Diffusion of Nicotinamide Adenine Dinuncleotide in Calcium Alginate Hydrogel Beads Doped with Carbon and Silica Nanotubes

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Alginate gel beads doped with nanotubes or nanoparticles were prepared using four kinds of dopants: carbon nanotubes (CNTs), silica nanotubes, graphite, or silica nanoparticles (SiO_2). The effective diffusion coefficients (D_e) of reduced nicotinamide adenine dinuncleotide (NADH) in hybrid gels were calculated using an unsteady-state model developed by Nguyen and Luong. The maximum D_e values of NADH in the four kinds of hybrid gels obey the following sequence: D_e (CNTs-alginate) > D_e (silica nanotubes-alginate) > D_e (SiO_2-alginate) > D_e (graphite-alginate). The effect of the dopant concentration on D_e was examined at five dopant concentration levels (0.3, 0.5, 0.9, and 1.25) g/L. For the CNTs-alginate and silica nanotubes-alginate gels, the maximum D_e values of NADH were obtained at 0.3 g/L and 0.9 g/L dopant concentrations, respectively. For the silica-alginate and graphite-alginate gels, the maximum D_e values of NADH were obtained at 0.5 g/L dopant concentration. The maximum D_e value of NADH in the CNTs-alginate gel is 50 % larger than the value for the pure alginate gel (D_{e0}). Owing to their more favorable diffusion characteristics, the doped alginate gels could be used as novel and effective immobilizing carriers for encapsulating biomolecules such as enzymes and cells.

Introduction

Calcium-alginate (Ca-alginate) hydrogel beads are one of the most commonly used carriers in the entrapment immobilization of biocatalyst owing to their significant advantages such as good biocompatibility, low cost, easy availability, and simplicity of preparation. However, some disadvantages are often associated with this carrier, including high biomolecule leakage, low mechanical strength, and serious swelling due to their open structure, large pore size, and high hydrophilicility.[1−3] These disadvantages significantly restrict the lifetime of these biomaterials and limit the application of alginate gels to high molecular weight compounds and whole cells or organelles.

To overcome these disadvantages, various methods have been proposed, including (1) covalent cross-linking with polymers, such as chitosan and poly(acrylic acid), and (2) coating the surface of alginate gel beads with other reagents, such as poly-L-lysine and glutaraldehyde.[4−7] However, these methods often suffer from the low efficiency, high toxicity, and complexity. [8]

Recently, Coradin and Livage[9] prepared a silica-alginate hybrid gel by impregnation of mesoporous silica particles with alginic acid solution. Compared to the pure Ca-alginate gel, the hybrid gel exhibited better stability upon aging and effectively limited enzyme leaching. Rege et al.[10] have prepared enzyme-containing polymer-carbon nanotubes (CNTs) composites by suspending CNTs and α-chymotrypsin (CT) in solutions of poly(methyl methacrylate) in toluene. The catalytic activity of CT-polymer-CNTs films was found to be higher than both CT-polymer and CT-polymer-graphite films.

In this paper, novel and efficient hybrid gels consisting of alginate gels doped with nanotubes or nanoparticles were prepared by incorporating nanotubes and nanoparticles into alginate gels. The nanotubes used in the present study included silica nanotubes and CNTs. The nanoparticles were silica (SiO_2) or graphite. Such novel hybrid gels might possess advantages such as better biocompatibility of the alginate and better stability of the mineral phase and also provide higher retention of biomolecules, improved mechanical properties, and in particular, better diffusion characteristics for substrates and products.

Many studies of the diffusion characteristics of pure Ca-alginate gel have been reported.[11−13] The diffusion properties of several substrates of varying molecular weights into and from the Ca-alginate gel beads were investigated by Tanaka et al.[11] They found that, for solutes with molar masses $< 2 \times 10^4$ g·mol^{-1}, there was no reduction in diffusion coefficients as compared to free diffusion in water. Larger solutes such as albumin, ρ-globulin, and fibrinogen could diffuse out of, but not into, the Ca-alginate gel beads. Chai et al.[12] have studied the diffusion of glucose, lactose, tyrosine, glutamic acid, lysine, and phenylalanine from bulk solution into Ca-alginate microcapsules. The results indicated that the effective diffusion coefficients (D_e) of the substrates were 2 % to 12 % smaller than values for pure water. The effective diffusion coefficients for selected mono- and disaccharides and organic acids were determined in homogeneous Ca-alginate gels with and without entrapped bacteria by Øyaas et al.[13] However, to the best of our knowledge, reports of the diffusion characteristic of components in alginate gel beads doped with nanotubes or nanoparticles have not been found.

Reduced nicotinamide adenine dinuncleotide (NADH) (709.4 g·mol^{-1}) and its oxidized form (NAD^+) are important coenzymes for more than 400 enzymatic redox reactions, in which they act as an electron and hydrogen donor or acceptor.[14,15] The redox rate of the immobilized enzyme is affected greatly by the diffusion characteristics of NADH in the immobilization carrier.

Using NADH as a model substrate, the diffusion characteristics of Ca-alginate gel beads doped with nanotubes or nanoparticles at different dopant concentrations were

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Table 1. Average Diameters (d) of Hybrid and Pure Alginate Gel Beads at Various Concentrations (C_d) of Dopant

C_d	d/mm				
g·L^{-1}	pure alginate	silica nanotubes-alginate	SiO_2-alginate	CNTs-alginate	graphite-alginate
0.00	2.73 ± 0.02				
0.30		2.41 ± 0.02	2.40 ± 0.03	2.54 ± 0.03	2.35 ± 0.02
0.50		2.38 ± 0.02	2.43 ± 0.03	2.58 ± 0.02	2.37 ± 0.02
0.90		2.46 ± 0.03	2.45 ± 0.03	2.38 ± 0.03	2.50 ± 0.02
1.25		2.62 ± 0.03	2.51 ± 0.02	2.63 ± 0.03	2.48 ± 0.02

investigated in the present study. A mathematical model developed by Nguyen and Luong[16] was employed to calculate the effective diffusion coefficients of NADH in the hybrid gels. The effect of the dopant concentration on the effective diffusion coefficient was also examined.

Experimental Section

Chemicals. Sodium alginate was purchased from Shanghai Tianlian Co. Carbon nanotubes were donated by Tsinghua University, and silica nanotubes were donated by Jilin University. Reduced nicotinamide adenine dinuncleotide (CAS Registry Number: 606-68-8) was obtained from Sigma. All other chemicals were reagent grade.

Preparation of Hybrid Alginate Gels. To prepare the hybrid alginate gel beads, a solution of sodium alginate (20 g/L) was mixed with each dopant, including silica nanotubes, CNTs, silica, and graphite in a series of concentrations (0.3, 0.5, 0.9, and 1.25) g/L. Each dopant was well-dispersed ultrasonically for 0.5 h prior to use. The inside and outer diameter of the CNTs are about 30 nm and 60 nm. For the silica nanotubes, the inside and outer diameter are around 25 nm and 50 nm. The sizes of the silica and graphite particles are about 120 nm and 150 nm, respectively.

The sol of the mixture of dopants-alginate was added dropwise through an injection needle (an i.d. of 0.7 nm) into a 0.2 mol/L $CaCl_2$ solution at a fixed dropping speed. A dropping height of 10 cm was used to ensure the formation of spherical droplets. The hybrid gel beads formed rapidly. After 3 h of aging, the beads were collected by filtration using Millipore microfiltration membranes (0.2 μm), rinsed with distilled water several times, and stored in the distilled water. All procedures were carried out at room temperature.

Gel-Bead Diameters. The gel-bead diameters were measured with vernier calipers after beads had been wiped dry with filter paper. The average diameters (d) of five kinds of gel beads and their corresponding uncertainties (see Supporting Information) are listed in Table 1.

Analytical Method. The concentrations of NADH were determined by using a UV−Vis spectrophotometer (U-2800, Hitachi, Japan) at 340 nm (optimum absorption wavelength). The error of the substrate concentration is less than ±1.5 μmol·L^{-1}.

Effective Diffusion Coefficients. A 50 mL constant-temperature (25 ± 0.1 °C) vessel was used for the diffusion experiments. About 2.5 mg of NADH was dissolved in 30 mL of a 0.1mol/L, pH 7.0, Tris-HCl buffer solution. At the beginning of each run, a known amount of hybrid gel beads was added rapidly into a well-stirred suspension, and 50 μL samples of solution were withdrawn from the bulk solution at specified time intervals. Due to the NADH concentration gradient, NADH diffuses into the gel beads, and the concentration of NADH in the bulk solution decreases. The effective diffusion coefficients are calculated from the change in the bulk solution concentration of NADH.

The hybrid gel beads can be assumed to be homogeneous spheres; the diffusion equation for a sphere can thus be applied. According to the unsteady-state model, the solute concentration within the sphere (C_r) can be described by eq 1:[16,17]

$$C_r = \frac{\alpha C_0}{1+\alpha} \times \left\{1 + \sum_{n=1}^{\infty} \frac{6\alpha(\alpha+1)\exp(-D_e q_n^{\,2} t/R^2)}{9 + 9\alpha + q_n^{\,2}\alpha^2} \frac{R}{r} \frac{\sin(q_n r/R)}{\sin q_n}\right\} \quad (1)$$

where R is the external radius of the sphere, r is the distance from the core, C_0 is the initial concentration of the bulk solution without gel beads, t is the time, and D_e is the effective diffusion coefficient in the sphere. α, the ratio of the liquid volume to the solid sphere volume, can be defined as follows:

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

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 equation:

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

With sufficient agitation, the liquid film resistance around the beads can be ignored. Under these conditions, the solute concentration just within the bead surface is equal to that in the bulk solution C. Equation 1 can then be expressed as[16]

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

The effective diffusion coefficients of NADH in the pure and hybrid alginate gel beads can be thus calculated using eq 4.

Results and Discussion

The diffusion characteristics of NADH in pure alginate gel and in four kinds of hybrid alginate gel beads including silica nanotube-alginate, SiO_2-alginate, CNTs-alginate, and graphite-alginate gel are investigated. To study the effect of the dopant concentration on the effective diffusion coefficient, for each hybrid gel, four kinds of gels with 0.3, 0.5, 0.9, and 1.25 g/L dopant concentrations were prepared. All diffusion data were measured at least three times, and the average values are shown in Figures 1 to 5.

As shown in Figures 1 to 5, the concentrations of NADH in the bulk solution decrease after the addition of hybrid

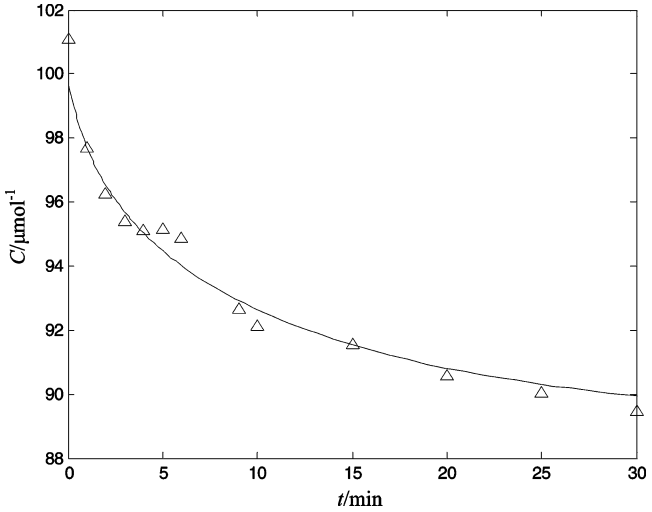

Figure 1. Diffusion of NADH from bulk solution into pure alginate beads.

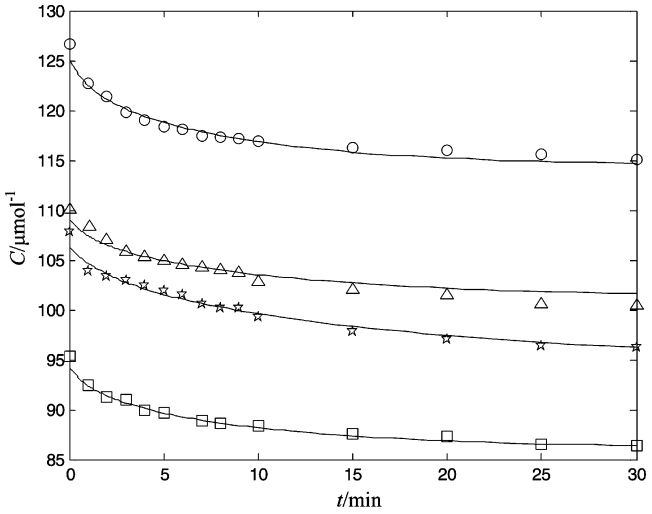

Figure 2. Diffusion of NADH from bulk solution into silica nanotubes-alginate beads with the different silica nanotubes concentrations: △, 0.3 g/L; □, 0.5 g/L; ○, 0.9 g/L; ☆, 1.25 g/L.

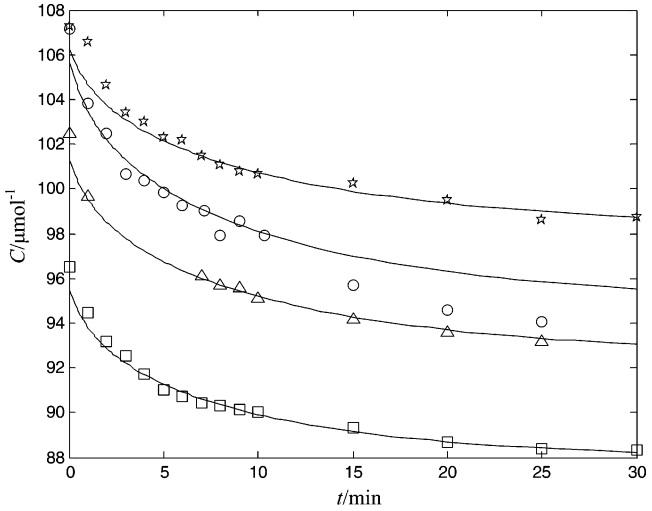

Figure 3. Diffusion of NADH from bulk solution into SiO_2-algiante beads with different SiO_2 concentrations: △, 0.3 g/L; □, 0.5 g/L; ○, 0.9 g/L; ☆, 1.25 g/L.

gel beads, and then the decreasing trends gradually slow down and level off after about 30 min.

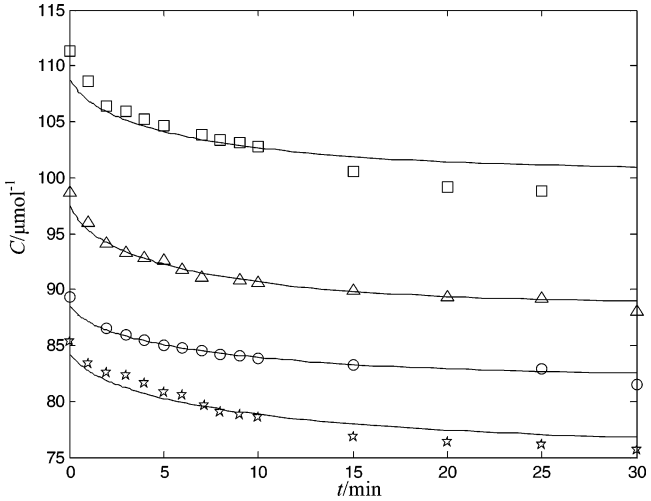

Figure 4. Diffusion of NADH from bulk solution into CNTs-alginate beads with different CNTs concentrations: △, 0.3 g/L; □, 0.5 g/L; ○, 0.9 g/L; ☆, 1.25 g/L.

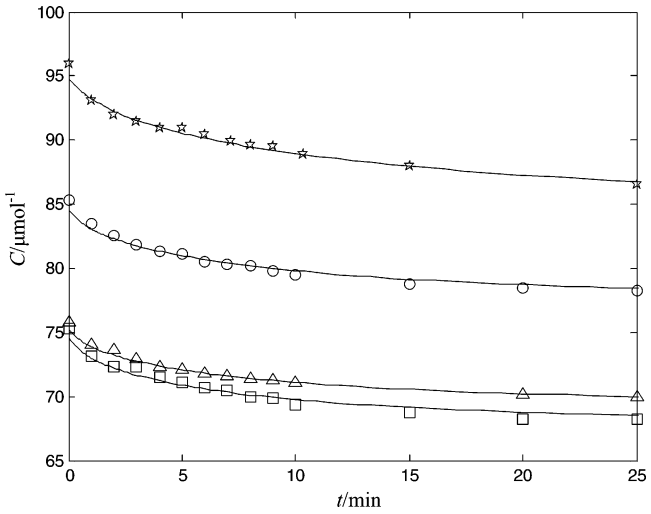

Figure 5. Diffusion of NADH from bulk solution into graphite-alginate beads with different graphite concentrations: △, 0.3 g/L; □, 0.5 g/L; ○, 0.9 g/L; ☆, 1.25 g/L.

To calculate the efficient diffusion coefficient, for each experiment, an assumed value of D_e is used together with eq 4 to calculate the concentration of NADH in the bulk solution. Then the result is compared with the experiment data to calculate the absolute error. The trial-and-error method is used until the absolute error is smaller than 0.0001 μmol·L^{-1}. The mean relative deviation of D_e among parallel experiments is about 10 %. The average values of D_e are listed in Table 2 (see also Supporting Information).

The curves in Figures 1 to 5 give the calculated results of the variation of NADH concentration in bulk solution with time by the unsteady-state model developed by Nguyen and Luong.[16] The good agreement between the calculated curves and the experimental results indicated that the model used could well describe the diffusion behavior of NADH in the doped alginate gels. The values of α, the effective diffusion coefficient of NADH in pure alginate gel (D_{e0}), the effective diffusion coefficients (D_e) of NADH in the four kinds of hybrid gels, and the ratios (D_e/D_{e0}) are listed in Table 2.

As shown in the Table 2, the D_e of NADH in each hybrid gel increases distinctly with an increase in the dopant concentration (C_d) at first, then reaches the maximum

Table 2. Effective Diffusion Coefficients (D_e and D_e/D_{e0}) of NADH in Alginate Gel Beads Doped with Nanotubes or Nanoparticles for an Alginate Concentration of 20 g·L^{-1} with Different Concentrations (C_d) of Dopant

hybrid gel	alginate concn / g·L^{-1}	C_d / g·L^{-1}	α	$D_e \times 10^{10}$ / m^2·s^{-1}	D_e/D_{e0}
pure alginate	20	0.00	7.45	1.84	
silica nanotubes-alginate	20	0.30	11.37	1.89	1.02
		0.50	9.34	2.08	1.13
		0.90	9.19	2.54	1.38
		1.25	6.77	1.11	0.60
SiO_2-alginate	20	0.30	9.31	1.81	0.98
		0.50	10.24	2.22	1.20
		0.90	7.78	2.16	1.17
		1.25	10.89	1.76	0.95
CNTs-alginate	20	0.30	8.89	2.81	1.52
		0.50	10.92	2.27	1.23
		0.90	11.61	2.10	1.14
		1.25	8.26	1.83	0.99
graphite-alginate	20	0.30	11.31	2.06	1.12
		0.50	9.66	2.14	1.16
		0.90	10.55	2.06	1.12
		1.25	8.06	1.41	0.76

value, and thereafter decreases with the further increase of C_d.

For the CNTs-alginate and silica nanotubes-alginate gels, the maximum D_e values of NADH are obtained at 0.3 g/L and 0.9 g/L dopant concentrations, respectively. While for silica-alginate and graphite-alginate gels, the maximum D_e values of NADH are both obtained at 0.5 g/L dopant concentration.

The maximum D_e values of NADH in the different hybrid gels obey the following sequence: D_e (CNTs- alginate) > D_e (silica nanotubes-alginate) > D_e (SiO_2-alginate) > D_e (graphite-alginate). The maximum D_e value of NADH in the CNTs-alginate is 50 % larger than the value for the pure alginate gel.

According to the Wei's simulation results[18] after the addition of CNTs to the polymer, the increase in the diffusion coefficient of the solute parallel to CNTs axis is about 30 % larger than the increase perpendicular to the tube axis in the CNTs-polymer composites. At low concentrations, the nanotubes are well-dispersed as individual nanotubes in the hybrid gels. NADH can diffuse parallel to the nanotube sidewall, resulting in the substantial increase of the D_e. As the concentration of nanotubes increases, nanotube ropes or bundles will form due to strong interactions. Hence, most of diffusion will take place perpendicular to the nanotube sidewall, which causes the decrease of the D_e.

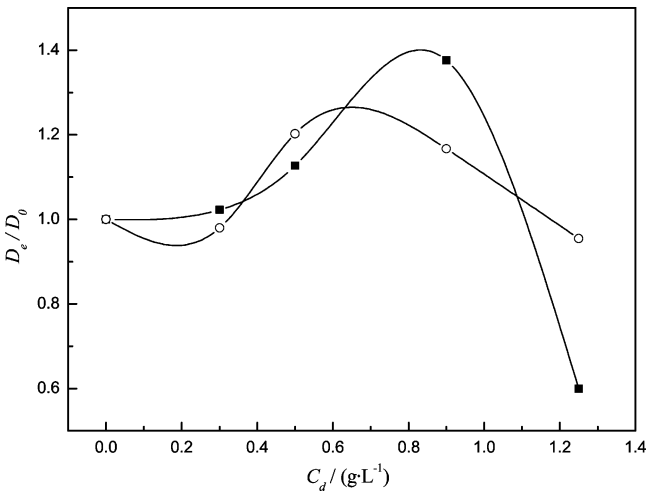

Figure 6. Effect of the silica nanotubes/SiO_2 concentrations C_d on D_e/D_{e0} of silica nanotubes-algiante (■)/SiO_2-alginate (○).

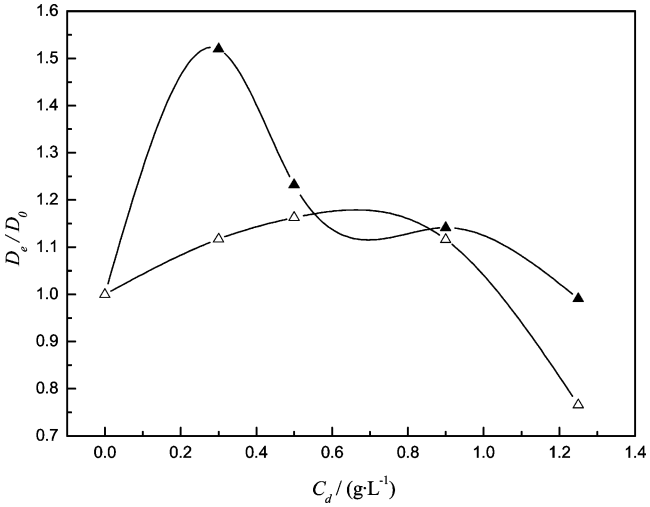

Figure 7. Effect of the CNTs/graphite concentrations C_d on D_e/D_{e0} of CNTs-algiante (▲)/graphite-alginate (△).

The plots of the D_e as a function of the silica nanotubes/SiO_2 and CNTs/graphite concentration in hybrid alginate gel are shown in Figures 6 and 7.

The dependence of D_e on dopant concentration must be characterized for the hybrid gel to accurately predict D_e of the hybrid gels over a wide range of dopant concentrations. A convenient way to describe the effect on D_e of the dopant concentration is to use simplified models. Numerous models have been proposed and applied to the cell immobilizing gel beads systems.[19] Several investigators have developed simple correlational models by fitting polynomials directly to the experimental data, such as the empirical models by Scott et al.[20] (eq 5) and by Korgel et al.[21] (eq 6):

$$\frac{D_{e,c}}{D_{e0}} = 1 - 0.9\phi + 0.27\phi^2 \quad (5)$$

$$\frac{D_{e,c}}{D_{e0}} = 1 - 2.23\phi + 1.40\phi^2 \quad (6)$$

where $D_{e,c}/D_{e0}$ is the ratio of the effective diffusion coefficient of solute in cell-containing gel ($D_{e,c}$) to that in pure gel ($D_{e,0}$). ϕ is the volume fraction of cells.

Similarly, a series of simple fourth-order or third-order polynomial can be applied to correlate the change in the effective diffusion coefficient with the dopant concentrations from these preliminary experimental data:

$$\frac{D_e}{D_{e0}} = 1 + \alpha C_d + \beta C_d^{\,2} + \chi C_d^{\,3} + \delta C_d^{\,4} \quad (7)$$

where D_e/D_{e0} is the ratio of the effective diffusion coefficients (D_e) of NADH in the hybrid alginate gels to that in pure alginate gel (D_{e0}). C_d is the dopant concentration.

The values of four constants α, β, χ, and δ are listed in Table 3. The four parameters are determined by a least-squares fit of the data shown in Figures 6 and 7. Such a correlation and similar correlation from others, should allow the prediction of diffusion characteristics for the doped alginate gel beads and the calculation of the optimal

Table 3. Values of α, *β*, *γ*, and *δ* of Each Hybrid Gel for Eq 7

dopant	α	*β*	*γ*	*δ*
silica nanotubes-alginate	−1.10	4.32	−2.96	0.00
SiO_2-alginate	−2.17	10.56	−13.30	4.97
CNTs-alginate	6.29	−21.92	25.20	−9.36
graphite-alginate	0.63	−0.38	−0.12	0.00

dopant concentration and D_e over a large range of dopant concentrations.

Conclusions

In summary, alginate gel beads doped with nanotubes or nanoparticles are prepared at four dopant concentration levels: 0.3, 0.5, 0.9, and 1.5 g/L. D_e and D_e/D_{e0} of NADH in the hybrid gels are evaluated. The maximum effective diffusion coefficient of NADH in the CNTs-alginate is 50 % larger than that in pure alginate gel. Due to the desirable diffusion characteristics, the doped alginate gels can be used as a novel and effective immobilization carriers for encapsulating biomolecules such as enzymes and cells.

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Supporting Information Available:

The data of diameters and their corresponding uncertainties, the data of D_e, and the mean relative deviation of D_e among parallel experiments. This material is available free of charge via the Internet at the http://pubs.acs.org.

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