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Effect of pectinolytic enzymes on the theophylline release from pellets coated with water insoluble polymers containing pectin HM or calcium pectinate

Rasmane Semdé [a], Karim Amighi [a], Michel J. Devleeschouwer [b], André J. Moës [a,*]

[a] *Laboratory of Pharmaceutics and Biopharmaceutics, Pharmacy Institute, CP 207, 1050 Brussels, Belgium*
[b] *Laboratory of Microbiology and Hygiene, Pharmacy Institute, CP 205/02, Free University of Brussels, Campus Plaine, 1050 Brussels, Belgium*

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Abstract

Theophylline pellets were coated with cellulosic (Aquacoat® ECD 30, Surelease® clear) or acrylic (Eudragit® NE30D, RS30D) polymer aqueous dispersions, containing 10% (related to the insoluble polymer content) of pectin HM or calcium pectinate, using a Uni-Glatt fluidized-bed coating apparatus. When commercial pectinolytic enzymes were added to the dissolution media (0.05 M acetate — phosphate buffer, pH 6.0), the release of theophylline from the coated pellets was generally slower than that observed in the media without enzymes. The enzymatic slowing down of the drug release, depending on the type of the aqueous polymer dispersion used, is more important with mixed Eudragit® NE/calcium pectinate coated pellets. The results obtained have been examined with regard to the validity of the approach based on the combination of pectins and the insoluble polymer aqueous dispersions intended for specific-delivery of drugs to the colon. The mechanism of the hydrophilic drug release from pellets coated with insoluble polymer aqueous dispersions containing an aqueous gel-forming polymer has been also discussed.

Keywords: Colonic drug delivery; Pectins; Insoluble polymers; Polymer aqueous dispersions; Coated pellets; Pectinolytic enzymes; Mechanism of drug release

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). Some polysaccha-

* Corresponding author. Tel.: +32-2-6505255; fax: +32-2-6505269.
E-mail address: amoes@ulb.ac.be (A. Moës)

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rides, degraded by the human colonic flora, have thus been investigated as colonic drug delivery carriers (Watts and Illum, 1997). Among these polysaccharides, pectins, chiefly consisting of partially methoxylated poly α-(1→4)-D-galacturonic acids, are completely digested by the colonic bacteria (Cummings et al., 1979). They are also film-forming polymers (Coffin and Fishman, 1993, 1994) and have a real colonic drug delivery potential (Rubinstein et al., 1993; Ashford et al., 1993, 1994; Rubinstein and Radai, 1995; Wakerly et al., 1996a).

The major problem encountered with pectins, in the field of targeted drug delivery systems, is their solubility and swelling properties in aqueous media. As a consequence, film-coatings consisting of pectins alone are unable to prevent the release of drugs during the transit through the stomach and the small intestine. However, some studies carried out on isolated films (Macleod et al., 1997; Semdé et al., 1998) or coated tablets (Wakerly et al., 1996b, 1997), have shown that the incorporation of appropriate amounts of pectins in the commercial insoluble polymer aqueous dispersions could be an interesting alternative. Indeed, it is thought that the integrity of such coatings during the transit from mouth to caecum could be better controlled by the insoluble polymers. On the other hand, the degradation of pectins by the colonic flora is expected to generate aqueous pores through the coating and therefore, to increase the drug release in the colon.

The aim of this work was to assess the suitability of such an approach for achieving specific-delivery of drugs to the colon, using theophylline pellets coated with ethylcellulose (Aquacoat® ECD 30, Surelease®) or acrylic polymer (Eudragit® RS30D and NE30D) aqueous dispersions, containing pectin HM or calcium pectinates. The theophylline release profiles from the coated pellets, at pH 6.0 and in presence of commercial pectinolytic enzymes, were compared to those obtained in absence of the enzymes. Furthermore, the water absorption kinetics of some isolated mixed films were determined, both in absence and in presence of the pectinolytic enzymes, in order to better understand the effect of these enzymes on the theophylline release and to elucidate the drug release mechanism.

2. Materials and methods

2.1. Materials

Theophylline pellets (mean diameter: 0.96 ± 0.14 mm), containing 81% of drug and obtained by extrusion-spheronization, were a gift from SMB Technology (Brussels, Belgium).

The aqueous ethylcellulose pseudolatexes used were Aquacoat® ECD 30 (FMC, 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, 30% w/w solid content) were gifts from Röhm Pharma (Darmstadt, Germany). Eudragit® RS is a slightly cationic hydrophilic polymethacrylate which 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® NE is a neutral acrylic polymer whose MFT is about 5°C. It forms soft and elastic films at room temperature without plasticizer (Amighi and Moës, 1997; Lehmann, 1997).

Dibutyl sebacate and triacetin, used as plasticizers, were supplied by Union Camp (USA) and Merck (Darmstadt, Germany). Pectinex® Ultra SP-L (pectinolytic enzymes, extracted from *Aspergillus niger* and having an activity of 26 000 PG/ml at pH 3.5) and pectin type 170, referred to as pectin LM (Low Methoxylated), were gifts from Novo Ferment (Dittingen, Switzerland) and Citrus colloids (Hereford, UK), respectively. Pectin from apple, referred to as pectin HM (High Methoxylated) was supplied by Fluka (Buchs, Switzerland). Silicone emulsion, polysorbate 80 and talc (mean particle size of ≈ 8 μm) were supplied from Vel SA (Belgium), Welphar (Belgium), and Aldrich chemical (England), respectively. 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 USP Pharmacopeia XXIII (1995).

2.2. Methods

2.2.1. Preparation of coating dispersions

Gels of 2% w/w pectin HM and 1.5% w/w calcium pectinates (Ca/Pectin LM ratios: 30 or 60 mg/g), pH 4.5, obtained, respectively from pectin HM and pectin LM, were prepared as described elsewhere (Semdé et al., 1998).

Appropriate amounts of the pectin HM or calcium pectinate gels were slowly added with stirring into each insoluble polymer aqueous dispersion (Aquacoat® 30D, Surelease®, Eudragit® RS30D or NE30D), previously mixed for 90 min, if required, with the plasticizer (20% w/w, related to the insoluble polymer content). The contents of pectin HM or calcium pectinate in each dispersion was 10% w/w (related to the insoluble polymer content). Talc, previously dispersed in distilled water containing silicon emulsion (antifoam), was then added and finally, the total solid content of each coating dispersion was adjusted with distilled water to the desired value. The compositions of the different coating dispersions are presented in Tables 1 and 2.

Table 1
Dispersions used for the preparation of the film coatings, containing 10% w/w (related to the insoluble polymer content) of pectin HM[a]

Formulation	Aquacoat® ECD 30	Surelease® clear	Eudragit® RS30D
Insoluble polymer (g, in dry basis)	160	160	160
Pectin HM (g)	16	16	16
Dibutyl sebacate (g)	32	–	–
Triacetin (g)	–	–	32
Talc (g)	–	–	32
Silicone emulsion (g)	1.6	1.6	1.6
Water ad (g)	1500	1500	1500
Solid content of the dispersion (% w/w)	14	11.8	16.1
Coating level (% w/w, mean ± SD, $n = 3$)	19.8 ± 0.9	21.7 ± 0.8	20.2 ± 0.4

[a] The coating levels of the different coated pellets are also mentioned.

Table 2
Dispersions used for the preparation of the film coatings, containing 10% w/w (related to the insoluble polymer content) of calcium pectinate[a]

Formulation	Aquacoat® ECD 30	Eudragit® NE30D	Eudragit® RS30D
Insoluble polymer (g, in dry basis)	135	68	160
Dibutyl sebacate (g)	27	–	–
Triacetin (g)	–	–32	
Calcium pectinates (g)	13.5	6.8	16.0
Talc (g)	–	17	–
Silicone emulsion (g)	1.4	0.7	1.6
Water ad. (g)	3000	1450	2900
Ca/pectin LM ratio (mg/g)	60	30	30
Solid content of the dispersion (% w/w)	6.0	6.4	7.2
Coating level (% w/w, mean ± SD, $n = 3$)	19.3 ± 0.7	8.0 ± 0.4	14.8 ± 0.2

[a] The coating levels of the different coated pellets are also mentioned.

Table 3
Operating conditions prevailing during the coating processes of the pellets

Operating parameters	Aquacoat® ECD 30	Surelease® clear	Eudragit® RS30D	Eudragit® NE30D
Initial weight of pellets (g)	800	800	800	800
Inlet temperature of drying air (°C)	60	60	45	36
Outlet temperature of drying air (°C)	38–40	36–38	28–30	28–30
Pneumatic spraying pressure (bar)	1	1	1	1
Spraying rates (g/min)	10–12	10–12	8–10	8–10

2.2.2. Coating processes

Known weights of pellets (800 g) were transferred into a fluidized-bed apparatus (Uni-Glatt, Glatt GmbH, Germany) equipped with a bottom-spray coating process in a Wurster column and then, coated with each dispersion (Tables 1 and 2) until the desired coating level was deposited. During the coating operations, the aqueous dispersions were continuously stirred in order to prevent the sedimentation of the insoluble particles. The operating conditions prevailing during the coating processes are given in Table 3.

After coating, the resulting coated pellets were cured at 60°C for 15 ± 1 h. These ageing conditions have been shown to be suitable to achieve the complete stabilization of ethylcellulose (Bodmeier and Paeratakul, 1994a), and Eudragit® RS and NE (Amighi and Moës, 1995, 1996, 1997) film-coatings.

The drug contents of the uncoated and coated pellets were then determined by UV-spectroscopy (Hitachi spectrophotometer, model 100-60), at 272 nm, after dissolution of theophylline from crushed pellets and suitable dilutions with distilled water ($n = 3$). The coating levels of the coated pellets (percentages of the film deposited), given in Tables 1 and 2, were finally determined (Amighi and Moës, 1996).

2.2.3. In vitro dissolution studies

The dissolution studies were carried out at 37°C, in absence or in presence of 3 ml of Pectinex® SP-L Ultra, using USP Pharmacopeia XXIII (1995), No. 2 dissolution apparatus (paddle). Samples of coated pellets ($n = 5$), equivalent to 100 mg theophylline, were placed into 900 ml 0.05 M acetate-phosphate buffer, pH 6.0 (compromise between the mean pH of the caecum and the optimum pH activity of the pectinolytic enzymes), containing 0.05% (w/w) polysorbate 80, used as wetting agent. The rotation speed of the paddle was set at 50 rpm. The dissolution medium was continuously withdrawn from the vessel, using a Gilson minipuls 2 peristaltic pump (Villiers-Le-Bel, France), passed through a filter (10 μm of porosity, Prolabo, France), then through a multicell Philips 8620 Series spectrophotometer (Philips analytical, Cambridge, UK) and finally, assayed at 280 nm. All these operations were controlled by the Philips PU 8605/60 Tablet Dissolution Monitoring System.

2.2.4. Water absorption kinetics of the isolated films

Film samples having a surface area of about 4 cm^2 and a thickness ranging from 130 to170 μm were obtained from Eudragit® RS30D or Eudragit® NE30D aqueous dispersions, containing 10% w/w pectin HM or calcium pectinate (Ca/Pectin LM ratio = 30 mg/g) and then, incubated in 25 ml 0.05 M acetate-phosphate buffer, pH 4.5, containing or not 50 μl of Pectinex® Ultra S-PL. The pH 4.5 value was chosen because it is close to the optimum pH activity of Pectinex® Ultra S-PL. The details about the preparation and description of the isolated films can be found elsewhere (Semdé et al., 1998). At timed intervals, the samples were removed, blotted dry between filter papers and immediately weighed. The percentages of the film water absorption were then calculated, using the following relationship: $100 \times (W_{ti} - W_d)/W_d$ (W_d and W_{ti} are the weights of dry and wet films at time ti, respectively). The percentages of the water absorption of the isolated films were plotted against time (mean $\pm$ S.D., $n = 5$).

3. Results and discussion

3.1. Results

Figs. 1 and 2 show the release curves of theophylline from the pellets coated with ethylcellulose (Aquacoat® ECD 30 or Surelease® clear) or polymethacrylate polymers (Eudragit® RS30D or Eudragit® NE30D), containing 10% w/w of pectin HM (Fig. 1) or calcium pectinate (Fig. 2). It can be observed that the release of theophylline from the coated pellets is highly influenced by the pectin derivative incorporated (pectin HM or calcium pectinate), the type of insoluble polymer used (ethylcellulose, Eudragit® RS or NE) and, for the same insoluble polymer, the type of

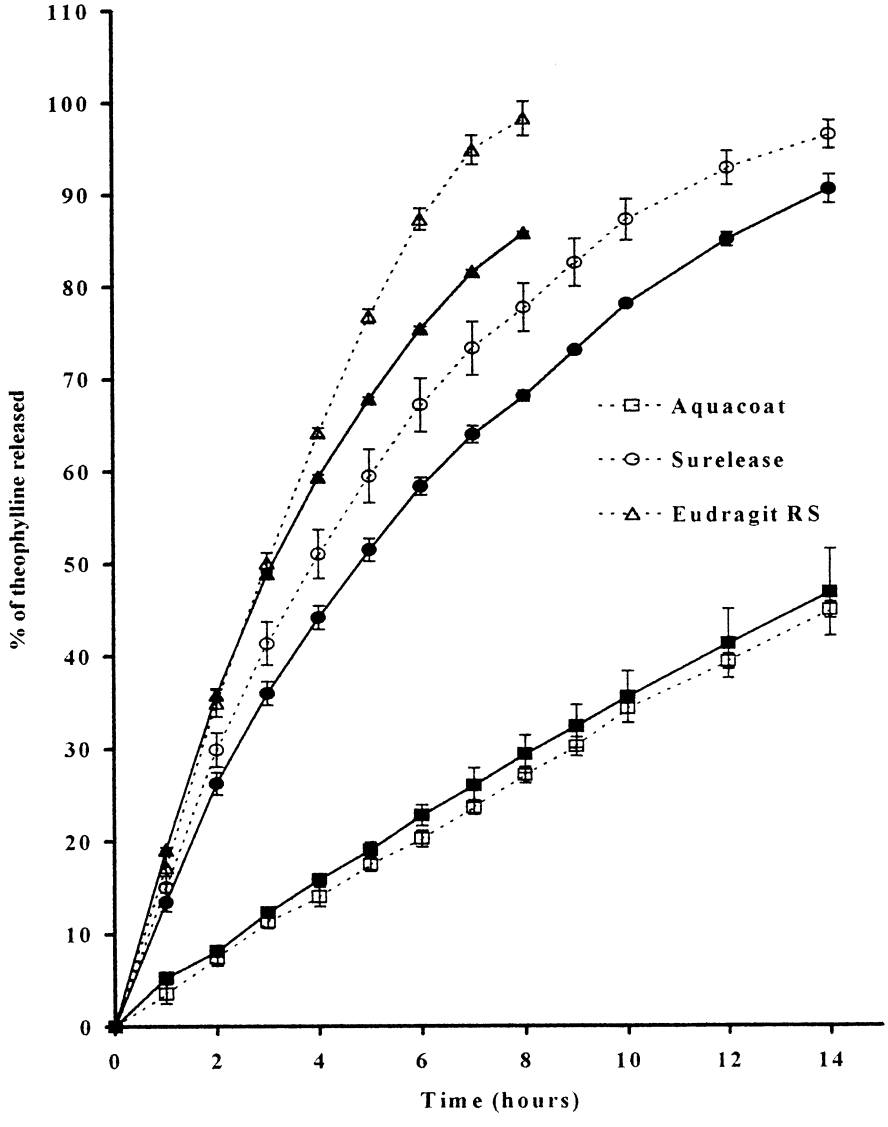

Fig. 1. Effect of pectinolytic enzymes on the theophylline release (mean ± SD, $n = 5$), at pH 6.0, from the pellets coated with Aquacoat® ECD 30, Surelease® clear or Eudragit® RS30D, containing 10% w/w (related to the insoluble polymer content) of pectin HM. Dotted lines: in absence of enzymes; full lines: in presence of enzymes.

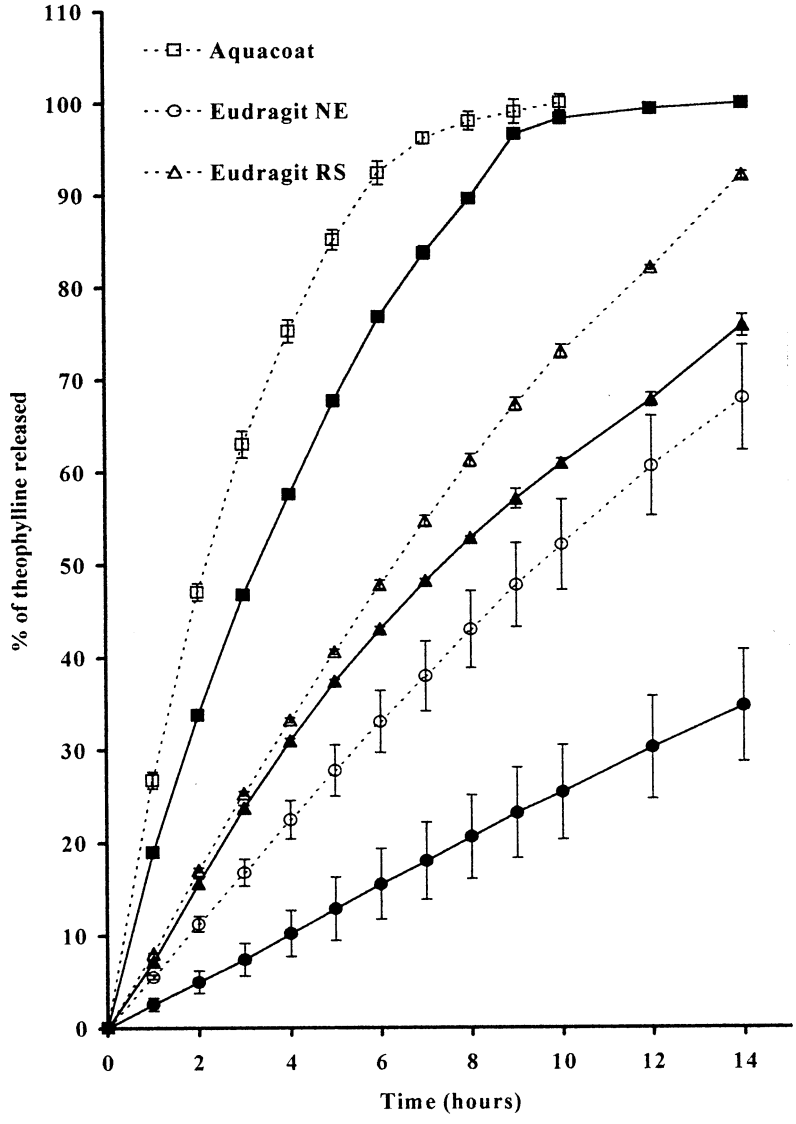

Fig. 2. Effect of pectinolytic enzymes on the theophylline release (mean ± SD, $n = 5$), at pH 6.0, from the pellets coated with Aquacoat® ECD 30, Eudragit® NE30D or Eudragit® RS30D, containing 10% w/w (related to the insoluble polymer content) of calcium pectinate. Dotted lines: in absence of enzymes; full lines: in presence of enzymes.

aqueous dispersion (Aquacoat® ECD 30 or Surelease® clear).

It can be observed also that the drug release from the coated pellets is influenced, at different degrees, by the presence or not of the pectinolytic enzymes in the dissolution media. Except in the case of the pellets coated with Aquacoat® ECD 30/Pectin HM blend, for which the release of theophylline is practically not affected by the pectinolytic enzymes (Fig. 1), the theophylline release rates from pellets coated with the various blends are lower in presence of the pectinolytic enzymes, compared to those observed in absence of the enzymes (Figs. 1 and 2). The examination of the results presented in Figs. 1 and 2 also shows that the slowing down of the theophylline

release by the pectinolytic enzymes is much more dramatic for the pellets coated with the Eudragit® NE/calcium pectinate blend. Dissolution tests were also performed on control samples, consisting of uncoated or Eudragit® NE30D, containing hydrosoluble but non degradable polymer (Eudragit® NE/HPMC 10:1, w/w), coated pellets in order to be sure that any change of the drug release, consequent to the action of pectinolytic enzymes, results mainly from the degradation and the leaching of pectin from film-coatings. Indeed, no significant effect of the pectinolytic enzymes on the theophylline release has been found (not shown).

Fig. 3 shows the water absorption profiles of Eudragit® NE or Eudragit® RS isolated films, containing 10% w/w of pectin HM or calcium pectinate. It can be observed that the water absorption profile of the isolated mixed films depends also on the type of insoluble polymer used (Eudragit® NE or Eudragit® RS), the pectin derivative incorporated (pectin HM or calcium pectinate) and more particularly, on the presence or not of the pectinolytic enzymes in the dissolution media. Indeed, for all mixed films studied, the percentages of water absorption, recorded in presence of the pectinolytic enzymes, are slower than those observed in absence of the enzymes. It is also interesting to notice that, as observed in the dissolution studies, the decrease of the water absorption, induced by the pectinolytic enzymes, is much more pronounced for the Eudragit® NE/calcium pectinate isolated films (Fig. 3).

3.2. Discussion

The results obtained from the dissolution studies (Figs. 1 and 2) are totally in contradiction with those expected, if we consider the colonic drug delivery approach, which is more specifically the topic of our development work (faster release of the drugs from the coated pellets in presence of the pectinolytic enzymes). In order to explain the slowing down of the theophylline release from the coated pellets by the pectinolytic enzymes, a release mechanism, as presented in Fig. 4, has been proposed for water-insoluble coated pellets, containing hydrophilic gel-forming polymers.

According to this release mechanism, pectin HM or calcium pectinates are homogeneously dispersed in the applied film-coatings. In contact with the dissolution media without pectinolytic enzymes, a substantial amount of pectin is retained in the coatings, and rapidly absorbs the surrounded water, since pectin HM and particularly calcium pectinate are hydrophilic and good gel-forming polymers (May, 1990). The pectin present in the coatings, which is now hydrated, can therefore swell and induce an increase of the swelling and the hydration of the film-coatings, the formation of hydrated pectin channels and probably, the appearance of distensions in the film-coatings. As a result, the diffusion of hydrophilic drugs such as theophylline through the film-coatings is improved. This release mechanism may be valid for water-insoluble polymer coated pellets, containing any hydrophilic and gel forming polymer (pectins, calcium pectinate, methylcellulose (MC), hydroxypropylmethylcellulose (HPMC)), which is not totally leached from the coatings into the dissolution media.

In presence of the pectinolytic enzymes, pectin HM or calcium pectinate are rapidly degraded and leached into the dissolution media from the film-coatings. As example, with the Eudragit® RS/Pectin HM (10:1, w/w) isolated films incubated for 8 h in 0.05 M acetate-phosphate buffer (37°C, pH 4.5), pectin is practically not released in absence of the pectinolytic enzymes while, the totality of pectin incorporated is rapidly leached, in presence of the enzymes (Semdé et al., 1998).

The enzymatic degradation and leaching of pectin or calcium pectinate from the coatings result not only in the suppression of the hydrated pectin channels and the distensions in the film-coatings but also, in the decrease of the swelling and the hydration of the film-coatings (Fig. 4). Indeed, as shown above (Fig. 3), when incubating in 0.05 M acetate — phosphate buffer (pH 4.5, 37°C), the water absorption obtained in presence of the pectinolytic enzymes is lower than that observed in absence of the enzymes for the isolated films prepared from the blends of Eudragit® RS/Pectin HM (10:1), Eudragit® NE/Pectin HM

(10:1) and more particularly, from Eudragit® NE/Calcium pectinate (10:1) blend. As a consequence, the diffusion of hydrophilic drugs such as theophylline through the film-coatings and therefore their release from the coated pellets occurs more slowly in presence of the pectinolytic enzymes.

On the other hand, after the enzymatic breakdown and leaching of pectin HM or calcium pectinate, the film-coatings can restructure, plug up the possible pores and reduce the free volume between polymer chains, and therefore, slow down the drug release. Such a restructuring of the film-coatings is possible, since the glass transition

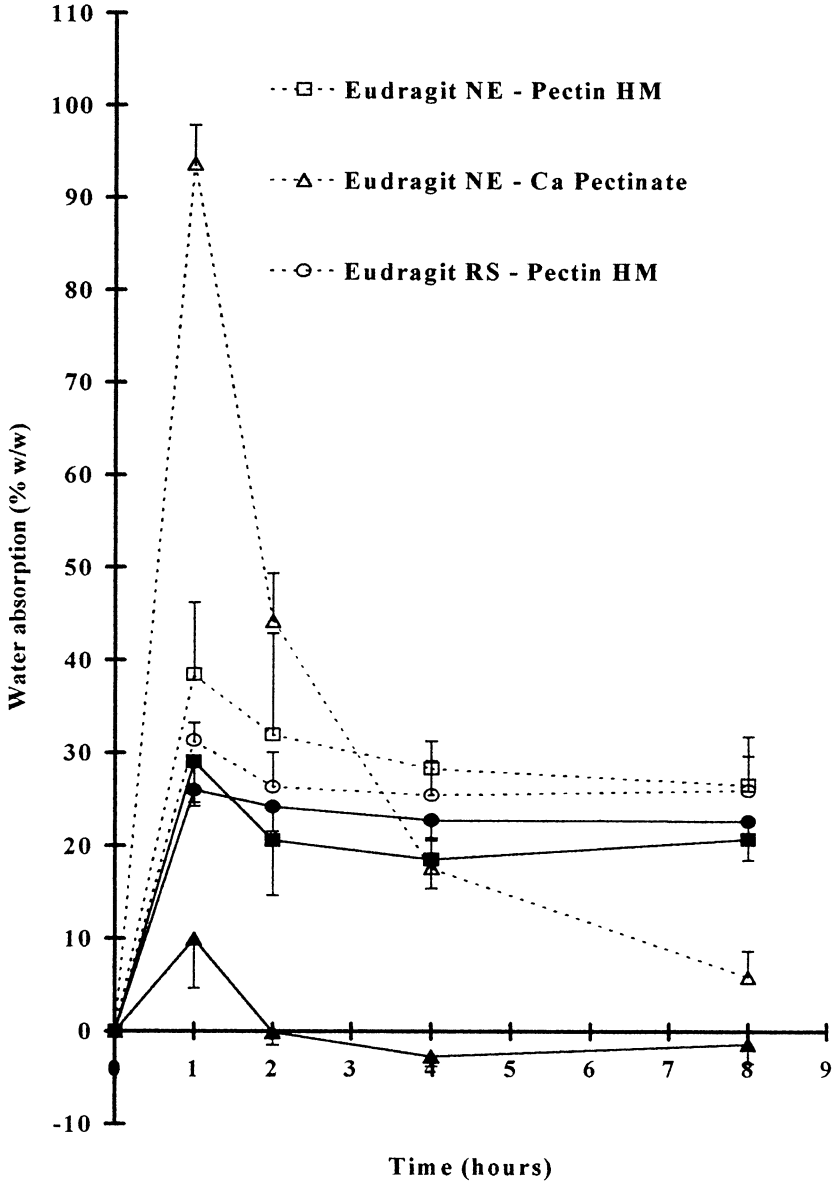

Fig. 3. Effect of pectinolytic enzymes on the water absorption (mean ± SD, $n = 5$) of Eudragit® NE or Eudragit® RS isolated films, containing 10% w/w (related to the insoluble polymer content) of pectin HM or calcium pectinate (Ca/Pectin LM ratio = 30 mg/g). Dotted lines: in absence of the enzymes; full lines: in presence of the enzymes.

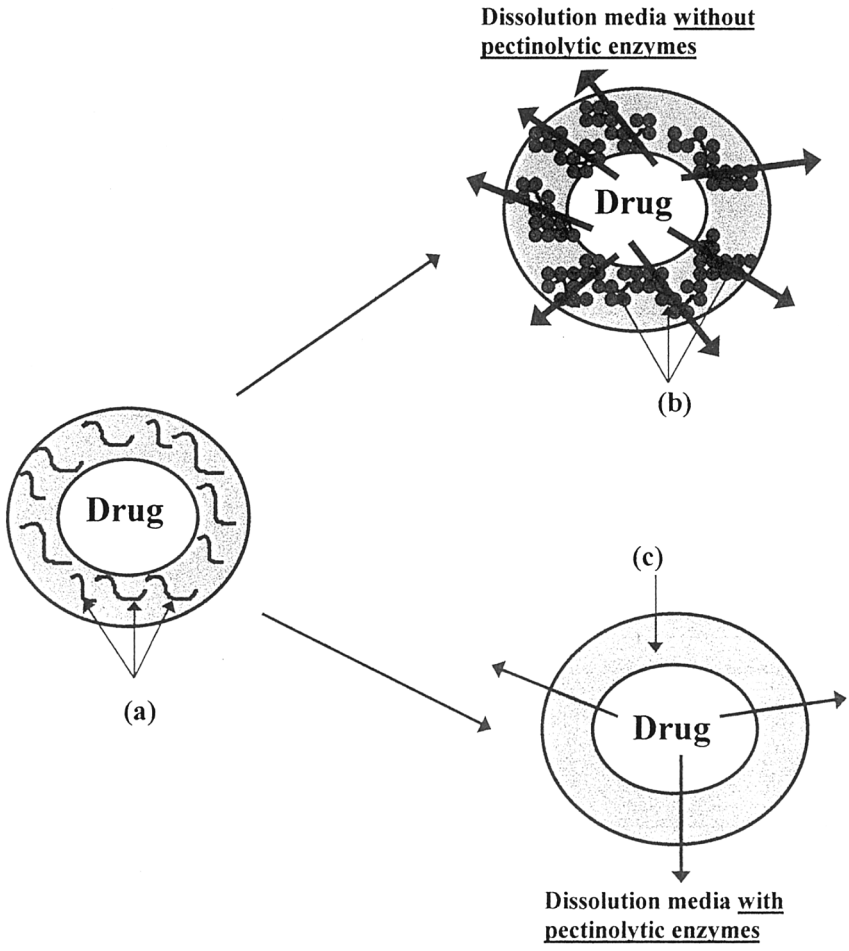

Fig. 4. Mechanisms by which the presence of pectinolytic enzymes results in the slowing down of the theophylline release from the pellets coated with blends of insoluble polymer aqueous dispersions/pectin HM or calcium pectinate. (a) Homogeneously dispersed pectin HM or calcium pectinate in the dry film-coatings. (b) In absence of pectinolytic enzymes: generation of aqueous pores and distensions, by the formation of hydrated pectin HM or calcium pectinate. The hydrophilic drugs, such as theophylline, can easily diffuse through these hydrophilic channels. (c) In presence of pectinolytic enzymes: rapid degradation and leaching of pectin HM or calcium pectinate from the film-coatings. The film-coatings, consisted of the insoluble polymers alone, are less permeable to the hydrophilic drugs such as theophylline.

temperatures (T_g) of the insoluble polymers (plasticized or not), used in this study, are lower or close to the temperature of the dissolution media (37°C). For example, the T_g of the Eudragit® RS polymer (plasticized with triacetin) and that of ethylcellulose (plasticized with dibutyl sebacate) are 27°C (O'Donnell and McGinity, 1997) and 44°C (Wheatley and Steuernagel, 1997), respectively. Moreover, the T_g and the mechanical properties of films are highly decreased in contact with the dissolution medium (hydrated form), increasing thus the film restructuring phenomenon. Among the insoluble polymers used, the Eudragit® NE polymer ($T_g = -8$°C) forms the softest and the most elastic film-coating (Bodmeier and Paeratakul, 1994b). Therefore, after the leaching of calcium pectinate, the Eudragit® NE film-coatings can more easily rebuild their structure than the others. Consequently, the slowing down of the theophylline release by the pecti-

nolytic enzymes is much more pronounced for the pellets coated with the Eudragit® NE/calcium pectinate blend (Fig. 2).

From the overall results, it clearly appears that the colonic drug delivery approach, based on the use of mixed film-coatings obtained from Aquacoat® ECD 30, Surelease® clear, Eudragit® RS30D or Eudragit® NE30D aqueous dispersions, containing pectin HM or calcium pectinate, is not valuable for the hydrophilic drugs such as theophylline. Based on the mechanism proposed in order to explain this observation (Fig. 4), it can be postulated that when the coating moiety degraded by the colonic flora is a hydrophilic water gel-forming polymer, the release of the hydrophilic drugs will be slower in the colon.

Therefore, in order to increase the drug release in the colon, the degradable moiety of the coating must be as less hydrophilic as possible. Indeed, the substantial reduction of the hydrophilicity and the water-swelling of amylose (another polysaccharide degraded by the colonic flora) by its complexion with butan-1-ol has allowed Milojevic et al. (1996a,b) to obtain promising results from pellets coated with the blend of amylose and ethylcellulose (1:4, w/w) aqueous dispersion (Ethocel®): the release of 5-aminosalicylic acid and glucose from these coated pellets was much faster in the media containing an inoculum of colonic bacteria, compared to that observed in the control media. Furthermore, 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).

4. Conclusion

The presence of the pectinolytic enzymes in the dissolution media results in the decrease of the theophylline release rate from the pellets coated with Aquacoat® ECD 30, Surelease® clear, Eudragit® RS30D or Eudragit® NE30D aqueous dispersions, containing pectin HM or calcium pectinate. A set of phenomena, including the hydrophilicity and the gel-forming properties of pectin HM and calcium pectinate, the elasticity and softness of the insoluble polymer used, is probably responsible of this behaviour. Anyway, systems based on this approach for targeting of drugs to the colon need to be rethought. As an example, we think that the substitution of pectin HM and calcium pectinate by complexes of pectin and non enzymatically degradable cationic polymers, such as Eudragit® E, RL and chitosans, should allow to provoke the increase of the theophylline release in presence of the pectinolytic enzymes. Results obtained from the theophylline pellets coated with Pectin HM/Eudragit® RL/Eudragit® NE ternary blends will be presented in a further paper.

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