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Effect of Degree of Esterification of Pectin and Calcium Amount on Drug Release from Pectin-Based Matrix Tablets

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ABSTRACT

The aim of this work was to assess the effect of 2 formulation variables, the pectin type (with different degrees of esterification [DEs]) and the amount of calcium, on drug release from pectin-based matrix tablets. Pectin matrix tablets were prepared by blending indomethacin (a model drug), pectin powder, and various amounts of calcium acetate and then tableting by automatic hydraulic press machine. Differential scanning calorimetry, powder x-ray diffraction, and Fourier transformed-infrared spectroscopy studies of the compressed tablets revealed no drug-polymer interaction and the existence of drug with low crystallinity. The in-vitro release studies in phosphate buffer (*United States Pharmacopeia*) and tris buffer indicated that the lower the DE, the greater the time for 50% of drug release (T_{50}). This finding is probably because of the increased binding capacity of pectin to calcium. However, when the calcium was excluded, the pectins with different DEs showed similar release pattern with insignificant difference of T_{50}. When the amount of calcium acetate was increased from 0 to 12 mg/tablet, the drug release was significantly slower. However, a large amount of added calcium (ie, 24 mg/tablet) produced greater drug release because of the partial disintegration of tablets. The results were more pronounced in phosphate buffer, where the phosphate ions induced the precipitation of calcium phosphate. In conclusion, both pectin type and added calcium affect the drug release from the pectin-based matrix tablets.

KEYWORDS: pectin, hydrogel matrices, matrix tablets, controlled-release, sustained-release, indomethacin

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INTRODUCTION

Hydrophilic polymer matrix systems are widely used in oral controlled drug delivery because of their flexibility to obtain a desirable drug release profile, cost-effectiveness, and broad regulatory acceptance.[1-3] The ability of the hydrophilic polymer matrices to release an entrapped drug in aqueous medium and to regulate the release of such drug by control of swelling and cross-linking makes them particularly suitable for controlled-release applications.[2] These matrices can be applied for the release of both hydrophilic and hydrophobic drugs and charged solutes. Recently, many controlled-release formulations based on hydrophilic polymer matrices have been developed.[3-6]

Pectins are hydrophilic polysaccharides derived from plant cell walls. They contain linear chains of (1→4) linked α-D-galacturonic acid residues.[7] These uronic acids have carboxyl groups, some of which are naturally presented as methyl esters. The degree of esterification (DE), which is expressed as a percentage of the esterified carboxyl groups, is an important means to classify pectins. High methoxy (HM) pectins (with DE >50%) require a relatively high concentration of soluble solids and a low pH for gel formation.[7-8] Low methoxy (LM) pectins (with DE <50%) form rigid gels by the action of calcium or multivalent cations, which cross-link the galacturonic acid chains.[7] The nontoxicity and the low production costs of pectins make them of great interest for the formulation of controlled-release dosage forms.[4-6]

In a previous study,[6] the effects of compression force, ratio of drug to pectin, and grade of HM pectin on drug release from matrix tablets were investigated. The drug release from the matrix tablets could be modified by grade of HM pectin and ratio of drug to pectin. DE, which is an important characteristic of pectin and may influence the drug release from the system, has not yet been examined. Therefore, in the present study, formulations containing indomethacin, either high or low methoxy pectins, alone or in combination with

Table 1. Composition of Pectin-Based Matrix Tablets*

Formulation	IMC (mg)	GP72 (mg)	GP36 (mg)	GP28 (mg)	Calcium acetate (mg)
GP72	75	600	-	-	0
GP72 Ca6	75	600	-	-	6
GP72 Ca12	75	600	-	-	12
GP72 Ca24	75	600	-	-	24
GP36	75	-	600	-	0
GP36 Ca6	75	-	600	-	6
GP36 Ca12	75	-	600	-	12
GP36 Ca24	75	-	600	-	24
GP28	75	-	-	600	0
GP28 Ca6	75	-	-	600	6
GP28 Ca12	75	-	-	600	12
GP28 Ca24	75	-	-	600	24

*IMC indicates indomethacin.

calcium acetate, were prepared and tested. The presence of calcium acetate was in relation to the importance of calcium ions to the gelling mechanism of pectin. The effects of the calcium amount and type of medium on drug release from matrix tablets were also investigated.

MATERIALS AND METHODS

Materials

HM pectin with DE of 72% (GENU pectin type B) and LM pectins with DE of 36% (GENU pectin type LM101 AS) and 28% (GENU pectin type LM104 AS-FS) were obtained from CP Kelco (Lille Skensved, Denmark) and are referred to as GP72, GP36, and GP28, respectively. Indomethacin (IMC), calcium acetate, and tris (hydroxymethyl) aminomethane buffer (Sigma 7-9, referred to as tris buffer) were purchased from Sigma Chemical (St Louis, MO). All other materials were used as supplied without further purification.

Preparation of Pectin-Based Matrix Tablets

Matrix tablets were prepared using 1:8 drug to polymer ratio. Each tablet contained 75 mg of indomethacin; 600 mg of pectins with different DEs (GP72, GP36, and GP28); and 0, 6, 12, and 24 mg of calcium acetate. All ingredients were passed through a 60-mesh sieve and thoroughly mixed in a blender for 15 minutes. The blend was compressed into tablet on an automatic hydraulic press (model 2I-15710, Perkin Elmer, Wellesley, MA) with 13-mm diameter flat-faced tooling. The tablets were compressed at a compression force of 80 kN. The summary of the formulations is shown in Table 1.

Physicochemical Characterization

Physical mixtures containing 1:8 drug to pectin ratio were prepared by mixing with vortex mixer for 5 minutes. The matrix tablets containing 75 mg of IMC and 600 mg of pectins were powdered to obtain suitable samples for physicochemical characterizations. Both physical mixtures and tablets were characterized in comparison with intact IMC and pectins.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) thermograms were obtained on a Du Pont 910 differential scanning calorimeter connected to a DuPont 9900 computer/thermal analyzer (TA Instruments, Delaware, DE). Approximately 2 mg of samples was sealed in the aluminum pan, and measurement was performed at a heating rate of 10°C/min. The temperature was calibrated with pure indium, with a melting point of 156.60°C. An empty pan was used as a reference.

Powder X-Ray Diffractometry

Powder x-ray diffraction (PXRD) was performed on a Rigaku Denki 2027 diffractometer (Rigaku Denki Co, Tokyo, Japan) using a scintillation counter. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 30 kV; current, 5 mA; time constant, 0.5 seconds; scanning speed, 4 degree/min; and count range, 2000 cps.

Fourier Transformed Infrared Spectroscopy

Infrared (IR) spectra were obtained by KBr disk method using computer-mediated Fourier transformed infrared spectroscopy (FTIR) (Nicolet 5ZDX, Middleton, WI). Each

Table 2. Summary of Factors for the 3 × 4 × 2 Statistical Factorial Design*

Factor	Level 1	Level 2	Level 3	Level 4
Degree of esterification (DE) of pectin	72%	36%	28%	NA
Calcium amount added (mg/tablet)	0	6	12	24
Dissolution medium	pH 6.2 phosphate buffer	pH 7.4 tris buffer	NA	NA

*NA indicates not applicable.

sample was mixed with KBr powder and then pressed by a hydrostatic press at a pressure of 5 ton/cm^2 for 5 minutes.

Matrix Tablet Evaluations

Tablet Thickness Testing

The thickness of the matrix tablets was determined using a Mitutoyo caliper (model 7301, Mitutoyo, Japan), and the results were expressed as mean values of 10 determinations.

Tablet Weight Variation Testing

In order to determine batch to batch variations, 20 matrix tablets were selected and accurately weighed using a Mettler analytical balance (Griefensee, Switzerland). The results were expressed as mean values of 20 determinations.

Hardness Determination

Ten matrix tablets were sampled and individually subjected to test for hardness using Stoke-Monsanto hardness tester (St Louis, MO). The tablet hardness was expressed in kilograms. The mean and standard deviation of the tablet hardness were calculated.

Friability Test

Tablet friability test was performed on 20 tablets at 25 rpm for 4 minutes using Erweka Abrasion Tester (Heusenstamm, Germany). The percentage of friability was calculated based on the weight lost after the test.

In Vitro Release Studies

To examine the effects of the DE of pectin and calcium amount on drug release, the dissolution studies were performed using *United States Pharmacopeia (USP)* dissolution apparatus I equipped with baskets, which was operated at the speed of 75 rpm. Nine hundred milliliters of either phosphate buffer *USP* (pH 6.2) or tris buffer (pH 7.4), as the dissolution medium, were placed in the glass vessel, the apparatus was assembled, and the dissolution medium was equilibrated to 37 ºC. The amount of drug release was measured at the suitable time interval and was then determined spectrophotometrically (model DU 605i, Beckman Instrument, Fullerton, CA) in a 1-cm cell at 318 nm. Each in-vitro release study was performed in triplicate.

Experimental Design and Statistical Analysis

The effect of the DE of pectin, calcium amount added in the formulation and dissolution medium on drug release behavior were investigated using a 3 × 4 × 2 complete factorial design. This design is shown in Table 2. The 3 factors tested were 3 levels of DE, 4 levels of calcium amount added, and 2 types of dissolution medium. Analysis of variance (ANOVA) and Levene's test for homogeneity of variance were performed using SPSS version 10.0 for Windows (SPSS Inc, Chicago, IL). Post hoc testing ($P < .05$) 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.

RESULTS AND DISCUSSION

Physical Properties of Pectin-Based Matrix Tablets

The comparison of the physical properties of the matrix tablets containing HM or LM pectins, alone or combination with different levels of calcium acetate, is shown in Table 3. The hardness, thickness, weight, and friability of the formulations ranged from 9.8 to 11.1 kg, 3.73 to 3.91 mm, 666.2 to 700.1 mg, and 0.37% to 0.93%, respectively.

Physicochemical Properties of Pectin-Based Matrix Tablets

The PXRD patterns of IMC, Pectin GP72, physical mixture of IMC and GP72, and tablet containing IMC and GP72 are shown in Figure 1. The results demonstrated that the IMC used was of γ-form.[9-10] The PXRD patterns of both the physical mixture and the tablet showed characteristic diffraction peaks of both IMC and pectin, indicating no polymorphic transformation. In the tablet, the intensity of diffraction peaks attributed to IMC considerably decreased, compared with that of intact IMC or physical mixture, demonstrating lower crystallinity of IMC in the tablet.

Table 3. Physical Properties of Pectin-Based Matrix Tablets*

Formulation	Physical Characteristics			
	Hardness (kg), n = 10	Thickness (mm), n = 10	Weight (mg), n = 20	Friability (%)
GP72	9.8 (0.6)	3.82 (0.01)	666.2 (2.8)	0.77
GP72 Ca6	10.3 (0.3)	3.82 (0.01)	673.6 (6.6)	0.93
GP72 Ca12	10.6 (0.4)	3.82 (0.01)	679.6 (13.5)	0.82
GP72 Ca24	10.5 (0.8)	3.91 (0.01)	692.6 (0.5)	0.91
GP36	11.2 (0.5)	3.73 (0.01)	673.2 (4.9)	0.39
GP36 Ca6	10.2 (0.3)	3.77 (0.01)	672.3 (4.7)	0.44
GP36 Ca12	11.1 (0.7)	3.80 (0.05)	679.8 (14.2)	0.58
GP36 Ca24	10.5 (0.1)	3.81 (0.01)	700.1 (0.8)	0.41
GP28	10.0 (0.4)	3.73 (0.01)	671.0 (10.2)	0.37
GP28 Ca6	10.2 (0.3)	3.73 (0.02)	672.2 (5.1)	0.65
GP28 Ca12	10.2 (0.3)	3.73 (0.01)	673.2 (9.1)	0.55
GP28 Ca24	10.2 (0.2)	3.81 (0.01)	697.5 (4.6)	0.43

*Values in parentheses represent SD.

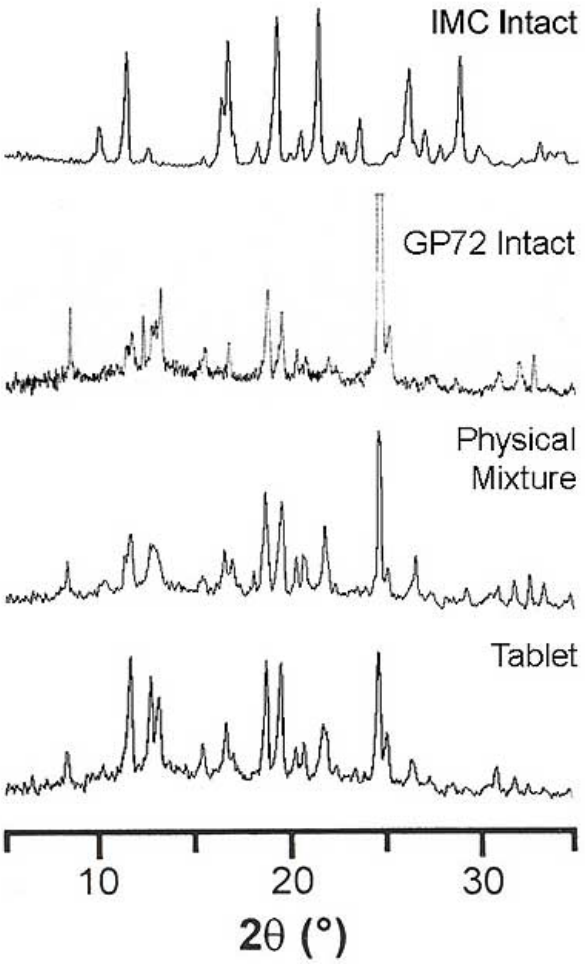

Figure 1. PXRD patterns of indomethacin (IMC), pectin (GP72), physical mixture of IMC and GP72, and tablet containing IMC and GP72.

The DSC thermograms of IMC, Pectin GP72, physical mixture of IMC and GP72, and the tablet containing IMC and GP72 are shown in Figure 2. The DSC curves of intact IMC demonstrated the melting point at 162.0°C. The thermograms of physical mixture and tablet showed melting peaks of IMC at 158.5°C and 158.0°C, respectively, and broad endothermic peaks around 180°C to 230°C. The thermograms of tablets containing GP36 or GP28 showed similar results to those of tablets containing GP72. As the polymorphic transformation was not observed in PXRD study, the lower melting point of IMC in both the physical mixture and the tablet was not attributable to the conversion of γ-form (melting point; 160°C-161.5°C) to β-form (melting point; 158°C-160.5°C). The pectins used consisted of polymer and sucrose as a gelling agent. Therefore, the lower melting point was probably due to the change in melting behavior of IMC in an environment of polymer and added substance while DSC measurement took place.

Figure 3 shows the IR spectra of IMC, Pectin GP72, physical mixture of IMC and GP72, and the tablet containing IMC and GP72. In the carbonyl frequency region, IMC showed strong peaks at 1716 and 1691 cm-1, which were attributed to carboxylic C = O stretching. Similar spectra that consisted of peaks corresponding to IMC and pectin were obtained in physical mixture and tablet. The results demonstrated no molecular interaction between pectin and IMC.

Effect of DE of Pectin on Drug Release

HM pectin (GP72) and LM pectin (GP36 and GP28) were used as a hydrophilic polymer material in the matrix tablets. The matrix tablets containing GP36 and GP28 were expected to release the drug faster than those containing

GP72, according to its higher hydrophilicity and solubility resulting from the larger number of ionized carboxyl groups.[11] Unexpected, in phosphate buffer, the effect of DE of pectin on drug release from matrix tablets without calcium acetate was not observed (Figure 4). This finding is probably due to the lack of gel formation of HM and LM pectins in phosphate buffer, resulting in comparable drug release from the matrix tablets containing pectins with different DEs. HM pectin requires a relative low pH for gel formation, while LM pectin requires the presence of divalent cations.[7]

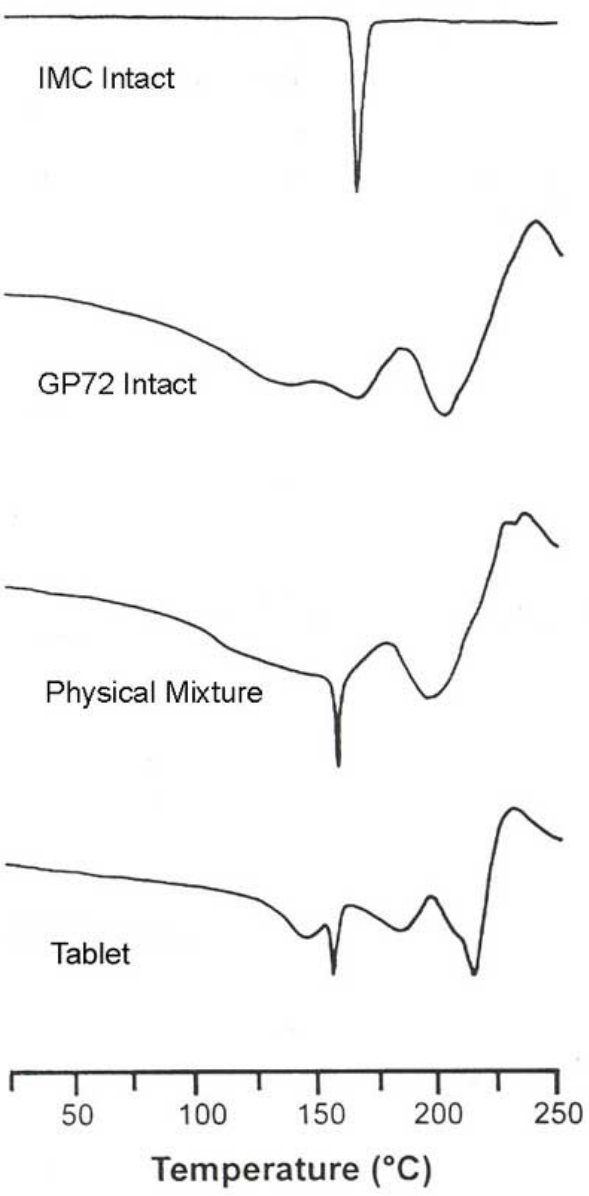

Figure 2. DSC thermograms of indomethacin (IMC), pectin (GP72), physical mixture of IMC and GP72, and tablet containing IMC and GP72.

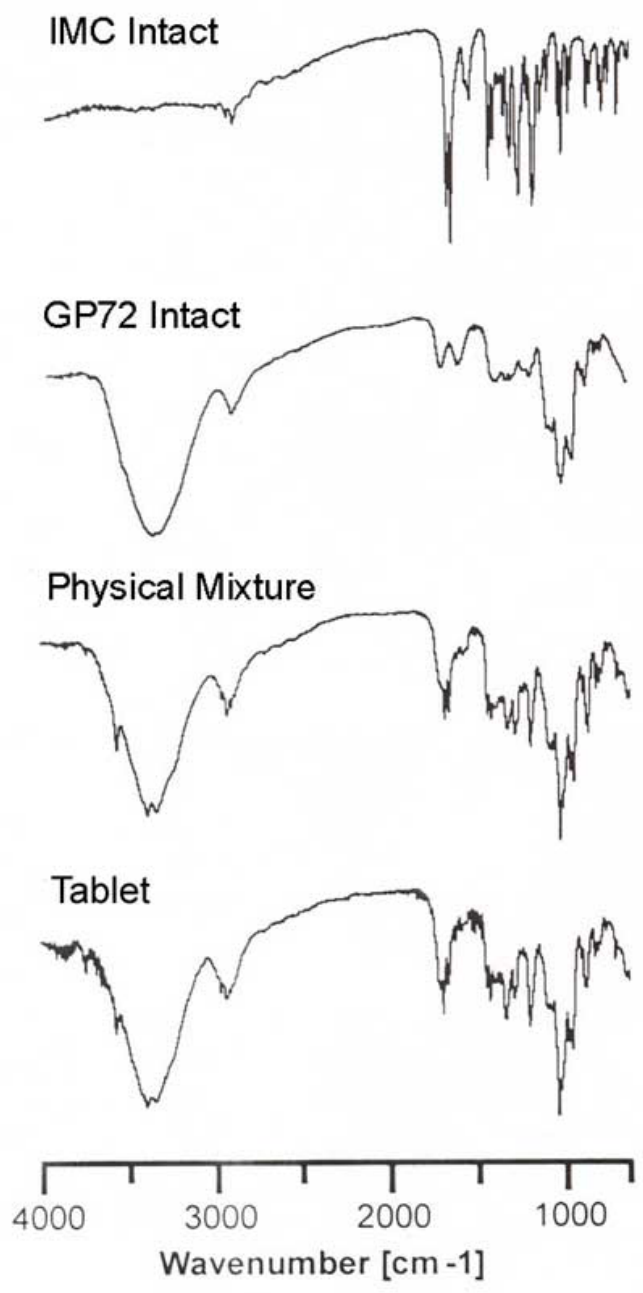

Figure 3. IR spectra of indomethacin (IMC), pectin (GP72), physical mixture of IMC and GP72, and tablet containing IMC and GP72.

Of interest, the drug release was dramatically decreased in the pectin-based matrix tablets containing calcium acetate (Figure 4). The effect of the addition of calcium acetate on drug release was more pronounced with decreased DE of pectin. The possible explanation is that LM pectin could form rigid gel with calcium ions[7] during the dissolution test. Water penetrates into matrix tablets and dissolves calcium acetate. Further, the dissolved calcium ions interact with pectin, thus forming a calcium-pectinate gel matrix. Therefore, the slower drug release in

the tablet with calcium acetate resulted from the retention of the drug in the calcium-pectinate gel matrix. This result was consistent to the previous studies.[12-13] The drug release from the matrix tablets containing GP28 was more retarded than those containing GP36. The lower DE caused the higher sensitivity to calcium ions.[7,14] Surprising, the addition of calcium acetate also retarded drug release from matrix tablets containing HM pectin; the retardation effect was, however, less extent than for those containing LM pectin. Pectin is generally able to form strong gel in the presence of calcium ions, although pectin with a high DE disfavors the interaction between the carboxyl groups and the calcium ions. The free carboxyl groups in the HM pectin molecule are distributed blockwise, therefore the gelation of this pectin is possible because of the "egg-box" structure, resulting from chelation of calcium ions in electronegative cavities formed by the carboxyl residues and hydroxyl groups.[15] So, the effect of the addition of calcium acetate on drug release from matrix tablets containing HM pectin could be observed.

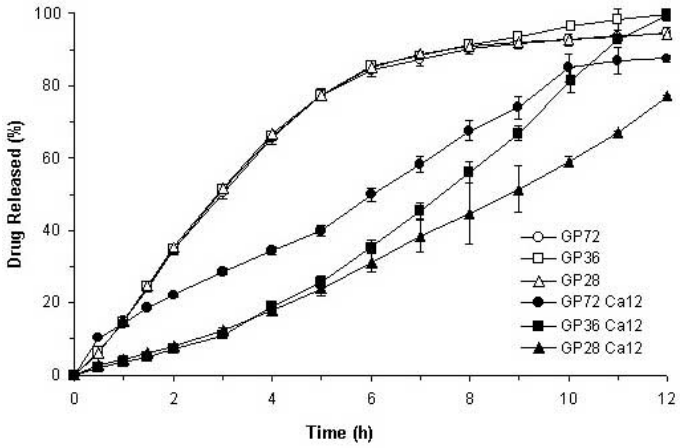

Figure 4. The effect of degree of esterification of pectin on indomethacin release from pectin-based matrix tablets. Error bars indicate SD; n = 3.

From the release profiles, the time (in minutes) to achieve release of 50% of the payload was measured and designated as T_{50}. The comparison of the T_{50} (in phosphate buffer and tris buffer) from matrix tablets containing different pectins is shown in Figure 5. When no calcium was added, the matrix tablets containing different pectins showed no significant difference in T_{50}. The longer T_{50} was observed in the matrix tablets containing pectin with lower DE at any level of added calcium acetate.

Effect of Calcium Amount on Drug Release

Besides DE of pectin, the effect of calcium amount on drug release from the matrix tablets was also investigated, as shown in Figure 6. Forming of calcium-pectinate gel reduces the solubility of pectin, especially LM pectin. Calcium forms cross-links between 2 pectin molecules in a section of the chain, which is free from methoxyl groups. Increasing the calcium acetate content in the tablets from 6 to 12 mg/tablet resulted in a reduced release rate (Figure 6) and longer T_{50} (Figure 5) . Similar results have previously been reported.[13,16] Increasing the amount of calcium leads to a greater degree of cross-linking and aggregation of the initial dimers giving higher gel strength and results in the slower drug release pattern.[16] However, a large amount of added calcium (24 mg/tablet) produced a greater drug release (Figure 6) and shorter T_{50} (Figure 5). This result can be explained by the influence of calcium on the gel formation. The gel strength increases with the addition of calcium up to a critical concentration.[12] Above this concentration, the gel strength weakens. This weakening is caused by excessive cross-linking by the calcium and hence formation of a nonhomogenous gel matrix. The matrix tablets containing a high amount of calcium acetate (24 mg/tablet) did not remain intact during the experiment. The tablet disintegrated partially. The larger surface area created will partly explained the faster drug release.

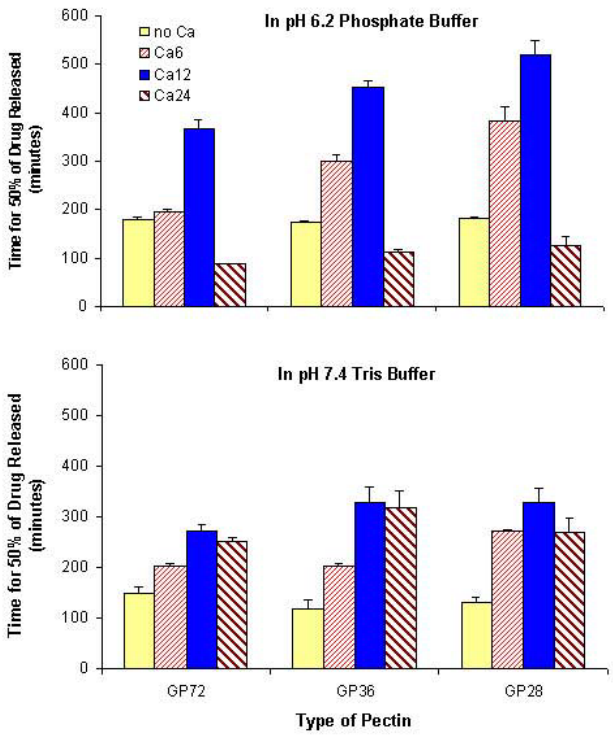

Figure 5. Comparison of time for 50% of drug released from pectin-based matrix tablets in pH 6.2 phosphate buffer and pH 7.4 tris buffer. Error bars indicate SD; n = 3.

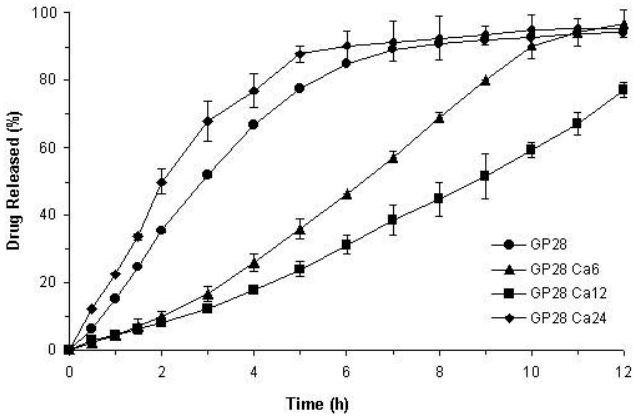

Figure 6. The effect of amount of calcium acetate added on indomethacin release from pectin-based matrix tablets. Error bars indicate SD; n = 3.

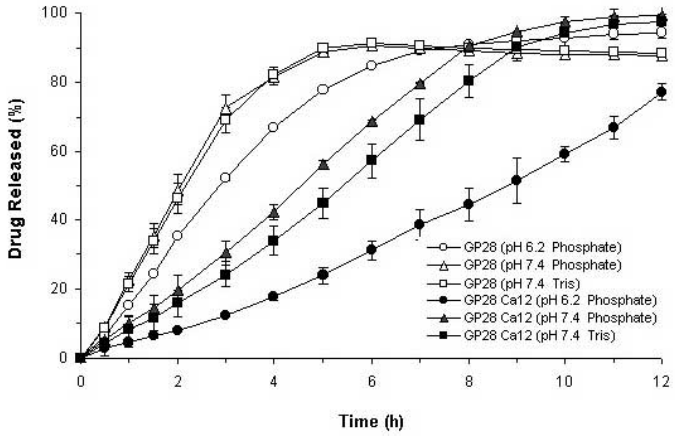

Figure 7. The effect of release medium on indomethacin release from pectin-based matrix tablets. Error bars indicate SD; n = 3.

Effect of Dissolution Medium on Drug Release

Figure 7 shows that the drug release from matrix tablets containing pectin (GP28) alone or combination with calcium acetate in pH 6.2 phosphate buffer was slower than that in pH 7.4 tris buffer or pH 7.4 phosphate buffer. Since, indomethacin is a weak acidic drug,[9] its solubility may increase with increasing pH. Thus, the higher drug release of pectin-based matrix tablets in pH 7.4 buffer is not surprising.

It can also be seen in Figure 7 that, in case of matrix tablets containing pectin alone, there was no significant difference between drug release in pH 7.4 phosphate buffer and pH 7.4 tris buffer. Drug release from matrix tablets containing 12 mg calcium, however, demonstrated faster release in pH 7.4 phosphate buffer than in pH 7.4 tris buffer. A possible explanation is the calcium ions in the matrix tablets were precipitated by phosphate ions (PO_4^{3-}) in phosphate buffer. The available residual calcium ions for gel formation with pectin decreased and were not enough for complete formation of calcium-pectinate gel. In contrast, there were no competitive ions to form salts with calcium ions in tris buffer. Therefore, there are more available calcium ions for the gel formation of calcium pectinate in tris buffer. It should be noted, however, that phosphate ions are essentially nonexistent in human intestinal fluids.[17] Therefore, using phosphate buffer as a medium for release studies may not reasonable if calcium ions are presented in the designed system.

The T_{50} of pectin-based matrix tablets was shorter in tris buffer than in phosphate buffer (Figure 5) according to the higher solubility of indomethacin in higher pH medium. It was noted that T_{50} of pectin-based matrix tablets decreased dramatically with increasing calcium acetate from 12 to 24 mg/tablet in phosphate buffer. This decreasing was slightly observed in tris buffer (Figure 5). This finding is probably because phosphate ions induced the precipitation of calcium phosphate as mentioned in the above paragraph. Since the pectin-based matrix tablets with a large amount of calcium acetate (24 mg/tablet) disintegrated partially, calcium phosphate could form more easily.

Statistical Analysis

The ANOVA results for T_{50} are presented in Table 4. The analysis of T_{50} revealed that the DE of pectin, calcium amount added in the formulation and dissolution medium statistically significantly influenced the drug release. Interactions between all factors were also relevant. The different DEs of pectin showed different T_{50} when 12 mg calcium was added but, at the same time, were not different when no calcium was added (Figure 5). These interactions also explain why the T_{50} of tablets containing calcium acetate of 24 mg was shorter than the T_{50} of tablets containing calcium of 12 mg and simultaneously longer than that of tablets with no added calcium, as demonstrated in Figure 4.

CONCLUSION

Changing DE of pectin or calcium amount in the pectin-based matrix tablet formulations could modify drug releases from the formulations. The results from this study enable us to state that the matrix tablets containing pectin are an interesting way of formulating oral controlled-

Table 4. Analysis of Variance Results for the Time for 50% of Drug Released*

Source	F Value	*P* Value
DE of pectin	95.239	< .001
Calcium amount	682.906	< .001
Medium	32.383	< .001
DE of pectin × calcium amount	28.859	< .001
DE of pectin × medium	24.776	< .001
Calcium amount × medium	308.799	< .001
DE of pectin × calcium amount × medium	8.537	< .001

*Alpha = 0.05. DE indicates degree of esterification.

release matrix tablets. The manufacturing processes are easy, inexpensive, and do not require special production equipment. It is, therefore, possible to achieve a firmer basis of their use.

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