral selector for enantioseparation by CE if this chiral IL has chirality on the cation as well as on the anion. We are, therefore, actively working on synthesis and applications of novel chiral ILs which have both chiral cation and chiral anion.

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