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Research paper

Enzymatic degradation of cross-linked high amylose starch tablets and its effect on in vitro release of sodium diclofenac

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Abstract

The influence of several physicochemical parameters on enzymatic hydrolysis and the in vitro release of sodium diclofenac (SDic) from cross-linked high amylose starch (Contramid) (CLA) tablets was evaluated. These parameters included pH, ionic strength of the medium, enzyme concentration, compression force and incorporation of gel-forming polymers such as hydroxypropyl methylcellulose (HPMC), poly(ethylene oxide) (PEO) and poly(vinyl alcohol) into the tablet. Pure CLA tablets were incubated in phosphate buffer (pH 6.8) containing α-amylase and the extent of enzymatic erosion was determined by gravimetry. Release of SDic from CLA tablets, in the presence of α-amylase, was measured using a USP type III dissolution apparatus. For low α-amylase concentrations (<2250 IU/l), the drug release was mainly diffusion-controlled. At higher α-amylase concentrations (>4500 IU/l) both diffusion and erosion contributed to the release of SDic. The hydrolysis kinetics of CLA tablets by α-amylase was biphasic. During the first phase (2–4 h), the hydrolysis rate was hyperbolically related to the α-amylase concentration but was practically α-amylase concentration-independent during the second phase. Enzymatic erosion and drug release kinetics appear to be relatively independent of ionic strength, pre-incubation time in simulated gastric fluid, and compression force of the tablets (6–34 kN). Incorporation of HPMC or PEO into the tablet resulted in a significant decrease of both tablet erosion and drug release rates.

Keywords: Amylose; Contramid; Cross-linked starch; α-Amylase; Controlled release; Enzymatic degradation

1. Introduction

Starch and its derivatives are widely used in pharmaceutical formulation as filler, binder, disintegrant and thickening agent [1]. Recently, cross-linked high amylose starch (CLA) has been introduced, under the trademark of Contramid, as a new class of controlled release excipient for the preparation of matrix tablets [2]. CLA is composed of two major polysaccharides, namely amylose (70%) and amylopectin (30%) cross-linked with phosphate. Both amylose and amylopectin undergo hydrolytic bond cleavage by α-amylase to form water-soluble oligosaccharides, resulting in polymer erosion [3]. After exposure of CLA tablets to an aqueous medium, an interfacial rubbery layer rapidly forms [4]. Permeability of drugs through this gel layer and its hydrolysis by α-amylase is expected to play an important role in controlling drug release. It was demonstrated that, depending on tablet composition and concentration of α-amylase in the matrix, drug release from CLA tablets can switch from a diffusion to an enzymatic erosion controlled mechanism [5].

Hydrolysis of starch by α-amylase has been extensively studied over the last two decades and its kinetic mechanism is fairly well understood [6–8]. This kinetics is affected by three major factors which are the type of α-amylase, properties and structure of the starch substrate, and mode of interaction between the enzyme and polymer. It has been reported that the nature and the distribution of hydrolysis products is solely dependent on the source of α-amylase [9]. Some of these hydrolysis by-products, such as maltose and maltotriose, strongly inhibit the hydrolytic action of α-amylase [10]. Crystallinity and surface area are considered to be the most important parameters affecting the kinetics of starch hydrolysis. Indeed, α-amylase can more easily degrade the amorphous regions than the crystalline regions [11–13]. Similarly, a high surface area tends to increase the accessibility of the enzyme molecules to the substrate, resulting in an increased rate of hydrolysis.

Another important factor which also affects the kinetics of starch hydrolysis is the mode of interaction between the

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enzyme and the polymeric chains. Hydrolysis of insoluble starch substrates differs distinctly from that of soluble substrates. Indeed, the hydrolysis of starch composed of mainly insoluble fractions involves four steps: (i) diffusion of enzyme molecules from the bulk aqueous phase to the solid surface, (ii) adsorption of the enzyme onto the solid substrate, resulting in the formation of the enzyme-substrate complex, (iii) hydrolysis of the α-(1 → 4)-glucose bond and finally, (iv) diffusion of the soluble degradation products from the solid substrate into the bulk aqueous phase [7,14]. The global reaction rate of the enzymatic degradation is controlled by the slowest step. Some authors have reported that the diffusion of α-amylase into a gel of amylose and amylopectin constitutes the limiting step during the first period of hydrolysis [15]. Others reported that adsorption of the enzyme onto the solid substrate is the main limiting parameter since α-amylase (*Bacillus* species) has less affinity for a solid substrate than for a soluble one [10]. Accordingly, by assuming that the degradation rate is proportional to the amount of adsorbed enzyme and that the enzyme adsorption occurs according to a Langmuir isotherm, the rate of hydrolysis (r) can be calculated using the following equation [16]

$$r = k\frac{[A][E]}{K_{eq} + [E]} \quad (1)$$

Where [E] and [A] are the enzyme and substrate concentration, respectively, k and K_{eq} are the specific rate constant and equilibrium constant between free and absorbed enzyme, respectively.

Finally, it has been reported in the literature that the hydrolysis rate of starch was drastically reduced as the reaction progresses. This was attributed in part to the transformation of starch into a less digestible form and/or inhibition of the enzymatic degradation by reaction by-products [10–17].

In this study, the enzymatic erosion of CLA tablets, and its impact on the release of sodium diclofenac (SDic) was investigated. The influence of some physicochemical parameters such as compression force, pH, ionic strength and α-amylase concentration in the medium, on the enzymatic hydrolysis and SDic release were studied. Incorporation of gel-forming polymers such as hydroxypropyl methylcellulose (HPMC), poly(ethylene oxide) (PEO) and poly(vinyl alcohol) (PVAL) into the tablets to protect CLA against α-amylase hydrolysis was also investigated.

2. Materials and methods

2.1. Materials

CLA (Contramid) batch #R-336GR was supplied by Rougier Inc. (Laval, Canada). α-Amylase (EC 3.2.1.1) batch #114HO654 from *Bacillus* species with a catalytic activity of 1260 IU/mg protein, was supplied by Sigma (St. Louis, MO). One unit is defined as the amount of enzyme liberating 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20°C. SDic pharmaceutical grade (Labochim, Milan, Italy) batch #170122 was used as a model drug. HPMC (Methocel K100M premium CR, Dow Chemical, Lake Jackson, TX) ($M_r \approx 1.5 \times 10^6$) batch #R80601001, PEO (Polyox WSR 303-NF grade, Union Carbide Danbury, CT) ($M_r \approx 8 \times 10^6$) batch #247497, and PVAL ($M_r \approx 150\,000$) (Aldrich, Milwaukee, WI) batch #17611LQ were used as gel-forming polymers.

2.2. Tablet preparation

Tablets containing 200 mg of pure CLA or a mixture of CLA:SDic (95:5 w/w) were directly compressed with a single station Stokes F4 instrumented tablet press (model 252, Stokes, Bristol, PA) using 8.73-mm flat-faced punches. The compression forces were measured by calibrated strain gauge (Korsch America Inc., Somerset, NJ) and the signals were amplified, digitized and analyzed with Korsch Data Acquisition Unit, CRS system (Korsch Pressen GmbH, Berlin, Germany). Tablets containing different concentrations of gel-forming polymers (HPMC, PEO and PVAL) were also prepared in order to study the protective effect of these polymers against enzymatic erosion. The CLA used in all experiments was previously granulated with water in a fluid bed granulator (Glatt GPCG-1, Ramsey, NJ) and the moisture content (H) of the granulated product was determined by thermogravimetry using a moisture analyzer (Sartorius MA30, Edgewood, NY). The thickness and diameter of the tablets were measured with an electronic digital micrometer (Max-cal, Tokyo, Japan) and the tablet crushing strength was recorded using a hardness tester (Vector corporation, Marion, IA).

2.3. Stability of α-amylase in solution

In order to monitor the stability of α-amylase activity over time, a stock solution containing 12 000 IU/l of α-amylase in phosphate buffer (50 mM, pH 6.8), was prepared and stored at 37°C in tightly closed glass container. The enzymatic activity was determined at different time intervals according to the USP procedure 'Assay for amylase activity of pancreatin', and the activity loss was calculated relatively to the initial activity at time zero. All experiments were performed in triplicate.

2.4. Enzymatic degradation

Enzymatic degradation studies of CLA tablets were conducted using a USP type III dissolution apparatus (Bio-Diss, Vankel, Edison, NJ). CLA tablets were placed in 250 ml phosphate buffer (50 mM, pH 6.8) containing different α-amylase concentrations (1000–18 000 IU/l). The stirring and temperature of the medium were set at 10 dips/min and 37°C, respectively. At predetermined time intervals, the tablets were removed from the α-amylase

solution, immersed for 10 min in 0.1 N HCl for enzyme deactivation, dried in an oven at 105°C for 12 h and weighed.

In another series of experiments, CLA tablets were also pre-incubated in 0.1 N HCl for different periods of time (15, 30, 60, 90 and 120 min). Then, they were removed and soaked for 2 and 4 h, in 250 ml phosphate buffer (50 mM, pH 6.8) containing 9000 IU/l of α-amylase. Each experiment was performed in triplicate and the enzymatic erosion was assessed using the following equation

$$\text{Degradation } (\%) = 100\frac{W_1(1-H) - W_2}{W_1(1-H)} \quad (2)$$

where W_1 is the weight of tablet before enzyme incubation, W_2 the weight of dry tablet after enzyme incubation, and H the percentage of moisture content of the granulated CLA.

2.5. Hydrolysis products

Hydrolysis products were identified by high performance liquid chromatography (HPLC) on a C18 silica gel μ-Bondapak column (30 × 0.4 cm, Waters Associates, Milford, MA) eluted with acetonitrile/water 8:2 (v/v) at a flow rate of 0.4 ml/min. Detection was done by differential refractometry. The column was calibrated using three oligosaccharide standards (glucose, maltose and maltotriose) purchased from Sigma (St Louis, MO).

2.6. Dissolution studies

In vitro dissolution studies were performed in a USP type III apparatus connected to a spectrophotometer (HP 8453 Hewlett Packard, Watdbronn, Germany). CLA tablets containing 5% (w/w) SDic and compressed at 20 kN were placed in 250 ml phosphate buffer (50 mM, pH 6.8) containing α-amylase at different concentrations. Temperature and stirring rate of the dissolution system were set at 37°C and 10 dips/min, respectively. SDic absorbance was measured at 256 nm. The dissolution profiles were compared to the reference using a similarity factor f_2 as proposed by Moore and Flanner [18] (Eq. (3))

$$f_2 = 50\log\left\{\left[1 + \frac{1}{n}\sum_{i=1}^{n}(R_t - T_t)^2\right]^{-0.5} 100\right\} \quad (3)$$

where R_t ant T_t are the cumulative percent of drug released for reference and test assay at time t, respectively, and n is the number of time points. The dissolution of CLA tablets without α-amylase was used as the reference. Note that f_2 is a measure of the similarity between two dissolution curves and its value varies between 0 and 100. A high f_2 value indicates high similarity between two release profiles. FDA suggests that two dissolution profiles are considered to be similar if the similarity factor f_2 falls between 50 and 100 [19].

In order to characterize the release mechanism of SDic from CLA tablets in the dissolution medium, data were fitted to the following equation proposed by Ritger and Peppas [20]

$$\frac{M_t}{M_\infty} = kt^n \quad (4)$$

Where M_t is the amount of drug released at time t, M_∞ is the overall drug released, k is the kinetic constant and n is the dimensionless number characterizing the release mechanism (for cylindrical shape, $n = 0.45$ for Fickian release, $0.45 < n < 1.0$ for anomalous release and $n = 1.0$ for zero order release).

3. Results

3.1. Stability of α-amylase in solution

The α-amylase activity in phosphate buffer pH 6.8 at 37°C was stable for at least 4 h, then it lost 2.6, 7.9 and 21.1% of the initial activity after 8, 16 and 24 h, respectively. Accordingly, it can be assumed that most of the enzyme activity was preserved during the first 16 h.

3.2. Hydrolysis products

Three soluble oligosaccharides, namely glucose, maltose and malotriose, produced by the action of α-amylase on CLA tablets were identified by HPLC. The relative proportion of these oligosaccharides was not determined.

3.3. Factors affecting the enzymatic erosion of CLA tablets and SDic release

3.3.1. α-amylase concentration

Pure CLA tablets, compressed at 20 kN, were incubated at 37°C in phosphate buffer (pH 6.8) containing α-amylase at different concentrations ranging from 1000 to 18 000 IU/l. The kinetics of the enzymatic erosion over a period of 24 h is shown in Fig. 1A. The enzymatic erosion profiles consisted of two distinct phases. During the first phase (0–2 h), the erosion increased sharply with incubation time and α-amylase concentration in the medium. After 2 h, it ranged from 8% (1000 IU/l) to 20% (18 000 IU/l). Data of the initial erosion rate (% polymer eroded/h) obtained during the first phase of the erosion (0–2 h), were fitted to Eq. (1) with the non-linear least squares method (Fig. 1B), using the SigmaPlot version 4.0 (SPSS Science Inc, Chicago, IL) software. During the second phase (4–24 h), the erosion rate was slower, the tablets eroded at a rate ranging from 1.5 to 3.1%/h, which was less dependent of α-amylase concentration (Fig. 1B). After 24 h incubation, the enzymatic erosion had reached values ranging from 47% (1000 IU/l) to 94% (18 000 IU/l).

Fig. 2 shows the release of SDic from CLA tablet in phosphate buffer at different α-amylase concentrations. These profiles were compared to the release kinetics in the absence of α-amylase. The results revealed no significant

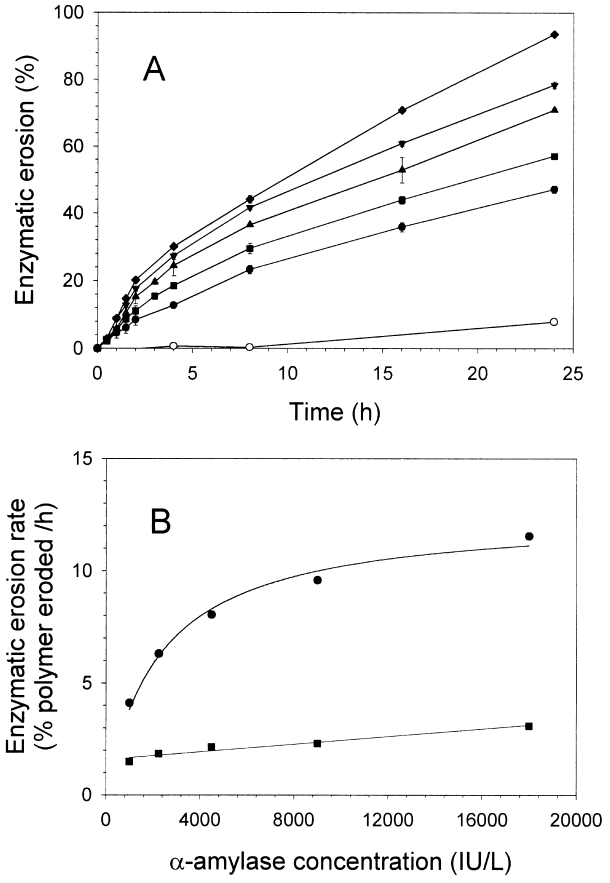

Fig. 1. (A) Kinetics of the enzymatic erosion of CLA tablets incubated in phosphate buffer (pH 6.8) containing α-amylase at different concentrations 0 IU/l (○), 1000 IU/l (●), 2250 IU/l (■), 4500 IU/l (▲), 9000 IU/l (▼) and 18 000 IU/l (◆). Mean ± SD ($n = 3$). (B) Relationship between the enzymatic erosion rate of the CLA tablet and the α-amylase concentration in the incubation medium during the first 2 h of incubation (●) and between 8 and 24 h of incubation (■).

difference between the release profiles when α-amylase concentrations were kept below 2250 IU/l as indicated by a high f_2 value (Table 1). Increasing the α-amylase concentration in the dissolution fluid to 4500 IU/l and above led to a significant increase in the release rate (Fig. 2). As shown in

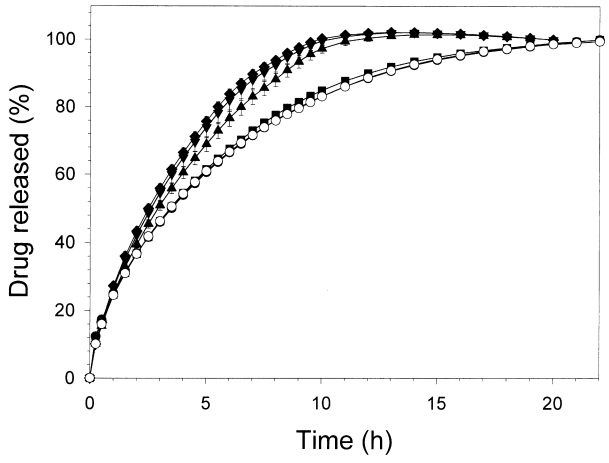

Fig. 2. Effect of the α-amylase concentration in phosphate buffer (pH 6.8) on the in vitro release of SDic: 0 (○), 1000 (●), 2250 (■), 4500 (▲), 9000 (▼) and 18 000 IU/l (◆). Mean ± SD ($n = 3$).

Table 1, the f_2 factor decreased to reach a value of 42 at an α-amylase concentration of 18 000 IU/l.

3.3.2. Compression force

Fig. 3 shows the influence of compression force on the enzymatic erosion of CLA tablets in phosphate buffer containing 9000 IU/l of α-amylase. Pure CLA tablets made at low compression forces are more porous and have a large surface area available for enzyme adsorption, leading to a high initial rate of hydrolysis. After 4 h incubation with α-amylase, tablets compressed at 3.3 kN lost about 57% of their initial weight. On the contrary, CLA tablets containing 5% SDic were more resistant to enzymatic degradation (data not shown). As shown in Fig. 4, drug release from those tablets was hardly affected by compression force. Increasing compression force up to a minimum value of approximately 6 kN did not significantly affect either crushing strength (Fig. 4, inset), enzymatic degradation (Fig. 3) or drug release (Fig. 4). The f_2 values given in Table 1 were in excess of 88 from 6 to 31 kN.

Table 1
f_2 values of different dissolution tests as a fuction of α-amylase concentration (AC), compression force (F) and incorporation of 10% of a gel-forming polymer (GFP) into CLA tablets[a]

	Reference	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
AC (IU/l)	0	1000	2250	4500	9000	18000
f_2 value		99.1	91.3	50.9	44.1	42.1
F (kN)	20	4	6	12	32	–
f_2 value		63.5	88.1	99.4	99.9	–
10% of GFP	No GFP	HPMC	PEO	PVAL	–	–
f_2 value		73.0	61.0	49.0	–	–

[a] HPMC, hydroxypropyl methylcellulose; PEO, poly(ethylene oxide); PVAL, poly(vinyl alcohol).

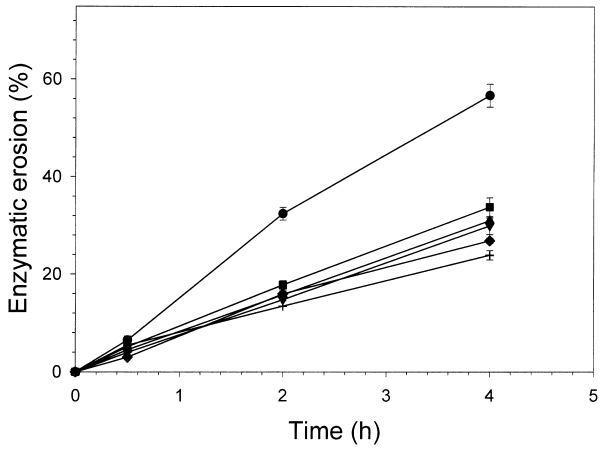

Fig. 3. Effect of compression force on the enzymatic erosion of CLA tablets incubated in phosphate buffer (pH 6.8) containing α-amylase at 9000 IU/l: 3.3 (●), 6 (■), 8 (▲), 12 (▼), 18 (◆) and (+) 34 kN. Mean ± SD ($n = 3$).

3.3.3. Ionic strength

The enzymatic erosion of the tablets incubated in phosphate buffer containing 9000 IU/l of α-amylase was evaluated at different ionic strengths ($\mu = 0.10$, 0.25 and 0.50). The results indicated that the erosion rate was barely affected by the ionic strength of the medium (data not shown).

3.3.4. Pre-incubation time in simulated gastric fluid

In order to simulate the effect of the residence time of the CLA tablets in the stomach before their transfer to the intestinal tract where they come in contact with α-amylase, tablets were pre-incubated in 0.1 N HCl for different periods of time. Results in Fig. 5 clearly show that the enzymatic erosion of the CLA tablet was not greatly affected by pre-incubation in the acidic medium.

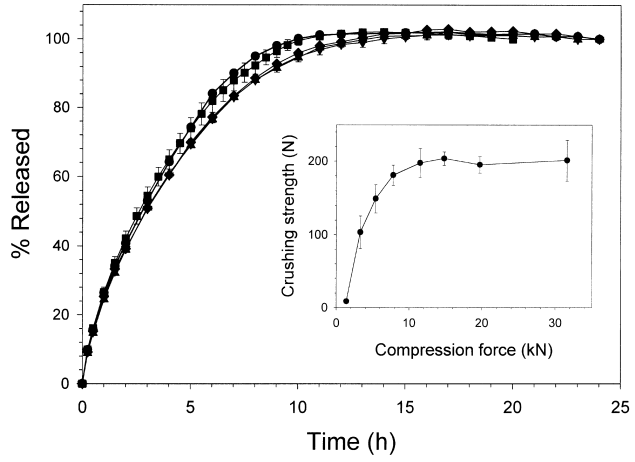

Fig. 4. In vitro release of SDic from CLA tablets compressed at 4 (●), 6 (■), 12 (▲), 18 (▼) and 34 kN (◆). Dissolution was carried out in phosphate buffer pH 6.8 containing α-amylase at 9000 IU/l. Inset: crushing strength vs. compression force. Mean ± SD ($n = 3$).

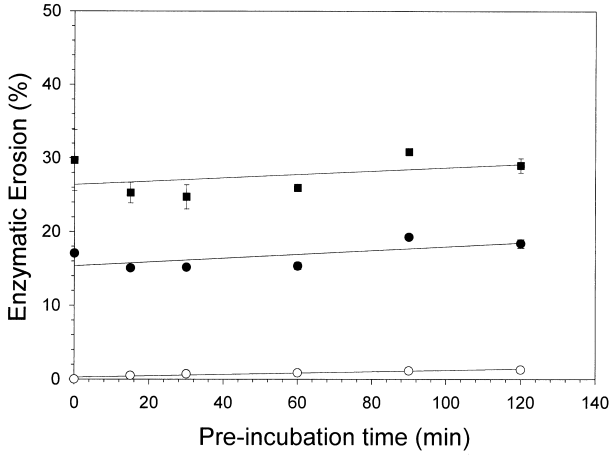

Fig. 5. Effect of pre-incubation time of CLA tablets in simulated gastric fluid (0.1 N HCl) on enzymatic erosion of CLA tablets incubated for 2 (●) and 4 h (■) in phosphate buffer (pH 6.8) containing α-amylase at 9000 IU/l. The control formulation was incubated 4 h in phosphate buffer without α-amylase (○). Mean ± SD ($n = 3$).

3.3.5. Incorporation of gel-forming polymer

In order to protect the CLA against α-amylase hydrolysis, one possible approach consists in reducing the diffusion of the enzyme to the CLA substrate. Three hydrophilic polymers, namely HPMC, PEO and PVAL which are able to form a gel immediately upon contact with water and which cannot be degraded by α-amylase, were incorporated in CLA tablets. Fig. 6 shows the enzymatic erosion of the tablets containing 20% (w/w) of gel-forming polymer. HPMC and PEO significantly reduced the hydrolysis rate of CLA. On the other hand, PVAL accelerated the enzymatic erosion of the tablets. The influence of HPMC concentration on the enzymatic erosion profile was also investigated (Fig. 7). After 4 h incubation, the enzymatic erosion of the tablets decreased from 30% to 10.5% with increasing HPMC concentrations from 0 to 30% (w/w).

Fig. 8 shows the release of SDic from CLA tablets containing 10% (w/w) of the three gel-forming polymers in phosphate buffer containing 9000 IU/l of α-amylase. As expected from the enzymatic erosion results, only HMPC and PEO reduced significantly (see f_2 values, Table 1) the SDic release in the presence of the enzyme. According to the FDA guidelines, the release profiles with HPMC and PEO in the presence of α-amylase were similar to that of the control formulation (dissolution of CLA tablets in phosphate buffer without α-amylase). The similarity factors f_2 were 73 and 61 for CLA tablets containing HPMC and PEO, respectively (Table 1).

4. Discussion

CLA (Contramid) was recently introduced as a new controlled release excipient. Upon hydration and in the absence of α-amylase, CLA tablets swell and form a rubbery gel interface with minimal erosion. The tablets

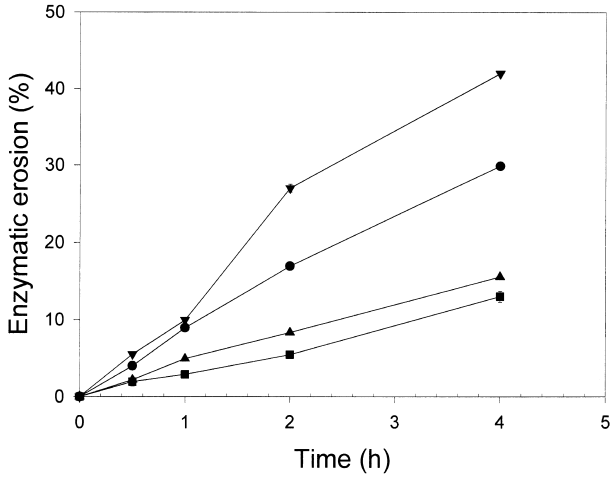

Fig. 6. Effect of the addition of 20% of a gel-forming polymer on the enzymatic erosion of CLA tablets incubated in phosphate buffer pH 6.8 containing α-amylase at 9000 IU/l: control (●), HPMC (■), PEO (▲) and PVAL (▼). Mean ± SD ($n = 3$).

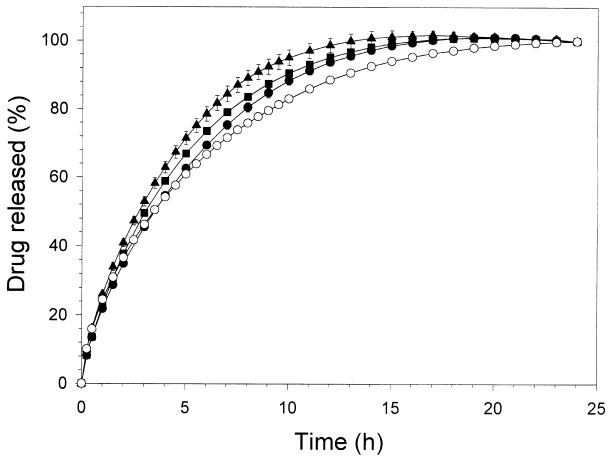

Fig. 8. In vitro release of SDic from CLA tablets containing 10% of a gel-forming polymer. Dissolution was carried out in phosphate buffer (pH 6.8) containing α-amylase at 9000 IU/l except for the control where α-amylase was absent in the dissolution medium. HPMC (●), PEO (■), PVAL (▲) and the control (○). Mean ± SD ($n = 3$).

loose approximately 10% of their weight after 24 h, which probably reflects the release of soluble uncross-linked amylose. In the presence of α-amylase, CLA tablets are enzymatically degraded into water soluble degradation products resulting in tablet weight loss. Among these degradation products, glucose, maltose and maltotriose were identified by HPLC. The kinetic profile of the enzymatic erosion of the CLA tablets consisted of two distinct phases. The duration of the first phase varied between 2 and 4 h depending on the concentration of α-amylase in the medium. Data of the initial erosion rate (% polymer eroded/h) obtained during the first phase of hydrolysis fitted well with the model based on the Langmuir isotherm adsorption (Eq. (1)). Unlike the Michaelis–Menten kinetics, the initial hydrolysis rate of CLA tablets is not a linear function of the enzyme concentration. A hyperbolic relationship was found suggesting that adsorption could be the limiting step of CLA enzymatic degradation. These results are in agreement with the study of Sattler et al [16] describing the enzymatic degradation of the cellulase–cellulose system. The relationship between the rate of hydrolysis of cellulose and cellulase concentration was found to obey to the Langmuir equation. During the second phase which begins after about 6 h of incubation, the erosion rate is slower and is less influenced by α-amylase concentration. Several factors could be responsible for this change in enzymatic erosion rate. These factors include the increase in the crystallinity of the substrate during hydrolysis [17] and the inhibition of α-amylase activity by the hydrolysis by-products [10–13].

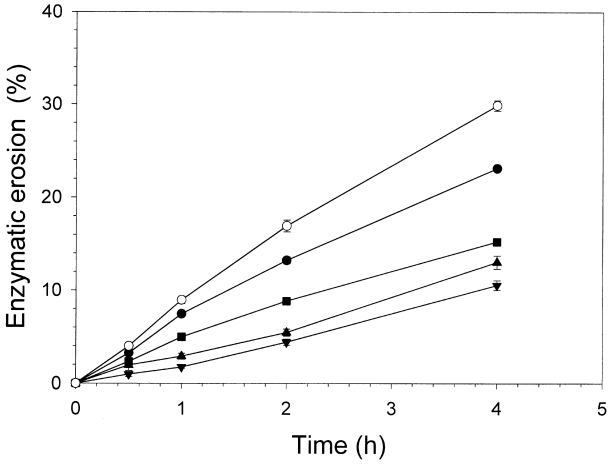

Fig. 7. Effect of the concentration of HPMC on the enzymatic erosion of CLA tablets incubated in phosphate buffer pH 6.8 containing α-amylase at 9000 IU/l: control (○), 5 (●), 10 (■), 20 (▲) and 30% (▼). Mean ± SD ($n = 3$).

SDic was released from CLA tablets in the phosphate buffer medium without α-amylase following an anomalous mechanism ($n = 0.57$) which is typical of a gel-type matrix. In the presence of α-amylase both diffusion and erosion mechanisms can operate simultaneously. The profiles of SDic release obtained at low α-amylase concentrations (<2250 IU/l) were similar to that obtained without the enzyme, as indicated by a high f_2 value (>91) (Fig. 1 and Table 1). These results suggest that the release of SDic from the CLA matrices at low α-amylase concentrations was mainly controlled by diffusion. However, at high α-amylase concentrations (>4500 IU/l) erosion became significant with increased release rate and linearity ($0.57 \leq n \leq 0.70$).

Varying the compression forces between 1.4 and 6 kN affected significantly the crushing strength of the tablets (Fig. 4, inset). Tablets prepared at a low compression force (3.3 kN) lost more than 57% of their weight within 4 h in the presence of α-amylase (9000 IU/l) (Fig. 3). In previous studies conducted on CLA tablets, it was found that the major changes in crushing strength and tablet poros-

ity occurred for compression forces between 1 and 5 kN [21,22]. The average pore diameters of dry CLA tablets were found to be 89, 27 and 19 nm for compression forces of 1, 3 and 5 kN, respectively. These pore sizes do not necessarily correspond to the exact pore size of the swollen material, since upon hydration, the swelling of CLA granules reduces the void volume, creating a heterogeneous gel having low and high gel density regions [23]. One can assume that α-amylase degrades more rapidly highly porous gels and especially the fraction of the gel with a low density of material. In contrast, tablets loaded with 5% SDic seem to be less affected by enzymatic degradation. This was also indirectly reflected in the SDic release rate which showed little variation with increasing compression forces. As mentioned previously, the total amount of SDic released in a phosphate buffer containing 9000 IU/l of α-amylase was governed by both diffusion and enzymatic erosion mechanisms. Decrease of compression force leads to an increase in the diffusion coefficient of the drug in the tablets and a slower increase of SDic release rate [4]. On the other hand, the decrease in compression force increases tablet thickness, creating a longer path length for drug diffusion, and this might explain why the overall release rate of SDic from CLA tablets is slightly affected by compression force.

At compression forces above 6 kN the physical characteristics of the dry tablets remained constant. The physical properties of the swollen tablets is also thought to remain constant since Ravenell et al. [23] reported that the stiffness of the swollen CLA tablets was not affected by compression forces greater than 6.5 kN. The pore size of the dry CLA tablets is known to be 17 nm [22] and upon hydration this porosity is expected to fall far below the hydrodynamic radius of α-amylase which is about 15 nm [12]. This implies that α-amylase would not diffuse easily through the swollen tablet prepared at compression forces above 6 kN, and its hydrolytic action will be limited to the surface of the tablets. This explains why the erosion rate and drug release kinetics did not change as observed in Figs. 3 and 4.

Enzymatic erosion of CLA tablets was barely affected by the ionic strength of the medium. This result was expected since both α-amylase activity and physical properties of the swollen tablets (water uptake and stiffness of the gel) are not affected by the presence of salts in the incubation medium at concentrations ranging from 0.1 to 0.5 M [23]. Also, pre-incubation of CLA tablets in an acidic medium had no effect on the hydrolysis of CLA by α-amylase (Fig. 5). The acidic medium taken up during tablet swelling is probably rapidly neutralized in phosphate buffer. Accordingly, the catalytic activity of α-amylase should not be altered by the transit of the tablets from the stomach to the small intestine.

Incorporation of gel-forming polymers such as HPMC and PEO into the tablets at a concentration of 20% decreased significantly the enzymatic erosion rate (Fig. 6). As reported by Chouinard and Lenaerts [24], these polymers swell and form a gel rapidly when in contact with water and thus, reduce the availability of the substrate. Furthermore, the dissolution results (Fig. 8) showed that 10% of HMPC or PEO in CLA tablets significantly reduced (see f_2 values, Table 1) the release rate of SDic in the enzymatic medium, compared with the CLA tablets without the gel-forming polymers. The dissolution profiles obtained in the presence of amylase were similar to the reference (dissolution of CLA tablets in phosphate buffer without α-amylase) as indicated by the high f_2 value (Table 1), suggesting that 10% of HPMC or PEO into the tablets was efficient in protecting CLA against α-amylase hydrolysis. PVAL, on the other hand, increased the enzymatic erosion of the tablet. This polymer has a relatively low molecular weight. Thus, it rapidly dissolves and is released from the tablet, thereby increasing the tablet porosity. In addition to creating passageways that can facilitate the penetration of the enzyme into the matrix, it also increases the effective adsorption surface area and thus increases the hydrolysis rate of CLA.

CLA is a highly compressible material, and chemically biocompatible with several active ingredients. Upon hydration with aqueous medium containing no α-amylase, CLA tablets swell and form a rubbery gel interface with minimal erosion. In the presence of α-amylase, CLA tablets are enzymatically degraded. The initial erosion rate increases with increasing α-amylase concentrations in the incubation medium until a plateau is reached. Pre-incubation of CLA tablets in acid medium, ionic strength of the incubation medium, and compression force in excess of 6 kN had little or no effect on the enzymatic erosion of the tablets and on SDic release. Incorporation of HPMC or PEO into the tablet reduced enzymatic erosion of CLA matrices and SDic release.

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References

[1] H. Röper, Applications of starch and its derivatives, Carbohydr. Eur. 15 (1996) 14–21.
[2] V. Lenaerts, Y. Dumoulin, M.A. Mateescu, Controlled release of theophylline from CLA tablets, J. Control. Release 15 (1991) 39–46.
[3] M.A. Mateescu, H.D. Schell, A new amyloclastic method for the selective determination of αamylase using cross-linked amylose as an insoluble substrate, Carbohydr. Res. 124 (1983) 319–323.
[4] M. Rahmouni, V. Lenaerts, J.C. Leroux, Solute transport across cross-linked high amylose starch (Contramid®) hydrogels, Proc. Int. Symp. Control. Release Bioact. Mater. 27 (2000) 724–725.
[5] Y. Dummoulin, L.H. Cartilier, M.A. Mateescu, Cross-linked amylose tablets containing α-amylase: an enzymatic-controlled drug release system, J. Control. Release 60 (1999) 161–167.
[6] P. Snow, K. O'Dea, Factors affecting the rate of hydrolysis of starch in food, Am. J. Clin. Nutr. 34 (1991) 2721–2727.

[7] P. Colonna, V. Leloup, A. Buleon, Limiting factors of starch hydrolysis, Eur. J. Clin. Nutr. 46 (1992) S17–S32.
[8] H. Jacobs, R.C. Eerlinger, H. Spaepen, P.J. Grobet, J.A. Delcour, Impact of annealing on the susceptibility of wheat, potato, and pea starches to hydrolysis with pancreatin, Carbohydr. Res. 305 (1998) 193–207.
[9] E.A. MacGregor, α-Amylase structure and activity, J. Protein Chem. 7 (1988) 399–415.
[10] V.M. Leloup, P. Colonna, S.G. Ring, α-Amylase adsorption on starch crystallites, Biotechnol. Bioeng. 38 (1991) 127–134.
[11] D.J. Gallant, B. Bouchet, A. Buleon, S. Perez, Physical characteristics of starch granules and susceptibility to enzymatic degradation, Eur. J. Clin. Nutr. 46 (1992) S3–S16.
[12] V.M. Leloup, P. Colonna, S.G. Ring, Physico-chemical aspects of resistant starch, J. Cereal Sci. 16 (1992) 253–266.
[13] V. Planchot, P. Colonna, D.J. Gallant, B. Bouchet, Extensive degradation of native starch granules by α-amylase from *Aspergilus sumnigapus*, J. Cereal Sci. 21 (1995) 163–171.
[14] R. Cui, C.G. Otes, The effect of retrogradation on enzyme susceptibility of sago starch, Carbohydr. Polym. 32 (1997) 65–72.
[15] R.C. Erlinger, H. Jacobs, L.A. Delcour, Enzyme-resistant starch. V. Effect of retrogradation of waxy maize starch on enzyme susceptibility, Cereal. Chem. 71 (1994) 351–355.
[16] W. Sattler, H. Esterbaur, O. Glatter, W. Steiner, The effect of enzyme concentration on the rate of the hydrolysis of cellulose, Biotechnol. Bioeng. 33 (1989) 1221–1234.
[17] P. Colonna, A. Buleon, F. Lemarié, Action of Bacillus subtilis α-amylase on native wheat starch, Biotechnol. Bioeng. 31 (1988) 895–904.
[18] J.W. Moore, H.H. Flanner, Mathematical comparison of curves with an emphasis on in-vitro dissolution profiles, Pharm. Tech. 20 (1996) 64–74.
[19] FDA, Centre for Drug Evaluation and Research, Guidance for Industry: Modified Release Solid Oral Dosage Forms; Scale-Up and Post-Approval Changes: Chemistry, Manufacturing and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation, September 1997.
[20] P.L. Ritger, N.A. Peppas, A simple equation for description of solute release II Fickian and anomalous release from swellable devices, J. Control. Release 5 (1987) 37–42.
[21] M. Rahmouni, M. Peyronnet, S. Gervais, F. Chouinard, Effect of moisture content and compression force on Contramid® controlled release tablets, AAPS Pharm. Sci. 1 (1999) S3987.
[22] I.S. Moussa, V. Lenaerts, L.H. Cartilier, Effect of some physical parameters on the swelling properties of cross-linked amylose matrices, Int. J. Pharm. 173 (1998) 35–41.
[23] F. Ravenelle, R.H. Marchessault, A. Légaré, M.D. Buschmann, Rheology of high amylose gelatinized starch excipient, Thirtieth Canadian High Polymer Forum, Quebec, Canada, 2000.
[24] F. Chouinard, V. Lenaerts, Protective effect of HPMC on cross-linked amylose controlled release tablets, Proc. Int. Symp. Control. Release Bioact. Mater. 24 (1997) 265–266.