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Amino Acid Bound Surfactants: A New Synthetic Family of Polymeric Monoliths Open Up Possibilities for Chiral Separations in Capillary Electrochromatography

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Abstract

By combining a novel chiral amino-acid surfactant containing acryloyl amide tail, carbamate linker and leucine head group of different chain lengths with a conventional cross linker and a polymerization technique, a new “one-pot” synthesis for the generation of amino-acid based polymeric monolith is realized. The method promises to open up the discovery of amino-acid based polymeric monolith for chiral separations in capillary electrochromatography (CEC). Possibility of enhanced chemoselectivity for simultaneous separation of ephedrine and pseudoephedrine containing multiple chiral centers, and the potential use of this amino-acid surfactant bound column for CEC and CEC coupled to mass spectrometric detection is demonstrated.

Amino-acid based surfactants synthesized from various hydrocarbon tails, linkers and amino acid head groups with D- or L-optical configurations are a uniquely tunable family of functional synthetic chiral materials.¹⁻³ They are generally designed to contain terminal double bond, which when dissolved and polymerized in aqueous micellar solutions constitute a covalently stabilized molecular micelle. With 15 years of work, the palette of useable vinyl terminated acyl and alkenoxy amino-acid based chiral molecular micelles has grown in breadth nuance, and their number of applications for chiral separations in micellar electrokinetic chromatography (MEKC) and MEKC coupled to mass spectrometry (MS) has increased. Still the number of research groups tinkering with these amino-acid based surfactant polymers is relatively small. The spectrum of this synthetic form of chiral materials must broaden if amino-acid based polymers are to fulfill their considerable potential in separation science.

Unlike the success of chiral surfactant forming molecular micelles, the design and discovery of amino-acid based chiral surfactants, which can form chiral nanoparticles and chiral polymeric monoliths to be used in capillary electrochromatography (CEC) separations until now has been challenging. However, this is about to change. Priego-Capote and colleagues reported an elegant approach to achieve chiral nanoparticles in which miniemulsion polymerization was performed for the synthesis of molecularly imprinted (MIP) nanoparticles (NPs).⁴ Using amino-acid surfactant, sodium *N*-undecenoyl glycinate as a functional monomer they developed MIP-NPs with a relatively narrow range of particle size

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Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at <http://pub.acs.org>

(30-150 nm). The long standing challenge of peak tailing of later eluting enantiomer was eliminated, and NPs provided fast separation with symmetrical peak shapes for both enantiomers of propranolol. Obtaining chiral polymeric monoliths in which amino-acid surfactants are bound to achiral monolithic backbone provide an exciting opportunity to study chiral recognition on a column surface. Although several class of chiral materials (cyclodextrins,^{5,6} vancomycin,^{7,8} alkaloids,⁹ cellulose,^{10,11} valine¹² and quini-dine)¹³⁻¹⁷ have been polymerized to generate polymer-based monolithic chiral stationary phase (CSP) for CEC, the design and discovery of new families of polymeric monolithic columns derived from synthetic chiral monomers is still needed to develop new and unique separations.

In the present study, the discovery of a novel surfactant-bound chiral polymeric monolith derived from acryloylamide tail, carbamate linker and amino acid (e.g., L-leucine) head group is demonstrated for chiral separations in CEC. Three polymerizable leucine based carbamate chiral surfactants with 8, 10, and 12 carbon alkyl chain [namely sodium 8-acrylamido-octenoxy carbonyl-L-leucinate (SAAOCL), sodium 10-acryl-amidodecenoxy carbonyl-L-leucinate (SAADCL), and sodium 12-acryl-amidododecenoxy carbonyl-L-leucinate (SAADoCL) were synthesized (Scheme 1)]. With an acryloylamide tail, the amino-acid form of the surfactants was conveniently co-polymerized with ethylene glycol dimethacrylate (EDMA) in a ternary porogenic solvent system to form a chiral monolith. The obtained mixed-mode negatively charged monolithic columns were characterized and evaluated for enantioseparation of cationic drugs in CEC. To the best of our knowledge, this is the first study, which has described the use of chiral surfactant bound polymeric monolithic column for CEC. It is well-known that the use of moving chiral pseudophase (in the micellar form) in MEKC-MS running buffer is challenging because of signal suppression by chiral unpolymerized micelles.¹⁸ Therefore, the development of surfactant-bound CSP could provide us with a unique opportunity to develop CEC-MS method for sensitive detection of chiral compounds.

EXPERIMENTAL SECTION

Synthesis of Surfactants

Full experimental details on the synthesis of acid form of acrylamido alkenoxy carbonyl-L-leucinate (SAACL) surfactants of three different chain lengths (shown in Scheme 1),^{2,19} as well as details on the preparation of monolithic columns for CEC-UV and CEC-MS are all described in Supporting Information. The ¹H-NMR and elemental characterization are shown Figure S1-S3 and Table S1, respectively. Triply deionized water was used in all experiments, including buffer preparation and synthesis.

Optimization of the Polymerization Mixture

As mentioned earlier, the major advantage of our procedure is a simple “one-pot” preparation of the chiral monolithic column by directly crosslinking the acid form of chiral surfactant monomer with a conventional crosslinker but without any charged achiral co-monomer. This is essential to improve the chiral recognition. The composition of the polymerization mixture is critical to the physical and chromatographic properties of the monolith. First, the percentage of C₁₀ monomer (i.e., AADCL) and cross linker were carefully optimized to yield the best monolithic CSPs for CEC. Preliminary experiment revealed that the best composition of the acid form of AADCL monomer and crosslinker was 15% w/w of each (data not shown). Next, different porogenic solvents such as ACN, MeOH, propanol, butanol, butanediol, decanol, dimethyl ether, and water were investigated as possible porogens in various proportions. All four AADCL columns (AADCL-1, ADDCL-2, AADCL3, and AADCL-4) containing a fixed weight percent of AADCL

monomer, EDMA and AIBN but various weight ratios of porogens (dimethyl ether, ACN, MeOH and water) were tested in different combinations as outlined in Table 1.

The AADCL-1 was chosen over AADCL-2 due to better chiral separation, whereas AADCL-3 and AADCL-4 were not homogenous. Thus, a monolithic column (i.e., AADCL-1, row 1 Table 1) containing a ternary porogen mixture of ACN and MeOH with small portion (i.e., 5% w/w) of water was considered as the optimum monolithic column because it provided the most homogenous monoliths with improved chiral selectivity. Two additional columns of chain lengths C₈ (i.e., 15 % w/w of AAOCL) and C₁₂ (i.e., 15% w/w AADoCL) were polymerized under the same optimum reagent compositions: 45% w/w ACN, 20% w/w MeOH and 5% w/w water, 15% w/w EDMA, 0.5% w/w AIBN, as the one used for AADCL-1 column.

RESULTS AND DISCUSSION

Characterization of the Monolithic Columns

Three columns for each type of surfactant monomer, i.e., AAOCL, AADCL and AADoCL were prepared and used to characterize the total porosity (ϵ_T) and specific permeability (K^o) values of the chiral monolithic columns. The % RSD values of ϵ_T and K^o ranged from 1.4-2.9 and 2.6-6.7, respectively, indicating a reasonable repeatability between the columns (Supporting Information, Table S2). Although a fixed (70% w/w) porogen ratio was used for each column, there was a slight decrease in column porosity with increasing chain length of the surfactant monomer [i.e., AAOCL (77.1%) > AADCL (73.2%) ~AADoCL (71.5%)]. The porosity values of AADoCL and AADCL is close to what we expect at a fixed weight ratio of 70% porogens to 30% monomer but the porosity of AAOCL was slightly higher than the percentage of the porogens in the polymerization mixtures. Overall, from the %RSD values reported in Table S2, one can conclude that the chain length of surfactant monolith does not significantly influence the total porosity of the monolithic bed.²⁰ The overlay plots of the back pressure versus the volumetric flow rate on the three monolithic columns are shown in Supporting Information, Figure S4. At low flow rates, the back pressure values on these three columns are very similar but the values slightly diverge at high flow rate and follow the trend: AADoCL>AAOCL>AADCL. The % RSD values for the measurement of column back pressure with the decrease in flow rate range from 10-15 for AAOCL, 3.7-9.7 for AADCL and 3.2-5.8 for AADoCL, respectively. However, the linearity of all three plots is very good ($R^2=0.99991-0.99998$), which means the mechanical stability of all three monolithic columns is excellent. The K^o values are very similar for AAOCL and AADCL columns but for AADoCL column K^o value is not only lower but also statistically significant (Supporting Information, Table S2). Perhaps, the lower K^o and high back pressure on AADoCL (compared to AAOCL and AADCL) could be ascribed to different pore diameter and changes in overall pore size distribution relative to the other two monolithic columns. The surface area of all three monolithic columns was measured using BET method. The AADoCL column has the highest surface area (38.2 m²/g), followed by AAOCL (24.7 m²/g) and AADCL (16.2 m²/g) columns. According to the experimental results obtained by Gu *et al.*, the BET surface area of the achiral surfactant-bound columns is in the range of 6-30 m²/g,²¹ which is comparable to the values of the BET surface area of chiral columns mentioned above.

The SEM images of the three monolithic columns are shown in the Supporting Information, Figure S5. From the three sets of SEM micrographs, one can easily conclude that the monolithic material was successfully formed in the capillary. The material is homogeneous and micropores are evenly distributed. However, for the three different monolithic columns with various chain lengths, no significant difference in SEM morphology was observed.

Mobile phase optimization

With a long C₈-C₁₂ hydrophobic alkyl chains and the polar anionic head group, the surfactant-bound monolithic CSPs are considered to mimic a mixed mode/cation exchange characteristics. Using the AADCL column and a model chiral cationic analyte, pseudoephedrine [(±)-PEP], experiments were carried out to examine how the mobile phase parameters effect the retention factor (calculated as average retention factor (k^*_{avg}) of two enantiomers, see Supporting Information], resolution, efficiency and selectivity factor (α).

Effect of Triethylamine

When the volume fraction of TEA was varied in the range of 0.1-1% (v/v) TEA at 5 mM NH₄OAc (pH 5), the electrochromatograms of the (±)-PEP enantiomers demonstrates decreasing k^*_{avg} from 4.50 to 2.91 using AADCL column (Figure 1 inset). At pH 5.0, the (±)-PEP is positively charged and the carboxylate group on the amino acid moiety of the chiral stationary phase (CSP) is anionic. Therefore, chromatographic (electrostatic and hydrophobic) interactions, as well as the electrophoretic effects will contribute to rate of transport. From the inset of Figure 1, one can observe that the chiral R_s of (±)-PEP first increases upon increasing % (v/v) of TEA from 0.1 % - 0.5 % (v/v) (due to significant increase in N_{avg}). However, the R_s values are very similar from 0.5 % (v/v) TEA to 1.0% (v/v) TEA. At 1.0 % (v/v) TEA (data not shown) in the mobile phase, the TEA cations show a higher affinity for cation-exchange sites of the stationary phase, decreasing adsorption and k^*_{avg} of (±)-PEP for the negatively charged CSP.²² Thus, 0.5% (v/v) was chosen as the optimum TEA concentration because it provided the highest R_s and N .

The mobile phase pH and operating voltage (Supporting Information, Figure S6 and S7) was also evaluated. The retention time increases with increasing pH but enantioresolution was only seen in the pH range from 4.0-5.5. At pH lower than 4.0 no enantioresolution was observed due to shorter retention whereas at pH > 5.5 very long retention time, broad peaks and unstable current were observed (data not shown). When voltage increases from +5 kV to +15 kV, the chiral R_s first increases and then decreases significantly but selectivity remains unchanged. In addition, N_{avg} drops at 15 kV (compared to 10 kV) consequently decreasing enantioresolution. As a trade-off between R_s and analysis time, applied voltage of 10 kV was chosen.

Effect of Acetonitrile

The % (v/v) ACN was varied from 60% (v/v) to 80% (v/v) to optimize the chiral separation of (±)-PEP. Lower than 60% (v/v) ACN was not studied due to high current and low chiral resolution. As shown in Figure 2, 60% (v/v) ACN causes shorter retention time, smaller k^*_{avg} , lower chiral R_s , and N . When the percentage of ACN increases in the mobile phase to 70% (v/v), the polar and ion-pairing interactions between (±)-PEP and the polar head group of AADCL CSP increases k^*_{avg} . Finally, using 80 % (v/v) ACN in the mobile phase, not only these interactions get even stronger but also the solubility of (±)-PEP in the mobile drops substantially (as evidenced from decrease in peak height and S/N (Figure 2 bottom electrochromatogram). As a compromise between chiral R_s and analysis time, 70% (v/v) was eventually chosen as a desirable volume percentage of ACN in the mobile phase. To further examine this unusual retention trend of (±)-PEP, the effect of % (v/v) ACN on k' of neutral alkyl benzene test mixture was studied (Figure S8, Supporting Information). In contrast to increasing k^*_{avg} observed for (±)-PEP, the injection of test mixture of alkyl benzene homologues showed a decrease in k' upon increasing ACN in the range of 60-80% (v/v) suggesting a typical mixed mode characteristics.

In addition, DMSO (a neutral and unretained compound) was injected at 60% (v/v)-80% (v/v) ACN. At 60% (v/v) ACN, the retention time of DMSO was the longest (~18 min). On the

other hand, at 70% (v/v) and 80% (v/v) ACN, the retention times of DMSO were very similar (~15 min) but shorter than 60% (v/v) ACN (data not shown). Therefore, the retention trends of ($\pm$)-PEP (Figures 1-2) as well as alkyl benzene test mixture (Figure S8) clearly suggest that the AADCL column mimic a mixed-mode /cation exchange characteristics.

Effect of Surfactant Chain Length

The effect of surfactant chain length was first examined by achiral CEC separation of alkyl benzene test solutes using AAOCL, AADCL, and AADoCL monolithic columns (Supporting Information, Figure S9). Overlaid electrochromatograms in Figure S9 suggest that the increase of surfactant chain length from C₈-C₁₂ increases the retention time and k' of five neutral alkyl benzenes. The methylene selectivity of each plot, was calculated from the antilogarithm of the slope of each graph of the natural logarithm of retention factor ($\ln k$) versus carbon number (n) of benzene and its four alkyl derivatives. As mentioned in inset of the revised Figure S9 of the Supporting Information, the methylene selectivity were 1.337 (± 0.002), 1.443 (± 0.008), 1.549 (± 0.032), for respective chain length of C₈, C₁₀ and C₁₂ monolithic columns. The good linearity further confirmed that the chiral surfactant-bound monolithic columns poses reversed-phase characteristics.²²⁻²³ The trend clearly shows strong hydrophobic interactions between the neutral alkylbenzenes with all three CSPs. Note, that the AADCL and AADoCL columns provide an overall better achiral R_s and N than AAOCL column. To the best of our knowledge, there is only report in which chiral methacrylate based monolithic column was tested for achiral selectivity.¹² Under mobile phase conditions of 80/20 ACN/H₂O and a column length of 25 cm, the respective methylene selectivity of 1.20 and 1.08 was reported when butyl methacrylate (BMA) or glycidyl methacrylate (GMA) moieties were copolymerized with a chiral monomer containing 2-hydroxyethylmethacrylate (*N*-L-valine-3,5-dimethylanilide carbamate).

Next, AAOCL, AADCL, and AADoCL columns were tested for chiral separation of ($\pm$)-PEP. Figure 3 compares the enantioseparation of ($\pm$)-PEP by the three monolithic columns. The inset of Figure 3 shows an expected gradual increase in k^*_{avg} of ($\pm$)-PEP with increasing chain length from C₈ to C₁₂ monolithic stationary phase. This may be attributed to the increase in hydrophobic region at one locus of the chiral center to ensure complete solvation of the phenyl moiety on ($\pm$)-PEP proving increasing chiral α with longer chain surfactant. Therefore, the chiral R_s of ($\pm$)-PEP with C₁₀ and C₁₂ columns are significantly higher compared to C₈ column. These results are opposite of what was observed in the literature by Peters et al.¹² The authors concluded that the substitution of BMA with a more hydrophilic co-monomer GMA substantially decreases the hydrophobic achiral selectivity but improve chiral separations.

In our studies, we did observe a saturation in chiral separation for ($\pm$)-PEP when using C-12 (AADoCL) column. Because there is concurrent increase in k^*_{avg} but only a slight increase in chiral R_s and α of ($\pm$)-PEP with AADoCL. Further studies are underway to investigate how the chain length of the surfactant-bound monolithic column is dependent on the charge state and hydrophobicity of a variety of other chiral analyte.

Comparison of β -CD versus Amino Acid Sufactant Monolithic Columns

Next, we compared the simultaneous separation of both enantiomers of ($\pm$)-PEP with its corresponding diastereomers [i.e., ($\pm$)-ephedrine (EP)] using β -CD versus AADCL monolithic column. Although close to baseline separation of ($\pm$)-PEP enantiomers is possible using GMA- β -CD-*co*-EDMA-*co*-AMPS column (Figure 4A), no enantioseparation is noted for ($\pm$)-EP enantiomers.

On the other hand, both ($\pm$)-PEP and ($\pm$)-EP were simultaneously enantioseparated using AADCL-*co*-EDMA monolithic column (Figure 4B). The enhanced chemoselectivity of the amino acid based AADCL monolith open up the possibility of performing simultaneous enantio-separation of the parent chiral drug and its structurally similar chiral metabolites in biological samples. In addition, the study opens up the potential of investigating various amino acid and dipeptide chiral surfactants as possible chiral monomers to develop various polymeric monolithic CSPs.

CEC-MS/MS Capability

The potential of surfactant-bound monolith column for its compatibility in CEC-MS/MS mode was tested. For example, the CEC-MS/MS for ($\pm$)-PEP and ($\pm$)-EP enantiomers were successfully achieved using AADCL-1 column (Figure 5). It is worth mentioning that the enantioselective CEC-UV implementing monolithic CSP are usually configured with an inner diameter of 100 μ m, a monolith filled segment of $\sim$ 25 cm for separation and an open segment of $\sim$ 8.5 cm for UV detection. However, it poses significant challenge when the same configuration is used for MS detection. In addition, due to lower permeability and significant back pressure of chiral monoliths, the CEC-MS experiments are generally hampered by the limited low flow rates and low inlet pressure, which result in longer preconditioning time. One modification to the CE-MS system was necessary to counteract this problem. For this experiment, it was necessary to place 30 cm segment of the capillary containing monolith at the MS end of the CE-MS instrument, and the open segment of 23 cm of the capillary at the inlet end of the instrument. Therefore, this combination of open segment and monolithic segment at the inlet and outlet end, respectively was necessary to achieve faster separation on the monolithic CSP via CEC-MS/MS. Comparing the bottom electrochromatogram shown in Figure 4 versus Figure 5 suggest that CEC-UV provided slightly higher *R*s and shorter retention times but CEC-MS/MS provided significantly higher *S/N* with nearly two-fold higher *N_{avg}*. In case of CEC-MS/MS, there was an empty segment of 23 cm from the inlet side of the column, which could have contributed to some band broadening due to suction effect by the nebulizer, but this effect is partially offset by the monolithic segment of 35 cm from the outlet end of the CEC-MS/-MS column. Although the ESI-MS parameters have not been extensively optimized, the *S/N* of 10,500 for ($\pm$)-PEP and 8,400 for ($\pm$)-EP suggest a limit of detection in the low ng/mL range is possible in CEC-MS/MS compared to 0.1 mg/mL in CEC-UV. On the other hand, to counteract the long analysis time in CEC-MS created by the need of at least 50-60 cm long capillary, it is important to carefully evaluate a range of negatively charged chiral surfactant monomers to enhance both electroosmotic flow and permeability of monolithic column for CEC-MS.

Run-to-Run and Column-to-Column Repeatability

The column-to-column repeatability study was carried out by preparing three different batches of polymerization mixtures containing the AADCL monomer (synthesized from the same batch) on three different days (Table S3, Supporting Information). A test mixture of ($\pm$)-PEP was injected 10 times for three consecutive days on three different AADCL columns. The statistical results are tabulated in Table S3. The % RSD values for the intraday repeatability of retention time, efficiency, and chiral resolution range from 2.6-7.2, 9.3-11.9, and 4.0-8.0, respectively. Representative electrochromatograms of intercolumn repeatability study shown in Figure S10 illustrates that the preparation of monolithic column is stable and robust. Further extensive studies with the goal to improve the reproducibility and repeatability of the monolithic columns using different monomer batches are currently in progress in our laboratory.

CONCLUSIONS

Three novel chiral surfactant-bound C₈-C₁₂ monolithic columns [(poly-(AAOCL-*co*-EDMA), poly-(AADCL-*co*-EDMA), and poly-(AADoCL-*co*-EDMA)] were successfully synthesized and characterized. As expected, at a fixed porogens ratio the surfactant chain length have essentially no influence on the porosity to any significant extent. However, there is statistically significant lower K^0 for AADoCL column compared to AAOCL and AADCL columns, suggesting that the pore size distribution could be different for AADoCL column. At a fixed weight ratio of porogen to monomer, chain length of the surfactant-bound monolithic column influence partitioning and chiral recognition. Baseline separation of model test analyte (±)-PEP was achieved by AADCL and AADoCL columns. The CEC conditions to deliver the best chiral resolution of (±)-PEP were found to be: 70% ACN, 0.5% TEA%, pH, 5.0; at +10 kV. Although, the C₁₂ carbon chain, provided the best *R_s* and α of (±)-PEP but the *N* was lower and the run times were slightly longer. Recent chiral screening in our laboratory suggest that overall AADCL surfactant column is more versatile than AAOCL and AADoCL columns in separating a wide range of positively charged chiral compounds (data not shown). Such results will be reported at a later date. Nevertheless, these results suggests that not only electrostatic ionic and polar interactions between the CSP and analyte is important, but partitioning with the appropriate chain length of amino acid surfactant is equally important for improved chiral recognition. In addition, the alkyl chain of the surfactant monomer when polymerized as a monolithic chiral column provided a unique feature of achiral selectivity, indicating reversed phase mechanism. This is very beneficial for simultaneous separation of (±)-PEP and (±)-EP enantiomers enhancing both enantioselectivity and chemoselectivity. On the other hand, β -CD based chiral monolithic column was unable to provide such chemoselectivity. In addition, the preliminary data on CEC-MS/MS for simultaneous separation and detection of (±)-PEP and (±)-EP with AADCL column suggest great potential of using this type of monolithic column configuration for sensitive detection in CEC-MS/MS.

This study not only provides a useful mixed-mode/cation exchange CSP for the separation of cationic chiral compounds and achiral separation of neutral compounds, but also open up the possibility to develop new amino acid bound CSPs, which could be optimized in terms of optical configuration (L or D), linker as well as head groups. Such studies will be important to understand what role the chemistry of amino-acid surfactant monomers may play when investigating chiral recognition on a solid surface in CEC compare to solution phase used in MEKC. For example, it would be interesting to compare chiral separations on surfactant bound “true-CSP” in CEC versus the use of molecular micelles derived from the same chiral surfactant as “pseudophase” in MEKC for a range of applications. Furthermore, we are also initiating studies, which will extend the application of chiral surfactant bound columns to micro- and nano-HPLC-MS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Chiral separation of (±)-pseudoephedrine (PEP) at different % (v/v) of TEA. CEC conditions: AADCL column, 100 μ m I.D., 33.5 cm total length, 25 cm monolithic segment, 8.5 cm open segment from the detection window to the outlet end. Voltage, +10 kV; high pressure, 6 bar applied at both ends of the column at 20 °C. Mobile phase: 70% (v/v) ACN and 30% (v/v) aqueous buffer containing 5 mM NH_4OAc , at pH 5.0; UV-detection at 200 nm. Analyte, (±)-PEP (1 mg/mL) dissolved in mobile phase; injection: 3 kV for 3 s.

Figure 2. Effect of volume fraction of ACN in the mobile phase on chiral CEC separation of (±)-PEP. Conditions: Mobile phase with various % (v/v) ACN and aqueous buffer containing 5 mM NH_4OAc , 0.5% (v/v) TEA (pH 5.0); Other conditions are the same as in Figure 1

Figure 3.

Effect of monomer chain length of monolithic columns on chiral CEC separation of (±)-PEP using the mobile conditions containing 5 mM NH_4OAc , 0.5% (v/v) TEA (pH 5.0), 70% (v/v) ACN. Other conditions are the same as Figure 1.

Figure 4.

Comparison between poly-(GMA- β -CD-*co*-EDMA -*co*-AMPS) column (A) and poly-(AADCL-*co*-EDMA) column (B) for simultaneous enantioseparation of ($\pm$)-PEP and ($\pm$)-EP enantiomers. The CEC column dimensions for the poly(GMA- β -CD-*co*-EDMA-*co*-AMPS) columns are the same as described in Figure 1. Mobile phase: 50% (v/v) ACN and 50% aqueous buffer, 5 mM NH_4OAc , 0.3% (v/v) TEA (pH 4.0). Mobile phase conditions for the AADCL column are the same as described in Figure 3. Both ($\pm$)-PEP and ($\pm$)-EP (1 mg/mL) are dissolved in 50/50 ACN- H_2O (v/v); injection, 5 kV for 3 s.

Figure 5.

CEC-ESI-MS/MS of (±) PEP and (±) EP with AADCL column. CEC conditions: 53 cm long column, 30 cm monolithic bed length. 0.3 kV/cm (15 kV); high pressure, 5 bar applied at inlet end of the column; Other conditions are the same as Figure 1. The (±) PEP and (±) EP (50 $\mu\text{g/mL}$) were injected at 5 kV for 3 s. The MRM product ions were observed at m/z 115.1 and 133.1 for (±) PEP and m/z (±) EP respectively; nebulizer pressure, 7 psi; drying gas flow rate, 5 L/min; drying gas temperature, 150 $^{\circ}\text{C}$; capillary voltage, 3500 V; fragmentor 90 V. Sheath liquid: 80/20 MeOH/ H_2O (v/v), 5 mM NH_4OAc (pH 6.8) delivered at a flow rate of 8 $\mu\text{L/min}$.

Scheme 1.

Synthesis of polymerizable monoliths: poly-(AAOCL-*co*-EDMA), poly-(AADCL-*co*-EDMA), or poly-(AADoCL-*co*-EDMA) monoliths with $n = 8, 10, \text{ or } 12$. Subscript $a, b,$ and c are number of subunits in the polymer.

Table 1

Composition of the polymerization mixtures used in the preparation of the surfactant-bound polymeric monolithic columns[†]

Column	Monomers (% w/w)				Porogen (% w/w)			Initiator (% w/w)		Comment
	Surfactant	AMPS	EDMA	Dimethylether	ACN	MeOH	H ₂ O	AIBN		
AADCL-1	15	0	15	0	45	20	5	0.5		Good separation
AADCL-2	15	0	15	0	50	20	0	0.5		
AADCL-3	15	0.5	15	0	45	20	5	0.5		Not homogenous
AADCL-4	15	0	15	35	0	35	0	0.5		

[†] All monolithic columns were prepared from the same batch of AADCL surfactant monomer.