Korean J. of Chem. Eng., **7**(1) (1990) 1-6

A KINETIC STUDY ON CALCIUM ALGINATE BEAD FORMATION

Hae-Sung KIM

Department of Chemical Engineering, Myong-Ji University,
Sudaemoonku, Seoul 120-728, Korea

(Received 16 November 1988 • accepted 16 October 1989)

Abstract—Ca-Alginate-Gel is one of the most widely used carriers for cell entrapment. Since gel formation can take place under mild conditions, entrapment in this matrix is very suitable for immobilization of viable cells and it has found most extensive application. Despite of the extended use there have been no kinetic data related to gel formation of alginate with calcium ion. In the present work the kinetic study was accomplished to more fully elucidate the transient structure transformations involved in the gel formation using shrinking-core model. The proposed kinetic model may be successfully extended to account for the transient behavior and complete gelling time as well as some useful information of the gelling conditions in the process of Ca-Alginate-Gel.

INTRODUCTION

Ca-Alginate-Gel is one of the most widely used carriers for cell entrapment. Alginate, the major structural polysaccharide of marine brown algae, contains β-D-mannopyranosyl uronate and α-L-gulopyranosyl uronate in regular (1-4)-linked sequences [1]. Both homopolymeric sequences are found together, although to different extents, in all alginate molecules. Mixed sequences containing both monomer are usually also present. One of the most important and useful properties of alginates is the ability to form gels in the presence of divalent cations, especially calcium. The calcium ions react preferentially with the polyguluronic sequences before reacting with the polymannuronic sequences [2]. It is quite likely that the mixed sequences play no direce role in the gelation with calcium except to join the associated sequences and hence provide a three-dimensional network of cross-linking in the gel [3]. Since gel formation can take place under mild conditions, entrapment in this matrix is very suitable for immobilization of viable cells and it has found most extensive application [4]. Despite of the extended use there have been no kinetic data related to gel formation of alginate with calcium ion. In the present work the kinetic study was accomplished to more fully elucidate the transient structure transformations involved in the gel formation using shrinking-core model and the proposed kinetic model may be extended to account for the transient behavior and complete gelling time as well as some useful information of the gelling conditions in the process of Ca-Alginate-Gel.

THEORETICAL BACKGROUND

Alginate gels are heteropolymer carboxylic acids consisting of β-D-mannuronic and α-L-guluronic units linked by 1,4-glycosidic bonds. It is formed by contacting alginate solution with calcium chloride solution. The calcium-alginate reaction should be considered as follows [3].

$$2\ NaAlg + Ca^{++} = CaAlg + 2\ Na^{+}$$

Almost instantly calcium alginate forms on the surface of the sodium alginate stream, and it maintains the shape it had when contacted with the calcium chloride solution. Initially the center is unreacted sodium alginate, but over a period of time the calcium ion will diffuse into the center and form a complete calcium alginate structure. During gelling process the boundary of calcium alginate gel will move toward the center from the surface while calcium ion penetrates the porous gelled layer, as shown in Fig. 1. Assuming dominant pore-diffusion kinetics and pseudo-steady-state approximation in the gelling process, the governing equations can be presented as follows.

$$O = \frac{\partial C}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}\left(D_e r^2 \frac{\partial C}{\partial r}\right) \tag{1}$$

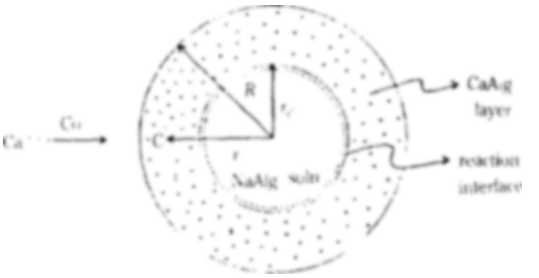

Fig. 1. Schematic diagram of a gelling bead.

B. C.

$$C=C_o \quad \text{at } r=R$$
$$C=0 \quad \text{at } r=r_c \tag{2}$$

The concentration distribution of Ca ion in the gelled layer is given such as

$$C/C_o=1-\frac{R\cdot r_c}{R-r_c}\left(\frac{1}{r}-\frac{1}{R}\right). \tag{3}$$

The transfer rate of Ca ion at the outer surface is obtained from the flux.

$$\frac{dN}{dt}=4\pi R^2 D_e\frac{dC}{dr}\Big|_{r=R}=4\pi D_e\frac{C_o}{1/r_c-1/R} \tag{4}$$

The rate of consumption of sodium alginate is given from the volume change of the gelled layer

$$-\frac{dW}{dt}=-\frac{d}{dt}\left(p\frac{4}{3}\pi r_c^3\right)=-4\pi p r_c^2\frac{dr_c}{dt}. \tag{5}$$

The transfer rate of Ca ion must be the rate of consumption of sodium alginate

$$\frac{dN}{dt}=-q\frac{dW}{dt} \tag{6}$$

$$4\pi D_e\frac{C_o}{1/r_c-1/R}=-4\pi pqr_c^2\frac{dr_c}{dt}. \tag{7}$$

So that,

$$\int_{R_o}^{r_c}-\left(\frac{1}{r_c}-\frac{1}{R}\right)r_c^2 dr_c=\frac{D_e C_o}{pq}\int_0^t dt. \tag{8}$$

For a gelling bead, defining

$$\phi=\frac{R}{R_o} \tag{9}$$

$$\gamma=\frac{r_c}{R_o} \tag{10}$$

$$\varepsilon=\frac{R^3-r_c^3}{R_o^3-r_c^3} \tag{11}$$

the resulting kinetic equation is as follows [5].

$$\frac{2C_oD_e}{pqR_o^2}t=1-\gamma^2-\frac{1-\phi^2}{1-\varepsilon}\equiv\psi \tag{12}$$

EXPERIMENTAL METHOD

1.75 per cent (W/V) of solution of sodium alginate was made by dissolving 10.5g of sodium alginate (Kelco, Keltone LV, MW = 25,000-50,000) in 600 ml of distilled water. The solution was filled into a cylindrical reservoir and compressed air was used to force the solution through 20G hypodermic needle mounted coaxially inside a 4.76 mm tube for concentric gas flow. The coaxial tube was used to blow off the alginate droplets at the controlled diameter of 2.42 ± 0.01 mm by concentric gas stream. The drop size was determined from the flowrate of alginate solution based on the time to fill 10 ml and the formation rate of alginate drop based on the number of drops to be collected in the calcium chloride solution during known formation time. The 7 or 8 alginate drops were formed in a few seconds and contacted during fixed time with calcium chloride solutions of known constant concentrations which were gently stirred in the apparatus shown in

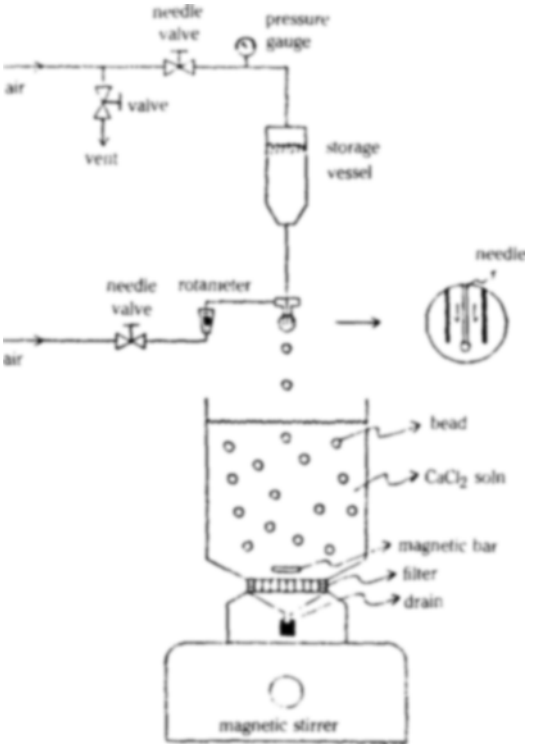

Fig. 2. Experimental apparatus for gelling bead.

Fig. 2. After known contact time the calcium ion was washed off carefully from the formed bead with distilled water. Instantly the bead size was measured on the microscope with 45 times magnification. All experiments were five times done repeatedly keeping constant all parameters other than the concentration of calcium chloride solutions at the room temperature (22° ± 1°C). With these procedures the bead size-time data were obtained for calcium chloride solution of 25 mM, 50 mM, 75 mM, 100 mM and 125 mM.

RESULTS AND DISCUSSION

When the alginate drops were contacting with calcium ions, almost instantly calcium alginate formed on the surface of the sodium alginate drops and it maintained the shape it had. Even with 3 mM calcium chloride solution it could be practically possible. Its shape was probably spherical but sometimes skewed in the low concentration range less than 1.75% (W/V) sodium alginate concentration. More than 50% of the beads had a perfect spherical shape for 1.75% (W/V) alginate concentration which was satisfactory for this work. The concentration of calcium chloride solution also had some effect on the shape and homogeneity of beads. The calcium chloride solution higher than 1 M often made some bulges on the surface of beads during gel formation of sodium alginate. The bulges seemed to be formed through a rapid release of the bound water driven by the osmotic pressure within alginate network. The homogeneity of bead structure was also examined by an optical microscope. Higher concentration of calcium chloride caused some water cavities (typically 15 μm × 40 μm) within the gelled layer of beads. The water cavities were relatively frequent in the concentration range of higher than 125 mM calcium chloride. Accordingly, the kinetic study was done for 1.75% (W/V) alginate drops in the concentration range of 25-125 mM calcium chloride.

Calcium alginate gel exhibites the phenomenon of syneresis, or loss of bound water. It is the spontaneous release of bound water with contraction of the gel volume, indicating that the polyguluronic sequences of alginate are making the junction zones in the gel network with calcium ions. Since the junction zones form the frame work of alginate gel, the number of junction zones can be interpreted as being progressive indication of the gel formation. The change of bead volume must be corresponding to the number of junction zones and the bead size was considered to be a progressive indication of gel formation in the Calcium-Alginate-Gel system.

Fig. 3 gives the dimensionless radius of alginate bead as a function of the dimensionless time in the gel formation process of Calcium-Alginate-Gel. The radius change of alginate bead appeared to indicate the transient process of gel formation and the gelation was completed at the dimensionless radius of 0.815 with the volume contraction coefficient of 0.541. The time for complete gelling was determined as the time for the dimensionless radius to be 0.815. As shown in Fig. 3 the result shows that the transient behavior of gel formation can be shown well regardless of calcium chloride concentration, on the ϕ versus θ plot with the dimensionless time based on the time for complete gelling.

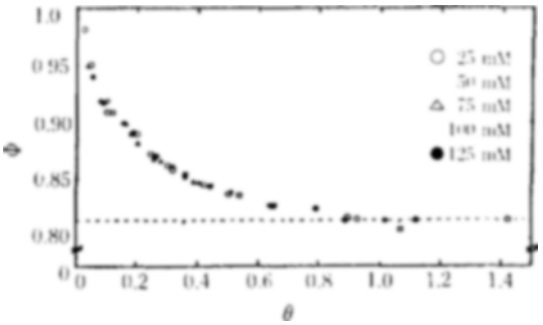

Fig. 3. Transient variation of dimensionless bead radius.

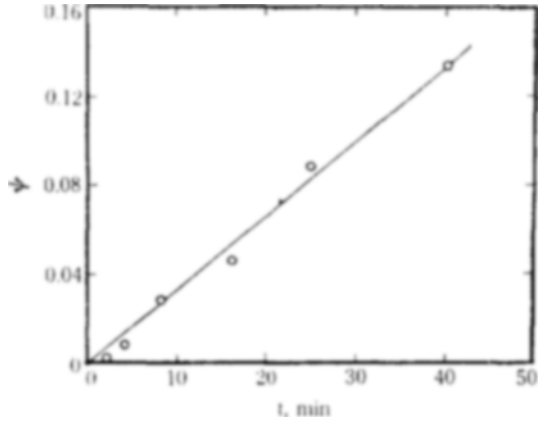

Fig. 4. ψ vs t plot for alginate gelation with 25 mM $CaCl_2$ solution.

In Fig. 4-8 the ψ versus t plots are shown to determine the effective diffusion coefficients of calcium ion in the gel formation process of sodium alginate with 25 mM, 50 mM, 75 mM, 100 mM and 125 mM calcium chloride. The experimental values of ψ were correlated linearly well with respect to the time t and the slopes of the ψ versus t plots were found by least-square method. The values of the slope were 3.34×10^{-3}

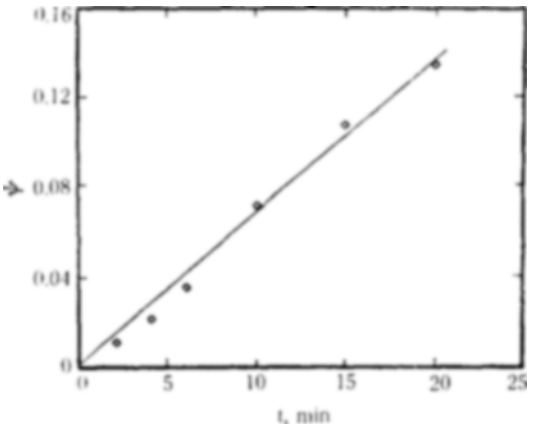

Fig. 5. ψ vs t plot for alginate gelation with 50 mM $CaCl_2$ solution.

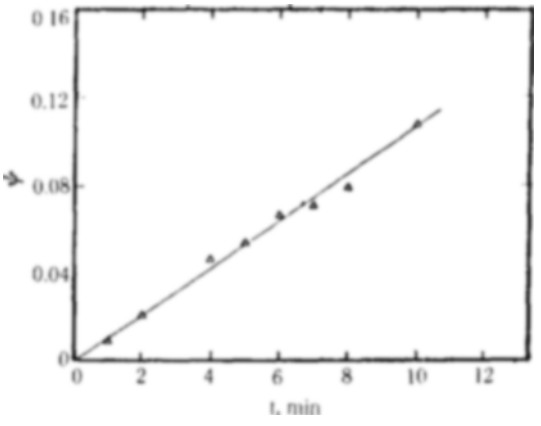

Fig. 6. ψ vs t plot for alginate gelation with 75 mM $CaCl_2$ solution.

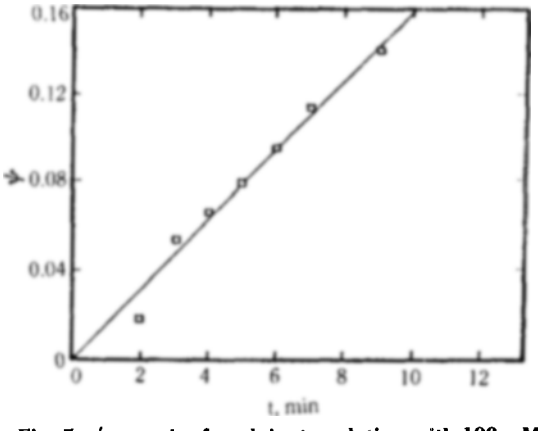

Fig. 7. ψ vs t plot for alginate gelation with 100 mM $CaCl_2$ solution.

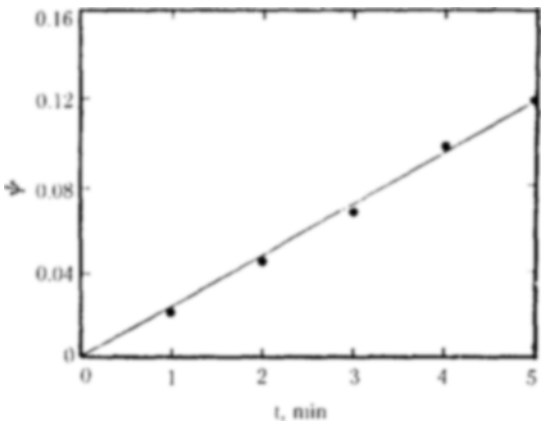

Fig. 8. ψ vs t plot for alginate gelation with 125 mM $CaCl_2$ solution.

min^{-1}, $6.81 \times 10^{-3} min^{-1}$, $1.07 \times 10^{-2} min^{-1}$, $1.60 \times 10^{-2} min^{-1}$ and $2.37 \times 10^{-2} min^{-1}$, giving the time for complete gelling 80.2 min, 39.4 min, 25.0 min, 16.8 min and 11.3 min, respectively for 25 mM, 50 mM, 75 mM, 100 mM and 125 mM calcium chloride. The slopes must be $2\ C_o D_e / pqr_o^2$ according to the proposed theoretical equation. The values of pq should be found to determine the effective diffusion coefficient of calcium ion. The main contribution to structural integrity of alginate gel comes from a buckled two-fold conformation of polyguluronate sequences whose the maximum calcium-binding capacity is known to be 150% of the stoichiometric equivalent in terms of "half egg-box" model [6,7]. The value of pq was estimated to be 8.35×10^{-6} moles Ca/cm^3 alginate solution for 1.75% (W/V) sodium alginate solution with 17.7% guluronic units since stoichiometrically 7.2% calcium is required based on the weight of sodium alginate for complete substitution [3]. With the above value of pq the effective diffusion coefficients of calcium ion were determined from the slopes of the ψ vs t plots and were 8.17×10^{-6} cm^2/min, $8.33 \times 10^{-6} cm^2/min$, 8.87×10^{-6} cm^2/min, 9.94×10^{-6} cm^2/min and 1.16×10^{-5} cm^2/min respectively for 25 mM, 50 mM, 75 mM, 100 mM and 125 mM calcium chloride solution.

Fig. 9 shows the effect of calcium chloride concentration on the effective diffusion coefficient of calcium ion within the calcium alginate layer during the gel formation process of sodium alginate. The effective diffusion coefficient appears to increase with increasing concentration of calcium chloride from the value of 8.17×10^{-6} cm^2/min at 25 mM calcium chloride solution. The effective diffusion coefficient at 25 mM calcium chloride solution is likely to be the effective diffusion coefficient at infinite dilution of calcium chlo-

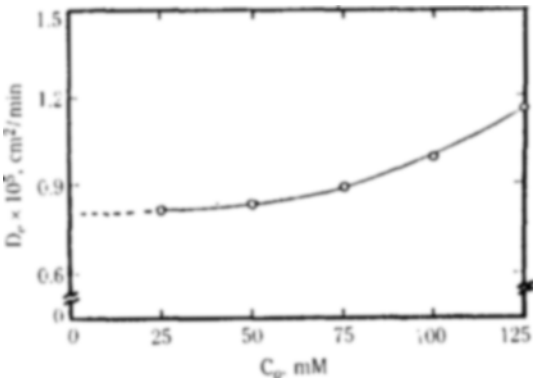

Fig. 9. Effect of $CaCl_2$ concentration on effective diffusion coefficient of calcium ion.

ride. Between 25 mM and 125 mM, the effective diffusion coefficient increased by about 42% compared to that at infinite dilution of calcium chloride. The increasing diffusion rate of calcium ion seemed to be probably due to macro-pore formation at higher concentration of calcium chloride because some water cavities were observed within the gelled layer by an optical microscope. The diameter of water cavity was on the order of 10 μm and three orders of magnitude larger than the maximal pore size of alginate gels measured by Klein et al. [8]. The macro-pore gave calcium ions freely-diffusing spaces and was believed to cause enhancement of diffusion rate of the calcium ions. The enhancement could be interpreted in terms of the fraction of macro-pore volume as follows [9],

$$\frac{D_e}{D_i}=\frac{\frac{2}{D_a}+\frac{1}{D_i}-2f\left(\frac{1}{D_a}-\frac{1}{D_i}\right)}{\frac{2}{D_a}+\frac{1}{D_i}-f\left(\frac{1}{D_a}-\frac{1}{D_i}\right)} \tag{13}$$

where D_a is the diffusion coefficient in the macro-pores, D_i is the diffusion coefficient in the micro-pores, f is the volume fraction of the macro-pores. The diffusion coefficient of calcium ion is probably two orders of magnitude smaller in the gelled layer than that in the water cavities, considering that the diffusivity of calcium ion is 8.04×10^{-4} cm^2/min in the pure water at 25°C [10]. Hence Eqn. (13) approximates to Eqn. (14).

$$\frac{D_e}{D_i}=\frac{1+2f}{1-f} \tag{14}$$

By the above equation, 42% enhancement of diffusion rate of calcium ion for 125 mM calcium chloride solution was estimated to be due to about 12% volume of macro-pores within the gelled layer. Accordingly, higher concentration than 125 mM calcium chloride could

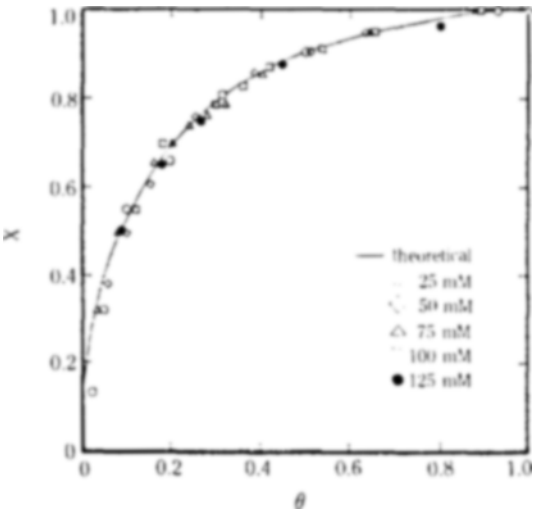

Fig. 10. Comparison of kinetic model to experimental data.

cause the inhomogeneity of discontinuous structure due to water cavities in the gel formation of sodium alginate.

In Fig. 10 the theoretical equation (12) was shown graphically with the fractional gelation x of sodium alginate bead as a function of dimensionless time to examine the validity of the proposed kinetic model. The fractional gelation was given as follows,

$$x=1-\gamma^3 \tag{15}$$

and the dimensionless time was based on the theoretical time for complete gelling. Agreement between experimental data and the theoretical equation was quite good for all five cases, considering the assumptions of dominant pore-diffusion kinetics and pseudo-steady-state approximation.

The kinetic model may be successfully extended to account for the effect of parameters, (C_o, R_o, p, q, etc.) on the transient behavior and complete gelling time of sodium alginate gelation. It also seems to given useful informations about the intermediate gelation of alginate beads. For example, by the x vs θ plot, 0.1 dimensionless time gives 53% conversion of alginate bead and 90% conversion is done during 0.5 dimensionless time in the Calcium-Alginate-Gel system. Such a useful information as intermediate gelation may be applicable to liquid-core bead formation for hybridoma cell entrapment.

CONCLUSION

In conclusion, it seems clear that there is a very

reasonable agreement between the gelation process of sodium alginate with calcium ion and the proposed kinetic equation based on shrinking-core model with assumptions of dominant pore-diffusion kinetics and pseudo-steady-state approximation. The kinetic model may be successfully extended to account for the transient behavior and complete gelling time as well as some useful information of the gelling conditions in the process of Ca-Alginate-Gel.

ACKNOWLEDGEMENT

The author gratefully acknowledges the financial support of The Ministry of Education of Korea and deeply appreciates Professor Janice A. Phillips for her advice on this study done at Bioprocessing Institute of Lehigh University, Bethlehem, PA, U.S.A..

NOMENCLATURE

- C : conc. of Ca ion in the gelled layer, [mol/l]
- C_o : conc. of Ca ion at the outer surface of the gelled layer, [mol/l]
- D_a : diffusion coefficient of Ca ion in the macro-pores, [cm^2/min]
- D_e : effective diffusion coefficient of Ca ion in the gelled layer, [cm^2/min]
- D_i : diffusion coefficient of Ca ion in the micro-pores, [cm^2/min]
- f : volume fraction of the macro-pores in the pore volume, [dimensionless]
- N : transferred moles of Ca ion, [mol/min]
- p : composition of sodium alginate in the solution, [g/cm^3]
- q : moles of Ca ion required for complete gelation based on the weight of sodium alginate, [mol/g]
- r : radial distance from the center of a bead, [cm]
- r_c : radial distance of the inner surface of the gelled layer from the center of a bead, [cm]
- R : radius of a spherical bead, [cm]
- R_o : initial radius of a spherical bead, [cm]
- t : gelling time, [min]
- W : weight of sodium alginate, [g]
- x : fractional gelation defined by Eqn. (15), [dimensionless]

Greek Letters

- γ : dimensionless core radius defined by Eqn. (10), [dimensionless]
- ε : gelled layer shrinkage defined by Eqn. (11), [dimensionless]
- θ : dimensionless time based on complete gelling time, [dimensionless]
- ϕ : dimensionless bead radius defined by Eqn. (9), [dimensionless]
- ψ : dimensionless variable defined by Eqn. (12), [dimensionless]

REFERENCES

1. Haug, A., Larsen, B. and Smidsrød, O.: *Acta Chem. Scand.*, **21,** 691 (1967).
2. Kohn, R. and Furda, I.: *Acta Chem. Scand.*, **22,** 3098 (1968).
3. Kelco Technical Bulletin: "Algin/Hydrophilic Derivatives of Alginic Acid", 2nd ed., Kelco, San Diego, Cal., 25 (1979).
4. Scott, C.D.: *Enzyme Microb. Technol.*, **9,** 66 (1987).
5. Carberry, J.J.: "Chemical & Catalytic Reaction Engineering", Prentice-Hall, Englewood Cliffs, NJ, 319 (1976).
6. Rees, D.A.: *Advan. Carbohydrate Chem. Biochem.*, **24,** 267 (1969).
7. Morris, E.R., Rees, D.A. and Thom, D.: *Carbohydrate Research,* **66,** 145 (1978).
8. Klein, J., Stock, J. and Vorlop, K.D.: *Eur. J. Appl. Microbiol. Biotechnol.*, **18,** 86 (1983).
9. Cussler, E.L.: "Diffusion", Cambridge University Press, Cambridge, NY, 186 (1984).
10. Anghileri, L.J. and Anghileri, A.M.T.: "The Role of Calcium in Biological Systems", Vol. 1, CRC Press, Boca Raton, Fla., 17 (1980).