Supplementary Material (ESI)

Polymerized organogel particles formed and imprinted by chiral gelators and their selective adsorption for phenylalanine racemates

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Experimental

Materials

N-Stearine-N'-stearyl-L-phenylalanine (bis18-L-Phe) was synthesized according to a method described previously.[1] ß-Hydroxyethyl methacrylate (HEMA, CP), polyethylene glycol dimethacrylates (PEG200DMA, AR), L-phenylalanine (L-Phe, AR) and D-phenylalanine (D-Phe, AR) were purchased from Sinopharm Chemical Reagent Co. 1-Hydroxy-cyclohexylphenyl ketone (Irgacure 184, AR) was purchased from Ciba Geigy Co. Propylene polyoxyethylene ether (ONIST AE-50) was purchased from Shanghai Chemical Co. All the other reagents used in the experiments were of analytical grades and used as received. Ultrapure water was produced by a Millipore Direct-Q system.

Preparation of polymerized imprinted organogel particles

Bis18-L-Phe (5 wt%) was first dissolved in a solvent mixture of HAMA and PEG200DMA (10/90, w/w), then Irgacure 184 as photoinitiator (0.5 wt% of the total mass of the mixture) was added. The mixture as the oil phase was heated until the solid completely dissolved. The aqueous phase consists of water and propylene polyoxyethylene ether as emulsifier (1wt% of the mass of oil phase). The ratio of oil phase and water phase was 3:7. The oil phase was added dropwise into the aqueous phase at 70–80 °C. The resultant suspension was allowed to cool to room temperature.

The resultant suspension of organogels was stirred and irradiated with a UV lamp (20 W) at 0 °C for 72 hours and then by a UV lamp (1000 W) for 10 min. Subsequently, the suspension of polymerized organogels was demulsified by water. Polymerized organogel particles were obtained by filtration, washing and drying. Finally, the polymerized imprinted organogel particles were refluxed with ethanol for 72 h to remove the template and dried in vacuum (the final product, i.e. the polymerized imprinted organogel particles are denoted as PI-gel particles).

Characterization of the particles

The particle size of PI-gels was measured by a laser particle size analyzer (Master Min, Malvern). The PI-gel particles were dispersed by ultrasonication in ethanol and then dropped on a glass plate. The morphology of the samples was observed by optical microscopy (BH-2, Olympus) after evaporation of ethanol and by field emission scanning electron microscopy (FE-SEM, Sirion 200 FEI). The sample was freeze-dried and coated with Au. The accelerating voltage was 10 KV.

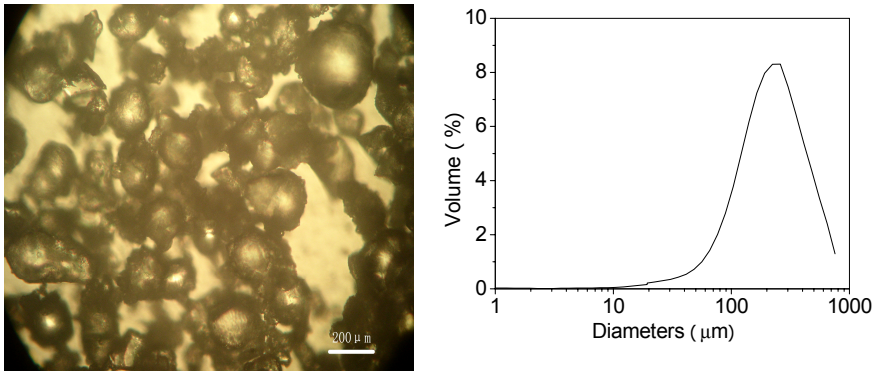

Figure S-1. Optical microscope photographs of PI-gel particles (scale bar is 200 μm) and particle-size analysis.

In general, the adsorption capacity of particles depends on their size and surface area. In order to evaluate the adsorption capacity of PI-gel particles more precisely, the prepared PI-gel particles have been sieved. Table 1 lists the size distribution of PI-gel particles after the sieving.

Table S-1 The diameter range of imprinting polymerized gel particles after sieving.

samples	Sieving (mesh)	diameters range (μm)
A	before sieving	average diameter 242*
B	50	< 355
C	100	150–355
D	150	102–150
E	> 150	< 102

* The average diameter of 242 μm was obtained from Figure 1.

Fig. S-2 shows the SEM images of the particle E before and after extraction with ethanol. The image A shows the surface morphology of the particle E before extraction with ethanol. A compact and less porous surface can be observed. In contrast, a porous surface can be clearly observed in the particle E after extraction with ethanol. The scale bars for both images are same (5 μm).

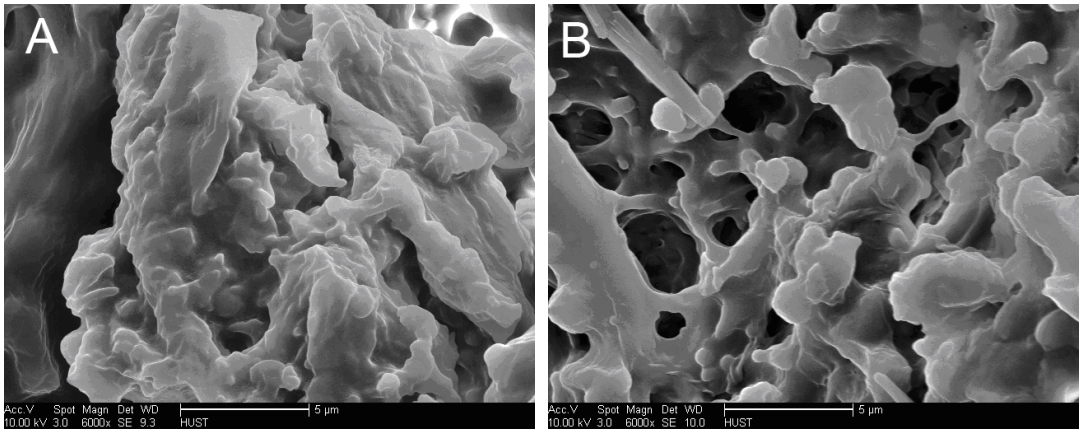

Fig. S-2 SEM images of PI-gel particle E before (A) and after (B) extraction with ethanol.

Characterization of PI-gel particles with and without bis-18-Phe

The existence of template in the polymer matrices can be characterized by using DSC technique. To verify no bis-18-Phe contained in polymer matrices, we measured DSC of the PI-gel particles before and after extraction with ethanol (Fig. S-3). As a reference, the DSC thermogram of a HEMA organogel in the presence of bis18-L-Phe was also measured (curve A). As shown in Fig. S-3, a sharp peak is observed at 63 °C (curve A), which can be attributed to the

dissociation of bis18-L-Phe aggregates. For the PI-gel particles before ethanol extraction, a broad peak was found (curve B), in which, the maximum heat effect value appears at 64 °C. This is in accord with the dissociation of bis18-L-Phe aggregates at 63 °C. Therefore, we attribute this broad peak to the dissociation of bis18-L-Phe aggregates. Why does a broad peak appear? This may be explained by assuming that bis18-L-Phe aggregates are interpenetrated and astricted in the cross-linked HEMA polymer network, resulting in a slow relaxation and dissociation process of bis18-L-Phe aggregates. By contrast, there is no heat effect peak found in case of PI-gel particles after ethanol extraction (curve C) because of complete removal of bis18-L-Phe aggregates. DSC analysis indicates that bis18-L-Phe can be used as a gelator to form polymerized organogels first, and can then be removed from the polymer matrices to form porous polymer materials. This is a novel strategy for the preparation of molecular imprinted polymers.

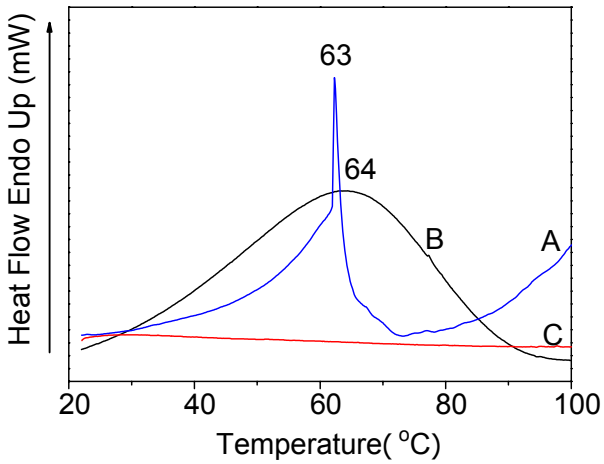

Fig. S-3 DSC thermograms of the unpolymerized HEMA gel (A) and the imprinted polymerized HEMA gel before (B) and after extracting (C) by ethanol.

Adsorption experiments

Solutions of D- and L-phenylalanine (C_0 = 4 mmol/L) were prepared by dissolving them separately in phosphate buffer (pH 7.4). D- or L-phenylalanine solutions (10mL) were added in a test tube filled with 100 mg of PI-gels and then incubated in an ultrasonic bath for a certain time at

37 °C. The concentrations of D- or L-phenylalanine after adsorption were determined by UV–Vis spectrophotometry (TU-1810, Beijing Purkinje General Instrument Co. Ltd). The detection wavelengths of D- and L- phenylalanine were 257 nm. The adsorption efficiency was calculated according to:

$$\alpha = \frac{C_0 - C_t}{C_0} \times 100\%$$

Herein, α is the adsorption efficiency of D- or L-phenylalanine on PI-gels. C_0 and C_t are the concentrations of D- or L-phenylalanine before and after adsorption, respectively.

Adsorption isotherms

The concentration of L-Phe (4 mmol/L) in the adsorption experiments was used according to the adsorption isotherm (Fig. S-2). Measurement of adsorption isotherms was carried out by determining saturated adsorption of varied concentration of L-phen phosphate buffers on 100 mg of PI-gel particles at 37 °C. As shown in Fig. S-4, the saturated adsorption was reached in the case of 4 mmol/L of L-Phe solution employed. Therefore, we use this concentration for all adsorption experiments.

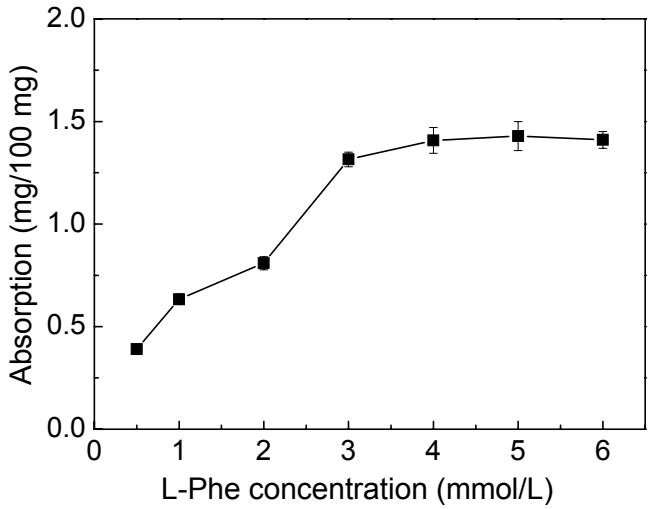

Fig. S-4. Adsorption isotherms of L-Phe on the PI-gel particles at 37 °C. The error bars are from three parallel experiment.

As a reference, the adsorption efficiency of PI-gel particles for the structurally related chiral amino acids such as L-histidine and L-tyrosine was also very low in comparison with L-phenylalanine (Fig. S-5).

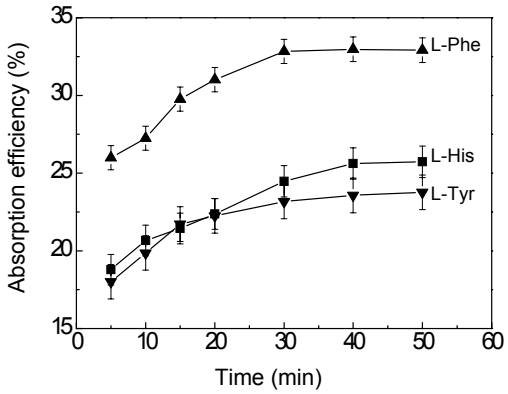

Fig. S-5 Adsorption efficiency of PI-gel particles (sample E) for the structurally related chiral amino acids such as L-histidine and L-tyrosine.

Column separation experiments

The PI-gel particles (about 2 g) with average diameters of 100 μm were filled in a sand funnel with an internal diameter of 10 mm and a height of 100 mm. The height of stationary phase was about 50 mm. The phosphate buffer solutions (pH 7.4) of racemic D- and L-phenylalanine (mass ratio was 1:1) were added to the column. The solutions from the bottom of the column were collected after designed intervals of time. The circular dichroism spectra (CD) of the samples were recorded on a J-810 spectrometer (Jasco) at 25 °C.

Similarly, the aqueous sodium carbonate solutions of racemic D- and L-phenylalanine (mass ratio 1:1) were prepared (the concentration of racemic phenylalanine and sodium carbonate were 0.15g/mL and 0.5 mol/L, respectively). The solutions (20 mL) were gradually added into the column. The blank aqueous sodium carbonate solution used as mobile phase was added to elute. The solutions were collected from the bottom of the column after designed intervals of time. The specific rotation ([α]) of the samples was measured by optical spectrophotometry (WZZ-2S Automatic Polarimeter, Shanghai Precision & Scientific Instrument Co. Ltd) at 25 °C and calculated according to:

$$[\alpha] = \frac{\alpha}{LC}$$

When the length (L) of the rotation tube is fixed, the specific rotation (α) and the concentration (C) are proportional. The specific rotation of D-phenylalanine is defined as "+" and the specific rotation of L-phenylalanine is defined as "–". Thus, the specific rotation of a racemic mixture is 0. When the amount of D- and L-phenylalanine is varied, the concentrations of both phenylalanines can be calculated by determining the α values of solutions collected from the bottom of column.

$$\Delta C = C_{D-Phe} - C_{L-Phe}$$

Herein, $C_{D\text{-}Phe}$ and $C_{L\text{-}Phe}$ are the concentrations of D- or L-phenylalanine after column separation, respectively.

1 X. Fu, Y. Yang, N. Wang, H. Wang, Y. Yang, A novel chiral separation material: polymerized organogel formed by chiral gelators for the separation of D- and L-phenylalanine, *J. Mol. Recognit*., 2007, **20**, 238–244.