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Application of cyclodextrin-modified gold nanoparticles in enantioselective monolith capillary electrochromatography

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ABSTRACT

β-cyclodextrin modified gold nanoparticles (CD-GNP) were employed as the stationary phase in monolith capillary electrochromatography (CEC) to facilitate enantioseparation. CD-GNP were covalently bound to the surface of the thiolated porous polymer monolithic column. The fabricated enantioselective monolithic column was characterized by a variety of spectroscopic methods. The column exhibited steady EOF mobility over pH values ranging from 4.6 to 9.7. Additionally, the column was stable under CEC separation conditions over 180 min. Moreover, the column exhibited good column-to-column reproducibility. The CD-GNP-modified monolithic column was employed in the efficient CEC separation of three pairs of drug enantiomers (chlorpheniramine, zopiclone and tropicamide). The results exhibit reproducible run-to-run enantioseparations and the monolith column can maintain its enantioselectivity for more than 1 month if the column is stored in a CD-GNP solution at 4 °C.

1. Introduction

Capillary electrochromatography (CEC) exploits both the high efficiency of CE and the selectivity of liquid chromatography stationary phase; CEC is a powerful technique in separation science [1–3] and the key for the separation process is the solute–stationary phase interaction. Developing an efficient stationary phase to enhance the solute–stationary phase interaction has been a major task in CEC studies for many years. Notably, the high colloidal stability and large surface area of nanoparticles have positioned them as promising stationary phase materials for CEC separations [4,5]. Over the past decade, different types of nanoparticles, including polymer [6,7], titanium oxide [8,9] and gold nanoparticles [10] as well as carbon nanotubes [11], have been utilized as the stationary phase for CEC separations. Nanoparticles have been shown to improve separation efficiency by forming a stable and large surface that interacts with the analytes.

Among various nanoparticles, GNPs are very attractive since their easy and inexpensive to prepare, they easily form active complexes with biological substances and they have controllable particle size and narrow size distribution [12,13]. GNPs have been used as novel stationary or pseudo-stationary phases in CEC techniques to efficiently separate DNA and proteins [10]. It has been well established that organic molecules containing an thiol (–SH) or amine (–NH) group can be easily adsorbed onto gold surfaces through covalent bonding, leading to well defined and stable arrays of chemically modified GNPs. Such chemically modified GNPs can be very useful in CEC separations, including enantioseparations.

Various research fields, including the pharmaceutical, clinical, environmental and food science, rely heavily upon CEC enantioseparation [14]. In recent years, the application of nanoparticles in CEC enantioseparation has attracted increased research interest. The large surface area of nanoparticles has been shown to be a key element in improving enantioselectivity. It is expected that chiral selectors (e.g., CD and protein) can be covalently bound to the surface of GNPs to form an efficient nanoparticle-based chiral stationary phase for CEC separations. Unfortunately, reports applying GNPs in CEC enantioseparations are rare; however, capillaries and microdevices that have been chemically modified with GNPs have shown great promise in enhancing enantioseparation performance. For example, Li et al. presented the first application of BSA-GNP conjugates. These conjugates have been employed as the chiral stationary phase in fabricated chiral OTCEC microdevices [15]. Lu et al. describes the development of a silica monolith stationary phase modified with BSA-GNP conjugates; this modified stationary phase was used in the CEC separation of phenylthiocarbamyl amino acids [16]. The application of CD-GNP as chiral selectors in pseudo-stationary phase CEC and OTCEC separation have been reported for

Abbreviations: β-CD, β-cyclodextrin; GNPs, gold nanoparticles; CD-GNP, β-cyclodextrin modified gold nanoparticles; CEC, capillary electrochromatography; PDDA, poly diallydimethylammonium chloride; TMSPM, 3-trimethoxysilyl propyl methacrylate; AIBN, 2, 2′-azobis (2-methylpropionitrile); EDMA, ethylene dimethacrylate; GMA, glycidyl methacrylate; EDS, energy dispersive X-ray spectroscopy; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TGA, thermogravimetric analysis.

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efficient enantioseparation of amino acid enantiomers and drug enantiomers [17,18].

In this work, a monolith stationary phase modified with CD-GNP conjugates was developed to function as the chiral stationary phase during CEC enantioseparation. The enantioselective monolithic column was prepared by introducing CD-GNP conjugates onto a poly (GMA-co-EDMA) monolith through covalent bonding. The stability and reproducibility of the enantioselective monolithic column were investigated. Three drug enantiomer pairs were separated using the modified monolithic column to demonstrate its performance during CEC enantioseparation. The results demonstrate the promise of using a monolithic column outfitted with a chiral selector comprising modified GNPs to achieve CEC enantioseparation.

2. Experimental

2.1. Chemicals and instruments

β-CD, p-toluenesulfonyl chloride, hydrogen tetrachloroaurate hydrate, sodium disulfite, trichloroethylene and sodium borohydride were obtained from Sigma Chemical (St. Louis, MO, USA). Poly dially dimethylammonium chloride (PDDA) (20%, w/w in water, MW= 200,000–350,000) was purchased from JingChun Reagent Inc. (Shanghai, China). 3-trimethoxysilyl propyl methacrylate (TMSPM), 2,2′-azobis (2-methylpropionitrile) (AIBN) and ethylene dimethacrylate (EDMA) were purchased from J&K Chemical Ltd. Glycidyl methacrylate (GMA), 1-dodecanol and cyclohexanol were purchased from Alfa Aesar. 2-Aminoethanethiol was obtained from TCI Co. Ltd. Chlorpheniramine, zopiclone and tropicamide were purchased from local pharmaceutical stores. Other reagents were of analytical grade and used without further purification.

The phosphate buffer was prepared by dissolving NaH_2PO_4 in deionized water; the pH of the buffer was adjusted by adding H_3PO_3. Stock solutions (1 mg/mL) of the bulk drug samples were prepared in deionized water. All the solutions were prepared daily and filtered through an inorganic 0.22-μm nylon membrane prior to use.

CE experiments were carried out on a CE apparatus (CL1020, Bei-jing Cailu Science Apparatus, China) equipped with a UV detector set at 214 nm. The scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) spectra of the monoliths were obtained using an XL30ESEM-FEG SEM microscope (SEI) integrated with an energy dispersive X-ray spectrometer. UV–vis and Fourier-transform infrared (FT-IR) spectra of the synthesized CD-GNPs were obtained on a Cary 500 UV–vis-NIR spectrophotometer (Varian, CA, USA) and a fluorescence spectrophotometer. Transmission electron microscopy (TEM) images were captured on an H-7500 TEM spectrometer (Hitachi, Japan). Porosity measurements were carried out using an ASAP 2020M automatic micropore and mesopore analyzer (Micromeritics, Norcross, GA, USA). Thermogravimetric analysis (TGA) was conducted using a Perkin-Elmer TGA-2 thermogravimetric analyzer.

2.2. Preparation of CD-GNP-modified monolithic columns

The inner wall of the capillary was first vinylized to enable covalent attachment of the monolith [19]. A bare capillary was

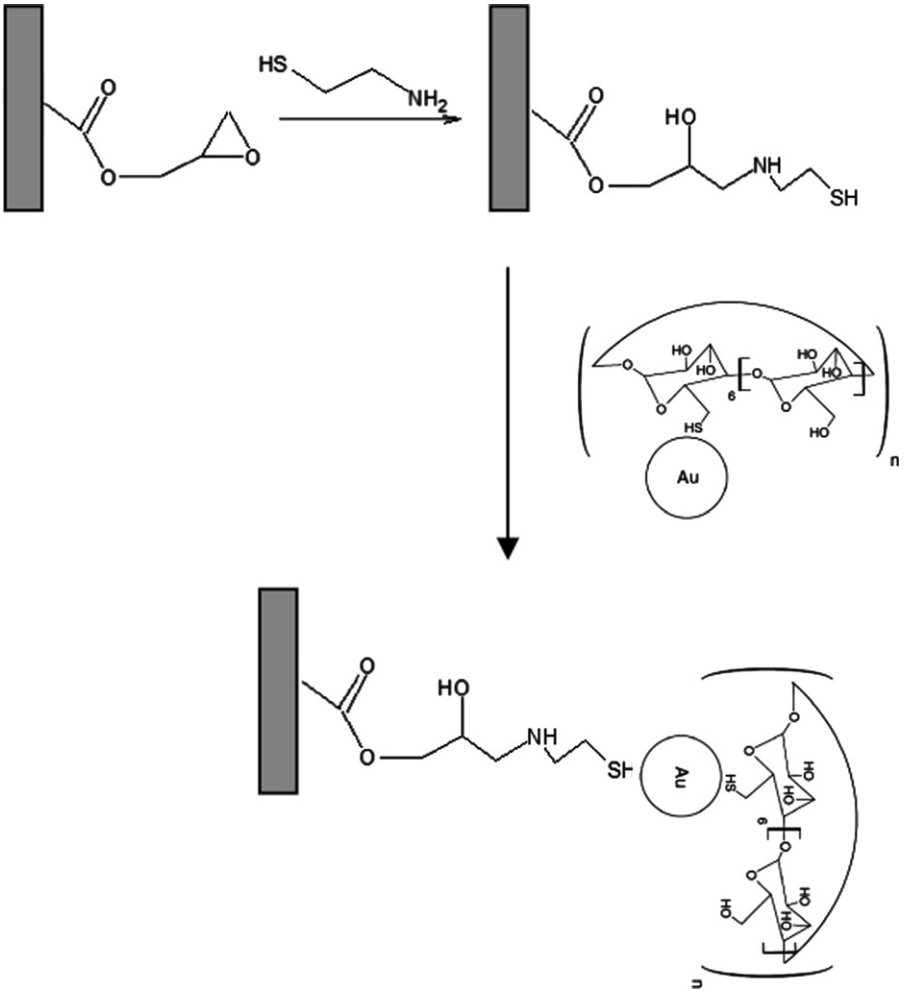

Fig. 1. Reaction scheme for the fabrication of the CD-GNP-modified monolithic column.

treated with 1.0 M HCl for 30 min, then flushed with 1.0 M NaOH for 1 h and rinsed successively with H_2O for 10 min and acetone for 20 min. The capillary was dried under nitrogen gas for 1 h. Using a syringe, a solution of 50% TMSPM (v/v) in acetone was loaded into the capillary from the injection end up to a length of 15 cm. Then, the capillary was sealed with rubber septa at both ends and placed in an oven at 25 °C for 24 h. The capillary was then washed with acetone and dried with a stream of nitrogen for 30 min.

After the capillary was pretreated, the method described in the literature [20,21] was used with little modifications to obtain the CD-GNP-modified monolithic columns. The reaction scheme is shown in Fig. 1. Briefly, a polymerization mixture containing 24% (w/w) GMA (functional monomer), 16% (w/w) EDMA (cross-linker), 54% (w/w) cyclohexanol, 6% (w/w) 1-dodecanol (porogens) and 1% (w/w) AIBN (initiator) (relative to the monomer content) was sonicated until the mixture was homogenous. The solution was then was purged with nitrogen for 10 min. The vinylized capillary was filled with the polymerization mixture from the injection end up to a length of 15 cm. The capillary was then sealed with rubber septa at both ends, and the polymerization reaction was carried out in a water bath at 40 °C for 40 h. Once the polymerization reaction was completed, the poly(GMA-co-EDMA) monolithic column was rinsed with methanol to remove any unreacted components. Thiol groups were then introduced to the monolithic column by pumping a 2.5 mol/L aqueous solution of cysteamine through the column for 30 min. The thiol-modified column was rinsed with H_2O for 20 min and then flushed with a 0.5 mg/mL aqueous solution of CD-GNP until surface saturation was achieved. Once the entire length of the monolithic column (about 15 cm) turned deep red and the liquid leaving the capillary outlet turned a pinkish red, the column stood for 1 h with both ends sealed. The column was then rinsed with water to remove the excess CD-GNP solution and stored in a refrigerator at 4 °C.

2.3. Porosity measurements

A polymerization reaction identical to the one performed in the capillary was also conducted in a glass vial to determine the pore volume and surface area of the polymer. The specific surface area was evaluated by the BET method using the adsorption data in a relative pressure (p/p_0) range of 0.06–0.25. The total pore volume was determined by the single point method by converting the adsorbed nitrogen volume at $p/p_0=0.979$ to the volume of liquid adsorbate.

2.4. Determination of gold content

TGA measurements were carried out under nitrogen at temperatures ranging from 60 to 800 °C. The heating rate was 10 °C/min. To determine the CD-GNP concentration by the TGA method, a capillary stripped from the outer polyamide coating was used to prepare the CD-GNP-modified monolithic column. Thus, the loss of mass at approximately 340 °C is attributed solely to the decomposition of monolithic polymer (CD-GNP cannot be burned off within this temperature range). The concentration of CD-GNP as well as the total amounts of CD-GNP and monolithic polymer can be determined by comparing the masses of the bare capillary, the CD-GNP-modified monolithic column and the column whose monolithic polymer has been decomposed by burning. The percentage of gold coverage was estimated by dividing the total amount of CD-GNP and monolithic polymer by the amount of CD-GNP in the monolith [21].

2.5. CE conditions

Fused-silica capillaries of 75 μm i.d. and 365 μm o.d. (Hebei Yongnian Optical Fiber Factory, China) were applied as the monolithic columns. The columns had a total length of 40 cm and an

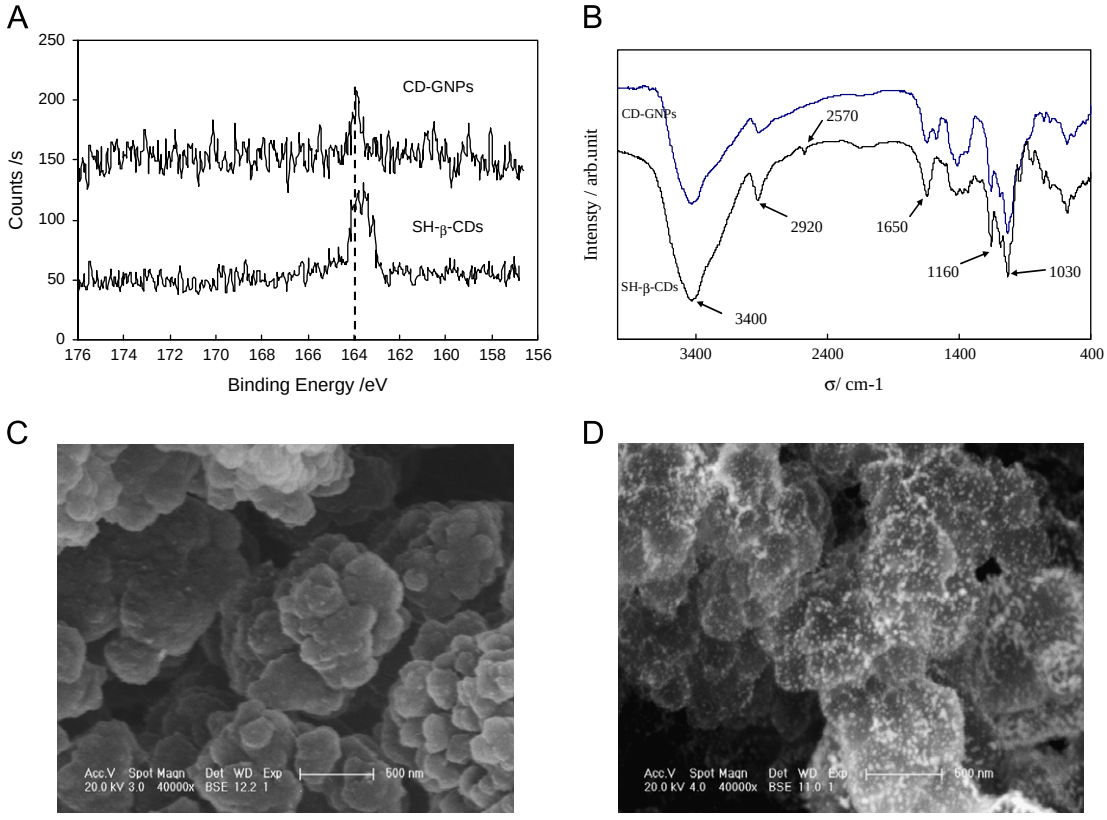

Fig. 2. (A) XPS and (B) FT-IR spectra of synthesized CD-GNPs and free SH-β-CD. (C) and (D), SEM photographs of the internal structures of the poly (GMA-co-EDMA) monolith (C) and its CD-GNP-modified analog (D). The white spots in (D) are the CD-GNPs.

effective length of 31 cm. Prior to use, the CD-GNP-modified monolithic column was flushed with running buffer. The capillary was subjected to pre-electrophoresis until the baseline of the detector output remained stable. Samples were injected electrokinetically at 5 kV for 3 s. CEC separations were performed at 20 °C. The detection wavelength was set at 214 nm for the analytes. Acetonitrile was used as the EOF marker. Phosphate buffer was used as the running buffer.

3. Results and discussion

3.1. Characteristics of the CD-GNP-modified monolithic column

CD-GNP were synthesized using a three-step procedure reported in a previous work [17]. The synthesized CD-GNP were characterized by various spectroscopic methods. As shown in Fig. 2(A), the EDS spectra of SH-β-CD and CD-GNP exhibit the same peak at a photoelectron energy of 163.6 eV. This value is assigned to the binding energy of the 2p electron of the sulfur atom and indicates the presence of an –SH group in both free CD and CD-GNP. The FT-IR spectrum of CD-GNP was essentially identical to that of SH-β-CD. As shown in Fig. 2(B),the FT-IR spectra of both CD-GNP and SH-β-CD exhibit characteristic peaks at 3400 cm^{-1} and 2920 cm^{-1} which are attributed to the O–H and C–H stretching vibrations of CD, and at 1650 cm^{-1}, 1160^{-1} and 1030 cm^{-1} which correspond to HOH, C–O and C–O–C glucose units of rings of CD, respectively. The only noticeable difference is the disappearance of the S–H stretching at 2570 cm^{-1} in the spectra of CD-GNP, as anticipated due to the mode of attachment of CDs to the gold surface. The results of EDS and FT-IR spectra confirm that the CD were well attached to the GNP surface, which was also demonstrated by ^{1}H NMR spectroscopy. (see Ref. [17] As it was reported [17] the synthesized CD-GNP have an average diameter of approximately 9.5 ± 2.5 nm according to measurements made using TEM and UV–vis spectroscopy. Notably, the CD-GNP are stable in both basic and acidic solution [17].

Fig. 2(C and D) shows the internal structures of the poly (GMA-co-EDMA) monolith and its CD-GNP-modified analog. The small white spots in Fig. 2(D) indicate that the coverage of CD-GNPs on the monolithic column is quite extensive. The amount of CD-GNPs loaded onto the monolithic column was determined by TGA and EDS and the results of the two measurements were relatively consistent with each other. The coverage was measured at 28.0% and 30.8% by the TGA method and EDS spectroscopy, respectively.

The surface area within a silica capillary is difficult to determine because of the extremely small sample size. To circumvent this problem, a larger amount of polymer monolith vial was prepared in a glass vial, then, BET isotherms were acquired to evaluate and pore volume of the monolithic column (without CD-GNP) were 15.17 m^2/g and 0.037 cm^3/g, respectively. The pore

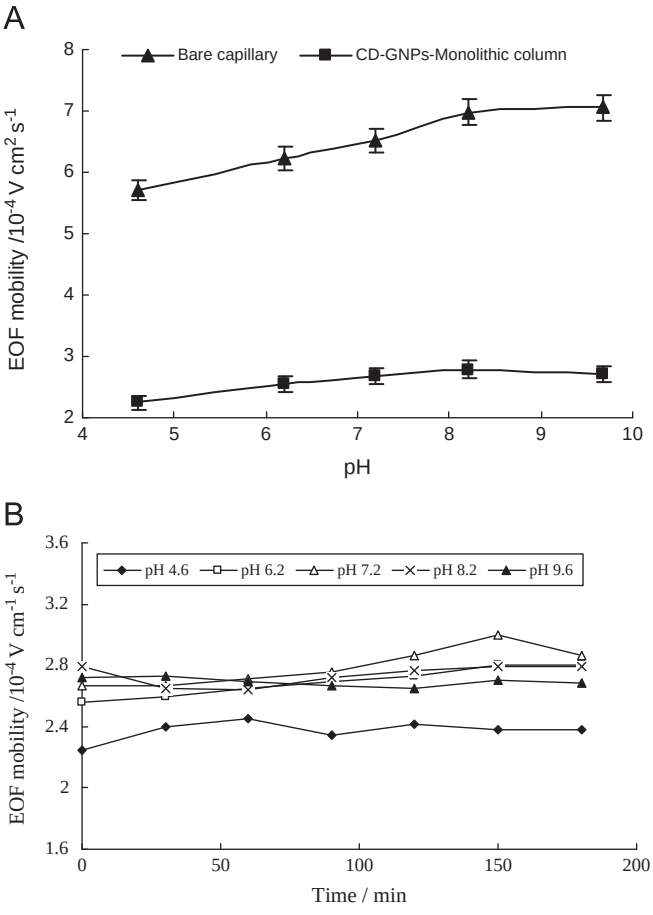

Fig. 3. (A) EOF mobilities of the bare capillary and the CD-GNP-modified monolithic column over a pH range of 4.6–9.7. The EOF from anode to cathode is denoted as positive. The running buffer was a 12.5 mM phosphate buffer solution with the ionic strength maintained at 50 mM by the addition of NaCl. Acetonitrile was used as the EOF marker. (B) The stability and reproducibility of the CD-GNP-modified monolithic column under CEC separation conditions (pH 4.6–9.7) over 180 min.

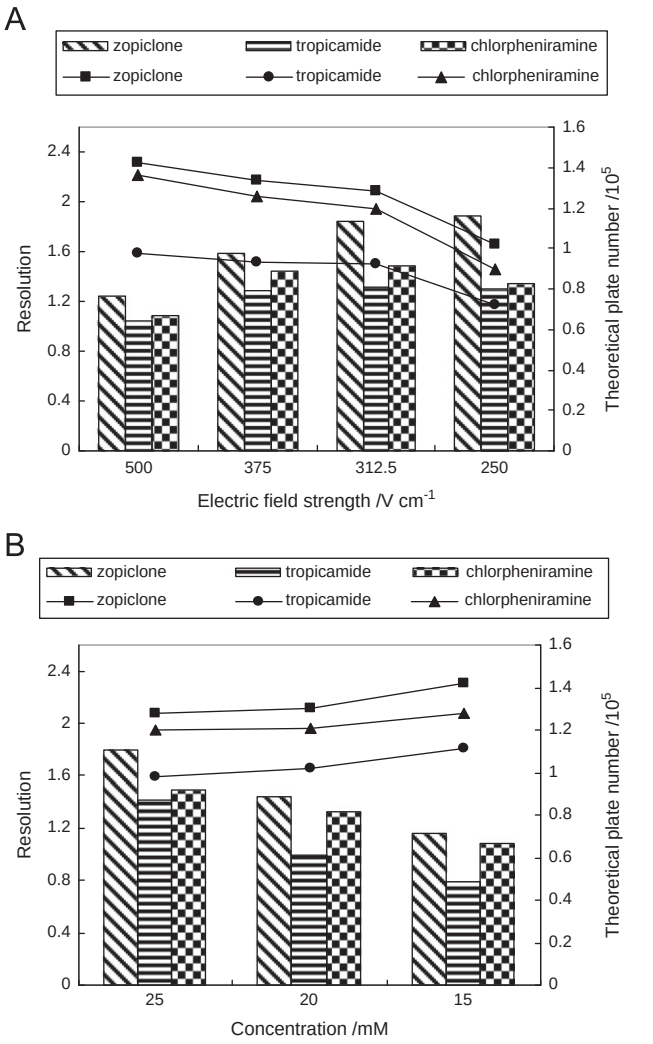

Fig. 4. Effect of (A) electric field strength and (B) buffer concentration on the CEC separations of three drug enantiomer pairs using CD-GNP-modified monolithic columns. In each figure, the bar graphs and the line graphs represent the resolution and the theoretical plate numbers of enantioseparation, respectively.

volume did not change significantly when the column was modified with CD-GNP; only a slight decrease to 0.033 cm^3/g was observed upon modification with the CD-GNP. The surface area, however, increased to 24.12 m^2/g upon attachment of the CD-GNP to the surface of the monolithic column.

3.2. EOF mobility of the CD-GNP-modified monolithic column

The EOF mobilities of the CD-GNP-modified monolithic column and the bare capillary over a pH range of 4.6–9.7 is shown in Fig. 3A. The pH value was adjusted by adding NaOH or H_3PO_3 to the 25 mM phosphate buffer solution. The ionic strength was maintained at 50 mmol/L by the addition of NaCl. The EOF on the bare capillary, which was generated by the negatively charged silanol groups on the silica wall, was positive (from anode to cathode). Because CD-GNP are negatively charged over the pH range, the EOF of the enantioselective monolithic column was also positive, as shown in Fig. 3. Unlike the EOF mobility of the bare capillary, that of the CD-GNP-modified monolithic column was stable within the pH range. The relative standard deviation (RSD) of the EOF in the pH value range from 4.6 to 9.7 was 7.2% for the CD-GNP-modified monolithic column. Because CD-GNP were fully ionized at all experimental pH values (the pKa value of β-CD is 12.2), it was expected that the corresponding EOF mobility would show much less pronounced dependence on pH compared with the bare capillary.

The delivery of mobile phases through a capillary requires a stable and reproducible column. Thus, reproducibility is also critical for CEC separation. To investigate the stability of the enantioselective monolithic column, the EOF mobility of the capillary was monitored over pH values ranging from 4.6 to 9.7 (Fig. 3B). EOF mobility with RSD varying from 1.1 to 2.1% for 180 min measurement period was shown. This result proved that the binding of CD-GNPs to the monolithic column was very stable. The column-to-column reproducibility of EOF mobility was investigated by testing six freshly prepared enantioselective monolithic columns. The result for each capillary was obtained based on three separation runs. Good column-to-column reproducibility was demonstrated, with an RSD of 4.3%. The results demonstrate the reliability and feasibility of enantioselective CEC separation using CD-GNP-modified monolithic columns.

3.3. Chiral separation of drug enantiomers using the CD-GNP-modified monolithic column

The performance of the CD-GNP-modified monolithic column for CEC enantioseparation was evaluated by analyzing three important drug enantiomer pairs, zopiclone (pK_a 6.7), chlorpheniramine (pK_a 9.2) and tropicamide (pK_a 5.4). The effect of electric field strength and buffer concentration on CEC enantioseparation using the CD-GNP-modified monolithic column was investigated. The results are shown in Fig. 4(A) and (B). As the electric field strength decreased, the resolution of each drug enantiomers increased. The slight decrease in resolution of chlorpheniramine and tropicamide at 250 V/cm was attributed to peak broadening at low electric field strength. On the contrary, the theoretical plate numbers of enantioseparation decreased as the electric field strength was decreased, especially for zopiclone and chlorpheniramine. As a compromise, a separation electric field strength of 312.5 V/cm was chosen. As shown in Fig. 4(B), the resolution of enantioseparation decreased monotonically when decreasing the buffer concentration from 25 to 15 mmol/L. The theoretical plate numbers remained almost the same at different buffer concentrations. Thus, the buffer concentration was chosen to be 25 mM for the CEC enantioseparation of each drug enantiomer pair.

In Fig. 5, a typical electropherogram of CEC separation for each drug enantiomer pair is shown using the CD-GNP-modified monolithic column. Efficient baseline separation of the drug enantiomers was achieved within 14 min. For zopiclone, tropicamide and chlorpheniramine, respectively, the separation resolutions (averaged over three runs) were 1.85, 1.32 and 1.48; the theoretical plate numbers were 1.28×10^5, 0.92×10^5 and 1.20×10^5; and the enantioselectivities were 1.040, 1.064 and 1.034. The limits of detection were 0.78 μg/

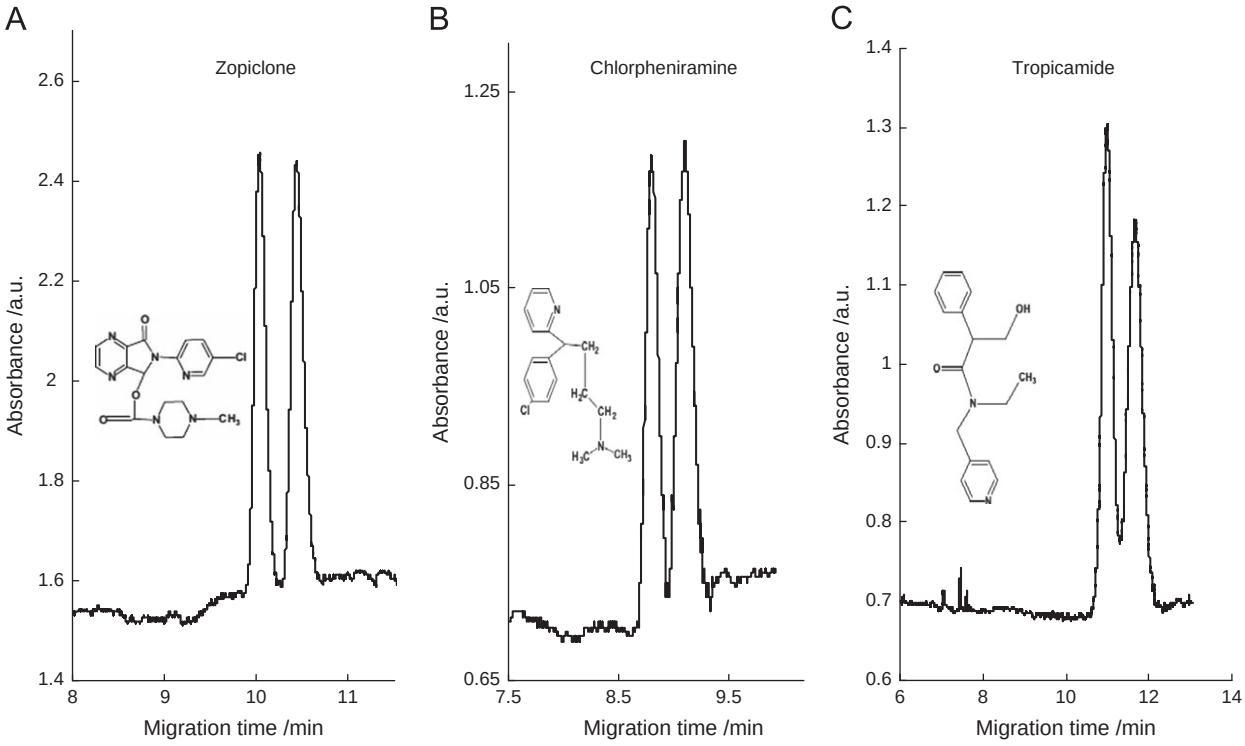

Fig. 5. Electropherograms of separations of three drug enantiomer pairs using the CD-GNP-modified monolithic columns. A 25 mM phosphate buffer (pH 3.0) was used as the running buffer. The separation electric field strength was 312.5 V/cm. Detection was carried out on-column at 214 nm. Samples were injected electrokinetically at 5 kV for 3 s.

mL, 0.39 μg/mL and 0.02 μg/mL (S/N=3) for zopiclone, tropicamide and chlorpheniramine, respectively. The calibration curves (dynamic ranges) are $y=37.7\times-28.6$ (15.6 μg/mL to 0.5 mg/mL) for zopiclone, $y=56.2\times-57.5$ (12.7 μg/mL to 0.5 mg/mL) for chlorpheniramine and $y=48.2\times-39.8$ (10.08 μg/mL to 0.5 mg/mL) for tropicamide, resp ectively.

The run-to-run reproducibility was studied by five consecutive separations. For each drug enantiomer, the RSDs of the retention time and the peak area were lower than 1.8% and 4.5%, respectively, whereas the RSD of the resolution was approximately 4.4%. The results indicate that the CD-GNP-modified monolithic column can be repeatedly used for CEC enantioseparation. The enantioselectivity can be retained for more than 1 month if the column is stored in a CD-GNP solution at 4 °C.

4. Conclusion

In this work, the application of CD-GNP as the chiral stationary phase in monolith CEC enantioseparation was demonstrated. An enantioselective monolith column can be easily fabricated by covalently attaching CD-GNP onto the surface of a thiolated porous polymer monolith column. The EOF mobility in the pH range of 4.6–9.7 indicated that the CD-GNP-modified monolithic column is stable during at least 180-min. Good column-to-column reproducibility with a RSD of 4.3% was also demonstrated. Efficient baseline separations for three pairs of drug enantiomers were achieved using the present method. The theoretical plate numbers were as high as 1.28×10^5, and the resolution was as high as 1.85. The results show the CD-GNP-modified monolithic column exhibits good run-to-run reproducibility for enantioseparation and can maintain enantioselectivity for more than 1 month if the column is stored in a CD-GNP solution at 4 °C. The present work demonstrates the reliability and feasibility of enantioselective CEC separation using CD-GNP-modified monolithic columns. The study suggests that the large surface area and strong adsorption characteristics of GNPs enable chemically modified GNPs to serve as suitable chiral stationary phases in CEC enantioseparations.

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