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Preparation of Molecularly Imprinted Polymers for Vanillin via Reversible Addition-Fragmentation Chain Transfer Suspension Polymerization

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ABSTRACT: Molecularly imprinted polymers (MIPs) for vanillin were synthesized by suspension polymerization using ethylene glycol dimethacrylate as cross-linker and methacrylic acid as functional monomer, respectively. The analysis of scanning electron microscopy and equilibrium binding experiments indicated that the MIPs can selectively separate the target analytes. Reversible addition-fragmentation chain-transfer (RAFT) technique was used to synthesize MIPs using benzyl dithiobenzoate as RAFT agent. The results showed smaller particle size, higher molecular adsorption, and considerable binding specificity toward vanillin than those prepared by suspension polymerization. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

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INTRODUCTION

Molecularly imprinted polymers (MIPs) are synthetic polymers with highly specific recognition ability for target molecules.[1] MIPs have gained great attention due to their predetermined recognition ability, stability, relative ease, and low cost of preparation and potential application to separate a wide range of target molecules.[2–6] MIPs are being used in capillary electrochromatography,[7,8] chemical sensors,[9,10] membrane separation,[11,12] solid-phase extraction,[13,14] and chromatographic separations.[15,16]

MIPs are obtained by polymerizing different functional monomers and cross-linkers in the presence of the template molecules. The template molecules and the functional monomers combine through noncovalent or covalent interactions and are then joined by using a cross-linking agent. After the removal of the template, binding sites of the functional groups are exposed, which are complementary to the template in size, shape, and consequently allow its selective uptake.[17–20]

Free radical polymerization is the most important method to prepare polymer. But conventional free radical polymerization lacks control for the reason of chain transfer and termination processes. The advent of "living" free radical polymerization has provided powerful tools to synthesize polymers with predictable molecular weight, narrow polydispersity, and well-defined molecular architecture.[21] Atom transfer radical polymerization and reversible addition-fragmentation chain-transfer (RAFT) polymerization are typical methods of controlled/living free radical polymerization.[22–28] More recently, the use of RAFT polymerization has been developed into a new approach to synthesize MIPs with the applicability to an extensive range of monomers, mild reaction conditions, and good control to the polymer structures and molecular weight. The controllability of RAFT polymerization to polymeric processes can attribute to the use of a reversible chain transfer agent and the resulting fast and dynamic equilibrium between active species and dormant species.[29–33]

In this study, MIPs for vanillin were prepared by conventional suspension polymerization with methacrylic acid (MAA) as functional monomer and ethylene glycol dimethacrylate (EDMA) as cross-linker. Hydrogen bonds between vanillin and MAA during polymerization can create networks with selective binding sites, as shown in Figure 1. The vanillin-imprinted polymers were also prepared by RAFT suspension polymerization. The morphology, template-rebinding properties, and binding selectivity of the obtained MIPs were characterized and also compared between conventional suspension polymerization and RAFT polymerization.

EXPERIMENTAL

Materials

Vanillin and MAA were purchased from Tianjin Chemical Reagent Institute (Tianjin, China). *O*-vanillin and EDMA were purchased

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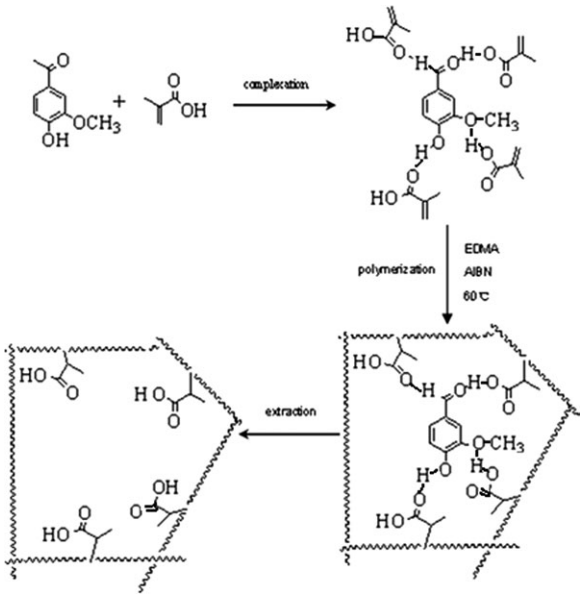

Figure 1. Schematic representation of synthesis of molecularly imprinted polymers (MIPs).

from Alfa Aesar. Polyvinyl alcohol (PVA), 2,2-azobis-siobutyronitrile (AIBN), and other reagents were of analytical grade.

Synthesis of RAFT Chain Transfer Agent

Bromide benzene of given concentration, excess magnesium (Mg), and proper iodine (I_2) were dissolved in tetrahydrofuran in a three-necked flask at 40°C and stirred for 45 min under the atmosphere of nitrogen. Then, carbon disulfide (CS_2) was slowly added drop wise to the stirred solution for 35 min. Following benzyl bromide, $ArCH_2Br$ was added to the above solution at the same dropping speed and maintained the temperature at 50°C. The flask was sealed, and the solution continued reaction for 24 h. After the reaction was finished, the mixture was washed by water and extracted by ether, respectively. Then the product was purified by chromatogram separation via silica gel as sorbent and the petroleum ether as eluting agent. Finally, the redness oil of product (benzyl dithiobenzoate, BDB) was obtained.

BDB was characterized by Mercury Plus-400 nuclear magnetic resonance spectrometer (Varian, Palo Alto, America), and the result is 4.58 (s, 2H, CH_2—Ph), 7.22–7.52 (m, 8H, ArH), and 8.00 (m, 2H, ArH).

Preparation of the MIPs

In this work, the molecularly imprinted polymers (MIPs) were synthesized by suspension polymerization. PVA (400 mesh) was dissolved in 50 mL water at 95°C in a three-necked flask. The flask was then purged under N_2 for 10 min. The template molecule vanillin and functional monomer MAA were dissolved in acetonitrile in a test tube, and then cross-linker EDMA and initiator AIBN were added. Then, the mixture was poured to the PVA solution. After purging with N_2 for 10 min, all the necks of the flask were sealed, and polymerization was carried out at 70°C for 24 h with stirring at 400 rpm. After polymerization, the products were washed by water at 90°C to remove the remnants and then purged through soxhlet extraction by methanol/acetic (9/1 v/v, 24 h) and methanol (24 h), respectively. Finally, the beads were dried at 60°C for 24 h to obtain the MIPs. The nonimprinted polymers (NMIPs) were prepared and purified in the same way, except the addition of vanillin.

Preparation of the RAFT-MIPs

Details of the preparation steps and techniques of the reversible addition-fragmentation chain-transfer-molecularly imprinted polymers (RAFT-MIPs) and the RAFT-NMIPs were the same as the preparation of the MIPs and the NMIPs, except that 70 mg chain transfer agent BDB with cross-linker EDMA and initiator AIBN was added together to the acetonitrile solution.

Fourier Transform Infrared Analysis

The polymers were characterized by Fourier transform infrared (FTIR) spectroscopy in a transmittance mode on a VECTOR FTIR spectrometer (Brucker, Germany). The FTIR spectra were recorded from 4000 to 400 cm at a resolution of 4 cm and 32 scans.

Morphology of the MIPs and the RAFT-MIPs

The particle size and size distribution of the molecularly imprinted polymers (MIPs) and the reversible addition-fragmentation chain-transfer (RAFT)-MIPs were determined by JSM6380LV scanning electron microscope (Japan Electronics, Japan). The surfaces of the samples were coated by gold sputtering before they were scanned at 20 kV.

Equilibrium-Binding Experiments

Binding experiments were performed both in ethanol solution (ethanol/water, 1/4 v/v) and in water media. To study the binding isotherms of the imprinted polymers, 40-mg imprinted polymers were equilibrated with initial concentration 1 mmol/L of vanillin 10 mL in a 50-mL one-necked flask. After 24-h thermostatic oscillation, the saturated polymers were separated by centrifugation (2000 rpm, 10 min). Then the supernatant fluid that contained the unabsorbed vanillin after equilibrium adsorption was diluted to 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, and 0.1 mmol/L. Finally, the concentrations were determined using a TU-1810 UV–vis spectrophotometer (Beijing Purkinjie General Instrument Company, Beijing, China). All processes were carried out at room temperature. Based on the results, Q was obtained based on the following eq. (1)[22]:

$$Q = \frac{(C_0 - C_t)V}{W} \quad (1)$$

where Q (mg/g) is the amount of vanillin bound to MIPs at equilibrium, C_0 (mmol/L) is the initial vanillin concentration,

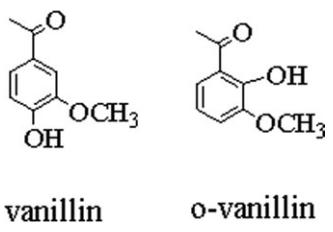

Figure 2. Schematic representation of the vanillin and *o*-vanillin structure.

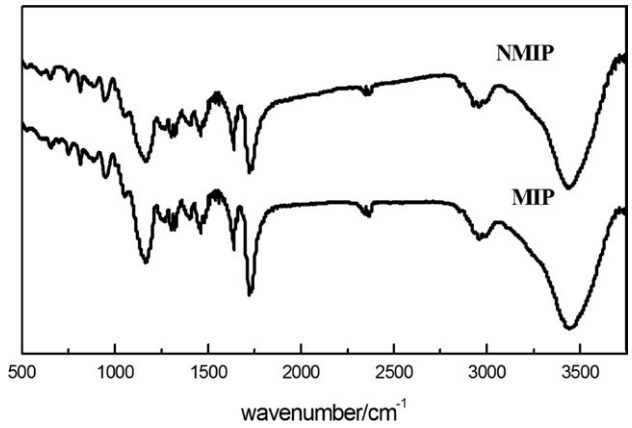

Figure 3. FTIR spectra of the NMIPs and the MIPs.

C_t (mmol/L) is the different balanceable vanillin concentration, V (mL) is the volume of vanillin concentration, and W (mg) is the weight of the polymer.

Selective Binding Experiments

The binding selectivity of the imprinted polymers was evaluated by measuring their rebinding capacities toward vanillin and its structurally related compound *o*-vanillin. Schematic representation of vanillin and o-vanillin structure is shown in Figure 2. Twenty micrograms of imprinted polymer were dispersed in 5 mL solution (ethanol/water, 1/4 v/v) containing 1 mmol/L vanillin and *o*-vanillin, respectively. The mixture was shaken at room temperature for 24 h. Then the saturated polymers were separated by centrifugation (2000 rpm, 10 min). The supernatant fluid that contained the unabsorbed vanillin and *o*-vanillin after selective adsorption was diluted to 0.08 mmol/L. Finally, the concentration of vanillin and *o*-vanillin was determined by UV–vis spectrophotometer at the conditions given earlier, respectively.

RESULTS AND DISCUSSION

Discussion of Infrared Spectrum

Figure 3 shows FTIR spectra of the NMIPs and the MIPs. In the NMIPs spectra, the peaks at 3440 cm^{-1}, 1730 cm^{-1}, and 1160 cm^{-1} were, respectively, attributed to the carbonyl O—H stretching vibration, the carbonyl C=O stretching vibration, and the carbonyl C—O stretching vibration. The peaks at 1450 cm^{-1} and 1390 cm^{-1} were, respectively, attributed to —CH_3 and —CH_2-bending vibration.

The characteristic peak of the MIPs that was similar to the NMIPs indicated that template molecule vanillin was completely extracted from the MIPs. The vanillin and MAA were bound through the hydrogen bond. For the molecular-imprinted polymer, the binding sites were produced *in situ* by polymerization of MAA and cross-linkers around the vanillin. The vanillin was eluted after polymerization, and the hydrogen bond was destroyed. The cavities of vanillin for specific adsorption were left.

Morphology of the MIPs and RAFT-MIPs

The morphology of the molecularly imprinted polymers (MIPs) and the reversible addition-fragmentation chain-transfer (RAFT)-MIPs was observed on SEM as shown in Figure 4. The MIPs microsphere exhibited a smooth surface, spherical shape, a mean particle size of $\sim$ 50 μm, and a relatively narrow size distribution. We can see clearly from the SEM images that the average particle size of the RAFT-MIPs was smaller than that of the MIPs, which can be attributable to the intrinsic characteristics of the controlled/living polymerization mechanism of RAFT. The domain size is often used to indicate the adsorption capacity of the MIPs, that is, the smaller the domain size is, the larger surface area of the systems is and the more adsorption of the systems is. Although the size distribution of the RAFT-MIPs was mostly relatively narrow, some particles of the RAFT-MIPs seemed to be inhomogeneous compared to those of the MIPs, and further studies are needed to overcome the problem.

Rebinding Properties of the MIPs/RAFT-MIPs

To evaluate the presence of imprinted sites in the obtained molecularly imprinted polymers (MIPs), the adsorption capacity of the imprinted polymers has been compared to that of their respective NMIPs. As shown in Figure 5, the MIPs and the reversible addition-fragmentation chain-transfer (RAFT)-MIPs exhibited a higher capacity for vanillin than the corresponding blank polymers in the mixed solvent of ethanol/water (1/4 v/v), which suggested the presence of selective binding sites in the

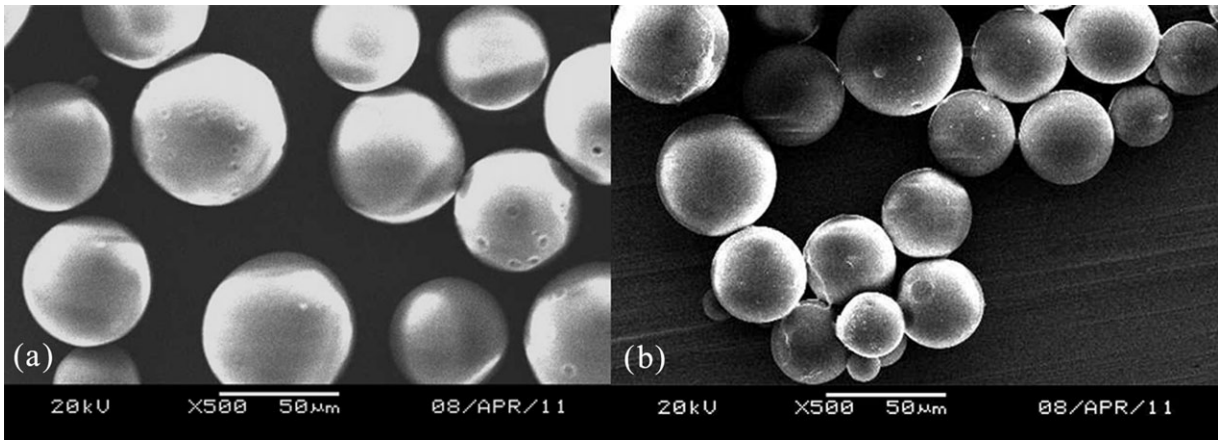

Figure 4. SEM images of the MIPs (a, ×500) and the RAFT-MIPs (b, ×500).

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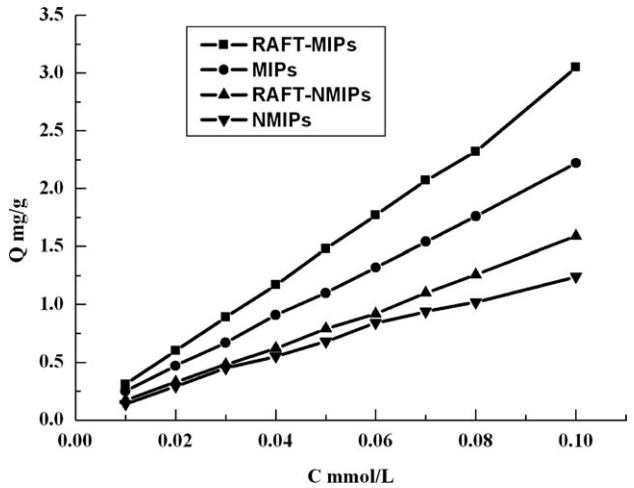

Figure 5. Amount of bound vanillin by the MIPs and the NMIPs in the mixed solvent of ethanol/water.

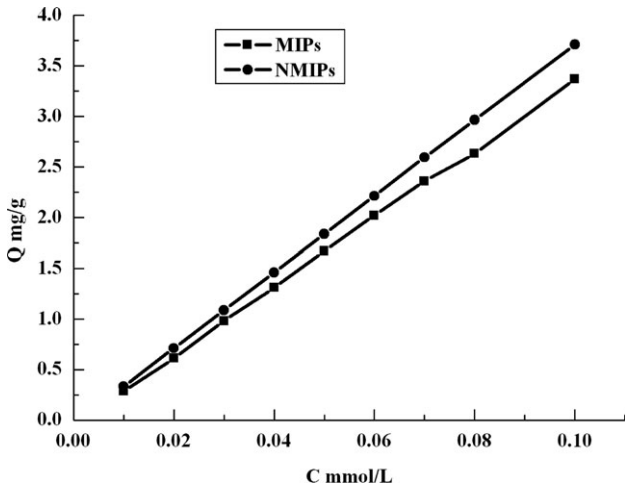

Figure 6. Amount of bound vanillin by the MIPs and the NMIPs in water solution.

obtained MIPs. Besides, the RAFT-MIPs showed higher equilibrium loading capacity than the MIPs in a wide range of vanillin concentrations. For example, in the 0.08 mmol/L vanillin ethanol/water solution, while the RAFT-MIPs bound 2.32 mg/g of vanillin, an equivalent amount of the MIPs bound 1.76 mg/g of vanillin. The results indicated that by controlled/living free radical polymerization, the number of binding sites was increased at approximately equivalent binding affinity. The changes may be a manifestation of shorter kinetic chain lengths and a more narrow dispersity of kinetic chains, which led to a more homogeneous network and potentially a more uniform cross-linking density. The kinetic chains with narrow size distribution would decrease the mesh size of the macromolecular structure and lead to a more uniform and higher population of appropriately sized imprinted macromolecular cavities.[34] In RAFT polymerization, the dithioester derivatives BDB as the chain-transfer agents can react with growing radicals and form the dormant intermediates. The dormant intermediates can release the new living radicals from the corresponding sulfur atoms because of self-cracking. The living radicals are in a dynamic equilibrium with the dormant species and a low-stationary concentration. The irreversible bimolecular termination reaction between radicals is reduced by low concentration of the living radicals. In these systems, the polymerizations have some nature of living polymerization, such as controlled molecular weights, narrow macromolecules weight distribution, shorter kinetic chain lengths, and narrow dispersity of kinetic chains.

In the binding experiments, we also found the MIPs, and the NMIPs showed no significant change in adsorption capacity under the aqueous media (Figure 6). Even the NMIPs exhibited a little better adsorption capacity than the MIPs in the higher vanillin concentrations. From Figures 5 and 6, we found that as the water content increased from 80 to 100%, the adsorption capacity of both the MIPs and the NMIPs increased. This is hypothesized to be due to the hydrophobic substrate of the template molecule vanillin. As the water content increased, the effects between the hydrophobic substrate and the MIPs/NMIPs could be enhanced. The nonspecific adsorption resulted in the unconspicuous change in adsorption capacity between the MIPs and the NMIPs. When the hydrophobic interaction was restrained in the mixed solvent of ethanol/water, the MIPs exhibited specific adsorption.

Scatchard Analysis

The equilibrium dissociation constant and the apparent maximum number of binding sites of the MIPs and the RAFT-MIPs can be estimated by a Scatchard analysis.[35] The Scatchard equation is:

$$Q/[\text{vanillin}] = (Q_{\max} - Q)/K_D \quad (2)$$

where $Q_{\max}$ (mg/g) is the apparent maximum number of binding sites, Q (mg/g) is the amount of vanillin bound to the MIPs at equilibrium, K_D (mg/mL) is the equilibrium dissociation constant, and [vanillin] (mg/mL) is the free vanillin concentration in the solution after adsorption. $Q_{\max}$ and K_D can be determined from the intercept and slope, respectively.

Q/[vanillin] was plotted versus Q, as shown in Figures 7 and 8. From the figures, the plots of the MIPs and the RAFT-MIPs

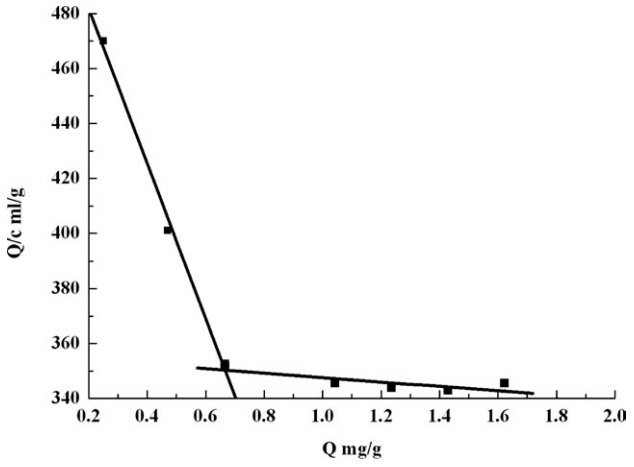

Figure 7. Scatchard plots of the binding of vanillin to the MIPs.

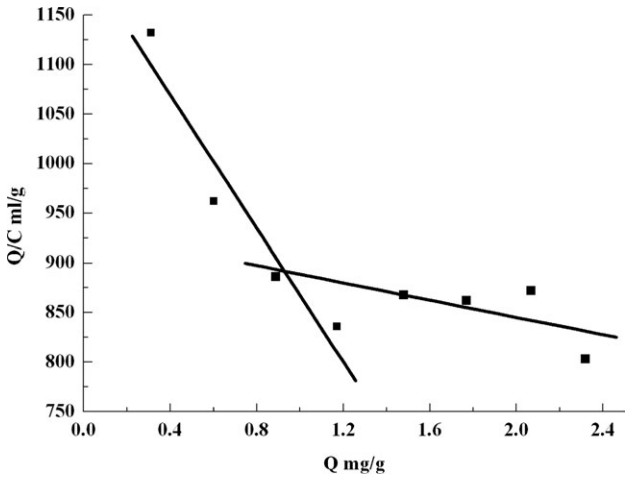

Figure 8. Scatchard plots of the binding of vanillin to the RAFT-MIPs.

that can be seen have two distinct sections that the plots could be regarded as straight lines, respectively. The results indicated that the binding sites of vanillin could be classified into two distinct groups with different specific binding properties. The linear regression equations for the MIPs are Q/[vanillin] = $-281.5Q + 538.1$ (type I-binding sites) and Q/[vanillin] = $-8.14Q + 355$ (type II-binding sites), respectively. Similarly, the linear regression equations for the RAFT-MIPs are Q/[vanillin] = $-336.5Q + 1203.9$ (type I-binding sites) and Q/[vanillin] = $-43.5Q + 932.4$ (type II-binding sites), respectively. The K_D and Q_{max} of the MIPs-binding sites were calculated as 0.0036 mg/mL, 1.94 mg/g and 0.123 mg/mL, 43.67 mg/g. The K_D and Q_{max} of the RAFT-MIPs-binding sites were 0.003 mg/mL, 3.61 mg/g and 0.023 mg/mL, 21.45 mg/g. For both MIPs and RAFT-MIPs, the dissociation of the type I binding sites is lower than those of the type II-binding sites, indicating that they are high-affinity sites in comparison with the low affinity of the type II-binding sites. In addition, the dissociation of the RAFT-MIPs-binding sites (both types I and II) is lower than those of the MIPs-binding sites, indicating that RAFT-MIPs-binding sites are high-affinity sites in comparison with the low affinity of the MIPs-binding sites.

Binding Specificity of MIPs

The binding selectivity of the molecularly imprinted polymers (MIPs) was determined by comparing the binding of the template with those of its analogues. The binding selectivity of the MIPs and the reversible addition-fragmentation chain-transfer (RAFT)-MIPs toward vanillin was also compared to the structurally related compounds *o*-vanillin. As can be seen clearly from Figure 9, both the MIPs and the RAFT-MIPs showed significantly lower binding capacities toward *o*-vanillin than vanillin, which indicated that the imprinted polymers had higher molecular recognition selectivity to the template. Moreover, the RAFT-MIPs showed more difference of binding capacities to vanillin and *o*-vanillin than the MIPs, suggesting more distinct binding specificity of the RAFT-MIPs. This could be attributable to the characteristic of the controlled/living free radical polymerization.

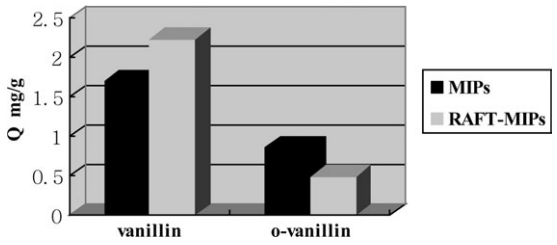

Figure 9. The binding specificity of the MIPs and the RAFT-MIPs.

CONCLUSIONS

MIPs for vanillin were successfully synthesized by conventional suspension polymerization and RAFT suspension polymerization. The morphology and adsorption capacity were examined. The results suggested that the MIPs and the RAFT-MIPs microsphere exhibited spherical shape with the relatively narrow size distribution and a higher adsorption capacity toward vanillin than the corresponding blank polymers in the solvent of ethanol/water. Moreover, the RAFT-MIPs showed smaller average particle size, more obvious molecular imprinting effects toward the template, and more considerable binding specificity than the MIPs, which could be thanked to the addition of the RAFT agent. These advantages make them highly promising in many practical applications.

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