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Diffusion of ions in a calcium alginate hydrogel-structure is the primary factor controlling diffusion

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ABSTRACT

The diffusion of solutes has been evaluated in an alginate hydrogel as a function of its structure. The role of solute and gel charge on the diffusion measurements were of particular interest. Diffusion coefficients were measured using fluorescence correlation spectroscopy as a function of solute charge and size, bulk solution ionic strength and pH, and gel density. Diffusion coefficients of fluorescent dextrans with hydrodynamic radii up to 6 nm were reduced by 30% in a 1.8% (w/w) hydrogel whereas they were reduced by only 2% in a 0.2% (w/w) hydrogel. The role of ionic strength was examined for various concentrations (0.1–100 mM) and compositions of ions (Na^+, Ca^{2+} or mixtures thereof). The diffusion coefficient of a small charged probe (rhodamine 6G, $R6G^+$) did not change significantly with increasing ionic strength when sodium was used as the counter ion. The diffusion coefficient was only moderately influenced by the charge of solutes (from +1 to −2). Similarly, pH variations from 3 to 9 had little impact on the diffusion coefficients of $R6G^+$ in the gel. On the other hand, the addition of Ca^{2+} had a significant impact on gel compactness, which led to a significant reduction in solute diffusion. For the calcium alginate hydrogels, structural modifications resulting from Ca binding were much more important than electrostatic effects due to modifications of the gel Donnan potential.

1. Introduction

The diffusive properties of solutes in gels are key to a large number of environmental (Chang & Huang, 1998; Chen, Hong, Wu, & Wang, 2002; Davis, Llanes, Volesky, & Mucci, 2003), pharmaceutical (Pillay, Dngor, Govender, Moopanar, & Hurbans, 1998) and biotechnological (Lim & Sun, 1980) applications. The diffusion of a solute through a hydrogel will depend upon both physical (e.g. obstruction (Amsden, 1998a; Giddings, Kucera, Russel, & Myers, 1968; Johansson, Skantze, & Lofroth, 1991)) and chemical (e.g. hydrogen bonding, electrostatic effects (Fatin-Rouge, Milon, Buffle, Goulet, & Tessier, 2003; Nilsson, Nordenskiold, Stilbs, & Braunlin, 1985)) interactions between the two phases, which are dependent upon the physicochemical properties of both the solute and the gel. For example, solute size (Petit, Zhu, & Macdonald, 1996) and charge (Johansson, Skantze, & Lofroth, 1993) and the extent of gel crosslinking (Amsden, 1998b) have been well documented to influence diffusion in hydrogels. For calcium alginate hydrogels, electrostatics has also been thought to play an important role in the diffusion of sodium (Lundberg & Kuchel, 1997) and bovine serum albumin (BSA) (Amsden, 2001) at various ionic strength and pH (Kalis, Davis, Town, & Van Leeuwen, 2009a). Indeed, a decreased mobility of charged solutes was attributed to an increased electrostatic interaction between the solute and the gel (Holte, Tonnesen, & Karlsen, 2007; Lundberg & Kuchel, 1997). Several recent papers (Davis, Kalis, Pinheiro, Town, & Van Leeuwen, 2008; Kalis et al., 2009a; Kalis, Davis, Town, & Van Leeuwen, 2009b) have confirmed a large Donnan potential in alginate gels, resulting in significant cation partitioning. Nonetheless, the precise role of gel (and solute) charge on diffusion is not clear. For example, ionic strength increases (or pH decreases) can lead to contrasting effects on solute mobility in gels by (i) decreasing the gel Donnan potential, leading to decreased partitioning of solutes with the gel (Sangi, Halstead, & Hunter, 2002); (ii) decreasing the double layer thickness on the pores, leading to an increase of the effective pore size in the hydrogel (Golmohamadi, Davis, & Wilkinson, 2012); (iii) reducing electrostatic repulsion among the gel fibers, leading to a compression of the gel structure and decrease in the physical pore size; or (iv) increasing the homocoagulation of the diffusing solutes, effectively decreasing diffusion (Wilkinson, Gendron, & Avaltroni, 2008). In order to quantitatively determine the effects of charge on solute diffusion, especially in highly charged gels such as alginate, further investigation is thus required.

In this paper, the diffusion coefficients of a number of charged probes with relatively small sizes were evaluated in order to assess the relative importance of charge interactions on diffusion through a negatively charged calcium alginate hydrogel. Ionic strength (I) and pH of the bulk medium and the nature of the diffusing solutes

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were systematically varied in order to determine the role of charge effects on diffusion in an alginate hydrogel.

2. Experimental

2.1. Materials

Medium viscosity alginate, D-glucono-δ-lactone (GDL), rhodamine 6G (R6G), calcium nitrate and calcium carbonate were purchased from Sigma–Aldrich. Rhodamine 110 (R110) and ultrapure nitric acid were acquired from Fluka. Fluorescently labeled dextrans (molar masses = 3k, 10k, 40k and 70k) and other fluorescent probes – tetramethylrhodamine, methyl ester (TMRM); Oregon green 488 carboxylic acid, succinimidyl ester (Oregon 1C); Oregon green 488 carboxylic acid (Oregon 2C) – were purchased from Invitrogen. For all fluorophores except Oregon 1C and 2C, small quantities of fluorophore were added to Milli-Q water ($R > 18\,M\Omega\,cm$) to obtain stock solutions in the micromolar concentration range. Oregon 1C and 2C were dissolved in 1 mM morpholineethanesulfonic acid (MES) at pH 7.2. Samples were prepared by dilution of the stock solutions into an electrolyte solution with a controlled pH and ionic strength in order to obtain a final probe concentration of 20 nM (pH: 3–9, I: 0.1–100 mM). Dilute HNO_3 (Fluka), sodium hydroxide (Sigma) and sodium nitrate (Fluka, analytical grade) were used to adjust the pH and ionic strengths of the solutions. pH was measured using a Metrohm 744 pH meter, calibrated with standard NBS buffers. All products were used without further purification.

2.2. Hydrogel preparation

The hydrogel was prepared according to the method of Draget, Ostgaard, and Smidsrod (1989). Simply, sodium alginate solutions were stirred overnight. The next day, calcium carbonate ($CaCO_3$) particles were dispersed into the viscous alginate solutions and degassed under vacuum. Finally, a freshly prepared solution of D-glucono-δ-lactone (GDL, 30 mM) was added to the mixture and stirred for 2 min, after which the gel was poured into cylindrical wells (for swelling measurements) or FCS coverslips (for FCS experiments). The cylindrical gel pieces (diameter = 1 cm; height = 0.5 cm) were left 24 h to solidify and then equilibrated in the desired experimental solutions for another 24 h. In a few selected experiments, hydrogels were first immersed in a 50 mM $Ca(NO_3)$–20 mM $Na(NO_3)$ mixture for 48 h prior to transfer into 20 mL of a pH and ionic strength controlled experimental solution for an additional 48 h (solutions were refreshed 3×).

2.3. Diffusion measurements

Diffusion coefficients of fluorescent solutes were measured by fluorescence correlation spectroscopy (FCS) using a Leica TCS SP5 laser scanning microscope equipped with an argon ion (Ar^+) laser (excitation at 488 or 514 nm) and a DPSS Nd:YVO_4 laser (excitation at 561 nm). An avalanche photodiode detector was used to quantify fluorescence intensity fluctuations in the small volume (ca. 1 μm^3) defined by the confocal optics of the instrument. Fluorescence was measured in the emission ranges of 500–530 nm or 607–683 nm. For any given set of calibrations/experimental measurements, the position of the laser from the bottom of the coverslip was kept constant.

Diffusion coefficients measured by FCS are attributed to the Brownian motion of a fluorescent solute (*self*-diffusion) after its equilibration with the hydrogel (i.e. chemical potential gradient equal to zero). Such measurements can be contrasted with measurements of *mutual* diffusion, which are directional and driven by concentration gradients. The characteristic time that a fluorescent probe spends in the confocal volume, τ, is determined from an autocorrelation function, $G(t)$:

$$G(t) = a + \left(\frac{1}{N}\right)\left(1 + \left(\frac{t}{\tau}\right)^{\delta}\right)^{-1}\left(1 + \frac{1}{p^2}\left(\frac{t}{\tau}\right)^{\delta}\right)^{-0.5} \quad (1)$$

where a is the limiting value of $G(t)$ for $t \to \infty$; N is the average number of fluorescent particles diffusing through the confocal volume, t is the measurement time, δ accounts for anomalous diffusion in the gel (Banks & Fradin, 2005) and p is the structural parameter (ratio of the transversal, ω_{xy}, to the longitudinal, ω_z, dimension of the confocal volume: $p = \omega_z/\omega_{xy}$). Diffusion coefficients are determined from the measured values of τ:

$$D = \frac{\omega_{xy}^2}{4\tau} \quad (2)$$

Prior to measurements, ω_{xy} is obtained from a calibration of the confocal volume using R110, which has a known diffusion coefficient of $4.42 \times 10^{-10}\,m^2\,s^{-1}$ (Wilkinson et al., 2008).

Fluorescent probes (typically 2×10^{-8} M) were added to the top of a small quantity (0.25 mL) of gel in each of the 8 wells of an FCS cell; solutions were refreshed at least three times over the 24 h period leading up to the FCS measurements. For each experimental condition, diffusion coefficients were measured in the bulk solutions and in the hydrogels under identical conditions. Diffusion was examined as a function of the pH and ionic strength of the bulk solution, the charge and size of the fluorescent probe and the weight fraction of the gel. For each experimental condition, results were obtained at a minimum of 8 different locations in the gel. In addition, experiments were repeated with freshly prepared gels on different days. Means and standard deviations were obtained from all of the repeated measurements (combination of different days and different gel locations). Acquisition times of 100 s were used to optimize the signal-to-noise ratio.

2.4. Swelling measurements

It was assumed that the swelling of the gel due to changes in the physicochemistry of bulk solution would have an influence on solute diffusion. Swelling was quantified using a swelling factor (S), which was determined from the ratio of the mass of the hydrogel before (m_1) and after (m_2) its equilibration in the experimental solutions:

$$S = \frac{m_2}{m_1} \quad (3)$$

As above, gels were allowed to equilibrate with external solutions for at least 48 h, with at least 3 renewals of the experimental solutions over this period.

3. Results and discussion

3.1. Effect of probe size and gel concentration on the diffusion coefficient

The diffusion coefficients of solutes in hydrogels are generally smaller than those in the bulk solution due to the presence of the polymer network that limits the free volume available for diffusion (Amsden, 1998a, 2001; Aslani & Kennedy, 1996; Martinsen, Storro, & Skjark-Braek, 1992; Muhr & Blanshard, 1982; Wang & Spencer, 1998). This effect was indeed seen for the diffusion of the dextran size standards for several different concentrations of alginate (Fig. 1). The diffusion coefficient of the largest dextran (hydrodynamic radius, r_h, of 6.2 nm) decreased by ca. 20% in the 0.2% (w/w) alginate and by ca. 50% in the 1.8% (w/w) hydrogel when compared to measurements in water (i.e. D_g/D_w of 0.8 and

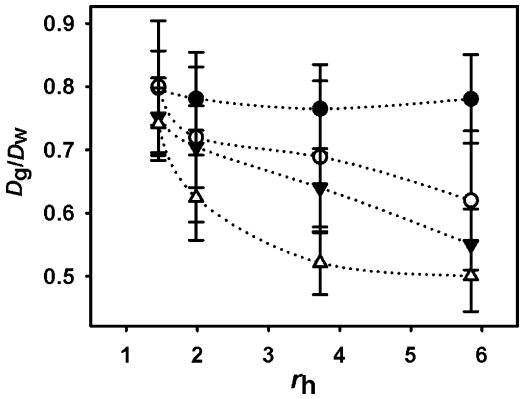

Fig. 1. Diffusion coefficient of dextrans (3–70 kDa) in the calcium alginate hydrogel as a function of their hydrodynamic radii, r_h, and for several densities (%, w/w) of alginate: 0.2% (●); 1% (○); 1.4% (▼) and 1.8% (△). Error bars correspond to standard deviations of n = 15–20 FCS measurements.

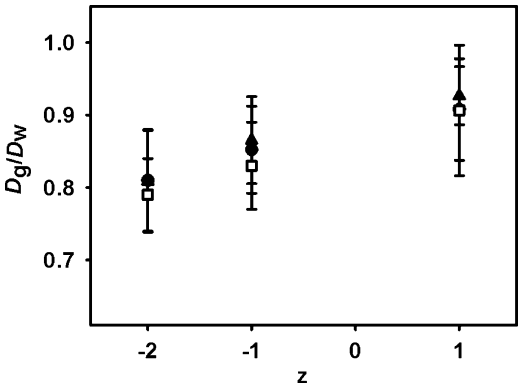

Fig. 2. Effect of solute charge on diffusion within a calcium alginate hydrogel (1%, w/w). Probes corresponded to: R6G$^+$ (z = +1), Oregon 1C (z = −1) and Oregon 2C (z = −2); I = 0.1 mM (□); 1 mM (●); 10 mM (▲); pH = 7.0, [Ca]/[Na] = 3/1. Error bars correspond to standard deviations compiled FCS measurements (n = 15–20 over several days).

0.5, respectively, where the subscripts refer to the values made in the gel and water). The size of the probe also had an important effect on the diffusion coefficient, especially at the higher gel densities. For example, in the 0.2% alginate, the diffusion coefficient only decreased by an additional 2% for an increase in the hydrodynamic radius of the dextrans from 1.5 nm to 6.2 nm, whereas for the 1.8% alginate, an additional 30% decrease in D_g was observed for the same increase in probe size. While there was little difference (7%) among the diffusion coefficients of the smallest probe (3 kDa dextran) between the least and most concentrated gel, a much larger effect (36%) was observed for the largest probe (70 kDa dextran).

Attempts to model the decrease in diffusion coefficients in the gels using literature models based solely upon obstruction or hydrodynamic effects (Philips, Amsden models) were not successful (Fig. S3). We hypothesized that by taking into account charge effects on gel, we might be able to better predict the diffusive properties of the alginate gel. Therefore, several experiments were undertaken to better quantify the role of gel charge on diffusion.

3.2. Effect of probe charge on diffusion coefficient

Hydrogels were prepared in 50 mM $Ca(NO_3)_2$ and 20 mM $NaNO_3$ (pH 7.0) and then transferred to experimental solutions with variable ionic strengths of 0.1, 1.0 or 10 mM (corresponding Debye lengths of 30, 9.6 and 3.0 nm, respectively), and a [Ca]/[Na] = 3 (Kalis et al., 2009a). Three charged fluorophores of similar sizes: R6G$^+$ (z = +1, r_h = 0.5 nm), Oregon 1C (z = −1, r_h = 0.65 nm) and Oregon 2C (z = −2, r_h = 0.58 nm) were used to probe the effect of solute charge on diffusion. At all three ionic strengths, the relative diffusion coefficient (D_g/D_w) decreased slightly as the charge of the solute was varied from +1 to −2 (Fig. 2). Nonetheless, the high value of the D_g/D_w ratio under all conditions, indicated that no significant chemical or physical interactions were occurring between the solutes and the hydrogel (Garbayo, León, & Viïlchez, 2002; Holte et al., 2007; Lannuccelli, Coppi, & Cameroni, 1996; Zhang, Nadezhina, & Wilkinson, 2011). The results were also consistent with the observation of a constant fluorescence intensity (data not shown), suggesting that little adsorption to the hydrogel was occurring for any of the probes, under any condition. A similar observation of an increasing diffusion coefficient with increasing probe charge (from −2 to +1) has been observed in a bacterial biofilm (Zhang et al., 2011) and has been attributed to a stronger electrostatic repulsion between the anionic probes and the negatively charged hydrogel. Note that while the accumulation of positively charged probes (and depletion of the negatively charged probes) in the hydrogel (Davis, Yezek, Pinheiro, & Van Leeuwen, 2005) would be expected to increase the concentration gradient between the gel and the surrounding bulk solution, such an increase should have little effect on *equilibrium* FCS measurements of the diffusion coefficient.

3.3. Effect of ionic strength on diffusion and gel structure

Diffusion coefficients for R6G$^+$ were evaluated at several ionic strengths. When $NaNO_3$ was employed as the electrolyte, diffusion coefficients were virtually constant over the entire range of ionic strength (0.1–100 mM; Fig. 3a). In contrast, when $Ca(NO_3)_2$ was employed as the electrolyte or when Ca^{2+} was mixed with Na^+, there was a small but perceptible decrease in D_g/D_w at the highest ionic strengths (Fig. 3b and c). For experiments performed at a constant ionic strength, the diffusion coefficient in the gel clearly decreased with the increasing ratio of Ca/Na (Fig. 4).

Ionic strength changes can lead to numerous and often contradictory effects on diffusion measurements (Golmohamadi et al., 2012). For example, an increase in ionic strength leading to an increase in charge screening may: (i) increase diffusion due to effectively larger effective pore sizes (reduction of the Debye layer) (larger D), (ii) decrease solute–gel interactions (R6G$^+$ is positively charged while the alginate is negatively charged) (larger D), (iii) increase homocoagulation of the diffusion probe (smaller D), or (iv) increase gel compactness (smaller D). With respect to the above potential explanations, simple calculations based upon the ionic strength suggest that the Debye layer (point i) would decrease from ca. 30 nm at 0.1 mM to ca. 1 nm at 100 mM (Supporting information), potentially leading to a large change in the *effective* pore size for charged substrates. The use of measured Donnan potentials to model diffusion in the gels showed that although significant solute accumulation was predicted at the lower ionic strengths (e.g. Donnan partition coefficient of ca. 44 at I = 0.13 mM), little effect on the diffusion was predicted (point ii, Fig. S3 and Table S2 (Kalis et al., 2009a)). These results are consistent with those presented in Fig. 2 that showed that solute charge had a relatively small effect on diffusion. Homocoagulation of the probe (point iii) was not observed under any of the experimental conditions – diffusion coefficients in water were constant across the range of examined ionic strength values.

Gel swelling (point iv) was measured as the ratio of the gel mass before and after equilibration in solutions of varying ionic strengths (0.1–100 mM) and composition ($NaNO_3$, $Ca(NO_3)_2$). Swelling is thought to lead to larger pore radii and increased interconnectivity (decreased tortuosity) of the gel. When both Na^+ and Ca^{2+} were added, the swelling ratio decreased with ionic strength, however,

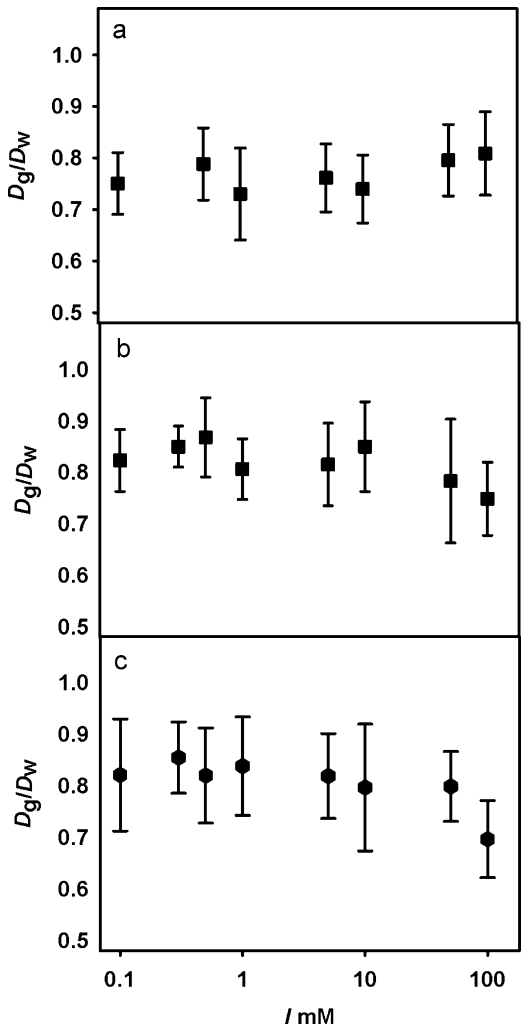

Fig. 3. Diffusion coefficients of the R6G$^+$ in the 1% (w/w) hydrogel with respect to that in the water (D_g/D_w) as a function of ionic strength, I, for a bulk solution containing (a) only $NaNO_3$; (b) only $Ca(NO_3)_2$; and (c) a mixture of $NaNO_3$ and $CaCO_3$ ([Ca]/[Na] = 3/1). The pH of the solutions was 7. Error bars correspond to standard deviations for compiled measurements (n = 30–40 FCS measurements over several days).

gels showed much greater shrinkage for $CaNO_3$ as compared to $NaNO_3$ (Fig. 5a). Indeed, the gel contraction observed with increasing $NaNO_3$ (Fig. 5a) is attributed to a decreasing Donnan potential due to charge screening (Kuo & Ma, 2001; Morris & Rees, 1978; Morris, Rees, Robinson, & Young, 1980; Rees, 1981; Saitoh, Araki, Kon, Katsura, & Taira, 2000; Stokke et al., 2000), whereas compression of the gel with increasing $Ca(NO_3)_2$ can be attributed to an increased crosslinking of the carboxylate functional groups (Davis et al., 2008; Kalis et al., 2009a). Swelling was also determined for gels that were held at a constant ionic strength (I = 100 mM) in the presence of an increasing ratio of Ca to Na (Fig. 5b). Consistent with the diffusion measurements (Fig. 3), a significant contraction of the gel was observed as the Ca/Na ratio increased. Interestingly, a similar gel contraction in the gel structure could be obtained by immersing an already formed gel in a 50 mM calcium nitrate–20 mM sodium nitrate mixture (Supporting information, Fig. S1); in contrast, no difference in the diffusion coefficients was measured.

Since different electrolyte compositions but similar I produced perceptibly different diffusion coefficients, the observed change in D_g was likely not due to changes to the Donnan potential of the gel (Kuo & Ma, 2001; Morris et al., 1980; Rees, 1981) but rather

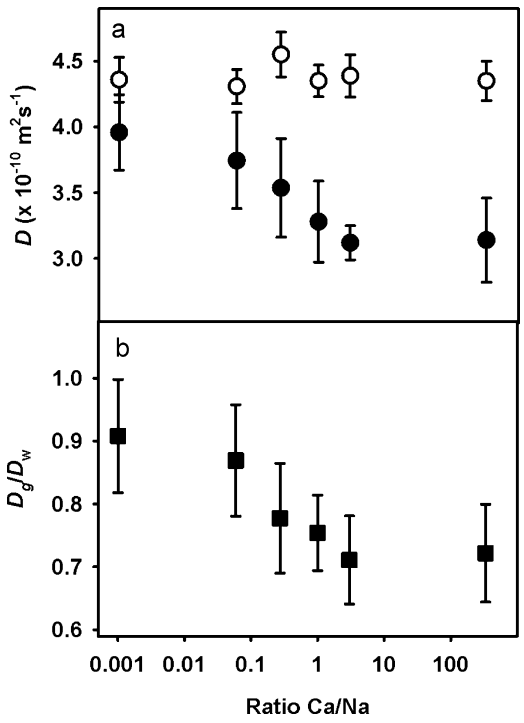

Fig. 4. (a) Diffusion coefficients of R6G$^+$ in water (○) and in the alginate hydrogel (●); (b) D_g/D_w (■) at various ratios of Ca/Na for a constant ionic strength of 100 mM. Error bars correspond to standard deviations for compiled measurements (n = 30 FCS measurements over several days).

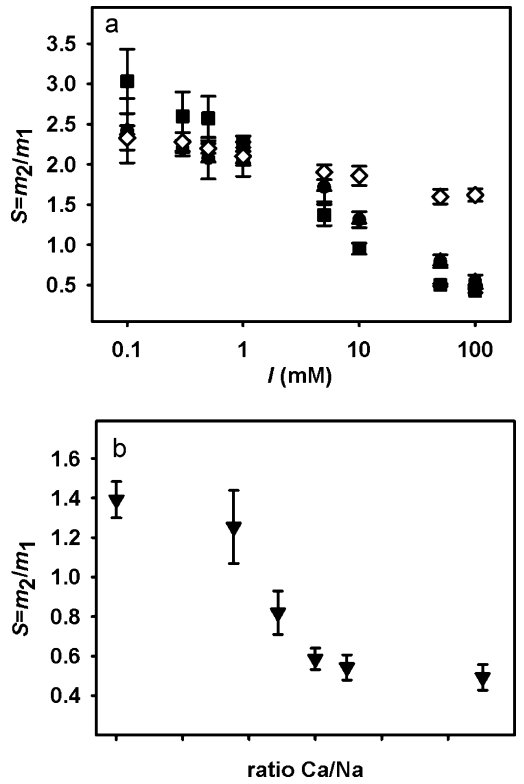

Fig. 5. (a) Swelling of calcium alginate (1%, w/w) in various ionic strengths with only $NaNO_3$ (◊), only $Ca(NO_3)_2$ (▲) and a mixture of $CaCO_3$ and $NaNO_3$ ([Ca]/[Na] = 3/1) (■) in the bulk solutions; (b) Swelling of a calcium alginate (1%, w/w) for a varying ratios of Ca^{2+} to Na^+ in the bulk solutions in a constant ionic strength of 100 mM. Error bars correspond to standard deviations for 2 gels.

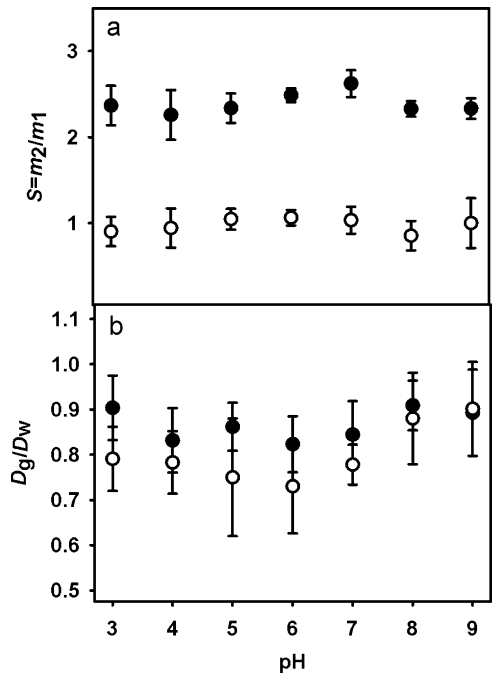

Fig. 6. (a) Swelling of the calcium alginate (1%, w/w) as a function of pH for ionic strengths of 1 mM (●) and 10 mM (○); (b) Ratio of D_g/D_w for R6G$^+$ in a 1% (w/w) calcium alginate gel across a wide range of pH, at ionic strengths of 1 mM (●) and 10 mM (○). For both experiments ([Ca]/[Na] = 3/1). Error bars correspond to standard deviations for compiled measurements (n = 18–20 for FCS measurements; n = 2 for swelling measurements).

its induced structural modifications (Davis et al., 2008; Kalis et al., 2009a; Kuo & Ma, 2001; Liu, Qian, Shu, & Tong, 2003; Saitoh et al., 2000). Note that since any gel contraction will also lead to increased charged density and an enhanced Donnan effect, it is impossible to completely separate the effects of ionic strength on diffusion. Nonetheless, since the directions of the expected effects on the diffusion coefficient were opposed (see above), we are able to conclude that structural effects predominate here. In other words, the significant effect of Ca on diffusion appears to be mainly due to Ca binding to carboxylates and the resulting structural modifications to the gel structure, rather than an effect on the electrostatic interactions between the probe and the gel.

3.4. Effect of pH on diffusion

The pH of the bulk solution can also affect the Donnan potential of the gel via protonation of carboxylate functional groups (Kalis et al., 2009b). Calcium alginate hydrogels were immersed in solutions across the pH range of 3.0–9.0 (I = 10 and I = 1 mM) for a constant Ca/Na ratio of 3. Although gels were significantly more compressed at 10 mM as compared to 1 mM (Fig. 6a), no significant differences in swelling could be attributed solely to the pH changes. pK_a values of M and G monomers that constitute the alginate are in the range of 3.38 and 3.65, respectively (Draget, Braek, & Smidsrod, 1994), and thus only the lowest examined pH would be expected to result in significantly increased protonation of the carboxylate functional groups on the alginate chains. Furthermore, it is possible that the protonation effect leading to decreased charge density of alginate (decreased swelling) (Kalis et al., 2009b) was compensated by a decreased concentration of bound calcium (increased swelling).

Similarly, little variation of the diffusion coefficients was observed as a function of pH, although D_g/D_w values were slightly higher at the extreme pH values (Fig. 6b). At low pH, a decrease in the gel Donnan potential could be responsible for increased diffusion of the cationic R6G due to an increase in the effective pore size of the gel (decrease in the charged double layer). At pH 8 and 9, a larger diffusion coefficient could be attributed to gel degradation due to β-elimination (Haug, 1964; Haug, Larsen, & Smidsron, 1963) or a shift in Ca speciation (formation of $Ca(OH)^+$, $Ca(HCO_3)^+$), leading to reduced Ca^{2+} complexation. Note that reduced Ca binding would be expected to lead to increased gel swelling, which was not observed. The significant effect of calcium on diffusion coupled to the weak effect observed for both Na^+ and H^+ strongly supports the contention that structural rather than charge effects are mainly controlling diffusion through the alginate gel.

4. Conclusion

Numerous factors influence the diffusion of ions in the alginate hydrogel. For example, the gel Donnan potential was previously shown to have a major influence on *predicted diffusive fluxes* within an alginate gel, due to its large Donnan potential (Kalis et al., 2009a). Mutual diffusive fluxes are mainly due to increased cation concentrations associated with the significant negative charge on the alginate gel (resulting in an increased concentration gradient). Since measurements of *diffusion* (as measured here) result from Brownian motion only, they are likely to depend strongly on the physicochemical structure of the gel. Indeed, diffusion coefficients were shown to vary by up to 30%, however, most of the variability was shown to be due to variations in the gel structure (swelling, compression) rather than charge effects. Correspondingly, Ca had the greatest effect on the gel structure and diffusivity while changes in H^+ and Na^+ resulted in only relatively small effects on the diffusion of charged solutes in the gel. Other effects, such as an increasing negative charge of the probe, were shown to result in significant, but smaller (ca. 10%) decreases in diffusion coefficients. As expected, probe size and gel structure had significant effects on diffusion; however, even these effects were not straightforward when looking at the diffusion of charged solutes. For example, while increasing the density of the calcium alginate gel will decrease its pore sizes and increase obstruction effects, especially for the larger probes (Favre, Leonard, Laurent, & Dellacherie, 2001; Gagnon & Lafleur, 2011; Liu et al., 2002), greater gel charge (and Donnan potential) will also result (Golmohamadi et al., 2012), potentially leading to increased mutual diffusion fluxes. Clearly, diffusive transport in charged gels such as the alginate can only be thoroughly understood by taking into account all of the effects resulting from charge (solute and gel), size (solute), structure (gel) and concentration gradients.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.01.046.

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