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Note

Production of alginate microspheres by internal gelation using an emulsification method

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Abstract

Alginate is a natural polysaccharide found in brown algae. Alginates are widely used in the food and pharmaceutical industries and have been employed as a matrix for the entrapment of drugs, macromolecules and biological cells. Alginate microspheres can be produced by the external or internal gelation method using calcium salts. The addition of calcium chloride solution in the final phase of production of microspheres by external gelation method using an emulsification technique causes the disruption of the equilibrium of the system being stirred, resulting in a significant degree of clumping of microspheres. Therefore, in this study, production of alginate microspheres by the internal gelation method using a modified emulsification technique was explored. The influence of calcium salt, added in varying amounts and at different stages, on the morphology of the microspheres was investigated. The effects of other hardening agents and different drying methods were also studied. © 2002 Published by Elsevier Science B.V.

Keywords: Alginate; Microspheres; Internal gelation; Emulsification

Alginate, a natural polysaccharide found in brown algae, is a linear 1, 4 linked copolymer of β-D-mannuronic acid (M) and α-L-guluronic acid (G). These monomers are arranged as blocks in a chain. The homopolymeric regions of M blocks and G blocks are interdispersed with regions of alternating structure (MG blocks) (Haug and Larsen, 1962; Haug et al., 1967). The composition, sequence of arrangement and molecular weight of the alginate determine the physical properties of the alginates. Alginates are widely used in the food and pharmaceutical industries and have been employed as a matrix for the entrapment of drugs (Tomida et al., 1993; Aslani and Kennedy, 1996), macromolecules (Kim and Lee, 1992; Mumper et al., 1994) and biological cells (Lim and Sun, 1980; Lee and Palsson, 1993). Alginate microspheres can be produced by the external (Chan et al., 1997) or internal (Poncelet et al., 1992) gelation method using calcium salts. In the production of alginate microspheres by the external gelation method using an emulsification technique, calcium chloride solution is added in the final stage of encapsulation. This will cause disruption of the equilibrium of the system being stirred, resulting in a significant degree of clump-

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ing of microspheres. In addition, a relatively large amount of calcium salt is required to produce sufficiently hard microspheres. Internal gelation using emulsification technique may provide an alternative method to produce discrete microspheres with the use of less calcium salt. Therefore, in this study, production of alginate microspheres by the internal gelation method using a modified emulsification technique (Chan et al., 1997) was explored. The influence of calcium salt, added in varying amounts and at different stages, on the morphology of the microspheres was investigated. The effects of other hardening agents and different drying methods were also studied.

Varying amounts of calcium carbonate (0.5, 0.8 and 1.0 g), dispersed in water, were incorporated into sodium alginate solution (Manucol® DM, Kel Co., ISP-Alginates Industries, USA) to give a final alginate concentration of 2% w/w. The alginate solution containing calcium carbonate was dispersed in isooctane containing 2.54 g of Span 85 (Sorbitan trioleate) (Sigma, USA) using a mechanical stirrer (IKA-WERK RW 20 DZM, Germany) at 1000 rpm. Five grams of solution containing 1.36 g of Tween 85 (Polyoxyethylene sorbitan trioleate) (Merck, Germany) was added after 10 min of stirring. Glacial acetic acid (1.1, 1.6 and 1.8 ml, respectively) was then added to react with the calcium carbonate to release the calcium ions for crosslinking with the alginate to produce microspheres. The alginate microspheres produced were subjected to different treatments, washed and collected by filtration (Fig. 1). The microspheres were then oven-dried at 45 °C or freeze-dried. Samples of the microspheres produced were withdrawn for microscopic examination and evaluation.

M1 microspheres were discrete and spherical before in vacuo filtration. They appeared distorted in shape after filtration and formed massive clumps after oven-drying. This could be attributed to the deformation of the alginate matrix subjected to the strong pressure of in vacuo filtration and the adhesion of the microspheres in close contact with one another during the drying process in the oven. Similar observations were made for M2 microspheres despite crosslinking with additional calcium ions of total calcium chloride concentration up to 35% w/v.

Glutaraldehyde has been employed as a crosslinking agent in the preparation of calcium alginate beads (Kulkarni et al., 2000) as well as a hardening agent for beads made from pectin (Sriamornsak and Nunthanid, 1998). Similarly, isopropyl alcohol has been employed to harden and dehydrate calcium alginate microspheres (Wan et al., 1992). Hence, relatively large amounts of glutaraldehyde and isopropyl alcohol were used in this study in an attempt to remove as much moisture as possible from the microspheres to prevent shape distortion and clumping, as well as, to further strengthen the matrix of the microspheres produced. The treated microspheres were found to be discrete and spherical after in vacuo filtration. However, they exhibited distorted shape and marked clumping upon drying in the oven. This showed that both glutaraldehyde and isopropyl alcohol were unable to produce adequate hardening of the microspheres.

Although, the amounts of calcium carbonate, glutaraldehyde and isopropyl alcohol used were more than sufficient for the intended function, it failed to produce microspheres of sufficient strength. Carbon dioxide was liberated from calcium carbonate in the presence of an acid. The above observations could be due to the carbon dioxide that was rapidly liberated. This might result in a porous microsphere matrix, which was weaker and succumbed to the pressure of filtration.

Freeze-drying was attempted to remove the moisture from the product and this constitutes its drying effect. As freeze-drying is also suitable for drying thermolabile materials and cause minimal structural changes to the product, it was employed to eliminate the adverse effects of in vacuo filtration and oven-drying on the structure of the alginate microspheres. The M5 and M6 microspheres produced with 0.5 g and 0.8 g of calcium carbonate were discrete but ‘millet’ in shape (Fig. 2a and b).

Significantly less clumping was observed when the microspheres were freeze-dried, as compared to oven-dried. Additional crosslinking with calcium chloride solution produced less clumping of the microspheres. The microspheres produced with 0.8 g of calcium carbonate showed less clumping than those produced with 0.5 g of calcium carbonate. Interestingly, 1.0 g of calcium carbonate produced distorted microspheres with a higher degree of clumping. This could be attributed to the production of more carbon dioxide when a greater amount of calcium carbonate was used, resulting in a more porous and weaker microsphere matrix. From this study, it was found that alginate microspheres produced by internal gelation were relatively weak. They deformed un-

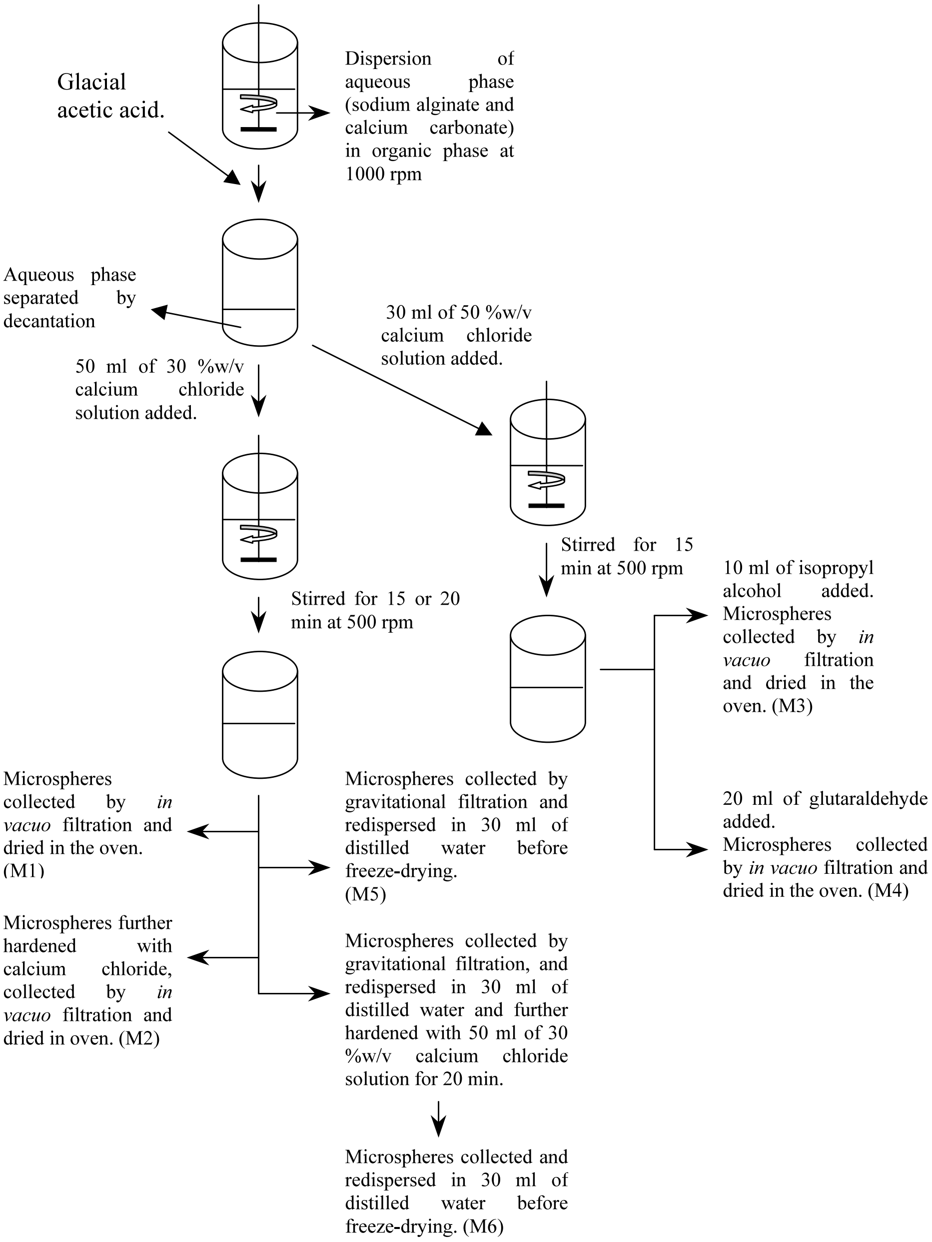

Fig. 1. Preparation of microspheres.

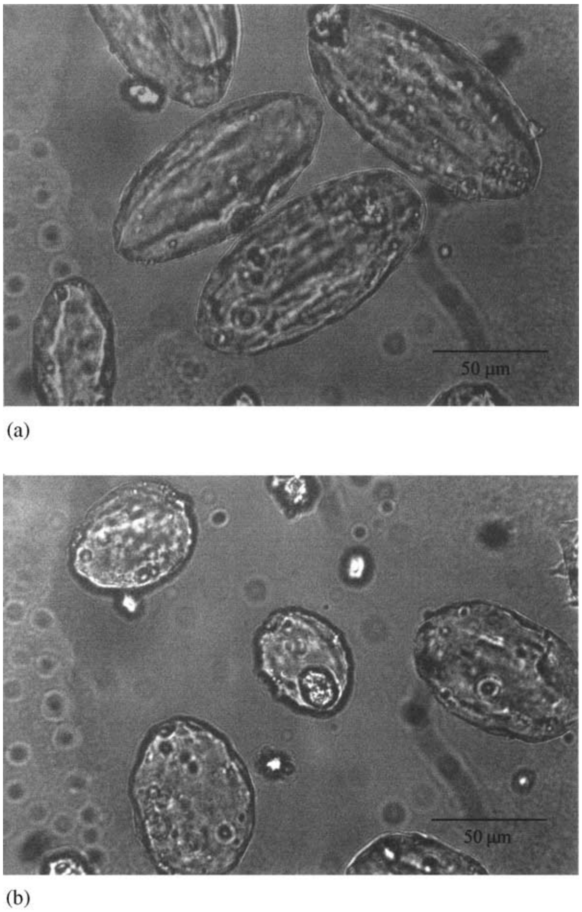

Fig. 2. Photographs of freeze-dried microspheres produced with (a) 0.5 g and (b) 0.8 g of calcium carbonate.

der the pressure of in vacuo filtration and formed clumps on oven-drying. Further hardening with calcium salt, isopropyl alcohol or glutaraldehyde had little effect on the strength of the matrix. The weak matrix could be attributed to the liberation of carbon dioxide from the calcium carbonate.

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