Diffusion Characteristics of Solutes with Low Molecular Weight in Sodium Alginate/Cellulose Sulfate−$CaCl_2$/ Poly(methylene-*co*-guanidine) Capsules

Li-Yang Zhang, Shan-Jing Yao,* and Yi-Xin Guan

Department of Chemical and Biochemical Engineering, Zhejiang University, Hangzhou 310027, People's Republic of China

Three kinds of poly(methylene-*co*-guanidine) (PMCG)−sodium alginate/cellulose sulfate capsules were prepared by using PMCG concentrations of mass percent volume of 0.2%, 0.5%, and 1.5%, respectively. The diffusivities of nine solutes with low molecular weight in the above capsules were measured. The diffusion coefficients of the solutes through the capsular membrane (D_1) and the combined diffusion coefficients between the capsular membrane and the intracapsular solution (D_m) were determined by established mathematical models. The calculated results were in good agreement with the experimental data, indicating that the mathematical model could well describe the diffusion characteristics in this capsule. D_1 is related to the PMCG concentration in encapsulation, and D_m has little relationship with the PMCG concentation. D_1 increased with the increase in PMCG concentration from mass percent volume of 0.2% to 1.5%, whereas D_m varied over a small range with the increase in PMCG concentration. The results of $D_1 \ll D_m$ indicated that the resistance in mass transfer focused on the capsular membrane and the diffusion through the capsular membrane is the controlling step in mass transfer.

1. Introduction

Encapsulation is designed to entrap bioactive materials such as enzymes or cells within a semipermeable membrane which should allow the free exchange of molecules important for cell survival and function such as nutrients and oxygen while retaining the larger molecular weight encapsulant. As one of the most important immobilizing techniques, it has been applied in the pharmaceutical, medical, food, and other industries.[1−4]

Obviously, the permeability of a capsule is one of its most important properties and will determine whether it can be applied in organ transplantation, cell culture, and other fields. The property of molecular weight cutoff (MWCO) is often used to evaluate the permeability of capsules. However, MWCO can only show the exclusion abilities of capsules for solutes with various molecular weights (MWs) and fails to describe the diffusion characteristics of solutes with MWs smaller than MWCO. Moreover, for hollow capsules the diffusion of solutes with small molecular weight (MW < MWCO) in membranes differs markedly from that in the solution inside of the capsule due to their different structures. So it is interesting to establish a suitable mathematical model to quantitatively describe the diffusion of small solutes in the capsule membrane and in the solution inside of the capsule. Though there have been some studies concerning diffusion models in gel beads,[5−7] it is still difficult to select a proper one because of few mathematical models available. Yao et al.[8] proposed an improved mathematical model for calculating the diffusion coefficient of solutes in NaCS−PDMDAAC capsules (sodium cellulose sulfate−poly(dimethyl-diallyl-ammonium chloride)).

Recently, a new multicomponent capsular system which is formed by a polyelectrolyte complex of sodium alginate (SA) and cellulose sulfate (CS) with poly(methylene-*co*-guanidine) (PMCG) in the presence of Ca^{2+} has been developed by Wang et al.[9−13] It allows the independent modification of parameters critical for immunoisolation and cell survival such as mechanical strength and membrane permeability in contrast to binary polyelectrolyte capsular systems including poly-L-lysine-alginate capsules. It has been successfully used to encapsulate islets to treat diabetic mice and shows a prosperous future in biotechnology fields. Wang et al.[9] studied the permeability of the capsule by determining its molecular weight cutoff. However, MWCO can only describe the capsular permeability roughly, so it is necessary to understand the capsular permeability comprehensively and accurately.

The aim in the present work is to evaluate the diffusion characteristics of different PMCG-coated SA/CS capsules prepared with different PMCG concentrations. A mathematical model developed by Yao et al.[8] will be used to calculate the combined diffusion coefficient in different PMCG−SA/CS capsules and the diffusion coefficient in the corresponding capsular membrane of small solutes (MW < MWCO).

2. Materials and Methods

2.1. Material. SA was purchased from Sigma Chemical Co. (St. Louis, MO). PMCG was purchased from Scientific Polymer Products Co. (Ontario, NY), and CS was prepared by the Institute of Bioengineering, Zhejiang University (China). All other chemicals were of reagent grade.

2.2. Preparation of Capsules. A mixture solution of mass percent volume of 2% SA in water and 2.5% CS in water was extruded dropwise through a syringe 7-gauge needle connected to an air pressure droplet generator at a pressure of 172 kPa. The drops were collected in a solution of mass percent volume of 1% $CaCl_2$ in water and reacted for 30 min to form the gel beads. After being washed with

* Corresponding author. Tel: 0086-571-87951982. E-mail: yaosj@che.zju.edu.cn.

10.1021/je0256075 CCC: $25.00
Published on Web 04/25/2003

deionized water, the gel beads were coated with mass percent volume of 0.2% to 5% PMCG (pH 6.0) by stirring for 5 to 20 min in PMCG solution, and the residual PMCG was removed by washing three times with deionized water. The microcapsules were subsequently treated with 50 mM sodium citrate for 6 min to liquefy the SA−CA gel inside the capsule. Finally the beads became capsules−hollow spheres. Then these capsules were washed with deionized water again and stored.

2.3. Capsule Diameter and Membrane Thickness. The volume of a group of capsules was measured to obtain the average capsular diameter (±0.02 mm). The membrane thickness (±1 μm) was determined under a microscope (XSP−18B optical microscope, Optical Instruments Corp. in Nanjing, China) by slicing the capsules after they were frozen.

2.4. Analysis. The concentration of glucose or lactose was measured with the dinitrosalicylic (DNS) colorimetric method.[14] The test tubes were immersed in a 95 °C water bath for 10 min to develop the characteristic red-brown color. The absorbance was measured at 550 nm after cooling with a precision of ±0.001. Ethanol or lactic acid content was measured by gas chromatography (CC-102, Analysis Device Corp. in Shanghai, China). The reproducibility of measurement was better than ±0.001.

The concentrations of amino acids were determined by spectrophotometer (722; Third Analysis Devices Corp. of Shanghai, China) at different wavelengths with a precision of ±0.001. The concentrations of L-tryptophan, L-phenylalanine, and L-tyrosine were analyzed at 280 nm, L-glutamic acid at 190 nm, and L-lysine at 364 nm.

2.5. Measurement of the Diffusion Coefficient in PMCG-Coated SA/CS Capsules. The measurement of the diffusion coefficient[15] was made in a jacketed vessel while stirring well with the temperature at (25 ± 0.1) °C. V milliliters of solution with the initial concentration of C_0 was in the vessel. A definite amount of solute-free capsules were rapidly added to the solution. The solute concentration in bulk solution would be decreased with time because the solute diffused from bulk solution into the capsules. By measurement of the concentration variation with time, the diffusivity can be determined. In the initial 5 min, a sample from bulk solution should be taken every 30 s. After that, the time intervals could be lengthened. After enough time, the concentration in bulk solution and the concentration inside the capsules would be close to the equilibrium.

2.6. Diffusion Coefficient Calculation. The relationship of concentration in bulk solution and time for solid spheres is described as follows by Crank:[16]

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

where C_L is the concentration of solute in bulk solution, C_0 is the initial concentration in bulk solution, R is the radius of a capsule, t is time, and D is the diffusion in a solid sphere. α is defined as follows:

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

where V is the volume of bulk solution and N is the number of spheres. q_n is the nonzero positive roots of the following equation:

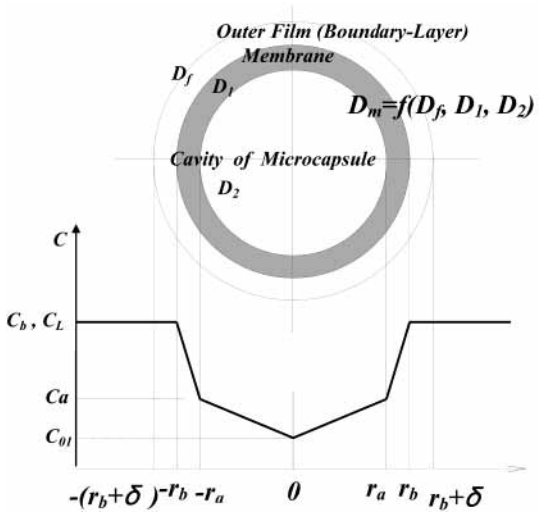

Figure 1. Diffusion of solutes in the PMCG−SA/CS capsule.

$$\tan q_n - \frac{3q_n}{3 + \alpha q_n^{\,2}} = 0 \quad (3)$$

Obviously, the diffusion coefficient D can be obtained by eq 1 according to the data of variation of concentration in bulk solution with time. However, eq 1 can only be suitable to the homogeneous solid beads and not to hollow capsules (spheres) where the diffusion coefficient of solute in the membrane is different from that in the intracapsule solution. In the mathematical model proposed by Yao et al.,[8] it was assumed that a hollow capsule is a quasi-solid bead. D can be replaced by D_m which is defined as the combined diffusion coefficient in the capsular membrane and intracapsular solution. Then eq 1 becomes

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

Just as for the NaCS−PDMDAAC capsule,[8] the diffusion process of solute in the PMCG-coated SA/CS capsules can also be shown in Figure 1, where r_a is the internal radius of the capsule, r_b is the external radius, D_f is diffusion coefficient in the outer liquid film around the capsule, D_1 is the diffusion coefficient in the capsule membrane, D_2 is the diffusion coefficient in the intracapsule solution, δ is the outer liquid film thickness, C_a is the concentration of solute in the internal surface of the capsule, and C_b is the concentration of solute in the external surface of the capsule.

At the condition of stirring well, the diffusion resistance in the liquid film can be neglected. D_m should satisfy the following conditions: If the beads are homogeneous solid spheres, $r_a = 0$, and thus $D_m = D_1$. If $r_b - r_a \to 0$, then $D_m = D_2$. If $D_1 = D_2$, then $D_1 = D_m = D_2$. If $D_1 \gg D_2$, the controlling step is the diffusion in the intracapsule solution and D_m mainly depends on D_2. If $D_1 \ll D_2$, the controlling step is the diffusion in the membrane and D_m mainly depends on D_1.

According to the theory of mass transfer, the relationship among D_m, D_1, and D_2 could be described as follows:

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

Table 1. Diameter, *D*, and Membrane Thickness, MT, of Capsules Prepared at Different Concentrations of PMCG

mass percent volume of PMCG/%	*D*/mm	MT/μm
0.2	3.02	27 to 40
0.5	2.51	43 to 55
1.5	2.21	86 to 100

When the liquid film resistance is neglected, eq 5 is simplified as

$$D_1 = \frac{r_b - r_a}{\frac{r_b}{D_m} - \frac{r_a}{D_2}} \quad (6)$$

where $r_b - r_a$ is the membrane thickness. In our experiments, the bulk solution is so dilute that the diffusion process in the intracapsule solution can be considered as that in pure water, that is, $D_2 = D_w$, where D_w is the diffusion coefficient of solute in pure water. Thus, D_m and D_1 can be calculated by eq 4 and eq 6, respectively.

This mathematical model has been applied to calculate the diffusion coefficients of some solutes in the NaCS/PDMDAAC capsule system, and satisfying results were obtained.

3. Results and Discussion

3.1. Effect of the PMCG Concentration on the Properties of PMCG-Coated SA/CS Capsules. To study the influence of the capsule properties on diffusivity, three kinds of capsules were prepared with mass percent volume of 0.2%, 0.5%, and 1.5% PMCG solution in water, respectively. As shown in Table 1, the diameter of the capsules decreased markedly whereas the membrane thickness increased distinctly with the increase in PMCG concentration from mass percent volume of 0.2% to 1.5%. Mechanical strength is closely dependent on membrane thickness. So mechanical strength was also enhanced with the increase in PMCG concentration. The electrostatic interaction of PMCG with SA/CS gel beads belongs to diffusion control. The reaction velocity is increased with the increase in PMCG concentration so that the membrane thickness will be larger in the same reaction time. Meanwhile, the capsules with thinner membranes are inclined to swell during storage so as to slightly increase the diameter.

3.2. Determination of the Diffusion Coefficient in PMCG-Coated SA/CS Capsules. The diffusion processes of different solutes in the capsules prepared at three different PMCG concentrations of mass percent volume of 0.2%, 0.5%, and 1.5% are shown. These solutes with low molecular weight are either substrates of microorganisms or metabolites including glucose (Figure 2), lactose (Figure 3), lactic acid (Figure 4), ethanol (Figure 5), L-glutamic acid (Figure 6), L-lysine (Figure 7), L-tryptophan (Figure 8), L-phenylalanine (Figure 9), and L-tyrosine (Figure 10). All data were measured repeatedly at least three times, and the average values are shown in the figures. The mean relative deviation of the experimental data from the model is 3.41%.

As shown in the figures, the solute concentrations in the bulk solutions decreased remarkably with addition of solute-free capsules. Then this decreasing trend gradually slowed with time and was close to equilibrium after 30 min. Moreover, the calculated results of variations of concentration in bulk solution by the developed model were in good agreement with the experimental data. This showed that the developed model could well describe the diffusivities in hollow capsules with homogeneous membranes.

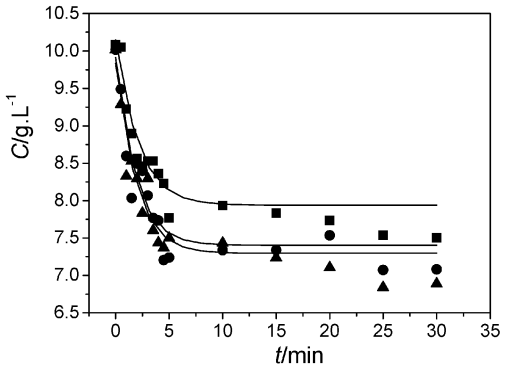

Figure 2. Diffusion of glucose in PMCG−SA/CS microcapsules with different properties at 25 °C: ■, 1.5% PMCG; ●, 0.5% PMCG; ▲, 0.2% PMCG; solid lines are calculated by eq 4.

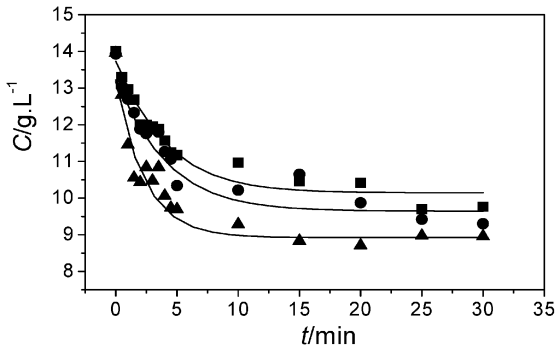

Figure 3. Diffusion of lactose in PMCG−SA/CS microcapsules with different properties at 25 °C: ■, 1.5% PMCG; ●, 0.5% PMCG; ▲, 0.2% PMCG; solid lines are calculated by eq 4.

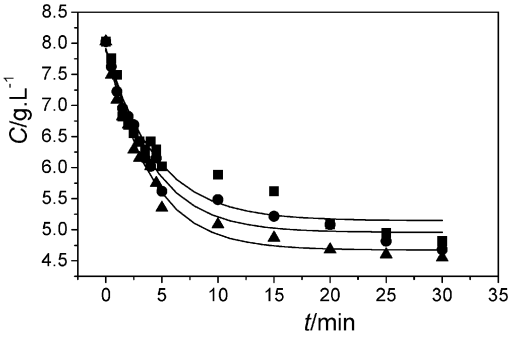

Figure 4. Diffusion of lactic acid in PMCG−SA/CS microcapsules with different properties at 25 °C: ■, 1.5% PMCG; ●, 0.5% PMCG; ▲, 0.2% PMCG; solid lines are calculated by eq 4.

The diffusion coefficients of nine solutes in pure water[17] are shown in Table 2. The values of α obtained from the experiments are listed in Table 3. The combined diffusion coefficients D_m and diffusion coefficients in the capsular membrane D_1 of nine solutes in three kinds of capsules are also listed in Table 2; D_m and D_1 were calculated by eq 4 and eq 6, respectively. D_1 increased distinctly with raising of the PMCG concentration from mass percent volume of 0.2% to 1.5%. These results indicated that the increasing membrane thickness with the raising of PMCG concentration resulted in a looser membrane structure and decreased the mass transfer (or diffusion) resistance. But the concentration of PMCG showed an uncertain influence on D_m. D_m in the capsule prepared with 0.5% PMCG is the largest, and the ranking of D_m at different PMCG concentrations is D_m (0.5%) > D_m (1.5%) > D_m (0.2%). This may indicate that above a certain PMCG concentration, the membrane thickness becomes thick enough to counteract the increase in diffusion rate.

Table 2. Diffusion Coefficients of Solutes in PMCG−SA/CS Capsules Obtained with Different Concentrations of PMCG at 25 °C

		0.2%		0.5%		1.5%	
solutes	D_w	D_m ($\times 10^{10}$) $m^2 \cdot s^{-1}$	D_1 ($\times 10^{10}$) $m^2 \cdot s^{-1}$	D_m ($\times 10^{10}$) $m^2 \cdot s^{-1}$	D_1 ($\times 10^{10}$) $m^2 \cdot s^{-1}$	D_m ($\times 10^{10}$) $m^2 \cdot s^{-1}$	D_1 ($\times 10^{10}$) $m^2 \cdot s^{-1}$
glucose	6.73	4.51	0.280	4.69	0.540	4.22	0.796
lactose	5.87	4.20	0.342	4.23	0.522	4.15	0.950
lactic acid	16.7	9.89	0.504	10.4	1.05	10.3	1.88
ethanol	12.8	6.43	0.271	6.72	0.514	6.59	1.00
L-glutamic acid	7.62	3.86	0.164	3.95	0.298	3.90	0.590
L-lysine	6.88	3.65	0.163	3.87	0.320	3.75	0.602
L-tryptophan	6.60	3.25	0.134	3.87	0.336	3.51	0.550
L-phenylalanine	7.05	3.86	0.178	4.08	0.349	3.78	0.597
L-tyrosine	6.95	3.05	0.126	3.22	0.200	3.11	0.399

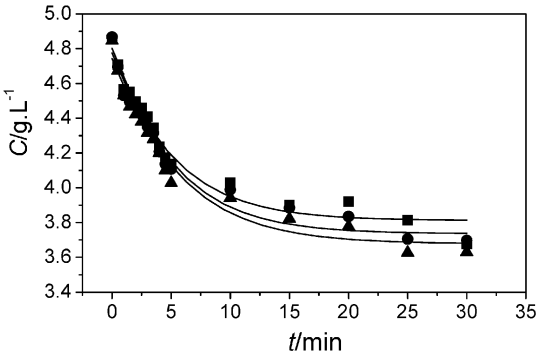

Figure 5. Diffusion of ethanol in PMCG−SA/CS microcapsules with different properties at 25 °C: ■, 1.5% PMCG; ●, 0.5% PMCG; ▲, 0.2% PMCG; solid lines are calculated by eq 4.

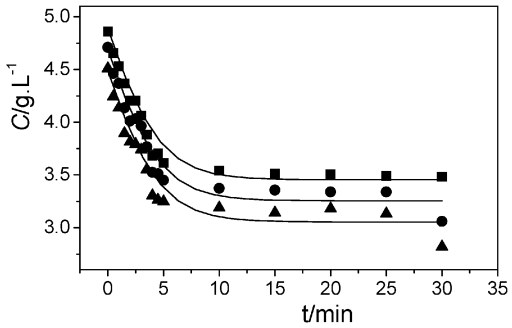

Figure 6. Diffusion of L-glutamic acid in PMCG−SA/CS microcapsules with different properties at 25 °C: ■, 1.5% PMCG; ●, 0.5% PMCG; ▲, 0.2% PMCG; solid lines are calculated by eq 4.

Table 3. Values of α

	α at the following mass percent volume of PMCG		
solutes	0.2%	0.5%	1.5%
glucose	2.47	2.70	3.60
lactose	2.21	2.43	3.08
lactic acid	1.48	1.69	1.71
ethanol	2.91	3.18	3.58
L-glutamic acid	2.62	3.01	3.22
L-lysine	1.32	1.49	1.57
L-tryptophan	1.89	2.03	2.28
L-phenylalanine	1.32	1.34	1.37
L-tyrosine	1.48	1.41	1.51

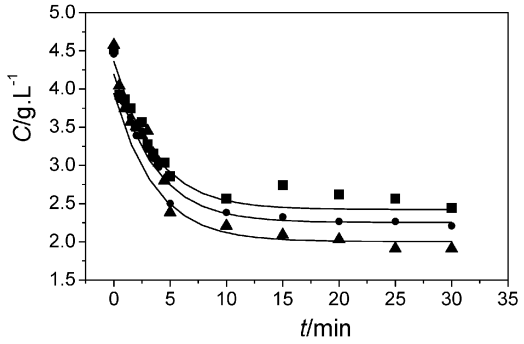

Figure 7. Diffusion of L-lysine in PMCG−SA/CS microcapsules with different properties at 25 °C: ■, 1.5% PMCG; ●, 0.5% PMCG; ▲, 0.2% PMCG; solid lines are calculated by eq 4.

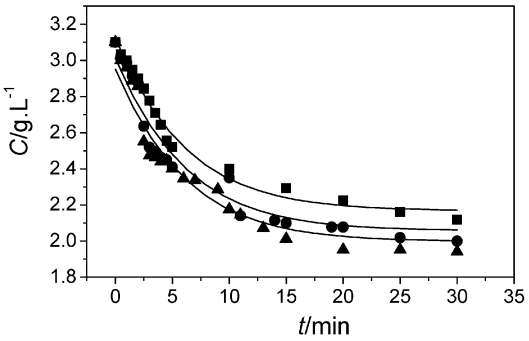

Figure 8. Diffusion of L-tryptophan in PMCG−SA/CS microcapsules with different properties at 25 °C: ■, 1.5% PMCG; ●, 0.5% PMCG; ▲, 0.2% PMCG; solid lines are calculated by eq 4.

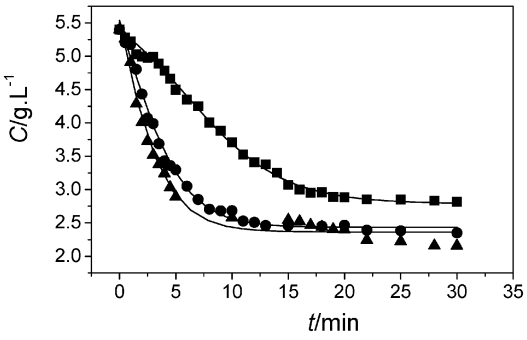

Figure 9. Diffusion of L-phenylalanine in PMCG−SA/CS microcapsules with different properties at 25 °C: ■, 1.5% PMCG; ●, 0.5% PMCG; ▲, 0.2% PMCG; solid lines are calculated by eq 4.

According to the reports of Yadav,[18] for interfacial polyreaction, the membrane of polymer will become thicker with an increase in monomer concentration, thus enhancing the diffusion rate in the membrane; the reasons may be as follows: The speed of polymerization can affect the molecular weight distribution and crystallinity of the polymer. The polyreaction with high speed is inclined to form an amorphous membrane which shows better diffusion characteristics than the crystalline polymer. The reason for this is that as the polymer molecules are precipitated on the interface at a high rate, they do not have enough time to form an ordinal crystal lattice and consequently tend to produce an amorphous membrane.

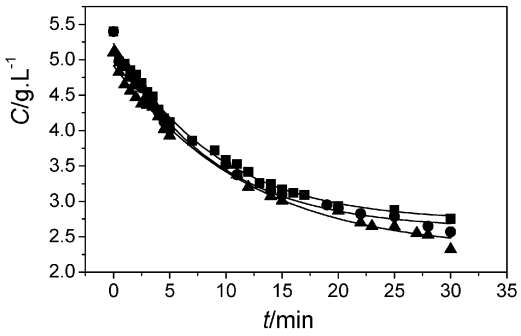

Figure 10. Diffusion of L-tyrosine in PMCG−SA/CS microcapsules with different properties at 25 °C: ■, 1.5% PMCG; ●, 0.5% PMCG; ▲, 0.2% PMCG; solid lines are calculated by eq 4.

Also, as seen in Table 2, the diffusion coefficients in the capsule membrane D_1 of the solutes were 2% to 16% of those in pure water and the combined diffusion coefficients D_m were 43% to 76% as large as those in pure water. This showed that the resistance in mass transfer focused on the capsular membrane and it might be the controlling step.

4. Conclusion

In summary, PMCG−SA/CS capsules with different properties were prepared with different PMCG concentrations at mass percent volume of 0.2%, 0.5%, and 1.5%. The combined diffusion coefficients D_m and the diffusion coefficients in the capsular membrane D_1 of small solutes were both calculated by the developed mathematical model. The results indicated the solutes with low molecular weight could freely diffuse into the capsules, but only the diffusion rate was smaller than that in pure water. Thus, the PMCG−SA/CS capsule has good diffusion characteristics and is suited to immobilize biomaterials such as cells and enzymes.

Literature Cited

(1) Cheng, W. T.; Chen, B. C.; Chiou, S. T.; Chen, C. M. Use of Nonautologous Microencapsulated Fibroblasts in Growth Hormone Gene Therapy to Improve Growth of Midget Swine. *Hum. Gene Ther.* **1998**, *9*, 1995−2003.

(2) Hortelano, G.; Al-Hendy, A.; Ofosu, F. A.; Chang, P. L. Delivery of Human Factor IX in Mice by Encapsulated Recombinant Myoblasts: A Novel Approach Towards Allogeneic Gene Therapy of Hemophilia B. *Blood* **1996**, *87*, 5095−5103.

(3) Alexakis, T.; Boadi, D. K.; Quong, D.; Groboillot, A.; O'Neill, E.; Poncelet, D.; Neufeld, R. J. Microencapsulation of DNA Within Alginate Microspheres and Crosslinked Chitosan Membranes for in Vivo Application. *Appl. Biochem. Biotechnol.* **1995**, *50*, 93−106.

(4) Groboillot, A. F.; Champagne, C. R.; Darling, G. D.; Poncelet, D.; Neufeld, R. J. Membrane Formation by Interfacial Cross-linking of Chitosan for Microencapsulation of *Lactococcus lactis*. *Biotechnol. Bioeng.* **1993**, *42*, 1157−1163.

(5) Merchant, F. J. A.; Margaritis, A.; Wallace, J. B. A Novel Technique for Measuring Solute Diffusivities in Entrapment Matrices Used in Immobilization. *Biotechnol. Bioeng.* **1987**, *30*, 936−945.

(6) Scott, D.; Woodward, C. A.; Thompson, J. E. Solute Diffusion in Biocatalyst Gel Beads Containing Biocatalysis and Other Additives. *Enzyme Microb. Technol.* **1989**, *11*, 258−263.

(7) Westrin, B. A.; Axelson, A. Diffusion in Gels Containing Immobilized Cells: A Critical Review. *Biotechnol. Bioeng.* **1991**, *38*, 439−446.

(8) Yao, S. J.; Cho, M. G. Diffusion Characteristics in Microcapsules. *Chin. J. Chem. Eng.* **1998**, *6* (2), 116−123.

(9) Wang, T. G. New Technologies for Bioartificial Organs. *Artif. Organs* **1998**, *22*, 68−74.

(10) Briššová, M.; LacÍk, I.; Powers, A. C.; Anilkumar, A. V.; Wang, T. Control and Measurement of Permeability for Design of Microcapsule Cell Delivery System. *J. Biomed. Mater. Res.* **1998**, *39*, 61−70.

(11) LacÍk, I.; Briššová, M.; Anilkumar, A. V.; Powers, A. C.; Wang, T. New Capsule With Tailored Properties for the Encapsulation of Living Cells. *J. Biomed. Mater. Res.* **1998**, *39*, 52−60.

(12) Hunkeler, D.; Prokop, A.; Powers, A. C.; Haralson, M.; DiMari, S.; Wang, T. A Screening of Polymers as Biomaterials for Cell Encapsulation. *Polym. News* **1997**, *22*, 232−240.

(13) Wang, T.; LacÍk, I.; Briššová, M.; Anilkumar, A. V.; Prokop, A.; Hunkeler, D.; Green, R.; Shahrokhi, K.; Powers, A. C. An Encapsulation System for the Immunoisolation of Pancreatic Islets. *Nat. Biotechnol.* **1997**, *15*, 358−362.

(14) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric Method for Determination of Sugar and Related Substances. *Anal. Chem.* **1956**, *28*, 350−356.

(15) Tanaka, H.; Matsumura, M.; Veliky, I. A. Diffusion Characteristics of Substrates in Ca−Alginate Gel Beads. *Biotechnol. Bioeng.* **1984**, *26*, 53−58.

(16) Crank, J. *The Mathematics of Diffusion*, 2nd ed.; Oxford University Press: London, 1975; p 93.

(17) Longsworth, L. G. Diffusion Measurement, at 25 °C, of Aqueous Solution of Amino Acids, Peptides and Sugars. *J. Am. Chem. Soc.* **1953**, *75*, 5705−5709.

(18) Yadav, S. K.; Khilar, K. C.; Suresh, A. K. Microencapsulation in Polyure Shell: Kinetics and Film Structure. *AIChE J.* **1996**, *42* (9), 2616−2626.

Received for review August 30, 2002. Accepted April 1, 2003. The authors are thankful for the financial support of the National Nature Science Foundation of China and the Foundation for University Key Teacher by the Ministry of Education.

JE0256075