Diffusion Coefficients in Intrahollow Calcium Alginate Microcapsules

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Intrahollow calcium alginate microcapsules were prepared with sodium alginate (SA) of different concentrations. Using a well-mixed and temperature-controlled vessel, the diffusivities of glucose, lactose, tyrosine, glutamic acid, lysine, and phenylalanine from bulk solution into the microcapsules were investigated and the diffusion coefficients were also calculated. The results indicate that all these substrates tested can diffuse easily through the microcapsule. The combined diffusion coefficients (D_m) are 2%−12% smaller than the diffusion coefficients in pure water while the diffusion coefficients in the microcapsule membrane (D_1) are 50%−94% smaller than those. In all cases, the diffusion coefficients are hardly affected by SA concentrations ranging from 8 g·L^{-1} to12 g·L^{-1} used in preparing the microcapsules.

Introduction

The encapsulation of biocatalysts within intrahollow calcium alginate microcapsules is a widespread immobilization technique, which encloses the biocatalyst in an aqueous solution inside a semipermeable membrane microcapsule.[1] The significant advantages of this system include biocompatibility, simplicity, and low cost. Moreover, it has such a specific liquid core−membrane (intrahollow) structure that the contact between substrate and biocatalyst can be achieved in the liquid core of the microcapsule.[2,3] Since the gel membrane does introduce an additional mass transfer resistance, there is a mass transfer limitation in the microcapsule system. The substrates must diffuse through the gel membrane before they can contact with the catalysts or cells, and the products and/or CO_2 must be able to diffuse out of the membrane. Thus the pore size of the gel is a critical parameter in selecting a carrier for a particular immobilization process.[4]

Since many applications entail the diffusion of common substrates and products into and out of the immobilization carrier, the diffusivities of calcium alginate gel bead/microcapsule have been studied by many researchers. The effective diffusion coefficients for selected mono- and disaccharides and organic acids were determined in the calcium alginate beads with and without entrapped bacteria. The concentration, the uronic acid composition of the alginate, and the conditions under which the beads are prepared, including the pH and the temperature, can affect the diffusion rate of serum albumin out of beads.[5] For solutes with MW $< 2 \times 10^{-4}$, no reduction in diffusion coefficients was found compared with the free diffusion in water. Larger solutes, such as albumin, γ-globulin, and fibrinogen, could diffuse out of, but not into, the beads.[6] Yao et al.[7] studied the diffusion of glucose from bulk solution into intrahollow calcium alginate microcapsules. The purpose of this work is to determine the diffusion coefficients of several low molecular weight substrates from bulk solution into intrahollow calcium alginate microcapsules. The effect of sodium alginate (SA) concentration on diffusivity is also examined.

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Table 1. Radius and Membrane Thickness of Intrahollow Calcium Alginate Microcapsules

SA conc/g·L^{-1}	r_b/mm	r_a/mm	$r_b - r_a$/mm
8	1.69 ± 0.15	1.46 ± 0.20	0.237 ± 0.04
10	1.60 ± 0.15	1.38 ± 0.20	0.222 ± 0.05
12	1.53 ± 0.20	1.32 ± 0.25	0.210 ± 0.05

Experimental Section

Sodium alginate and sodium carboxymethylcellulose (CMC) were obtained from Shanghai Bioengineering Company (Shanghai, China). Calcium chloride ($CaCl_2$) and all substrates tested were of reagent grade. To prepare the intrahollow calcium alginate microcapsules, SA of three concentrations (8 g·L^{-1}, 10 g·L^{-1}, and 12 g·L^{-1}) was introduced as the anionic solution. The cationic solution was a mixture of $CaCl_2$ (100 g·L^{-1}) and CMC (12 g·L^{-1}) in the proportion 1:4. Here CMC was used as a nongelling polymer to modulate the viscosity and density of the cationic solution in order to ensure the spherical shape of the microcapsule. The $CaCl_2$/CMC solution, forced by a peristaltic pump, was dropped into a 200 mL well-stirred SA solution through an injection needle (3 mm internal diameter). A dropping height of 10 cm was used to ensure a spherical droplet. After 10 min of membrane formation, the microcapsules were filtrated, washed, and hardened for 10 min in 0.2 M $CaCl_2$ solution. Finally, the microcapsules were washed again to remove the excess $CaCl_2$. All of the above procedures were carried out at room temperature.

After the microcapsule surface had been dried with filter paper, the external radius r_b (±0.02 mm) of the microcapsule was measured with a caliper. To estimate the membrane thickness ($r_b - r_a$) (±1 μm), the microcapsules were cut in half and measured in four different locations on the membrane with a light microscope (XSP-18B optical microscope, Optical Instruments Corporation in Nanjing, China). The average value was used as the membrane thickness. The internal radius r_a (±0.005 mm) of the microcapsule can be obtained by subtracting the membrane thickness from the external radius. The radius, the membrane thickness, and the corresponding largest absolute error (using the average value as the basis) are listed in Table 1.

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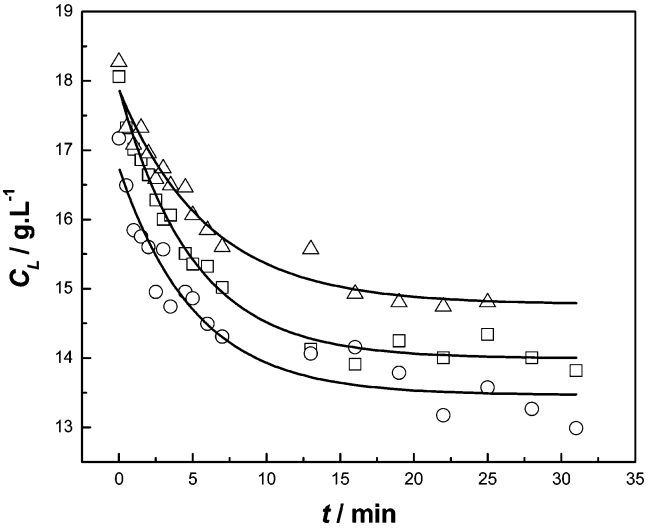

Figure 1. Diffusion of glucose from bulk solution into intrahollow calcium alginate microcapsules: □, 8 g·L^{-1} SA; ○, 10 g·L^{-1} SA; △, 12 g·L^{-1} SA.

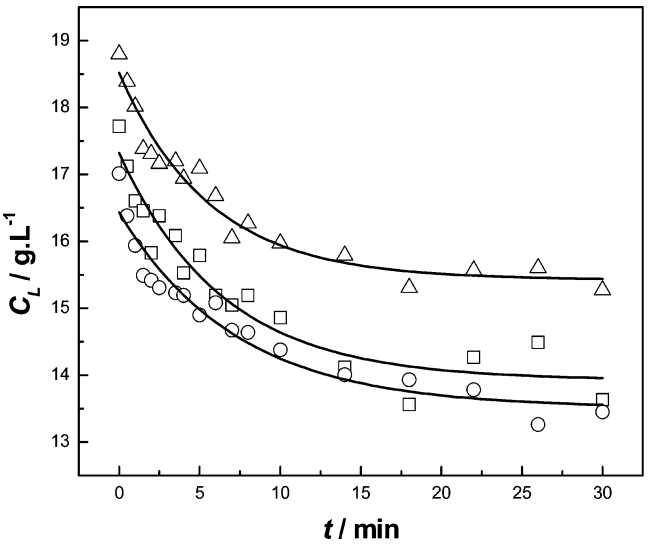

Figure 2. Diffusion of lactose from bulk solution into intrahollow calcium alginate microcapsules: □, 8 g·L^{-1} SA; ○, 10 g·L^{-1} SA; △, 12 g·L^{-1} SA.

The concentrations of glucose and lactose were measured by the dinitrosalicylic colorimetric method (DNS): immerse the test tube in a 95 °C water bath for 10 min and measure the absorbance at 550 nm with a precision of ±0.001.[8] The concentrations of amino acids were determined by using a spectrophotometer (Ultrospec 3300 pro UV/visible spectrophotometer, Amersham Biosciences, Piscataway, NJ) at their optimum absorption wavelength with a precision of ±0.001. The phenylalanine and tyrosine were analyzed at 280 nm, the glutamic acid was analyzed at 190 nm, and the lysine was analyzed at 364 nm. The reproducibility of the absorbance was better than ±0.002. Considering the error of the standard curve, the error of the substrate concentration is less than 2%.

A 200 mL well-stirred constant-temperature (25 ± 0.1 °C) vessel was introduced to the diffusion experiment. And a thousand substrate-free microcapsules were suspended in the substrate solution (initial concentration C_0). Due to the concentration gradient, the solute could diffuse into microcapsules and the concentration of the bulk solution would decrease. Therefore, the diffusion coefficients could be calculated from the change of the concentration.

In the case of diffusion from bulk solution into a solid sphere, the substrate concentration within the sphere C_r can be described by the following equation[4,6]

$$C_r = \frac{\alpha C_0}{1+\alpha}\left[1 + \sum_{n=1}^{\infty} \frac{6(1+\alpha)e^{-Dq_n^2 t/R^2}}{9 + 9\alpha + q_n^2\alpha^2} \frac{R}{r} \frac{\sin(q_n r/R)}{\sin q_n}\right] \quad (1)$$

where R is the radius of the sphere, r is the distance from the core of the sphere, t is time, and D is the diffusion coefficient. α is the ratio of the liquid volume to the solid sphere volume. It can be defined as follows:[4]

$$\alpha = \frac{V}{N\left(\frac{4}{3}\pi R^3\right)} \quad (2)$$

where V is the volume of the bulk solution and N is the number of spheres. q_n are the nonzero positive roots of the following equation[4,6]

$$\tan q_n = \frac{3q_n}{3 + \alpha q_n^2} \quad (3)$$

When it comes to the intrahollow microcapsule, the diffusivity of the substrate through the microcapsule membrane (diffusion coefficient D_1) is different from that through the liquid core of the microcapsule (diffusion coefficient D_2). Thus, the diffusion of substrate in an intrahollow microcapsule is more complex than that in a homogeneous gel bead. To solve this problem, the microcapsule is assumed as a sphere, and the diffusion coefficient D in eq 1 is substituted by the combined diffusion coefficient D_m, which is defined as the combination of the diffusivity of the microcapsule membrane and the diffusivity of the core solution.

Under the condition of adequate stirring, the liquid film resistance around the microcapsule can be ignored. Therefore, the concentration of substrate on the microcapsule surface is equal to that in the solution C_L. Then eq 1 can be rewritten as follows[4]

$$C_L = C_{r=R} = \frac{\alpha C_0}{1+\alpha}\left[1 + \sum_{n=1}^{\infty} \frac{6(1+\alpha)}{9 + 9\alpha + q_n^2\alpha^2} \exp\left(-\frac{D_m q_n^2 t}{R^2}\right)\right] \quad (4)$$

Under the experimental conditions, the radial diffusion in the microcapsules is assumed to be the exclusive diffusion and the diffusion resistance in the liquid film around them can be neglected. Our system could approximately satisfy the assumption of the mass transfer model. According to the model of Yao,[8] the relationship among D_m, D_1, and D_2 could be defined as follows[7]

$$D_m = \frac{r_b}{\frac{r_b - r_a}{D_1} + \frac{r_a}{D_2}} \quad (5)$$

where r_a is the internal radius while r_b is the external radius of the microcapsule. The solution used in our experiments is so dilute that the diffusion process in the liquid core of the microcapsule can be considered as that

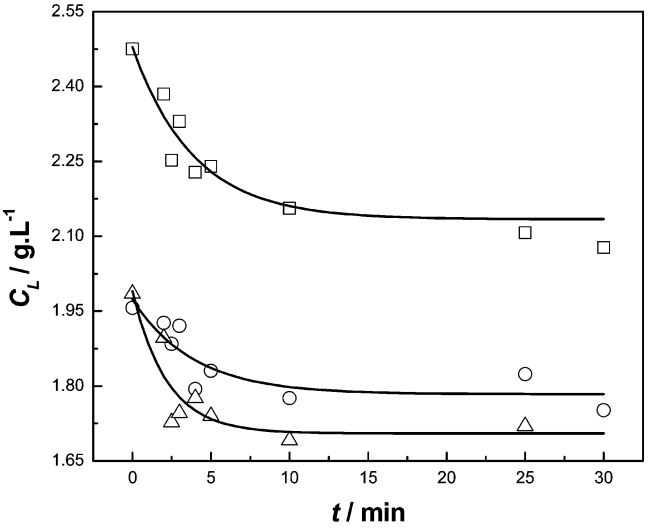

Figure 3. Diffusion of tyrosine from bulk solution into intrahollow calcium alginate microcapsules: □, 8 g·L^{-1} SA; ○, 10 g·L^{-1} SA; △, 12 g·L^{-1} SA.

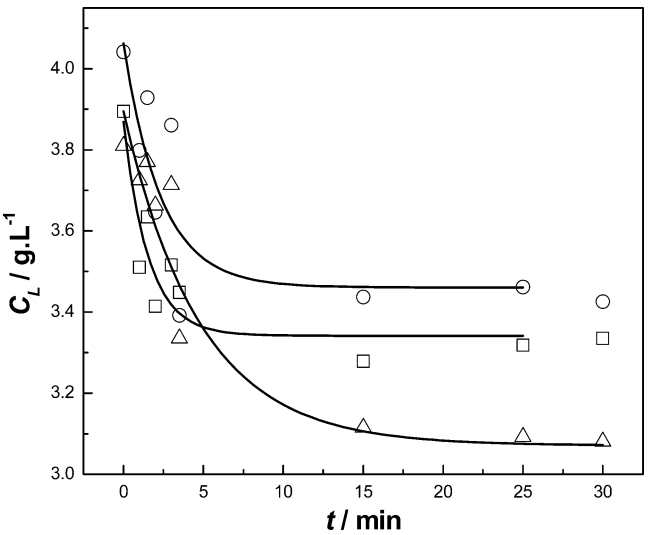

Figure 4. Diffusion of glutamic acid from bulk solution into intrahollow calcium alginate microcapsules: □, 8 g·L^{-1} SA; ○, 10 g·L^{-1} SA; △, 12 g·L^{-1} SA.

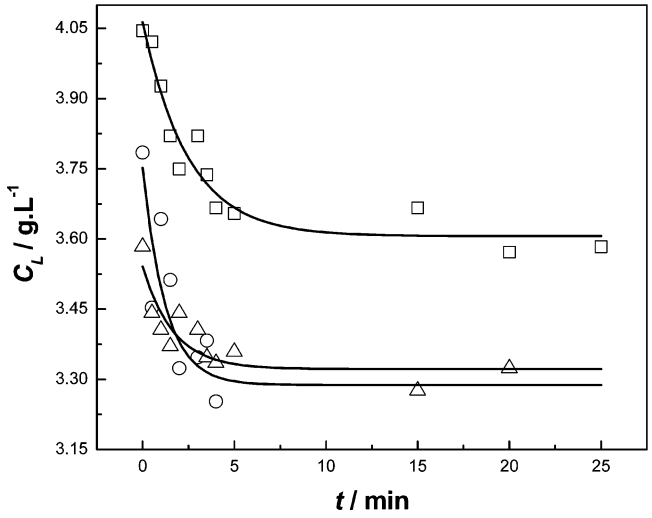

Figure 5. Diffusion of lysine from bulk solution into intrahollow calcium alginate microcapsules: □, 8 g·L^{-1} SA; ○, 10 g·L^{-1} SA; △, 12 g·L^{-1} SA.

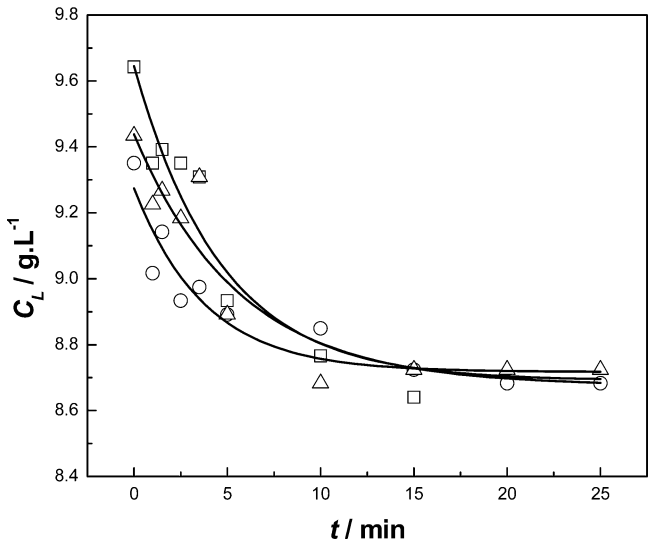

Figure 6. Diffusion of phenylalanine from bulk solution into intrahollow calcium alginate microcapsules: □, 8 g·L^{-1} SA; ○, 10 g·L^{-1} SA; △, 12 g·L^{-1} SA.

in pure water (diffusion coefficient D_w): $D_2 = D_w$. Obviously, D_m and D_1 can be calculated according to this model.

Results and Discussion

Several ecumenic substrates, such as glucose, lactose, glutamic acid, lysine, phenylalanine, and tyrosine, were involved. For each substrate, microcapsules prepared with SA solutions of three concentrations were used. All diffusion data were measured at least three times, and the average values are shown in Figures 1−6.

Diffusion experiments showed that the concentration of the bulk solution decreased remarkably after the addition of microcapsules, and then the decrease gradually slowed until it leveled off in 30 min. To calculate the diffusion coefficient, for each experiment, an assumed value of D_m was used together with eq 4 to calculate the concentration of the bulk solution. Then the result was compared with the experimental data to compute the standard deviation. The trial-and-error method was continued until the smallest standard deviation was found. The mean relative deviation of experimental data from the model is less than 2%, and the error of D_m among parallel experiments is about 4.2%. According to eq 5, the diffusion coefficients in the microcapsule membrane are calculated. The average values of D_m and D_1 are listed in Table 2.

The combined diffusion coefficients D_m of all the substrates are 88% to 98% as large as their diffusion coefficients in pure water. This means that these low molecular weight substrates tested can diffuse almost as freely from bulk solution into the microcapsules as in water. The diffusion coefficients in the microcapsule membrane D_1 are much smaller, about 50%−94% as large as those in pure water. Since the resistance to mass transfer in a liquid core solution is lower than that of the microcapsule membrane, it appears that the mass transport in the membrane is the main controlling factor of the diffusion.

The combined diffusion coefficients obtained in this work are similar to other investigators' results. Tanaka et al.[6] studied the diffusion of several solutes into and from the calcium alginate bead, and they found that the diffusion of glucose through calcium alginate beads is nearly the same as the diffusion in pure water. Dembczynski et al.,[9] who studied the diffusion from the hydrogel−membrane liquid−core capsules into the solution, reported the diffu-

Table 2. α, D_m, and D_1 of Substrates in Intrahollow Calcium Alginate Microcapsules at 25 °C

substrate	$10^{10}D_w$ / $m^2 \cdot s^{-1}$	SA conc / $g \cdot L^{-1}$	a	$10^{10}D_m$ / $m^2 \cdot s^{-1}$	$10^{10}D_1$ / $m^2 \cdot s^{-1}$
glucose	6.8	8	3.74	6.2	4.0
		10	4.47	6.2	3.8
		12	5.08	6.0	3.5
lactose	5.9	8	3.69	5.7	4.7
		10	4.41	5.3	3.5
		12	5.24	5.5	4.0
tyrosine	7.0	8	8.13	6.7	5.3
		10	9.81	6.8	6.2
		12	6.73	6.8	6.2
glutamic acid	7.6	8	6.00	7.5	6.8
		10	4.68	7.2	5.3
		12	4.06	7.3	6.0
lysine	6.9	8	8.24	6.7	5.7
		10	7.12	6.7	5.7
		12	12.70	6.8	6.5
phenylalanine	7.1	8	9.13	7.0	6.7
		10	13.77	7.0	6.7
		12	11.22	6.8	5.7

sion coefficient of glucose is 6.6×10^{-10} $m^2 \cdot s^{-1}$, just a little larger than our result. This suggests that the diffusivity of a particular substrate in the investigated microcapsules is of the same range as that in gel beads. The values of combined diffusion coefficients all approach their diffusion coefficients in pure water, which confirms that the intrahollow calcium alginate microcapsule, as an immobilization carrier, has good mass transfer property.

To contrast the diffusivities of microcapsules prepared with SA solutions of different concentrations, three kinds of concentrations (8 $g \cdot L^{-1}$, 10 $g \cdot L^{-1}$, and 12 $g \cdot L^{-1}$) were used for each substrate. However, for a given substrate, the D_m and D_1 values of three kinds of microcapsules are very similar. This means that the influence of SA concentration in a certain range on the diffusion process is slight. Such behavior can be explained by two reverse factors. An increase in polymer concentration will favor a smaller microcapsule and a thinner membrane, which increases D_m, but will also result in a more dense gel structure, which decreases D_m. The two factors compete with each other and finally result in a similar combined diffusion coefficient.

Conclusion

Substrates of low molecular weight, such as glucose, lactose, and amino acids, can diffuse nearly freely from bulk solution into intrahollow calcium alginate microcapsules. By the mathematical model, the combined diffusion coefficients of the substrates are about 2%−12% smaller than their diffusion coefficients in pure water, and the diffusion coefficients in the microcapsule membrane are 6%−50% smaller than those. This result means that, as an immobilization carrier, the microcapsule has good diffusivity. For any substrate, when the concentration of SA ranges from 8 $g \cdot L^{-1}$ to 12 $g \cdot L^{-1}$, the diffusion coefficients are similar.

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