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Impact of cross-linker on alginate matrix integrity and drug release

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Abstract

Sodium alginate, a biopolymer, was employed in the formulation of matrix tablets. They cracked or laminated at acidic pH, compromising their dissolution performance. Improved mechanical strength and reduced barrier permeability of calcium alginate gel provided the rationale for cross-linking the alginate matrix to sustain drug release. Studies had suggested that the incorporation of soluble calcium salts in alginate matrix tablets could sustain drug release at near-neutral pH due to *in situ* cross-linking. However, results from the present study showed otherwise when gastrointestinal pH conditions were simulated. Significant reduction in drug release rate was only observed when an external calcium source was utilized at low concentration. High calcium ion concentrations caused matrix disintegration. In contrast, matrices pre-coated by calcium alginate could sustain drug release at pH 1.2 followed by pH 6.8 for over 12 h. The presence of cross-linked barrier impeded matrix lamination and preserved matrix structure, contributing to at least three-fold reduction in drug release at pH 1.2. Zero order release as well as delayed burst release could be achieved by employing appropriate grade of alginate and cross-linking conditions.

Keywords: Lamination; Cross-linking; Disintegration; Barrier preservation; Modified release

1. Introduction

Sodium alginate is a biopolymer that is widely used as an encapsulation matrix due to its ability to form hydrogels upon cross-linking. Its ability to gel under mild conditions makes alginate the polymer-of-choice in food, pharmaceutical and biotechnological applications. Alginates are linear unbranched copolymers of β-D-mannuronic acid (M) and α-L-guluronic acid (G) units. The M and G monomers are $1 \rightarrow 4$ linked by glycosidic bonds, forming homopolymeric M- or G-blocks and heteropolymeric MG blocks. In the presence of polyvalent cations such as Ca^{2+} or Al^{3+}, cross-linking occurs to form gels. The cations act as bridges between the anionic polymer chains, constituting junction zones, forming a hydrogel network. Ca^{2+}, a commonly used cross-linker, preferentially interacts with G-blocks due to structurally favorable chelation sites formed by the corrugated chains (Braccini et al., 1999). Hence, selective ion binding is linked to the content of G-blocks (Smidsrød, 1974). Due to selective ion binding, cross-linking of alginates of different chemical compositions results in gels with different properties (Skjåk-Bræk, 1992).

Cross-linking of alginate has been employed to prepare delivery systems such as beads, microspheres and film-coatings (Lee et al., 2005; Chan et al., 2006). Sustained-release from such delivery systems has met with limited success, particularly with highly water-soluble drugs (Østberg et al., 1994; Chan et al., 1997) due to the high porosities of these matrices (Klein et al., 1983; Hills et al., 2000). In contrast, sodium alginate matrix tablets have been shown to sustain the release of a highly water-soluble drug (Liew et al., 2006). It was also observed that certain grades of alginate gave rise to faster drug release in acid due to crack formation or lamination. Crack formation can potentially limit the use of alginate matrix tablets for oral drug delivery.

Researchers have employed cross-linking to retard drug release from alginate matrix tablets. Azarmi et al. (2003) reported reduced drug release rates at pH 7.4 with increasing calcium chloride dihydrate concentration (0.75–19%, w/w) incorporated into alginate matrices. Nokhodchi and Tailor

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(2004) observed slower drug release only at high (21%, w/w) calcium salt content. At intermediate calcium salt contents (11.8 and 16.7%, w/w), initial burst release was enhanced followed by slower drug release. Drug release was rapid at low calcium salt concentration (3.8%, w/w) and this was attributed to insufficient cross-linking to produce an insoluble barrier. In contrast, another study reported enhanced drug release from alginate matrices containing calcium gluconate at pH 7.4 (Güngör et al., 2003). The impact of calcium gluconate was not clearly elucidated as the amount of sodium alginate was not kept constant among formulations. In addition, the use of microcrystalline cellulose as the diluent could have interfered with alginate–calcium interaction. When calcium gluconate was added into sodium alginate matrix capsules (5–20%, w/w), faster drug release occurred in acid due to channeling effect of the soluble salt (Pongjanyakul and Puttipipatkhachorn, 2007). In contrast, addition of 0.5% (w/w) calcium acetate into alginate matrix tablets retarded drug release in acid, suggesting cross-linking in the presence of H^+ (Sriamornsak et al., 2007). It was also noted that calcium acetate did not affect matrix morphology. In yet another study, calcium alginate-coated tablets were found to produce zero order drug release in water (Bhagat et al., 1991). To date, studies on drug delivery from matrices containing cross-linkers or cross-linked alginate matrix tablets under conditions simulating changes in gastrointestinal pH are limited. Moreover, the impact of cross-linking on the integrity of alginate matrix tablets in gastric pH has not been well-documented.

The effect of acidic media on alginate matrices warrants an investigation as oral tablets will be exposed to the acidic gastric juices. Besides, sodium alginate is pH-sensitive. At pH below the pK_a of M (3.38) and G (3.65) monomers, soluble sodium alginate is converted to insoluble alginic acid (Haug, 1964). The pH-sensitivity of alginate resulted in pH-dependent hydration, swelling and erosion kinetics, giving rise to pH-dependent drug release from these matrices (Chan et al., 2007). Alginate matrix tablet cracked at acidic pH. It had been suggested that calcium alginate gel is stronger than the corresponding alginic acid gel (Draget et al., 1994, 2006) and therefore less susceptible to mechanical damage due to swelling pressure. Hence, in the present study, it was hypothesized that matrix lamination could be minimized by cross-linking, thereby retarding drug release at gastric pH.

Cross-linking can be achieved by incorporating calcium salts into alginate matrices or by immersing sodium alginate matrices in solutions containing Ca^{2+}. The availability of Ca^{2+} from incorporated calcium salts depends on the amount added and their solubilities. Hence, calcium salts of different solubilities were added at two concentrations in this study. Dissolution studies for matrices containing calcium additives were carried out at pH 1.2 followed by pH 6.8 to simulate changes in gastrointestinal pH. In addition, dissolution was carried out at pH 6.8 to remove competition with H^+ as well as to prevent acid-induced crack formation. The impact of external calcium source was examined by performing dissolution studies of sodium alginate matrices in calcium chloride solutions of varying concentrations. Further studies on externally cross-linked matrices were also carried out to assess their potential for sustaining drug release under simulated gastrointestinal pH conditions. As cross-linking is influenced by the MG content of alginates, high-M (Manucol SS/LL) and high-G (Manugel DMB) alginates were employed in these investigations.

2. Materials and methods

2.1. Materials

Two grades of sodium alginate (ISP-Alginates Industries, USA) with different MG ratios but comparable viscosity and median particle size were used. These were Manucol SS/LL (60% M, 40% G) and Manugel DMB (37% M, 63% G) (Lawson, 2003, personal communication); each had kinematic viscosities of 108 and 115 $mm^2\,s^{-1}$ (1%, w/w solution in water at 37 °C), as well as median particle sizes of 75 and 82 μm, respectively. Chlorpheniramine maleate (BP grade, China) was used as a highly water-soluble model drug. Calcium salts used were dibasic calcium phosphate (Emcompress, Edward Mendell, USA), calcium gluconate monohydrate, calcium carbonate and calcium chloride dihydrate (Merck, Germany). Magnesium stearate (Merck, Germany) was used as the lubricant. Sodium chloride (Merck, Germany) was used in the investigation on ionic strength effect. Methylene blue and bromophenol blue (J.T. Baker, USA) were used as dye and pH-indicator, respectively.

2.2. Preparation of matrix tablets

The amounts of sodium alginate and model drug were kept constant at 306.5 and 40 mg per tablet, respectively. Calcium gluconate, dibasic calcium phosphate or calcium carbonate was incorporated into alginate matrices at 5% (w/w) (18.4 mg/tablet) and 20% (w/w) (87.7 mg/tablet). Weighed amounts of sodium alginate, drug and calcium salt were randomly mixed in a bag for 10 min. Magnesium stearate (1%, w/w) was then added, followed by further mixing for 2 min. The resultant powder mixture was individually weighed and compressed into tablets using a single punch machine (F3, Manesty, UK) with 9.5 mm diameter flat punches. Tablets with porosities of 0.20–0.25 were made. Control matrices (without calcium salt) were prepared with or without methylene blue (2 mg/tablet). Matrices were also made with bromophenol blue (2 mg/tablet) to visualize changes in matrix micro-environmental pH. All matrices were stored in a desiccator for at least 3 days to allow for tablet relaxation before use.

2.3. Preparation of calcium alginate-coated matrices

Each Manugel DMB control matrix (without calcium salt) was immersed in 500 ml of 0.1 or 0.01 M calcium chloride solution with gentle stirring at 30 rpm and 25 °C for 0.5, 1 or 1.5 h. The cross-linked matrices were oven-dried at 40 °C overnight and stored in a desiccator for a day prior to dissolution testing.

2.4. Dissolution studies

Drug release from matrix tablets was evaluated by dissolution testing using paddles at 50 rpm and 37 ± 0.5 °C (USP Appa-

ratus II, Vankel, USA). Dissolution studies were divided into two parts. The first part evaluated the influence of calcium salts incorporated into alginate matrices (internal calcium salts) at pH 1.2 followed by pH 6.8 (USP method A), as well as in pH 6.8 media alone. According to USP method A, dissolution was first carried out in 750 ml of 0.1N hydrochloric acid (pH 1.2) for 2 h, followed by addition of 250 ml of 0.2 M sodium phosphate solution, preheated to 37 °C. Concentrated hydrochloric acid (2 M) was used for minor adjustment of the dissolution media pH to 6.8 ± 0.1 when necessary. The pH 6.8 media employed consisted of 750 ml of 0.1N hydrochloric acid and 250 ml of 0.2 M sodium phosphate, adjusted to pH 6.8 ± 0.1 with 2 M hydrochloric acid. The second part of the dissolution studies investigated the effects of external Ca^{2+} on drug release behavior of alginate matrices without adding calcium salt in the tablets. Appropriate quantities of calcium chloride dihydrate were dissolved in distilled water to prepare 0.01, 0.05, 0.1, 0.2 and 0.5 M solutions. Dissolution of control matrices was also carried out in sodium chloride solutions to investigate the effect of ionic strength.

At suitable time intervals, samples were collected and assayed spectrophotometrically (UV-1201, Shimadzu, Japan) at 266 nm for samples in acid, and at 262 nm for samples in buffer or water (or calcium chloride or sodium chloride solutions), using the appropriate Beer's plots. For each formulation, at least triplicate dissolution runs were carried out and the averaged results reported.

2.5. Measurement of liquid transport by gravimetry and image analysis

Manugel DMB matrices containing a water-soluble dye, methylene blue, were used to study liquid penetration in the absence and presence of external calcium source. These matrices were immersed in distilled water and 0.01 or 0.1 M calcium chloride solutions at 37 ± 0.5 °C. Each matrix tablet was placed on a stainless steel mesh to facilitate matrix retrieval. At appropriate time intervals, hydrated matrices were retrieved from the liquid medium, gently blotted to remove excess liquid and weighed (W_w).

The matrices were then immersed in liquid nitrogen for 5–10 s to prevent matrix deformation upon cutting. Images of the cross-sectioned matrices were captured with a digital camera (E-300, Olympus, Japan) and analyzed using an imaging software (Image-Pro Plus, Media Cybernetics, Inc., USA). At least three matrices were measured for each time point. Matrix swelling was expressed as a percentage of the initial diameter or thickness of the matrix; the hydrated and dry core areas were reported as the actual measurements.Hydrated matrices retrieved at each time point were oven-dried at 60 °C to constant weight to determine the extent of matrix erosion, which was calculated using Eq. (1):

$$\%\ \text{matrix erosion} = 100\frac{W_i - W_d}{W_i} \quad (1)$$

where W_i and W_d are the initial and the final dry weights of the matrix, respectively.

Subsequently, liquid uptake per unit weight of matrix remaining was computed according to the following Eq. (2):

$$\%\ \text{liquid uptake} = 100\frac{W_w - W_d}{W_d} \quad (2)$$

2.6. Curve-fitting and statistical analysis

The rate of drug release was determined by curve-fitting to zero order or Higuchi's square-root equations using GraphPad Prism 3.0 for comparative purposes. Analysis of variance (ANOVA) was carried out to compare drug release rate at $\alpha = 0.05$ using SPSS version 11.0, followed by post hoc tests when $P < 0.05$ and more than two group means were compared. The Dunnett test or Bonferroni test was performed when comparison was made against the control or against each experimental group, respectively.

3. Results and discussion

3.1. Influence of calcium salts incorporated into matrix tablets on drug release

3.1.1. Dissolution at pH 6.8

The incorporation of 20% water-soluble calcium gluconate accelerated drug release from alginate matrices, and the effect was greater for Manugel DMB matrices (Table 1A). Rapid release of Ca^{2+} resulted in rapid formation of calcium alginate, before sodium alginate particles could swell and fuse together to form a continuous barrier. Less extensive cross-linking in high-M alginate allowed better overall hydration and polymer coalescence. Formation of non-binding calcium alginate in the midst of channeling action by soluble calcium gluconate resulted in aggregates of calcium alginate, which detached readily from the matrix surface. Hence, drug release was accelerated as cross-linking contributed to increased matrix erosion rather than reinforcing barrier function. These findings are in contrast to previous studies (Azarmi et al., 2003; Nokhodchi and Tailor, 2004) which employed soluble calcium chloride dihydrate as the cross-linker. In the present study, matrices containing calcium chloride were not used as the matrices were too "wet" and sticky due to the hygroscopicity of the calcium salt. This made handling of the matrices difficult and might give rise to inaccurate results.

Dibasic calcium phosphate and calcium carbonate are practically insoluble in pH 6.8 media. Despite being insoluble, both additives produced contrasting effects on drug release. Dibasic calcium phosphate did not affect drug release from both types of alginate matrices. In contrast, addition of calcium carbonate increased drug release rate from Manugel DMB matrices. Contact angles of water on pure compacts of dibasic calcium phosphate or calcium carbonate were 31° and 30°, respectively (Odidi and Newton, 1993), indicating that both calcium salts had similar wettability and were not likely to affect matrix wetting to different extents. Dibasic calcium phosphate and calcium carbonate are sparingly soluble in water (0.2 and 0.014 g/L, respectively) (Baden, 2000). However, in pH 6.8 media, the

Table 1
Effect of calcium salt inclusion on drug release rates from Manugel DMB or Manucol SS/LL matrix at (A) pH 6.8 and (B) pH 1.2 (2 h) followed by pH 6.8

Type of matrix	Rate of drug release (% min^{-1})[a]	
	Manugel DMB	Manucol SS/LL
(A)		
Control	0.09 (0.00)	0.12 (0.00)
5% calcium gluconate	0.10 (0.00)	0.10 (0.00)
20% calcium gluconate	1.24 (0.04)[b]	0.25 (0.01)[b]
5% dibasic calcium phosphate	0.10 (0.00)	0.11 (0.00)
20% dibasic calcium phosphate	0.10 (0.00)	0.11 (0.00)
5% calcium carbonate	0.14 (0.01)	0.10 (0.00)
20% calcium carbonate	0.22 (0.01)[b]	0.10 (0.00)

Type of matrix	Rate of drug release (% min^{-1})[a]			
	Manugel DMB		Manucol SS/LL	
	pH 1.2	pH 6.8	pH 1.2	pH 6.8
(B)				
Control	0.51 (0.01)	0.13 (0.01)	0.23 (0.01)	0.11 (0.00)
5% calcium gluconate	0.71 (0.01)[b]	[c]	0.24 (0.01)	0.07 (0.00)
20% calcium gluconate	1.31 (0.06)[b]	[c]	0.41 (0.01)[b]	0.58 (0.06)[b]
5% dibasic calcium phosphate	0.54 (0.01)	0.18 (0.01)[b]	0.32 (0.01)[b]	0.08 (0.00)
20% dibasic calcium phosphate	0.41 (0.01)[b]	0.17 (0.01)[b]	0.43 (0.01)[b]	0.15 (0.01)
5% calcium carbonate	0.48 (0.01)	0.19 (0.01)[b]	0.27 (0.01)	0.13 (0.01)
20% calcium carbonate	0.36 (0.01)[b]	0.21 (0.02)[b]	0.26 (0.01)	0.23 (0.01)

Values in parentheses represent standard errors.
[a] Drug release rate was determined by curve-fitting to zero order equation up to 90% drug release.
[b] Significantly different compared to control ($P < 0.05$).
[c] Curve-fitting was not carried out as 80–90% of drug was released at pH 1.2.

abundance of PO_4^{3-} would suppress the ionization of dibasic calcium phosphate due to common ion effect. Although limited, Ca^{2+} from the ionization of calcium carbonate within the matrix could have resulted in cross-linking. Considering the limited solubility of calcium carbonate, cross-linking was likely to be restricted to the immediate vicinity of the dissolving salt, resulting in microscopic calcium alginate patches which hindered uniform hydration of surface alginate, thereby compromising barrier formation. Increased drug release was only observed with the high-G matrices containing calcium carbonate as Ca^{2+} preferentially binds to guluronate segments. The apparent lack of gel barrier disruption in Manucol SS/LL matrices was due to the higher manuronate content which was relatively insensitive to Ca^{2+} (Smidsrød, 1974).

3.1.2. Dissolution at pH 1.2 followed by pH 6.8

In general, incorporation of calcium salts into alginate matrices did not significantly reduce drug release rates at pH 1.2 and pH 6.8 (Table 1B). Dissolution of calcium gluconate caused channeling which increased acid gel porosity, causing faster drug release. Even though Ca^{2+} was available, interaction with alginate was not favorable due to competition with H^+ at pH 1.2. Addition of dibasic calcium phosphate increased drug release rates from Manucol SS/LL matrices at pH 1.2. The relatively insoluble particles (solubility of ~1% (w/w) at pH 1.2) disrupted barrier formation by preventing polymer particle coalescence during hydration. On the other hand, 20% dibasic calcium phosphate reduced drug release from Manugel DMB matrices. Given that these matrices cracked extensively at pH 1.2 (Fig. 1A), the influence of dibasic calcium phosphate on barrier formation might be negligible. On the contrary, these insoluble particles could have reduced matrix wetting at 20% concentration, slowing drug release. Addition of 20% calcium carbonate retarded drug release from Manugel DMB matrices at pH 1.2. The surface layer of carbon dioxide bubbles generated by the reaction between calcium carbonate and acid provided a secondary diffusion barrier and was only effective at 20% additive level. At pH 6.8, drug release rates increased or remained unchanged in the presence of calcium additives, as explained in the preceding section.

3.2. Effect of external calcium source on drug release from alginate matrices

Dissolution of sodium alginate matrices (without calcium salt) was carried out in calcium chloride solutions to determine whether sustained drug release could be attained from externally cross-linked alginate matrices. Without cross-linking, drug release from Manugel DMB and Manucol SS/LL matrices was complete at 9 and 7 h, respectively, whereas drug release could be sustained for at least 24 h with *in situ* cross-linking (Fig. 2).

3.2.1. Influence of calcium ion concentration on drug release

Besides varying the availability of Ca^{2+} for cross-linking, varying Ca^{2+} concentrations also affected the ionic strength of the dissolution media. Hence, the influence of ionic strength on drug release was investigated by conducting dissolution studies

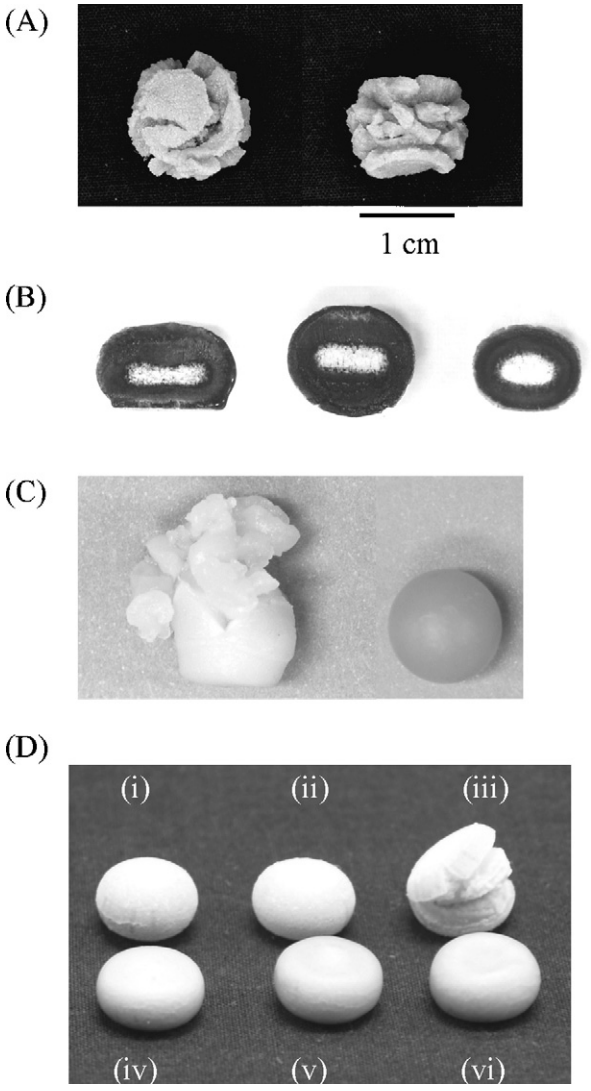

Fig. 1. (A) Axial and radial view of Manugel DMB control matrices at 110 min at pH 1.2; (B) axial cross-sections of Manugel DMB matrices containing methylene blue at 110 min in water (left), 0.01 M (center) and 0.1 M (right) calcium chloride solutions. The dark grey area represents the hydrated area; (C) appearance of Manucol SS/LL (left) and Manugel DMB (right) matrices after 24 h dissolution in 0.01 M calcium chloride solution and (D) images of matrices at 110 min of dissolution at pH 1.2 for calcium alginate-coated Manugel DMB matrices cross-linked in 0.1 M calcium chloride solution for (i) 1.5, (ii) 1, (iii) 0.5 or 0.01 M calcium chloride solution for (iv) 1.5, (v) 1 and (vi) 0.5 h, respectively.

in sodium chloride solutions of equivalent ionic strengths using Manugel DMB matrices. Clearly, drug release was affected by solution ionic strength (Fig. 3). However, drug release kinetics differed in the presence of cross-linkers (Fig. 2A). Dissolution profiles during early stages of drug release were examined to elucidate the influence of ionic concentration on the mechanism of diffusion barrier formation. In general, drug release during the initial hour was slower at lower ionic concentrations (Fig. 2A; Fig. 3). This suggests that barrier formation was influenced by ionic strength, in addition to cross-linking. A reduction in polymer–solvent interaction was postulated to decrease polymer solubility with increasing solution ionic strength (Cho et al., 2006). Hence, higher concentration of ions in the vicinity

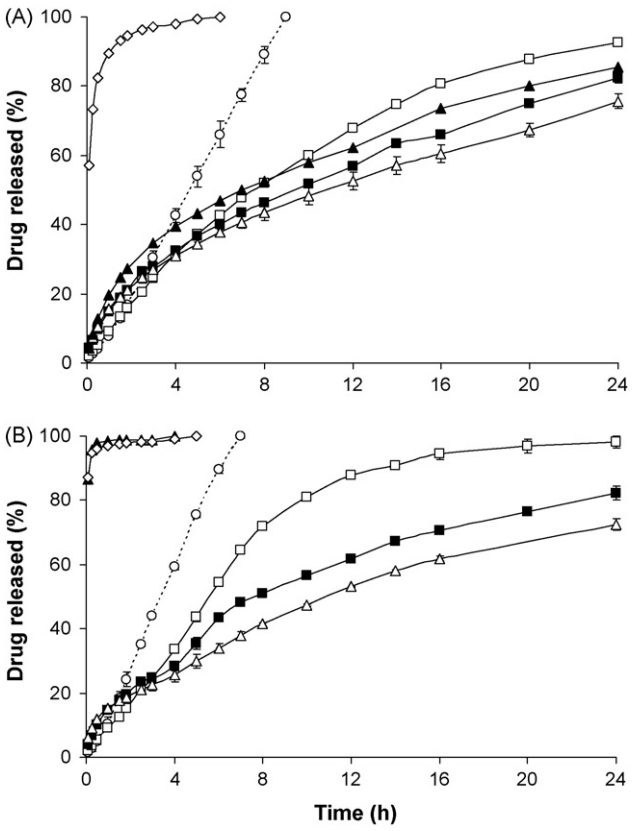

Fig. 2. The influence of external calcium source at 0 M (water) (◯), 0.01 M (□), 0.05 M (■), 0.1 M (△), 0.2 M (▲) and 0.5 M (◊) on drug release profiles from (A) Manugel DMB and (B) Manucol SS/LL matrices. Vertical bars represent standard error of mean.

reduced the hydration rate of surface alginate due to competition for water of hydration, and subsequently slowed down the formation of an intact gel barrier. Furthermore, as Ca^{2+} content increased, alginate was more rapidly cross-linked which

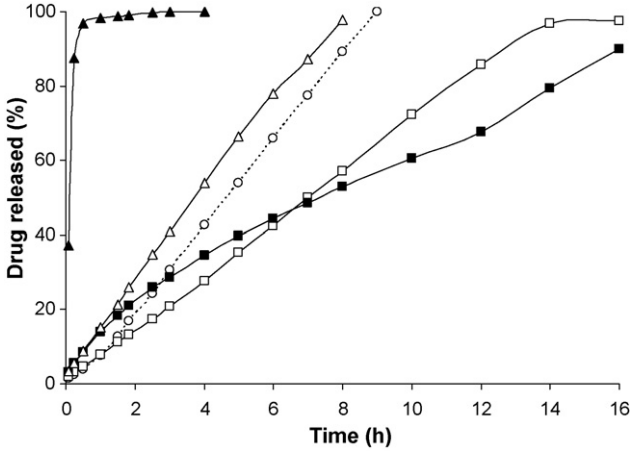

Fig. 3. The influence of ionic strength (adjusted using sodium chloride) on drug release from Manugel DMB matrices. Ionic strengths used were 0 M (water) (◯), 0.03 M (□), 0.3 M (■), 0.6 M (△) and 1.5 M (▲). These were equivalent to calcium chloride concentrations of 0, 0.01, 0.1, 0.2 and 0.5 M, respectively. Ionic strength, $I = 1/2 \sum c_i z_i^2$ where c_i is the ionic concentration in units of molarity and z_i is the number of charges on the ion (Maron and Lando, 1974).

further hindered polymer swelling. Initial polymer hydration, swelling and coalescence were important to occlude surface pores and minimize barrier permeability. The permeability of the initial barrier formed was generally greater at higher Ca^{2+} concentration, as indicated by the corresponding faster initial drug release.

The importance of rapid polymer hydration in establishing a functional diffusion barrier was illustrated by matrix disintegration at high salt concentrations. In 0.5 M calcium chloride and 1.5 M sodium chloride solutions, alginate hydration and subsequent barrier formation was severely compromised. Without quick polymer swelling to occlude surface pores, liquid uptake by capillary forces was enhanced. At the same time, rapid formation of non-binding calcium alginate aggregates further compromised barrier formation. Without an integral barrier, rapid liquid influx into the matrix interior was possible and subsequent development of swelling pressure from within forced the matrix to disintegrate.

3.2.2. Liquid penetration study to elucidate mechanism of drug release

To further examine the influence of Ca^{2+} concentration on barrier formation, solvent penetration studies were performed on Manugel DMB matrices using a dye (Fig. 1B). Overall liquid uptake was markedly reduced by cross-linking (Fig. 4A) due to the insolubility of calcium alginate. Greater extent of liquid uptake by matrices in 0.01 M solution produced higher overall matrix swelling compared to matrices in 0.1 M solution (Fig. 4B). This was not the case for control matrices as polymer dissolution reduced the apparent matrix swelling. Image analysis of matrix cross-sections (Fig. 1B) also showed that matrices immersed in 0.01 M solution were more extensively hydrated as indicated by larger hydrated area in these matrices (Fig. 5). As the apparent dry core area was similar for matrices immersed in both 0.01 and 0.1 M solution, the larger hydrated area of matrices in 0.01 M cross-linker solution implied that the liquid imbibed by matrices during this stage was used to swell the alginate polymer within the hydrated area. This suggests that higher cross-linker concentration reduced the extent of polymer hydration, as mentioned in the preceding section, giving rise to faster initial drug release (Fig. 2).

Reducing drug release rates from cross-linked matrices can be attributed to minimal matrix erosion (Fig. 4C), which increased drug diffusion path length. Erosion of control matrices (Fig. 4C) maintained a constant diffusion barrier thickness, producing pseudo-zero order drug release (Fig. 2). Moreover, increasing extent of cross-linking resulted in tighter polymer networks that retarded drug release. Polymer chains contract during transition from sol to gel state (Woelki and Kohler, 2003). Interaction with Ca^{2+} draws alginate chains closer together, facilitating hydrogen bonding which promotes gel consolidation (King, 1983). This was shown by matrix consolidation upon complete wetting of the matrix core (4.2 and 12.3% reduction in cross-sectional area from 3 to 6 h for matrices in 0.1 and 0.01 M calcium chloride solution, respectively) (Fig. 5). The minimal drug loss (1.4 and 2.1% in 0.1 and 0.01 M calcium chloride solution, respectively) and minimal change in matrix erosion (0.8 and 1.7% in 0.1 and 0.01 M calcium chloride solution, respectively) during this stage (3–6 h) were unlikely to have caused the reduction in total cross-sectional area.

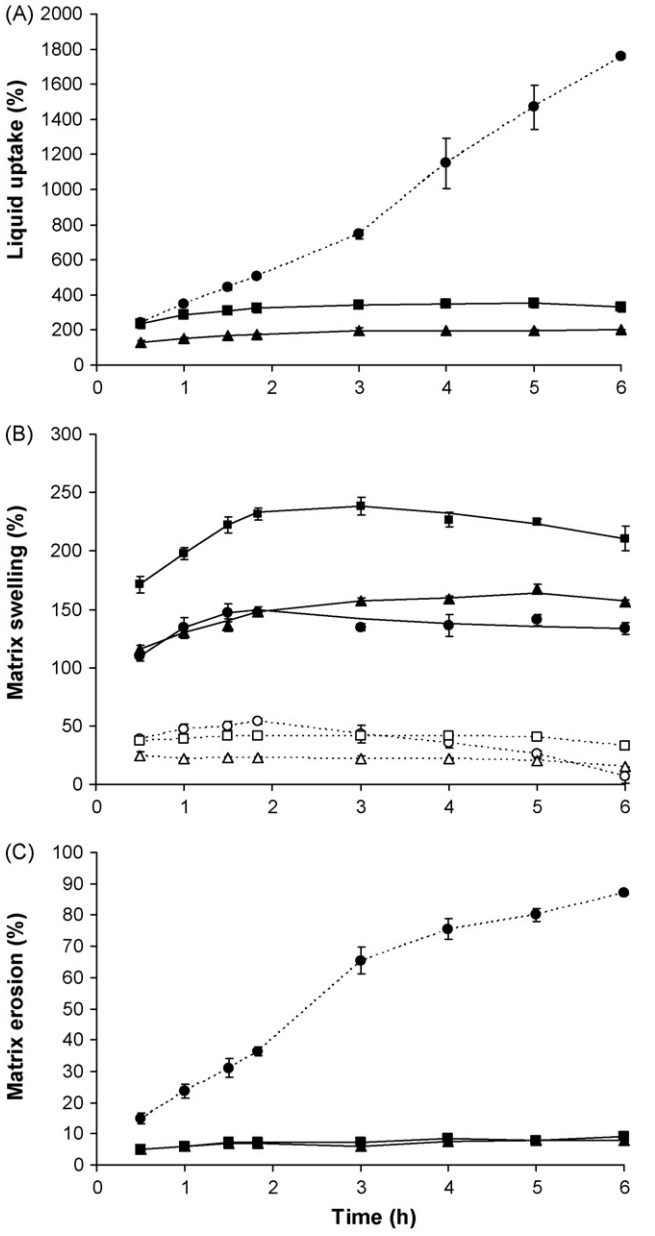

Fig. 4. The profiles of (A) liquid uptake, (B) matrix swelling and (C) matrix erosion of Manugel DMB matrices in water (◯, ●) as well as in 0.01 M (□, ■) and 0.1 M (△, ▲) calcium chloride solutions. Swelling in the axial and radial directions is denoted by closed and open symbols, respectively.

Reduction in cross-sectional dry core area occurred linearly to similar extents in all three media (Fig. 5), suggesting that liquid penetration was not the rate-limiting factor in matrix wetting. However, the rates of polymer hydration and swelling were dependent on the availability of free water molecules, which was determined by media composition. At low solute concentration, polymer hydration was relatively unhindered, as shown by larger hydrated areas in water and 0.01 M calcium chloride solution (Fig. 5). Rapid hydration of polymer facilitated the formation of an intact gel barrier for better initial retardation of drug release.

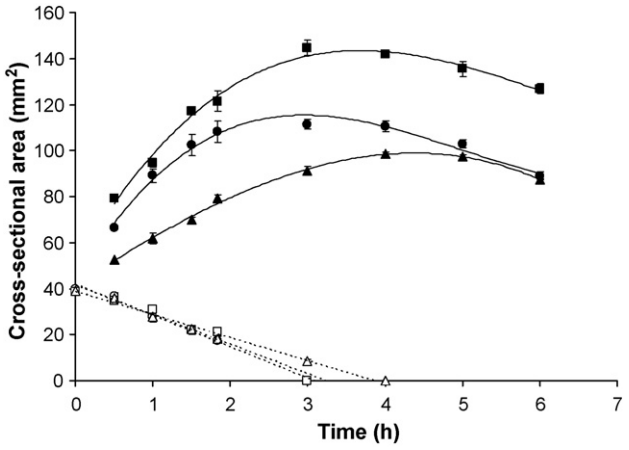

Fig. 5. Hydrated (closed symbol) and apparent dry core (open symbol) area of matrix cross-sections in water (◯, ●) as well as in 0.01 M (□, ■) and 0.1 M (△, ▲) calcium chloride solutions.

Therefore, it is not the rate of liquid penetration but the rate of polymer hydration that determines drug release characteristics from a matrix system.

3.2.3. Influence of alginate grade on drug release

Manugel DMB matrices showed slower overall drug release than Manucol SS/LL matrices in 0.01 and 0.05 M calcium chloride solutions (Table 2). The latter swelled and cracked extensively (Fig. 1C), causing faster drug release. In contrast, Manugel DMB matrices remained intact (Fig. 1C). Calcium alginate formed from high-G alginates are mechanically stronger (Skjåk-Bræk, 1992; Mancini et al., 1999) and hence, more capable of withstanding swelling pressure. Despite crack formation, drug release was sustained (Fig. 2). Rupture of the cross-linked barrier caused leakage of viscous sodium alginate from the inner hydrated layer, which gelled upon contact with Ca^{2+}. This resulted in the formation of a gelatinous mass at the cracked region (Fig. 1C) which effectively sealed the crack, preserving the diffusion barrier. The presence of hydrated sodium alginate within the matrix was likely as the incoming Ca^{2+} were sequestered upon contact with available binding sites in alginate while water molecules continued to imbibe further into the sodium alginate matrix. This 'self-sealing' mechanism enabled continued drug release retardation from such matrices.

Table 2
Influence of alginate grade on drug release rate from alginate matrices undergoing dissolution in calcium chloride solution

Calcium chloride concentration (M)	Higuchi rate constant (% $min^{-0.5}$)[a]	
	Manugel DMB	Manucol SS/LL
0.01	3.20 (0.02)	5.24 (0.12)
0.05	2.20 (0.02)	2.41 (0.10)
0.1	2.10 (0.05)	2.14 (0.05)
0.2	2.07 (0.07)	–

Values in parentheses represent standard errors.
[a] Curve-fitting to the Higuchi equation was carried out for 20–80% drug release ($R^2 > 0.98$).

At 0.1 M Ca^{2+} concentration, the effect of alginate MG ratio on drug release was not apparent as ionic strength effect comes into play. Reduced rate of polymer hydration at higher ionic strength could have masked the influence of alginate MG ratio on gel barrier property. Interestingly, Manucol SS/LL matrices disintegrated at 0.2 M Ca^{2+} concentration while Manugel DMB matrices remained intact. More extensive cross-linking of minimally hydrated polymer in the latter probably kept the matrices intact.

3.3. Dissolution performance of calcium alginate-coated matrices

Results have shown that prolonged release of a highly water-soluble drug from alginate matrices can be achieved via *in situ* cross-linking using an external calcium source (Fig. 2). In addition, the integrity of Manugel DMB matrices was preserved. Calcium alginate-coated matrices were therefore prepared and tested under simulated gastrointestinal pH conditions. Such studies have not been reported. Manugel DMB was used in this part of the study as it showed minimal rupturing tendency relative to Manucol SS/LL matrices during dissolution in calcium chloride solution. Manugel DMB matrices were immersed in 0.1 and 0.01 M calcium chloride solution for 0.5, 1 or 1.5 h and oven-dried overnight. Matrices were also immersed in distilled water to account for the effects of cross-linking as well as drug loss. However, these matrices stuck to the mesh due to the adhesive nature of sodium alginate and cracked upon drying. Hence, further investigations on these matrices were not carried out and Manugel DMB matrices without coating were used as the control.

Dissolution studies showed markedly reduced drug release rates from the cross-linked matrices in the acidic phase (Fig. 6). Drug release at 2 h decreased from 60 to 22% or less with cross-linking. Matrices cross-linked in 0.1 M calcium chloride solution showed lower drug release rates in acid than matrices treated in

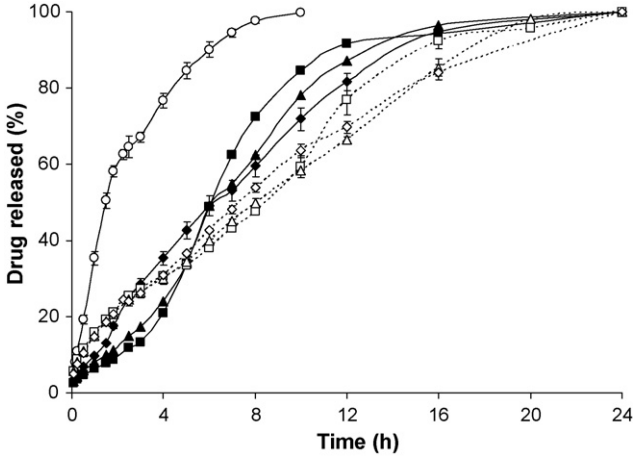

Fig. 6. Drug release from calcium alginate-coated Manugel DMB matrices in pH 1.2 (2 h) followed by pH 6.8 media. Matrices were previously cross-linked in 0.1 M (closed symbol) and 0.01 M (open symbol) calcium chloride solutions for 1.5 h (□, ■), 1 h (△, ▲) or 0.5 h (◊, ♦) and dried prior to dissolution testing. Dissolution profiles of control matrices are denoted by (◯).

Table 3
Drug loss during immersion in cross-linking solution and drug release rate from calcium alginate-coated matrices

Calcium chloride concentration (M)	Duration of immersion in cross-linking solution (h)	Drug loss (%)	Drug release rate in acid (% $min^{-0.5}$)[a]
0.1	0.5	7.7 (0.2)	1.29 (0.06)[b]
	1	11.4 (0.3)	0.99 (0.02)[b]
	1.5	14.9 (0.7)	0.75 (0.03)[b]
0.01	0.5	3.4 (0.3)	1.91 (0.06)
	1	6.8 (0.5)	1.83 (0.05)
	1.5	9.9 (1.2)	1.86 (0.03)

Values in parentheses represent standard errors.
[a] Drug release rate was determined by curve-fitting to the Higuchi equation.
[b] Significantly different compared to each other.

0.01 M calcium chloride solution. Drug loss during cross-linking from these matrices was higher than from those cross-linked in 0.01 M solution (Table 3). Greater extent of drug depletion from the outer layer of the matrix could have reduced the initial drug release rate due to increased diffusion path length as well as reduced drug concentration gradient for diffusion. In addition, matrices cross-linked for a longer duration had lower drug release rates in the acidic phase ($P < 0.05$) but this was only observed for matrices cross-linked in 0.1 M calcium chloride solution. Apparently, the amount of drug loss during cross-linking for different durations in 0.01 M calcium chloride did not affect drug release at pH 1.2 significantly ($P > 0.05$). The different duration of cross-linking did not influence drug release too, probably due to minimal formation of cross-linkages within the short treatment duration at low Ca^{2+} concentration. This suggests that drug loss in the reported range was unlikely to have an effect on drug release rate. Furthermore, drug release from matrices cross-linked in 0.01 M solution for 1.5 h was faster than that from matrices cross-linked in 0.1 M solution for 0.5 h, even though drug loss from the former was greater than the latter (Table 3). Hence, the lower rate of drug release from matrices treated in 0.1 M calcium chloride solution as well as the decreasing drug release rate with increased treatment duration at this concentration was more likely to be due to lower barrier permeability brought about by more extensive cross-linking rather than drug depletion. This observation was also made in previous studies on alginate film permeability (Julian et al., 1988; Chan et al., 2006). For a highly water-soluble drug, drug release is governed by the thickness and consistency of the diffusion barrier, and not just the mere distance of the drug from the bulk media.

The cross-linked matrices remained relatively intact during dissolution at pH 1.2 (Fig. 1D).

In contrast, control matrices laminated extensively, leading to high drug release rates at this pH (Fig. 1A). Clearly, preservation of a continuous barrier in cross-linked matrices during dissolution contributed significantly towards drug release retardation at pH 1.2. The matrix structure was preserved even with gradual conversion of calcium alginate to alginic acid. Preservation of the matrix structure also affected the shape of the drug release profiles at pH 1.2. Drug release from control matrices showed linear kinetics (Table 1B) which can be attributed to increasing surface area exposed to dissolution media with time, brought about by matrix lamination. In contrast, drug release from cross-linked matrices followed square-root kinetics (Table 3), indicating diffusion-controlled drug release. This was not surprising since the calcium alginate-coated matrix retained its shape throughout dissolution at this pH (except for matrices cross-linked for 0.5 h in 0.1 M calcium chloride solution).

Burst release was observed shortly after pH change and in the late acidic phase for matrices cross-linked in 0.1 M calcium chloride solution (Fig. 6). Burst release coincided with the appearance of cracks as shown in Fig. 1D (iii) for matrices cross-linked for 0.5 h. The gradual conversion of calcium alginate to alginic acid at pH 1.2 coupled with increasing matrix expansion caused the weaker alginic acid barrier to crack. As the swelling of the sodium alginate core was likely to be greater than the outer alginic acid layer, an internal pressure was generated which caused the barrier to crack. These cracks continued to propagate with increased swelling pressure as the matrices entered the buffer phase, eventually triggering burst release as the cracks penetrated the core. The sudden increase in drug release that occurred in the late acidic phase (Fig. 6) did not persist as the exposed core was gradually sealed by the swelling sodium alginate layer. On the contrary, matrices cross-linked in 0.01 M calcium chloride solution remained intact for longer periods and only developed cracks in the early buffer phase (4–5 h). However, the cracks formed did not propagate as they were rapidly sealed by the swelling alginate core. Matrices cross-linked in 0.01 M cross-linker solution for 1 h showed zero order kinetics from 20 to 80% drug release.

Evidence of the matrix core consisting mainly of sodium alginate was obtained from cross-sectional images of hydrated calcium alginate-coated matrices containing a pH indicator, bromophenol blue (Fig. 7). This indicator turns yellow when pH goes below 3, corresponding closely to the pH below which more than 50% of sodium alginate is converted to alginic acid. The blue coloration (represented by dark grey) of the matrix core beneath the coat (Fig. 7) indicates that the micro-environmental pH within the core was above 4.6. Therefore, the matrix core consisted mainly of sodium alginate. The coat surrounding the matrix was able to protect the core from being converted to alginic acid. The incoming protons from the acidic dissolution media underwent ionic exchange with calcium alginate to form alginic acid while water molecules continued to imbibe into the matrix.

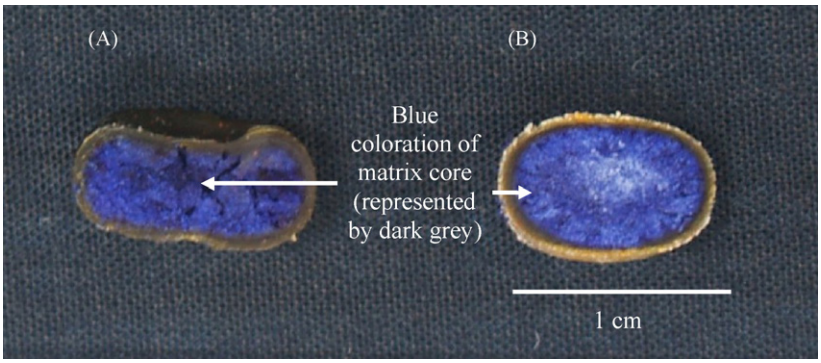

Fig. 7. Cross-sectional images of calcium alginate-coated matrices hydrated for 1 h in pH 1.2 dissolution medium. Manugel DMB matrices containing bromophenol blue were pre-coated in (A) 0.01 M or (B) 0.1 M calcium chloride solutions, respectively for 1 h.

4. Conclusion

The influence of the type and amount of cross-linker, internal/external cross-linking as well as dissolution media pH were examined in this study. In general, the incorporation of calcium salts into sodium alginate matrix tablets did not significantly promote drug release retardation. Mechanisms such as channeling effect of soluble calcium salts, disruption of polymer particle coalescence by insoluble particles, competition with H^+, and even cross-linking itself interfered with the formation of an integral diffusion barrier. Significant drug release retardation was only achieved at low concentrations of external Ca^{2+}. At higher Ca^{2+} concentrations, matrix disintegration occurred resulting in dose-dumping. Gravimetric and image analysis studies showed that rapid initial polymer hydration was critical in reducing the extent of initial drug released by promoting rapid polymer swelling to form an effective diffusion barrier. This process was impeded by high cross-linker concentration, resulting in faster initial drug release. During external cross-linking, high-G alginate matrices remained intact while high-M matrices cracked. The latter continued to sustain drug release as the ruptured barrier was repaired via a 'self-sealing' mechanism. Calcium alginate-coated matrices significantly reduced drug release at pH 1.2, mainly by preserving matrix structure. Hence, the cross-linked barrier effectively resisted crack development at acidic pH.

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