Physicochemical Characterization of a Pectin/Calcium Matrix Containing a Large Fraction of Calcium Chloride: Implications for Sigmoidal Release Characteristics

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ABSTRACT: The physicochemical properties of pectin/calcium matrix tablets were studied with an emphasis on the effect of over 50% of calcium chloride to better understand the fundamentals of the drug-release mechanisms. The absence of the adsorption of methylene blue onto the calcium-containing pectin matrix served as evidence of the calcium-ion-induced *in situ* crosslinking of pectin chains. Visual and scanning electron microscopy observation showed the reinforced robustness of the pectin/calcium matrix, whereas cryo-SEM revealed the formation of a pectin aggregate network as a result of calcium-induced crosslinking. The aggregate network underwent gradual erosion to a lower density, which indicated the erosion of the matrix. However, little change in the texture of the pectin/calcium matrix was observed at earlier times, which was interpreted as the initial stages of drug-release retardation. The apparent viscosity of the pectin aqueous dispersion increased biphasically as the calcium amount increased. The initial sharp increase was interpreted as calcium-induced crosslinking, whereas the following gradual increase was ascribed to a possible salt effect. We concluded that the salt effect and calcium-induced crosslinking contributed to a series of physical changes of the pectin matrix, which correlated to the sigmoidal release of indomethacin from the pectin/calcium matrix tablet. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 2418–2428, 2009

Key words: biomaterials; crosslinking; drug delivery systems; gelation; matrix

INTRODUCTION

Pectin, a naturally occurring polysaccharide, possesses gelling properties and has long been used in the food industry as a gelling or thickening agent and as an excipient for pharmaceutical purposes.[1,2] By virtue of its gelling and microbially degradable properties, pectins alone or those hybridized with other polymers have been used in drug-delivery systems, especially colon-specific ones.[3–6] The gelation of pectin is determined by several factors, including the origin and concentration of pectin, degree of esterification, degree of amidation, modification of the hydroxyl groups, solution pH, temperature, and presence of cations.[7–10] It is usually difficult to achieve tight control of drug release by the pectin matrix itself because of its high hydrophilicity. For this reason, calcium salts of pectin have been developed and have shown favorable results. The calcium ions bind to polyguluronate or polygalacturonate molecules through egg–box complexes[11] with the polysaccharide chains in analogous 2_1 conformations,[12,13] which reduces the solubility and induces the crosslinking of the carbohydrate chains. The calcium-induced associations of pectin chains are stable at low solution pH and are able to resist extensive hydration *in vivo* in the gastrointestinal tract. Calcium pectinate has been formulated into matrix tablets or compression-coated tablets and evaluated both *in vitro* and *in vivo* for potential colon-specific delivery.[3,4,14–18] The calcium level influences the pectin gel strength, which increases with calcium ions added to a critical concentration, and, thereby, influences the drug release.[4,18] Calcium and pectin association can also be achieved through *in situ* crosslinking systems.[19–21] Calcium acetate in various amount (<5% w/w) has been incorporated into pectin matrix tablets as a crosslinking agent to achieve sustained drug release.[19] The *in situ* crosslinking system is simple to manufacture, and one can easily adjust the degree of gelation by changing the calcium level. Moreover, the incorporation of calcium salt into the pectin matrix may enhance its susceptibility to enzymes at the proximal colon,[22] which strengthens its potential use in colon-specific drug delivery.

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In previous studies,[20,23] we investigated the sigmoidal release behavior of a water-insoluble drug, indomethacin, from an *in situ* crosslinking pectin/calcium chloride ($CaCl_2$) matrix tablet. It was unusual that a significant retarding effect on drug release was observed, despite the large fraction of $CaCl_2$ (>50%) that was used. The sigmoidal release was characterized by slow release at earlier stages and faster release at later stages, which is common in coated systems but rare in matrix systems. When the power-law equation[24,25] was used to characterize the sigmoidal release profiles, values of the exponent n as high as 1.20 were observed. The good correlation between the matrix erosion and drug release indicated erosion-controlled release mechanisms. These findings were strengthened by another study with a hydroxypropyl methylcellulose/pectin/$CaCl_2$ matrix tablet.[21]

It is logical to conclude that the calcium-induced crosslinking of pectin chains is crucial for achieving retardation of drug release. However, only a small amount of calcium was sufficient to saturate the pectin chains,[19] and the calcium level in the pectin/calcium matrix[20,21] seemed to be far above the crosslinking saturation levels or to be excessive. It is interesting that $CaCl_2$, a highly water-soluble salt that may perform as a tunneling agent, exerted a strong retardation of drug release at extremely high levels, up to over 50%. Although a mechanism of the relaxation of the crosslinking degree was assumed previously[20] to explain the sigmoidal release characteristics, a large fraction of $CaCl_2$ in the pectin matrix seemed to have an extra effect in addition to calcium-induced crosslinking. Herein, we performed a systemic investigation of the physicochemical properties of this unique pectin matrix containing a large fraction of $CaCl_2$. The association of these properties with the sigmoidal release patterns gave us a clue to the fundamentals of the effect of $CaCl_2$. Methylene blue (MB) adsorption by the pectin matrix was first studied to find evidence of calcium-induced crosslinking, and the effects of $CaCl_2$ on the morphological changes in the hydrated pectin matrix were studied with visual observation, scanning electron microscopy (SEM), and environmental scanning electron microscopy [i.e., cryo scanning electron microscopy (cryo-SEM)]. Calcium release from the matrices and calcium-induced gelation of the pectin dispersions were also studied to facilitate the elucidation of the effect of excessive calcium.

EXPERIMENTAL

Materials

Micronized indomethacin (<5 μm) was a gift from Sine Pharmaceuticals (Shanghai, China). Pectin (Pectin HM 70) and poly(vinyl pyrrolidone) (PVP K30) were gifts from International Specialty Products (Shanghai, China). $CaCl_2$ and sodium chloride (NaCl) were analytically pure and were purchased from Shanghai Chemical Regent Corp. (Shanghai, China). Distilled water was prepared with a Milli-Q water-purifying system (Millipore, Molsheim, France). All other chemicals were analytical grade.

Preparation of the *in situ* crosslinked pectin matrix tablets

In situ crosslinking pectin matrix tablets containing 10 mg of indomethacin were prepared by a wet granulation/compression method, as described previously.[20] Briefly, indomethacin, pectin, and $CaCl_2$ were mixed thoroughly and milled continuously to make a paste after the addition of a 10% (w/v) PVP K30 ethanol solution as a binder. The wet mass was forced through a 20-mesh sieve and dried at 50°C for 3 h. The granules were lubricated with magnesium stearate at 1% (w/w) and compressed into flat 8-mm tablets with a ZDY-8 model single-punch compressor (Far East Pharmaceutical Machinery Co., Shanghai, China). Similarly, matrix tablets containing pectin only or pectin and NaCl instead of $CaCl_2$ were prepared as controls. For better comparison, the hardness of the tablets was controlled within a narrow range of 6–8 kg of tensile strength, whereas the thickness of the tablets varied slightly because of various tablet weights.

Adsorption of MB by the pectin matrix

MB is a cationic molecule with a high affinity to negatively charged solids. Adsorption of MB onto the samples is proportional to the quantity of anionic groups and, therefore, can be related to the crosslinking density.[26,27] The same apparatus used in the release study[20] was adapted to test the adsorption of MB. The pectin matrix tablet was first sealed in a dialysis bag (molecular weight cutoff = 12,000–14,000) and put into a rotation basket. After that, it was immersed in a total of 900 mL of a 0.011 mmol/L MB solution thermostatically maintained at 37°C and stirred at a speed of 100 rpm. At certain time intervals after immersion, 5-mL samples were withdrawn, and the disappearance of MB from the MB solution was measured at a wavelength of 665 nm with a Spectrumlab 54 UV spectrophotometer (Shanghai Lengguang Technologies, Ltd., Shanghai, China). Each sample was measured in triplicate.

Visual observation of the pectin matrix tablets

Dynamic changes in the morphology of the pectin tablet and pectin/$CaCl_2$ (75/75 mg) tablet were evaluated and compared. For ease of observation, the samples were immersed in 200 mL of deionized water and allowed to hydrate and swell under

stationary conditions at room temperature. The morphological changes were observed directly and recorded by a digital camera (Canon S80, Tochigi, Japan). The hydration process may have differed from that in a real release study.

SEM and cryo-SEM study

The morphology of the hydrated pectin and pectin/$CaCl_2$ (75/75 mg) matrix tablets was also evaluated by SEM (Hitachi S-520, Tokyo, Japan) and cryo-SEM (FEI Quanta 200F, Hillsboro, OR). The pectin matrix tablets were first subjected to hydration under conditions that simulated the release study.[20] At certain time intervals, the samples were collected and processed for SEM and cryo-SEM observation.

Before SEM observation, the samples were dehydrated first *in vacuo* and gold-sputtered. As the dehydration process may have altered the structure of the pectin matrix network, it was also evaluated by cryo-SEM without dehydration. The pectin matrix tablets collected at different release time points from the release tester were prefrozen with liquid nitrogen and broken. Suitable cross-sectional segments of the tablets were immediately transported to the cryo-SEM facility without further treatment. A Peltier-cooled stage allowed us to regulate the segment temperature at 0°C, and the pressure was reduced to 5 Torr while a relative humidity of 70% was maintained.

Calcium release study

Calcium release was evaluated with procedures similar to those used the drug-release study.[20] The pectin matrix tablets were put into 900 mL of distilled water thermostatically maintained at 37 ± 0.5°C and stirred at a speed of 100 rpm. At specific time intervals, 10 mL of the release sample was collected, filtered (Millex AP, Millipore, 0.4 μm), and assayed for calcium. Meanwhile, an equal volume of distilled water was added to keep a constant volume. Each sample was measured in triplicate.

Calcium was determined with an atomic absorption spectrometer (Varian Spectr AA 240, Palo Alto, CA) equipped with a 10-cm single element hollow cathode lamp at a wavelength of 422.7 nm in a rich air–acetylene flame.[28,29] The instrumental parameters were as follows: lamp current = 10 mA, slit width = 0.7 nm, air flow 13.5 L/min, and acetylene flow = 2.21 L/min. Good linearity between the absorbance (A) and calcium concentration (C) within the range 0.25–5.0 μg/mL was observed: $A = 0.05803C + 0.01044$ ($n = 5$, $r = 0.9968$). The accuracies at all concentration levels were within 100 ± 5%. The intraday/interday precisions at concentration levels of 0.25, 1.0, and 5.0 μg/mL were 4.26/6.59, 3.31/4.97, and 4.50/5.22%, respectively.

Viscosity measurement

As the pectin/calcium matrix underwent dynamic hydration, swelling, erosion, and release of both the incorporated drug and calcium, it was difficult to quantify the effect of calcium on the physical properties of the pectin matrix. In this study, a 2% (w/v) pectin aqueous dispersion was used as a simple model to simulate the situation of the pectin/calcium matrix with a large amount of calcium that was far above the crosslinking saturation level. The apparent viscosity of the pectin dispersion was measured by an NDJ-5S digital rotating viscometer (Shanghai Hengping Scientific Instruments Co., Ltd., Shanghai, China) to identify the effect of calcium on the pectin gel strength. To a volume of 200 mL of pectin aqueous dispersion, different amounts of $CaCl_2$, dissolved in 4 mL of distilled water were added and stirred mechanically for 15 min and settled for 5 min before measurement. A #3 probe was used, and the operating rotating speeds were set to 12, 30, and 60 rpm, respectively.

RESULTS

In a previous study,[20] the release of the model drug indomethacin from the pectin/$CaCl_2$ matrix tablet was evaluated. At different calcium levels, especially with a large fraction of $CaCl_2$, sigmoidal release was observed, whereas the pectin matrix without $CaCl_2$ showed a near-zero-order drug release without an obvious initial release lag. The release percentages for the formulation containing pectin/$CaCl_2$ in a ratio of 75/75 mg were about 2, 4, 10, 24, 45, 57, 70, 79, 87, and 89% at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, and 12 h, respectively, whereas those for the formulation containing 75/25 mg pectin/$CaCl_2$ were about 5, 9, 37, 61, 71, 85, 91, and 97% at 0.5, 1, 2, 3, 4, 5, 6, and 8 h, respectively.[20] Because indomethacin was sparingly soluble in water, drug release was predominantly controlled by the erosion rate of the pectin/calcium matrix. The calcium-induced retardation of drug release was actually based on calcium-induced physical changes of the pectin/calcium matrix. In this study, the physicochemical properties of these two formulations were investigated to elucidate the effect of excessive calcium because both contained calcium above the crosslinking saturation level.

Evidence of the calcium-induced crosslinking of the pectin matrix

MB adsorbed to the surface of the hydrated pectin matrix of opposite charges, which contrasted strongly with the background with an appearance of dark blue. The pectin/calcium matrix, nevertheless, remained unstained, even at much lower calcium levels (pectin/$CaCl_2$ = 75/25 mg). It was evident that calcium cations bonded to the pectin chains and shielded the

system from MB binding. When $CaCl_2$ was substituted with NaCl, the binding of MB was also observed, which served as counterevidence for the *in situ* crosslinking of pectin chains by calcium ions.

The association of MB with pectin is a process of adsorption and desorption until a balance is reached.[26,27] Ions, Ca^{2+} or Na^{+} in this study, of the same charge may compete with MB for pectin binding sites. Because divalent Ca^{2+} is a stronger binder than the MB cation, saturation-level calcium is enough to shield the pectin matrix completely from MB adsorption. For weaker binders such as monovalent Na^{+}, where pectin binding sites cannot be saturated by Na^{+}, MB cations substitute for Na^{+} to give the pectin matrix its blue color.

The adsorption of MB onto the pectin chains were easily quantified by measurement of the absorbance reduction of the MB solution. We assumed that the absorbance would not change if there was no MB adsorption at all and that a decrease in absorbance would indicate the association of MB with the pectin matrix. Figure 1 shows the dynamic change in the absorbance of the MB solution. There was virtually no change at all in the absorbance of the MB solution for the matrix containing calcium. The pectin matrix without calcium showed a gradual decrease in absorbance, and the pectin/NaCl matrix also showed a decrease in absorbance but to a lesser extent. It was evident that, even at a pectin/$CaCl_2$ ratio of 75/25 mg, there was no binding of MB onto the pectin/calcium matrix. Therefore, it was reasonable to conclude that the two pectin/$CaCl_2$ (75/75 and 75/25 mg) matrices all had sufficient calcium above the crosslinking saturation level. Because MB bonded to free pectin only, the two formulations showed the same results of no MB binding. To facilitate easy understanding, the part of calcium that was above the saturation level was defined as *excessive calcium.* With reference to the previous result[20] that no acceptable release lag time was achieved under such $CaCl_2$ levels of 25 mg (pectin = 75 mg), the excessive calcium might have had a close relationship with the release lag. In other words, the large fraction of excessive calcium was the determining factor that led to the sigmoidal release characteristics.

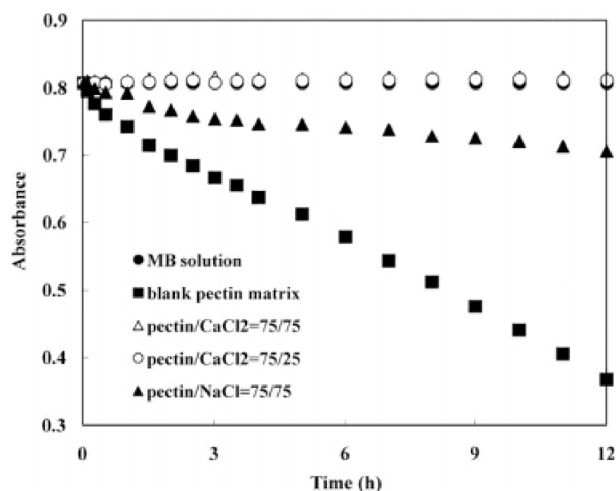

Figure 1 Dynamic changes in the absorbance of MB solutions after adsorption onto pectin matrices.

There seemed to be a continuous increasing tendency of adsorption for the *in situ* crosslinking pectin/calcium matrix. Unlike systems studied by other researchers,[26,27] the matrix tablet system underwent gradual hydration, swelling, and erosion, and the pectin counterparts ready for MB association were in a state of continuous increase. While erosion continued, more and more pectin chains might have been exposed and took in more MB, which was the major reason that a continuous adsorption was observed.

Visual observation of the matrix tablet

Under stationary hydration conditions, both matrix tablets of pure pectin and pectin/calcium underwent gradual hydration and obvious morphological changes. Figure 2 shows the dynamic change in the hydrated pectin matrices, and a significant visual difference was observed for these two matrix systems. The most significant difference between the two pectin systems was in changes at the erosion front. The outer surface of the pure pectin matrix became loose after 1 h [Fig. 2(B–F)], whereas that of the pectin/calcium matrix was tight [Fig. 2(H–L)]. As hydration proceeded, the outer layer of the pure pectin matrix became tenuous, and significant erosion was observed at the gel/water junction after 3 h [Fig. 2(C)]. Nevertheless, the pectin/calcium matrix kept its integration, and no obvious changes at the gel/water junction were observed [Fig. 2(H–L)]. It was evident that calcium exerted a significant effect on the hydration behavior of the pectin matrix. The calcium-containing matrix seemed to be stronger to withstand prolonged hydration.

SEM and cryo-SEM observation

To investigate the inner morphology of the hydrated matrices, the same matrix tablets of pure pectin and pectin/calcium were observed by SEM and cryo-SEM. Figure 3 shows the SEM photographs of the pectin and pectin/calcium matrix tablets collected at different times from the release tester. Because the pure pectin matrix underwent faster erosion, only samples before 2 h of release were successfully mounted for SEM observation. The pectin/calcium matrix eroded more slowly and could be processed for SEM observation for 4 h. Both the pure pectin matrix [Fig. 3(I–K)] and the pectin/calcium matrix

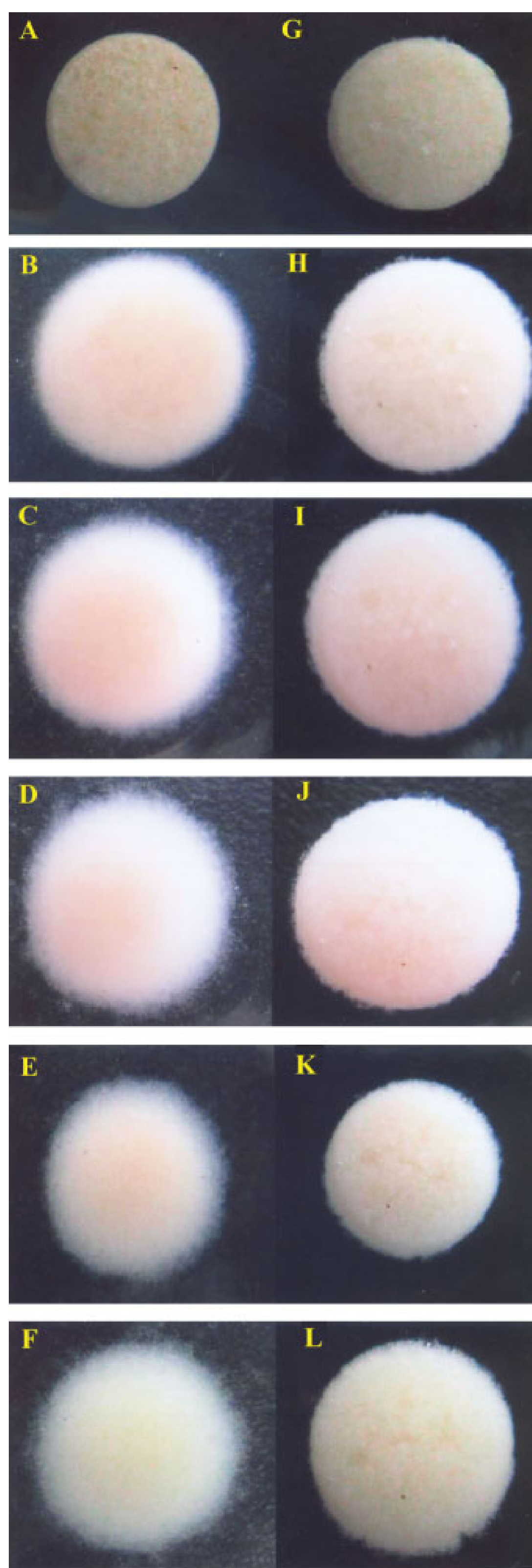

Figure 2 Photographs of dynamic changes in the morphology of pectin [(A) 0, (B) 1, (C) 3, (D) 5, (E) 7, and (F) 9 h] and pectin/$CaCl_2$ [(G) 0, (H) 1, (I) 3, (J) 5, (K) 7, and (L) 9 h] matrix tablets under stationary hydration conditions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

[Fig. 3(A–D)] showed continuous surfaces. The cross sections of the pure pectin matrix appeared to be discontinuous and full of large cavities, and integrity was lost at 2 h [Fig. 3(L–N)]. The pectin/calcium matrix appeared to be more compact in the cross-section show, and integrity was kept until 4 h after release [Fig. 3(E–H)].

The SEM sampling process involved a step of vacuum dehydration, which may have destroyed the original structure of the hydrated matrix. This is why the cross section of the pure pectin matrix showed a lower density. However, the cross section of the pectin/calcium matrix was higher in density, which indicated that the pectin matrix lost more solid content than the pectin/calcium matrix during the release process. It was evident that enhanced gel robustness was achieved through the calcium-induced crosslinking.

The main advantage of cryo-SEM imaging over conventional SEM is that samples can be observed in a hydrated state to reflect their real structure without any need for a dehydration and coating process.[30] The cryo-SEM photographs of the pure pectin and pectin/calcium matrix tablets are shown in Figure 4. The pure pectin matrix showed a uniform and continuous texture [Fig. 4(A,B)] in the hydrated fraction, and no obvious difference was observed at different time points within 2 h. The pure pectin matrix hydrated completely within 2 h, whereas the pectin/calcium matrix hydrated completely within 0.5 h because of the tunneling effect of $CaCl_2$. The cryo-SEM show of the pectin/calcium matrix was much different from that of the pure pectin matrix. The microstructure of the pectin gel was affected greatly by the addition of calcium ions. As a result of calcium-induced crosslinking, a pectin chain aggregate network was observed at different sampling points. At the beginning of matrix hydration, the gel structure was denser with limited porosity, and strands of connectives were seen. As the pectin matrix underwent gradual erosion, the network also underwent a series of changes. The filaments of pectin chain aggregates became thinner and thinner, and there was also an increase in porosity. The strands of connectives began to break after 2 h [Fig. 4(E–G,J–L)], and the network structure became extremely tenuous at 4 h [Fig. 4(G,L)]. The results indicate that changes in the texture of the matrix were limited at earlier stages, which interpreted the release lag at earlier times.

Calcium release

Upon hydration of the pectin/calcium matrix, calcium began to release into the outer medium. The monitoring of calcium release provided useful information about the matrix erosion and drug-release

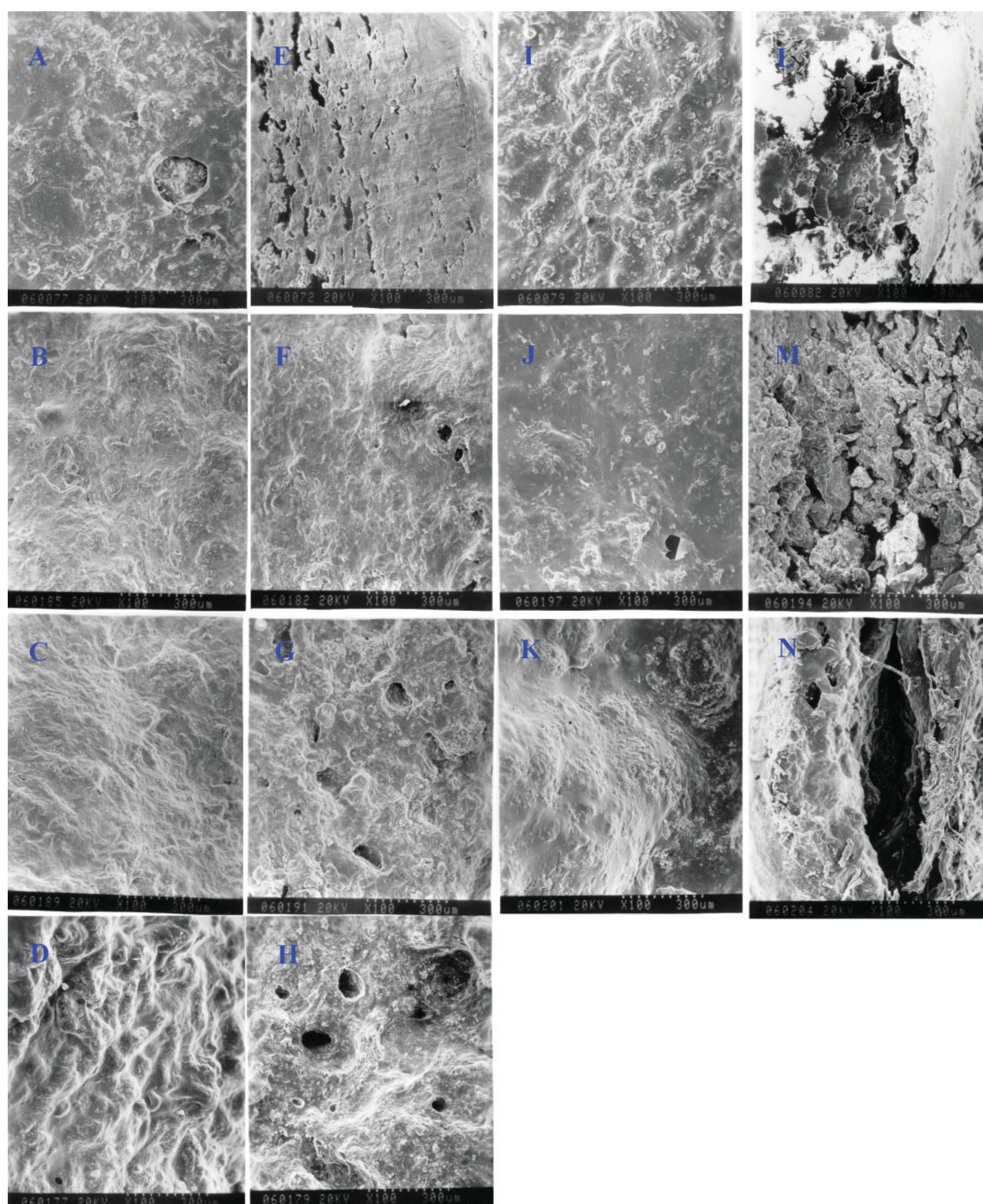

Figure 3 SEM photographs of pectin/$CaCl_2$ [surface view: (A) 0.5, (B) 1, (C) 2, and (D) 4 h; cross-sectional view: (E) 0.5, (F) 1, (G) 2, and (H) 4 h] and pectin [surface view: (I) 0.5, (J) 1, and (K) 2 h; cross-sectional view: (L) 0.5, (M) 1, and (N) 2 h] matrix tablets after a definite time in the release medium. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

mechanisms. Figure 5 shows the results of calcium-ion release from the matrix tablets prepared with pectin/$CaCl_2$ at 75/25 and 75/75 mg ratios. Calcium ions were released quickly from the pectin/$CaCl_2$ matrix tablets. The matrix tablet containing less calcium (75/25) released about 90% total calcium at 2 h, whereas the release was 77% for the matrix containing more calcium (75/75). Although the 75/75 matrix contained three times more calcium than the 75/25 matrix, calcium release, expressed as percentage release, was not faster. This effect could not be fully explained by a crosslinking mechanism because the calcium release of the 75/75 matrix was similar or quicker than that of the 75/25 matrix if there was no extra stress exerted on the pectin matrix by excessive calcium.

In our previous study,[20] we assumed that the initial erosion percentage was due to the quick escape of $CaCl_2$ to the release medium, which was confirmed in this study. However, the erosion of the pectin in the matrix itself, which controlled drug release, decreased as the calcium level in the matrix increased.[21] The excessive calcium seemed to bring about extra stress on the pectin matrix and led to a

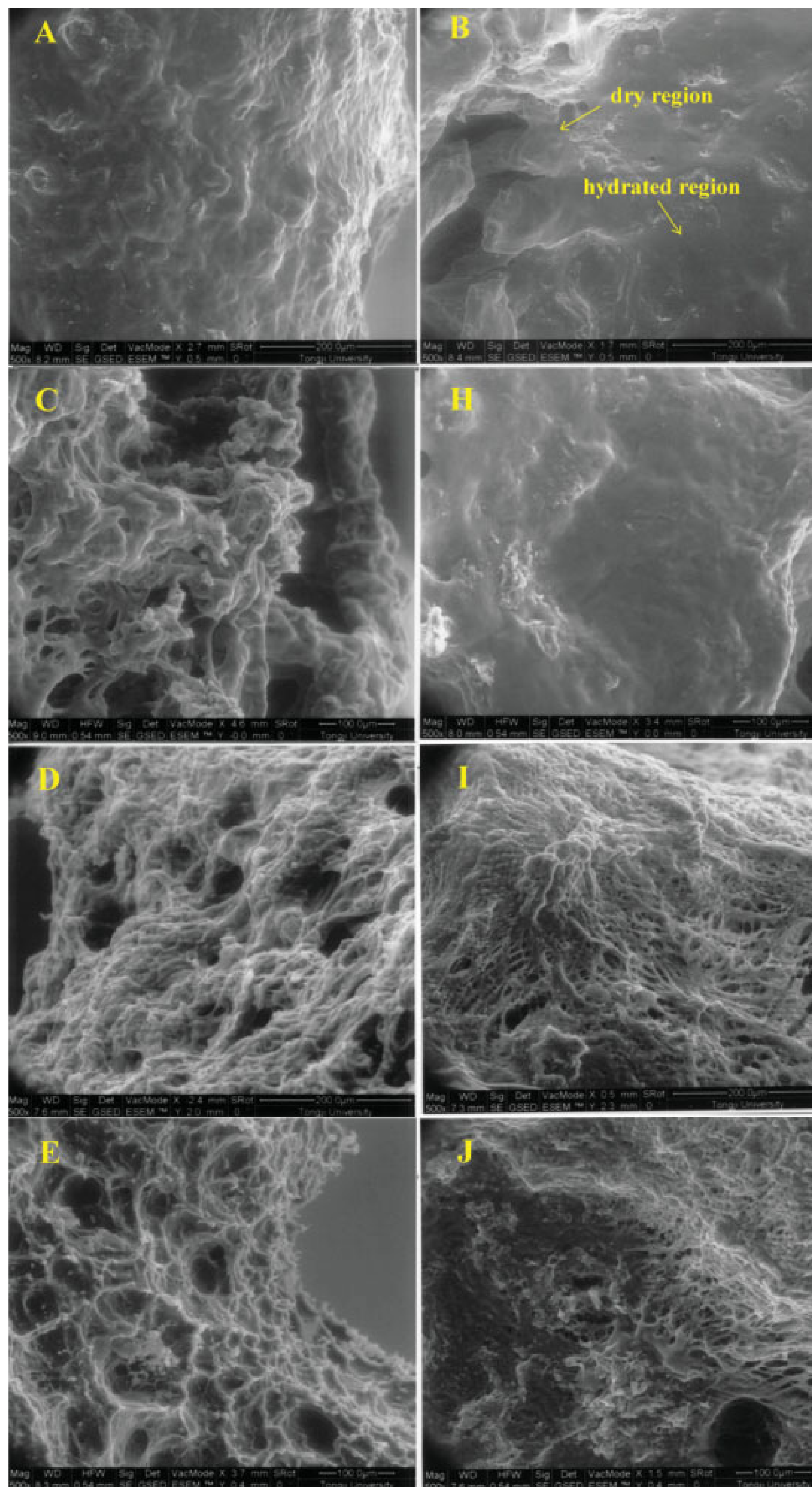

Figure 4 Cryo-SEM photographs of cross-sectional views of pectin [near the surface: (A) 1 h; near the center: (B) 1 h] and pectin/$CaCl_2$ [near the surface: (C) 0.5, (D) 1, (E) 2, (F) 3, and (G) 4 h; near the center: (H) 0.5, (I) 1, (J) 2, (K) 3, and (L) 4 h] matrix tablets after a definite time in the release medium. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

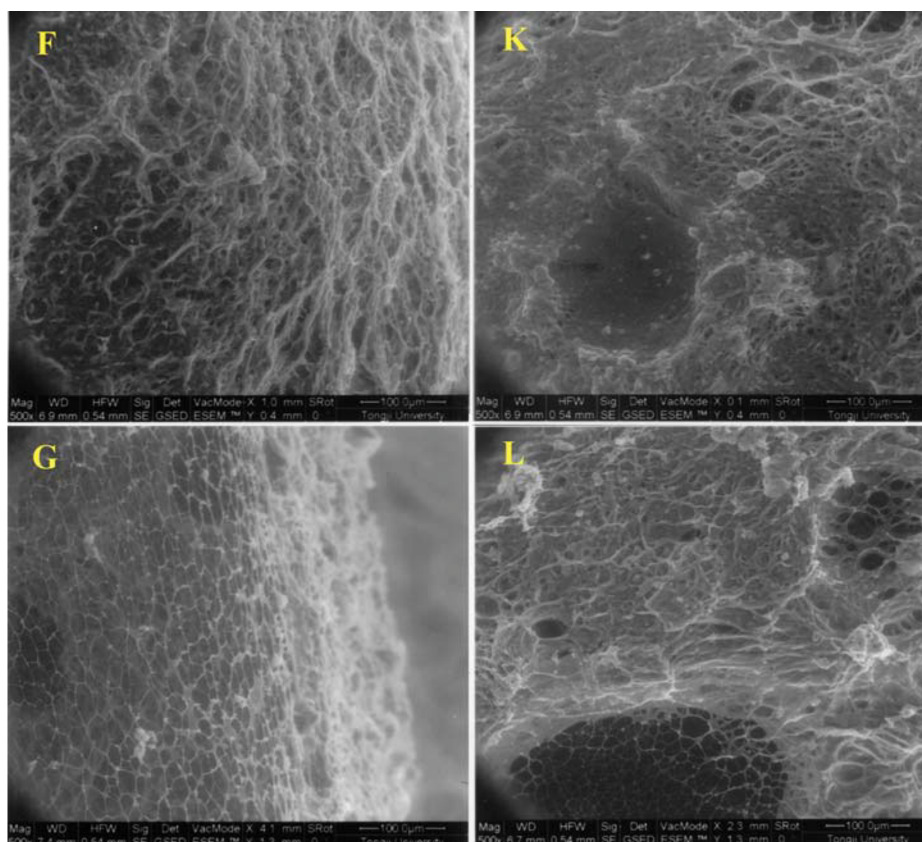

Figure 4 (*Continued from the previous page*)

more significant retardation of drug release, as we observed previously.[20]

Gel strength as a function of the calcium concentration

The gelation of low-methoxy pectin with the addition of Ca^{2+} has been well studied.[31,32] The affinity between calcium ions and pectin chains increases with decreasing average degree of methyl esterification of the pectic polysaccharide and increasing length of the unsubstituted galacturonan backbone. Although the affinity of high-methoxyl pectins for Ca^{2+} is not as high as that for low-methoxyl pectins, the gelation of high-methoxyl pectin on the addition of Ca^{2+} has also been reported.[10,33] It has been

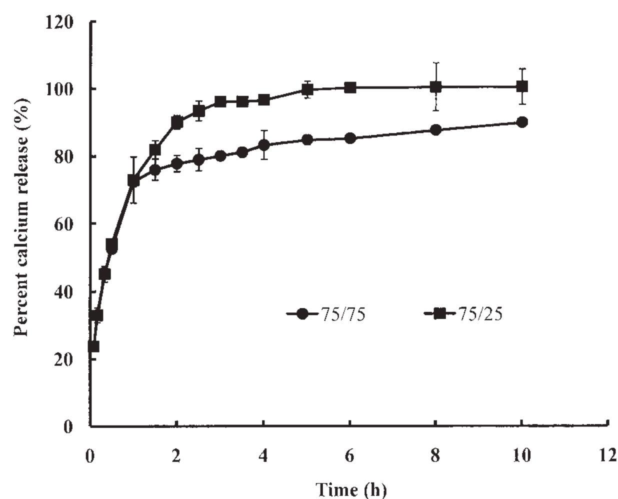

Figure 5 Release profile of calcium from pectin/$CaCl_2$ (75/75 or 75/25) matrix tablets (mean ± standard deviation, $n = 3$).

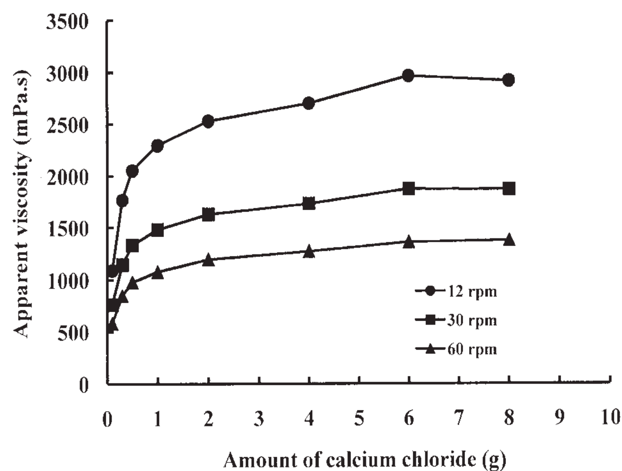

Figure 6 Apparent viscosity of pectin solutions determined at different revolution speeds as a function of added $CaCl_2$.

observed that, when the concentration of free calcium ions decreases, the dissociation of calcium crosslinks will occur and will result in the significant swelling and erosion of the pectin gel.[10]

Here, we studied the relationship between the gel strength of the pectin aqueous dispersion and calcium at extreme levels to simulate the pectin/$CaCl_2$ ratio in the matrix. The apparent viscosities of the 2% (w/v) pure pectin solution were 131.7 and 128.3 mPa s at 30 and 60 rpm, respectively. When $CaCl_2$ was added to the pectin solution, a significant increase in the apparent viscosity was observed. Figure 6 shows the relationship between the amount of $CaCl_2$ added and the apparent viscosity determined at different shear rates. Even at a $CaCl_2$ levels as low as 0.1 g, the apparent viscosity increased remarkably to 1088, 763, and 581 mPa s at shear rates of 12, 30, and 60 rpm, respectively. The curve was typically biphasic. Within the range 0.1–0.5 g of $CaCl_2$ addition, the apparent viscosity increased sharply, whereas a gradual increase was observed within the range 0.5–8.0 g. There seemed to be a turning point at about 0.5 g of $CaCl_2$ addition.

The results of this investigation provide useful information on the effect of excessive $CaCl_2$ on the pectin gel. We assumed that the sharp increase was a result of the calcium-induced crosslinking of the pectin chains. When $CaCl_2$ was added at an amount of 0.5 g (pectin/$CaCl_2$ = 75/9.4), the pectin seemed to be saturated by the calcium ions. At $CaCl_2$ levels over 0.5 g, the apparent viscosity kept increasing but at a slower speed. The excessive $CaCl_2$ continued to strengthen the pectin gel. If we followed the apparent viscosity change from higher to lower calcium levels, a gradual decrease in viscosity in the initial stage, followed by a sharp decrease in the apparent viscosity, was observed. This situation correlated well with the sigmoidal release pattern of the *in situ* crosslinking pectin/calcium matrix system.

DISCUSSION

The result of MB adsorption confirmed the *in situ* crosslinking of the pectin chains by calcium ions. The large fraction of $CaCl_2$ in the matrix led to significant physical changes in the morphology of the pectin matrices, as indicated by visual, SEM, and cryo-SEM observations. The physicochemical characterization also indicated that the calcium in the pectin matrix performed biphasically. Here, the total amount of calcium in the pectin matrix could be divided into two parts, that is, the part for crosslinking and the part above the crosslinking saturation level, or the excessive calcium part. Evidence showed that the excessive calcium in the pectin matrix exerted extra stress on the pectin gel, which was temporarily specified as the salt effect. The following points are discussed to support this assumption.

First, the excessive calcium has been proven to work as a retardation factor on indomethacin release in previous studies.[20,21] Although it was difficult to calculate the molar equivalent of calcium needed to saturate pectin, a pectin/$CaCl_2$ ratio of 75/25 seemed to be excessive[19] and resulted in the complete association of calcium with pectin. However, pectin/calcium matrices with less $CaCl_2$ failed to show release lag at the beginning stage. The extra calcium had a close relationship with the initial release lag.

Second, the excessive calcium strengthened the pectin gel. Although the observation with the pectin aqueous dispersion did not virtually reflect the *in situ* crosslinking matrix, some conclusions did provide useful information for interpreting the effect of excessive calcium. As observed in the apparent viscosity study, when a large amount of $CaCl_2$ was added to the dispersion, there was a gradual increase in the pectin gel strength. It could not be simply explained by calcium-induced crosslinking, and the properties of $CaCl_2$ as an electrolyte may have contributed to this effect. Similarly, the observation with the pectin aqueous dispersion may have been extrapolated to the *in situ* crosslinking matrix system. However, it could be explained inversely by descending calcium concentration. Upon contact with water, the pectin/calcium matrix hydrated and formed a gel. In the gel layer, pectin was in a state of calcium-induced crosslinking, whereas indomethacin was in an undissolved state. The calcium in the pectin gel was at a level higher than crosslinking saturation. Although calcium ions continued to release into the surrounding medium, calcium inside continued to dissolve and compensate to keep the status of high calcium concentration over saturation until the right point of calcium saturation without excessive calcium. As we learned from simulating the pectin dispersion system, this was crucial in keeping the pectin gel in a stable strengthened status, which was directly related to pectin matrix erosion and drug release. A small decrease in calcium over the saturation level led to minor relaxations of the salt stress and the pectin gel strength. The duration of maintenance calcium above the crosslinking saturation level was directly related to the initial lag release of indomethacin from the pectin/calcium matrix tablet. In another words, the time delay of the sigmoidal release profile was determined by how long the calcium in the pectin matrix could be maintained over the crosslinking saturation level. Calcium ions kept releasing quickly from the pectin matrix (Fig. 5) and reached a level of crosslinking saturation without excessive calcium, which was a turning point when a continuous decrease in calcium level led to a relaxation of the crosslinking degree and resulted in a significantly reduced pectin gel strength.

In the drug-release profile, this point corresponded to the turning point from lagged release at the initial stage to the accelerated release stage. The fraction of calcium to achieve crosslinking and that to keep the oversaturation status together achieved the sigmoidal drug-release profile.

Third, the observation with the pectin matrix containing NaCl provided an interesting comparison with excessive calcium. To highlight the effect of $CaCl_2$, we studied a pectin matrix containing NaCl as counterevidence. As pectin could only be crosslinked by multivalent cations, sodium ions did not seem to have the ability to crosslink pectin. As expected, NaCl failed to show a similar effect to $CaCl_2$ on the pectin matrix. However, we obtained new findings with the pectin/NaCl matrix. As pointed out in the previous study,[20] the pectin matrix containing NaCl somehow also showed the retardation of indomethacin release but to a limited degree in the beginning hour, which was contrary to our expectation that it would only function as a tunneling agent. The MB adsorption study also indicated that the matrix containing NaCl had the ability to resist MB binding but to a limited degree. What was the effect of NaCl on the pectin matrix? The crosslinking of pectin by sodium ions was not the answer. Some properties of the NaCl salt itself may have contributed to this effect. The excessive $CaCl_2$ possibly functioned similarly to NaCl.

Although the underlying mechanisms of the salt effect still await elucidation, we tried to use this theory to provide a reasonable interpretation of the sigmoidal release characteristics. The calcium-induced crosslinking was basic and resulted in drug-release retardation, whereas the excessive calcium helped to maintain this action for several hours. The sigmoidal release profile could be divided into two distinct phases that correlated directly to the dynamic variation of the calcium level in the matrix. In the first stage, calcium was over the crosslinking saturation level, and a decrease in the calcium concentration only led to the relaxation of the salt stress, which only resulted in a slight weakening of the gel strength. That is why we observed a much slower drug-release rate at the beginning. The duration of this phase depended on the time for which the calcium could be maintained over the crosslinking saturation level. In the second stage, when the calcium level dropped below the saturation point, calcium release caused the relaxation of crosslinking,[20] which led to a sharp decrease in the gel strength and, therefore, accelerated matrix erosion and drug release.

CONCLUSIONS

Calcium-induced crosslinking of pectin chains led to a variety of changes in the physicochemical properties of the pectin/$CaCl_2$ matrix tablet. The most obvious changes were in the surface and inner morphologies of the hydrated tablet. The matrices containing calcium showed reduced hydration and erosion rates compared with pure pectin matrices. The matrix containing only a small amount of calcium (pectin/$CaCl_2$ = 75/25) showed complete shielding of MB adsorption as indicated by the saturation of the pectin chains by calcium cations. The excessive calcium salt exerted additional retardation on the matrix behavior, which was assumed to be the salt effect. Calcium-induced crosslinking was the basis for the retardation of drug release, whereas the excessive calcium managed to maintain this crosslinking saturation status for a definite duration. The calcium release at the initial stages only resulted in a gradual reduction in the matrix performance, and a slower release rate was observed consequently. At later stages, the calcium level dropped below saturation, and a significant reduction in the pectin gel was assumed, which resulted in accelerated drug release.

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