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Swelling and diffusion studies of calcium polysaccharide gels intended for film coating

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ABSTRACT

The aim of this paper was to study the swelling and diffusion behaviors of calcium polysaccharide gel (CaPG) films prepared by an interfacial complexation technique, a new gel formation method that allowed calcium ions to diffuse from a source to form gel films with polysaccharide (i.e., alginate or pectin). The dynamic swelling behavior of CaPG films showed that swelling was a function of time. Most CaPG films showed a maximum amount of water absorption during the first few hours. The films swelled less in water and acidic media but extensively swelled in 0.1 M NaCl. The rehydration of the dry films in the acidic media or the 0.1 M NaCl solution also lead to the extraction of most of the calcium ions from the CaPG within 4 h or less. Partitioning and diffusion of a model drug, theophylline (TPL), were measured through CaPG films equilibrated in different media. The partition and diffusion coefficients of TPL through CaPG films were found to vary, depending upon polysaccharide type, concentration and equilibration medium. The results suggest that both partition and pore mechanisms operated concurrently in the transport of TPL through CaPG films equilibrated in different media.

1. Introduction

Pectin and alginate are naturally occurring polysaccharides that are regarded as safe for human consumption and have been used successfully for many years in food and beverage industries as thickening agents, gelling agents and colloidal stabilizers. They are finding increasing applications in the pharmaceutical and biotechnology industries; the application of alginate in modified release formulations has recently been reviewed (Coviello et al., 2007). Pectin is a partial methyl ester of (1-4)-α-D-galacturonic acid (Gal) interrupted with (1-2)-α-L-rhamnose units and other neutral sugars. Low methoxy pectin, with a degree of esterification less than 50%, forms gels with divalent ions such as calcium (Rolin, 1993). Alginate is a linear chain containing (1-4)-β-D-mannuronic acid (M) and its C5 epimer (1-4)-α-L-guluronic acid (G), arranged as homopolymeric blocks (poly-M and poly-G) and as mixed blocks (MG) (Smidsrød and Draget, 1996). In general, gel formation has been demonstrated to result from specific interactions between (calcium) cations and blocks of Gal and G residues in pectin and alginate, respectively (Kohn, 1975).

The poly-G and the MG blocks assume a buckled ribbon conformation in solution, and calcium ions can reside in a cavity between two adjacent guluronates in the poly-G or MG blocks and form a cross-link with the poly-G or MG blocks of another alginate molecule (Donati et al., 2005). The poly-M does not contribute to cross-linking with divalent ions (Mørch et al., 2006). Although the cross-linking reaction has been traditionally described in terms of a 2/1 helical conformation (the so-called 'egg-box model') (Smidsrød and Draget, 1996), this has been recently the subject of debate (Li et al., 2007; Sikorski et al., 2007). Molecular modeling studies (Braccini and Perez, 2001) have suggested that (for Gal) the strong "egg-box" associations are supplemented by weaker associations between the chains. In addition, a recent study has shown that a few calcium ions are able to stabilize numerous binding sites; one calcium ion is able to stabilize 18 poly-Gal dimeric units (Donati et al., 2006).

The cross-linking properties of pectin and alginate with metal ions have enabled them to be used as a matrix or membrane for the entrapment and/or delivery of a variety of drugs (e.g., Novosel'skaya et al., 2000; Coviello et al., 2007). Calcium polysaccharide gel (CaPG) coated pellets have been developed to control drug delivery (e.g., Sriamornsak et al., 1997, 2006; Sriamornsak and Kennedy, 2006a). These gels were applied as a film coat by interfacial complexation onto spherical cores to achieve sustained release properties. The release characteristics of film-coated sustained release formulations are strongly dependent on the properties of the film, e.g., film permeability and its mechanical strength (Arwidsson, 1991). Aulton (1982) suggested that evaluation of free films could be used to predict the properties of applied films and will be ben-

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eficial since the number of variables involved in the system is reduced.

Although calcium gel films have been used to investigate the diffusion of small model drugs, these were prepared by immersing a piece of dry cast alginate film in aqueous calcium (or zinc) solution (Julian et al., 1988; Aslani and Kennedy, 1996; Lim and Kennedy, 1996). However, these studies may not represent the characteristics of films deposited onto pellets by interfacial complexation (Sriamornsak et al., 1997). A novel technique of CaPG film formation which mimics the conditions during the interfacial complexation coating process has been reported and some physico-chemical properties of the films have been investigated (Sriamornsak and Kennedy, 2006b). In the present study, free films of calcium polysaccharide gels (CaPG) prepared using this technique will be characterized with swelling and diffusion studies. The permeation and partitioning studies will be performed on different CaPG films equilibrated in different media, using theophylline as the model drug. Therefore, the aim of this work is to attempt to elucidate the relationships that exist between the chemical nature of the polysaccharide, the swelling and diffusion medium, the swelling of the films and molecular transport through the films.

2. Materials and methods

2.1. Materials

Low and medium viscosity sodium alginates (ALV and AMV, respectively) obtained from *Macrocystis pyrifera* were purchased from Sigma Chemical Co. (USA). Low viscosity sodium alginates obtained from *Laminaria hyperborae* leaves and stipes (i.e., LVM and LVG, respectively) were purchased from Pronova Biomedical (Norway). Low methoxy pectin with 28% esterification was obtained from two sources. A commercial pectin (LMA) with 20% amidation (GENUpectin type LM-104 AS-FS) was the generous gift of CP Kelco (Denmark). A potassium salt of esterified pectin from citrus fruit (LMC) was purchased from Sigma Chemical Co. (USA). Theophylline (TPL) (Sigma Chemical Co., USA) was used as received. All other chemicals were of analytical reagent grade and used as received without further purification. Deionized water was used throughout all experiments.

2.2. Manufacture of CaPG films

CaPG films were manufactured by a new method described previously (Sriamornsak and Kennedy, 2006b). In summary, polysaccharide solutions (2% w/w) were stirred in deionized water and then left undisturbed overnight. The polysaccharide solutions (30 g) were poured into the mould which was previously levelled (Niveau-level, E.D.A., France). One litre of 0.34 M calcium chloride was poured into the bath to start gelation at 25 °C. A gelation time of 60 min and hardening time of 30 min was used. Excess calcium was rinsed off with deionized water. The gel film was then washed by soaking in deionized water for 3 h, where water was changed every 30 min for the first 2 h. After washing, the gel was left overnight in water under ambient conditions (about 25 °C). The gel film was cut into pieces, dried in a hot air oven at 50 °C for 48 h and then stored in a desiccator over silica gel at about 25 °C until used.

2.3. Swelling studies

Dried gel discs were cut, accurately weighed and placed in small vials with 10 mL of either water, 0.1 M HCl, simulated gastric fluid USP without pepsin (SGF) or 0.1 M NaCl. They were agitated and equilibrated at 37 °C in such a way that unrestricted swelling could occur three-dimensionally. Swelling was assessed gravimetrically and by dimensional changes. At specified times a disc was gently removed from the hydration medium, dried with filter paper and weighed; the same disc was used at each time. The percentage water uptake by the gel disc was calculated by Eq. (1):

$$\%\,\text{Water uptake} = \frac{(W_t - W_o)}{W_o} \times 100 \quad (1)$$

where W_t is the wet weight of the gel disc at time t and W_o is the initial dry weight of the disc. These experiments were performed in triplicate. The swelling behavior of the same gel discs was also monitored by measuring changes in the diameter and thickness of dried and swollen gels using a thickness gauge (Mitutoyo Corporation, model 7309, Japan) and a linear scale.

2.4. Diffusion studies with theophylline

Dry CaPG films were immersed in either water, 0.1 M HCl, SGF or 0.1 M NaCl for 2 h (at 37 °C) and then in water for another 24 h. The diffusion studies were performed using side-by-side water-jacketed glass half-cells in which the film was held vertically between the half-cells, leaving a diffusion area of 12.57 cm^2 (custom made by LAB Supply, Australia). The receptor cell was filled with 100 mL of deionized water and 100 mL of TPL solution (approximately 6 mg/mL, but accurately assayed) was filled into the donor cell. During diffusion studies, both donor and receptor solutions were stirred at 500 rpm and temperature was maintained at 37 °C. The UV spectrophotometer (Cary 1E, Varian, Australia) had a flow through system where, at the specified time intervals, the solution was pumped (Model 17-2300, Vankel, USA) from the receptor cell of the diffusion apparatus into the spectrophotometer and returned to the receptor cell under control of Cary WinUV software (Varian, Australia). Absorbance measurements were taken at specified intervals and converted to concentration of drug using a linear Beer's law plot. No samples were removed from the receptor cell, and therefore, there were no sampling losses. A new equilibrated CaPG film was used for each diffusion experiment, with triplicates for each experiment. The thickness of each piece of film was measured 6 times with the thickness gauge mentioned above and mean was used as the estimate of the value of h in Eq. (3). Sink conditions (i.e., less than 1% of initial drug quantity entering the receptor cell) were maintained for all experiments.

2.5. Determination of partition coefficient (K_p) in CaPG films

CaPG films were equilibrated in water, 0.1 M HCl, SGF and 0.1 M NaCl for 2 h at 37 °C and then in water for another 24 h. A solution depletion method (Gilbert et al., 1988) was used to determine the K_p of TPL between the equilibrated gel and the medium. The equilibrated gels were placed in 10 mL of drug solution containing 0.320 mg/mL of TPL. The concentration of the surrounding solution was monitored using UV-spectroscopy over a 24 h period. The partition coefficient, K_p, was calculated by Eq. (2) (Varshosaz and Falamarzian, 2001):

$$K_p = \frac{c_g}{c_s} = \frac{V_s[c_i - c_{eq}]}{V_g c_{eq}} \quad (2)$$

where c_g is the concentration of the solute in the gel film, c_s is the concentration in the solution, c_i is the initial concentration of the solute in the solution, c_{eq} is the concentration of solute in the solution after equilibrium has been reached, and V_s and V_g are the volumes of the solution and the gel film, respectively.

2.6. Calcium analysis and displacement

The calcium content of CaPG films was analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES; Liberty

Series II and Plasma 96 software, Varian, Australia) at a plasma temperature of 10000 K and wavelength maximum of 317.9 nm. Preliminary tests showed that up to 1600 mg/L of the polysaccharides in 2% sodium citrate solutions did not interfere with the analysis of calcium. Calcium content in CaPG films was determined by dissolving each film (about 8 mg in weight and 10 mm in diameter) in 10 mL of 2% sodium citrate solution. Each determination was performed in duplicate.

In order to study the calcium displacement by other cations, dry films were placed in 500 mL of either water, 0.1 M HCl, SGF or 0.1 M NaCl, in a dissolution vessel (VK7000, Vankel, USA). The temperature was 37 °C and the paddles were rotated at 50 rpm. At specific times, hydrated films were manually collected and dissolved in 10 mL of 2% sodium citrate solution and the calcium analyzed by ICP-AES. All studies were done in duplicate.

2.7. Statistical analysis

Analysis of variance (ANOVA) and Levene's test for homogeneity of variance were performed using SPSS version 10.0 for Windows (SPSS Inc., USA). *Post hoc* testing ($P < 0.05$) of the multiple comparisons was performed by either the Scheffé or Games-Howell test depending on whether Levene's test was insignificant or significant, respectively.

3. Results and discussion

3.1. Rehydration and swelling behavior of CaPG films

Alginate and pectin are hydrophilic polysaccharides that form gels in the presence of divalent ions such as calcium and which may be used as coatings on pharmaceutical dose-forms (e.g., Sriamornsak et al., 1997, 2006; Sriamornsak and Kennedy, 2006a). Depending on the particular polysaccharide examined, the films that have been produced have different initial thicknesses. This information is shown in Fig. 1. It is apparent that the pectin films are slightly thinner than the alginate films, suggesting that as the pectin gels collapse the molecules are able to achieve a more compact arrangement. This may be because the estimated molecular weights of the pectins are substantially less than the alginates used in this study (Sriamornsak, 2002).

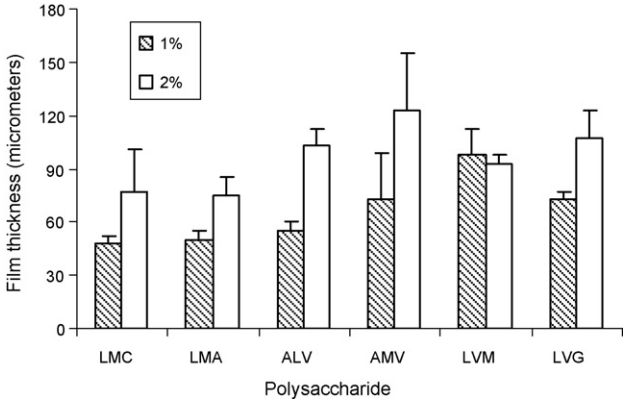

Fig. 1. Plot of the mean thickness of the dry films of both 1 and 2% polysaccharides. The abbreviations are defined in Section 2.1. ($n = 6$, S.D. shown.)

The transport of water through the dry films depends on the intrinsic rigidity of the polysaccharide as well as the extent of cross-linking. Since hydration and swelling are the prelude to drug diffusion, the initial studies investigated the effect of polysaccha-

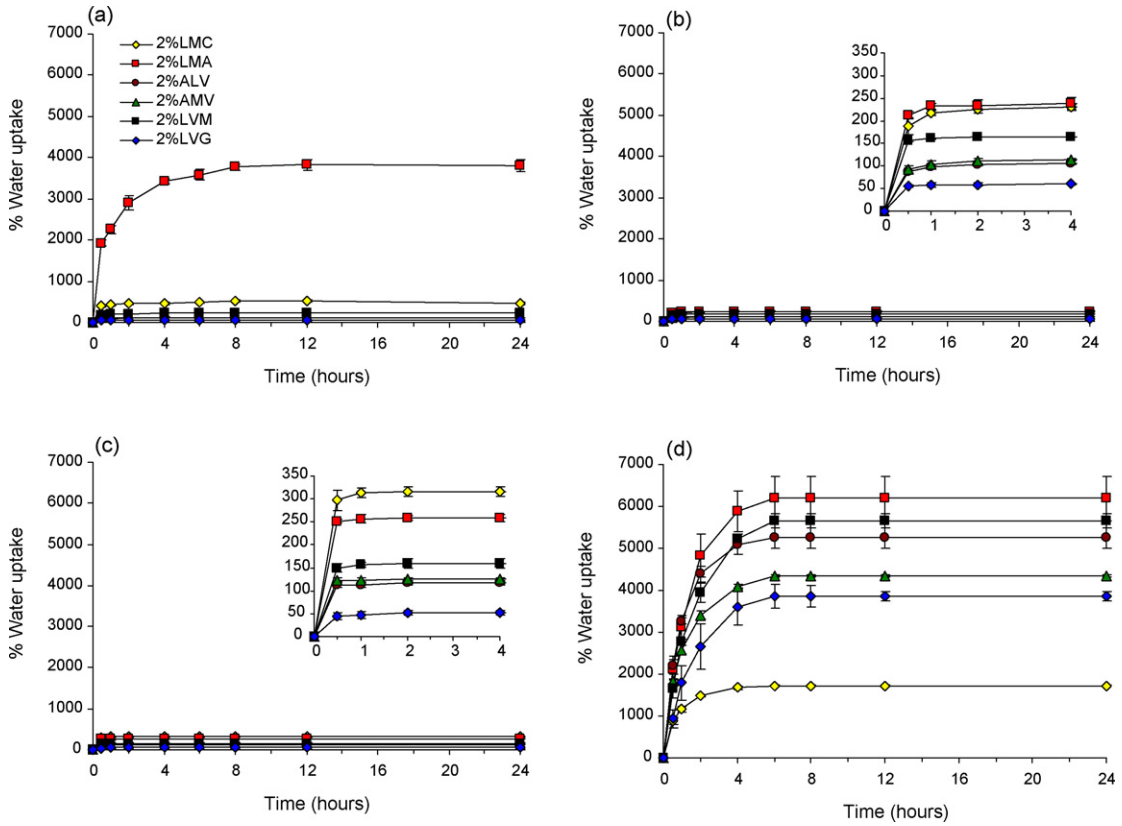

Fig. 2. Plot of the swelling kinetics of calcium polysaccharide gel films (2%), measured as the percentage water uptake at 37 °C, in (a) water, (b) 0.1 M HCl, (c) SGF, and (d) 0.1 M NaCl. ($n = 3$; S.D. shown.)

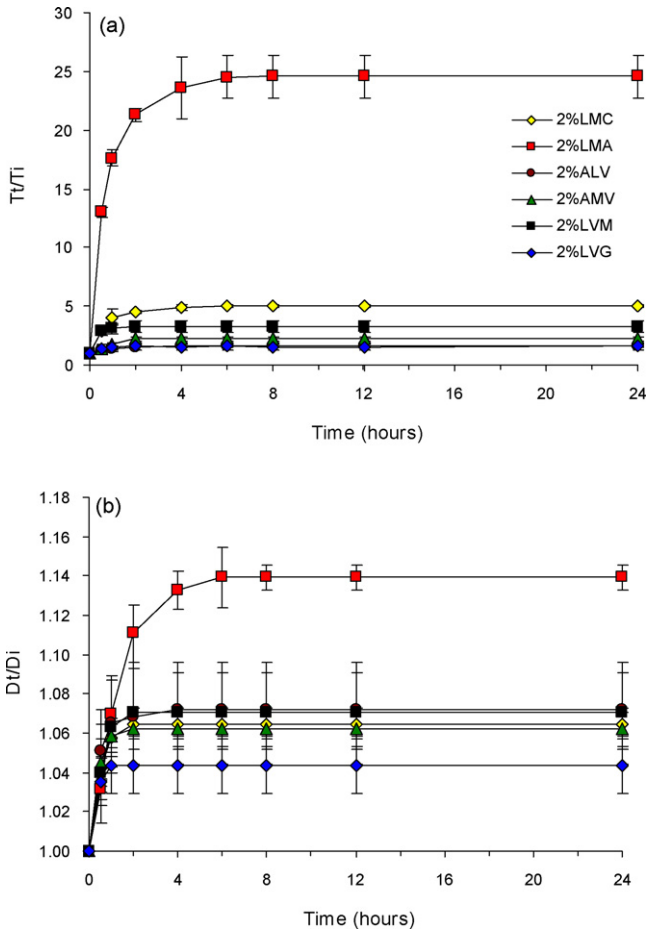

Fig. 3. Plot of the swelling kinetics in water of calcium polysaccharide gel films (2%), measured as (a) the relative change in thickness (Tt/Ti), and (b) the relative change in diameter (Dt/Di) at 37 °C. (n = 3; S.D. shown.)

ride type and concentration on the extent of water uptake and the resultant dimensional changes. Fig. 2 shows the effect of different media on the hydration kinetics of 2% CaPG films. Although the results for the 1% CaPG films displayed similar trends, in many film/medium combinations the 1% films swelled a little less than the corresponding 2% film (data not shown).

In water, all calcium alginate films reached hydration equilibrium in about 2 h. Calcium pectinate gel films swelled more extensively and more slowly, with the CaLMA films swelling most and slowest. Compared to immersion in water, all CaPG films swelled less when placed in acidic media (i.e., 0.1 M HCl and SGF), and reached equilibrium within 2–4 h (Fig. 2b and c). Although all the CaPG films swelled most extensively in 0.1 M NaCl (Fig. 2d), they needed about 6 h to reach equilibrium.

Fig. 3 shows the dimensional changes (axial and radial) of CaPG films immersed in water; the major change for the CaPG films was swelling in the axial direction. The data for normalized increases in thickness (Fig. 3a) revealed that there were rapid axial increases within 1 h for alginates and 2 h for pectins. Subsequently, the dimensional changes slowed and equilibrated within 2–4 h, except for LMA which took about 6 h. Radial swelling (presented as normalized results in Fig. 3b) was small by comparison. In all other media, axial swelling was substantially greater than radial swelling (data not shown). Preferential axial expansion may be due to the release of stresses developed during the anisotropic drying process that resulted in the largely axial direction of gel collapse when the film was dried.

Fig. 4 shows the effect of the media on the equilibrium swelling of all CaPG films, measured as the percentage water uptake after exposure to the specified medium for 24 h. Several results are notable. Swelling was largely unaffected by the concentration of the gel used. The swelling was 23–80 times greater when 0.1 M NaCl was used in comparison to water and the acidic media. For most alginates, the swelling in water and acidic media were very similar. The exception was the LVM, where the swelling in water was significantly greater than in the acid media. Both pectins also swelled more extensively in water than in the acidic media.

When CaPG films were contacted by the aqueous media, a number of processes occurred simultaneously. The film surface was wetted by the medium and the pectin or alginate molecules hydrated, slowly disentangled and the films swelled. However, the expansion of the gel film due to penetration of water is limited by the extent of entanglement and the retractive force within the gelled network. The latter is influenced by the rigidity of the polysaccharide, the extent of calcium cross-linking and any additional inter- or intra-molecular associations. A reduction in the extent of cross-linking would be expected to lead to a reduced retractive force and would allow more water to be absorbed. Therefore, the greater swelling of pectin films in water, compared to alginate films, could be due to a lower number of calcium cross-links. Table 1 shows the calcium content of the six polysaccharides after the films were extensively washed in water to remove unassociated calcium ions. On a dry weight basis the LMA had the lowest calcium content, followed by the LMC and then the alginates. These results correlate with the results in Figs. 1a, 2 and 3a, where the rank order of water uptake or axial swelling was LMA > LMC > LVM > (ALV ~ AMV) > LVG.

Although the calcium contents of ALV, AMV and LVM were similar, the calcium content of LVG was a little higher. The lower swelling of high G alginate (LVG) films may be due to the higher calcium content and the expected presence of long G-blocks (Moe et al., 1995) that may lead to more effective cross-linking. It has been shown that G-blocks are the most important junction forming structures in alginate gels under equilibrium and non-equilibrium conditions (Draget et al., 1996; Braccini et al., 1999) especially when calcium is used as the cross-linking ion (Mørch et al., 2006). The calcium content of the calcium pectinate films were lower than the calcium alginates and the calcium content in CaLMA was substantially less than CaLMC. The latter may be due to the presence of amide groups in LMA, leading to a decrease of free carboxyl groups to cross-link with calcium ions (Kim et al., 1978). Despite having a reduced calcium binding capacity, LMA formed stable gels through the formation of hydrogen bonds between the amidated regions (Alonso-Mougán et al., 2002).

Immersion of the CaPG films in electrolyte solutions allowed ion-exchange reactions to be established and Fig. 5 shows that during exposure for up to 4 h, there was extensive displacement of calcium from the LMC, LMA and LVG CaPG films; the behavior of the AMV, ALV and LVM (data not shown) were intermediate between the LMC and LMA. Within 5 min exposure, both acidic media had extracted at least 95% of the calcium from all the CaPG films; these data are not shown in Fig. 5. In water, the greatest calcium loss was about 20% in the case of CaLMA. However, the addition of 0.1 M NaCl caused significantly greater losses of 70–90% of the calcium content irrespective of the type of CaPG. The exchange of the bound calcium ions with the sodium ions in solution, lead to the partial formation of hydrophilic and poorly cross-linked alginate or pectinate films, and allowed the gel network to expand in consequence of extensive water uptake into the gel films. This is apparent in Figs. 2(d) and 3.

The acidic media reduced the calcium content of all CaPG films by more than 95% after exposure for only 5 min. This is consistent with the results of Lim and Kennedy (1996). Exposure of CaPG to

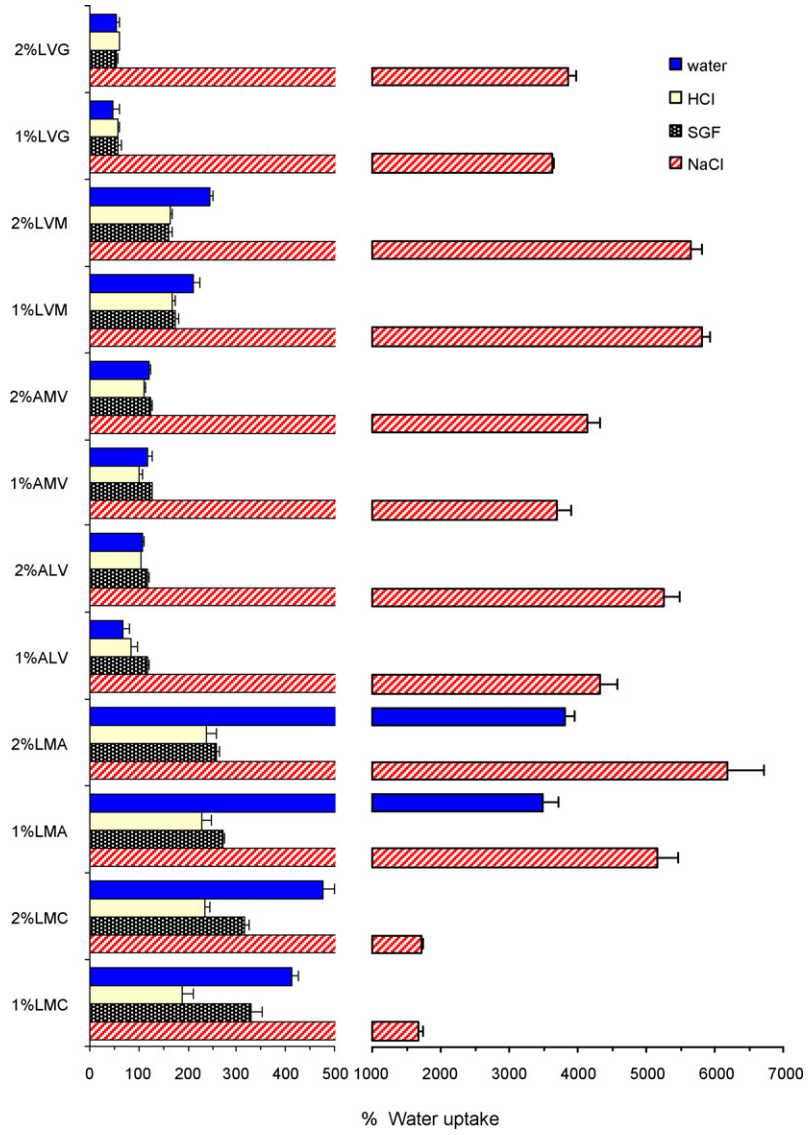

Fig. 4. Equilibrium swelling in different media of calcium polysaccharide gel films at 24 h, expressed as the percentage water uptake at 37 °C. ($n = 3$; S.D. shown.)

acidic media is slightly different to the 0.1 M NaCl, in that although the calcium is severely depleted from the films, the product of the exchange reaction is hydrophilic, but water insoluble, alginic or pectinic acid. It is well known that alginic acid can form stable gels (Draget et al., 1994, 1996). The swelling of the calcium alginate gel films in acidic media was generally similar to that in water, except the CaLVM films which swelled less than in water. The calcium pectinate gel films, swelled significantly less in acidic media than in water. Peppas et al. (2000) suggested that low swelling could be expected in the absence of ionized groups, i.e., when the pH of the medium is below the pK_a of the ionizable group. This is likely in these gels, since the pK_a of carboxyl group of M, G (of alginate) and Gal (of pectin) are about 3–4 (Smidsrød and Draget, 1996; Ralet et al., 2001).

Table 1
Calcium contents of dried CaPG films ($n = 2$; range shown)

Polysaccharide	Calcium content ± range (micromoles/mg dry weight)
LMA[1]	1.137 ± 0.007
LMC[1]	1.526 ± 0.025
AMV[2]	2.057 ± 0.018
LVM[2]	2.062 ± 0.063
ALV[2]	2.067 ± 0.003
LVG[2]	2.145 ± 0.019

Notes: 1 = pectinate gel, 2 = alginate gel.

In the 0.1 M HCl and 0.1 M NaCl solutions, the rank order of swelling was LMA > LVM > (ALV ~ AMV) > LVG. The response of the LMC was inconsistent. Although in the 0.1 M HCl, the LMC and LMA swelled equally, the LMC swelled least of all the CaPG films in 0.1 M NaCl. In SGF, containing both HCl and NaCl, the LMC swelled most. The behavior of the alginates is consistent with their calcium content, the M/G ratio and the size of the G blocks. It is known that the gelling mechanisms of pectins are influenced by factors such as the distribution of charge along the pectin main chain (Löfgren et al., 2006) and this will be related to the insertion of the α-L-rhamnose and other neutral sugars into the main chain. It is likely that these differences between the LMA and LMC contribute to the different response of the pectins to the electrolytes used in this study.

3.2. Partition coefficient of TPL in CaPG films

The K_p of TPL in CaPG films is defined as the ratio of the equilibrium concentration (i.e., at 24 h) of TPL within the gel film to that

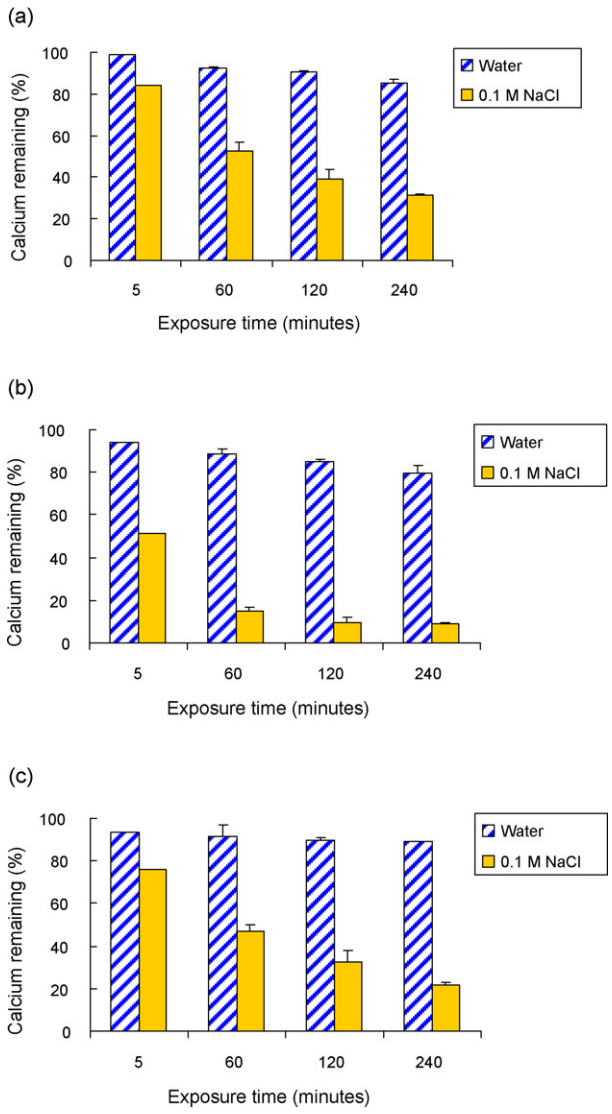

Fig. 5. Effect of exposure time in water or 0.1 M NaCl on the extraction of calcium ions from dry CaPG films at 37 °C, (a) LMC, (b) LMA, and (c) LVG. ($n = 3$; S.D. shown.)

in the solvent as shown in Eq. (2). Preliminary tests showed equilibrium was established within 24 h (Sriamornsak, 2002). Table 2 shows that the measured equilibrium K_p of TPL into CaPG films in different media range from 0.60 to 39.24. The K_p of TPL in CaPG films equilibrated in 0.1 M NaCl were less than one; this is probably due to the extensive swelling of the films equilibrated in 0.1 M NaCl, that allowed a free distribution of TPL between solvent in the gel film and bulk solvent. Wisniewski and Kim (1980) suggested that K_p close to one should be interpreted as representing uniform distribution between the membrane solvent and bulk solvent.

Table 2
Partition coefficients (K_p) of theophylline into calcium polysaccharide gel films equilibrated in different media, at 24-h soaking in theophylline solution ($n = 3$)

Polysaccharide	Partition coefficient, $K_p \pm$ S.D.			
	Water	0.1 M HCl	SGF	0.1 M NaCl
1%LMC	6.54 ± 1.31	2.72 ± 0.41	ND	ND
2%LMC	2.32 ± 0.53	1.68 ± 0.35	2.15 ± 0.16	0.60 ± 0.07
1%LMA	8.04 ± 1.73	2.80 ± 0.66	ND	ND
2%LMA	2.42 ± 0.78	1.73 ± 0.23	2.22 ± 0.06	0.62 ± 0.04
1%ALV	19.14 ± 0.94	7.71 ± 0.97	18.63 ± 1.98	ND
2%ALV	10.42 ± 1.43	5.19 ± 1.11	13.26 ± 0.73	0.71 ± 0.02
1%AMV	14.40 ± 2.61	9.36 ± 0.34	19.21 ± 1.98	ND
2%AMV	10.49 ± 0.82	5.98 ± 0.70	11.22 ± 0.56	0.70 ± 0.05
1%LVM	15.87 ± 1.51	6.65 ± 1.72	13.47 ± 2.46	ND
2%LVM	7.61 ± 1.11	3.30 ± 0.32	7.05 ± 0.27	0.68 ± 0.03
1%LVG	20.13 ± 0.55	7.72 ± 0.75	39.24 ± 3.38	ND
2%LVG	17.12 ± 1.33	9.45 ± 1.26	23.43 ± 2.81	0.90 ± 0.01

ND = Not determined.

The partitioning results are in general agreement with reports of K_p for low molecular weight drugs in hyaluronate membranes (Hunt et al., 1990) and for xanthine drugs (e.g., theobromine) in hydrophobic/polyelectrolyte hydrogel membranes (Varshosaz and Falamarzian, 2001). Caram-Lelham et al. (1997) and Hugerth and Sundelof (2000) suggested that the extent of interaction between amphiphilic drugs and polysaccharides (e.g., alginate, κ-carrageenan and dextran sulfate) depended on the hydrophobicity of the amphiphiles as well as the rigidity and charge density of the polysaccharides. Most of the K_p listed in Table 2 is greater than one, and this phenomenon has been attributed to specific interactions between the drug and the polymer chain (Varshosaz and Falamarzian, 2001). The large K_p for TPL shown in Table 2 (especially in the calcium alginate films equilibrated in water or acidic media) may be due to the hydrophilic nature of TPL that allowed interaction with the hydrophilic domain of the polysaccharide network, possibly through hydrogen bonding with the carbonyl groups in TPL. The K_p for calcium gel films of pectin were smaller, suggesting that the interaction between the drug and pectin films was less than in alginate films.

Most values of K_p showed a reduction as the concentration of polysaccharide was increased from 1 to 2% w/w. A similar reduction was also observed by Grassi et al. (2001). An increase in the concentration of polysaccharide may restrict the availability of free water (in the gels) and reduce the ability of TPL to partition into the gels. Since the gels were prepared using calcium chloride alone (i.e., without added sodium chloride), they may be non-homogenous and possess a more dense gel network at the periphery (Smidsrød and Draget, 1996). This non-homogeneity may be more profound for the 2% gels and may have restricted the extent of TPL partitioning into the gels.

3.3. Transport of TPL in CaPG films

The diffusion studies reported here were performed with a test film sandwiched vertically between two identical stirred half-cells. A drug solution was added to the donor cell and the concentration of diffusant appearing in the receptor cell was monitored. Under these conditions, and assuming that steady-state diffusion was occurring, Fick's first law of diffusion may be written to show the relationship between the amount permeated (Q) and time, as shown below.

$$\frac{Q}{A} = \frac{DK_pCt}{h} \quad (3)$$

Q/A is the amount of drug diffused per unit area (mg/cm^2), D is the diffusion coefficient (cm^2/s), K_p is the partition coefficient, C is the concentration of diffusant in the donor cell (mg/cm^3), t is time (s) and h is the film thickness (cm). The permeability coefficient (P) is given by the product of K_p and D.

Fig. 6 shows a plot of the cumulative amount of drug diffused (Q/A) through a 2% CaLMA film versus time; all other films showed similar behavior. The slope of the linear portion of the flux curve was used to calculate D. All diffusion studies reported here were carried out for 90–180 min which is at least six times greater than the typical apparent lag times of less than 15 min. The equilibrated 1% gel films of LMC (in all media), LMA (in acidic media) and all the CaPG in 0.1 M NaCl gave soft, swollen and fragile gels that easily tore when handled. Therefore, diffusion experiments were not performed with these films.

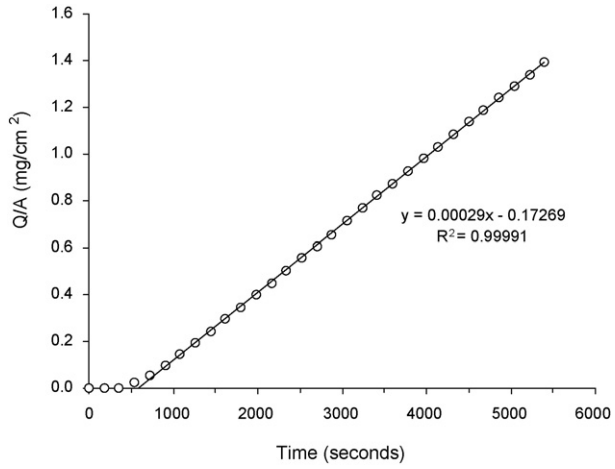

Fig. 6. Typical plot showing the amount of theophylline diffused through a calcium polysaccharide gel film (2%LMA) at 37 °C after exposure to water.

In all cases the cumulative flux at the conclusion of each experiment was greater for the 1% w/w gel compared to the 2% w/w gel (data not shown). The permeability coefficients (P) of TPL through the 2% films of all the CaPG (except LMC and LMA in 0.1 M NaCl) exposed to the four media are shown in Fig. 7. The data for 1% gel films are not shown, and although many more combinations of CaPG and medium produced films that were too fragile to handle, the overall trend of the available results was similar to Fig. 7.

The flux of TPL through the LMA was unaffected by a change in pH from water to either of the acidic media, and in all cases the flux through LMA was significantly greater than through any of the alginates. In all media, the LVG showed significantly lower values of P, compared to the other alginates that all behaved in a similar manner. The main point of difference was that LVM in the 0.1 M NaCl was significantly more permeable than the ALV or the AMV. For the alginate CaPG films, the flux was greater in the acidic media than water, but there was no significant difference between the two acid media. These results broadly concur with our previous investigations on the use of these same CaPG as coatings applied to spherical pellets (Sriamornsak et al., 1997; Sriamornsak and Kennedy, 2006a). In those studies, we showed that the alginates slowed release more effectively than the pectinates. For the alginates in water, we showed that the rank order of release rate was LVM < (ALV ≈ AMV) < LVG, and generally that all the electrolytic media accelerated release compared to water. The flux results also broadly agree with the data of Østberg et al. (1994), who showed

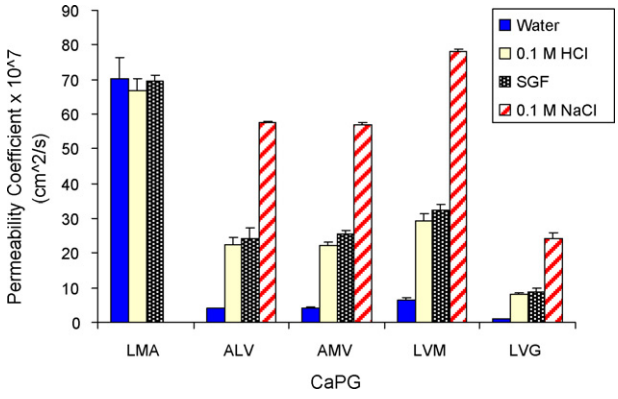

Fig. 7. The effect of water, 0.1 M HCl, SGF and 0.1 M NaCl on the permeability coefficients (P) of theophylline in CaPG films at 37 °C. (n = 3; S.D. shown.)

that the release of theophylline from calcium alginate gel beads (matrices) was significantly more rapid in 0.1 M HCl and SGF than in water. Comparisons between our work and that of Østberg et al. must be done cautiously, since their study focused on release of drug from gel matrices in which the drug was suspended at the time of gelation.

The value of the diffusion coefficient (D) for TPL in water at 37 °C has been reported as $\approx 8.2 \times 10^{-6}$ cm^2/s (Grassi et al., 2001). Fig. 8 shows the estimates of D for TPL in all stable CaPG films; the values of D ranged from 1.15×10^{-5} to 5.13×10^{-9} cm^2/s, depending upon polysaccharide type, polysaccharide concentration and the equilibration medium. Therefore, most gel and medium combinations resulted in a slowing of TPL diffusion. This is consistent with previous findings in similar hydrogels that have reported values of D for several small molecules in different biopolymers, and where the D values of nine solutes in 2% calcium alginate gel beads were found to be lower compared to water (Oyaas et al., 1995a,b).

In general, the D values were different among those CaPG films made with different polysaccharide concentrations and types. Fig. 8 shows a comparison between TPL diffusion coefficients in different CaPG films equilibrated in different media. The D values of TPL in the 1% CaPG films were lower than in the 2% films. This is probably due to the 1% films being less porous, since the 1% films were less hydrated than the 2% films (see Fig. 4). Julian et al. (1988) have shown that increased hydration leads to increased values of D in some calcium alginate films. It is also likely that in the less swollen gels there is increased likelihood of an interaction between the drug and polysaccharide and this is supported by our observation that the K_p of the 1% CaPG films, in most cases, were greater than those of 2% CaPG films.

For CaPG films equilibrated in water, D in LMA films was high, while in the alginates it was lower and less variable (Fig. 8a). This may be due to the high swelling of calcium films of LMA in water that is likely to be caused by a reduced degree of cross-linking of LMA with calcium ions compared to the alginates. The latter is supported by the calcium content in dried calcium films of LMA, which was less than that of alginates (see Table 1). Calcium loss was higher for LMA (about 20%) compared to alginates (about 10–14%) after 4 h exposure to water, and this would lead to an increase in diffusion of TPL through LMA films.

The calcium films of LMA equilibrated in acidic media (Fig. 8b and c) also had higher D values than those of alginates. The loose, sheet-like structure of calcium films of LMA in acidic media previously reported by Sriamornsak and Kennedy (2006b), together with higher displacement of calcium ions from the gel, allowed the drug to diffuse more easily than in calcium alginate gel films. Fig. 8 also shows that, for calcium alginate gel films equilibrated in all media, LVG gave the lowest D. This could be explained by a high content of long G-blocks in the LVG, resulting in a strong bonding with calcium ions. Different types of high M alginates (namely, ALV, AMV and LVM) which are similar in gross composition showed different D values. Calcium films of ALV and AMV were less permeable than LVM. This may be due to the difference in the monosaccharide sequence of the alginates. The alginates derived from *Macrocystis pyrifera* (i.e., ALV and AMV) gave more permeable gels than high G alginate but gave less permeable gel than that of alginate with similar M/G ratio, i.e., LVM. This is due to the unusual arrangements of the monosaccharides (M or G) in *Macrocystis* alginate, that is characterized by a high content of random MG block together with few but very long G-blocks (Grasdalen, 1983).

The D values of TPL in CaPG films equilibrated in acidic media were much higher than those equilibrated in water. The acid gels formed by the calcium-proton exchange in acidic media would allow drug to diffuse more easily. Lim and Kennedy (1996) reported that the P values of TPL through calcium alginate gel

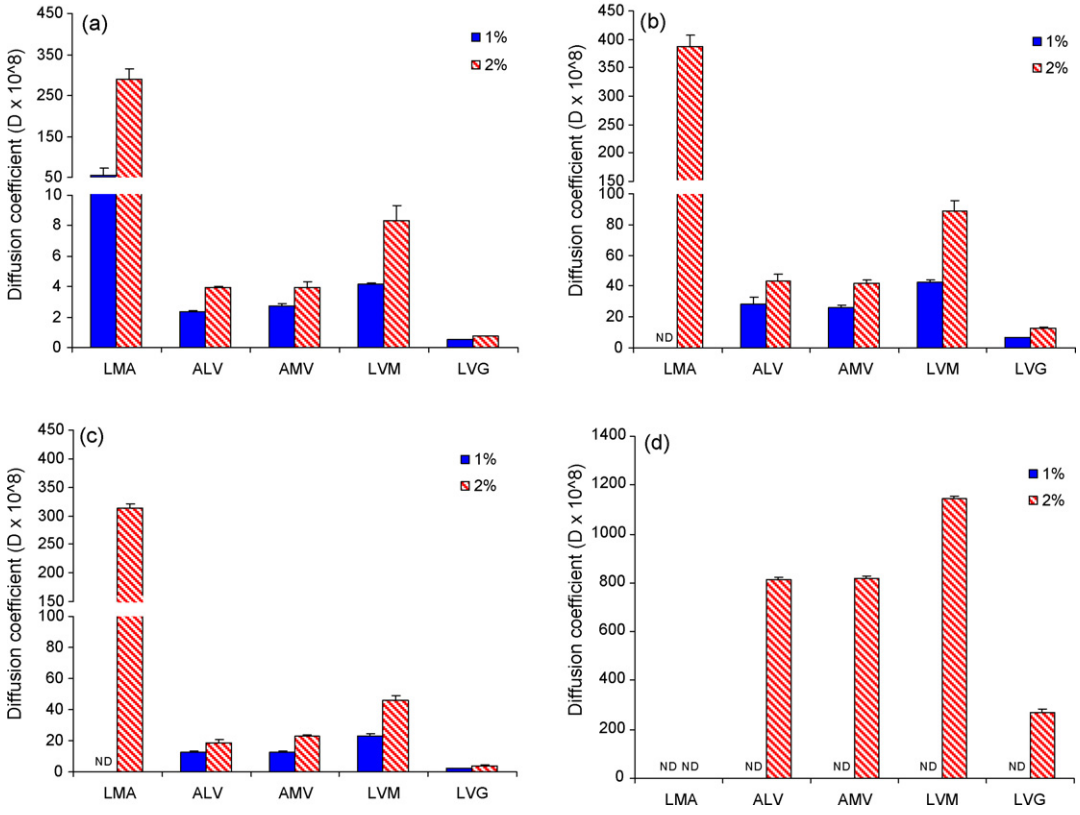

Fig. 8. Diffusion coefficients (D) of theophylline in different calcium polysaccharide gel films at 37 °C in (a) water, (b) 0.1 M HCl, (c) SGF, and (d) 0.1 M NaCl. (ND = not determined; $n = 3$, S.D. shown.)

films, made by immersing a dry sodium alginate (3%ALV) piece in aqueous calcium chloride, increased about 15-fold after the films were simply exposed to SGF for 5 min. However, in the present study, the acid equilibrated gel films were immersed in water for another 24 h after the acid exposure to achieve equilibrium swelling and minimize error due to swelling or thickness changes during the diffusion studies. It was found that the P values of the acid-treated gel films (1 or 2%ALV) were significantly higher (about 1.5 times) than those reported in previous work (Lim and Kennedy, 1996). This may be partly explained by differences in film processing conditions in the two studies.

The CaPG films equilibrated in 0.1 M NaCl showed the highest D. The likely reason is the CaPG films swelled extensively in 0.1 M NaCl. The high swelling was probably due to the partial calcium–sodium ion exchange, causing a loose porous structure, and inducing a high water uptake into the gel films. As the gel films were highly hydrated, the drug can freely pass through the water fraction of the hydrated gel films. It was found that D in CaPG films equilibrated in 0.1 M NaCl was slightly higher than that reported by Grassi et al. (2001). This may be due to the difference in processing conditions used by Grassi et al. (2001).

Diffusive transport of solutes in a polymeric membrane has been described in terms of two mechanisms: the partition and pore mechanisms (Thacharodi and Rao, 1992, 1993). The two mechanisms may not operate exclusively but one may be expected to dominate over the other for a given drug/membrane pair. In those membranes where the partition mechanism predominates, the solute dissolves in the polymer itself and progresses across the membrane via diffusion in the polymer fractions. Permeability is then mainly determined by the solubility of the solute in the polymer. In the pore mechanism the solute is presumed to diffuse through microchannels within the membrane structure. The diffusion would then be determined by the average pore size in relation to the molecular volume of the solute and the solubility of the solute in the solvent used for the diffusion studies. Thacharodi and Rao (1993), using cross-linked chitosan membranes, suggested that a poorly water-soluble drug (nifedipine) was transported across the membrane by diffusion through the pores and partitioning in the gelled structure. They also showed that a highly water-soluble drug (propanolol hydrochloride) was transported across chitosan membranes predominantly by the pore mechanism, i.e., mainly via microchannels, as there was no change in the K_p with increasing degree of cross-linking in the gelled structure (Thacharodi and Rao, 1992).

In the current study, it is likely that both mechanisms, the partition and pore mechanisms, operated when TPL diffused through CaPG films equilibrated in different media. In those gel films equilibrated in 0.1 M NaCl where the $K_p < 1$ and the films extensively swelled, the pore mechanism may dominate over the partition mechanism. On the other hand, in those gel films equilibrated in water and acidic media where the films were stronger and showed high K_p values, the partition mechanism probably predominated. In this case, the drug dissolved in the gel film and progressed across the film via diffusion in the polysaccharide fractions, together with some interactions between the drug and the hydrophilic domain of the polysaccharide network as previously discussed.

4. Conclusions

The partition, permeability and diffusion coefficients of TPL in CaPG films varied with the polysaccharide type and concentration

and the equilibration medium. The *P* values of TPL were reduced as the concentration of polysaccharide was increased, and importantly the broad trend of the results agreed with results showing the release of TPL from spherical pellets coated with the same CaPG. Some of the CaPG were able to substantially reduce the release rate of TPL (compared to uncoated pellets). These results show that the CaPG may offer potential in the development of sustained release delivery systems that are able to be manufactured under very mild conditions and also showed that investigation of the transport properties within free films of the CaPG may offer some insight into the development of the coated pellets.

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