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Fabrication of chiral amino acid ionic liquid modified magnetic multifunctional nanospheres for centrifugal chiral chromatography separation of racemates

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Abstract

As the rapid development of nanotechnology, the magnetic nanospheres modified with special chiral selective ligands show a great potentiality in enantiomeric separation. In this study, magnetic nanospheres modified with task-specific chiral ionic liquid were designed for the separation of chiral amino acids. These modified magnetic nanospheres were effective in a direct chiral separation of five racemic amino acids (D- & L-cysteine, D- & L-arginine, D- & L-leucine, D- & L-glutamine and D- & L-tryptophan). Furthermore, a new online method for complete separation of the enantiomers via the magnetic nanospheres was established with centrifugal chiral chromatography using a spiral tube assembly mounted on a type-J coil planet centrifuge. One kind of chiral compounds, D- & L-tryptophan was resolved well using this method. These results demonstrated that the modified nanospheres display a good chiral recognition ability, and can be used as a potential material for chiral separation of various racemates.

Graphic Abstract

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Keywords

fabrication; chiral amino acid ionic liquid; magnetic nanospheres; centrifugal chiral chromatography; D- & L-tryptophan

1. Introduction

The separation of chiral compounds enantiomers has been widely investigated due to their prevalence in the pharmaceutical industry, agrochemicals, food additives and so on [1]. The enantiomers have identical physical and chemical properties except for optical rotation, whereas they exhibit different biological and pharmacological properties. So the chiral discrimination of racemates into individual enantiomers is a very significant task. Racemates' separation remains a challenge due to their identical properties in a chiral environment, and research on specialized separation techniques continues to be developed to resolve individual enantiomers [2]. Proteins, ligands, ion exchangers, and antibiotics were widely used in the chiral separation because they provide a chiral environment [3, 4]. Though there have been a number of chiral separation methods, such as micellar electrokinetic chromatography [5], chiral high-performance liquid chromatography [6], and capillary electrophoresis chromatography [7–9], researches on specific chiral selectors as well as faster and lower cost separation of enantiomers still are necessary.

Chiral amino acids have broad applications in the synthesis of many drugs, plant protectants and fine chemicals. Therein, tryptophan is one of many important chiral compounds, which can only be synthesized by microorganisms or plants and plays an essential role in the physiological and pharmaceutical metabolisms in human body [10, 11]. D-tryptophan and L-tryptophan exhibit different medical activities and biological functions. Therefore, it is essential to develop a simple and effective method for chiral separation of D- & L-tryptophan. Zhu et al. used a modified nano-TiO₂ for the separation of L-tryptophan [12], Chan and co-workers employed a ChiroSil-SCA simulated moving bed chromatographic process for separation of tryptophan enantiomers [13]. These methods have different abilities for chiral recognition.

Magnetic nanoparticles of Fe₃O₄ combined with functional molecules have been widely applied for drug delivery [14], catalysis [15], magnetic response image [16] and bioanalysis [17] due to their good biocompatibility, superparamagnetic property, low toxicity and easy

preparation [18]. It is recognized that nanoparticles composed of magnetic cores with chiral ligands on their surface simultaneously possess both magnetic property and chiral recognition ability. A proper chiral selector integrated with magnetic nanospheres may be considered as a good alternative for the separation of racemates. More recently, Uddin et al. used carboxymethyl- β -cyclodextrin-modified magnetic nanospheres for adsorption of three chiral aromatic amino acids [19]. Wei and co-workers employed cellulose-phenylcarbamate anchored magnetic silica microspheres for the separation of five racemates [20]. Yang et al. employed teicoplanin-modified hybrid magnetic mesoporous silica nanoparticles for the separation of racemic compounds [21]. Hence, magnetic nanospheres modified by a general chiral ligand had a great potential in chiral recognition.

Chiral ionic liquids possessing chiral cation and/or chiral anion have been demonstrated to display superior capability in the general field of chiral recognition due to its highly organized structure [22, 23]. For instance, silica gel supported pyrrolidine-based chiral ionic liquid showed high enantioselectivity in asymmetric Michael addition reaction [24]. Armstrong et al. used chiral ionic liquids as stationary phases in gas chromatography for the separation of different compounds including alcohols, diols, sulfoxides, epoxides, and acetylated amines [25]. Amino acid-based chiral ionic liquids have displayed their potential in separation of amino acids as well [26] and demonstrated great potential for ligand exchange chiral separation [27]. The unique chiral ionic liquids can separate numerous racemic compounds because of the formation of hydrophobic interaction, hydrogen bonding, steric repulsion, π - π , dipole-dipole, and ionic interactions between chiral ionic liquids and racemates.

According to previous studies, chiral ionic liquids are promising in the separation of amino acids due to their good environmental and biological compatibility, so chiral ionic liquid 1-ethyl-3-methyl-imidazolium L-proline (EMIMLpro) consisting of imidazolium cation with a natural amino anion, was chosen as a functional legend to design more task-specific chiral magnetic nanospheres in the current study. The natural amino anion (L-proline) possesses the similar physical and chemical properties (such as structure, water-soluble, polarity) with target amino acids. Thus, the separation of chiral amino acids via chiral ionic liquids with L-proline would show great advantages compared with traditional chiral selectors. Using automatic digital polarimeter analyses, the direct separation of several amino acids by the chiral magnetic nanospheres has the versatile trend for separation of racemates. Furthermore, a new online method for complete resolution of the enantiomers via the magnetic nanospheres was established for the first time with centrifugal chiral chromatography using a spiral tube assembly mounted on a type-J coil planet centrifuge. One kind of chiral amino acid, D- & L-tryptophan was resolved well using this method.

2. Experimental

2.1. Materials

1, 6-Diisocyanatohexane (HMDI) was purchased from Company J&K. The chiral ionic liquid EMIMLpro was obtained from Shanghai Cheng Jie Chemical Co., LTD. D- & L-cysteine, D- & L-arginine, D- & L-leucine, D- & L-glutamine, D- & L-phenylalanine, D-

tryptophan, L-tryptophan, and D- & L-tryptophan was purchased from Sigma Chemical Company (St. Louis, MO, USA).

All the solvents and other chemicals were of analytical grade commercially available, including ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), succinic acid, urea, 1, 2- propylene glycol, concentrated hydrochloric acid (12 M HCl), ammonium hydroxide ($\text{NH}_3 \cdot \text{H}_2\text{O}$), tetraethyl orthosilicate (TEOS) and dimethylsulfoxide (DMSO). There was no need to purify the reagents more.

2.2. Synthesis of magnetic nanoparticles (Fe_3O_4)

Magnetic nanoparticles Fe_3O_4 were synthesized by a solvothermal reaction [28]. A mixture consisting of 0.81 g (3 mmol) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 1.8 g (30 mmol) urea, and 0.12 g (1 mmol) succinic acid was completely dissolved in 30 ml of propylene glycol by ultrasonication. Then, the obtained clear yellow solution was transferred to a Teflon-lined stainless steel autoclave, sealed and heated at 200°C for 8 h. After the completion of reaction, autoclave was cooled down to room temperature. The formed black magnetic nanoparticles were transferred into a beaker and washed 5 times at room temperature. For each wash, 50 ml of ethanol was added and the solution was ultrasonicated for 10 min. Then the magnetic nanoparticles were immobilized for 5 min by putting the beaker onto a magnet. The supernatant-cleaning solution was removed. The clean product was kept in ethanol.

2.3. Synthesis of silica-coated magnetic nanospheres ($\text{Fe}_3\text{O}_4 @ \text{SiO}_2$)

The above produced Fe_3O_4 nanoparticles were suspended in 1 M HCl aqueous solution (50 ml) and ultrasonicated for 10 min. Fe_3O_4 nanoparticles were washed three times with deionized water and the supernatant was decanted. The magnetically collected Fe_3O_4 nanoparticles were then dispersed in the 200 ml mixture of ethanol and deionized water at the ratio of (4:1, v/v), along with an addition of 5 ml of concentrated ammonia. The suspension was treated with ultrasonication for 30 min, followed by the dropwise addition of 100 μl of TEOS diluted in 20 ml of ethanol. That mixture was mechanically stirred for 4 h. The pH adjusted to 7 using 1 M HCl to terminate the reaction and the product was collected with the help of magnet, washed with ethanol and water several times, finally kept in ethanol.

2.4. Fabrication of ionic liquid modified magnetic nanospheres ($\text{Fe}_3\text{O}_4 @ \text{SiO}_2 @ \text{HMDI-EMIMLpro}$)

The above obtained magnetic nanospheres ($\text{Fe}_3\text{O}_4 @ \text{SiO}_2$) 0.2 g and the EMIMLpro ionic liquid 0.4 g were dispersed in 60 ml of the DMSO by sonication. Then the mixture was stirred at 80°C for 4 h. After stirring, 1 ml of HMDI was added in batches, and the mixture was allowed to react for 48 h. The resultant magnetic nanospheres were separated from the solvent by magnet, washed 5 times with methanol and then the product $\text{Fe}_3\text{O}_4 @ \text{SiO}_2 @ \text{HMDI-EMIMLpro}$ was dried under vacuum at 70°C for 24 h.

2.5. Direct separation of chiral amino acids via $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanoparticles

Racemate samples ($2 \text{ mg}\cdot\text{mL}^{-1}$) were prepared by dissolving the five chiral amino acids into 25 mL deionized water, respectively. Then, 150 mg chiral magnetic nanoparticles $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ was added into one of the above five racemic solutions, respectively. After vibration at room temperature for 5 min by vortex, the supernatant was collected with the help of a magnet within 60 s and analysed using an automatic digital polarimeter, along with the other chiral amino acids samples.

2.6. Separation of D- & L-tryptophan via ionic liquid modified magnetic nanospheres using centrifugal chiral chromatography

The apparatus (P.C. Inc. Potomac, MD, USA) used in the present study is a type-J coil planet centrifuge equipped with a separation column and a counterweight symmetrically mounted on the rotary frame at a distance of 10 cm from the central axis of the centrifuge. In this planetary centrifuge, the separation column revolves around the central axis of the centrifuge while it synchronously rotates around its own axis at the same direction. This particular type of planetary motion permits the flow tubes to rotate without twisting, hence the effluent eluted through the rotating column without use of the conventional rotary-seal device which often produces complications such as liquid leakage and contamination. The separation column was made up of a spiral tube purchased from CC Biotech, Rockville, MD, USA. It has 4 spiral interwoven grooves, each 2.8 mm wide and 5 cm deep with 4 transfer radial grooves. The corner of the each spiral tube was rounded to prevent kinking. The separation coil was made in our laboratory at NIH as follows: PTFE tubing of 1.6 mm ID (SW14) (Zeus Industrial Products, Orangeburg, SC, USA), flat-twisted and accommodated tightly into the spiral tube support by squashing it with a tool which fits to the radial grooves. There are ten numbers of spiral layers and the total capacity is about 80 ml.

The chiral separation was performed as follows: First the spiral column was entirely filled with 70 ml of ethanol containing 610 mg ionic liquid magnetic nanospheres, followed by injecting 0.5 ml of sample solution containing 0.75 mg D- & L-tryptophan from the external tail terminal of the spiral column. Then the column was eluted with ethanol-water using a linear gradient elution mode from 100% to 5% ethanol for 180 min and held 5% ethanol for 60 min at a flow rate ($1.0 \text{ mL}\cdot\text{min}^{-1}$), while the apparatus was rotated at 800 rpm. Similarly, the success experiment was achieved using the two-phase solvent system composed of n-butyl alcohol/water (1:1, v/v): First the spiral column was entirely filled with 70 ml of upper phase containing 600 mg ionic liquid magnetic nanospheres, followed by injecting 0.5 ml of sample solution containing 0.75 mg D& L-tryptophan from the external tail terminal of the spiral column, the lower phase as mobile phase. The effluent from the outlet of the column was continuously monitored with a UV spectrophotometer (LKB Uvicord IIs, LKB Instruments, Stockholm, Sweden) at 280 nm and chromatogram was recorded using a strip-chart recorder (Millipore, Bedford, Boston, MA, USA). The fractions were collected in test tubes at 4 min/tube with a fraction collector (LKB Instrument).

A standard addition method was used for the identification of each isomer.

2.7. Characterization

Transmission electron microscope (TEM, Philip Tecnai 20, Netherland) was used to test the size and morphology of nanospheres. Using Cu K α radiation ($\lambda = 1.54056 \text{ \AA}$), X-ray diffraction (XRD) carried out on Bruker D8 Advance. (Germany). Fourier transform infrared (FT-IR) spectra were obtained using the KBr method on Nicolet 8700 (Thermal Fisher, USA). Ultraviolet spectrum was recorded on U-3010 (Hitachi, Japan). The magnetic properties of nanospheres were measured by vibrating sample magnetometer (VSM, Lake Shore 7410, USA). Thermogravimetric analysis (TGA, Mettler Toledo 1100SF, Sweden) was utilized for powder samples with an increasing rate of $20^{\circ}\text{C} \cdot \text{min}^{-1}$ using a thermogravimetric analyzer under nitrogen up to 800°C . Elemental analysis (Elementar, Vario EL cube, Germany) was performed to measure the relative C, H, N composition of ionic liquid modified magnetic nanospheres. The chiral recognition abilities of the functional MNPs were estimated on a WZZ-2S automatic digital polarimeter (Pudong Optical Instruments, Shanghai, China) with a 10 cm sample tube under a sodium lamp ($\lambda = 589 \text{ nm}$).

3. Results and Discussion

The fabrication of ionic liquids modified magnetic nanospheres consists of three steps as illustrated below in Fig. 1. Firstly, magnetite nanoparticles Fe_3O_4 were synthesized via solvothermal reduction method as reported previously [28]. In this reaction, 1, 2- propylene glycol acted as the solvent and reducing agent, ferric salt used as the iron source, succinic acid was the catalyst and urea provided the hydroxyl ion. Subsequently, magnetite nanoparticles were coated with silica, which was generated from the hydrolysis and condensation of TEOS through a sol-gel method to form $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanospheres. Consequently, the spacer HMDI was employed to connect chiral ionic liquids EMIMLpro onto the surface of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanospheres to form chiral magnetic nanospheres $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$.

The morphology and structure of the synthesized magnetic nanospheres were studied using TEM as shown in Fig. 2a. From the TEM observations, it was found that these magnetite clusters had a mean diameter of 80 nm consisting of nanoparticles with an average crystallite size of about 13 nm, which was calculated by the Scherrer equation from the x-ray diffraction results (Supporting Information, Fig. S1 and Table S1). As seen from Fig. 2b, the TEM image revealed that core/shell-structured $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanospheres were prepared successfully.

The spacer reagent HMDI was utilized to connect ionic liquids EMIMLpro onto the surface of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanospheres, as reported here for the first time. In order to verify the combination of EMIMLpro with $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanospheres, FT-IR spectra of $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanospheres were analyzed in Fig. 3. Comparing FT-IR spectra of Fe_3O_4 (Supporting Information, Fig. S2) with FT-IR spectrum of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanospheres, the Si-O-Si vibration was observed at 1091cm^{-1} in FT-IR spectrum (Fig. 3) of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanospheres, indicating that silica was formed on the surface of magnetite. Characteristic absorptions of EMIMLpro moiety were observed at 3060, 1621 and 1576cm^{-1} in $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$, corresponding to unsaturated C-H of the

imidazolium ring, carboxyl group and bending vibration of N-H group, respectively. In addition, peaks at 592 cm^{-1} and 1091 cm^{-1} of the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ moiety appeared, implying the successful combination of EMIMLpro with $\text{Fe}_3\text{O}_4@\text{SiO}_2$. Ultraviolet spectra of the $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ microspheres (Supporting Information, Fig. S3) further confirmed the connection between the EMIMLpro moiety and $\text{Fe}_3\text{O}_4@\text{SiO}_2$ microspheres due to the apparent blue shift of the wavelength of EMIMLpro.

Moreover, thermogravimetric analysis was performed to show the relative content of the organic shell in $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanospheres. As shown in Fig. 4, $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ displayed a distinct mass-loss of 36.55% during the first stage between $200\text{--}350^\circ\text{C}$ due to the combustion of EMIMLpro. Mass-loss of 13.67% was further observed during the second stage between $350\sim 550^\circ\text{C}$ arising from the loss of HMDI, giving rise to a high total organic mass release of about 50% between 200°C and 550°C . The organic compound was decomposed completely above 600°C . Elemental analysis reflected the composition of $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$. The data were as follows: C 34.54%, H 5.67 %, and N 11.02 %. The magnetic properties of the magnetic nanospheres were investigated on a vibrating sample magnetometer. It was found that the magnetic saturation values of Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ were $72.364\text{ emu}\cdot\text{g}^{-1}$, $32.969\text{ emu}\cdot\text{g}^{-1}$ and $12.013\text{ emu}\cdot\text{g}^{-1}$ respectively from the characterization results (shown in Fig. 5). An obvious decrease of magnetic saturation value was observed in $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ due to the high organic content. Because magnetic $\text{Fe}_3\text{O}_4@\text{SiO}_2$ core was covered with the thick nonmagnetic organic HMDI-EMIMLpro layer and thus led to a decrease in magnetization per gram. Though the magnetization value of $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ decreased to $12.013\text{ emu}\cdot\text{g}^{-1}$, strong magnetic response to a magnetic field was observed along with the complete separation from solution within 30 s, which is of great importance in the separation process.

Direct separation of five different chiral amino acids via $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanospheres was conducted to preliminarily investigate the chiral recognition ability of $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanospheres, and the separation results were analysed using automatic digital polarimeter (shown in Table 1). Before treatment with $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanospheres, all five chiral amino acids showed no optical activity because the amounts of optical (+)-enantiomers and (–)-enantiomers were equal in their solutions. After a 5 min period of interaction with $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanospheres by vortex, the supernatants of all five chiral amino acids showed a certain optical rotation. The cysteine, arginine and glutamine showed negative optical rotation. The rest, leucine and tryptophan, showed the positive optical rotation. The result suggested that the interaction between $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanospheres and the (+)-enantiomers was stronger than that with the (–)-enantiomers cysteine, arginine and glutamine, whereas the opposite results were obtained for leucine and tryptophan. This indicates that $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanospheres possess a strong discriminating ability for chiral amino acids. The reason is that the chiral ionic liquid EMIMLpro onto the surface of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanospheres play an important role in chiral separation. When $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanospheres were utilized in direct enantioseparation, it might interact with racemates through hydrogen bonding, $\pi\text{--}\pi$ interaction and/or hydrophobicity interaction. However, the offline separation mode using

magnetic nanomaterials was unable to achieve the ideal chiral separation. Therefore, a novel online separation technology combined with centrifugal chiral chromatography was established in our work.

The online chiral separation of tryptophan racemates was performed using a type-J planetary centrifuge by eluting the mobile phase from the outer terminal towards the inner terminal of the spiral column in a head to tail elution mode. Under this condition, magnetic nanospheres were well retained in the column due to their high density and relatively large diameter compared with nanoparticles, while the fluctuating centrifugal force by planetary motion of the column vibrates the nanospheres to enhance the solute partitioning. When ethanol was used as eluent, the racemates were almost retained in the column, while when water used as eluent, a single peak of the racemates was produced at the void volume without resolution. We also did experiment using isocratic elution with 80% ethanol and 20% ethanol, respectively. There are no peaks using 80% ethanol, and D- tryptophan and L-tryptophan just has been resolved with low resolution using 20% ethanol. Therefore, a linear gradient between ethanol and water was applied to separate enantiomers according to their different chiral affinity to the magnetic nanospheres. This is the first application of the spiral tube assembly for chiral separation using magnetic chiral amino acid ion liquid nanospheres. These nanospheres are also easy to disperse in the upper phase of butanol and water solvent system for separation of other low hydrophilic chiral compounds. These nanospheres are very efficient for chiral separation of racemates due to their huge surface area to accommodate chiral selectors. However, they are too small to be used for the column chromatography. Although the type-J planetary centrifuge can produce enough centrifugal force to retain the heavy iron microspheres in the column, the method is not applicable to small particles with low density, such as carbon nanotubes.

Fig. 6a shows the chromatographic separation of D- & L-tryptophan using the ethanol-water elution with a linear gradient elution mode from 100% to 5% ethanol for 180 min and held 5% ethanol for 60 min at a flow rate ($1.0 \text{ ml} \cdot \text{min}^{-1}$). Fig. 6b shows the chromatographic separation using n-butyl alcohol/water (1:1, v/v) solvent system with the lower phase as mobile phase, where the retention of stationary phase for this butanol/water solvent system was 63%. A standard addition method was used for the identification of each isomer. In this method, after separation of racemates of D- & L-tryptophan using gradient elution from 100% ethanol to 5% ethanol, D-tryptophan and L-tryptophan have the same peak area, then each isomer was added respectively, the corresponding peak area would be increased. D-tryptophan was added into the D- & L-tryptophan racemates, the peak area of first peak (D-tryptophan) was larger than before. Similarly, L-tryptophan was added into the D- & L-tryptophan racemates, the peak area of L-tryptophan was larger than that before. For n-butyl alcohol/water (1:1, v/v) solvent system, the detection method was the same as above. In Fig. 6a, using 180 min gradient from 100% ethanol to 5% ethanol, the D-tryptophan has been eluted out with the concentration of 28.75% ethanol, while L-tryptophan has been eluted out with the concentration of 5% ethanol. Under other gradient elution mode like 100 min gradient elution from 80% ethanol to 20% ethanol and 20% ethanol hold 60 min, the separation time has decreased, but with low separation resolution (See Fig. S4). D-tryptophan with a little affinity to the chiral selector eluted shortly after the void volume while L-tryptophan, which has higher affinity to the chiral selector, was substantially

delayed in elution, resulting in complete separation of D- & L-tryptophan (peak resolution is 1.5). As the increase of the amount of MNPs, the retention time of L-tryptophan increased, while D-tryptophan changed little. When the spiral column was entirely filled with 70 ml of ethanol containing 1200 mg ionic liquid magnetic nanospheres, followed by injecting 0.5 ml of sample solution containing 0.75 mg D- & L-tryptophan from the external tail terminal of the spiral column under other conditions unchanged, the retention time of D-tryptophan was still 135 min, while the retention time of L-tryptophan was 190 min.

For the n-butyl alcohol/water (1:1, v/v) solvent system using same detection method, the elution order of D- & L-tryptophan was reversed in Fig. 6b. The result shows that L-tryptophan was eluted ahead of D-tryptophan, the reason of which might be that different water content in mobile phase of solvent system has an effect on the elution order. L-tryptophan is slight higher water solubility than D-tryptophan. In this separation, the mobile phase always was water containing a little amount of butanol, while in the previous separation, the mobile phase changed gradiently from 100% ethanol to 95% water. In another sample D- & L- phenylalanine test, it showed the same results that the elution order of D- & L- phenylalanine was changed and shorter retention time in the second separation was obtained when ethanol-water and butanol-water two solvent systems were used respectively (Fig. S5). The recognition mechanism of $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ was mainly ascribed to the functional chiral ligand EMIMLpro which structure was presented in Fig. 7. This ligand provides π - π interaction through the conjugated imidazolium ring as well as ion-pairing interaction, hydrogen bonding site via the carbonyl O atom, hydrophobicity interaction by the alkyl side chain of proline [29, 30]. Moreover, hydrogen bonding in addition to electrostatic force between the cation and anion stabilized its ordered structure [29].

4. Conclusions

In summary, novel chiral amino acid ionic liquid modified magnetic nanospheres were successfully fabricated for the first time by grafting EMIMLpro onto the surface of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanospheres. The modified magnetic nanospheres have high bonded content of the EMIMLpro. A new method of chiral separation via the functional nanospheres was successfully established. Complete resolution of task-specific separation of tryptophan racemate via the $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanospheres was eventually achieved by centrifugal chiral chromatography using a spiral tube assembly mounted on a type-J coil planet centrifuge. The functional nanospheres newly synthesized in this study have high selectivity to separate chiral compounds. They are promising materials for chiral separation of racemates, because they can provide a huge surface area to accommodate chiral selectors and are easy to be recycled through an external magnetic field. Our research provides an understanding of the function of chiral amino acid ionic liquid modified magnetic nanospheres. Further research on separation of other racemates will continue.

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Highlights

1. $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$ nanospheres were fabricated for the first time.
2. The materials were used as chiral selector in centrifugal chiral chromatography.
3. Task-specific separation of D- & L-tryptophan was achieved completely.
4. They may also be used for the chiral separation of various other racemates.

Fig. 1.

Illustration of fabrication of $\text{Fe}_3\text{O}_4@ \text{SiO}_2@ \text{HMDI-EMIMLpro}$. TEOS is tetraethyl orthosilicate; DMSO is dimethylsulfoxide; HMDI is 1, 6-diisocyanatohexane; EMIMLpro is 1-ethyl-3-methyl-imidazolium L-proline.

Fig. 2.
Transmission electron microscope images of (a) Fe₃O₄; (b) Fe₃O₄@SiO₂.

Fig. 3. Fourier transform infrared spectra of Fe₃O₄@SiO₂ and Fe₃O₄@SiO₂@HMDI-EMIMLpro. HMDI is 1, 6-diisocyanatohexane; EMIMLpro is 1-ethyl-3-methyl-imidazolium L-proline.

Fig. 4. Thermogravimetric analysis curves of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$. HMDI is 1, 6-diisocyanatohexane; EMIMLpro is 1-ethyl-3-methyl-imidazolium L-proline. HMDI is 1, 6-diisocyanatohexane; EMIMLpro is 1-ethyl-3-methyl-imidazolium L-proline.

Fig. 5.
Hysteresis loops of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{HMDI-EMIMLpro}$.

Fig. 6. Chromatographic separation of D- and L-tryptophan, (a) ethanol-water elution (b) n-butyl alcohol/water (1:1, v/v) solvent system, lower phase is mobile phase.

Fig. 7.
Structure of chiral ionic liquid EMIMLpro. EMIMLpro is 1-ethyl-3-methyl-imidazolium L-proline.

Table 1

Separation results of racemates recorded on an automatic digital polarimeter

Samples	The specific rotation $[\alpha]_D^{25}$, 2 mg·mL ⁻¹ in H ₂ O	
	Racemic samples before treatment with magnetic beads	Supernatants collected after interaction with magnetic beads
D- & L-cysteine	-0.000	-5.30
D- & L-arginine	-0.000	-19.50
D- & L-leucine	-0.000	+8.15
D- & L-glutamine	-0.000	-11.35
D- & L-tryptophan	-0.000	+22.80