Effect on Rebinding Behavior with Different Composition and Structure of the Dually Imprinted Alginate Polymer Microspheres Using Proteins and o/w Emulsion Drops as Dual Templates

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ABSTRACT: In this study, the dually imprinted alginate polymer microspheres are prepared using proteins and o/w emulsion drops as dual templates with different structure and composition such as the content of hydroxylethyl cellulose (HEC), crosslinking degree, and porous size, etc. The rebinding tests are carried on egg white albumin (EA) protein imprinted alginate polymer microspheres with interpenetrating networks (IPNs) under various conditions like ionic strength, temperature, protein concentration, and pH value. Results showed that the IPNs with alginate and HEC by covalent bond influence the rigidity and swelling properties. Porous structure formed by introducing emulsion drops into alginate hydrogel has effect on the permeability and specific surface area of the polymer microspheres. The variation rule of rebinding behavior according to different HEC content and pore diameters reveals macromolecular imprinting effect to be different with pure pore creating effect compared with the emulsion imprinting effect. Moreover, the macromolecular and emulsion dually imprinting-rebinding tests verify the coexisting macromolecular imprinting effect and pore creating effect of emulsion imprinting. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 3516–3526, 2010

Key words: dually imprinted polymer microsphere; porous; alginate; hydroxylethyl cellulose; rebinding behavior; macromolecular imprinting; emulsion imprinting

INTRODUCTION

Molecularly imprinted polymer microspheres (MIPMs) are attracting more and more attention because MIPMs exhibit regular sphericity, preferable surface, and better rebinding property.[1–3] The protein macromolecular imprinting using hydrogel polymer matrix have been showed enhanced protein rebinding property on account of the soft and wet property with specific network structures. The molecular (protein) imprinting process in hydrogel matrix is shown in Scheme 1. The rebinding behavior of the protein-macromolecularly imprinted polymer microspheres could be influenced by some structural factors.[4–8] As for the protein imprinted hydrogel polymer microspheres, the protein is rebound on the functional sites on the interface between the hydrogel polymer matrix and the medium. Structural factors like crosslinking density and porous diameter will have effects on the molecular movement and configuration, subsequently influent the molecular imprinting efficiency. The crosslinkage will exert effects on protein diffusion and exchange inside the matrix, and also on the swelling and dissociation of the microspheres, the porosity factors such as internal pores and channels will also significantly influence the property of the polymer microspheres.[9–11] In recent years, we have proposed and developed a new principle of target interaction model (PTIM) in intermolecular interactions between protein and the hydrogel polymer matrix to obtain an optimized specific rebinding property of macromolecularly imprinted polymer. We provided a strategy to gain the optimum specific rebinding property via achieving corresponding target interaction model in every process of gelling, removing, and rebinding, according to the principle of target interaction model by designing or adjusting of the structure and property of hydrogel polymers and proteins.[12,13]

In our previous works, macromolecularly imprinted alginate hydrogel polymer microspheres are prepared, and an improved imprinting efficiency is achieved as the porous structure introduced by the o/w emulsion.[14–16] Afterwards the hydroxyl ethyl cellulose (HEC) is applied to form interpenetrating networks (IPNs), and the imprinting efficiency (IE) is further increased because of the microspheres stabilization. On the basis of another article, the rebinding behavior of the macromolecularly imprinted hydrogel polymer microspheres is

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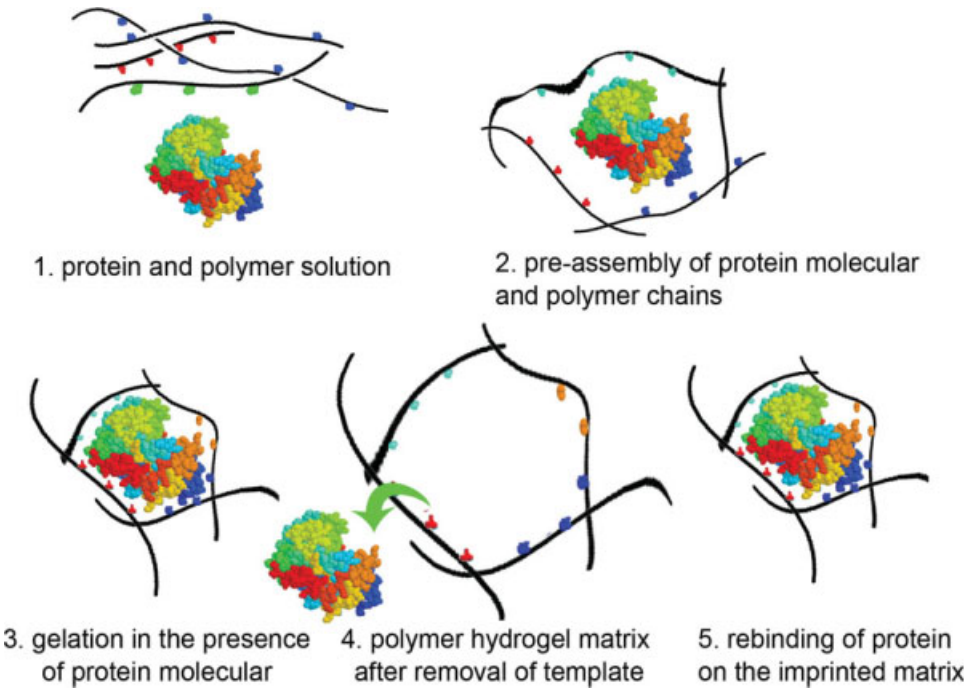

Scheme 1 Schematic representation of the molecular imprinting process. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

studied, and it's showed that the macromolecular imprinting effect is from the cooperation of the imprints specificity and the macromolecular pore-creating effect.[17] The rebinding process is presumed to be influenced by the crosslinkage density and pore dimension. In the crosslinked porous hydrogel polymer system, on one side, the degree of crosslinking can be modified by the HEC content, and the molecular transferring and rebinding are changed consequently. On the other side, the pores statistics diameter could be changed by alter the emulsion composition and surfactant content, and the specific surface area and the functional groups density are influenced. In this article, the PTIM was used as guideline to discuss the specific rebinding property with controlling the structures of protein and polymer via tuning of ionic strength, concentration and acidity of protein, temperature and pore diameters, etc., the imprinting efficiencies of the MIPMs with different composition and structure (crosslinking degree is represented by swelling ratio, equilibrium pore diameters, etc.) are studied detailedly.

EXPERIMENTAL

Materials

Sodium alginate (Sodium–Alg, SA, Mn = 35,000, Mw = 218,000) is purchased from Beijing Xudong Chemical Reagent Factory. HEC HD30000 is from Shangdong HEDA Co. Egg white albumin (EA, isoelectric point pH = 4.7) is obtained from Fluka Chemie Gmbh. Tris-(hydroxymethyl) aminomethane (Tris), analytical grade, is from Institute of Biological Engineering, Chinese Academy of Medical Science. Polyvinylpyrrolidone K30 (PVP) is from Tianjin Tiantai Fine Chemical Reagent Co. Calcium Chloride Anhydrous, Glutaraldehyde (GA, 50% in H_2O), Chloroform, Hexane, Tween60, Liquid paraffin, Span 85, Alcohol (100%) and Ethyl ether (100%), chemical grade, were all from Tianjin No.2 Chemical Reagent Factory, China. Span80, chemical grade, is from Dazhong pharmaceutical factory, Shanghai. The reagents above were used without further purification. Alginate solution, cellulose ether solution, and crosslinking agent were freshly prepared as needed in deionized water.

Equipments

Constant temperature vibrating incubator (HZQ-F) is from Donglian Electronic Technology Developing Co. ZDJ-5 automatic potential titrator and DDS-307 conductometer are from Shanghai Precision and Scientific Instrument Co. The Spectrophotometer UV/Vis U-1800 instrument is from Hitachi Co. Japan. Microscopes are the Axiovert 25C inverted microscope from Carl Zeiss Co. Germany and the BXX—X51M, Olympus from Japan.

The rebinding behavior of the MIPMs with various HEC content

The crosslinking degree of the microspheres is determined by the HEC content added to the alginate

hydrogel. The dually imprinted Calcium Alginate microspheres are prepared as described elsewhere,[15–17] and their rebinding property are studied in the following skills.

The rebinding controlled by ionic strength

The EA protein solutions with various ionic strengths are prepared by adding NaCl or $CaCl_2$ into 20 μmol/L EA aqueous solutions, and the acidity is adjusted to pH = 4.7. Equal amount of dually imprinted, emulsion imprinted, molecular imprinted, and nonimprinted microspheres are applied in the protein solutions. The adsorbance of the microspheres is detected by UV spectrometer, and the equilibrium rebound quantity is calculated as follows:

$$Q = (C_0 - C_t)V/W \quad (1)$$

Wherein Q is the rebound quantity, C_0 is the beginning concentration of EA, C_t is the equilibrium concentration, V is the volume of the EA solution and W is the mass of the applied microspheres. The imprinting efficiency (IE) is calculated as follows:

$$\mathrm{IE} = Q/Q_N \quad (2)$$

Wherein Q_N is the rebound quantity on the nonimprinted microspheres.

With the aim of investigating the swelling effect on the imprinting-rebinding process, the swelling ratio of the microspheres in different ionic strength is measured as described elsewhere.[15]

The rebinding controlled by temperature

The rebinding test is carried out in the vibrating incubator. The C_0 and C_t of the protein solution under certain temperatures is detected by UV spectrometer. The rebound quality Q and the imprinting efficiency E under various temperature are calculated according to Formula (1) and (2).

In the swelling experiment, the microspheres are placed in the same solution as in the above experiment, and the swelling ratio within 25– 47°C is detected. Concretely, the set of microspheres and solution together with the beaker is incubated under 25°C, and the equilibrium weight is measured. Then the temperature is adjusted to 27°C, and the work is proceeded with similarly.

The rebinding controlled by protein concentration

A series of EA solutions with various concentrations (from 10 to 22 μmol/L) are applied in the rebinding experiment. The adsorption is detected by UV spectrometer and Q and E are worked out. Then the imprinting efficiency curve according to concentration and swelling ratio is determined.

The rebinding controlled by acidity

The protein solutions with various pH ranging from 3 to 11 are prepared as follows. $CaCl_2$–$Ca(OH)_2$ aqueous solution is used to prepare basic solution, and HCl–$CaCl_2$ to prepare the acid solution, in which the ions contained are Ca^{2+}, H^+ and OH^-. The Ca^{2+} ionic strength maintained the same throughout all of the solutions with different pH.

MIPMs with different HEC content are applied in the above EA solutions under 25°C. The rebound quantity is detected by UV spectrometer, and the imprinting efficiency curve according to pH is determined. Meanwhile the equilibrium swelling ratio in the solutions with different pH is also determined.

The rebinding behavior of the MIPMs with different pore diameters

The pore diameter of the dually imprinted microspheres determines the specific surface area and the rebinding sites distribution. Therefore, the porosity will exert effects on the environmental responsibility of the microspheres. In this work, the pore diameter of the microspheres is modified by changing ratio of the porogen emulsion. Three of the microspheres with better pore monodispersity are chose to be imprinted, and the emphasis is put on the ionic and temperature responsibility of the dually imprinted microspheres.

Preparation of the alginate emulsion with different droplets diameters

The pore diameter of the microspheres could be controlled by regulating the porogen drops (paraffin) content of the alginate emulsion. The mass percent of alginate sol applied is 3%, and EA protein solution with the concentration of 2×10^{-5} mol/L is adjusted to pH = 4.87. The homogeneous and stable emulsion may be prepared following the emulsifying procedure introduced elsewhere, and the

TABLE I
Composition of Sodium Alginate/HEC, and the Equilibrium Swelling Ratio in 0.9% NaCl Solution

Samples	2% HEC (mL)	3% SA (mL)	Ratio of HEC/SA (w/w)	Equilibrium swelling ratio
HEC0	0	20	0%	2.9
HEC1	0.3	20	1%	2.0
HEC2	0.6	20	2%	1.7
HEC3	0.9	20	3%	1.4
HEC5	1.5	20	5%	1.1

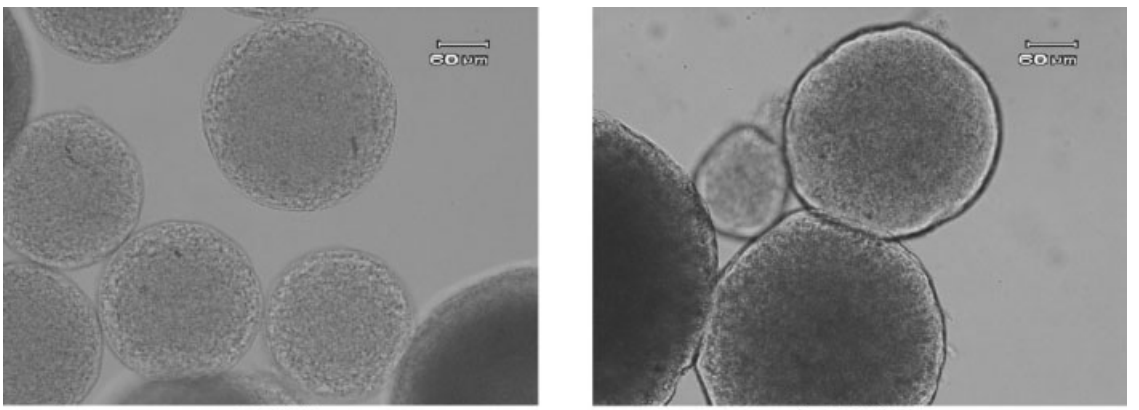

Figure 1 Morphology of alginate polymer microspheres.

emulsion imprinted microspheres is prepared by inverse suspension gelation method.[15–18]

The ionic strength effect on the imprinting efficiency

The dually imprinted or emulsion imprinted microspheres with various pore diameter are applied in the EA solutions with different ionic strength (Na^+ and Ca^{2+}). The microspheres are allowed to swell until equilibrium under different ionic strength, and the rebound quality is detected by the UV spectrometer. The imprinting efficiency is calculated by Formula (1) and (2) as described earlier in eq. (1).

The temperature effect on the imprinting efficiency

The dually imprinted or emulsion imprinted microspheres with various pore diameter are incubated in 20× 10e-6mol/L EA and 3% $CaCl_2$ solution (pH = 4.87), and the whole set is placed in the constant temperature vibrating incubator. The rebinding is proceeded till equilibrium under 25°C, and the imprinting efficiency is determined as described earlier in eq. (1). In like manner, the imprinting efficiency under other temperature (from 25 to 42°C) is also detected.

In this process, EA solutions of different concentration (10 –22 μmol/L), and different acidity (pH = 3–11) are also used to study the temperature effect on the imprinting efficiency.

RESULTS AND DISCUSSION

MIPMs with different HEC content

Swelling test in microspheres with different HEC content

Small amount of hydroxyl ethyl cellulose (HEC) is introduced into alginate hydrogel to improve the rigidity and reduce the swelling ratio in the Na^+ and Ca^{2+} solution. The HEC content in the alginate solution and the corresponding swelling ratio are shown in Table I. The swelling ratio R was calculated as follows:

$$R = (W_t - W_0)/W_0 \quad (3)$$

Wherein R is the swelling ratio, W_0 is the original weight and the W_t is the equilibrium weight.

It is suggested the equilibrium swelling ratio is reduced as the HEC increases. Moreover, other proof for the HEC effect may be the microscope photograph (Fig. 1). Micropsheres with 1% HEC take on good sphericity, and samples with more than 3% HEC have a rough lineament duel to the HEC chain restricting the alginate matrix as the beads swells.

The rebinding process controlled by ionic strength

In the study of ionic effect, a series of BSA solutions with different concentration of NaCl or $CaCl_2$ were

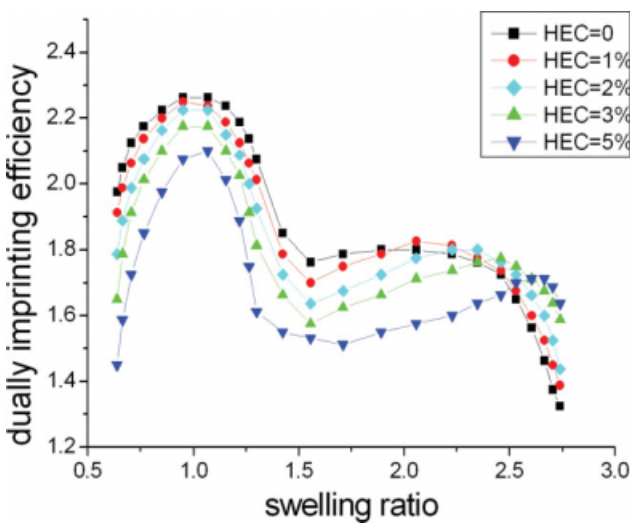

Figure 2 Dually imprinting efficiency as the function of swelling ratio for microspheres with different HEC concentration. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

prepared with the ionic strength ranging from 0 to 0.5 mol/kg. The ionic strength was calculated as follows:

$$I = \sum \frac{1}{2} bz^2 \quad (4)$$

Wherein I is the ionic strength, b is the solvent molality (mol/kg) of electrolyte and z is the charge number.

The alginate microspheres has different swelling ratio in solutions with Ca^{2+} and Na^{+} of different concentration. In this work, the concentration of Ca^{2+} and Na^{+} ion is measured in the form of ionic strength. When the swollen state changes, the imprinting cavities and specific sites configuration are changed, leading to different rebinding affinities. Therefore, the molecular imprinting efficiency is affected. Besides, the emulsion imprintings will also change their dimension when the matrix swells. As a result, we change the bead swelling ratio by means of adjusting ionic strength, and consequently control the molecular, emulsion and dually imprinting efficiency.

The ionic controlled dually imprinting efficiency (dIE) in the microspheres with various HEC content is detected under different swollen states. Each curve has characteristic peaks for the molecular imprint and the emulsion imprint, as is shown in Figure 2. The first peak (the left one) is considered to be the intrinsical peak of the molecular imprint. The curve is sharpen and lowered at the apex as the HEC increases due to the improved rigidity of the matrix. An imprinted matrix with good rigidity is not easy to be distorted and provides complementarity to the rebound molecules and the functional sites. The second peak is the intrinsical peak of the emulsion imprint, which is suggested to be shift right as the HEC increases. The reason is considered to be the HEC restraining the beads volume and surface from expanding and the apex emulsion imprinting efficiency is delayed to even more swollen states.

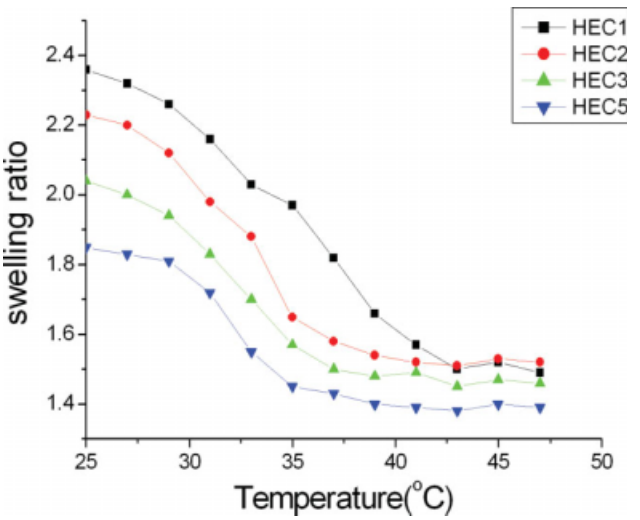

Figure 3 Swelling ratio as the function of temperature for micospheres of different HEC concentration. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The rebinding process controlled by temperature

The alternation in the equilibrium swelling ratio by temperature is on the account of change in the HEC solution content within the matrix. The HEC used in the microspheres is expected to influence the transfer behavior through the hydrogel as the temperature changes. The curve of equilibrium swelling ratio according to temperature is shown in Figure 3. Compared with the dIE curve in Figure 4, it is suggested that the inflexion point of swelling ratio is related to the molecular imprint structure as is explained elsewhere.[17]

The thermodynamic energy desorbs the rebound molecules, and the dIE is enhanced as the swelling ratio increases consequently. Meanwhile the amount of dIE inclination is decreased as HEC mounts up, suggesting that the desorption process in the following equation is inhibited.

$$\underset{\text{(desorption)}}{\text{diffusion to solution}} \xleftarrow[\text{kinetics energy(concentration gradient)}]{\text{thermodynamic energy}} \underset{\text{(non-specific adsorption)}}{\text{diffusion into microshperes}} \quad (5)$$

The rebinding kinetics in EA solutions

The rebinding experiment is performed in series of EA solution varied in concentration. Microspheres with different HEC content are applied in the imprinting and rebinding process so as to study the structural factors in the interaction between hydrogel and protein molecules. The dIE curve according to EA concentration (pH = 4.7) at 25°C is shown in Figure 5.

The dIE is enhanced as the protein increases in concentration, due to the kinetic equilibrium demand of the concentration and adsorption. The imprinting efficiency is lowered as HEC increases because of the descent in emulsion imprinting efficiency (eIE) at lower swelling ratio. Moreover, the inflexion point of the curve shifts to different concentration as the HEC content changes and reaches the maximum protein concentration (18 μmol/L) when the HEC content is 2%. The inflexion point suggests an alternation in the molecular imprint rebinding property, i.e., the point when the

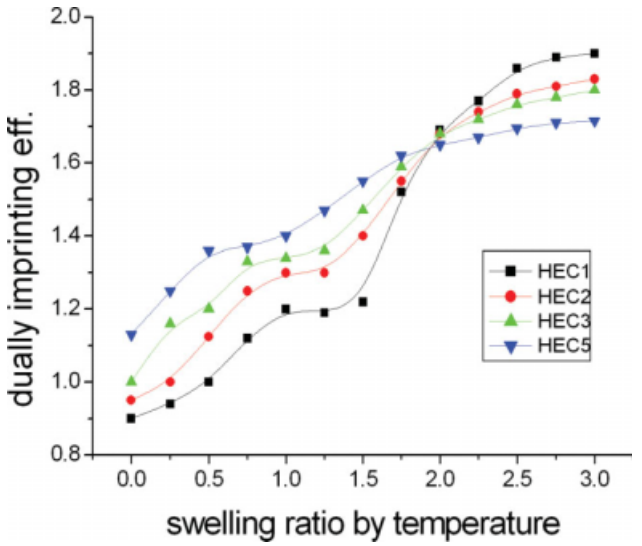

Figure 4 Dually imprinting efficiency for microspheres of different HEC concentration as the function of swelling ratio controlled by temperature. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

molecular imprinting efficiency (mIE) begins to maintain invariable. As for the dually imprinted system, the inflexion point is considered to be the distinction between the two types of imprints. Before the point (lower concentration), the dIE is the collaboration of the molecular imprints and the emulsion imprints; while after the point, the growth in the dIE is mainly on the count of emulsion imprints.

The facts that the inflexion point of dIE curve shifts at different HEC content could be explained at several aspects. The imprinted hydrogel rebinds molecular by means of specifically structured functional sites and shape complementary cavities. As a result, the rebinding procedure on the molecularly imprinted hydrogel could be analogous with the monolayer adsorption model. Protein is assumed to be rebound exclusively on the vacant sites and cavities. The microspheres with less HEC may have more functional sites but less complementary cavities due to the deficiency of hydrogel rigidity, resulting in the lowered dIE. Centrally, higher HEC content provide the hydrogel with better rigidity that is required by the cavities, but more HEC occupied the room that should have been to the alginate; moreover, more [SBond]OH groups are consumed in the covalent bond forming process, followed by a less amount of functional sites and consequently a lowered imprinting efficiency. On all account, an optimum imprinting efficiency is achieved by a proper HEC content when the number of functional sites, and complementary cavities are in best proportion.

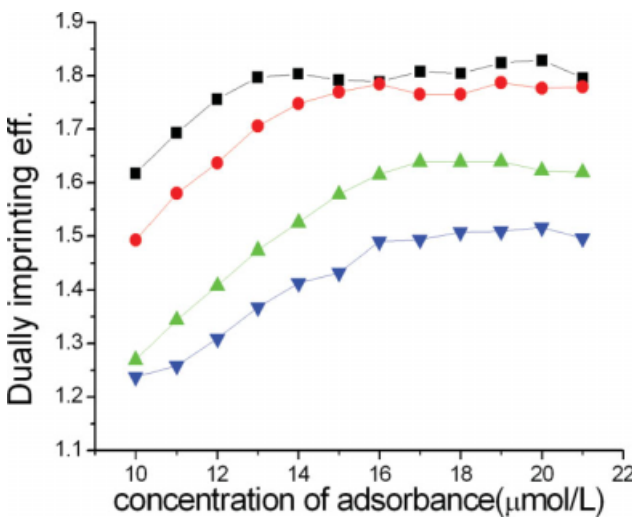

Figure 5 Dually imprinting efficiency as the function of the concentration of adsorbance (■): HEC1; (●): HEC2; (▲): HEC3; (▼): HEC5. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The acidity effect on the imprinting efficiency of microspheres with different HEC content

Microspheres with different HEC content are put in $CaCl_2$–$Ca(OH)_2$ solution and allowed swelling to equilibrium under 25°C. Figure 6 shows the swelling ratio of microspheres samples according to pH. In the acid surrounding, most of the carboxylate radical of the alginate chain exists in the carboxylic acid form ([SBond]COOH), which forms more hydrogen bounds with each other and hydroxyl groups ([SBond]OH) to restrain the microspheres from swelling. Whereas in basic surrounding, the static

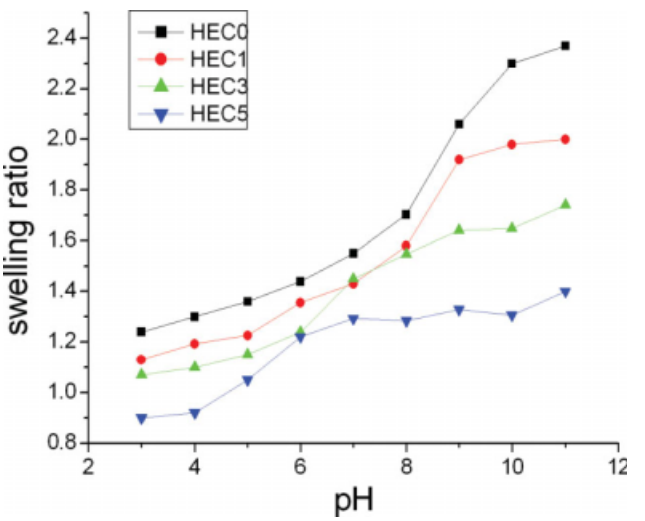

Figure 6 Equilibrium swelling ratio of microspheres with different crosslinkage in different pH solution. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

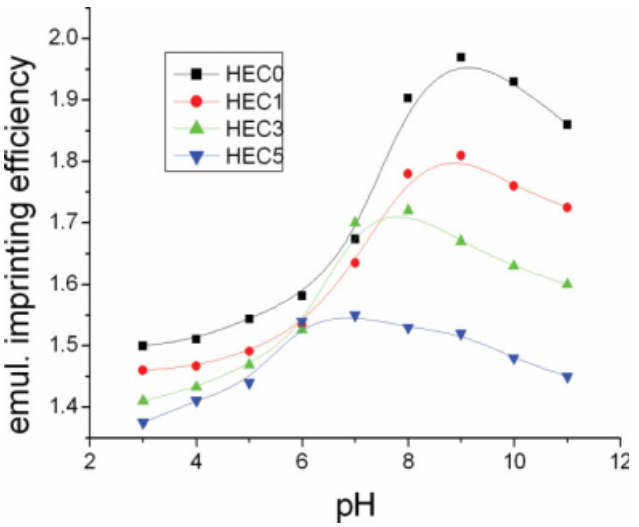

Figure 7 Emulsion imprinting efficiency of microspheres with different crosslinkage as the function of pH. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

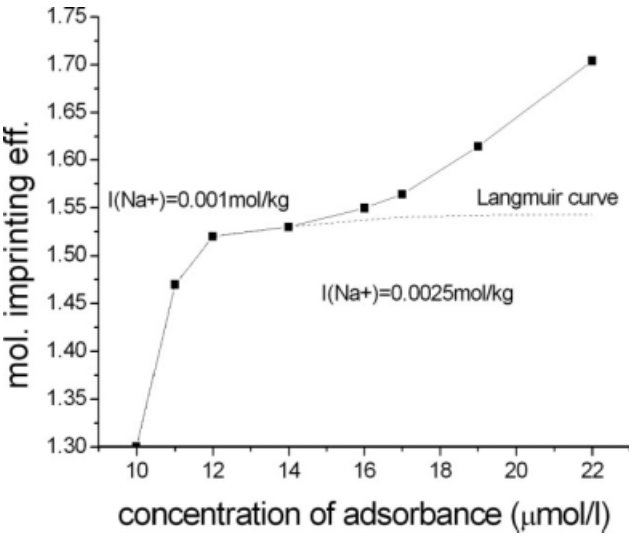

Figure 9 Adsorption isotherm curve of MIPMs as Langmuir curve (■): molecular imprinting efficiency; (·): the Langmuir adsorption.

repulsion between the carboxyl groups ([SBond]COO^-) causes the microspheres a more swollen state.

The molecular and emulsion imprinting efficiency according to pH are shown in Figures 7 and 8. The emulsion imprinted microspheres rebound protein mainly by means of surface effect, therefore, the eIE is lowered as the HEC restrains the hydrogel from swelling. While the extremum of mIE is enhanced by HEC as the highly crosslinked rigid matrix provides better functional sites specificity and cavity complementary.

Each eIE curve has one apex value at certain pH condition (Fig. 7) owning to the microspheres swelling as the basicity increases, which is suggested in Figure 6. The location where the eIE begins to mount up, and approaches peak is found to accord with the swelling ratio curve, which proves the emulsion imprints rebound to be an adsorbing process based on surface effect. The emulsion imprinting efficiency varies in accordance with the swollen state in different acidity surrounding.

Contrastively the molecular imprinting efficiency curve is characteristic for the two-peak shape (Fig. 8), marked as peak A (the left) and peak B (the right). Each curve has the same peak A location, i.e.,

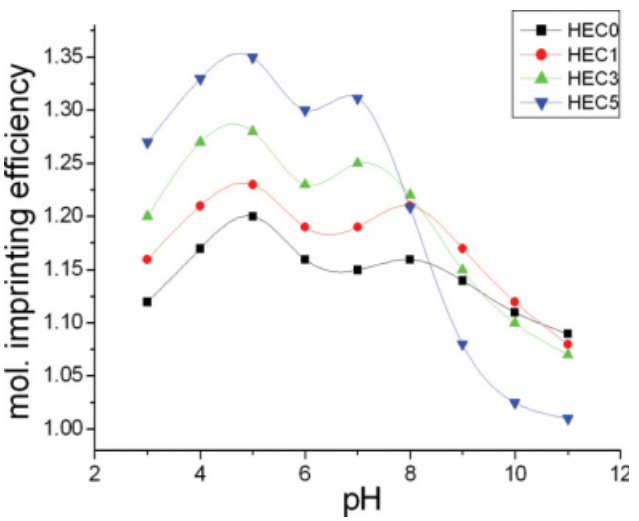

Figure 8 Molecular imprinting efficiency of microspheres with different crosslinkage as the function of pH. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

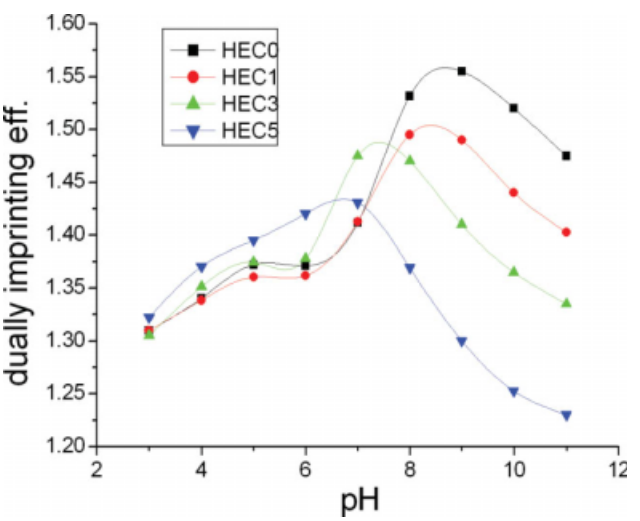

Figure 10 Dually imprinting efficiency of microspheres with different crosslinkage as the function of pH. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II
Preparation of the O/W Emulsion, the Static Pore (Emulsion Drop) Diameters and Standard Deviation

Sample no.	3% SA (mL)	Paraffine (g)	Surfactants	Mean diameter (μm)	SD (μm)
1	20	0.5	0.05 g Span85 + 0.10 g Tween60	2.17	0.59
2	20	1.2	0.10 g Span85 + 0.20 g Tween60	3.21	1.12
3	20	1.5	0.15 g Span85 + 0.20 g Tween60	4.96	0.33
4	20	3.0	0.20 g Span85 + 0.40 g Tween60	6.15	1.53

pH = 4.7, the isoelectric point of EA point. The protein solution applied in preparation of imprinted microspheres is adjusted to pH = 4.7 with the aim of the most stabilized EA configuration. The condition in which the beads prepared is suggested to be most favorable for the rebinding effect. The peak B shifts to different pH value as the acidity effects differently on the hydrogel systems varied in HEC content. The alginate hydrogel swells with the growth of alkalescency during pH = 7 and pH = 9 accompanied by a mild increasing in the mIE. Considering the porous loose structure of the hydrogel matrix, and the inconstancy configuration of the protein, the rebinding process actually is presumed to be the rebinding of dimer or multimer protein on the expanded imprints. What proves is the adsorption dynamic of the microspheres with 5% HEC. Molecular imprinted beads are allowed to adsorb till equilibrium in a series of protein solutions with the concentration between 10 and 22 μmol/L. The curve is found to deviate from the Langmuir monolayer adsorption formula (Fig. 9), indicating the molecular imprints rebinding is a multilayer adsorption process under certain swollen state.

As for the dIE-pH curve (Fig. 10), the two peaks may be considered as the characteristic peak of eIE and the characteristic left peak of mIE. The characteristic left peak of mIE in the dually imprinted system has the same location in the beads with different HEC content, suggesting the eIE is enhanced by the stabilized EA configuration at the isoelectric point. While the right peak of the mIE is

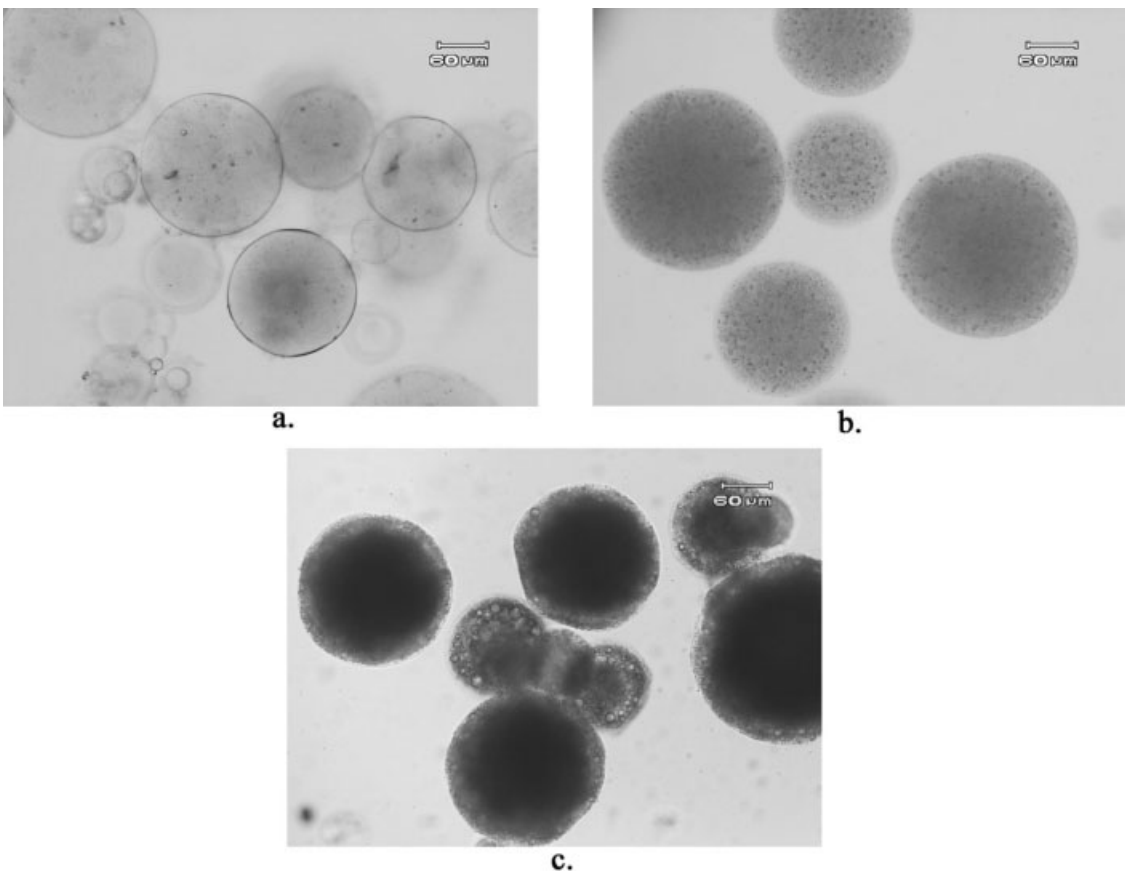

Figure 11 Morphology of the emulsion imprinted microspheres (a) Nonimprinted microspheres; (b) Emulsion imprinted microspheres made from No.3 sample ($d = 4.96 \pm 0.33$ μm); and (c) Emulsion imprinted microspheres made from No.4 sample ($d = 6.15 \pm 1.53$ μm).

overlaid in the eIE, and both of them are altered in the location as HEC content changes.

The molecularly imprinted hydrogel is found to rebind macromolecules through specific arranged sites, as the different microspheres are enhanced in the mIE at isoelectric point. Moreover, the molecular imprints rebinding process is also found to involve the macromolecular porous effects, as is indicated in the above experiment with different HEC content, the mIE changes similarly with eIE as the swollen state changes.

Microspheres with different pore diameters

The pore diameter is controlled by the proportion of oil and aqueous phase. The rebinding behavior of dually or emulsion imprinted microspheres varied in the pore morphology is studied as the function of temperature, ionic strength, and acidity (pH). The composition of the suspended system is showed in Table II, and the accordingly prepared microspheres are observed by microscope, as is shown in Figure 11. In Table II listed the diameters and the standard deviation (SD) of the pores. The smallest pores are found in the sample No. 1 prepared with lest liquid paraffine (0.5 g) while a surfactant dosage of 0.15 g Span85+0.2g Tween60 seems to provide the narrowest distribution of pore diameters.

The ionic strength effect on rebinding

The swelling state is varied within 0.5 ~ 3.0 by adjusting the Na^+ and Ca^{2+} ionic strength, and the emulsion and dually imprinting efficiency are determined as the function of swelling ratio. The eIE of the emulsion-imprinted beads according to swelling ratio is shown in Figure 12. The beads with the largest pores have the lower eIE at insufficient swollen state but possess the greatest increment as swelling processes.

At lower swelling state, the beads with smaller pores rebind protein more efficiently than the larger, suggesting a more prominent surface effect on adsorption. Concretely, the smaller pore is considered to be superior in the larger specific surface area and quantity. Moreover, the greater curvature of the smaller pores facilitates the solvent enrichment on the surface.

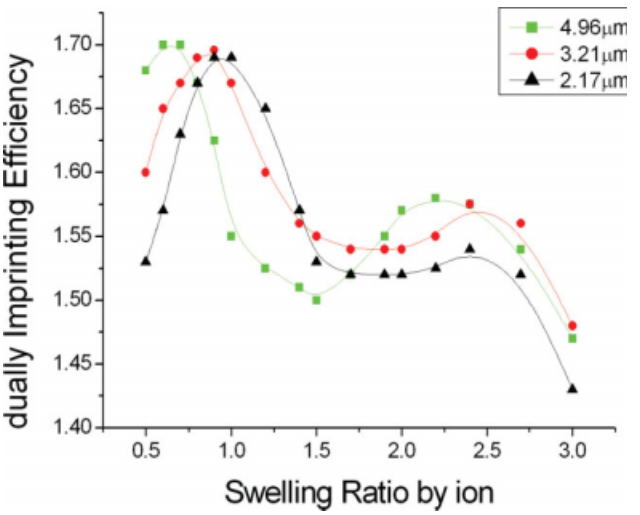

Figure 13 Dually imprinting efficiency of microspheres of different pore diameters as the function of swelling ratio controlled by ion strength. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

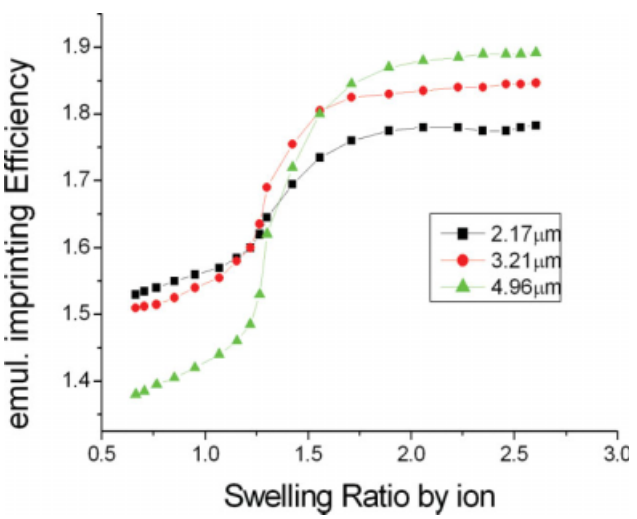

Figure 12 Emulsion imprinting efficiency of microspheres of different pore diameters as the function of swelling ratio controlled by ion strength. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

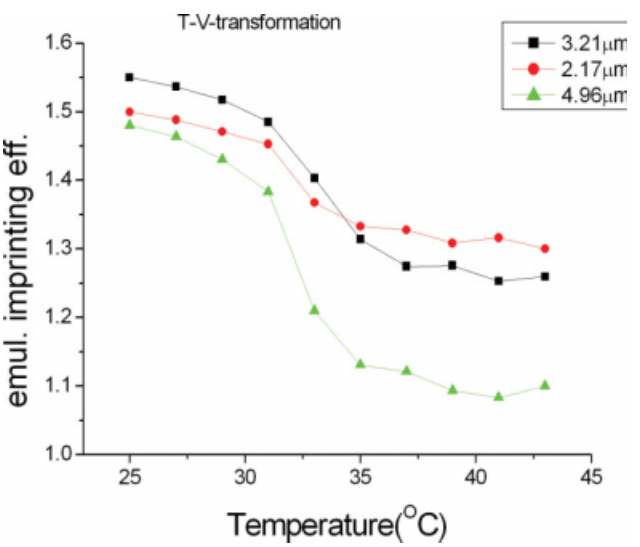

Figure 14 Emulsion imprinting efficiency of microspheres of different pore diameters as the function of temperature. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE III
Equilibrium Swelling Ratio of Alginate Microspheres as the Function of Temperature (HEC3)

Temperature (°C)	25	27	29	31	33	35	37	39	41	43	45	47
Swelling ratio	2.14	2.12	2.12	2.05	1.89	1.57	1.47	1.30	1.28	1.34	1.28	1.31

During further swollen state at about 1.5 swelling ratio, the unsubstantial and highly solvated inner part in the microspheres with larger pores begins to fracture as the matrix extending and more perforative cavities are formed, which brings about a faster diffusion. Consequently a higher eIE is detected in the beads with larger pores.

The dIE curves are two-peak shaped according to the swelling ratio, as is shown in Figure 13. The left peak is characteristic for the molecular imprints rebinding behavior, which is similar in the intensity but shifted to different swelling ratio due to the porosity effect on the protein diffusion dynamic. Beads with smaller pores possess a more compact hydrogel structure and less solvent content. It costs longer interval for the protein reaching the rebinding sites and requires a more swollen state, while the larger pore allows the beads to rebind protein at a lower swelling ratio and reach its climax much early. What is noticeable, is the peak value of the mIE that is almost constant in each sample even the porosity varies. The porosity exerts much less effect on the molecular imprints than on the emulsion imprints (Fig. 12), or rather, the molecular imprints rebinding counts mainly on specific sites and complementary cavities, even though the macromolecular steric effect may exert influences to some extent.

The location and the intensity of the eIE peaks (the right peak) are both changeable by the pore diameter. Higher emulsion imprinting efficiency is found in the microspheres with larger pores, which is similar with that of the emulsion imprinted system (Fig. 12). The succedent descent in the dIE is due to the molecular imprints deterioration under highly swollen state.

The temperature effect on the rebinding

The microspheres swollen state is suggested to change because of the balance among the solvation effect, the covalent bound, and the ionic bound, resulting in the variety of pore scale and hydrogel density, consequent exerts effect on the emulsion or dually imprinting efficiency. The beads are also found to swell to different level as are prepared with different pore diameter. The rebinding properties of emulsion and dually imprinted microspheres are tested as the temperature varied from 25 to 47°C.

The emulsion imprinting efficiency according to temperature is shown in Figure 14. Under from 25 to 33°C, the highest eIE is found in the microspheres with moderate pore diameter, suggesting a rebinding specificity for the dimension in the emulsion imprints. Besides the microspheres with the smallest pores tend to maintain eIE as the temperature rises from 35 to 43°C. The reason is that the microspheres prepared with smaller pores possess better tensiometric property and larger specific surface area than those of larger pores under higher temperature, during which the microspheres are at relatively contracted or less swollen state, as is shown Table III.

The rebinding property of the dually imprinted polymer microspheres is also studied under different temperature. The emulsions of proper proportion are applied so as to induce imprints with different pore diameters. The dIE behavior is found similar with that of the eIE except for an apex value, as the arrow pointing out in Figure 15. The apex is considered to be molecular imprinting specific point, and is achieved when the swollen state provides the molecular imprints with the best sites specificity and shape complementary. The corresponding temperature is the proper condition that the optimum swollen state requires. It is indicated that the microspheres with smaller pores achieves the apex at the lower temperature, for the beads with smaller pores is structurally compact, and more swollen voids are required for efficient diffusion.

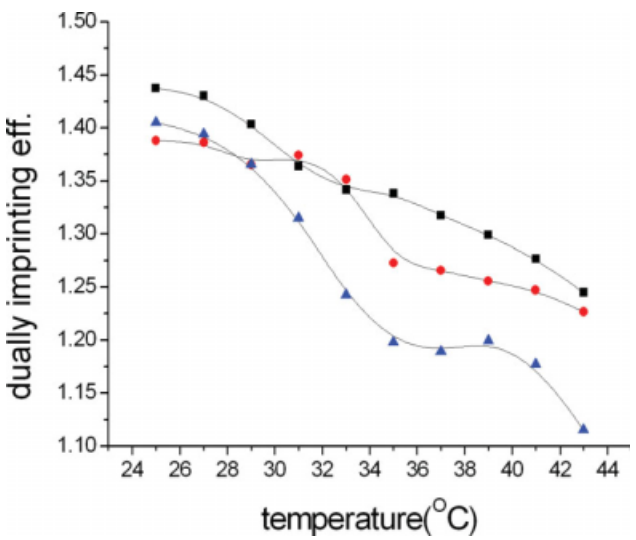

Figure 15 Dually imprinting efficiency of microspheres of different pore diameters as the function of temperature pore diameter: (■): d3.21μm; (●): d2.17μm; (▲): d4.96μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The characteristic peaks detected in the dIE curve indicate a distinct property in the rebinding process of molecular imprints with that of the emulsion imprints. As macromolecular templates, protein acts too as a porogen when imprinting within the hydrogel. Holes that formed are endowed with rebinding and reactive properties just similar with those of the emulsion imprints, as is illustrated in Figure 10. Nevertheless, as molecules that possess multifunctional groups and delicacy configuration, protein templates imprint hydrogel with the rebinding sites specificity and the cavity shape complementary, by which the macromolecular imprinting effect is distinguished from mere pore-creating effect.

Undoubtedly, the specificity of rebinding property of the protein-macromolecularly imprinted polymer need further investigate and confirm by different ways and means.[4,9–11,19,20]

CONCLUSIONS

Dually imprinted alginate hydrogel polymer microspheres with various structures are prepared in this research, and the structural factors on the rebinding properties are studied. The sample with the best mechanical strength and uniform pores were gained by using 5% HEC/SA (w/w) and 0.15 g Span85 + 0.20 g Tween60, the rebinding capacity and the imprinting efficiency were not necessarily the best according to experimental conditions. The imprinted microspheres with (5% HEC) have the greatest mIE (about 1.35) at the pH of 4.7. However, microspheres with no HEC blended were found to have the highest eIE (about 1.97). Moreover, the eIE of microspheres with large pores (4.96 μm) were found the greatest at fully swollen state (about 1.89 at SR>1.75). Nevertheless, the greatest dIE is detected in the sample with a pore diameter of 3.21 μm at lower temperature (about 1.44 at 25°C). Results showed that the HEC content exerts effects on the rebinding property through the alternation to the equilibrium swelling ratio. The swelling capacity is reduced, as the matrix is restricted by the crosslinked IPNs, resulting in the descent of the mass diffusivity. Therefore, the transfer and the rebinding of the protein macromolecules are retarded. The junctions not only prevent the inner transfer of the macromolecules but also exert steric effect on the rebinding sites. As the increasing in the crosslinkage and matrix rigidity, the mIE is enhanced, whereas the eIE decreased, which suggests that the association in the matrix improves the imprint stability and reduces the surface effect and diffusivity. It is also confirmed that the protein rebinding behavior is also dependent on the sites recognition of template and matrix, apart from the macromolecular pore-creating effect. The rebinding property of the macromolecularly imprinted hydrogel polymer proves to be different with that of the molecularly imprinted rigid material. Macromolecular rebinding behavior is studied by introducing emulsion imprinted pores to the MIPMs. As molecular aggregates, the emulsion templates in this research are not mere pore-creating agent for higher emulsion imprinting efficiency but also reference structures to macromolecular imprints. Protein templates, as kinds of macromolecular templates, are distinguished from classical small molecular templates in their multifunctional groups and massive steric effect.

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