Published in final edited form as:

J Chromatogr A. 2014 September 19; 1360: 296–304. doi:10.1016/j.chroma.2014.07.047.

Combined use of chiral ionic liquid surfactants and neutral cyclodextrins: Evaluation of ionic liquid head groups for enantioseparation of neutral compounds in capillary electrophoresis

Yijin Liu and Shahab A. Shamsi

Department of Chemistry, Center of Diagnostics and Therapeutics, Georgia State University, Atlanta, GA-30303

Abstract

Cyclodextrins (CDs) are most commonly used chiral selectors in capillary electrophoresis (CE). Although the use of neutral CDs and its derivatives have shown to resolve plethora of charged enantiomers, they cannot resolve neutral enantiomers. The use of ionic liquids (ILs) surfactants forming successful complex with CDs present itself an opportunity to resolve neutral enantiomers. In this work, the effect of IL head groups and their complexation ability with heptakis (2,3,6-tri-O-methyl)- β -cyclodextrin (TM- β -CD) was studied for the separation of neutral enantiomers by CE. First, cationic IL type surfactants with different chiral head groups were synthesized. Physicochemical properties such as critical micelle concentration was determined by surface tension, whereas aggregation and polarity were determined by fluorescence spectroscopy. The complexation ability of ILs with TM- β -CD was characterized in the gas phase by CE-mass spectrometry. The influence of the type of ILs head group and its concentration on chiral resolution, resolution per unit time and selectivity were investigated for four structurally diverse neutral compounds. The binding constants of the neutral analytes to the IL-CD complex were estimated by y-reciprocal method. The hydrophobicity of the side chain of the IL head group displayed significant effect on the binding constants and enantioseparations.

Keywords

Enantioseparation; Capillary electrophoresis; Neutral compounds; Ionic liquids type surfactants; 2,3,6-tri-O-methyl- β -cyclodextrin

1. Introduction

The separation of chiral compounds continues to be the center of significant interest not only in the development of pharmaceutical drugs and therapeutics [1], but also in agricultural

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industry (herbicides and pesticides) as well as biomarkers [2, 3]. This interest can be greatly attributed to the increasingly awareness that chirality of living organisms show different biological responses such as different pharmacological activity and pharmacokinetic profile to one of the enantiomer [4–5]. Capillary electrophoresis (CE) has been a useful technique for chiral separation due to its very high efficiency, which provides baseline resolution of enantiomers even when the selectivity is in the range of 1.01–1.02. Other advantages of CE include compact and relatively simple instrumentation as well as small volume requirement of sample and chiral selector it consumes. This latter advantage is very important for developing chiral assay of enantiomers in biological samples or using exotic and expensive chiral selectors. Neutral and derivatized cyclodextrins (CDs) have been employed successfully as chiral selectors in CE [6–9]. However, in several cases, baseline resolution is often difficult and enantiomeric separation of neutral compounds is not achieved using CDs alone. Under such circumstances, other effective chiral reagents could be added to the CDs to improve enantio-separations.

Applications of ionic liquids (ILs) as separation media in CE are of rising interest in the past several years [10–13]. The ILs can also form surfactant, which has long hydrocarbon chain and a polar head group. The IL surfactants are mostly organic salts in the liquid state at room temperature. At present, the combined use of IL and CD in CE has drawn increased interest because of their ability to provide improved chiral separations for applications in cyclodextrin modified CE methods. Our group published the first CE enantioseparation of acidic analytes using two chiral ILs type surfactants as a single chiral selector [14]. Since then, a variety achiral ILs including, imidazolium based [15–17] and dodecyl trimethyl ammonium chloride [18] have been reported in combination with CD derivatives for the enhancement of the enantiomeric resolution of charged drugs. In addition, a fundamental model is developed on the interactions of heptakis-(2,3,6-tri-O-methyl)- β -cyclodextrin (TM- β -CD) with L-UCLB to rationalize enhanced separation of anionic profens [19–20]. However, to the best of our knowledge there are no reports on the CE application of IL surfactants combined with neutral CDs for chiral separations of neutral compounds.

In this study, IL type cationic surfactants with five different chiral head groups such as *N*-undecenoxy-carbonyl-L-alaninol bromide (L-UCAB), *N*-undecenoxy-carbonyl-L-valinol bromide (L-UCVB), *N*-undecenoxy-carbonyl-L-leucinol (L-UCLB) bromide, *N*-undecenoxy-carbonyl-L-isoleucinol (L-UCILB) bromide and *N*-undecenoxy-carbonyl-L-ephedrine bromide (L-UCEB) (Figure 1A) were synthesized and characterized. The complexes form between TM- β -CD and various IL surfactants were observed in gas phase and confirmed by CE-MS. The figures of merit (resolution, resolution per unit time and selectivity) of four representative neutral chiral compounds (Figure 1B): (i) 1,1'-bi-2-naphthol (BOH), (ii) 7,8,9,10-tetrahydro-benzo[a]pyren-7-ol (THBP), (iii) 2,2,2-trifluoro-1-(9-anthryl) ethanol (TFAE) and (iv) trans-stilbene oxide(TSO) were compared for enantioseparation. The IL head groups and concentrations were varied to find the optimum separation conditions for each neutral enantiomeric pair. The binding constant (*K*) of the neutral enantiomers to the TM- β -CD-IL was measured to further investigate the interactions between the neutral analyte and the TM- β -CD-IL complex.

2. Materials and methods

2.1 Reagents and chemicals

Two neutral compounds (BOH and THBP), HPLC-grade acetonitrile (ACN), ammonium formate (99%, NH_4COOH), formic acid (95%), acetic acid (99%), dimethyl sulfoxide (99%, DMSO) and TM- β -CD (90%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The other two neutral compounds (TFAE and TSO) were purchased from TCI AMERICA (Tokyo, Japan). Sodium acetate (NaOAc), sodium hydroxide (NaOH) (50% w/w), sodium phosphate dibasic heptahydrate and sodium phosphate monobasic monohydrate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Triply deionized (DI) water used in this experiment was obtained from Barnstead Nanopure II water system (Barnstead International, Dubuque, IA, USA).

The reagents ω -undecylenyl alcohol, 2-bromoethylamine hydrobromide, pyridine anhydrous, L-leucinol, L-alaninol, L-valinol, L-isoleucinol, (1S,2R)-ephedrine hydrochloride, formic acid (95%), formaldehyde (37% (wt/wt) solution in water) used for the synthesis of ILs were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Triphosgene was obtained from TCI AMERICA (Tokyo, Japan). Sodium sulfate anhydrous and sodium bicarbonate were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2 Synthesis and characterization of L-UCAB, L-UCVB, L-UCLB, L-UCILB and L-UCEB

The surfactant L-UCLB, L-UCAB, L-UCVB, L-UCILB and L-UCEB (Fig. 1A) were synthesized according to a previously reported procedure by Rizvi and Shamsi [14]. Briefly, the (undec-10-enyl-2-bromoethylcarbamate) intermediate was first synthesized by dropwise addition of chloroformate over equimolar solution of bromoethylamine hydrobromide and sodium carbonate [14]. Next, the second intermediates (*N,N*-dimethyl alaninol, *N,N*-dimethyl valinol, *N,N*-dimethyl leucinol, *N,N*-dimethyl isoleucinol and *N,N*-dimethyl ephedrine) were synthesized by reductive alkylation of primary amine of alaninol, valinol, leucinol, isoleucinol and ephedrine according to the Eschweiler-Clark reaction [21,22]. The chiral ILs with difference head groups were synthesized by reacting the first intermediate with all five aforementioned second intermediates under reflux in acetone for 72 hrs to form L-UCAB, L-UCVB, L-UCLB, L-UCILB and L-UCEB, respectively. After 72 hours, the reaction mixture was concentrated by evaporating acetone, and the resulting fluid was dissolved in water and extracted with ethyl acetate. Finally, the aqueous solution of ILs were stirred for at least 1 day to remove trace ethyl acetate and lyophilized at -50°C collector temperature and 0.05 mBar pressure for 3 days. Except for alanine, all four cationic surfactants were IL at room temperature. Data for ^1H -NMR and ESI-MS of L-UCLB is reported elsewhere [14]. The ILs was characterized by ESI-MS in positive ion mode (Figure S1). The ^1H -NMR spectra of four ILs were recorded on a Bruker Avance 400 MHz spectrometer. The chemical shifts (δ) in ppm; multiplicities are indicated as: s (singlet), d (doublet), t (triplet) and m (multiplet). **L-UCAB**, ^1H -NMR: δ 1.18 (s, 13H), 1.38 (d, 3H), 1.54 (m, 2H), 1.99 (d, 2H), 3.06 (d, 6H), 3.46 (d, 2H), 3.51–3.62 (m, 4H), 3.82 (m, 1H), 4.01 (t, 2H), 4.89 (m, 2H), 5.85 (m, 1H). **L-UCVB**, ^1H -NMR: δ 0.94 (m, 1H), 1.22 (s, 12H), 1.54 (s, 2H), 1.96 (d, 2H), 3.14 (d, 6H), 3.56 (d, 4H), 3.97 (d, 2H), 4.83 (m, 1H), 5.70 (m, 2H). **L-UCILB**, ^1H -NMR: δ 0.92 (m, 6H), 1.20 (s, 12H), 1.52 (s, 3H), 3.13 (d, 6H), 3.38 (s, 1H),

3.55 (m, 4H), 3.96 (m, 4H), 4.80 (m, 2H), 5.67 (m, 1H). **L-UCB**, $^1\text{H-NMR}$: δ 1.09 (m, 12H), 1.43 (m, 3H), 1.96 (m, 2H), 2.12 (s, 2H), 3.11 (s, 3H), 3.18 (s, 3H), 3.36 (m, 4H), 3.77 (s, 2H), 4.82 (m, 2H), 5.09 (d, 1H), 5.49 (s, 1H), 5.64 (m, 1H), 7.09–7.39 (m, 5H) (Figure S2-S5). All monomers of IL synthesized in this study were found to be 99% pure or better as estimated from elemental analysis.

2.2.1 Determination of Critical Micelle Concentration—A sigma 703 digital tensiometer (KVS Instruments, Monroe, CT, USA) was used to determine the CMC of all five ILs. This method employs a platinum ring with defined geometry. The DuNoüy ring was first immersed in the liquid. The ring was slowly lifted from a liquid surface and the force required to raise the liquid is related to the surface tension (γ). The γ value (mN/cm^2) displayed on the digital readout is recorded. Solutions of a series of concentration from 0.0625 mM to as high as 12 mM were made in triply DI water and their surface tension values were measured. A graph of surface tension (mN/cm^2) versus concentration of each ILs was plotted. The CMC was determined as the intersection point of two straight lines, which fits the experimental values before and after the abrupt change of slope (Figure S6). Each measurement of the surface tension value was repeated three times to determine the CMC values of all ILs reported in Table 1.

2.2.2 Determination of Aggregation Number—Fluorescence measurements were conducted on a Perkin Elmer LS 55 Fluorescence spectrometer (PerkinElmer Instruments, Norwalk, CA, USA) at room temperature. The aggregation number of the ILs was determined using pyrene and cetylpyridinium chloride as fluorescent probe and quencher, respectively [23, 24]. The stock solution of pyrene was prepared in methanol at a concentration of 1 mM. The quencher was prepared in triply DI water at a concentration of 2 mM. In a typical measurement, 2 mL of 20 mM solution of IL was prepared in triply DI water in a small vial. Next, 2 μL of pyrene from the stock solution was pipetted into this vial, mixed well and sonicated for 90 min and stored in a dark place to equilibrate overnight. Next day 1 mL aliquot of IL was mixed with the quencher solution to make a mixture, which contains 1×10^{-3} mM probe, 1 mM quencher and 10 mM IL (solution 1). The other 1 mL aliquot of IL was diluted with triply DI water to give 1×10^{-3} mM probe and 10 mM IL (solution 2). The solution 1 was added to the solution 2 at an increment of 50 μL and allowed to stabilize for 20 min before each measurement. The excitation and emission wavelengths were set at 335 nm and 393 nm, respectively. After addition of each aliquot of the solution 1, the decrease in emission spectra of the probe was recorded. A graph of the logarithm of the intensity ratio (I_0/I) versus the quencher concentration [Q] was plotted (Figure S7). The aggregation number is obtained from the slope of the plot (Table 1, column 2).

2.3 CE instrumentation

All CE experiments were carried out on an Agilent CE system (Agilent Technologies, Palo Alto, CA, USA) equipped with an on-line diode array detector and 0–30 kV high-voltage power supply. The temperature of the sample carousel was maintained at 16°C (to prevent analytes evaporation, which were dissolved in high percentage of organic solvent) by Fisher ISOTEMP 3016S refrigerating circulator obtained from Fisher Scientific (Pittsburgh, PA,

USA). The Agilent ChemStation software was employed for instrumental control, data acquisition, and data analysis. The fused silica capillary, used in the experiment, with 64.5 cm total length and 56.0 cm effective length (375 μm O.D., with 50 μm I.D) was obtained from Polymicron Technologies (Phoenix, AZ, USA).

2.4 CE conditions and preparation of buffers and analyte solutions

For CE-MS experiments used to characterize ILs-CD complex, a mixture of five ILs was prepared at a concentration of 0.1 mM each. This mixture was profiled using 25 mM NH_4COOH as running buffer at pH 2.5. Next, the ILs-CD complex was profiled using the aforementioned buffer containing 5mM TM- β -CD. For chiral separations, the stock solutions of BOH, TSO and TFAE were prepared in pure ACN at 6.4 mg/mL and THBP stock solution was prepared in pure ACN at 3.2 mg/mL. All chiral analytes were diluted to 1.6 mg/mL in 80/20 ACN/ H_2O (v/v) and pressure injected at 5 mbar for 10 s. The separations were carried out under the normal polarity mode with an applied voltage of +30 kV. The background electrolytes (BGEs) for enantiomeric separations of BOH, TSO and TFAE was 10 mM NaOAc solution, which were prepared by dissolving NaOAc $\cdot$ 3 H_2O in triply DI water and then adjusted to pH 5.0 using acetic acid. The BGEs for TSO consisted of 10 mM each of $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer at pH 7.0. The final running CD-MEKC buffer solutions were prepared by addition of 30 mM TM- β -CD and ILs with five different head groups at various concentrations to the BGE solution. Before each experiment, the bare fused silica capillary was pretreated with 1M NaOH at 25°C for 60 min followed by 30 min flush of triply DI water. During the experiments, the temperature of the cartridge was maintained at 20°C. As a preconditioning step, the capillary was rinsed with the running buffer containing TM- β -CD and ILs for 3 min. After each run, the capillary was flushed with triply DI water for 3 min, 1M NaOH for 30 min and then triply DI water for another 10 min. This ensure repeatable migration time with RSD <2%.

3. Results and Discussion

3.1 CE-MS characterization of ILs and the ILs-CD complex

The structures of IL surfactants with different head groups are shown in Figure 1A. The ILs such as L-UCAB and L-UCVB have small non-polar side chain with one and two methyl group attached to α -carbon and β -carbon, respectively. Although one would expect that the C-11 alkyl chain, which was kept similar in all ILs will have the strongest effect on hydrophobicity, the presence of isobutyl, secondary butyl and phenyl groups in side chains of L-UCLB, L-UCILB, L-UCB still impart some differences in hydrophobicity. The L-UCLB and L-UCILB are isomers with only difference in their structure is the position of methyl groups. In addition, both L-UCILB and L-UCB have two chiral centers.

A mixture of five of the aforementioned IL surfactants was profiled by CE-MS. The resulting extract ion chromatograms (EIC) of ILs without and with TM- β -CD in the formate buffer are shown in Fig. 2A and 2B, respectively. Two major trends are noted. First, the migration time increases slightly with the increase in hydrophobicity of side chain of IL head group and follows the order: (L-UCAB<L-UCVB<L-UCLB=L-UCILB<L-UCB). Second, note that in the presence of TM- β -CD all IL forms a heavier CD-IL complex but

eluted faster than the free forms of IL. Furthermore, no distinct peaks are seen in panel B for the extract ions of the free form of IL surfactants at m/z 343, 371, 385, and 419 but significant and distinct peaks of the complex form of ILs with TM- β -CD are seen at higher m/z values in Figure 2B. This suggests 1:1 complex formation between IL and TM- β -CD in the gas phase. The panel C of Fig. 2, shows that the effective mobility values of injected IL surfactants in the presence of TM- β -CD are larger compared to values obtained in the formate buffer only (i.e., without TM- β -CD). Although one would expect the complex form to have lower mobility than the free form, this is offset by the increase in electro osmotic (EOF) (Fig. 2 panel C, inset bar plots) in the presence of TM- β -CD resulting in faster elution time. No clear explanation is available for this unexpected trend at this time.

3.2 Physicochemical properties of ILs

Physicochemical properties of the chiral amino acid derived cationic ILs type surfactants are summarized in Table 1. The data shows that the CMCs and aggregation of the five IL surfactants decreases with increase in the size of the polar head group. For example, L-UCAB with the smallest polar head group had the largest CMC (1.30 mM + 0.11) but also the largest aggregation number (134 +7). This trend suggests that strong intramolecular hydrogen bonds among smaller head group surfactants are more favorable in the micellar form [25, 26]. In addition, note that the CMCs of L-UCLB and L-UCILB are very close because they are stereoisomers having very similar hydrophobicity of the side chain (isobutyl versus sec butyl groups). A general trend observed is the decrease in aggregation number with increasing size and hydrophobicity of the IL head groups. Thus, L-UCB has better ability to stabilize smaller aggregates than the other four ILs. Because L-UCB is the most hydrophobic among the five surfactants, it is expected to have the strongest interaction with the neutral chiral molecules [25]. In addition, note that the aggregation number of L-UCLB in this work is 91, which is slightly lower than previously reported value of 97 [14]. This could possibly be due to the measurement obtained on two different fluorescence instruments.

The polarity (I_1/I_3) values of L-UCAB, L-UCVB and L-UCLB are very close to form any concrete conclusions (Table 1, last column). Interestingly, L-UCLB and L-UCILB have very similar CMC values but the hydrophobicity/polarity comparison suggest that the latter is slightly more hydrophobic surfactant. Significantly higher hydrophobicity was observed for L-UCB, indicating that pyrene enters the microenvironment core of L-UCB, while it stays close to the head groups of L-UCAB, L-UCVB and L-UCLB surfactants forming micelles.

3.3 Optimization of the enantioseparation parameters

The enantioseparations of four structurally diverse neutral analytes (BOH, TFAE, THBP, and TSO) were examined. First, enantioseparation were compared with TM- β -CD only. Second, each of the five chiral head group surfactant was mixed with TM- β -CD at various molar concentrations. The experimental parameters, which correspond to the shortest run time with baseline resolution of each neutral enantiomer were optimized to determine the optimum surfactant concentration and the best surfactant head group.

3.3.1 Comparison of enantioseparations with and without IL—Figure 3 shows the representative electropherograms for enantioseparation of BOH. Clearly, there is no chiral separation of BOH when the buffer contains only TM- β -CD. This is expected as both BOH and TM- β -CD are electrically neutral at pH 5.0. The uncharged TM- β -CD essentially creates an environment where BOH elutes with the EOF. However, the addition of only 1 mM of all head groups of chiral ILs as a modifier to 30mM TM- β -CD not only showed increased retention but also increased chiral resolution. Note that from charge-to-mass ratio, smallest head group surfactant (i.e., L-UCAB) and the largest head group surfactant (i.e., L-UCB) when added to TM- β -CD provided fastest and slowest elution of BOH enantiomers, respectively. It is worth mentioning that 1 mM L-UCAB and 30 mM TM- β -CD when added to the 10 mM sodium acetate buffer, partial resolution of BOH is obtained, but no reversal of direction of EOF was observed. It is well known in the literature that the CMC of cationic surfactant is decreased several folds upon addition of salt solution [27]. Therefore, in the presence of 10 mM sodium acetate all surfactants seems to reach or exceeds the CMC, but there seem to be less monolayer coverage and more “admicelles” formation on the capillary surface. As discussed in details by Lucy et al [28], admicelles is a pair of surfactant molecules with one positive charged end directed towards the negatively charged wall of the silica surface and the other end is directed outward in the buffer solution. Note that the elution order of BOH in Figure 3 using ILs of different head group is the same as the migration order and effective mobility of the ILs-CD complex observed in the CE-MS (Figure 2, panel A and panel B).

All five chiral head groups of surfactants provided some enantioresolution of BOH. However, the enantioresolutions for the same chiral analyte using L-UCAB and L-UCVB are relatively low compared to using IL with the other three head groups (i.e., L-UCLB, L-UCILB and L-UCB). While at 1 mM of all surfactants, the enantioselectivity of BOH values remains fairly constant from 1.01–1.04 good efficiency in the range of 56,000–126,000 plates/m was observed using all five IL-TM- β -CD pseudophases. Therefore, it appears that the nature of IL surfactant head group has a significant impact on the enantioseparation capability when used in combination with TM- β -CD.

3.3.2. Optimization of type of IL head groups and concentrations—The effect of concentration of ILs with all five head groups on R_s and R_s /time of four neutral enantiomeric pairs is shown in Figure 4(A–H). The aforementioned trends were studied by increasing the ILs concentration from 1 mM to 7 mM at a fixed concentration of TM- β -CD using 10 mM NaOAc buffer pH 5.0 for BOH, TFAE and THBP, and at 10 mM phosphate buffer pH 7.0 for TSO.

As shown in Fig. 4A–D increasing the concentration of ILs increases the chiral R_s of all four neutral analytes, irrespective of the type of chiral head group. However, this increase is more pronounced for IL containing the largest cationic head group (e.g., L-UCB), which provide highest R_s for 3 out of 4 neutral analytes at 4 mM or 7 mM, irrespective of pH. The R_s /tr, on the other hand, can be favorably applied when the R_s is so large that even lower R_s values are acceptable. In such cases, faster effective mobility obtained with IL of a certain head group has a positive effect, namely reduction in analysis time for neutral chiral enantiomers. Therefore, first an ideal IL head group and optimum IL concentration should be optimized to

achieve the maximum resolution in minimum time (i.e. optimum R_s/tr). Second, if resolution values are very large and high throughput screening is desired, then baseline R_s with fastest possible run time should be considered. We investigated the optimum conditions of the two afore-mentioned separation strategies in the enantioseparation of four selected neutral analytes as discussed in the next few paragraphs.

For the four R_s/tr_2 plots shown in Fig. 4E–H, the best IL head group for each neutral analyte was not the one at the highest IL concentration. For instance, comparing Fig. 4A versus Fig. 4E, 5mM L-UCB and 5 mM L-UCLB provided the highest R_s value of 16.0 and 15.0 with tr_2 of 64.9 min and 58.8 min, respectively for BOH. However, in the presence of 5 mM L-UCLB a R_s of 11.0 with tr_2 of only 34 min was obtained. Thus, R_s/tr is ~1.3 folds higher with leucine compared to ephedrine and isoleucine head groups. Based on this result, 5mM L-UCLB is considered the optimum head group and the optimum concentration for the chiral separation of BOH. Similarly, comparing Fig. 4B versus Fig. 4F, 7 mM L-UCB gave the highest R_s value of 7.5 with tr_2 of 94 min for TFAE (R_s/tr ~0.08). Decreasing the concentration of L-UCB to 5 mM, decreases the R_s to 4.4 and tr_2 to 51min but slightly higher R_s/tr value of ~0.09 was obtained. Because the R_s of TFAE using 5 mM L-UCB is similar to 5 mM L-UCLB, but migration time is 7 min longer with the former IL. Thus, 5mM L-UCLB is the optimum head group and concentration for the chiral separation of TFAE as R_s/tr of ~0.10 is the highest possible ratio achieved (Fig. 4F). The highest R_s value of 4.3 and R_s/tr_2 of 0.06 were achieved for THBP in Figure 4C and 4G, respectively at 7 mM L-UCILB. On the other hand, 3mM L-UCLB provided a R_s of 2.5 in 28.6 min (R_s/tr = 0.09). Because the t_R of THBP using 7 mM L-UCILB was 2.3 times longer while the R_s was only about 2 times higher compared to 3mM L-UCLB, the optimum IL concentration and type of head group for the separation of THBP enantiomers is still 3 mM L-UCLB.

Finally, comparing Fig. 4D and Fig. 4H, the highest R_s value of 4.8 was obtained for TSO using 7 mM L-UCB in ~80 min (R_s/tr = 0.06). However, 5 mM L-UCAB realized baseline resolution of 2.1 in only 20 min, (R_s/tr ~.11). In addition, we noted that even though L-UCB gave much higher resolution but its separation efficiency was much lower than that of L-UCAB. This is probably due to significantly higher selectivity (α) value using former IL compared to the later. Therefore, for TSO enantiomers, the optimum concentration and head group for enantioseparations was determined to be 5 mM L-UCAB (Fig. 4H). Furthermore, the effect of concentration of ILs with all five head groups of four enantiomeric pairs on α has almost the same trend as that on R_s , which is the α increases generally with the increase of the concentration of ILs (plots shown in Fig. S8, supplementary data). The only exception noted was TSO which shows essentially no change in α value with the increase in concentration of L-UCAB surfactant.

As mentioned earlier, for the enantiomer analysis, separation scientists are encountered with two situations: (i) (case a) in which highest possible R_s/tr is desired; and (ii) (case b) where baseline resolution with the shortest run time is the goal. When mixtures of multiple chiral analytes are simultaneously analyzed. For example, in monitoring and understanding the role of both parent and active metabolites, highest R_s/tr is desired for separation and accurate quantitation of parent drugs and its chiral metabolites (i.e., case a) [29, 30]. However, in case b such as screening of chiral drug [31], identifying an active chiral

compound among large libraries of potential drug candidates [32], baseline resolution is sufficient because the ultimate goal is fastest possible run time (i.e., highthroughput screening).

Fig. 5 shows the optimum ILs head group and concentration for chiral separation of the three analytes following case b (left electropherograms, Figure 5a, 5c and 5e), but also following case a (right electropherograms, Figure 5b, 5d and 5f), which provided the optimum electropherograms at the highest R_s/t_R values determined from the optimization plots in Fig. 4. For example, ($\pm$)-BOH had a very high R_s value of 11.0 with a migration time of 35 min when using 5 mM L-UCLB/30mM TM- β -CD (Figure 5b, right). However, it still had a sufficient R_s of 3.2 with a run time of ~26 min using 3 mM L-UCAB/30 mM TM- β -CD (Figure 5a, left). Note that the equivalent run times can also be obtained for the BOH using only 1 mM L-UCVB but with slightly lower R_s value (Figure 3 bottom electropherogram). Similar representative electropherograms of appropriate chiral IL suitable under optimum R_s/t_r and fastest separation times with baseline resolution are shown for TFAE and THBP, in respective right and left column, of Figure 5. Interestingly, fastest run time is obtained after screening of the IL head groups, whereas optimum R_s/t_r is achieved mostly using leucine head group. However, the results on enantiomers of TSO (Fig. 5g) is different from the other three chiral compound because 5 mM L-UCAB is the only surfactant head group, which provided both the fastest run time and highest R_s/t_R values.

3.4 Estimation of binding constants between various ILs-CD complex and the neutral analytes

The binding constants (K) of the four chiral analytes were calculated from the experimentally measured mobilities of each enantiomer by y-reciprocal method assuming 1:1 binding between IL and CD [20,33]. Representative example of fitting curves for two neutral enantiomeric pairs (THB and TFAE) using 1–7 mM L-UCLB, 10 mM NaOAC, pH 5.0 at fixed concentration of 30 mM TM- β -CD is shown in Figure 6. For the four model analytes, the correction coefficients (R^2) was in the range from 0.998 to 0.999 with L-UCLB, whereas most of the R^2 fell in the range of 0.997 to 0.999 using the other four ILs. The high degree of correlation by y-reciprocal plot suggests 1:1 complexation model is acceptable between the IL/TM- β -CD-complex and the chiral analyte.

Table 2 shows the binding constants between the neutral enantiomers with various ILs-TM- β -CD complex. In general, the binding constants (K_1 or K_2) of the four neutral chiral analytes with larger polar head group surfactants (i.e., L-UCLB, L-UCILB- L-UCVB) are higher compared to the values obtained with L-UCAB-and L-UCVB surfactants containing small polar head groups. The enantiomers of BOH and TSO display the same trend in K_1 and K_2 values i.e., L-UCVB>L-UCILB>L-UCLB>L-UCAB, which is consistent with the R_s trends shown in Fig. 4A and 4D, respectively for the two analytes. Interestingly, for THBP enantiomers, the K value increases in the order: L-UCILB>L-UCLB>L-UCVB~L-UCAB, which is again consistent with the R_s trend shown in Fig. 4C. Similar correlation between R_s trends shown in Fig. 4B versus the tabulated values in Table

2 (row 2) is seen for TFAE, which follows the order: L-UCB>L-UCLB>L-UCILB>L-UCVB>L-UCAB.

4. Conclusions

The five ILs type surfactants of different amino acid head groups were synthesized and characterized. Their 1:1 complexation in the gas phase with TM- β -CD was confirmed by CE-MS. Moreover, the physicochemical properties of the ILs show that the CMCs of the five IL surfactants and aggregation number decreases with increase in the size of polar head group. The combination of TM- β -CD with cationic ILs type surfactants of different amino alcohol head groups was successfully applied to the chiral separation of four neutral compounds by optimizing the ILs head group and IL concentrations in the low mM concentration regime.

The IL head group has a significant effect on both *R_s* and run time of enantiomers. For example, the enantiomers of BOH, TFAE and THBP were resolved at fastest run time using L-UCAB, L-UCILB and L-UCVB surfactants, respectively but highest *R_s/tr* value was always obtained with L-UCLB. However, L-UCAB was the only surfactant, which provided both fastest run time and optimum *R_s/tr* for enantiomers of TSO. Thus, the optimum ILs head group and concentration is analyte dependent. The optimum head group should be screened for each analyte when fastest run time with baseline resolution is desired. The binding constants between the ILs-CD complexes and the enantiomers were estimated using y-reciprocal linear method assuming 1:1 binding. The *K* values fell in the range from 3,800 to 71,100 for all the four chiral analytes, In most cases, the *K* of each enantiomer increases with increasing size of the head group indicating strong contribution from hydrophobic interaction imparted by the amino alcohol side chain of the IL surfactants [32]. In addition, the *K* values correlate well with the trends in chiral *R_s* values of the four chiral analytes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge the support of the National Institutes of Health for funding this research through grant number (R01-GM-062314).

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Highlights

- Evidence of complex formation between neutral CD and charged IL surfactant with various amino alcohol head groups were observed in CE-MS
- Effect of IL surfactant head groups on CMC, aggregation, and polarity were studied
- Effect of IL head groups and IL concentration on chiral resolution, and resolution/time were studied to optimize enantioseparation of neutral enantiomers
- The binding constant of IL-TM- β -CD-analyte complex correlates well with trends in chiral resolution of enantiomers

Figure 1.

Chemical structure of A: TM- β -CD and ionic liquids (ILs) with various head groups [L-UCAB, L-UCVB, L-UCLB, L-UCILB, L-UCB], and B: structures of neutral chiral analytes.

Figure 2.

CE-MS extracted ion electropherograms (EIC) of IL surfactants (panel A), and ILs-CD complex (panel B) obtained in the absence and presence of TM-β-CD, respectively in 25 mM ammonium formate buffer (pH 2.5). A plot of effective mobility versus ILs surfactant type is shown in Panel C. The left electropherograms represent the protonated $[M+H]^+$ ion profile of the five injected ILs, monitored at m/z 343 for L-UCAB, m/z 371 for L-UCVB, m/z 385 for L-UCLB and L-UCILB and m/z 419 for L-UCEB. The positive $[M+H]^+$ ion profile of ILs-CD complex is shown in the right electropherograms, which were monitored at m/z 1772 for L-UCAB+CD, m/z 1800 for L-UCVB+CD, m/z 1814 for L-UCLB/L-UCILB+CD and m/z 1848 for L-UCEB+CD. The inset bar plot in panel C compares the EOF mobility with and without TM-β-CD in 25 mM ammonium formate buffer (pH 2.5). Thiourea was used as an EOF mobility marker, which was monitored as protonated compound in ESI-MS at m/z 77.

Figure 3. Enantioseparation of BOH using 30 mM TM- β -CD only and 30 mM TM- β -CD with 1mM of IL surfactant with various head groups. Running buffer: 10mM NaOAc, pH 5.0. For other conditions see experimental section.

Figure 4.

Trend plots illustrating the concentration of IL with different head groups added to 30mMTM- β -CD on the chiral R_s and R_s/tr_2 of (A,E) BOH, (B, F) TFAE, (C, G) THBP, (D, H) TSO. Conditions: 10mM NaOAc buffer pH 5.0 for BOH, TFAE and THBP, and at 10mM phosphate buffer pH 7.0 for TSO. Other conditions are the same as described in experimental section.

Figure 5.

Electropherograms of the neutral chiral analytes obtained at the fastest run times (a, c and e); and at the highest possible R_s/tr values (b, d, f.). The chiral separation of TSO in (g) represent the conditions for both optimized run times and optimized R_s/tr .

Figure 6.

The y-reciprocal plots for the four chiral solutes used to estimate the binding constant (K) between the analytes and IL-CD complex (μ_{eff} the effective mobility; μ_{free} : the effective mobility of the free solute)

Table 1

Physicochemical properties of the five chiral cationic ILs type surfactants

	Physicochemical properties of the ionic liquid surfactants		
	CMC (mM) ^a	Aggregation number ^b	Polarity(I_1/I_3) ratio ^c
L-UCAB	1.30±0.11	134±7	1.03±0.03
L-UCVB	0.94±0.06	117±4	1.09±0.01
L-UCLB	0.86±0.01	91±2	1.10±0.05
L-UCILB	0.85±0.06	75±5	1.23±0.05
L-UCEB	0.70±0.08	51±10	1.27±0.04

^a Critical micelle concentration was determined by surface tension^b Aggregation number was determined by the fluorescence quenching experiment using pyrene as a probe and cetylpyridinium chloride as a quencher.^c Polarities of the surfactants were determined using ratio of the fluorescence intensity (I_1/I_3) of pyrene.

Table 2

The estimated values of K_1 and K_2 of four neutral enantiomers by γ -reciprocal method^a

		L-UCAB-CD	L-UCVB-CD	L-UCLB-CD	L-UCILB-CD	L-UCEB-CD
THBP	K_1 (M^{-1})	5,100	7,700	9,000	11,200	7,900
	K_2 (M^{-1})	6,200	8,500	9,200	13,900	9,000
TFAE	K_1 (M^{-1})	5,400	13,700	27,000	26,500	28,000
	K_2 (M^{-1})	6,900	14,900	48,400	51,000	71,100
BOH	K_1 (M^{-1})	3,800	4,300	4,700	8,500	9,100
	K_2 (M^{-1})	4,900	5,500	7,100	8,900	9,300
TSO	K_1 (M^{-1})	18,400	18,800	19,000	21,500	24,200
	K_2 (M^{-1})	20,000	22,900	20,000	25,000	56,900

^aThe concentration of IL was varied at levels of 1 mM, 3 mM, 5 mM, 7 mM using a fixed concentration of 30 mM TM- β -CD. Other CE conditions are same as described in the experimental section.