Published in final edited form as:

Electrophoresis. 2009 August ; 30(16): 2812–2819. doi:10.1002/elps.200800851.

Combined use of chiral ionic liquid and cyclodextrin for MEKC: Part I. Simultaneous enantioseparation of anionic profens

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Abstract

The enantiomers of five profen (PROF) drugs were simultaneously separated by MEKC with the combined use of 2, 3, 6-tri-O-methyl- β -cyclodextrin (TM- β -CD) and chiral cationic ionic liquid (IL), *N*-undecenoxy-carbonyl-L-leucinol bromide (L-UCLB), which formed micelles in aqueous buffers. Enantioseparations of these PROF drugs were optimized by varying the chain length and concentration of the IL surfactant using a standard recipe containing 35 mM TM- β -CD, 5 mM sodium acetate at pH 5.0. The batch-to-batch reproducibility of L-UCLB was tested and found to have no significant impact in terms of enantiomeric resolution, efficiency and migration time. Finally, this method was successfully applied for the quantitative determination of ibuprofen in pharmaceutical tablets.

Keywords

TM- β -CD; L-UCLB; Profen; Simultaneous enantioseparation

1 Introduction

Separations of chiral compounds on analytical-scale are subject to immense interest. This is mainly because currently there are strict regulatory issues for the preparation of enantiomerically pure compounds. Therefore, dedicated analytical assay for enantiomeric purity are essential for drug screening in the pharmaceutical industry. In addition, chiral discrimination has also been shown to be increasingly important in agricultural, environmental and biological sciences [1]. Capillary electrophoresis (CE) has proved to be an effective tool for chiral discrimination, studying chiral interactions and measurement of binding constants of enantiomers. This is due to high efficiency, short analysis time and requirement of only a small quantity of exotic chiral selector. Among the various chiral reagents, native cyclodextrins (CDs) and their derivatives [2] macrocyclic antibiotics [3] and polymeric surfactants [4] have proven to be very successful for CE. However, the utility of the aforementioned reagents as a single chiral selector to resolve enantiomers is not always satisfactory. Hence, the utility of more than one chiral reagent in CE to improve the enantioseparation has drawn increase attention in recent years [5–7].

Ionic liquids (ILs) are defined as materials consisting only ionic components with low melting points (i.e., at or close to room temperature) [8–9]. Very recently, our group reported the first

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The authors have declared no conflict of interest.

use of two new cationic ILs type surfactants as a single chiral selector in micellar electrokinetic chromatography (MEKC) [10]. Subsequently the cationic IL, *N*-undecenoxy-carbonyl-L-leucinol bromide (L-UCLB) and *N*-undecenoxy-carbonyl-L-pyrrolidinol bromide (L-UCPB) and their polymers were synthesized, characterized and compared for the enantioseparation of two negatively charged enantiomers (α -bromo-phenyl acetic acid, and phenoxy propionic acid). The primary interaction of aforementioned ILs is thought to involve electrostatic attractive interactions. Proposed secondary interactions include steric, hydrophobic and hydrogen-bonding interactions.

Very recently, the combined use of various CD derivatives and two chiral ILs (ethyl- and phenyl-choline of bis (trifluoromethylsulfonyl) imide) was evaluated for enantiomeric separations by CE. Although no clear trend was observed, enhancement in chiral selectivity and resolution of two profens (e.g., carprofen and suprofen) were achieved, which suggests synergistic effect [11]. A similar report on the use of chiral IL, S-[3-(chloro-2-hydroxypropyl) trimethylammonium][bis(trifluoromethyl)sulfonyl]amide has been reported in combination with 1-*S*-octyl- β -D-thiogluco-pyranoside (OTG) and/or sodium cholate for the enantioseparation of ibuprofen and flurbiprofen, respectively [12].

This work is focused on the combined use of cationic IL-type surfactants (L-UCLB) and heptakis 2,3,6-tri-O-methyl- β -CD (TM- β -CD) as a dual chiral selector for MEKC. Because L-UCLB did not provide any significant enantioselectivity for model class of analytes (PROFs) under the reversed electroosmotic flow (EOF) MEKC, the influence of L-UCLB was studied in combination to TM- β -CD under the normal EOF MEKC. As demonstrated in this paper, the presence of L-UCLB would widen the scope of application of TM- β -CD. For example, the widening of the chiral window was obtained upon addition of L-UCLB. This implies that simultaneous enantioseparation of the five structurally similar aryl-propionic acids (PROFs) could be achieved in a high throughput fashion. The enantiomeric resolution, selectivity and the total analysis time were compared using concentration and chain length of ILs. In addition, batch-to-batch reproducibility of IL surfactants for the simultaneous enantioseparation was also compared. To the best of our knowledge, this is the first study in which the simultaneous enantioseparation of PROF drug is achieved using a mixture of IL and TM- β -CD as a dual chiral selector

2 Materials and methods

2.1 Reagents and chemicals

The five PROF compounds (ibuprofen, fenoprofen, indoprofen, suprofen, and ketoprofen), dimethyl sulfoxide (DMSO, anhydrous, 99.9+ %), and TM- β -CD were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium acetate was purchased from Fisher Scientific (Chemical Manufacturing Division, Fair Lawn, NJ, USA). Acetic acid, dichloromethane, ethyl acetate, and acetone were purchased from Caledon Laboratories (Georgetown, ON, Canada). Deionized water was prepared with a Barnstead "NANO-pure" ultrapure water system (Dubuque, Iowa, USA). The ibuprofen tablets (200 mg) were purchased from CVS pharmacy.

The reagents ω -undecylenyl alcohol, 2-bromoethylamine hydrobromide, pyridine anhydrous, *S*-(+)-leucinol, formic acid (≥ 95), and formaldehyde (37 wt. % solution in water) used for the synthesis of ILs were purchased from Sigma-Aldrich (St. Louis, MO, USA). 7-Octen-1-ol and triphosgene were obtained from TCI (Tokyo Kasei Kogyo Co. LTD, Tokyo, Japan). Hydrochloric acid and sodium sulfate anhydrous were purchased from EMD Chemicals (Gibbstown, NJ, USA). Sodium bicarbonate was purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). Sodium hydroxide (50% w/w) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Three ILs, L-UCLB, *N*-butoxycarbonyl-L-leucinol bromide (L-

BCLB), and *N*-octenoxycarbonyl-L-leucinol (L-OCLB, Figure 1A) were synthesized following a previously optimized procedure [10].

2.2 CE instrumentation

All CE experiments were performed on an Agilent CE system (Product Number G1602A, Agilent Technologies, Santa Clara, CA, USA) equipped with an on-line diode array detector (DAD) for the absorbance measurements at 214 nm. The temperature control of the sample carousel at 16 °C was maintained by Fisher ISOTEMP 3016S refrigerating circulator (Fisher Scientific, Pittsburgh, PA, USA). The 3D-CE Chemstation software (Agilent Technologies, Santa Clara, CA, USA) was used for the instrumental control, data acquisition, and data analysis. The fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with dimensions of 64.5 cm total length, 56.0 cm effective length, 50 µm i.d., and 365 µm o.d. were used throughout the study. The exact values of the total length and effective length of the capillaries were measured and used for the calculation of binding constants.

2.3 CE conditions and methods

2.3.1 Enantioseparation of profen drugs using L-UCLB, L-OCLB, and L-BCLB—

The concentration of each PROF stock solution prepared in pure MeOH was 2.0 mg/mL. A fixed aliquot of ibuprofen, fenoprofen, indoprofen, suprofen, and ketoprofen racemates were respectively dissolved in a binary solvent mixed with MeOH and H₂O in the ratio of 70:30 (v:v). The 5 mM NaOAc solution was prepared by dissolving NaOAc·3H₂O in triply DI water. The pH of this solution was adjusted to 5.0 using 15.1 µL of 17.4 M HOAc solution. A bare fused silica capillary was first preconditioned for 60 minutes with 1 M NaOH at 50 °C, and then flushed for 30 min with triply DI water at 16 °C. Before each injection, the capillary was flushed with the separation buffer for 5 min. After each run, the capillary was postconditioned with DI water for 5 min, 0.1 M aqueous NaOH solution for 10 min, and DI water for 5 min. The sample was hydrodynamically injected at 3 mbar, 8 seconds. The separations were carried out under an applied voltage of +30 kV and a temperature of 16 °C. The absorbance of PROF drugs in the CE buffer was monitored at 214 nm.

2.3.2 Quantitative analysis of ibuprofen enantiomers in the commercial tablet—

To prepare ibuprofen tablet for analysis, tablet solutions were prepared using a developed method [13]. Briefly, one ibuprofen tablet (labeled at 200 mg ibuprofen) was weighed and ground to fine powder, weighed again and dissolved in 25 mL binary solvent of MeOH and H₂O in the ratio of 70:30 (v:v). A 2.0 mL aliquot of this solution was sonicated for 30 min and centrifuged for 10 min. The supernatant was collected and diluted four folds with the same solvent and filtered through 0.45 µm filter as the tablet sample. The racemic ibuprofen solution as external standard sample was made at concentrations of 0.3, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL. The calibration plots of *R*- and *S*-enantiomer were obtained and used to determine the concentration of each enantiomer in the tablet sample.

3. Results and discussion

3.1 Evaluation of synergistic effect between L-UCLB and TM-β-CD

With a goal to extend the enantioselective capability of L-UCLB, a series of structurally similar anionic drugs (e.g., PROFs, shown in Figure 1B) was investigated. No enantio-selectivity was observed for any PROF drug when using L-UCLB as a single chiral selector. It is well-documented that among the several native and derivatized CDs, the TM-β-CD is the best chiral selector for the separation of PROFs [11,14]. Next, we investigated the association between L-UCLB and TM-β-CD to search for possible synergistic effects. As shown in Figure 2A, TM-β-CD exhibits reasonable enantio-selectivity for individual anionic PROFs, but it was less successful in completely resolving the PROF enantiomers in complex mixtures of structurally

similar PROFs. For example, only slight resolution was observed for ibuprofen enantiomers (1,1'). In addition, the later eluting enantiomers of indoprofen (3') co-eluted with the first eluting enantiomer of suprofen (4). Hence, TM- β -CD only partially separated the enantiomeric mixture of PROFs with a narrow chiral window. However, upon addition of 1.5 mM L-UCLB to the CE buffer containing TM- β -CD, all enantiomers of PROFs were baseline resolved (except for ibuprofen, which provided R_s value of 1.32) with a significant widening of the chiral window (Figure 2B). Furthermore, the peak efficiency improved by 3–10 times (data not shown). We hypothesize that these improvements in simultaneous enantioseparation of PROFs are due in large part to the interaction between TM- β -CD and L-UCLB, which in turn reduces the interaction of the L-UCLB with the capillary wall.

3.2 Influence of ionic liquid concentration on resolution and migration time

Figure 3 shows the electropherograms illustrating the effect of low mM concentrations of L-UCLB as an additive to the TM- β -CD buffer. The increasing concentrations of L-UCLB from 1.0 to 3.0 mM had a significant influence on the resolution and migration time of anionic PROFs. As shown in Figure 3A–C, the chiral and achiral resolutions of all PROFs increased, comparing to the ones in Figure 2A. In addition, the electroosmotic mobility decreased dramatically over the same concentration range from $1.998 \times 10^{-4} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ to $8.964 \times 10^{-5} \text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ due to adsorption of L-UCLB to the capillary wall. The inset plot in Figure 3 shows that the increasing concentration of L-UCLB caused the highest change in resolutions of fenoprofen racemate (from 2.6 to 5.0) and the smallest change in the resolution of ibuprofen racemate (from 1.1 to 1.7). Furthermore, the achiral resolution between critical pair of indoprofen and suprofen (3'/4) also increased from 1.7 (1.0 mM L-UCLB) to 1.9 (3.0 mM L-UCLB). However, the continuous increase in migration times made the buffer with concentration higher than 3.0 mM L-UCLB not applicable. The optimum concentration of L-UCLB for simultaneous enantioseparation seems to be 2.0 mM, which provides a good compromise between analysis time and resolution values.

3.3 Batch-to-batch reproducibility of L-UCLB for the enantioseparation

We also investigated the batch to batch reproducibility of the L-UCLB for the simultaneous enantioseparation. Figure 4A–C is a representative series of electropherograms for the simultaneous enantio-separation of PROFs using three batches of L-UCLB. All three electropherograms were generated under the same buffer conditions using the same capillary. The %RSD of electroosmotic mobility was 0.84%. The %RSD for the average efficiency was 7.53%, whereas the %RSD for the migration time of the PROFs range from 1.06% to 2.56%. The %RSD of the resolution between two consecutive PROF peaks in Figure 4 (i.e., from peak 1 to 5') were 8.47%, 8.68%, 6.76%, 3.66%, 3.27%, 5.00%, 3.09%, 3.20%, 4.79%, and 7.76%. Therefore, it can be concluded that the simultaneous enantioseparation approach is sufficiently reproducible with different batches of L-UCLB synthesized in our laboratory.

3.4 Influence of chain length

Previously, the polymeric anionic surfactants with leucinate head group and C₈–C₁₁ chain length were evaluated for the simultaneous enantioseparation of eight β -blockers [15]. The eight carbon chain chiral surfactant provided the overall best enantioselectivity. However, the anionic chiral polymeric surfactants are not very suitable for separation of anionic chiral analytes. To study the effect of the chain length, we synthesized other cationic ILs such as L-OCLB with eight carbon chain and L-BCLB with four carbon chain (Figure 1A). Note that the major difference between these three chiral surfactant-type IL (L-UCLB, L-OCLB, and L-BCLB) is the length of the hydrocarbon chain attached to the leucinol head group via carbamate linkage. Figure 5 compares the simultaneous enantioseparation of five profen drugs using the same concentration of L-UCLB (Figure 5A), L-OCLB (Figure 5B), and L-BCLB (Figure 5C).

As expected, the migration times of all profens and EOF in Figure 5A are longer with the use of eleven carbon chain IL micelles than the use of eight and four carbon chain IL micelles (Figure 5B and 5C). For example, the migration time of the latest peak (*S*-ketoprofen) eluted at 52.3 min when using L-UCLB. However, it eluted at 44.8 min when using L-OCLB, and only 24.7 min when using L-BCLB. Unexpectedly, the chiral resolutions and selectivities of the racemic profen pairs did not change significantly when using L-UCLB and L-OCLB. The chiral resolutions of racemic profens were lower when using L-BCLB but the selectivities did not change significantly. This study suggests that L-UCLB, L-OCLB, and L-BCLB performed similar binding interactions with TM- β -CD. Moreover, the L-UCLB provided widest elution window compared to other two ILs. The measurement of elution window ($\Delta t = |t_{mc} - t_0|$) is 3.3 min for L-UCLB, 2.9 min for L-OCLB, and only 0.9 min for L-BCLB. This trend is in accordance with the decrease of chain length of the ILs.

3.5 Quantitative analysis of ibuprofen enantiomers in the commercial tablet

The *R*- and *S*-enantiomers in the ibuprofen racemate were used as external standards for each enantiomer in the tablet sample. The concentration of standard ibuprofen racemate ranges from 0.3 mg/mL to 2.5 mg/mL. The peak areas of each enantiomer at different concentration of standard solutions were used for the linear regression and calculation of the tablet sample concentration. Figure 6A shows the electropherogram of ibuprofen tablet sample and representative ibuprofen standard (Figure 6B, 1.0 mg/mL racemate, 0.5 mg/mL for each enantiomer). It is possible to see similar analysis time of the enantiomers with no significant changes in migration time. The two inset graphs in Figure 6A show the calibration plots of *R*- and *S*-ibuprofen enantiomer, respectively. The *R* square values of *R*-ibuprofen and *S*-ibuprofen are 0.9973 and 0.9974, respectively. The concentrations of *R*- and *S*-ibuprofen in the tablet sample obtained from the calibration plots are 0.72 ± 0.02 mg/mL and 0.70 ± 0.02 mg/mL, respectively. Therefore, the amounts of *R*- and *S*-ibuprofen in the commercial ibuprofen tablet are calculated as 100.1 ± 3.2 mg/mL and 96.9 ± 3.2 mg/mL, respectively. The comparison of the experimental results and commercial labels are listed in the inset table of Figure 6B. The % error for the total racemate is 1.5%, which is acceptable.

4 Concluding remarks

The simultaneous enantioseparation of five profens was achieved by the addition of L-UCLB in the low concentration range (1.5 to 2.0 mM) in the TM- β -CD buffer. The resolutions (both achiral and chiral resolutions) and migration times of the profens were very sensitive to the concentration of L-UCLB. The chiral resolution values of several profens (e.g. indoprofen, and ketoprofen) reported in [11] are less than 1.0. On the other hand, the resolution values reported in our manuscript are significantly higher in the range of 2.0–2.4 for the same two and other profens (see Figure 4). Unlike the literature report on the effects of the chain length of anionic surfactants, decreasing length of the hydrocarbon chain of the cationic IL surfactants did not show any significant influence on the chiral resolutions. However, the migration times decreased as expected. The final method permits to simultaneously resolve the enantiomers of the PROFs with an analysis time of ~45 min. Three batches of L-UCLB synthesized during various months in our laboratory were tested for the simultaneous enantioseparation of PROFs under the same conditions. The migration times, efficiencies, and resolutions of the PROFs showed little deviation, which implies that the batch-to-batch manufacture of L-UCLB synthesized in our laboratory provides reproducible enantioseparation. The use of L-UCLB was applicable to the quantitative determination of ibuprofen in commercial samples. The following paper (Part II) expands this study by applying the fundamental concepts of enzymatic reactions to determine the mode of inhibition of L-UCLB.

Acknowledgments

The present work was supported by the National Institute of health (2R01-GM062314) and the Petroleum Research Fund (47774-AC7).

List of abbreviations

CD	cyclodextrin
DMSO	dimethyl sulfoxide
L-OCLB	<i>N</i> -octenoxycarbonyl-L-leucinol
L-UCLB	<i>N</i> -undecenoxy-carbonyl-L-leucinol bromide
PROF	profen
TM-β-CD	2,3,6-tri-O-methyl- β -cyclodextrin

References

1. Gal, J. Chirality in Drug Research. Francotte, E.; Lindner, W., editors. Vol. 33. Wiley VCH; Weinheim, Germany: 2006. p. 3-24.
2. Maruszak, W.; Schmid, MG.; Gubitz, G.; Ekiert, E.; Trojanowicz, M. Methods in Molecular Biology. In: Gubitz, G.; Schmid, MG., editors. Chiral Separation Methods and Protocols. Vol. 243. Humana Press Inc; Totowa, N.J: 2004. p. 275-289.
3. Gasper MP, Berthod A, Nair UB, Armstrong DW. Anal Chem 1996;68:2501–2514. [PubMed: 8694258]
4. Shamsi SA, Cedillo B, Haddadian F, Warner IM. Anal Chem 2003;75:379–387. [PubMed: 12585461]
5. Han Y, Chen Yi. Electrophoresis 2007;28:2765–2770. [PubMed: 17657762]
6. Huang, Lu; Lin, J.; Xu, L.; Chen, G. Electrophoresis 2007;28:2758–2764. [PubMed: 17592615]
7. Mwongela SM, Numan A, Gill NL, Agbaria AR, Warner IM. Anal Chem 2003;75:6089–6096. [PubMed: 14615986]
8. Seddon KR. J Chem Technol Biotechnol 1997;68:351–356.
9. Wilkes JS, Levisky JA, Wilson RA, Hussey CL. Inorg Chem 1982;21:1263–1264.
10. Rizvi SA, Shamsi SA. Anal Chem 2006;78:7061–7069. [PubMed: 17007537]
11. François Y, Varenne A, Juillerat E, Villemin D, Gareil P. J Chromatogr A 2007;1155:134–141. [PubMed: 17222416]
12. Tran CD, Mejac I. J Chromatogr A 2008;1204:204–209. [PubMed: 18440541]
13. Persson-Stubberud K, Åström O. J Chromatogr A 1998;798:307–314. [PubMed: 9542142]
14. Martín-Biosca Y, Garcia-Ruiz C, Marina M. Electrophoresis 2001;22:3216–3225. [PubMed: 11589282]
15. Rizvi SA, Shamsi AS. Electrophoresis 2005;26:4172–4186. [PubMed: 16217829]

Figure 1. Structural formulas of A: ionic liquids L-UCLB, L-OCLB, and L-BCLB used as chiral selectors, and B: the racemic arylpropionic acids (profens) used as analytes. Asterisks mark the chiral center.

Figure 2.

Simultaneous enantioseparation of profen (PROF) drugs in the absence (A), and presence (B) of L-UCLB. CE conditions: 5 mM NaOAc, 2.63 mM HOAc (pH 5.0) containing 35 mM TM- β -CD (A), and 35 mM TM- β -CD, 1.5 mM L-UCLB (B). Fused silica capillary, 64.5 cm total length and 50 μ m i.d.; separation temperature 16 $^{\circ}$ C; applied voltage +30 kV; pressure injection: 3mbar, 8 sec. UV detection at 214 nm. Peaks identification: 11'= R, S ibuprofen; 22' = R,S fenoprofen; 33'= R, S indoprofen; 44'= R, S suprofen; 55'= R, S ketoprofen.

Figure 3.

Effect of concentration of L-UCLB for the simultaneous enantioseparation of PROF drugs. All other conditions are same as described in Figure 2(B) except variable concentration of L-UCLB was used. 1.0 mM L-UCLB (A), 2.0 mM L-UCLB (B), 3.0 mM L-UCLB (C). The inset plot shows the resolutions of profen drugs as a function of L-UCLB concentrations.

Figure 4.

Effect of batch-to-batch reproducibility on the simultaneous enantioseparation of PROF drugs. 5 mM NaOAc, 2.63 mM HOAc, pH 5.0; 35 mM TM- β -CD buffer, 3.0 mM L-UCLB, A: batch091507, B: batch092107, C: batch102207. All other conditions are same as described in Figure 2(B).

Figure 5.

Comparison of L-UCLB, L-OCLEB, and L-BCLB ionic liquids for the simultaneous enantioseparation of profen drugs. 5 mM NaOAc, 2.63 mM HOAc, pH 5.0; 35 mM TM-β-CD buffer; 3.0 mM L-UCLB, L-OCLEB, or L-BCLB. All other conditions are same as described in Figure 2(B).

Figure 6. Representative electropherograms of (A) ibuprofen tablet sample, 1.42 mg/mL racemate, and (B) standard ibuprofen, 1.0 mg/mL racemate. Peak R: *R*-ibuprofen, peak S: *S*-ibuprofen. Other conditions are same as described in Figure 2(B).