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RESEARCH PAPER

Chitosan Microspheres Prepared by Emulsification and Ionotropic Gelation

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ABSTRACT

An attempt was made to prepare chitosan microspheres by an emulsion-phase separation technique but without the usual use of glutaraldehyde as a cross-linking agent. Instead, ionotropic gelation was employed in a w/o emulsion. The effect of formulation factors was examined. The results showed that microspheres so formed were spherical, free-flowing, and had smooth surfaces. The rate of addition of counterions was important. Gelation of chitosan droplets should take place before the destabilizing effect of the counterions occurred. This effect is associated with the increase in aqueous phase volume when the counterion solution is incorporated.

INTRODUCTION

Chitosan is the deacetylated derivative of chitin, a natural polysaccharide found in the shells of crustaceans. Chitosan has been widely studied for use in drug delivery systems and in agricultural and cosmetic products. As a drug carrier, chitosan has the advantage of being biocompatible. It has mostly been formulated into drug-containing spheres by the extrusion of a chitosan–drug solution/suspension into a solution containing counterions such as pentasodium tripolyphosphate. Ionotropic gelation of the chitosan yields soft beads that may then be hardened for drying.

Smaller chitosan microspheres have also been formulated by emulsion–phase separation techniques (1–4). In such cases, an aqueous solution of chitosan is emulsified into an immiscible solvent and glutaraldehyde added as a cross-linking agent to solidify the dispersed chitosan droplets. A controlled release of incorporated drug has been achieved with these cross-linked chitosan microspheres.

However, residual glutaraldehyde in the chitosan microspheres can cause irritation to mucosal membranes, and it is difficult to ensure that the chitosan microspheres are free from traces of the cross-linking agent. Instead of using glutaraldehyde, we attempt to form, using the

emulsification technique, chitosan microspheres via ionotrophic gelation. This report gives an account of such a method, and the effect of formulation parameters on the characteristics of chitosan microspheres so formed.

MATERIALS

Chitosan (Tokyo Kasei Kogyo, Co. Ltd., Japan) and sorbitan trioleate or Span 85 (Honeywill-Atlas, US) were used as obtained. Except for *n*-hexane which was of industrial grade, all other reagents and solvents were of analytical grade.

METHODS

The chitosan microspheres were prepared by an emulsion–phase separation method. The dispersed phase consisted of 40 g of an aqueous solution of chitosan prepared by evaporating down to the required chitosan concentration, a solution containing 1% w/w chitosan in 2% v/v acetic acid. The continuous phase consisted of 150 g of iso-octane containing an appropriate amount (2–4% w/v) of Span 85 as the emulsifier.

The dispersed phase was added to the continuous phase under agitation (Heidolph RZR50, Germany) to form a w/o emulsion. After a specified emulsification time (10–30 min), 15 ml of a NaOH solution was added at the rate of 5 ml at 30-min intervals. Stirring was continued for a specific time period (2–4 hr) after the addition of the last 5 ml of NaOH solution. The microspheres were removed by filtration, washed with 40 ml *n*-hexane followed by 120 ml distilled water, and dried at 60°C under vacuum.

The dried microspheres were characterized for size by sieve analysis. The sieves (Endecotts Ltd) used have aperture sizes of 63 mm, 125 mm, 250 mm, 500 mm, and 1000 mm. The amount of microspheres retained by each sieve was weighed and the weight percentage of microspheres in each size range calculated.

The surface topography of the microspheres was examined under a scanning electron microscope (Jeol JSM 5200 SEM, 15 kV). Samples were mounted on studs, coated for 120 sec with a layer of gold using a sputter coater (Bio-Rad SC 502).

The morphology of chitosan in the microspheres was studied using differential scanning calorimetry (Perkin Elmer DSC-4), with a heating rate of 20°/min and a heating range of 20–320°C.

RESULTS AND DISCUSSION

Studies involving the preparation of alginate microspheres using an emulsification technique have been reported (5–8). The method described for the preparation of chitosan microspheres was adapted from a procedure used to prepare calcium alginate microspheres (5). In the latter method, two surfactants were needed to give satisfactory calcium alginate microspheres. Span 85, the hydrophobic surfactant, helped to stabilize the w/o emulsion, while Tween 85, a hydrophilic surfactant, was added to the counterion solution. In the system used for preparing chitosan microspheres, it was found that the presence of Tween 85 in the counterion solution destabilized the emulsion and chitosan microspheres could not be successfully formed. On the other hand, the use of only one surfactant, Span 85, dissolved in the continuous phase, gave satisfactory chitosan microspheres.

In most of the studies in which chitosan beads have been prepared via a droplet extrusion technique, the tripolyphosphate ions were used as counterions for the gelation of chitosan. However, when 1 M sodium tripolyphosphate solution was added to the w/o emulsion in this study, weak chitosan microspheres showing a high degree of aggregation were obtained. The results were unsatisfactory. Based on an earlier study (9), which showed that the hydroxide ions may also be used as counterions in the preparation of chitosan beads, a 1-M NaOH solution was used in place of the tripolyphosphate ions. Although the microspheres prepared were weak, they showed little aggregation. The stronger basicity of the NaOH solution compared to the sodium tripolyphosphate solution appeared to reduce the degree of aggregation amongst the microspheres.

The rate of addition of the NaOH solution to the w/o emulsion was important. When 15 ml of the 1M NaOH solution was added to the emulsion, there was a destabilizing effect on the emulsion as a result of the sudden increase in volume ratio of dispersed:continuous phases. Coalescence of chitosan droplets appeared to precede the gelation effect caused by the counterions because an amorphous mass was obtained. On the other hand, when the rate of addition of the NaOH solution was reduced to 5 ml at 30-min intervals, the increase in volume ratio of dispersed:continuous phases was gradual. Gelation of the chitosan droplets occurred before the destabilizing effect caused by the increase in volume of aqueous phase, and discrete microspheres were formed.

The batches of chitosan microspheres prepared are listed in Table 1. The following formulation parameters were studied: the concentration of chitosan in 2% v/v acetic acid, concentration of Span 85 in iso-octane, concentration of sodium hydroxide as the counterion, the period of emulsification prior to addition of NaOH, the period of stirring after addition of NaOH, and the speed of stirring. The batch B microspheres was regarded as a control batch and served as a basis of comparison with other batches.

Microspheres produced from the 2% and 3% w/w chitosan solutions were spherical, free-flowing with a low degree of aggregation, and had smooth surfaces [Figs. 1(b) and 1(c)]. On the other hand, those produced with the 1% chitosan solution were a mixture of spherical, ellipsoidal, and irregularly shaped particles that tended to form aggregates [Fig. 1(a)].

With the 1% chitosan solution, the dispersed chitosan droplets in iso-octane had a low chitosan volume, resulting in weak microspheres upon interaction with NaOH because of the relatively thin wall of gelled chitosan present per microsphere. Collision of the microspheres with each other consequential to agitation forces may cause tearing at the point of contact in the weak wall, resulting in the fusion of ungelled material between the microspheres. Depending on the extent of fusion, irregular microspheres may result or aggregates of microspheres may be formed. Droplets of 2 and 3% chitosan solution contained sufficient chitosan to form relatively strong walls of gelled chitosan upon interaction with NaOH. The microspheres formed with these two solutions were less susceptible to aggregation upon collision with each other.

Due to the high degree of aggregates that could not be easily separated by pressing with a spatula, the size range of microspheres prepared with the 1% chitosan solution was not determined. Fig. 1 shows that 2 and 3% chitosan solution gave microspheres of similar size and size distribution, although the 2% solution gave more microspheres in the small size range of up to 250 mm. In both cases, the majority of the microspheres were relatively large, with 80% (2% solution) to 90% (3% solution) of them being greater than 250 mm in diameter. Increasing the concentration of the surfactant from 2 to 4 to 6% w/w resulted in larger microspheres which had the appearance of collapsed spheres (Fig. 2). Size analysis indicated that the batches of microspheres produced with 4 and 6% Span 85 were relatively monodispersed.

Sodium hydroxide is a strong base. When NaOH was added to the w/o emulsion of chitosan solution in iso-octane, the base caused the gelation of chitosan from solution by an acid–base reaction. The concentration of NaOH used was important for the preparation of chitosan microspheres. When a 0.5-M NaOH solution was used as the gelling agent, very soft aggregates of spherical microspheres were harvested from the emulsion. Upon drying at 60°C in vacuo, the microspheres lost their spherical structure and fused to form an amorphous mass. Using the same conditions of preparation,

Table 1

Preparations of Chitosan Microspheres

Code	Chitosan Conc. (% w/w)	Span 85 Conc. (% w/w)	NaOH Conc. (M)	Emulsification Time (min)	Stirring Time (hr)	Stirring Speed (rpm)
A	1	2	1	10	2	1000
B	2	2	1	10	2	1000
C	3	2	1	10	2	1000
D	2	4	1	10	2	1000
E	2	6	1	10	2	1000
F	2	2	0.5	10	2	1000
G	2	2	3	10	2	1000
H	2	2	1	20	2	1000
I	2	2	1	30	2	1000
J	2	2	1	10	3	1000
K	2	2	1	10	4	1000
L	2	2	1	10	2	500
M	2	2	1	10	2	1500

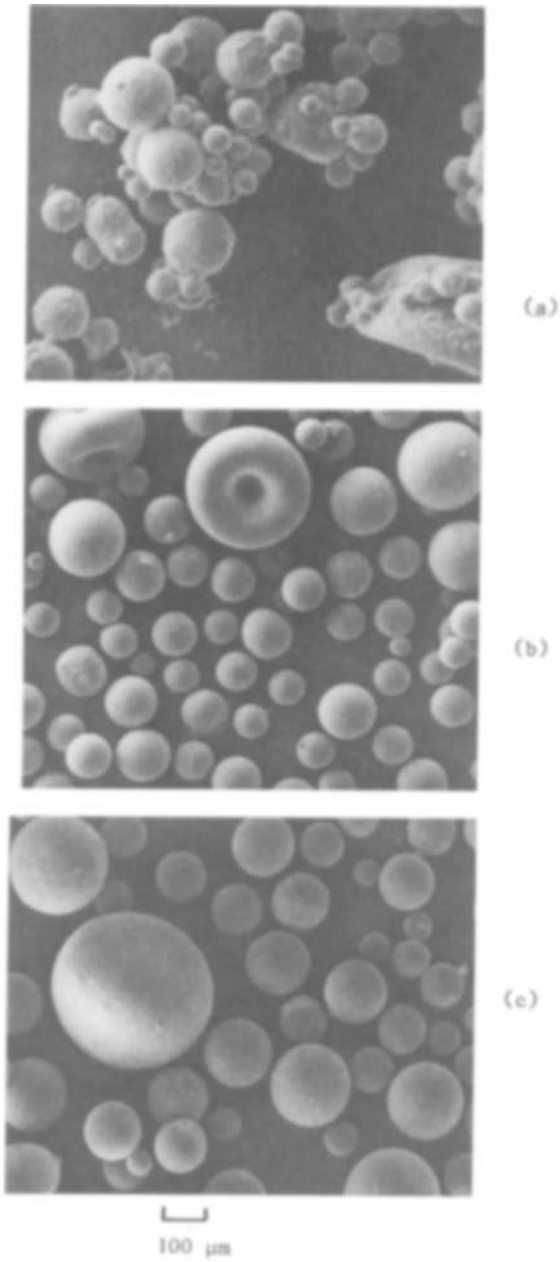

Figure 1. Chitosan microspheres with varying concentration of chitosan, (a) 1%, (b) 2%, (c) 3% w/w.

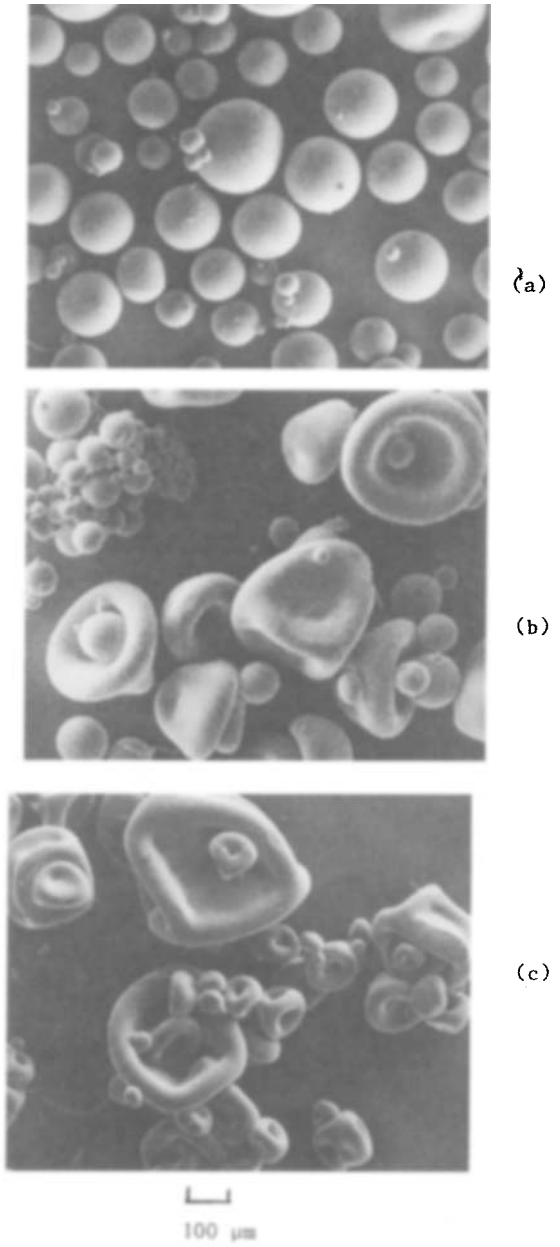

Figure 2. Chitosan microspheres with varying concentration of Span 85 (a) 2%, (b) 4%, (c) 6% w/w.

but with a 1-M NaOH solution, gave firm, spherical, and free-flowing microspheres that retained their shape upon drying. A three-fold increase in NaOH concentration to 3 M was also not desirable. Instead of spherical particles, large, rough, and elongated particles were obtained. These results showed that an optimum amount of NaOH must be present to react with the chitosan to give satisfactory microspheres. Too small an amount of NaOH will not give microspheres of sufficient integrity. Too large an amount of NaOH encouraged aggregation of the microspheres.

Increasing the period of emulsification from 10 to 20 to 30 min changed the size distribution of the microspheres. There was a predominance (about 50%)

of microspheres in the size range of >1000 mm when the emulsification time was 20 min. The most probable size range for the microspheres was reduced to 250–1000 nm upon increasing the emulsification time to 30 min. SEM micrographs show that as the emulsification time was prolonged to 30 min, the microspheres produced had collapsed structures. This phenomenon was observed also with an increase in the concentration of Span 85 in the continuous phase. Lengthening the period of emulsification led to a greater tendency to aggregation. The latter point should be noted in reviewing the size distribution of microspheres in this batch.

All the microspheres formed after 2, 3, and 4 hr of stirring have smooth walls, were discrete, spherical, and free-flowing. Size analysis showed that changes in the duration of stirring did not give rise to monodispersed microspheres. However, as the duration of stirring was increased from 2 to 4 hr, the proportion of smaller microspheres increased. Three different stirring speeds were investigated; 500, 100, and 1500 rpm. SEM photomicrographs showed that all three speeds produced spherical microspheres. A higher degree of aggregation was observed in microspheres formed at a speed of 1500 rpm.

Comparing the size and size distribution of microspheres prepared at 500 and 1000 mm, the former produced relatively monodispersed microspheres: 70% of the microspheres were within the size range of 500–1000 rpm. The latter produced polydispersed microspheres where there was an increase in the proportion of smaller microspheres having a diameter of less than 125 mm. Stirring an emulsion at high speed produced turbulent eddies which tend to break up the dispersed droplets. A higher speed of stirring increased the turbulent stress, thereby reducing the size of the dispersed chitosan droplets and resulting in the formation of smaller microspheres. Furthermore, the viscosity of chitosan decreased at a higher shear rate because the polymer is a pseudoplastic material (10). A less viscous solution could be dispersed more effectively, hence smaller microspheres would be formed.

Size distribution depends on the distribution of the turbulent forces throughout the emulsion; the more uniform the stirring force distribution, the narrower the size distribution of the droplets. Shear stress is higher at the tip of the propeller than at the center. A faster stirring speed increased this difference, thereby providing a less uniform distribution of energy throughout the mixing vessel, and thus gave rise to microspheres of a wider size distribution.

When the stirring speed was increased from 1000 to 1500 rpm, microspheres of narrower size distribution were produced. The percentage of larger microspheres (500–1000 mm) increased to 54.1% of the total microspheres produced. At such a high stirring speed, the chitosan droplets that were formed initially would be very small as a result of turbulent stress. However, because of the very high speed in which these small chitosan droplets were moving, they would be thrown at one another at great force. This, together with the large surface area of the droplets, may encourage coalescence of the droplets, resulting in large microspheres being formed ultimately. Indeed, the SEM photomicrograph shows several small micrographs together with very large ones, with some of the small microspheres incorporated into the larger microspheres.

DSC analysis of the microspheres indicated that chitosan remains an amorphous polymer in the microspheres. Changing the formulation parameters as discussed above did not appear to affect the polymer morphology in the microspheres.

REFERENCES

1. J. Akbuga and G. Durmaz, Int. J. Pharm., 11, 217–222 (1994).
2. S. Y. Lin and P. C. Lin, Chem. Pharm. Bull., 40(9), 2491–2497 (1992).
3. E. E. Hassan, R. C. Parish, and J. M. Gallo, Pharm. Res., 9(3), 390–397 (1992).
4. B. C. Thanoo, M. C. Sunny, and A. Jayakrishnan, J. Pharm. Pharmacol., 44, 283–286 (1992).
5. L. S. C. Wan, P. W. S. Heng, and L. W. Chan, in *Proceedings of Recent Developments in Pharmaceutics and Pharmaceutical Technology*, Chiba University, Japan, 1990, pp. 243–255.
6. L. S. C. Wan, P. W. S. Heng, and L. W. Chan, J. Microencapsulation, 9, 309–316 (1992).
7. L. S. C. Wan, P. W. S. Heng, and L. W. Chan, Int. J. Pharm., 95, 77–83 (1993).
8. L. W. Chan, L. S. C. Wan, and T. L. Foo, in *Proceedings of Advances in Pharmaceutical Sciences*, Chiba University, Japan, 1994, pp. 40–44.
9. L. Y. Lim, L. S. C. Wan, and T. W. Wong, in *Proceedings of Advances in Pharmaceutical Sciences*, Chiba University, Japan, 1994, pp. 71–75.
10. O. Skaugrud, Manuf. Chem., 60, 31–35 (1989).