Evaluation of an Enzyme-Containing Capsular Shaped Pulsatile Drug Delivery System

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Purpose. To develop an enzymatically-controlled pulsatile drug release system based on an impermeable capsule body, which contains the drug and is closed by an erodible pectin/pectinase-plug.
Methods. The plug was prepared by direct compression of pectin and pectinase in different ratios. In addition to the disintegration times of the plugs, the lag times and the release profiles of the pulsatile system were determined as a function of pectin:enzyme ratio, the pH of the surrounding medium, and the addition of buffering or chelating agents.
Results. The disintegration time of the plug, respectively the lag time prior to the drug release was controlled by the pectin:enzyme ratio and the plug weight. The inclusion of a buffering agent within the plug lead to a plug disintegration independent of the surrounding pH. The addition of Na-EDTA hindered the formation of non-soluble calcium pectinate in the presence of calcium ions in the environment. The addition of effervescent agents to the capsule content resulted in a rapid emptying of the capsule content after plug degradation.
Conclusions. A pulsatile drug delivery system based on an erodible pectin plug containing a pectinolytic enzyme was developed. The drug release was controlled by the enzymatic degradation and dissolution of pectin.

KEY WORDS: controlled drug release; enzymatically-controlled drug release; oral drug delivery; pectin; pulsatile drug release.

INTRODUCTION

Besides the dominant role of peroral controlled release dosage forms, which release the drug continuously during their gastrointestinal passage, novel systems like gastroretentive or pulsatile drug delivery systems have gained increasing interest in recent years. Pulsatile systems release the drug rapidly within a short period of time after a specified lag time during which no drug has been released (1). Ideally, pulsatile drug delivery systems should address the chronopharmacological needs of certain diseases (2). Most pulsatile systems have a reservoir-type structure, whereby a drug reservoir (e.g., a tablet) is surrounded by a barrier coating. The coating dissolves (3), erodes (4,5), or ruptures (6–9) after a specified lag time (time-controlled drug release systems) or as a result of special environmental conditions (site-controlled systems, e.g., controlled by the pH-value of intestinal fluids (10,11) or by enzymes being specifically present in the colon (12,13). The drug is then released rapidly. Polysaccharides (e.g., guar gum, amylose, dextran, pectin) (14–17), polysaccharide-derivatives (18), and synthetic polymers like azo polymers (19) have been used as barrier coatings or matrix materials, which are degraded by enzymes present in the colon.

Several single unit pulsatile dosage forms based on a capsular design have been developed, whereby an insoluble and either water-impermeable (Pulsincap®-system) (20) or semi-permeable (Port®-system) (21) capsule half is filled with drug/excipients and is closed with a plug, which is ejected from the capsule body after a specified lag time, either after swelling in contact with aqueous fluids, or by being pushed out because of the rising internal osmotic pressure. In a recent publication, a capsular device with an erodible plug has been presented (22,23). The lag time prior to the release was controlled by the erosion rate of compressed or congealed plug materials.

The objective of the present study was to develop and evaluate an alternative pulsatile drug delivery system consisting of a drug-containing, impermeable capsule body closed with an enzyme-degradable plug. The degradation of the plug material was not controlled by enzymes being present in the gastrointestinal tract, but by an enzyme being directly incorporated into the plug. The enzyme-degradable plug consisted of the natural polysaccharide pectin, which is widely used in the food industry as a thickening agent, and a pectinolytic enzyme mixture, Rohapect® D5S (24).

MATERIALS AND METHODS

Materials

The following materials were obtained from commercial suppliers and used as received: pectin (Pektin Classic AU 702, Herbstreith & Fox, Neuenbürg, Germany), liquid pectinolytic enzyme, Rohament® PL, (enzyme activity: >28000 polygalacturonase units/mg), pectinolytic enzymes, Rohapect® D5S, (supplier's information: granulated mixture of pectintranseliminase and endopectinlyase with an enzyme activity of >27.7 PTF/mg, one PTF correlates to the amount of enzyme which gives an increase in extinction of 0.01/min at 235 nm at pH 5.8 and 30°C with a 0.5% aqueous pectin solution), liquid cellulase, Rohalase® 7069 (enzyme activity > 1404 cellulase units/mg) (all Röhm Enzym GmbH, Darmstadt, Germany), pepsin (enzyme activity: 8.7 U/g from hog stomach, Fluka Chemie GmbH, Neu-Ulm, Germany), zein (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany), hydroxypropyl methylcellulose (HPMC) (Methocel® E5, K4M, K15M and K100M, Colorcon Limited, Orpington, UK), mannitol (Roquette Frères GmbH, Lestrem, France), ethylcellulose (EC) (Ethocel® 7, Dow Benelux N.V., Rotterdam, Netherlands), cellulose acetate (CA) (CA-398-10, Eastman Fine Chemicals, Kingsport, TN, USA), triethyl citrate (TEC) (Morflex, Inc., Greensboro, NC, USA), anhydrous calcium chloride ($CaCl_2$) and magnesium stearate (Caelo, Caesar & Loretz GmbH, Hilden, Germany), colloidal silicium dioxide (Aerosil® 200, Degussa AG, Hanau, Germany), potassium dihydrogen phosphate (KH_2PO_4), Titriplex® III (Na-EDTA), sodium bicarbonate, anhydrous citric acid, methyl red, methyl orange and congo red (E. Merck, Darmstadt, Germany), poly(propylene) tube as the impermeable capsule (Recker GmbH, Berlin, Germany), ethanol, acetone, (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany), chlorpheniramine maleate (CPM) (Dolorgiet GmbH, Bonn, Germany).

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Methods

Preparation and Filling of the Capsule Bodies

Cut pieces of a poly(propylene) tube (inner diameter: 8 mm, length: 15 mm) were closed at one end with compressed ethylcellulose plugs (150 mg, 100 N, 8 mm diameter) and additionally sealed with a 10% w/w ethanolic ethylcellulose solution (20% w/w TEC based on the polymer) to give the impermeable capsule body to be filled with the drug:excipient mixture. Chlorpheniramine maleate, the model drug, and mannitol, the filler, (sieved through a 800 μm sieve) were blended for 15 min in a Turbula®-mixer (W. A. Bachhofen Maschinenfabrik, Basel, Switzerland), followed by hand-filling of the mixture into the capsule bodies (fill weight = 450 mg; drug content = 10% w/w). For the effervescent filling, mannitol was partly replaced by 10 or 20% w/w of a mixture of $NaHCO_3$: citric acid (1:0.76 w/w) (fill weight = 500 mg, the mixture containing effervescent agents had a higher density compared to the drug-mannitol blend).

Preparation of the Degradable Plugs and the Complete Drug Delivery System

The powder (pectin and pectinolytic enzyme in different ratios, in special cases also KH_2PO_4 and Na-EDTA) (sieved through a 800 μm sieve) was blended in a Turbula®-mixer for 15 min and for an additional 5 min after the addition of 0.5% w/w magnesium stearate and 0.5% w/w Aerosil®. The plugs were prepared by direct compression with a single punch press (EKO, Korsch, Berlin, Germany) (diameter: 8 mm, hardness: 50 N, weight: 100, 150, 200 mg) (Fig. 1A).

For the preparation of the two-layer tablets (150 mg), the first layer consisting of a powder blend (75 mg) of Methocel® K4M or pectin and mannitol (1:1) was hand-filled into the die and pre-compressed, followed by hand-filling of the second layer, which consisted of freeze-dried enzyme and mannitol (3:2) and a final compression (Fig. 1B).

The compressed plugs were placed manually within the orifice of the drug-filled poly(propylene) capsule body, with the top of the capsule body and the plug being even. The enzyme-free layer of the two-layer-plug was on top of the capsule.

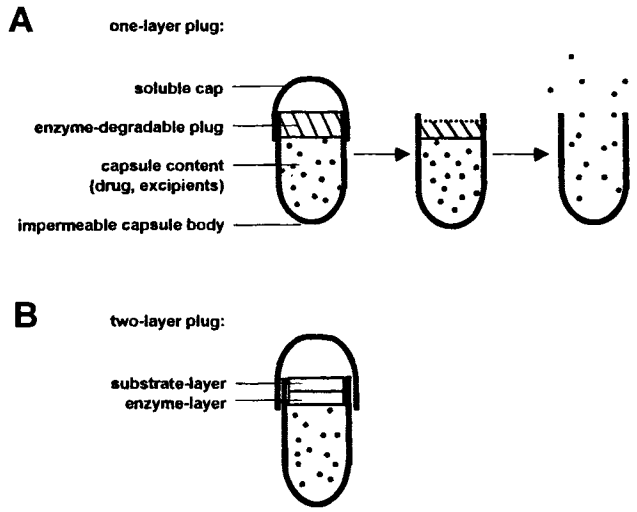

Fig. 1. Schematic diagrams of (A) a one-layer pulsatile DDS with an impermeable capsule body and an enzyme-degradable plug and (B) a pulsatile DDS with a two-layer plug.

Disintegration and Drug Release Studies

The disintegration time of the plugs (150 mg, 50 N) was determined with a disintegration tester (Erweka ZT 3, Erweka GmbH, Heusenstamm, Germany) (900 ml 0.1 M pH 7.4 phosphate buffer USP XXIII, 0.1 M HCl, simulated gastric fluid USP XXIII, pH 5.8 phosphate buffer USP XXIII or 2.5 mM $CaCl_2$-solution, 37°C, n = 3).

For the release studies, the drug delivery system was attached to a wire in order to retain it at the bottom of a transparent plastic dissolution flask (Nalgene®), which was placed in a horizontal shaker (GFL® 3033, Gesellschaft für Labortechnik mbH, Burgwedel, Germany) (500 ml 0.1 M pH 7.4 phosphate buffer USP XXIII unless otherwise mentioned, 37°C, 90 rpm, n = 3). Samples were taken at predetermined time points and analysed for drug content with a UV-spectrophotometer (Shimadzu UV-2101PC, Shimadzu Europa GmbH, Duisburg, Germany) at 261 nm.

Preliminary Studies on the pH Within Hydrated Plugs

Pectin or a mixture of pectin and KH_2PO_4 (9:1 w/w) were blended with three different solid pH-indicators: methyl red (red < pH 4.4, yellow orange > pH 6.2), methyl orange (red < pH 3.1, yellow orange > pH 4.4), and congo red (blue violet < pH 3.0, red orange > pH 5.2). The powder was wetted with demineralized water and the color change was recorded.

Preparation of Ca-Pectinate

A suspension of 6 g pectin in 16 ml ethanol (96% v/v) was mixed with an aqueous $CaCl_2$ solution (10% w/w) and stirred for 30 min. The resulting suspension was filtered, washed with ethanol and dried at 40°C overnight.

RESULTS AND DISCUSSION

Various capsular-shaped drug delivery systems have been presented in the literature, whereby the drug is released in a pulsatile fashion from an insoluble capsule half after ejection/erosion of a plug, which closes the capsule half. In this study, a capsular-shaped system with an enzyme-degradable plug was developed. The plug, which consists of a mixture of the enzyme-degradable polymer and the enzyme, acts as a release barrier. The polymer is degraded by the enzyme after contact with aqueous fluids (Fig. 1A). Ideally, no drug is released prior to the complete plug degradation, leading to a defined lag time prior to the drug release.

Various polysaccharide:enzyme mixtures (HPMC:cellulase, zein:pepsin, pectin:pectinase) were evaluated. The cellulases were available in liquid form, but had to be present in the plug in the solid state. Two approaches were investigated, first, a wet granulation of the degradable polymer with the enzyme solution and second, the freeze-drying of the enzyme solution. The latter was the method of choice because of the possible degradation of the polymeric substrate during the wet granulation step. A non-sticking cake was obtained after freeze-drying the enzyme solution containing mannitol as a bulking/

cryo-protective agent. The addition of mannitol was necessary in order to obtain a cake, which could be ground and compressed together with the polymer into a plug. After the successful drying of the enzyme solution, different plug compositions with HPMC were evaluated in the form of one-layer or two-layer plugs. The one-layer system (150 mg) was prepared by compression of a homogenous mixture of freeze-dried cellulase/mannitol (3:2) and Methocel® K4M (1:1) (Fig. 1A). In the two-layer system, Methocel® K4M/mannitol (1:1) (75 mg) were compressed into a top layer and freeze-dried cellulase/mannitol (3:2) (75 mg) into the sub-layer (Fig. 1B). The two-layer plugs were prepared to separate the polymeric substrate from the enzyme and to protect the enzyme from possibly degrading enzymes in GI-fluids. However, the lag-time prior to drug release from the two layer-DDS was longer than 6 h and the subsequent drug release occurred in a sustained and not pulsatile manner because the plug material was not completely emptied from the orifice. Therefore, the two-layer system was not suitable and the one-layer system was further investigated. The disintegration time of an enzyme-free Methocel® K4M-mannitol-(1:1)-plug was more than 480 min, whereas the disintegration time decreased to 145 min with the addition of cellulase (plug composition: Methocel® K4M: freeze-dried cellulase-mannitol (3:2) in a ratio of 1:1). Unfortunately, with HPMC, the results were not reproducible due to difficulties of incorporating the freeze-dried cellulase-mannitol homogeneously into the plug matrix. Another investigated polymer-enzyme combination was zein and pepsin, both are available as solids. In preliminary disintegration studies, the compressed zein swelled in aqueous media and was degraded in acidic medium in the presence of pepsin. The drawback of this enzyme-polymer-system is the dependence of the degradation process not only on the amount of pepsin present in the plug, but also on the physiological pepsin in the stomach. An enteric coat could protect the zein plug from exposure to gastric fluids, however, pepsin has its pH-optimum at lower pH-values, which are not reached in the intestine. A buffering agent would be required in the plug.

The optimal enzymatically degradable plug material selected was pectin, and the corresponding enzyme was Rohapect® D5S; both are available in the solid state. Pectin is a natural polysaccharide consisting of mainly α-1,4-linked galacturonic acid units in the backbone with some units being esterified with methanol. Depending on the natural source, neutral sugars, such as arabinose and galactose are present in the side chains and rhamnose is a possible unit in the main chain (25). Pectins are classified with respect to their molecular weight and their degree of esterification, both influencing the gelation properties. The pectin used in this study had a low degree of esterification (38.4%), leading to a higher sensitivity to enzymatic degradation because of a better access to the bonds of unesterified galacturonic acid units, in particular for enzymes of the polygalacturonase type (26). Additionally, the chosen pectin had a relatively high molecular weight, which dissolved slower in aqueous media and therefore had a better barrier function as plug material. The pectinolytic enzyme is frequently used in the juice and vine production to degrade soluble and insoluble pectins present in the cell walls of fruits, resulting in a solution of low viscosity. After fermentation, it remains in the product and is destroyed by heat inactivation.

Several studies have evaluated pectin as a potential carrier material for colonic drug delivery (17,27). The natural polymer is not degraded in the upper parts of the gastrointestinal tract, but is specifically degraded by enzymes in the colon. Two types of polygalacturonase enzymes were identified: a) a lyase (pH-optimum at pH 8), which requires calcium-ions as cofactor, degrading pectin to oligosaccharides (Michaelis constant, k_m = 40–70 μg/ml); and b) a hydrolase (pH-optimum pH 4–6), which degrades pectin to galacturonic acid units (k_m = 350–400 μg/ml) (28). In this study, the degrading enzyme composition was directly blended with pectin and compressed in a plug. Storage at room temperature without activity loss was possible (24) and no additional processing steps, like freeze-drying or granulation, were necessary.

Before studying the complete drug delivery system, the optimal plug composition was found by determining the plug disintegration time in different media. The disintegration times of pectin-enzyme-tablets (ratio of 9.5:0.5; weight of 150 mg) were approx. 200 min in 0.1 M HCl, 135 min in pH 5.8 buffer and 100 min in pH 7.4 buffer (Fig. 2). In addition to the pH-dependent activity of the enzyme, these results were consistent with the decreased solubility of pectin at low pH-values, at which the galacturonic acid units are protonated. In pH 7.4 buffer, the tablets initially maintained their integrity, then they swelled and continuously degraded.

The optimal pectinase activity is reported to be at pH 4–8 (29), while there is almost no enzymatic activity at pH 3–3.5. The pH of an aqueous pectin solution and hence the pH in the direct vicinity of the pectinolytic enzyme within the hydrated plug is between 3–3.5. A buffering agent, KH_2PO_4, was therefore included in the plug to increase the pH-value of the hydrated plug. Physical mixtures of pectin/KH_2PO_4 (9:1) with different pH-indicators were examined in order to estimate the pH within the hydrated plug. The following results were obtained after addition of some droplets of purified water to the powder blends: methyl orange - a mixture of yellow (> pH 4.4) and red (< pH 3.1) dots indicated different pH regions throughout the mixture; congo red blue color, methyl red - a red color, corresponding to a pH between pH 4.4 and 5.2. The addition of KH_2PO_4 improved the enzymatic activity within the plug by adjusting the pH near the enzyme's pH-optimum. After the addition of 10% w/w KH_2PO_4 to the plug mixture, the disintegration times were significantly lower in 0.1 M HCl and pH 7.4 buffer, while the values remained the same at pH 5.8 (Fig.

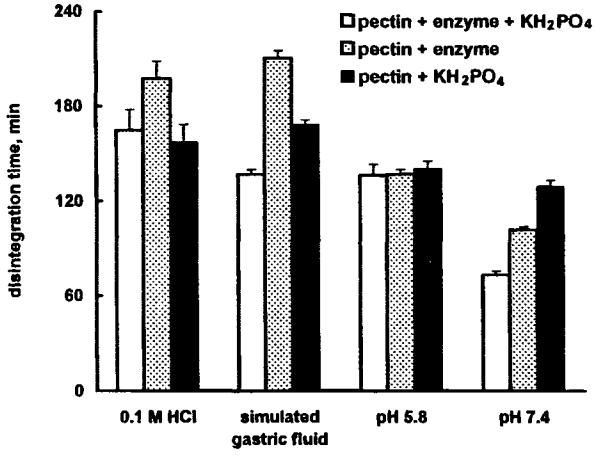

Fig. 2. Disintegration time of pectin-enzyme plugs (9.5:0.5) with and without KH_2PO_4 (10% w/w) and enzyme-free pectin-KH_2PO_4 (10% w/w) plugs.

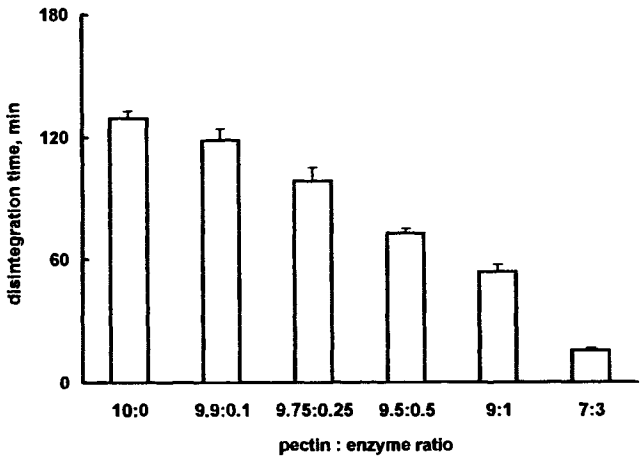

Fig. 3. Disintegration time of plugs as a function of the pectin:enzyme ratio (ratio of 10:0, 9.9:0.1, 9.75:0.25, 9.5:0.5, 9:1, 7:3) with KH_2PO_4 (10% w/w).

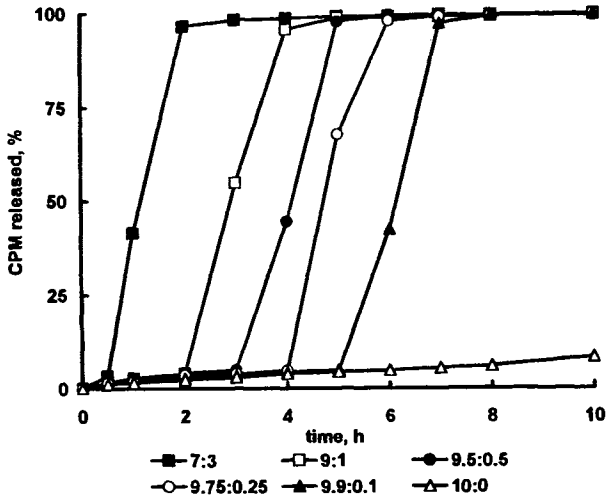

Fig. 4. Chlorpheniramine maleate release as a function of the pectin:enzyme ratio within the plug.

2). Tablets without pectinolytic enzyme, but with 10% w/w KH_2PO_4 were also investigated. Here, no changes in disintegration times were observed, proving that the difference in disintegration time was due to the enzyme and not to a higher pectin solubility at a higher pH or the influence of the water-soluble KH_2PO_4. There was no difference in disintegration time between enzyme-containing and enzyme-free plugs in 0.1 M HCl (disintegration time: 164 min vs. 157 min) because of the low enzyme activity, while a distinct difference was observed at pH 7.4.

The protein nature of the pectinolytic enzyme might lead to its degradation in the gastrointestinal environment, e.g., by pepsin present in gastric fluids. Therefore, the disintegration time in the presence of pepsin (simulated gastric fluid USP XXIII) was determined. The disintegration times for plugs (9.5:0.5 pectin:enzyme) with additional KH_2PO_4 were lower (137 min vs 164 min), whereas without KH_2PO_4, the disintegration time remained almost the same (210 min vs. 197 min in 0.1 M HCl) (Fig. 2). Pepsin has its pH-optimum at pH 1, where the pectinolytic enzyme was inactive.

Other proteolytic luminal enzymes of the gastrointestinal tract might also attack the incorporated pectinolytic enzyme. However, the luminal enzymes are large molecules, which might not be able to diffuse into the hydrated/gelled plug that forms at pH-values between pH 3 and 6.5 or in the presence of calcium ions, and therefore probably would not inactivate the pectinolytic enzyme within the plug but, if at all, only on the exposed surface. The pectinolytic enzyme would also be trapped within the gelled matrix. At pH-values below 3, the pectin matrix does not hydrate and gel, the pectinolytic enzyme could dissolve and possibly leach from the plug prior to hydration and pepsin might also penetrate the plug and inactivate the enzyme. A protective enteric coat would be essential for a future drug delivery system to avoid varying release profiles due to the pH-influences on the pectin solubility and to minimize the influence of proteolytic enzymes in the stomach on the enzyme activity within the plug.

The disintegration time of pectin:enzyme plugs decreased with increasing enzyme concentration (Fig. 3). At pH 7.4, the disintegration time of compressed plugs (150 mg) decreased from 120 to 15 min with an increasing enzyme concentration within the plug (pectin:enzyme ratio of 9.9:0.1 up to 7:3 w/w). Enzyme-free pectin tablets dissolved/disintegrated after 130 min. The pectin:enzyme ratio was therefore an easy tool to influence the disintegration time and therefore, also the lag time of the complete drug delivery system.

Next, the drug release from the complete drug delivery system was investigated. It consisted of an impermeable capsule body containing the model drug chlorpheniramine maleate and mannitol as inert excipient, which was closed with the enzyme-degradable plug (100 mg). After a lag time, the complete capsule content was released within a 1 to 2 hour period (Fig. 4). The lag time decreased from 312 min at a pectin:enzyme ratio of 9.9:0.1 to 30 min at a ratio of 7:3 (Fig. 5) and was easily controlled by varying the enzyme concentration within the plug. This correlated well with the observed disintegration times of the plugs. The baseline of the release profile was not exactly at 0% because of the absorption of the dissolved pectin at the wavelength used for the determination of the drug. No drug release occurred prior to the almost complete disintegration of the plug. The plug eroded from the surface with the penetrating dissolution medium, which hydrated the pectin and induced the

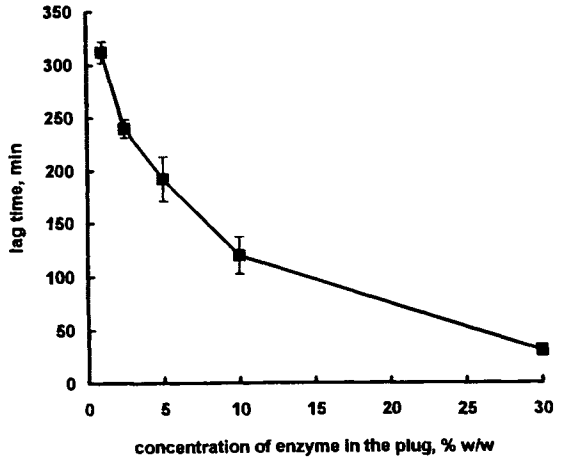

Fig. 5. Lag time of the drug delivery system as a function of the enzyme concentration within the plug.

enzyme activation. The plug completely disintegrated and no remaining parts or a viscous pectin solution hindered the complete release of the capsule content.

Plugs of different weight, respectively height (weights of 100, 150 and 200 mg with a corresponding plug height/thickness of 1.57, 2.52 and 3.32 mm) were investigated at a pectin-enzyme-ratio of 9:1 w/w. The lag time prior to drug release increased with a higher plug weight (Fig. 6).

In related studies (22), the composition of the capsule content affected the lag time and the emptying of the capsule. The lag time and the time necessary for the release of the drug content after the erosion of the plug were reduced by including effervescent agents within the capsule content. Observing the release profile in Fig. 4, it was obvious the drug was released over a 1 to 2 hour period after erosion of the plug, shorter release times were therefore desirable. The addition of effervescent agents to the capsule content did not affect the lag time, but resulted in the desired, steeper release profile. At a concentration of 20% effervescent agents, the emptying of the capsule content lasted only about 5–10 min from the first detected gas bubbles until the last solid particles disappeared from the inside of the capsule.

Besides the composition of the capsule content, the position of the capsule in the dissolution vessel had an influence on the release behavior. In the beginning, the capsules were always fixed on the bottom of the dissolution vessel in an upright, vertical position. This position, however, retarded the drug release because of a lower agitation and little exchange of the medium within the capsule and the external phase. A wire was attached to the capsule to overcome this problem and the capsule was kept in a horizontal position with the orifice at the side within the dissolution vessel.

The less soluble calcium salt of pectin has also been used as a carrier material. It has an additional retarding effect because of its lower solubility. A comparison of the release profiles of the drug delivery systems prepared with either pectin or calcium pectinate plugs revealed that the desired pulsatile release profile was only obtained with pectin plugs (results not shown). The calcium pectinate plugs disintegrated slower and not completely, parts of the plug remained in the capsule opening and acted as a barrier for the release of the capsule content. Additionally, the reproducibility of the release onset was poor. Calcium pectinate was therefore not a suitable plug material.

Calcium ions are present in gastrointestinal fluids and their influence on the disintegration time of the pectin plug had to be examined. The disintegration time was determined in 2.5 mM $CaCl_2$ solution, a concentration which corresponds to physiological conditions (29). The pectin plugs swelled in the medium and the disintegration time in the presence of calcium ions was 145 min compared to a disintegration time of 95 min in demineralized water and of 72 min in pH 7.4 phosphate buffer (Fig. 7A). The lag time was also prolonged in 2.5 mM $CaCl_2$ solution when compared to pH 7.4 phosphate buffer (Fig. 7B). Pectin with a low degree of methoxylation forms a gel in the presence of low amounts of calcium ions, whereas a higher calcium ion concentration leads to the precipitation of calcium pectinate. If the interaction of calcium ions and pectin was undesired, a complexing agent could be incorporated in the plug. Na-EDTA was homogeneously mixed within the plug matrix at concentrations of 0, 1, 5, 10, 20, or 30% w/w. The disintegration time of the plugs decreased with increasing Na-EDTA concentration (Fig. 8). The plugs containing Na-EDTA

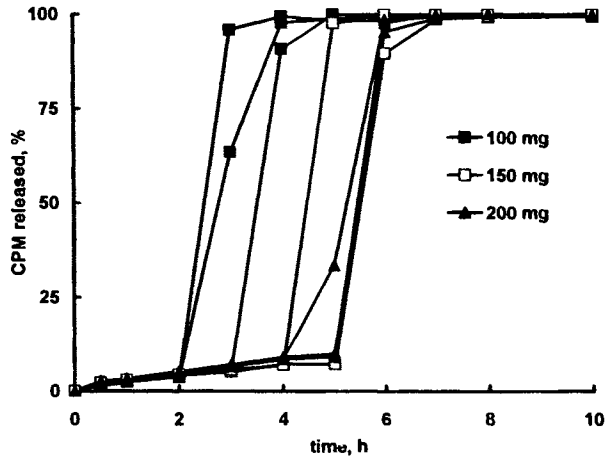

Fig. 6. Chlorpheniramine maleate release as a function of the plug weight (100, 150, 200 mg) (plugs with a pectin:enzyme ratio of 9:1).

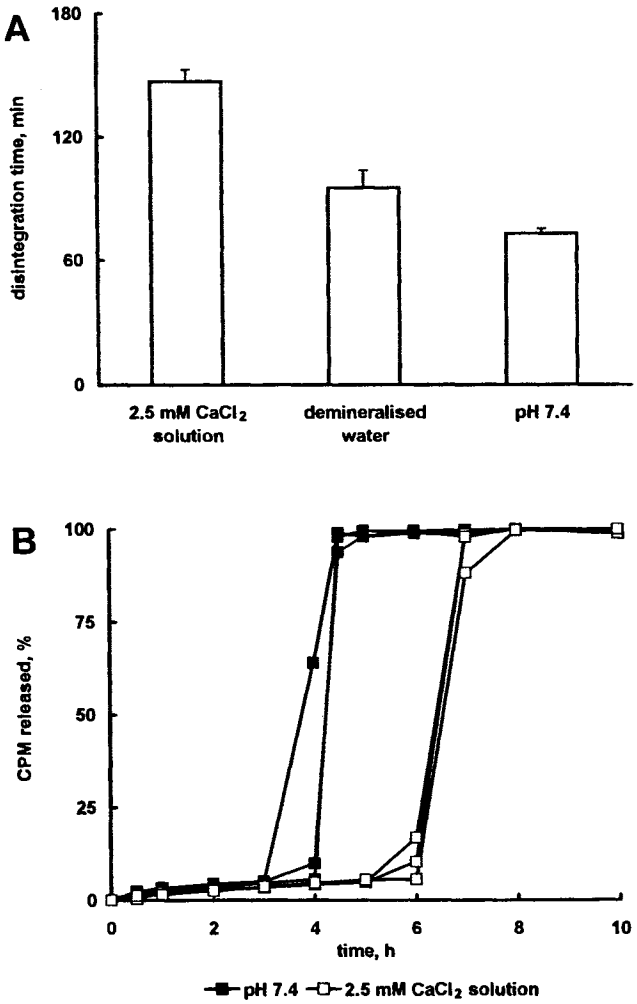

Fig. 7. A. disintegration time of pectin-enzyme plugs (pectin:enzyme ratio of 9.5:0.5) in different media (2.5 mM $CaCl_2$ solution, pure water or phosphate buffer pH 7.4 USPXXIII), B. effect of release medium on the chlorpheniramine maleate release (pectin-enzyme ratio of 9.5:0.5).

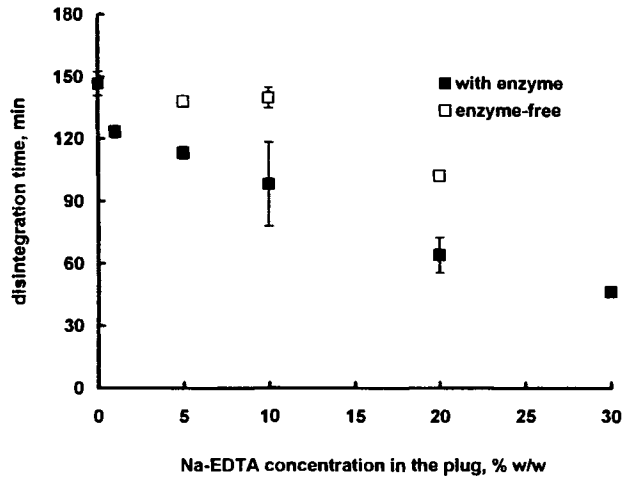

Fig. 8. Disintegration time of pectin-enzyme plugs (pectin:enzyme ratio of 9.5:0.5, 150 mg weight) in 2.5 mM $CaCl_2$ solution with and without enzymes as a function of the Na-EDTA concentration.

showed very little swelling when compared to Na-EDTA free plugs. The disintegration of the plug matrix was faster with increasing Na-EDTA concentration because of the absence of gel formation. The disintegration times of pectin-EDTA-tablets without enzyme were higher when compared to the values of plugs with the pectinolytic enzyme, indicating no negative influence of EDTA on the enzyme activity.

The major advantage of the discussed system compared to the previously developed system with erodible/soluble plugs is the complete degradation of the polymer. With other polymers as plug material, a highly viscous polymer solution sometimes remained in the orifice of the capsule body, which possibly retarded the drug release from the capsule body, while the viscous solution of pectin is completely degraded by pectinolytic enzymes and a non-hindered diffusion and release of the content was possible.

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