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Reaction of pectin and glycidyl methacrylate and ulterior formation of free films by reticulation

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Abstract

In this work, low-methoxyl pectin was chemically modified by reaction with glycidyl methacrylate (GMA) to give a material with low hydrosolubility. After physio-chemical characterization by FT-IR, DSC, and TGA analyses, the methacrylated/modified pectin (Pect-GMA) was crosslinked after the addition of sodium persulfate (SP), that actuates as initiator, at 50 °C for 24 and 48 h either in the presence or not of aqueous polymethacrylate dispersion (Eudragit® RS 30 D) to obtain free films by Teflon® plate "casting" process. Different Pect-GMA/Eudragit® RS 30 D ratios and SP concentrations were used. The free films were characterized by the determination of water vapor transmission (WVT), the swelling index (I_{eq}%) in simulated gastric (SGF) and intestinal (SIF) fluids, and by scanning electron microscopy (SEM). The presence of ionized groups in Pect-GMA turned the films pH-dependent because I_{eq}% of swollen crosslinked Pect-GMA films was larger at pH 6.8 than at pH 1.2. This was confirmed by the large pore size observed in the micrographs of SIF-swollen lyophilized films. In this way, films containing Pect-GMA and Eudragit® RS 30 D, a time-dependent polymer, may present a synergistic action that favors specific biodegradation of the film in distal end of the gastrointestinal tract (GIT) by enzymes produced by the colonic microflora, enabling the modification of the release kinetics of drugs.

Keywords: Methacrylated pectin; Films cast from modified pectin; Pectin-Eudragit® RS 30 D; Modified release

1. Introduction

Currently, many therapeutically promising molecules that are available through the pharmaceutical and biotechnology industries (insulin, endorphins and their analogues, contraceptive hormones, calcitonin, growth hormones, oral vaccines, etc.) have very limited oral administration and are destroyed by the stomach acid and or metabolized by enzymes of the small intestine. However, these extremely important and needed chemical entities do not stand the physiological requirements of the inhospitable environment of the gastrointestinal tract (pH variations, digestive enzymes, biliary salts). The colon can be viewed as the preferred absorption site for oral administration of protein and peptide drugs, because of the relatively low proteolytic enzyme activities. These compounds are very sensitive to the gastrointestinal environment and therefore cannot be administered orally due to the negligible activity of brushborder peptidase activity and much less activity pancreatic enzymes in the colon in comparison to the small intestine (Irache et al., 2005; Dupuis et al., 2006; Ibekwe et al., 2006; Orlu et al., 2006). Colon-specific delivery systems can provide local treatment of several inflammatory diseases that affect the colon, ulcerative colitis, Crohn's disease, colon carcinoma's and infections with a site-specific delivery system. In this way, the therapy will be more effective, once that it requires lower dose and the undesirable side effects would be reduced.

Different approaches have been explored in order to develop colon-specific drug release, including the synthesis of pro-drugs, the use of pH or time-dependent degradable coatings, as well as biodegradable systems. In the case of the time controlled release

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forms, the drug is released after a specific time interval based on the expected transit time for the device to reach the colon. But due to large variations in pH and transit times, neither principle is very reliable in terms of colon-specific drug release. Therefore, the specific enzymatic activity of the colon environment has been explored thoroughly.

Several natural polymers, such as those found in the diet, are preferred over synthetic materials for colonic delivery because they are safer and more available. The polysaccharides have recently been proposed as appropriate excipients for the development of colon-specific devices for oral administration based on their microbial biodegradability. A large number of these polysaccharides and oligosaccharides may form the basis for a suitable colonic biodegradable carrier.

The application of the polysaccharides as pharmaceutical excipients has received great attention in the prioritized conception of reservoir or matrices systems. The development of materials and the equipment for the such processes are believed as challenges and are promising sources of solutions regarding new drug delivery systems for therapeutic purposes. The main purpose of these efforts is centered on the application of polymeric materials to the development of more and more efficient systems for the transport and modified release of drugs with high site specificity. Furthermore, these systems may be extremely useful when it is sought to delay the release/absorption of the active principle in cases of pathologies that present a night circadian rhythm (application of chronopharmacology such as in night asthma, angina pectoris, and arthritis (Ibekwe et al., 2006; Mastiholimath et al., 2007; Orlu et al., 2006).

Recent research has suggested the development of new drug-coating materials made of natural polymers associated with synthetic polymers (aqueous dispersion) already established in the drug industry (Surelease®, Aquacoat®, ECD® and acrylic polymers such as Eudragit® RS 30 D, NE 30 D, RL 30 D and FS 30 D). These aqueous–base polymers have the advantage of avoiding the use of organic solvents, which minimizes the complexity of processes and industrial facilities, eliminating explosion risk and environmental and human hazards (Sinha and Kumria, 2003; Ibekwe et al., 2006; Bunhak et al., 2007a,b). Additionally, these materials ensure the non-toxicity of the pharmaceutical formulations while keeping with the good fabrication practices and the new environmental regulations.

Aqueous dispersions of Eudragit® RS 30 D have been extensively used in industrial applications and broadly investigated in the development of new materials aiming at the formulations of new oral systems with modified release (controlled and sustained). The polymers available in aqueous form have a potential spectrum of application in pellet, microparticle, and nanoparticle coating in addition to the coating of the classic solid pharmaceutical forms (Peeters and Kinget, 1993; Bunhak et al., 2007a; Oliveira and Cavalcanti, 2007).

Besides offering protection against external factors such as light, air, and humidity and ensuring stability to the pharmaceutical forms, coatings based on methacrylate copolymers such as Eudragit® RS 30 D afford release profiles with low permeability and controlled drug diffusion (Petereit and Weisbrod, 1999; Akhgari et al., 2006).

This growing interest in the use of natural hydrophilic polymers as drug carriers is justifiable by their large availability in nature (plants, micro-organisms, animals and algae) and their resulting low cost. These materials have a broad variety of structures and properties, high stability, good gellation, biocompatibility, non-toxicity, and can be easily chemically and/or biochemically modified. Additionally, they are resistant to the passage through the upper gastrointestinal tract and still have good biodegradability by the exclusive resident anaerobic microflora of the colonic environment (Sinha and Kumria, 2001; Friend, 2005; Orlu et al., 2006).

Found mainly in cell walls of several vegetal species, pectin is responsible for maintaining plant structure and support. This polysaccharide is mainly a linear polymer chemically constituted by D-galacturonic acid monomers in α-(1–4) bonds, occasionally interrupted by L-rhaminose α-(1–2) bonds. However, other monomers also may make part of the side chains such as neuter sugars as D-galactose, L-arabinose, D-xylose, L-rhamnose, L-fucose, and traces of 2-*O*-methylfucose (Pérez et al., 2000, 2003; Silva and Braga, 2004). Moreover, pectin has high molecular weight, ranging from 50,000 to 180,000 g/mol. Depending on the degree of substitution of D-galacturonic acid carboxyl groups by methoxyl groups ($-OCH_3$), pectin may be classified as high (over 50%) or low (below 50%) methoxylated/esterified. Thus, pectin may be described as a "canonic" structure for presenting a rather heterogeneous and complex chemical structure (Pérez et al., 2000; Pérez et al., 2003; Chourasia and Jain, 2003).

However, the greatest challenge found in the use of pectin in the development of drug coatings is to overcome its solubility in aqueous medium, which may contribute to the premature and local undesirable release of the active principle as a polysaccharide in the support system. An alternative to reduce the high solubility of polysaccharides is to chemically modify them without affecting their biodegradability by the colonic microflora (Sinha and Kumria, 2003; Friend, 2005).

In this work, we proposed the chemical modification of pectin by the reaction with glycidyl methacrylate (GMA). The objective of this modification reaction is the introduction of vinylic groups in the polysaccharide structure. These vinylic groups will later react through free radicals and generate polymeric chains and produce polysaccharide hydrogels (Dijk-Wolthuis et al., 1995; Vervoort et al., 1997; Reis et al., 2003, 2006).

Later, based on the data available in recent literature, it was possible to propose the use of methacrylated pectin (Pect-GMA) with reduced hydrosolubility to develop free films with potential pharmaceutical use in association with polymethacrylate Eudragit® RS 30 D in aqueous dispersion (Cavalcanti et al., 2002; Liu et al., 2003; Codagnone et al., 2004; Bunhak et al., 2007a,b). This association aimed at improving the filmogenic property of the new material and ensuring its broader application in site-specific drug release field. This can be achieved because the chemically modified pectin must be throroughly degraded by the enzymes secreted by the colonic microflora (mainly *Bacterioids*, *Bifidobacteria* and *Eubacteria*), making the drug available exclusively at the target site (Dongowski et al., 2002; Chourasia and Jain, 2003).

2. Experimental

2.1. Materials

Pectin-USP Spectrum® (CAS 9000-69-5), glycidyl methacrylate (Acros Organics®), and sodium persulfate (Sigma–Aldrich®), Eudragit® RS 30 D (methacrylate–acrylate copolymer with quaternary ammonium groups USP/NF/ Degussa Pharma/Germany) kindly provided by the firm Almapal (São Paulo/Brazil); simulated gastric (SGF, pH 1.2) and intestinal (SIF, pH 6.8) fluids according to USP (28th Ed).

2.2. Modification of the polysaccharide with glycidyl methacrylate

According to the methodologies proposed by Dijk-Wolthuis et al. (1995), Vervoort et al. (1997) and Reis et al. (2003, 2006), it was initially prepared a polysaccharide aqueous solution at 2.5% (Pectin-USP Spectrum®, 6.7% methoxylation). After the complete solubilization of the polysaccharide, it was added 4.8 mL of GMA (pH 3.5) and maintained under mechanical agitation for 24 h at 50 °C. Afterwards, it was added with ethanol to precipitate the modified pectin. After filtration, the modified pectin was lyophilized. The material obtained was triturated and stored in desiccators for later physical–chemical characterization and obtaining the free films.

As shown in the scheme in Fig. 1, Li et al. (2003) and Reis et al. (2006) suggested that the addition of GMA to pectin in acid aqueous medium (pH 1.2) occurs by opening the epoxide ring of GMA without the formation of glycidol, which results from the transesterification reaction in aprotic solvent at alkaline pH.

2.3. Infrared spectroscopy (FT-IR)

Unmodified and modified polysaccharide samples were analyzed by infrared spectroscopy (spectrophotometer FT-IR-BOMEN-MB-100-Michelson®) as a first attempt to characterize the probable structural modification produced. The samples were prepared by the potassium bromide pellet technique (KBr) with 1% sample for analysis in the 4000 and 400 cm^{-1} region.

2.4. Thermal analysis (DSC and TGA)

The differential scanning calorimetry (DSC) analyses were carried out in a Shimadzu DSC-50 apparatus with approximately 6 mg of sample (unmodified and modified pectin) under nitrogen flow of 50 mL/min in the temperature range of 0–500 °C at 10 °C/min. The equipment was calibrated with indium and zinc standards.

Thermogravimetric analyses (TGA) were carried out in a Shimadzu® TGA-50 apparatus with 6 mg samples in the temperature range of 25–1000 °C at 10 °C/min and with nitrogen flow at 50 mL/min. All samples were kept in vials with dehydrated silica gel (110 °C/1 h) until the moment of analysis.

2.5. Production of methacrylated pectin films

Pect-GMA was reticulated with sodium persulfate (SP) at 50 °C for either 24 or 48 h either in the presence or not of polymethacrylate aqueous dispersion (Eudragit® RS 30 D). The films were produced by Teflon® plate casting, as shown in Fig. 2. For the films without Eudragit® RS 30 D, the Pect-GMA solution was mixed with different amounts of SP as shown in

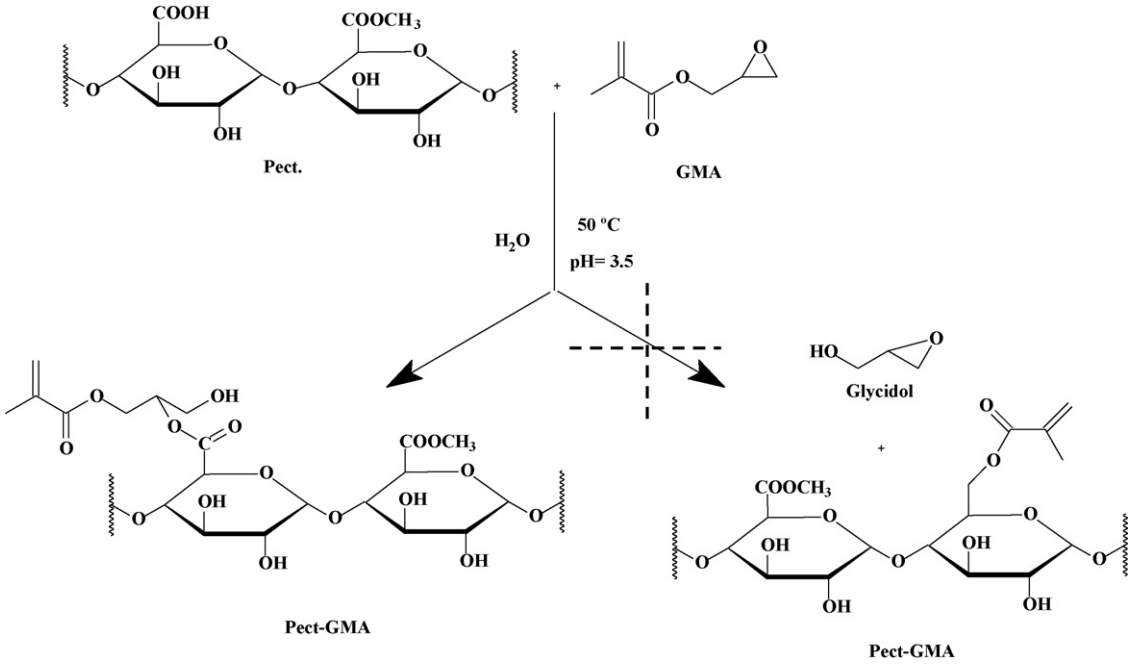

Fig. 1. Schematic representation of the reaction between pectin and GMA (adapted from Reis et al., 2006).

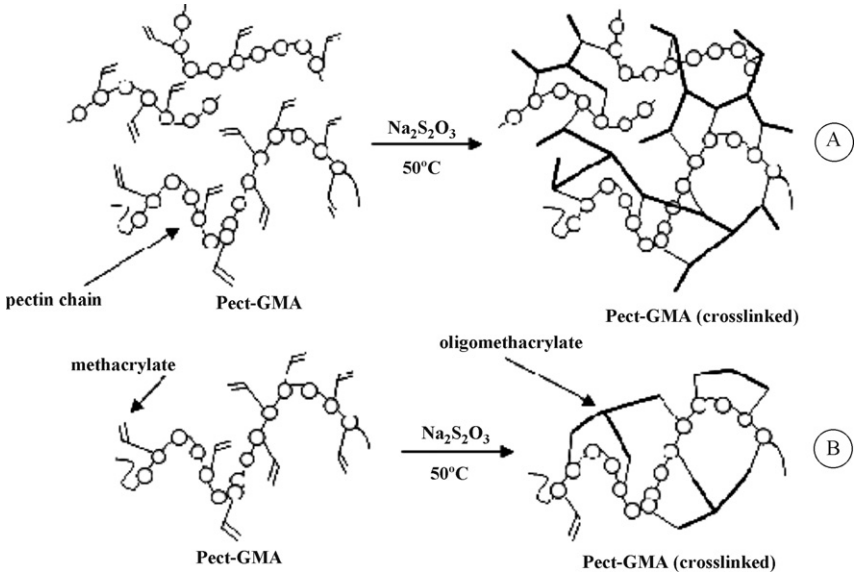

Fig. 2. Schematic representation of the crosslinking reaction of the Pect-GMA. In (A) intramolecular and intermolecular crosslinks and (B) only intermolecular crosslinks (adapted from Franssen et al., 1999).

Table 1 and agitated for approximately 2 h at room temperature ($T = 25 \pm 2.0$ °C). After the complete homogenization of the different mixtures, samples with 10 mL of each solution were poured onto a Teflon® plate and placed in a leveled oven at 50 °C (minimum temperature for reticulation and film formation) for either 24 or 48 h. The previous procedure was used to prepare Pect-GMA/Eudragit® RS 30 D films; however, with varying Pect-GMA/Eudragit® RS 30 D proportions for a final polymer mass in solution of 2% (w/v). To avoid the incorporation of air and bubble formation in the film, the Pect-GMA/Eudragit® RS 30 D/SP mixtures were homogenized under reduced pressure obtained with a vacuum pump.

2.6. Macroscopic characterization of the film morphology

It is fundamental to verify the macroscopic morphologic characteristics of the films, especially the presence of air bubbles and/or cracks, as the film integrity will ensure the reproducibility and the execution of the other analyses, particularly the permeability assays. Film thickness was determined with a Mitutoyo® micrometer at five random film surface points. Next, the selected films were stored in a desiccator with silica gel until the moment of the other assays.

Table 1
Proportion of components in free films (Pect-GMA and Eudragit® RS 30 D).

Film	Dry time (h)	Concentration of sodium persulfate (M)
Different composition of free films (Pect-GMA)		
n1	24	0.01
n2	24	0.001
n3	48	0.01
n4	48	0.001
Different compositions of free films (Pect-GMA:Eudragit® RS 30 D)		
No. 1 (90:10)	48	0.01
No. 2 (70:30)	48	0.01
No. 3 (90:10)	48	0.001
No. 4 (70:30)	48	0.001
No. 5 (90:10)	24	0.01
no. 6 (70:30)	24	0.01
No. 7 (90:10)	24	0.001
No. 8 (70:30)	24	0.001

2.7. Permeability/water vapor transmission study (WVT)

The study of WVT was carried out according to method B of ASTM designation E96-66 using Payne permeability cups (Braive Instruments, Liège, Belgium, in Fig. 4). Demineralized water (10 mL) was put into one of the cups, and the film was subsequently attached to the device. The cup with the film was then weighed and stored in a desiccator filled with silica gel. After 24, 48, 72, 96 and 120 h of storage the cups were reweighed in order to determine the permeated amount of water (mass loss %). The different values of mass loss were fitted to Eq. (1) and standardized to a 24-h time period, establishing the WVT for each polymeric composition tested (Cavalcanti et al., 2002, 2005; Bunhak et al., 2007a,b; Oliveira and Cavalcanti, 2007; Lamim et al., 2006):

$$\mathrm{WVT} = \frac{g24}{t\,a} \quad (1)$$

where g represents mass loss, t time measured in hours during which the weight loss occurred and a the exposed area of the film, which was 10 cm^2. Measurements were made in triplicate.

2.8. Determination of swelling-in-equilibrium indexes (I_{eq}%)

Films with different formulations were carefully cut with surgical scissors (professional model F/1) to approximately 1.0 cm^2 and distributed onto Petri dishes. Later, the Petri dishes were placed in an oven at 50 °C for 10 h for total humidity loss.

The dishes were removed and kept in desiccators with silica gel until the hydration experiment. The dry samples of the different associations were initially weighed and immediately immersed in recipients with either SGF (pH 1.2) or SIF (pH 6.8) without digestive enzymes at 37 °C. The samples were removed from the simulation media with tweezers 1 h after immersion and were carefully dried between two filter paper sheets and weighed again. The same procedure was adopted for the other samples, always in triplicate. The swelling-in-equilibrium index is a parameter that correlates the absorbed fluid mass (in this case SGF or SIF) by the film and the dry film mass. It was considered that swelling in equilibrium occurred when the swollen film mass variation as a function of time was zero. To calculate $I_{eq}\%$, the following equation was used:

$$I_{eq}\% = \frac{M_s - M_d}{M_d} \times 100 \quad (2)$$

where M_s is the mass of the swollen film in equilibrium and M_d is the mass of the dry film. $I_{eq}\%$ was determined following procedures similar to those in literature established by Cavalcanti et al. (2002), Codagnone et al. (2004), Bunhak et al. (2007a,b), Oliveira and Cavalcanti (2007) and Akhgari et al. (2006).

2.9. Scanning electron microscopy (SEM)

Free film samples, either dry or immersed in either SGF or SIF were frozen with liquid nitrogen after the swelling assays ($I_{eq}\%$) and lyophilized at −55 °C (Lyophilizer Martin Christ®, Alpha 1-1/DL) for 6 h in an attempt to preserve their morphological characteristics in both conditions. The micrographs of the free films were obtained with scanning electron microscope (Shimadzu model SS-550—Superscan) at 12 keV. All micrographs were obtained from gold-coated fracture surfaces. All samples were properly kept in vials with dehydrated silica gel (110 °C/1 h) until the moment of analysis.

2.10. Statistical analysis

The level of significance between the swelling indexes and water vapor transmission for the different associations of Pect-GMA films were statistically determined with GraphPad Prism® (version 2.01, 1996). The different $I_{eq}\%$ and WVT coefficients obtained under different circumstances (simulated gastrointestinal tract media) were initially evaluated by variance analysis (ANOVA) at significance level of $p < 0.05$. When the values gave significant parameters, we applied the data means to Tukey's multiple comparison tests to compare the role of the different polymer compositions. The values for $p < 0.05$ were also considered significant. The WVT assay results were treated with Statistica® (version 7.0, 2004) by surface response analysis.

3. Results and discussion

3.1. Infrared spectroscopy

Fig. 3 gives the FT-IR spectra of pectin (Spectrum®, low methoxylation) and of Pect-GMA. The presence of the two

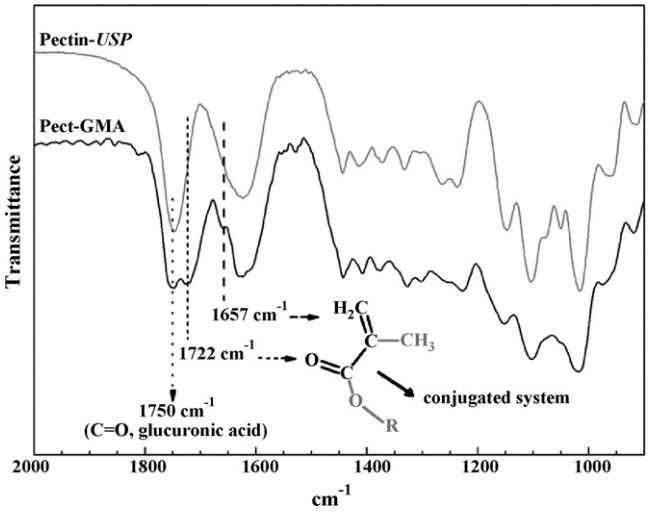

Fig. 3. FT-IR spectra of the natural pectin (Pect-USP) and the modified pectin (Pect-GMA).

bands in the Pect-GMA spectrum characterizes the chemical modification of pectin with GMA as follows: first band at 1657 cm^{-1} attributed to (C=C) vibrations of the GMA vinylic groups. The second band, in the 1722 cm^{-1} region of the Pect-GMA spectrum, was attributed to the axial deformation of C=O conjugated ester groups, also from GMA. These results agree with those of the investigation of the chemical modification of galactomannan and Arabic gum with GMA by Reis et al. (2003, 2006).

3.2. Thermal analysis (DSC and TGA)

The comparison of the thermal behaviors of Pect-GMA and unmodified pectin (low methoxylation) based on DSC thermograms, Fig. 5, reveals that the intensity of the dehydration endothermic peak of modified pectin (60–150 °C) suddenly decreases and shifts to lower temperatures in comparison to that of unmodified pectin. This result suggests that Pect-GMA presents a smaller amount of carboxyl groups available to interact with water molecules since the incorporation of GMA reduces the hydrophilicity of pectin. Such evidences are supported by similar results obtained by Zohuriaan and Shokrolahi (2004) in the evaluation of the thermal behavior of unmodified and modified gums. Additionally, the results analyzed based on Fig. 5 showed an exothermic peak in the thermograms of the modified product (Pect-GMA) and of unmodified pectin at higher temperatures (235 and 241 °C, respectively). Based on the analysis of the thermograms obtained for methylcellulose, Zohuriaan and Shokrolahi (2004) concluded that in general, temperatures as high as the ones recorded in their work are associated to dehydration, depolymerization, and pyrolysis due to the evolution of H_2O, CO, and CH_4. However, in polysaccharides with carboxyl groups in their constitution such as unmodified pectin and Pect-GMA, these thermal transitions are probably related to the observed generation of CO_2. The results obtained in this work agree with those of the investigation of xantan, tragacante, and sodium alginate by Zohuriaan and Shokrolahi

Fig. 4. System adapted from Payne's permeability cups (Braive Instruments, Liège, Belgium), according to 'B' method of ASTM guidelines E96-66.

(2004). It was also possible to observe that the released energy (which is directly proportional to the peak areas) attributed to the exothermic transitions observed for modified pectin (Pect-GMA) is 234 J/g and that it is larger than that of unmodified pectin (219 J/g). This increase is probably due to the structural modification performed. Zohuriaan and Shokrolahi (2004) and Iijima et al. (2000) reported that the glass transition temperature (T_g) could not be recorded in similar thermograms due to the humidity, which probably interfered with this part of the endothermic curve.

Fig. 6 presents the TGA curves of unmodified and GMA-modified Pectin. It can be observed an initial mass loss between 0 and 200 °C in this thermogram both for unmodified and modified pectin attributable to the desorption of humidity from the polysaccharide structure. The same behavior was observed in previous works such as that by Zohuriaan and Shokrolahi (2004), who studied unmodified and modified polysaccharides. The main decomposition step starts at approximately 181 °C for unmodified pectin and at 173 °C for modified pectin (Pect-GMA). These values, obtained from the first derivative curve versus temperature (1stDer × T, not shown), suggest that Pect-GMA presents slightly lower thermal stability than that of unmodified pectin.

3.3. Macroscopic characteristics of the films

In the macroscopic evaluation of the film morphology, the following were considered (i) phase separation, (ii) presence of cracks, (iii) presence of air bubbles, (iv) transparence, and (v) flexibility. The results are given in Table 2. The film transparence and flexibility were suitable for the assays. However, it is worth noting that as shown in Table 2, the films with Eudragit® are less flexible. This characteristic is probably related to the high glass transition temperature of the polymethacrylate in the film formulation. The flexibility observed in Pect-GMA films without Eudragit® remained constant, regardless of the variation of the concentration of SP and the drying time at 50 °C necessary for reticulation and film formation. We can also suggest that the increase in concentration of Pect-GMA in the compositions obtained seems not to induce macroscopic changes related to transparence and flexibility, as shown in Table 2.

It is interesting to observe in Table 3 that there is a significant variation in standard film thickness, which is probably related to the drying time of either 24 or 48 h. Pect-GMA films

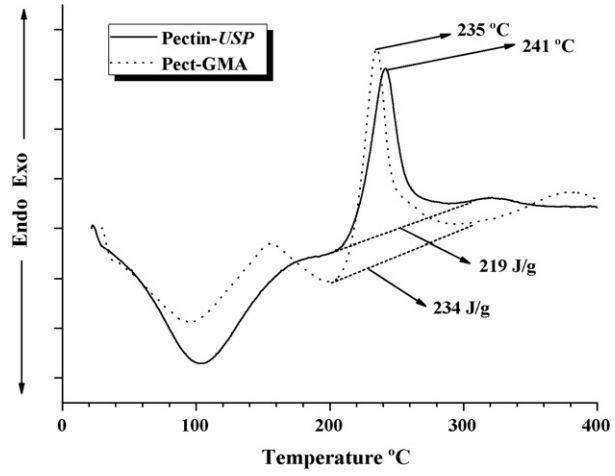

Fig. 5. Comparison of DCS thermograms of the natural pectin (pectin-USP) and the modified pectin (Pect-GMA).

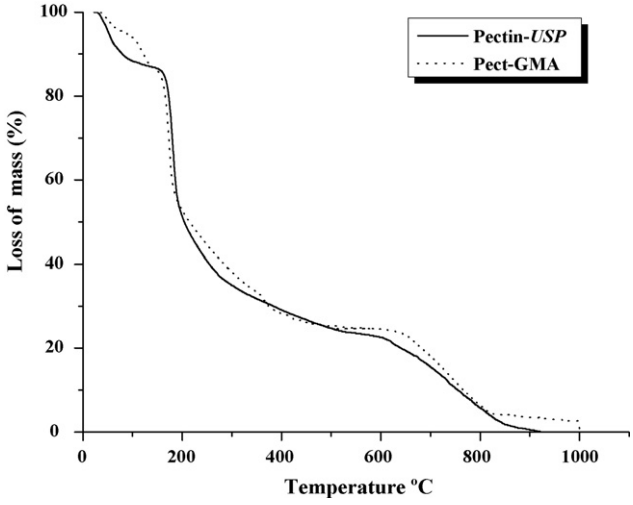

Fig. 6. Thermogravimetric curves for natural pectin (pectin-USP) and modified pectin (Pect-GMA).

Table 2
Main macroscopic characteristics observed in different formulations of free films (Pect-GMA: Eudragit® RS 30 D)

Dry time (h)	Concentration [SP] (M)	Separation of phase	Presence of crack	Bublle of air	Transparence	Flexibility
Compositions of free films (Pect-GMA)						
24	0.01	0	0	0	+++	+++
24	0.001	0	0	0	+++	+++
48	0.01	0	0	0	+++	+++
48	0.001	0	0	0	+++	+++

Dry time (h)	Concentration [SP] (M) & Pect-GMA:Eudragit® RS 30 D	Separation of phase	Presence of crack	Bublle of air	Transparence	Flexibility
Different compositions of free films (Pect-GMA:Eudragit® RS 30 D)						
No. 1 (48)	[0.01] 90:10	0	0	0	+++	++
No. 2 (48)	[0.01] 70:30	0	0	0	+++	++
No. 3 (48)	[0.001] 90:10	0	0	0	+++	++
No. 4 (48)	[0.001] 70:30	0	0	0	+++	++
No. 5 (24)	[0.01] 90:10	0	0	0	+++	++
No. 6 (24)	[0.01] 70:30	0	0	0	+++	++
No. 7 (24)	[0.001] 90:10	0	0	0	+++	++
No. 8 (24)	[0.001] 70:30	0	0	0	+++	++

0, no change observed; +, lightly present; ++, moderately present; +++, strongly present.

obtained with 24-h drying/reticulation are thicker than those obtained with 48 h, regardless of the concentration used. As shown in Table 3, the variations were not observed in Pect-GMA Eudragit® RS 30 D film thickness compared to each other and to Pect-GMA films, regardless of the concentrations of SP and modified polysaccharide and the drying/reticulation time. Thus, one can suggest that there was coalescence during film formation. Crosslinking was more intense for 48-h drying/reticulation, which results in thinner Pect-GMA films. It can also be inferred that even the smallest SP amount used is excessive, as this factor (SP) did not influence the thickness of the films obtained. Additionally, as shown in Table 2, the analysis of the macroscopic characteristics reveals that the films obtained are homogeneous for the different formulations. This demonstrates that the methodology used is appropriate and compatible with a good dispersion of the constituents.

3.4. Permeability/water vapor transmission study

The determination of water vapor transmission is a simple and easy method to make a preliminary evaluation of permeability of polymeric films when compared to the traditional diffusion methods. WVT results provide valuable information on the protection against environmental humidity during the storage of several materials (paper, plastic, packages, etc.), including polymeric materials used in drug-coating technology (Akhgari et al., 2006; Bunhak et al., 2007a).

As shown in Fig. 7, the values of WVT of Pect-GMA films are influenced by the drying/reticulation time and the SP concentration. WVT is not affected by drying time for large SP concentrations. These results suggest that there is effective film reticulation for 0.01 mol L^{-1} of SP and 24 h due to the larger generation of free radicals, which are responsible for the attack

Table 3
Values of thickness of obtained free films ($n = 5$)

Dry time (h)	Concentration of [PS] (M)	Thickness (mm)	S.D.
Different compositions of free films (Pect-GMA)			
24	0.01	0.114	±0.02
24	0.001	0.116	±0.01
48	0.01	0.063	±0.00
48	0.001	0.063	±0.01

Dry time (h)	Concentration of [SP] (M) and associations	Thickness (mm)	S.D.
Different compositions of free films Pect-GMA:Eudragit® RS 30 D)			
No. 1 (48)	[0.01] 90:10	0.058	±0.010
No. 2 (48)	[0.01] 70:30	0.074	±0.008
No. 3 (48)	[0.001] 90:10	0.085	±0.020
No. 4 (48)	[0.001] 70:30	0.068	±0.013
No. 5 (24)	[0.01] 90:10	0.050	±0.008
No. 6 (24)	[0.01] 70:30	0.058	±0.011
No. 7 (24)	[0.001] 90:10	0.078	±0.011
No. 8 (24)	[0.001] 70:30	0.070	±0.018

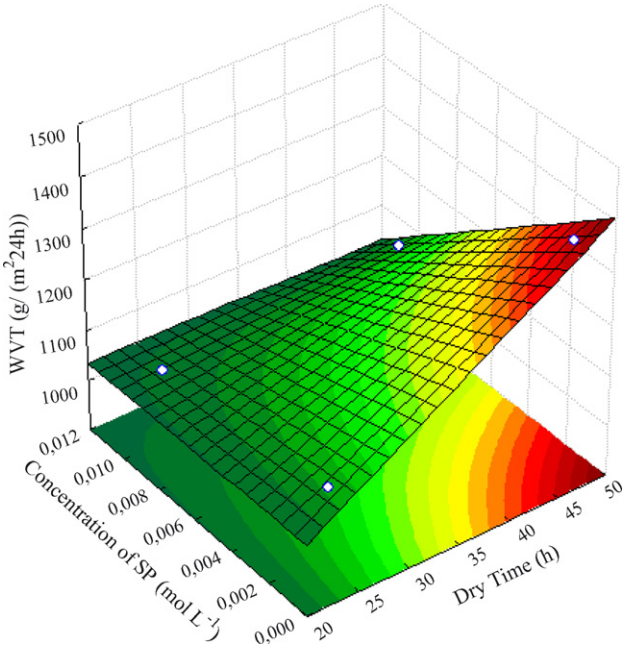

Fig. 7. Response surface plot for water vapor transmission through free films containing only Pect-GMA (concentration of SP: 0.01 and 0.001 mol L^{-1} and dry of time: 24 and 48 h) ($n = 3$).

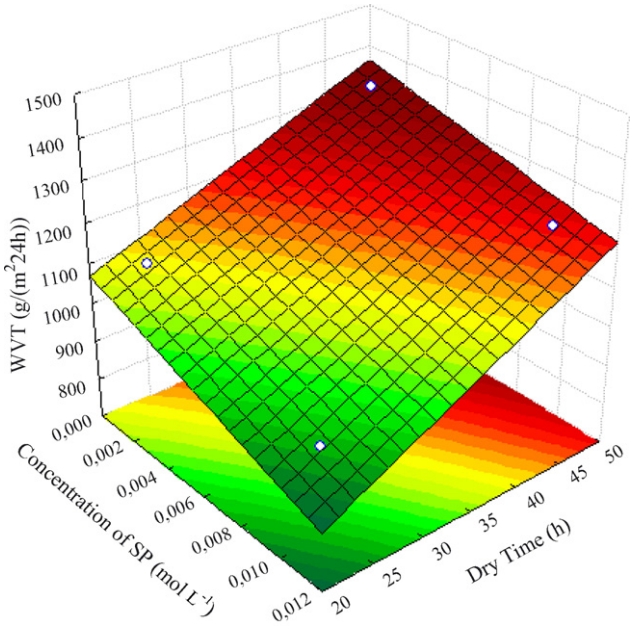

Fig. 9. Response surface plot for water vapor transmission through free films constituted by Pect-GMA: Eudragit® RS 30 D (concentration of SP: 0.01 and 0.001 mol L^{-1} and dry of time: 24 and 48 h) ($n = 3$).

of the double bonds of vinylic groups present in Pect-GMA (Reis et al., 2003, 2006; Vervoort et al., 1998). Film exposure for longer than 24 h does not result in subsequent decrease in WVT values in this case. If the SP concentration is kept at 0.001 mol L^{-1}, the WVT values of films dried/reticulated for 48 h are larger than those obtained for films dried/reticulated for 24 h. This result indicates that cracks may have been formed during film coalescence, which allowed easier permeation of water vapor.

As shown in Figs. 8 and 9, the evaluation of the WVT values of Pect-GMA/Eudragit® RS 30 D films also reveals that the increase in drying time affected WVT value positively. However, it can be observed a reduction in WVT in Pect-GMA/Eudragit®

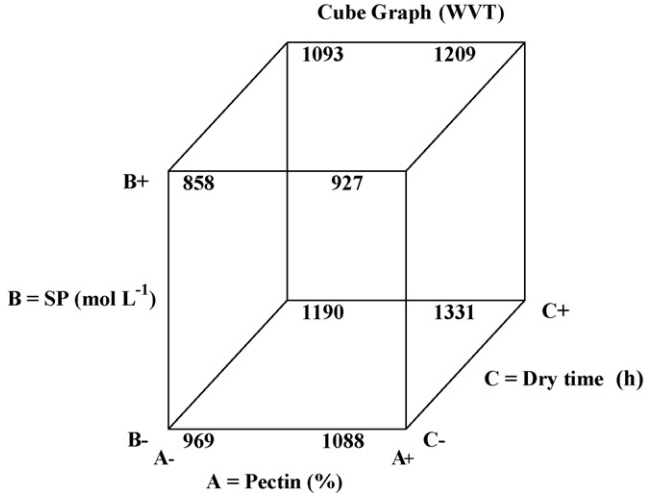

Fig. 8. Values of WVT ((g/m^2) 24 h)) obtained from different formulations of free films constituted by Pect-GMA: Eudragit® RS 30 D.

RS 30 D films prepared with larger concentrations of SP. These results agree with those observed for Pect-GMA films. Another important observation is that the increase in Pect-GMA concentration resulted in lager WVT ratios, as shown in Fig. 7.

This behavior is similar to the one observed by Bunhak et al. (2007a,b), who incorporated condroitine sulfate, unmodified and also reticulated, to the formation of Eudragit® RS 30 D and ethyl cellulose (Surelease®) free films. The authors observed that the increase in permeability is proportional to the polysaccharide concentration. Accordingly, Codagnone et al. (2004) investigated the formation of Surelease® free films associated to phosphated Guar gum and observed an increase in WVT proportional to and dependent on the concentration of the incorporated polysaccharide. From the results presented in this work, one can be suggested that the increase in Pect-GMA from 70% to 90% (in mass) in films afforded an increase of the hydrophilicity of the system due to the largest number of hydroxyl groups available for the interaction with molecules of water.

Besides the hypothesis mentioned above, it may be suggested that the increase of Eudragit® RS 30 D from 10–30% (in mass) reduced the hydrophilic portion of the films, since that copolymer (acrylic and methacrylic acid esters) is insoluble in water. Besides, this increase of the copolymer (10–30%) in the films provided larger number of ionic interactions between the carboxyl groups of Pect-GMA (unreacted with GMA) with the quaternary ammonium groups of Eudragit® RS 30 D, what still decreases more the hydrophilic character of the films. This probably affected the transport of water molecules through the film surface, as it can be observed through the lower WVT ratios (permeability) according to Fig. 8. Semde et al. (1998) who proposed a similar interpretation of the phenomena when evaluating films containing Eudragit® RS 30 D associated to low methoxyl pectin and calcium pectinate. In that case, smaller WVT ratios can

contribute to larger stability of several pharmaceutical products during storage.

Besides the hypothesis mentioned above, it may be suggested that the addition of a larger concentration of methacrylated pectin reduced the hydrophilic portion of the films (due to the incorporation of GMA). This ability of Pect-GMA may have affected the molecule transport through the film surface and led to lower WVT ratios.

3.5. Determination of the swelling-in-equilibrium index

The hydrophilicity of free films is essential to allow the access of enzymes of bacteria in the distal end of the GIT and to facilitate the degradation of the material when in contact with physiological fluids. Thus, the evaluation of the swelling index allows evaluating hydrophilicity and may also assist in the elaboration of a mathematical model capable of describing the release kinetics (Mulhbacher et al., 2004).

Based on WVT results, film no. 6, made up of Eudragit® RS 30 D/Pect-GMA (70:30), dried/reticulated for 24 h from 0.01 M of SP, was selected for the swelling index assay (I_{eq}%) in SGF and SIF as it presented the lowest WVT. The swelling indexes of this film in SGF and SIF are given in Fig. 10. It is patent that the I_{eq}% of the film swollen in the two fluids varies. In SGF, the medium with low pH (1.2) interferes with the Pect-GMA hydration significantly; as it has an anionic characteristic due to the presence of carboxyl groups (not reacted with GMA). According to Mulhbacher et al. (2004), with the increase in pH, it is expected an increase in the swelling of anionic polymers such as Pect-GMA. In SIF, the ionization of the carboxyl groups of Pect-GMA provokes repulsion of the chain segments and leads to the expansion of the polymer network. This results in a larger space within the polymeric matrix that may be occupied by water molecules.

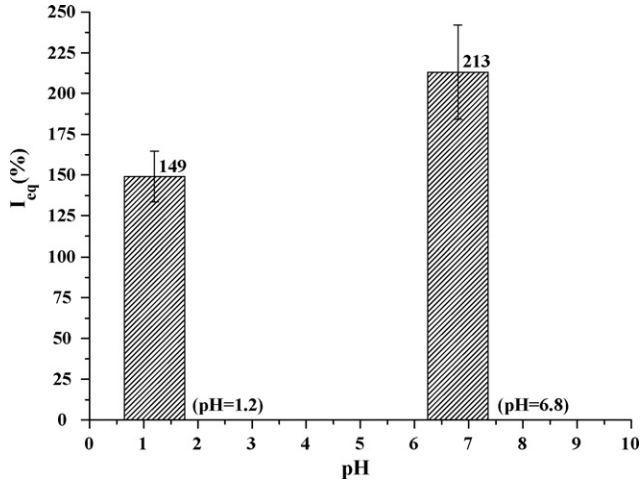

Fig. 10. Swelling index (I_{eq}%) for free film no. 6 (Pect-GMA: Eudragit® RS 30 D; 70:30, 0.01 mol L^{-1} of SP and 24 h of dry time) in simulated gastric fluid (pH 1.2) and in simulated intestinal fluid (pH 6.8) ($n = 3$).

These results suggest that the presence of Pect-GMA turns the free film obtained pH-dependent, preventing the early release

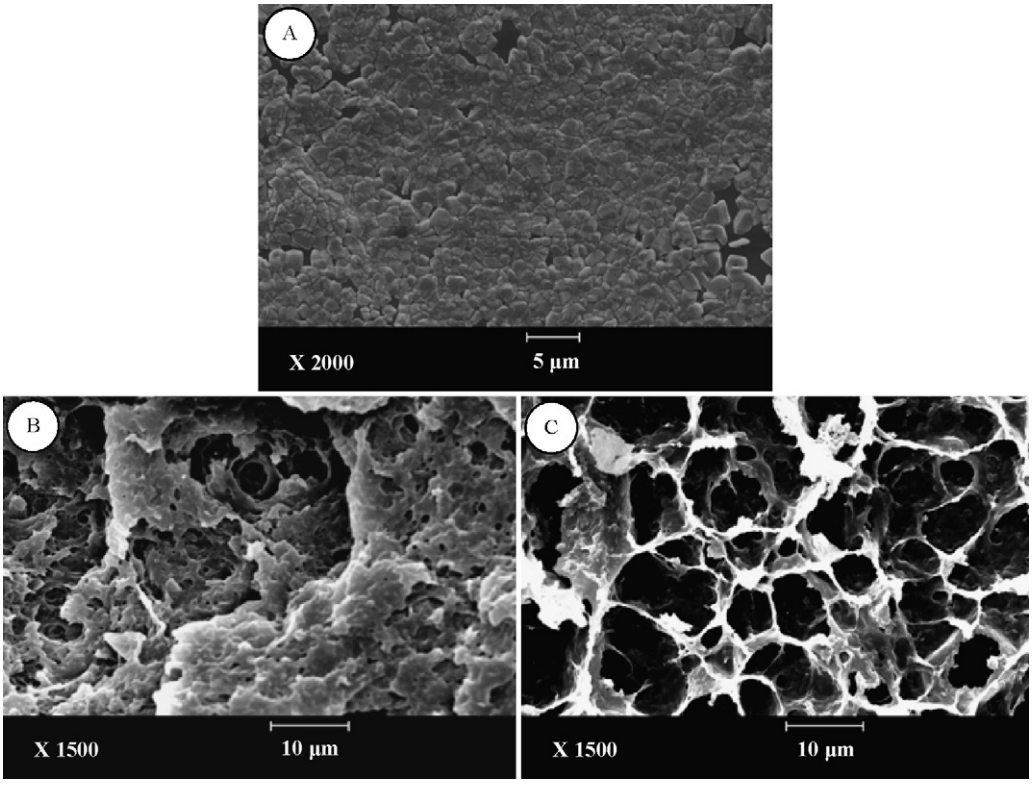

Fig. 11. Scanning electron micrographs of films of Pect-GMA: Eudragit® RS 30 D (70:30, 0.01 mol L^{-1} of SP and 24 h of dry time). In (A) dry samples, (B) pH 1.2 and (C) pH 6.8.

of the drugs in upper regions of the GIT. However, when in contact with biologic fluids with pH close to neuter (characteristic of the GIT distal end), the membrane presents excellent hydration capacity, which makes drug release easier. Furthermore, the presence of Pect-GMA makes the film vulnerable to the enzymes produced by the colonic microflora, making it potentially relevant as an oral solid system coating with modified drug release kinetics.

3.6. Scanning electron microscopy

Fig. 11 presents the micrographs obtained for film no. 6 lyophilized after the swelling assays. It is possible to observe the changes in the morphological characteristics when compared to the dry film micrograph.

After swelling in SGF (pH 1.2), it is possible to observe a rather heterogeneous surface, with the clear presence of Pect-GMA between regions rich in Eudragit® RS 30 D (Fig. 11B). In contrast, after swelling in SIF, the film presents flaws in some regions (Fig. 11C), probably due to the larger relaxation of the polymeric chains as previously discussed. Based on this observation, it is possible to suggest that this phenomenon is related to the larger hydration of the film during the SIF (I_{eq}%) assay, as shown in Fig. 10.

4. Conclusion

Our results suggest that the modification/reticulation methods of (low methoxylation) pectin used in this study resulted in a new material (Pect-GMA) with reduced solubility suitable for the development of free films associated to Eudragit® RS 30 D polymethacrylate. The free film formed with Pect-GMA associated to Eudragit® RS 30 D produced a material with pharmacotechnical potential with attractive characteristics for the development of new drug oral administration systems. When applied as a pharmaceutical coating, this material may prevent the early release of the drug in the proximal GIT, besides ensuring the effective control of the target-site-specific release of drugs due to the prospective specific degradation of the film by enzymes produced by the colonic microflora. However, further *in vitro* and/or *in vivo* colon-specificity assays may shed light on the effective application of this new material.

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