The Imprinting Induce-Fit Model of Specific Rebinding of Macromolecularly Imprinted Polymer Microspheres

Xiaoguang Ying,[1,2] Guoxiang Cheng,[2] Xiao Li[1]

[1]*College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350108, China*
[2]*School of Materials Science and Engineering, Tianjin University, Tianjin 300072, China*

Received 8 November 2010; accepted 29 January 2011
DOI 10.1002/app.34263
Published online 10 June 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: The rebinding behavior of protein-imprinted hydrogel is different from classical small molecularly imprinted polymer especially in protein aggregating state and interaction changeability between protein and imprinted sites. BSA-imprinted calcium alginate-hydroxy ethyl cellulose microspheres were prepared, and the rebinding behavior was studied. An "imprinting induce-fit model" was proposed to describe the rebinding property of protein-imprinted hydrogel. Three kinds of different interacting forms between protein and imprinted hydrogel were discovered by Scatchard analysis. Slogistic fit analysis of rebinding rate coefficient was carried out, and the imprinted hydrogel was found capable of promoting rebinding through induce-fit behavior. Higher imprinting efficiency was found in microsphere samples with lower crosslinking density. Rebinding regression equation was established, and the rebinding quantity was computable with parameters including BSA aggregate concentration [P_n], dissociating rate (k_1), and single molecule rebinding rate (k_2). The experimental and calculated rebinding concentration were compared, and errors between −9.43% and 5.59% were found. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 1847–1856, 2011

Key words: proteins; templates; hydrogels; molecular imprinting; modeling

INTRODUCTION

Protein-imprinted hydrogels have been widely used in many fields such as biosimulation sensors, separation, purification and diagnosis, high-performance liquid chromatography, macromolecular electrophoresis, and genetic engineering.[1–7] Protein molecules have hydrogen bonds, coordination bonds, and hydrophobic regions with high motility. When applied as templating and targeting molecules of imprinted materials, protein can form aggregations of two or more units.[8] The configuration variability and aggregation of protein are two important factors affecting the preassembling and rebinding properties.

Bovine serum albumin (BSA) is popular as protein template in molecular imprinting because of its functional and structural stability,[9] especially where hydrogel is used as imprinted material, such as alginate,[10] agarose,[11] PVP,[12] and chitosan[9]. It has been reported that BSA exists in the form of dimer and multimer in aqueous solution besides single molecule. The dimer percentage of commercial BSA is about 6.4–13.8%.[13] Samples with even higher purity are detected of multimer aggregation state.[14,15] Aggregation has great impact on the imprinting and recognition behavior.

The lock-and-key theory is an elemental assumption of antigen-antibody relationship in the field of immunology and drug design.[16] It has been found that the "pockets" of many antibody molecules are softer than ever expected, making them suitable for antigen with completely different conformations.[17] On the contrary, some of the precisely structured artificial ligands cannot fit the targets with even slightly changes in conformation.[18] The induce-fit model was proposed by D. E. Koshland in 1958, believing that the best conformation of enzyme was induced during the process when enzyme and substrate were contacting. The approaching, stimulating, and binding of substrates lead to certain changes in enzyme conformation before they are adjusted to meet both sides and are finally recognized.

We have recently realized the induce-fit model being applicable to BSA-imprinted hydrogel to a certain extent. The rebinding process of BSA on imprinted hydrogel is analogous to interaction

Correspondence to: X. Ying (yxg@fzu.edu.cn).
Contract grant sponsor: Talent Fund of Fuzhou University; contract grant number: 0460022305.
Contract grant sponsor: Teaching and Research Award Program for Outstanding Young Teachers in Higher Education Institutions of MOE, PRC; contract grant number: 2002-123.
Contract grant sponsor: Key Research Project of Ministry of Education, PRC; contract grant number: 02041.

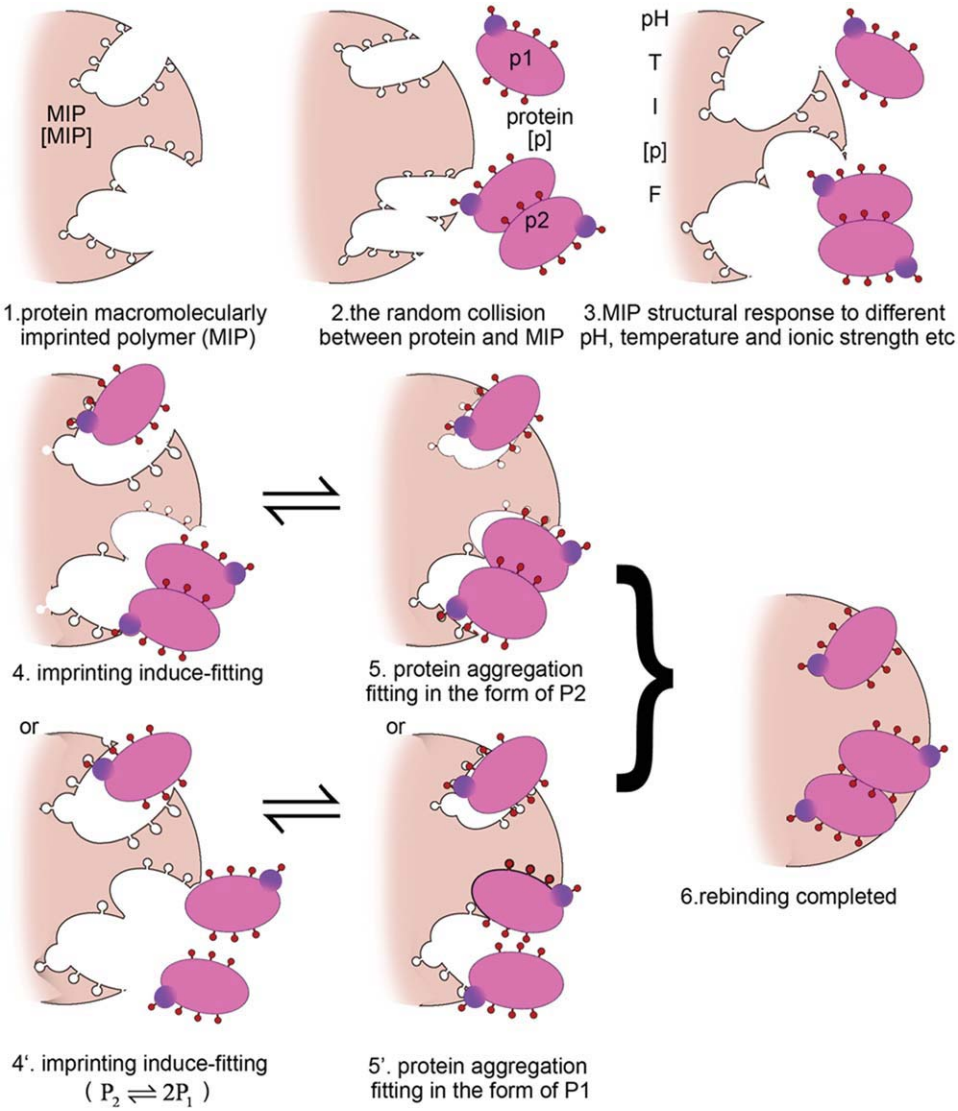


Figure 1 The imprinting induce-fit model of protein imprinting hydrogel; P_1 and P_2 are protein molecule and dimer, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

between enzyme and substrate. Imprinted hydrogel may change its conformation to fit BSA when it is approaching. Protein will adapt to modified conformation of the imprints and finally be recognized (Fig. 1). However, there are also differences with enzyme-substrate system. Imprinted hydrogel is not dissociated enzyme but solidlike matrix with polymeric chains and segments, spatial crosslinking meshes, and more concentrated specific sites. This rebinding model, namely, the "imprinting induce-fit model" (IIF model), is important for characterizing the rebinding between protein molecule and hydrogel. In this work, quantitative investigations concerning this model are carried out by calculating rebinding quantity and imprinting efficiency of imprinted hydrogel microspheres with different swelling ratio. Rebinding kinetics is also studied, and the rebinding quantity regression equation is established.

MATERIALS AND METHODS

Materials and instruments

BSA (fraction V, M_r = 68,000, pI = 4.70, and electrophoretically pure) and ovalbumin (OVA, M_r = 43,000, pI = 4.71, and electrophoretically pure) were from Fluka@. Sodium dodecyl sulfonate (SDS, $C_{12}H_{25}NaO_3S$, M = 272.38, and analytically pure), calcium chloride, hydroxyl ethyl cellulose (HEC, HD 30,000), sodium alginate (SA, M_n = 35,000, M_w = 218,000, and chemically pure), chemically pure, were from Eco-aware Technology Co., Tianjin.

Inverse optical microscopy (Carl Zeiss Axiovert 25C,Germany) was used for morphology observation. Micro-nano Particle Size Analyzer (BI-90Plus, Brookhaven Instruments, US Co.) was used for BSA aggregation measurement; UV–vis spectrophotometer (UV-2550PC, SHIMADZU, Japan) and real-time

TABLE I
Different Compositions of SA–HEC Mixture

Sample ID	HEC1	HEC2	HEC3	HEC4
SA solution volume (mL)	19.7	19.4	19.1	18.5
HEC solution volume (mL)	0.3	0.6	0.9	1.5
HEC/SA (w/w %)	1.52%	3.09%	4.71%	8.11%

Conductivity Meter (DDSJ-308A, Shanghai Precision and Scientific Instrument, China) were used for BSA concentration determination.

Preparation of BSA-imprinted microspheres

The crosslinking density of microspheres was adjusted by adding small amount of HEC and calculated according to equilibrium swelling ratio.[10] Protein-imprinted microspheres with different HEC content were prepared as follows. First, HEC aqueous solution (2% w/w %) was prepared in 50°C water bath; sodium alginate was dissolved in SDS–BSA solution ([BSA] = 4.2 μ*M*, [SDS] = 0.002 mol/L, and pH = 4.70) at 25°C to form 2% (w/v %) with BSA template. Then, HEC and SA solution were mixed thoroughly with different ratios according to Table I. At last, hydrogel microspheres were prepared with the mixtures by inverse phase-suspending gelling method[10] and eluted to remove templates for further use. Control samples were prepared following similar steps except no protein was contained in SA solution.

Preparation of BSA–SDS solution for rebinding tests

SDS is a protein denaturant being widely used in SDS–PAGE experiments for protein denaturation, separation and molecular weight determination,[8,9,19] gel electrophoresis chromatography,[20] and protein template eluting.[21] Protein solutions with SDS have been studied, and quantitative investigations have been conducted about the effects on BSA conformation. Protein was conformationally simplified at the denaturing effect of SDS and was believed to exhibit a rodlike conformation, etc.[22] Aggregation description of SDS–BSA complex was also made by surface tension tests.[23]

SDS aqueous solutions from 0 to 0.002 mol/L were prepared. BSA was dissolved in SDS solutions to form SDS–BSA complex with different aggregating state. The concentrations of BSA were from 0 to 14.5 μ*M* as shown in Table II. All the SDS–BSA solutions were adjusted to pH4.7 to achieve the best rebinding property.[24]

Rebinding tests in solutions with different BSA aggregation

Accurately weighted 1.000 g microspheres were mixed with 20 mL of SDS–BSA solution prepared at step 2.3 and kept in water bath at 25°C. The rebinding quantities Q_{rB} and Q_{rO} were calculated based on BSA and OVA concentrations before and after rebinding:

$$Q_{rB} = (C_{0B} - C_{tB})V/W; Q_{rO} = (C_{0O} - C_{tO})V/W \quad (1)$$

where C_{0B} and C_{0O} are beginning concentrations (μmol/L) of BSA and OVA, C_{tB} and C_{tO} are concentrations (μmol/L) after rebinding, V is protein solution volume (20.0 mL), and W is microspheres weight (g). Protein concentration was detected by UV absorption at 280 nm (Ultra U-1800, HITACHI). Rebinding quantity (Q_{rB} and Q_{rO}) is composed of specific rebinding by imprints (Q_{rBS} and Q_{rOS}) and nonspecific rebinding by surface absorption (Q_{BN} and Q_{ON}). Therefore, the specific rebinding quantities of BSA (Q_{rBS}) and OVA (Q_{rOS}) are calculated as follows:

$$Q_{rBS} = Q_{rB} - Q_{BN}; Q_{rOS} = Q_{rO} - Q_{ON} \quad (2)$$

where Q_{rOS} is specific rebinding quantity of OVA on BSA-imprinted microspheres. Q_{rO} is rebinding quantity of OVA. The imprinting efficiency (IE) was calculated as follows:

$$IE = Q_{rBS}/Q_{rOS} \quad (3)$$

The data of specific rebinding quantity for modeling are all calculated as Q_{rBS} (formula 2) in this work. As for the determination of rebinding thermodynamic properties, BSA concentration (C_{tB}) was recorded every 2 min. The real-time rebinding quantity and imprinting efficiency were calculated according to formulas (2) and (3).

RESULTS AND DISCUSSION

The preparation of protein-imprinted calcium alginate microspheres

BSA-imprinted calcium alginate microspheres were prepared by inverse phase suspending gelling method (Fig. 2). Microspheres prepared with different HEC content possess statistic diameters from 158 ± 6.2 μm to 161 ± 10.5 μm, as shown in Figure 3. Number–average diameters (D [μm]) and polydispersity index were determined from pictures of 1000 beads. HEC content has little effect on beads' scale

TABLE II
BSA–SDS Complex Preparation for Rebinding Tests

Sample ID	I	II	III	IV
BSA (imol/L)	0–7.5	0–4.5	4.9–6.5	6–14.5
SDS (mol/L)	0	0.002	0.002	0.0011

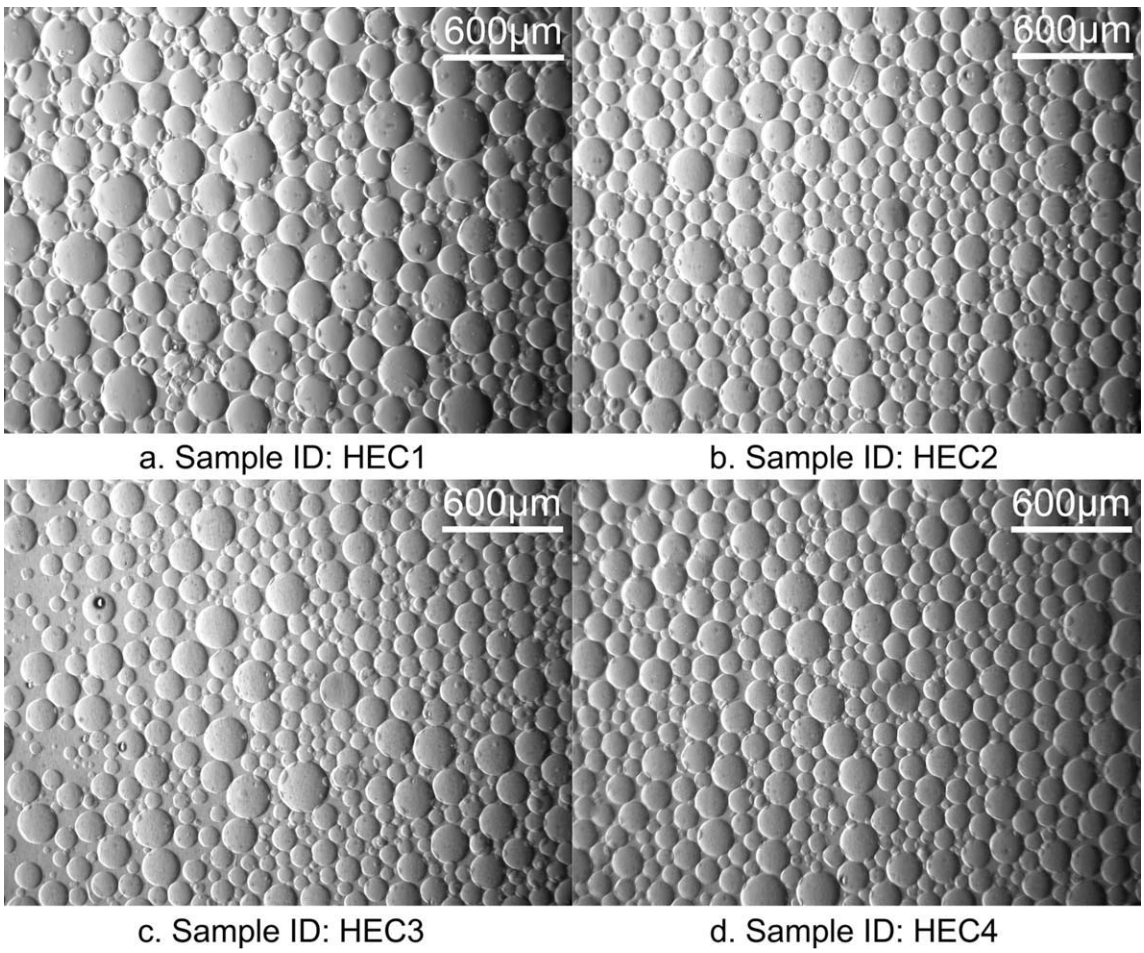
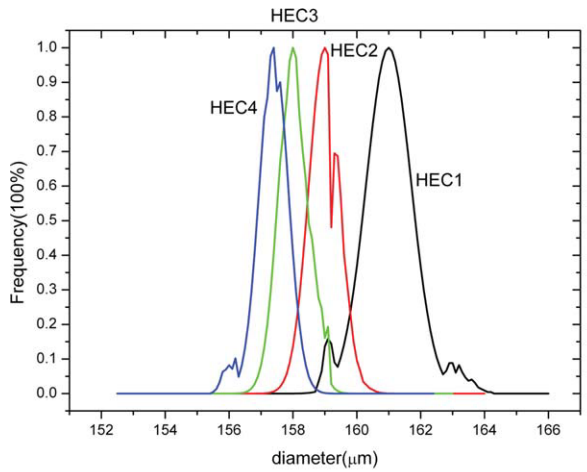


Figure 2 BSA-imprinted microspheres prepared by inverse phase suspending gelling method. The BSA template concentration is 6 μ*M*; HEC contents [HEC/SA (w/w %)] are (a) 1.52%; (b) 3.09%; (c) 4.71%; (d) 8.11%.



Sample ID	HEC1	HEC2	HEC3	HEC4
Mean *D*(μm)	161.0	159.0	158.0	157.4
PDI	0.685	0.465	0.337	0.244

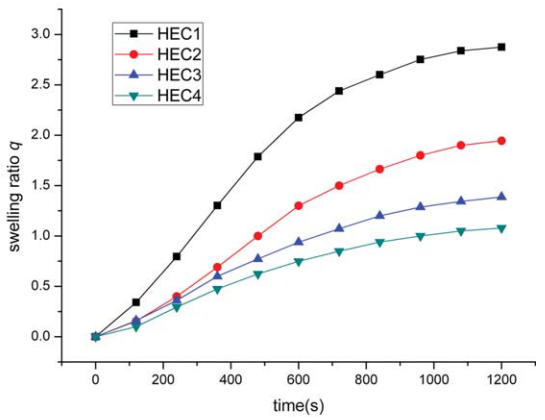


Figure 3 Swelling ratio and diameter distribution of microspheres with different HEC content. HEC contents [HEC/SA (w/w %)] are (a) 1.52%; (b) 3.09%; (c) 4.71%; (d) 8.11%. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE III
Calculation of the Liberated Imprints Percentage of Microspheres by UV–Vis Measurement

Mass of BSA template (g)	Eluting process				Percentage of liberated imprints
0.0264		First	Second	Third	86.36%
	BSA concentration in eluting solution (g/mL)	8.14×10^{-4}	2.91×10^{-4}	1.89×10^{-5}	
	Eluting solution volume (mL)	20.02	21.06	19.67	
	Mass of eluted BSA (g)	0.0163	0.0061	0.0004	
		$\Sigma = 0.0228$			

but exerts a significant impact on swelling property. Swelling ratio was detected by immersing microspheres in deionized water and interval recording weight (Fig. 3). The swelling ratio q was calculated as follows:

$$q = (W_t - W_0)/W_0 \quad (4)$$

where q is swelling ratio, W_0 is microspheres weight before swelling, and W_t is the weight when swelling reaches equilibrium.

The template elution percentage is estimated by UV–vis measurement of BSA concentration in the eluate (Table III). The BSA is hardly detectable after eluating three times, and templates are considered to have been fully removed. The percentage of liberated imprints is calculated as the ratio of eluted template to total protein amount. According to Table III, 86.36% of the templates have been eluted; others are trapped in the hydrogel.

The existence of noncovalent interaction between protein and hydrogel is proved by the strength and location changes in ν_{O-H} at 2930 cm^{-1} and ν_{C-O} at 1030–1130 cm^{-1} as shown in FTIR (Fig. 4). Hydrogen bonds are formed between —OH groups when rebinding, leading to higher intensity of ν_{O-H} peaks.

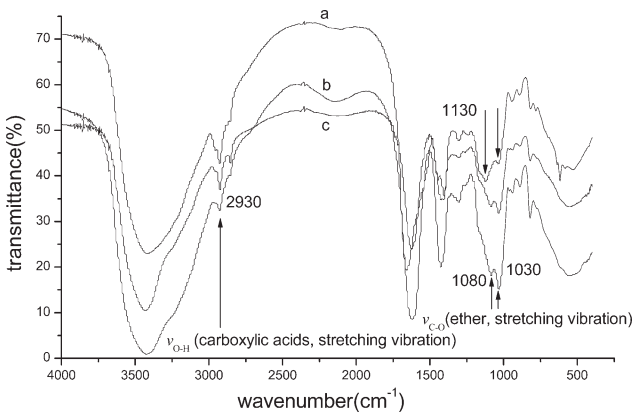


Figure 4 FTIR of BSA and polymer after rebinding process products. (a) FTIR of PIMs after rebinding in 3 μ*M* BSA solution; (b) FTIR of PIMs after rebinding in 6 μ*M* BSA solution; (c) superposition of BSA and SA FTIR.

The electron cloud density of carboxyl groups is also affected by the formation of hydrogen bonds, and peaks shift to lower wave numbers.

Specific rebinding quantity and determination of BSA aggregation standard curve

BSA–SDS solutions for rebinding are prepared based on Table II. The specific rebinding quantity (Q_{rBS} andQ_{rOS}) is shown in Figure 5 as a function of protein concentration. Rebinding curves in BSA solution show much higher growth rates than in OVA solution. Rebinding quantity grows as BSA concentration increases and finally approaches equilibrium. According to different concentrations of SDS and BSA in the four samples, protein exists in different aggregating state such as rodlike, folded, or multimer. The determination of the aggregating proportion is important in understanding this unique isothermal adsorption curve. We applied particle size and UV adsorption when inspecting BSA solution of different concentrations.

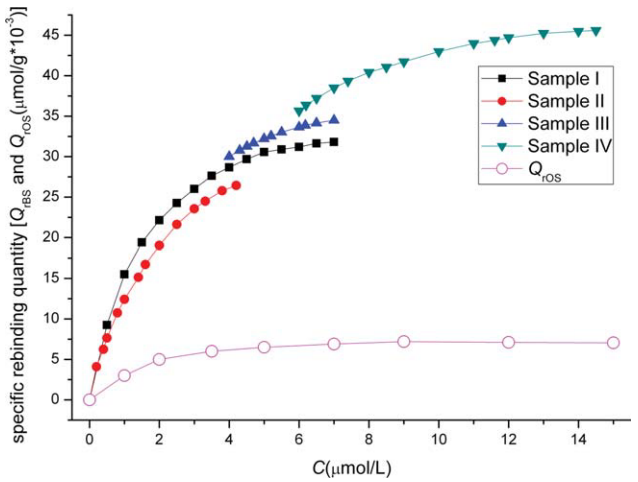


Figure 5 Specific rebinding quantity-time curve of BSA-imprinted microspheres. Microspheres: 1.0 g; BSA volume 20 mL; the adsorbate BSA–SDS complex component (samples I–IV) is listed in Table II; Q_{rOS} is OVA rebinding quantity on BSA-imprinted microspheres. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

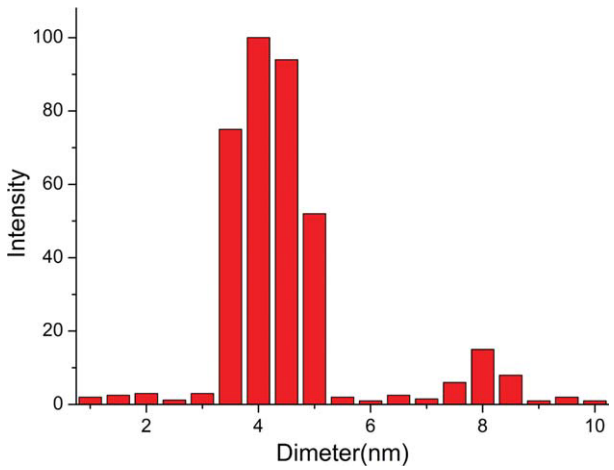


Figure 6 Dynamic laser scattering analysis of BSA solution. The ratio of single molecule to dimer is 11.07. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Protein particle size distribution is estimated by dynamic laser scattering (DLS) as shown in Figure 6. Diameter peaks are found at 4 and 8 nm. It has been reported that the ratio of BSA multimer (not including dimer) is usually lower than 3.2% in a total concentration of 1.52×10^{-8} mol/L.[15] Therefore, the two peaks represent the single molecule and dimer composition of BSA solution. According to particle intensity and UV measurement, the concentrations of single molecule (e.g., $[P_1]$) and dimer (e.g., $[P_n]$) at different total concentrations of BSA (e.g.) are available. The standard curves are worked out and used in determining $[P_1]$ and $[P_n]$ in solutions with different [BSA] as shown in Figure 7. Take [BSA] = 7.50 μM, for example, the intersections of vertical dash line and the two standard curves represent the value of $[P_1]$ and $[P_n]$.

Rebinding of BSA with different aggregation states: Scatchard analysis

Protein exists in the form of several aggregation states in aqueous solution. Rebinding behaviors are affected significantly by aggregation states in the aspects of rebinding kinetics and interaction forms. The specific rebinding concentration MIP is expressed as formula (4).

$$\underset{\text{(imprinted\_sites)}}{\text{MIP}} + \underset{\text{(protein)}}{\text{P}} \xleftrightarrow{K_{rb}(\text{rebinding})/K_{ds}(\text{dissociation})} \underset{\text{(complex)}}{\text{MIP}-\text{P}} \quad (4)$$

With the aim of investigating aggregation rebinding properties, BSA solution is adjusted to particular aggregating states by adding SDS. According to formula 4, the equilibrium rebinding constant K_{rb} $(\text{mol/L})^{-1}$ represents the rebinding ability, of which the reciprocal is the equilibrium dissociation constant (K_{ds}). B_{max} (mol/g) is the maximum specific rebinding quantity. P is the targeting protein (single molecule and aggregate) with a concentration of C (i.e., $[P] = C$). The amount of specific rebinding protein is recorded as B (mol/g), that is, $[\text{MIP-P}] = B$. The specific rebinding constant is calculated as follows:

$$K_{rb} = \frac{[\text{MIP}-\text{P}]}{[\text{MIP}][\text{P}]}; [\text{MIP}] = B_{max} - B; K_{rb} = \frac{B}{[B_{max} - B]C}$$

$$\frac{B}{C} = -K_{rb}B + K_{rb}B_{max} \quad (5)$$

The B/C-B curve is worked out by Scatchard analysis (Formula 5). The values of K_{rb} and B_{max} are the slope and intercept of the curve. Linear curve with only one slope indicates identical imprints and rebinding form. A nonlinear curve with two or more slopes implies several types of rebinding forms.

The rebinding data of different SDS–BSA solutions are analyzed by Scatchard method as shown in Figure 8. Fitting lines of B/C-B are worked out accordingly. The slopes indicate different interactions between target and imprinted matrix. The intersections suggest changes in the rebinding forms of different aggregates as described in Table IV. It can be seen from Figure 8(a) that BSA solution of lower concentration (0–7.5 μM) with no SDS has three aggregating states. The simplest B/C-B curve is found in sample II containing 0.002 mol/L SDS and at most 4.2 μM BSA as shown in Figure 8(b). All data points are gathered around one slope, indicating only one type of interaction occurs between protein and hydrogel. In this experiment, BSA solution for preparing MIP microspheres is all made from sample II in order that only one kind of imprint is

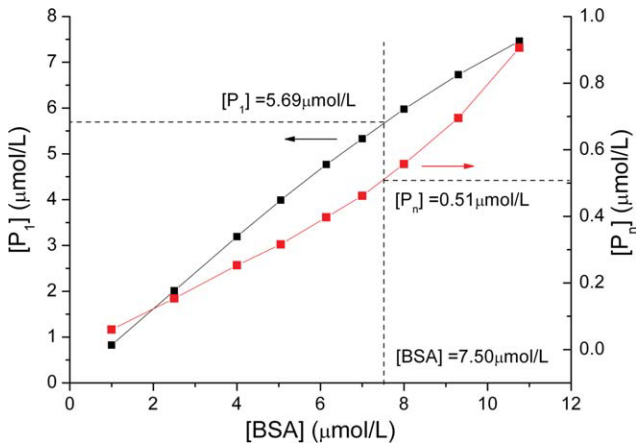


Figure 7 Concentration standard curve of single molecule and multimer (mainly dimer) in BSA solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

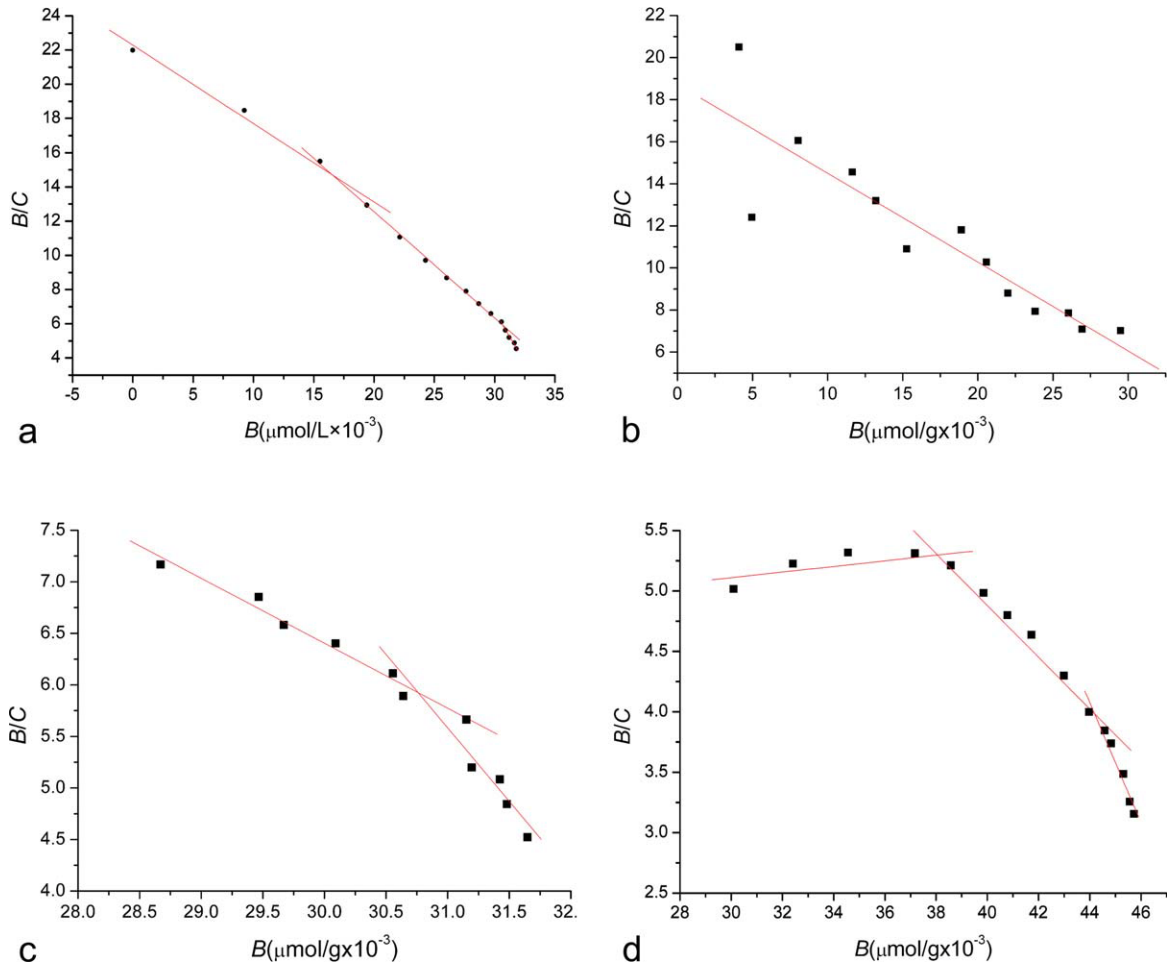


Figure 8 Scatchard analysis of the specific rebinding data of BSA-imprinted microspheres; the rebinding was performed in solutions of (a) Sample I; (b) Sample II; (c) Sample III; and (d) Sample IV, respectively. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

generated. Different types of aggregates are supposed to be rodlike, folded, and dimer forms according to DLS analysis and other reports.[13–15,22] Other rebinding forms are found in protein samples with aggregates of rodlike, folded, and multimer states as shown in Figure 8(c,d).

The induce-fit model of imprinted hydrogel with different crosslinking degrees

The main purpose of "induced fit" model is studying the rebinding behavior of soft hydrogel with BSA-imprinted structure. Compared to chemistry reacting systems, protein-imprinted hydrogel has complex variations and numerous influencing factors. The experimental data from single batch cannot reflect its characteristic comprehensively; moreover, the yield is not easily obtained in many cases. The "induced fit" model is applied to inspect protein-imprinted hydrogel system quantitatively by establishing functional expression. It can be used not only in calculation for rebinding quantity, but also preparing-imprinted matrix with certain crosslinking density and controllable rebinding progress.

In this work, microspheres are prepared by calcium alginate blended with small amount of HEC

TABLE IV
BSA–SDS Complex and the Aggregations

Sample ID	BSA (μmol/L)	SDS (mol/L)	Molecular conformation
I	0–7.5	0	Folded conformation of the single molecule
II	0–4.5	0.002	Rodlike conformation
III	4.9–6.5	0.002	Rodlike + folded conformations
IV	6–14.5	0.0011	Rodlike + folded + multimer (mainly dimer)

TABLE V
Crosslinking Coefficient and IE of MIPs with Different HEC Content

Sample ID	HEC1	HEC2	HEC3	HEC4
HEC content [w(HEC)/w(SA)%]	1.52%	3.09%	4.71%	8.11%
Equilibrium swelling ratio (q)	2.90	2.00	1.40	1.10
Crosslinking coefficient ($q^{5/3}$)	5.90	3.17	1.75	1.17
IE	2.81	2.65	2.33	2.02

with the aim of regulating swelling capacity. The crosslinking density is represented by M_c (average molecular weight between crosslinking) in the form of q(swelling ratio) according to Flory–Huggins lattice model theory[25–27]:

$$\overline{M}_C \propto q^{5/3}$$

Table V gives the equilibrium swelling ratio and IE of MIP microspheres with different HEC content in BSA solution of sample II. Because of the soft nature of hydrogel microspheres, IE is time-varying rather than having a constant value. Considering separately the aggregation dissociating rate (k_1) and single-molecule rebinding rate (k_2), the independent variables are concentrations of protein aggregation ([P_n]) and single protein ([P_1]) (formula 6). Rebinding rate coefficient k_2 (formula 6) is used as the parameter related with induce-fit behavior. If "IIF" behavior exists in this rebinding system, it will be reflected in the deviation of k_2 from fixed value.

$$\mathrm{P_n} \xrightarrow{k_1} x\mathrm{P_1};\ \mathrm{P_1} \xrightarrow[+\mathrm{MIP}]{k_2} \mathrm{MIP-P} \tag{6}$$

Take sample (HEC3) for example, according to formula 6, the experimental k_2 is calculated from protein concentrations [P_1] recorded by real-time conductivity meter (DDSJ-308A) as follows:

$$\ln\frac{[\mathrm{P_1}]_{t1}}{[\mathrm{P_1}]_{t2}} = k_2(t_2 - t_1);\ (k_2 > 0)$$

A Slogistic fit curve is worked out (formula 7) for regression analysis. Because the ideal k_2 is constant, the lower horizontal asymptotic line is chosen as original k_2 curve [$k_{2(t\ =\ 0)}$]. Thus, the difference between higher and lower asymptotic lines [$\Delta k_2 = k_{2(t\ \geq\ 240)} - k_{2(t\ =\ 0)}$] indicates the effect of "IIF" behavior as shown in Figure 9.

$$k_2 = \frac{a}{1 + be^{-ut}} + 0.0022 \tag{7}$$

$$a = 0.0038 \pm 1.73 \times 10^{-7};\ b = 115.19 \pm 0.376;\ u = 0.0471 \pm 1.19 \times 10^{-6}$$

In this regression function, all the parameters have mathematical meanings. Parameter a is Δk_2; b is related with inflection point coordinates of the "S"-shaped curve; u is related with the slope at the inflection point.

As interpreted in Figure 9, $\Delta k_2 = k_{2(t=240)} - k_{2(t=0)} = 0.0038$ suggests an increment in rebinding coefficient k_2 when BSA is adsorbed on imprinted microspheres. Δk_2 is considered to be attributed to "IIF" effect. Because of protein-inducing effect, the polymer chain keeps adjusting its conformation so as to maintain a matching form, leading to a deviation toward higher rebinding rate[$k_{2(t\ \geq\ 240)}$]. Further analysis reveals higher Δk_2 in samples with lower crosslinking density, indicating greater IIF ability (Table VI).

Calculation of the rebinding properties of protein aggregates

It has been proved by DLS and other reports that BSA solution contains single molecule (P_1) and dimer (P_2) as well as a trace amount of multimer aggregates. The assembling equation is represented with the concentration if P_1 and P_n as shown in formula 8.

$$\mathrm{P_1} \xleftrightarrow{\text{assemble/disassemble}} \mathrm{P_n} \tag{8}$$

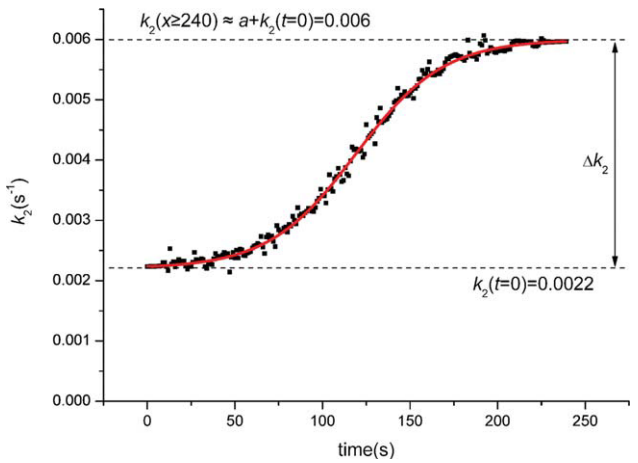


Figure 9 Slogistic fit of rebinding rate coefficient k_2 of sample HEC3 ([w(HEC)/w(SA)%] = 4.71%) lower k_2 curve: $k_{2(t\ =\ 0)} = 0.0022$; higher k_2 curve: $k_{2(t\ =\ 240)} = 0.006$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE VI
Crosslinking Coefficient and Δk_2 of MIPs with Different HEC Content

Sample ID	HEC1	HEC2	HEC3	HEC4
HEC content [w(HEC)/w(SA)%]	1.52%	3.09%	4.71%	8.11%
Equilibrium swelling ratio (q)	2.90	2.00	1.40	1.10
Crosslinking coefficient ($q^{5/3}$)	5.90	3.17	1.75	1.17
Δk_2	13.4×10^{-3} s^{-1}	8.82×10^{-3} s^{-1}	3.8×10^{-3} s^{-1}	3.7×10^{-3} s^{-1}

The aggregating reaction of BSA is a reversible process. Given equilibrium constant (k_1) and rebinding rate (k_2), the reaction process can be simulated by calculating. On the contrary, the rebinding experimental data can also be used to inspect BSA aggregation states.

Total BSA concentration is calculated as follows:

$$[\mathrm{BSA}] = [P_1] + \Sigma n[P_n]$$

According to DLS analysis, $[P_1]$ and $[P_n]$ have the relationship as follows:

$$[P_n]/[P_1] = I_n/I_1$$

where I_n and I_1 are relative intensity of multimer (mainly dimer) and single protein detected by DLS. Total BSA concentration and single molecule concentration ([BSA] and $[P_1]$) are calculated as follows:

$$[\mathrm{BSA}] = [P_1]/[P_1] \times \sum nI_n/I_1$$
$$[P_1] = [\mathrm{BSA}]/(1 + \sum nI_n/I_1) \quad (9)$$

The first-order consecutive reaction of rebinding is established according to formula 2:

$$\mathrm{P_n} \xrightarrow{k_1} \mathrm{P_1} \xrightarrow[+\mathrm{MIP}]{k_2} \mathrm{P-MIP}$$
$$t = 0[P_n]_0 0\,0$$
$$t = t[P_n]\,P_1[\mathrm{P-MIP}]$$

$[P_n]$ is calculated with k_1 as follows:

$$[\mathrm{P_n}] = [\mathrm{P_n}]_0 e^{-k_1 t} \quad (10)$$

$[P_1]$ is generated at the rate of k_1 and consumed at the rate of k_2, calculated as follows:

$$[\mathrm{P_1}] = \frac{k_1 \sum [\mathrm{P_n}]_0}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right) \quad (11)$$

Thus, the rebinding concentration [P-MIP] is calculated with the aggregating number x (e.g., how many BSA molecule in one aggregate)as follows:

$$[\mathrm{P-MIP}] = x[P_n]_0 - x\sum[P_n] - [P_1] \quad (12)$$

An expression of [P-MIP] is then formulated by substituting (10) and (11) in Eq. (12):

$$[\mathrm{P-MIP}] = x\sum[\mathrm{P_n}]_0 \times \left[1 - e^{-k_1 t} - \frac{k_1}{x(k_2 - k_1)}\left(e^{-k_1 t} - e^{-k_2 t}\right)\right] \quad (13)$$

Formula (13) gives the final expression of rebinding concentration [P-MIP] with the beginning concentration of BSA aggregate $[P_n]_0$ and rebinding time (t). For simplifying calculation, here, $\Sigma[P_n]_0$ is considered to be entirely converted from $[P_1]_0$. In BSA aqueous solution, $\Sigma[P_n]_0$ can be considered approximately equal to $0.5[P_1]_0$, that is, half the total concentration of BSA solution. The reaction constants k_1 and k_2 are obtained by (10) and (11):

$$\frac{1}{y-1}\left(\frac{1}{[\mathrm{P_n}]_2^{y-1}} - \frac{1}{[\mathrm{P_n}]_1^{y-1}}\right) = k_1 t; \ln\frac{[\mathrm{P_1}]_1}{[\mathrm{P_1}]_2} = k_2 t \quad (14)$$

In a protein aggregation system with mainly single and dimer molecules, we have the presumption that:

$$y = 1/x = 1/2, n = 2$$

Take the rebinding data listed in Table VII, for example. The total BSA concentration ([BSA]) is 10.0 μM. $[P_1]_1$ and $[P_1]_2$ are determined by concentration sampling at 0 and 120 s; $[P_n]_1$ and $[P_n]_2$ are located accordingly using the standard curve in Figure 7. Therefore, k_1 and k_2 are calculated by formula (14) using data in Table VII:

$$k_1 = 7.18 \times 10^{-3}, k_2 = 8.01 \times 10^{-3}$$

Thus, the specific rebinding quantity is calculated by formula (13) using k_1 and k_2, compared to experimental data as listed in Table VIII. The calculated

TABLE VII
Concentrations of the Components in BSA Solution at 0 and 120 s

Time	$t_1 = 0$ s	$t_2 = 120$ s
[BSA] (μmol/L)	$[\mathrm{BSA}]_1 = 10.0$	$[\mathrm{BSA}]_2 = 3.46$
$[P_n]$ (μmol/L)	$[P_n]_1 = 0.81$	$[P_n]_2 = 0.22$
$[P_1]$ (μmol/L)	$[P_1]_1 = 7.24$	$[P_1]_2 = 2.77$

TABLE VIII
Comparison of Experimental and Calculated Q_{rBS}

Time (s)	5	10	20	50	105
[P-MIP] (ìmol/l)	0.180	0.360	0.721	1.789	3.596
Calculated Q_{rBS} results (μmol/g×10^{-3})	3.60	7.20	14.4	35.8	71.9
Experimental Q_{rBS} data (μmol/g×10^{-3})	3.58	7.41	15.9	38.3	69.2
Calculation errors	5.59%	−2.83%	−9.43%	−6.53%	3.90%

Note: Calculated Q_{rBS} = [P-MIP] × 20 mL/1.000 g.

results of Q_{rBS} were obtained with a relative error from −9.43% to 5.59%. The main sources of error are estimation of BSA aggregation and dissociation constant, aggregation curve plotting (Fig. 7) by DLS analysis, and the effect of a small amount of multimer existing in the protein solution besides dimer and single molecules.

CONCLUSIONS

BSA-imprinted alginate hydrogel microspheres were prepared, and rebinding performance was tested in SDS–BSA solution. Scatchard analysis shows that BSA target molecules were rebinding in at most three aggregating forms. "IIF" model was proposed for protein-imprinted hydrogel systems, and researches were performed by Slogistic fit of rebinding rate coefficient compared to ideal rebinding rate constant. The induce-fit behavior of imprinted hydrogel is found promoting protein rebinding. Better promoting effect is found in hydrogel greater swelling capacity (larger M_c). The yield regression equation is also established according to BSA dissociation and rebinding constant, which shows a relative error from −9.43% to 5.59%.

The "IIF" model is a novel theory proposed for the interaction rules of BSA-imprinted hydrogel systems. It suggests that variable binding interactions between protein targets and imprinted polymer are different from classical small molecule imprinted materials. The "IIF" model is a candidate method for evaluating and testing BSA rebinding behavior in imprinted hydrogel matrix. The IIF estimation method is established based on rebinding rate and is promising in helping preparation of imprinted materials with designed rebinding properties. Yield regression will be used for calculating product concentration and predicting BSA rebinding process. However, it is not clear if the "IIF" model is applicable in the cases of other protein imprinting. Further works should be focused on "IIF " model calculations for other protein templates in common usage such as cytochrome C, lysozyme, and ovalbumin.

References

1. Zhang, H.; Ye, L.; Mosbach, K. J Mol Recogn 2006, 19, 248.
2. Watterson, J. H.; Piunno, P. A. E.; Krull, U. J Anal Chim Acta 2002, 457, 29.
3. Gore, M. A.; Karmalkar, R. N. J Chromatogr B 2004, 804, 211.
4. Hiratani, H.; Fujiwara, A.; Tamiya, Y. Biomaterials 2005, 26, 1293.
5. Venkatesh, S.; Sizemore, S. P.; Byrne, M. E. Biomaterials 2007, 28, 717.
6. Ogisoa, M.; Minourab, N.; Shinboa, T. Biomaterials 2006, 27, 4177.
7. Bolisaya, L. D.; Culverb, J. N.; Kofinas, P. Biomaterials 2006, 27, 4165.
8. Gorbenko, G.; Ioffe, V.; Kinnunen, P. Biophys J 2007, 93, 140.
9. Fu, G.-Q.; Zhao, J.-C.; Yu, H.; Liu, L.; He, B. React Funct Polym 2007, 67, 442.
10. Zhang, F.-J.; Cheng, G.-X.; Ying, X.-G. React Funct Polym 2006, 66, 712.
11. Ito, H.; Nakamura, M.; Saito, K.; Sugita, K.; Sugo, T. J Chromatogr A 2001, 925, 41.
12. Feczko, T.; Toth, J.; Gyenis, J. Colloids Surf A—Physicochem Eng Aspects 2008, 319, 188.
13. Hunter, A. K.; Carta, G. J Chromatogr A 2001, 937, 13.
14. Asadpour, R.; Alavi-Shoushtari, S. M.; Rezaii, S. A. Ansari, M. H. Kh. Anim Reprod Sci 2007, 102, 308.
15. Janatova, J.; Fuller, J. K.; Hunter, M. J. J Biol Chem 1968, 243, 3612.
16. Sackmann, E.; Goennenwein, S. Prog Theor Phys Suppl 2006, 165, 78.
17. Notkins, A. L. Trends Immunol 2004, 25, 174.
18. Ohtaka, H.; Freire, E. Prog. Biophys Mol Biol 2005, 88, 193.
19. Renata, K.; Kobayashi, T.; Gaziri, L. C. J.; Emerson, J. V.; Marilda, C. V. J Microbiol Methods 2007, 68, 654.
20. Jiang, N.; Zhou, W.-X.; Zhang, Y.-X.; Zhang, X. M.; Wang, J.; Liu, B. Y. Chem J Chin Univ 2006, 27, 1462.
21. Gai, Q.-Q.; Liu, Q.-Y.; Li, W.-Y.; He X. W.; Chen, L. X.; Zhang, Y. K. Chem J Chin Univ 2008, 29, 64.
22. Shweitzer, B.; Zanette, D.; Itri, R. J Colloid Interface Sci 2004, 277, 285.
23. Sonia, F. S.; Zanette, D.; Fischer, H.; Rosangela, I. J Colloid Interface Sci 2003, 262, 400.
24. Peng, Z. G.; Hidajat, K.; Uddin, M. S. J Colloid Interface Sci 2004, 271, 277.
25. Flory, P. J. J Chem Phys 1941, 10, 51.
26. Fried, J. R. Polymer Science and Technology, Chapter 3: Conformation, Solutions and Molecular Weight, 2003; 2nd ed. Prentice Hall College Div; United States.
27. Huggins, M. L. J Chem Phys 1941, 9, 440.