Preparation and Properties of Salicylic Acid-Imprinted Polymers from Emulsions

*Qiu Li, Wei-ying Zhang, Xiao Li**

Summary: Molecularly imprinted polymers with specific recognition to salicylic acid (SA-MIPs) were prepared by oil-in-water emulsion polymerization using salicylic acid as template, acrylamide as functional monomer, and ethylene glycol dimethacrylate as crosslinker. The morphology and size distribution of the SA-MIPs were detected by SEM and photon cross correlation spectrometry. Equilibrium binding experiments and Scatchard analyses were carried out to investigate the selectivity of the SA-MIPs. The results show that the SA-MIPs exhibit a higher affinity and selectivity to salicylic acid than to *m*-hydroxybenzoic acid and sulfosalicylic acid. Two classes of binding sites were produced in the SA-MIPs and the equilibrium dissociation constants were estimated to be 2.03 and 9.97 mmol/L, respectively.

Keywords: emulsion polymerization; molecularly imprinted polymers; recognition; salicylic acid

Introduction

Molecular imprinting is an approach developed recently to prepare functional polymers with specific recognition to target molecules.[1] As the common strategy to prepare molecular imprinting polymers (MIPs), target molecule (the template)–functional monomer complexes are first formed by using the non-covalent or covalent interaction between the template and functional monomers. The complexes are then copolymerized with crosslinkers to yield a three-dimensional network.[2,3] After extracting the template, the cavities with complementary size, shape, and arrangement of functional groups to the template are left in the MIPs. The specific affinity of the MIPs to the template is attributed to the 'memory effects' of the cavities. To date, MIPs have been widely used in a number of applications that require selective recognition, such as the stationary phase for chiral separation and solid-phase extractions, antibody/receptor mimics, enzyme mimics, recognition elements in biosensors, and catalyst of chemical reactions.[3–6]

In general, MIPs are synthesized by bulk or solution polymerization in organic media, followed by grinding and sieving.[7,8] The grinding and sieving process is time-consuming and detrimental to some of the binding sites. The ground MIP particles are also irregularly shaped, which results in low efficient column packing for chromatography. Therefore, direct production of uniform-sized MIP particles is desired. Suspension polymerization and dispersion polymerization have been used to prepare spherical MIPs,[9,10] but emulsion polymerization applied in imprinting is rarely reported. In this paper, spherical MIPs with specific recognition to salicylic acid (SA-MIPs) were synthesized by polymerization in oil-in-water emulsions with salicylic acid as a template and acrylamide as a functional monomer.

Experimental Part

Materials

Salicylic acid (SA), acrylamide (AM), *m*-hydroxybenzoic acid (m-HBA), sodium

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dodecyl sulfate (SDS), methyl α-methacrylate (MMA), alkylphenol polyoxyethylene ether (OP-10), potassium persulfate (KPS), and sulfosalicylic acid (SSA) were obtained from local sources and were of reagent grade. Ethylene glycol dimethacrylate (EDMA) was purchased from Fluka. All chemicals were used as received without further purification.

Preparation of SA-MIPs

The SA-MIPs were prepared by emulsion polymerization as follows. The template (SA, 0.1166 g) and functional monomer (AM, 0.12 g) were dissolved in EDMA (1.2 g) and MMA (4.68 g) overnight. The solution was then transferred to a three-necked flask and the emulsifiers (SDS/OP-10, 0.08 g/0.16 g) and distilled water (20 g) were added to form the oil-in-water emulsion. The emulsion was degassed by purging with nitrogen for 30 min. Potassium persulfate (0.03 g) was then added and the flask was subsequently sealed. Polymerization was performed at 70 °C for 5 h. After polymerization, the latexes were de-emulsified with methanol to obtain the polymer particles. The particles were washed with methanol/acetic acid (9/1, v/v) until the template could no longer be detected by fluorescence spectrophotometry, and finally dried in a vacuum.

Non-imprinted polymers (non-MIPs) were synthesized under the same conditions, but in the absence of the template.

Determination of Properties

The properties of the MIPs were directly evaluated in terms of the adsorption capacity and separation factor. Batchwise binding experiments were conducted for the SA-MIP particles. Particles (0.03 g) were added to 3 mL of SA aqueous solutions in a certain concentration (C_0, mmol/L) in a Erlenmeyer flask. The flask was placed on a rocking bed at room temperature until the adsorption reached equilibrium. The mixture solution was withdrawn and centrifuged at 12000 rpm. The concentration of SA in the supernatant (C, mmol/L) was analyzed using a Cary Eclipse fluorescence spectrophotometer (Varian Inc, USA). The amount of SA bound to the polymer particles (Q, μmol/g) was then calculated. To discuss the selectivity, the static partition coefficient K_d and separation factor α were defined as follows:[11]

$$K_d = C_p/C_s \quad (1)$$

$$\alpha = K_{di}/K_{dj} \quad (2)$$

where, C_p is the concentration of substrate bound to MIPs in equilibrium (mmol/g), C_s is the concentration of substrate left in solution (mmol/L), K_{di} is the static partition coefficient of SA, and K_{dj} is the static partition coefficient of competitor.

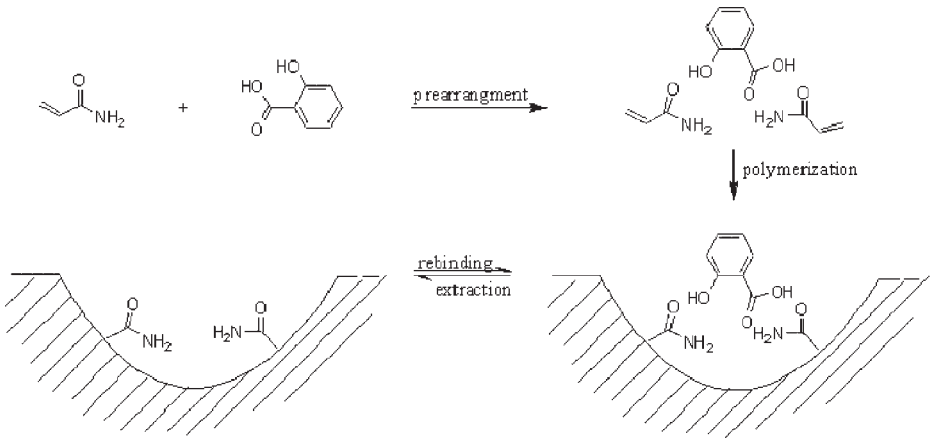

Figure 1.
Schematic representation of synthesis and recognition process of SA-MIPs.

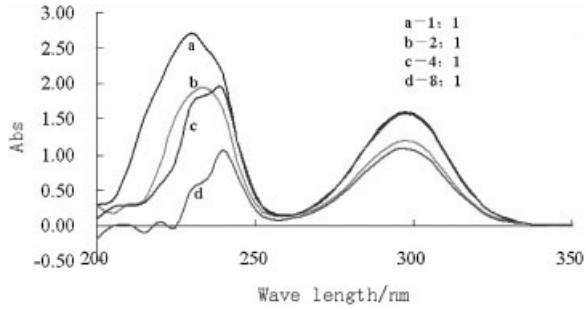

Figure 2.
UV spectra of AM/SA mixture at different molar ratio.

Characterization

UV subtractive spectra were obtained using a Cary50 UV-Visible spectrophotometer (Varian Inc, USA) with AM solution as reference. The particle morphology was observed on a XL30 ESEM-TMP scanning electron microscope (Philips-FEI Co., Holand). The size distribution of the particles was examined by a Nanophox photon cross correlation spectrometer (Sympatec GmbH, Germany).

Results and Discussion

Synthesis and Recognition Process of SA-MIPs

The scheme for the synthesis and recognition process of SA-MIPs is depicted in Figure 1. First, SA and AM are prearranged by the formation of hydrogen bonds. The polymerization is then initiated in the presence of EDMA. For the hydrophilic functional monomer and the hydrophobic template, the bonding sites lie in the surface of the polymer particles. Lastly, the SA is removed from the particles by washing with solvent. The vacant imprinted sites are then available for rebinding of the template, which results in a higher binding capacity over non-MIPs.

Interaction between Template and Functional Monomer

The interaction between the template and the functional monomer was investigated by using UV subtractive spectroscopy techniques. The UV spectrum of AM was subtracted and the obtained subtractive spectra of the AM/SA mixture are shown in

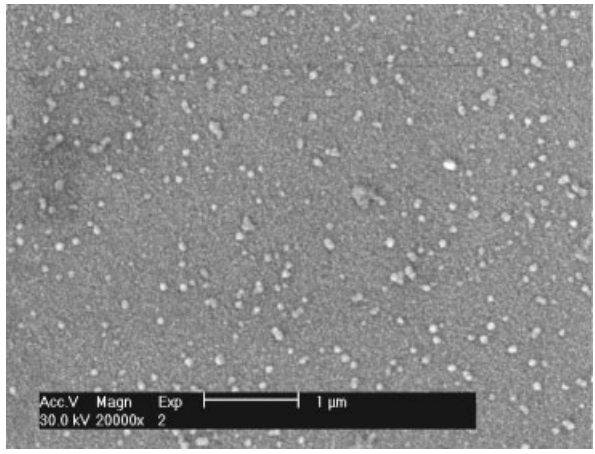

Figure 3.
SEM photograph of SA-MIP particles.

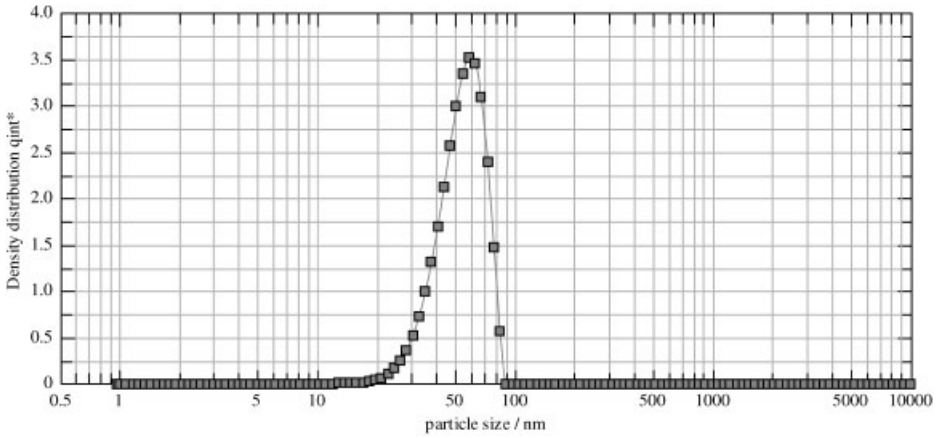

Figure 4.
Size distribution of SA-MIP particles.

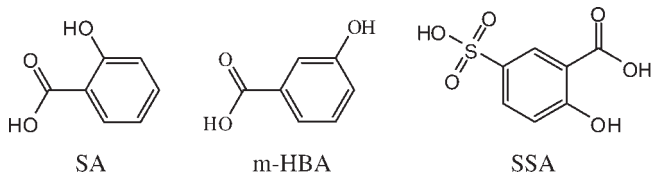

Figure 5.
The structures of SA and competitive substrates.

Figure 2. The wavelength that corresponds to the adsorption peak at the lower wave band increased with increasing AM/SA molar ratio. This indicated that hydrogen bonds and ionic interactions maybe exist between SA and AM.

Particle Size and Distribution of SA-MIPs

The typical morphology and size distribution of the SA-MIP particles are shown in Figure 3 and Figure 4. The particles were uniformly sized and the average diameter was around 50 nm.

Binding Selectivity of SA-MIPs

The selective binding performance of the SA-MIPs was studied by the static adsorption of salicylic acid and the competitive substrates, *m*-hydroxybenzoic acid (m-HBA) and sulfosalicylic acid (SSA), which are structurally close to SA (Figure 5). The results are shown in Table 1. The separation factors of SA-MIPs for SA relative to m-HBA and SSA were up to 2.25 and 3.04, respectively. Meanwhile, the separation factors of non-MIPs were only 1.18 and 2.44, respectively. Therefore, the SA-MIPs showed higher binding selectivity for SA than the non-MIPs.

Adsorption Kinetics of SA-MIPs

The adsorption kinetics of SA-MIPs is depicted in Figure 6. Initially, the amount of adsorbed SA increased rapidly, and then the adsorption rate slowed down at the later stages. The adsorption reached equilibrium in about 80 min.

Table 1.
The binding selectivity of SA-MIPs.

Absorbent	K_d			α	
	SA	m-HBA	SSA	m-HBA	SSA
SA-MIPs	155	69	51	2.25	3.04
non-MIPs	122	103	50	1.18	2.44

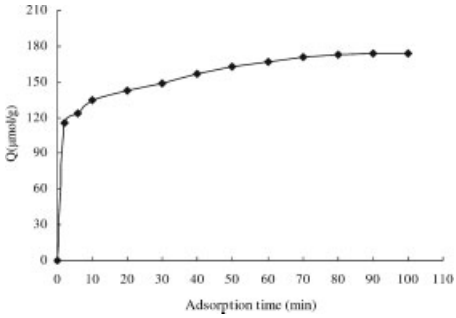

Figure 6.
Adsorption kinetics curve of SA-MIPs.

Binding Isotherm and Scatchard Analysis

The binding isotherm is presented in Figure 7. The adsorption capacity increased until equilibrium, with an increase of the initial concentration of SA. The binding isotherm was further analyzed by fitting to the Scatchard equation:[12–14]

$$Q/C = (Q_{max} - Q)/K \quad (3)$$

where, K is the equilibrium dissociation constant of the binding sites, and Q_{max} is the apparent maximum amount of adsorption (μmol/g).

The Scatchard analysis (Figure 8) shows that two classes of binding sites are produced in the SA-MIPs. The binding constants can be evaluated on the basis of the slopes and intercepts of the fitting lines. The equilibrium dissociation constants K_1 and K_2 were estimated to be 2.03 and 9.97 mmol/L, respectively, which represent high-affinity binding sites and low-affinity binding sites, respectively. The apparent maximum amount of adsorption Q_{max1} and Q_{max2} were calculated to be 411 and 1436 μmol/g, respectively.

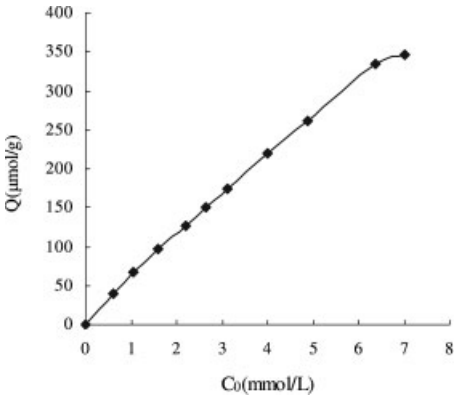

Figure 7.
Binding isotherm of SA-MIPs.

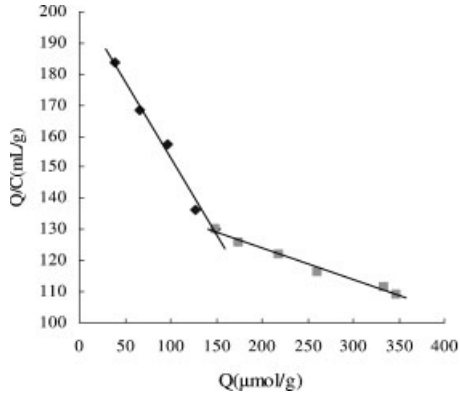

Figure 8.
Scatchard plot for binding isotherm.

Conclusions

Uniform-sized spherical molecularly imprinted polymers with specific recognition to salicylic acid were prepared by oil-in-water emulsion polymerization with salicylic acid as the template and acrylamide as the functional monomer. The average diameter of the SA-MIPs was about 50 nm. The interaction between the template and functional monomer was confirmed by UV subtractive spectra. The adsorption kinetics showed that the adsorption of SA to the SA-MIPs was rapid and reached equilibrium in about 80 min. The Scatchard analysis indicated two classes of binding sites existing in the SA-MIPs.

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