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# Caffeine Molecular Imprinted Microgel Spheres by Precipitation Polymerization

**Dexian Wang*,**, Seung Pyo Hong*, Gengliang Yang** and Kyung Ho Row*,†**

*Center for Advanced Bioseparation Technology, Department of Chemical Engineering, Inha University, 253 Yonghyun-Dong, Nam-Ku, Incheon 402-751, Korea
**Department of Chemistry, Hebei University, Baoding 071002, P. R. China
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**Abstract**−Imprinted uniform microgel spheres were prepared by precipitation polymerization. Acetonitrile was used as the dilute solvent with MAA as the monomer, EDMA as the crosslinker and caffeine as the print molecule. Comparison of caffeine adsorption on molecular imprinted and blank microgel spheres was made. Langmuir model was used to fit the adsorption data. It was found that the caffeine imprinted microgel spheres show specific binding sites to the target molecules. A binding study of caffeine on imprinted microgel spheres was made by Scatchard analysis; the dissociation constants ($K_D$) and the maximum binding capacity were $K_D=1.84\times10^{-4}$ mol/L, $Q_{max}=16.98$ μmol/g for high affinity binding site and $K_D=1.33\times10^{-3}$ mol/L, $Q_{max}=46.84$ μmol/g for lower affinity binding site, respectively. This microgel spheres can be useful affinity adsorbents in further applications.

Key words: Molecular Imprinted Polymers, Microgel Spheres, Caffeine

## INTRODUCTION

The technique of molecular imprinting consists of the self-assembly of a functional monomer and a template molecule in solution followed by the co-polymerization of the functional monomers and an excess amount of appropriate crosslinking monomers. After dissolution of the small molecule, the resulting network polymer exhibits significantly higher affinity for the molecule used as the template than for similar molecules, including closely related isomers [Zhou et al., 1999; Sajonz et al., 1998; Owens et al., 1999; Chen et al., 2001; Zheng et al., 2002]. Molecular imprinted polymer (MIP) has been applied to chiral separation [Spivak and Shea, 1999; Matsui et al., 1998], solid extraction [Bereczki et al., 2001], biomimic sensor [Ansell et al., 1996; Kriz et al., 1997] and membrane separation [Lang et al., 1999; Yoshikawa et al., 1998]. The most successful non-covalent imprinting systems are based on commodity methacrylic monomers, such as methacrylic acid (MAA) because its carboxyl group is the most commonly hydrogen-bonding and acidic functional group in molecular imprinting, cross-linked with ethyleneglycol dimethacrylate (EDMA).

Generally, MIPs are prepared in the form of a macroporous monolith that is then ground, sieved and floated in solvent to obtain the appropriate particle sizes. The grinding, sieving and floating process is time-consuming and low yield producing. Further, the MIPs particles obtained by this way are irregular in shape and show a wide size distribution. This is the main reason that although the selectivity is usually high, MIPs are generally associated with a poor chromatographic efficiency and the elution of broad and asymmetric peaks [Sellergren and Shea, 1995]. Uniformly sized and monodispersed particles had been made by suspension polymerization and seed polymerization or multi-step swelling process [Mayees and Mosbach, 1996; Hosoya et al., 1996; Haginaka and Kagawa, 2002]. However, the above methods either require the use of special dispersing phases/surfactants or are too complicated. Precipitation polymerization, which is high in yield, easy to handle and shows specific binding sites to the target molecules, for preparing uniform MIPs microspheres has been reported [Ye et al., 2001; Ye and Mosbach, 2001].

Here we present a new work of microgel spheres prepared by precipitation polymerization with caffeine as the imprinted molecule. Experiments show that these microgel spheres show specific binding sites to the imprinted molecule.

## EXPERIMENTAL

### 1. Chemicals

Caffeine and methacrylic acid (MAA) were from Sigma (ST Louis, MO, USA). α, α′-Azobis (isobutyronitrile) (AIBN) was the product of Junsei Chemical Co., Ltd. (Japan). Ethylene glycol dimethacrylate Ethylenglykol-dimethacrylat (EDMA) was from Fluka (Buchs, Switzerland). All the above reagents were used directly without further treatment. Acetonitrile and methanol are HPLC grade and from Duksan Pure Chemical Co., LTD (Ansan, Korea). Acetic acid (analytical grade) was from Oriental Chemical Industries (Incheon, Korea).

### 2. Preparation of Molecularly Imprinted Microgel Spheres

In a 250 ml glass flask, 6.98 mmol (0.6 g) monomer MAA, 12 mmol (2.376 g) crosslinker EDMA, 0.24 g reaction initiator AIBN were dissolved in 65 ml acetonitrile. The solution was put into a sonication bath for 10 min to dissolve the chemicals well, sparged with helium for 10 min to remove oxygen, then the flask sealed under helium. Polymerization was performed in a water bath with the temperature maintained at 60 °C for 24 h. After the polymerization, the microspheres were collected by centrifugation at 10,000 rpm for 10 min and put into an oven to dry. Blank microgel spheres were made with the same procedure just in the absence of caffeine template.

†To whom correspondence should be addressed.
E-mail: rowkho@inha.ac.kr

### 3. HPLC Analysis

Analysis of caffeine was carried by a liquid chromatography system containing a Waters 600s Multisolvent Delivery System and a Waters 616 pump (Waters, Milford, MA, USA), a detector of Waters 2487 Dual Absorbance (Waters, Milford, MA, USA) and Rheodyne injection valve (20 μl sample loop). Millennium 3.2 (Waters, Milford, MA, USA) was used as the data acquisition system. Quantitative determination was based on a 3.9×150 mm Waters stainless steel column packed with LiChrospher 100 RP-18 (12 μm) (Merck, Germany) particles, mobile phase methanol-water (50/50, v/v) at a flow rate of 0.8ml/min, UV wavelength at 270 nm.

### 4. Adsorption and Scatchard Analysis

First, caffeine imprinted microgel spheres were washed by using a methanol-acetic acid mixture (4/1, v/v) to remove the template molecule; then the residual acetic acid was removed with methanol, and 30 mg of the imprinted and blank microgel spheres were put into two 10 ml test tubes, respectively. Then 3.0 ml of caffeine acetonitrile solution with the concentration varying from 0.15 to 2.5 mmol/L was added. The mixture was incubated under temperature for 24 h and then was transferred to centrifugation for 10 min at 4,000 rpm. The concentration of free caffeine was determined by HPLC. Bound caffeine to the microgel spheres was obtained by the difference between the initial and the free concentration of caffeine.

## RESULTS AND DISCUSSIONS

### 1. Preparation of Microgel Spheres

Producing conventional imprinted polymer monolith requires a large amount of cross-linking monomer. Phase separation occurs during the cross-linking process when the limit of solubility of the growing polymer is exceeded. Polymers with a wide range of porosity can be prepared by controlling the point of phase separation, which, in turn, depends on the nature and the volume of porogen used and the concentration of crosslinker [Sherrington, 1998]. Increasing the amount of solvent gradually causes the growing polymer chain to be unable to occupy the entire reactor volume, and therefore form a dispersion of macrogel particles. Further dilution can lead to a decrease in the size of gel particles [Ye and Mosbach, 2001]. Fig. 1 schematically shows the change of polymer structure from macroporous monolith to macrogel particles, and finally to microgels by diluting the reaction system.

In this work, acetonitrile was used as the diluting solvent and the concentration of acetonitrile was 96% of the reactive mixture. After polymerization and centrifugation, it was found that the yield of the microgel spheres was 91.3% for both imprinted and blank microgel spheres. In Figs. 2, 3 and 4, the SEM pictures of the grounded particles by traditional bulk polymerization and the obtained microgel spheres by precipitation are illustrated. It can be seen that the microgels can be regarded as spherical and particle size shows

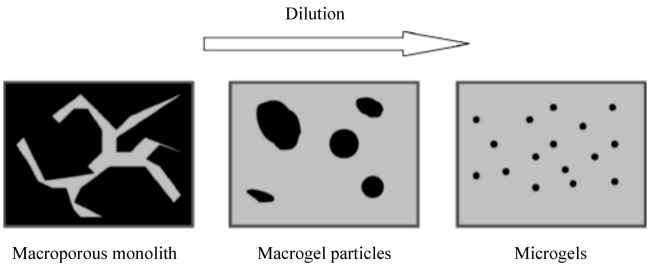


**Fig. 1. Change of polymer structure from macroporous monolith to discrete microgels by dilution of the reaction solution.**

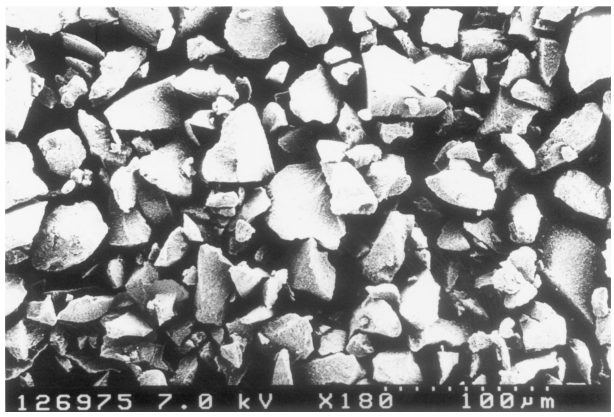


**Fig. 2. SEM of molecular imprinted polymer particles prepared by traditional bulk polymerization and grounding-seiving-sendimentation proceders.**

**Fig. 3. SEM of blank microgel spheres. Molar ratio of MAA : EGDMA is 6.98 : 12, solvent (acetonitrile) fraction is 96% (v/v).**

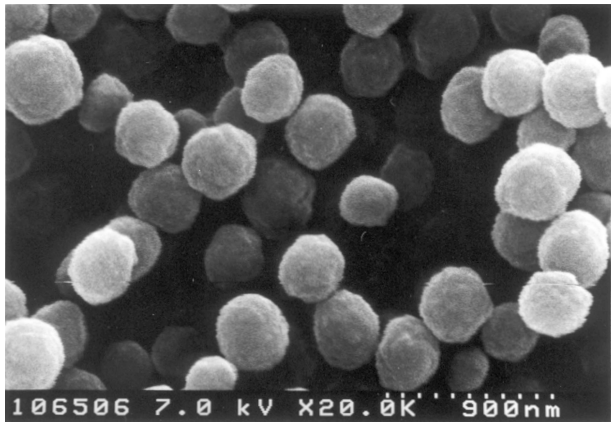


**Fig. 4. SEM of caffeine imprinted microgel spheres. Molar ratio of MAA : EGDMA : Caffeine is 6.98 : 12 : 0.86, other condition is same as that of blank.**

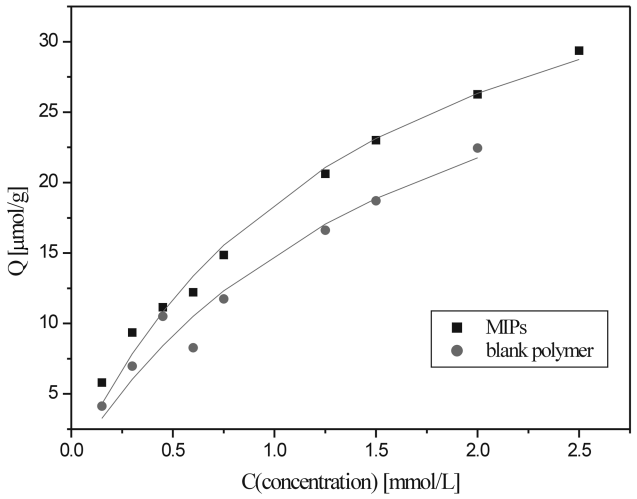


**Fig. 5. Adsorption of caffeine on imprinted and blank microgel spheres fitted with Langmuir isotherm model.**

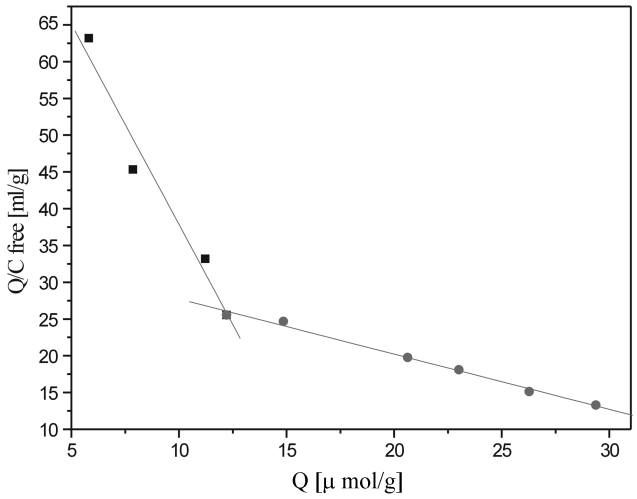


**Fig. 6. Scatchard plot of caffeine imprinted microgel spheres for caffeine.**

**Table 1. Constant of the adsorption data fitted by Langmuir model**

| | a | b |
|---|---|---|
| Caffeine imprinted microgel spheres | 31.6 | 0.701 |
| Blank microgel spheres | 23.7 | 0.589 |

an average of 400 nm for caffeine imprinted microgels, and an average of 800 nm for blank microgels, which indicates the template can affect the polymerization and result in smaller particles.

**2. Adsorption Analysis**

Fig. 5 shows the adsorption of caffeine on the imprinted and blank microgel spheres. The caffeine concentration changed from 0.15-2.5 mmol/L. Langmuir isotherm model was used to fit the obtained adsorption data:

$$q = \frac{aC}{1+bC} \quad (1)$$

where C is the free equilibrium concentration of caffeine, q is the amount of caffeine absorbed on the polymer, a and b are constants. The constants fitted by Langmuir isotherm model can be seen in Table 1. It can be seen that the imprinted microgel spheres show higher affinity to the target molecule.

**3. Scatchard Analysis**

Binding parameters of caffeine on the imprinted microgel spheres were determined by Scatchard analysis [Zhou et al., 1999; Zhang et al., 2001]. The Scatchard equation is as follows:

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

where Q is the amount of caffeine bound to the polymer, $Q_{max}$ is the maximum binding capacity, $K_D$ is the equilibrium dissociation constant, [Caffeine] represents the equilibrium concentration of caffeine.

Fig. 6 is the plot according to the Scatchard equation. There are two distinct sections within the plot that can be regarded as straight lines, which indicates that there exist two classes of binding sites in the imprinted microgel spheres [Matsui et al., 1995]. This is consistent with the common description about MIPs. It is known that the imprinted polymer surface and also the imprinted microgels are often regarded as heterogeneous and there are two kinds of binding sites on the imprinted polymer surface [Zhou et al., 1999; Sajonz et al., 1998; Chen et al., 2001]: one is selective or high affinity with high binding energy, and the other is nonselective or low affinity with low binding energy. In low concentration range, the adsorption on selective binding sites is stronger than that on nonselective binding sites [Chen et al., 2001]. From the slope and intercept of its Scatchard plot, the dissociation constants ($K_D$) and the maximum binding capacity of the imprinted microgel spheres were $K_D = 1.84 \times 10^{-4}$ mol/L, $Q_{max} = 16.98$ μmol/g for high affinity binding site and $K_D = 1.33 \times 10^{-3}$ mol/L, $Q_{max} = 46.84$ μmol/g for lower affinity binding site, respectively.

## CONCLUSION

Microgel spheres with high yield (product rate was 91.3%) have been prepared by precipitation polymerization method. Through adsorption and Scatchard analysis, the caffeine imprinted microgel spheres showed high specific affinity to the target template molecule. These microgel spheres can be useful affinity adsorbents in further applications.

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