

Amino-functionalized silica nanoparticles for improved enantiomeric separation in capillary electrophoresis using carboxymethyl- β -cyclodextrin (CM- β -CD) as a chiral selector

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Abstract We report on the use of amino-modified silica nanoparticles (SiNPs) as an additive to the background electrolyte solution to enhance the chiral selectivity of in capillary electrophoresis that is induced by the presence of a small quantity of carboxymethyl- β -cyclodextrin (CM- β -CD). The modified SiNPs were characterized by transmission electron microscopy, elemental analysis and their zeta potential. The method was applied to the separation of four alkaline drugs (ephedrine, chlorpheniramine, propranolol and amlodipine). The addition of the modified SiNPs to the background electrolyte results in a distinct improvement in the separation power, especially when the capillary was pretreated with high concentration of particle suspensions prior to separation. The effects of fractions of modified SiNPs and organic modifier, of the thickness of the SiNP coating layer on the capillary wall were investigated. Under optimum experimental conditions, all the racemates investigated were separated with improved resolution, thus indicating the potential of the method in the field of enantiomeric separation.

Keywords Amino-modified silica Nanoparticles · Enantioseparation · Capillary electrophoresis

Abbreviations

CE	Capillary electrophoresis
CEC	Capillary electrochromatography
CM- β -CD	Carboxymethyl- β -cyclodextrin

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DDW	Double-distilled water
EOF	Electroosmotic flow
HCNPs	High concentration NPs
HP- β -CD	Hydroxypropyl- β -cyclodextrin
HPLC	High performance liquid chromatography
NPs	Nanoparticles
NH ₂ -SiNPs	Monoamino moiety modified silica nanoparticles
PSPs	Pseudo-stationary phases
<i>R_s</i>	Resolution
SiNPs	Silica nanoparticles

Introduction

Enantiomeric analysis is of interest in pharmaceutical, toxicological and clinical analysis. In most cases, the pharmacological activity is restricted to one of the enantiomers, whereas the other form can exhibit unwanted side effects, antagonistic activities or even toxic effects [1,2]. Therefore, the enantioseparation of chiral compounds is of great importance. Instrumental techniques that have been widely used for the separation of enantiomeric compounds include high performance liquid chromatography (HPLC) [3], gas chromatography [4], supercritical fluid chromatography [5], capillary electrophoresis (CE) [6] and capillary electrochromatography (CEC) [7]. With the advantages of high efficiency, rapid and low consumption of reagents, CE has become a powerful tool in chiral separation [3,6,8].

Applications of nanoparticles (NPs) in separation science are of increasing interest due to their unique properties, such as favorable surface-to-volume ratio and easy modification

[9,10]. In 1989, Wallingford and Ewing reported the first application of sulfonated polymer NPs in CE [11], in which five different catechol amines were successfully separated. Since then, a variety of NPs including silica, gold, carbon, and polymer have to date been employed in CE, which has been reviewed by Nilsson [12], Palmer [13] and Duan [14]. In the past few years, several kinds of NPs have been successfully introduced into chiral CE. Na et al. [15] reported the use of polystyrene NPs to enhance enantiomeric separation of propranolol by capillary electrophoresis with hydroxypropyl- β -cyclodextrin (HP- β -CD) as chiral selector. In their succeeding work, they also used other kinds of NPs (multi-walled nanotubes (MWNTs), TiO₂ and Al₂O₃) modified with single layer β -CD as chiral selector to enhance enantioseparation of clenbuterol by capillary electrophoresis [16]. Choi et al. [17] used sulfonated β -CD as a chiral selector and Ag colloid as an additive in an attempt to improve the chiral separation of arylalcohols. The result showed that addition of Ag colloid to the background electrolyte improved the resolution significantly. Recently, Li et al. [18] reported the application of thiolated β -CD modified gold NPs in CE for the efficient enantioseparation of four dinitrophenyl-labeled amino acid enantiomers and three drug enantiomers. Soon after that, a novel chiral column was fabricated by the same group through electrostatic assembly of poly (diallyldimethylammonium chloride) followed by self-adsorption of negatively charged β -CD modified gold NPs. Under open-tubular CEC mode, three representative drug enantiomers were successfully resolved with good repeatability [19].

Silica nanoparticles (SiNPs) own many advantages over other types of nanoparticles, such as good biocompatibility, no swelling in aqueous and organic solvents, and easy post-modification with different functional groups, etc. [20] In the past decade, functional SiNPs have received considerable attention and had been successfully used as pseudo-stationary phases (PSPs) for the enhanced separation of organic acids and bases [21,22], drugs [23] and proteins [24] in CE mode. There is no paper up to date reporting the use of functionalized SiNPs as running buffer additives to enhance the enantioselective separation in CE. The reports on different kinds of NPs applied to enhance the enantiometric separation of basic drugs in CE are summarized in Table 1. In Table 1, crystalline material mesoporous silica nanoparticles on the inner wall of an open-tubular capillary as the support for coating chiral selector of cellulose tris (3,5-dimethylphenyl-carbamate) was carried out by Dong et al. [25].

In this work, the prepared NH₂-SiNPs were added in background electrolyte solution in the presence of very small amount of CM- β -CD (6.45×10^{-2} mmol L⁻¹). The enantioresolution for these drug racemates i.e., ephedrine, chlorpheniramine, propranolol and amlodipine was greatly improved, especially when the capillary column was

Table 1 Figures of merit of comparable method for enantiometric separation of basic drugs in CE

Material/Method	Chiral selector	Limit Of Detection (LOD)	Range of pH value	Merits	Analytes	Reference
Carbon nanoparticles / electrokinetic chromatography	Dextrin	–	Sulconazole :2.1–3.5 ketoconazole :2.5–5.0	The first use of carbon nanoparticles as PSPs to modify chiral separation system employing dextrin as chiral selector	Sulconazole ketoconazole	[9]
Polystyrene nanoparticles / CE	Hydroxypropyl- β -cyclodextrin	–	2.1–4.5	Greatly decreasing the required amount of HP- β -CD and also improving the separation efficiency	Propranolol	[15]
Crystalline material mesoporous silica nanoparticles / CEC	Cellulose tris (3,5-dimethylphenyl-carbamate)	–	6.8	Much higher enantioselectivity	Tetrahydropalmatine, pindolol	[25]
Surfactant coated carbon nanotubes/ electrokinetic chromatography	Carbon nanostructures	0.2–0.45 μ g/mL	–	Rapid and simple; being a very interesting alternative to other chiral selectors like CDs or microemulsion electrokinetic chromatography based chiral methods	(\pm)-ephedrine, (\pm)-norephedrine (\pm)-N-methylephedrine	[26]

pretreated with high concentration of NH_2 -SiNPs suspension solution prior to separation.

Experimental

Chemicals

All chemicals were analytical grade unless noted otherwise. Double-distilled water (DDW) purified by a Nanopure II system (Barnstead, USA) was utilized throughout the experiments. Tetraethoxysilane (98 %) and 3-amino propyltriethoxysilane (99 %) were purchased from Tianjin Chemical Reagent Research Institute (<http://www.ectcr.com/>, Tianjin, China). Ammonia (25 %) was purchased from Tianjin Guangfu Fine Chemical Research Institute (<http://tjguangfu.cn.china.cn/>, Tianjin, China). Methanol, ethanol and acetonitrile were all of analytical grade and purchased from Concord Technology Co., Ltd. (<http://tjconcord.company.lookchem.cn/>, Tianjin, China). Phosphoric acid (85 %), sodium hydroxide, sodium dihydrogen phosphate and hydrochloric acid (36–38 %) were from Kewei Co., Ltd. (<http://tjnankai011792.11467.com/>, Tianjin, China). Carboxymethyl- β -cyclodextrin (CM- β -CD, >98 %, molecular weight ≈ 1551 , degree of substitution ≈ 7.05) was purchased from Binzhou Zhiyuan Bio-Technology Co., Ltd. (<http://www.bzzysw.com/index.asp>, Shandong, China). Stock solutions of racemic ephedrine, chlorpheniramine, propranolol and amlodipine (structures are shown in Fig. S1) were extracted from tablets with methanol. The concentrations of stock solution were 2.0, 2.4, 1.0, 0.25 mg mL^{-1} for racemic ephedrine, chlorpheniramine, propranolol and amlodipine respectively. The sample solutions for injection were prepared at a certain concentration by diluting stock solutions with background electrolyte solution.

Instrumentation

Transmission electron microscope micrographs of sample were taken using Tecnai G2F20 electron microscope (FEL, USA) with a maximum accelerated voltage of 200 kV. The samples tested were prepared by dipping carbon membrane coated copper grinds (300-mesh) (Beijing Da Yi Instrument Technology Co. Ltd.) into the dispersion of NPs in ethanol. Elemental analysis was performed by elemental analyzer (vario EL CUBE, German). The zeta potential and dynamic light scattering measurements were made on Zetasizer Nano ZS with 633 nm He-Ne laser (Malvern Instruments, Worcestershire, UK). A high speed centrifuge with the model of TG 16-WS (Xiangyi Centrifuge Co. Ltd., Hunan, China) was used to collect the final product after synthesis. All CE experiments were carried out on a TH-3000 HPCE-HPLC amphibious system equipped with a CXTH-3000 data

handling software (Chuang Xin Tong Heng Science and Technology, Beijing, China). The UV detector was operated at 214 nm unless stated otherwise.

Synthesis of the NH_2 -SiNPs

In general, there are two ways for synthesizing organosilica materials, namely, post-modification and one-pot co-condensation. Comparing to post-modification, co-condensation is much preferred for the formation of core-shell SiNPs with a high and uniform surface coverage of organic units [27,28].

To a solution of TEOS (2 mL) in 50 mL of ethanol, a mixture of NH_4OH (2 mL) and DDW (1.4 mL) was added dropwise with vigorous stirring. When the solution started to become turbid (~ 15 min), 220 μL 3-amino propyltriethoxysilane was added with a constant flow rate (~ 2.0 mL min^{-1}). After reaction for 18 h, the resulting products were separated by centrifugation at 9055rcf for 15 min and then washed with ethanol and DDW repeatedly for several times. The final product was dried at 80 $^\circ\text{C}$ for 8 h under vacuum.

For comparison, pure SiNPs were also prepared by nearly the same procedure except without adding 3-amino propyltriethoxysilane.

Procedures

A capillary with the total length of 49 cm and the effective length of 37 cm was made by scraping off 4–5 mm of the polymer outside the capillary at an appropriate place. Prior to the first use, the capillary was successively rinsed by methanol, DDW, 1 mol L^{-1} NaOH, DDW, 0.1 mol L^{-1} NaOH, DDW, 0.1 mol L^{-1} HCl, DDW and buffer solution for 20 min each. A high concentration NPs (HCNPs) method was used to further treat the capillary prior to chiral separation, i.e., the previously pretreated capillary was flushed with high concentration NH_2 -SiNPs suspension (5.0 mg mL^{-1}) for 5 min, then the pressure was released and the capillary was allowed to stand for 10 min. The procedure was repeated for three times unless state otherwise before the capillary column was washed with plenty of water to wash out excessive NH_2 -SiNPs.

Enantioseparation was performed at applied voltage of 10 kV, and the temperature of capillary was maintained at 25 $^\circ\text{C}$. The electroosmotic flow (EOF) was determined by using thiourea as the neutral marker unless stated otherwise.

Stock solution of 30 mmol L^{-1} phosphate background electrolyte was prepared by dissolving a certain amount of sodium dihydrogen phosphate in DDW. Prior to use, it was adjusted to the appropriate pH value with concentrated KOH or H_3PO_4 . All solutions were kept at 4 $^\circ\text{C}$ in a refrigerator. The running buffer is 30 mM phosphate background electrolyte (pH 3.0) containing 6.45×10^{-2} mmol L^{-1} CM- β -CD with

0.3 mg mL⁻¹ NH₂-NPs added. Before use, all solutions were filtered through a 0.45 μm nylon membrane and degassed by ultrasonication.

Results and discussion

Characterization of NH₂-SiNPs

The resultant NH₂-SiNPs were treated with 5 % (wt %) ninhydrin in ethanol. The color of the suspension turned blue within a few minutes, indicating the successful grafting of amine moiety on the surface of SiNPs.

Transmission electron microscopy provides information about the size distribution and shape of particles. The NPs obtained are spherical and uniform in particle size (~120 nm). Dynamic light scattering is useful for determining the size of an ensemble of dispersed nanoparticles. As can be seen in Fig. S2, the average diameter is 139 nm with particle dispersion index (PDI) 0.120. It is about 20 nm larger than that obtained by transmission electron microscopy, probably because of the contributions from hydration layers on the surface of the NPs.

The results of elemental analysis for pure SiNPs and NH₂-SiNPs are summarized in Table 2. Basing on the N content for NH₂-SiNPs, it could be deduced that the surface concentration of aminopropyl group was about 0.72 mmol g⁻¹.

The measurement of the Zeta-potential of the aqueous suspension of NPs is an indirect, but useful way to confirm the existence of functional groups on the surface of NPs. Fig. S3 shows the curves of the zeta potential vs pH. Every plot was measured three times, the RSDs were in the range of 1.1–3.5 %. In the pH range of 2.5–9.0, NH₂-SiNPs have more positive zeta potentials than those of pure SiNPs. The positive surface charge provided by amino group also results in a shift of the isoelectric point from 3.4 to 5.2.

Enantioseparation of racemic drugs

Effect of background electrolyte pH on enantioseparation

It is well known that background electrolyte pH plays an important role in the separation of ionizable compounds in CE,

because it determines the extent of ionization of analytes, as well as the net electric charges on the inner wall of capillary column which in turn determines the direction and magnitude of EOF. Using CM-β-CD (6.45 × 10⁻² mmol L⁻¹) as chiral selector, the effect of pH (from 2.5 to 6.0) on the resolution of the four drug racemates was investigated (Fig. S4). Considering other factors, such as column performance and separation time, we finally choose pH 3.0 for ephedrine and chlorpheniramine, and pH 5.0 for propranolol and amlodipine in the following experiments.

Enantioseparation of ephedrine and chlorpheniramine

Effect of the concentration of NH₂-SiNPs on enantioseparation

When used as PSPs, NPs are suspended in the electrolyte and are continuously pumped through the capillary by the EOF during separation, so it is a key issue that NPs utilized should be well dispersed in background electrolyte solution. Otherwise, unreproducible results would be obtained. NH₂-SiNPs can be well dispersed in buffer solution without the assistance of other reagents such as urea and surfactant, probably due to the existence of hydrophilic amino groups on the surface. Fig. S5 shows the effect of NH₂-SiNPs concentration on the chiral separation of ephedrine. When an optimum amount of NH₂-SiNPs (0.3 mg mL⁻¹) was added, the resolution (*R_s*) values increased from 0.86 to 1.05 for ephedrine and from 0.88 to 1.12 for chlorpheniramine, respectively.

Effect of organic additives on enantioseparation

The addition of organic modifiers to the background electrolyte can affect several parameters such as the stability constants of the inclusion-complexes, the viscosity of the background electrolyte, the EOF and also the analysis time, which in turn would definitely influence the separation results [8]. Na et al. [15] investigated the effect of methanol content on the separation of propranolol, and found 15 % methanol content would be optimum, and any increase above 15 % would result in the reduction in *R_s*.

Using chlorpheniramine as model compound, the effect of organic modifiers on the enantioresolution was also investigated (Fig. S6). It was found that increasing the methanol or acetonitrile content would result in a decrease of resolution. It seems organic modifiers may compete with solute to enter into the CD cavity, which would definitely impair the enantiomer recognition process [15, 29].

Table 2 Elementary analysis of pure SiNPs and NH₂-SiNPs

NPs	C% / RSD% (n=3)	H% / RSD% (n=3)	N% / RSD% (n=3)
Pure SiNPs	1.82/ 2.64	2.39/ 1.91	0.06/ 4.16
NH ₂ -SiNPs	4.97/ 7.21	2.94/ 2.93	1.06/ 3.00

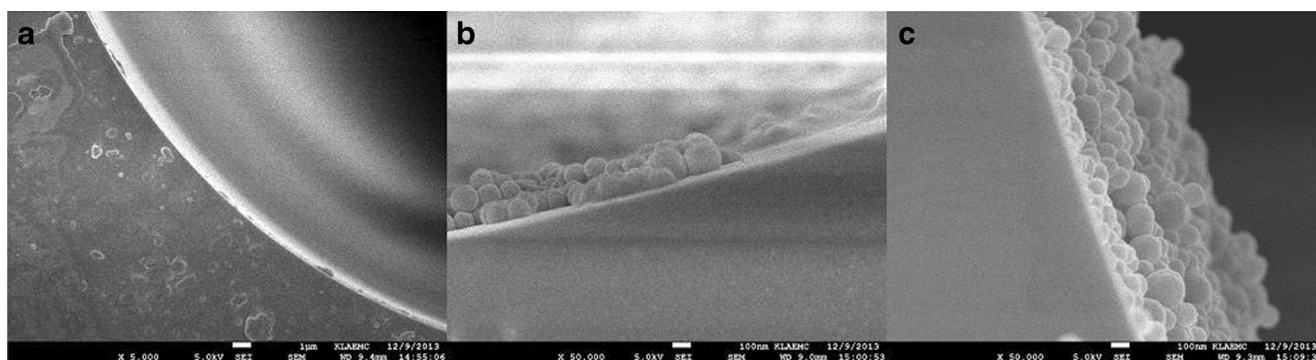


Fig. 1 SEM images of bare capillary wall (a), being pretreated with HCNPs for one time (b) and three times (c)

Effect of thickness of coating layer of NH_2 -SiNPs on enantioseparation

It had been confirmed in several papers [21,27] that NPs surface modified with amino group can be adsorbed on the inner surface of capillary column through electrostatic interaction. With the increase in the concentration of the positively charged NPs in background electrolyte, an incremental reversed anodic EOF (i.e., from cathode to anode) could be obtained under lower pH conditions, and this may be favorable for CE separation [27]. We also tested the EOF values of the current separation system with the change of the pH of background electrolyte, while keeping other conditions constant (30 mM phosphate, 6.45×10^{-2} mmol L^{-1} CM- β -CD with 0.3 mg mL^{-1} NH_2 -SiNPs added). However, no reversed EOF was obtained in the pH range of 2.5 to 6.0 before treating the capillary column with HCNPs. It seems that the interactions between CM- β -CD and NH_2 -SiNPs can suppress the adsorption of NH_2 -SiNPs to the inner surface of capillary column.

Basing the observations above, we tried to treat capillary with HCNPs method (as shown in section 2.4) prior to chiral separation. The capillary column thus treated was then equilibrated with background electrolyte (30 mM phosphate (pH 3.0), 6.45×10^{-2} mmol L^{-1} CM- β -CD with 0.3 mg mL^{-1} NH_2 -SiNPs added) until a flat baseline was

observed. An EOF measurement showed an anodic EOF was obtained with a repeatable value of $1.19 \times 10^{-8} \text{ m}^2 \text{ s}^{-1} \text{ V}^{-1}$, which may indicate the formation of NH_2 -SiNPs coating on the inner surface of capillary column.

To further verify the existence NH_2 -SiNPs coating, we carried out a microscopic test. Figure 1 shows the formation of NH_2 -SiNPs coating on the inner wall of capillary. Clearly, the inner wall of the bare fused silica capillary was very smooth as shown in Fig. 1a. However, rough inner surfaces were observed on the NH_2 -SiNPs coating capillary column as in Fig. 1b and c. When increasing the number of repetitive coating, the thickness would increase accordingly. We also investigated the influence of the thickness of NH_2 -SiNPs coated on the inner wall of capillary on enantioresolution. Figure 2 shows the electropherograms of enantioselective separation of ephedrine and chlorpheniramine on HCNPs coating capillaries with different thickness. It was clearly shown that the *R_s* value was measured to be dependent on the repetitive coating times. In a certain range, the more times the capillary was pretreated with NH_2 -SiNPs, the better the enantioresolution would be. For example, the *R_s* values for chlorpheniramine racemates were determined to be 1.16, 1.52, 2.95 respectively when the separation capillary was repetitively treated 0, 1, 3 times with HCNPs. However, the column efficiency would decrease and the migration time would be much longer when the number of repetitive coatings was further increased.

Fig. 2 The electropherograms of enantioselective separation of ephedrine (a) and chlorpheniramine (b) on HCNPs coating capillaries with different thickness. Experimental conditions: 30 mM phosphate background electrolyte (pH 3.0) containing 6.45×10^{-2} mmol L^{-1} CM- β -CD with 0.3 mg mL^{-1} NH_2 -NPs added. Detection wavelength, 214 nm; injection, 10 kV \times 5 s; separated voltage, + 10 kV

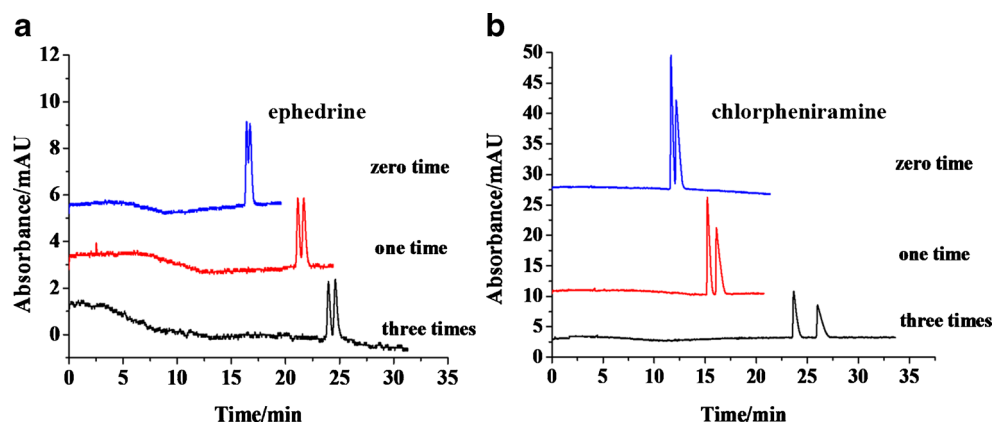


Fig. 3 The electropherograms of enantioseparation of chlorpheniramine on the capillary column pretreated with HCNPs. Experimental conditions: 30 mM phosphate background electrolyte (pH 3.0) containing 6.45×10^{-2} mmol L⁻¹ CM- β -CD and 0.3 mg mL^{-1} NH₂-NPs (a), after being flushed with 30 mM phosphate background electrolyte (pH 3.0) without CM- β -CD and NH₂-NPs for 15 min (b), 0.5 h (c) and 1 h (d). The other conditions are the same as mentioned in Fig. 2

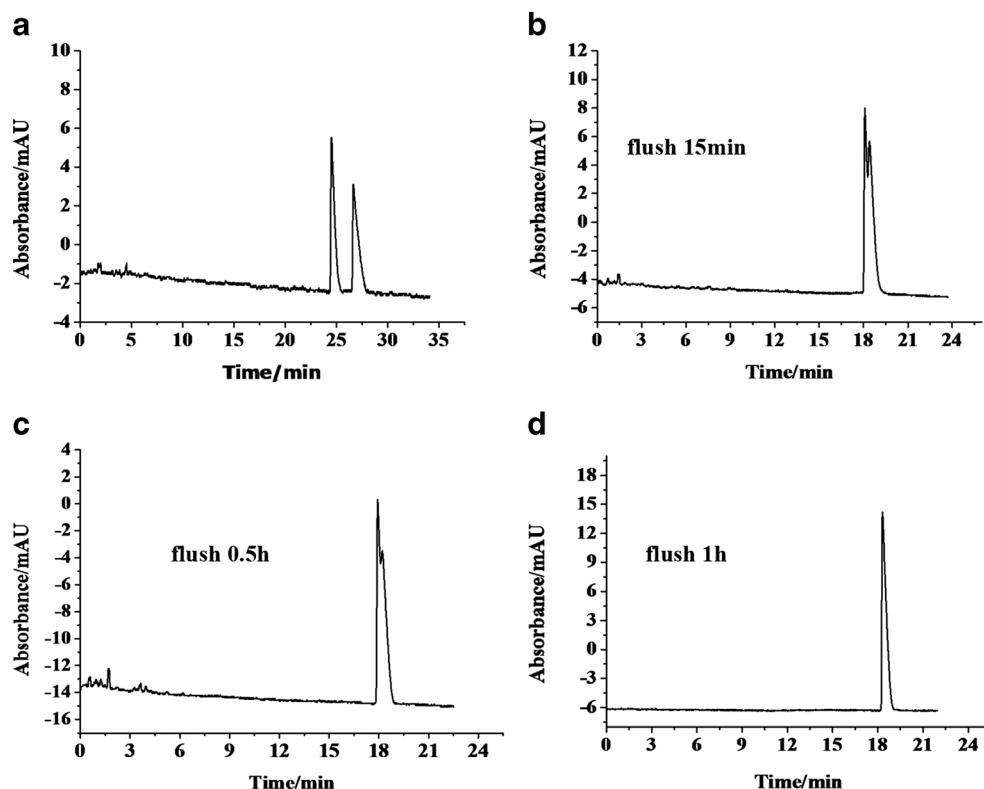
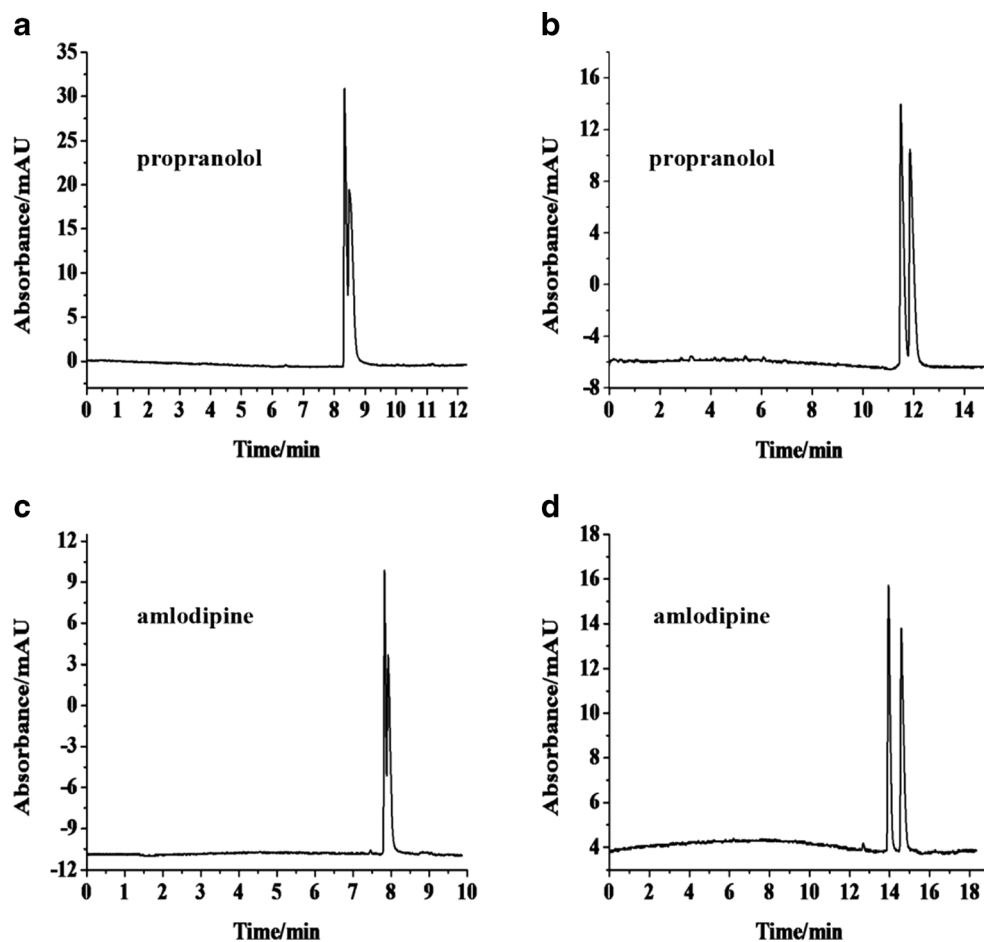


Fig. 4 Typical electropherograms of the enantioseparation of propranolol (a, b) and amlodipine (c, d). Experimental conditions: 30 mM phosphate background electrolyte (pH 5.0) containing 6.45×10^{-2} mmol L⁻¹ CM- β -CD on bare capillary column (a, c); 30 mM phosphate background electrolyte (pH 5.0) containing 6.45×10^{-2} mmol L⁻¹ CM- β -CD, with 0.3 mg mL^{-1} NH₂-NPs added (b, d) on HCNPs coating capillary column. Detection wavelength, 214 nm; injection, 10 kV \times 5 s for propranolol and 10 kV \times 2 s for amlodipine; separated voltage, +10 kV



Considering the migration time, column efficiency and resolution, 3 times treatment of the capillary with HCNPs was chosen for the subsequent separation.

Working principle and the mechanism for improved enantioseparation

NPs may interact with chiral selectors to form complex, and this would be beneficial to enantioseparation [15,16]. NH₂-SiNPs may provide a large surface for CM-β-CD adsorption (mainly through electrostatic interaction), allowing a larger contact and interaction between CM-β-CD and analytes. NH₂-SiNPs can be adsorbed on the inner surface of capillary column through electrostatic interaction. When the capillary is further treated with the HCNPs, a dynamic coating layer will be formed on the inner wall of capillary column. The cathodic EOF (i.e., from anode to cathode) could be reversed, and this may be favorable for CE separation. The coating layer may absorb chiral selector of CM-β-CD to act as a chiral stationary phase (CSP) in the chiral separation process.

To verify this, another experimental was carried out, and the experimental results are shown in Fig. 3. It can be concluded from Fig. 3 that NH₂-SiNPs coating layer is beneficial for the improved separation. When NH₂-SiNPs were removed from the buffer solution, the already-formed coating still took effect. However, the coating thus formed was dynamic and it would disappear gradually when be flushed with buffer solution not containing NH₂-SiNPs and chiral selector.

The repeatability of enantiomeric separation

To test the run-to-run repeatability, using chlorpheniramine as model compound, we investigated the repeatability of the enantiomeric separation by 4 sequent injections (Fig. S7). The RSDs for retention time (the first enantiomer), α (selective factor) and R_s were 2.87, 0.17 and 3.73 %, respectively. These data suggested the separation system was stable.

Enantioseparation of other drug racemates

The enantioseparation of other drug racemates, such as propranolol and amlodipine, were also tested using the same method established above. For both compounds, only partial separation can be obtained when using the background electrolyte containing 6.45×10^{-2} mmol L⁻¹ CM-β-CD. When adding NH₂-SiNPs in buffer solution, the enantioseparation was greatly improved (R_s from 0.9 to 1.47 and from 0.93 to 2.59 for propranolol and amlodipine respectively) on HCNPs coating capillary column at the cost of a slightly longer analysis time (Fig. 4). For both compounds, an obvious improvement in resolution can be obtained, which predicates the

validity of the presently established method in the field of chiral separation.

Conclusion

When NH₂-SiNPs were dispersed in buffer solution, they would provide a large surface for CM-β-CD adsorption through electrostatic interaction, allowing a larger contact and interaction between CM-β-CD and analytes. And when the capillary was further treated with HCNPs, a dynamic coating layer would be formed on the inner wall of capillary column. The coating layer would absorb chiral selector of CM-β-CD and then acted as a chiral stationary phase in the chiral separation process. Under the synergistic effect of both factors, several representative chiral drugs were successfully resolved at a very low concentration level of chiral selector of CM-β-CD.

The strategy established here is most likely to be extended to other kinds of chiral selectors in chiral analysis. Further research work in relation to this is currently under way in our lab.

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Conflict of interest The authors have declared no conflict of interest.

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