School of Pharmacy, University of Oslo, Norway

Effect of charge and size of diffusing probe on the diffusion through calcium alginate gel matrices

Ø. HOLTE, H. H. TØNNESEN, J. KARLSEN

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Øyvind Holte, School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, N-0316 Oslo, Norway
oholte@farmasi.uio.no

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The diffusion of latex particles and low molecular weight fluorescein analogues in homogenous, well-defined calcium alginate gel matrices was studied. The experimental results indicate that the different types of diffusion probes are retarded by different mechanisms in the gel. The positively charged low molecular weight substance experienced some degree of retardation by the gels, but the diffusion rate of the uncharged low molecular weight substance was little influenced by gel composition. A wide range of diffusion rates was observed for the particles in the various gel compositions.

1. Introduction

Calcium alginate gels have been investigated for the potential use in pharmaceutical formulations and other biomedical applications (Dumitriu 2002; Shilpa et al. 2003; Skaugrud et al. 1999; Tonnesen and Karlsen 2002). The polymer is biocompatible, and the gelling conditions are mild. Therefore, calcium alginate gels could be suitable for the encapsulation and controlled release of biologically active molecules, including proteins. The possible use of alginate gels as drug reservoirs or controlled drug release matrices requires that the drug is sufficiently retarded by the gel. The diffusion behaviour of various kinds of substances in or from calcium alginate gels has been widely investigated (Chai et al. 2004; David et al. 2004; Fundueanu et al. 1999; Gombotz and Siong Fong 1998; Ho and Neufeld 2001; Klein et al. 1983; Kuga 1981; Murata et al. 2000; Shiraishi et al. 1993; Stewart and Swaisgood 1993). The experiments referred to above were performed using inhomogeneous calcium alginate beads. Thus, the exact composition of the diffusion rate-controlling calcium alginate bead shell is not known. Molecular weight cut-off values were reported for large diffusion probes, and some authors report diffusion characteristics dependent on release medium or rate of flow or stirring. The aim of this study was to investigate the effect of calcium alginate gel composition on the diffusion characteristics of various types of diffusion probes. In a previous work, we have developed an experimental design suitable for the study of the effective diffusion of model substances in homogenous gel cylinders (Holte et al. 2006). Effective diffusion coefficients of high- and low molecular weight substances were reported. In this paper, we report the effective diffusion coefficients of particles, and low molecular weight substances having different electrical charges, in homogeneous well-defined calcium alginate gel compositions.

2. Investigations and results

2.1. Diffusion of fluorescein sodium and fluorescein amine

The effective diffusion coefficients of fluorescein sodium and fluorescein amine in the various calcium alginate gel compositions are listed in Table 1. Concerning the diffusion rates of fluorescein sodium, the variation in diffusion coefficients among the different gel compositions is small compared with the error margins of the experiments. A

Table 1: Effective diffusion coefficients (D_e) of fluorescein sodium (FNa) and fluorescein amine (FNH_2)

Alginate (%)	F_G	Calcium (mM)	FNa (n = 54)		FNH_2 (n = 41)	
			Temp (°C)	$D_e \cdot (10^{-10}\ m^2/s)$	Temp (°C)	$D_e \cdot (10^{-10}\ m^2/s)$
1	0.349	15.0	21.0	2.13 (1.2–2.6)	20.0	3.18 (2.8–4.0)
1	0.349	60.0	20.0	2.85 (1.8–3.2)	21.0	2.05 (1.3–3.0)
1	0.694	15.0	20.0	1.46 (1.1–1.8)	21.2	5.13 (4.5–5.5)
1	0.694	60.0	20.7	4.03 (3.9–4.2)	21.0	5.17 (4.5–6.0)
2	0.444	37.5	19.2	2.11 (1.4–3.0)	20.0	1.78 (1.5–2.1)
3	0.349	15.0	20.5	2.73 (1.8–3.4)	21.2	1.23 (0.75–2.0)
3	0.349	60.0	21.0	1.87 (1.1–2.8)	20.5	1.70 (1.3–2.1)
3	0.694	15.0	21.0	2.18 (1.5–3.0)	21.0	3.63 (3.4–4.0)
3	0.694	60.0	20.7	2.05 (1.8–2.5)	21.5	6.88 (6.0–9.0)

Values are the means of 3–6 experiments, error margins are minimum and maximum values, respectively. F_G is the fraction of guluronic acid residues in the alginate sample.

PLS regression model was established from 54 experimental results using one principal component. However, the correlation between the statistical model and the observed data was only 0.65, and not more than 31% of the X variance and 42% of the Y variance was explained by the model. It was therefore difficult to identify any trends regarding the different gel compositions' ability to retard the diffusion of fluorescein sodium by use of this model. The statistical model was discarded in the case of the fluorescein sodium results. A six-fold variation in diffusion coefficients was observed among the different gels for the diffusion rates of fluorescein amine (Table 1). A PLS regression model was established from 41 experimental results, using two principal components. The correlation between the statistical model and the observed data was 0.95. 51% of the X variance and 90% of the Y variance was explained by the model. The statistical model was therefore regarded as valid for the fluorescein amine results. The regression coefficients are presented in Fig. 1. Response surfaces of the diffusion coefficients of fluorescein amine in the various calcium alginate gels are presented in Fig. 2 and Fig. 3. The diffusion of fluorescein amine through gels made from M-rich alginate is two to three times slower than the diffusion through G-rich calcium alginate gels. At high calcium concentrations, the diffusion rate of fluorescein amine is little affected by alginate concentration (Fig. 2). At low levels of calcium, however, the diffusion rate of fluorescein amine is somewhat slower at high levels of alginate (Fig. 3).

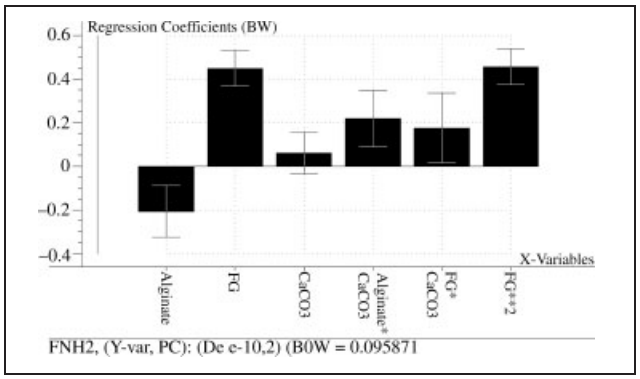

Fig. 1: Normalized regression coefficients from the PLS analysis of the fluorescein amine diffusion measurements. Variables are alginate concentration (%), alginate F_G value and calcium concentration (mM). Error bars are confidence intervals, determined by the Jackknifing method

2.2. Diffusion of latex particles

The diffusion rates of 100 nm latex particles in the gels were too small to be investigated under the experimental conditions used in this study, and therefore only smaller particles (20 nm) were further studied. Only G-rich alginate was used for the latex particle diffusion measurements. Preliminary experiments using M-rich alginate gels resulted in too slow latex particle diffusion to be investigated, even for the 20 nm particles. It was not possible to increase latex particle diffusion rate by reducing alginate concentration, as these gels would not have the proper gel strength to withstand the experimental conditions. The ef-

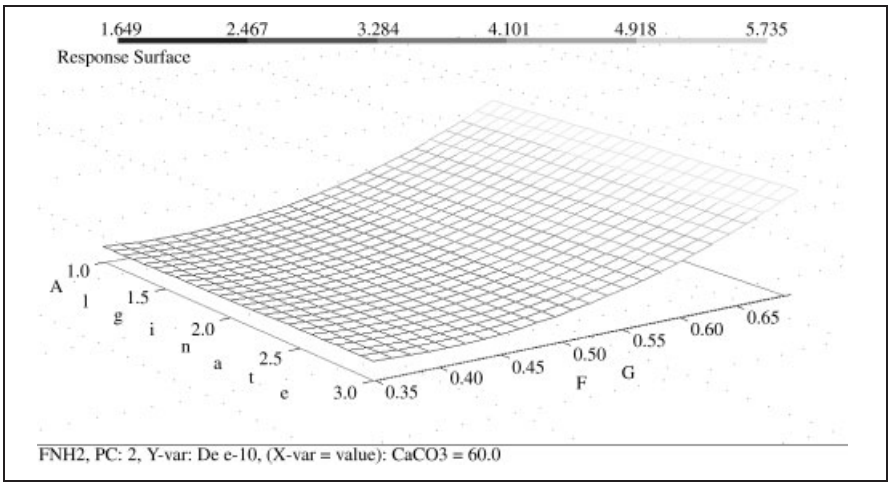

Fig. 2:
Response surface of the diffusion coefficients of fluorescein amine through the gels versus alginate concentration (%) and F_G value. Calcium concentration is 60 mM

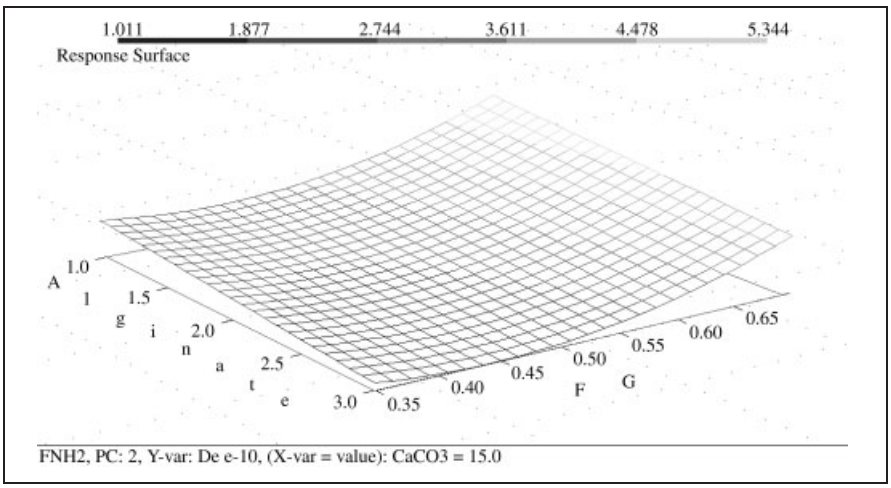

Fig. 3:
Response surface of the diffusion coefficients of fluorescein amine through the gels versus alginate concentration (%) and F_G value. Calcium concentration is 15 mM

fective diffusion coefficients of 20 nm latex particles in the various G-rich calcium alginate gel compositions are listed in Table 2. A 36-fold variation in diffusion coefficients was observed among the different gels for the diffusion rates of the particles. A PLS regression model was established from 38 experimental results, using three principal components. The correlation between the statistical model and the observed data was 0.87. 67% of the X variance and 76% of the Y variance was explained with the model, and the model was regarded as acceptable for the particle diffusion results. The regression coefficients are presented in Fig. 4. Alginate concentration in the gel was most important for particle diffusion rate. Calcium concentration also affected particle diffusion rate, but to a lesser extent than alginate. The effect of temperature was not significant, but the regression coefficient was included in the model to account for its significant interaction effect with alginate concentration. Response surfaces of the diffusion coefficients of the particles in the various calcium alginate gels are presented in Fig. 5. The fastest particle diffusion rates were observed in gels having small amounts of alginate and high concentrations of calcium. The slowest particle diffusion rates were observed in gels having large amounts of alginate.

Table 2: Effective diffusion coefficients (D_e) of 20 nm latex particles

Alginate (%)	Calcium (mM)	Temperature (°C)	$D_e \cdot (10^{-10}\ m^2/s)$
0.25	3.75	19.5	0.60 (0.4–0.9)
0.25	7.5	19.0	0.90 (0.6–1.5)
0.25	11.25	19.5	1.23 (1.0–1.5)
0.375	7.5	18.0	0.80 (0.6–1.0)
0.375	15	19.0	1.08 (0.8–1.3)
0.5	7.5	20.0	0,49 (0.4–0.6)
0.5	15	19.5	0.43 (0.4–0.5)
0.75	7.5	19.0	0.22 (0.1–0.4)
0.75	15	17.5	0.16 (0.1–0.2)
1	15	19.5	0.033 (0.03–0.04)

The alginate F_G value is 0.694. Values are the mean of 3–5 experiments, error margins are minimum and maximum values, respectively. n = 38

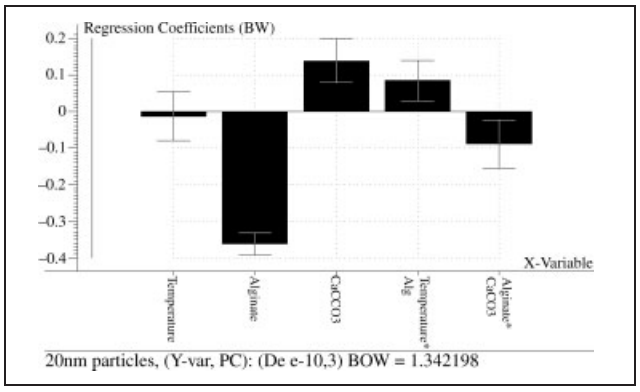

Fig. 4: Normalized regression coefficients from the regression analysis of the 20 nm particles diffusion measurements. Variables are temperature (°C), alginate concentration (%) and calcium concentration (mM). Error bars are confidence intervals, determined by the Jackknifing method

3. Discussion

The different diffusion characteristics of the two fluorescein analogues could be attributed to the different molecular charges. At the neutral pH of the gels, fluorescein sodium possesses no net charge, whereas fluorescein amine has a positively charged amine group. The positively charged fluorescein amine could possibly interact with the negatively charged alginate in the gel, leading to a reduced diffusion rate. Slow diffusion through gels as a result of such ionic interactions was previously reported for drug/gel compositions (Iannuccelli et al. 1996; Segi et al. 1989). The poor statistical model obtained from the fluorescein sodium diffusion experiments is not suitable for making definite conclusions regarding the diffusion behaviour of fluorescein sodium in the calcium alginate gels investigated. On the contrary, it seems that the diffusion rates of fluorescein sodium are little influenced by the gel composition. Assuming that the fluorescein sodium diffuses freely through the gel, experiencing little physical interaction with the alginate network, this is not surprising. Although the range of gel compositions investigated is great in terms of gel strength, the aqueous phase in the gel network is relatively constant. The water content is virtually the same although the alginate concentration is tripled from one to three per cent. On the other hand, the diffusion of the positively charged fluorescein amine is affected by the gel composition. If the diffusion rate of fluorescein amine through the gel is reduced because of ionic interactions between the fluorescein amine and the alginate network, the slowest diffusion should be observed in the gels having the most alginate available for ionic interaction. Assuming that the strong calcium binding to

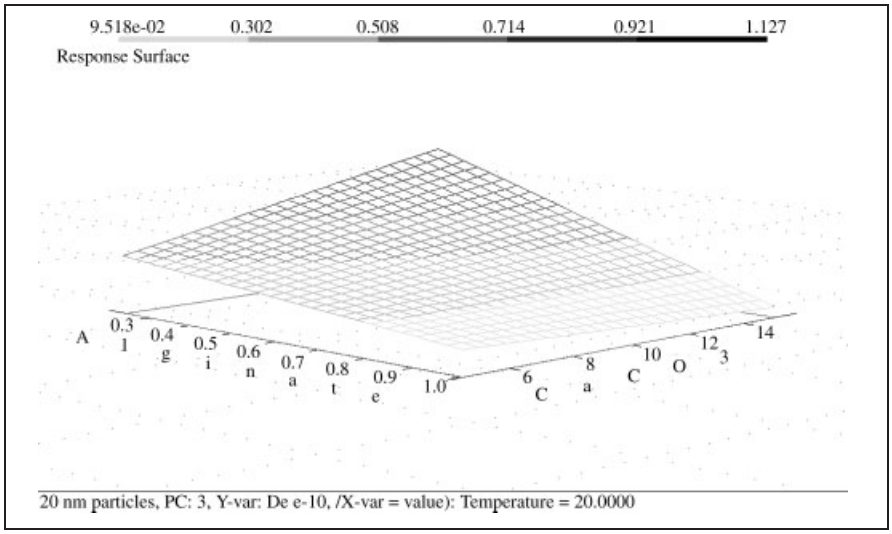

Fig. 5:
Response surfaces of the diffusion coefficients of 20 nm particles through the gels versus alginate concentration (%) and calcium concentrations (mM). Temperature is set to 20 °C

alginate is little affected by the presence of fluorescein amine, the alginate available for fluorescein interaction is directly dependent on the relative amounts of alginate and calcium in the gel, and the alginate type. G-rich alginate, designated by a large F_G value, will bind calcium more strongly than M-rich alginate (Draget 2000). Thus, in a G-rich calcium alginate gel, the amount of unbound alginate available for ionic interactions with positively charged molecules like fluorescein amine will be smaller than in a corresponding M-rich alginate gel. Indeed, from Fig. 1, the following can be deduced: The diffusion rate of fluorescein amine is slowest in calcium alginate gels made from M-rich alginate. Furthermore, the diffusion rate is slower in the gels having large amounts of alginate, and small amounts of calcium. The latex particles used in this study bear a negative surface charge to prevent aggregation of the particles. Thus, no ionic interaction is expected between the particles and the alginate in the gels. Pore sizes of alginate gels in the order of 5–20 nm have been reported in the literature (Fundueanu et al. 1999; Stewart and Swaisgood 1993). Although the gel compositions are different in our study, the latex particles are expected to be in the same order of size as the alginate gel pore size. Diffusion of the particles in the gel requires that the gel pore size is larger than the particles, or that the alginate network is flexible enough to allow the temporary formation of larger pores. The largest alginate gel pore sizes are expected in the gels having low levels of alginate and high levels of calcium. Indeed, the fastest latex particle diffusion is observed in such gels. The slowest latex particle diffusion is observed in gels having large amounts of alginate (Fig. 5). Although no data are presented on latex particle diffusion in M-rich alginate gels, it seems that the diffusion rate of the particles in such gels is lower than in corresponding G-rich alginate gels. The latex particle diffusion rates in M-rich alginate gels were too small to be investigated under the given experimental conditions. M-rich calcium alginate gels are expected to have smaller pore sizes than corresponding G-rich calcium alginate gels, and a slower diffusion rate of particles in such gels is reasonable. The diffusion probes investigated in this study could be regarded as models of drugs and other biologically active compounds. Particulate materials could include drug suspensions and drugs adsorbed to inert particles. Also, some polymeric materials like globular proteins exhibit particulate behaviour. The possible use of calcium alginate gels as drug reservoirs or controlled drug release matrices requires that the drug can be adequately retarded by the gel formulation. Then the preferred drug release rate might be achieved by formulating the drug in a suitable gel composition. The results presented above indicate that calcium alginate gels are not suitable for the controlled delivery of uncharged low molecular weight drugs if drug release rate is to be determined by diffusion through the gel matrix. However, a positively charged drug, or particulate material might benefit from such a formulation.

4. Experimental

4.1. Chemicals

Samples of sodium alginate (Protanal SF 120, lot number 477021, F_G 0.694; Protanal LV 120 D, lot number 940040, F_G 0.349) were supplied by FMC BioPolymer (Drammen, Norway). Fluorescent labelled latex particles (Postnova (Landsberg, Germany)), fluorescein sodium and fluorescein amine (both Fluka (Buchs, Switzerland)), calcium carbonate ($CaCO_3 \cdot 2\,H_2O$) and sodium chloride (NaCl) (both NMD (Oslo, Norway)) and glucono-δ-lactone (GDL) (Calbiochem (Darmstadt, Germany)) were obtained commercially.

4.2. Gel preparation

Calcium alginate gels were prepared by internal gelation, as described by Draget et al. (1991). Sodium alginate was dissolved in water, and a solution of fluorescein analogue or a suspension of latex particles was added. Calcium carbonate was suspended in the alginate solution. A freshly prepared solution of glucono-δ-lactone (GDL) was added to the mixture. The reduction of pH resulting from the slow hydrolysis of GDL leads to the dissolution of the calcium salt, leading to calcium alginate gel formation. Ultimately, the pH of the gels is neutralised by the dissolved carbonate. The gel was put in a refrigerator overnight to assure that the reaction was complete before diffusion experiments were performed. The gels contained the following concentrations of said diffusion probe: fluorescein sodium 0.013 mg/ml, fluorescein amine 0.65 mg/ml, latex particles 3 mg/ml.

4.3. Diffusion experiments

The effective diffusion of the various diffusion probes from one face of a loaded gel cylinder into a stirred solution of finite volume was studied. The diffusion measurements were performed as described previously (Holte et al. 2006) using Franz diffusion cells (PermeGear, Hellertown, PA, USA; diffusion area 1 cm^3, 8 ml volume) equipped with a home-made gel matrix holder consisting of a steel mesh (1.7 mm) supported by an O-ring. Gel cylinders were cut to fit tightly into the Franz cell to avoid liquid flow along the sides of the gel cylinder. The gel was sealed with a plastic foil at one end, thus allowing macroscopic diffusion of the diffusion probe in one direction only. The diffusion of model substance out of the gel was studied by monitoring the concentration in the receiving solution at predetermined time intervals. Small volumes (100 μl) were withdrawn from the receiving solution and replaced with water. Samples were diluted with appropriate buffer solutions before quantification. The concentration of model substance in the samples was determined by measurement of the fluorescence intensities (fluorescein analogues: λ_{ex} 492 nm, λ_{em} 512 nm; latex particles: λ_{ex} 260 nm, λ_{em} 404 nm; Perkin Elmer LS 50 B luminescence spectrometer) and compared to a standard curve ($R > 0.995$).

4.4. Calculation of diffusion coefficients

Effective diffusion coefficients (D_e) of the diffusion probes in the gels were calculated from the concentration profiles of the probes in the receiving solutions. The method described by Crank (1975) for solute diffusion from a cylinder into a stirred solution of finite volume was used. Eq. (1) was fitted to experimental data by inserting different values of D_e until the model giving the best fit was obtained.

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2+q_n^2} \exp\left(-D_e q_n^2 t/l^2\right) \qquad (1)$$

M_t and M_∞ are amounts of diffusing solute in the receiving compartment at time t and at equilibrium between the gel and the solution, respectively. α is the ratio of the volumes of solution and gel, respectively, and q_n is the positive root of ($\tan q_n = -\alpha q_n$). The first six values of q_n were obtained from Crank (1975) and used in the calculations. This was sufficient to achieve a proper model to fit the experimental data. D_e is the effective diffusion coefficient of the diffusing solute.

4.5. Data handling

In the fluorescein experiments a 2^3 full factorial design was performed with 3–6 parallels of each experiment and 2 centre points. The variables investigated were alginate concentration in the gel (1 or 3% w/w), calcium concentration in the gel (15 or 60 mM) and alginate type (F_G 0.349 or 0.694). In the latex particle experiments, the variables investigated were alginate type (F_G 0.349 or 0.694), alginate concentration in the gel (0.25–1% w/w) and calcium concentration in the gel (3.75–15 mM). The measurements were performed at room temperature. The temperature was monitored throughout the experiments and accounted for during the data analysis. The data were analysed with partial least squared projections to latent structures (PLS) using the Unscrambler 9.0 software (Camo, Trondheim, Norway). The variables were normalised before analysis, and confidence intervals of the regression coefficients obtained were determined by the jack-knifing method performed by the computer software. Traditional standard deviations are not available in PLS, but the confidence intervals obtained by this method are comparable to a significance level of $P < 0.05$.

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References

Chai Y, Mei L, Wu G-L, Lin D, Yao S (2004) Gelation conditions and transport properties of hollow calcium alginate capsules. Biotechnol Bioeng 87: 228–233.

Crank J (1975) The mathematics of diffusion. Oxford University Press, Bristol, UK.

David B, Doré E, Jaffrin MY, Legallais C (2004) Mass transfers in a fluidized bed bioreactor using alginate beads for a future bioartificial liver. Int J Artif Organs 27: 284–293.
Draget KI (2000) Alginates. In: Phillips GO, Williams PA (ed.) Handbook of hydrocolloids, CRC Press, Cambridge.
Draget KI, Østgaard K, Smidsrød O (1991) Homogenous alginate gels: a technical approach. Carbohydr Polym 14: 159–178.
Dumitriu S (2002) Polysaccharides as biomaterials. In Polymeric Biomaterials, S Dumitriu editor, Marcel Dekker Inc., New York, USA. Pages 1–61.
Fundueanu G, Nastruzzi C, Carpov A, Desbrieres J, Rinaudo M (1999) Physico-chemical characterization of Ca-alginate microparticles produced with different methods. Biomaterials 20: 1427–1435.
Gombotz WR, Siong Fong W (1998) Protein release from alginate matrices. Adv Drug Deliver Rev 31: 267–285.
Ho J, Neufeld RJ (2001) Retention and release of low molecular weight DNA from alginate beads. STP Pharma Sci 11: 109–115.
Holte Ø, Tønnesen HH, Karlsen J (2006) Measurement of diffusion through calcium alginate gel matrices. Pharmazie 61: 30–34.
Iannuccelli V, Coppi G, Cameroni R (1996) Biodegradable intraoperative system for bone infection treatment. I. The drug/polymer interaction. Int J Pharm 143: 195–201.
Klein J, Stock J, Vorlop KD (1983) Pore size and properties of spherical calcium alginate biocatalysts. Eur J Appl Microbiol Biotechnol 18: 86–91.
Kuga S (1981) Pore size distribution analysis of gel substances by size exclusion chromatography. J Chromatogr 206: 449–461.
Murata Y, Sasaki N, Miyamoto E, Kawashima S (2000) Use of floating alginate gel beads for stomach-specific drug delivery. Eur J Pharm Biopharm 50: 221–226.
Segi N, Yotsuyanagi T, Ikeda K (1989) Interaction of calcium-induced alginate gel beads with propranolol. Chem Pharm Bull 37: 3092–3095.
Shilpa A, Agrawal SS, Ray AR (2003) Controlled delivery of drugs from alginate matrix. J Macromol Sci-Pol R C43: 187–221.
Shiraishi S, Imai T, Otagiri M (1993) Controlled-release preparation of indomethacin using calcium alginate gel. Biol Pharm Bull 16: 1164–1168.
Skaugrud Ø, Hagen A, Borgersen B, Dornish M (1999) Biomedical and pharmaceutical applications of alginate. Biotechnol Genet Eng 16: 23–39.
Stewart WW, Swaisgood HE (1993) Characterization of calcium alginate pore diameter by size-exclusion chromatography using protein standards. Enzyme Microb Technol 15: 922–927.
Tonnesen HH, Karlsen J (2002) Alginate in drug delivery systems. Drug Dev Ind Pharm 28: 621–630.