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Formation of Calcium Alginate Gel Capsules: Influence of Sodium Alginate and $CaCl_2$ Concentration on Gelation Kinetics

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The formation kinetics of calcium alginate gel capsules is studied. An increase in the concentration of alginate gives rise to a reduction in membrane thickness, while an increase in the concentration of calcium chloride leads to the formation of a thicker film. Experimental data are adjusted to the binomial diffusion equation.

[**Key words:** calcium alginate, encapsulation, gelation kinetics]

The encapsulation of biocatalysts within hydrogels, and more specifically in calcium alginate gel capsules, is an immobilization technique that has now found widespread application. It consists of enclosing the biocatalyst in an aqueous solution inside a semipermeable membrane capsule (1). This technique offers all the advantages of immobilization in calcium alginate gels: biocompatibility, simplicity and low cost (2). Nevertheless, the main advantage of this immobilization technique lies in the specific particle structure, in which contact between substrate and biocatalyst can be achieved in an appropriate way since the biocatalyst is in solution within the core capsule.

Of all the different biopolymers that can be employed in the formation of the semipermeable membrane in capsules, alginate is one of the most frequently used owing to the fact that its immobilization is carried out under very mild conditions (3). In molecular terms, alginate consists of a family of unbranched binary copolymers of (1→4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) of widely varying composition and sequential structure (4). Alginates are true block copolymers composed of homopolymeric regions of M and G, termed MM and GG blocks, respectively, interspersed with regions of alternating structure (MG blocks) (5). In solution, alginates behave like flexible coils. However, upon interaction with divalent metal ions—such as Ca^{2+}—they form an ordered structure. Grant *et al.* showed that the gelation process involves cooperative binding of calcium ions between aligned GG blocks of two alginate chains (6). The gelation of calcium alginate is an almost instantaneous and irreversible process, which is governed by the relative rate of diffusion of calcium ions and polymer molecules into the gelling zone (7, 8). This fact indicates that the gelation process can be accurately expressed by the relationships used for other diffusion-limited reaction systems (9–12).

Since the gelling properties of an alginate depend strongly upon its monomeric composition, block structure, molecular size and concentration of polymer and calcium ions (13–15), the main objective of this work was to study the gelation kinetics of capsules of calcium alginate gel under different operational conditions. Knowledge of gelation kinetics allows easy control over some capsule characteristics, such as thickness and permeability to different substrates of the gel membrane.

Calcium alginate gel capsules were prepared by extrusion, using a simple one-step process similar to that described by Nigam *et al.* (1). Alginic acid salt (molecular weight of 100.000–200.000) obtained from brown algae (*Laminaria hyperborea* blades) was purchased from Fluka BioChemika (Fluka art. no. 71238, Switzerland). The specifications of this product are the most suitable for the immobilization of microorganisms and enzymes: pH (1% in water) 6.0–7.5; loss on drying ≤15%; ash ≤27%. A medium-viscosity sodium salt of carboxymethylcellulose (CMC) was obtained from Fluka BioChemika (Fluka art. no. 21902). According to the manufacturer's specifications, a 2% w/v solution has a viscosity range of 400–1000 mPa·s at 25°C, a degree of substitution between 0.60 and 0.95, a loss on drying at 110°C of <15% and a pH range (1% in water) of 6.5–8.0.

Several sodium alginate solutions of different concentrations were prepared, according to the particular experiment to be performed, and were used as anionic solutions. For the preparation of cationic solutions, CMC was dissolved in different solutions of $CaCl_2$ to give a 3% w/v CMC solution. CMC was used as a non-gelling polymer to modulate the viscosity and density of cationic solutions in order to ensure the spherical shape of the capsules.

Droplets of $CaCl_2$-CMC solution (5–20 ml depending on the number of capsules to be obtained) were added dropwise through a silicone tube, using a peristaltic pump, into 200 ml of sodium alginate solution. The sodium alginate solution was constantly stirred at 330 rpm using a magnetic stirrer situated at the bottom of the vessel, in order to keep the droplets from sticking together and to minimise external mass transfer resistance. A dropping height of 10 cm was used to ensure that spherical droplets were formed. The inner diameter of the silicon tube was 1.6 mm. The time between consecutive drops was maintained at a rate lower than 1% of the residence time of the capsules in the anionic solution, in order to ensure that capsules were all formed over the same period.

Once the $CaCl_2$-CMC solution was added dropwise into the alginate solution, a capsular membrane was formed instantaneously around each droplet due to the cross-linking of the interfacial alginate molecules with calcium cations. In each experiment, different times of gelation or periods in which capsules were formed were

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selected. Prior to the removal of capsules, sodium alginate solution was diluted more than fourfold by adding the required amount of distilled water. This step dilutes the alginate solution outside the capsules and reduces the possibility of capsules joining together when they are in close contact, as well as helps terminate the gelation process.

After diluting the sodium alginate solution, the capsules were filtered off using a Büchner funnel. With the aim of stabilising the calcium alginate membrane, the capsules were immediately transferred to a 1.3% w/v calcium chloride solution and incubated for 15 min. Finally, after rinsing the capsules with distilled water to remove excess calcium chloride, they were stored in water until measurements were carried out. All experiments were carried out at 25°C.

The formation kinetics of the capsules was characterized by measuring the temporal evolution of the membrane thickness and diameter of the capsules. Measurement of the external diameter was carried out using a caliper. Membrane thickness was studied by cutting the capsules in half and carrying out measurements in at least four different locations on the membrane. The image processing software MIP 4 ADVANCED allowed us to perform the measurement of the membrane thickness on an image of each half-capsule, captured by a video camera connected to a microscope. The diameters and gel layer thicknesses reported here represent the average of the measurements performed on ten capsules obtained under the same experimental conditions.

The effect of sodium alginate concentration on capsule formation kinetics was studied by fixing the cationic solution at 2.6% w/v $CaCl_2$ and 3% w/v CMC. As can be seen in Fig. 1, the thickness of the membrane increases rapidly within the first 15 min of the process. Indeed, within the first 10 min, membrane thickness is about 50% of its maximum value. After this first stage, the thickness of the gel film increases more slowly, and finally levels off at its maximum value. This temporal evolution was also exhibited by the external diameter; however, the core diameter—obtained from the external diameter and membrane thickness—was always of the same order of magnitude (5.6 ± 0.1 mm) and was independent of the operational conditions. This kind of behaviour indicates that gel film formation occurs from outside the core of the capsule. All these results can be easily explained by taking into consideration the gel formation process, which is assumed to be controlled by the diffusion of the two components involved in it. In this regard, the fact that the metallic cation has a smaller size than the polymer molecules means that it is mainly the cation that diffuses between the alginate chains, binding to unoccupied binding sites on the polymer. Thus, once the cationic solution is added dropwise into the alginate solution, a capsular membrane forms instantaneously around the droplet, and this will grow along the flux direction of the Ca^{2+} ions. When the mass of Ca^{2+} ions contained within the core capsule is exhausted, the gelation process is finished. As far as the initial moments of the gel formation process are concerned, all the binding sites for Ca^{2+} ions present in alginate chains are unoccupied, so cations can bind rapidly to the polymer. However, when diffusing through a gel that has already formed where all the binding sites are occupied, there is no opportunity for Ca^{2+} ions to bind until it reaches available binding sites in the gelling zone. Thus, calcium ions must diffuse through the gel film to react with sodium alginate during the formation of capsules. This means that the maximum growth of the gel film is realized within the first 15 min of the process, where the resistance to diffusion caused by the gel film is not significant.

In other respects, on increasing sodium alginate concentration, the thickness of the membrane decreases at a given gelation time. This effect is presumably due to the fact that on increasing the number of biopolymer molecules per unit solution volume in the vicinity of the core capsule, the number of binding sites for Ca^{2+} ions also increases. As a result, a more densely cross-linked gel structure will probably form and, consequently, it will have a smaller thickness. In connection with this phenomenon, we observed qualitatively that the capsules obtained from 0.75% w/v sodium alginate solutions were more resistant, from a mechanical point of view, than those obtained from less concentrated solutions.

The effect of $CaCl_2$ concentration on capsule formation kinetics was studied by fixing the anionic solution at 0.5% w/v sodium alginate (Fig. 2). On increasing calcium chloride concentration, the thickness of the membrane increases at a given gelation time. This result can be explained by the fact that an increase in the mass of calcium ions initially contained in the core capsule will

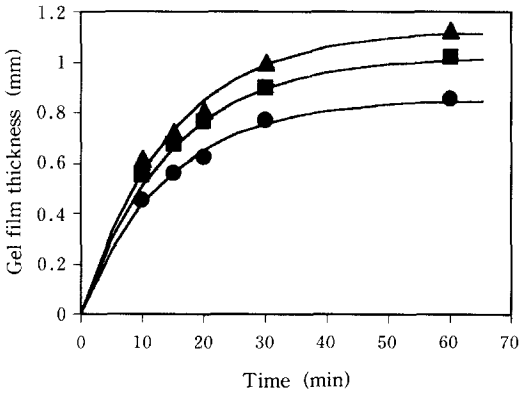

FIG. 1. Thickness of gel layer capsules as a function of time and sodium alginate concentration. Symbols: ▲, sodium alginate 0.25% w/v; ■, sodium alginate 0.5% w/v; ●, sodium alginate 0.75% w/v.

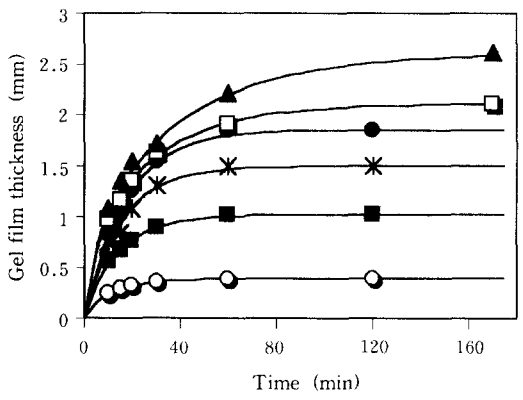

FIG. 2. Thickness of gel layer capsules as a function of time and $CaCl_2$ concentration. Symbols: ○, $CaCl_2$ 1.3% w/v; ■, $CaCl_2$ 2.6% w/v; *, $CaCl_2$ 4% w/v; ●, $CaCl_2$ 5.5% w/v; □, $CaCl_2$ 7% w/v; ▲, $CaCl_2$ 9% w/v.

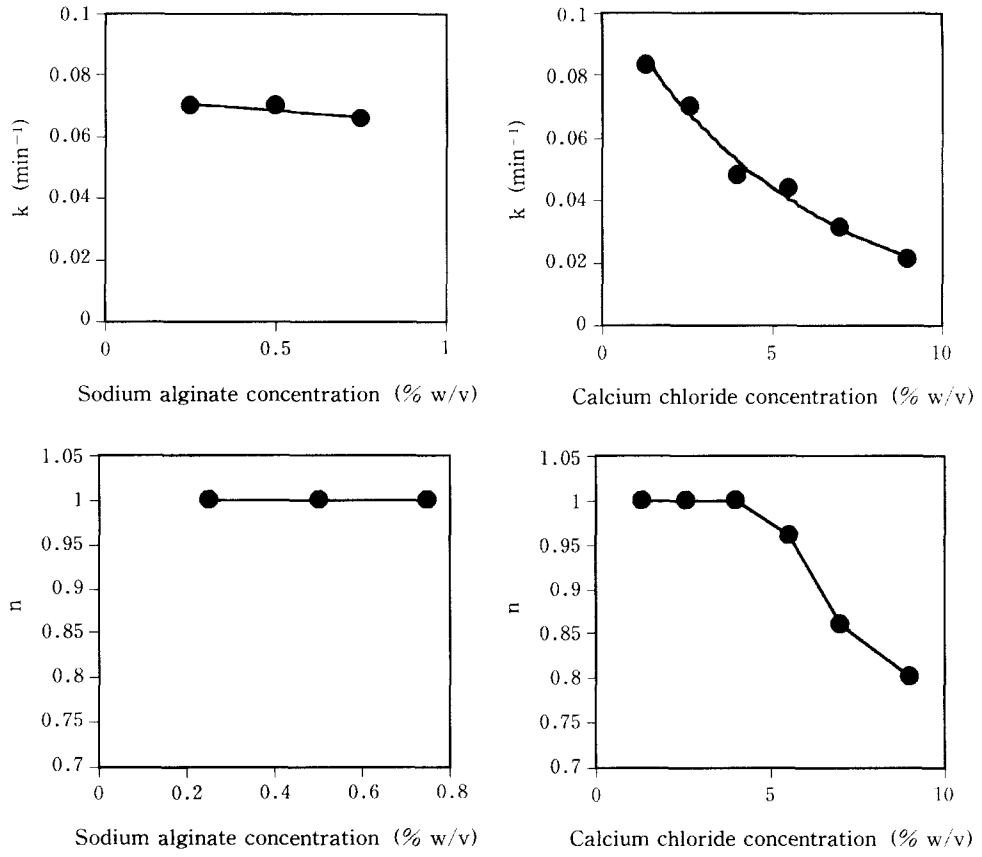

FIG. 3. Parameters of gelation kinetics model obtained for different sodium alginate and $CaCl_2$ concentrations.

result in a larger concentration gradient between the core and the outside solution. This situation will favour the diffusion of Ca^{2+} ions from the core. Moreover, the time required to obtain the maximum gel film thickness is considerably longer when calcium chloride concentration exceeds 5.5% w/v. This result confirms that the membrane thickness increases continuously until complete consumption of calcium ions contained in the core capsule has been achieved.

With the aim of modelling the gelation kinetics of the capsules, the data of gel film volume were adjusted to the binomial diffusion equation derived by Yamagiwa *et al.* (10) for describing the process of forming a spherical coating around a biocatalyst carrier:

$$V = V_{max}[1-\exp(-k \cdot t)]^n$$

where V and V_{max} are the volumes of gel membrane formed at times t and $t \rightarrow \infty$ respectively, k is the gelation rate constant, and n is the heterogeneous structural resistance constant, which is indirectly proportional to the diffusion resistance. Specifically, the value of n is lower than unity for all diffusion-limited reaction systems (16). Gel membrane volumes were calculated from external diameter and membrane thickness measurements. The values of n and k were determined by a non-linear regression method based on the Marquardt algorithm (17), considering the maximum volume of gel film to be that reached after 60 or 165 min, depending on the particular experiment. The resulting values of the parameters of the gelation kinetics model, k and n, are given in Fig. 3. As shown in Fig. 3, the gelation rate constant and the heterogeneous structural resistance are independent of biopolymer concentration within the range of concentrations tested. Moreover, n is unity in the range of alginate concentration studied. This indicates that the resistance to diffusion that Ca^{2+} ions suffer during gel formation is negligible under these particular conditions and the gelation reaction is almost a first-order reaction. Yamagiwa *et al.* (9) reported that n was almost unity for calcium alginate gels made from 0.2–0.8% w/v sodium alginate solutions. In the same manner, Chrastil (9) reported that the structural diffusion resistance, n, did not depend on alginate concentration between 2 and 3% w/v. However, calcium chloride concentration does affect the values of the two kinetic parameters significantly. For example, the value of the structural resistance constant (n) is unity when $CaCl_2$ concentration is in the range 1.3 to 4% w/v, but tends to decrease when more concentrated solutions are used. This reduction in n values is considered to be due to the increase in diffusional resistance that Ca^{2+} ions suffer in their flux through a thicker membrane (constant, n, decreases with increasing resistance). The same type of behaviour was observed by Yamagiwa *et al.* (10), who reported that n was almost unity with calcium chloride concentrations up to 4% w/v but decreased with increasing concentrations above 4% w/v. In the same way, the gelation rate constant (k) decreases with increasing calcium chloride concentration, with this reduction being more marked within the range of 1.3% to 4% w/v. This behaviour could be due to the relationship that exists between the diffusivity of calcium ions and calcium alginate concentration in the gelling zone. Moreover, as previously indicated, it is necessary to consider the increased diffusion resistance

caused by the formation of a thicker membrane gel.

REFERENCES

1. **Nigam, S. C., Tsao, I.-F., Sakoda, A., and Wang, H. Y.:** Techniques for preparing hydrogel membrane capsules. Biotech. Tech., **2(4)**, 271–276 (1988).
2. **Christenson, L., Dionne, K., and Lysaught, M.:** Biomedical application of immobilized cells, p. 7–41. *In* Goosen (ed.), Fundamentals of animal cell encapsulation. CRC Press, Boca Raton, M.F.A. (1993).
3. **Jen, A. C., Wake, M. C., and Mikos, A. G.:** Review: hydrogels for cell immobilization. Biotechnol. Bioeng., **50**, 357–364 (1995).
4. **Haug, A.:** Fractionation of alginic acid. Acta Chem. Scand., **13**, 601–603 (1959).
5. **Grasdalen, H., Larsen, H., and Smidsrød, O.:** ^{13}C-NMR studies of monomeric composition and sequence in alginate. Carbohydr. Res., **89**, 179–184 (1981).
6. **Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C., and Thom, D.:** Biological interactions between polysaccharides and divalent cations: the egg-box model. FEBS Lett., **32**, 195–200 (1973).
7. **Bergstrom, E., Goodall, D. M., and Norton, I. T.:** Kinetics of ion binding and structural change in alginates, p. 501–505. *In* Phillips, G. O., Williams, P. A., and Wedlock, D. J. (ed.), Gums and stabilisers for the food industry. IRL Press, Oxford (1990).
8. **Skjak-Braek, G., Grasdalen, H., and Smidsrød, O.:** Inhomogeneous polysaccharide ionic gels. Carbohydr. Polym., **10**, 31–54 (1989).
9. **Chrastil, J.:** Gelation of calcium alginate. Influence of rice starch or rice flour on the gelation kinetics and on the final gel structure. J. Agric. Food Chem., **39**, 874–876 (1991).
10. **Yamagiwa, K., Shimizu, Y., Kozawa, T., Onodera, M., and Ohkawa, A.:** Formation of calcium-alginate gel coating on biocatalyst immobilization carrier. J. Chem. Eng. Jpn., **25**, 723–728 (1992).
11. **Mouquet, C., Aymard, C., Gulbert, S., Cuvelier, G., and Launay, B.:** Influence of initial pH on gelation kinetics of texturized passion fruit pulp. Food Sci. Technol., **30**, 129–134 (1997).
12. **Lin, S. H.:** Gelation of sodium alginate in a batch process. Chem. Eng. Sci., **46**, 651–655 (1991).
13. **Martinsen, A., Skjak-Braek, G., and Smidsrød, O.:** Alginate as immobilization material, I. Correlation between chemical and physical properties of alginate gel beads. Biotechnol. Bioeng., **33**, 79–89 (1989).
14. **Velings, N. M. and Mestdagh, M. M.:** Physico-chemical properties of alginate gel beads. Polym. Gels Networks, **3**, 311–330 (1995).
15. **Ouwerx, C., Velings, N. M., Mestdagh, M. M., and Axelos, M. A. V.:** Physico-chemical properties and rheology of alginate gel beads formed with divalent cations. Polym. Gels Networks, **6**, 393–408 (1998).
16. **Chrastil, J.:** Enzymic product formation curves with the normal or diffusion limited reaction mechanism and in the presence of substrate receptors. Int. J. Biochem., **20**, 683–693 (1988).
17. **Marquardt, D.:** An algorithm for least-squares estimation of non linear parameters. J. Soc. Ind. Appl. Math., **11**, 431–441 (1963).