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Synthesis, characterization and application of polysodium *N*-alkylenyl α -D-glucopyranoside surfactants for micellar electrokinetic chromatography-tandem mass spectrometry

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Abstract

Sugar based ionic surfactants forming micelles are known to suppress electrospray ionization of various compounds due to decrease in surface tension upon micelle formation [1]. For the first time, poly (sodium *N*-alkenyl- α -D-glucopyranoside, (poly- α -D-SUGP) based surfactants with different chain lengths and head groups have been successfully synthesized, characterized and applied as compatible chiral selector for MEKC-ESI-MS/MS. First, the effect of polymerization concentration of the monomer, sodium *N*-undecylenyl- α -D-glucopyranoside 4,6-hydrogen phosphate (α -D-SUGP), was evaluated by enantioseparation of one anionic compound [1,1'-binaphthyl-2,2'-diyl-hydrogen phosphate (BNP)] and one zwitterionic compound (dansylated phenylalanine) in MEKC-UV to find the optimum molar surfactant concentration for polymerization. Next, MEKC-UV and MEKC-MS was compared for the enantioseparation of BNP. The influence of polymeric glucopyranoside-based surfactant head groups and carbon chain lengths on chiral *Rs* was evaluated for two classes of cationic drugs (ephedrine alkaloids and β -blockers). Finally, enantioselective MEKC-MS of ephedrine alkaloids and β -blockers were profiled at their optimum pH 5.0 and 7.0, respectively using 20 mM NH₄OAc, 25 mM poly- α -D-SUGP at 30 kV and 25 °C under optimum spray chamber conditions. The LOD for most of the enantiomers ranges from 10 ng/mL-100 ng/ml with *S/N* of at least 3.0.

Keywords

Enantioseparations; poly(sodium *N*-alkenyl- α -D-glucopyranoside) surfactants; ephedrine alkaloids; beta-blockers; MEKC-ESI-MS/MS

1. Introduction

Increasing concerns on the resolution, sensitivity and structural identification of pharmaceutical drugs boosted the recent development of different types of chiral stationary phases for HPLC-MS [2–4]. However, over the years CE-MS, has emerged as a good alternative [5–7] because this microscale hyphenation technique provide efficient and low cost assays, which is particularly attractive in clinical settings where reduced requirement of

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biological samples and solvent consumptions is a contributing factor in future analysis of biological activity, *in vivo* studies and toxicity evaluation of pharmaceutical drugs.

The technique of CE-MS can be employed in two main approaches. The first approach is most common in which simply the chiral selector or chiral surfactant is added to the volatile buffer solution (aka. EKC-MS or MEKC-MS) [5–7]. The second approach involves the use of bonded or immobilized chiral selector on the capillary column (aka. CEC-MS), which presents high sensitivity and greater sample loading capacity [8–10]. There is potential in both EKC-MS or MEKC-MS as well as CEC-MS to improve with the development of new chiral selectors for the separation and sensitive detection of a large number of chiral compounds. Nevertheless, in all three aforementioned approaches of CE-MS, exotic chiral selector could be used to understand chiral separation and developed new chiral assays at much lower cost and high efficiency compared to HPLC-MS.

Among the different chiral selectors added to the volatile buffers in EKC-MS, cyclodextrins [11] and crown ethers [12] are useful at low mM concentrations, perhaps due to prevent ion suppression of the low molecular weight chiral selectors. Polymeric amino acid based surfactants (aka. molecular micelles) are a promising class of chiral selector, which has significant advantages for MEKC-MS over conventional low-molecular-mass chiral selectors (e.g., cyclodextrins and crown ethers). First, they are covalently stabilized micellar aggregates with zero CMC, which can be used over a wide concentration range in MEKC-MS [13]. Second, due to high molecular weight they are not fragmented in the gas phase of ESI-MS [7,14,15]. Thus, the key to development of EKC-MS or MEKC-MS approaches is to identify chiral selectors, which provides high separation selectivity and are MS compatible for sensitive detection of chiral compounds.

In this work, the synthesis, characterization and application four new sugar based polymeric chiral surfactant is described for MEKC-MS. Although unpolymerized dodecyl-glucopyranoside-based surfactants was previously reported for chiral separations in MEKC-UV [16], to the best of our knowledge, poly(sodium *N*-alkylenyl- α -D-glucopyranoside) based surfactants have never been studied as chiral selectors for MEKC-MS. To achieve high separation selectivity, we investigated the polymerization concentration of the surfactants monomers, two different head groups and two different chain lengths of the surfactant as well as buffer pH. The chiral separation of BNP using the polymeric glucopyranoside-based surfactants in MEKC with UV and MS detection is compared. As illustrated, the use of optimum polymeric glucopyranoside-based surfactant in MS gave higher enantioselectivity and efficiency with lower limit of detection for ten racemates.

2. Materials and methods

2.1 Reagents and chemicals

Racemic mixture and individual isomers of ephedrine alkaloids (norephedrine, pseudoephedrine, ephedrine, and methylephedrine), β -blockers (atenolol, metoprolol, carteolol, and talinolol) and 1,1'-binaphthyl-2,2'-diylhydrogen-phosphate (BNP), analytical-grade ammonium acetate (as 7.5 M NH_4OAc solution) and HPLC grade methanol (MeOH) were all obtained from Sigma-Aldrich (St. Louis MO, USA). Racemic mixture of dansyl

phenylalanine (DNS-PA) was purchased from Santa Cruz Biotechnology (Dallas, Texas, USA). Sodium phosphate dibasic heptahydrate and sodium phosphate monobasic monohydrate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ammonium hydroxide and acetic acid were supplied by Fisher Scientific (Springfield, NJ, USA). All four vinyl surfactant monomers (i.e., octenyl and undecylenyl phosphated and sulfated sugar surfactants) were polymerized under 20 Mrad of ^{60}Co γ -radiation by Phoenix Memorial Laboratory (University of Michigan, Ann Arbor, MI). Other reagents used for synthesis of sugar surfactants could be found in the supporting information (S-1a).

2.2 Synthesis of *N*-undecylenyl and *N*-octenyl- α -D-glucopyranoside 4,6-hydrogen phosphate sodium salt, *N*-undecylenyl and *N*-octenyl- α -D-glucopyranoside 6-hydrogen sulfate, sodium salt

Both phosphated and sulfated sugar surfactants with eight and eleven chain lengths were synthesized with some modifications of the literature protocols [16,17,18] as detailed below. Synthesis of compounds 2 and 3. The compounds 2 and 3 were synthesized according to step 1 of Scheme 1. A solution of 10-undecen-1-ol (3.9 mL, 0.0195 mol) or 7-octene-1-ol (2.9 mL, 0.0195 mol), and 8.0 mL (0.065 mol) boron trifluoride etherate were added to a stirring solution of 5g (0.013 mol) β -D-glucose pentaacetate in 50 mL dry dichloromethane under nitrogen. The resulting solution was then stirred for 72 hrs under a blanket of nitrogen at room temperature. The mixture was neutralized by 250 mL saturated sodium bicarbonate solution (30 g, 0.36 mol) and then washed with 200 mL H_2O . The lower organic layer was then dried with 10g Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography [SiO_2 , hexane-EtoAc, 1:3] to yield pure compound 2, *N*-undecylenyl α -D-glucopyranoside pentaacetate (1.9g, 29%) and compound 3, α *N*-octylenyl- α -D-glucopyranoside pentaacetate (1.5 g, 25 %).

Synthesis of compounds 4 and 5: The intermediate 2 and 3 were deacetylated in the presence of 20 mL anhydrous methanol and 1mL sodium methoxide solution (25%) under nitrogen for 3 hrs in ice bath. The resulting reaction was then neutralized by 2g Amberlyst®15 hydrogen resin, filtered and concentrated in vacuo. The residue was purified by column chromatography [SiO_2 , EtoAc-MeOH, 10:0.5] to yield the pure intermediate 4, undecylenyl α -D-glucopyranoside (1.1 g, 87%) and 5, *N*-octylenyl α -D-glucopyranoside (0.82 g, 87 %).

Synthesis of compounds 6 and 7: To synthesize product 6 and 7, phenyl dichlorophosphate (0.55 mL, 0.0037 mol) and dry triethylamine (0.69 mL, 0.0050 mL) was added to the solution containing intermediate 4 (1.1g, 0.0033 mol) or intermediate 5 (0.82 g, 0.0028 mol) in 20 mL of dichloromethane. The resulting solution was then stirred for 22 hrs under nitrogen to yield intermediate 6, *N*-undecylenyl α -D-glucopyranoside 4,6-phenyl phosphate and 7, *N*- octylenyl α -D-glucopyranoside 4,6-phenyl phosphate. The resulting reaction was concentrated in vacuo and then purified by column chromatography [SiO_2 , hexane- EtoAc, 2:1] to yield the pure intermediate 6 (1.0 g, 64%) and 7 (0.73 g, 61 %).

Synthesis of final products 8 and 9: The product 8 sodium *N*-undecylenyl- α -D-glucopyranoside 4, 6-hydrogen phosphate and product 9, sodium *N*-octylenyl- α -D-

glucopyranoside 4, 6-hydrogen phosphate were formed by dissolving intermediates 6 (1.0 g, 0.0021 mol) and 7 (0.73 g, 0.0017 mol) in 20 mL THF and by stirring for 24 hrs in the presence of 5 mL NaOH (10%) solution. Finally, THF was removed by vacuo and the resulting solution of each product was neutralized by 1 M HCl and extracted with ethyl acetate for 3 times. Next, the bottom aqueous solution of α -phosphate sugar surfactants were stirred for 1 day to remove trace ethyl acetate and lyophilized at -50°C collector temperature and 0.05 mbar pressure for 2 days to yield pure final product 8 (0.8 g, 90%) and 9 (0.56 g, 88%)

Synthesis of compounds 10 and 11: To synthesize product 10 and 11, sulfur trioxide pyridine complex (0.63 g, 0.0040 mol) was added to the stirring solution containing 20 mL pyridine anhydrous and intermediate 4 (1.1 g, 0.0033 mol) or intermediate 5 (0.82 g, 0.0028 mol). The resulting reaction was stirred for overnight under nitrogen to yield intermediate 10, *N*-undecylenyl α -D-glucopyranoside 6-hydrogen sulfate and 11, *N*-octylenyl α -D-glucopyranoside 6-hydrogen sulfate. The resulting reaction was concentrated in vacuo and then purified by column chromatography [SiO_2 , EtOAc-MeOH- H_2O , 10:2:1] to yield the pure intermediate intermediate 10 (0.9 g, 66%) and 11 (0.59 g, 64%).

Synthesis of final products 12 and 13: The product 12 sodium *N*-undecylenyl- α -D-glucopyranoside 6-hydrogen sulfate, and product 13, sodium *N*-octylenyl 6-hydrogen sulfate, monosodium salt was formed by the addition of 1 mL NaOH (10%) solution and was lyophilized at -50°C collector temperature and 0.05 mbar pressure for 2 days to yield pure final product 12 (0.9 g, 95%) and 13 (0.59 g, 95%).

2.3 Characterization of α -D-Sugar Surfactants

The synthesized α -D-sugar surfactants with different chain lengths and head groups were characterized by nuclear magnetic resonance (^1H -NMR) spectroscopy and electrospray ionization mass spectrometry (ESI-MS). The details of the characterization of intermediates and products of the four synthesized sugar surfactants could be found in the supporting information.(Fig. S1–S13).

2.4 CE-MS instrumentation

2.4.1 MEKC-UV and MEKC-MS conditions—The direct infusion ESI-MS experiments to obtain the deprotonated molecular ions $[\text{M}-\text{H}]^-$ of synthesized anionic sugar phosphated and sulfated surfactants could be found in Figure S.13 a–d. The results are discussed in section 3.3 were carried out on an Agilent G1600 CE system (Agilent Technologies, Palo Alto, CA, USA) equipped with a single quadrupole mass spectrometer. A typical procedure involves flushing 1mg/mL of each synthesized surfactant (dissolved in triply deionized water) through a 60-cm long, 50 μm i.d., fused silica capillary at 50 mbar to the ESI sprayer interface and monitoring the ESI-MS spectrum of the synthesized surfactant. The details of MEKC-UV and MEKC-MS/MS instrumentation could be found in the supporting information.

2.4.2 Preparation of running MEKC-MS buffer and analyte solutions—For chiral separations of DNS-PA and BNP in MEKC, the stock solutions were prepared in pure

MeOH at 2.0 mg/mL and then were diluted to 1.0 mg/mL in MeOH/H₂O (50/50, 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 +20 kV. The background electrolytes (BGEs) for enantiomeric separation of DNS-PA consisted of 12.5 mM each of NaH₂PO₄/Na₂HPO₄ buffer at pH 7.0. The final running MEKC buffer solution was prepared by addition of 45 mM poly- α -D-UGP at various polymerization concentrations (20 mM, 50 mM, 75 mM and 100 mM) to the BGE solution. The BGE for comparison of enantiomeric separation and sensitivity of BNP by MEKC-UV and MEKC-MS/MS was prepared in 20 mM NH₄OAc solution by diluting 7.5 M NH₄OAc stock solution in triply DI water and then adjusting to pH 10.8 by 13.4 M ammonium hydroxide. The final running MEKC buffer solution was prepared by adding 15 mM poly- α -D-SUGP to the BGE solution.

All MEKC-UV and MEKC-MS/MS experiments were performed under the normal polarity mode with an applied voltage of +20 kV. The ephedrine alkaloids, β -blockers and BNP were injected by applying a pressure of 5 mbar for 10 s. The final running MEKC-MS buffer for enantiomeric separation of BNP is the same as that in MEKC experiment. The BGEs for enantiomeric separation of ephedrine alkaloids and β -blockers were 25 mM NH₄OAc prepared by diluting the 7.5 M NH₄OAc stock solution in triply DI water and then adjusted to pH 5.0 by acetic acid or to pH 7.0 by ammonium hydroxide, respectively. The final running MEKC-ESI-MS/MS buffer solutions for screening of enantioseparation were prepared by addition of 30 mM polymeric UGP, OGP, UGS or OGS to the BGE solutions. The composition and the flow rate of the sheath liquid were MeOH/H₂O (80/20, %v/v) containing 5 mM NH₄OAc at pH 6.8 and 5 μ L/min, respectively. Spray chamber parameters for BNP: nebulizer pressure: 3 psi, drying gas temp.: 250 °C, drying gas flow: 6 L/min; capillary voltage: -3000 V. For ephedrine alkaloids and β -blockers: nebulizer pressure: 3 psi, drying gas temp.: 200 °C, drying gas flow: 8 L/min; capillary voltage: +3000 V. The fragmentor voltage, collision energy and product ion formations for these analytes were optimized by optimizer software using Agilent LC-MS in flow injection mode. The details are tabulated in Table 1. The ESI-MS/MS detection was performed in the multiple reaction monitoring (MRM) negative ion mode. The sheath liquids were prepared by mixing 5 mM NH₄OAc aqueous solution with MeOH/H₂O (80/20, %v/v) for optimization study for BNP, ephedrines and β -blockers.

3. Results and Discussion

3.1 Physicochemical Properties

Surface tension measurements were performed to evaluate the surface activity of all four α -D-glucopyranoside based surfactant in water at various molar concentrations. The surface tension of surfactants gradually decreases with increase in surfactant concentration and then reaches a plateau region, signaling micelle formation and the concentration of the break point, which represents the critical micelle concentration (CMC). As shown in Table 2, the values of CMC decreases in the following order: α -D-SOGS > α -D-SOGP > α -D-SUGS > α -D-SUGP, suggesting that sulfate head group sugar surfactants have higher CMC than phosphate head group surfactants at equivalent surfactant chain length. This is perhaps due to increase in hydrogen bonding interactions, which decreases the repulsion between

phosphate head groups within a micelle. The trend for chain length is in accordance with increased hydrophobicity owing to the extension of hydrocarbon chain from C₈ to C₁₁. The CMC is lower for longer chain (i.e., α -D-SUGS and α -D-SUGP), because it facilitates micelles formation at lower concentration and hence lower CMC. Aggregation number and polarity of both unpolymerized micelle and polymerized form of the four surfactants were calculated as reported earlier for amino acid surfactants [19]. With increase in chain length of α -D-glucopyranoside surfactants for each head group, aggregation increases and polarity decreases.

3.2 Effect of Polymerization concentrations on chiral resolution and run time

It is well-known that ionic molecular micelles facilitates the polymerization to produce high molecular weight polymers. The chiral recognition ability of molecular micelle was characterized using two model test solutes [DNS-PA and BNP] using optimum pH and optimum buffer type by varying the analytical concentration of α -D-SUGP monomers. The polymerization experiment was performed by varying the analytical concentrations at 20 mM, 50 mM, 75 mM and 100 mM β -D-SUGP at a fixed dose of 20 Mrad of ⁶⁰Co- γ -radiation. Several interesting trends are noted in Fig. 1 when comparing the resolution and retention of two enantiomeric pairs. First, note that due to high reactivity, α -D-SUGP monomers, even when polymerized at 20 mM (i.e., 5x CMC) provides significant retention of both chiral analyte. Second, micelles formed using higher analytical concentration of α -D-SUGP always resulted in longer run times irrespective of the type of chiral solute. This trend is primarily due to increase in aggregation number and hence hydrophobicity of the micelle. Note, that this effect of increasing migration time is less pronounced for BNP, (Fig. 1A right panel) compared to DNS-PA (Fig 1B left panel).

Our hypothesis regarding the data shown in Fig. 1 is that when poly- α -D-SUGP micelles are used for chiral separation, the achiral hydrophobic tail, which is linked to a chiral linker (i.e., sugar moiety) does not participate in chiral separation, but it does contribute to experimentally observed retention of both enantiomers. The relatively higher increase in retention of DNS-PA compared to BNP suggests that the former enantiomeric pair is mainly retained by extensive contribution from non-enantioselective hydrophobic site of the molecular micelles. Consequently, the increase in enantiomeric resolution is smaller for DNS-PA than for BNP. Third, the enantioselectivity of BNP was observed at all surfactant concentration used for polymerization. On the other hand, the DNS-PA requires the use of micelles, which are formed using at least 12.5 times the CMC of α -D-SUGP to show any hint of chiral resolution ($R_s=0.8$). To test the hypothesis on the data acquired above, a quantitative assessment of enantioselective versus non-enantioselective interaction in MEKC will be performed in our future communication using linear free energy relationship.

3.3 Direct infusion ESI-MS of model test analyte

Our surface tension data suggest that sugar based surfactants forming polymeric micelles are less surface active (surface tension ~ 54 mN/m) and its surface tension is fairly constant in the studied range from 1–50 mM, compared to the corresponding unpolymerized micelles (surface tension ~ 30 mN/m at the CMC) (data not shown). Thus, the lower surface activity of polymerized sugar micelles should reduce interferences in electrospray process.

Therefore, these findings may have significant implications for sensitive CE-MS analysis of chiral compounds. Fig. 2 compares the positive ion ESI-MS spectra (arbitrary y-units) of model test analyte, protonated DNS-PA ($m/z = 397$) in the absence (2A) and presence of monomeric (unpolymerized α -D-SUGP micelle, 2B) and polymeric (poly- α -D-SUGP, 2C).

Consistent with the surface tension data, significant ESI-MS suppression of protonated form of model test analyte, DNS-PA was observed and the signal abundance of this compound reduces from 39872 to only 3695, which is ~10% in the presence of 50 mM of unpolymerized (α -D-SUGP, Fig. 2B). However, as evident from the right panel, the use 50 mM poly- α -D-SUGP in the positive ion ESI-MS also suppresses the signal intensity of DNS-PA, but still 57% of the signal intensity (abundance = 22184) was observed (Fig. 2C). This suggest that unlike commonly used low molecular weight selectors (e.g., sulfated β -CD, vancomycin, crown ether), direct infusion of the poly- α -D-SUGP into the ESI-MS does not contaminate the ion source. Furthermore, no frequent MS cleaning is required. The absence of any monomer peak when infused with 50 mM poly- β -D-SUGP and no polymer peak observed at any upper m/z of the ESI-MS (Fig. 1C) confirmed that the covalent bond formed between sugar surfactant monomers indeed generate a high molecular weight micelle, which is difficult to ionize during electrospray process. While 50 mM of chiral selector is only used for weakly binding analyte, we can state that for strongly binding analyte we could achieve excellent S/N , allowing trace level chiral drug detection as demonstrated in the next section. Even with off-line MALDI-TOF we were unable to see any dimer or trimer peak of poly- α -D-SUGP. Direct infusion of poly- α -D-SUGP in the electrospray at much lower concentration (e.g., 25 mM), the signal intensity of DNS-PA was restored to 80% of its original value (data not shown).

3.4 Comparison of MEKC-UV with MEKC-MS

The UV absorbance is the most commonly used detection method for majority of chiral separations in MEKC. Although chiral MEKC-UV is inherently less sensitive than MEKC-MS, the use of low molecular weight chiral selectors in MEKC-ESI-MS is detrimental and has several limitations [20]. For example, the low molecular weight selector produces abundant gas phase molecular ions, which participate in charge competition as well as ion pair formation in the sprayer suppressing the analyte signal. It should be noted that the high molecular weight chiral polymeric surfactant (aka. molecular micelles, MoMs) are non-volatile but they are not fragmented in the ESI-source. The benefit of using high molecular weight (e.g., >10 kDa) MoMs as chiral selector for MS is that the formation of covalent bond between vinyl terminated monomers in MoMs prevent fragmentation in the electrospray. Furthermore, MoMs are difficult to ionize and they migrate in a direction, which is opposite to EOF. Thus, the use of higher molecular weight chiral selector despite of being non-ionizable and non-volatile could provide very useful information about the mass and structure associated with enantioseparation of unknown chiral compounds. On the other hand, UV cannot solve such identification problems. In fact, when samples containing contaminants are analyzed in MEKC-UV, several unknown peaks appears or coelutes in the electropherogram.

The binaphthyl derivative such as BNP is extensively used as asymmetric ligands in the synthesis of chiral catalysts and also employed as chiral probe solute in MEKC to evaluate the enantioselectivity of chiral stationary phase [21]. As is shown clearly in Fig. 3A and B, baseline chiral *Rs* has been achieved for BNP using poly- α -D-SUGP with ammonium acetate buffer in MEKC-UV and MEKC-MS/MS, respectively. However, MEKC-MS/MS using high molecular weight poly- α -D-SUGP provide higher efficiency and signal to noise ratio (*S/N*) even when injected at concentration ten folds lower than MEKC-UV. The levels of repeatability, which is expressed as the precision obtained under same operating condition was briefly investigated. Using BNP as a model test analyte for 10 consecutive runs, the %RSD of migration time, peak area and resolution were 2.3, 20.5 and 2.2, respectively without any normalization (Fig. S14, supporting information).

3.5 Effect of chain lengths and head groups of sugar surfactant on chiral separations

Four polymeric sugar surfactants with two different head groups (phosphate and sulfate) and two chain lengths (C_8 and C_{11}) were selected to separate ephedrine alkaloids under the same CE conditions to find out the optimum head group and chain length. The bar plots in Fig. 4A and 4B compares the chiral *Rs* of four ephedrine alkaloids and four β -blockers, respectively when the four polymeric sugar surfactants were used as chiral selector in MEKC-MS/MS. From Fig. 4A, poly- α -D-SUGP has the highest chiral *Rs* values among all of the four surfactants for all ephedrine alkaloids. Note, poly- α -D-SUGS gave baseline chiral *Rs* for norephedrine only, whereas partial *Rs* was obtained for methylephedrine and norephedrine using poly- α -D-SUGS and poly- α -D-SOGS, respectively. However, no chiral *Rs* was observed when using poly- α -D-SOGP.

Similarly, in Fig. 4B, the highest chiral *Rs* value was again achieved by poly- α -D-SUGP for the four β -blockers. Using the other three polymeric surfactant, only atenolol provided partial chiral *Rs* irrespective of the chain length and head group of surfactants. Therefore, it could be concluded that poly- α -D-SUGP is the optimum chiral selector among the four surfactants for the enantioseparations of ephedrine alkaloids and β -blockers. Furthermore, it is noted that poly α -D-SUGP with phosphate head group and C_{11} chain length provides better enantioresolution than poly α -D-SUGS using sulfate head group with equivalent chain length with regard to enantioseparations of BNP (data not shown). This could be explained by the fact that phosphate head group has a more rigid bicyclic structure than sulfate group [16], which contributes to higher enantioseparation and better interaction between poly α -D-SUGP and the analytes.

3.6 Effect of buffer pH

The effective charge on the chiral analyte and chiral polymeric surfactant as well as the magnitude of the EOF are greatly affected by the pH of the volatile BGE. According to previous study in our group using amino acid based polymeric surfactants [19, 22–23], the enantioseparation of ephedrine alkaloids and β -blockers were achieved at pH 6.0 and 8.8, respectively using ammonium acetate buffer. Thus, pH ranges were investigated from 5.0 to 6.0 for ephedrine alkaloids and 6.0 to 9.5 for β -blockers using poly- α -D-SUGP.

Fig. 5A shows the chiral R_s of ephedrine alkaloids vs. buffer pH. The R_s value of four ephedrine alkaloids decreased with the increase of pH from 5.0–6.0. This is because increasing pH increases the EOF resulting in faster electroosmotic mobility and weaker interactions with poly- α -D-SUGP. The chiral R_s of β -blockers vs. buffer pH is shown in Fig. 5B. As stated earlier, the increase in EOF of ammonium from 5.0 to 6.0 caused decreasing enantioseparation of ephedrine alkaloids. However, the β -blockers seem to encounter the most favorable (fastest) EOF at pH 7.0 compared to pH 6.0. Further increase in pH from 7.0 to 9.5 (by titration with NH_4OH) caused an increase in ionic strength because at higher pH values the buffer solution also contains excess of ammonium ions and hydroxide ions resulting in much slower electroosmotic mobility. Consequently increasing migration time and decreasing the chiral R_s . Thus, the majority of β -blockers provided highest R_s value at pH 7.0.

3.6.1 Optimum chiral MEKC-MS/MS of ephedrine alkaloids and beta blockers

—Using 25 mM poly- α -D-SUGP at optimum pH 5.0 in MEKC-MS/MS the enantioresolutions and enantioselectivity of four ephedrine alkaloids follows the decreasing order: (methylephedrine > ephedrine ~ norephedrine > pseudoephedrine) in MEKC-MS/MS (Fig. 6). The enantiomer pair of ephedrine and pseudoephedrine have the same precursor and the daughter ions. Thus, neither mass (accurate mass) nor MS/MS could distinguish the two pairs of enantiomers. The simultaneous baseline separation based on MEKC retention of pseudoephedrine from ephedrine prevent any cross talk of daughter ions. This would allow the two isomers to be quickly identified and quantitated in biological samples. The pseudoephedrine enantiomers has partial R_s of 0.9 but still it was well resolved from ephedrine enantiomers. The other three enantiomers were baseline separated with methylephedrine being the longest retained and norephedrine is the shortest retained among all four chiral analytes.

Fig. 7 shows the electropherograms of the enantioseparations of four β -blockers: atenolol, metoprolol, carteolol and talinolol using the optimum 25 mM poly- α -D-SUGP at optimum pH 5.0. Interestingly, polymeric UGP is capable of separating these β -blockers based on their hydrophobicity ($\log P$). Note that the migration time increases in the order of increasing retention: atenolol < carteolol < metoprolol < talinolol, which is consistent with the $\log P$ (atenolol: 0.34 < carteolol: 1.34 < metoprolol: 1.63 < talinolol: 2.98). However, R_s and α trends does not seem to follow the $\log P$ order. The chromatograms shown in Table S.1 (supporting information) indicates that LOD is analyte dependent and ranges from 10–100 ng/mL, which could be further improved using preconcentration techniques.

4 Concluding remarks

Four polymers of α -D-glucopyranoside based surfactants were synthesized, characterized and screened for chiral analysis in MEKC-MS. The use of polymers instead of the monomers of the same surfactants shows significantly enhanced signal intensity in ESI-MS. The addition of poly- α -SUGP at pH 5.0 and pH 7.0 to the MEKC-MS buffer provided the optimum separation of two classes of analytes such as ephedrine alkaloids and β -blockers, respectively. While only 10 runs were performed to test the repeatability of chiral analysis, the results could be improved further using absolute migration time and standardization of

peak areas. Furthermore, the use of a chemically modified capillary with buffer containing sugar surfactant polymers as pseudophase are in progress in our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

poly-α-D-SUGP	poly(sodium <i>N</i> -undecylenyl α -D-glucopyranoside 4,6-hydrogen phosphate)
poly-α-D-SUGS	poly(sodium <i>N</i> -undecylenyl α -D-glucopyranoside 6-hydrogen sulfate)
poly-α-D-SOGP	poly(sodium <i>N</i> -octylenyl α -D-glucopyranoside 4,6-hydrogen phosphate)
poly-α-D-SOGS	poly(sodium <i>N</i> -octylenyl α -D-glucopyranoside 6-hydrogen sulfate)
α-D-SUGP	sodium <i>N</i> -undecylenyl α -D-glucopyranoside 4,6-hydrogen phosphate
α-D-SUGS	sodium <i>N</i> -undecylenyl α -D-glucopyranoside 6-hydrogen sulfate)
α-D-SOGP	sodium <i>N</i> -octylenyl α -D-glucopyranoside 4,6-hydrogen phosphate
α-D-SOGS	sodium <i>N</i> -octylenyl α -D-glucopyranoside 6-hydrogen sulfate)
BNP	1,1'-binaphthyl-2,2'-diylhydrogen-phosphate
DNS-PA	dansylated phenylalanine

References

1. Ishihama Y, Katayama H, Asakawa N. Anal Biochem. 2000; 287:45–54. [PubMed: 11078582]
2. Zhang X, Luo F, Luo Z, Lu M, Chen Z. J Chromatogr A. 2014; 1359:212–223. [PubMed: 25108768]
3. Li Y, Dong F, Liu X, Xu J, Li J, Kong Z, Chen X, Liang X, Zheng Y. J Chromatogr A. 2012; 1224:51–60. [PubMed: 22209355]
4. Yu J, Han KS, Lee G, Paik MJ, Kim KR. J Chromatogr B. 2010; 878:3249–3254.
5. Wuethrich A, Haddad PR, Quirino JP. Electrophoresis. 2014; 35:2–11. [PubMed: 24265218]

6. Wang X, Hou J, Jann M, Hon Y, Shamsi SA. J Chromatogr A. 2013; 1217:207–216. [PubMed: 23246089]
7. He J, Shamsi SA. J Sep Sci. 2009; 32:1916–1926. [PubMed: 19479771]
8. Zhang Y, Huang L, Chen Q, Chen Z. Chromatographia. 2012; 75:289–296.
9. Gu CY, Shamsi SA. Electrophoresis. 2011; 32:2727–2737. [PubMed: 21983821]
10. Zheng J, Bragg W, Hou JG, Lin N, Chandrasekaran S, Shamsi SA. J Chromatogr A. 2009; 1216:857–872. [PubMed: 19108837]
11. Giuffrida A, Leon C, Garcia-Canas V, Cucinotta V, Cifuentes A. Electrophoresis. 2009; 30:1734–1742. [PubMed: 19360784]
12. Xia S, Zhang L, Lu M, Qiu B, Chi Y, Chen G. Electrophoresis. 2009; 30:2837–2844. [PubMed: 19655329]
13. He J, Shamsi SA. J Chromatogr A. 2009; 1216:845–856. [PubMed: 19110258]
14. Hou J, Zheng J, Shamsi SA. J Chromatogr A. 2007; 1159:208–216. [PubMed: 17499757]
15. Rizvi SAA, Zheng J, Apkarian RP, Dublin SN, Shamsi SA. Anal Chem. 2007; 79:879–898. [PubMed: 17263313]
16. Tickle DC, Okafo NG, Camilleri P, Jones RFD, Kirby AJ. Anal Chem. 1994; 66:4121–4126.
17. Li X, Turanek J, Knotigova P, Kudlackova H, Masek J, Pennington DB, Rankin SE, Knutson BL, Lehmler HJ. New J Chem. 2008; 32:2169–2179.
18. Didier D, Florence D. J Phytochemistry. 1994; 36:289–298.
19. Rizvi SAA, Shamsi SA. Electrophoresis. 2003; 24:2514–2526. [PubMed: 12900863]
20. Shamsi SA. Anal Chem. 2001; 73:5103–5108. [PubMed: 11721906]
21. Billiot E, Thibodeaux S, Warner IM. Anal Chem. 1999; 71:4044–4049. [PubMed: 10500490]
22. Rizvi SAA, Shamsi SA. Electrophoresis. 2005; 26:4172–4186. [PubMed: 16217829]
23. Hou J, Zheng J, Rizvi SAA, Shamsi SA. Electrophoresis. 2007; 28:1352–1363. [PubMed: 17465416]

Figure 1.

Effect of polymerization concentrations (mM) of sodium *N*-undecylenyl α -D-glucopyranoside 4,6-hydrogen phosphate (α -D-SUGP) surfactant monomers on chiral R_s of zwitterionic compound (DNS-PA, left) and anionic compound (BNP, right) in MEKC. Conditions: Panel (A): 56 cm effective length (375 μ m O.D., 50 μ m I.D.) fused silica capillary; sample concentration: 1.0 mg/mL in MeOH/-H₂O (50/50). Buffer: 12.5 mM NaH₂PO₄ + 12.5 mM Na₂HPO₄, pH 7.0, 45 mM poly- α -D-SUGP. Applied voltage: +20 kV, injection: 5 mbar, 10 s. Panel (B) sample concentration: 1.0 mg/mL in MeOH/H₂O (50/50). Buffer: 20 mM NH₄OAc, pH 10.8, 15 mM poly- α -D-SUGP. Conditions for applied voltage, injection and capillary dimensions are the same as panel A. Peak identification: 1 = *R*-DNS-PA, 1' = *S*-DNA-PA, 2 = *R*-BNP, 2' = *S*'-BNP.

Figure 2. Comparison of ESI-MS signal of DNS-PA obtained by direct infusion positive ion ESI-MS in the presence of (A) no surfactant, (B) 50 mM unpolymerized α -D-SUGP, and (C) 50 mM poly- α -D-SUGP.

Figure 3.

Comparison of MEKC-UV (A), and MEKC-MS/MS (B) for chiral separation of BNP. Conditions: (A) 56 cm effective length (375 μ m O.D., 50 μ m I.D.) fused silica capillary. BNP concentration: 1.0 mg/mL in MeOH/H₂O (50/50). Buffer: 20 mM NH₄OAc, pH 10.8, 15 mM poly- α -D-SUGP. Applied voltage: +20 kV, injection: 5 mbar, 10 s. The capillary dimension in (B) are the same as (A) except for 60 cm effective length. BNP concentration: 0.1 mg/mL in MeOH/H₂O (50/50, v/v). Spray chamber parameters: nebulizer pressure: 3 psi; drying gas temp: 250 $^{\circ}$ C, drying gas flow rate: 6 L/min; capillary voltage: -3000 V; fragmentor voltage: 200 V, collision energy: 41 eV, MRM transition: 347.1 $\rightarrow$ 79.1. Sheath liquid: MeOH/H₂O (80/20, v/v), 5 mM NH₄OAc, pH 6.8 with a flow rate of 5 μ L/min. Peak identification: 1 = R-BNP, 1' = S'-BNP.

Figure 4.

Bar plots illustrating the effect of chain length and head groups of sugar surfactants on chiral R_s of ephedrine alkaloids (top, A) at pH 5.0 and β -blockers (bottom, B) at pH 7.0 in MEKC-MS/MS. The * indicates $R_s = 0$. Conditions: 75 cm long (375 μ m O.D., 50 μ m I.D.) fused silica capillary. Buffer: 25 mM NH_4OAc , pH 5.0, 30 mM *poly- α -D-SUGP*, *poly- α -D-SUGS*, *poly- α -D-SOGP* and *poly- α -D-SOGS*. Applied voltage, +20 kV, injection, 5 mbar, 10 s. Spray chamber and sheath liquid conditions are the same as Fig. 3. Sample concentration: 10 μ g/mL in MeOH/ H_2O (10/90, v/v). MEKC-MS conditions are the same as Fig. 3 except the optimum fragmentor voltage, collision energy and MRM of ephedrines and β -blockers are listed in Table 2.

Figure 5.

(A) Bar plots illustrating the effect of pH on chiral R_s of ephedrine alkaloids (top, A) and β -blockers (bottom, B) in MEKC-MS/MS. Buffer conditions and spray chamber parameters are the same as in Fig. 4 and 3, respectively except 30 mM poly- α -D-SUGP was used at pH 5.0–6.0 in (A), and 6.0–9.5 in (B). The * indicates $R_s = 0$.

Figure 6.

Electropherograms for enantioseparation of ephedrine alkaloids using sugar surfactant with optimum head group and chain length at optimum pH 5.0 in MEKC-MS/MS. The MEKC conditions and spray chamber parameters are the same as Fig. 5. Peak identifications: 1 = (*1R,2S*)-(-) norephedrine, 1' = (*1S,2R*)-(+)-norephedrine, 2 = (*1R,2R*)-(-) pseudoephedrine, 2' = (*1S,2S*)-(+)-pseudoephedrine; 3 = (*1R,2S*)-(-)-ephedrine, 3' = (*1S,2R*)-(+)-ephedrine; 4 = (*1R,2S*)-(-)-*N*-methylephedrine, 4' = (*1S,2R*)-(+)-*N*-methylephedrine.

Figure 7.

Electropherograms for enantioseparation of β -blockers using sugar surfactant with optimum head group and chain length at optimum pH 7.0 in MEKC-MS/MS. The MEKC conditions and spray chamber parameters are the same as Fig. 5. Peak identifications: 1,1' = atenolol, 2,2' = carteolol, 3,3' = metoprolol, 4,4' = talinolol. For each β -blocker the *R*-enantiomer always eluted earlier than *S*-enantiomer.

Scheme 1.

Synthesis of poly(sodium *N*-alkylenyl α -D-glucopyranoside 4,6-hydrogen phosphate) (poly- α -D-SAGP) and poly(sodium *N*-alkylenyl α -D-glucopyranoside 6-hydrogen sulfate) (poly- α -D-SAGS).

Fragmentor voltage, collision energy and product ion formations for the analytes from optimization experiments using flow injection ESI-MS

Table 1

Analyte	fragmentor voltage (V)	collision energy (eV)	MRM transition (m/z)
BNP	200	41	347.1 → 79.1
pseudoephedrine	88	25	166.1 → 115.1
ephedrine	88	25	166.1 → 115.1
norephedrine	64	17	152.2 → 117
methylephedrine	98	21	180.2 → 147.2
atenolol	137	25	267.2 → 145.2
metoprolol	107	17	268.2 → 116.2
carteolol	83	17	293.2 → 237.2
talinolol	98	13	364.3 → 308.3

Data collected using Agilent Mass Hunter optimizer software operated in flow injection LC-MS

Physicochemical properties of monomers *N*-alkylenyl α -D-glucopyranoside monomers and polymers

Table 2

monomers				polymers		
	CMC (mM) ^{a)}	Aggregation # ^{b)}	Polarity (I_1/I_3) ^{c)}	CMC (mM) ^{a)}	Aggregation # ^{b)}	Polarity (I_1/I_3) ^{c)}
α -D-SOGP	20 $\pm$ 2	86	1.019	0	50	1.023
α -D-SUGP	3.8 $\pm$ 0.1	137	0.999	0	62	1.000
α -D-SOGS	58 $\pm$ 4	71	1.007	0	34	1.039
α -D-SUGS	11 $\pm$ 1	124	0.978	0	47	0.988

- ^{a)}Critical micelle concentration is determined by the surface tension measurements
- ^{b)}Aggregation number is determined by the florescence quenching experiment using pyrene as a probe and cetyl pyridinium chloride as a quencher.
- ^{c)}Polarities of the surfactants are determined using ratio of the fluorescence intensity (I_1/I_3) of pyrene.