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3 **Preparation of Surface Imprinted Material of Single Enantiomer**
4 **of Mandelic Acid with a New Surface Imprinting Technique and Study on**
5 **its Chiral Recognition and Resolution Properties**
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14 Baojiao Gao * , Lulu Chen, Yanbin Li
15 Department of Chemical Engineering,
16 North University of China,
17 Taiyuan 030051, People' s Republic of China
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29 * To whom correspondence should be addressed

30 Tel: 86-0351-3921414

31 Fax: 86-0351-3922118

32 E-mail: gaobaojiao@126.com

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Abstract A surface imprinted material of the single enantiomer of mandelic acid with high performance was successfully prepared with a new surface imprinting technique of synchronously graft-polymerizing and molecule imprinting, and its enantiomeric recognition and resolution properties were investigated. Micro-sized silica gel particles were first modified with coupling agent γ -mercaptopropyl trimethoxysilane (MPMS), obtaining the modified particles MPMS-SiO₂ on which mercapto groups were introduced. A surface initiating system of -SH/BPO was constituted with the mercapto group (-SH) on MPMS-SiO₂ particles and dibenzoyl peroxide (BPO) in *N,N*-dimethyl formamide (DMF) solution. In DMF solution, (R)-mandelic acid molecule as the template and the functional monomer hydroxyethyl methylacrylate (HEMA) were combined together by right of multi-site hydrogen bonds. The free radicals produced on MPMS-SiO₂ particles initiate HEMA molecules around (R)-mandelic acid molecules and the crosslinking agent *N,N'*-Methylenebisacrylamide (MBA) to produce graft/crosslinking-polymerization. At the same time, the template (R)-mandelic acid molecules were enveloped within the thin grafted polymer layer on the surfaces of SiO₂ particles, obtaining (R)-mandelic acid surface imprinted material MIP-PHEMA/SiO₂. The experimental results show that MIP-PHEMA/SiO₂ particles have excellent enantiomeric recognition and resolution ability. The binding capacity of MIP-PHEMA/SiO₂ particles for (R)-mandelic acid reaches up to 278 mg/g. As the resolution experiment of a racemic mixture was carried out with MIP-PHEMA/SiO₂ particles as solid adsorbent, relative another enantiomer, (S)-mandelic acid, their selectivity coefficient for (R)-mandelic acid is 5.02. As a consequence, the two enantiomers were well separated, and the optical purities (*ee* values) of the supernatant and eluant get up to 44% (corresponding to (S)-mandelic acid excess) and 85% (corresponding to (R)-mandelic acid excess), respectively.

Keywords: Mandelic acid; Hydroxyethyl methylacrylate; Molecule surface Imprinting; Chiral Recognition, Chiral Resolution

1. Introduction

Many drugs are racemic mixtures with chiral isomers. The enantiomers have the same chemical or physical properties but a different biological natures and pharmacological activities, and it will lead to the significant differences in their biological, pharmacological and toxicological effects [1-3]. Therefore, enantioseparation is important and indispensable in various research fields, such as pharmaceutical, clinical, forensic and food analysis [4-7], and now approximately half of all commercial drugs are chiral [8]. Enantiomers of mandelic acid and their derivatives have been considered as important substances because they are utilized extensively for synthetic purposes as well as in stereochemical investigations [9,10]. For example, (R)-mandelic acid is used as a versatile intermediate for preparation of semisynthetic cephalosporins, penicillins, anti-tumor agents and anti-obesity agents [11], whereas (S)-mandelic acid is an important organic molecule precursor for the introduction of a stereogenic center in stereoselective transformations, and is also a versatile intermediate for pharmaceuticals [12]. The two mandelic acid enantiomers are also good resolving agents and are employed in the resolution of racemic alcohols and amines [13]. For the enantioseparation of mandelic acid, although there are several methods such as diastereomeric crystallization, chromatography and enzymatic resolution [14-16], etc. However, among these methods, some disadvantages such as expensive cost, complicated operation, weak generative capacity and enzyme instability and so on limit their further application to various extents. Therefore, the separation techniques appropriate for large-scale, low costs and highly effective purification of mandelic acid enantiomers from the racemic mixture still needs to be developed greatly. At present, the chiral-separation technique with molecular imprinted polymers (MIPs) as separation medium has got great attention and development [17-19] because the ease of preparation, ability to scale-up, low material costs and pre-determined selectivity of MIPs make them advantageous in comparison with other chiral separation

systems.

Molecular imprinting is a technique for creation of polymeric matrices containing tailor-made receptor sites. The molecularly imprinted polymers show specific molecular recognition selectivity and high affinity towards the template molecule because inside MIPs there is a great deal of target-specific binding cavities, which are complementary to the template molecule in size, shape and functional groups. MIPs have been wide applied in many fields involving the recognition, separation, purification and enrichment of substances as well as the removal of toxic substances [20-24]. Similarly, the application of MIPs in chiral separation of enantiomers has been expanded in a broad range of chiral compounds, such as various pharmaceutical drugs, amino acids and their derivatives and peptides [25-27]. For the separation of substances, molecularly imprinted solid-phase extraction (MISPE) with MIPs as selective solid adsorbent is a strong technique, and in the separation of small-molecule substances, it has been widely applied. In chiral separation of enantiomers, MISPE is also a promising technique, but the researching in the field is still few.

The performance of the MIPs prepared by the conventional method, entrapment way, is poor, such as a low binding capacity associating with crushing and grinding the imprinted polymeric monolith to obtain the particles with suitable size, a slow mass transfer rate and time-consuming process. In order to overcome the above disadvantages, in recent years, the surface molecular imprinting technique, which can establish imprinting sites on the surface of the solid particles or membranes [28-30], has been developed, and the prepared imprinted materials have more accessible sites, faster mass transfer rate and more effective recognition capacity for the template molecules. Our group also devotes our great effort to develop surface molecular imprinting technique, and established a novel surface imprinting method of synchronously graft-polymerizing and molecule imprinting on inorganic particles and polymeric microspheres via the action of surface initiating systems, obtaining various surface imprinted materials with

high performance[31,32]. For successfully conducting this novel surface imprinting process, the key point is to create a good surface initiating system so that the graft/crosslinking-polymerization of the functional monomer and the molecule imprinting of the template can smoothly be carried out on the surfaces of solid particles or microspheres. The novelty of the proposed methodology of molecule surface imprinting is very evident, and the advantages over other imprinted materials used previously lie in: (1) the imprinted caves are more accessible to the template molecules because they are distributed within the thin polymer layer around the solid particle as a core, resulting in the good comprehensive properties of the imprinted materials; (2) as compared the imprinted materials prepared by the conventional method, there are a great deal of the imprinted caves for the surface imprinted materials because of the great specific surface of the solid particles, leading to excellent binding affinity and recognition selectivity; (3) the surface imprinted materials have fast binding rate as fast desorption rate, and it very favorable to their practical application.

The above successful investigation results inspired the authors to apply this novel surface imprinting method in the chiral separation of drugs. In this study, a surface initiating system, -SH/BPO, was constituted on the interface of micron-sized silica gel particles and a solution containing initiator BPO and functional monomer. (R)-mandelic acid imprinted material was prepared with 2-hydroethyl methylacrylate (HEMA) as functional monomer and with micron-sized silica gel particles as the supporting material, resulting in the surface imprinted particles, MIP-PHEMA/SiO₂. Its chiral recognition selectivity and chiral discrimination mechanism were investigated in depth, and the chiral resolution ability was estimated by several methods. For (R)-mandelic acid, the surface imprinted material MIP-PHEMA/SiO₂ displays a selectivity coefficient of 5.02 relative to another enantiomer, (S)-mandelic acid, and it possesses fine enantioseparation property and can be used in MISPE for the resolution of mandelic acid enantiomers from raceme. This study result has supplied important reference data for the chiral resolution of enantiomers of

chiral compounds through MISPE technique with the surface imprinted materials as solid adsorbents, and such surface imprinted materials can be well prepared by using the novel surface imprinting method of synchronously graft-polymerizing and molecule imprinting on solid particles. Therefore, this study is significant not only in the development of surface imprinting but also in the fields of medicine, pharmaceutical science and bio-technology.

2. Experiments

2.1. Materials and instruments

Silica (120~160 mesh, about 125 μm in diameter) was received from Qingdao Ocean Chemical Co., Ltd. (China) was received. γ -Mercaptopropyl trimethoxysilane (MPMS) was supplied by Nanking Chuangshi Chemical Aux Co., Ltd. (China). Hydroxyethyl methacrylate (HEMA) was purchased from Suzhou Nanhang Chemical Co., Ltd. (China), and was purified by vacuum distillation before use. N,N'-Methylenebisacrylamide (MBA) was purchased from Shanghai Yuanye Bio-Technique Co., Ltd.(China); Dibenzoyl peroxide (BPO) was obtained from Shanghai Zhongli Chemical Plant (China), and was purified by recrystallization from ethanol. (R)-Mandelic acid and (S)-mandelic acid were purchased from Shanhai Hongbang Medical Science and Technology Co., Ltd. (China). N,N-dimethylformamide was obtained from Qingdao Shijixin Chemical Reagent Co., Ltd. (China). All of the other chemicals were purchased from Chinese companies.

The instruments used in this study were as follows: Perkin-Elmer1700 infrared spectrometer (Perkin-Elmer Company, USA); LEO-438VP scanning electronic microscope (SEM, LEO Company, UK); STA449 thermogravimetry analyzer (TGA, Netzsch Company, German); Unic-2602 UV spectrophotometer (Unic Company, Shanghai); Autopol-III polarimeter (Rudolph Company, USA); THZ-92C constant temperature shaker equipped with gas bath (Shanghai Boxun Medical Treatment Equipment Factory,

Shanghai, China).

2.2. Estimating interaction between polymer of HEMA and mandelic acid

2.2.1. Preparation and characterization of grafted particles PHEMA/SiO₂

The monomer HEMA was first allowed to be graft-polymerized on micron-sized silica gel particles in a manner of surface-initiated graft-polymerization, forming the grafted particles PHEMA/SiO₂, and so the interaction between the functional monomer HEMA and the template mandelic acid molecule was estimated through examining the adsorption property of the grafted particles PHEMA/SiO₂ for mandelic acid. The preparation procedure of PHEMA/SiO₂ particles is described briefly as follows.

(1) Obtaining modified particles MPMS-SiO₂ By using the method described in Ref. [33], the silica gel particles were first modified by coupling agent γ -mercaptopropyl trimethoxysilane (MPMS) so that mercapto group was chemically introduced onto the surfaces of silica gel particles, obtaining the modified particles MPMS-SiO₂. The main procedures are as follows. The activated silica gel particles were reacted with MPMS in toluene at 110 °C for 12 h, and the product particles were washed and dried under vacuum, getting modified particles MPMS-SiO₂. The mercapto group content on the modified particles was determined by TGA [33], and it was about 1 mmol/g.

(2) Surface-initiated graft-polymerization of HEMA The modified particles MPMS-SiO₂ were added into a four-necked flask equipped with a mechanical agitator, a reflux condenser, a thermometer and a N₂ inlet, followed by adding 70 mL of DMF as solvent and 4.5 mL of monomer HEMA. N₂ was bubbled for 30 min to exclude air. Initiator BPO (0.0483) was added, and the graft-polymerization was carried out under N₂ atmosphere at the constant temperature of 65 °C for 16 h with stirring. The resultant particles were extracted with acetone in a soxhlet extractor for 24h to remove the polymer attaching physically to the particles. By drying to constant weight under vacuum, the grafted particles PHEMA/SiO₂

were obtained.

(3) Characterization of grafted particles PHEMA/SiO₂ The infrared spectra of the grafted particles PHEMA/SiO₂ was determined with KBr pellet method to confirm their structure. The grafting degree of PHEMA on PHEMA/SiO₂ particles was determined with thermal gravimetric analysis (TGA) and the difference between the weight-loss ratio of the grafted particles PHEMA/SiO₂ and that of the modified particles MPMS-SiO₂ was just the grafting degree. The result was indicated that the grafting degree of PHEMA/SiO₂ particles was 21.34g/100 g.

2.2.2. Examining adsorption property of grafted particles PHEMA/SiO₂ for mandelic acid

The DMF solutions of mandelic acid (raceme) with different concentrations (in a range of 0.3~3.0 g/L) were prepared. Before performing the isothermal adsorption experiments, the adsorption kinetics experiment was conducted to determine the time in which the adsorption of mandelic acid on PHEMA/SiO₂ particles reached equilibrium, and it was about 4 h. Mandelic acid solutions (25 mL) with different concentrations were placed into a number of conical flask, and they were shaken for 4 h in a constant temperature oscillator. After the isothermal adsorption experiments, the mandelic acid concentrations in the supernatants were determined by ultraviolet spectrophotography ($\lambda=268$ nm), and the equilibrium adsorption amounts, Q_e (mg/g), were calculated according to Equation (1) (see Section 2.4.1), and the adsorption isotherms were plotted.

2.3. Preparation and characteristic of (R)-mandelic acid surface imprinted particles MIP-PHEMA/SiO₂

2.3.1. Preparation of MIP-PHEMA/SiO₂ particles

DMF(70 mL), HEMA(4.5 mL) and (R)-mandelic acid (1.97 g) were added in a four-necked flask equipped with a mechanical agitator, a reflux condenser, a thermometer and a N₂ inlet, followed by adding

1.2 g of the modified particles MPMS/SiO₂ and 1.50 g of MBA, and N₂ was bubbled for 30 min to exclude air. The temperature of this system was raised to 45 °C, followed by adding 0.0483 g of initiator BPO. The synchronously graft/crosslinking-polymerizing and molecule imprinting were carried out at the constant temperature of 45 °C for 24 h. The product particles were filtered off and washed repeatedly with a mixed solution of methanol and glacial acetic acid in a volume ratio of 8:2 to remove the template molecules, (R)-mandelic acid molecules. Finally, the particles were dried under vacuum, obtaining (R)-mandelic acid -molecule surface imprinted material MIP-PHEMA/SiO₂, on which the content of the thin imprinted polymer layer was determined by TGA and was 27.42 g/100g (see below).

Under the same reaction conditions, the non-imprinted material NMIP-PHEMA/SiO₂ was prepared in the absence of the template (R)-mandelic acid. The content of the thin grafted and crosslinked polymer layer on NMIP-PHEMA/SiO₂ particles was 26.73 g/100g, and obviously it is similar with that of the thin imprinted polymer layer on MIP-PHEMA/SiO₂ particles.

2.3.2. Characterization of MIP-PHEMA/SiO₂ particles

(1) The infrared spectrum of MIP-PHEMA/SiO₂ particles was determined to confirm their structure changes. (2) The amount of the thin imprinted polymer layer on MIP-PHEMA/SiO₂ particles was determined by TGA, and the difference between the weight-loss ratio of the imprinted particles MIP-PHEMA/SiO₂ and that of the modified particles MPMS-SiO₂ was just the amount of the thin imprinted polymer layer. (3) The morphology of MIP-PHEMA/SiO₂ particles was examined with SEM, and the surface imprinting of (R)-mandelic acid was further confirmed by comparing the morphologies of both SiO₂ particles and MIP-PHEMA/SiO₂ particles.

2.4. Examining enantiomeric recognition and separation potency of MIP-PHEMA/SiO₂ particles

2.4.1. Determining binding isotherm

The batch method was first used to examine the enantiomeric recognition property of MIP-PHEMA/SiO₂ particles, and namely the isothermic binding experiments of MIP-PHEMA/SiO₂ particles for two enantiomers, (R)-mandelic acid and (S)-mandelic acid, were first carried out, respectively. (R)-mandelic acid (S)-mandelic acid solutions in a concentration range of 0.3~3.0 g/L were prepared with DMF as solvent, respectively. Numbers of 25 mL of (R)-mandelic acid solutions with different concentrations were taken and transferred into several conical flasks with plug. MIP-PHEMA/SiO₂ particles (about 0.03g) weighted accurately were added into these solutions, respectively. Based on the binding kinetics experiment (the binding equilibrium time was about 4 h), these mixtures were shaken on a constant temperature oscillator for 4 h, and after centrifuging, and the equilibrium concentrations of (R)-mandelic acid in the supernatants were determined by UV spectrophotometry, respectively. The equilibrium binding amounts of MIP-PHEMA/SiO₂ towards (R)-mandelic acid were calculated according to Eq. (1), and the binding isotherm was figured. Similarly, the isothermic binding experiments of MIP-PHEMA/SiO₂ particles towards another enantiomer, (S)-mandelic acid, were also performed, and the binding isotherm was also plotted.

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

where C_0 (g/L or mg/mL) is the initial concentration of one enantiomer of mandelic acid and C_e (g/L or mg/mL) is its equilibrium concentration; V (mL) is the volume of this enantiomer solution; m (g) is the mass of the added MIP-PHEMA/SiO₂ particles; Q_e (mg/g) is the equilibrium binding quantity of MIP-PHEMA/SiO₂ particles for this enantiomer of mandelic acid.

2.4.2. Determining dynamic binding curve

The enantiomeric recognition property of MIP-PHEMA/SiO₂ particles was also examined with column method. MIP-PHEMA/SiO₂ particles (1.45 g) were packed into a piece of glass pipe with an

internal diameter of 10 mm, and here the bed volume (BV) of the packed column was just 2 mL. A (R)-mandelic acid solution with a concentration of 0.5 g/L was allowed to gradually flow through the packed column at a rate of five bed volumes per hour (5 BV/h) in a countercurrent manner. The effluents with three bed volume (3 BV) interval were collected, and the (R)-mandelic acid concentrations in these effluents were determined by UV spectrophotometry. The dynamic binding curve of (R)-mandelic acid on the column packed with MIP-PHEMA/SiO₂ particles was plotted, and the leaking binding amount and saturated binding amount of (R)-mandelic acid were calculated with the data of the concentration and bed number of the effluents, respectively. Similarly, the dynamic binding experiment of (S)-mandelic acid on MIP-PHEMA/SiO₂ column was conducted, and its dynamic binding curve was also figured.

2.4.3. Estimating enantioseparation ability of MIP-PHEMA/SiO₂ particles

(1) Determining chiral selectivity coefficient A racemate solution of mandelic acid with a concentration of 0.5 g/L was prepared with DMF as solvent. This solution (25 mL) was taken and transferred into a conical flask with plug, and 0.03 g of MIP-PHEMA/SiO₂ particles was added. This mixture was shaken on a constant temperature shaker for 4 h. Meantime, the competitive adsorption of two mandelic acid enantiomers on MIP-PHEMA/SiO₂ particles was allowed to be carried, or the chiral resolution of two mandelic acid enantiomers from the racemic mixture was allowed to be performed. Afterwards, the system was separated by filtration. The total concentration of mandelic acid in the supernatant was determined still by UV spectrophotometry. At the same time, the optical rotation and specific rotation of the supernatant were determined. It was found that at that time, the supernatant had produced optical activity (the specific rotation [α] was +63.58) and the rotatory direction was identical to that of (S)-mandelic acid. The result reveals that MIP-PHEMA/SiO₂ particles have more adsorption for (R)-mandelic acid, and namely, MIP-PHEMA/SiO₂ particles produce a resolution effect for the racemic

mandelic acid solution. The equilibrium concentrations of (R)-mandelic acid and (S)-mandelic acid in the supernatant were calculated according to Eq. (2).

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

Where $C_{S,e}$ (g/L) and $C_{R,e}$ (g/L) are the equilibrium concentrations of the two enantiomers, (S)-mandelic acid and (R)-mandelic acid, in the supernatant; $[\alpha]$ is the specific rotation of the supernatant; $[\alpha]_{S,S}$ is the standard specific rotation of (S)-mandelic acid ($[\alpha]_{S,S} = +153^\circ$); $C_{T,e}$ is the total equilibrium concentration of mandelic acid, which is the total sum of the equilibrium concentrations of the two enantiomers, (S)-mandelic acid and (R)-mandelic acid.

Based on the determined equilibrium concentrations of the two enantiomers, the distribution coefficient for each enantiomer was calculated according to Eq. (3) [34,35].

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

where K_d (mL/g) represents the distribution coefficient (mL/g) of one enantiomer; Q_e (mg/g) is the equilibrium binding amount of this enantiomer on MIP-PHEMA/SiO₂ particles; C_e (mg/mL) is its equilibrium concentration in the supernatant.

Relative to another enantiomer, (S)-mandelic acid, the selectivity coefficient of MIP-PHEMA/SiO₂ particles for (R)-mandelic acid can be calculated from the distribution coefficient data of the two enantiomers and according to Eq. (4)

$$k = \frac{K_d(R)}{K_d(S)} \quad (4)$$

where k is the selectivity coefficient of MIP-PHEMA/SiO₂ particles for (R)-mandelic acid, and the value of k marks the chiral recognition ability of the imprinted material MIP-PHEMA/SiO₂ for (R)-mandelic acid.

(2) Estimating enantioseparation ability After the above competitive adsorption experiment (or the chiral solution experiment) of the racemic mandelic acid solution, the optical rotation and specific rotation of the supernatant were determined as described above, and the optical purity or the enantiomeric excess (ee) of the supernatant was calculated according to Eq. (5). The separated MIP-PHEMA/SiO₂ particles, on which one enantiomer of mandelic acid had been adsorbed selectively, was washed with a mixed solution of methanol and glacial acetic acid (V:V=8:2) three times, these eluants were incorporated. The optical rotation and specific rotation of the incorporated eluant were also determined, and the optical purity of the eluant was calculated also according to Eq. (5). The enantioseparation ability of the imprinted particles MIP-PHEMA/SiO₂ was estimated from the specific rotation and optical purity data of the supernatant and eluant.

$$ee = \frac{[\alpha]_{obs}}{[\alpha]_{max}} \times 100 \quad (5)$$

where $[\alpha]_{obs}$ is the specific rotation of the sample solution, and $[\alpha]_{max}$ is the specific rotation of the corresponding single chiral isomer.

3. Results and Discussions

3.1. Chemical structure and of grafted particles PHEMA/SiO₂ and interaction between it and mandelic acid

3.1.1. Chemical structure of grafted particles PHEMA/SiO₂

The silica gel particles were first modified with coupling MPMS to chemically introduce mercapto groups on the surfaces of silica gel particles, resulting in the modified particles MPMS-SiO₂. A redox initiating system -SH/BPO was constituted by the mercapto groups on MPMS-SiO₂ particles and BPO in

DMF solution. The mercapto groups accelerate BPO to decompose to produced benzoyloxy-radicals, and at the same time, the hydrogen protons of the mercapto groups are transferred onto benzoyloxy-radicals, resulting in that a great deal of sulfur free radicals are produced on the surfaces of the modified silica gel particles [36]. These sulfur free radicals initiate monomer HEMA molecules in DMF solution to produce graft-polymerization on the surfaces of silica gel particles so that the grafted particles PHEMA/SiO₂ are obtained. The above process as well as the structure of PHEMA/SiO₂ particles is schematically presented in Figure 1.

Figure 1

3.1.2. Characterization of PHEMA/SiO₂ particles

Figure 2 gives the infrared spectra of SiO₂ particles, the modified particles MPMS-SiO₂ and the grafted particles PHEMA/SiO₂.

Figure 2

In the spectrum of SiO₂, the wide band at 3440 cm⁻¹ is associated with the silanol groups as well as the adsorbed water. In the modified particle MPMS-SiO₂, the band at 3440 cm⁻¹ has been weakened greatly, and at the same time, the stretching vibration absorption of S-H bond of mercapto group has appeared at 2566 cm⁻¹, showing that the coupling agent MPMS has been chemically linked onto the surfaces of SiO₂ particles. In the spectrum of grafted particles PHEMA/SiO₂, the characteristic absorption band of carbonyl group of ester group has appeared at 1735 cm⁻¹, and the asymmetric stretching vibration band of C-H bond

of methyl group appears at 2945 cm^{-1} . Furthermore, the absorption band at wide band at 3440 cm^{-1} is again strengthened because of the introduction of alcohol hydroxyl groups of the grafted polymer PHEMA. The above data demonstrate that the monomer HEMA has been graft-polymerized on the surfaces of silica gel particles and the grafted particles PHEMA/SiO₂ has formed. It needs to be pointed out that all of the above various absorption bands of MPMS-SiO₂ as well as that of PHEMA/SiO₂ look very weak because of the effect of the strong absorption background of SiO₂.

3.1.3. Adsorption property of PHEMA/SiO₂ for mandelic acid

Figure 3 presents the adsorption isotherms of SiO₂ and PHEMA/SiO₂ particles, on which the grafting degree of PHEMA was 21.34g/100 g, for mandelic acid in DMF solution, respectively.

Figure 3

Figure 3 the adsorption action of SiO₂ particles for mandelic acid is very weak. While the grafted particles PHEMA/SiO₂ display very strong adsorption ability for mandelic acid, and the saturated adsorption amount reaches to 216.6 mg/g (such high adsorption capacity is relatively rare). This strong adsorption action comes from the strong hydrogen bonding between host-guest. It can be seen from Scheme 1 that the grafted macromolecule PHEMA contains a great deal of carbonyl group of ester group and alcohol hydroxyl group, whereas in mandelic acid molecule there are one carboxyl group, one alcohol hydroxyl group and one benzene ring (see Scheme 2). It is obvious that multi-site hydrogen bonds including common hydrogen bonds and π -type hydrogen bond can be formed between PHEMA/SiO₂ and mandelic acid molecule as shown in Figure 4, and these hydrogen bonds together drive PHEMA/SiO₂ particles to produce strong adsorption for mandelic acid. Therefore, it can be concluded that there are also

multi-site hydrogen bonds between the monomer HEMA and mandelic acid molecule. The described hydrogen bonding just is the basis of preparing the imprinted material of the enantiomer of mandelic acid with HEMA as functional monomer.

Figure 4

3.2. Physical chemistry process to prepare (R)-mandelic acid surface imprinted material

(1) In DMF solution of the single enantiomer of mandelic acid, (R)-mandelic acid, monomer HEMA molecules will be combined around (R)-mandelic acid molecule to form host-guest complex by right of the multi-site hydrogen bonds, which are located around the asymmetric carbon atom; (2) A surface initiating system -SH/BPO is constituted by the mercapto group on the modified particles MPMS-SiO₂ and BPO in the solution, and a great deal of sulfur radicals will be produced; (3) These radicals will initiate HEMA molecules around (R)-mandelic acid molecule and the crosslinker MBA molecules to produce graft/crosslinking-polymerization, and a thin crosslinked polymer layer will be formed on the surfaces of silica gel particles. (4) Meanwhile, (R)-mandelic acid molecules are enveloped into the crosslinked networks, namely, (R)-mandelic acid imprinting is realized, and after removing these template molecules, a mass of (R)-mandelic acid-imprinted caves are left within the thin polymer layer, forming (R)-mandelic acid surface imprinted material MIP-PHEMA/SiO₂. The physical chemistry process to prepare (R)-mandelic acid surface imprinted material as described above is schematically expressed in Figure 5.

Figure 5

3.3. Characterization of (R)-mandelic acid surface imprinted material MIP-PHEMA/SiO₂

3.3.1. IR spectrum

Figure 6 presents the IR spectrum of the imprinted particles MIP-PHEMA/SiO₂ as well as that of the grafted particles PHEMA/SiO₂.

Figure 6

As compared the IR spectrum of MIP-PHEMA/SiO₂ with that of PHEMA/SiO₂, the similarities for two is that in both spectra there are the same three absorption bands at 1735 cm⁻¹ of carbonyl group of ester group, at 3400 cm⁻¹ of alcohol hydroxyl group and at 2945 cm⁻¹ of C-H bond of methyl group. These bands are attributed to the characteristic absorptions of HEMA unit of the grafted and crosslinked polymer layer. Besides, in the spectrum of MIP-PHEMA/SiO₂, there are two new bands at 1680 and 1545 cm⁻¹ comparison with that of PHEMA/SiO₂. The former is ascribed to stretching vibration absorption of carboxyl group of amide group, namely amide I band of amide group, the latter is assigned to in plane bending vibration absorption of N-H bond of amide group, namely amide II band of amide group. These characteristic absorptions of amide group come from MBA unit of the grafted and crosslinked polymer layer. The above spectrum data fully demonstrate that the graft/cross-linking polymerization of HEMA and MBA has been produced by the initiating of the surface initiating system of -SH/BPO, and the (R)-mandelic acid surface-imprinted layer have formed on the surfaces of silica gel particles, namely (R)-mandelic acid surface-imprinted material MIP-PHEMA/SiO₂ has been prepared.

3.3.2. Morphology

Figure 7 (A) and Figure 7 (B) present the SEM images of raw silica gel particles and the imprinted

particles MIP-PHEMA/SiO₂.

It can be clearly observed that the surfaces of raw silica gel particles are rough and scraggy, whereas the surfaces of the imprinted particles MIP-PHEMA/SiO₂ become smoother and flatter. This is the result of graft/crosslinking polymerization of HEMA and MBA, and the thin grafted and crosslinked polymer layer (or the imprinted polymer layer) plays a role of filling up, and makes the silica gel particles to produce morphology change.

Figure 7

3.3.3. TGA analysis

The amount of the thin imprinted polymer layer on MIP-PHEMA/SiO₂ particles was determined through the weight loss at 800 °C in air using thermogravimetric analysis (TGA). Figure 8 shows the thermal weight loss curves of the modified particles MPMS/SiO₂ and the imprinted particles MIP-PHEMA/SiO₂.

Figure 8

The amount of the thin imprinted polymer layer on MIP-PHEMA/SiO₂ particles was determined through the weight loss at 800 °C in air using thermogravimetric analysis (TGA). Fig. 3 shows the TGA curves of the modified particles MPTS-SiO₂ and the grafted particles PMAA/SiO₂. The weight loss of the modified particles is 10.65%, and the weight loss of the grafted particles is 38.36%. Therefore, the amount of the thin imprinted polymer layer on MIP-PHEMA/SiO₂ particles is approximately 27.42g/100g.

3.4. Enantiomeric recognition and resolution properties of MIP-PHEMA/SiO₂

3.4.1. Binding isotherms of MIP-PHEMA/SiO₂ particles

In order to estimate the enantiomeric recognition properties of (R)-mandelic acid surface-imprinted material MIP-PHEMA/SiO₂, binding experiments were first performed with the batch method. Figure 9 Fig. 6 gives the adsorption isotherms of the non-imprinted material NMIP-PHEMA/SiO₂, on which the content of the thin grafted and crosslinked polymer layer was 26.73 mg/g, for the two single enantiomers, (R)-mandelic acid and (S)-mandelic acid, and whereas Figure 10 displays the binding isotherms of the imprinted material MIP-PHEMA/SiO₂, on which the content of the thin imprinted polymer layer was 27.42 mg/g, the two single enantiomers, respectively.

Figure 9

Figure 10

It can be seen from Figure 9 that for both single enantiomers, (R)-mandelic acid and (S)-mandelic acid, the non-imprinted particles NMIP-PHEMA/SiO₂ have very strong adsorption ability, and the adsorption capacities are very high (271mg/g) and perfectly identical, indicating that NMIP-PHEMA/SiO₂ particles have no any adsorption selectivity for two enantiomers. It is well known that all enantiomers of a chiral compound have identical physicochemical property except for different optical rotation property so that the non-imprinted particles NMIP-PHEMA/SiO₂ for two enantiomers of mandelic acid consequentially produce the same adsorption action, resulting in the same adsorption capacity.

However, Figure 10 shows that the imprinted particles MIP-PHEMA/SiO₂ produce the different

binding behaviors for the two single enantiomers. The adsorption capacity of (S)-mandelic acid decreases greatly from 271mg/g to 91mg/g, whereas the adsorption capacity of (R)-mandelic acid still remains a high value (about 278mg/g), demonstrating that the (R)-mandelic acid-imprinted material MIP-PHEMA/SiO₂ has excellent recognition selectivity and fine binding affinity for the template enantiomer (R)-mandelic acid, whereas for the non-template enantiomer (S)-mandelic acid, its binding ability are poor. As compared the binding capacity and the recognition selectivity of the imprinted particles MIP-PHEMA/SiO₂ for template isomer with that of other mandelic acid enantiomer-imprinted particles, it can be found the property of the imprinted particles prepared in this work are excellent. For example, Monier et al. prepared enantioselective S-mandelic acid imprinted chitosan particles [37], the maximum adsorption capacity capacities of (S)-mandelic acid and (R)-mandelic acid on the imprinted particles were 100 and 64 mg/g, respectively. It is obvious that both the binding capacity and the recognition selectivity of imprinted particles for the template isomer are low. Another study of Monier et al. is about (L)-glutamic acid –imprinted particles, and the maximum adsorption capacities of (L)-and (D)-glutamic acid on THE imprinted particles were also only 42 and 26 mg/g, respectively[38], showing the poor recognition selectivity of the imprinted particles for the template isomer.

The above experiment facts display a good enantiodiscrimination ability of MIP-PHEMA/SiO₂ particles. Within the thin polymer layer on the surfaces of MIP-PHEMA/SiO₂, a great deal of (R)-mandelic acid-imprinted caves is distributed. These chiral caves are highly matched with (R)-mandelic acid molecule in space structure and spatial arrangement of action sites, namely in conformation. Therefore, these chiral caves have special chiral discrimination ability for (R)-mandelic acid, and can produce strong binding action, leading to high binding capacity. These chiral caves are not matched with (S)-mandelic acid molecules in conformation, and it is difficult for (S)-mandelic acid

molecules to enter into these caves, resulting in lower binding capacity. In a word, it has been confirmed by the isothermic binding experiments that the (R)-mandelic acid surface imprinted particles MIP-PHEMA/SiO₂ possess fine enantiodiscrimination ability.

3.4.2. Dynamic binding behavior of MIP-PHEMA/SiO₂ particles

Dynamic binding experiments of MIP-PHEMA/SiO₂ material were also conducted with the column method so as to further demonstrate its enantiodiscrimination ability. Figure 11 and Figure 12 present the dynamic adsorption curves of NMIP-PHEMA/SiO₂ and the dynamic binding curves of MIP-PHEMA/SiO₂, namely the breakthrough curves, for two single enantiomers, respectively.

Figure 11

Figure 12

Figure 11 displays that as the solutions of (R)-mandelic acid and (S)-mandelic acid solutions with the same concentration (2mg/mL) flow upstream through the column packed with NMIP-PHEMA/SiO₂ particles, respectively, the breakthrough curves of the two solutions are completely identical, and the leaking volumes for both are about 93BV. However, Figure 12 shows that the breakthrough curves of the two enantiomeric solutions are obviously different as the two enantiomeric solutions flow through the column packed with MIP-PHEMA/SiO₂, respectively. (S)-mandelic acid solution begins to leak at 27 BV, whereas the leaking volumes of (R)-mandelic acid solution is still greater, about 93 BV. By calculation, the leaking and saturated adsorption amounts of (S)-mandelic acid are only 70.13 mg/g and 85.21 mg/g, respectively, whereas for (R)-mandelic acid, they get up to 225.97 mg/g and 270.74 (it is similar with the

value of binding capacity in the batch experiment), respectively. The above dynamics binding data once again confirm that MIP-PHEMA/SiO₂ particles possess excellent enantiodiscrimination ability for the template enantiomer, (R)-mandelic acid, and it still comes from highly matching of the imprinted chiral caves within the thin polymer layer on MIP-PHEMA/SiO₂ particles with (R)-mandelic acid molecules in conformation.

3.4.3. Enantioselectivity of MIP-PHEMA/SiO₂

Competitive adsorption experiments (or chiral resolution experiments) of raceme solution were carried out with NMIP-PDMAEMA/SiO₂ and MIP-PDMAEMA/SiO₂ particles as solid adsorbents, respectively. The distribution coefficients (K_d) of the two enantiomers and the selectivity coefficients (k) of the two materials for (R)-mandelic acid relative to (S)-mandelic acid are summarized In Table 1.

Table 1

From the data in Table 1, it can be found that the selectivity coefficient of NMIP-PHEMA/SiO₂ for the template enantiomer (R)-mandelic acid relative to (S)-mandelic acid is equal to 1.0, suggesting that the non-imprinted particles NMIP-PDMAEMA/SiO₂ have no any enantiomeric recognition selectivity. While the selectivity coefficient of MIP-PHEMA/SiO₂ for (R)-mandelic acid relative to (S)-mandelic acid is 5.02, displaying a obvious recognition selectivity for (R)-mandelic acid. The above result of the competitive adsorption experiments again reveals that the (R)-mandelic acid surface imprinted material MIP-PHEMA/SiO₂ has a good enantiomeric recognition selectivity. As a result, it will has a fine chiral resolution ability for the racemic mixture, and the further discussion is developed below.

3.4.4. Enantioseparation ability of MIP-PHEMA/SiO₂

After the above competitive adsorption experiments (or enantiomeric resolution experiments) with the two materials, MIP-PHEMA/SiO₂ and NMIP-PDMAEMA/SiO₂, respectively, the optical rotation and specific rotation of the supernatants were determined, and the optical purities of the supernatants were calculated. At the same time, the separated MIP-PHEMA/SiO₂ and NMIP-PDMAEMA/SiO₂ particles were washed with eluant, and the optical rotation and specific rotation of the collected eluants were also determined, and the optical purities of the eluants were also calculated (see Section 2.4.3). The data of the relevant specific rotation and optical purity are listed in Table 2.

Table 2

It can be found from the data in Table 2 that the supernatant and eluant for the adsorption system with NMIP-PDMAEMA/SiO₂ particles as solid adsorbent have no optical activity, indicating that the non-imprinted material has no chiral resolution ability for the racemic mixture. However, both the supernatant and eluant for the adsorption system with MIP-PDMAEMA/SiO₂ particles as solid adsorbent produce optical activity, clearly demonstrating that the imprinted material MIP-PDMAEMA/SiO₂ has stronger enantioseparation ability. The optical activity (positive value) of the supernatant is corresponding to an enantiomeric excess of the (S)-mandelic acid, and the optical purity of the supernatant is 44%. The optical activity of the eluent (negative value) is corresponding to an enantiomeric excess of (R)-mandelic acid, and the optical purity of the eluant is 85%, displaying excellent chiral resolution ability for the racemic mixture. In a word, the above data fully confirm that the (R)-mandelic acid surface imprinted material MIP-PHEMA/SiO₂ has excellent enantioseparation ability. At the same time, these data also fully

demonstrate that the novel surface imprinting technique of synchronously graft-polymerizing and molecule imprinting on solid particles can be successfully applied in the preparation of single enantiomer surface imprinted materials, and it is promising to effectively conduct chiral resolution by using MISPE technique with such imprinted materials as solid adsorbents.

4. Conclusions

The new surface imprinting technique of synchronously graft-polymerizing and molecule imprinting on solid particles was successfully applied in the preparation of the surface imprinted material of the single enantiomer, (R)-mandelic acid. The prepared (R)-mandelic acid surface imprinted material MIP-PHEMA/SiO₂ has excellent enantiomeric recognition and resolution ability as well as good binding affinity for (R)-mandelic acid. The binding capacity of MIP-PHEMA/SiO₂ particles for (R)-mandelic acid reaches up to 278 mg/g. The result of the resolution experiments of a racemic mixture with MIP-PHEMA/SiO₂ particles as solid adsorbent shows relative another enantiomer, (S)-mandelic acid, the selectivity coefficient of MIP-PHEMA/SiO₂ particles for (R)-mandelic acid is 5.02. Furthermore, both the supernatant and eluant produce optical activity, and their optical purities are 44% and 85%, respectively, indicating that the two enantiomers were well separated. There are two key factors for successfully conducting surface imprinting of the single enantiomer of mandelic acid. (1) Firstly, there should be strong interaction between the functional monomer and the template molecule. By right of the multi-site hydrogen bonds located around the asymmetric carbon atom of (R)-mandelic acid, the host-guest complex between (R)-mandelic acid and monomer HEMA is formed. (2) Secondly, in the imprinting system, a surface initiating system needs to be constituted. The mercapto group on the modified particles MPMS-SiO₂ and BPO in the solution constituted a surface initiating system -SH/BPO so that a great deal of sulfur radicals produced on the surfaces of SiO₂ particles. It is these radicals that initiate the

graft/crosslinking-polymerization of HEMA molecules around (R)-mandelic acid molecules, and make synchronously graft-polymerizing of HEMA and molecule imprinting of (R)-mandelic acid to be realized, resulting in the (R)-mandelic acid surface imprinted material with high performance.

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Figure legend

Figure 1 Chemical process to prepare grafted particles PHEMA/SiO₂

Figure 2 FTIR spectra of grafted particles PHEMA/SiO₂ and other two kinds of particles

Figure 3 Adsorption isotherm of MIP-PHEMA/SiO₂ particles for mandelic acid

Temperature: 25°C; Solvent: DMF

Figure 4 Schematic illustration of interaction model between grafted particles PHEMA/SiO₂ and mandelic

acid via multi-site hydrogen bonds

Figure 5 Schematic expression of chemical reaction process to prepare MIP-PHEMA/SiO₂

Figure 6 FTIR spectrum of MIP-PHEMA/SiO₂ particles

Figure 7 SEM photographs of SiO₂ and MIP-PHEMA/SiO₂ particles

Figure 8 TGA curves of MPMS-SiO₂ and MIP-PHEMA/SiO₂ particles

Figure 9 Adsorption isotherm of NMIP-PHEMA/SiO₂ particles for two single enantiomers of mandelic acid

Temperature: 25°C; Solvent: DMF

Figure 10 Binding isotherms of MIP-PHEMA/SiO₂ for two single enantiomers of mandelic acid

Temperature: 25°C; Solvent: DMF

Figure 11 Dynamic adsorption curves of NMIP-PHEMA/SiO₂ for two single enantiomers of mandelic acid

Temperature: 25°C; Initial concentration: 2 g/L; Flow rate: 5BV/h

Figure 12 Dynamic binding curves of MIP- PDMAEMA/SiO₂ for two single enantiomers of mandelic acid

Temperature: 25°C; Initial concentration: 2 g/L; Flow rate: 5BV/h

Table 1 Distribution coefficient and selectivity coefficient data

Table 2 Results of chiral resolution of mandelic acid raceme solution using MIP-PHEMA/SiO₂ particles

(1)Surface modification of silica gel particles with coupling agent MPMS

(2)Production of free radical on surfaces of modified particle MPMS

(3)Surface-initiated graft polymerization of HEMA on silica gel particles

Figure 1

Figure 2

Figure 3

Figure 4

(1) Forming complex between monomer HEMA and R-mandelic acid

(2) Production of free radical on surfaces of modified particle MPMS

(3) Synchronously grafting/crosslinking and imprinting on surfaces of silica particles

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

Figure 12

Table 1 Distribution coefficient and selectivity coefficient data

Adsorbing material	NMIP-PHEMA/SiO ₂		MIP-PHEMA/SiO ₂	
	(R)-mandelic acid	(S)-mandelic acid	(R)-mandelic acid	(S)-mandelic acid
<i>K_d</i> /(m L/g)	799.8	799.6	2011.8	403.9
<i>k</i>	1.0		5.02	

Table 2 Results of chiral resolution of mandelic acid raceme solution using MIP-PHEMA/SiO₂ particles

Adsorb material	Specific rotation [α]		Optical purity/% (ee)	
	Supernatant	Eluant	Supernatant	Eluant
MIP-PHEMA/SiO ₂	+63.58 ° ^a	-128.76 ° ^b	44	85
NMIP-PHEMA/SiO ₂	0	0		

^a The specific rotation of chiral isomer (S)-mandelic acid in DMF is +153.00 ° ; ^b The specific rotation of chiral isomer (R)-mandelic acid in the mixed solution of methanol and glacial acetic acid is -156.00 °