Research Article

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Phosphated crosslinked pectin as a potential excipient for specific drug delivery: preparation and physicochemical characterization

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Abstract

Pectin was chemically modified with different amounts of trisodium trimetaphosphate (STMP) in aqueous solution (pH = 12), thereby giving a material with reduced water solubility. The physiochemical characterization of this new material was carried out through Fourier transform infrared and thermogravimetric analyses. Phosphated pectin (Pect-STMP) together with prebiotic (oligosaccharide) were incorporated into an aqueous dispersion of polymethacrylate (Eudragit® RS 30 D) in order to obtain free films using a casting process (50 °C) on a Teflon plate. The free films were evaluated using water vapour transmission, average swelling index in simulated gastric fluid (SGF) and simulated intestinal fluid, scanning electron microscopy and a diffusion study with theophylline in buffer solution with and without pectinolytic enzyme. The results suggest that the new material can be used in the coating process for oral solid-reservoir systems, to prevent the premature release of drugs in SGF (pH = 1.2). Furthermore, the presence of both Pect-STMP and oligosaccharide favours the specific degradation of the pellicle by the action of the enzymes produced by colonic microflora. The material obtained in this work has the potential to be applied in devices for drug delivery in the colon, making possible modified release of drugs. Nevertheless, subsequent colon-specific experiments *in vivo* need to be carried out in order to confirm the possible application of this new material.
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Keywords: phosphated pectin; prebiotic; trisodium trimetaphosphate (STMP); Eudragit® RS 30 D; modified release

INTRODUCTION

Conventional oral drug delivery systems are, usually, unable to guarantee the stability of the drug and the protection of the gastric mucous membrane, in addition to being unable to release the drug in specific areas of the gastrointestinal tract (GIT). Such systems produce a random release and/or the premature absorption of the active ingredients, thus causing the degradation of the drugs in the upper part of the GIT. Moreover, the oral administration of peptides and proteins, developed by the pharmaceutical and biotechnological industries, encounters a major barrier when the drugs reach the GIT, as the active molecules break down and/or become inactive, thus rendering their oral bioavailability unfeasible.[1]

Specific-target-site release systems show excellent biopharmaceutical and pharmacokinetic advantages compared to conventional pharmaceutical systems. These advantages include reductions in required drug dosages, protection of drugs against degradation, maintenance of bioavailability (thereby avoiding variations in dosages administered) and minimizing possible side effects. In addition, they have led to reductions in long and inconvenient posology programmes, resulting in greater patient adherence to treatments, and the optimization of pharmacological effects.[2,3]

The formula of pseudolatex aqueous dispersions (ethylcellulose or acrylic polymer, commercial Surelease®, Aquacoat®, ECD®, Eudragit® NE 30 D, RS 30 D, RL 30 D and FS 30 D) has been the main choice in the industrial application of oral dispensing devices in order to meet the required specific rates of drug delivery. The greatest advantage of such a versatile formula in the manufacture of pharmaceutical oral solid systems is that it is possible to avoid: (1) the use of organic solvents, (2) the increased complexity of the industrial facilities required to produce the organic solvents and (3) the associated industrial risk of environmental damage caused by residues of the manufacturing processes.[2,4–6] In addition, toxicity is prevented in the pharmaceutical formulas, thus complying with good manufacturing practices and environmental laws.

Some filmogenic acrylic polymers stand out because of their effectiveness in carrying drugs to specific areas of the GIT. The most prominent are those whose solubility is pH dependent (e.g. Eudragit® FS 30 D). However, some types of GIT pathologies can cause considerable alterations to the normal range of physiological

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pH. An illustrative example is that of ulcerative colitis, where colonic pH reaches values of around 4.7. Thus, the use of formulations coated with pH-dependent polymers aiming at the release of drugs at alkaline pH levels can present severe therapeutic limitations.[7]

In the light of such physiological and pathological characteristics of the GIT, the use of natural non-cellulosic hydrophilic polysaccharides, particularly when one considers the abundance of microflora in the distal areas of the GIT, has been arousing great interest among researchers.[7,8]

The pectin found in several vegetable species, especially in cellular walls, is responsible for the maintenance of structures and the sustenance of plants. This polysaccharide, known predominantly as a linear polymer, is chemically constituted of α-(1–4)-bond D-galacturonic acid units, occasionally interrupted by α-(1–2)-bond L-rhamnose units ('smooth' regions). However, other units, such as the neutral sugars D-galactose, L-arabinose, D-xylose, L-ramnose, L-fucose and a small presence of 2-*O*-methylfucose, can also make up part of the side chains ('hairy' regions).[9–11] It has a high molecular weight and, depending on the degree of esterification by methoxyl groups ($-OCH_3$), which are present in the D-galacturonic acid units, pectin can be classified as highly (above 50%) or poorly (below 50%) methoxylated/esterified. Thus, pectin can be described in terms of a 'canonical' structure, considering that it has quite a heterogeneous and complex chemical structure.[9,10,12]

In general, polysaccharides, including pectin, are able to pass unaffected through the upper portion of the GIT, showing biodegradability only in the colonic environment, due to the anaerobic microflora resident in this region.[3,7] However, the greatest challenge encountered when using pectin in the development of pharmaceutical coatings is to overcome its high solubility in aqueous media, which can contribute to an undesirable premature release of an active ingredient. An alternative to reducing the high solubility of polysaccharides is to chemically modify them, while still protecting their characteristic of biodegradation by colonic microflora.[2,7]

Therefore, the objective of the study reported here was to chemically modify pectin through reaction with trisodium trimetaphosphate (STMP). STMP is an effective crosslinking agent used in the food industry that does not show any toxicity in humans.[13] STMP reacts with polysaccharide hydroxyl groups, binding itself to the polymeric chains, leaving the carboxylic groups of some polysaccharides free to undergo future modifications. According to Lack *et al.*,[14] the reaction mechanism for polysaccharide crosslinking by STMP involves two steps: the opening of the STMP cycle by an alcohol moiety followed by reaction with a second alcohol moiety leading to the crosslinking of polysaccharides. This reticulation reaction promotes a decrease in polysaccharide solubility, as the number of hydroxyls free to interact with water is reduced.[13,15–17]

Crosslinked pectin was added to Eudragit® RS 30 D (a pH-independent polymer with low permeability) aqueous dispersion to produce films, following methodologies found in the literature.[4,5,12,18–20] The prebiotic α-gluco-oligosaccharide, commercially known as Bioecolians® (Solabia, France), was added to the dispersion before the coalescence of latex particles in order to improve the specificity of the obtained material (phosphated pectin + Eudragit® RS 30 D) to colonic microflora. Thus, the phosphated pectin together with oligosaccharide should be degraded only by colonic microflora, mainly *Bacterioid, Bifidobacterium* and *Eubacterium*.[12,21]

MATERIALS AND METHODS

Materials

Pectin Genu® (type USP-B, degree of esterification 72%, kindly supplied by CPKelco, Limeira/SP), Eudragit® RS 30 D (Alemanha, Evonik Röhm GmbH) and triethyl citrate (TEC; Morflex®, USA), both kindly supplied by Almapal (São Paulo/SP), theophylline anidre (Henrifarma®, São Paulo), STMP (Sigma Aldrich), Bioecolians® (α-gluco-oligosaccharide, kindly supplied by Solabia, France), Pectinex® Ultra SP-L (from *Aspergillus niger*), simulated gastric fluid (SGF; pH = 1.2), simulated intestinal fluid (SIF; pH = 6.8), simulated colonic fluid (SCF; pH = 6.0) and other reagents of analytical grade were used in the study.

Crosslinking of pectin with STMP

Pectin was crosslinked with increasing amounts of STMP, in basified aqueous solution (pH = 12, with 2 mol L^{-1} NaOH), following the methods proposed previously,[13,15–17,19] according to Fig. 1. Initially, five aliquots (200 mL) of pectin solution (2% w/v) were placed under constant magnetic agitation for 2 h which provided maximum homogenization. During that period, the pH was monitored and readjusted to 12, whenever necessary. After this procedure, increasing volumes (1, 2, 4, 12 and 20 mL) of STMP solution (10% w/v) were added, and the mixtures remained under agitation for 1 h at room temperature (25 ± 2 °C). After this, the dispersions were poured into Petri dishes and dried at 50 °C until the casting process was over. The dry products obtained (A, B, C, D and E, respectively), were placed into beakers (500 mL) containing a mixture of distilled water and ethanol (90 : 10). After adjusting the pH to 7 by adding HCl (0.01 mol L^{-1}), the products remained at rest for a period of 48 h, with the mixtures being renewed after 24 h (for the removal of any substance that did not react or that could interfere in the making of films).[22] At the end of this process, the products were filtered and dried at 50 °C. Finally, the dry products were stored in desiccators for further analysis and to obtain films.

Characterization of pectin and phosphated pectin (Pect-STMP) through Fourier transform infrared (FTIR) analysis

Samples of pectin and Pect-STMP were analysed using FTIR spectroscopy (FTIR-BOMEN-MB-100-Michelson® spectrometer), in order to determine whether the expected structural modification had indeed occurred. Sample analyses were carried out using KBr pellets, containing 1% of sample, in the range 4000–400 cm^{-1}.

Characterization of pectin and Pect-STMP through TGA

A Shimadzu® TGA-50 system was used to analyse 6 mg samples for TGA. The temperature of the analysis ranged from 25 to 1000 °C, at a rate of 10 °C min^{-1}, in a nitrogen atmosphere with flow of 50 mL min^{-1}. All samples were properly conditioned in flasks containing dehydrated silica gel (110 °C h^{-1}) up to the time the analyses were carried out.

Preparation of films

Films were prepared starting from aqueous dispersions using a conventional method for thermoplastic and thermorigid polymers known as the 'casting process', according to the formulae in Table 1.

Initially, four different dispersions were prepared by varying the concentrations of Pect-STMP-A (2.44% w/w of STMP/pectin) or Pect-STMP-E (33.33% w/w of STMP/pectin) with Eudragit® RS

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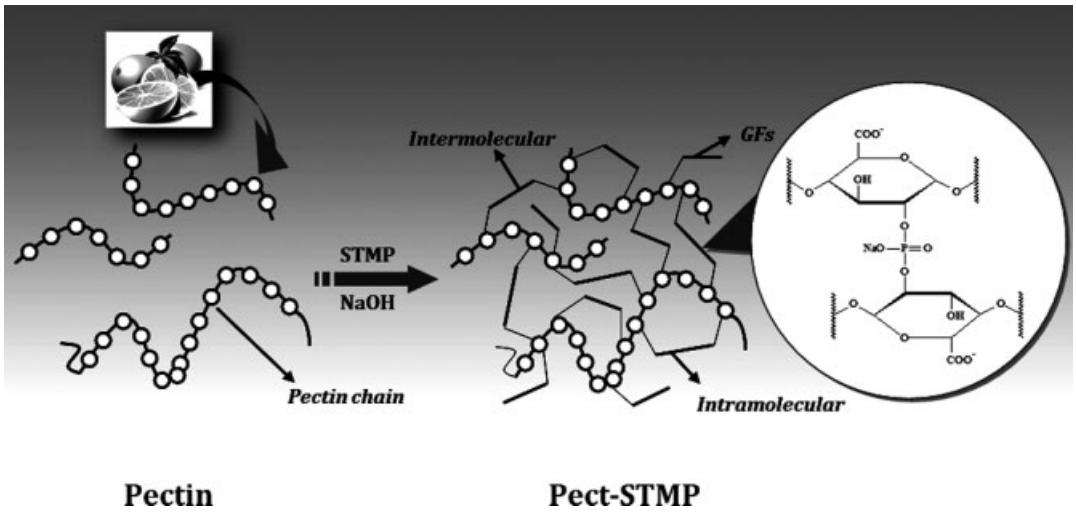

Figure 1. Schematic of the crosslinking of pectin, showing three possibilities of phosphorus incorporation: intermolecular, intramolecular and monografted (GFs). (Adapted from Franssen *et al*.[38]).

Table 1. Proportions of each component in the films (Eudragit® RS 30 D : Pect-STMP : oligosaccharide)

Eudragit® RS 30 D (%)	Pect-STMP-A and -E (%)	Oligosaccharide (%)	Triethyl citrate (%)
100	0	0	20
90	5	5	20
85	10	5	20
80	15	5	20

30 D and α-gluco-oligosaccharide, while always maintaining the total solids content of the dispersions at 4% (w/v). The proportions (Eudragit® RS 30 D : Pect-STMP-A or Pect-STMP-E : oligosaccharide) tested were 90 : 5 : 5, 85 : 10 : 5, 80 : 15 : 5 and 100 : 0 : 00, with the last being the control, as shown in Table 1. The plasticizer chosen was TEC, added at a concentration of 20% in exclusive relation to Eudragit® RS 30 D mass.

The dispersions containing Eudragit® RS 30 D with the addition of TEC (20%) were kept under magnetic agitation for 30 min at room temperature (25±2.0 °C). After completing the homogenization process, varying amounts of Pect-STMP and oligosaccharide aqueous solution – following the formulae given in Table 1 – were gently added to the synthetic polymer dispersions under constant agitation. The mixtures were maintained under magnetic agitation for an additional 60 min at room temperature. A vacuum pump was used throughout the homogenization process to avoid the incorporation of air and the formation of undesired bubbles in the polymeric mixtures.

After complete homogenization, 10 mL of each dispersion was poured onto a Teflon plate, and dried in an air oven at 50 °C for approximately 15 h, until the formation of a film.[4,5,18,19,23]

Macroscopic and thickness analysis of films

The samples were macroscopically examined for the presence of air bubbles and cracks, as the integrity of the films is important to ensure reproducibility in the execution of other analyses, particularly in the study of permeability. The dry thickness of the films was measured at five locations using a micrometer (Mitutoyo®). The selected films were then stored in a desiccator for further assays.[4,5,18,19,23]

Water vapour transmission analysis of films

The evaluation of permeability, through polymeric films, in order to determine water vapour transmission (WVT) is considered to be a simple method, in comparison to traditional diffusion methods. This method provides valuable information on the protection of a coated dosage system against environmental humidity during storage.[4,24]

This study was carried out in accordance with the 'B' method of ASTM guidelines E96-66, using a system adapted from Payne's permeability cups (Braive Instruments, Liège, Belgium). Distilled water (10 mL) was put into one of the cups and 10 cm^2 of the film was subsequently attached to the device. The cup with the film was then weighed and stored in a desiccator with silica gel at 25 °C. The cups were reweighed after 24, 48, 72, 96 and 120 h to determine the amount of permeated water (mass loss percentage). At each time period, the silica gel was substituted with dehydrated silica (110 °C h^{-1}). The different mass loss values were fitted to the following equation and standardized to a 24 h time period, establishing the WVT for each polymeric composition used:

$$\mathrm{WVT} = \frac{m}{At} \times 24\ \mathrm{h} \qquad (1)$$

where m is mass loss (g), t is time (h) and A is the film area (0.001 m^2).[4,5,15,18,23,25]

A randomized complete 2^2 factorial design (with four replicas) was built to evaluate the WVT through free films. Both the STMP (used during the crosslinking reaction) and Pect-STMP (used in membrane preparation) amounts were selected as the independent variables. The WVT was used as response (or dependent variable). The limits of inferior (−1) and superior (+1) levels were selected as follows: 2.44 and 33.33% w/w (STMP/pectin) for STMP; 5 and 15% for Pect-STMP. The data were evaluated by means of Statistica 6.0® software.

Determination of swelling index (I_i) of films

In order to guarantee the access of the bacterial enzymes that exist in the distal portion of the GIT, it is necessary to know the degree

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of hydrophilicity of the free films when in contact with physiologic fluids. Moreover, the swelling analysis can represent a first step in the development of a mathematical model capable of predicting the release kinetics.[26]

To this end, a 1 cm^2 piece of each free film was dried in an air oven at 50 °C for approximately 15 h until reaching total loss of humidity. After this period, the dry samples of the different films were accurately weighed (±0.0001 g) and immediately immersed in flasks with SGF (pH = 1.2) and SIF (pH = 6.8) at 37 °C, without the presence of digestive enzymes (USP XXVIII, 2006). At specific time intervals (1, 5, 10, 30 and 60 min), the swollen samples were removed from the medium, excess surface water was removed by light blotting with filter paper and they were then weighed (±0.0001 g). To quantify the swelling process, the swelling index (I_i) was calculated as follows:

$$I_i\,(\%) = \frac{m_{sw} - m_o}{m_o} \times 100 \qquad (2)$$

where m_{sw} is the weight after swelling and m_o is the weight of the dried polymer film. The average swelling index (I_{im}) of five swelling experiments was calculated for each film composition used.[4,5,18,19,23,24]

SEM analysis of film morphology

A morphological analysis was carried out on the Pect-STMP films using a Shimadzu model SS 550 SEM instrument operating at 12 keV. After the determination of I_i, the swollen samples were frozen in liquid nitrogen and then lyophilized by freeze-drying (Martin Christ®, Freeze Dryer, Alpha 1-1/DL) for 6 h.

All of the micrographs were obtained by sputter coating the samples with gold before SEM analysis. It was assumed that the morphology of the swollen samples was preserved during the freeze-drying process. All samples were properly conditioned in flasks containing dehydrated silica gel (110 °C h^{-1}) until the time the analyses were carried out.

Study of theophylline diffusion through films

Membranes constituted of Eudragit® RS 30D : Pect-STMP-E : oligosaccharide (80 : 15 : 5) used in the permeability experiments were cut into circular pieces and placed between the two cells of a diffusion device, as shown in Fig. 2. Compartment A (donor cell) was filled with SCF with the addition of 2 µL mL^{-1} of Pectinex® Ultra SP-L (from *Aspergillus niger*) to the medium of pH = 6.0 containing a theophylline solution; compartment B (acceptor cell) was filled with the same buffer without diffusant. A control study was performed in parallel without pectinolytic enzyme. The temperature of the cells was kept at 37 °C throughout the experiments and each compartment was stirred continuously with a magnetic stirrer. At predetermined time intervals (30, 60, 90, 120, 150 and 180 min), samples of 3 mL were taken from the receptor cells and replaced with fresh medium. The increase of the theophylline concentration in the acceptor cell was measured as a function of time using UV-visible spectrophotometry at 271 nm.[24,27]

To quantify the diffusion process, the permeation rate of the theophylline (P) was calculated as follows:

$$P = \left(\frac{dc}{dt}\right)\frac{V\delta}{cA} \qquad (3)$$

where dc/dt is the slope (drug permeated *versus* time), V is the volume of the acceptor cell (70 mL), c is the concentration of drug in the donor cell (0.25 mg mL^{-1}) and A and δ are, respectively, the diffusion area (2.54 cm^2) and thickness of the membrane (0.0116 ± 0.0006 cm). All of the experiments were carried out in triplicate.[24]

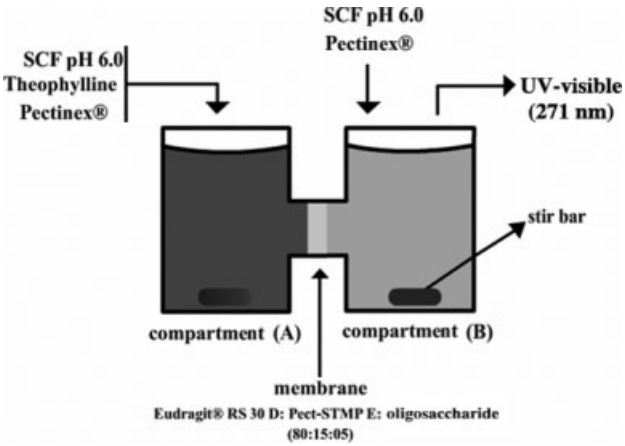

Figure 2. Schematic of the diffusion cell.

RESULTS AND DISCUSSION

FTIR analysis

This method allows analysis and comparison of the bonding and the characteristic functional groups of natural pectin (Pect-USP) and pectin phosphated with STMP (Fig. 3). In the FTIR spectra of Pect-STMP-A and -E, a significant decrease in the intensity of the absorption bands between 3660 and 3100 cm^{-1}, which are caused by O–H stretching, is observed. The number of inter- and intramolecular hydrogen bonds to the carboxylic groups probably decreases due to the crosslinking of the O–H groups with the STMP.

In addition, it is possible to observe other absorptions, which are attributed to the presence of phosphate groups, near 1327 cm^{-1} (P═O) and 954 cm^{-1} (P–O–P).

Another observation that can be made is the occurrence of a peak at 1425 cm^{-1}, which is related to the formation of P–O–C bonds between the polysaccharide and the STMP. This peak is not visible in the spectrum of the crosslinker or of Pect-USP. However, it is visible in the spectra of the reticulated products (Pect-STMP-A and -E), which suggests the formation of phosphated pectin. Cavalcanti *et al.*[15] also observed similar absorptions during the synthesis and characterization of chondroitin sulfate crosslinked with STMP.

Other evidence of Pect-STMP formation is the occurrence of a peak at 1620 cm^{-1}, which is attributed to COO^- groups resulting from neutralization with NaOH during the reaction. This evidence is based on a similar observation by Nurjaya and Wong,[28] who investigated calcium pectinate beads. On the other hand, in the Pect-USP spectrum, it is also possible to observe the presence of two peaks, at 1745 cm^{-1} (C═O) and especially at 1634 cm^{-1} (C═O, esterified), the latter characterizing esterified polysaccharide that was not subject to ester hydrolysis (saponification reaction) during crosslinking with STMP in basified medium. Singthong *et al.*,[29] working with low methoxy pectin extracted from *Cissampelos pareira*, also found similar spectral differences compared to high methoxy pectin (76.5%).

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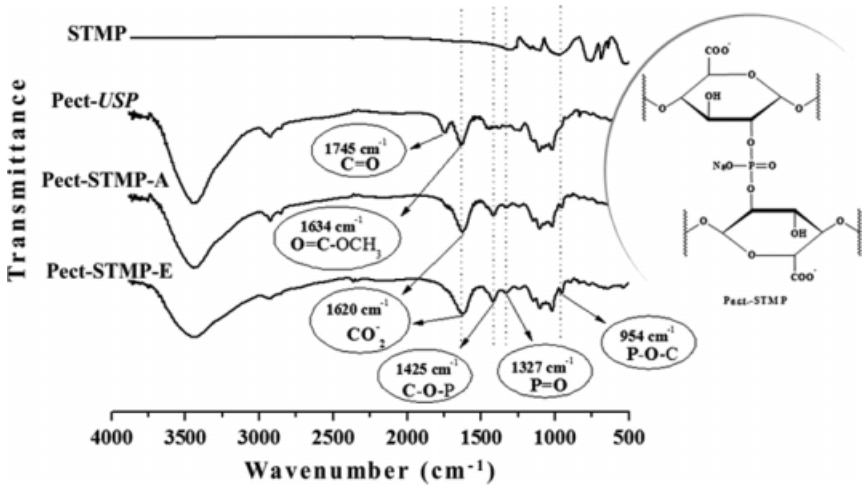

Figure 3. FTIR spectra of STMP, Pect-USP, Pect-STMP-A (2.44% w/w STMP/pectin) and Pect-STMP-E (33.33% w/w STMP/pectin).

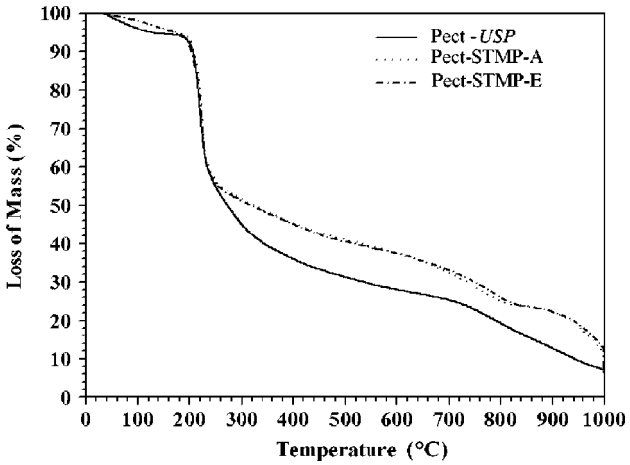

Figure 4. TGA curves for natural pectin (Pectin-USP) and phosphated pectin (Pect-STMP-A and Pect-STMP-E).

Table 2. Average thickness values of films studied

Film composition	Thickness (± SD[a]) (mm)
100 : 0 : 0	0.081 (± 0.023)
90 : 5 : 5 (A)	0.123 (± 0.006)
90 : 5 : 5 (E)	0.128 (± 0.009)
85 : 10 : 5 (A)	0.116 (± 0.016)
85 : 10 : 5 (E)	0.115 (± 0.005)
80 : 15 : 5 (A)	0.123 (± 0.012)
80 : 15 : 5 (E)	0.116 (± 0.006)

[a] SD, standard deviation; $n = 5$.

TGA results

The TGA results are shown in Fig. 4. Based on this analysis, it is possible to observe and compare the thermal behaviour of the pure pectin sample with that of Pect-STMP-A and -E. In the thermograms, the early minor weight loss in samples (in the range 0–200 °C) is attributed to desorption of moisture, as hydrogen-bound water, from the polysaccharide structure. This was observed in previous studies carried out by others authors,[30] when natural and modified polysaccharides were analysed. However, this weight loss is greater for natural pectin than for Pect-STMP-A and Pect-STMP-E. This suggests that the pectin has undergone crosslinking and, consequently, has a smaller amount of hydroxyl groups to interact with the water molecules. Significant differences related to the increase in STMP concentration between Pect-STMP-A and Pect-STMP-E are not observed at any time.

The main decomposition of polysaccharides usually happens above 200 °C. According to the first derivative TGA curve (DrTGA, not shown), the temperature of the thermal decomposition peak for Pect-USP is around 219 °C, whereas it is around 224 °C for the modified samples. It is also observed that Pect-STMP-A and -E have larger residual masses above 260 °C compared to natural pectin. This is probably related to a greater amount of pyrolysed residue, generated by polysaccharide reticulation.

The thermal decomposition of natural pectin is greater in the first reaction stage, thus generating a smaller amount of pyrolysed material, which shows a larger percentage weight loss, as can be seen in the thermogram in Fig. 4. A previous investigation[30] showed the same pattern of thermal decomposition and weight loss through TGA that was carried out with methylcellulose and sodium carboxymethyl.

Macroscopic and thickness analysis of films

The macroscopic analysis of the polymeric compositions synthesized for the present study showed no phase separation, no cracks and no air bubbles, guaranteeing reproducibility when the methodology is applied. Transparency only slightly decreases from pure Eudragit to the different formulations. The flexibility decreases continuously from the pure formulation, which is proportionally influenced by the increase in concentration of Pect-STMP. The plasticizer (20%) favours the coalescence of Eudragit® RS 30 D, which associated to Pect-STMP and oligosaccharide under the conditions applied. Petereit and Weisbrod[31] and Aydinli and Tutas[32] attribute this improved coalescence to the capacity that plasticizers have in decreasing the glass transition temperature of latex particles and, consequently, increasing the mobility of polymeric chains, thereby improving the mechanical property of films.

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Table 3. Average water vapour transmission (WVT_m) values ($n = 5$)

Film composition	WVT_m (g (m^2 24 h)$^{-1}$)	± Standard error of the mean
100 : 0 : 0	402.0640	11.0188
90 : 5 : 5 (A)	542.4922	10.1035
90 : 5 : 5 (E)	476.2920	8.8350
85 : 10 : 5 (A)	706.6554	11.1060
85 : 10 : 5 (E)	639.6040	7.6195
80 : 15 : 5 (A)	806.262	8.1130
80 : 15 : 5 (E)	741.4346	10.6018

An interesting observation (Table 2) is that film thickness increases with the incorporation of Pect-STMP when compared to the control (100 : 0 : 0, Eudragit® RS 30), yet it is sensitive neither to Pect-STMP content (5 to 15%) nor to the crosslinking with Pect-STMP-A and -E, showing no further increase in the thickness of the compositions tested. Previous studies[5,33] also reported similar effects, in which the modified polysaccharide generated an increase in the thickness of the free films formed.

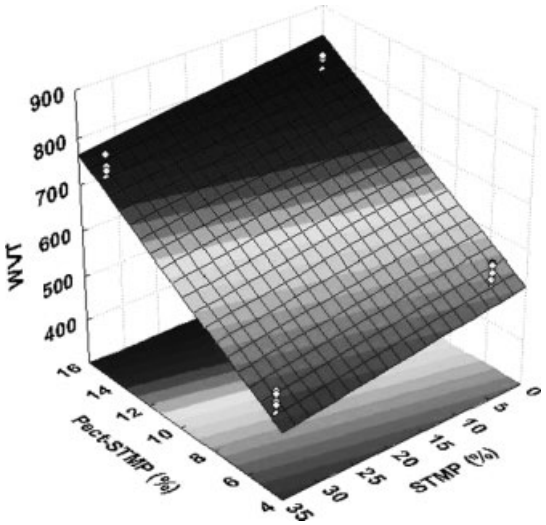

Figure 5. Response surface plot of randomized complete 2^2 factorial design (with four replicas) for WVT through films made from various amounts of STMP and Pect-STMP. The WVT data refer only to an interval of 24 h.

WVT analysis of films

Table 3 gives the average WVT (WVT_m) values for the different film compositions. An increase in the Pect-STMP content generates an increase in the WVT_m value when compared to the control film (100 : 0 : 0). This phenomenon can be explained by an increase in the hydrophilicity of the system, caused by the addition of the polysaccharide.

The analyses of variance (ANOVA) for the randomized complete 2^2 factorial design (with four replicas) suggests that the main effects of the amounts of both STMP and Pect-STMP are statistically significant for WVT values (not shown). It is found that the P values for a confidence interval of 95% are lesser than 0.05. In addition, the adjustment of linear model applied can be verified through value of R^2_{adj} (0.9750). Figure 5 shows response surface plot generated from randomized complete 2^2 factorial design (with four replicas). The circular open points indicate experimental WVT data. Films with larger WVT values may be achieved by decreasing the amounts of STMP and increasing the amount of Pect-STMP in the preparation.

Rosina *et al.*[34] reported that when they produced films with Eudragit® RS 30 D associated to a polymer extracted from *Nelumbo nucifera* root, they observed an increase in the WVT rate, which was proportional to and dependent on the increase in polysaccharide concentration. Bunhak *et al.*,[4,5] using either Eudragit® RS 30 D or ethylcellulose (Surelease®) associated to chondroitin sulfate, also found similar results for permeability, the values of which were also proportional to the increase in polysaccharide concentration. The same trend of increasing WVT values can be observed in Table 3. Compounds involving phosphated pectin (Pect-STMP-A and Pect-STMP-E) and oligosaccharide in concentrations of 90 : 5 : 5, 85 : 10 : 5 and 80 : 15 : 5 show greater permeability compared to the control (100 : 0 : 0).

The behaviour of the films in relation to the degree of reticulation of the phosphated pectin was also evaluated, through the increase in STMP (Table 3). From previous investigations,[13,15–17] it can be concluded that linking pectin hydroxyl groups with STMP promotes a reduction in the hydrophilicity of the films.

Characteristics related to changes in permeability are of great interest, and are connected to the increase in modified polysaccharide concentration, to the increase in the degree of reticulation of the polysaccharide and also to the addition of prebiotic to the compound. This combination of materials will probably provide filmogenic material with greater vulnerability to specific fermentation in the colonic medium, which can improve the control of both fluid and drug diffusion.

Swelling index (I_i) study

The average swelling index (I_{im}) was the parameter used to evaluate the degree of hydration of the films. Figures 6(A) and (B) show that both pH values and Pect-STMP contents in the films influence the I_{im} values. The films containing more Pect-STMP (80 : 15 : 5) have lower I_{im} values in SGF (Fig. 6(A)) when compared with the films containing less Pect-STMP (90 : 5 : 5). On the other hand, the films containing more Pect-STMP (80 : 15 : 5) in SIF (Fig. 6(B)) assume high I_{im} values. This variation in the I_{im} values with pH can be attributed to two factors: (i) the pectin phosphate content in the films and (ii) the degree of ionization of the carboxylate and phosphate groups of the phosphated pectin present in the films. As reported by Mulhbacher *et al.*,[26] with increasing pH of the solution, the swelling volume of anionic polymers is expected to increase, as happens to phosphate pectin. Similar events were also observed by Gliko-Kabir *et al.*[17] when evaluating the swelling index of phosphated guar gum films in SGF and SIF.

A significant swelling (40–60%) is observed in the first minutes for formulae containing 15% of Pect-STMP (80 : 15 : 5 (A) and 80 : 15 : 5 (E)), followed by an accentuated decrease (20–30%) in the hydration kinetics of these films after 30 min, as shown in the SIF (pH = 6.8) graph (Fig. 6(B)). This result is probably related to the leaching of Pect-STMP fragments and oligosaccharide, which leads to a decrease in hydration levels due to weight loss, thus suggesting that they are released to the solution in near-neutral pH conditions (SIF). Leaching of pectin and/or oligosaccharide from the films is qualitatively observed through precipitate formation, after adding

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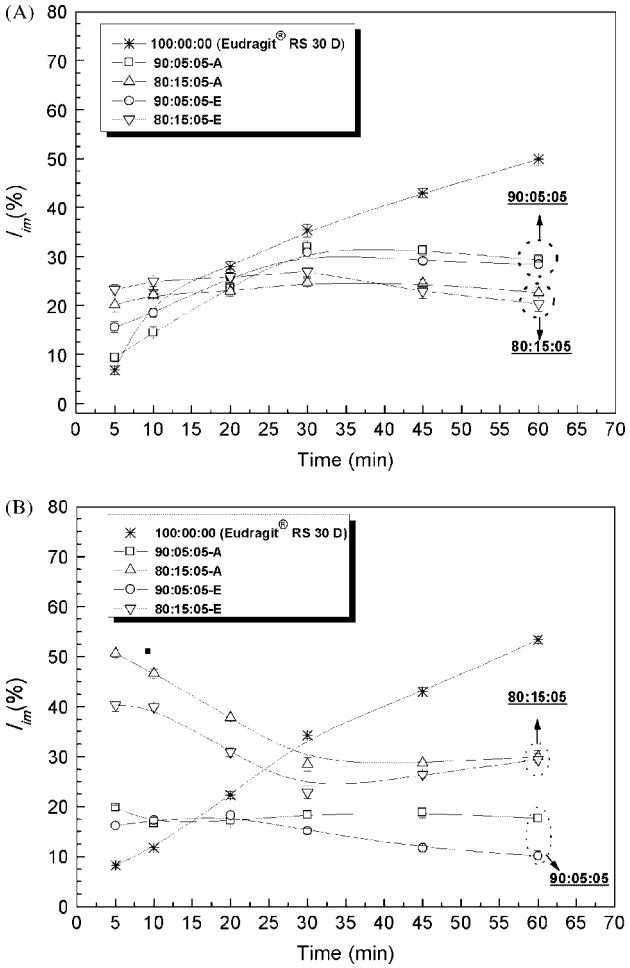

Figure 6. Average swelling index (I_{im}) for the films in (A) SGF and (B) SIF at 37 °C ($n = 5$).

acetone to the media at the end of the experiment. A similar result was observed by Semdé *et al.*[35] during the evaluation of films containing Eudragit® RS 30 D and calcium pectinate. This probably happened because in acid medium (pH = 1.2) most carboxylate and phosphate groups present in the Pect-STMP structure are protonated and thus there are many hydrogen bonds in the films. The hydrogen bond complex (between Eudragit and Pect-STMP) would restrict the movement or relaxation of network chains. A compact network would be formed and, therefore, no leaching of the Pect-STMP and oligosaccharide is observed (Figs 6(A) and 7(B) and (E)). In SIF (pH = 6.8), the free carboxylate and phosphate groups would be ionized, which would break hydrogen bonds and generate electrostatic repulsion among polymer chains. This repulsive force would push the network chain segments apart and leaching of the Pect-STMP and oligosaccharide occurs, so a lower swelling ratio would be observed (Figs 6(B) and 7(C) and (F)).[20,36]

In addition, Figs 6(A) and (B) show that starting from a determined STMP concentration, the polymeric net probably collapses, due to an increase in the number of intramolecular phosphate links, to the detriment of intermolecular links (which are responsible for the mobility of the polymeric chains). This is reflected by a reduced swelling capacity. Both Lack *et al.*[13] and Gliko-Kabir *et al.*[17] have previously described this phenomenon in studies undertaken using the same crosslinker (STMP) to obtain reticulated polysaccharide.

From a pharmacokinetics point of view, this fact can be seen as being extremely positive, as the release rate of various drugs can be modulated in order to assist pharmacotechnical and/or therapeutic needs.

SEM for film morphology

Analysing the micrographs obtained from the film samples, after carrying out the swelling experiment in SGF and SIF (Fig. 7), changes in the morphological characteristics can be observed in comparison to samples of dry film (Figs 7(A) and (D)). When submitted to SGF (pH = 1.2), a quite heterogeneous surface, with a marked presence of Pect-STMP and oligosaccharide, is observed (Figs 7(B) and (E)). In contrast, when the films are immersed in SIF (pH = 6.8), the samples show some flaws in certain areas (Figs 7(C) and (F)). As previously discussed for the swelling index analysis, such flaws are probably related to the leaching of Pect-STMP and oligosaccharide, a phenomenon related to the greater relaxation of the polymeric network when in contact with SIF.

Diffusion study

The concentration of theophylline diffusing through the membrane in SCF (pH = 6.0) as a function of time is shown in Fig. 8. As can be seen, the membrane shows an increase in theophylline permeability with time for both conditions, with ($P = 1.01 \times 10^{-4}$ cm min^{-1}) and without ($P = 4.54 \times 10^{-5}$ cm min^{-1}) pectinolytic enzyme. However, the lower drug permeability that is observed in the first minutes (30 and 60 min) of the experiment in the absence of enzyme probably occurs only due to a larger space between the polymeric chains and the leaching of the Pect-STMP and the oligosaccharide, as described above in our discussion of the swelling study.

Figure 8 shows enhanced drug permeability in the presence of the pectinolytic enzyme in the first and more markedly in the last minutes of the investigation probably due to Pect-STMP degradation. As a consequence, more pores are created through which theophylline molecules can permeate. As regards this degradation, a similar result was observed by Gliko-Kabir *et al.*[37] during the evaluation of guar gum hydrogel crosslinked with STMP.

Another point, in accordance with the observations of Semdé *et al.*,[27] is that the degradable moiety of the coating must be as least hydrophilic as possible to obtain promising drug release in the colon. According to their findings, when the coating moiety degraded by the colonic flora is a hydrophilic water gel-forming polymer (pectin or calcium pectinate), the release of hydrophilic drugs will be slower in the colon.

Thus, our results reveal that this new material constituted of Eudragit® RS 30 D (time-dependent polymethacrylate), Pect-STMP and oligosaccharide has potential in coating systems for specific colon delivery because of its potential biodegradability in the presence of pectinolytic enzyme.

In addition, in accordance with our swelling study carried out in SGF (pH = 1.2) and the results of others authors,[13,17] we can suggest that our material when applied in the development of oral drug delivery systems can prevent the premature release of a drug.

CONCLUSIONS

The methodology proposed in the present study regarding the crosslinking of pectin led to the generation of a new material, Pect-STMP, with reduced solubility, suitable for the development of films

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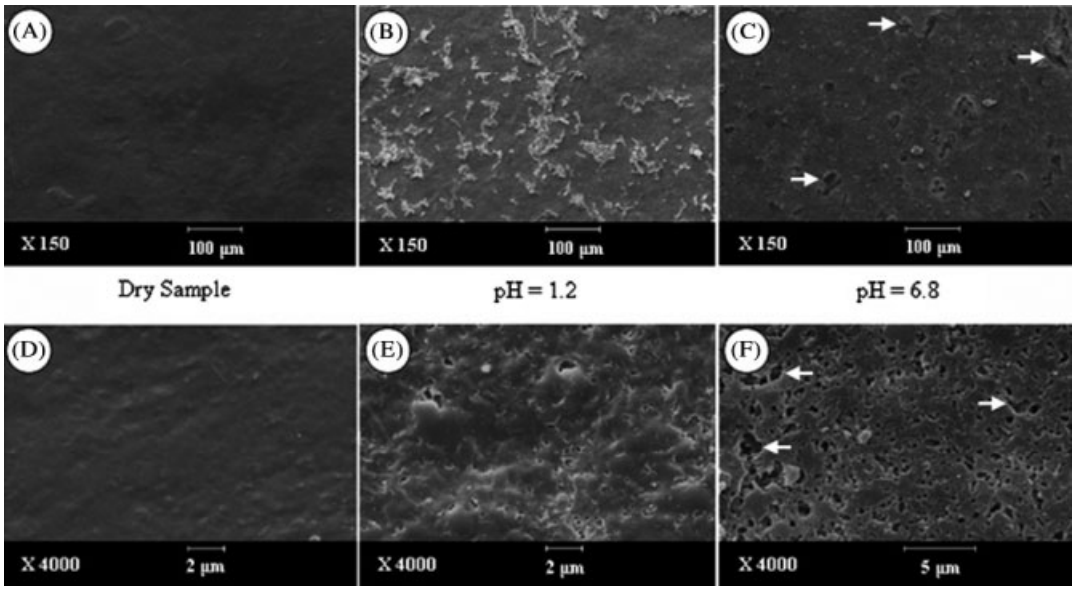

Figure 7. SEM micrographs of film containing 80 : 15 : 5 Eudragit® RS 30 D : Pect-STMP-E : oligosaccharide. (A, D) Dry samples, (B, E) samples in SGF (pH = 1.2) and (C, F) samples in SIF (pH = 6.8). The white arrows indicate erosion.

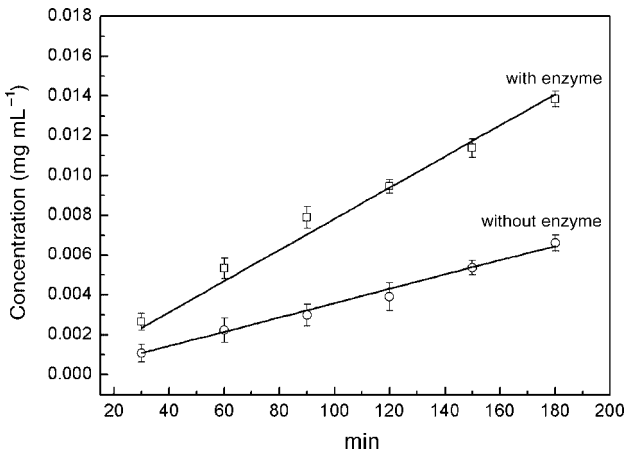

Figure 8. Concentration of theophylline diffusing through a membrane constituted of Eudragit® RS 30 D : Pect-STMP-E : oligosaccharide (80 : 15 : 5) at 37 °C in SCF (pH = 6.0); $n = 3$. The straight lines with and without pectinolytic enzyme have R^2 values of 0.9967 and 0.9972, respectively.

through an association with Eudragit® RS 30 D polymethacrylate and oligosaccharide.

The results of this study have shown that the addition of Pect-STMP and oligosaccharide to Eudragit® RS 30 D polymethacrylate in aqueous dispersions promoted changes to both the water vapour permeability and hydration properties of the films formed, compared to the control films (100 : 0 : 0, Eudragit® RS 30 D). The changes were shown to be dependent on, as well as proportional to, the increase in the concentration Pect-STMP added to the films.

Therefore, the films obtained, especially that constituted of Eudragit® RS 30 D : Pect-STMP-E : oligosaccharide (80 : 15 : 5), are regarded as being suitable for preventing the premature release of drugs in the upper part of the GIT, when used for oral solid system coatings. Moreover, the presence of Pect-STMP and oligosaccharide enables the specific degradation of the films through the action of enzymes produced by the colonic microflora, making the kinetics of modified drug release with a specific delivery characteristic possible. Nevertheless, subsequent colon-specific experiments *in vivo* need to be carried out in order to confirm the possible application of this new material.

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