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A physico-chemical approach to production of alginate beads by emulsification-internal ionotropic gelation☆

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Abstract

Some physico-chemical parameters influencing production and size of alginate beads by emulsification-internal gelation method have been examined: guluronic-acid content and viscosity of alginate samples, grain size and type of insoluble calcium compounds producing internal gelation, pH and gelation time, and the stability of water in oil emulsion. It is demonstrated that the emulsification-internal gelation is promising for large-scale immobilisation within alginate gels. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Alginate beads; Gelation; Physico-chemical parameters

1. Introduction

Immobilisation of cells and enzymes by physical entrapment in polymer (alginate) hydrogels is largely used in bioencapsulation technology. Extrusion of an alginate solution into a calcium chloride bath is the most commonly used technique. It leads to large beads: 2 mm. For drug encapsulation, material containing alginate is spray-dried. The obtained beads are small (200–500 μm) but the size distribution is very large.

An alternative to these techniques is the emulsification method which allows the encapsulation of a bioactive substance in small beads. Smaller diameters of beads allow better fermentation performance and minimise bead rupture due to gas formation and accumulation.

Bead formation through emulsion techniques has not been adequately investigated with ionic polysaccharides. One adaptation involves a hot carrageenan/oil emulsion that is dropped into cold water [1]. This technique involves high temperatures which may be incompatible with thermally labile material. An emulsion of an alginate aqueous solution in oil can be added with $CaCl_2$ solution, and thereby leads to bead formation. However, particle size cannot be easily controlled and the beads tend to coagulate into large masses before hardening properly.

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An internal gelation method was proposed in which calcium ions are slowly liberated within the ionic polysaccharide via spontaneous breakdown of gluconolactone, resulting in acidification of an alginate slurry containing a calcium salt [2]. The inconvenience of this technique is the cast of the calcium alginate gel, since it takes up to 5 min for the solution to harden. The gradual hardening would render this method unsuitable for gelation within an emulsion due to aggregation of the beads.

In the present study, the internal gelation of an alginate solution is achieved through the rapid release of calcium ions from an insoluble dispersed calcium complex in the aqueous phase [3]. Calcium ion release is initiated via gentle acidification with an oil-soluble acid. Calcium is liberated in situ from complex form ('calcium vector'), allowing gelation of alginate. Small beads (< 1 mm) are produced in soft conditions and at large scale. Since no toxic reagents or solvents are used, biological and food applications may be considered. This paper gathers some fundamental considerations useful for improvement of results in terms of size dispersion. Those improvements are shown by some experimental data.

2. Experimental

2.1. Materials

Sodium alginate samples (Grinsted Products, Denmark; Sanofi Bio Industries, France) with different guluronic acid contents and viscosities, canola oil (Canada Packers, Canada), surfactants (Span 80 and Tween 80 from Sigma), calcium carbonate (Baker, Canada) were used. The characteristics of the sodium alginate samples used in this work have been summarized in [4]. Reagent-grade calcium citrate, calcium chloride, sodium bicarbonate and acetic acid were purchased from Anachamia (Canada).

2.2. Methods

2.2.1. Bead production

Sodium alginate was dissolved and intensively mixed to yield of 1–4% alginate solution. Solutions stood for at least 1 h to allow desaeration, and buffer was added to adjust the pH to the desired value (typically 8.0). Before use, the encapsulant was uniformly dispersed into the alginate (ratio up to 1:1). This alginate preparation (20 ml) was mixed with 1 ml suspension of insoluble calcium salt (500 mM Ca^{2+} equivalent) resulting in a suspension of the 25 mM calcium salt in alginate solution (Fig. 1(a)). The carbonate, citrate, monohydrogenophosphate, oxalate and tartrate salts of calcium were tested.

The alginate/calcium-salt mixture (21 ml) was dispersed within 100 ml canola oil by stirring at 200–500 rpm for 15 min (Fig. 1(b)).

With continuous agitation, 20 ml canola oil containing 80 μM glacial acetic acid is then added to the emulsion, liberating divalent calcium for gelation of the alginate polyanions (Fig. 1(c)).

After 5 min, the oil-bead suspension was added with gentle mixing to 150 ml of 50 mM calcium chloride solution (Fig. 1(d)). The external gelation of beads begins to be realised as a result of the coalescence between the aqueous droplets containing pregelled alginate beads and those containing the calcium chloride solution.

After adding into a water in oil (w/o) emulsion of some critical volume of calcium chloride solution, the phase inversion in the emulsion occurs (Fig. 1(e)). Phase inversion time is controlled by electro-conductivity measurements. After complete partitioning of beads to the aqueous phase, the oil is discarded, the beads are washed with 1% Tween 80 and filtered on a 30 μm sieve.

2.2.2. Other methods

Volume size distribution of beads were obtained using a Malvern 2605-Lc particle size analyser. The alginate viscosity was measured at 20°C using a Brookfield viscometer model LVT with an adapter UL for small volumes (16 ml).

3. Results and discussion

Size distribution of beads formed by emulsification techniques is determined by numerous physico-chemical factors. Important factors are concentration and molecular structure of alginate,

presence of surfactants, type and form of the calcium vector, its concentration, the initial and final pH values and mechanical dispersive power producing emulsification. These factors affecting viscosity of the discontinuous phase, interfacial tension and dispersive forces, determine the rates of dispersion and coalescence processes in the emulsion and finally the droplets and beads size.

3.1. Size distribution of alginate beads

Fig. 2 illustrates a typical size distribution for alginate beads formed by the process of dispersion/internal gelation. The size distribution curves are usually polymodal with two main peaks: primary peak A represented 65–95% and secondary peak B ~25% of the total bead volume. Several smaller satellite peaks (S) under 150 μm represented up to 10% of the bead volume.

Dominance of peak A or B depends on the type of alginate and calcium vectors used and, in some cases, the peaks A or B can be eliminated (Fig. 3). Satellite beads formation is most likely due to turbulence in the reactor; thus reducing turbulence may minimise or eliminate satellite peaks [5]. The increased rate of the internal gelation may

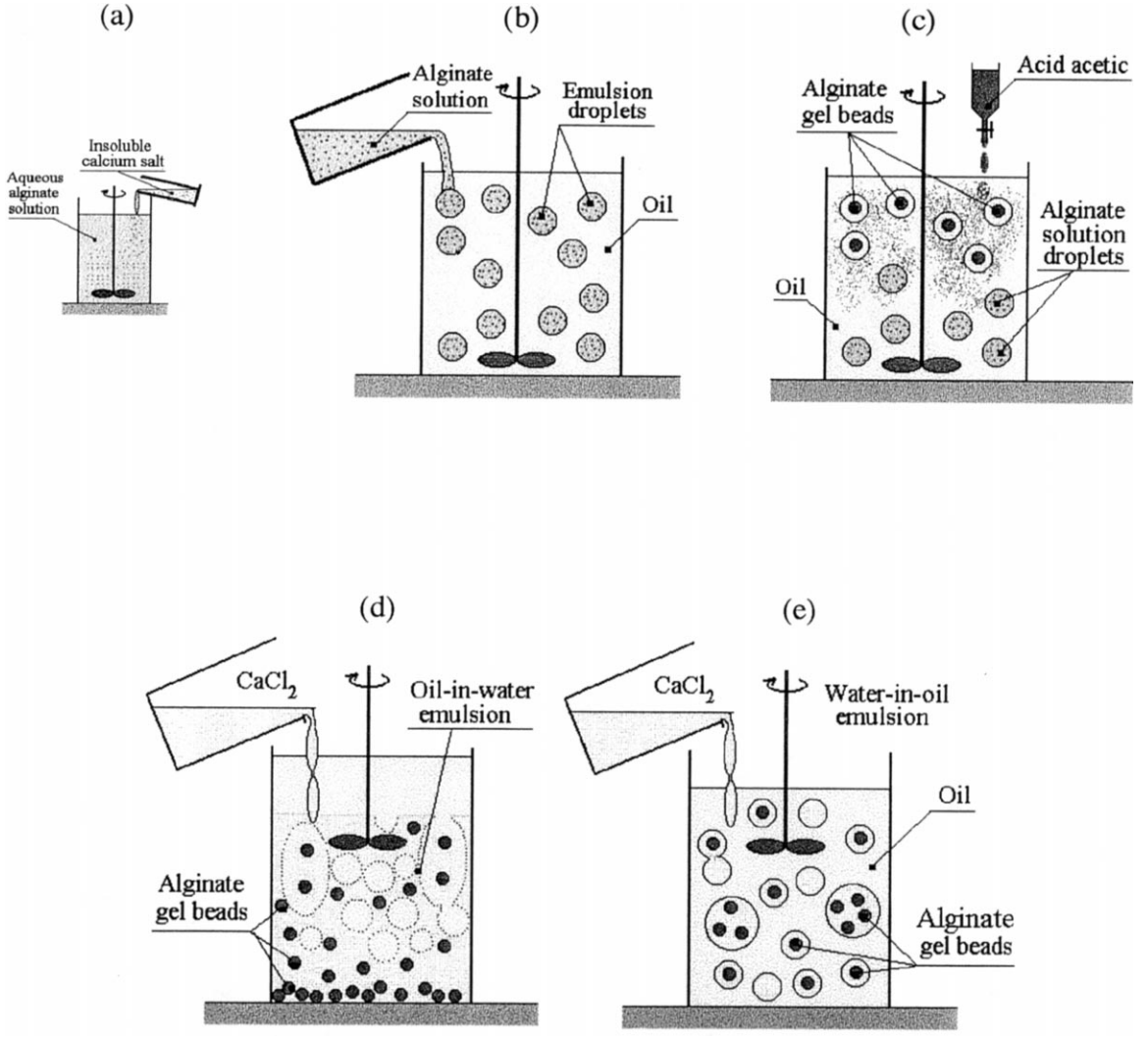

Fig. 1. Different stages of the preparation of alginate beads by emulsification/internal gelation method (for explanation see text).

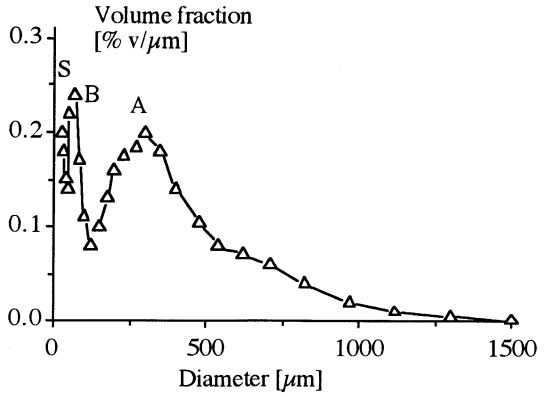

Fig. 2. Typical size distribution curve for alginate beads. (Calg = 1.8%; alginate of high guluronic acid content; cross-linker 50 mM Ca^{2+}; Cspan 80 = 2%; 400 rpm [5]).

Dominance of peak A or B depends on the type of alginate and calcium vectors used and, in some cases, the peaks A or B can be eliminated (Fig. 3). Satellite beads formation is most likely due to turbulence in the reactor; thus reducing turbulence may minimise or eliminate satellite peaks [5]. The increased rate of the internal gelation may also contribute to the satellite beads formation: due to the high gel viscosity the small droplets will have less tendency to coalesce with larger droplets, and will contribute largely to the final size distribution. Internal gelation is related with release of calcium ions inside the droplets.

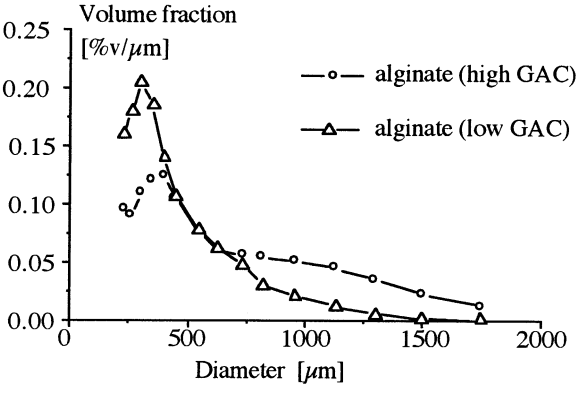

Fig. 3. Size distribution curves of beads made from alginate with high (1) and low (2) guluronic acid content (GAC), calcium vector = calcium carbonate [8].

3.2. Release of calcium ions from insoluble complexes

The release of calcium may be written as:

$$Ca_xA_z\downarrow \leftrightarrow x\cdot Ca^{2+} + z\cdot A^{-x}$$

where A represents the anion, and the free calcium concentration $[Ca^{2+}]$ is given by the expression

$$[Ca^{2+}] = \sqrt[x+z]{L\left(\frac{x}{z\varphi_0}\right)^z}$$

where L is the solubility product, φ_0 is the partition coefficient between acid and base forms, which may be calculated from pH and from acidity constants of A [6].

Fig. 4 gives the free calcium concentration as a function of the pH for various calcium complex forms. Selection of a suitable calcium vector for internal gelation depends on the range of initial to final pH values desired. Typically, neutral pH values are appropriate for live cell immobilisation. Acid-tolerant cultures such as lactic acid bacteria may be immobilised at a lower pH range (7 initial to 5 final). Over the pH range of interest, the concentration of free calcium must initially be low with a rapid release of calcium upon pH decrease. A pK_a value of the anions in the working range 6.5–7.5 is optimal for cell immobilisation.

So, oxalate and tartrate were unacceptable, as calcium is not released within a suitable pH range. Release of calcium occurs in a narrower pH range with calcium carbonate than with calcium citrate. At pH 7–8, the calcium concentration with cal-

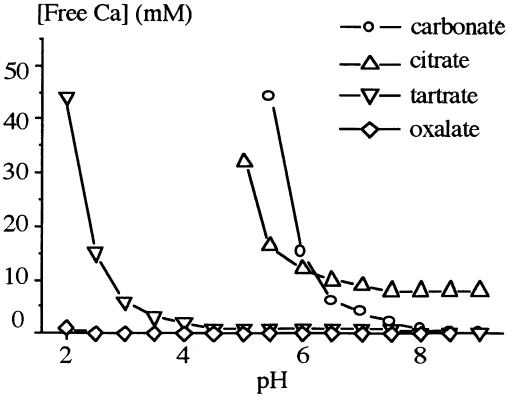

Fig. 4. Free calcium concentration versus pH for different calcium vectors.

3.3. Effect of alginate composition

The alginate composition is also an important parameter in alginate bead formation. In molecular terms alginate is unbranched binary copolymer of 1-4 linked β-D-mannuronic acid (M) and α-L-guluronic acid (G). It is known [7] that calcium primary cross-links the guluronic residues. With increasing the guluronic acid content (GAC) in the alginate samples, the viscosity of their aqueous solutions increases and gels are more strong.

In the emulsification step, these alginates result in premature gelation, giving larger beads with larger dispersion (Fig. 3). At the same time, the molecular mass of alginates which is usually characterised by the intrinsic viscosity of their solutions, has little influence on the beads size distribution [5] (Fig. 5).

3.4. Recuperation of gel beads

Recovering of gel beads from the oil after their internal pregelation (Fig. 1(c)) and external gelation by the addition of the aqueous $CaCl_2$ solution (Fig. 1(d)) is an important step of the process. Both, separation time and volume of the aqueous phase may limit the development of the process at large scale.

The use of the oil soluble surfactant (Span 80) permits the remarkable reduction in size of alginate gel beads as the result of decreasing the interfacial tension and preventing the droplets coalescence [5]. At the same time, this may result in the intensive formation of very small satellite droplets and the hydrophobisation of gel beads leading to their aggregation. The excessive stabilisation of the water-in-oil emulsion may hinder its destabilisation and inversion which is necessary to recover the gel beads (Fig. 1(e)).

The increase of the volume ratio φ of the aqueous phase ($CaCl_2$ solution) in the preliminary obtained water-in-oil (w/o) emulsion (Fig. 6(a)) leads finally to the oil-in-water (o/w) emulsion (Fig. 6(f)) via the phase inversion stage (Fig. 6(c) and (d)) which corresponds to the minimum emulsion stability and the intensive coalescence of water droplets. This moment is favourable for the separation of gel beads from the oil to the macroscopic aqueous phase appeared after breaking the emulsion (Fig. 1(e)). But with addition of some more aqueous phase in the system, a stable oil-in-water emulsion begins to form (Fig. 6(e)) which stability increases with increasing φ. Then gel beads extraction from the oil phase becomes impossible.

The control of phase inversion in the emulsion system may be realised by electroconductivity measurements. With increasing the volume ratio φ of the aqueous phase, the electroconductivity κ of the emulsion increases slowly at the beginning and sharply at the moment of the phase inversion in the system (Fig. 7). The control of phase separation of the emulsion system by electroconductivity measurements allows optimum large-scale separation technology.

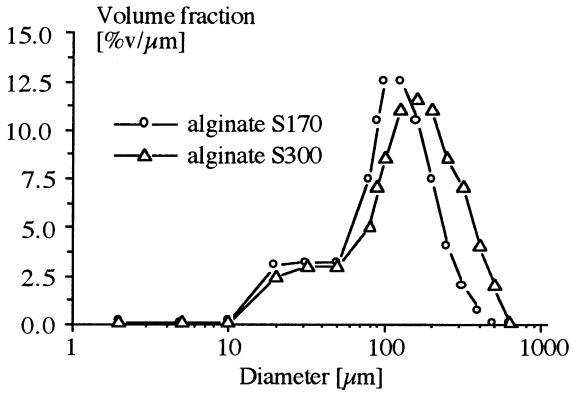

Fig. 5. Effect of the molecular mass of the alginate samples on the size distribution of gel beads.

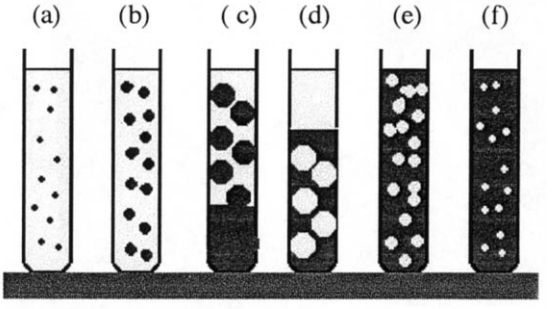

Fig. 6. Transformation of w/o emulsion to o/w emulsion with increasing aqueous phase ratio φ.

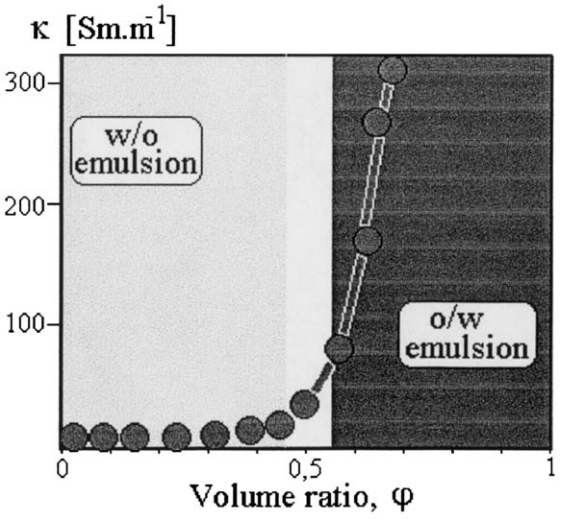

Fig. 7. Electroconductivity κ of the emulsion as function of the volume ratio φ of the aqueous phase.

4. Conclusion

Emulsification/internal gelation is a promising method for large-scale bioencapsulation within alginate gel beads. First it allows to use soft conditions to make the beads (neutral pH, work at room temperature, use of non-toxic reagent). Second the continuous production is possible with the use of static mixers, what simplifies the scale-up. This study shows that critical points of the process are: the calcium vector, the calcium grain size, the mannuronic/guluronic content of alginate and the volume ratio of the aqueous phase.

Acknowledgements

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