ELSEVIER

International Journal of Pharmaceutics 174 (1998) 233–241

international journal of pharmaceutics

Leaching of pectin from mixed pectin/insoluble polymer films intended for colonic drug delivery

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Received 17 March 1998; received in revised form 27 July 1998; accepted 2 August 1998

Abstract

Investigations intended to combine pectin HM or calcium pectinates with commercially available aqueous polymer dispersions for colon-specific drug delivery have been conducted on isolated films. The mixed films were prepared from Aquacoat® ECD 30, Surelease® clear, Eudragit® RS30D or Eudragit® NE30D containing 5, 10 or 15% w/w (related to insoluble polymer content) of pectin HM or 10% w/w of calcium pectinates. The kinetics of pectin leaching from the mixed films, incubated in 0.05 M acetate–phosphate buffer (pH 4.5, 37°C) in the absence of pectinolytic enzymes, showed that pectin HM or calcium pectinates were quickly released from the different films except from the mixed pectin/Eudragit® RS films. Moreover, in these cases, the leaching of pectin from Eudragit® RS films containing up to 10% w/w (related to Eudragit® RS polymer content) of pectin HM or pectin LM, was significantly faster in the presence of enzymes than in absence. These results indicate that the associations of pectin HM or LM and Eudragit RS are likely to give more suitable coating materials for colon-specific drug delivery than the other combinations.

Keywords: Colonic drug delivery; Pectin HM; Calcium pectinates; Eudragit®; Aquacoat®; Surelease®

1. Introduction

Targeting of drugs to the colon by the oral route could be achieved by different approaches including matrix and coated systems, for which the drug release is controlled by the gastrointestinal pH, transit times or intestinal flora (Watts and Illum, 1997). The method by which the drug release will be triggered by the colonic flora appears to be more interesting with regard to the selectivity (Rubinstein, 1990). A number of syn-

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0378-5173/98/$ - see front matter
PII S0378-5173(98)00269-5

thetic azo polymers and natural or modified polysaccharides (chondroitin sulphate, guar gum, locust gum, inulin, dextrans, starch, amylose, pectins) degraded by the human colonic flora, have thus been investigated as colonic drug delivery carriers (Watts and Illum, 1997).

The major problem encountered with the native degradable polysaccharides is their high solubility in aqueous media. As a consequence, film coatings consisting of these polymers alone will be unable to prevent the release of drugs during the transit through the stomach and the small intestine. As example, Sriamornsak et al. (1997a,b) have shown that theophylline pellets coated with calcium pectinate release about 80% of their drug content within 4 h whereas the mouth to caecum transit time of solid dosage forms is generally known to be largely higher than this duration (Davis et al., 1984, 1986).

However, the incorporation of hydrophilic degradable polysaccharides in water-insoluble film-forming polymers, such as cellulosic or acrylic polymers could provide a promising alternative. Indeed, studies on isolated films obtained from Eudragit® RS incorporating inulin HP (highly polymerized) have shown that such films, incubated in presence of human faeces, exhibited a higher permeability than those incubated in the control medium (Vervoort and Kinget, 1996). The association of amylose-butan-1-ol complex and ethylcellulose aqueous dispersion (Ethocel®) in the 1:4 (w/w) ratio, has also been used to coat 5-aminosalicylic acid and glucose pellets. The drug release from these coated pellets was much faster in the medium containing an inoculum of mixed human faecal bacteria, compared to that observed in the control medium without the same inoculum (Milojevic et al., 1996a,b). Furthermore, the in vivo studies have also demonstrated that the site of glucose release from amylose-ethylcellulose coated [^{13}C]glucose microspheres was specifically the colon (Cummings et al., 1996).

Pectins are hydrophilic linear polysaccharides extracted from plant cell walls, chiefly consisting of partially methoxylated poly α-(1$\rightarrow$4)-D-galacturonic acids. They are generally regarded as non toxic material, mainly used as gelling agents (May, 1990) and are completely digested by the colonic bacteria (Cummings et al., 1979). They have also film forming properties (Coffin and Fishman, 1993, 1994). The potential of pectin HM (Ashford et al., 1993, 1994) and calcium pectinates (Rubinstein et al., 1993; Rubinstein and Radaï, 1995) for colon-specific drug delivery being also reported, it has been thought that coatings prepared from cellulosic or acrylic insoluble polymer aqueous dispersions (commercially available for controlled release formulations) incorporating appropriate amounts of pectins should be more suitable for targeting of drugs to the colon. The integrity of such coatings could be better controlled by preventing a too fast swelling and solubilization of pectin during the transit from mouth to caecum. On the other hand, the coating degradability by the colonic flora is maintained, which is expected to generate drug release pores or the coating disintegration during the colonic transit.

The aim of this work was to investigate the suitability of such an approach for achieving specific delivery of drugs to the colon using isolated mixed films, prepared from ethylcellulose (Aquacoat® ECD 30, Surelease®) or acrylic polymer (Eudragit® RS30D and NE30D) aqueous dispersions containing pectin HM or calcium pectinates. For this purpose, a kinetic study of the pectin leaching from these mixed films, incubated in acetate–phosphate buffer (pH 4.5, 37°C), in the presence or in the absence of commercial pectinolytic enzymes was conducted.

2. Materials and methods

2.1. Materials

The aqueous ethylcellulose pseudolatexes used were Aquacoat® ECD 30 (F.M.C., Newark, NJ, USA) and Surelease® clear (Colorcon, Orpington, UK). Aquacoat® ECD 30 (30% solid content) is stabilized with sodium lauryl sulfate and cetyl alcohol and requires the addition of plasticizers prior to use. Surelease® clear (25% solid

content) contains ammonium oleate and fumed silica as stabilizers and dibutyl sebacate as a plasticizer. The insoluble acrylic ester polymers in form of aqueous dispersions (Eudragit® RS30D and NE30D) were gifts from Röhm Pharma (Darmstadt, Germany). Eudragit® RS30D is a slightly cationic hydrophilic polymethacrylate and requires the addition of 10–20% of plasticizers in order to reduce the minimum film forming temperature (MFT) below 20°C (Amighi and Moës, 1996). Eudragit® NE30D is a neutral acrylic polymer, its MFT is around 5°C, and a very soft and flexible film is formed at room temperature without plasticizer (Amighi and Moës, 1997).

Dibutyl Sebacate (DBS) and triacetin, used as plasticizers, were supplied from Union Camp (USA) and Merck (Darmstadt, Germany). Pectinex® Ultra S-PL (pectinolytic enzymes, extracted from *Aspergillus niger* and having an activity of 26000 PG/ml at pH 3.5) and pectin type 170, referred to as pectin LM (low methoxyl pectin), were gifts from Novo Ferment (Dittingen, Switzerland) and Citrus Colloid (Hereford, UK), respectively. Pectin from apple, referred to as pectin HM (high methoxyl pectin) was supplied from Fluka (Buchs, Switzerland). All other materials used were of analytical reagent grade.

The degrees of methoxylation (DM) and the galacturonic acid contents of pectin HM (59.9 ± 0.6 and $81.8 \pm 0.9\%$; $n = 4$) and pectin LM (38.6 ± 1.1 and $82.3 \pm 1.3\%$; $n = 4$) were determined according to the method of the US Pharmacopeia XXIII (1995).

2.2. Methods

2.2.1. Preparation of pectin HM and calcium pectinate gels

A gel of 2% (w/w) pectin HM (used as received) was obtained by stirring at 500 rpm (propeller stirrer, Ika Werk, Germany) pectin HM with distilled water until complete dissolution. This gel was used for the preparation of mixed films containing pectin HM (Table 1).

Gels of 1% (w/w) calcium pectinates (calcium/pectin ratios: 0, 30, 60 mg/g) were obtained from pectin LM, previously purified (elimination of low molecular weight additives like dextrose). The method of purification, similar to that used for the quantification of sugar from pectin (US Pharmacopeia XXIII, 1995) was the following: 20 g of pectin LM were stirred for 1 h with 400 ml of a mixture of 95% ethanol/water/30% hydrochloride acid aqueous solution (60:40:5, v/v). After filtration, the powder was washed with six fractions of 60 ml of the previous solvent mixture, followed by 95% ethanol/water (60:40, v/v) until the complete elimination of chloride ions. Finally, after washing with 95% ethanol, the powder was dried for one night at room temperature in a vacuum oven and then for 1 h at 105°C. The low molecular weight impurity content, extracted from the received pectin LM, was about 48%.

The purified pectin LM was used to prepare 1% (w/w) calcium pectinate aqueous gels as follows: 1.5% (w/w) pectin LM solution (pH value was adjusted to 4.5 with 1 M NaOH solution) was heated, with stirring, to 80°C and then, an

Table 1
Dispersions used for the preparation of films containing 5, 10 or 15% w/w (related to the insoluble polymer content) of pectin HM

Formulation	Aquacoat® ECD 30	Surelease® clear	Eudragit® NE30D	Eudragit® RS30D
Insoluble polymer (g in dry basis)	3	3	3	3
Pectin HM (g)	0.15 or 0.3	0.15 or 0.3	0.15 or 0.3	0.15, 0.3 or 0.45
Dibutyl sebacate DBS (g)	0.72	—	—	—
Triacetin (g)	—	—	—	0.72
Water ad (g)	32.3 or 33.5	32.3 or 33.5	32.3 or 33.5	32.3, 33.5 or 34.8
Solid content (% w/w) of the dispersion	12	10	10	12
Pectin HM (in % w/w of insoluble polymer)	5 or 10	5 or 10	5 or 10	5, 10 or 15

Table 2
Dispersions used for the preparation of films containing 10% w/w (related to the insoluble polymer content) of calcium pectinates

Formulation	Aquacoat® ECD 30	Eudragit® RS30D	Eudragit® NE30D
Insoluble polymer (g in dry basis)	3	3	3
Dibutyl sebacate (DBS) (g)	0.72		—
Triacetin (g)	—	0.72	—
Calcium pectinates (g)	0.3	0.3	0.3
Water ad. (g)	60	60	60
Ca/pectin ratio (mg/g)	0 or 30	0, 30 or 60	0, 30 or 60
Solid content (% w/w) of the dispersion	6.7	6.7	5.5

aqueous solution of 3.31% (w/v) $CaCl_2.2H_2O$ was slowly added until obtaining 0, 30 or 60 mg/g as calcium/pectin ratios. Finally, the concentration of calcium pectinates was adjusted to 1% (w/w) with distilled water. After cooling and standing for one night at 4°C, these gels were used for the preparation of mixed films containing calcium pectinates (Table 2).

2.2.2. Film preparation

Appropriate amounts of 2% (w/w) pectin HM or 1% (w/w) calcium pectinate gels were slowly added with stirring into each aqueous polymer dispersion (Aquacoat®, Surelease®, Eudragit® NE30D or RS30D), previously mixed for 90 min, if required, with 24% w/w (related to the film former) of plasticizer (Tables 1 and 2). After adjusting the total solids content of the dispersions with distilled water to the desired value, 10 g of each blend containing pectin HM or 20 g of those containing calcium pectinates were cast in petri dishes (9-cm diameter). The dishes were covered with funnels in order to prevent too fast evaporation rates. After the complete evaporation of water (one night at 60°C or room temperature for Eudragit® NE dispersion), the films were cured for 24 h at 60°C, removed from the dishes and stored in a dessicator at room temperature until use. Samples of about 4 cm^2 surface area were taken from the different films, weighed and their thickness was measured using a micrometer ($n = 6$).

2.2.3. Leaching of pectin HM or calcium pectinates from the films

Film samples having a thickness ranging from 130 to 170 μm were selected and incubated (at 37°C) in 25 or 50 ml of 0.05 M phosphate–acetate buffer, pH 4.5, containing 0.05% of tween 80. At predetermined time intervals, the release of pectin HM, calcium pectinates or their degradation products into the buffer was quantified, using the method of Bitter and Muir (1962) which allows the specific assay of uronic acids. In short, galacturonic acid contained in the buffer samples is condensed, at 100°C, in concentrated sulphuric acid containing 0.025 M sodium tetraborate, into its furfural derivative. The carbazole reagent is then added to give a coloured product, whose absorbance (at 530 nm) is proportional to the galacturonic acid concentration. Calibration graphs with galacturonic acid standards (4–40 μg/ml) and blank films without pectin were used to determine the amounts of pectin in solution.

The percentage of pectin HM or calcium pectinates leached into the buffer was then calculated and plotted versus time (mean $\pm$ S.D., $n = 3$).

The effect of pectinolytic enzymes on the leaching of pectin HM or calcium pectinates incorporated in the films was studied in the same way except that, the incubation buffer contained 200 μl/100 ml of Pectinex® Ultra S-PL. The pH of 4.5 was chosen because it is close to the optimum pH activity of Pectinex® Ultra S-PL.

3. Results and discussion

Fig. 1 shows the leaching of pectin HM and calcium pectinates from mixed films prepared with Aquacoat® ECD 30 aqueous dispersion. As can be observed, these films were unable to prevent the leaching of pectin, in absence of pectinolytic en-

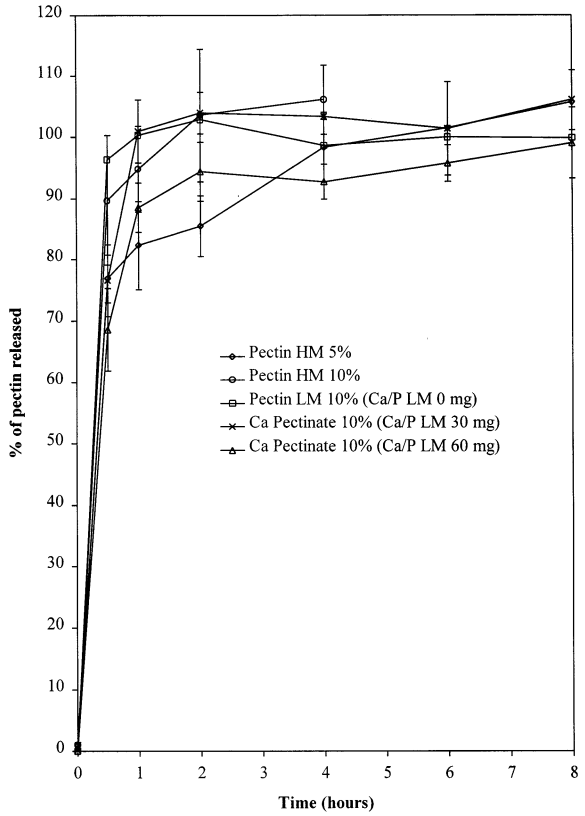

Fig. 1. Leaching of pectin (mean ± S.D., $n = 3$) into pH 4.5 acetate–phosphate buffer (without pectinolytic enzymes) from Aquacoat® films containing 5 or 10% w/w (related to the ethylcellulose content) of pectin HM or 10% w/w of calcium pectinates. Ca/P LM, calcium/pectin LM ratio.

zymes, even when the pectin content is low (pectin HM 5% w/w). Indeed, practically all the pectin content of the film is released within 30 min. The leaching rate of calcium pectinates from Aquacoat® films slightly decreases as the calcium/pectin ratio increases. This phenomenon can be attributed to the decrease of the pectin water-solubility, resulting from the physical cross-linking between the carboxylic functions of pectin molecules and calcium ions (Ca^{2+}). Nevertheless, although the water-solubility of pectin can be reduced by the calcium cross-linkage and despite Macleod et al. (1997) having recently shown that films obtained from Aquacoat® ECD 30 containing up to 20% w/w (related to the ethylcellulose content) of pectin HM show good mechanical properties, combination of pectins and Aquacoat® ECD 30 remains unsuitable for colonic drug delivery since, all the mixed films prepared from such a combination leached the totality of pectin within 1 h in absence of enzymes.

The release of pectin from Surelease® films appears to be highly dependent on the level of pectin HM in the mixed films (Fig. 2). Pectin is rapidly and almost completely released (within 30 min) in the incubation medium without enzymes from films containing high level of pectin HM (10% w/w). However, in the same incubation conditions, films obtained from Surelease® containing a lower amount of pectin HM (5% w/w) released only about 50% of their total pectin content within approximately 4 h. This initial 'burst' release probably resulted from pectin molecules localized in the vicinity of the film surface, and the

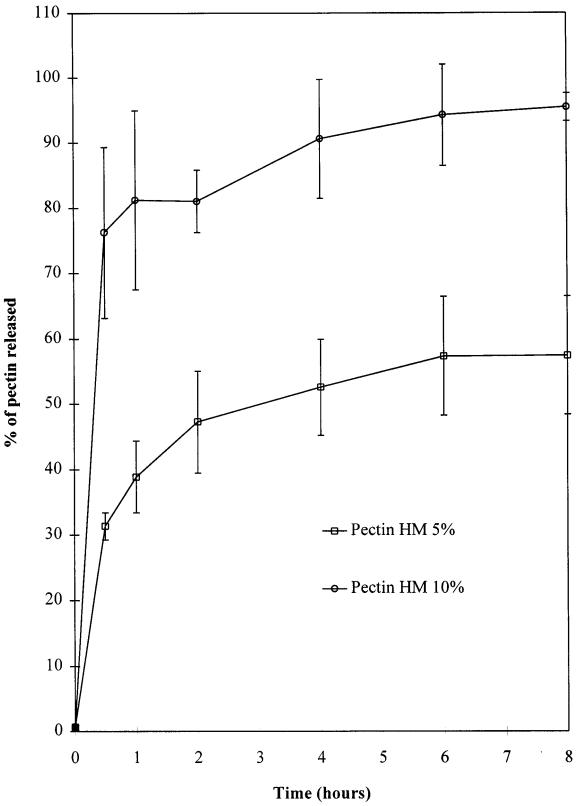

Fig. 2. Leaching of pectin (mean ± S.D., $n = 3$) into pH 4.5 acetate–phosphate buffer (without pectinolytic enzymes) from Surelease® films containing 5 or 10% w/w (related to the ethylcellulose content) of pectin HM.

remaining pectin content is entrapped in the film structure for more than 8 h. The proportion of the pectin trapped in the film for the low amount of pectin (5% pectin HM) is probably resulted from the lower porosity of the film comparatively to that containing high amount (10% pectin HM).

The incorporation of pectin HM in Eudragit® NE30D films gave similar results to those obtained with films prepared from Surelease® and pectin HM (Fig. 3). Moreover, with Eudragit® NE30D films containing 10% of calcium pectinates and in absence of enzymes, the pectin release was slower in presence of the cross-linking agent (30 and 60 mg/g) than with pectin LM (0 mg/g Ca). This retardation depends on the calcium/pectin ratio and appears to be more effective

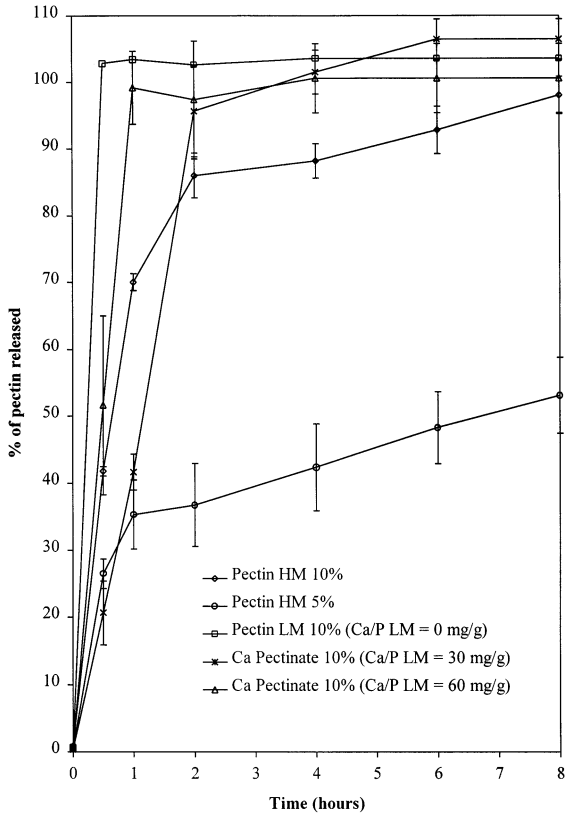

Fig. 3. Leaching of pectin (mean ± S.D., $n = 3$) into pH 4.5 acetate–phosphate buffer (without pectinolytic enzymes) from Eudragit® NE films containing 5, 10 and 15% w/w (related to the Eudragit NE polymer) of pectin HM or 10% w/w of calcium pectinate. Ca/P LM, calcium/pectin LM ratio.

for Eudragit® NE30D films containing calcium pectinate where the calcium/pectin ratio was 30 mg/g.

All these films can be considered as matrix polymeric systems from which the pectin macromolecules can diffuse out. The small molecular size substances like water-soluble plasticizers are known to diffuse from polymeric films obtained from aqueous polymer dispersions such as Aquacoat®, Eudragit® RS30D and RL30D (Bodmeier and Paeratakul, 1992). The leaching of water-soluble plasticizers (PEG-600) from cellulose acetate films is considered to be responsible of the formation of aqueous channels, through which encapsulated drugs can diffuse during the dissolution step (Guo, 1994).

On the other hand, the leaching of macromolecules from insoluble polymer films is generally believed to occur to a lesser extent. Lindstedt et al. (1991) have studied the release of hydroxypropylmethylcellulose (HPMC, used as pore forming agent) from mixed HPMC-ethylcellulose free films and have shown that the leaching of this hydrophilic polymer is only observed with films containing more than 24% w/w of HPMC. Sato and Kim (1984) investigated the diffusion of macromolecules through polymer membranes. They found that macromolecules like Insulin, Cytochrome c and Albumin diffuse through different types of polymer membranes or matrices not only via bulk water channels but also through the matrix polymer network. The extent and the rate of the macromolecule permeation were found to be dependent on the type of film-forming polymer as well as the method of the preparation of the membrane. They have postulated that the mechanism of macromolecular diffusion through polymer membranes is mainly a partition type phenomenon rather than a pore type permeation. The latter predominate in the case of permeation of hydrophilic small molecular size substances. Finally, they have also shown that the partition mechanism of the macromolecular diffusion is amplified when the film hydration is low.

In accordance with these statements, our results have shown that pectin HM or calcium pectinates, dispersed in a molecular state in the polymer films

investigated, can diffuse out of the films. The rate of the diffusion depends on the size of pectin molecule (pectin or calcium pectinate), on the type of film-forming polymer (ethylcellulose or acrylic polymer) and for the same polymer, on the type of aqueous dispersion (Aquacoat® and Surelease®). As example, calcium pectinate molecules (physical cross-linking of pectin LM with calcium), being less hydrophilic and bigger than those of pectin LM (Ca/P LM 0 mg) are generally released more slowly from the different films (Fig. 3).

Fig. 4 shows the release of pectin from Eudragit® RS films in absence of enzymes. As can be seen, only a very slow release of pectin into the buffer is observed with films containing 5 or 10% of pectin HM, while the pectin leaching is more progressive with films incorporating 15% of pectin HM, for which about 40% of the initial pectin content is released after 8 h. The cationic quaternary ammonium groups of Eudragit® RS can interact with the anionic groups (carboxylic acids) of pectin HM to form pectin-Eudragit® RS complexes which prevent the leaching of pectin. This explanation is also supported by the behaviour of calcium pectinate/Eudragit® RS films (Fig. 4). Whereas the non cross-linked pectin (pectin LM, 0 mg/g Ca) is firmly bonded to the films, the entirety of pectin content is released within 30 min from the Eudragit® RS films containing 10% of calcium pectinate (calcium/pectin ratio = 30 mg/g). A total of 30 mg of calcium per g of pectin seems therefore to be sufficient to obtain an efficient physical cross-linkage and to prevent the Eudragit® RS30D from interacting with pectin LM molecules. As a result, the behaviour of Eudragit® RS films incorporating calcium pectinate (calcium/pectin ratio = 30 mg/g) becomes similar to that observed with mixed pectin HM or calcium pectinates/neutral polymer films (Aquacoat®, Surelease®, Eudragit® NE), with regard to the leaching out of pectin.

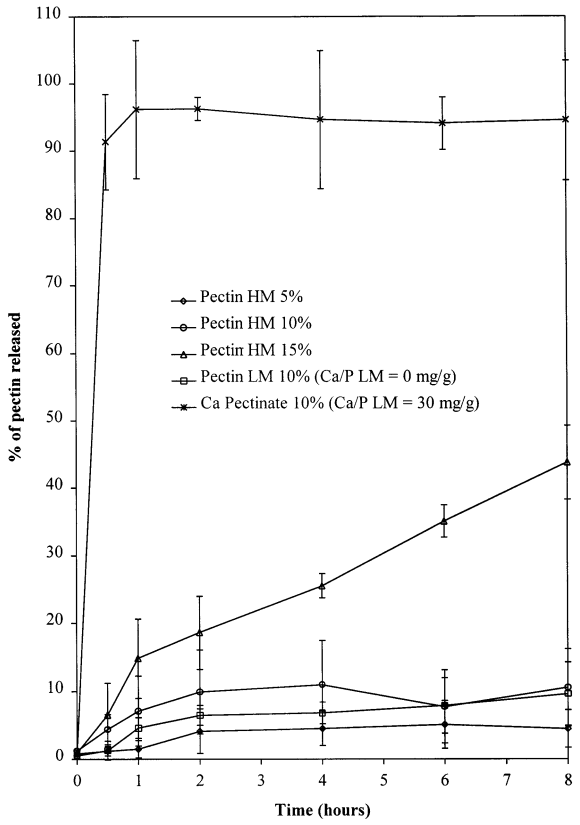

Fig. 4. Leaching of pectin (mean ± S.D., $n = 3$) into pH 4.5 acetate–phosphate buffer (without pectinolytic enzymes) from Eudragit® RS films incorporating 5, 10 or 15% w/w (related to the Eudragit RS polymer) of pectin HM or 10% w/w of calcium pectinate. Ca/P LM, calcium/pectin LM ratio.

Finally, in the buffer containing enzymes (Fig. 5), the rate and the extent of pectin leaching from Eudragit® RS films are dependent on the pectin content of the films. Films containing 5% of pectin HM appear to be not permeable enough to the pectinolytic enzymes since no significant release of pectin could be observed from these films incubated both in the absence and presence of enzymes. On the other hand, pectin was released much faster from Eudragit® RS films containing more than 10% of pectin HM (or LM) in presence of enzymes (Fig. 5) compared to that observed in the medium without enzymes (Fig. 4). The films with the highest content of pectin appear therefore to be accessible to the pectinolytic enzymes.

4. Conclusion

In this study we have demonstrated that, as in the case of the azo polymer approach for colon-

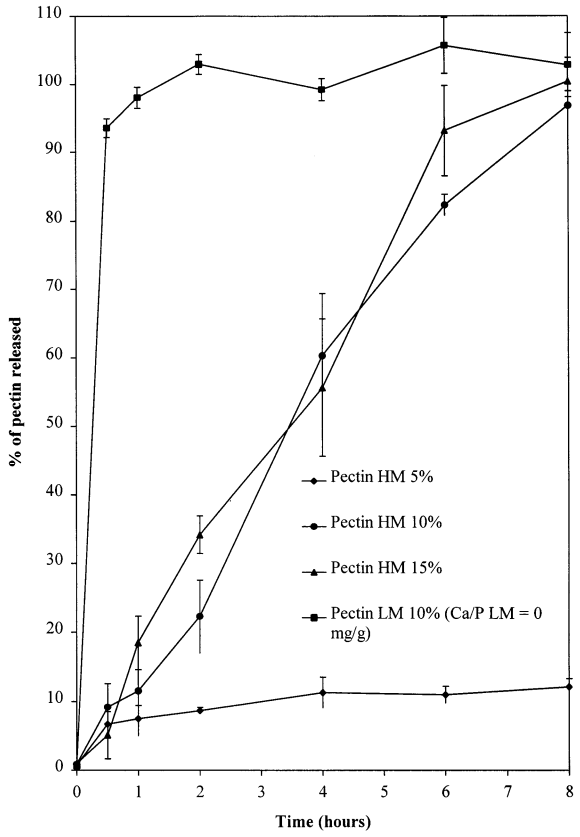

Fig. 5. Leaching of pectin (mean ± S.D., $n = 3$) into pH 4.5 acetate–phosphate buffer containing pectinolytic enzymes, from Eudragit® RS films incorporating 5, 10 or 15% w/w (related to the Eudragit RS polymer) of pectin HM or 10% w/w of pectin LM (can be considered as calcium pectinate with calcium/pectin LM ratio = 0 mg/g).

specific drug delivery (Semdé et al., 1998), it is firstly preferable to verify and understand the mechanism from which the approach of mixed pectin/cellulosic or acrylic polymer coatings as colonic drug delivery carriers is based. The kinetics of leaching from isolated films incorporating pectin HM or calcium pectinates indicate that the association of pectin HM or LM with Eudragit® RS30D are likely to give more suitable colonic coating materials for colon-specific drug delivery than the other combinations. In vitro dissolution studies using coated pellets are currently in progress.

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