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Preparation and recognition performance of uric acid-imprinted material prepared with novel surface imprinting technique

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ABSTRACT

Acrylonitrile (AN) was first graft-polymerized on the surfaces of crosslinked polyvinyl alcohol (CPVA) microspheres by initiating of cerium salt, and then the grafted polyacrylonitrile (PAN) was transformed to polyamidoxime (PAO) via amidoximation transform reaction, resulting in the functional microspheres PAO/CPVA. By adopting the novel surface-molecular imprinting technique put forward by us, uric acid molecule-imprinted material MIP-PAO/CPVA was prepared with glutaraldehyde as crosslinking agent The binding character of MIP-PAO/CPVA towards uric acid was investigated in depth with both batch and column methods and using guanine as a contrast substance whose chemical structure is similar to uric acid to a certain extent. The experimental results show that the surface imprinted material MIP-PAO/CPVA has excellent binding affinity (a great binding capacity of 104 mg/g) and high recognition selectivity for the template molecule, uric acid. The selectivity coefficient of PAO/CPVA microspheres (non-imprinted material) for uric acid relative to guanine is only 1.273, displaying no recognition selectivity for uric acid. However, after imprinting, the selectivity coefficient of MIP-PAO/CPVA for uric acid in respect to guanine is remarkably enhanced to 14.00, displaying the excellent recognition selectivity and binding affinity towards uric acid molecules.

1. Introduction

Uric acid (UA) is the primary product of purine nucleotide metabolism. Abnormal levels of uric acid are associated with a number of clinical situations, such as gout, hyperuricemia, Lesch–Nyan disease, cardiovascular and kidney diseases [1–4]. The cumulated uric acid in blood can be removed effectively by using blood purification techniques so that the toxicosis symptom of patients can be mitigated. In the field of biomedicine engineering, it is an attractive research subject to prepare polymeric adsorption material with high performance of eliminating excess endogenesis toxin [5–8], and it is also a key of developing blood purification techniques such as hemoperfusion. Different polymer adsorption materials were used to eliminate small molecular endogenesis catabolism products, such as uric acid. Some researchers adopted adsorption resins to remove the cumulated uric acid [9–11] in blood. However, the researched adsorption resins have lower adsorption capacity, and it is more important that the adsorption selectivity of the adsorption resins is always absent. Besides, the biocompatibility and hemocompatibility are also important issues.

There are two kinds of solid adsorbents with high adsorption selectivity for the target substances, and they are molecularly imprinted materials and immunoadsorbents. Molecularly imprinted polymers (MIPs) are an artificially synthesized macromolecular material, in which a great deal of specific cavities designed for a target molecule (namely, the template molecule) is distributed and these cavities are complementary in shape, size and functional groups to the target molecule. Therefore, MIPs have specific molecular recognition ability and high binding affinity [12–15] for the target molecules, 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. We conceive with reason that molecularly imprinted materials can be also introduced into the blood purification treatment, and they will be able to effectively remove toxic molecules from blood. In fact, the related exploratory research was carried out by a few of the researchers [16]. In the present work, we prepared uric acid-imprinted material with crosslinked polyvinyl alcohol microspheres as matrix, which are biocompatible or rather hemocompatible [17,18], and investigated in detail the binding characteristic of the imprinted material for uric acid.

The conventional method to prepare MIPs, entrapment way, has some disadvantages, such as time-consuming and complicated preparation process, 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 poor binding capacity and lower binding kinetic of MIPs towards the template molecules. In order to

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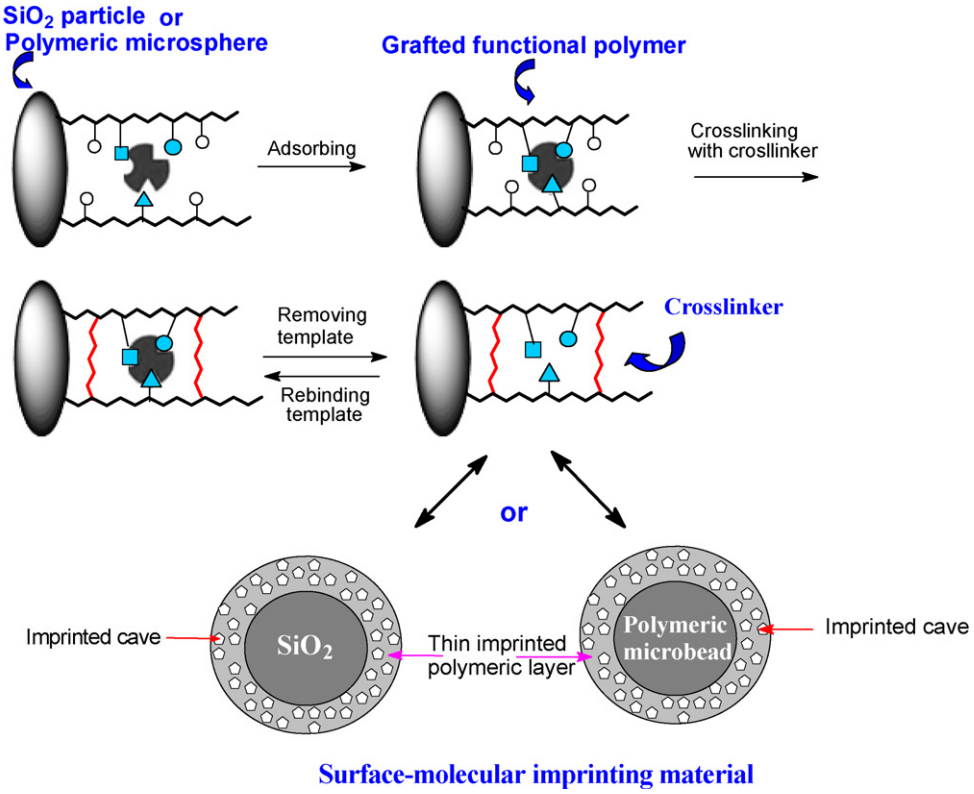

Fig. 1. Schematic expression of surface-molecular imprinting process.

overcome these drawbacks, many researchers developed surface-molecular imprinting techniques [19–21], and tried to make the imprinted caves to lie on the surfaces of solid particles. The surface imprinted materials are more effective for recognizing the template molecules.

In the previous study [19,22,23], 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 the intermolecular interaction between the grafted functional macromolecules and template molecules; (3) Post-imprinting of template molecules is conduced towards the grafted polymers using a special crosslinking agent that 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. This novel surface imprinting method is based on the interactions between the grafted functional polymers and template molecules rather than based on the interactions between the functional monomers and template molecules like as the conventional imprinting method, and this is an obvious difference between the two methods. The surface-molecular imprinting process can be expressed schematically in Fig. 1.

In our current investigation, the surface imprinting of uric acid was conducted with crosslinked polyvinyl alcohol (CPVA) microspheres as matrix particles that are biocompatible. Polyacrylonitrile (PAN) was first grafted on the surfaces of CPVA microspheres in the "grafting from" manner, obtaining the grafted microspheres PAN/CPVA, and then, the grafted PAN was transformed into poly(amidoxime) (PAO) via amidoximation transformation reaction, resulting in the functional microspheres PAO/CPVA. Finally, by using the new surface imprinting technique described above, with uric acid as template and with glutaraldehyde as crosslinker, the surface imprinting was performed, resulting in the uric acid molecule-imprinted material, MIP-PAO/CPVA. The results of the binding experiments indicate that by right of the strong interactions between the grafted PAO macromolecules and uric acid molecules, hydrogen bonding and electrostatic interaction, the surface-molecular imprinting of uric acid on PAO/PVA microspheres was conducted successfully, and the imprinted material MIP-PAO/PVA has excellent binding affinity and high recognition selectivity towards uric acid molecules. It can be expected with confidence that by adopting the novel surface imprinting method put forward by us and with the polymeric microspheres that are biocompatible or rather blood compatible as matrix, the various adsorption materials with high performance used in blood purification can be prepared. Therefore, the study results in this work supply valuable references for developing blood purification materials.

2. Experiments

2.1. Materials and instruments

Polyvinyl alcohol (PVA, a polymerization degree of 2200, Shanwei Chemical Engineering Ltd., Province Shanxi, China) was received. Acrylonitrile (AN, Fuchen Chemical Reagent Plant, Tianjin, China) was of analytical grade, and was purified by vacuum distillation before use. Ammonium cerous sulfate (ACS Tientsin Bodi Chemical Engineering Ltd., Tientsin, China) was of analytical grade.

Hydroxylamine hydrochloride (HAHC, Chengyuan Trade Ltd., Tianjin, China) was of analytical grade. Uric acid (Alfa Aesar) was of analytical grade. Guanine (Sinopharm Chemical Reagent Ltd., Beijing) was a biochemical reagent. Glutaraldehyde (50% of aqueous solution, Tientsin Baishi Chemical Engineering Ltd., Tientsin, China) was of analytical grade. Other reagents were all commercial chemicals with analytically 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 (PerkineElmer Company, USA), Zetasizer Nano-Zeta potential analyzer (Malvern Instrument Company, UK), PHS-2 acidimeter (The Second Analytical Instrument Factory, Shanghai, China), TG16-WS high-speed centrifuge with desk type (Changsha Xiangyi Centrifuge Factory, Province Jiangsu, China) and THZ-92C constant temperature shaker equipped with gas bath (Boxun Medical Treatment Equipment Factory, Shanghai, China).

2.2. Preparing functional microspheres PAO/CPVA

2.2.1. Preparing crosslinked polyvinyl alcohol microspheres

By directly crosslinking and balling of line-type polyvinyl alcohol (PVA) in an inverse suspension system, crosslinked polyvinyl alcohol (CPVA) microspheres were prepared, and the typical procedure was as follows. Disperser Span 60 (Sorbitan monostearate) was dissolved in liquid paraffin, constituting the oil phase (continuous phase). An aqueous PVA solution was mixed with glutaraldehyde solution, comprising the water phase (dispersed phase). The water phase was poured into the oil phase, and the system was fully dispersed by stirring, resulting in an inverse suspension system. After adding a few of HCl solution as the catalyst, the crosslinking reaction (an aldolization and forming ether reaction) and balling process were allowed to be carried out for 7 h at the constant of 65 °C, obtaining semitransparent microspheres CPVA with excellent sphericity and with a mean diameter of 150 μm.

2.2.2. Preparing grafted microspheres PAN/CPVA

By referencing the procedures ascribed in Ref. [19], the graft-polymerization of acrylonitrile on the surfaces of CPVA microspheres was conducted, and the typical process was as follows. CPVA microspheres with a given amount were placed in a four-necked flask equipped with a mechanical stirring, a condenser and a N_2 inlet. The solvent *N*,*N*-dimethylfomamide (DMF) was added, and CPVA microspheres were allowed to be soaked and swelled in DMF for 10 h. AN, ACS and sulfuric acid were added in turn. The graft-polymerization of AN was performed under N_2 atmosphere at the constant temperature of 45 °C for 6 h. By filtrating, the grafted microspheres PAN/CPVA were collected, soaked and washed with DMF and distilled water to remove a little of polyacrylamide physically attached to the microspheres, and dried under vacuum to constant weight. The grafting degree of PAN of PAN/CPVA microspheres was determined with weighing method, and the prepared and used PAN/CPVA microspheres in this study have a grafting degree of 27 g/100 g.

2.2.3. Preparation of functional microspheres of PAO/CPVA

Via the amidoximation transform reaction, the grafted PAN on PAN/CPVA microspheres was transformed into poly(amidoxime) (PAO), resulting in the functional microspheres PAO/CPVA, and the detailed processes were as follows. Twenty milliliters of HAHC solution of 1 M were mixed with 10 mL of Na_2CO_3 solution of 1 M, and the mixed solution was added into a four-necked flask, followed by adding 1 g of the grafted microspheres PAN/CPVA. N_2 was bubbled through the system, and the temperature was raised to 70 °C. The amidoximation transform reaction was allowed to be carried out at the constant temperature of 70 °C for 4 h with stirring. After finishing the reaction, the resultant microspheres were filtered out, washed repeatedly with distilled water, and dried under vacuum, obtaining the functional microspheres PAO/CPVA, on which poly(amidoxime) macromolecules were grafted, namely, the grafted PAN had transformed into the grafted PAO. The following characterizations were conducted for the functional microspheres PAO/CPVA.

(1) The infrared spectrum was determined with KBr pellet method to confirm the chemical structure of PAO/CPVA. (2) Weighing method was used to determine the amount of the AN chain unit which has converted into amidoxime chain unit, and according to Eq. (1), the conversion (Conv.) of nitrile group (mol %) was calculated.

$$\text{Conv.} = \frac{(m_2 - m_1)/M'}{m_1 \times \text{GD}/M \times 100} \times 100 \quad (1)$$

where $m_1(g)$ was the mass of the grafted microspheres PAN/CPVA; $m_2(g)$ was the mass of the microspheres after amidoximation conversion, namely the mass of PAO/CPVA microspheres; M' (30.03) was the molar mass difference between amidoxime chain unit and acrylonitrile chain unit; GD (g/100 g) was the grafting degree of the grafted microspheres PAN/CPVA; M (53.06) was the molar mass of the acrylonitrile chain unit. The prepared and used PAO/CPVA microspheres in this study have a nitrile group conversion of 72%. (3) The zeta potential of PAO/CPVA microspheres was measured with electrophoresis method: the microspheres were ground, and the finely divided particles were dispersed into water; the pH value of the dispersed system was adjusted with diluted HCl solution and NaOH solution; the zeta potential of PAO/CPVA microspheres was determined with the dispersed systems with different pH values, obtaining the zeta potential curve of PAO/CPVA microspheres, namely the relationship curve between zeta potential and pH value.

2.3. Examining adsorption property of PAO/CPVA for uric acid

2.3.1. Isothermic adsorption experiment of microspheres PAO/CPVA for uric acid

Based on the adsorption dynamics experiment (adsorption equilibrium time was about 5 h), the isothermic adsorption experiments of the functional microspheres PAO/CPVA for uric acid were carried out to examine the adsorption property of PAO/CPVA microspheres for uric acid. Aqueous solutions of uric acid in a concentration range of 0.1–3.0 mg/mL were prepared, and the adsorption experiments were carried out in a constant temperature shaker. The uric acid concentrations in the supernatants were determined with tungsten blue-spectrophotometry at 680 nm [24], the equilibrium adsorption amounts were calculated according to Eq. (2), and the adsorption isothermers were plotted.

$$Q_e = \frac{V(C_0 - C_e)}{m} \quad (2)$$

where C_0(mg/mL) was the concentration of uric acid in the initial solution; C_e (mg/mL) was the concentration of uric acid in the supernatant; V (mL) was the volume of the uric acid solution; m (g) was the mass of the adsorbent PAO/CPVA microspheres; Q_e (mg/g) was the equilibrium adsorption amount of uric acid.

2.3.2. Examining effect of pH value of medium on adsorption ability of PAO/CPVA for uric acid

The pH values of uric acid solutions were adjusted with diluted solutions of HCl and NaOH, obtaining uric acid solutions with different pH values. The isothermal adsorption experiments of PAO/CPVA for uric acid in these solutions were carried out to examine the effect of pH value on the adsorption ability of PAO/CPVA microspheres for uric acid and to investigate the interactions between the grafted PAO and uric acid molecules.

2.4. Preparation of uric acid-imprinted microspheres MIP-PAO/CPVA

PAO/CPVA microspheres with a given amount were added into the aqueous uric acid solution with a concentration of 3 mg/mL, and the pH value of the solution was adjusted to pH 7 with diluted NaOH solution. The mixture was shaken on a constant temperature shaker for 5 h until PAO/CPVA microspheres were fully swelled and the adsorption for uric acid reached equilibrium. After filtrating, the PAO/CPVA microspheres, which had adsorbed uric acid in a saturation state, were dried under vacuum. Two grams of PAO/CPVA microspheres adsorbing uric acid in a saturated state were placed in 100 mL of a uric acid solution of 3 mg/mL (to prevent the adsorbed uric acid to be desorbed). The pH value of the solution was adjusted to pH 7, and 0.5 mL of glutaraldehyde solution was added. The crosslinking reaction was performed at 50 °C for 8 h. The resultant microspheres were filtered off, washed repeatedly with aqueous NaOH solution to remove the template, uric acid molecules, and finally dried under vacuum, obtaining the uric acid molecule-imprinted material MIP-PAO/CPVA.

2.5. Study on binding characteristic of MIP-PAO/CPVA for uric acid

2.5.1. Evaluating binding property of MIP-PAO/CPVA for uric acid

The binding behavior of MIP-PAO/CPVA for uric acid was examined with batch method (static method) and column method (dynamic method), respectively.

2.5.1.1. Batch method. Based on the determination of the binding dynamics behavior of MIP-PAO/CPVA for uric acid (the equilibrium binding time was also 5 h), the binding isotherm was measured. Numbers of 50 mL of uric acid solutions with different concentrations were taken and transferred into several conical flasks. MIP-PAO/CPVA microspheres with the same mass (0.2 g) were added into these solutions, respectively. These mixtures were shaken on a constant temperature shaker at 30 °C, centrifuged after reaching binding equilibrium, and the equilibrium concentrations of uric acid in the supernatants were determined, respectively. The equilibrium binding amounts of MIP-PAO/CPVA towards uric acid were calculated also according to Eq. (2), and the binding isotherm was figured.

2.5.1.2. Column method. For the column method, the experimental procedures are explained as follows. A certain amount (1.04 g) of MIP-PAO/CPVA microspheres was packed into a piece of glass pipe with an internal diameter of 1.0 cm, and the bed volume (BV) of the packed column was 2 mL. The uric acid solution with a concentration of 1 mg/mL was allowed to gradually flow through the packed column at a rate of four bed volumes per hour (4 BV/h) in the countercurrent manner. The effluents with two bed volume (2 BV) interval were collected, and the uric acid concentrations of these effluents were determined with spectrophotometry. The dynamic binding curve was plotted, and the leaking adsorption amount and saturated adsorption amount of uric acid were calculated with the data of the concentrations and bed number of effluents, respectively.

2.5.2. Selectivity experiments

In this work, guanine was selected as a contrast substance to examine the recognition selectivity of MIP-PAO/CPVA microspheres towards uric acid. Guanine is a purine base, and is an essential constituent of both RNA and DNA. Both uric acid and guanine are purine derivatives, so the chemical structure of guanine is similar to uric acid to a certain degree. The molecular structures of the two substances are schematically expressed in Fig. 2. For the sake of comparing the binding selectivity of MIP-PAO/CPVA for uric acid in respect to guanine, the static binding isotherm and dynamic binding curve of MIP-PAO/CPVA as well as PAO/CPVA for guanine were also determined with spectrophotometry (at 244 nm), separately.

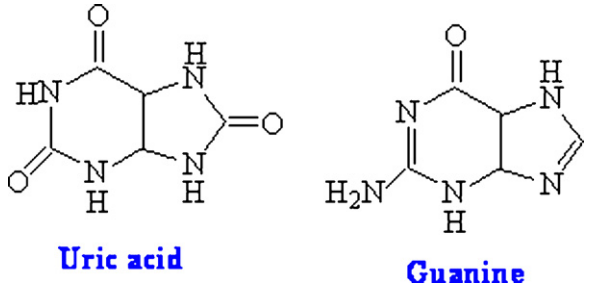

Fig. 2. Schematic expression of chemical structures of uric acid and guanine.

In order to further show the binding specificity of MIP-PAO/CPVA for uric acid (UA), the competitive adsorption of uric acid with respect to guanine (GU) was studied. A binary mixed solution of GU/UA was prepared, and the concentrations of the two substances in the mixed solution were the same (1 mg/mL). 0.2 g of MIP-PAO/CPVA was added into 50 mL of the mixed solution, and the static adsorption experiments were performed. After binding equilibriums were reached, the concentrations of the two substances in the supernatant were determined, respectively. The distribution coefficient for each substance was calculated according to Eq. (3), which was originated from Ref. [25].

$$K_d = \frac{Q_e}{C_e} \tag{3}$$

where K_d represents the distribution coefficient (ml/g); Q_e (mg/g) is the equilibrium binding amount; C_e (mg/mL) is the equilibrium concentration.

The selectivity coefficient of MIP-PAO/CPVA for UA with respect to the competition species, GU, can be obtained from the equilibrium binding data according to Eq. (4)

$$k = \frac{K_d(\mathrm{UA})}{K_d(\mathrm{GU})} \tag{4}$$

where k is the selectivity coefficient, and the value of k allows an sufficient estimation of selectivity of MIP-PAO/CPVA for uric acid. A relative selectivity coefficient k' is also defined as expressed in Eq. (5) [25], and the value of k' can reveal the enhanced extent of the adsorption affinity and selectivity of the imprinted material MIP-PAO/CPVA towards the template molecule with respect to the non-imprinted material PAO/CPVA.

$$k' = \frac{k_{\mathrm{impr}}}{k_{\text{non-impr}}} \tag{5}$$

where k_{impr} is the selectivity coefficient of MIP-PAO/CPVA for uric acid with respect to the competition species, guanine, and $k_{\text{non-impr}}$ is the selectivity coefficient of PAO/CPVA for uric acid also with respect to guanine.

2.5.3. Desorption experiment

A certain amount of MIP-PAO/CPVA microspheres adsorbing uric acid in a saturation state was packed into a piece of glass pipe with an internal diameter of 1.0 cm, 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 the countercurrent manner. The effluents with two volume (2 BV) interval were collected, and the concentration of uric acid in the effluents was determined with spectrophotometry.

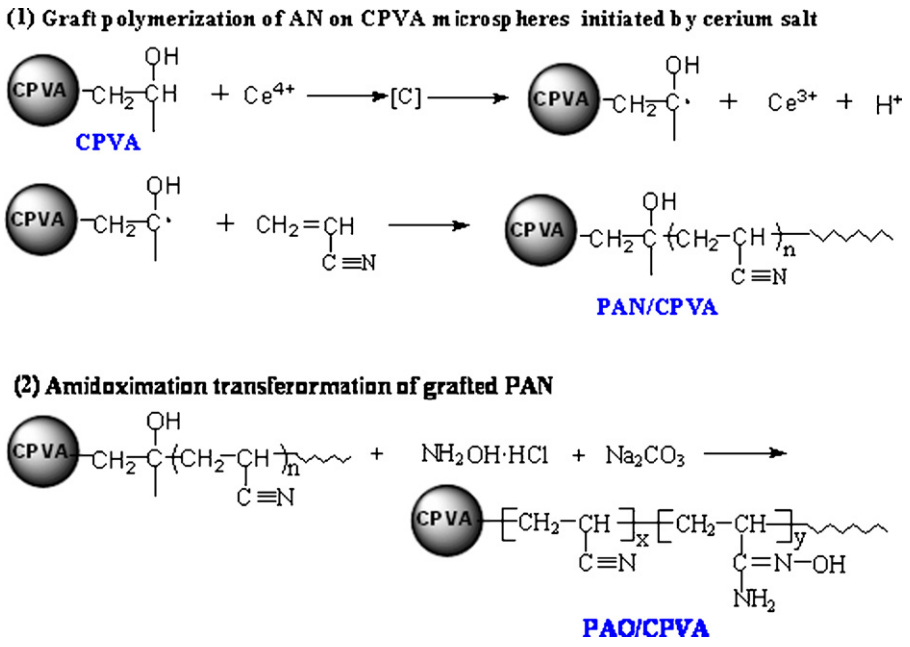

Fig. 3. Schematic illustration of preparation process of functional microspheres PAO/CPVA.

The dynamic desorption curve was plotted, and elution property of MIP-HEMA/NVP was evaluated.

3. Results and discussions

3.1. Preparation, chemical structure and surface electrical property of functional microspheres PAO/CPVA

3.1.1. Preparation process

There is a mass of hydroxyl groups on the surfaces of CPVA microspheres. These hydroxyl groups with Ce^{4+} ion can constitute a redox initiating system, and thereby, the graft-polymerization of AN on the surfaces of CPVA microspheres is initiated and carries out in a manner of surface-initiated grafting, forming the grafted microspheres PAN/CPVA. With the action of hydroxylamine which is produced by the reaction between HAHC and Na_2CO_3, the nitrile groups of the grafted PAN are transformed into amidoxime groups, resulting in the functional microspheres PAO/CPVA. The entire preparation process of PAO/CPVA microspheres can be schematically illustrated in Fig. 3.

3.1.2. Infrared spectrum

The infrared spectra of the three kinds of microspheres are given In Fig. 4. In the spectrum of CPVA, various characteristic absorption bands of CPVA which has undergone aldolization and forming ether reaction can be found. In the spectrum of the grafted microspheres PAN/CPVA, the absorption band of alcoholic hydroxyl group at 3444 cm^{-1} has been weakened greatly indicating the consummation of the hydroxyl groups, and a new band at 2250 cm^{-1} has appeared. This new band should be attributed to the characteristic absorption of nitrile group (it needs to be pointed that the vibration absorption of nitrile group –C≡N is very weak). The above spectrum changes show that monomer AN has been graft-polymerized on the surfaces of CPVA microspheres, and the grafted microspheres PAN/CPVA have been formed. In the spectrum of the functional microspheres PAO/CPVA, the band of nitrile group at 2250 cm^{-1} has been disappeared, and two new bands at 1660 and 960 cm^{-1} have appeared. The former should be ascribed to the absorption of the stretching vibration of C=N bond in amidoxime groups, whereas the later is corresponding to the absorption of the stretching vibration of N–O bond in amidoxime groups. Furthermore, the absorption of the stretching vibration of amino group $–NH_2$ in amidoxime groups exhibits at 3300 cm^{-1}. The above spectrum changes suggest that the amidoximation transform of the grafted PAN have occurred, and the functional microspheres PAO/CPVA, on which poly(amidoxime) macromolecules are grafted, have been produced.

3.1.3. Zeta potentials of PAO/CPVA microspheres

Fig. 5 presents the zeta potential curves of the grafted microspheres PAN/CPVA and the functional microspheres PAO/CPVA. In Fig. 2, the following facts can be seen clearly. (1) The zeta potential of PAN/CPVA is negative and is a small number value. (2) The zeta potential of PAO/CPVA is positive in a wider pH range, but it is a large number value. This suggests that after the amidoximation transform reaction, the zeta potential of the microspheres has been changed fundamentally, and implies that positive charges with a high density are present on the surfaces of the functional microspheres PAO/CPVA. The positive charges with a high density are originated from the protonation of N atoms of amino groups $–NH_2$ in amidoxime groups of PAO/CPVA. (3) The zeta potential

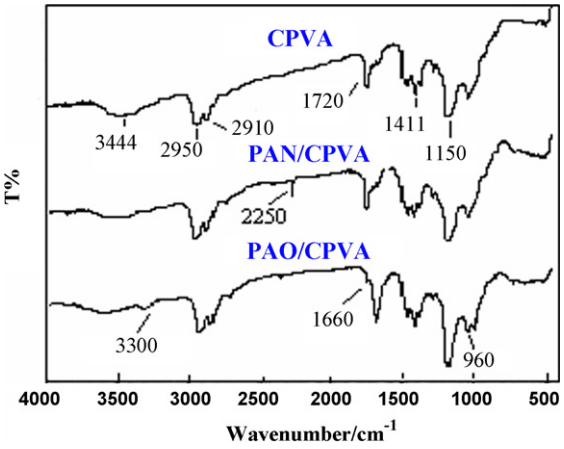

Fig. 4. Infrared spectra of three kinds of polymeric microspheres.

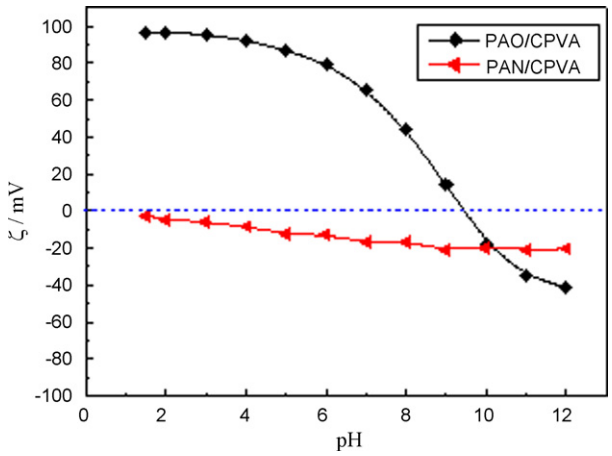

Fig. 5. Variation of zeta potentials of PAN/CPVA and PAO/CPVA microspheres with pH value.

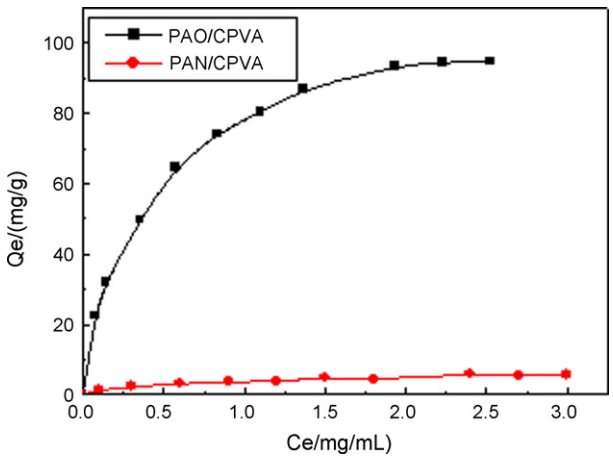

Fig. 6. Adsorption isotherms of two kinds of microspheres for uric acid. Temperature: 30 °C; pH 7

of PAO/CPVA decreases gradually with increasing pH value of the medium, and it is the result of the synergism of the decrease of the protonation degree of amino groups in amidoxime groups of PAO/CPVA and the increase of the dissociation degree of oxime hydroxyl groups (amidoxime groups are amphoteric [26], and the dissociation of oxime hydroxyl groups produces negative ions). After pH > 9, the zeta potential of PAO/CPVA becomes negative because amino groups in amidoxime groups are severely deprotonized and the dissociation degree of oxime hydroxyl groups in amidoxime groups of PAO/CPVA increases.

3.2. Adsorption property and mechanism of PAO/CPVA towards uric acid

3.2.1. Adsorption isotherms of PAO/CPVA microspheres for uric acid

The isothermal adsorption experiments of uric acid were carried out under the same conditions using two kinds of polymeric microspheres, the grafted microspheres PAN/CPVA and functional microspheres PAO/CPVA, respectively, and Fig. 6 presents the adsorption isotherms. It is can be seen clearly from Fig. 6 that the grafted microspheres PAN/CPVA nearly have no adsorption ability for uric acid, whereas the functional microspheres PAO/CPVA possess very strong adsorption ability for uric acid, having a maximum adsorption capacity of 95 mg/g and displaying the high affinity of PAO/CPVA microspheres towards uric acid. The high adsorption affinity should attributes to the hydrogen bonding and electrostatic interaction between PAO/CPVA microspheres and uric acid molecules, and the adsorption mechanism will be further discussed below.

In uric acid molecule, intra-molecular proton transfer reaction can occur [27], and the reaction gives rise to the tautomer, 2,6,8-trihydroxypurine. There is a tautomeric equilibrium between uric acid (purine form) and 2,6,8-trihydroxypurine, and the equilibrium will be induced to move towards certain directions under different conditions. Besides, the three hydroxyl groups in 2,6,8-trihydroxypurine molecule have certain acidity to a certain extent, so there is a ionization equilibrium between 2,6,8-trihydroxypurine and purine-oxygen ion in the aqueous solution, and the equilibrium will move towards different directions under various pH conditions. The above two equilibriums can be expressed in Fig. 7.

It is obvious that the hydrogen bond interaction between the three hydroxyl groups as well as the amino group and imino group in 2,6,8-trihydroxypurine molecule and the oxime hydroxyl groups as well as the amino groups in amidoxime groups of PAO/CPVA will be formed. Furthermore, by inducing of hydrogen bond interaction, the tautomeric equilibrium will move in the direction to produce 2,6,8-trihydroxypurine, and more 2,6,8-trihydroxypurine molecules will be adsorbed on the surfaces of PAO/CPVA microspheres. It is more important that according to the ionization equilibrium of 2,6,8-trihydroxypurine, the negative purine-oxygen ions also exist in the aqueous solution, especially in the aqueous solution with a higher pH value. It is obvious that PAO/CPVA microspheres carrying positive charges with high density will consequentially produce strong electrostatic interaction for the negative purine-oxygen ions. Therefore, PAO/CPVA microspheres will produce strong adsorption action for uric acid by the synergism of electrostatic interaction and hydrogen bonding. Because the zeta potential of PAO/CPVA microspheres is positive and a larger number value in a wider pH range, it can be considered that the electrostatic interaction is the main driving force and it should be principally responsible for the strong adsorption of PAO/CPVA microspheres for uric acid.

3.2.2. Effect of pH value on adsorption action of PAO/CPVA microspheres towards uric acid

The isothermal adsorption experiments of PAO/CPVA for uric acid were carried out in uric acid solutions with different pH values as described in Section 2.3.2, and Fig. 8 gives the saturated adsorption amount as a function of pH value of the solution.

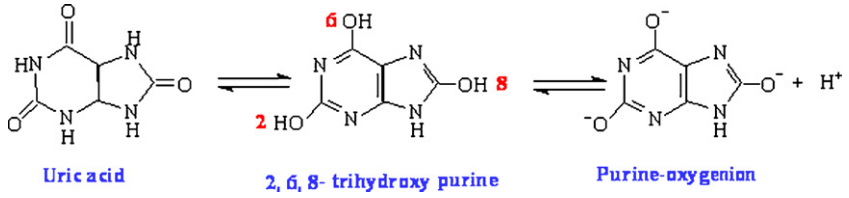

Fig. 7. Tautomeric equilibrium of uric acid and ionization equilibrium of trihydroxypurine.

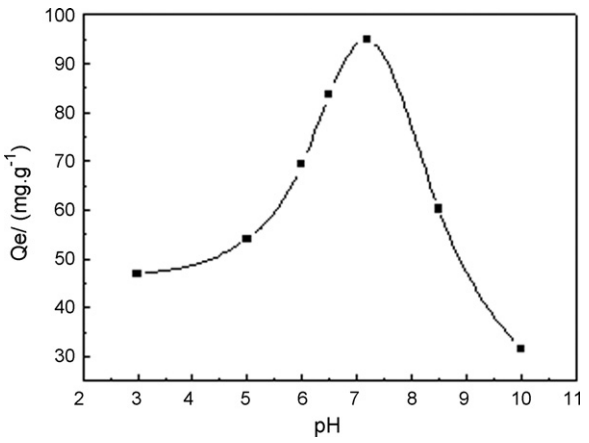

Fig. 8. Effect of pH value on adsorption capacity of PAO/CPVA microspheres for uric acid. Temperature: 30 °C.

It can be found from Fig. 8 that the adsorption capacity of PAO/CPVA for uric acid initially increases and then decreases with the increase of pH value, and a maximum appears at pH 7, implying that in the neutral solution, PAO/CPVA microspheres have the strongest adsorption action for uric acid. The difference of adsorption capacity of PAO/CPVA for uric acid at different pH values is caused by the variation of the action force between them, whereas the action force variation is originated from the change of the exist form of the amidoxime group of PAO/CPVA as well as uric acid molecule at different pH values. The mechanism of the variation of the action force between them is interpreting as follows.

In acidic solution, the most of uric acid molecules exist as purine form, and according to the ionization equilibrium equation shown in Fig. 3, it is nearly impossibly that uric acid molecules exist as purine-oxygen ions. Therefore, the electrostatic interaction cannot be produced between PAO/CPVA microspheres and uric acid. Besides, all N atoms of amino groups in both amidoxime groups and uric acid molecule are protonized, so the hydrogen bonding between PAO/CPVA and uric acid is also weaker. The above two factors result in low adsorption capacity of PAO/CPVA for uric acid in acidic solution. Along with the increase of pH value, the tautomeric equilibrium of uric acid will move gradually in the direction of forming 2,6,8-trihydroxypurine, resulting in the strengthening of the hydrogen bond interaction between PAO/CPVA microspheres and uric acid. It is more important that along with the increase of pH value, the ionization equilibrium of 2,6,8-trihydroxypurine will move gradually in the direction of forming purine-oxygen ion, resulting in the strengthening of electrostatic interaction between PAO/CPVA and uric acid. So here increasing pH value of the medium consequentially leads to the enhancement of the adsorption capacity.

In basic solution, the protonation degree of N atoms of amino groups in the amidoxime groups of PAO/CPVA will be weakened greatly, leading to the gradual decrease of the positive zeta potential of PAO/CPVA microspheres, and or rather leading to the zeta potential to be changed into negative value due to the dissociation of oxime hydroxyl groups in the amidoxime groups of PAO/CPVA. Therefore in basic solution, the electrostatic attraction interaction between PAO/CPVA microspheres and uric acid will probably transformed into electrostatic repulsion interaction, leading to low adsorption capacity of PAO/CPVA for uric acid. By this token, only in neutral solution, the combination of the electrostatic interaction and hydrogen bonding results in strong adsorption action of PAO/CPVA microspheres for uric acid.

From the above experimental facts, the following essentials can be summarized. (1) There are strong interactions between PAO/CPVA microspheres and uric acid molecules, and they are electrostatic interaction and hydrogen bonding. Just these interactions lay a solid foundation for the surface-molecular imprinting of uric acid on the surfaces of PAO/CPVA microspheres. (2) In the neutral solution of pH 7, the interactions between PAO/CPVA microspheres and uric acid molecules are the strongest. (3) It suggests that the optimum pH for uric acid molecule imprinting should be at pH 7, and the imprinted material prepared under this pH condition should have excellent binding performance for uric acid. In the imprinting experiment of Section 2.5, this pH condition was adopted.

3.3. Preparation processes of uric acid-imprinted material MIP-PAO/CPVA microspheres

There is a great deal of hydroxyl groups on the surfaces of the crosslinked polyvinyl alcohol microspheres (CPVA). According to the redox initiation mechanism of cerium salt [28,29], the complex reaction between Ce^{4+} ion and the hydroxyl group on CPVA will first occur, forming a complex [C]. Subsequently, an oxidation process of a single-electron transfer will produce and the complex [C] was disproportionated soon, forming free radicals on the carbon atoms bearing the hydroxyl groups. So the graft-polymerization of monomer AN on CPVA microspheres is initiated, resulting in the grafted microspheres PAN/CPVA. The amidoximation transform reaction of the grafted PAN macromolecules is conducted by the action of hydroxylamine which is produced by the reaction of HAHC with Na_2CO_3, and the grafted PAN is transformed into the grafted poly(amidoxime), resulting in the formation of the functional microspheres PAO/CPVA.

As the adsorption of uric acid on PAO/CPVA microspheres reached saturation, the crosslinking agent glutaraldehyde was added. The Schiff base reaction between the primary amine groups of amidoxime groups of the grafted PAO and glutaraldehyde will be carried out favorably, leading to the crosslinking of PAO macromolecules. As a result, uric acid molecules were enveloped in the crosslinking networks, and the imprinting of uric acid was realized. As the template molecules were washed away, large numbers of uric acid molecule-imprinted caves remained within the thin polymer layer on the surfaces of PAO/CPVA microspheres, thereupon, the uric acid-imprinted material MIP-PAO/CPVA was obtained. The entire preparation processes of MIP-PAO/CPVA are schematically expressed in Fig. 9, and the possible combining model of the grafted PAO macromolecules with uric acid molecule also schematically illustrated in Fig. 9.

In the spectrum of MIP-PAO/CPVA, the band about at 1660 cm^{-1}, which should be ascribed to the vibration absorption of N=C bond in amidoxime as described in Section 3.1.2, has strengthened greatly, and it is caused by the formation of N=C bond in Schiff base reaction of crosslinking process. The spectrum data suggested that the crosslinking and imprinting process has been successfully carried out, and confirmed that uric acid-imprinted material MIP-PAO/CPVA has been formed.

3.4. Binding characteristic of MIP-PAO/CPVA for uric acid

3.4.1. Binding isotherms and dynamics binding curves

The adsorption experiments with the batch method were first performed, and Figs. 10 and 11 give the adsorption isotherms of PAO/CPVA (non-imprinted material) and the binding isotherms of MIP-PAO/CPVA (imprinted material) for uric acid and guanine, respectively. Because the solubility of guanine in water is smaller, in Figs. 10 and 11, the range of the guanine concentration is narrower than that of uric acid.

It can be seen from Fig. 10 that the adsorption capacity of PAO/CPVA for uric acid is greater than that for guanine. The possi-

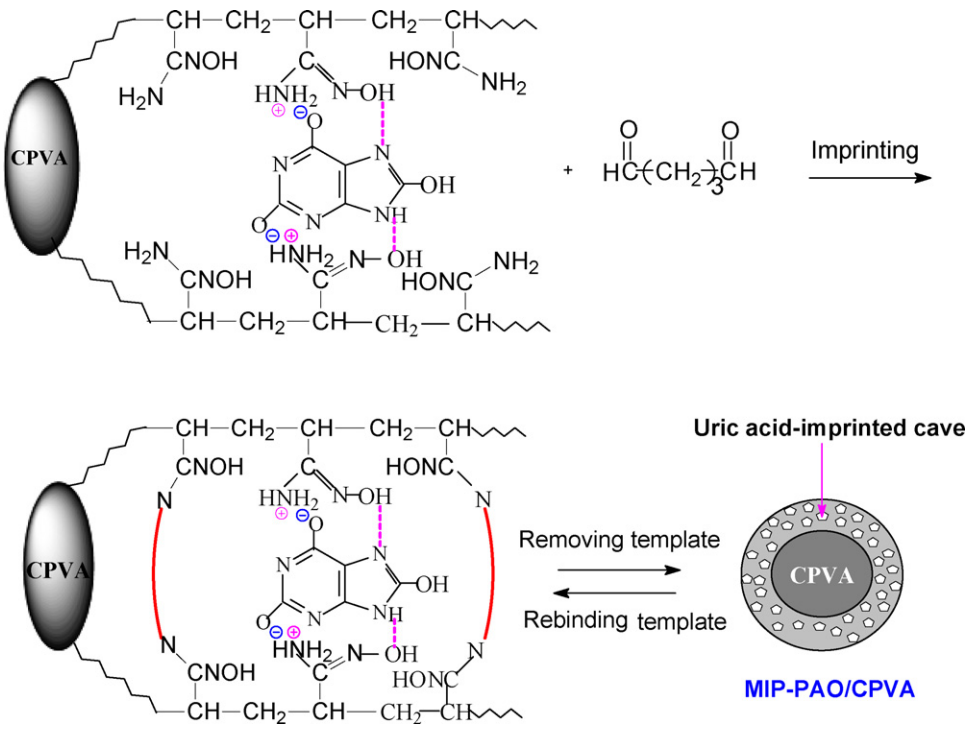

Fig. 9. Schematic expression of preparation process of MIP-PAO/CPVA.

ble reason for this is the electrostatic attraction interaction between PAO/CPVA microspheres and guanine is absent in comparison with uric acid, and it can be estimated from the chemical structures of both uric acid and guanine shown in Fig. 2. However, the adsorption capacity difference of PAO/CPVA for uric acid and guanine is not very notable (95 mg/g for uric acid and 65 mg/g for guanine, respectively), namely, PAO/CPVA microspheres have no obvious adsorption selectivity for uric acid. In contrast, for the imprinted microspheres MIP-PAO/CPVA, a remarkable difference of the binding capacity for the two substances is displayed, as shown in Fig. 11. The maximum binding amount of uric acid is 104 mg/g, whereas for guanine, the corresponding binding amount is only 7.0 mg/g. This fact clearly demonstrates that the uric acid-imprinted material MIP-PAO/CPVA has excellent binding affinity and high recognition selectivity for the template molecule, uric acid. This result also shows clearly that the surface-molecular imprinting of uric acid conducted on the surfaces of PAO/CPVA microspheres is successful. It can be expected with confidence that the uric acid-imprinted material MIP-PAO/CPVA will be a promising solid adsorbent of uric acid in the blood purification treatment because of its biocompatibility and recognition selectivity for uric acid. In regard to the recognition selectivity of MIP-PAO/CPVA, further discussions are as follows.

In order to further investigate the binding characteristic of MIP-PAO/CPVA for uric acid, the adsorption experiments with the column method were also performed. Figs. 12 and 13 display the dynamic adsorption curves of PAO/CPVA and the dynamic binding curves of MIP-PAO/CPVA for uric acid and guanine, respectively.

It can be observed from Fig. 12 that as the solutions of uric acid and guanine with the same concentration (1 mg/mL) flow upstream through the column packed with PAO/CPVA microspheres, respectively, the leaking volume of uric acid (42 BV) is greater than (32 BV) that of guanine, again displaying that the adsorption ability of PAO/CPVA microspheres for uric acid is stronger than that for gua-

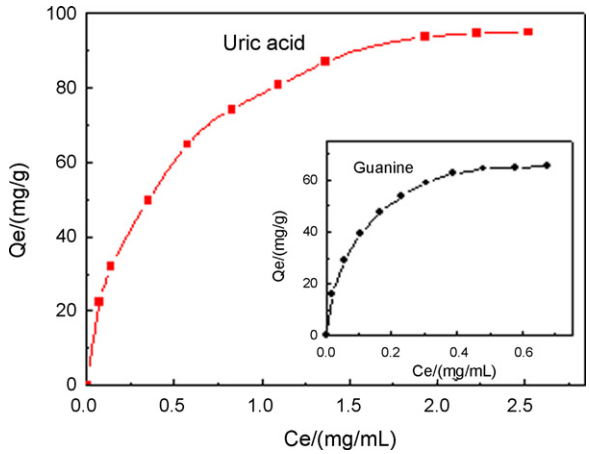

Fig. 10. Adsorption isotherms of PAO/CPVA for uric acid and guanine. Temperature: 30 °C; pH 7

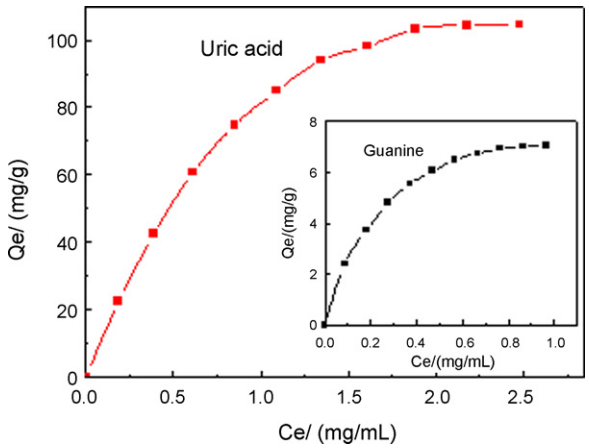

Fig. 11. Binding isotherms of MIP-PAO/CPVA for uric acid and guanine. Temperature: 30 °C; pH 7

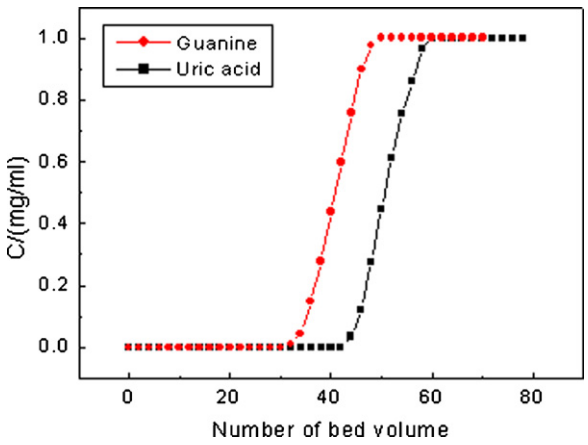

Fig. 12. Dynamic adsorption curves of PAO/CPVA for uric acid and guanine. BV: 2 mL; temperature: 20 °C; initial concentration: 1 mg/mL; flow rate: 4 BV/h

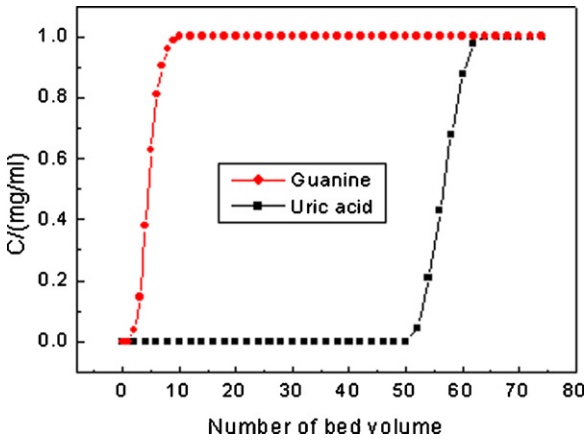

Fig. 13. Dynamic binding curves of MIP-PAO/CPVA for uric acid and guanine. BV: 2 mL; temperature: 20 °C; initial concentration: 1 mg/mL; flow rate: 4 BV/h

nine. However, the difference of their leaking volumes is not very notable. This fact again shows that PAO/CPVA has no adsorption selectivity for uric acid. In contrast, it is observed in Fig. 13 that the dynamic binding curve of MIP-PAO/CPVA for uric acid is obviously different from that for guanine. The leaking volume of uric acid is 52 BV and far greater than that of guanine (only about 3 BV). By calculating, the leaking and saturated adsorption amounts of uric acid are 96.16 mg/g and 123.08 mg/g, respectively, whereas for guanine, they are only about 5.77 mg/g and 15.38 mg/g, respectively. Obviously, MIP-PAO/CPVA nearly does not recognize and does not bind guanine molecules. In contrast, it has excellent recognition selectivity for uric acid, and it will be further discussed below.

3.4.2. Recognition selectivity of MIP-PAO/CPVA for uric acid

Competitive adsorption experiments of MIP-PAO/CPVA microspheres were conduced in a binary solution of uric acid and guanine. In Table 1, the data of the distribution coefficients K_d, selectivity coefficients k and relative selectivity coefficients k' are summarized.

From the data in Table 1, the following facts can be found. (1) The selectivity coefficient of the non-imprinted material, PAO/CPVA, for uric acid in relation to guanine is very low, and is only 1.273. This implies that the adsorption abilities of PAO/CPVA for the two substances, uric acid and guanine, are approximate and the adsorption selectivity is absent. (2) The selectivity coefficient of MIP-PAO/CPVA for uric acid with respect to guanine is remarkably enhanced, and gets up to 14, displaying a very high recognition selectivity of MIP-PAO/CPVA for uric acid. (3) The relative selectivity coefficients of MIP-PAO/CPVA is 10.99, indicating a remarkable enhancement of the adsorption affinity and selectivity of the imprinted material MIP-PAO/CPVA for the template molecule, uric acid, in relation to non-imprinted material PAO/CPVA. The above facts again clearly reveal that MIP-PAO/CPVA has high recognition selectivity and binding affinity for uric acid.

The reason for the above facts can be explained as follows. Although guanine is also a purine derivative like as uric acid, the functional groups contained in its molecule are different from that in uric acid molecule as shown in Fig. 2, so that the uric acid-imprinted cavities within the thin polymeric layer of PAO on the surfaces of MIP-PAO/CPVA microspheres are not matched with guanine molecules in size, shape and spatial arrangement of action sites. Obviously, the high recognition selectivity and excellent binding ability of MIP-PAO/CPVA for uric acid come from a mass of the suited imprinted caves for uric acid within the thin polymeric layer on the surfaces of the microspheres.

3.4.3. Relationship between recognition property of MIP-PAO/CPVA and reactant ratio during imprinting

It is well known that for conventional imprinting method, there is no a certain relationship between the added amount of the functional monomer and the added amount of the crosslinker, namely, there is no a certain feed ratio of the monomer to the crosslinker. However, for the novel surface imprinting method, there is a optimal reactant ratio of the amidoxime chain unit of the grafted functional macromolecule PAO to the crosslinker, and under the optimal feed ratio condition [30], the prepared imprinted material will have the best re-binding behavior for the template molecule. This suggests that in this surface imprinting system, there is a quantificational relationship among the grafted functional macromolecule (chain unit)-template molecule-crosslinker molecule [30]. This is a significant findings and it profoundly reflects the intrinsic scientificity of this novel surface imprinting process.

In the preparation process of MIP-PAO/CPVA, the crosslinking reaction occurs between the terminal aldehyde groups of glutaraldehyde and the amino groups in amidoxime groups of PAO/CPVA. By varying the molar ratio of the amidoxime groups of PAO/CPVA to glutaraldehyde, in whose molecule there are two terminal aldehyde groups, the crosslinking and imprinting process was performed. The selectivity coefficient of MIP-PAO/CPVA as a function of the molar ratio of amidoxime (AO) group to glutaraldehyde is displayed in Fig. 14. It can be found that as the ratio of n (AO): n (glutaraldehyde) is equal to 3:1, the selectivity coefficient of MIP-PAO/CPVA is the greatest and it implies

Table 1
Distribution coefficient and selectivity coefficient data.

Adsorbent material	PAO/CPVA		MIP-PAO/CPVA	
	Uric acid	Guanine	Uric acid	Guanine
K_d (mL/g)	107.361	84.324	112.013	7.999
k	1.273		14.004	
k'	10.99			

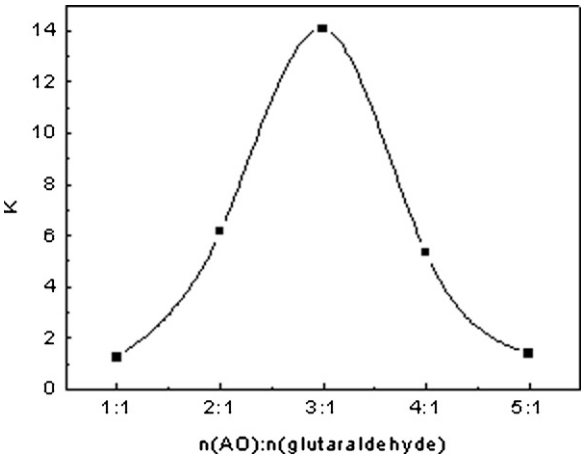

Fig. 14. Selectivity coefficient as a function of molar ratio of amidoxime group to glutaraldehyde. Temperature: 50 °C; pH 7

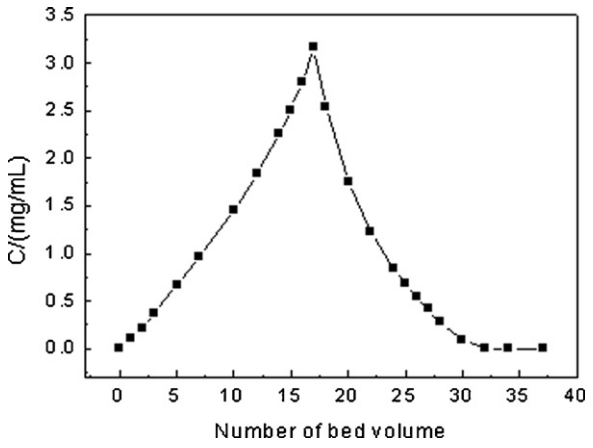

Fig. 15. Elution curve of uric acid on MIP-PAO/CPVA column. Temperature: 20 °C.

that at this reactant ratio, the imprinting result is the best. This fact is closely correlative with the number of average action sites between the grafted PAO chains and uric acid molecules. A possible interaction mode can be observed from Fig. 9. Two amidoxime groups on two polymeric chains interact with one uric acid molecule by right of electrostatic and hydrogen bond interactions including four interaction sites. As a result, two glutaraldehyde molecules and six amidoxime groups are needed for imprinting one uric acid molecule. Among the six amidoxime groups, two interact physically with one uric acid molecule as described above, whereas other four amidoxime groups are reacted chemically with two glutaraldehyde molecules as crosslinker. So in the optimal crosslinking and imprinting system, the molar ratio of amidoxime group to glutaraldehyde should be 3:1. Therefore, at this reactant ratio, the prepared MIP-PAO/CPVA should have the most imprinted caves, and have the best recognizing selectivity for uric acid. The experimental result shown in Fig. 14 is completely consistent to the mode of intermolecular interaction speculated above.

As the reactant ratio is greater than 3:1, the crosslinking agent is lacking, leading to less imprinting caves inside the polymer layer on the surfaces of MIP-PAO/CPVA microspheres and poorer recognizing ability of MIP-PAO/CPVA for uric acid. Whereas as the reactant ratio is smaller than 3:1, namely, as the crosslinking agent is overmuch, greater crosslinking degree will be produced. As a consequence, the uric acid molecules adsorbed on the functional microspheres PAO/CPVA will be pushed aside partially, and less imprinted caves inside the polymer layer also will be led to. By this token, in this new surface imprinting system, it seems that there are definite average interaction sites between the template molecule and the grafted functional polymer. Only when the amount of the crosslinking agent is matched with the number of the average interaction sites, a good imprinting result can be obtained. This is greatly different from the conventional imprinting method.

3.5. Elution property of MIP-PAO/CPVA

An aqueous NaOH solution of 0.1 M was used as the eluent. The eluent upstream passes through the column packed with MIP-PAO/CPVA adsorbing uric acid in a saturated state. The dynamic desorption curve is given in Fig. 15. It can be seen in Fig. 15 that the desorption curve is cuspidal and without trailing formation. By calculating, the desorption ratios in 26 BV and in 30 BV reach 96.8% and 99.1%, respectively. These desorption data indicate that the uric acid molecules combined on MIP-PAO/CPVA microspheres are easy to be desorbed or eluted, namely, MIP-PAO/CPVA microspheres have excellent eluting property. This implies that the surface imprinted material MIP-PAO/CPVA is easy to be recovered and reused.

4. Conclusions

In this paper, uric acid-imprinted material MIP-PAO/CPVA with high performance has been prepared successfully with CPVA microspheres as the matrix microspheres and with the novel surface-molecular imprinting technique put forward by us. The imprinted microspheres MIP-PAO/CPVA not only have high binding affinity and specific recognition selectivity for the template, uric acid, but also have biocompatibility probably owing to the biocompatibility of the matrix microspheres CPVA. Therefore, it can be expected with reason that the uric acid-imprinted material MIP-PAO/CPVA will be a promising uric acid solid adsorbent in the blood purification treatment. It can be anticipated that other medical materials used in the blood purification can be prepared by using the novel surface imprinting technique and using those polymeric microspheres with biocompatibility and blood compatibility as the matrix microspheres. As long as there are strong intermolecular interactions between the functional macromolecules grafted onto the surfaces of the matrix microspheres and template molecules, the surface-molecular imprinting process can be favorably carried out by employing a special crosslinker, in whose molecule there are two reactive end groups. The research results of this work have supplied a new clew and theoretical reference for preparing the blood purification materials with high performance.

References

[1] S.-G. Wu, T.-L. Wang, Z.-Y. Gao, H.-H. Xu, B.-N. Zhou, C.-Q. Wang, Biosens. Bioelectron. 23 (2008) 1776.
[2] D. Yao, A.G. Vlessidis, N.P. Evmiridis, Anal. Chim. Acta 478 (2003) 23.
[3] J.-C. Chen, H.-H. Chung, C.-T. Hsu, D.-M. Tsai, A.S. Kumar, J.-M. Zen, Sens. Actuators B 110 (2005) 364.
[4] D. Sun, Y. Zhang, F.-R. Wang, K.-B. Wu, J.-W. Chen, Y.-K. Zhou, Sens. Actuators B 141 (2009) 641.
[5] H. Kutsuki, Biophys. Acta 1753 (2005) 141.
[6] D.F. Stamatialis, B.J. Papenburg, M. Gironés, S. Saiful, S.N.M. Bettahalli, S. Schmitmeier, M. Wessling, J. Membr. Sci. 308 (2008) 1.
[7] V.A. Davankov, L.A. Pavlova, M.P. Tsyurupa, D.R. Tur, J. Chromatogr. A 689 (1997) 117.
[8] P. Tomme, A. Boraston, B. McLean, J. Kormos, A.L. Creagh, K. Sturch, N.R. Gilkes, C.A. Haynes, R.A.J. Warren, D.G. Kilburn, J. Chromatogr. B 715 (1998) 283.
[9] C.-J. Liu, X.-Y. Liang, X.-J. Liu, Q. Wang, L. Zhan, Appl. Surf. Sci. 254 (2008) 6701.
[10] V. Wernert, O. Schäf, H. Ghobarkar, R. Denoyel, Micropor. Mesopor. Mater. 83 (2005) 101.
[11] V. Davankov, L. Pavlova, M. Tsyurupa, J. Brady, M. Balsamo, E. Yousha, J. Chromatogr. B 739 (2000) 73.
[12] W. Luo, L.-H. Zhu, C. Yu, H.-Q. Tang, H.-X. Yu, X. Li, X. Zhang, Anal. Chim. Acta 618 (2008) 147.
[13] X.-Z. Shi, A.-B. Wu, G.-R. Qu, R.-X. Li, D. Zhang, Biomaterials 28 (2007) 3741.

[14] F.G. Tamayo, E. Turiel, A. Martín-Esteban, J. Chromatogr. A 1152 (2007) 32.
[15] V. Pichon, F. Chapuis-Hugon, Anal. Chim. Acta 622 (2008) 48.
[16] D. Silvestri, N. Barbani, C. Cristallini, P. Giusti, G. Ciardelli, J. Membr. Sci. 282 (2006) 284.
[17] W.-C. Lin, D.-G. Yu, M.-C. Yang, Colloids Surf. B 47 (2006) 43.
[18] H.-T. Li, Y.-M. Zhang, X.-F. Chen, K.-Y. Shi, Z. Yuan, B. Liu, B. Shen, B.-L. He, React. Funct. Polym. 58 (2004) 53.
[19] B.-J. Gao, J.-H. Lu, Z.-P. Chen, J.-F. Guo, Polymer 50 (2009) 3275.
[20] H.-M. Liu, C.-H. Liu, X.-J. Yang, S.-J. Zeng, Y.-Q. Xiong, W.-J. Xu, Anal. Chim. Acta 628 (2008) 87.
[21] J. Otero-Romanía, A. Moreda-Piñeiroa, P. Bermejo-Barrera, A. Martin-Esteban, Anal. Chim. Acta 630 (2008) 1.
[22] B.-J. Gao, F.-Q. An, Y. Zhu, Polymer 48 (2007) 2288.
[23] B.-J. Gao, J. Wang, F.-Q. An, Q. Liu, Polymer 49 (2008) 1230.
[24] B.-J. Gao, P.-F. Jiang, H.-B. Lei, Mater. Lett. 60 (2006) 3398.
[25] A. Ersöz, R. Say, A. Denizli, Anal. Chim. Acta 502 (2004) 91.
[26] F. Eloy, R. Lenaers, Chem. Rev. 62 (1962) 155.
[27] W.K. Chen, C.H. Lu, J. Xu, Y.C. Yang, J.Q. Li, Struct. Chem. 2 (2002) 186 (in Chinese).
[28] H. Arslan, B. Hazer, Eur. Polym. J. 35 (1999) 1451.
[29] C. Yagci, U. Yildiz, Eur. Polym. J. 41 (2005) 177.
[30] B.-J. Gao, J. Wang, Y. Yang, Chromatographia 69 (2009) 1062.