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Short communication

Enantioselective open-tubular capillary electrochromatography using cyclodextrin-modified gold nanoparticles as stationary phase

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ABSTRACT

Enantioselective open-tubular CEC (OTCEC) with thiolated β-CD modified gold nanoparticles (CD-GNPs) as stationary phase was developed. The enantioselective OT capillary column was fabricated by electrostatic assembly of poly(diallydimethylammonium chloride) (PDDA) followed by self-adsorption of negatively charged CD-GNPs. The enantioselective capillary column has a steady EOF mobility over a wide pH range of 3.0 to 9.2 (RSD 4.8%), and is quite stable over 240 min with very good column to column reproducibility. Efficient enantioseparation of the presented method was demonstrated by analyzing three drug enantiomers. Our results show that the column exhibits good run-to-run repeatability for enantioseparations and can maintain the enantioselectivity for more than 1 month if the column was stored in CD-GNPs solution at 4 °C.

1. Introduction

Capillary electrochromatography (CEC), which can exploit the high efficiency of CE and the selectivity of LC phases, has emerged as a powerful technique in enantioseparation during the past decade [1–4]. Enantionselective open-tubular CEC (OT-CEC), in which the chiral selector is coated onto the inner wall of the capillary as stationary phase, is an alternative mode to enantioselective packed column CEC, and is viewed as a promising approach to avoid various problems associated with packed column CEC, such as the difficulties of end-frit fabrication and obtaining uniformly packed columns. After the first report of enantionselective OTCEC by Mayer and Schurig [5], this technique has been used in many studies to achieve enantioseparations using various enantioselective OT columns [6–15]. The greatest disadvantage of OTCEC is that the phase ratio and sample capacity are relatively poor due to the limited amount of stationary phase coating. Special and efficient coating strategies are thus required. Several options, such as polymer coatings, porous silica layers, etching and sol–gel techniques, have been advanced to increase the surface area and the interaction between the solutes and the coated phase [16,17].

In the past two decades, there are increasing reports in the literature using nanoparticles for chemical separation using CE or micro-chip [18–21], due to their unique physical and chemical properties. Although not many in OTCEC, studies have shown the promise to enhance separation efficiency by using modified nanoparticles as stationary phase. Different kinds of nanoparticles, including polymer, titanium oxide, gold nanoparticles and carbon nanotubes, were investigated as stationary phases in OTCEC [22–28]. Because of the growing applications of nanoparticles in separation science, it is interesting to investigate the use of nanoparticles as supports to immobilize chiral selectors in OTCEC for efficient enantioseparation. Surprisingly, there are very few reports employing nanoparticles in OTCEC enantioseparation [29–31]. In this article, we present the application of thiolated β-CD modified gold nanoparticles (CD-GNPs) as a novel stationary phase of OTCEC for efficient enantioseparation. A simple, reliable and regenerate immobilization procedure was utilized. The stability and reproducibility of the chiral chromatographic column were investigated. Three drug enantiomers were chirally separated using the presented method. The results showed the promising separation of chiral compounds using enantioselective OTCEC with CD-GNPs as stationary phase.

2. Materials and methods

2.1. Chemicals

β-CD, p-toluenesulfonyl chloride, hydrogen tetrachloroaurate hydrate, sodium disulfite, trichloroethylene, sodium borohydridewere were obtained from Sigma Chemical (St. Louis, MO, USA). PDDA (20%, w/w in water, Mw = 200,000–350,000)

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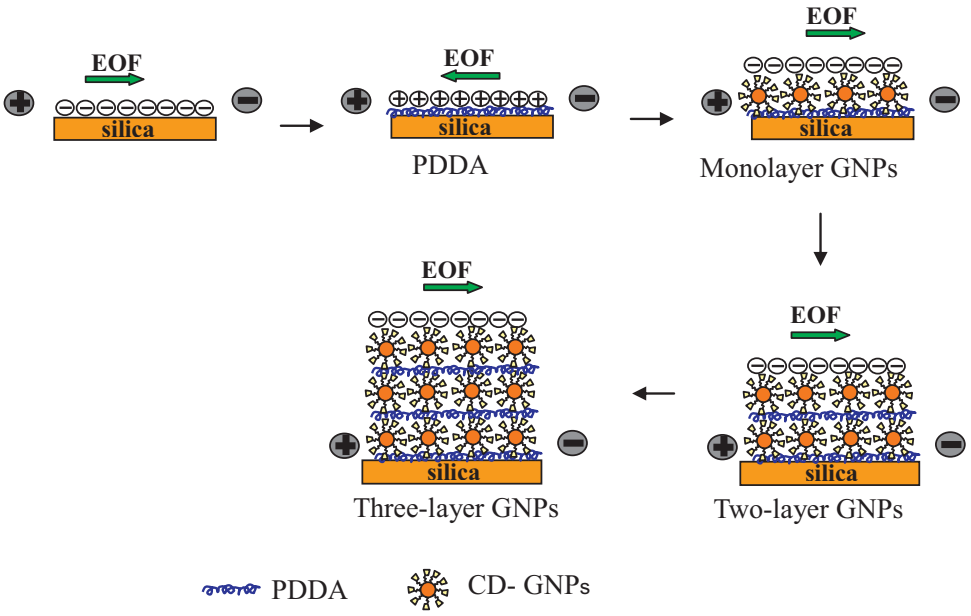

Fig. 1. Schematic drawing of the strategy to prepare capillary columns coated with mono-layer and multi-layer CD-GNP films.

was purchased from JingChun Reagent Inc. (Shanghai, China). Chlorpheniramine, zopiclone and tropicamide were purchased from HeZhong MedicalBiology Engineering (Wuhan, China), ShunFeng Pharmaceutical (Guangdong, China) and ZhuoFeng Pharmaceutical (Zhengzhou, China), respectively. Other reagents were analytical grade and used without further purification.

Phosphate and borate buffers were prepared by dissolving NaH_2PO_4 in deionized water, and pH value was adjusted by H_3PO_3 for phosphate buffer and ionic strength was adjusted by adding NaCl. Stock solutions of the bulk drug samples (1 mg/mL) were prepared in deionized water, and diluted tenfold with CE run buffer. All the solutions were prepared daily and filtered through an inorganic 0.2-μm membrane prior to use.

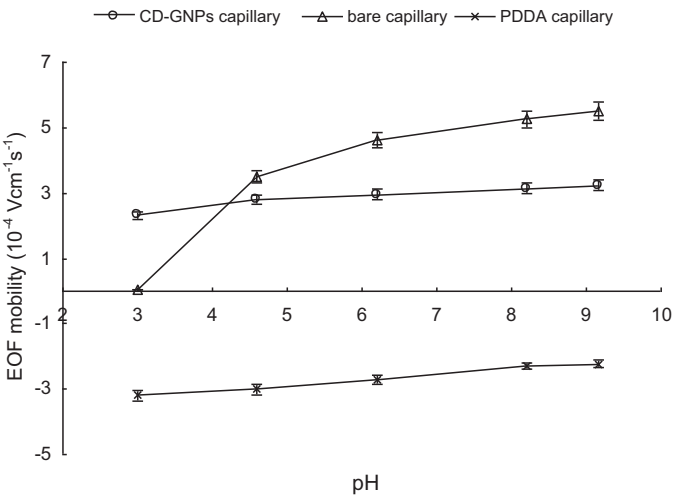

Fig. 2. EOF mobilities of the CD-GNPs capillary, the PDDA coated capillary and the bare capillary in the pH range from 3.0 to 9.2. EOF from anode to cathode is denoted as positive. Running buffer was 12.5 mM phosphate buffer solution with the ionic strength maintained at 50 mM by adding NaCl to the solution. Acetonitrile was used as the EOF marker.

2.2. Instruments

CE experiments were carried out on CE apparatus (CL1020, Beijing Cailu Science Apparatus, China) with UV detector at 214 nm. Fused-silica capillaries of 50 μm i.d. and 365 μm o.d. (Hebei Yongnian Optical Fiber Factory, China) were applied and the total length was 50 cm (effective length 41 cm). SEM pictures of capillaries were obtained using an XL30ESEM-FEG SEM Microscope (SEI).

2.3. CE conditions

Prior to use, the untreated fused-silica capillary was successively flushed with 0.1 M NaOH, H_2O for 15 min. The PDDA and CD-GNPs coated column was flushed with H_2O and buffer for 15 min each. The capillary was pre-electrophoresis under certain voltage until the baseline of the detector output remained stable. Samples were injected hydrodynamically at a height differential of 10 cm for 2 s.

2.4. Preparation of CD-GNPs coated columns

CD-GNPs were synthesized using a three-step procedure, as reported in our previous work [32]. Fig. 1 shows the strategy employed to prepare capillary columns coated with mono-layer and multi-layer CD-GNP films. The protocol was based on the conventional CE procedure [33,34] with a few modifications here. Briefly, the untreated capillaries were successively rinsed with 1 M NaOH for 5 min, 0.1 M NaOH for 1 h and deionized water for 30 min. Once preconditioned, the capillaries were sequentially filled and flushed with PDDA solution (2%, w/w) containing 0.1 M NaCl for 1 h, then CD-GNPs solution (1.8 mg/mL) for 1 h, after each step, the capillaries were washed with deionized water for 5 min. For preparation of multi-layer CD-GNPs, PDDA monolayer and CD-GNPs monolayer coating procedures were repeated. The coated capillary columns were stored in CD-GNPs solution at 4 °C after use. To regenerate the coated layers, the columns were successively flushed with 1 M NaCl, 0.1 M HCl, and 0.1 M NaOH, and then the preparation protocol was repeated.

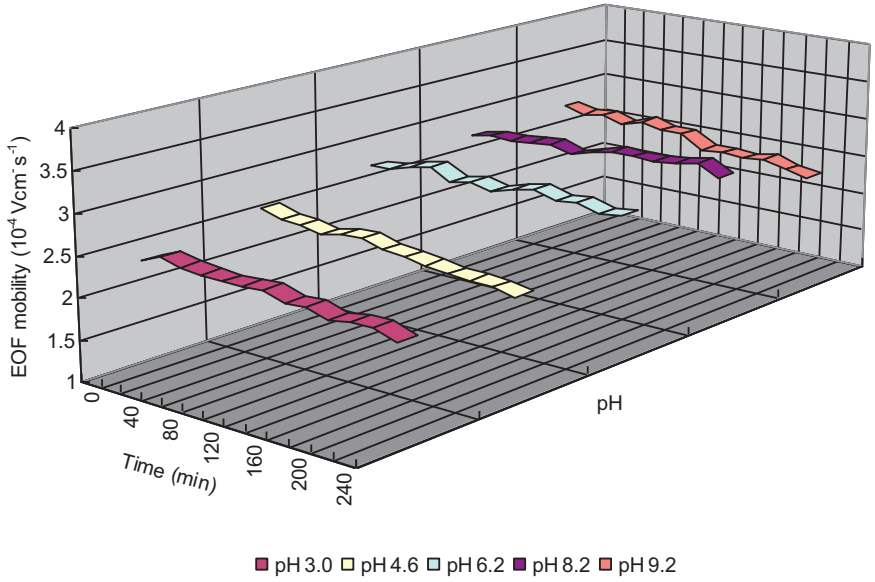

Fig. 3. Stability and reproducibility of CD-GNPs coated OTCEC capillary under OTCEC separation conditions (pH 3.0–9.2) over 240 min.

3. Results and discussion

3.1. Characteristics and performance of the CD-GNPs capillary

Characteristics the synthesized CD-GNPs by several spectroscopic methods was described in our previous study [32]. The results demonstrate that the chiral selectors are well attached to the surface of the GNPs. The average diameter of GNPs is estimated to be 9.5 nm (±2.5 nm).

The modification process on the inner surface of the capillaries was investigated using SEM spectroscopy. It is indicated that the surface area of the inner wall is greatly increased after coating with CD-GNPs (the BET surface area estimated by N_2 adsorption isotherms was 8×10^{-4} m^2 for CD-GNPs coated capillary (length of 40 cm), but no BET value for bare capillary at all). The new surface is quite uniform and well defined, which can still be observed in the SEM photography after one-week use (total 100 runs), indicating that there is no obvious peeling off during CE runs.

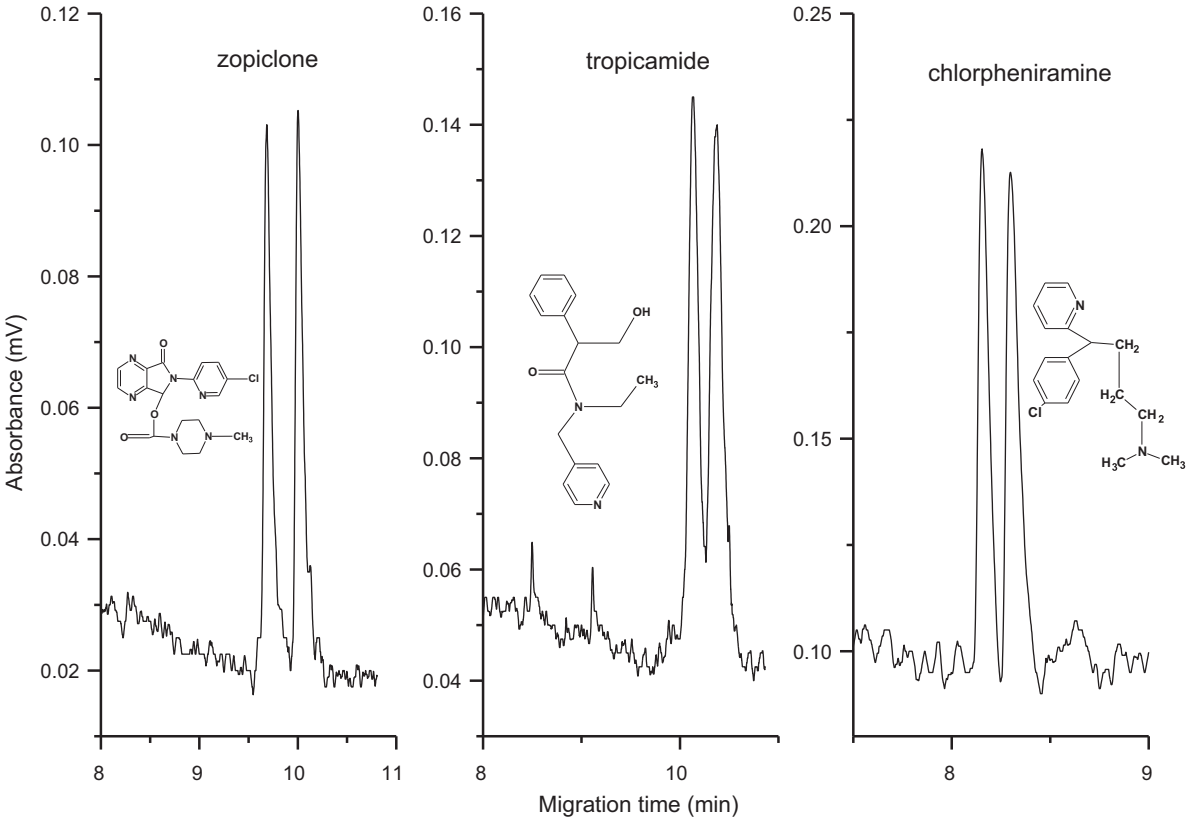

Fig. 4. Electropherograms of separation of the drug enantiomers using CD-GNPs coated OTCEC. Phosphate buffer with concentration of 12.5 mM and pH of 3.0 was used as the running buffer. The separation electric field strength was 300 V/cm.

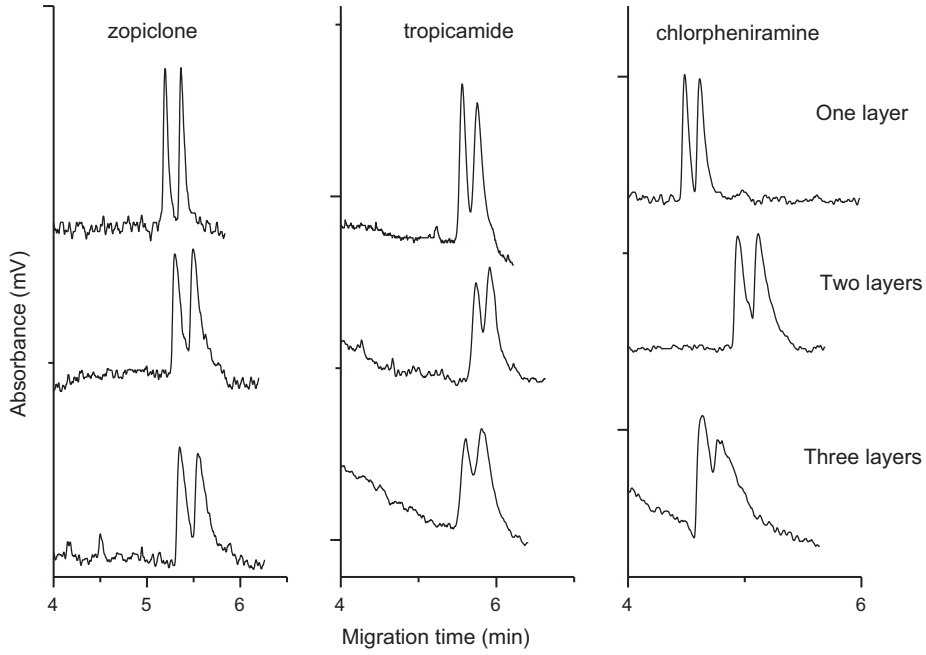

Fig. 5. Electropherograms of enantioselective OTCEC separation of the drug enantiomers using one-layer, two-layer and three-layer CD-GNPs immobilized capillary columns.

Fig. 2 shows EOF mobilities of the CD-GNPs capillary, the PDDA coated capillary and the bare capillary in the pH range from 3.0 to 9.2. The EOF flow on the bare capillary, which is generated by the negatively charged silanol groups on the silica wall, is a positive EOF (from anode to cathode). For the coated capillary, alternate absorption of oppositely charged substances on the inner wall of the fused-silica capillary causes charge neutralization and overcompensation that may lead to EOF reversal. The EOF flow of the PDDA coated capillary is reversed due to the residual of the positively charged PDDA. The EOF flow of the CD-GNPs coated capillary changes back to a positive one since CD-GNPs are negatively charged over the pH range in our experiment (pK_a of β-CD is 12.2). The alternation of the direction of EOF flow serves as another support of successful formation of open tubular capillary with CD-GNPs as stationary phase.

Unlike that of the bare capillary, the EOF mobilities of the coated capillaries are quite stable within the pH range. The RSD of the EOF at pH 3.0–9.2 is 6.1% for the PDDA coated capillary, and 4.8% for the CD-GNPs coated capillary. Since PDDA and CD-GNPs layers are fully ionized at all experimental pH values, it is therefore expected that the corresponding EOF mobility will show much less pronounced dependence on pH than that of the bare capillary.

The stability of the enantioselective open tubular capillary was investigated by monitoring the EOF mobility of the capillary over the pH range of 3.0 to 9.2 using the "three-plug" [35,36] method. During the interval between EOF measurements, 20 kV electric voltage (400 V/cm) was applied across the coated capillary. It can be seen that the EOF mobility is very stable under OTCEC separation conditions during the investigation period (Fig. 3). The RSDs of the EOF at pH 3.0–9.2 are 4.2–5.8% for 240 min measurement and are as small as 1.2–1.9% for 120 min measurement. It proves that the binding of CD-GNPs to PDDA as well as the binding of PDDA to silica capillary wall are very tight.

The column to column reproducibility of EOF mobility was investigated by testing six freshly prepared enantioselective open tubular capillaries. The result for each capillary was obtained based on three separation runs. The RSD of the EOF mobility is 3.9%, indicating good column to column reproducibility of enantioselective open tubular capillaries.

3.2. Enantioseparation of drugs using OTCEC with CD-GNPs as stationary phase

Three drug enantiomers, chlorpheniramine (pK_a 9.2), zopiclone (pK_a 6.7) and tropicamide (pK_a 5.4), were analyzed to investigate the performance of the fabricated chiral capillary for OTCEC enantioseparation with CD-GNPs as stationary phase. The separation electric field strength was optimized and was set as 300 V/cm.

Fig. 4 shows the electropherograms of OTCEC separation of the drug enantiomers. Efficient baseline separation of the drug enantiomers was achieved within 11 min. The separation resolution is 2.69 for zopiclone, 1.37 for tropicamide and 1.62 for chlorpheniramine. The theoretical plate numbers are 1.4×10^5, 1.0×10^5 and 1.2×10^5, and the enantioselectivities are 1.036, 1.041 and 1.028, for zopiclone, tropicamide and chlorpheniramine, respectively. Here, the enantioselectivity is defined as migration separation factor calculated as $\alpha = (t_2 - t_1)/(t_1 - t_0)$, where t_2, t_1 and t_0 are the migration times of the second enantiomer, the first enantiomer and the neutral marker, respectively. The limits of detection are 8.8 μg/mL, 6.3 μg/mL and 5.4 μg/mL for zopiclone, tropicamide and chlorpheniramine, respectively. The calibration curves (dynamic ranges) are $y = 37.746x - 28.625$ (15.6 μg/mL–0.5 mg/mL zopiclone), $y = 56.281x - 57.542$ (12.7 μg/mL–0.5 mg/mL chlorpheniramine) and $y = 48.278x - 39.826$ (10.08 μg/mL–0.5 mg/mL tropicamide), respectively.

To test the run-to-run reproducibility, six consecutive separations of three pairs of the drug enantiomers were performed. The RSDs of the retention and peak area are lower than 2.0% and 4.1%, respectively, while the RSD of the resolution is around 3.0%. The results indicate that the CD-GNPs coated open tubular capillary can be repeatedly used for enantioselective OTCEC separation. The enantioselectivity without degradation of the separation performance retains for more than 1 month if the column was stored in CD-GNPs solution at 4 °C.

Enantioselective OTCEC separation using multilayer CD-GNPs coated capillary was also investigated. The EOF mobility increases as the thickness of CD-GNPs is increased, as observed in Layer-By-Layer assembly study reported by Liu et al. [36]. There is no significant improvement of either stability or reproducibility

of multi-layer CD-GNPs on the capillary. Fig. 5 shows the electropherograms of enantioselective OTCEC separation of the drug enantiomers using different layer of CD-GNPs on the capillary. It is unfortunately to find that the more layers of CD-GNPs are coated on the capillary, the worse the enantioselectivity and the separation efficiency are. Since GNPs have high UV absorbance at 214 nm, the detection sensitivity is dramatically reduced as the layers of CD-GNPs coated on the capillary is increased. To obtain the same UV absorbance signal, higher concentration of samples should be loaded in multi-layer assembly, thus leading to more asymmetric peak and lower separation efficiency.

4. Conclusion

In this paper, we reported the application of CD-GNPs as stationary phase for enantioselective OTCEC separation. The CD-GNPs coated capillary, which was easily fabricated by electrostatic assembly of PAAD followed by self-adsorption of CD-GNPs, forms a quite uniform and well defined new surface on the inner wall of the capillary and is stable enough to serve as stationary phase for enantioselective OTCEC separation. By investigating the EOF mobility of the capillary, we have shown the good stability and reproducibility of the CD-GNPs coated capillary. Efficient baseline separation of three drug enantiomers was achieved using the presented method. The chiral capillary exhibits good run-to-run repeatability for enantioseparations and retains enantioselectivity for more than 1 month if the column is stored in CD-GNPs solution at 4 °C.

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