

Preparation of Uniform Calcium Alginate Gel Beads by Membrane Emulsification Coupled with Internal Gelation

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ABSTRACT: With the objective of making calcium alginate gel beads with small and uniform size, membrane emulsification coupled with internal gelation was proposed. Spherical gel beads with mean size of about 50 μm , and even smaller ones in water, and with narrow size distribution were successfully obtained. Experimental studies focusing mainly on the effect of process parameters on bead properties were performed. The size of the beads was mainly dependent on the diameter of the membrane pores. High transmembrane pressure made for large gel beads with wide size distribution. Low sodium alginate concentration pro-

duced nonspherical beads, whereas a high concentration was unsuitable for the production of small beads with narrow distribution. Thus 1.5% w/v was enough. A high surfactant concentration favored the formation of small beads, but the adverse effect on mass transfer should be considered in this novel process. © 2002 Wiley Periodicals, Inc. *J Appl Polym Sci* 87: 848–852, 2003

Key words: polyelectrolytes; gels; drug delivery systems; membranes; gelation

INTRODUCTION

On the basis of the polyelectrolyte complex reaction between polycation and polyanion materials, alginate–chitosan microcapsules with biocompatibility and biodegradability may be prepared under mild conditions, even physiological conditions, so they are suitable for the application in biomedical fields. Especially in recent years, there has been increasing interest in the study of the use of alginate–chitosan microcapsules as the drug-delivery systems of proteins and polypeptides.^{1–5}

With respect to microparticles (microcapsules and microspheres) as drug carriers, it has been found that their distribution in the body is strongly dependent on their size^{6,7} and that the release of drugs is different depending on their size distribution,⁸ therefore, it is important to control the size and the size distribution of microparticles. So far, the droplet method^{9,10} and the emulsification method^{11–14} of calcium alginate (Ca-alginate) gel bead production, which is the first step of microcapsule production, have been established. For the droplet method, the bead size cannot be less than 200 μm , despite uniform bead size, and it is unsuitable for mass production. However, for the

emulsification method, although the beads can be prepared in a desired size range, the shortcomings of the method include the appearance of a wide peak, and even several peaks that suggest wide size distribution, and the high shearing force due to stirring against the activity maintenance of biological materials.

Membrane emulsification has been proven a technology with characteristics of monodisperse droplets, controlled size, mild conditions, low energy consumption, and mass production^{15,16} so that it shows wide application prospects for many industry fields. In this study, to overcome the wide size distribution of the conventional emulsification method, membrane emulsification was adopted and coupled with internal gelation, and attempts to prepare Ca-alginate gel beads with small and uniform size were carried out.

EXPERIMENTAL

Materials

Sodium alginate was purchased from the Chemical Reagent Corp. (Shanghai, China). The viscosity was over 0.02 Pa s when dissolved to form a 1% (w/v) aqueous solution at 20°C, and the powder was smaller than 200 mesh. Calcium carbonate (40 nm) was kindly provided by the Beijing University of Chemical Engineering. The surfactant Sorbitan monooleate (Span 80) was obtained from Lushun Chemical Factory (Dalian, China). Others were analytical reagents. Nickel membrane was purchased from the Eighth Institute of Nuclear Industry (Shanghai, China). The average mem-

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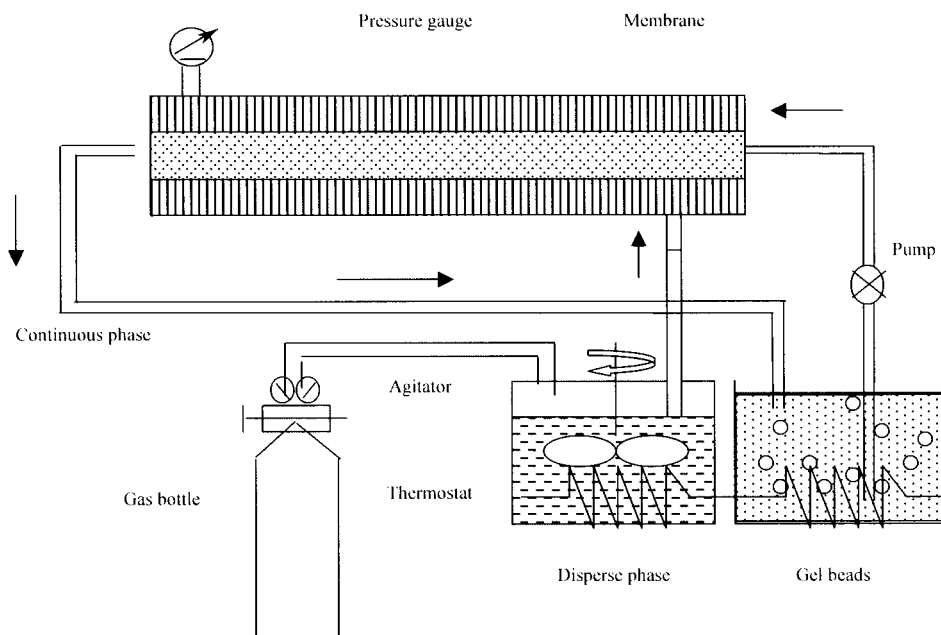


Figure 1 Illustration of membrane emulsification coupled with the internal gelation process.

brane pore sizes (D_m 's) were 2.9, 3.8, 4.4, and 5.2 μm , respectively.

Preparation of Ca-alginate gel beads

Sodium alginate was weighed and dissolved in 100 mL of 0.9% w/v NaCl solution to form solutions with concentrations of 0.5, 1.0, 1.2, 1.5, and 2.0% w/v; correspondingly, the solutions were stored overnight before use to facilitate deaeration. Then, calcium carbonate (25 mM Ca^{2+} equivalent) was added into 40 mL of sodium alginate solution and mixed with a magnetic stirrer to form the disperse phase. The continuous phase was composed of paraffin oil containing 1.5% v/v surfactant. The membrane, pretreated by soaking in paraffin oil for at least 8 h, was installed in the apparatus (Fig. 1). The disperse phase was extruded through the microporous membrane under N_2 pressure, and droplets were whirled and carried away from the inner surface of membrane by the continuous phase, which was circulated to form a water-in-oil (W/O) emulsion. Fifteen minutes later, glacial acetic acid was added into the emulsion, dissolving CaCO_3 to release Ca^{2+} . The gelation was then initiated. After the gelation was maintained for at least 30 min, the beads were separated by gravity action in an equivalent volume of 0.1M CaCl_2 solution. Finally, the beads were rinsed with 1% Tween 80 solution and distilled water successively, and then stored in distilled water.

Microscopic observation

The sphericity and surface smoothness of Ca-alginate gel beads under different conditions were observed

with an optical microscope (XDS-1 inverted biological microscope, Chongqing Optical Instrument Factory, Chongqing, China). The symbols ++, +, and - represent good, common, and bad sphericity, respectively, and surface smoothness of the beads.

Determination of the size and size distribution of Ca-alginate gel beads

The size and size distribution of Ca-alginate gel beads under different conditions were determined with a laser diffraction particle analyzer (LS100 Q, Beckman-Coulter Corp., Miami, FL). The SD and CV represent the standard deviation and coefficient variation, respectively. The size distribution was estimated with the SPAN (size distribution) factor,¹⁷ which is defined as

$$\text{SPAN} = (D_{90\%} - D_{10\%}) / D_{50\%}$$

where $D_{90\%}$, $D_{50\%}$, and $D_{10\%}$ are the mean diameters at which 90, 50, and 10% (vol %) of the particles are counted and calculated. A high SPAN indicates a wide distribution in size, whereas a low value indicates a narrow size distribution.

RESULTS AND DISCUSSION

Morphology and size distribution of Ca-alginate gel beads

Figure 2 is the optical photograph of Ca-alginate beads prepared mean membrane pore size of 2.9 μm . It is shown that the relatively uniform beads with good

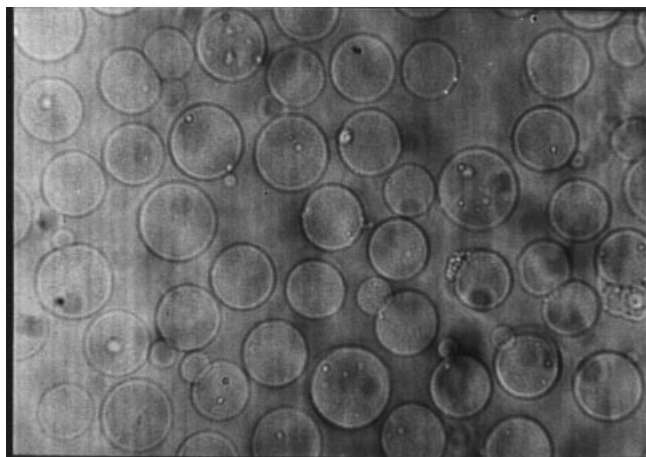


Figure 2 Photographs of Ca-alginate beads prepared by membrane emulsification coupled with internal gelation ($D_b = 55.3 \mu\text{m}$; magnification $50\times$).

sphericity and smooth surface could be easily obtained by the novel method. Moreover, the narrow peak shown in Figure 3 also demonstrates the narrow size distribution of the beads (SPAN = 0.7). The mean size of beads was $55.3 \mu\text{m}$, counted and calculated automatically by the software of the particle analyzer.

Effect of the mean diameter of the membrane pores (d_p)

The effect of the mean d_p , one of the important parameters of membrane structure, on the mean size of the Ca-alginate beads is plotted in Figure 4. When membranes with various d_p 's ($D_m = 2.9, 4.4,$ and $5.2 \mu\text{m}$, respectively) were used, the mean sizes of the Ca-alginate beads in water (D_b 's) were $44.2, 97.8,$ and $146.0 \mu\text{m}$, respectively. The size of beads was depen-

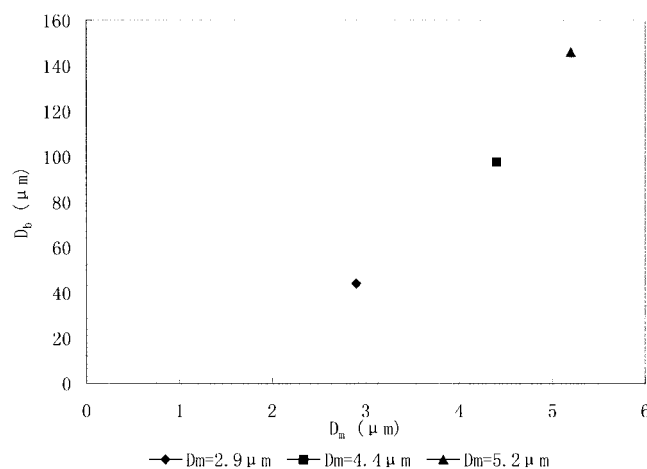


Figure 4 Effect of the mean d_p 's on the mean size of Ca-alginate beads.

dent on the size of the membrane pores to a certain extent. Therefore, it is important to select a proper d_p 's so that the bead sizes can be controlled.

Effect of transmembrane pressure

Theoretically, a minimum transmembrane pressure (also called *critical pressure*) for membrane process is needed to overcome the capillary pressure of membrane pores so that the disperse phase can permeate through the membrane and form emulsion. This can be calculated from the equation for capillary pressure:

$$P_c = \frac{4\gamma \cos \theta}{d_p}$$

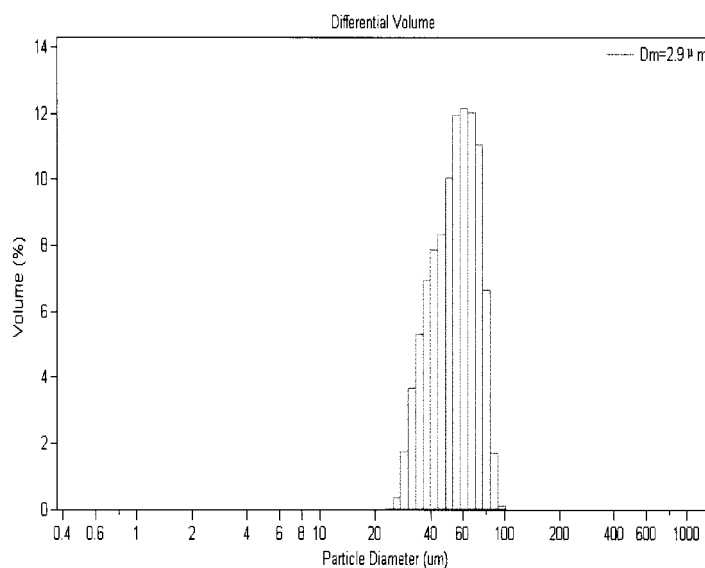


Figure 3 Size distribution of Ca-alginate beads with Ni membrane ($D_m = 2.9 \mu\text{m}$).

TABLE I
Effect of Transmembrane Pressure (ΔP) on the Sphericity, Size, and Size Distribution (SPAN) of Ca-Alginate Beads

ΔP (MPa)	D_b (μm)	Sphericity	SD (μm)	CV (%)	SPAN
0.05	44.0	++	11.8	26.9	0.7
0.07	49.0	+	14.9	30.5	0.8
0.10	55.3	++	14.8	26.7	0.7
0.15	121.0	+	47.6	39.3	1.1

where P_c is the capillary pressure, γ is the w/o interfacial tension, and θ is the contact angle between the disperse phase and the membrane surface.

In this study, the disperse phase was a solution with high viscosity, containing mostly nanoparticles; thus, the applied transmembrane pressure needed to be high enough to ensure the passing of the disperse phase through the membrane pores.

The effect of transmembrane pressure on the sphericity, size, and size distribution of the Ca-alginate beads is summarized in Table I. With the increase in the transmembrane pressure, the size of the Ca-alginate beads increased; so the high transmembrane pressure opposed the formation of Ca-alginate beads of small size. Moreover, when a higher pressure was applied, the SPAN values appeared higher, which implies that the size distribution of Ca-alginate beads was wide.

According to Darcy’s law, an increase in transmembrane pressure usually results in an increase of the flux of disperse phase. When high transmembrane pressure is applied, a large volume of droplets is formed at the opening of the membrane pores in unit time; thus, large Ca-alginate beads are accordingly produced after the gelation reaction. However, when a low transmembrane pressure is applied, a small volume of droplets will be formed, resulting in small Ca-alginate beads.

Effect of sodium alginate concentration

Table II shows the effect of sodium alginate concentration on the sphericity, size, and size distribution of

TABLE II
Effect of Sodium Alginate Concentration (C_{Alg}) on the Sphericity, Size, and Size Distribution (SPAN) of Ca-Alginate Beads

C_{Alg} (% w/v)	D_b (μm)	Sphericity	S.D. (μm)	CV (%)	SPAN
0.5	/	/	/	/	/
1.0	/	/	/	/	/
1.2	56.0	++	15.3	27.2	0.7
1.5	74.0	++	26.0	35.2	1.0
2.0	97.1	++	80.0	82.4	1.3

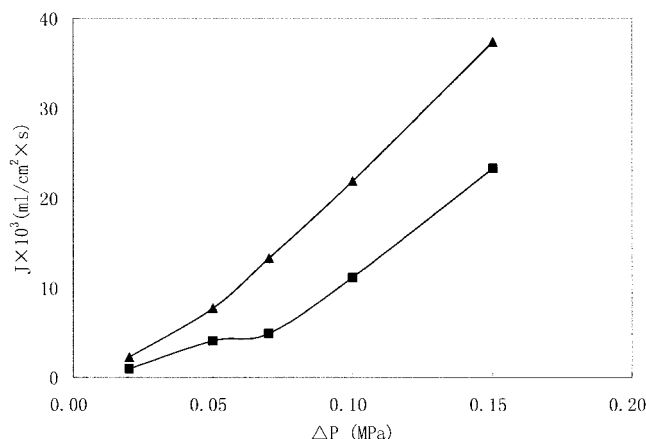


Figure 5 Flux of sodium alginate solution with different concentrations: (▲) 1.2% NaAlg; (■) 1.5% NaAlg.

Ca-alginate beads. When sodium alginate concentration was below 1.0% (w/v), almost no spherical beads were formed, probably due to the lack of enough carboxyl groups for gelation. The mean size and SPAN values of beads increased with the increase of sodium alginate concentration. As shown in Figure 5, when the sodium alginate concentration was high, the flux decreased; thus, the detached time of droplets from membrane pores was prolonged at the same transmembrane pressure, resulting in larger droplets. Therefore, a high sodium alginate concentration is unsuitable for the production of small beads with narrow distributions.

Effect of surfactant concentration

For any emulsification process, a surfactant is usually needed for two main functions. One is to lower the interface tension between the water phase and the oil phase and to make the formation of emulsions easy; the other is to stabilize emulsion droplets against coalescence.

The effect of surfactant concentration on the sphericity, size, and size distribution of Ca-alginate beads is shown in Table III. The size of Ca-alginate beads fell remarkably with the increase in surfactant concentration from 0.5 to 2.0% (v/v), and the size distribution

TABLE III
Effect of Surfactant Concentration (C_s) on the Sphericity, Size, and Size Distribution (SPAN) of Ca-Alginate Beads

C_s (% w/v)	D_b (μm)	Sphericity	SD (μm)	CV (%)	SPAN
0.5	538	++	218.0	40.5	1.2
1.0	305	+	128.0	42.0	1.0
1.5	178	+	97.9	54.9	1.3
2.0	115	+	76.7	66.8	1.9

was wide. Considering that one of the functions of the surfactant is to be adsorbed at the surface of the disperse phase to form a film against coalescence among emulsion droplets, it was not enough to completely cover the surface of the disperse phase at a low surfactant concentration, which gave rise to a decline in the stability of the droplets. Then, coalescence occurred, and large-sized droplets formed. On the other hand, it is somewhat different with simple membrane emulsification. When coupled with internal gelation, oil-soluble acid should diffuse through the w/o interface to initiate the gelation reaction; thus, a high surfactant concentration will cause mass transfer resistance to H^+ , relatively prolong the gelation process, and result in a low production of gel beads.

CONCLUSIONS

Spherical Ca-alginate beads with small and uniform size were successfully prepared with membrane emulsification coupled with internal gelation technology. By adjusting process parameters, we could control the size and size distribution of beads. Further research on drug loading and controlled release with alginate-chitosan microcapsules prepared by this technology will be carried out.

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