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Designing and Preparation of Ferulic Acid Surface-imprinted Material

and its Molecular Recognition Characteristics

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Abstract Ferulic acid (FA) is a phenolic acid with styrene-type, and it has many important bioactivity and

pharmacological function. In this work, for the presence of a polymerizable double bond in FA molecule,

FA surface-imprinting was realized successfully by adopting the new surface imprinting technology of

"pre-graft polymerizing and post-crossling/imprinting". Dimethylaminoethyl methacrylate (DMAEMA)

was first graft-polymerized onto the surfaces of micron-sized silica gel particles in the manner of surface

initiated graft-polymerization, obtaining the grafted particles PDMAEMA/SiO$_2$, and then FA

surface-imprinting was successfully carried out with dibromohexane as crosslinker, obtaining FA

surface-imprinted material MIP-PDMAEMA/SiO$_2$. This imprinting process completely avoided the

problem of taking part in the polymerization of the polymerizable double bond in FA molecule. The

binding and recognition characteristics of MIP-PDMAEMA/SiO$_2$ particles towards FA were investigated

with batch method, column method and competitive adsorption method. The experimental results show that

in acidic solution, there are strong interactions between PDMAEMA/SiO$_2$ particles and FA molecule, and

they are electrostatic interaction as main driving force, cation-π interaction as well as hydrogen bonding,

constituting a supermolecule system.   On this basis, FA surface-imprinting was smoothly performed. The

surface-imprinted particles MIP-PDMAEMA/SiO$_2$ have special recognition selectivity and excellent

binding affinity for FA. The selectivity coefficients of MIP-PDMAEMA/SiO$_2$ particles for FA relative to

chlorogenic acid and caffeic acid, which were used as two contrast phenolic acid compounds, are 6.47 and

2.75, respectively. The binding capacity of MIP-PDMAEMA/SiO$_2$ particles for FA reaches up to 142 mg/g.

MIP-PDMAEMA/SiO$_2$ particles still have excellent elution property, and it is convenient for their reusing.

Keywords Dimethylaminoethyl methacrylate; Ferulic acid; Molecule surface-imprinting; Surface-initiated

graft-polymerization; Molecule recognition

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1. Introduction

Molecular imprinting is a technology to elaborately set up recognition sites in polymer matrix. A great deal of caves that are highly matched with template molecule in size, shape and chemical functionality is distributed within the molecularly imprinted polymers (MIPs), and so MIPs have specific molecular recognition ability and high binding affinity for template molecules. Therefore, MIPs are described as artificial antibodies or receptors [1-4], and so MIPs are a group of biomimetic substances. MIPs can specifically recognize and bind the template molecule at the molecular level from mixed systems. Such characteristic of MIPs let them have very important applications in many areas of science and technology. Especially, in the various areas involving the recognition, separation, purification, enrichment and removal of substances from the mixtures, the study on MIPs has received an astonishing amount of attention [5-9], and the molecular imprinting solid-phase extraction (MISPE) technology has been developed greatly.

Phenolic acids are a large class of natural compounds with many bioactivities and pharmacological activities, whereas ferulic acid (4-hydroxy-3-methoxycinnamic acid) is considered as one of the most important phenolic acids. It belongs to cinnamic acid-type phenolic acids or styrene-type phenolic acids because there is a polymerizable double bond in its molecule like as that in styrene molecule. Ferulic acid (FA) exhibits beneficial physiological effects such as antioxidant, anti-microbial, anti-thrombosis and anti-cancer activities [10-13]. Thus, it is widely used in food, pharmaceutical and cosmetic industries. Ferulic acid is present in many crop residues such as wheat bran (0.5%), sugar-beet pulp (0.9%) and corn kernel (5%), and so [14,15]. FA is cross-linked with lignin and polysaccharides via ester and ether bonds, forming lignin/phenolics–carbohydrate complexes in crop residues, and it can release by alkaline hydrolysis with dilute NaOH solution so as to form the crude extracts. However, the separation and

purification of FA from the alkaline extracts is challenging. The composition of the crude extracts is very

complicated, and so the separation of ferulic acid is very difficult. For the separation and purification of FA

from the crude extracts, some solid adsorbents such as active charcoal, macroporous resin and anion

exchange resin were used [16-18]. However, all of these solid adsorbents exists a common weakness, and it

is that their adsorptions are without selectivity, leading low separation efficiency. Up to now, MIPs are the

solid adsorption materials with the best adsorption selectivity, and if MIPs are introduced into the

extraction and separation of phenolic acid, the separation efficiency of these natural compounds will be

improved greatly. Unfortunately, up to now, FA-imprinted materials have not been reported.

The traditional method to prepare MIPs is entrapment way. The monomer, crosslinking agent and

template molecule are dissolved in the solvent, and by polymerization, monolithic crosslinked polymers are

obtained. And then by crushing, grounding and sieving, fine particles with appropriate size are acquired so

as to suit for use. During the crushing and grounding process, a great deal of the imprinted caves is

destroyed, and besides, the matrix is thick and the pore canal is deep within the imprinted particles.

Consequently, some serious drawbacks are led to, such as small binding capacity, poor site accessibility,

greater diffusion resistance and slow mass transfer, and these drawbacks severely limit the applications of

MIPs [19-21]. In order to overcome the above drawbacks, in recent years, molecule surface-imprinting

technology has been developed greatly, and researchers try to constitute imprinted caves on the surfaces of

solid particles or membranes [22-24]. Our group also contributes our effort to the development of the

surface-imprinting technique, and a new surface imprinting technique of "pre-graft polymerizing and

post-crossling/imprinting" was put forward. Based on this method, various MIPs with high performance

were successively prepared [25-28].

As described above, there is polymerizable double bond in the molecule structure of FA, whose

structure is shown in Scheme 1 below, and so for the molecule imprinting of FA, the general

polymerization methods cannot be adopted. It is an important reason for that the FA-imprinted polymers

as well as that of other compounds containing polymerizable double bond are seldom reported so far.

However, the "pre-graft polymerizing and post-crossling/imprinting" method of ours is just well fits to the

molecule imprinting of FA, and the above difficult for FA imprinting can be effectively overcome. In this

work, an elaborate molecular made could make us to use the new surface imprinting technique of "pre-graft

polymerizing and post-crossling/imprinting" for realizing the imprinting of FA. Ternary amine monomer

dimethylaminoethyl methacrylate (DMAEMA) was first graft-polymerization on the surfaces of

micron-sized silica gel particles, and then the grafted macromolecule PDMAEMA produced strong

adsorption action for FA molecule by right of the strong electrostatic interaction and cation-π interaction as

well as hydrogen bonding, constituting a supramolecular system. Finally by using dibromohexane as

crosslinker, FA imprinting was realized. That way, the problem that the polymerizable double bond in FA

molecule would take part in the polymerization during the imprinting process was completely avoided, and

the FA imprinted material with high performance was prepared successfully, fully displaying the

superiority of the new surface imprinting technique of "pre-graft polymerizing and

post-crossling/imprinting". The imprinted material has excellent molecular recognition characteristic for

FA molecule, and is good a material of solid phase extraction of FA from the crude extraction. To our

knowledge, this is the first report on the molecular imprinting of the compounds containing polymerizable

double bond, and the similar study has not been reported.

The research result in this work has important scientific significance and application value for

preparing surface-imprinted materials of phenolic acid compounds with styrene-type as well as that of other

compounds, in whose molecules polymerizable doubles are contained, and can effectively accelerate the

extraction and separation of natural pharmacologic active substances from natural plant extracts.

2. Experiments

2.1. Materials and instruments

Silica (120～160 mesh, about 125 μm in diameter,300-450 m^2/g of surface area) were obtained from Qingdao Ocean Chemical Limited Company (China); γ-Aminopropyltrimethoxysilane (AMPS) was supplied by Nanking Chuangshi Chemical Aux Ltd.(China); Dimethylaminoethyl methacrylate (DMAEMA) was purchased from JiangsuFeixian chemical Co., Ltd. (China), and was purified by vacuum distillation before use; Ferulic acid, chlorogenic acid and caffeic acid were supplied by Shaanxi Luqing bio-engineering Limited company (China); Dibromohexane was obtained from Shanghai Zhixin Chemical Co., Ltd. (China); Glacial acetic acid (with a purity of 98%) and methanol were purchased from by Tientsin University Chemical Reagent Plant (China), and they were prepared to form a mixed solution in a volume of 1:9 proportions to be used an eluent of the template ferulic acid molecules; Other reagents were all commercial chemicals with analytical pure and purchased from Chinese companies.

The instruments used in this study were as follows: Unic-2602 UV/Vis spectrophotometer (Unic Company, Shanghai); Perkin-Elmer 1700 infrared spectrometer (Perkine-Elmer Company, USA), LEO-438VP scanning electronic microscope (SEM, LEO Company, UK); Zetasizer Nano-Zeta potential analyzer (Malvern Instrument Company, UK); PHS-2 acidimeter (The Second Analytical Instrument Factory, Shanghai, China); THZ-92C constant temperature shaker equipped with gas bath (Boxun Medical Treatment Equipment Factory, Shanghai, China); STA449 thermogravimetry analyzer(TGA, Netzsch Company, German), air atmosphere, a heating rate of 10℃/min.

2.2. Preparation and characterization of grafted particles PDMAEMA/SiO$_2$

The grafted particles PDMAEMA/SiO$_2$ was prepared by using the surface-initiating method [29],

and the main procedure is described as follows. The silica gel particles were surface-modified with the

coupling agent AMPS, obtaining modified particles AMPS-SiO$_2$, on whose surface primary amino groups

-NH$_2$ were contained. The modified particles AMPS-SiO$_2$ were added into the aqueous solution containing

monomer DMAEMA, crosslinker and initiator (NH$_4$)$_2$S$_2$O$_8$, and a surface initiating system of -NH$_2$/S$_2$O$_8^{2-}$

was constituted. Free radicals were produced on the surfaces of the modified particles AMPS-SiO$_2$, and the

graft-polymerization of DMAEMA was carried out under N$_2$ atmosphere and at a constant temperature of

35℃ for 6 h, resulting in the grafted particles PDMAEMA/SiO$_2$. Their FTIR was determined with KBr

pellet method. The grafting degree of PDMAEMA was determined by TGA like as that in Ref. [29], and it

was 24 g/100g. The zeta potentials of the grafted particles PDMAEMA/SiO$_2$ at different pH values were

recorded with a potential analyzer, and the zeta potential curve was plotted.

2.3. Preparation and characterization of FA surface-imprinted material MIP-PDMAEMA/SiO$_2$

The grafted particles of PDMAEMA/SiO$_2$ of about 0.2 g weighted accurately were placed into an

aqueous FA solution with a concentration of 5mmol/L and with pH 4, and the adsorption of the grafted

particles for FA was conducted in a constant temperature oscillator for 4 h, and the adsorption was made to

reach saturation. The resultant particles were collected by filtering, and dried under vacuum. Such particles

were placed into an aqueous FA solution with a concentration of 5 mmol/L and with pH=4 to avoid

desorption, and 2.2 mL of bromohexane was added. The crosslinking reaction between the grafted

macromolecules PDMAEMA was performed at 40 ℃ with stirring for 8 h. After finishing the reaction, the

resultant particles were washed repeatedly with a mixed solution of acetic acid and methanol (V:V=1:9) to

remove the template FA molecules, finally washed with distilled water, and dried under vacuum. The

obtained particles were namely the FA surface-imprinted particles MIP-PDMAEMA/SiO$_2$. The FTIR of the

imprinted particles was determined with KBr pellet method to confirm the structure change, and their

morphology was observed by SEM.

For comparison, non-imprinted particles NMIP-PDMAEMA/SiO₂ were also prepared under the same condition only in absence of FA, namely the grafted macromolecules PDMAEMA of PDMAEMA/SiO₂ particles that did not adsorb FA were crosslinked, obtaining particles NMIP-PDMAEMA/SiO₂.

2.4. Investigating recognition and binding characteristics of MIP-PDMAEMA/SiO₂ particles for FA

2.4.1. Evaluating binding property of MIP-PDMAEMA/SiO₂ particles with static method

FA solutions with different concentrations were prepared in a range of 1-5 mmol/L, and their pH values were adjusted to pH=4 with diluted HCl solution. FA solutions of 15 mL with different concentrations were placed into a series of conical flasks with cover, and 0.02 g of MIP-PDMAEMA/SiO₂ particles weighted accurately was added into these solutions, respectively. These mixtures were shaken in a constant temperature oscillator for 4 h (The adsorption kinetic experiment was first conducted, and the adsorption equilibrium time was determined as 4 h) and the binding process was allowed to reach equilibrium. By standing, the supernatants were taken and their concentrations were determined by spectrophotometry at 310 nm. The equilibrium binding amount of FA, Q_e (mmol/g), was calculated according to Eq. (1), and the isothermal binding curve was plotted.

$$Q_e = \frac{(C_0 - C_e)V}{1000m} \qquad (1)$$

where C_0（mmol/L）and C_e（mmol/L）are the initial and equilibrium concentration of FA, respectively, V (mL) is the volume of FA solution, and m (g) is the mass of the used adsorbent MIP-PDMAEMA/SiO₂ particles.

Chlorogenic acid (CHA) is a depside, and it can be formed by esterification reaction between caffeic acid and quinate. CHA is also a native compound with many biological activities, and there exists

the similarities with certain degree between the chemical structures of CHA and FA. Caffeic acid (CA) is also a phenolic acid with pharmacological activity, and its chemical structure is very similar to that of FA. Therefore, in this work, CHA and CA were selected as two contrast substances to investigate the molecular recognition characteristics of MIP-PDMAEMA/SiO$_2$ particles for FA. The chemical structures of the three phenolic acid compounds are presented in Scheme 1. By using the same method described above, the isothermal binding experiments of MIP-PDMAEMA/SiO$_2$ particles for CHA and CA were performed, respectively, and their isothermal binding curves were also plotted. The concentrations of CHA and CA in the supernatants were also determined by spectrophotometry, and the characteristic absorption wavelengths were at 322 nm for CHA and at 286 nm for CA, respectively.

Scheme 1

2.4.2. Evaluating binding property of MIP-PDMAEMA/SiO$_2$ particles with dynamic method

At room temperature, MIP-PDMAEMA/SiO$_2$ particles (about 1.18 g) were 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 FA solution with a concentration of 5 mmol/L (pH=4) was allowed to flow gradually through the packed column at a rate of five bed volumes per hour (5 BV/h) in countercurrent manner. The effluents with three volumes (3 BV) interval were collected, and the concentrations of FA in these effluents were determined. The dynamic binding curve was plotted, and the break binding amount and saturated binding amount were calculated with the data of the concentrations and bed number of these effluents. The dynamic binding curves of MIP-PDMAEMA/SiO$_2$ particles towards two contrast substances were also determined with the same method.

2.4.3. Experiments of binding selectivity

Two binary mixed solutions, FA/CHA and FA/CA, were prepared, and in the two solutions, the

concentration of each component was the same, 2 mmol/L. The two solutions of 15 mL were placed into

two conical flasks with cover, followed by adding 0.02 g of the MIP-PDMAEMA/SiO$_2$ particles weighted

accurately. The two systems were shaken in a constant temperature oscillator for 4 h. After standing and

layering, the concentrations of various components in the two supernatants were determined by dual

wavelength UV spectrophotometry, and the distribution coefficient for each substance in the two systems

was calculated according to Eq. (2) [30,31].

$$K_d = \frac{Q_e}{C_e} \qquad (2)$$

where K_d represents the distribution coefficient (L/g) of a substance; Q_e (mmol/g) is the equilibrium

binding quantity of this substance; Ce (mmol/L) is the equilibrium concentration of this substance.

The selectivity coefficient k of MIP-PDMAEMA/SiO$_2$ particles for FA relative to the two

competition species, CHA and CA (assigned as B), can be obtained from the distribution coefficient data

according to Eq. (3) [30, 31]. The value of k marks the recognition selectivity of MIP-PDMAEMA/SiO$_2$ for

the template FA, and it is one of the most important parameters of imprinted materials.

$$k = \frac{K_d(\text{FA})}{K_d(B)} \qquad (3)$$

where k is the selectivity coefficient of MIP-PDMAEMA/SiO$_2$ particles for FA, and B represents CHA or

CA.

2.5. Desorption experiment

MIP-PDMAEMA/SiO$_2$ particles with a given amount, on which the adsorption of FA had reached

saturation, were packed into a piece of glass pipe with an internal diameter of 10 mm, and the bed volume

(BV) of the packed column was 2 mL. A mixed solution of acetic acid and ethanol (V:V=1:9) was used as

eluent and was allowed to upstream flow through the column at a rate of four bed volumes per hour

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(4BV/h), and the desorption of FA was made to occur. The effluents with one-bed volume interval were collected, and the concentrations of FA in these effluents were determined by spectrophotometry. The dynamic desorption curve was plotted, and the elution property of FA on MIP-PDMAEMA/SiO$_2$ particles was estimated.

3. Results and Discussions

3.1. Chemical structure of grafted particles PDMAEMA/SiO$_2$ and interaction between them and FA

3.1.1. Chemical process to prepare grafted particles PDMAEMA/SiO$_2$

In this work, surface-modification of silica gel particles was carried out with the coupling agent AMPS as reagent, obtaining the modified particles AMPS-SiO$_2$, on which primary amino groups, –NH$_2$, were contained. A surface-initiating system, –NH$_2$/S$_2$O$_8$, was constituted, and free radicals were produced on the surfaces of the particles [29]. These free radicals initiated the monomer DMAEMA to produce graft-polymerization, resulting in the grafted particles PDMAEMA/SiO$_2$. In the infrared spectrum of PDMAEMA/SiO$_2$ particles (see Fig. 4 below), the stretching vibration absorption of the carbonyl group of the ester group appears obviously at 1732 cm^{-1}, and the vibration absorption of the C-N bond appears at 1395 cm^{-1} as compared with the spectrum of SiO$_2$, fully demonstrating that the graft-polymerization of DMAEMA on the surfaces of silica gel particles has been realized, and the grafted particles PDMAEMA/SiO$_2$ have been obtained. The entire chemical process to preparing the grafted particles PDMAEMA/SiO$_2$ is shown schematically in Scheme 2.

Scheme 2

3.1.2. Surface electrical property of grafted particles PDMAEMA/SiO$_2$

Fig. 1 gives the zeta potential curves of the grafted particles PDMAEMA/SiO$_2$ and SiO$_2$ particles.

From Fig. 1, the following facts can be seen. (1) In a greater range of pH, the zeta potential of SiO$_2$

particles is negative. (2) However, in a greater range of pH, the zeta potential of the grafted particles

PDMAEMA/SiO$_2$ is positive, and at pH 4, there is a maximum value of about 45 mV. The positive zeta

potential of the grafted particles comes from the protonated N atoms in the DMAEMA units of the grafted

macromolecules PDMAEMA, and here PDMAEMA becomes a cationic polyelectrolyte, implying that the

grafted particles PDMAEMA/SiO$_2$ carry negative charge in acidic solution. (3) After pH 4, the zeta

potential declines with the increase of pH value because of the deprotonation of N atoms of the grafted

macromolecule PDMAEMA.

Fig. 1

3.1.3. Strong adsorption action of PDMAEMA/SiO$_2$ particles for FA

It is a key issue for the molecule imprinting system that there exists strong interaction between

host-guest, i.e., between functional monomer or functional polymer and template molecule. For this work,

the "pre-graft polymerizing and post-crossling/imprinting" method was used to prepare FA-imprinted

particles, and so the interaction between the grafted particles PDMAEMA/SiO$_2$ and FA was first studied.

The isothermal adsorption experiment of the grafted particles for FA was conducted, and the adsorption

isotherm at pH 4 is presented in Fig. 2.

Fig. 2

It can be seen from Fig.2 that the adsorption ability of silica gel particles for FA is very poor, or silica gel particles nearly do not adsorb FA molecule. However, the grafted particles $PDMAEMA/SiO_2$ have very strong adsorption ability for FA in an acidic solution, and the adsorption capacity reaches up to 0.74 mmol/g (144 mg/g), suggesting that there exist strong interaction between the grafted particles $PDMAEMA/SiO_2$ and FA. In an acidic solution, the grafted particles $PDMAEMA/SiO_2$ carry positive charge because of the protonation of N atoms of the grafted macromolecule PDMAEMA as described above and indicated in Fig.1. The carboxyl groups in FA molecule can ionize basically in a acidic solution of pH=4 (the ionization constant of cinnamic acid as the precursor of FA is in a range of pH 4.37-4.44, and it can be taken as a reference), and so strong electrostatic interaction between the grafted particles $PDMAEMA/SiO_2$ and FA inevitably is produced. Besides, cation-π interaction between benzene ring of FA and the protonated N atoms of the grafted macromolecule PDMAEMA will be also produced. Perhaps there is still hydrogen bond interaction between the phenolic hydroxyl group of FA and the ternary amino group of PDMAEMA. Obviously, there exists the synergy of the three interactions between the host-guest. It is the synergy that leads to very strong adsorption ability of the grafted particles for FA molecule, and this is the fundamental basis of imprinting FA on the surfaces of the grafted particles $PDMAEMA/SiO_2$. Among the three interactions, the electrostatic interaction is main driving force. The interaction model of $PDMAEMA/SiO_2$ with FA molecule is schematically shown in Scheme 3 (see below). It is the strong secondary bond forces between $PDMAEMA/SiO_2$ particles and FA molecules that lay a sufficient foundation for realizing surface-imprinting of FA molecule by adopting the novel surface imprinting method of "pre-grafting and post-crosslinking/imprinting".

In order to determine the optimum pH value for surface-imprinting, the isothermal adsorption experiments of the grafted particles $PDMAEMA/SiO_2$ for FA at different pHs were conducted, and Fig. 3

gives the saturated adsorption amount as function of pH value.

Fig. 3

It can be observed that the adsorption capacity of the grafted particles PDMAEMA/SiO$_2$ for FA first

increases and then decreases with the increase of pH value, and at pH 4, there is a maximum adsorption

capacity. This change rule reflects the variation of the interaction between host-guest with pH value of the

medium. At lower pH, the protonation degree of the N atoms of the grafted macromolecule PDMAEMA is

high, whereas the ionization degree of the carboxyl groups of FA is low but increases gradually with the

increase of pH value, leading to the strengthening of the electrostatic interaction between host-guest and to

the increase of the adsorption capacity with the increase of pH value. However, after pH＞4, the

protonation degree of the N atoms of the grafted macromolecule PDMAEMA becomes weaker and the

electropositivity of the particles PDMAEMA/SiO$_2$ begins to become poor as shown in Fig. 1. Consequently,

after pH＞4, the electrostatic interaction as well as cation-π interaction between host-guest begins to

weaken with the increase of pH value, resulting in the decrease of the adsorption capacity. It is possible that

there exists the strongest interaction between host-guest at pH=4, and so pH=4 is selected as the suitable

pH value for the following surface-imprinting process.

3.2. Chemical process to prepare imprinted particles MIP-PDMAEMA/SiO$_2$ and their characterization

3.2.1 Chemical process to prepare imprinted particles MIP-PDMAEMA/SiO$_2$

At pH 4, under the synergy action of three interactions, electrostatic interaction, cation-π interaction

and hydrogen bonding, the grafted particles PDMAEMA/SiO$_2$ produce strong adsorption for FA molecule,

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and such adsorption was made to reach saturation. And then dibromohexane as crosslinker was added into

the system, and the crosslinking reaction (quaterisation reaction) between the grafted macromolecule

PDMAEMA and dual bromine end groups of dibromohexane was allowed to occur. As a result, the grafted

macromolecules PDMAEMA are crosslinked with dibromohexane lost bromine as linkage, and the

adsorbed FA molecules are wrapped into the crosslinking networks so that the imprinting of FA is realized.

After washing out the template FA molecules, a great deal of FA imprinted caves left in the thin polymer

layer on the surfaces of silica gel particles, whereupon FA surface imprinted material

MIP-PDMAEMA/SiO$_2$ is formed. The preparation process of MIP-PDMAEMA/SiO$_2$ particles can be

schematically illustrated in Scheme 3.

Scheme 3

3.2.2 Characterization of imprinted particles MIP-PDMAEMA/SiO$_2$

(1) Infrared spectrum.   Fig. 4 gives the infrared spectra of three particles, SiO$_2$, the grafted

particles PDMAEMA/SiO$_2$ and the imprinted particles MIP-PDMAEMA/SiO$_2$.

Fig. 4

As compared with the spectrum of SiO$_2$ particles, in the spectrum of the grafted particles

PDMAEMA/SiO$_2$, three new absorption bands appear at 1732, 1395 and 2960 cm^{-1} respectively. As

described above, the first two bands belong to the characteristic vibration absorptions of carbonyl group of

carboxyl group and C-N bond of the ternary amino group of the grafted macromolecule PDMAEMA,

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respectively, and the last is attributed to the stretching vibration absorption of methyl group –CH$_3$ and

methylene group –CH$_2$- of the grafted macromolecule PDMAEMA, indicating the formation of the grafted

particles PDMAEMA/SiO$_2$. By comparing the spectrum of MIP-PDMAEMA/SiO$_2$ particles with that of

PDMAEMA/SiO$_2$, the following facts can be found. The characteristic absorption of C-N-C bond in the

quaternary ammonium structure appears at 2720 cm^{-1}, and the characteristic vibration absorption of C-N

bond of the ternary amino group has disappeared. Besides, the absorptions of methylene at 2927 and 2853

cm^{-1} are strengthened greatly because of the formation of the crosslinking linkage of dibromohexane lost

bromine. These spectrum data demonstrate that the quaterisation reaction between the grafted

macromolecule PDMAEMA and dibromohexane has been produced, and the imprinting of FA has been

realized.

It needs to be pointed out that all of the various absorption bands of PDMAEMA/SiO$_2$ and

MIP-PDMAEMA/SiO$_2$ look very weak. The reason for this is that the absorption background of SiO$_2$ is

very strong, and so it negatively affects the absorption bands of PDMAEMA/SiO$_2$ and

MIP-PDMAEMA/SiO$_2$.

(2) SEM image   Fig. 5(A) and Fig. 5(B) present the SEM images of raw silica gel particles

and the imprinted particles MIP-PDMAEMA/SiO$_2$. It can be clearly observed that the surfaces of raw silica

gel particles are rough and scraggy, whereas the surfaces of the particles MIP-PDMAEMA/SiO$_2$ become

smoother and flatter. It is caused by the filling up action of the grafted and crosslinked macromolecules.

Fig. 5(A)

Fig. 5(B)

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3.3. Recognition and binding characteristic of imprinted particles MIP-PDMAEMA/SiO$_2$for FA

3.3.1. Isothermal binding property

The adsorption experiments with batch method were first performed using the non-imprinted

particles NMIP-PDMAEMA/SiO$_2$ and the imprinted particles MIP-PDMAEMA/SiO$_2$, respectively, and

Fig. 6 and Fig. 7 give the adsorption isotherms of NMIP-PDMAEMA/SiO$_2$ and the binding isotherms of

MIP-PDMAEMA/SiO$_2$ for FA, CHA and CA, respectively.

Fig. 6

Fig. 7

It can be seen that for all of the three phenolic acid compounds, the non-imprinted particles

NMIP-PDMAEMA/SiO$_2$ have high adsorption capacity, and it is a range of 0.70-0.74 mmol/g, and so

NMIP-PDMAEMA/SiO$_2$ particles have no adsorption selectivity. The reason for this lies in that there also

exist the synergetic of three interactions, electrostatic interaction, cation-π interaction and hydrogen

bonding, between the non-imprinted particles NMIP-PDMAEMA/SiO$_2$ and the three phenolic acid

compounds, leading to high adsorption capacity.

However, Fig. 7 displays that the binding isotherm of FA on the imprinted particles

MIP-PDMAEMA/SiO$_2$ is greatly different from that of CHA and CA. The binding capacity of

MIP-PDMAEMA/SiO$_2$ particles for FA still remains high (0.73 mmol/g, 142 mg/g). However, the binding

capacities of CHA and CA decline dramatically, and they decrease to 0.19 mmol/g for CHA and 0.31

mmol/g for CA, respectively. Therefore, this experimental result fully displays that the imprinted particles MIP-PDMAEMA/SiO$_2$ possess specific recognition selectivity and excellent binding affinity for FA, whereas their recognition and binding ability for CHA and CA is very poor (especially for CHA), or the imprinted particles nearly do not recognize and bind CHA.

The reason for the above fact can be explained as follows. For MIP-PDMAEMA/SiO$_2$ particles, a great quantity of the imprinted caves of FA molecule is distributed within the thin polymer layer on the surfaces of silica gel particles. These caves are highly matched with FA molecule in size, shape and spatial arrangement of action sites, and it leads to the specific recognition ability and strong binding action of MIP-PDMAEMA/SiO$_2$ particles towards FA molecule. However, it can be seen from Scheme 1 that although carboxyl group and phenol structure are also contained in CHA molecule, the size of CHA molecule is larger than that of FA and there is a certain difference of spatial structure between CHA and CA molecules. Hence, FA-imprinted caves are unmatched with CHA molecules in size, shape and spatial arrangement of action sites, and CHA molecules are difficult to inter into these caves or these imprinted caves are difficult to accept and bind CHA molecules, leading to very low binding capacity of MIP-PDMAEMA/SiO$_2$ particles for CHA. For CA, although its molecule structure is closed to that of FA and its molecule size is smaller than that of FA, there is still difference of substituent group on phenol ring. This difference affects the matching in steric configuration, and limits the binding ability of the imprinted caves for CA molecule. The binding ability of MIP-PDMAEMA/SiO$_2$ particles for CA is higher than that for CHA because of greater similarity between the molecular structures between CA and FA, but is still much lower than that for the template FA.

3.3.2. Dynamic binding property

The adsorption experiments with column method were also conducted using the non-imprinted

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particles NMIP-PDMAEMA/SiO$_2$ and the imprinted particles MIP-PDMAEMA/SiO$_2$, respectively, and Fig.

8 and Fig. 9 display the dynamic adsorption curves of NMIP-PDMAEMA/SiO$_2$ and the dynamic binding

curves (or leaking curves) of MIP-PDMAEMA/SiO$_2$for FA and CHA, respectively.

Fig. 8

Fig. 9

Fig. 8 displays that as the solutions of FA and CHA with the same concentration (5 mmol/L) flows

upstream through the column packed with the non-imprinted particles NMIP-PDMAEMA/SiO$_2$,

respectively, both their leaking volumes are greater and are closed to each other, and they are 51BV for

CHA and 54BV for CA, respectively. The result of dynamic adsorption experiments once again confirms

that the non-imprinted particles NMIP-PDMAEMA/SiO$_2$ have no adsorption selectivity.

However, it can be observed clearly that for the column packed with the imprinted particles

MIP-PDMAEMA/SiO$_2$, the leaking curve of CHA is obviously different from that of FA. The leaking

volume of CHA is only 12BV, and it implies that once the solution of CHA goes on the column, it fast

leaks, showing that the column packed with the imprinted material MIP-PDMAEMA/SiO$_2$ basically does

not recognize and does not bind the contrast substance CAH. However, the leaking volume of the template

FA still remains high (54 BV) and is much higher than that of CAH. Obviously, the column packed with the

imprinted particles MIP-PDMAEMA/SiO$_2$ exhibits the special recognition selectivity and excellent binding

affinity. The reason for this still lies in that a great deal of FA-imprinted caves are distributed within the

thin polymer layer on the surfaces of MIP-PDMAEMA/SiO$_2$ particles, and these imprinted caves are highly

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matched with FA molecule and unmatched with CHA molecule in size, shape (steric configuration) and action sites. By calculating, the leaking and saturated adsorption amounts of CHA are 0.12 mmol/g and 0.18 mmol/g, respectively, whereas the leaking and saturated adsorption amounts of FA reach up to 0.54 mmol/g and 0.74 mmol/g, respectively.

The dynamic adsorption curves of NMIP-PDMAEMA/SiO$_2$ and the dynamic binding curves (or leaking curves) of MIP-PDMAEMA/SiO$_2$for FA and CA are also presented in Fig. 8 and Fig. 9 as inserted small diagrams, respectively. Fig. 8 displays that for NMIP-PDMAEMA/SiO$_2$ particles-packed column, the leaking volumes of FA and CA are also closed to each other (190 BV for FA and 195 BV for CA), indicating that the non-imprinted particles NMIP-PDMAEMA/SiO$_2$ have no adsorption selectivity. However, Fig. 9 shows that for the MIP-PDMAEMA/SiO$_2$ particles-packed column, the leaking curve of CA is different from that of FA. The leaking volume of CA is only 75 BV and that of the template FA still remains high (195 BV), exhibiting the special recognition selectivity MIP-PDMAEMA/SiO$_2$ particles-packed column for FA.

3.3.3. Recognition selectivity of MIP-PDMAEMA/SiO$_2$ for FA

Two binary mixed solutions, FA/CHA and FA/CA, were prepared, and the competitive adsorption experiments of MIP-PDMAEMA/SiO$_2$ particles were conducted in them. Table 1 and Table 2 summarize the data of the distribution coefficients K_d and selectivity coefficients k.

Table 1

Table 2

The following facts can be found from the data of Table 1 and Table 2. (1) As the competitive adsorption experiments were carried out in the binary mixed solutions by using the non-imprinted particles NMIP-PDMAEMA/SiO$_2$, each component has strong competitive adsorption ability so that both the selectivity coefficients of the NMIP-PDMAEMA/SiO$_2$ particles for FA relative to CHA (1.18) and relative to CA (0.95) are closed to 1. Obviously, there is no any recognition selectivity. (2) As the competitive adsorption experiments were carried out by using the imprinted particles MIP-PDMAEMA/SiO$_2$, the selectivity coefficients of the MIP-PDMAEMA/SiO$_2$ particles for FA relative to CHA and relative to CA are 6.47 and 2.75, respectively, displaying that MIP-PDMAEMA/SiO$_2$ particles possess recognition selectivity, especially relative to CHA, the high selectivity coefficient (6.47) shows the molecule recognition specificity of MIP-PDMAEMA/SiO$_2$ particles.

The molecule recognition specificity of the imprinted materials is very important in the separation of the target substance from the crude plant extracts. Generally, the crude extracts of phenolic acids contain multiple components. If the above ferulic acid-imprinted material is used as solid absorbent and the solid phase extraction is performed, the MIP-PDMAEMA/SiO$_2$ particles will specifically recognize and bind FA in the crude extract. It can be imagined that if multi-column packed with MIP-PDMAEMA/SiO$_2$ particles are combined, the effective separation and purification of FA can well realized finally. In a word, by adopting the method of "pre-graft polymerizing and post-crosslinking/imprinting", the surface imprinted materials of styrene-type phenolic acid compounds with high performance can be prepared. By take advantage of the special recognition selectivity and excellent binding property of such imprinted materials for the target substance, it can be expected that the target pharmacologic activity constituents are effectively separated from crude extracts via molecularly imprinted solid-phase extraction.

3.4. Effect of used amount of crosslinker on recognition selectivity of MIP-PDMAEMA/SiO$_2$

In the traditional imprinting method, the suitable used amount of crosslinker needs to be determined by experiments. However, in the new surface imprinting method of "pre-grafting and post-crosslinking/imprinting", the suitable used amount of crosslinker can be estimated basically from the binding model of the grafted macromolecule with the template molecule and from the number of average action sites the grafted macromolecule towards the template molecule [32]. It can be seen from Scheme 3 that three ternary ammonium groups of the grafted macromolecule PDMAEMA interact with one ferulic acid molecule by right of electrostatic interaction and cation-π interaction as well as hydrogen bonding, namely three chain units interact with one FA molecule. Besides, for one imprinted cave, the crosslinking action needs to spend four chain units of the grafted macromolecule PDMAEMA. Therefore, for imprinting one FA molecule, seven chain units of the grafted macromolecule PDMAEMA and two dibromohexane molecules as crosslinker are needed. As a result, a suitable ratio of the chain unit of the grafted macromolecule PDMAEMA to the crosslinker can be estimated, and it should be equal to 3.5:1.

The FA molecule surface-imprinting was conducted with different used amounts of the crosslinking agent, dibromohexane, by fixing other reaction conditions, and Fig. 10 gives the selectivity coefficient of MIP-PDMAEMA/SiO$_2$ particles for FA relative to CHA as a function of the used amounts of dibromohexane (it is expressed as the molar ratio of the chain unit of the grafted macromolecule PDMAEMA on PDMAEMA/SiO$_2$ particles to dibromohexane).

Fig. 10

It can been found from Fig.10 that the selectivity coefficient of MIP-PDMAEMA/SiO$_2$ particles first increases and then decreases with the increase of the molar ratio, and there is a maximun selectivity

coefficient of 6.47 as this ratio is 3.5:1, implying that with this ratio, the imprinting effect is the best. Obviously, the pre-estimated result from the binding model between host-guest is in good agreement with the experimental value. The above facts show that the new surface imprinting method of "pre-grafting and post-crossling/imprinting" is greatly different from the conventional imprinting method, and in this new surface imprinting system, there is a basic characteristic of "feed quantifying", and the suitable used amount of the crosslinker can be pre-estimated.

3.5. Elution property of MIP-PDMAEMA/SiO$_2$ particles

The elution experiment of MIP-PDMAEMA/SiO$_2$ particles was conducted to estimate their elution property. A mixed solution of acetic acid/methanol (V:V=1:9) was used as eluent, and it was allowed to upstream passes through the column packed with MIP-PDMAEMA/SiO$_2$ particles, which had adsorbed FA in a saturated state. Fig. 11 gives the dynamic elution curve.

Fig. 11

Fig. 11 indicates that the elution curve is cuspidal and without tailing phenomenon. By calculating, the desorption ratios in 16 BV and 18 BV reach 98.9% and 99.6%, respectively, indicating that the FA molecules combined on MIP-PDMAEMA/SiO$_2$ particles are easy to be washed off and the surface-imprinted material has excellent elution property. The reason is that those FA molecule-imprinted caves are distributed within the thin polymer layer on the surfaces of MIP-PDMAEMA/SiO$_2$ particles, and there is a small diffusion resistance for template molecules, so the bounded FA molecules are easy to be eluted.

4. Conclusions

PDMAEMA macromolecules are first grafted on micro-sized silica gel particles by adopting surface initiated graft-polymerization method, obtaining the grafted particles PDMAEMA/SiO$_2$. In acidic solution, there are strong interactions between the grafted particles PDMAEMA/SiO$_2$ and ferulic acid, and they are electrostatic interaction and cation-π interaction as well as hydrogen bonding. Therefore, the grafted particles PDMAEMA/SiO$_2$ can produce strong adsorption for ferulic acid. On this basis, FA surface imprinting was performed smoothly by using the new surface imprinting method of "pre-graft polymerizing and post-crossling/imprinting", obtaining FA imprinted particles MIP-PDMAEMA/SiO$_2$ and completely avoiding the issue of taking part in the polymerization of the polymerizable double bond in FA molecule. The imprinted particles MIP-PDMAEMA/SiO$_2$ have specific recognition selectivity and excellent binding affinity for FA molecule. It can be expected that MIP-PDMAEMA/SiO$_2$ can be used as solid adsorbent with high performance, and ferulic acid can be separated from the crude plant extract by using the molecularly imprinted solid-phase extraction method.

References

[1] Kryscio D R, Peppas N A. Acta Biomaterialia, Volume 8, Issue 2, February 2012, Pages 461-473.

[2] Prasad B B, Sharma P S, Lakshmi D. Journal of Chromatography A, 1173(2007, Pages 18-26.

[3] Ng S M, Narayanaswamy R. Sensors and Actuators B: Chemical, Volume 139, Issue 1, 20 May 2009, Pages 156-165.

[4] Karimian N, Turner A P F, Tiwari A. Biosensors andBioelectronics, 59(2014)160–165.

[5] Idriss Bakas, Akhtar Hayat, Sergey Piletsky, Elena Piletska, Mohamed M. Chehimi, Thierry Noguer, Régis Rouillon

[6] Akdamar H.A, Sarıözlü N Y, Özcan A A, Ersöz A, Denizli A, Say R. Materials Science and Engineering C, 29 (2009) 1404–1408.

[7] Uzun L, Say R, ünal S, Denizli A. Journal of Chromatography B, 877 (2009) 181–188.

[8] Yuan Y, Wang Y-Z, Huang M-d, Xu R, Zeng H, Nie C, Kong J-H. Analytica Chimica Acta, 695 (2011) 63–72.

[9] Lin Y, Shi Y, Jiang M, Jin Y, Peng Y, Lu B, Dai K. Environmental Pollution, 153 (2008) 483–491.

[10] Du N, Cao S-W, YuY-Y. Journal of Chromatography B, 879 (2011) 1697–1703.

[11] Srivastava S, Singh A P, Rawat A K S. Asian Pacific Journal of Tropical Biomedicine, (2012)S12-S14.

[12] Ou S-Y, LuoY-L, Xue F, Huang C-H, Zhang N, Liu Z-L. Journal of Food Engineering, 78 (2007) 1298–1304.

[13] Max B, Torrado A M, Moldes A B, Converti A, Domínguez J M. Biochemical Engineering Journal, 43 (2009) 129–134.

[14] Buranov A U, Mazza G. Food Chemistry, 115 (2009) 1542–1548.

[15] Salgado J M, Max B, Rodríguez-Solana R, Domínguez J M. Industrial Crops and Products, 39 (2012) 52– 61.

[16] Soto M L, Moure A, Domínguez H, Parajó J C. Journal of Food Engineering, 105 (2011) 1-27.

[17] Dávila-Guzman N E, Cerino-Córdova F J, Diaz-Flores P E, Rangel-Mendez J R, Sánchez-González M N, Soto-Regalado E. Chemical Engineering Journal, 183 (2012) 112– 116.

[18] Ou S-Y, Luo Y-L, Xue F, Huang C-H, Zhang N, Liu Z-L. Journal of Food Engineering, 78 (2007) 1298–1304

[19] YangY-Z, Liu X-G, Guo M-C, Li S, LiuW-F, Xu B-S. Colloids and Surfaces A, 377 (2011) 379–385.

[20] Milj T E, Prathish K P, Rao T P. Journal of Hazardous Materials, 188 (2011) 384–390.

[21] Gong X-Y, Cao X-J. Journal of Biotechnology 153 (2011) 8–14.

[22] Zhu G-F, Fan J, GaoY-B, Gao X, Wang J-N. Talanta, 84 (2011) 1124–1132.

[23] Liu H-M, Liu C-H, Yang X-J, Zeng S-J, Xiong Y-Q, Xu W-J. analytica chimica acta, 628 (2008) 87–94.

[24] Kochkodan V, Hilal N, Melnikc V, Kochkodan O, Remizovska A. Journal of Membrane Science, 377 (2011) 151– 158.

[25] Gao B-J, Lu J-H, Chen Z-P, Guo J-F. Polymer 50 (2009) 3275-3284.

[26] Gao B-J, Chen Y-X, Men J-Y. Journal of Chromatography A, 1218 (2011) 5441-5448.

[27] Gao B-J, Wang X-H, Zhang Y-Y. Material Chemistry and physics, 2013,140: 478-486.

[28] Gao B-J, Bi C-C, Fan L . Applied Surface Science, 332(2015) 430–439.

[29] Gao B-J, Shi N , Shi X-J. Journal of Polymer Research, 20 (2013) 260–267.

[30] Ersöz A, Say R, Denizli A. Analytica Chimica Acta, 502 (2004) 91–97.

[31] Say R, Ersöz A, Türk H, Denizli A. Separation and Purification Technology, 40 (2004) 9–14.

[32] Gao B-J, Wang J, Yang Y. Chromatographia 69 (2009) 1353-1361.

Figure legend

Scheme 1 Schematic expression of chemical structures of ferulic acid, chlorogenic acid and caffeic acid

Scheme 2 Schematic expression of chemical process to preparing the grafted particles PDMAEMA/SiO$_2$

Scheme 3 Schematic expression of preparation process of imprinted particles MIP-PDMAEMA/SiO$_2$

Table 1 Distribution coefficient and selectivity coefficient data for FA/CHA system

Table 2 Distribution coefficient and selectivity coefficient data for FA/CA system

Fig. 1 Variation of zeta potential of PDMAEMA/SiO$_2$ and SiO$_2$ particles with pH value

Fig. 2 Adsorption isotherm of PDMAEMA/SiO$_2$ for FA

   Temperature: 25 □; pH=4

Fig. 3 Adsorption capacity of PDMAEMA/SiO$_2$ for FA as a function of pH value

   Temperature: 25℃

Fig. 4 FTIR spectra of SiO$_2$, PDMAEMA/SiO$_2$ and MIP-PDMAEMA/SiO$_2$ particles

Fig. 5 (A) SEM image of SiO$_2$ particles

Fig. 5 (B) SEM image of MIP-PDMAEMA/SiO$_2$ particles

Fig. 6 adsorption isotherms of NMIP-PDMAEMA/SiO$_2$ particles for FA, CHA and CA

   Temperature: 25 ℃; pH=4

Fig. 7 Binding isotherms of MIP-PDMAEMA/SiO$_2$ for FA, CHA and

   Temperature: 25 ℃; pH=4

Fig. 8 Dynamic adsorption curves of NMIP-PDMAEMA/SiO$_2$ for FA , CHA and CA

   Temperature: 25 ℃; pH=4; Initial concentration: 5 mmol/L for FA and CHA system, 2 mmol/L for

   FA and CA system; Flow rate: 5 BV/h

Fig. 9 Dynamic binding curves of MIP-PDMAEMA/SiO$_2$ for FA , CHA and CA

   Temperature: 25 ℃; pH=4; Initial concentration: 5 mmol/L for FA and CHA system, 2 mmol/L for

   FA and CA system; Flow rate: 5 BV/h

Fig. 10 Selectivity coefficient as a function of molar ratio of chain unit of PDMAEMA to crosslinker

   Temperature: 25 ℃; pH=4
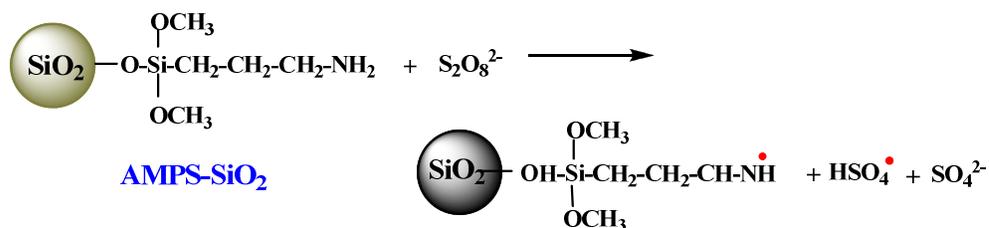
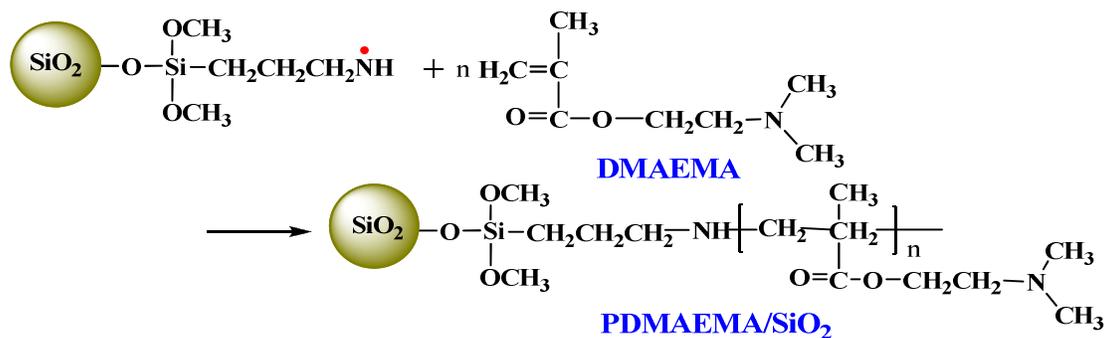Fig. 11 Elution curve of FA on MIP-PDMAEMA/SiO$_2$ column

   Temperature: 25 ℃

Ferulic acid

Caffeic acid

Chlorogenic acid
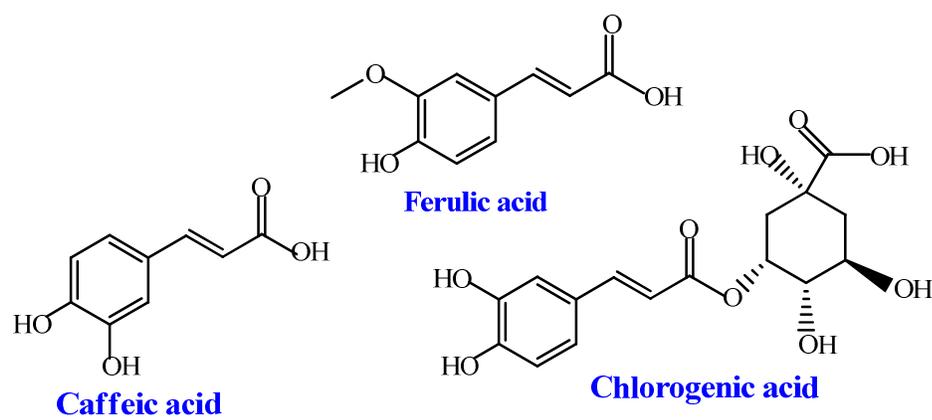
Scheme 1

(1) Production of free radical on surfaces of modified silica gel particles

$$\text{SiO}_2 - \overset{\overset{\displaystyle OCH_3}{|}}{\underset{\underset{\displaystyle OCH_3}{|}}{\text{O-Si}}}\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2 \quad + \quad \text{S}_2\text{O}_8{}^{2-} \longrightarrow$$

AMPS-SiO₂

$$\text{SiO}_2 - \overset{\overset{\displaystyle OCH_3}{|}}{\underset{\underset{\displaystyle OCH_3}{|}}{\text{OH-Si}}}\text{-CH}_2\text{-CH}_2\text{-CH-N}\overset{\bullet}{\text{H}} \quad + \text{HSO}_4{}^{\bullet} \; + \; \text{SO}_4{}^{2-}$$

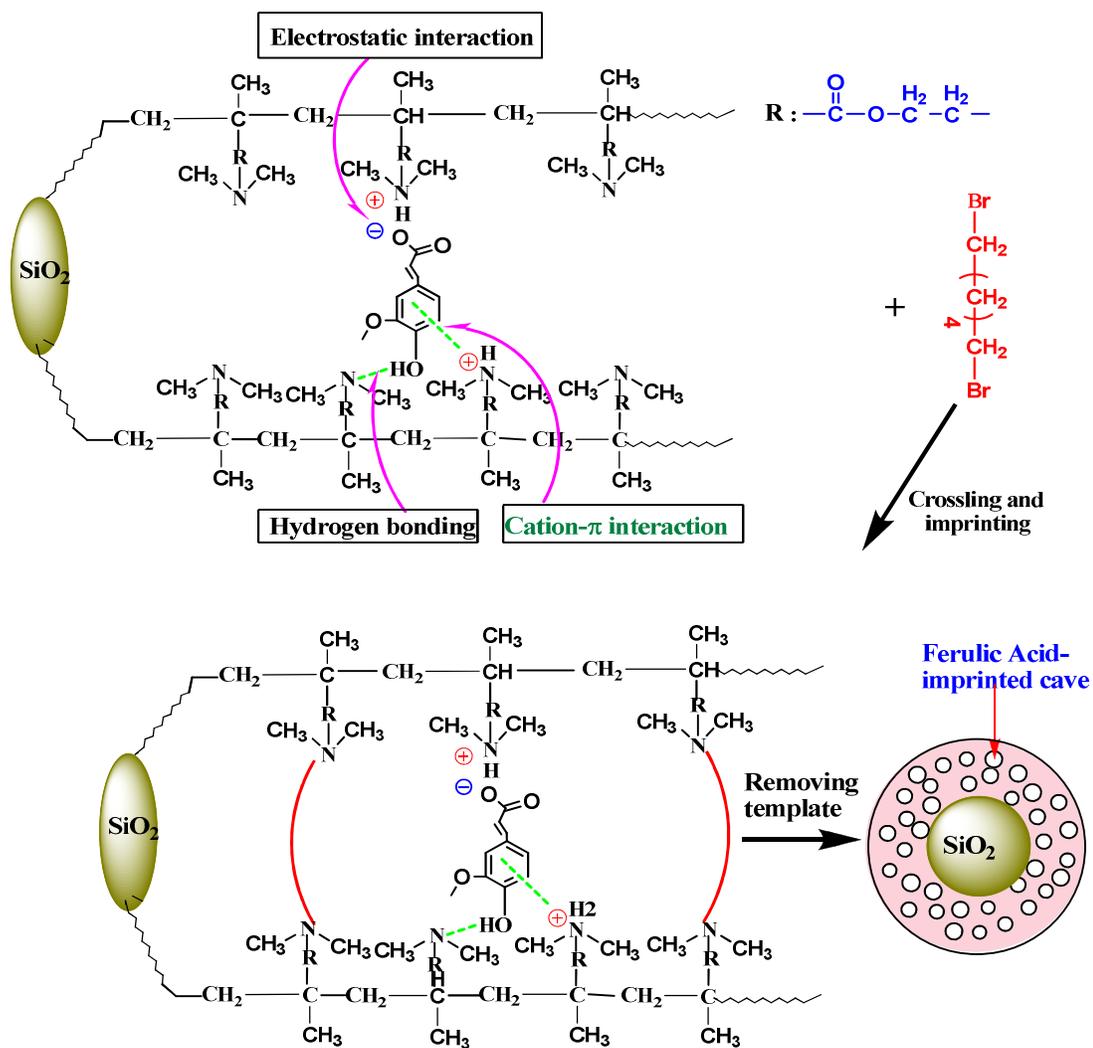**(2) Surface-intiated graft-polymerization of DMAEMA on silica gel particles**

$$\text{SiO}_2 - \overset{\overset{\displaystyle OCH_3}{|}}{\underset{\underset{\displaystyle OCH_3}{|}}{\text{O-Si}}}-\text{CH}_2\text{CH}_2\text{CH}_2\overset{\bullet}{\text{N}}\text{H} \quad + \; n \; \text{H}_2\text{C}=\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle O=C-O-CH_2CH_2-N}{|}}{C}}\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{<}}$$

DMAEMA

$$\longrightarrow \quad \text{SiO}_2 - \overset{\overset{\displaystyle OCH_3}{|}}{\underset{\underset{\displaystyle OCH_3}{|}}{\text{O-Si}}}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{NH}\left[\text{CH}_2-\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle O=C-O-CH_2CH_2-N}{|}}{\text{CH}_2}}\right]_n \overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{<}}$$

PDMAEMA/SiO₂

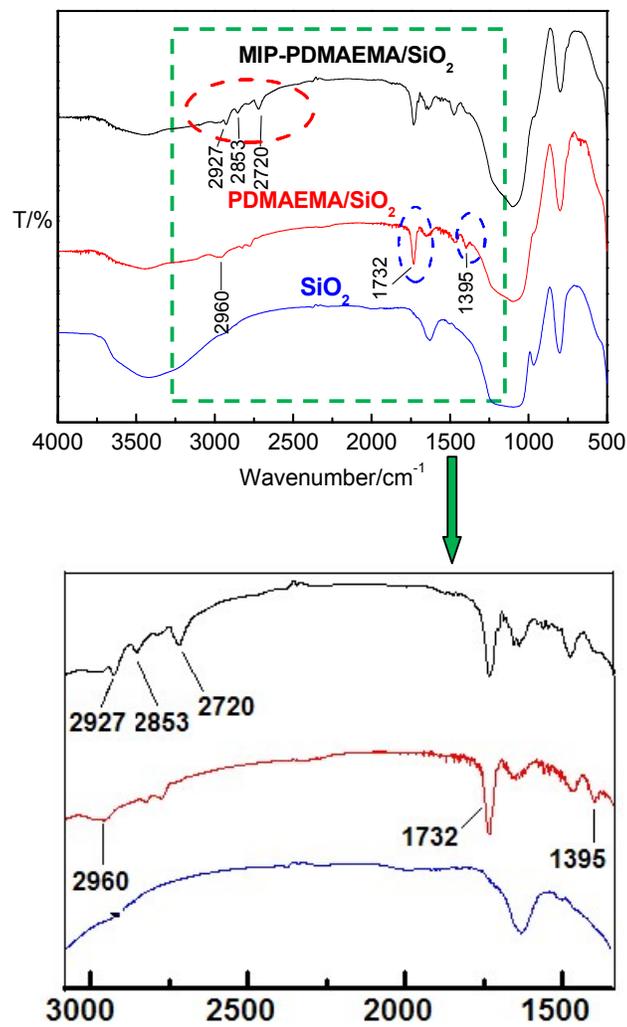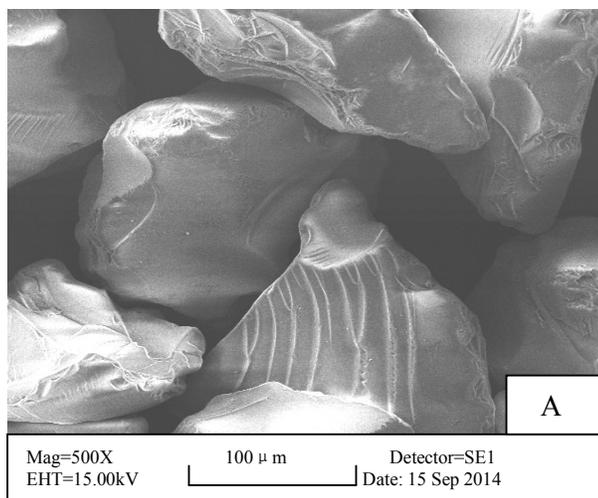**Scheme 2**

Fig. 1

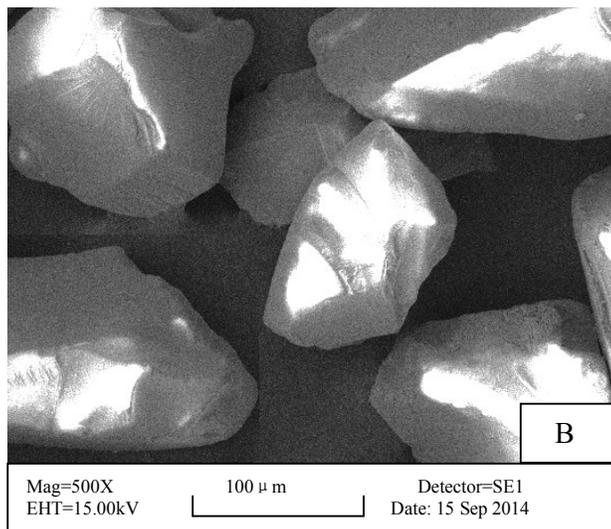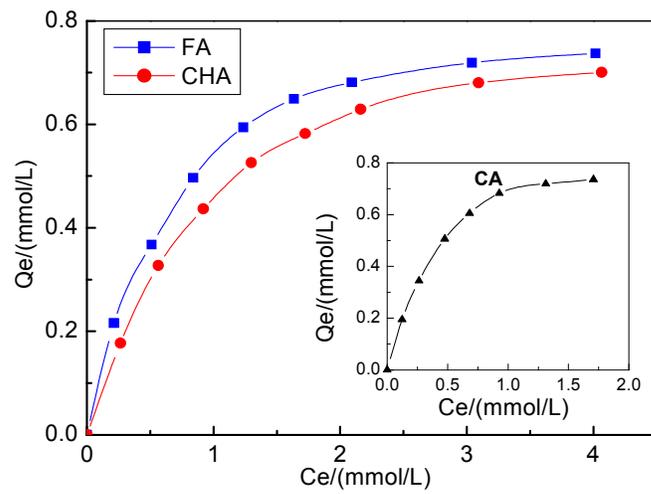**Fig. 2**

Fig. 3

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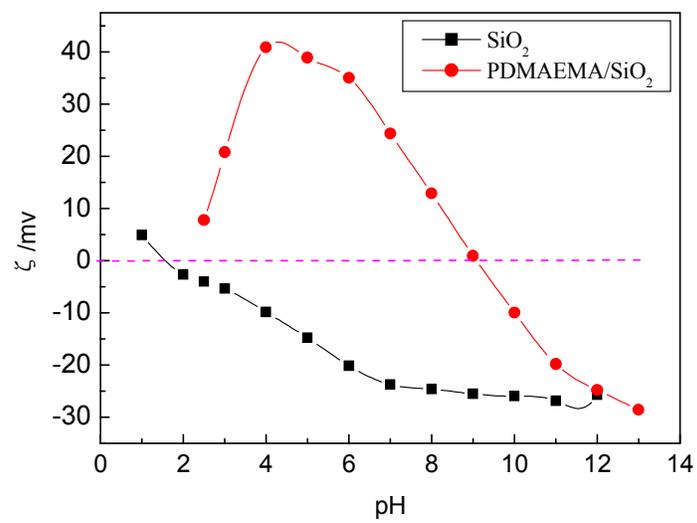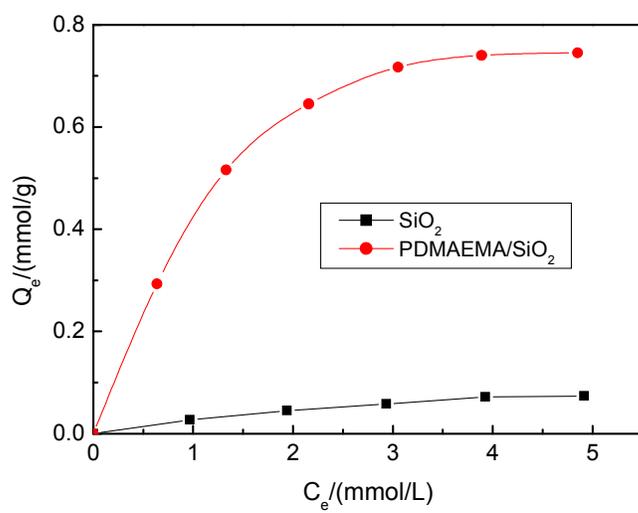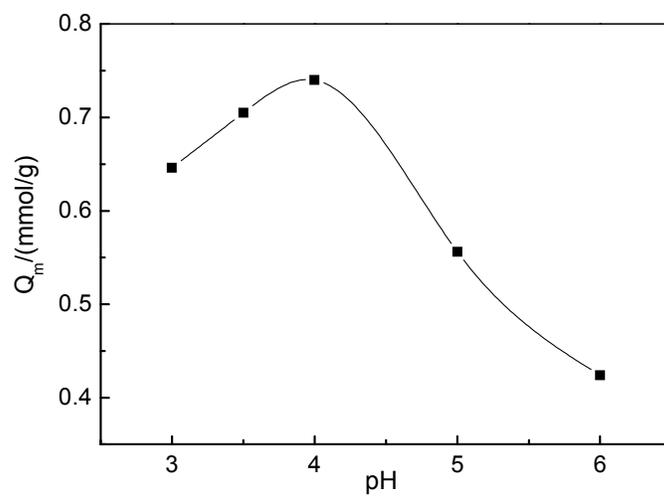
Scheme 3

Fig. 4

Fig. 5 (A)

Fig. 5 (B)

Fig. 6

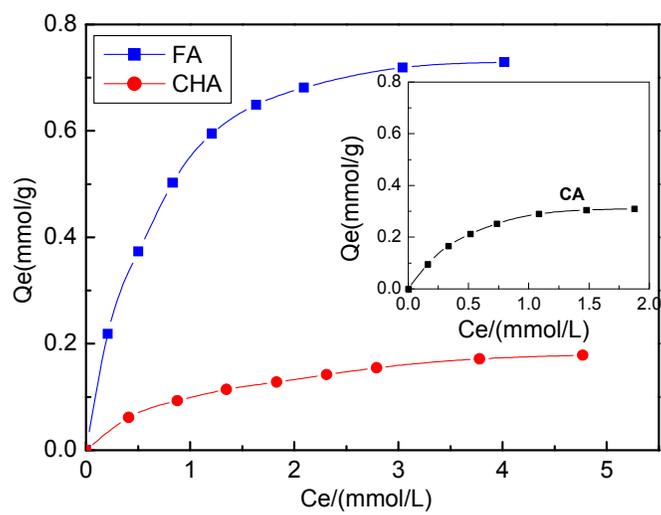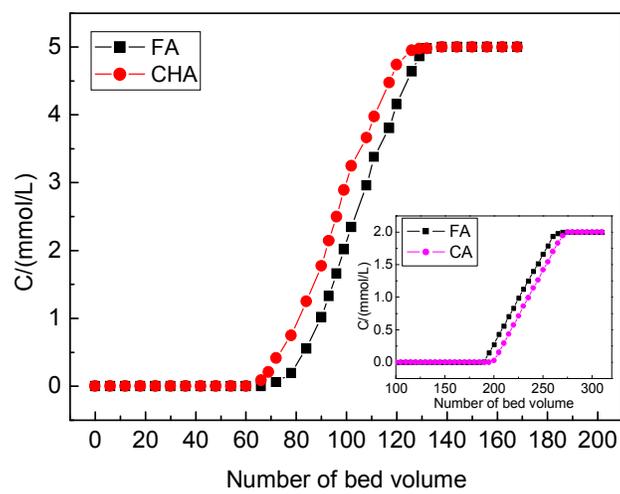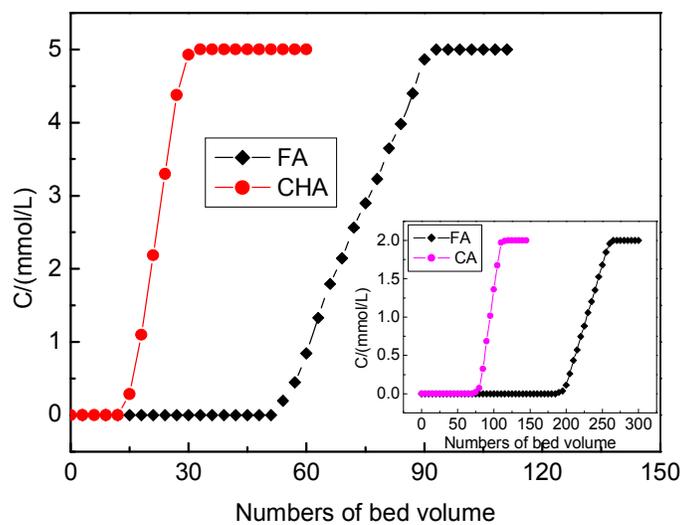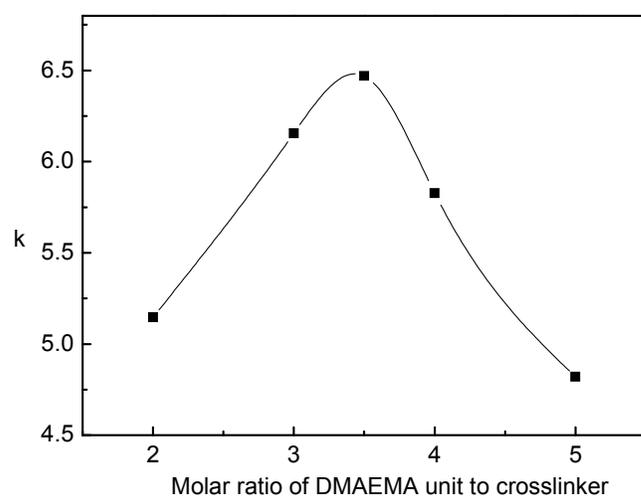**Fig. 7**

Fig. 8

Fig. 9

Table 1

Adsorbing material	PDMAEMA/SiO$_2$		MIP-PDMAEMA/SiO$_2$	
Adsorbate	FA	CHA	FA	CHA
K_d/(L/g)	0.397	0.337	0.398	0.0615
k	1.178		6.47	

Table 2

Adsorbing material	PDMAEMA/SiO$_2$		MIP-PDMAEMA/SiO$_2$	
Adsorbate	FA	CA	FA	CA
K_d/(L/g)	0.735	0.778	0.736	0.267
k	0.946		2.752	

Fig. 10
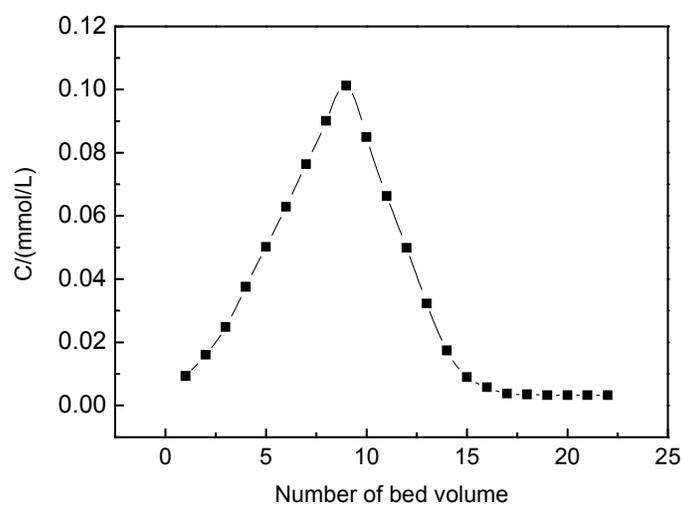
Fig. 11

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Graphical Abstract

Designing and Preparation of Ferulic Acid Surface-imprinted Material and its Molecular Recognition Characteristics

Baojiao Gao, Liqin Zhang, Li Fan