

Preparation of Hydroxyapatite/Ca-Alginate Composite Microspheres via Inverse Suspension Crosslinked Method

Chao Liu,¹ Xiujie Ji,² Kongyin Zhao,³ Guoxiang Cheng³

¹School of Materials Science and Engineering, Hebei University of Technology, Tianjin 300130, China

²Department of Chemistry, Tianjin University, Tianjin 300072, China

³School of Materials Science and Engineering, Tianjin University, Tianjin 300072, China

Received 12 October 2006; accepted 14 November 2006

DOI 10.1002/app.25851

Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Composite microspheres were prepared with hydroxyapatite (HA) and Na-alginate (ALG) via inverse suspension crosslinked method (ISCM). Chloroform/hexane (1 : 2 (volume)) was used as continuous phase, ethyl cellulose (EC) as disperser, mixture of HA powder and ALG solution as dispersed phase, and calcium chloride as crosslinking agent. Morphology of the composite microspheres and HA distribution state were observed by optical microscope. According to the compositions studied, the sample with 0.2 g of EC gives the best continuous phase. The sample with 25 mL/min of adding

speed and 120 r/min of stirring speed gives the best adding and stirring conditions. Two kind of the composite microspheres of 50/50 and 70/30 HA/ALG were prepared via ISCM. They are with specific gravity of 1.06 and 1.15, and water content of 88 and 81%, respectively. The size of the dried microspheres is mainly in the range of 125–425 μm . © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 104: 2034–2038, 2007

Key words: composite microspheres; hydroxyapatite; calcium alginate; inverse suspension crosslinked method

INTRODUCTION

Alginate/phosphate composites are widely used as carriers of drugs,^{1,2} proteins,³ DNA and cell,⁴ or implant material and bone fillers.^{5,6} The composite ways of polymeric matrices with ceramic particles, including deposition of ceramic phases *in situ*⁷ and mixture of polymeric matrices and presynthesized ceramic phases, have also been investigated. Hydroxyapatite (HA) and tricalcium phosphate are the most common ceramic phases have been used in these composites. For the polymeric matrices, both synthetic⁸ and natural origin have been employed, the latter including collagen, chitosan, gelatin, and alginate.³

Alginate-based spherical particles were usually prepared by dropping or spraying dilute sodium algi-

nate solutions containing the dispersed particles into calcium chloride solutions.^{2,9–11} The drop method cannot match the needs of speed and efficiency. Spray with device is efficient, but it needs the specialized device. Considering these factors, we think that it is valuable to develop a novel preparation method of alginate-based spherical particles with the traits of simpleness, quickness, and efficiency.

This investigation describes inverse suspension crosslinked method (ISCM) for the preparation of the HA/Ca-alginate composite microspheres. HA powder was presynthesized by hydrothermal reaction. The morphology factors of ISCM, including disperser, adding speed, stirring speed, and composite ratio, were discussed. HA distribution, specific gravity, and water content of microspheres were studied. The particle size distributions of the dried microspheres were investigated in this study. As a carrier of protein, the dried microspheres were under study by us.

MATERIALS AND METHODS

Materials

Sodium alginate (ALG, \overline{M}_w and \overline{M}_n is 21.8×10^4 and 3.5×10^4 , respectively) was from Beijing Xudong Chemical Factory (Beijing, China). Ethyl cellulose (EC) was obtained from Tianjin Second Chemistry Factory (Tianjin, China). Calcium chloride, chloroform, and

Correspondence to: C. Liu (liuchao_tj@yahoo.com) or G. Cheng (gxcheng@tju.edu.cn).

Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 50373032.

Contract grant sponsor: Teaching and Research Award Program for Outstanding Young Teachers in Higher Education Institutions of MOE, P.R.C.; contract grant number: 2002-123.

Contract grant sponsor: Excellent Ph.D. Dissertation Foundation of Tianjin University; contract grant number: JJ0219.

Journal of Applied Polymer Science, Vol. 104, 2034–2038 (2007)
© 2007 Wiley Periodicals, Inc.

TABLE I
Fixed Parameters in Preparation

Samples	Na-alginate (g)	Water (mL)	Chloroform (mL)	Hexane (mL)
a-g	0.75	25	20	40

hexane were provided by Kewei Company of Tianjin University (Tianjin, China). ALG and EC were chemical grade, and the others were analytical grade. Deionized water was used throughout the experiment. HA powder was prepared by us via hydrothermal reaction.

Preparation of composite microspheres via ISCM

HA powder was dispersed in the solution of sodium alginate in water (dispersed phase) and added to a stirred solution of EC in 1 : 2 (volume) of chloroform/hexane (continuous phase). After the inversed suspension was stirred at room temperature (r.t.) for 0.5 h, the solution of calcium chloride in water (2.5% (weight)) was added to crosslink the microspheres. The microspheres were stirred at r.t. for 2 h further, and then washed with water and preserved in water. The detail preparation parameters are listed in Tables I and II.

Observation of microspheres

The samples for observation were prepared as follows: For the wet composites, they were dropped onto the microscope slide together with water and covered with cover glass. For the dried composites, they were dispersed on the microscope slide and covered with cover glass (no water added). The morphology of composites and distribution state of HA were observed by optical microscope (OM), Olympus BX51TF.

Specific gravity and water content of the microspheres

The specific gravity of the microspheres was measured by Archimedean method. It can be calculated

as follows:

$$SG_s = \frac{W_s}{V_d \rho_w} \quad (1)$$

where SG_s is the specific gravity of microspheres, W_s is the weight of microspheres (g), V_d is the water displacement (mL), and ρ_w is the density of water (1 g/mL).

Microspheres were dried at 65°C to no weight change. The water content of the microspheres was evaluated as follows:

$$\frac{W_w - W_d}{W_w} \times 100\% \quad (2)$$

where W_w is the weight of wet microspheres and W_d is the weight of dry microspheres.

Particle size distribution of the dried microspheres

The wet microspheres were immersed in ethanol for 12 h. This treatment made the microspheres partially dehydrate and maintain spherical shape. The treated microspheres were dried at 65°C for 24 h. The dried microspheres were sieved and weighed.

RESULTS AND DISCUSSION

Process of microspheres preparation via ISCM

ISCM can be subdivided in to two steps and five stages (Fig. 1). The two steps are inversed suspension (step I) and crosslink (step II). The first two stages belong to step I, and the last three stages belong to step II. The five stages are as follows.

- A. *Predispersion of HA/ALG microspheres via inversed suspension*: In this stage, the dispersed phase was added into the continuous phase under stirring. A W/O (water in oil) suspension was formed.
- B. *Stabilization of HA/ALG microspheres by EC molecules adsorption*: EC, dissolved in the continuous phase, partially adsorbed on the surface of the predispersed microspheres. This increased the stability of the microspheres.

TABLE II
Controlled Parameters in Preparation

Samples	Hydroxyapatite (g)	Ethyl cellulose (g)	Adding speed (mL/min)	Stirring speed (r/min)
a	0.75	0	25	120
b	0.75	0.1	25	120
c	0.75	0.2	25	120
d	1.75	0.2	25	120
e	0.75	0.2	50	120
f	0.75	0.2	25	240
g	3.00	0.2	25	120

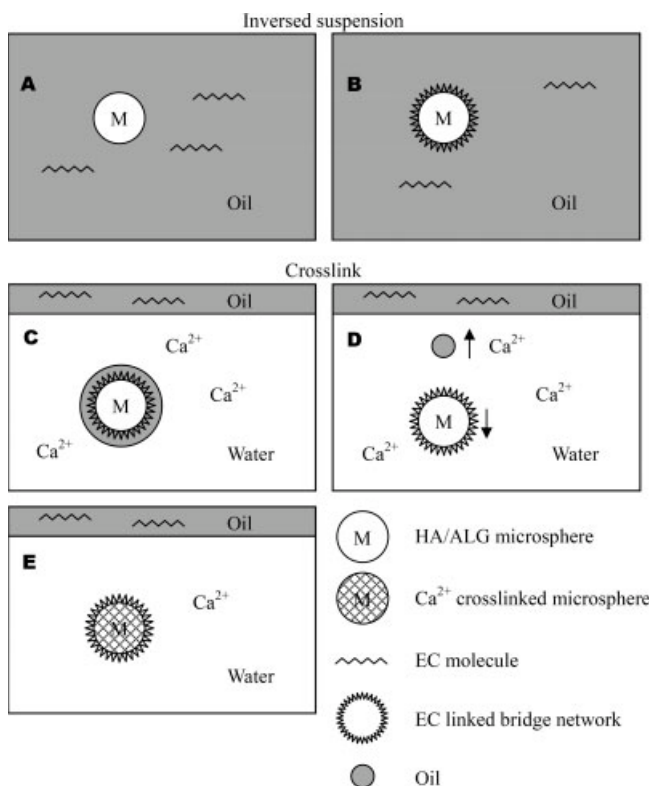


Figure 1 Schematic showing process of ISCM. (A) predisposition of HA/ALG microspheres via inversed suspension; (B) stabilization of HA/ALG microspheres by EC molecules adsorption; (C) addition of crosslinking agent; (D) oil–water separation; and (E) Ca^{2+} crosslink.

- C. *Addition of crosslinking agent:* In this stage, CaCl_2 solution was added in the inversed suspension. The microspheres with oil membrane went into the CaCl_2 solution.
- D. *Oil–water separation:* The oil membrane was separated with the microspheres. As a result, the microspheres were exposed to CaCl_2 solution.
- E. *Ca^{2+} crosslink:* Ca^{2+} penetrated into the microspheres. And then the Na-alginate molecules were crosslinked by Ca^{2+} . Finally, the cross-linked microspheres were obtained.

Influence factors of ISCM

Disperser factor

EC (0.1 and 0.2 g) was used as disperser in the samples *b* and *c*, respectively. Sample *a* is the control sample. It can be seen that with the amount of disperser increasing, the composite morphology was changed from irregular distortion to regular sphericity [Fig. 2(a–c)]. And for EC-free sample *a*, a lot of HA powder came into the external aqueous phase when CaCl_2 solution was added to the inversed suspension. This means that with the decrease of EC, not only will the shape of the microspheres be dis-

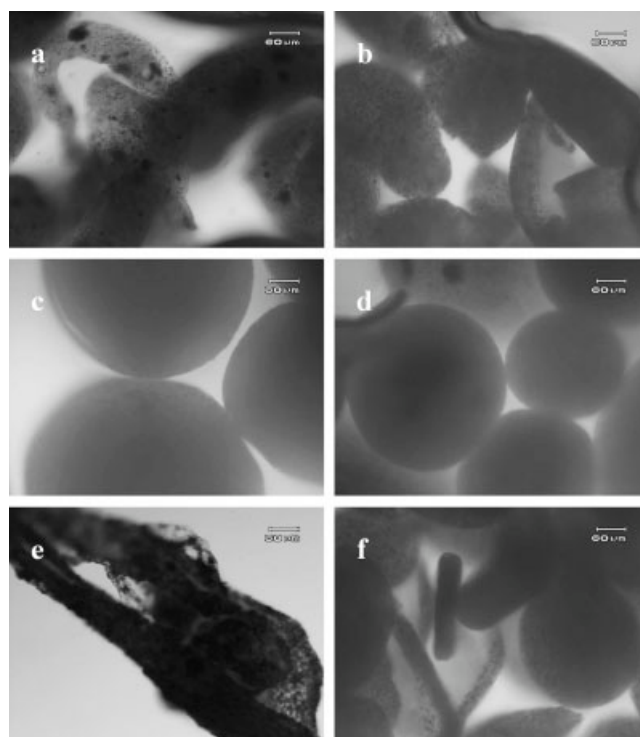


Figure 2 Optical microscopy photographs of samples *a–f* (the scales are 60 μm).

torted but also the composite ratio of HA/ALG will drop. In other words, the microspheres become instable with the decrease of EC.

We think that the adsorption of EC molecules on the microspheres surfaces is the key for stability to be improved. One of possible adsorption spots may be the hydrophobic segments of alginate molecules

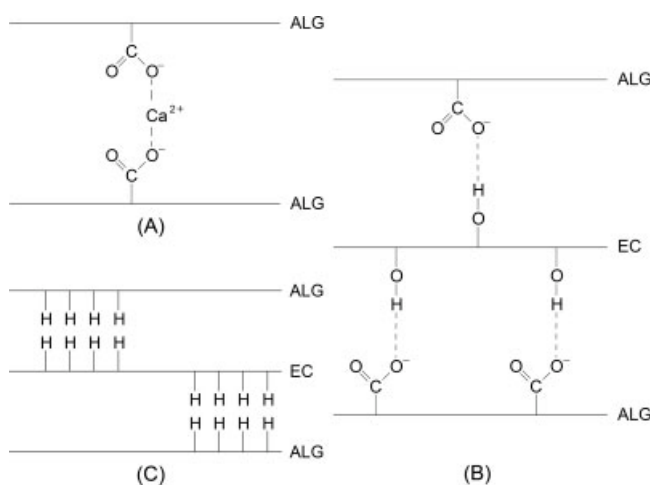


Figure 3 Sketch map of the relative interactions contributing to the stabilization of the microspheres. (A) Ca^{2+} cross-linked ALG; (B) hydrogen bond interactions between EC and ALG; and (C) hydrophobic interactions between EC and ALG.

and EC molecules. This kind of adsorption is based on hydrophobic interaction. Meanwhile hydrogen bond interaction between EC and ALG is another possible factor on stability improvement.^{12,13} Through these two kinds of interactions, the EC molecules play a linked bridge role on the surface of the microsphere. Once the EC linked bridge network formed on the microsphere surface, the ability of antishearing and antismwelling of the microsphere will be improved and the designed composite ratio will also be ensured. Moreover, with the increase of EC in the continuous phase, EC molecules were given more chances to adsorb on the surface of the microsphere. As a result, the ability of antishearing and antismwelling of the microsphere was improved further.

The relative interactions contributing to the stabilization of the microspheres are sketched in Figure 3.

Composite ratio factor

HA (0.75, 1.75, and 3 g) was used in the samples *c*, *d*, and *g*, respectively, which corresponded to 50/50, 70/30, and 80/20 HA/ALG. As shown in Figure 2(c,d), the shapes of 50/50 and 70/30 composites are all spherical. And with the increase of composite ratio, the diameter of the microspheres decreased. When the composite ratio is up to 80/20 (sample *g*), no microspheres or distorted microspheres can be gained, except for the fine powdered deposit.

This indicates that alginate sol play the key role of the microspheres forming in the initial stage through providing adequate cohesion for the composite microspheres. Under the same stirring speed, the size of microspheres will decrease with the drop of cohesion. When the amount of alginate sol decreases

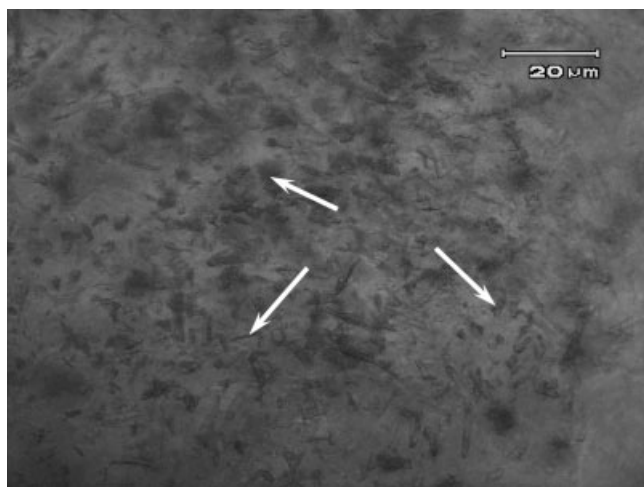


Figure 4 Amplificatory OM image of composite microsphere of sample *c*; the arrows indicated were HA (the scale is 20 μm).

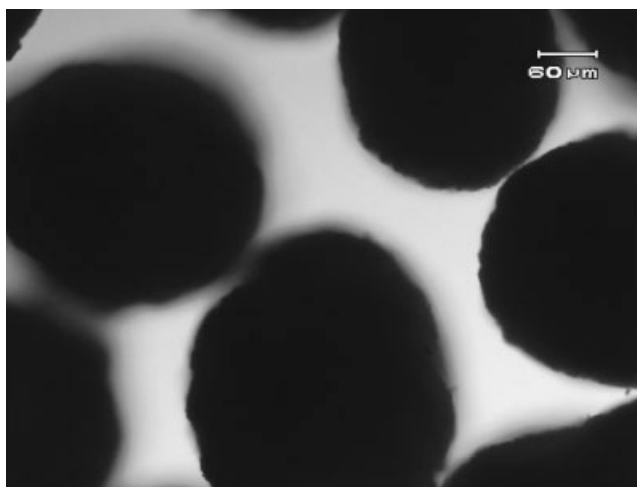


Figure 5 The dried composite microspheres of sample *c* (the scale is 60 μm).

to a critical value, a critical cohesion is achieved, and the microspheres will breakdown.

Adding speed factor

Compared to the sample *c* [Fig. 2(c)], the sample *e* [Fig. 2(e)] has a silk-like morphology when the adding speed of dispersed phase increases from 25 to 50 mL/min. Under the condition of stable addition, adding speed will directly influence the size of predisposed microspheres. When the adding speed is 50 mL/min, the predisposed microspheres are bigger and easy to be distorted by the shearing force. When the adding speed is 25 mL/min, the predisposed microspheres are smaller and less influenced by the shearing force, and so the spherical shape can be obtained. Accordingly, adding speed of 25 mL/min is more suitable than 50 mL/min for the preparation of the spherical composite.

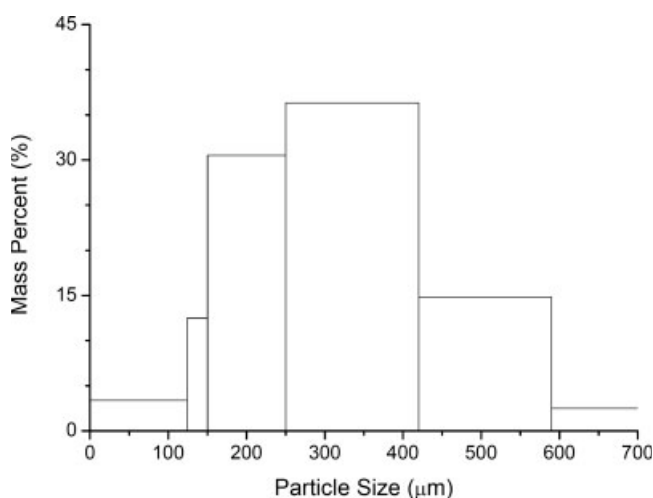


Figure 6 Particle size distribution of dried sample *c*.

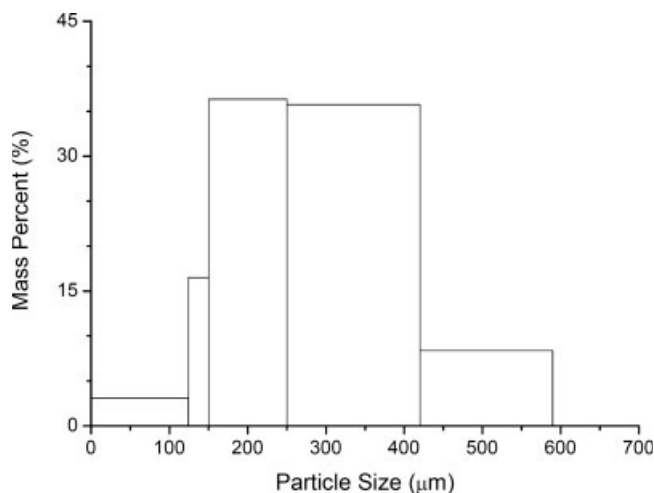


Figure 7 Particle size distribution of dried sample *d*.

Stirring speed factor

As shown in Figure 2(c,f), there are obviously difference in the morphology of the sample *c* and *f* due to the change of stirring speed. When stirring speed is 120 r/min, no microspheres distortion can be found. When the stirring speed is up to 240 r/min, the most of microspheres have distortions to a different extent and the undistorted microspheres are smaller than the stirring speed is 120 r/min. This means the high stirring speed is harmful to the spherical morphology of the microspheres. On the basis of this experiment, we found that stirring speed of 120 r/min is better than 240 r/min.

Characterization of microspheres

A typical microsphere surface of the sample *c* is shown in Figure 4. From Figure 4, we can see that the HA (dark) is well dispersed. The composite microspheres of 50/50 and 70/30 HA/ALG have specific gravity of 1.06 and 1.15, respectively. And these two kinds of microspheres have water content of 88 and 81%, respectively.

The microspheres were air dried after partial dehydration by ethanol. This treatment prevents the adhesion of the microspheres and makes them maintain spherical shape (as shown in Fig. 5). And then, the dried microspheres were sieved to separate them in six size distributions: <124, 124–150, 150–250, 250–420, 420–590, and >590 μm. Figures 6 and 7

show the particle size distributions of the dried sample *c* and *d*, respectively. From the comparison of the two figures, it is easy to find that the distribution of the dried sample *d* is shifted to a smaller particle size than the dried sample *c* (this result is agreement with the observed result in earlier section). In a whole, the sizes of the two dried microspheres are mainly in the range of 125–425 μm.

CONCLUSIONS

50/50 and 70/30 HA/ALG composite microspheres were prepared via ISCM. This method is more simple, quick, and efficient for the preparation of alginate-based microspheres than the drop and the spray methods. The two kinds of microspheres are with specific gravity of 1.06 and 1.15, and water content of 88 and 81%, respectively. The microspheres have good stability, and HA is well dispersed. The size of the dried microspheres is mainly in the range of 125–425 μm. EC plays a key role in stabilization of the composite microspheres in ISCM. EC molecules maybe adsorb on the surface of microspheres through hydrogen bond and hydrophobic interaction, and form linked bridge network which made the microspheres stable. The proper composite ratio, adding speed, and stirring speed will ensure the microspheres have good morphology.

References

- Paul, W.; Sharma, C. P. *J Mater Sci Lett* 1997, 16, 2050.
- Sivakumar, M.; Rao, K. P. *J Biomed Mater Res* 2003, 65A, 222.
- Ribeiro, C. C.; Barriasa, C. C.; Barbosa, M. A. *Biomaterials* 2004, 25, 4363.
- Lin, H. R.; Yeh, Y. J. *J Biomed Mater Res* 2004, 71B, 52.
- Ishikawa, K.; Miyamoto, Y.; Takechi, M.; Toh, T.; Kon, M.; Nagayama, M.; Asaoka, K. *J Biomed Mater Res* 1997, 36, 393.
- Ohtsuki, C.; Hosoya, K.; Kawai, T.; Kamitakahara, M.; Ogata, S.; Miyazaki, T.; Tanihara, M. *Key Eng Mater* 2005, 17, 779.
- Leveque, I.; Rhodes, K. H.; Mann, S. *J Mater Chem* 2002, 12, 2178.
- Nagata, F.; Miyajima, T.; Yokogawa, Y. *Chem Lett* 2003, 32, 784.
- Bodmeier, R.; Wang, J. J. *J Pharm Sci* 1993, 82, 191.
- Lee, D. W.; Hwang, S. J.; Park, J. B.; Park, H. J. *J Microencapsul* 2003, 20, 179.
- Misirli, Y.; Ozturk, E.; Kursaklioglu, H.; Denkbaz, E. B. *J Microencapsul* 2005, 22, 167.
- Zhou, J.; Zhang, L. *J Polym Sci B: Polym Phys* 2001, 39, 451.
- Yang, G.; Zhang, L.; Peng, T.; Zhong, W. *J Membr Sci* 2000, 175, 53.