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Constructing chiral caves and efficiently separating enantiomers of glutamic acid with novel surface-imprinting technique

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ABSTRACT

Dimethylaminoethyl methacrylate (DMAEMA) was first graft-polymerized onto the surfaces of micron-sized silica gel particles in the manner of "grafting from" in a solution polymerization system, obtaining the grafted particles PDMAEMA/SiO_2. Then, the molecular imprinting towards the grafted PDMAEMA was conducted with one enantiomer of glutamic acid (Glu), L-Glu, as template molecule and with 2,2′-dichlorodiethylether (DCEE) as crosslinking agent by adopting the novel surface-molecular imprinting technique established by our research group, and the single enantiomer (L-Glu) molecule-imprinted material MIP-PDMAEMA/SiO_2 was obtained. With another enantiomer of glutamic acid, D-Glu, as the contrast compound, the recognition property of MIP-PDMAEMA/SiO_2 for L-Glu was investigated in depth with both static and dynamic methods, and its ability to separate L-Glu and D-Glu in the racemic solution was examined. The experiment results show that the surface-imprinted material MIP-PDMAEMA/SiO_2 has fine recognition selectivity and binding affinity for L-Glu, whereas its ability to combine D-Glu is poor. The selectivity coefficient of MIP-PDMAEMA/SiO_2 for L-Glu with respect to D-Glu is equal to 3.30, displaying an excellent chiral separation result. It is obvious that in this study, the substance separation at the molecular configuration level has been realized successfully.

1. Introduction

Amino acids are the main structural components of proteins and enzymes. Chiral separation of amino acid enantiomers is of great importance in diverse fields, such as life science, pharmaceuticals, agrochemicals, foods, feeds, perfumes, and so forth [1–4] since amino acid enantiomers show different physiological activities depending on their absolute configurations.

Currently, the separation of racemic mixtures of amino acids is performed by several conventional methods [5–7,1,8] such as preferential crystallization, enzymatic kinetic resolution, chemical resolution, membrane and chromatography including gas chromatography (GC), high performance liquid chromatography (HPLC), capillary electrophoresis (CE), chiral ligand-exchange chromatography (CLEC) as well as other chromatographic methods [9–12]. Even though various chromatographic techniques have the advantages of high separation efficiency and good selectivity (especially CLEC [13,14]), in many cases, they are limited to the application in analytical chemistry area and are difficult to achieve large-scale separation. While for the above several non-chromatographic methods, there are some obvious and different drawbacks. Some of them are confined to a narrow application scope or contain too many steps leading to poor efficiency, and others are expensive and the process is difficult to be amplified. Although a great effort has been made in the separation of amino acid enantiomers, there is still a very great difficulty in the amino acid enantiomer separation with low cost, high efficiency and large-scale production [15,16]. So it is a primary challenge for researchers to develop new techniques of amino acid enantiomer separation, which not only have the characteristic of high efficiency and low cost, but also can be scaled up. If there are some suitable solid adsorbents that can recognize and combine one enantiomer of some amino acid, the chiral separation of amino acids can be realized efficiently and in large scale via solid-phase extraction (SPE). However, up to now, such solid adsorbents are difficult to be sought [17].

Molecularly imprinted polymers (MIPs) are a kind of artificially synthesized functional macromolecule materials, and within them a great deal of specific cavities designed for a target molecule (namely, the template molecule) is distributed and these cavities are complementary to the target molecule in shape, size and functional groups. Therefore, MIPs have specific molecular recognition ability and high binding affinity [18–21] for the target molecule, and are described as artificial antibodies or receptors. In recent years, MIPs as highly selective solid adsorbents have been widely used in various fields, especially in the separation and purification area, and the molecular imprinting solid-phase extraction (MISPE) technique is developed greatly [22,23]. In the separation field of amino acid

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enantiomers, MIPs have also attracted much attention and there are many relevant reports in the literature [24–27]. However, in this regard, there are two obvious limitations: (1) the method to prepare MIPs is the conventional method, entrapment way, and performance of the prepared MIPs are poor; (2) in most cases, MIPs are only used as chromatographic stationary phase in analysis, and their properties are far from the property of solid extractors. So up to now, by using MIPs, the solid extraction on large-scale for the separation of amino acid enantiomer still cannot be realized.

The goal of this work is to prepare MIPs with high performance and to use them in the recognition and separation of amino acid enantiomers via MISPE method so as to finally realize highly efficient scale separation of amino acid enantiomers.

The conventional method to prepare MIPs, entrapment way, has some disadvantages, such as time-consuming because of the tedious post-treatment including grinding and screening, less recognition sites inside matrices particles obtained via crushing and grinding the imprinted polymeric monolith, and greater diffuse barrier for the template molecules coming from thick matrices, leading to the poor recognition property, lower binding capacity and slower binding kinetic of MIPs towards the template molecules. In order to overcome these drawbacks, many researchers developed surface-molecular imprinting techniques [28–31], and tried to make the imprinted caves to lie on the surfaces of solid particles. The surface imprinted materials are more effective for recognizing and combining the template molecules.

In the previous study, we put forward a novel approach of surface imprinting on the surface of silica gel particles and polymeric microspheres based on graft polymerization, and its essential is as follows. (1) Functional polymers are pre-grafted (in the manner of "grafting from" or "grafting to") on the surface of micron-sized silica gel particles or polymeric microspheres, and a thin layer (or a film) of the grafted polymer is formed on the surfaces of the solid particles; (2) The adsorption of the grafted particles towards template molecules reaches saturation by right of intermolecular interaction between the grafted functional macromolecules and template molecules; (3) Post-imprinting of template molecules is conduced towards the grafted polymers using special crosslinking agent which has two reactive end groups. After the removal of the template molecules, a mass of the imprinted caves capable of recognizing and re-binding the template molecules is left and distributed within this thin polymer layer, resulting in the imprinted material with high performance such as more accessible sites, fast mass transfer, high binding affinity and specific recognition selectivity for template molecules. The prepared imprinted materials with such high performance were used for the recognition and separation of pesticide molecules, organism metabolites, alkaloid molecules and heavy metal ions, and the investigation results were all highly satisfactory [32–35]. In our current investigation, we try to construct chiral caves on the surfaces of micron-sized silica gel particles by using the novel surface-imprinting technique so as to realize the separation of amino acid enantiomers, namely to achieve the substance separation at the molecular configuration level.

Poly (dimethylaminoethyl methacrylate) (PDMAEMA) was first grafted on the surfaces of silica gel particles in the "grafting from" manner, obtaining the grafted particles PDMAEMA/SiO_2, and then by using the new surface imprinting technique described above with L-Glu as template and with 2,2′-dichlorodiethylether (DCEE) as crosslinker, the surface imprinting was performed, resulting in the L-Glu molecule-imprinted material, MIP-PDMAEMA/SiO_2. The recognition property of the imprinted material MIP-PDMAEMA/SiO_2 towards L-Glu and its ability to separate two enantiomers, L-Glu and D-Glu in raceme, were investigated in depth. The results of the binding and separating experiments indicate that by right of the strong interactions between the grafted PDMAEMA macromolecules and L-Glutamic acid molecules, electrostatic interaction and hydrogen bonding, the surface-molecular imprinting of L-Glu on PDMAEMA/SiO_2 particles was conducted successfully, and the imprinted material MIP-PDMAEMA/SiO_2 has fine recognition selectivity and binding affinity towards L-Glu, whereas its ability to combine D-Glu is poor. The selectivity coefficient of PDMAEMA/SiO_2 for L-Glu in the raceme solution with respect to D-Glu reaches 3.30, displaying a fine chiral separation property. The preliminary investigation result shows that it can be expected with confidence that by adopting the novel surface imprinting technique, the highly efficient separation of amino acid enantiomers as well as that of chiral drug enantiomers can be realized by using our novel surface-molecular imprinting technique.

2. Experiments

2.1. Materials and instruments

Silica (120–160 mesh, about 125 μm of diameter, 300–450 m^2/g of surface area, Ocean Chemical Limited Company, Qingdao City, China) was of reagent grade. γ-Methacryloylpropyl trimethoxysilane (MPS, Nanking Chuangshi Chemical Aux Ltd., Province Jiangsu, China) was of analytical grade. *N*,*N*-Dimethylaminoethyl methacrylate (DMAEMA, Feixiang Chemical Limited Company, Province Jiangsu, China) was of analytical grade, and was purified by vacuum distillation before use. Ammonium persulphate (APS, Fushu Chemical Engineering Inc., Shanghai) was of analytical grade. L-Glutamic acid (L-Glu) and D-Glutamic acid (D-Glu) (Huaibei Xinqi Aminoacid Limited Company, Province Jiangsu, China) was of analytical grade. 2,2′-Dichlorodiethylether (DCEE, Leping Yongxin Chemical Industry Limited Company) was of reagent grade. Other reagents were all commercial chemicals with analytical pure and purchased from Chinese companies.

The instruments used in this study were as follows: Unic-2602 UV/vis spectrophotometer (Unic Company, Shanghai), PerkinElmer 1700 infrared spectrometer (PerkinElmer Company, USA), Zetasizer Nano-Zeta potential analyzer (Malvern Instrument Company, UK), Autopol-III polarimeter (Rudolph Company, USA), PHS-2 acidimeter (The Second Analytical Instrument Factory, Shanghai, China), THZ-92C constant temperature shaker equipped with gas bath (Boxun Medical Treatment Equipment Factory, Shanghai, China) and TG16-WS high-speed centrifuge with desk type (Changsha Xiangyi Centrifuge Factory, Province Jiangsu, China).

2.2. Preparing functional grafted particles PDMAEMA/SiO_2

The functional grafted particles PDMAEMA/SiO_2 were prepared according to the described procedure in our previous study [36], and the main experimental steps were as follows. Silica gel particles were first surface-modified with coupling agent MPS, and the polymerizable double bonds were introduced on the surfaces of silica gel particles, forming the modified particles MPS-SiO_2. The modified particles MPS-SiO_2 (1 g) were added into a four-necked flask equipped with a mechanical agitator, a reflux condenser, a thermometer and a N_2 inlet, followed by adding 100 mL of distilled water and 5 g of monomer DMAEMA (the concentration of the monomer was 5 wt%). The content was stirred and the modified particles MPS-SiO_2 were dispersed fully. N_2 was bubbled for 30 min to remove air. The content in the flask was heated to 75 °C, and 0.06 g of initiator APS was added. The graft polymerization was performed under N_2 atmosphere at 75 °C for 10 h. The resultant particles were extracted with ethanol in a Soxhlet extractor for 24 h to remove the polymer physically attaching to the particles and dried under vacuum, and finally the grafted particles PDMAEMA/SiO_2 were obtained.

The following characterisation of PDMAEMA/SiO_2 particles were conducted [36]. The FTIR spectrum of PDMAEMA/SiO_2 particles was determined with KBr pellet method. The grafting degree (GD, g/100 g) of PDMAEMA on the grafted particles PDMAEMA/SiO_2 was determined with acid–base titration (the grafted particles PDMAEMA/SiO_2 used in this work have a grafting degree of 22.00 g/100 g). The zeta potential of the grafted particles was determined according to the following procedure. The particles PDMAEMA/SiO_2 were first ground; then, the finely divided particles were allowed to be dispersed into distilled water; finally, the zeta potential of these particles in the dispersed system was measured with a potential analyzer under different pH conditions, resulting in the zeta potential curve.

2.3. Examining adsorption property of PDMAEMA/SiO_2 particles for glutamic acid

2.3.1. Isothermic adsorption experiments of PDMAEMA/SiO_2 particles for glutamic acid in aqueous medium

Aqueous solutions of L-Glu and D-Glu in a concentration range of 0.03–0.30 g/L were prepared, respectively. Based on the adsorption dynamics experiment (adsorption equilibrium time was about 2.5 h), the isothermic adsorption experiments of the functional particles PDMAEMA/SiO_2 for glutamic acid enantiomers were carried out, respectively. The isothermic adsorption experiments were carried out in a constant temperature shaker, and the glutamic acid concentrations in the supernatants were determined by spectrophotometry (diacetone–formaldehyde coloration method [37]). The equilibrium adsorption amounts, Q_e (mg/g), were calculated according to Eq. (1) (see Section 2.5.1) and the adsorption isotherms were plotted.

2.3.2. Isothermic adsorption experiments of PDMAEMA/SiO_2 particles for Glu in aqueous media with different pH values

The pH values of L-Glu aqueous solutions were adjusted with diluted HCl or NaOH solution, and the isothermic adsorption experiments of PDMAEMA/SiO_2 particles for Glu were carried out under different pH conditions to examine the effect of pH value on the adsorption ability of PDMAEMA/SiO_2 particles for Glu and to investigate the interactions between the grafted particles and Glu molecule.

2.4. Preparation and characteristic of L-Glu-imprinted particles MIP-PDMAEMA/SiO_2

PDMAEMA/SiO_2 particles (0.12 g) were added into 200 mL of an L-Glu aqueous solution with a concentration of 0.24 g/L, and the pH value of the solution was adjusted to pH 4 with diluted NaOH solution. The mixture was shaken on a constant temperature shaker for 2.5 h until PDMAEMA/SiO_2 particles were fully swelled and the adsorption of PDMAEMA/SiO_2 particles for L-Glu reached equilibrium. After filtrating, the PDMAEMA/SiO_2 particles were dried under vacuum. The above PDMAEMA/SiO_2 particles (2.44 g), which had adsorbed L-Glu in a saturated state, were placed in 50 mL of water–ethanol mixture (V:V = 7:3) in which L-Glu with a concentration of 0.24 g/L was contained (to prevent the adsorbed L-Glu to be desorbed). The pH value of the solution was adjusted to pH 4, and 0.1 mL of crosslinker DCEE was added. The crosslinking reaction was performed at 35 °C for 24 h. The resultant particles were filtered off and washed repeatedly with diluted aqueous solution of NaOH to remove the template, L-Glu molecules. Finally, the particles were washed with distilled water and dried under vacuum, obtaining the single enantiomer (L-Glu) molecule-imprinted material MIP-PDMAEMA/SiO_2. The infrared spectrum of MIP-PDMAEMA/SiO_2 was determined to confirm its structure changes.

2.5. Examining recognition properties of MIP-PDMAEMA/SIO_2 for two enantiomers of Glu

2.5.1. Evaluating binding property of MIP-PDMAEMA/SIO_2 for two enantiomers

The binding behavior of MIP-PDMAEMA/SIO_2 for the two enantiomers of Glu was examined with batch method (static method) and column method (dynamic method), respectively.

2.5.1.1. Batch method. Similarly, based on the determination of the binding dynamics behavior of MIP-PDMAEMA/SIO_2 for Glu (the binding equilibrium time was also about 2.5 h), the isothermic binding experiments of MIP-PDMAEMA/SiO_2 particles for glutamic acid enantiomers were carried out, respectively. Numbers of 50 mL of L-Glu solutions with different concentrations (0.03–0.30 g/L) were taken and transferred into several conical flasks with plug. MIP-PDMAEMA/SiO_2 particles with the same mass (0.03 g) were added into these solutions, respectively. These mixtures were shaken on a constant temperature shaker at 30 °C and centrifuged after reaching binding equilibrium, and the equilibrium concentrations of L-Glu in the supernatants were determined by spectrophotometry, respectively. The equilibrium binding amounts of MIP-PDMAEMA/SiO_2 towards L-Glu were calculated still according to Eq. (1), and the binding isotherm was figured.

$$Q_e = \frac{V(C_0 - C_e)}{m} \quad (1)$$

where Q_e (mg/g) was the equilibrium binding amount of L-Glu; C_0 (g/L) was the concentration of L-Glu in the initial solution; C_e (g/L) was the concentration of L-Glu in the supernatant; V (mL) was the volume of the L-Glu solution; m (g) was the mass of MIP-PDMAEMA/SiO_2 particles. According to the same method, the equilibrium binding amounts of MIP-PDMAEMA/SiO_2 particles for another enantiomer of Glu, D-Glu, were determined and the corresponding binding isotherm was plotted.

2.5.1.2. Column method. A given amount (1.1 g) of MIP-PDMAEMA/SiO_2 particles was packed into a piece of glass pipe with an internal diameter of 10 mm, and the bed volume (BV) of the packed column was 2 mL. The L-Glu solution with a concentration of 0.48 g/L was allowed to gradually flow through the packed column at a rate of four bed volumes per hour (4 BV/h) in a countercurrent manner. The effluents with two bed volume (2 BV) interval were collected, and the L-Glu concentrations in these effluents were determined by spectrophotometry. The dynamic binding curve was plotted, and the leaking adsorption amount and saturated adsorption amount of L-Glu were calculated with the data of the concentration and bed number of the effluents, respectively. By using the same way, the dynamic binding experiment of MIP-PDMAEMA/SiO_2 particles for D-Glu was carried out, and the dynamic binding curve was also determined and plotted.

2.5.2. Competitive binding experiment (or resolution experiment) for two enantiomers

In order to further examine the recognition character of MIP-PDMAEMA/SiO_2 particles towards L-Glu, a competitive adsorption experiment of MIP-PDMAEMA/SiO_2 for L-Glu and D-Glu was conducted, and the procedures are explained as follows. A racemate solution of Glu with a concentration of 0.2 g/L, in which the concentrations of both L-Glu and D-Glu were 0.1 g/L, was prepared, and here the optical rotation of the solution was zero. 50 milliliters of the racemate solution were taken and placed into a conical flask with plug, and 0.03 g of MIP-PDMAEMA/SiO_2 particles was added. The mixture was shaken on a constant temperature shaker for 2.5 h, and the adsorption was allowed to reach equilibrium. After centrifugal separation, the total concentration of Glu in the supernatant

was determined by spectrophotometry, and at the same time, the optical rotation and specific rotation of the supernatant were determined by using a polarimeter. It was found that at that time, the supernatant produced optical activity (the specific rotation $[\alpha]$ was −5.54°) and the rotatory direction was identical to that of D-Glu. The result reveals that MIP-PDMAEMA/SiO_2 particles have more adsorption for L-Glu than D-Glu, and namely, MIP-PDMAEMA/SiO_2 particles produce a resolution effect for the racemate solution of Glu. The equilibrium concentrations of L-Glu than D-Glu in the supernatant were calculated according to Eq. (2).

$$\left.\begin{aligned} C_{D,e} &= \left(\frac{[\alpha]}{[\alpha]_{D,S}} + \frac{1-([\alpha]/[\alpha]_{D,S})}{2}\right) \times 100 \times C_{T,e} \\ C_{L,e} &= C_{T,e} - C_{D,e} \end{aligned}\right\} \quad (2)$$

where $C_{D,e}$ (g/L) and $C_{L,e}$ (g/L) are the equilibrium concentrations of the two enantiomers, D-Glu and L-Glu, in the supernatant; $[\alpha]$ is the specific rotation of the supernatant; $[\alpha]_{D,S}$ is the standard specific rotation of D-Glu ($[\alpha]_{D,S} = -32.2°$); $C_{T,e}$ is the total equilibrium concentration of Glu, which is the sum total of the equilibrium concentrations of D-Glu and L-Glu.Subsequently, the distribution coefficient for each enantiomer was calculated according to Eq. (3). This equation was originated from Ref. [38].

$$K_d = \frac{Q_e}{C_e} \quad (3)$$

where K_d (mL/g) represents the distribution coefficient (mL/g) of one enantiomer; Q_e (mg/g) is the equilibrium binding amount of the corresponding enantiomer; C_e (mg/mL) is the equilibrium concentration of this enantiomer in the supernatant.

The selectivity coefficient of MIP-PDMAEMA/SiO_2 for L-Glu with respect to another enantiomer, D-Glu, can be obtained from the distribution coefficient data of the two enantiomers according to Eq. (4)

$$k = \frac{K_d(\text{L-Glu})}{K_d(\text{D-Glu})} \quad (4)$$

where k is the selectivity coefficient of L-Glu, and the value of k allows an sufficient estimation of the recognition selectivity of MIP-PDMAEMA/SiO_2 for L-Glu [38] and an adequate evaluation of its resolution ability for two enantiomers.

2.5.3. Desorption experiment

A certain amount (1.1 g) of MIP-PDMAEMA/SiO_2 particles adsorbing L-Glu in a saturation state was packed into a piece of glass pipe with an internal diameter of 10 mm, and the bed volume (BV) of the packed column was 2 mL. An aqueous solution of NaOH with a concentration of 0.1 M was used as eluent, and was allowed to gradually flow through the column at a rate of four bed volumes per hour (4 BV/h) in a countercurrent manner. The effluents with one volume (1 BV) interval were collected, and the concentrations of L-Glu in the effluents were determined by spectrophotometry. The dynamic desorption curve was plotted, and elution property of MIP-PDMAEMA/SiO_2 was evaluated.

3. Results and discussions

3.1. Chemical structure and zeta potential of grafted particles PDMAEMA/SiO_2

PDMAEMA/SiO_2 particles are a kind of functional grafted particles. There is a great quantity of grafted macromolecules PDMAEMA on the surfaces of micro-sized silica gel particles. PDMAEMA is one kind of polymethacrylates, but tertiary amine groups are contained in its chain units. The N atoms of these amino groups will be protonated in acidic solution as well as in neutral solution. Therefore, PDMAEMA can be regarded as a cationic polyelectrolyte to some extent.

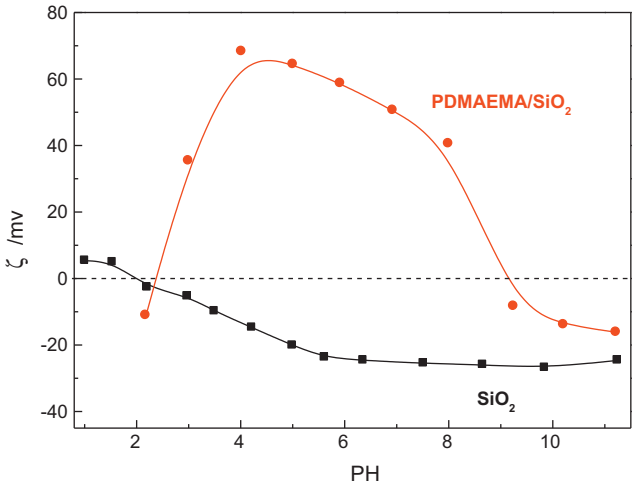

Fig. 1. Variation of zeta potential of PDMAEMA/SiO_2 and SiO_2 particles with pH value.

Fig. 1 gives the zeta potential curves of PDMAEMA/SiO_2 and SiO_2 particles. From Fig. 1, the following facts can be seen. (1) In a greater range of pH, the zeta potential of SiO_2 particles is negative, namely, in general, silica gel particles have negative charges. (2) After the grafting of PDMAEMA, in a greater range of pH, the zeta potential of the grafted particles PDMAEMA/SiO_2 is positive, and further its absolute value is greater. This implies that there are positive charges with high density on the surfaces of PDMAEMA/SiO_2 particles, and it arises from the protonation of N atoms of the tertiary amine groups of the grafted PDMAEMA macromolecules. (3) At pH = 4, the zeta potential of the grafted particles PDMAEMA/SiO_2 has a maximum, and then the zeta potential declines with the increase of pH. The possible reason for this is that the protonation degree of the N atoms of the tertiary amine groups of the grafted PDMAEMA gradually is weakened as pH is over 4.

3.2. Interaction between functional particles PDMAEMA/SiO_2 and Glu molecules

3.2.1. Adsorption of PDMAEMA/SiO_2 towards Glu molecules in aqueous medium

Fig. 2 presents the absorption isotherms of SiO_2 and PDMAEMA/SiO_2 particles for the two enantiomers of Glu at pH 4, respectively.

It is displayed obviously that silica gel particles do not adsorb Glu basically. However, after PDMAEMA macromolecules are grafted onto the surfaces of silica gel particles, the grafted particles PDMAEMA/SiO_2 produce very strong adsorption towards Glu (the saturated adsorption amount reaches 68 mg/g), indicating that there are strong interactions between PDMAEMA/SiO_2 particles and Glu molecules. The strong interactions come from electrostatic interaction and hydrogen bonding. Glu is an acidic amino acid, and its isoelectric point is equal to 3.22. Apparently, at pH = 4, Glu molecule has negative charge, whereas there are positive charges with a high density on the surfaces of PDMAEMA/SiO_2 particles. It is inevitable that strong electrostatic interaction will be produced between PDMAEMA/SiO_2 particles and Glu molecules. At the same time, hydrogen bonding will be produced with each other, and in this interaction system, the amino groups in Glu molecule are as doner groups of hydrogen bonding and the unprotonated tertiary amine groups of PDMAEMA/SiO_2 are as acceptor groups of hydrogen bonding at pH = 4. The synergism of the two action

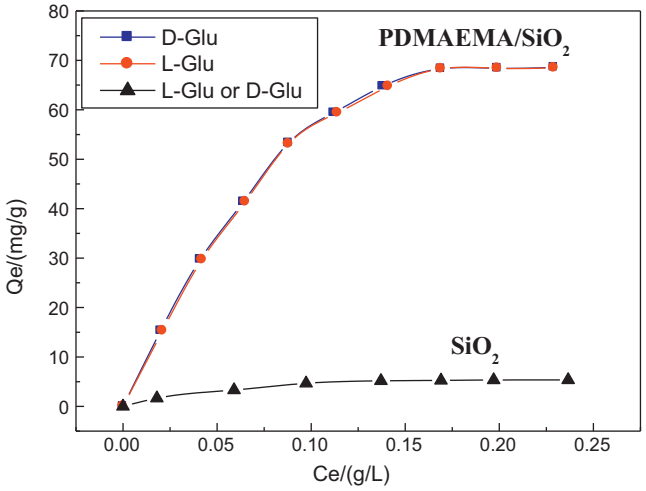

Fig. 2. Adsorption isotherm of PDMAEMA/SiO_2 for glutamic acid. Temperature: 30 °C; pH = 4.

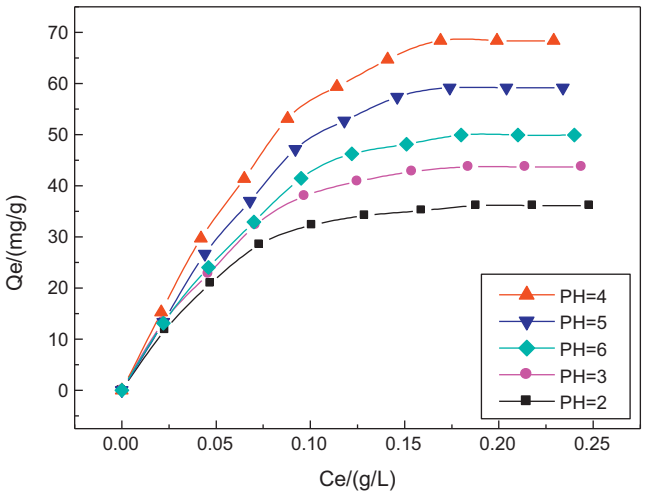

Fig. 4. Adsorption isotherm of PDMAEMA/SiO_2 for Glu molecule at different pH values. Temperature: 30 °C.

forces (the electrostatic interaction is the main driving force) results in the strong adsorption of PDMAEMA/SiO_2 particles towards Glu molecules. The adsorption mechanism of PDMAEMA/SiO_2 particles towards Glu molecules can be schematically expressed in Fig. 3.

3.2.2. *Effect of medium pH on adsorption property of PDMAEMA/SiO_2 for Glu*

The isothermal adsorption experiments of PDMAEMA/SiO_2 for Glu were carried out in Glu solutions with different pH values as described in Section 2.3.2, and Fig. 4 gives the adsorption isotherms under different pH conditions. In order to more clearly display the effect of medium pH on the adsorption property of PDMAEMA/SiO_2 particles for Glu, the saturated adsorption amounts are taken from Fig. 4, and they are expressed as a function of pH value of the solutions as shown in Fig. 5.

The following facts can be found in Figs. 4 and 5. At lower pH, the adsorption capacity of PDMAEMA/SiO_2 towards Glu is very low and then increases with the increase of pH value; at pH = 4, the adsorption capacity reaches a maximum; subsequently, the adsorption capacity turns to decline with the increase of pH value. The reason for this can be explained as follows: (1) As pH value is lower, the carboxyl group in Glu molecule nearly does not dissociate although the N atoms of amino groups of the grafted PDMAEMA macromolecules are highly protonated. Therefore, here the electrostatic interaction between PDMAEMA/SiO_2 particles and Glu molecules is very weak, leading to very low adsorption capacity, and at that time, the adsorption of Glu on PDMAEMA/SiO_2 particles is mainly caused by hydrogen bonding. It needs to be pointed out that here the status of the donor and acceptor of hydrogen bonding may be different from the case at pH 4 (in Fig. 6). (2) The dissociation degree of the carboxyl group in Glu molecule increases with elevating pH value, and this will result in the strengthening of the electrostatic interaction between PDMAEMA/SiO_2 particles and Glu molecules. Further, at that time, the hydrogen bonding still exists (see Fig. 6). The cooperation of the two effects leads to rapidly increasing of the adsorption capacity. (3) As pH value is over 4, the protonation degree of the N atoms of the tertiary amine groups of PDMAEMA is weakened gradually and the zeta potential of PDMAEMA/SiO_2 particles declines step by step, and this will lead to the weakening of the electrostatic interaction between PDMAEMA/SiO_2 particles and Glu molecules, resulting in that the adsorption capacity turns to decrease.

In a word, there is strong interaction between PDMAEMA/SiO_2 particles and Glu molecules. Moreover, at pH = 4, the interaction is the strongest. That lays a solid foundation for imprinting the single enantiomer (L-Glu) of Glu on the surfaces of PDMAEMA/SiO_2 particles.

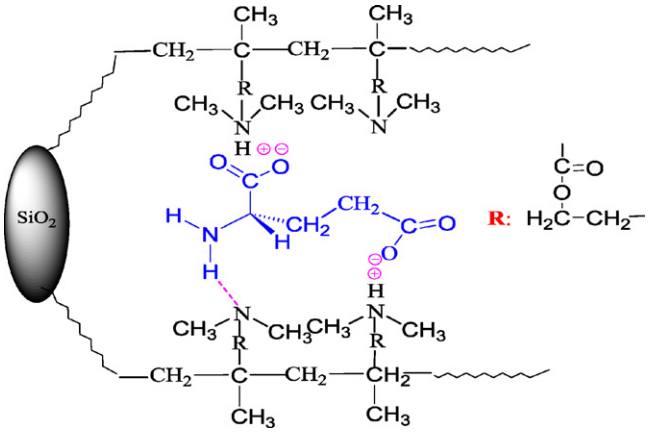

Fig. 3. Schematic expression of adsorption action of particles PDMAEMA/SiO_2 towards Glu molecule.

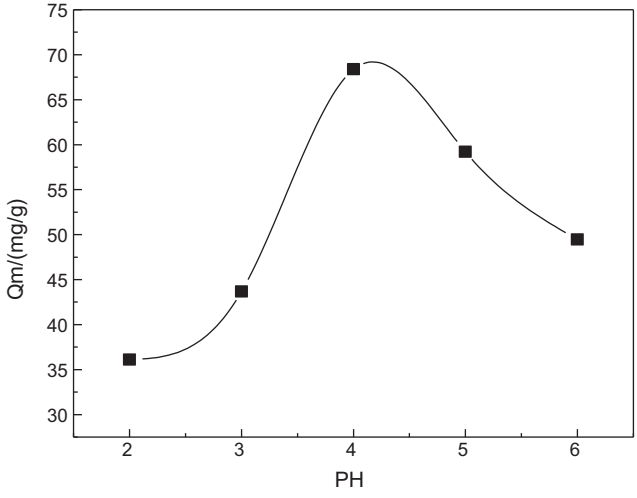

Fig. 5. Relationship curve between saturated adsorption amount and pH.

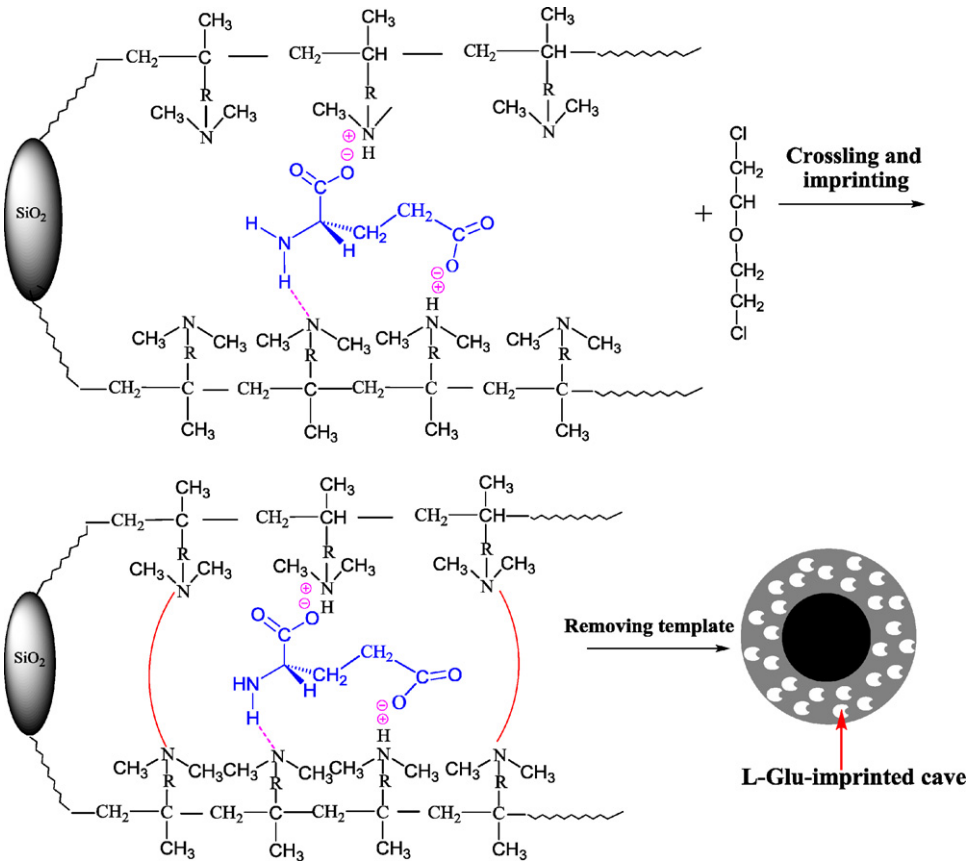

Fig. 6. Schematic illustration of preparation process of MIP-PDMAEMA/SiO_2.

3.3. Preparation processes of L-Glu-imprinted material MIP-PDMAEMA/SiO_2

As above mentioned, there are strong electrostatic interaction and hydrogen bonding between PDMAEMA/SiO_2 particles and Glu molecules. By right of the two secondary bond forces, the grafted particles PDMAEMA/SiO_2 can produce strong adsorption towards Glu molecules. As the adsorption of the single enantiomer (L-Glu) of Glu on PDMAEMA/SiO_2 particles reached saturation, the crosslinking agent DPEE was added, and thereupon the quaternarization between the tertiary amine groups of the grafted PDMAEMA and chloroalkane end groups of DPEE will be carried out, leading to the crosslinking of PDMAEMA macromolecules. As a result, L-Glu molecules were enveloped in the crosslinking networks, and the imprinting of L-Glu was realized. As the template molecules were washed away, large numbers of L-Glu molecule-imprinted caves, chiral caves, remained within the thin polymer layer on the surfaces of the particles. Thereupon, the L-Glu-imprinted material MIP-PDMAEMA/SiO_2 was obtained. The entire preparation processes of MIP-PDMAEMA/SiO_2 are schematically expressed in Fig. 6.

Fig. 7 gives the spectra of the grafted particles PDMAEMA/SiO_2 and the imprinted particles MIP-PDMAEMA/SiO_2. As compared the spectrum of MIP-PDMAEMA/$SiO_2$with that of PDMAEMA/SiO_2, it can be found that in the spectrum of MIP-PDMAEMA/SiO_2, the absorption band of methylene group $-CH_2-$ at 2850 cm^{-1} had been strengthened greatly, and it is caused by a great deal of methylene groups in the crosslinking bridges formed by dichloroether (whereas the absorption band of ether C–O–C at 1100 cm^{-1} was covered by the strong absorption background of SiO_2). This fact suggested that the crosslinking and imprinting process has been successfully carried out. It needs to be pointed out that that all various absorption bands of the two particles look very weak because of the affect of the strong absorption background of SiO_2.

3.4. Molecular recognition character and binding property of MIP-PDMAEMA/SiO_2 for two enantiomers

3.4.1. Binding isotherms and dynamics binding curves

The adsorption experiments in the batch method were first performed. Fig. 2 has given the adsorption isotherms of the grafted particles PDMAEMA/SiO_2 (non imprinted material) for the two enantiomers, L-Glu and D-Glu, while at present, Fig. 8 displays the

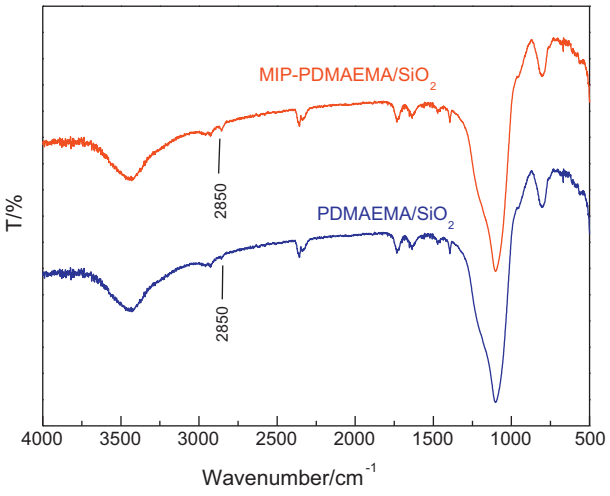

Fig. 7. FTIR spectra of two kinds of particles.

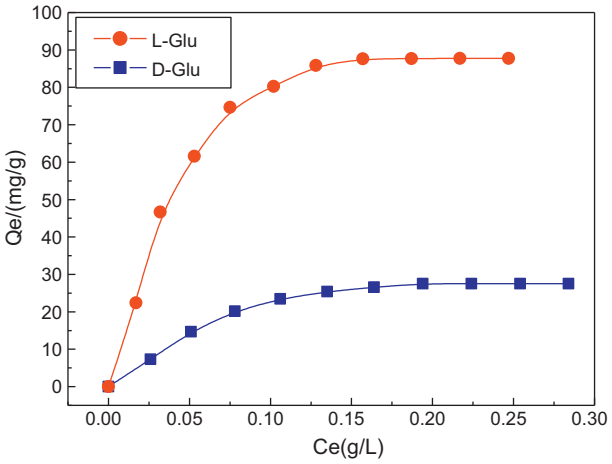

Fig. 8. Binding isotherms of MIP-PDMAEMA/SiO_2 for L-Glu and D-Glu. Temperature: 30 °C.

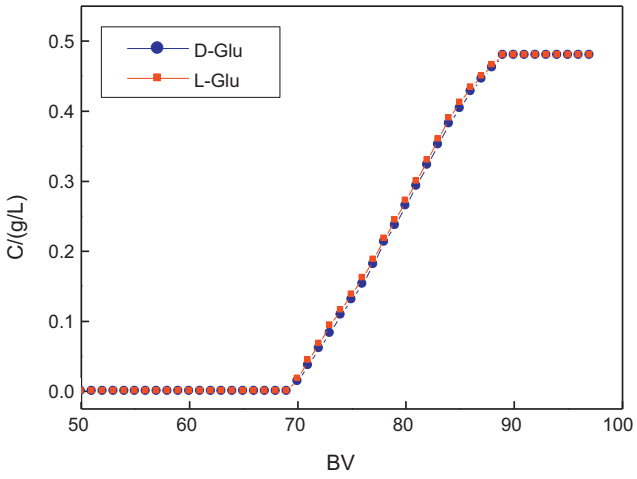

Fig. 9. Dynamic adsorption curves of PDMAEMA/SiO_2 for L-Glu and D-Glu. BV: 2 mL; temperature: 30 °C; initial concentration: 0.48 g/L; flow rate: 4 BV/h.

binding isotherms of MIP-PDMAEMA/SiO_2 (imprinted material) for the two enantiomers, respectively.

It can be seen from Fig. 2 that the adsorption abilities of grafted particles PDMAEMA/SiO_2 for two enantiomers are perfectly identical, and it is apparent that the PDMAEMA/SiO_2 particles have no any selectivity for two enantiomers. It is well known that all enantiomers of a chiral substance have identical physicochemical property except for different optical rotation properties. Therefore, the grafted particles PDMAEMA/SiO_2 have the same adsorption properties for L-Glu and D-Glu.

However, different experimental facts are shown in Fig. 8. After imprinting L-Glu on the surfaces of the grafted particles PDMAEMA/SiO_2 and forming L-Glu-imprinted material MIP-PDMAEMA/SiO_2, a remarkable difference of the binding capacity of MIP-PDMAEMA/SiO_2 for the two enantiomers is displayed, as shown in Fig. 8. The maximum binding amount of L-Glu is 87.73 mg/g, whereas for D-Glu, the corresponding binding amount is only 27.50 mg/g. This fact clearly demonstrates that the L-Glu-imprinted material MIP-PDMAEMA/SiO_2 has obvious recognition selectivity and fine binding affinity for the template enantiomer L-Glu. The reason for this is explained as follows. A great deal of L-Glu-imprinted caves is distributed within the thin polymer layer on the surfaces of MIP-PDMAEMA/SiO_2. These chiral caves are highly matched with L-Glu molecules in space structure and spatial arrangement of action sites, but are not matched with D-Glu molecules. Therefore, these chiral caves have special recognition ability for L-Glu, and can produce strong binding action, leading to higher binding capacity. These chiral caves are not matched with D-Glu molecule in molecular configuration, so have poorer binding ability for D-Glu, resulting in lower binding capacity.

In order to further investigate the binding character of MIP-PDMAEMA/SiO_2 for two enantiomers, the adsorption experiments in the column method were also performed. Figs. 9 and 10 display the dynamic adsorption curves of PDMAEMA/SiO_2 and the dynamic binding curves of MIP-PDMAEMA/SiO_2 for two enantiomers, respectively.

It can be observed from Fig. 9 that as the solutions of L-Glu and D-Glu with the same concentration (0.48 mg/mL) flows upstream through the column packed with PDMAEMA/SiO_2 particles, respectively, the breakthrough curves of the two solutions are perfectly identical, and the leaking volumes for both are 69 BV. By calculation, the leaking and saturated adsorption amounts are 60.22 mg/g and 68.24 mg/g, respectively. However, it can be seen from Fig. 10 that for the column packed with MIP-PDMAEMA/SiO_2, the breakthrough curve of L-Glu solution is distinctly different from that of D-Glu solution, and the following facts can be observed in Fig. 10. (1) D-Glu solution begins to leak at 76BV, whereas the leaking volumes of L-Glu solution is 88BV, having a difference of 12 BV. (2) D-Glu solution completely breaks through at 98BV, whereas the breakthrough volume of L-Glu solution is 111 BV. (3) By calculation, the leaking and saturated adsorption amounts of D-Glu are 66.32 mg/g and 76.20 mg/g, respectively, whereas for L-Glu, they are 76.80 mg/g and 88.04 mg/g, respectively. The above dynamics binding data once again show that MIP-PDMAEMA/SiO_2 particles possess fine recognition selectivity and binding affinity for L-Glu with respect to another enantiomer, D-Glu. In regard to the recognition selectivity of MIP-PDMAEMA/SiO_2 particles, a further discussion will be presented below.

3.4.2. *Recognition selectivity of MIP-PDMAEMA/SiO_2 for template enantiomer L-Glu*

A raceme solution with a concentration of 0.2 g/L, in which the concentrations of both L-Glu and D-Glu were 0.1 g/L, was prepared, and competitive adsorption experiments on MIP-PDMAEMA/SiO_2 particles were conduced. In Table 1, the data of the distribution coefficients K_d and selectivity coefficients k are summarized.

From the data in Table 1, it can be found that the selectivity coefficient of MIP-PDMAEMA/SiO_2 for the template enantiomer L-Glu with respect to D-Glu is equal to 3.30, demonstrating that MIP-

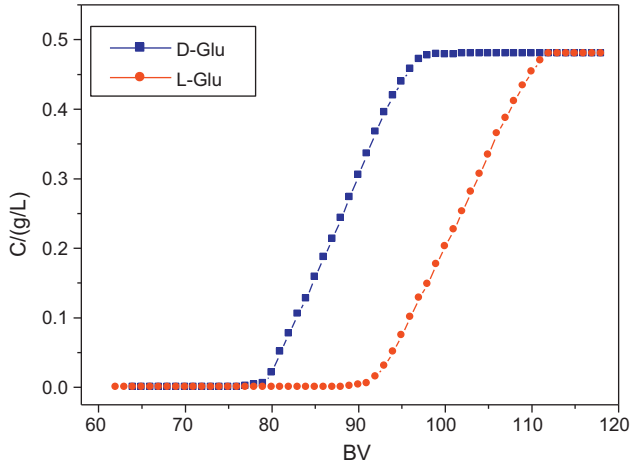

Fig. 10. Dynamic binding curves of MIP-PDMAEMA /SiO_2 for L-Glu and D-Glu. BV: 2 mL; temperature: 30 °C; initial concentration: 0.48 g/L; flow rate: 4 BV/h.

Table 1
Distribution coefficient and selectivity coefficient data.

Adsorb material	MIP-PDMAEMA/SiO_2	
	L-Glu	D-Glu
K_d/(mL/g)	1011.57	306.38
k	3.30	

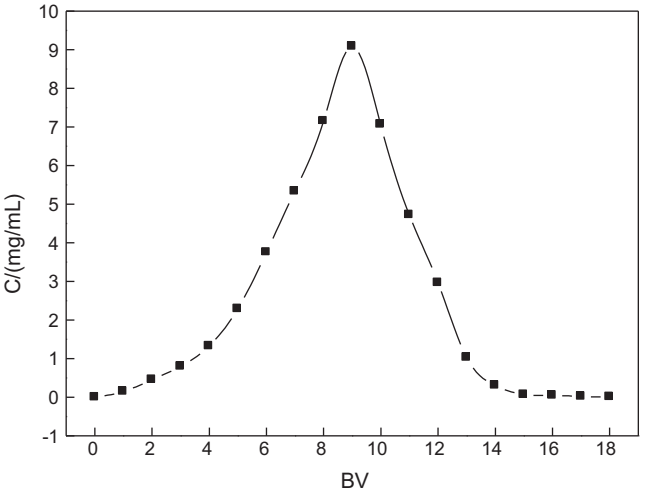

Fig. 11. Elution curve of L-Glu on MIP-PDMAEMA/SiO_2 column. Temperature: 30 °C.

PDMAEMA/SiO_2 particles have obvious recognition selectivity for L-Glu. The result of the competitive adsorption experiment fully reveals that the L-Glu-imprinted material, MIP-PDMAEMA/SiO_2, has good resolution ability for the two enantiomers of Glu, namely, by using MIP-PDMAEMA/SiO_2 particles, the substance separation at molecular configuration level has been realized. Consequently, it is shown that the novel surface-molecular imprinting technique can be used in the preparation of solid extraction materials for realizing effective resolution of enantiomers of amino acids. It is expected that via further molecular design, the surface-molecularly imprinted materials with higher performance (higher selectivity coefficient) can be prepared. At the same time, through well-designing separation technology, for example concatenation separation, a highly effective separation of enantiomers of amino acids and even that of enantiomers of chiral drug can be realized.

3.5. Elution property of MIP-PDMAEMA/SiO_2

An aqueous NaOH solution of 0.1 M was used as the eluent. The eluent upstream passes through the column packed with MIP-PDMAEMA/SiO_2 adsorbing L-Glu in a saturated state. The dynamic desorption curve is given in Fig. 11. It can be seen in Fig. 11 that the desorption curve is cuspidal and without trailing formation. By calculating, the desorption ratios in 14 BV and in 16 BV reach 98.10% and 99.23%, respectively. These desorption data indicate that the L-Glu molecules combined in the chiral caves are easy to be desorbed or eluted because these caves are distributed within the thin polymer layer on the surfaces of the particles. The dynamic desorption curve displays that the MIP-PDMAEMA/SiO_2 particles have excellent eluting property, and it is beneficial to the recovery and reuse of the surface-imprinted material.

4. Conclusions

PDMAEMA macromolecules are first grafted on micro-sized silica gel particles by adopting "grafting from" method, and the grafted particles PDMAEMA/SiO_2, which have strong adsorption action towards Glu, were prepared. The surface-molecular imprinting of single enantiomer (L-Glu) of Glu was conducted successfully on the surfaces of PDMAEMA/SiO_2 particles by using the novel surface-molecular imprinting technique and with 2,2′-dichlorodiethylether as crosslinker, obtaining L-Glu-surface-imprinted material MIP-PDMAEMA/SiO_2. Because a great deal of the chiral caves of L-Glu is distributed within the thin polymeric layer on the surfaces of the particles, MIP-PDMAEMA/SiO_2 particles possess obvious recognition selectivity and good binding affinity for the template molecule, L-Glu. These chiral caves are not matched with another enantiomer of Glu, D-Glu, in space structure and spatial arrangement of action sites, and so MIP-PDMAEMA/SiO_2 particles have poor binding ability for D-Glu. MIP-PDMAEMA/SiO_2 particles display a good resolution property for the raceme solution of Glu, namely, the substance separation at the molecular configuration level has been successfully achieved. The research results in this work have supplied a valuable reference for preparing solid extraction adsorbent with high performance for the chiral separation of enantiomers of amino acids as well as for the chiral separation of enantiomers of chiral drugs.

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