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Oral delayed-release system based on Zn-pectinate gel (ZPG) microparticles as an alternative carrier to calcium pectinate beads for colonic drug delivery

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Abstract

A new oral timed-release system was developed for colon-targeted delivery of drugs. The system which consists of ketoprofen-loaded Zn-pectinate gel (ZPG) microparticles together with pectin/dextran mixtures in a tablet form, has been investigated, in vitro, using conditions chosen to simulate the pH and times likely to be encountered during transit to the colon. In order to find the suitable ZPG microparticles, the formulations were prepared by utilizing 2^3 factorial design and the effect of various formulation factors on the release and surface characteristics of the microparticles was studied. The results obtained implied that the release of ketoprofen from ZPG microparticles was greatly extended with the pectinate microparticles, which were prepared with 2.5 or 3% w/v pectin, 2.75% w/v $Zn(CH_3COO)_2$ and 2.5% w/v drug. Additionally, the analysis of variance results showed that the release of ketoprofen in simulated intestinal fluid (S.I.F., pH 7.4) was strongly affected by crosslinking agent concentration and initial drug amount, but not particularly affected by the amount of pectin added. The investigated drug concentration factor has significantly increased the drug entrapment efficiency (EE). The optimum colonic drug delivery ZPG/tablet system provided the expected delayed-release sigmoidal patterns with a lag-time of 4.125–4.85 h and $t_{50\%}$ (the time for 50% of the drug to be released) at 7.45–8.70 h, depending on pectin/dextran ratio employed. The results also demonstrated that the untableted ZPG microparticles exhibited drug release profiles which were able to retard the release of ketoprofen in S.I.F. (pH 7.4) to be 5.28–37.82 times (depending on formulation parameters), lower than the conventional calcium pectinate beads. Therefore, this approach suggests that ZPG microparticles and their modified-release formulations are promising as useful controlled-release carriers for colon-targeted delivery of drugs.

Keywords: Colonic-targeting; Pectinate; Microparticles; Matrix tablets; Ketoprofen; Factorial design

1. Introduction

A particular challenge in pharmaceutical field is development of site-specific dosage forms that are able to control time of delivery for the release of

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active ingredients in the lower part of the small intestine, or in the colon. In particular, such a preparation could be used as a carrier for the anti-inflammatory drugs employed to treat inflammatory bowel diseases (Ashford and Fell, 1994; Watts and Illum, 1997) and for the systemic absorption of orally administered polypeptides susceptible to enzymatic digestion in the upper gastrointestinal tract (Mackay and Thomlinson, 1993; Watts and Illum, 1997; Sriamornsak, 1999).

The approaches utilized in achieving colonic delivery of drugs include use of prodrugs (Tozer et al., 1991), pH-sensitive polymer coatings (Ashford et al., 1993; Marvola et al., 1999), time-dependent formulations (Gazzaniga et al., 1994, 1995), bacterial degradable coatings (Siew et al., 2000), time/pH-controlled deliveries (Ishibashi et al., 1998), and intestinal luminal pressure-controlled colon delivery capsules (PCDC) (Yoshikawa et al., 1999). In addition, the use of biodegradable polymers such as azo-polymers and polysaccharides (e.g. pectins and dextrans) for colon targeting has been described in the literature (Hovgaard and Brondsted, 1995; Watts and Illum, 1997). Though pectin is a heterogeneous polysaccharide, it contains linear chains of $(1\rightarrow4)$-linked α-D-galacturonic acid residues. These uronic acids have carboxyl groups, some of which are naturally presented as methyl esters and others which are reacted with ammonia to produce carboxamide groups. The degree of esterification (DE) and degree of amidation (DA), which are both expressed as a percentage of carboxyl groups (esterified or amidated), are important means to classify pectin (Rolin, 1993). The ability of amidated low methoxy (LM) pectin (with $DE < 50\%$) to form rigid gels with divalent cations has been used in the production of calcium pectinate gel beads, intended for controlled-release delivery of conventional drugs and also as a carrier for colonic delivery of proteins (Sriamornsak et al., 1997; Sriamornsak and Nunthanid, 1998; Sriamornsak, 1999). However, the use of ca-pectinate beads has some drawbacks due to their rapid in vitro release (Sriamornsak et al., 1997).

Pectins or pectin-based systems were formulated into hydrogel matrix tablets (Sungthongjeen et al., 1999; Turkoglu et al., 1999) and compression-coated tablets (Fernández-Hervás and Fell, 1998). To overcome the problem of high dissolution of pectin in the upper gastrointestinal tract or its relatively thick compression coats, the pectin has been combined with chitosan (Macleod et al., 1999) or with an insoluble polymer, such as ethylcellulose (Wakerly et al., 1996), to produce a film coat. In addition, combinations of calcium salts and pectin have been used to prepare matrix tablets for colonic drug delivery (Rubinstein et al., 1993; Wakerly et al., 1997). The rationale for this is that ca-pectinate (the insoluble salt of pectin) is not degraded by gastric or intestinal enzymes (Sandberg et al., 1983), but will be degraded by colonic pectinolytic enzymes (Rubinstein et al., 1993).

Ketoprofen (biological half-life: 1–2 h) is a good candidate for the development of enteric-coated products (Qureshi et al., 1994; Sancin et al., 1999) due to its well-known gastrotoxicity. Gazzaniga et al. (1994) prepared oral delayed-release system for colonic specific delivery. The system consists of ketoprofen-containing cores (minitablets) coated with three successive polymeric layers that are designed to dissolve at different pH conditions.

Although the above-mentioned controlled-release approaches are thought quite practical in terms of producibility, the site-selectivity and lag-time period of drug delivery as well as production cost and time should be further improved.

The present study was therefore undertaken to establish the feasibility of creating a potential colonic delivery system that consists of drug-loaded Zn-pectinate gel (ZPG) microparticles or their martix tablets with pectin/dextran mixtures. In this regard, the design of these ZPG microparticles and their potential for controlled release drug delivery in comparison with the conventional ca-pectinate gel beads are investigated. The release of ketoprofen, as a model drug, from matrix tablets under conditions mimicking mouth-to-colon transit is assessed and analysed in terms of the composition of tablets.

2. Materials and methods

2.1. Materials

Amidated LM pectin with DE of 36% and DA of 14% (GENU pectin type LM-104 AS-FS) was obtained from Copenhagen pectin (Denmark). Ketoprofen (mol. wt. 254.29) was donated from courtesy of Amriya-Rhone Poulenc (Alexandria, Egypt). Dextran (mol. wt. 100,000–200,000), medium viscosity grade sodium carboxymethylcellulose (NaCMC), magnesium stearate (Mg St), zinc acetate and calcium chloride were purchased from the Sigma Chemical Company (St. Louis, USA). All other materials used in the dissolution studies were of analytical reagent grade and were used as received.

2.2. Formulations design and characterization

2.2.1. Factorial design experiments

ZPG microparticles were prepared based on the 2^3 full factorial design. The independent variables are pectin concentration (X_1), $Zn(CH_3COO)_2$ concentration (X_2) and drug concentration (X_3). The independent variables and their levels are shown in Table 1(a). On the other hand, the time for 50% of the drug to be released [$t_{50\%}$ (hour), S.I.F. (pH 7.4)] (Y_1) and the drug entrapment efficiency (EE) of the microparticles [(EE%) (Y_2)] are the dependent variables (response parameters). Table 1(b) shows the independent and dependent variables. Fitting a multiple linear regression model to a 2^3 factorial design gave a predictor equation which was a first-order polynomial, having the form (Cohran and Cox, 1957):

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3,$$

where Y, level of a given response (dependent variable); b, regression coefficients for the first-order polynomial; and x, level of the independent variable.

Statistical analysis of results was performed using analysis of variance (ANOVA) and regression coefficients of all factors were calculated (Table 2).

Table 1
A 2^3 factorial design parameters and experimental conditions

Factors	Low level (−)	High level (+)			
(a) The independent variables and their levels					
(A) Pectin concentration (% w/v)	2.5	3.0			
(B) Concentration of $Zn(CH_3COO)_2$ (% w/v)	2.0	2.75			
(C) Drug concentration (% w/v)	2.5	4.5			
(b) Formulation of the microparticles utilizing 2^3 factorial design					
Code	X_1	X_2	X_3	Y_1[a]	Y_2[a]
M1	2.5	2.0	2.5	9.80 ± 0.424	74.58 ± 1.301
M2	3.0	2.0	2.5	5.433 ± 0.9504	79.00 ± 0.402
M3	2.5	2.75	2.5	21.10 ± 3.818	78.66 ± 2.16
M4	3.0	2.75	2.5	26.84 ± 2.468	73.94 ± 2.037
M5	2.5	2.0	4.5	3.75 ± 0.100	91.66 ± 0.276
M6	3.0	2.0	4.5	4.40 ± 0.0866	89.47 ± 0.167
M7	2.5	2.75	4.5	5.32 ± 0.6602	83.95 ± 0.882
M8	3.0	2.75	4.5	7.4 ± 0.3123	86.97 ± 0.921

The independent variables are pectin concentration (X_1), $Zn(CH_3COO)_2$ concentration (X_2) and the drug concentration (X_3). Drug release ($t_{50\%}$ (hour), S.I.F. (pH 7.4)) (Y_1) and drug EE% (Y_2) are the dependent variables. EE% (w/w) = 100 × measured concentration/theoretical concentration.

[a] Mean ± S.D., $n = 3$.

Table 2
Results of analysis of variance for 2^3 factorial experiments (run in triplicate)

Code	Source of variation[a]	Experiment 1	Experiment 2	Experiment 3	d.f.	Mean square	F ratio
M1	(1)	10.10	9.5	9.8			
M2	(a)	6.40	5.40	4.50	1	0.6991	0.375
M3	(b)	18.40	23.80	21.10	1	57.86	31.008*
M4	(ab)	24.10	27.50	28.90	1	5.54	2.97
M5	(c)	3.85	3.65	3.75	1	74.54	39.95*
M6	(ac)	4.50	4.35	4.35	1	0.0782	0.0420
M7	(bc)	5.45	5.90	4.60	1	32.97	17.67*
M8	(abc)	7.15	7.30	7.75	1	3.13	1.677
	Experiment error				16	1.866	
	Significance level based on d.f.				1		

The effect of independent variables (pectin concentration (a), $Zn(CH_3COO)_2$ concentration (b), drug concentration (c)) on the time for 50% of the drug to be released ($t_{50\%}$ (hour), S.I.F., pH 7.4) (Y_1).

[a] (1) Refers to all factors at their low levels; if factor A is at its high level, and B and C are at their low level, the combination is denoted as (a), etc.

* $P < 0.01$.

2.2.2. Preparation of pectinate gel microparticles

The ionotropic gelation technique of using drug entrapped in the beads (Sriamornsak and Nunthanid, 1998) was redesigned using the method as follows: pectin solution at a concentration of 2.5 or 3% w/v was prepared initially. Then an appropriate amount of the model drug, ketoprofen (from 2.5 to 4.5% w/v) was dispersed in this stirred solution until a uniform dispersion was obtained. Finally, this homogenous, bubble-free slurry was added dropwise, at an average rate of 1 ml/min, with the disposable syringe (a nozzle of 1 mm inner diameter) into 20 ml of a gently agitated solution of the crosslinking agent ($CaCl_2$ or $Zn(CH_3COO)_2$). The falling distance was 5 cm. The gelled microparticles thus formed were allowed to stand in the crosslinking solution, unless otherwise noted, to be cured for 24 h. This curing period was chosen based on a preliminary study. The microparticles were separated, washed with deionized water and dried in air for 48 h. Diameter of dried microparticles was about 0.9–1.02 mm (measured from the scanning electron micrographs). All batches were prepared in triplicate. A number of different variables were investigated for optimization of ZPG microparticle properties prepared by using 2^3 factorial design experiments (Table 1).

2.2.3. Scanning electron microscopy (SEM)

Morphological examination of the pectinate gel microparticles was conducted using a JEOL scanning electron microscope (JSM-5200, Jeol Ltd., Japan) at 15 kV. Pectinate microparticles were coated with gold for 10 min under vacuum by using SPI Sputter™ Coating Unit (SPI Supplies, Division of Structure Probe Inc., PA, USA).

2.2.4. Equilibrium swelling studies of microparticles

A known weight (100 mg) of various drug loaded-Ca or ZPG microparticles was placed in enzyme-free simulated intestinal fluid (S.I.F.: KH_2PO_4/NaOH buffer, pH 7.4) and allowed to swell for the required period of time (using the dissolution apparatus with the dissolution basket assembly) at 37 ± 0.2 °C. The microparticles were periodically removed, blotted with filter paper and their changes in weight (after correcting for drug loss) were measured during the swelling until equilibrium was attained. Finally, the weight of the swollen microparticles was recorded after a time period of 4 h and the swelling ratio (SR) was then calculated from the formula:

$$SR = (We - Wo)/Wo,$$

where Wo is the initial weight of the microparticles and We is the weight of the microparticles at equilibrium swelling in the medium. Each determination was performed in triplicate.

2.2.5. Preparation of tableted microparticles

Tableted Zn-pectinate microparticles (750 mg) containing both microparticles (code: M4) (equivalent to 25 mg of ketoprofen) and excipient mixtures (pectin and dextran or sodium carboxymethylcellulose) were prepared by direct compression. The formulation composition of matrix tablets is listed in Table 3. Ketoprofen microparticles or the drug alone in case of control tablets, excipients and Mg St (2% w/w) (as a lubricant) were uniformly mixed and compressed into tablets. Compression was carried out using a laboratory hydraulic press (Carver Press, model 3912, Carver Inc., Wabash, USA) with a 13-mm diameter flat-faced punch set at a compression pressure of 1 ton/inch^2, for 1 min. Three batches were prepared for each formulation and only those tablets that were within ± 10 mg of the target weight were used in dissolution studies.

2.2.6. Physical characteristics of tablets

The prepared tablets were tested for weight (AB 104; Mettler Toledo, Switzerland), thickness (Starrett portable dial hand micrometer; L.S. Starrett Co., Athol, MA, USA), hardness (Erweka type TB T/S hardness tester; Erweka-Apparatebau, GmbH, Germany) and for friability (Roche friabilator type TA_3: Erweka-Apparatebau, GmbH, Germany). The results are expressed as mean values of five determinations $\pm$ S.D. (Table 3).

2.2.7. Determination of the drug content

An accurately weighed amount of microparticles (50 mg) or tablet was broken in 5 ml of enzyme-free simulated intestinal fluid (S.I.F.: KH_2PO_4/NaOH buffer, pH 7.4) initially, and then the drug was extracted with the same medium for a period of 24 h. The solution was filtered and ketoprofen was assayed spectrophotometrically (Shimadzu, Double-Beam Spectrophotometer 150-02, Japan) at 259 nm. Each determination was made in triplicate.

2.2.8. Drug release studies

The release of ketoprofen from pectinate gel microparticles was investigated using an in vitro USP rotating paddle dissolution apparatus (Model DT-06, Erweka, Germany). The dissolution studies were performed in enzyme-free simulated intestinal fluid (S.I.F.: KH_2PO_4/NaOH buffer containing 0.02% Tween 80, pH 7.4) at a rotation speed of 50 rpm and a temperature of 37 ± 0.2 °C. Drug release was measured from accurately weighed amounts of the pectinate microparticles, equivalent to 20 mg of ketoprofen, added to 500 ml of dissolution medium. Samples withdrawn from S.I.F. at various time intervals were analyzed spectrophotometrically at 259 nm. All dissolution runs were performed in triplicate.

For tablets, simulation of gastrointestinal transit conditions was achieved by using different dissolution media. Thus, drug release studies were conducted in simulated gastric fluid without pepsin (S.G.F.: HCl/NaCl solution containing 0.02% Tween 80, pH 1.2) for the first 2 h. Then, the dissolution medium was replaced with enzyme-free S.I.F. (pH 7.4) and tested for drug release for 3 h, and finally enzyme-free S.I.F. (KH_2PO_4/NaOH buffer containing 0.02% Tween 80, pH 6.5) was used for 7 h (or until the tablets dissolved completely) to mimic colonic pH conditions.

3. Results and discussion

3.1. Preparation, release characteristics and drug EE of the ZPG microparticles containing ketoprofen

When an aqueous solution of LM pectin containing ketoprofen was dropped into counter ion (zinc) solutions, gelled spheres were produced instantaneously by ionotropic gelation in which intermolecular crosslinks were formed between the negatively charged carboxyl groups of LM pectin and the positively charged counter ion. The resulting microparticles were spherical, with mean diameters of 1.9–2.2 mm, and efficiently encapsulated ketoprofen (Table 1(b)). The mean diameter of dried drug-loaded ZPG microparticles ranged between 0.9 and 1.02 mm.

Table 3
Composition and characteristics of the matrix tablets containing ketoprofen-loaded ZPG microparticles

Formulation code	Composition (%)		Polymers ratio	Physical characteristics[a]				Release characteristics[a]		
				Thickness (mm)	Hardness (kg)	Friability (%)	HFR[b]	Lag time (h)	$t_{50\%}$ (h)	% Drug released after 5 h of dissolution testing[c]
T_1	Drug	3.54								
	Pectin	56.68		4.48	4.88			2.15	4.7	56.13
	Dextran	37.78	1.5:1	±	±	1.299	3.760	±	±	±
	NaCMC	–		0.05	1.24			0.086	0.263	0.06
	Mg St	2.0								
T_2	M4	9.34								
	Pectin	88.66		4.95	0.75			4.925	8.363	7.725
	Dextran	–	–	±	±	50.25	0.015	±	±	±
	NaCMC	–		0.0580	0.354			0.966	1.27	1.05
	Mg St	2.00								
T_3	M4	9.34								
	Pectin	44.33		4.63				4.125	7.450	13.61
	Dextran	44.33	1:1	±	>15	0.1522	–	±	±	±
	NaCMC	–		0.05				0.645	0.969	0.783
	Mg St	2.00								
T_4	M4	9.34								
	Pectin	53.20		4.8	9.13			4.85	8.70	9.24
	Dextran	35.46	1.5:1	±	±	0.2912	31.35	±	±	±
	NaCMC	–		0.082	1.83			1.06	0.86	1.08
	Mg St	2.00								
T_5	M4	9.34								
	Pectin	53.20		4.98	6.13				7.00	42.5
	Dextran	–	1.5:1	±	±	1.35	4.541	–	±	±
	NaCMC	35.46		0.05	0.177				1.28	1.35
	Mg St	2.00								
T_6	M4	9.34								
	Pectin	53.20		4.85	10.06				10.80	25.70
	Dextran	17.73	3:1:1	±	±	0.555	18.13	–	±	±
	NaCMC	17.73		0.058	3.32				0.496	0.69
	Mg St	2.00								

[a] Mean ± S.D.
[b] HFR refers to hardness/friability ratio.
[c] In vivo simulated conditions: S.G.F., pH 1.2 (2 h); S.I.F., pH 7.4 (3 h) and S.I.F., pH 6.5 (7 h).

The solubility of ketoprofen, being a propionic acid derivative, is pH dependent, it increases rapidly at pH values higher than the pK_a-value of the drug (pK_a = 5.02–5.937). Solubility of the drug in S.G.F. (pH 1.2) and S.I.F. (pH 7.4) was determined to be 7.415 and 0.1332 mg/ml, respectively. Because of the above-mentioned facts, the dissolution tests of the pectinate microparticles were conducted at a pH of 7.4, which mimicks the pH in the end of the small intestine.

Different processing variables such as type of crosslinking agent and crosslinking time were investigated and their effects on the release of ketoprofen are shown in Figs. 1 and 2.

The drug release properties in S.I.F. (pH 7.4) of ZPG microparticles (prepared with 2.5% w/v pectin, 2% w/v $Zn(CH_3COO)_2$ and a crosslinking time of 2 h) were compared to those of the ca-pectinate gel (CPG) microparticles prepared at the same preparative conditions (Fig. 1). The release of ketoprofen from the ZPG or CPG microparticles was significantly slower than the dissolution of ketoprofen powder. The result was due to the application of a rate controlling polymer matrix. Fig. 1 shows substantially great differences among the release profiles of ZPG and CPG microparticles in S.I.F. The Zn^{2+}

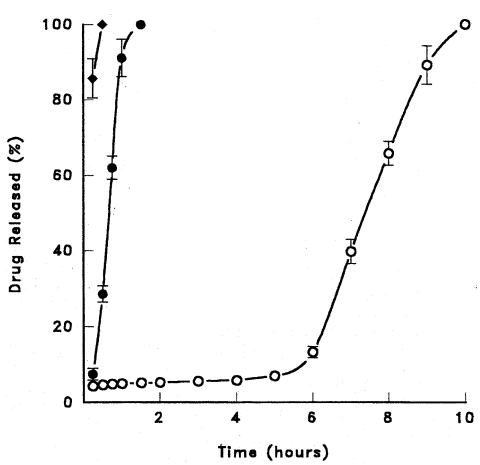

Fig. 1. Effect of crosslinking agent type on drug release from pectinate gel microparticles in S.I.F. (pH 7.4). Crosslinking agent (2% w/v): ○, $Zn(CH_3COO)_2$; ●, $CaCl_2$; ◆, free drug. Preparation conditions: crosslinking time: 2 h; 2.5% w/v pectin; drug concentration: 2.5% w/v.

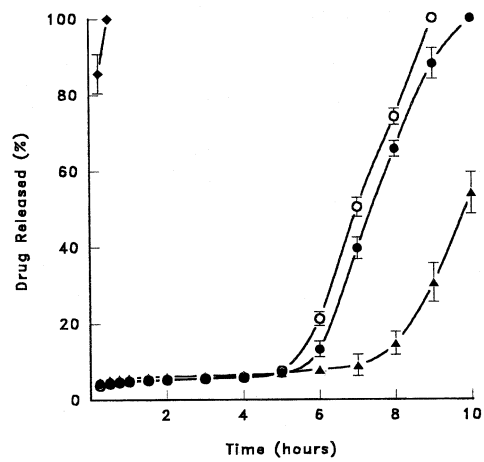

Fig. 2. Effect of crosslinking time on drug release from ZPG microparticles in S.I.F. (pH 7.4). Crosslinking time: ○, 0.5 h; ●, 2 h; ▲, 24 h; ◆, free drug. Preparation conditions: crosslinking agent: 2% w/v $Zn(CH_3COO)_2$; 2.5% w/v pectin; drug concentration: 2.5% w/v.

crosslinked pectinate microparticles remained intact and showed the most retarding effect on ketoprofen release with a $t_{50\%}$ (the time for 50% release of drug) of around 7.33 h, compared with pectinate microparticles crosslinked with calcium ($t_{50\%}$: 35 min). The results have shown that the payload in the ZPG and CPG microparticles prepared at the same crosslinking agent concentration (2% w/v), are nearly similar (38.9–39.08%). Therefore, it appears that differences in release rate could be explained by the difference in degree of crosslinking of the two gel types, which could affect the swelling rate of the microparticles during drug release and consequently the penetration of the solvent into the microparticles. The extent of swelling of the microparticles in S.I.F. (pH 7.4) was dependent of the crosslinking agent type. The Zn^{2+} crosslinked pectinate matrix showed the smallest degree of swelling (SR value = 0.98) with no disintegration during a period of more than 18 h, compared with ca-pectinate matrix which reached the highest swelling ratio (SR value = 6.02) after immersion in pH 7.4 solution for 1 h. In addition, the Ca-pectinate microparticles were completely broken in this pH after 1.5 h. On the other hand, the microparticles prepared using higher pectin and $CaCl_2$ concentrations (3% and

2.75 w/v, respectively) with a crosslinking time of 24 h exhibited lower swelling ratio (SR value = 4.14) but did not show a remarkable reduction in drug release ($t_{50\%}$: 50 min) (figure not shown). These findings suggest that CPG microparticles are subject to erosion in the lower intestine than those of the formulations prepared with zinc ions. The results obtained are consistent with those of Sriamornsak (1998) who has shown that the calcium ions form the loose linkages with carboxyl groups in the chains of LM pectin (PG36). A similar tendency has been reported for paracetamol release from calcium and zinc alginate beads (Aslani and Kennedy, 1996). Thus, it can be assumed that zinc forms more extensive crosslinking due to its mutal interaction with LM pectin. This results in a reduction in both the extent of rehydration and the molecular porosity. However, rapid ketoprofen release from ca-pectinate microparticles in phosphate buffer has been presumed to be due to greater solvent penetration into the ca-pectinate network, followed by greater ion exchange between Ca^{2+} and Na^{+} or K^{+} ions. These ions might displace calcium in the gelled structure and partially forming soluble pectin regions, which are more permeable (Kim and Lee, 1992; Sriamornsak, 1998).

Fig. 2 depicts the drug release profiles of ketoprofen from ZPG microparticles hardened with zinc for various times (0.5–24 h). As the crosslinking time increased, the drug release rate in S.I.F. (pH 7.4) decreased. These properties are probably explained by the promotion of stronger crosslinks between pectin chains by the zinc ions.

However, it is noteworthy first that the changing enzymic and pH conditions on passage through the stomach and down the small intestine make drug protection a significant problem. It was previously reported that gelation of LM pectin droplets in the presence of counter ion (Ca^{2+}) may provide a valuable approach to the formation of a multiparticulate system for colonic delivery (Munjeri et al., 1997; Sriamornsak, 1998). This may be true for a large molecule (e.g. protein drugs) which cannot diffuse through the pores of the matrix bead, but can be released due to enzymatic degradation of the matrix (Batycky et al., 1997; Sriamornsak, 1999). In contrast, smaller drug molecules can pass through the matrix pores easily, especially in the acidic environment where soluble and more permeable pectinic acid regions are formed in the presence of hydrogen ions (Sriamornsak and Nunthanid, 1998).

Concluding from the results mentioned above, the Zn^{2+} crosslinked pectinate (ZPG) microparticles are, therefore, considered more suitable than Ca-pectinate matrix for using as a colonic delivery carrier for conventional drugs. Hence, attempts to maximize the benefit of these promising ZPG microparticles was made by using factorial design (with three independent variables) as follows.

3.1.1. Effect of pectin concentration

It was seen from Table 1(b) and Fig. 3 that release from ZPG microparticles made at 3% w/v pectin concentration (code M4) was lower than that of microparticles prepared with 2.5% w/v pectin concentration (code M3). However, when the results of ANOVA for 2^3 factorial experiments were investigated in Table 2, the investigated factor and its interactions did not have significant effects on $t_{50\%}$ values ($P < 0.01$). The F ratio value of pectin concentration was also the lowest one as compared with the values of other factors.

3.1.2. Effect of zinc concentration

The release of ketoprofen from ZPG microparticles was clearly dependent upon zinc concentration (Table 1(b) and Fig. 4). The increase of the concentration of zinc from 2 to 2.75% w/v resulted in slower drug release patterns, especially with microparticles (M4) prepared at low drug concentration (2.5% w/v). The $t_{50\%}$ values of such microparticles (M4) in S.I.F. (pH 7.4) was 4–5-fold greater than those of microparticles coded M2, M6 and M8 (Table 1b) and the release was greatly extended with about 75% of the payload being released within 30 h. In fact, the increase of amounts of cation will lead to a greater degree of crosslinking due to the increase in the number and/or strength of crosslinks between pectin and counter ions as well as, aggregation of the initial dimers, thereby giving a higher gel strength (Wakerly et al., 1997). Similar results have previously been reported on ca-pectinate gel beads containing indomethacin (Sriamornsak and Nunthanid, 1998).

The variations in the release properties of ZPG microparticles can be verified by the scanning electron micrographs given in Fig. 5(A, B). Microparticles prepared at 2% w/v $Zn(CH_3COO)_2$ concentration (M2) are relatively spherical in shape and have numerous cracks and bigger pores, as well as larger and deeper, surface folds than those prepared with 2.75% w/v $Zn(CH_3COO)_2$ (code: M4) (Fig. 5(A, B), × 35, × 350, × 1000). The high magnification (× 1000) also shows the sponge-like and less-porous morphology of the outer-wall, as well as, the absence of cracks or fissures on the surface of microparticles prepared with 2.75% w/v $Zn(CH_3COO)_2$. This result correlated well with the decreased release rates of these microparticles.

From the results of the 2^3 factorial design experiments (Table 1(b) and Table 2), the following polynomial model can be derived for the effect of formulation factors (X_1: pectin concentration, X_2: $Zn(CH_3COO)_2$ concentration, X_3: drug concentration) on the $t_{50\%}$ value (Y_1).

$$Y_1 = 10.51 + 0.512X_1 + 4.658X_2 - 5.287X_3 + 1.441X_1X_2 + 0.1713X_1X_3 - 3.52X_2X_3 - 1.083X_1X_2X_3.$$

The fitted model and analysis of variance (ANOVA) showed a statistically significant effect of zinc concentration (X_2) ($P < 0.01$) on the $t_{50\%}$ values. The effectivity coefficient of X_2 has a positive sign, thereby, indicating an increasing effect on the corresponding response. However, X_3 (drug concentration) and its interaction (X_2X_3) showed a significant decreasing effect ($P < 0.01$) on the $t_{50\%}$ value (Table 2).

3.1.3. Effect of the amount of drug added

The release of ketoprofen was more extended with microparticles prepared with 2.5% w/v drug concentration ($t_{50\%}$: 21.1–26.84 h) than those of the 4.5% w/v drug ($t_{50\%}$: 5.32–7.4 h) (Table 1(b) and Fig. 3). The increase in drug release from microparticles containing high drug concentration may be due to the decrease in the polymer/drug ratio. The findings indicated similarity with the data of indomethacin/ca-pectinate beads reported by Sriamornsak and Nunthanid (1998). The surface topography of the microparticles showed that microparticles prepared with 4.5% w/v drug concentration (M8) are larger in size and have porous and very rough walls (Fig. 5C, × 35, × 350). At the × 1000 magnification, the surface appeared to be

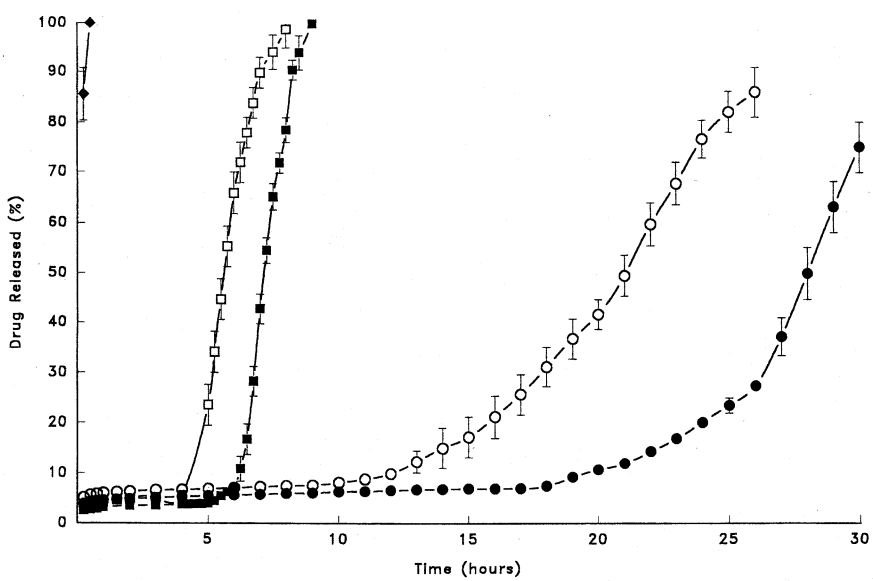

Fig. 3. Effect of pectin concentration on drug release from ZPG microparticles in S.I.F. (pH 7.4). Drug concentration (2.5% w/v): ○, 2.5% w/v pectin (M3); ●, 3% w/v pectin (M4). Drug concentration (4.5% w/v): □, 2.5% w/v pectin (M7); ■, 3% w/v pectin (M8); ◆, free drug. Preparation conditions: 2.75% w/v $Zn(CH_3COO)_2$, crosslinking time: 24 h.

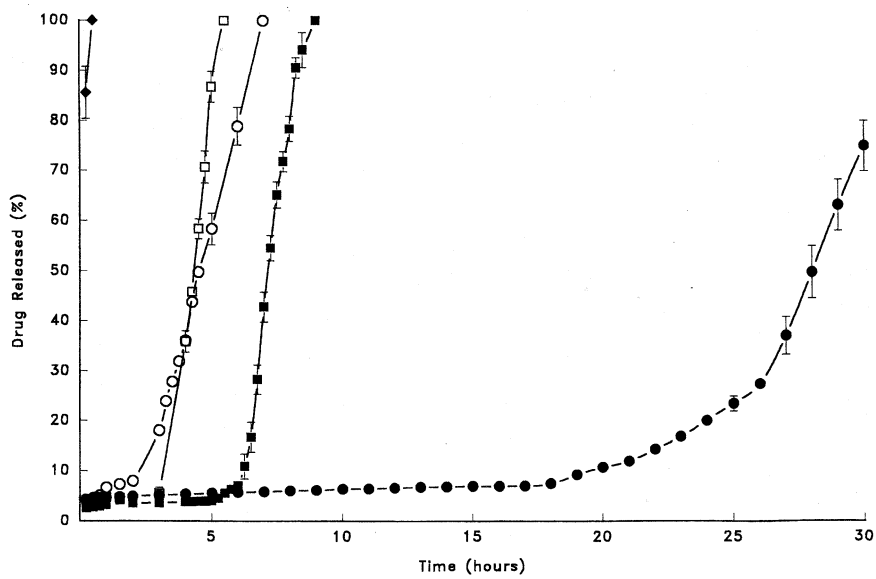

Fig. 4. Effect on $Zn(CH_3COO)_2$ concentration on drug release from ZPG mocroparticles in S.I.F. (pH 7.4). Drug concentration (2.5% w/v): ○, 2% w/v $Zn(CH_3COO)_2$ (M2); ●, 2.75% w/v $Zn(CH_3COO)_2$ (M4). Drug concentration (4.5% w/v): □, 2% w/v $Zn(CH_3COO)_2$ (M6); ■, 2.75% w/v $Zn(CH_3COO)_2$ (M8); ◆, free drug. Preparation conditions: 3% w/v pectin, crosslinking time: 24 h.

ruptured and loosely bound giving rise to a heavily structured and macroporous morphology (Fig. 5C), thus explaining the faster release rates of these microparticles.

The drug EE of the ZPG microparticles were varied from 73.94 to 91.66% (Table 1b). The increase of the amount of drug added from 2.5 to 4.5% caused a significant increase in drug EE for these microparticles. This may be due to the increase of the drug/pectinate weight ratio in the matrix.

The effect of the independent factors on drug EE was also evaluated with analysis of variance (ANOVA) (data not shown). The amount of drug added (X_3) had a significant effect on the drug EE ($P < 0.01$) whereas, other factors or their interactions did not affect the drug EE significantly.

3.2. Physical and release characteristics of the matrix tablets containing ketoprofen-loaded ZPG microparticles

The selected ZPG microparticles coded M4 were compressed into tablets with various polymer mixtures (pectin–dextran, pectin–NaCMC and pectin–dextran–NaCMC) in order to prepare ZPG/tablet system for colonic drug delivery.

The comparison of the hardness, thickness, and friability of different matrix tablets prepared at 1 ton compression pressure is presented in Table 3. Among the polymers used, dextran was the best compressible type in a mixture with pectin (friability %: 0.15–1.3). Pure pectin resulted in the highest friability % (50.25) and the lowest hardness values at the study compression pressure. It was concluded that addition of dextran to pectin or pectin–NaCMC matrices improves their mechanical strength (HFR: 31.35 and 18.13, respectively) (Table 3).

Table 3 and Fig. 6 demonstrate the release behavior of ketoprofen from six individual tablets during the dissolution testing under simulated GI conditions involving the transit times and pH changes. The in vitro release of ketoprofen from tablets is characterized by the $t_{50\%}$ release time, a lag time and the cumulative percent of drug re-

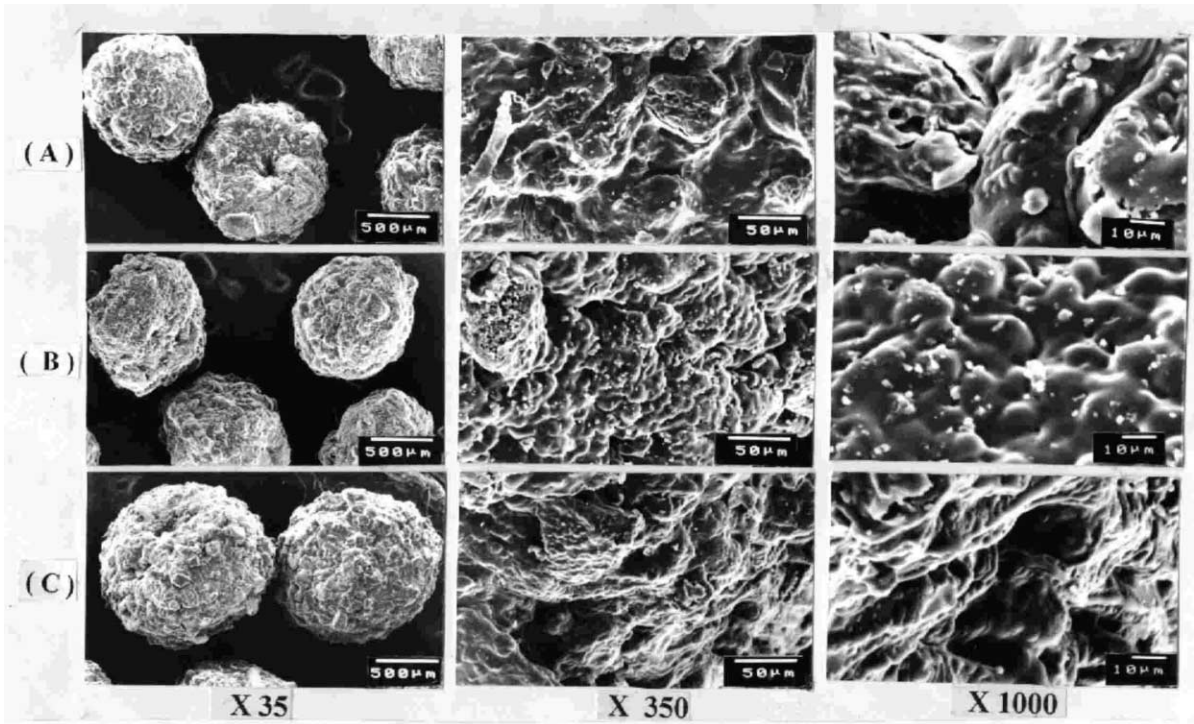

Fig. 5. Scanning electron micrographs for the influences of crosslinking agent and drug concentrations on surface morphology of ketoprofen-loaded ZPG microparticles. (A) ZPG microparticles (M2) (2% w/v $Zn(CH_3COO)_2$, drug concentration: 2.5% w/v). (B) ZPG microparticles (M4) (2.75% w/v $Zn(CH_3COO)_2$, drug concentration 2.5% w/v). (C) ZPG microparticles (M8) (2.75% w/v $Zn(CH_3COO)_2$, drug concentration 4.5% w/v). Preparation conditions: 3% w/v pectin, crosslinking time: 24 h.

leased after 5 h of the dissolution testing. Obviously, the release of ketoprofen from control tablets (T_1) prepared from drug powder and pectin/dextran mixture (at a ratio of 1.5:1) was fairly rapid with at least 56% of the drug load being released within 5 h, compared with only 7.7–13.6% for formulations (T_2, T_3, T_4) containing drug-loaded microparticles (Table 3). This result indicated that exposure time in the stomach of these formulations (T_2–T_4) would not markedly affect the dissolution performance during transit in the intestine. The lag time values (the intercept of the extrapolated straight line portion of the sigmoidal release curve with the time axis) of such delayed-release formulations were 2–2.3-fold greater than those of the control tablets which released about 8% of their drug load in S.G.F. However, the tablets made with the ZPG microparticles, especially code T4

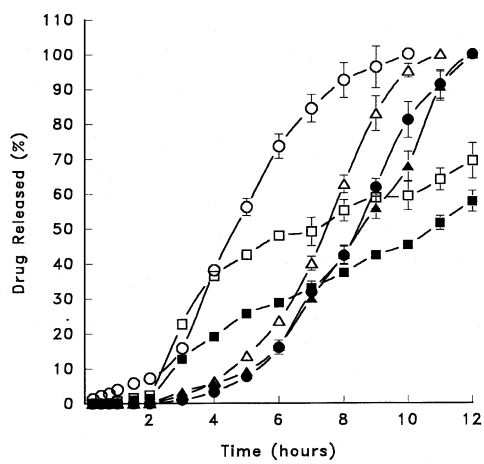

Fig. 6. The release of ketoprofen from matrix tablets containing drug-loaded ZPG microparticles under conditions simulating gastrointestinal transit times and pH. Matrix tablets: ○, T_1; ●, T_2; △, T_3; ▲, T_4; □, T_5; ■, T_6. Dissolution media: S.G.F., pH 1.2 (2 h); S.I.F., pH 7.4 (3 h) and S.I.F., pH 6.5 (7 h). See Table 3 for tablets codes key.

($t_{50\%}$: 8.7 h, pectin/dextran ratio of 1.5:1) remained intact and retained more than 98% of their drug load throughout the testing period in S.G.F. The higher concentration of pectin, in this formulation will form a more resistant gelatinous layer to water penetration, drug diffusion from microparticles, and hence release (Sungthongjeen et al., 1999). Therefore, these tablets were expected to be protected in the stomach, and would be delivered to the intended target site (lower small intestine or colon) after a predetermined lag time.

Drug release from hydrophilic matrices such as those used in the current study will be controlled by the rate of hydration of the matrix and the properties of the gel formed on hydration, which influence drug diffusion and gel erosion. Rapid hydration is required to establish the gel layer and prevent the release of an initial burst of drug. Thereafter, the thicker the gel layer, the longer is the diffusional path for the drug molecules, and the stronger the gel the less will be its susceptibility to erosion (Wakerly et al., 1997). In addition, as the gastric emptying time differs with individual dosage form or physiological conditions such as gastrointestinal motility, the dissolution behavior after gastric emptying time may be altered due to the time of exposure to gastric pH. Considering the results obtained, it is clear that the changes in tablets of ZPG microparticles, as visually inspected during dissolution testing, were a result of a combination of swelling and erosion. Pectin or pectin/dextran mixture was hydrated rapidly to form a viscous gel which did not visibly erode in S.G.F. (pH 1.2). In the simulated small intestinal fluid (S.I.F., pH 7.4), these matrices released a small portion of the drug (7–13.6%) slowly within 3 h. The increased drug release rate in the medium simulating the colon pH (S.I.F., pH 6.5) is most probably attributed to the gradual erosion and dissolution of the tablet matrix. When the erosion front reaches the gel microparticles, they will begin to erode and release the drug in the intestinal fluid.

On the other hand, the use of mixtures of pectin or pectin/dextran blend with NaCMC (codes T_5 and T_6) resulted in slight cracking and erosion of the matrix in S.G.F. This was followed by an initially substantial linear drug release (up to 43% of drug released within 3 h in S.I.F., pH 7.4), particularly in the presence of high concentration of NaCMC and absence of dextran in the matrix. Thereafter, high swelling and slow erosion of the matrix were observed. Results might be attributed to the increase in the gelatinous layer thickness of NaCMC based-matrix system in alkaline media, which accounts for the slower release of drug from these matrices afterwords (Fig. 6). Obviously, these matrices are not suitable for colonic delivery because they would result in the release of a high level of the drug before entry into the colon.

4. Conclusions

The approach presented in this study focuses on the use of zinc as opposed to calcium to form insoluble gels of pectin that can be used for colonic delivery.

In this regard, ZPG microparticles or their tablets with pectin/dextran mixtures were investigated for a potential oral colonic delivery of drugs. In fact, ZPG microparticles as an oral delayed-release system after filling into capsules, as well as, pectin/dextran mixtures as directly compressible materials were not reported in the literature before for colon targeting.

The optimum oral-delayed release formulations offered a greater degree of protection from premature drug release in the simulated upper GI tract conditions. Hence, the approach presented in this study provides another alternative to target conventional or protein drugs to the colon, and can be used to large scale manufacturing without any sophisticated equipments. In vivo evaluation studies of the prepared formulations and their susceptibility to degradation in presence of pectinolytic enzymes are currently in progress.

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