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Note

Cross-linking mechanisms of calcium and zinc in production of alginate microspheres

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Abstract

Calcium chloride and zinc sulphate were used to cross-link alginate microspheres prepared by an emulsification method. The microspheres cross-linked by a combination of these two salts showed different morphology and slower drug release compared with those cross-linked by the calcium salt alone. From viscosity study, it was found that zinc cations interacted with the alginate molecules to a greater extent than calcium cations. The varying effects of the salts on the properties of the microspheres were largely attributed to their ability to interact with the alginate molecules.

Keywords: Alginate; Calcium; Zinc; Viscosity; Microspheres

Alginic acid is a naturally occurring polysaccharide found in all species of brown algae and some species of bacteria. It is a linear polymer composed of α-L-guluronic acid (G) and β-D-mannuronic acid (M) units in varying proportions and sequential arrangements. Soluble sodium alginate can be cross-linked with divalent or polyvalent cations to form an insoluble alginate. Due to this property, alginate can be employed as a controlled release device for some drugs (Kim and Lee, 1992). Calcium cations are commonly used to cross-link sodium alginate. They have been reported to bind preferentially to the polyguluronic acid units (GG) of alginate in a planar two-dimensional manner, producing the so-called "egg-box" structure. Zinc and calcium cations have been reported to bind at different sites of the alginate molecule (McDowell, 1978). Some researchers have also speculated that zinc cations are less selective and hence produce more extensive cross-linking of alginate (Aslani and Kennedy, 1996).

In the present study, the effects of zinc sulphate as a cross-linking agent on the properties of alginate microspheres were investigated. The alginate microspheres were prepared using an emulsification method (Wan et al., 1994). An aqueous phase, consisting of 2.5% w/w sodium alginate (DM Manucol®, ISP-Alginates, USA) and 1% w/w sulphaguanidine (BP grade, passed through a

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75 μm sieve before use), was dispersed in isooctane with the aid of surfactants and a mechanical stirrer (IKA-WERK RW20DZM, Germany). The fine globules of sodium alginate produced were congealed by addition of equivalent amounts of individual and combinations of calcium chloride and zinc sulphate, respectively. The microspheres formed were collected by filtration, washed and oven dried at 40 °C.

The morphology of the microspheres mounted in glycerin was studied using a microscope (Olympus BH-2, Japan) connected to a video camera (CCD-1R1A, Sony, Japan) and a monitor (PVM-145E, Sony, Japan). The horizontal chord of each microsphere image was measured and the mean size of the microsphere was calculated. The degree of aggregation was expressed as the percentage number of aggregated microspheres over the total number of discrete and aggregated microspheres. At least 300 microspheres were measured. The drug release profiles were determined by dissolution studies using the paddle method (Method II, USP, Hanson Model 72, USA). One hundred milligrams of microspheres were put in 1 l of distilled water at 37 ± 0.5 °C under paddle speed of 50 rpm. Filtered samples were collected at specified time intervals and assayed spectrophotometrically at 259 nm for the drug. Three dissolution runs were carried out for each batch of microspheres and the results were averaged.

The total molar amount of cations used was kept constant. It was found that alginate microspheres could be successfully produced by the emulsification method using calcium chloride, but not zinc sulphate as the sole cross-linking agent. The products obtained using zinc sulphate consisted of lumps with a size of about 1 mm. A combination of zinc sulphate and calcium chloride produced microspheres which could be easily harvested. These microspheres generally had smaller mean size and markedly lower degree of aggregation compared with calcium alginate microspheres (Table 1). They had the largest fraction (23.3%) in the size range of 10–20 μm, while the calcium alginate microspheres had the largest fraction (19.3%) in the size range of 30–40 μm. The mean size of the microspheres decreased proportionally with an increase in molar percentage of zinc cations used ($r^2 = 0.937$).

The drug release profiles of the microspheres followed Higuchi's square root model ($r^2 \geq 0.997$), indicating that drug release was governed by diffusion of drug molecules through liquid filled channels of the matrix. It was found that the rate of drug release also decreased with an increase in the molar percentage of zinc cations used. However, the relationship was not linear ($r^2 = 0.775$). CaZn-3 microspheres, which were produced from the highest molar percentage of zinc cations, showed the lowest rate of drug release despite having the smallest mean size. Although CaZn-3 microspheres were produced from a higher molar percentage of zinc cations than CaZn-2 microspheres, their release rate was only

Table 1
Composition and properties of the alginate microspheres

Code	Type and amount of cross-linking agent	Molar ratio of cations		Mean size (μm)	Degree of aggregation (%)	K_H[a]
		Ca	Zn			
Ca-1	50 g of 40% $CaCl_2$	1	–	65.9	12	14.7
CaZn-1	40 g of 34% $CaCl_2$ 30 g of 25% $ZnSO_4$	2	1	71.2	5	13.7
CaZn-2	40 g of 30% $CaCl_2$ 20 g of 20% $ZnSO_4$	1	1	65.7	4	7.9
CaZn-3	25 g of 28% $CaCl_2$ 50 g of 20% $ZnSO_4$	1	2	51.2	4	7.7

[a] Higuchi's release constant.

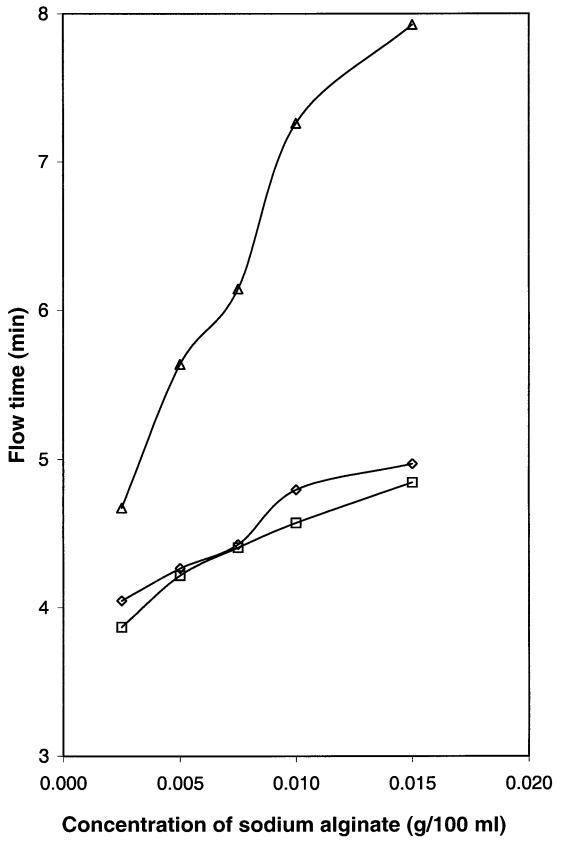

Fig. 1. Influence of increasing concentration of sodium alginate (–△–); sodium alginate and calcium salt (constant ratio, –◇–) and sodium alginate and zinc salt (constant ratio, –□–) on the flow time of the solutions.

slightly lower. This could be attributed to the much smaller mean size and hence larger specific surface area for drug release from CaZn-3 microspheres. Nevertheless, the results suggested that a larger proportion of zinc cations produced a more extensively cross-linked and less permeable alginate matrix. The proportion between calcium and zinc cations is an important factor affecting drug release. Replacing half the molar amount of calcium cations with zinc cations could successfully retard drug release by 50%. Further increase in the proportion of zinc cations would markedly decrease particle size, which would limit the effect on release retardation by increased surface area. The use of zinc cations alone would cause formation of lumps instead of microspheres.

Viscosity studies using dilute sodium alginate solutions, with and without additives, were carried out to investigate the extent of interaction of alginate molecules with zinc and calcium cations, respectively. A U-tube viscometer (Size A, BP) was used to determine the viscosities. The temperature of the whole system was kept at 37 ± 0.5 °C. Different concentrations of sodium alginate solutions were made by dissolving the appropriate amount of sodium alginate in exactly half the amount of distilled water required. An equivalent amount of distilled water containing a known amount of calcium or zinc salt was mixed with the sodium alginate solution and allowed to react for 10 min under constant agitation using a magnetic stirrer. Flow times were determined in triplicates for each mixture and results were averaged.

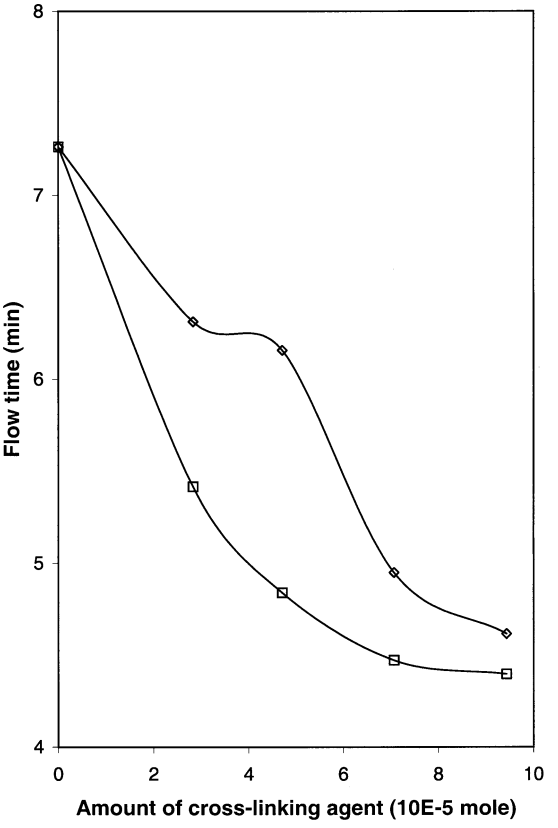

Fig. 2. Viscosity changes of 0.0100 g/100 ml alginate solution with different amounts of calcium (–◇–) and zinc (–□–) salts.

The change in flow time of sodium alginate solution containing a small amount of calcium or zinc salt was used to illustrate the extent of interaction of sodium alginate with calcium and zinc cations, respectively (Fig. 1). A greater decrease in flow time indicated a greater extent of interaction. The ratio of the amount of sodium alginate and the molar amount of calcium or zinc salt was kept constant. Mixtures consisting of higher concentrations of sodium alginate exhibited longer flow times (Fig. 1). Those with zinc salt had shorter flow time than the corresponding mixtures with calcium salt. The effects of different amounts of calcium or zinc salt on the flow time of 0.0100 g/100 ml sodium alginate solution were further investigated (Fig. 2). The flow time curve for zinc showed a steeper slope which leveled off at around 8×10^{-5} mole. Hence, the extent of interaction of zinc cations with alginate was greater than that of calcium cations. As both zinc and calcium cations are divalent, it could be inferred that the zinc cations interacted with more and/or different sites of the alginate molecule compared with calcium cations. The above effect explained the formation of smaller and less permeable alginate microspheres with a higher proportion of zinc cations. Interestingly, the zinc salt could not be employed as the sole cross-linking agent in this study as its high activity resulted in extensive uncontrolled cross-linking, leading to the formation of lumps of zinc alginate.

In conclusion, calcium and zinc cations exerted varying effects on the morphology and drug release profiles of alginate microspheres. A combination of zinc and calcium cations could be employed to produce discrete alginate microspheres with more sustained drug release. These effects were attributed to the greater extent of interaction between zinc cations and the alginate molecules.

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