Study on Preparation of Protein-Imprinted Soft-Wet Gel Composite Microspheres with Magnetic Susceptibility and their Characteristics. I. Preparation and Particle Morphology

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ABSTRACT: Protein-imprinted soft-gel composite microspheres with magnetic susceptibility (MS-PIGMs) were prepared by inverse suspension polymerization, using Fe_3O_4 particles as magnetically susceptible component, acrylamide (AM) and *N*,*N*′-methylenebisacrylamide (BisAM) as polymeric matrix components, toluene as solvent, and bovine serum albumin (BSA) and lysozyme (Lyz) as templates, respectively. The surface morphology of MS-PIGMs was observed by environmental scanning electron microscope (ESEM) and scanning electron microscope (SEM). The effects of the kinds and amount of dispersants, stirring rate, the amount and adding methods of initiator, the amount of Fe_3O_4 and monomer concentration on particle morphology of MS-PIGMs, as well as the effects of crosslinking degree on swelling ratio and particle morphology in wet condition were investigated in detail. ESEM and SEM photographs showed that the resulting MS-PIGMs were all spheroid form and had large quantity of regularly distributed pores in wet condition, which close in dry condition, and the experimental results indicated that all the affecting factors had obvious effects on particle morphology of MS-PIGMs. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 684–694, 2006

Key words: molecularly imprinted polymers; magnetically susceptible polymeric composite microspheres; inverse suspension polymerization; soft-gel composite microspheres; protein; Fe_3O_4; preparation; particle morphology

INTRODUCTION

Molecular imprinting is a method originated from bionic to prepare polymers with predetermined recognition specificity to specific substances (template molecules) by manual method.[1,2] These kinds of polymers were named molecularly imprinted polymers (MIPs). MIPs prepared by using proteins as templates could serve as substitute for antibodies, enzymes, or other native biological structures, as well as cell scaffold materials, and so they exhibit extensive application prospect in biotechnology, medicine, and other fields.[3–6]

When magnetically susceptible components, such as Fe, Co, Ni, or their oxides are encapsulated inside MIPs, MIPs will have magnetically susceptible characteristics, and can be separated easily and conveniently from the system they are located in by external magnetic fields after they finish their "active" adsorption and recognition.[7,8]

Up to now, molecular imprinting has been studied extensively and has made contribution in many fields, but the selection of templates are mainly focused on relatively low molecular weight compounds, such as sugars, steroids, amino acid derivatives, and drugs.[7,9–13] Molecular imprinting using proteins as templates is very difficult because molecular weights of proteins are very large, and they usually have bioactivity and thus denature easily. So studies on protein-imprinted polymers are not as good as that on small molecule compounds. There are not many studies on using polymerization methods to prepare protein-imprinted polymeric microspheres (PIPMs) directly, and there are still fewer studies on the preparation of PIPMs with magnetic susceptibility (MS-PIPMs).

MIPs obtained by the methods in existence are mostly rigid polymers, and so the elution of template proteins, which are non-rigid and "huge" in volume, from the matrix after polymerization and their reenter "imprinted cavities" in the course of recognition are unfavorable and difficult. Moderate crosslinked poly-

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TABLE I
The Recipe for Preparation of BSA-PIGMs

Run No.	Dispersant (g)	Tol/H_2O (ml)	AM/BisAM/Fe3O4/K2S2O8/NaHSO3 (g)	BSA (μmol)	Adding manner of intiator	Stiring rate (r/min)
A–Z	Variety	80–100/30	6.0–9.5/0–2.0/0–0.80/0.10/0.05	0.5	Dropwise or at starting	200–360

acrylamide (PAM) gel microspheres are favorable to the elution and adsorption of template proteins, since their crosslinked network can be changed according to the change of circumstance.

In this study, protein-imprinted soft-gel composite microspheres with magnetic susceptibility (MS-PIGMs) were prepared by inverse suspension polymerization (ISP), using Fe_3O_4 particles as magnetically susceptible component, acrylamide (AM) and *N*,*N*′-methylenebisacrylamide (BisAM) as polymeric matrix components, toluene as solvent, and bovine serum albumin (BSA) and lysozyme (Lyz) as templates, respectively. The factors affecting the preparation processes and particle morphology of MS-PIGMs were investigated in detail. Characteristics of the resulting MS-PIGMs will be published in another paper.

EXPERIMENTAL

Reagents

Magnetite Fe_3O_4 particles (Fe_3O_4, 0.5–1.0 μm) were obtained from Institute of Chemistry and Metallurgy, Chinese Academy of Sciences. BSA and Lyz were purchased from Chinese Academy of Medical Sciences. Acrylamide (AM), *N*,*N*′-methylene bisacrylamide (BisAM), ethyl cellulose (EC), toluene, tween85 (Tw), Span80 (Sp), calcium stearate (CS), and hydroxy ethyl cellulose (HEC) were all chemical reagents. Toluene (Tol), potassium persulfate ($K_2S_2O_8$), and sodium bisulfite ($NaHSO_3$) were all analytical reagents. All materials were used without further purification. Double distilled water was used throughout.

Preparation of MS-PIGMs

Polymerization recipe

The experiments were carried out in a 250-mL three-necked flask, and the polymerization recipes for the preparation of BSA-imprinted soft-gel composite microspheres with magnetic susceptibility (BSA-PIGMs) are given in Table I, and that of Lyz-imprinted soft-gel composite microspheres with magnetic susceptibility (Lyz-PIGMs) are given in Table II, according to the best preparation condition of BSA-PIGMs.

Preparation process

ISP were carried out in a 250-mL three-necked round-bottom flask equipped with a reflex condenser, nitrogen inlet, and stirrer. The flask was immersed in a thermostatical water bath at the reaction temperature. The detailed procedure may be given as follows: (1) EC was added to Tol, then heated and stirred at 60–70°C for 2–3 h. When EC dissolved completely, the solution was allowed to cool to room temperature (if using other dispersants by adding them directly into Tol). (2) BSA, AM, and BisAM were added to 20-mL distilled water, and Fe_3O_4 was added after the BSA, AM, and BisAM dissolved. The contents were then transferred into the flask. (3) $K_2S_2O_8$ and $NaHSO_3$ were dissolved in 10-mL distilled water and then added into the flask (according to the method given in Table I). (4) The reaction system was purged with nitrogen, and the reaction lasted for 2 h at room temperature. (5) Tol in upper layer of the system was removed after leaving it undisturbed for 10 min. MS-PIGMs were then obtained by adding some acetone to the system and stirring for 10 min, followed by removal of acetone and repeated washing with distilled water.

Lyz-PIGMs were prepared using the similar process as for BSA-PIGMs, according to polymerization recipe given in Table II.

The nonimprinted magnetically susceptible soft-gel composite microspheres (Non-PIGMs) were prepared in the same manner as for Lyz-PIGMs in the absence of protein templates.

TABLE II
The Recipe for Preparation of Lyz-PIGMs

EC (g)	Tol/H_2O (ml)	AM/BisAM/Fe3O4/K2S2O8/NaHSO3 (g)	Lyz (μmol)	Adding manner of intiator	Stiring rate (r/min)
0.20	100/30	9.0/1.0/0.20/0.10/0.05	0.5	Dropwise	280

Elution of protein templates

The same elution process was used by BSA-PIGMs, Lyz-PIGMs, and Non-PIGMs. The detailed procedures were as follows: An aqueous solution of Ace (10%, v/v) and SDS (1%, w/v) was first prepared. MS-PIGMs were then immersed in it, stirred for 24 h, and followed by filtration. MS-SMIPs were washed repeatedly with distilled water until the latter remained completely neutral. They were then vacuum dried in an oven at 60°C until they showed a constant weight.

Determination of swelling ratio

MS-PIGMs (1 g) were added to a 50-mL conical flask, followed by the addition of 25 mL distilled water, with the temperature kept at 25°C for 24 h. The swelled MS-PIGMs were filtered and the water on their surface was sucked by filter paper. The MS-PIGMs were then weighed. This process was repeated until they showed a constant weight.

Swelling ratio (SR) was defined using Formula. (1)

$$SR = (W_{wet} - W_{dry})/W_{dry} \quad (1)$$

Wherein W_{wet} is the weight of saturated swelled MS-PIGMs, and W_{dry} is that of dry MS-PIGMs.

Analysis methods

Scanning electron microscope

The surface morphology of dry MS-PIGMs was characterized by Hitachi *S*-3000N scanning electron microscope (SEM).

Environmental scanning electron microscope

The surface morphology of wet MS-PIGMs was characterized by PHILLIP XL30 enviormental scanning electron microscope (ESEM).

RESULTS AND DISCUSSION

Selection and design of polymerization system

As mentioned earlier, proteins are natural biomacromolecules with complicated steric structure and bioactivity. Steric structure made them difficult to slip in and out of a polymer network, and thermodynamic factors made them difficult to yield well-defined recognition sites.[14] As a result, proteins could not be imprinted using the same ways as that possible with small molecules, and so even the concepts of imprinting and recognition mechanism should be changed. Obviously, it was impossible to rely on merely several bonding sites (usually two or three bonding sites in imprinting and recognition of small molecules) to "tie" huge protein molecules. Only relying on large quantity of bonding sites can this purpose be realized. So the corresponding changes of polymer matrices and imprinting methods must be considered to overcome these difficulties.

Soft-wet polyacrylamide gel microspheres with moderate crosslinking degree provided this possibility, for they had many advantages, which are given as follows: (1) They could change their crosslinking network and the size of gel pores by shrinking and expanding according to their existent circumstance; (2) There was very good accessibility between template proteins and them, as their macromolecular chains had high flexibility; (3) There were many amido bonds in their macromolecular chains, and they could form a large quantity of hydrogen bond with peptide bond (amido bond), and thus was of great advantage to tie protein molecules; (4) Polyacrylamide gel was known to be chemically inert and biocompatible,[15] for they could not react with protein and could not denature protein. This was proved by the fact that polyacrylamide gel with low crosslinking degree was usually used in gelelectrophoresis to separate enzyme and other proteins and could retain their bioactivity; and (5) The copolymerization condition of AM and BisAM was mild (copolymerization could be carried out at room temperature) and could keep bioactivity of proteins.

AM and BisAM were all water soluble monomers, and the selected proteins in this study could also be dissolved in water, so ISP could be adopted to prepare soft-wet gel microspheres directly and meanwhile realizing the imprinting to proteins. Tol was a nonpolar organic solvent and was almost completely immiscible with water, and thus was suitable for using as dispersion medium of ISP. In addition, polyacrylamide had good compatibility with Fe_3O_4 and was favorable to the encapsulation of Fe_3O_4 in the preparation of MS-PIGMs.

For all the aforementioned reasons, PAM was selected as polymer matrix and ISP was adopted to prepare MS-PIGMs.

Particle morphology of MS-PIGMs

Particle morphology of MS-PIGMs in wet condition

Figure 1 shows ESEM photographs of single particle and its surface magnification of the resulting MS-PIGMs in wet condition. It could be obviously seen from Figure 1(a) that the resulting MS-PIGMs were of spheroid form and there were a large quantity of regularly distributed pores on its surface, which could be clearly seen from Figure 1(b). These pores provided the convenience for protein molecules to slip in and out of gel microspheres.

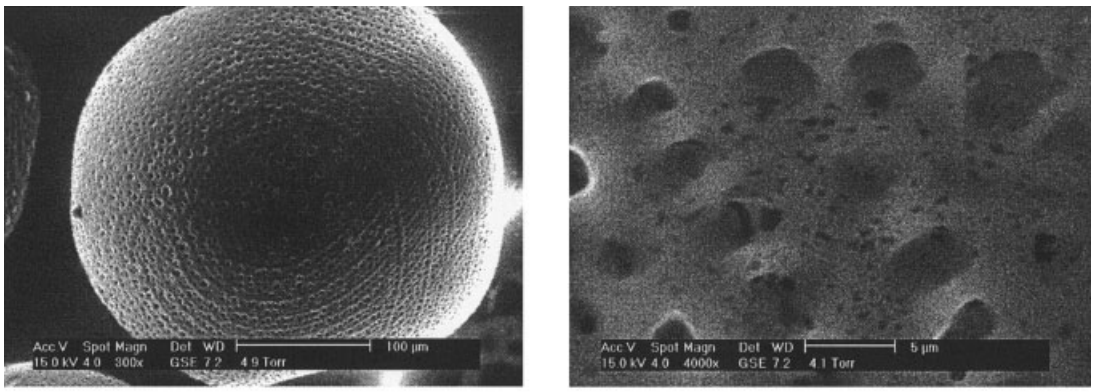


Figure 1 ESEM photographs of MS-PIGMs in wet condition.

Particle morphology of MS-PIGMs in dry condition

Figures 2(a) and 2(b) shows SEM photographs of single particle and its surface magnification of the resulting MS-PIGMs in dry condition. From Figure 2(a) we could see that the resulting MS-PIGMs are of spheroid form even after being dried, but there are no visible pores on its surface. This result showed that the pores were closed, accompanying the dehydration and shrinking of gel microspheres, and this could be confirmed by Figure 2(b).

Some examples of MS-PIGMs with different morphology

It was found that formation of particles with highly regular morphology was very difficult, as the conglomeration of particles occurred very easily, this in turn forming congeries. So it was very important to avoid the conglomeration of particles in the preparation of MS-PIGMs. Some examples of MS-PIGMs with regular shape and conglomeration with different extent are shown in Figure 3, of which Figures 3(a) and 3(b) are the photographs of regular MS-PIGMs with different magnification times; Figures 3(c) and 3(d) are the photographs of MS-PIGMs with slight and severe conglomeration, respectively.

It could be seen from these photographs that there was great difference in the morphology of MS-PIGMs prepared at different conditions. The authors thought that it was related to the formation mechanism of particles.

The polymerization process of MS-PIGMs included two stages: (1) As dispersion phase, aqueous solution of monomer in ISP system was dispersed into pieces by the shearing action of stirring and separated to form large numbers of little "waterpolos" by dispersion medium (Tol), and waterpolos were stabilized using adsorbing dispersant. (2) Monomers within waterpolos began to polymerize subsequently and turned into anhydrous gel microspheres gradually. These little waterpolos were not absolutely steady in the course of polymerization, and could be incorporated and redispersed because of the mechanical ac-

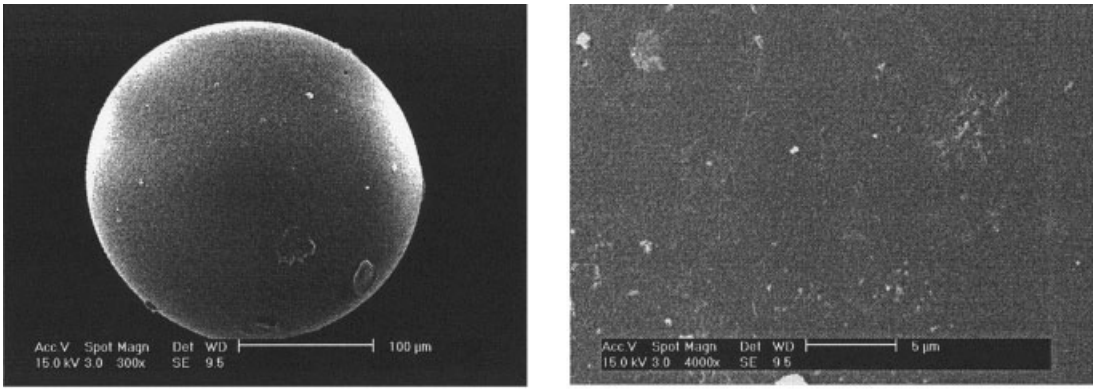


Figure 2 SEM photographs of MS-PIGMs in dry condition.

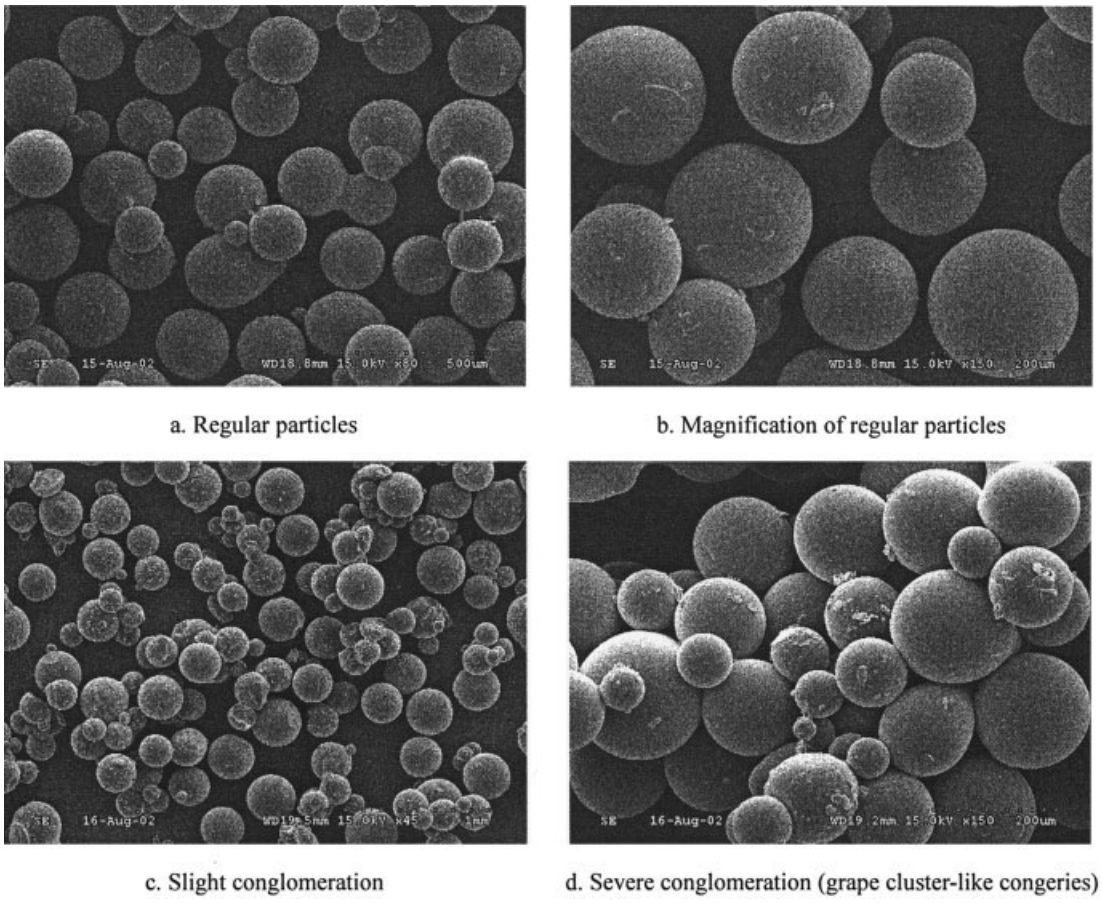


Figure 3 SEM photographs of MS-PIGMs with different morphology.

tion, static magnetic attraction, dispersive action of dispersant, and so on. So these little waterpolos were in dynamic equilibrium, and the final particle morphology was dependent on the combined action of many affecting factors. The effects of each factor on particle morphology would be discussed in detail hereinafter.

Influencing factor of particle morphology of MS-PIGMs

Effect of kinds and amount of dispersants on particle morphology

For ISP, the kind and amount of dispersant usually had very notable effects on particle morphology, particle size, and size distribution, and so the effects of dispersant on particle morphologies were investigated in detail, and the experimental conditions and results are shown in Table III.

It could be seen from Table III that EC was suitable as dispersant of this ISP system, and when the amount of EC was 0.20 g (Run G), regular microspheres could be obtained [see in Fig. 4(a)]. When the amount of EC was 0.15 g (Run I), the resulting product was microspheres in morphology but coalesces [see in Fig. 4(b)]. This was because the resulting microspheres could not exist in reaction system independently and steadily on account of the insufficiency of dispersant, with consequent congeries formation. When the amount of EC was 0.25 g (Run B), the production was partly lump

TABLE III
Particle Morphologies Prepared by Different Kinds and Amount of Dispersant

Run no.	EC (g)	HEC (g)	SP + TW (g)	EC + CS (g)	Product morphology
B	0.25	—	—	—	Part lump, part emulsion
G	0.20	—	—	—	Regular microspheres
I	0.15	—	—	—	Microspheres but coalesce
C	—	0.10	—	—	Lump
D	—	1.0 + 0.5	—		Emulsion
E	—	—	—	0.10 + 0.10	Rice grain-like huge particle

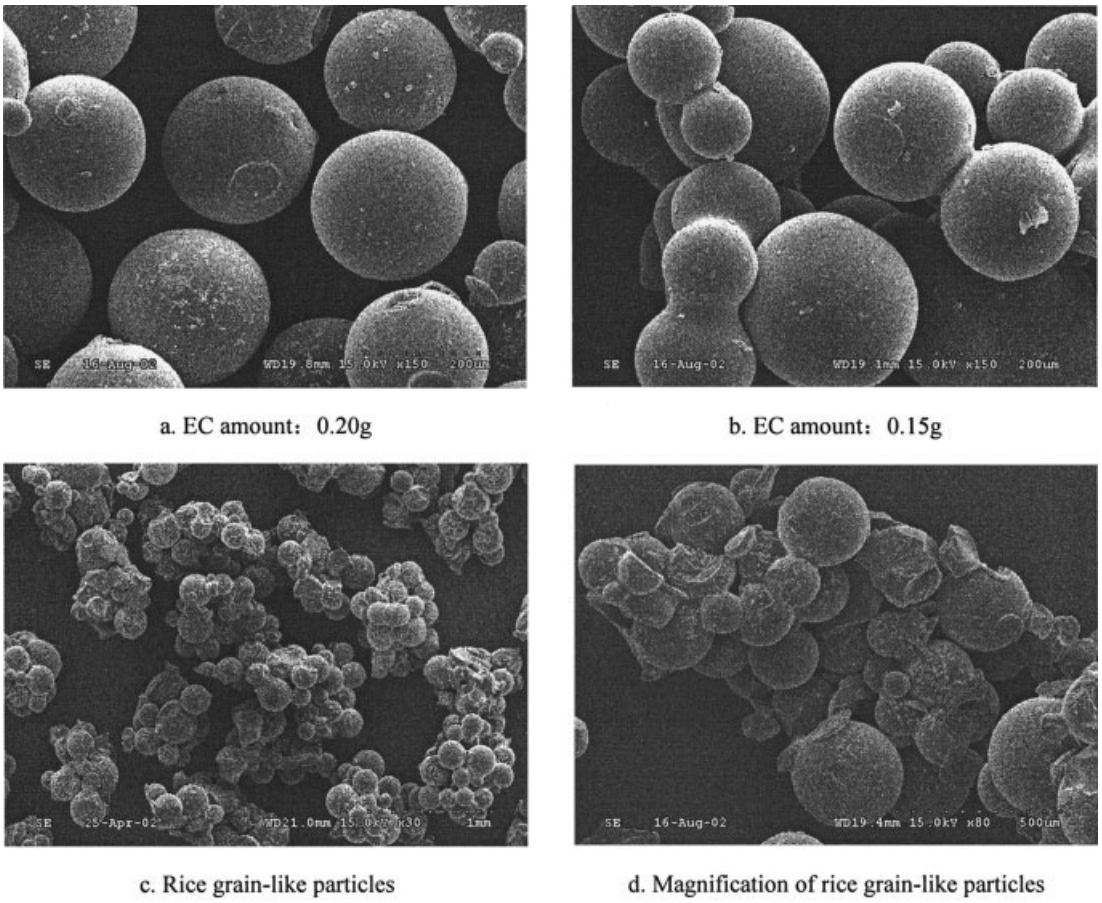


Figure 4 SEM photographs of MS-PIGMs prepared by different amount of EC.

and partly emulsion. The extent of product coalesce should be lightened along with the increasing amount of dispersant in theory, but the fact was that the extent of product coalesce was aggravated but not lightened. The phenomenon was probably aroused by the formation of inverse micellae and inverse emulsion subsequently in dispersant (EC) excess condition. With growth of inverse emulsion particles, the part of dispersant being used to stabilize ISP particles was deprived, and thus the stability of ISP particles was lost, which in turn led to the formation of lumps. The fact that the resulting product was lump when HEC was used as dispersant (Run C) illustrated that HEC was not suitable for this ISP system. This might be because HEC could not form a protective layer in the oil–water interface, for it could dissolve only in water. SP and TW (D) could form micellae and thus could form inverse emulsion; therefore, regular microspheres could not be formed. Rice grain-like huge particles were formed when EC and CS were used at the same time (Run E), because the amount of EC was too less and CS could not form a protective layer in the oil–water interface although it was dispersed into tiny granules in water. These rice grain-like huge particles were congeries made up of numerous small particles, (see Figures 4(c) and 4(d) for details).

Effect of stirring rate on particle morphology

The effect of stirring rate on particle morphology was studied in detail, and the experimental conditions and particle morphologies are shown in Table IV.

It could be seen from Table IV that the product morphology was different at different stirring rate, and the influencing extent of stirring rate on product morphology was different for different amounts of dispersant. When the amount of EC was 0.20 g and stirring rate was 200 rpm (Run F), the product was regular microspheres in morphology but with slight

TABLE IV
Production Morphologies Prepared at Different Stirring Rate

Run no.	EC (g)	Stirring rate (r/min)	Product morphology
F	0.20	200	Regular microspheres, slight coalesce
G	0.20	280	Regular microspheres, no coalesce
H	0.15	200	Regular microspheres, slight coalesce
I	0.15	280	Regular microspheres, severe coalesce
J	0.15	360	Regular microspheres, severe coalesce

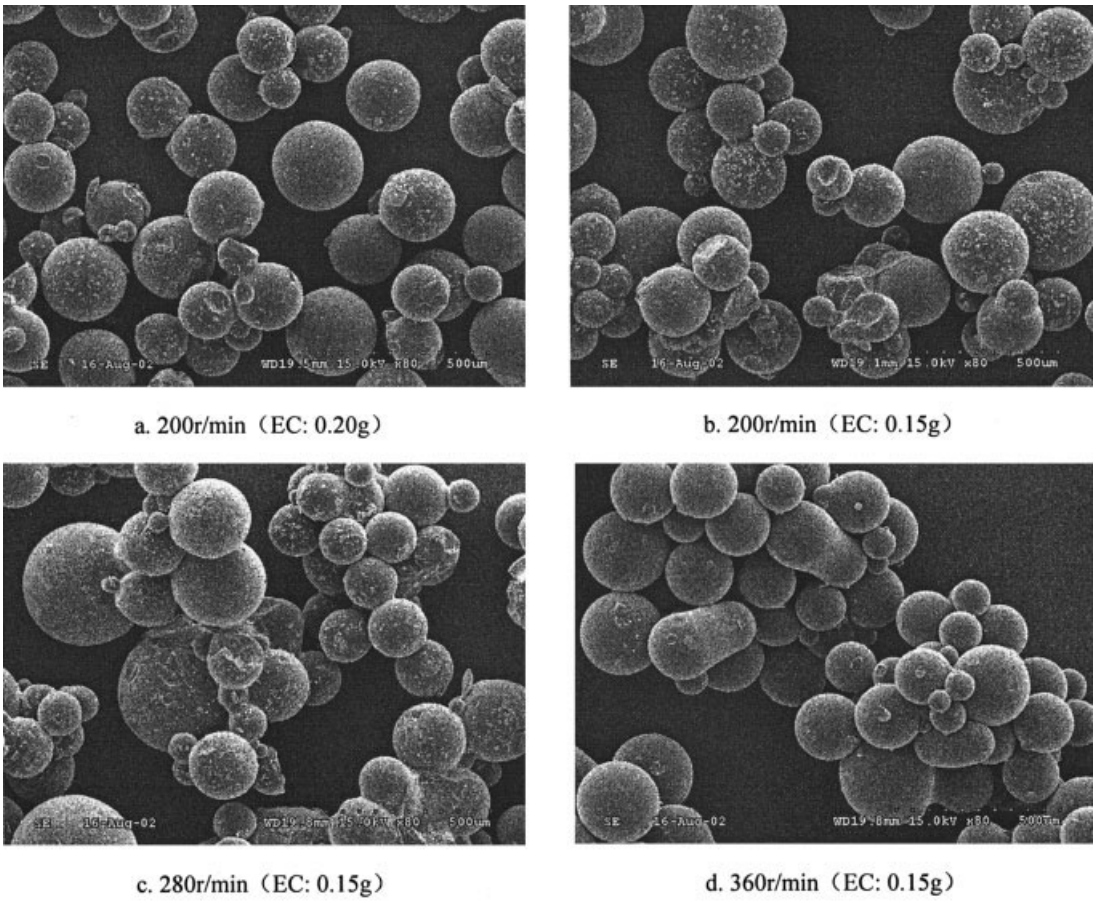


Figure 5 SEM photographs of MS-PIGMs prepared by different stirring rate.

coalesce [see in Fig. 5(a)]; when stirring rate was 280 rpm (Run G), the product was regular microspheres and almost with no coalesce [see in Fig. 3(a)]. These results showed that stirring rate had only slight effect on product morphology when the amount of dispersant was appropriate. When the amount of EC was 0.15 g and stirring rate was 200 rpm (Run H), the product was regular microspheres but with slight coalesce [see in Fig. 5(b)]; when stirring rate was 280 rpm (Run I), the product was still regular microspheres but coalesce extent was aggravated compared with Run I; and when stirring rate was 280 rpm (Run J), severe coalesce occurred and some "nonspherical" particles occurred meanwhile [see in Fig. 5(d)]. These results indicated that stirring rate had obvious effect on product morphology when the amount of dispersant was not enough.

The reason why stirring rate had different effects on particle morphology at different amounts of dispersant was that the faster the stirring rate, the smaller was the waterpolo, and larger the specific surface area of waterpolos, the more the dispersant needed. These results must be exhibited at different amount of dispersant, and the less the amount of dispersant was, the more obvious was the influencing extent was.

Effect of amount and adding methods of initiator on particle morphology

As mentioned earlier, above, the copolymerization of AM and BisAM occurred easily and very fast. So the effect of the amount and adding methods of initiator on particle morphology was investigated in detail, and the experimental conditions and particle morphologies are shown in Table V.

From the comparison of Run K, Run L, and Run M in Table V, it could be seen that in the same condition of initiator adding methods (initiator was added at starting, mixing with monomers), product morphologies were obviously different with different amount of initiator. When the amount of initiator was bigger (Run K), the product was lump; when the amount of initiator was decreased, the product was irregular huge particles; and when the amount of $NaHSO_3$, which was used as reducing agent in initiator, was further decreased, the reaction could not be carried out. When comparing Run L and Run N, it could be seen that the product morphologies were fully different when the initiator-adding methods were different and the amounts were the same.

TABLE V
Particle Morphologies Prepared by Different Amount and Adding Manner of Initiator

Run no.	$K_2S_2O_8$ (g)	$NaHSO_3$ (g)	Adding manner	Product morphology
K	0.15	0.05	At starting	Lump
L	0.10	0.05	At starting	Irregular huge particles
M	0.10	0.03	At starting	Not reaction
N	0.10	0.05	Dropwise	Regular microspheres

In the method of adding initiator at the beginning, the reaction went too fast when the amount of initiator was large, and in this circumstance the reaction had begun when the aqueous containing monomers and initiator was not dispersed into uniform waterpolos adequately and thus resulted in the formation of lump. This situation was improved to a certain extent by decreasing the amount of initiator but it could not be solved completely. One still could not obtain regular microspheres; the product was either irregular huge particles or the reaction could not be carried out. For this reason, the initiator was added dropwise during the course of reaction in Run M, and regular microspheres were obtained successfully by predispersing of dispersion phase (i.e., before the reaction occurred).

Effect of the amount of Fe_3O_4 on particle morphology

In general, the more the magnetic component content in materials was, the stronger their magnetic response was. Therefore, the amount of Fe_3O_4 was hoped to have as much as possible in MS-PIGMs. But the amount of Fe_3O_4 had remarkable effect on product morphology for the existence of static magnetic attraction. For this reason, the effect of amount of Fe_3O_4 on particle morphology was investigated via experiments, and the experimental conditions and particle morphologies are shown in Table VI.

It could be obviously seen from Table VI that the resulting product morphology was obviously different with the amount of Fe_3O_4. When the amount of Fe_3O_4 was 0.20 g (Run W), the product was regular microspheres in morphology and it was the same as in the absence of Fe_3O_4 (Run A) [see Figure 6(a)]. When the amount of Fe_3O_4 was 0.40 g (Run X), the product was still microspheres in morphology but with severe coalesce [see Figure 6(b)]. While the amount of Fe_3O_4 was added up to 0.60 g (Run Y) or 0.80 g (Run Z), the product morphologies could not be obtained and the product was irregular huge particles and lumps, respectively. These results illustrated that the amount of Fe_3O_4 was too much in the preparation of MS-PIGMs, and the better amount of Fe_3O_4 in this system was below 0.4 g.

It was found that there was much white attachment on the surface of MS-PIGMs. The author speculated that they were congeries of Fe_3O_4, and it was conformed by their size and shape [Figures 6(c) and 6(d)].

This result was due to static magnetic attraction between Fe_3O_4 particles essentially. Static magnetic attraction was relatively smaller when the adding amount of Fe_3O_4 was smaller, and it could be overcome by the combined action of stirring, dispersant, and dispersion medium action. Static magnetic attraction was remarkably enhanced along with the increase of adding amount of Fe_3O_4, and the aforementioned action was not strong enough to overcome static magnetic attraction, thus the coalesce of Fe_3O_4 particles occurred. Congeries of Fe_3O_4 attaching on the surface of MS-PIGMs probably due to the adding amount of Fe_3O_4 was too big to be capsulated by the copolymer of AM and BisAM and thus exposed on the surface of MS-PIGMs.

Effect of monomer concentration on particle morphology

PAM was a kind of water absorbent gel, and so the product morphology could be affected by monomer concentration, especially crosslinker monomer con-

TABLE VI
Production Morphologies Prepared by Different Amount of Fe_3O_4

Run no.	Monomer (g)	Fe_3O_4 (g)	Fe_3O_4 content (%)	Product morphology
A	10	0	0	Regular microspheres
W	10	0.20	1.96	Regular microspheres
X	10	0.40	3.84	Microspheres, severe coalesce
Y	10	0.60	5.66	Irregular huge particles
Z	10	0.80	7.41	Lump

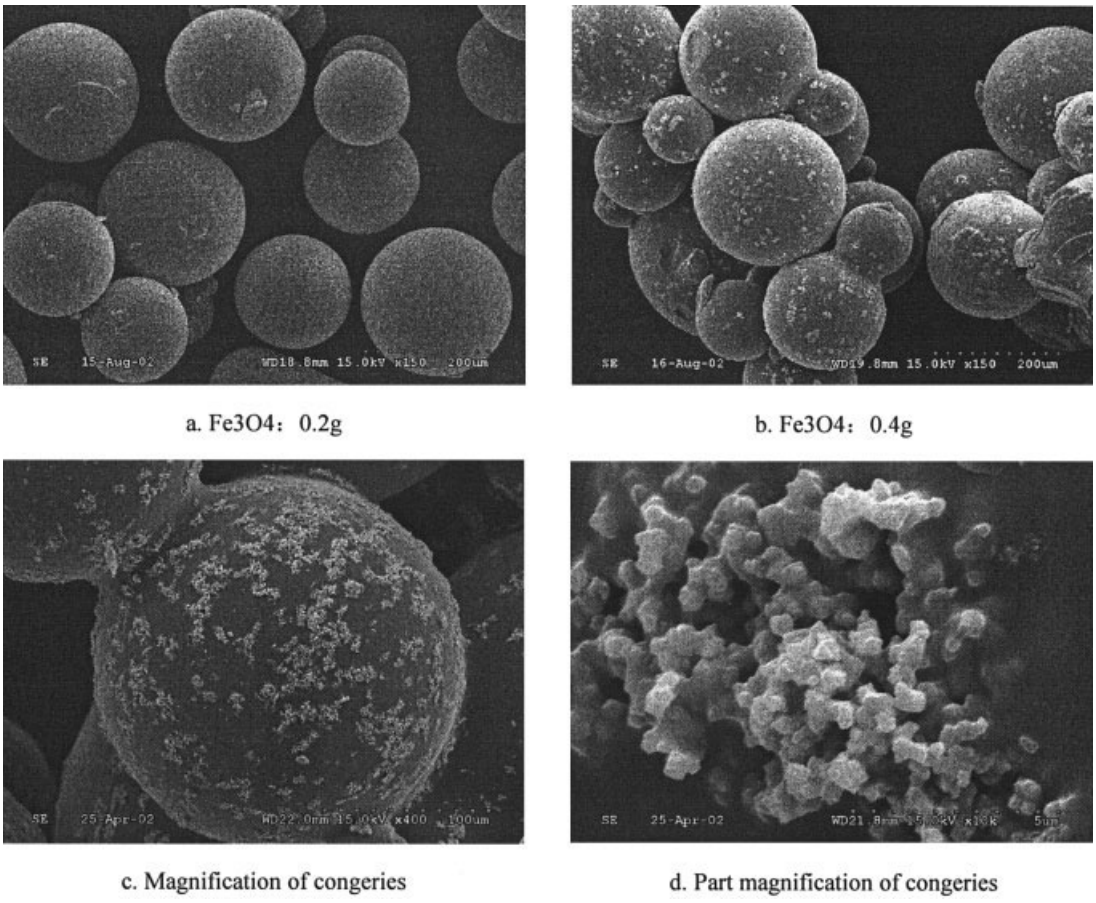


Figure 6 SEM photographs of MS-PIGMs prepared by different amount of Fe_3O_4.

centration. For this reason, the effect of monomer concentration on particle morphology was investigated, and the experimental conditions and particle morphologies are shown in Table VII.

It could be seen clearly from Table VII that monomer concentration of aqueous phase in reaction system had obvious effect on product morphology. The lower the monomer concentration, the more difficult was for the product to form microspheres, and vice versa. When monomer concentration arrived at 25% (Run R), regular microspheres were obtained.

When monomer concentration was too low and no crosslinker monomer was added, the polymeric gel swell completely and even be dissolved. Numerous macromolecular chains in a waterpolo could not form an organic whole, but many waterpolos could be united to form a lump. When the crosslinker monomer (BisAM) was added, the swelling of polymeric gel was restricted to some extent, and if the monomer concentration was too low, a large quantity of water was left in the polymeric gel even though a saturation in the swelling of the polymeric gel was reached. So the resulting gel could not be acted upon directly by the interfacial tension between oil and water, resulting in the formation of lumps or huge, irregularly shaped particles.

TABLE VII
Production Morphologies Prepared by Different Monomer Concentration

Run no.	AM (g)	BisAM (g)	H_20 (mL)	Monomer concentration (%)	Product morphology
O	6.0	—	30	16.7	Lump
P	6.0	0.6	30	18.0	Part lump, part huge particles
Q	6.0	1.0	30	18.9	Irregular huge particles
R	9.0	1.0	30	25.0	Regular microspheres

TABLE VIII
Swelling Ratio of MS-PIGMs with Different Crosslinking Degree

Run no.	AM (g)	BisAM (g)	Crosslinking degree (%)	SR
S	9.5	0.5	5	5.33
T	9.0	1.0	10	3.16
U	8.5	1.5	15	2.18
V	8.0	2.0	20	2.03

Effect of crosslinking degree on swelling ratio

Crosslinking degree was an important index, for it had effect on swelling ratio (SR) of gel microspheres and thus had effect on the maintenance of imprinting cavities directly. The effect of crosslinking degree on SR of MS-PIGMs was investigated, and the experimental conditions and results are given in Table VIII.

Table VIII indicated clearly that SR of MS-PIGMs decreased along with the increase of crosslinking degree. The reason was obvious that the less the crosslinking degree was, the sparser was the crosslinking network of gel microspheres, the more the absorbing water, the bigger the SR was, and *vice versa*.

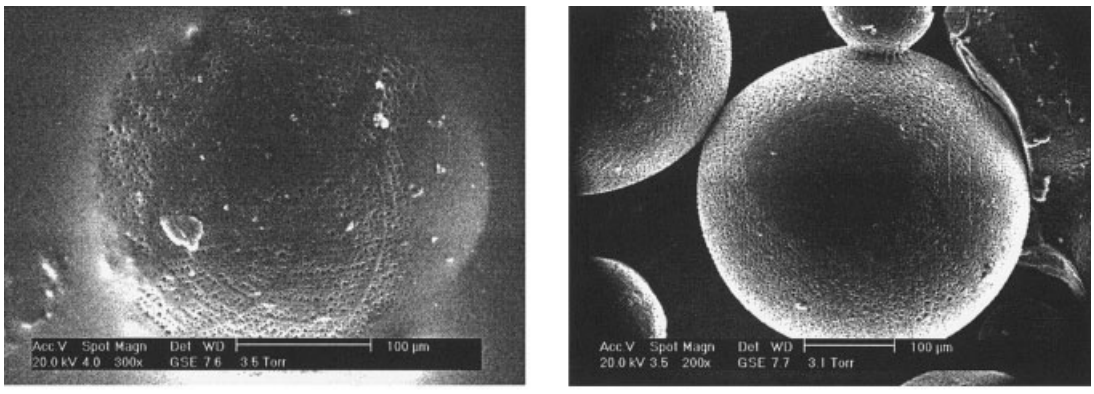
In general, the recognition of MS-PIGMs to their templates was carried out in saturated swelling condition, while the SR of MS-PIGMs was also determined in saturated swelling condition, according to the definition of SR (Formula (1)), so the SR of MS-PIGMs in case of template recognition should be the same as that in case of template imprinting, namely at the time when MS-PIGMs are being prepared, to keep the size of imprinting cavities and ensure the recognition specificity of MS-PIGMs. In the present system, the SR of MS-PIGMs at the time when MS-PIGMs are prepared should be 3.0, according to their polymerization recipe (see Table I), and monomer conversion is assumed 100%. It could be seen from Table VIII that only SR of MS-PIGMs was 3.16 (Run T), with 10% crosslinking degree had the nearest SR to that of MS-PIGMs being imprinted. Therefore, 10% crosslinking degree was preferred in this system.

Effect of crosslinking degree on particle morphology in wet condition

As mentioned above, the pores on the surface of MS-PIGMs in wet condition provided the convenience for template protein molecules to slip in and out of gel microspheres. Therefore, MS-PIGMs with different crosslinking degree in wet condition (corresponding to the state of recognition) were observed by ESEM, and the effect of crosslinking degree on surface morphology, especially on surface pores of MS-PIGMs, was investigated.

Figures 7(a) and 7(b) are the ESEM photographs of wet MS-PIGMs with 15 and 20% crosslinking degree respectively, and ESEM photograph of wet MS-PIGMs with 10% crosslinking degree is given in Figure 1(a).

It could be seen from Figures 7(a) and 7(b), and Figure 1(a) that surface morphologies, especially surface pores of particles, were obviously different in case of different crosslinking. When crosslinking degree was 10%, MS-PIGMs could be swelled adequately by absorbing water, for their crosslinking network of polymer chains was sparser, and thus their surface pores looked big and deep. When crosslinking degree was 15%, the swelling of MS-PIGMs by absorbing water was restricted, as density of their crosslinking network of polymer chains was increased, and thus the size and depth of surface pores being reduced to some extent; and when crosslinking degree was 20%, the swelling of MS-PIGMs by absorbing water was further restricted, for density of their crosslinking network of polymer chains being further increased, and the size and depth of surface pores being further reduced. It could be speculated according to the change



a. Crosslinking degree: 15%

b. Crosslinking degree: 20%

Figure 7 ESEM photographs of wet MS-PIGMs with different crosslinking degree.

of surface pores that the inner tunnel of MS-PIGMs would be adjusted along with the difference of crosslinking degree. Therefore, mass transfer of protein in MS-PIGMs would be affected definitely by the effect of crosslinking degree on surface pores and inner tunnel.

CONCLUSIONS

1. Protein-imprinted soft-gel composite microspheres with magnetic susceptibility (MS-PIGMs) were prepared by inverse suspension polymerization, using Fe_3O_4 particles as magnetically susceptible component, acrylamide (AM) and *N*,*N*′-methylenebisacrylamide (BisAM) as polymeric matrix components, and BSA and Lyz as templates, respectively. The resulting MS-PIGMs were all of spheroid form, and had large quantity of regularly distributed pores in wet condition, which close in dry condition.
2. The kinds and amount of dispersants, stirring rate, the amount and adding methods of initiator, the amount of Fe_3O_4 and monomer concentration on particle morphology of MS-PIGMs, as well as crosslinking degree had obvious effects on particle morphology. The polymerization recipe was as follows: toluene 100 mL, H_2O 30 mL, EC 0.2 g, AM 9.0 g, BisAM 1.0 g, $K_2S_2O_8$ 0.10 g, $NaHSO_3$ 0.05 g, Fe_3O_4 0.2 g, and protein 0.5 μmol. The preferred process was as follows: stirring at 280 rpm, initiator was added dropwise, and the reaction system was purged with nitrogen and the reaction lasted for 2 h at room temperature.

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