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Sigmoidal release of indomethacin from pectin matrix tablets: Effect of in situ crosslinking by calcium cations

Xiuli Wei [a,b], Ningyun Sun [a], Baojian Wu [a], Chunhua Yin [b], Wei Wu [a,*]

[a] *School of Pharmacy, Fudan University, Shanghai 200032, PR China*
[b] *The School of Life Sciences, Fudan University, Shanghai 200433, PR China*

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Abstract

Sigmoidal release pattern is therapeutically beneficial for timed release and colonic drug delivery, and is always observed in coated systems. In this study, sigmoidal release from pectin matrix tablets with indomethacin as a model drug was investigated. The underlying mechanisms are calcium cation-induced in situ crosslinking that retard the initial drug release to a limited percentage. Power law equation *n* values were estimated for sigmoidal release profiles. Results indicated that calcium chloride incorporated in pectin matrix functioned as retarding mechanisms on drug release. Larger amount of calcium chloride led to slower drug release and matrix erosion. Even at extremely high levels, retarding on drug release and matrix erosion rate was obvious, which highlighted the effect of calcium-induced in situ crosslinking as calcium chloride was a freely water-soluble salt. The sigmoidal release profiles were characterized by power law equation with high correlation coefficients of about 0.99 or over. Power law *n* values increased up to as high as 1.20 when calcium chloride content kept increasing. Erosion correlated well with release in almost all pectin matrix tablets indicating erosion-controlled mechanisms. It is concluded that large amount of calcium induces in situ crosslinking of pectin matrix and leads to sigmoidal release of indomethacin, and power law *n* values, sometimes larger than 1.0, are suitable to be used to describe sigmoidal release profiles.

Keywords: Pectin; Calcium chloride; Indomethacin; Matrix tablets; In situ crosslinking; Power law

1. Introduction

Sigmoidal or bimodal release profile is characterized by slower release at initial stage followed by increased release at later stage (Narisawa et al., 1994). This kind of release profile may be therapeutically beneficial for timed release or site-specific delivery of drugs (Maggi and Conte, 1997). For diseases influenced by circadian rhythms such as ischemic heart disease, asthma and arthritis, incremental release rate may be helpful to prevent exacerbation of nocturnal or early morning symptoms (Lemmer, 1991). Colonic delivery of drugs through sigmoidal release mechanisms is aimed to treat local diseases of the colon or to maintain sustained blood drug levels (Macleod et al., 1999).

Sigmoidal release is usually observed in press-coated or film-coated systems with tablets or pellets as substrate. This kind of release pattern needs triggering mechanisms such as degradation of the polysaccharide coating by colonic microbial degradation (Krishnaiah et al., 2002a; Macleod et al., 1999) or organic acid-induced enhancement of drug release (Narisawa et al., 1994). For reasons of simple manufacturing process, polysaccharide-based matrix tablets have also been studied for sigmoidal release with microbial triggering mechanisms (Krishnaiah et al., 2002b; Tugcu-Demiroz et al., 2004). One of the drawbacks of these systems is the relatively larger initial release rate compared with that of the coated systems. The crucial point in developing matrix tablets with sigmoidal release characteristics should be tight controlling on initial release rate. However, release rate at the initial stage of a common matrix system with a residing diffusion/erosion mechanisms is typically fast. It seems that alternative retarding mechanisms other than diffusion/erosion is desired to further reduce initial drug release.

Pectin, a naturally occurring polysaccharide of high hydrophilicity, has been used for drug delivery systems, especially

* Corresponding author. Tel.: +86 21 5423 7833; fax: +86 21 6417 0921.
E-mail address: wuwei@shmu.edu.cn (W. Wu).

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colon-specific ones (Ashford et al., 1993; Munjeri et al., 1997; Vandamme et al., 2002). To warrant tight controlling on drug delivery, calcium pectinate which is more water-resistant has been developed for colonic drug delivery purposes (Adkin et al., 1997; Rubinstein et al., 1993; Rubinstein and Radai, 1995). Calcium binding to pectin reduces solubility and induces crosslinking of carbohydrate chains, typical of changes in chain conformation and in the manner of chains packing (Morris et al., 1982; Powell et al., 1982). The calcium-induced associations of pectin chains are stable at low solution pH and able to resist extensive hydration in the gastrointestinal tract. Effect of incorporating calcium ions in drug delivery systems has also been studied by several authors. For pectin gel, the gel strength increases with calcium ion added to a critical concentration (Ashford et al., 1994). Calcium acetate in various amounts has been incorporate in pectin matrix tablets as in situ crosslinking agent to sustain in vitro drug release (Sungthongjeen et al., 2004). However, only relatively small amount of calcium was reported to have such effect. The in situ crosslinking pectin matrix system is advantageous over calcium pectinate-based ones in that the degree of gelation is adjustable simply by change the amount of calcium salt initially added. Moreover, incorporation of calcium salts into pectin matrices should enhance its susceptibility to enzymes at proximal colon, because many pectinases were supposed to be stimulated by or have an absolute requirement for calcium ions for their activity (Miller and MacMilan, 1970).

In this study, large amount of calcium chloride was incorporated into pectin matrix tablets to induce in situ crosslinking of pectin molecules. Effect of variables such as incorporating calcium chloride into pectin matrix, pectin/calcium ratio and drug/matrix material ratio on release characteristics were studied. Release kinetics was characterized by the power law equation. Indomethacin, a non-steroid anti-inflammatory drug frequently studied by other authors (Rubinstein et al., 1993) for colon-specific delivery was incorporated as a model drug.

2. Materials and methods

2.1. Materials

Micronized indomethacin (<5 μm in diameter) was purchased from Fengyan Pharmceuticals (Anhui province, China). Pectin HM (high methoxylated) 70 and PVP K30 were kindly gifted from International Specialty Products (Hong Kong). Calcium chloride ($CaCl_2$) and Sodium chloride (NaCl) were of analytical purity and purchased from Shanghai Chemical Regent Corp. (Shanghai, China). Sodium dodecyl sulfate (SDS) was purchased from Farco Chemical Supplies (Hong Kong). All other chemicals were of analytical grade.

2.2. Preparation of in situ crosslinking pectin matrix tablets

Matrix tablets containing 10 mg of indomethacin, pectin and calcium chloride in varying ratios were prepared by wet granulation/compression method using 10% (w/v) PVP ethanol solution as binder. In detail, all ingredients including pectin and calcium chloride were sieved through 80-mesh sieve and indomethacin was sieved through 200-mesh sieve before being mixed homogeneously in a Turbula T2 F shaker-mixer (Glen Mills Inc., USA). The mixed powder was transferred to a mortar, and 10% PVP K30 ethanol solution was added and the mixture was milled continuously to make paste. The wet mass was forced through a 20-mesh sieve and dried at 50 °C for 3 h. Magnesium stearate in 1% was added and mixed thoroughly with the granules. The lubricated granules were compressed into flat 8 mm tablets using a ZDY-8 model single punch compressor (Yuandong Pharmaceutical Machinery Co., Shanghai, China). Similarly, matrix tablets containing only pectin, pectin and sodium chloride instead of calcium chloride were prepared. The tablet formulations were given in Table 1. All the tablet batches were monitored for weight, crushing strength, friability and drug content uniformity. To avoid effect of variation in tablet hardness on release characteristics, crushing strength (shown in Table 1) was con-

Table 1
The composition, properties and power law correlation details of pectin matrix tablets

Formulation no.	Ingredients (mg)				Weight (mg)	Crushing strength (kg)	Friability (%)	Power law correlation			
	Indomethacin	Pectin	$CaCl_2$	NaCl				Correlation time span (h)	K	n	r
For evaluation of the effect of $CaCl_2$											
(1) Pectin/$CaCl_2$	10	75	75	0	158.7	6.0	0.05	0.5–8	0.075	1.20	0.989
(2) Pectin/NaCl	10	75	0	75	162.4	6.2	0.08	0.5–5	0.139	1.20	0.971
(3) Pectin	10	75	0	0	86.3	7.0	0.10	0.5–5	0.237	0.89	0.986
For evaluation of effect of pectin/$CaCl_2$ ratio											
(4) 75/15	10	75	15	0	102.5	6.1	0.09	0.5–5	0.220	0.93	0.980
(5) 75/25	10	75	25	0	109.8	7.3	0.08	0.5–6	0.193	0.90	0.980
(6) 75/50	10	75	50	0	137.4	6.4	0.04	0.5–7	0.129	1.03	0.990
(1) 75/75	10	75	75	0	158.7	6.0	0.05	0.5–8	0.075	1.20	0.989
(7) 75/100	10	75	100	0	188.6	6.8	0.04	0.5–8	0.077	1.18	0.990
For evaluation of effect of drug/(pectin + $CaCl_2$) ratio											
(8) 10/100	10	50	50	0	114.1	6.3	0.07	0.5–6	0.142	1.04	0.966
(1) 10/150	10	75	75	0	158.7	6.0	0.05	0.5–8	0.075	1.20	0.989
(9) 10/200	10	100	100	0	212.3	7.5	0.08	0.5–7	0.072	1.21	0.991

trolled to a narrow range. The tablets were sealed in glass bottles before tests.

2.3. Determination of indomethacin

Concentrations of indomethacin in tablets and release media were determined by a reported HPLC method with slight modification (Nováková et al., 2005). The Agilent 1100 series HPLC system consisted in a quaternary pump, a degasser, an autosampler, a column heater, and a tunable wavelength UV detector. The separation was performed at 40 °C using a C18 column (Diamonsil®, 5 μm, 4.6 mm × 150 mm, Dikma, China) guarded with a refillable precolumn (C18, 1.0 mm × 20 mm, Alltech, USA). The mobile phase was a mixture of acetonitrile and 0.3% acetic acid in the ratio of 58/42 pumped at a flow rate of 1.0 ml/min. Detection wavelength was 320 nm. Good linearity was observed between the concentration and peak area of indomethacin in the range of 0.01–100 μg/ml with a high correlation coefficient ($r > 0.9996$). Retention time under present HPLC conditions was 7.36 min. Limit of quantification was about 10 ng/ml. At spiked concentration of 0.1, 5 and 50 μg/ml, accuracy was 101.3%, 100.5% and 100.1%; intra-day precision was 3.06%, 2.87% and 1.95%; inter-day precision was 3.33%, 2.19% and 2.68%. Specific standard curve was constructed for the estimation of indomethacin in tablets and release media.

2.4. Release study

Release tests were performed in 900 ml of distilled water thermostatically maintained at 37 ± 0.5 °C based on a Chinese Pharmacopoeia (2005 Ed.) Method I. Basket rotational speed was set to 100 rpm. At predetermined time intervals, 5 ml of samples were withdrawn and filtered (Millex® AP, Millipore, 0.4 μm). The filtrate was analyzed by HPLC for indomethacin as described above. Meantime, an equal volume of the same medium was added to keep constant volume. As indomethacin was poorly water soluble, sodium dodecyl sulfate at a concentration of 1% (w/v) were added to release medium to keep sink conditions.

2.5. Characterization of sigmoidal release profiles by power law equation

In order to characterize the release profiles, experimental data were fitted to the power law equation (Korsmeyer et al., 1983; Ritger and Peppas, 1987):

$$\frac{M_t}{M_\infty} = Kt^n \tag{1}$$

where M_t is the amount released at time t, M_∞ the overall amount released, K a constant incorporating the properties of the macromolecular polymeric system and the drug, and n is the diffusion exponent that depends on the transport mechanism and the shape of the matrix tested. The n values of 0.5, 0.5–1.0, 1.0 and >1.0 indicates Fickian, non-Fickian (anomalous), Case II and Super Case II transport (Peppas and Brannon-Peppas, 1994). For cylindrical systems like tablets, the n values of 0.45 and 0.89 represents pure diffusion or erosion-controlled release, respectively (Ritger and Peppas, 1987).

2.6. Erosion study

Tablet erosion was evaluated by a method similar to that reported by Miyazaki et al. (2004) using a release test assembly. Tablets were placed in the basket and subjected to erosion in 900 ml of release medium at 37 ± 0.5 °C and a rotation speed of 100 rpm. At appropriate times, the tablets were recovered and dried to constant weight in a vacuum dryer at 60 ± 2 °C. The amount of indomethacin released was also monitored simultaneously by HPLC as described above to calculate the amount of drug remaining in the tablet. Three different tablets were measured for each time point, and fresh tablets were used for each individual time point. The percent matrix weight loss at each time point was calculated from the equation:

$$\text{percentweightloss}\,(\%) = \left[\frac{W_0 - (W_t - (X_0 - X_t))}{W_0}\right] \times 100 \tag{2}$$

where W_0 and X_0 are the initial weights of matrix and drug loaded, respectively, W_t the dried weight of the tablet and X_t is the amount of indomethacin released at time t.

3. Results and discussion

3.1. Results of tablet preparation

Properties such as weight, crushing strength and friability of pectin matrix tablets are summarized in Table 1. All tablets were of high quality and complied with pharmaceutical standards.

The mixed powder of indomethacin, pectin and calcium chloride was not freely flowable, so a wet granulation/compression method was used to prepare pectin matrix tablets. Indomethacin in micronized form was used throughout the experiment for enhanced dissolution rate.

3.2. Effect of calcium ion-induced crosslinking on pectin matrix

Release of indomethacin from pectin matrix was studied in distilled water. To verify the effect of calcium ions on pectin matrix, sodium chloride was added in lieu of calcium chloride at the same weight percentage and release profiles were compared. Fig. 1 gives the results of indomethacin release from matrix tablets prepared with pectin/calcium chloride, pectin/sodium chloride or only pectin. Indomethacin was released quickly from pure pectin matrix with t_{50} of about 2 h, and within 5 h, release was almost complete. Release from the calcium-based matrix showed sigmoidal characteristics. In the first 2 h, release of indomethacin was restrained greatly to less than 10%. In the following 2–8 h, near zero-order release was observed. Sodium-based matrix release only 20% of the total drug at 2 h, but release rate accelerated with similar profile to pure pectin-based system after 2 h. The significantly reduced release rate should be

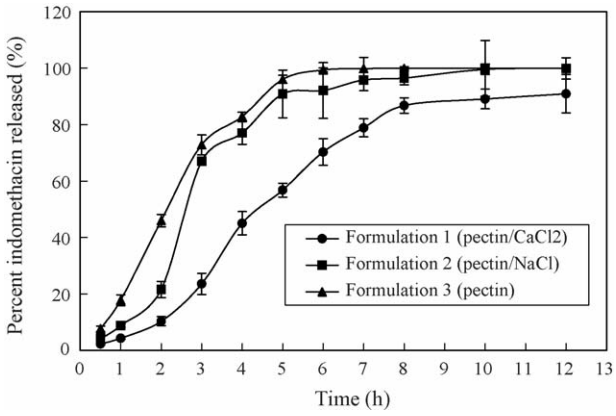

Fig. 1. Release profiles of indomethacin from matrix tablets prepared with pectin/calcium chloride, pectin/sodium chloride and pure pectin.

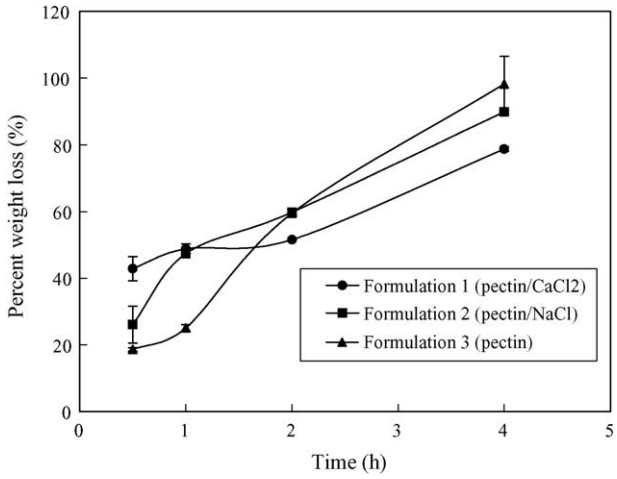

Fig. 2. Erosion profiles of matrix tablets prepared with pectin/calcium chloride, pectin/sodium chloride and pure pectin.

the result of calcium cation-induced crosslinking that strengthens the pectin gel. Sodium ion, which was weak in inducing crosslinking of pectin molecules, only showed slight effect on retarding drug release (Liu et al., 2003). As both calcium chloride and sodium chloride are highly water-soluble salts, it is easy to make one think that these salts may function as channeling agent and lead to faster drug release as observed for lactose in hydroxypropylmethylcellulose matrix (Sako et al., 2002). On the contrary, large amount of calcium chloride in pectin matrix resulted in reduced erosion and release rate. This highlighted the effect of in situ crosslinking induced by calcium cations. It is indicated that the interaction between calcium ions and pectin chain be the main mechanisms that controlling the sigmoidal release of indomethacin from pectin matrix.

The power law equation has been frequently used to characterize release profiles of matrix drug delivery systems. In the present study, we employed the same equation to characterize sigmoidal release. Power law correlation results are summarized in Table 1. Release of indomethacin from pure pectin matrix can be described by near zero-order equation with n value of 0.89 indicating erosion-controlled mechanisms. When single (Na^+) and double (Ca^{2+}) valent cations were incorporated, n value increased to as high as 1.20. Similar n values of larger than 1.0 have also been reported by other authors (Alur et al., 1999; Ferrero et al., 2000; Munday and Cox, 2000; Ranga Rao et al., 1988) to describe sigmoidal release pattern. In these reports, the n values higher than 1.0 was ascribed to a Super Case II transport, in which drug release seemed to be controlled by polymer relaxation (Korsmeyer et al., 1983). However, in the present study, the situation is somewhat different. It cannot be simply explained by polymer relaxation, because pure pectin matrix did not show signs of Super Case II transport with an n value of 0.89. For pectin/$CaCl_2$ matrix, at the beginning of the hydration process upon contact with water, calcium cations would be liberated in high concentration and exert the most significant effect on pectin matrix, thus perform the strongest controlling on retarding of drug release. Gradually, calcium cations would escape the matrix and result in "relaxation of crosslinking degree". So, increased release rate could be observed because of the gradual subsiding of the retarding force.

To further decipher the mechanisms controlling in situ crosslinking matrix, in vitro erosion was also investigated and correlated to in vitro release data. Fig. 2 indicates that erosion profiles of the three formulations prepared with pectin/$CaCl_2$, pectin/NaCl and pure pectin were totally different. At the beginning 0.5 h after immersion in water, pure pectin matrix showed the least erosion percentage, while pectin/$CaCl_2$ matrix showed the fastest erosion rate. As the erosion process continued, this situation seemed to proceed to inverse direction. After 2 h, percent matrix weight loss of pectin/$CaCl_2$ matrix was only about 51%, approximately 10% increase from 0.5 h. For pure pectin matrix, percent weight loss saw a 30% increase to about 60%. The results suggested that $CaCl_2$ in pectin matrix function as an erosion retarding material. It is known that calcium chloride, being highly water-soluble, dissociates to release calcium cations upon contact with water. The free calcium cations make the pectin molecules to crosslink, which results in rigidity of the pectin gel and reduced matrix dissolution rate. Similar mechanisms have been applied by Miyazaki et al. (2005) to gelate dilute pectin solutions for oral in situ gelling sustained release systems. Replacing calcium chloride with sodium chloride at the same level led to faster erosion rate at later times. However, there was discrepancy at the beginning of the erosion process among the three matrixes. That about 40% of erosion percentage of pectin/$CaCl_2$ matrix did not virtually mean that much loss of pectin matrix, and may lay reason on quick escape of $CaCl_2$ to the release medium. Multiple regression was performed for percent matrix weight loss and percent indomethacin released simultaneously monitored during the erosion study, and linearity was observed between them (Fig. 3) with high coefficient (r) of over 0.99 both for pure pectin and pectin/$CaCl_2$ matrix tablets. Although erosion was only tested for a limited 4 h due to difficulty in accurate weighing of the eroded matrix after that, the synchronization of erosion and release may partly reveal erosion-controlled mechanisms (Ng et al., 2000). It is concluded that release profiles with power law n values larger than 1.0 may also be explained by erosion-controlled mechanisms. Retarding on erosion rate by calcium cations

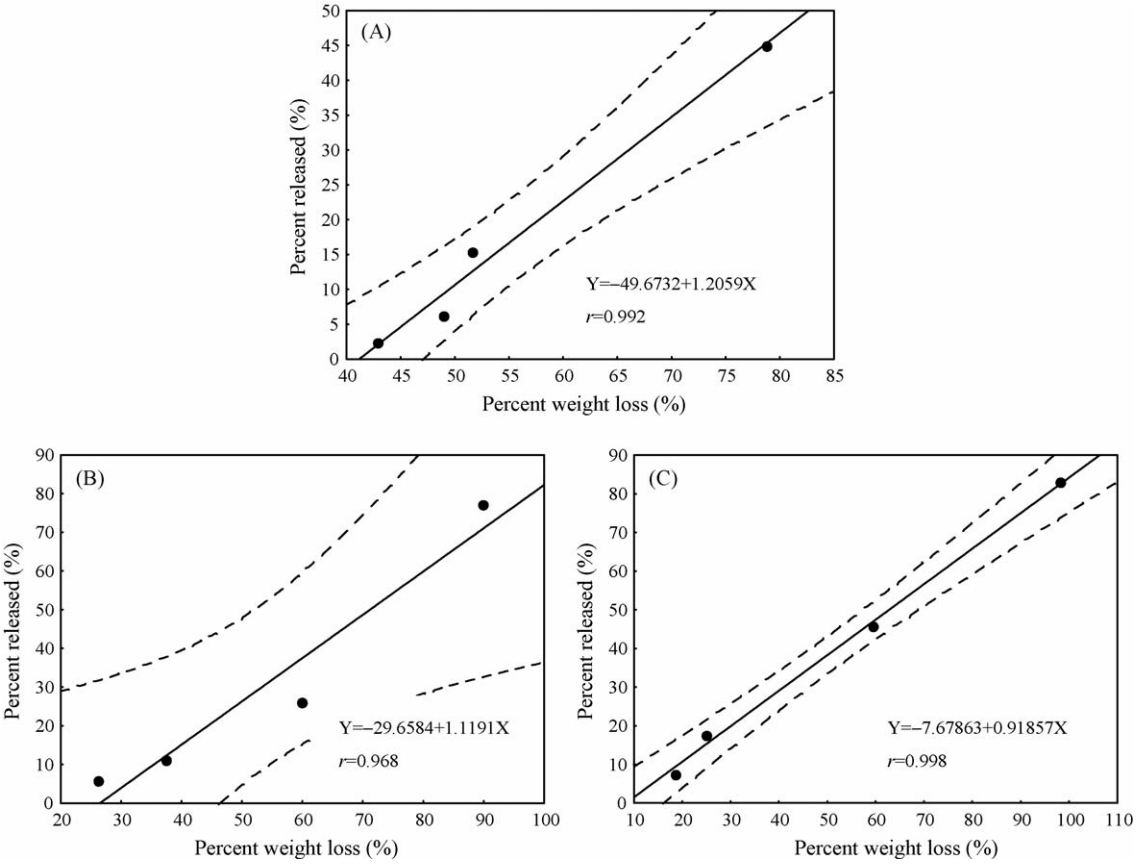

Fig. 3. Correlation of percent indomethacin released as a function of percent matrix weight loss with 95% confidence levels. (A) Formulation 1 (pectin/$CaCl_2$); (B) formulation 2 (pectin/NaCl); (C) formulation 3 (pectin).

may be one of the mechanisms to result in sigmoidal release patterns.

3.3. Effect of pectin/$CaCl_2$ ratio

As discussed by Ashford et al. (1994), pectin gel strength increased to a critical point as calcium amount increased. Only relatively small amount of calcium acetate has been evaluated to be able to induce sustained release (Sungthongjeen et al., 2004). In the present study, different results were observed incorporating large amount of calcium chloride in pectin matrix.

Fig. 4 shows the release profiles of pectin matrix tablets at various pectin/$CaCl_2$ ratios (w/w) with pectin weight remains constant. In all cases, sigmoidal release profiles were observed. Pectin/$CaCl_2$ ratio influences release rate significantly. Higher levels of $CaCl_2$ led to decreased release rate of indomethacin except that at extremely high levels release rate seemed not to decrease any more. Power law equation correlation was performed on each of the release profiles and details are presented in Table 1. Conventionally, application of the power law is confined to the first 60% of release. Under situations of non-classical diffusion, the power law can be applied to describe the 'entire' drug release curve (Rinaki et al., 2003). In this study, up to over 80% release data were fitted to the power law equation with high correlation coefficients. The n values seem to be positively correlated to calcium chloride level in matrix tablet. At lower pectin/$CaCl_2$ ratio of 75/15, 75/25 and 75/50, n values lies approximately in the range of $0.89 < n < 1.0$, which is indicative of erosion-controlled mechanisms. At extremely high $CaCl_2$ levels, n values increased to as high as 1.20.

In our preliminary study, less than 10% (w/w) calcium chloride in pectin matrix tablets has been studied to have only limited effect on indomethacin release. The largest amount of incorpo-

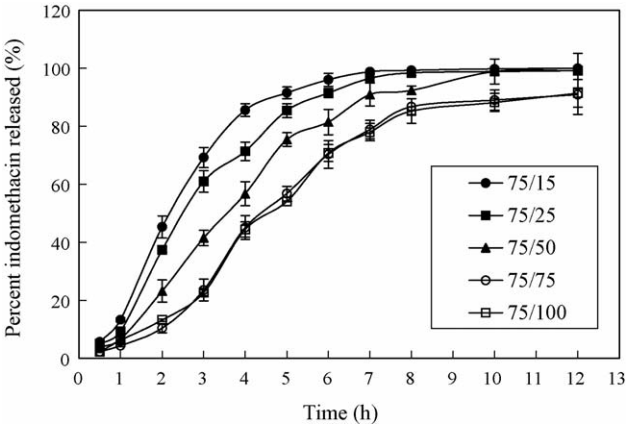

Fig. 4. Release profiles of indomethacin from matrix tablets prepared at various pectin/calcium chloride ratios.

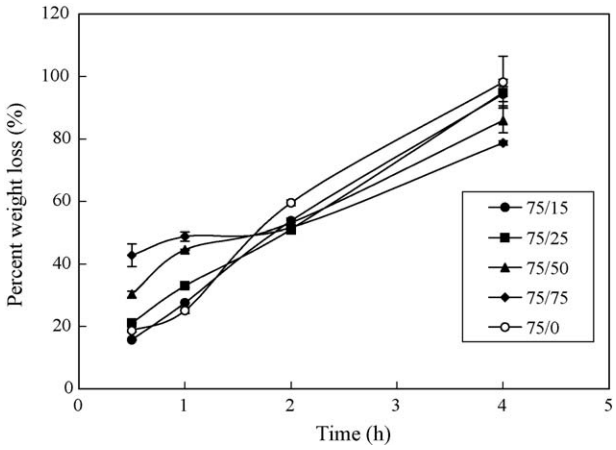

Fig. 5. Erosion profiles of matrix tablets prepared at various pectin/calcium chloride ratios.

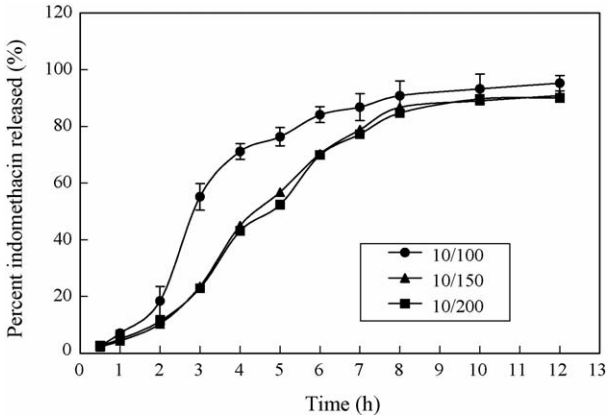

Fig. 6. Release profiles of indomethacin from matrix tablets prepared at various drug/(pectin + calcium chloride) ratios.

rated calcium in acetate that studied by Sungthongjeen et al. (2004) was about 3.4%. Contrary to findings by Ashford et al. (1994) and Sungthongjeen et al. (2004), large amount of calcium chloride did not show enhancing effect on release rate. It is suggested that the critical concentration of calcium (Ashford et al., 1994) do not comply with the matrix tablet system we studied here. A possible explanation of this disparity was that the present study used calcium chloride of high dissociation degree instead of calcium acetate, and calcium ions will be liberated to the pectin gel in high concentration. These findings open up perspective of application of large amount of calcium salts to control the release of active agents from pectin matrix tablets.

Erosion profiles of pectin matrix tablets in various pectin/$CaCl_2$ ratios are shown in Fig. 5. $CaCl_2$ amount negatively correlates to initial weight loss at 0.5 h. As discussed before, $CaCl_2$ seemed to release quickly at the beginning, and larger amount would lead to larger fraction of initial loss of weight. Gradually, erosion rate for larger $CaCl_2$ content began to slow down. Before about 1.5 h, percent weight loss negatively correlated to $CaCl_2$ content. During the time span of 1.5–2.0 h, the erosion curve seemed to cross and after 2 h, percent weight loss positively correlated to $CaCl_2$ content. Erosion rate was the lowest for matrix of greatest $CaCl_2$ content and for pure pectin matrix it became the highest. It is indicated that calcium cations exerted retarding effect on matrix erosion rate in a dose-dependent way.

3.4. Effect of drug/(pectin + $CaCl_2$) ratio

Drug/polymer ratio in matrix tablets has been studied as one of the most important factors influencing drug release. Here, effect of indomethacin to matrix material regarding pectin and $CaCl_2$ as a whole in ratio of 1/1 on drug release was investigated. Release profiles are shown in Fig. 6. At the three overall matrix weights, indomethacin release is sigmoidal. Smaller drug/(pectin + $CaCl_2$) ratio resulted in faster drug release. However, for the two matrix tablets in larger drug/(pectin + $CaCl_2$) ratio, release profiles were not significantly different. Power law n values (Table 1) are all bigger than 1.0.

4. Conclusion

Sigmoidal release of the model drug indomethacin from pectin matrix tablets was achieved by calcium cation-induced in situ crosslinking of pectin chains. The significant retarding effect on both release and erosion rate by highly water-soluble salt of $CaCl_2$ highlighted the effect of calcium cations. Power law n values bigger than 1.0 were employed to characterize sigmoidal release profiles. The residing mechanisms of drug release from pectin/$CaCl_2$ system were erosion and may be the result of "relaxation of the crosslinking degree" by calcium cations. It was also indicated that the effect of calcium cation-induced in situ crosslinking was dose-dependent.

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