Combined Use of Ionic Liquid and Hydroxypropyl-β-Cyclodextrin for the Enantioseparation of Ten Drugs by Capillary Electrophoresis

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ABSTRACT In the present study, hydroxypropyl-β-cyclodextrin and an ionic liquid (1-ethyl-3-methylimidazolium-L-lactate) were used as additives in capillary electrophoresis for the enantioseparation of 10 analytes, including ofloxacin, propranolol hydrochloride, dioxopromethazine hydrochloride, isoprenaline hydrochloride, chlorpheniramine maleate, liarozole, tropicamide, amlodipine benzenesulfonate, brompheniramine maleate, and homatropine methylbromide. The effects of ionic liquid concentrations, salt effect, cations, and anions of ionic liquids on enantioseparation were investigated and the results proved that there was a synergistic effect between hydroxypropyl-β-cyclodextrin and the ionic liquid, and the cationic part of the ionic liquid played an important role in the increased resolution. With the developed dual system, all the enantiomers of 10 analytes were well separated in resolutions of 5.35, 1.76, 1.85, 2.48, 2.88, 1.43, 5.45, 4.35, 2.76, and 2.98, respectively. In addition, the proposed method was applied to the determination of the enantiomeric purity of *S*-ofloxacin after validation of the method in terms of selectivity, repeatability, linearity range, accuracy, precision, limit of detection (LOD), and limit of quality (LOQ). *Chirality 25:409–414, 2013.* © 2013 Wiley Periodicals, Inc.

KEY WORDS: capillary electrophoresis; enantiomeric purity determination; enantioseparation; hydroxypropyl-β-cyclodextrin; ionic liquid

INTRODUCTION

The growing attention paid to drug enantioseparation stems from the fact that the desired pharmacological activity is exhibited by only one enantiomer, while the other enantiomer may be toxic, less active, and/or possess undesirable effects.[1] Therefore, the search for efficient and effective methods for obtaining a chiral drug in a single-isomer form and the control of its purity is very important not only to avoid unwanted pharmaceutical and/or toxicological side effects but also to guarantee its therapeutic efficacy and safety.[2]

Compared with classical high-performance liquid chromatography and gas chromatography, capillary electrophoresis (CE) provides a number of advantages in chiral separations due to its high efficiency, speed, and high resolution power.[3] Cyclodextrins (CDs) and derivative CDs, which have exhibited high enantioselectivity to various racemates,[4] are the most commonly used chiral selectors in CE. However, there are still some cases where partially overlapping peaks of enantiomers are observed when using CDs or derivatized CDs alone. Accordingly, the utility of more than one additive in CE to improve the enantioseparation has drawn increased attention in recent years. Among the numerous additives available, ionic liquids (ILs) have been of considerable interest to analytical chemists because of their unique chemical and physical properties.[5] François and coworkers[6] have examined a combination of ILs and classical chiral selectors (di- or tri-methyl-β-CD) using CE in order to evaluate the synergistic effect of the two selectors. Wang et al.[7] have applied a dual system, made up of trimethyl-β-CD and *N*-undecenoxylcarbonyl-L-leucinol bromide, for the simultaneous enantioseparation of five profens using micellar electrokinetic chromatography. Huang et al.[8] have developed approaches to improve the chiral resolution for the simultaneous enantioseparation of β-agonists by CE, using CD inclusion complexation modified with ionic liquids. Rousseau et al.[9] have determined the enantiomeric purity of a synthetic intermediate of a new drug in NACE using a single-isomer anionic CD derivative combined with a chiral IL.

Ofloxacin (1), propranolol hydrochloride (2), dioxopromethazine hydrochloride (3), isoprenaline hydrochloride (4), chlorpheniramine maleate (5), liarozole (6), tropicamide (7), amlodipine benzenesulfonic acid (8), brompheniramine maleate (9), and homatropine methylbromide (10) are widely used chiral drugs; their structures are shown in Figure 1. Most of their pharmacological activities are exhibited predominantly by one enantiomer or one of the enantiomers is toxic. For example, the antibacterial activity of *S*-ofloxacin is 8–128 times higher than that of *R*-ofloxacin and is approximately two times higher than that of the racemate.[10,11] *S*-propranolol is 60–100 times more potent as a β-blocker than *R*-propranolol[12] while one enantiomer of dioxopromethazine hydrochloride can cause severe photoallergic contact dermatitis followed by long-lasting photosensitivity.[13,14] Similar reports have been published for other analytes.[15–21] Therefore, the enantioseparation of these chiral drugs is of great importance in pharmaceutical chemistry.

This work has focused on the combined use of hydroxypropyl-β-cyclodextrin (HP-β-CD) and 1-alkyl-3-methylimidasolium-based ILs as a dual chiral selector using CE for the enantioseparation of the 10 model drugs. After optimization of the separation conditions using HP-β-CD as a selector, the effect of different concentrations of IL added to

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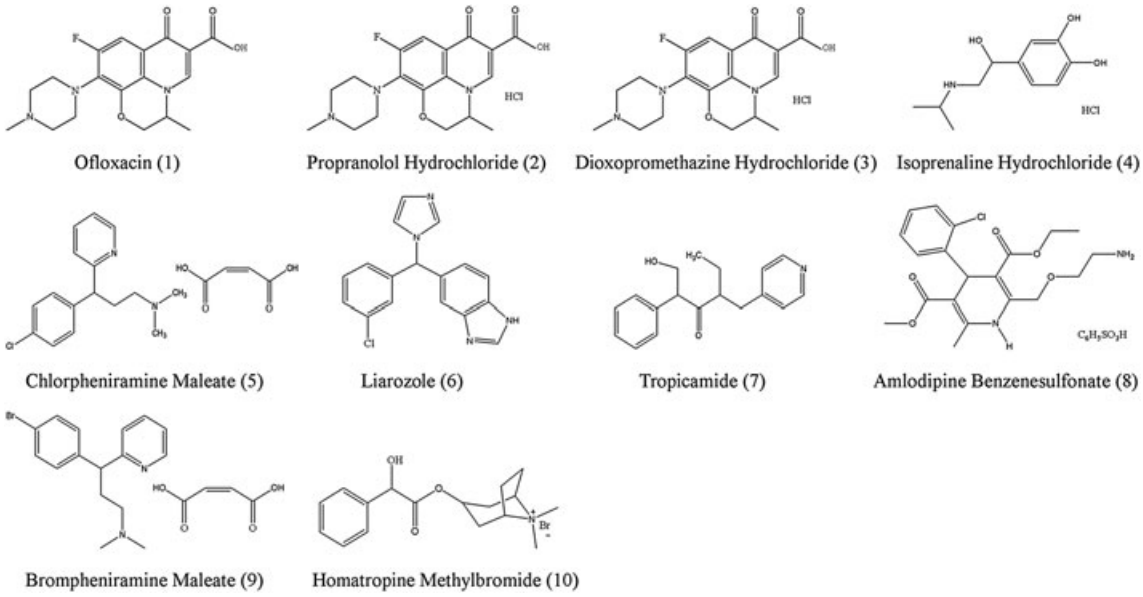

Fig. 1. Chemical structures of the 10 investigated drugs.

background electrolyte (BGE) was investigated. Then, the possible mechanisms involved in the dual-separation system were discussed. Finally, the developed CE method was used to determine the enantiomeric purity of *S*-ofloxacin. The present work will help widen the application of this dual system consisting of CDs and ILs for enantioseparation.

MATERIALS AND METHODS

Apparatus

All experiments were carried out on a Beckman P/ACETM MDQ Capillary Electrophoresis System (Fullerton, CA, USA), equipped with a diode-array detector and controlled by 32 Karat Software.

Chemicals and Reagents

RS-ofloxacin, *S*-ofloxacin, *RS*-propranolol hydrochloride, and *RS*-dioxopromethazine hydrochloride were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). *RS*-isoprenaline hydrochloride, *RS*-chlorpheniramine maleate, *RS*-liarozole, *RS*-tropicamide, *RS*-amlodipine benzenesulfonic acid, *RS*-brompheniramine maleate, and *RS*-homatropine methylbromide were provided by Sigma Chemical Co. (St. Louis, MO, USA). *R*-ofloxacin was provided by the Pharmaceutical Chemistry Laboratory of Shenyang Pharmaceutical University. *S*-ofloxacin bulk drug was purchased from Apeloa Kangyu Pharmaceutical Co., Ltd. (Dongyang, China) while 1-ethyl-3-methylimidazolium-L-lactate ([EMIm][L-lactate]), 1-ethyl-3-methylimidazolium-D-lactate ([EMIm][D-lactate]), 1-ethyl-3-methylimidazolium-Br ([EMIm]Br), and 1-heptyl-3-methylimidazolium-L-lactate ([HMIm][L-lactate]) were obtained from Shanghai Cheng Jie Chemical Co. (Shanghai, China). Hydroxypropyl-β-cyclodextrin (HP-β-CD, purity >90%, average degree of substitution 6.6) was obtained from TianJin Bodi Chemical Plant (Tianjin, China); phosphoric acid and sodium lactate of chromatography grade were also supplied by the Tianjin Bodi Chemical Plant (Tianjin, China).

Electrophoretic Technique

An untreated, bare fused silica capillary column (Hebei Optical Fiber, China) with dimensions of 50 cm × 50 μm (effective length 41 cm) was used throughout the study. Before the first use, the new capillary column was activated by flushing it with 1 mol · L^{-1} NaOH at 30 psi for 15 min. Each day the capillary was rinsed at a pressure of 20 psi with 0.1 mol · L^{-1} NaOH for 3 min, then water for 2 min, and conditioned with BGE for 10 min. Between each run, it was flushed with BGE at 25 psi for 2 min. The study was conducted at a voltage of +20 kV and samples were injected at the positive electrode into the capillary in pressure mode. Acetone was used as neutral marker to determine the electroosmotic flow (EOF).

Sample stock solutions of the 10 model compounds were prepared by dissolving 10 mg of each analyte in 10 ml of methanol and they were then stored in a refrigerator. The stock solutions were diluted tenfold with buffer solution to obtain sample solutions.

The enantioseparation parameters using CE were calculated and the effective electrophoretic selectivity, α_{eff}, was calculated using the following equation:

$$\alpha_{eff} = \mu_{ep2}/\mu_{ep1}$$

where μ_{ep1}, μ_{ep2} are the effective mobilities of enantiomers 1 and 2. The resolution, *Rs*, for a pair of enantiomers was calculated by

$$Rs = 2(t_2 - t_1)/(W_1 + W_2)$$

where W_1 and W_2 are the peak widths at the baseline of each enantiomer.

RESULTS AND DISCUSSION

Development of the Dual System

HP-β-CD is a neutral chiral selector widely used in enantioseparation. With HP-β-CD as a chiral selector, the enantioseparation conditions of the 10 analytes were optimized in a pilot study. The best resolution and selectivity were achieved using a buffer consisting of 50 mM NaH_2PO_4-H_3PO_4 (pH = 2.75), containing HP-β-CD at concentrations of 40, 30, 50, 40, 50, 50, 30, 50, 40, and 40 mM for each analyte, respectively. To establish a generic CE system for the enantioseparation of all the analytes, the concentration of HP-β-CD was selected as 40 mM. As shown in Figure 2, HP-β-CD exhibited reasonable enantioselectivity for the 10 model drugs under the given conditions, but it was less successful in completely resolving all 10 pairs of enantiomers, especially for the enantiomers of propranolol hydrochloride (2), dioxopromethazine hydrochloride (3,) and liarozole (6). To improve the efficiency of the enantioseparation, [Emim][L-lactate], a form of commonly used 1-alkyl-3-methylimidazolium-based IL, at a concentration of 30 mM, was added to the optimized BGE containing HP-β-CD. In the presence of [Emim][L-lactate], there were significant improvements in the

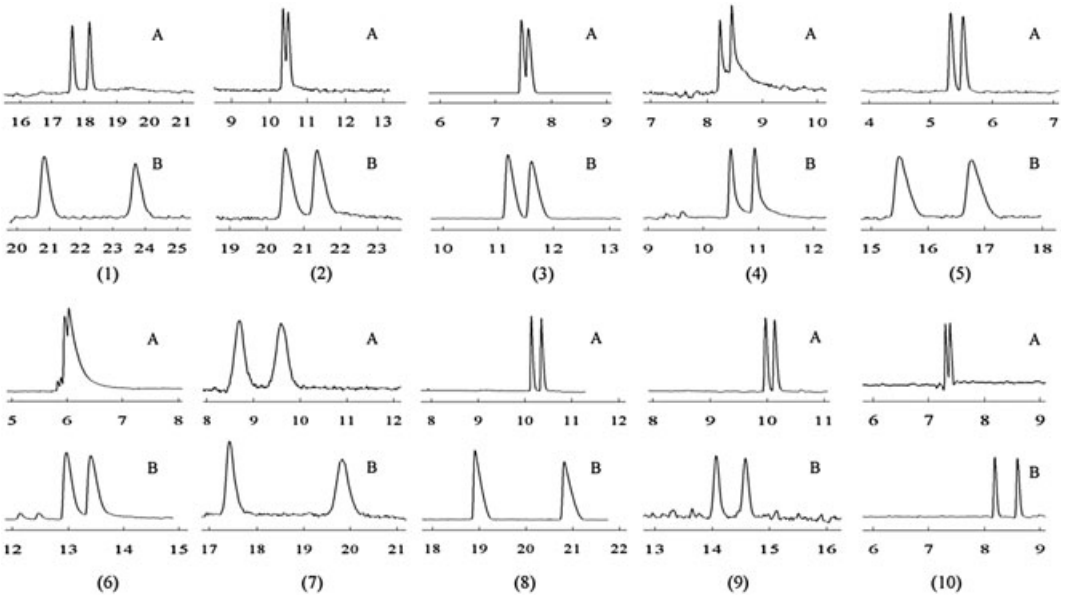

Fig. 2. Electropherograms of the 10 analytes in the absence (A) and presence (B) of [EMIm][L-lactate] in BGE (BGE: 40 mM HP-β-CD, 50 mM NaH_2PO_4-H_3PO_4, pH 2.75 with 30 mM [Emim][L-lactate] added); ofloxacin (1), propranolol hydrochloride (2), dioxopromethazine hydrochloride (3), isoprenaline hydrochloride (4), chlorpheniramine maleate (5), liarozole (6), tropicamide (7), amlodipine benzenesulfonate (8), brompheniramine maleate (9), and homatropine methylbromide (10).

resolutions of all enantiomers of the 10 analytes. Consequently, the dual system consisting of HP-β-CD and [Emim][L-lactate] was adopted for the enantioseparation of the analytes.

Effect of the IL Concentration

The effect of the IL concentration on the enantioresolution was then investigated with 40 mM of HP-*β*-CD in the 50-mM NaH_2PO_4-H_3PO_4 buffer (pH = 2.75), at concentrations of 0, 10, 30, and 50 mM. As shown in Table 1, on increasing the [Emim][L-lactate] concentration from 0 to 30 mM, the migration time and chiral resolutions of all the enantiomers steadily increased, which is a common phenomenon for ILs.[8] However, a further increase in concentration (from 30 to 50 mM) resulted in a longer migration time for most of the analytes and a decrease in the resolution of all the analytes. As a result, the optimum concentration of [Emim][L-lactate] for the 10 enantiomers was selected as 30 mM with the highest α_{eff} and *Rs* values and a satisfactory migration time.

The increase in the migration time following the addition of IL might be related to the adsorption of $[Emim]^+$ in the capillary wall, which would decrease the electroosmotic mobility or even reverse the direction of the EOF (the values are shown in Table 1),as mentioned by other authors.[8,9,22] The increase in the migration time with an increase in the IL concentration provides more opportunities for an interaction between the analytes and HP-β-CD, so the resolution was increased. The decrease in resolution from 30 to 50 mM of IL might be explained by the interaction of IL with HP-β-CD, leading to a reduction in the inclusion of analytes in the cavity of HP-β-CD.[8] An increase in Joule heat might also contribute to the reduced resolution.

Exploring Possible Mechanisms

In order to explore the possible mechanisms involved in the enantioseparation upon IL addition to BGE, further experiments were carried out.

Salt effect. IL is a natural salt.[5] To differentiate a salt effect from a specific effect due to the nature of the IL cations, the same experiment was conducted with sodium lactate instead of [EMIm][L-lactate], as reported by François et al.[6] Following the addition of 30 mM of sodium lactate to HP-β-CD, the enantioresolutions were higher than in the experiment with HP-β-CD alone, but were lower compared with the experiment involving addition of [EMIm][L-lactate] (Table 1). The effective electrophoretic selectivity (α_{eff}), a parameter independent of the electroosmotic mobility, was then calculated in order to highlight a possible synergistic effect between IL (or sodium lactate) and HP-β-CD. It could also be seen from Table 1 that higher α_{eff} values were obtained in the presence of [EMIm][L-lactate], compared with the experiments using HP-β-CD alone, especially in the case of ofloxacin, isoprenaline, chlorpheniramine maleate, amlodipine, and homatropine methylbromide. However, little change in α_{eff} was observed after addition of sodium lactate to HP-β-CD. It is also worth noting that no enantioseparation was obtained with [Emim][L-lactate] alone. Therefore, the simultaneous change in α_{eff} and *Rs* upon adding [Emim][L-lactate] seemed to indicate that [Emim][L-lactate] and HP-β-CD exhibited a synergistic effect.[9] This synergistic effect might be produced as follows: the enantiomers might be included in HP-β-CD to form inclusion complexes[23] and then they might associate with the positively charged alkylimidazolium groups either coated on the capillary or free in the BGE. These associations might be carried out by hydrophobic hydrogen bonding or ion-dipole/ ion-induced-dipole interactions between enantiomers and ILs [8].

Influence of the cationic part. Jiang et al.[24] have demonstrated that the alkyl group of ILs plays an important role in the separation process and Rizvi et al.[7,25,26] have reported that the chain length of the cationic part of ILs affects the quality of the enantioseparation. Thus, we compared [EMIm][L-lactate] and [HMIm][L-lactate] in the same experiment. The analytes exhibited a longer migration with better resolution following the addition of [HMIm][L-lactate] with a longer alkyl chain

TABLE 1. The effect of adding [Emim] L-lactate] and sodium lactate on the α_{eff} and *Rs* values of 10 chiral analytes (BGE: 40 mM HP-β-CD, 50 mM NaH_2PO_4-H_3PO_4, pH 2.75)

	[Emim][L-lactate]														Sodium lactate					
C (mM)	0				10				30				50				30			
μ_{EOF}(10^{-9} m^2 $^{-1}S^{-1}$)	3.43				2.84				−4.63				−5.52				1.11			
	t_1	t_2	α_{eff}	*Rs*	t_1	t_2	α_{eff}	*Rs*	t_1	t_2	α_{eff}	*Rs*	t_1	t_2	α_{eff}	*Rs*	t_1	t_2	α_{eff}	*Rs*
Ofloxacin	17.8	18.2	1.03	2.91	20.3	21.4	1.05	3.53	20.9	23.8	1.08	5.35	23.8	26.0	1.05	3.42	19.9	20.8	1.05	3.03
Propranolol hydrochloride	10.4	10.5	1.01	0.93	16.1	16.3	1.01	1.30	20.7	21.4	1.02	1.76	22.2	22.8	1.02	1.68	14.4	14.9	1.03	1.58
Dioxopromethazine hydrochloride	7.5	7.7	1.03	1.02	10.7	10.9	1.02	1.38	11.2	11.6	1.03	1.85	11.3	11.7	1.02	1.39	9.5	9.7	1.02	1.19
Isoprenaline hydrochloride	8.3	8.5	1.02	1.58	10.1	10.5	1.04	2.38	10.6	11.1	1.05	2.48	10.9	11.2	1.02	2.26	10.5	10.9	1.04	2.06
Chlorpheniramine maleate	5.4	5.5	1.01	1.65	10.3	10.6	1.02	1.86	15.6	16.8	1.05	2.88	19.5	20.1	1.02	2.05	11.4	11.9	1.03	2.07
Liarozole	6.0	6.1	1.01	0.61	8.3	8.5	1.02	0.86	12.9	13.6	1.03	1.43	16.0	16.6	1.03	1.30	10.2	10.6	1.02	1.17
Tropicamide	8.8	9.6	1.11	2.07	13.4	15.0	1.11	2.22	17.3	19.9	1.11	5.45	22.8	27.2	1.10	4.89	15.7	16.3	1.11	4.09
Amlodipine benzenesulfonic acid	10.2	10.4	1.02	1.49	15.5	16.3	1.05	4.06	19.0	20.9	1.06	4.35	21.1	23.1	1.05	3.68	14.7	15.4	1.04	3.71
Brompheniramine maleate	10.0	10.2	1.03	1.63	13.4	14.0	1.04	2.08	14.1	14.8	1.05	2.76	16.9	17.7	1.03	2.17	13.5	14.1	1.04	2.07
Homatropine methylbromide	7.2	7.3	1.01	0.78	7.4	7.6	1.03	2.00	8.2	8.7	1.05	2.98	9.1	9.3	1.02	1.61	7.7	8.0	1.02	2.04

in the cationic part. Taking ofloxacin as an example, [HMIm][L-lactate] exhibited a better resolution (5.98) at the expense of a longer analysis time (24.1 and 27.2 min for the *S*-isomer and *R*-isomer, respectively), which was in agreement with the literature evidence mentioned above. This behavior was to be expected, since shorter alkyl chains were less hydrophobic than longer ones and they formed a less stable bilayer inside the capillaries, wherease a stable bilayer inside the capillaries might allow a more stable environment for the separation of analytes.[26]

Influence of the anionic part. [EMIm][L-lactate] is a chiral anionic IL. To explore whether the chirality of the anionic part of IL affected the enantioseparation, we conducted experiments using [EMIm][DL-lactate] instead of [EMIm][L-lactate]. The addition of racemic IL to the BGE resulted in the same migration time and resolution of all the analytes as in the case of the L-isomer addition. As far as amlodipine benzenesulfonate, chlorpheniramine maleate, and ofloxacin were concerned, the elution orders of the enantiomers were not altered (*R*-amlodipine benzenesulfonate, *S*-chlorpheniramine maleate, and *S*-ofloxacin migrated more quickly). Furthermore, the separation performances of the analytes after addition of IL with the anionic part of Br were compared with that of the L-lactate. Following the addition of [Emim]Br, significant improvements in resolution were observed for all the enantiomers. The extent of the improvement was not exactly the same as that in the presence of [EMIm][L-lactate], but the differences were not significant. It seemed that the chirality and nature of the anionic part of IL had little influence on the enantioseparation in our study.

METHOD APPLICATION

Method Validation

Based on the work above, the developed method was subsequently validated according to ICH Guideline Q2(R1) with respect to selectivity, linearity range, LOD, LOQ, repeatability, precision, stability, and accuracy, with reference to the enantiomeric purity determination of ofloxacin. The limit for the impurity, *R*-ofloxacin, was set at not more than 0.2% which was far lower than for the HPLC method proposed in the Chinese Pharmacopoeia.[27]

Selectivity. The homogeneity of the peaks of ofloxacin enantiomers was examined by injecting solutions of racemic ofloxacin, then the peak identification was achieved by injection of *R*-ofloxacin or *S*-ofloxacin solution alone. The first migrating peak was identified as the *S*-enantiomer and the second migrating peak as the *R*-enantiomer (impurity). A typical electropherogram is shown in Figure 2 (1B). In addition, 10% methanol was also injected to ascertain that no peaks were observed at the migration times of the enantiomers in the solvent.

Linearity, LOD, and LOQ. When developing assays for the determination of related substances as well as stereoisomeric impurities, it is always desirable to validate the impurities in the presence of a large excess of the parent compound. Consequently, for the linearity measurement, five standard solutions containing *R*-ofloxacin were prepared at concentrations ranging from 1.60 to 8.00 $\mu g \cdot ml^{-1}$ in the presence of 800.00 $\mu g \cdot ml^{-1}$ *S*-ofloxacin (corresponding to 0.2–1.00%, respectively). Then the calibration curve was determined to be $Y = 3063\ X + 127.9$ ($r = 0.9993$) by plotting the peak area (Y) versus the concentration of *R*-ofloxacin (X). The LOD

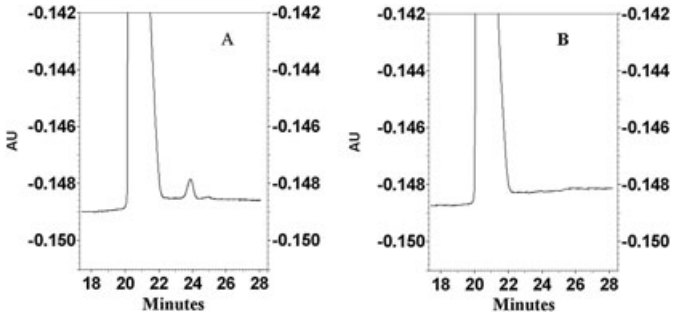

Fig. 3. Electropherograms of ofloxacin enantiomers (A: 0.2% *R*-ofloxacin in *S*-ofloxacin standard solution; B: *S*-ofloxacin bulk samples) (BGE: 40 mM HP-β-CD, 50 mM NaH_2PO_4-H_3PO_4, pH 2.75, and 30 mM [Emim][L-lactate]).

and LOQ for *R*-ofloxacin were determined as the lowest concentration producing signal-to-noise ratios of at least 3:1 and 10:1, respectively. The results for *R*-ofloxacin were determined as 0.53 $\mu g \cdot ml^{-1}$ and 1.60 $\mu g \cdot ml^{-1}$, respectively.

Precision and stability. Since the electropherogram of the *S*-ofloxacin bulk sample did not show any *R*-enantiomer impurity, a known quantity of the *R*-enantiomer (1.60 $\mu g \cdot ml^{-1}$) was added to the *S*-ofloxacin bulk sample (800.00 $\mu g \cdot ml^{-1}$) to prepare a simulated sample (corresponding to 0.2%) for validation of the precision and stability. To validate the precision of the proposed method, both repeatability and intermediate precision were investigated. Repeatability was studied by performing sequentially a series of six injections of simulated samples. The RSD values were 1.8% and 4.2% for the migration time and peak area, respectively. Intermediate precision was determined by analyzing the simulated samples by repeating the experiments on three successive days. The RSD values were 4.2% and 5.4% for the migration time and peak area, respectively. The RSD values for both repeatability and intermediate precision were below the acceptable maximum value of 10%, illustrating the good precision of the developed method. The stability of the simulated sample was analyzed at 0 h, 2 h, 4 h, 8 h, and 12 h within a single day. The variation in the area was 4.1% (RSD) which proved that the *R*-ofloxacin solution was stable over a 12 h period.

Accuracy. Recovery tests were carried out to investigate the accuracy of the proposed method by standard addition of a reference (adding a known concentration of *R*-ofloxacin solution to the *S*-ofloxacin bulk sample solution to obtain a final solution containing 1.60 $\mu g \cdot ml^{-1}$ *R*-ofloxacin, corresponding to an impurity content of 0.2%). Six samples were prepared. The recoveries of *R*-ofloxacin were 97.9, 97.2, 104.6, 105.5, 99.7, and 102.9 %, respectively, with an RSD value of 3.5%.

Enantiomeric Purity Determination of Ofloxacin

The method was used to determine the amount of chiral impurity (*R*-ofloxacin) in bulk samples of 800.00 $\mu g \cdot ml^{-1}$ *S*-ofloxacin (Fig. 3B). The result was then compared with that of a standard solution containing 800.00 $\mu g \cdot ml^{-1}$ *S*-ofloxacin and 1.60 $\mu g \cdot ml^{-1}$ *R*-ofloxacin (with an impurity content of 0.2%, Fig. 3A). It was clear that the concentration of the chiral impurity in *S*-ofloxacin bulk samples was well below 0.2%.

CONCLUSION

A CE method employing a dual system of HP-β-CD and [EMIm][L-lactate] has been developed for the enantioseparation of 10 model compounds. Following the addition of IL to BGE containing HP-β-CD, the resolutions of all the analytes were improved significantly. The possible mechanisms involved were discussed and the results proved that there was a synergistic effect between HP-β-CD and IL and that the cationic part of IL played an important role in the increased resolution. Finally, the developed method was validated and found to be sensitive and efficient for enantiomeric purity determination of ofloxacin. It allowed the determination of 0.2% *R*-ofloxacin in the presence of a large amount of the *S*-form.

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