Carbohydrate Polymers 99 (2014) 140–149

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Films from resistant starch-pectin dispersions intended for colonic drug delivery

Andréia Bagliotti Meneguin [a], Beatriz Stringhetti Ferreira Cury [b,*], Raul Cesar Evangelista [b]

[a] Graduate Program in Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, São Paulo State University-UNESP, km 1 Araraquara-Jaú Road, Araraquara, SP, Brazil
[b] Department of Drugs and Pharmaceuticals, Faculty of Pharmaceutical Sciences, São Paulo State University-UNESP, km 1 Araraquara-Jaú Road, Araraquara, SP, Brazil

ARTICLE INFO

Article history:
Received 16 February 2013
Received in revised form 23 July 2013
Accepted 27 July 2013
Available online 8 August 2013

Keywords:
Free film
Resistant starch
Pectin
X-ray diffraction
Dissolution
Enzymatic digestion.

ABSTRACT

Free films were obtained by the solvent casting method from retrograded starch-pectin dispersions at different polymer proportions and concentrations with and without plasticizer. Film forming dispersions were characterized according to their hardness, birefringence and rheological properties. The polymer dispersions showed a predominantly viscous behavior ($G'' > G'$) and the absence of plasticizers lead to building of stronger structures, while the occurrence of Maltese crosses in the retrograded dispersions indicates the occurrence of a crystalline organization. Analyses of the films included mechanical properties, thickness, superficial and cross sectional morphology, water vapor permeability, liquid uptake ability, X-ray diffractometry, *in vitro* dissolution and enzymatic digestion. The high resistant starch content (65.8–96.8%) assured the resistance of materials against enzymatic digestion by pancreatin. Changes in the X-ray diffraction patterns indicated a more organized and crystalline structure of free films in relation to isolated polymers. Increasing of pectin proportion and pH values favored the dissolution and liquid uptake of films. Films prepared with lower polymer concentration presented better barrier function (WVP and mechanical properties).

1. Introduction

The spatial control of drug release along the gastrointestinal tract (GIT) represents an important subject of research in the field of drug delivery systems since specific targeting to an organ or tissue can improve the bioavailability of many drugs, especially those that present stability, solubility and/or permeability problems in the drastic environmental conditions of the GTI upper segments, mainly in the stomach. However, to find a successful approach to overcome this problem still remains a great challenge. The colon provides conditions, such as pH near to neutral, slow transit time and decreased proteolytic activity that make it a favorable environment for delivering drugs, mainly proteins and peptides.

Coating of solid dosage forms with polymeric materials whose solubility is dependent on pH or with those that are able to be degraded by colonic microbiota represents relevant strategies exploited in order to achieve this drug targeting (Maior, Reis, Muniz, & Cavalcanti, 2008; Mennini, Furlanetto, Cirri, & Mura, 2012).

The colon contains more than 400 species of microorganisms with a population of 10^{11}–10^{12} CFU mL^{-1} that produce a wide range of enzymes with reductive or hydrolytic activities involved in fermentation process of carbohydrates and proteins that are nondigestible in the upper portions of the GIT (Yang, 2008; Yang, Chu, & Fix, 2002). This great variety and quantity (five orders of magnitude higher than in other GIT segments) of colonic microorganisms and its minor inter-individual variability in relation to pH values and transit time make the microbially triggered delivery systems a more reliable strategy to reach the targeting of drugs to the colon (Das & Ng, 2010; Kakoulides, Smart, & Tsibouklis, 1998).

The development of novel materials intended to control drug release to fulfill the different therapeutic needs is a fundamental aspect. In this sense, the use of blends of already known polymers represents a rational approach to obtain materials with modulated properties to enable them for specific therapeutic goals, avoiding the high costs involved in the synthesis and characterization of completely new materials (Carbinatto, Castro, Cury, Magalhães, & Evangelista, 2012; Ebude & Jones, 2004; Lecomte, Siepmann, Walther, Macrae, & Bodmeier, 2005; Patel & Patel, 2007; Prezotti, Meneguin, Evangelista, & Cury, 2012; Silva, Bierhalz, & Kieckbusch, 2009; Wang, Hu, Du, & Kennedy, 2010).

Starch is a safe and nontoxic excipient, however, native starch does not present physical and chemical properties suitable to some

* Corresponding author. Tel.: +55 16 33016961; fax: +55 16 33016960.
E-mail addresses: curybsf@fcfar.unesp.br, bstringhe@yahoo.com.br (B. Stringhetti Ferreira Cury).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.07.077

specific uses and its modification by physical, chemical and enzymatic processes is necessary in order to modulate its properties (Zavareze & Dias, 2011). Thus, starch derivatives have been used in formulations of tablets, microparticles and coating films in order to reach desired drug release rates (López, García, & Zaritzky, 2008; Marinich, Ferrero, & Jiménez-Castellanos, 2012; Rashid, Al-Remawy, Leharne, Chowdhry, & Badwan, 2011; Teacă, Bodîrlău, & Spiridon, 2013; Tuovinen, Peltonen, et al., 2004; Tuovinen, Ruhanena, et al., 2004).

Resistant starch (RS) is the starch fraction that resists digestion in stomach and duodenum, but is degraded by colonic microbiota (Htoon et al., 2010). These properties make it a promising material to be used in the design of colon-specific drug delivery systems. Resistant starch is classified in different subtypes and among them retrograded starch (RS 3) is highlighted due to its thermal stability and low solubility (Haralampu, 2000). The retrogradation process occurs by hydrothermal treatment of starch, in which the pregelatinized starch (amorphous) changes to a more organized crystalline form during storage (Chung, Lim, & Lim, 2006; Thompson, 2000; Yuan, Thompson, & Boyer, 1993). This recrystallization occurs *via* hydrogen bonds and inter/intra molecular van der Waals forces, as a spontaneous process in an attempt to reach a thermodynamically more stable configuration (Chung et al., 2006; Liu, Yu, Chen, & Li, 2007; Tako, 1996; Yuan et al., 1993).

High amylose starch (HAS), a modified starch containing high content of amylose, has been considered as a favorite material to obtain high contents of RS (Dimantov, Greeberg, Kesselman, & Shimoni, 2003; Fishman, Coffin, Unruh, & Ly, 1996; Htoon et al., 2009), because the amylose forms an amorphous matrix in which the crystallites are embedded and thus protected against the fast exposition to digestive enzymes (Cui & Oates, 1997).

Several studies in the food area about the influence of some polysaccharides on the retrogradation of starch of different botanical origins have reported distinct behaviors. The retrogradation of rice starch can be improved by maltodextrin's presence (Lii, Lai, & Liu, 1998), while amylopectin chains of high molecular weight and xyloglucans enhance the retrogradation process of wheat starch and tapioca, respectively (Kohyama, Matsuki, Yasui, & Sasaki 2004; Temsiripong, Pongsawatmanit, Ikeda, & Nishinari, 2005). To xyloglucan's presence, however, was attributed the retrogradation delay of gelatinized corn starch (Yoshimura, Takaya, & Nishinari, 1999). The lag of retrogradation was also observed for the sago starch in the presence of galactomannans and for potato starch in the presence of guar gum and xanthan gum (Ahmad & Willians, 2001; Lee, Baek, Cha, Park, & Lim, 2002). More recently, Babic et al. (2006) studied the addition effect of different hydrocolloids in tapioca starch retrogradation and concluded that in general it promoted the inhibition of this phenomenon.

The variance of above behaviors have been attributed to variations of botanical origins, as well as of polymer concentration, temperature and time of retrogradation process (Zhou, Wang, Zhang, Du, & Zhou, 2008) and its show the necessity of new studies in this field.

Pectin is another polysaccharide suitable for use in preparations intended to target the drug to the colon, because it remains as macromolecular aggregates in upper portions of GIT and is degraded by colonic enzymes (Dev, Bali, & Pathak, 2011; Friend, 2005; Liu, Fishman, Kost, & Hicks, 2003). However, the great disadvantage of this polysaccharide lies in its high solubility in acid media that could promote the premature undesirable release of the drug (He, Du, Cao, Xiang, & Fan, 2008; Sinha & Kumria, 2001).

The influence of pectin in the retrogradation of starch has not been studied and this approach represents a promising strategy to prepare a new material with application in pharmaceutical field. Thus, mixtures of HAS and pectin at different ratios were submitted to retrogradation process and the RS content was estimated. These materials were also characterized by hardness, rheological and birefringence analysis and then used to prepare free films. Free films properties were evaluated (macroscopic observation, thickness, liquid uptake ability, water vapor transmission, mechanical strength) and the potential for using in the design of colonic drug delivery systems was investigated by analyses of dissolution and resistance against digestion by pancreatic enzyme.

2. Materials and methods

2.1. Materials

High amylose starch (type Hylon VII – 68% amylose, lot:HA9140) was a gift of the National Starch & Chemical (New Jersey, EUA), pectin (type LM-5206CS – DE < 50%, lot: S74431) was provided by CP Kelco (Copenhagen, Dinamarca), glycerin (99.5%, lot: 0807381) was purchased from Vetec (Duque de Caxias, Brazil), propylene glycol (99.5%) was provided by Synth (Diadema, Brazil), sodium hydroxide (lot: 611648) was supplied by Grupo Química (Rio de Janeiro, Brazil), 37% hydrochloric acid (lot: 29957) was provided by Quimis (Diadema, Brazil), potassium phosphate monobasic (98.0–100.5%, lot: 1002890) was obtained from Vetec (Duque de Caxias, Brazil), pancreatin (lot: 0903372) was purchased from Vetec (Duque de Caxias, Brazil), 3,5-dinitrosalicylic acid (purity ≥ 98.0%, lot: 125k3664) was provided by Sigma–Aldrich Co. (St. Louis, USA), sodium chloride was supplied by Synth (Diadema, Brazil), purified water (Milli Q, Millipore).

2.2. Retrogradation of high amylose starch (HAS) mixed with pectin

The retrogradation process of starch mixed with pectin was carried out in alternating thermal cycles of 4 °C and 30 °C during 16 days (2 days at each temperature) (Park, Baik, & Lim, 2009). In this sense, with the aqueous dispersions (5 or 10%) of HAS were prepared under mechanical stirring during 30 min and autoclaved at 121 °C (120 min). These pregelatinized dispersions were mixed at different ratios (1:1 or 1:4) to the aqueous dispersions of pectin, previously prepared in the same concentrations (5 or 10%) and submitted to thermal cycles.

2.3. Hardness analysis of film forming dispersions

The hardness of aqueous dispersions prepared in previous item was analyzed on a TA-XT2 Texture Analyzer (Stable Micro Systems). Each sample (10 g) was introduced in a cylindrical glass tube (16 mm × 100 mm) and compressed twice ($v = 0.50$ mm s^{-1}; depth 10 mm) by the cylindrical analytical probe (10 mm), allowing a relaxation time of 5 s between the compressions. The analyses were performed at room temperature, in five replicates.

2.4. Viscoelastic properties of film forming dispersions

The viscoelastic properties of samples were evaluated by dynamic oscillatory assays on a controlled stress rheometer (Haake Rheostress 1) (Gebruder Haake, Germany) equipped with cone-plate device (C35/2°Ti; $D = 35$ mm; gap = 105 mm). A circulating water bath (HAAKE C25P) at 37 °C and a software (Rheowin 3) for data acquisition were used. The mechanical spectra were obtained at constant stress (0.5 Pa) under angular velocity range of 0.6–623 rad s^{-1}, after the determination of the viscoelasticity linear range.

Table 1
Concentrations and proportions of polymers used for preparing the free films with and without plasticizers.

Concentrations of filmogenic dispersions	HAS:Pectin ratio		Plasticizer
	1:1	1:4	
5%	511G	514G	Glycerol
	511P	514P	Propyleneglycol
	511W	514W	Without plasticizer
10%	–	1014G	Glycerol
	–	1014P	Propyleneglycol
	–	1014W	Without plasticizer

2.5. Birefringence of film forming dispersions

Birefringence of mixtures of pregelatinized or retrograded HAS with pectin was investigated on a Leica MZ APO™ microscope at 400× magnification under polarized light. Samples not birefringent were observed under normal light.

2.6. Free films preparation

Free films were prepared by solvent casting. Glycerin or propylene glycol (5% in relation to polymer mass) was added as plasticizer to the film forming dispersions and the preparations kept under magnetic stirring for 120 min. A pre-determined volume of plasticized dispersions (0.2043 mL cm^{-2}) was poured into a Petri dish and dried in oven-dryer at 40 °C overnight. Films without plasticizer were also prepared as controls. After drying, the films were peeled from the Petri dish surface and kept for subsequent studies in a desiccator containing silica gel. The free films were labeled according polymer concentration (5 or 10%) and HAS:pectin ratio (1:1 or 1:4). The G, P and W suffixes were used to identify films containing glycerin, propylene glycol and those without plasticizer, respectively (Table 1).

2.7. Enzymatic digestion and resistant starch content of free films

Pieces of film (about 100 mg) were incubated in 2 mL of 0.1 M phosphate buffer (pH 7.1) during 30 min at 100 °C in order to eliminate RSI and RSII fractions (Englyst, Wiggins, & Cummings, 1982). After that, the preparations were cooled down until 37 °C and incubated with 0.5 mL of a pancreatin solution (0.15 g mL^{-1}) for different times (20, 60, 120, 150 and 180 min). In order to stop the enzymatic activity, 80% ethanol was added to the sample and the glucose content was quantified by the reaction with 3,5-dinitrosalicylic acid (DNS) (Bernfeld, 1955), by using a standard glucose curve. The amount of hydrolyzed starch at 20 min was determined as RDS (rapid digestible starch) and that hydrolyzed between 20 and 120 min as SDS (slowly digestible starch). The resistant starch content was calculated according to the Eq. (1) (Zhang & Wang, 2009):

$$\text{RS}\ (\%) = \frac{(\text{total starch} - \text{RDS} - \text{SDS})}{\text{total starch}} \times 100 \quad (1)$$

2.8. Macroscopic observations and thickness analysis of free films

The free films were inspected in relation to transparency, flexibility and occurrence of bubbles and fissures. The thickness was evaluated with a digital micrometer MDC-Lite (Mitutoyo®) in five random positions of each film, in sextuplicate.

2.9. Field emission gun scanning electron microscopy (FEG-SEM)

The morphology of surface and transversal section of free films was evaluated by FEG- SEM (JEOL JSM-7500F). The photomicrographs were taken at 1000× and 10,000× magnifications. The films were attached to the slab surfaces with double sided adhesive tape and then coated with a layer of carbon, allowing surface and cross-section visualization. For cross-section observations films were cryofractured by immersion of the sample in liquid nitrogen. All samples were examined using an accelerating voltage of 10 kV.

2.10. Mechanical properties of free films

The mechanical properties of free films were evaluated on a texture analyzer TA-XT2 (Stable Micro Systems) with a spherical-ended puncture probe (25 mm) and the film sections were fixed on a metallic holder with circular hole (D = 50 mm). The probe moved down at 1 mm s^{-1} and during the test the velocity was kept constant (0.1 mm s^{-1}). The trigger force was 0.005 kg and the force *versus* displacement curves were recorded until the film rupture and used to determine the puncture strength (P_s), elongation at break (E_b) and perforation energy (E_p) parameters according equations below (Limmatvapirat, Limmatvapirat, Puttipipatkhachorn, Nuntanid, & Luangtana-Anan, 2007; Muschert et al., 2009; Sungthongjeen, Puttipipatkhachorn, Paeratakul, Dashevsky, & Bodmeier, 2004):

$$P_S = \frac{F}{A} \quad (2)$$

where F (N) is the force required to rupture the film and A (m^2) is the sectional area of the film ($A = 2rh$, where r is the hole radius and h is the film thickness).

$$E_b\% = \frac{\sqrt{r^2 + d^2} - r}{r} \times 100 \quad (3)$$

where r (mm) is the radius of the exposed film on the orifice plate and d is the displacement.

$$E_p = \frac{AUC}{V} \quad (4)$$

Where AUC is the area under the curve force *versus* displacement and V is the film volume ($V = \pi r^2 h$, where r is the hole radius and h is the film thickness) placed on the orifice plate.

2.11. Liquid uptake ability of free films

The profile of liquid uptake of free films was evaluated on an Enslin device (Prezotti et al., 2012; Voigt, 2000). Pieces of films (about 1 cm^2) were accurately weighed and carefully poured on the sintered glass filter of funnel. The changes on liquid volume on the graduated pipette of the device due to sample absorption were measured at 1, 2, 5, 10, 30, 60, 90 and 120 min. Different media were tested in order to simulate the pH variations throughout the GIT segments: simulated gastric fluid (0.1N HCl, pH 2.0), simulated intestinal fluid (0.1 M phosphate buffer, pH 7.4) and simulated colonic fluid (0.1 M phosphate buffer, pH 6.0), all of them without enzymes. The tests were performed in triplicate and the results were expressed as medium absorbed (%) in relation to initial mass of sample.

2.12. Water vapor permeability (WVP) of free films

WVP was evaluated gravimetrically, based on the procedure described by Akhagari, Farahmand, Garekani, Sadeghi, and Vandamme (2006). Circular pieces of films, which thickness were previously measured, were fixed on the top of glass cups (1.1 cm opening) containing 10 mL of water (100% RH gradient at 25 °C).

The set was accurately weighed and stored in a desiccator containing gel silica (0% RH). The RH inside the cell was always higher than outside, and water vapor transport was determined from the loss of weight of the cup. Changes in the weight of the cup were plotted as a function of time at 24, 48, 72, 96 and 120 h. The slope of each line was calculated by linear regression ($r^2 > 0.99$), and the water vapor transmission rate (*WVTR*) was measured from the slope of the straight line ($g\,h^{-1}$) by the test area (m^2). All values for *WVTR* were corrected for the effect of gradient of concentration established in the stagnant air gap inside the cup by Eq. (5) (Gennadios, Weller, & Gooding, 1994):

$$WVTR_C = WVTR\left[\frac{(p_{w0} - p_{w2})}{(p_{w1} - p_{w2})}\right] \quad (5)$$

where *WVTR* is the measured water vapor transmission rate ($g\,m^{-2}\,h^{-1}$), p_{w0} is the partial pressure of water vapor in air at the surface of distilled water (Pa), p_{w1} is the partial pressure of water vapor at underside of film (Pa), p_{w2} is the partial pressure of water vapor at the film surface outside the cup (Pa).

After the permeation tests, WVP ($g\,mm\,m^{-2}\,h^{-1}\,Pa^{-1}$) was calculated using Eq. (6):

$$WVP = \frac{WVTR_c \times L}{\Delta p} \quad (6)$$

where *WVTRc* is the corrected water vapor transmission rate ($g\,m^{-2}\,h^{-1}$), *L* is the average film thickness (mm), and Δp is water vapor pressure difference between the dry atmosphere and pure water.

2.13. X-ray diffraction (XRD) analysis

Crystallinity patterns of samples (isolated polymers – HAS or pectin, 1:1 physical mixtures, 511 W, 514 W and 1014 W films) were evaluated from their diffractograms recorded on a X-ray diffractometer (Siemens® – Model D5000; Germany), using nickel-filtered Cu Kα radiation (λ = 1.5406 Å) (tube operating at 40 kV and 30 mA). The scanning regions were collected from 4 to 70° (2θ) in step size of 0.05° (2θ).

2.14. Dissolution of free films

The tests of dissolution of free films were performed on a Hanson Research (New Hanson SR-8 Plus) dissolution station, by using apparatus V (USP, 2007). Pieces of films (about 4 cm^2) were attached on the transdermal patch holder. The set was accurately weighed and introduced in the bottom of vessels containing 0.1N HCl (pH 1.6) or 0.1 M phosphate buffer (pH 7.4) stirred at 50 rpm, at 37 °C. After 120 and 180 min, respectively, the films were removed from the vessels and dried in oven-drier (40 °C) until constant weight. The dissolution was determined gravimetrically by the difference between the film masses before the test and after drying.

2.15. Statistical analysis

When applied, the results were treated by one-way analysis of variance to assess the significance of the differences between data. Tukey–Kramer post-test was used to compare the means of different treatment data (Origin 7.0 software). Results with $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Hardness of dispersions

Hardness is an important property for the characterization of starch dispersions, since it is sensitive to several parameters, such as retrogradation (Yang et al., 2010), temperature, presence of other substances like polysaccharides or plasticizers (Gunaratne, Ranaweera, & Corke, 2007; Mandala, Palogou, & Kostaropoulos, 2002; Qiao, Tang, & Sun, 2011). According to Table 2, the highest hardness values were presented by dispersions containing higher HAS concentration (1014) and, among them, the samples without plasticizer (1014 W) showed to be the most resistant against deformation. These results are consistent since the contribution of high amylose to the increase of hardness values of retrograded gels is well established (Teng, Chin, & Yusof, 2011) and the plasticizers affect the original rigidity of polymer chains (Vieira, Silva, Santos, & Beppu, 2011). On the other hand, the lowest values of hardness were exhibited by 511 and 514 dispersions and at this low polymer concentration, the plasticizer addition did not influence this parameter ($p > 0.05$).

Table 2
Hardness (N), strength (*S*) and viscoelastic exponent (*n*) values of retrograded high amylose and pectin dispersions.

Dispersions	Hardness (N)	*S*	*n*
511G	0.01608 ± 0.00051	0.0997	0.6971
511P	0.01604 ± 0.00066	0.1674	0.5105
511W	0.01604 ± 0.00040	0.6769	0.3756
514G	0.01576 ± 0.00082	0.0013	2.3222
514P	0.01544 ± 0.00048	4.73×10^{-7}	6.1891
514W	0.01586 ± 0.00038	2.72×10^{-7}	7.0123
1014G	0.01720 ± 0.00084	0.1040	0.9879
1014P	0.01817 ± 0.00030	0.3053	0.8120
1014W	0.01858 ± 0.00045	0.0129	1.2876

3.2. Viscoelastic behavior of film forming dispersions

Structural changes in starch dispersions occurred due to recrystallization can be monitored by rheological tests that allow the characterization of microstructures (Xie, Halley, & Avérous, 2011). The mechanical spectra of samples are presented in Fig. 1, in which it can be observed that G'' values were higher than G' values along the whole frequency range, indicating the predominance of viscous behavior of samples related to the low level of organization of their structures (Khondkar, Tester, Hudson, Karkalas, Morrow, 2007; Lawal et al., 2011). The 511 W samples showed exceptional behavior since G'' values superposed the G' values, mainly in low-media frequency values, demonstrating some balance of viscous and elastic components, which can be attributed to the absence of plasticizer, substance that reduces the elastic modulus and affects the viscosity properties (Xie et al., 2011).

The lower G' values of 514 samples (about 1000×) in relation to 511 samples show that the increase of pectin proportion resulted in weaker structures. The predominance of viscous modulus over the elastic modulus as consequence of weak gel character of pectin dispersions was previously reported (Sriamornsak & Wattanakorn, 2008).

The values of G' of samples 1014 were intermediate to those of samples 511 and 514, demonstrating the influence of both polymer concentration and polymer proportion on the elasticity of dispersions. The reduction of G' values caused by the increase of pectin proportion was already observed (Khondkar et al., 2007). In order to establish a quantitative analysis of the gel structure, the G' data were fitted with a "Power Law" (Eq. (7)) and *n* exponent values were related to the mechanical strength of the polymer structures in an analogous manner done by Saxena, Kaloti, and Bohidar (2011) a study of cross-linked hydrogels of agar and gelatin.

$$G' = S\omega^n \quad (7)$$

where G' is the storage modulus, ω is the oscillatory frequency, *S* is the gel strength and *n* is the viscoelastic exponent.

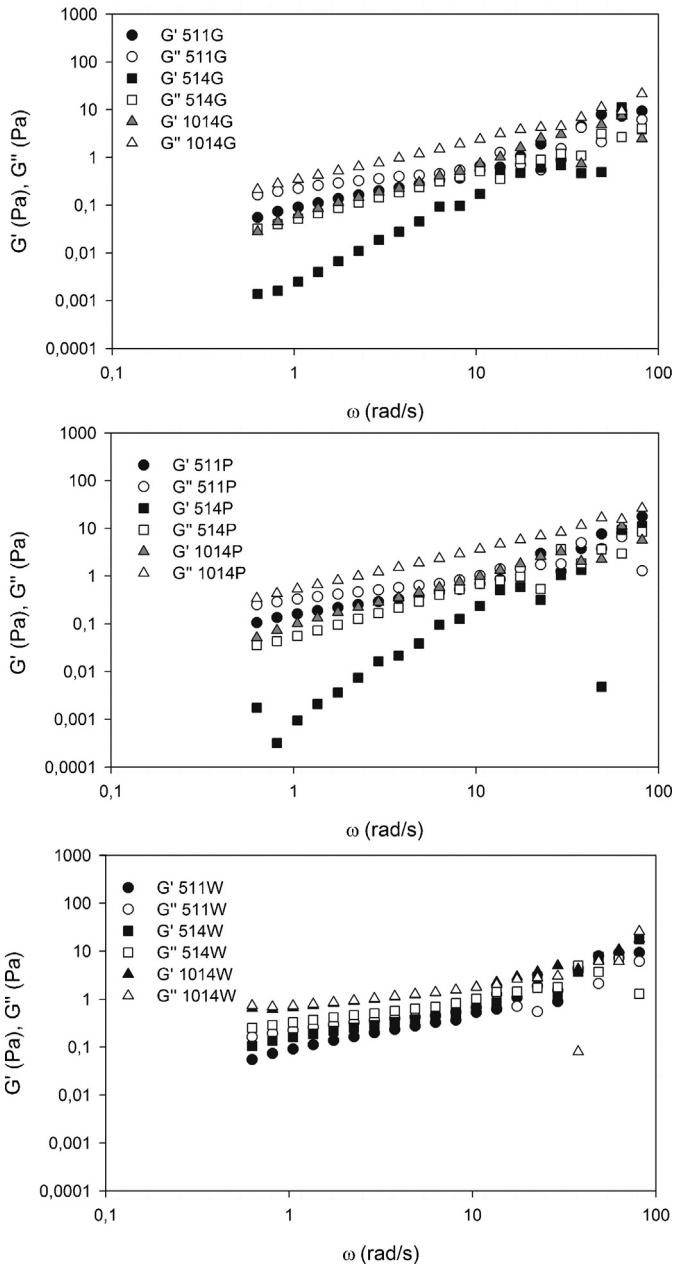

Fig. 1. Mechanical spectra of retrograded high amylose and pectin dispersions.

In this sense, the gel structure will be stronger as the *S* values become higher while lower *n* values indicate that the network stability increases (Saxena, Kaloti, & Bohidar, 2011). The values exhibited in Table 2 show that the 511 W sample originated stronger gels according to its higher *S* values and lower *n* values, confirming the mechanical spectra data, where this sample exhibits higher *G′* value. On the other hand, the weaker structures should be attributed to 514 sample (low *S* values, high *n* values), emphasizing the contribution of pectin to the formation of weaker structures.

3.3. Birefringence of film forming dispersions

Birefringence of HAS/pectin dispersions was evaluated after gelatinization or retrogradation processes (Fig. 2). Starch is a semi-crystalline polymer presenting characteristic patterns of birefringence, which can be lost after hydrothermal treatments. Gelatinized HAS/pectin dispersions at 121 °C for 120 min (Fig. 2a–c), exhibited a lack of birefringence, attributed to the heating in excess of water, promoting the rupture of granules and the fusion of the crystallites (Xie, Liu, & Cui, 2006). On the other hand, the Maltese crosses (Fig. 2d–f) exhibited by dispersions submitted to the thermal cycles of retrogradation process evidence the birefringence of these samples because the starch recrystallization allows the packaging of amylopectin crystallites in a radial and symmetrical manner in the granules (Ambigaipalan et al., 2011; Qin et al., 2012). The samples 1:1 (Fig. 2d) presented high birefringence, while the samples 1:4 (Fig. 2e and f) exhibited a decrease of this property, probably due to the lower proportion of resistant starch in these latter samples (Table 3).

3.4. Macroscopical observation and thickness determination of free films

The free films were continuous, transparent and flexible. However, the higher polymer concentration (10%) induced the occurrence of bubbles in the films, which can be attributed to the higher viscosity of the dispersions, preventing the overall release of air bubbles even after they have been subjected to an ultrasonication process. As expected, the films prepared with higher polymer concentration were also thicker, with values ranging between 0.112 and 0.166 mm, because after the solvent evaporation, a polymeric mass precipitates and, consequently, the thickness is directly related to the polymer concentration (Bertuzzi, Vidaurre, Armada, & Gottifredi, 2007). Films prepared with lower polymer concentration had their thicknesses between 0.035 and 0.055 mm.

3.5. Field emission gun scanning electron microscopy (FEG-SEM)

The photomicrographs exhibited in the Fig. 3 reveal that films without plasticizer (Fig. 3f and i) presented a more irregular and a more porous surface. The same features were presented by 1014 films, probably due to the building of a more packed and rigid structure, both factors being responsible for their brittle aspect. The films of the G and P series (Fig. 3a, b, d, e, g, h) exhibited a smoother and continuous surface that can be attributed to the polymer-plasticizer interactions as well as the ability of plasticizers to decrease the intermolecular forces throughout the polymer chains, increasing their motion and, consequently, the film flexibility (Lin, Chen, & Run-Chu, 2000; Vieira et al., 2011). At lower polymer concentration (Fig. 3e and f), the increase of pectin proportion is responsible for the surface roughness.

The transversal sections (embedded into the left-hand corner of the surface images) show that the plasticizer addition did not affect the morphology of the films. However, the polymer proportion affected this parameter since the increase of pectin proportion resulted in a discontinuous matrix, containing randomly dispersed pores. The increase of polymer concentration also originated porous structures but in a more packed arrangement (Alves, Mali, Beléia, & Grossmann, 2007).

3.6. Mechanical properties of free films

Suitable mechanical properties of free films are fundamental to ensure the success of a coating procedure, avoiding fissures or cracks during processing, transport and storage (Murillo-Martínes, Pedroza-Islas, Lobato-Calleros, Martínez-Ferez, & Vernon-Cartier, 2011). The 511 and 514 films were stronger (higher P_s values) than those prepared with higher polymer concentration, because the former have a looser network with larger intermolecular spaces that allow an extensive conformational rearrangement until the yield stress is reached (Felton, 2007). The reduction of mechanical properties by increase of polymer concentration was also related by Al-Hassan and Norziah (2012) in studies about starch-gelatin

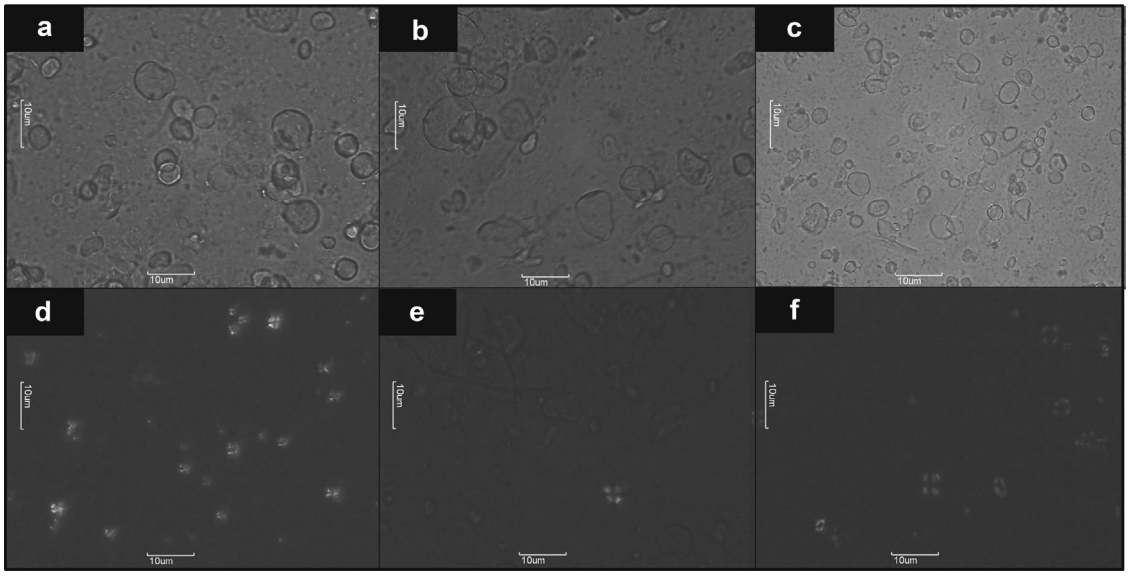

Fig. 2. Micrographs of different high amylose/pectin dispersions under normal and polarized light.

edible films. The results of E_b (Table 3) are in agreement with P_s since the higher energy values spent in perforation were presented by the 511 and 514 films, the same that showed to be stronger. Generally, the values of E_p, of both samples were similar ($p > 0.05$).

3.7. Liquid uptake ability of free films

The study of liquid uptake of films enables insights about their hydrophilicity, essential to allow the access of enzymes in the terminal segments of GIT and the subsequent degradation of them in these biological media (Ahmed & Ayres, 2011; Maior et al., 2008; Maroni, Zema, Del Curto, Foppoli, & Gazzaniga, 2012). The films generally presented lower liquid uptake in acid media than at pH 7.4 (Table 4), a typical behavior of anionic polymers, for which the increase of pH values promotes the ionization of carboxyl groups and consequent repulsion of chains with network dilation, favoring the water entrance (Mulhbacher, Ispas-Szabo, & Mateescu, 2004; Swarbrick, 2006). 511P, 514P and 1014G films exhibited higher values of liquid uptake in the simulated enteric medium (pH 7.4), indicating that the presence of plasticizers favors this behavior, since these compounds due to polar nature lead to an increase of both the free volume among adjacent molecules (Farris, Schaich, Liu, Cooke, Piergovanni, & Yam, 2011). The high hydrophilicity of pectin also improved the liquid uptake ability of the films.

The films prepared with low concentration of polymer (511 and 514) presented higher ability to take up liquid than those prepared with higher polymer proportion, fact that can be attributed to the entanglement level of the structures, the looser networks favoring the water entrance while the harder structures hindering water molecule diffusion (Sriamornsak, 2002).

3.8. Water vapor permeability (WVP)

The study of water vapor permeability provides important insights about the vapor transfer mechanisms and the polymeric interactions that can be occur in the film coating. The permeability of a film is influenced by several factors, as hydrophobic and hydrophilic nature of material, presence of cracks and pores, and tortuousity of pathways through the film (Akahgari, Farahmand, Garekani, Sadeghi, & Vandamme, 2006; Bertuzzi et al., 2007).

Despite the films of resistant starch/pectin are intended for drug delivery in the colon, the study of WVP is an important tool due the interaction that occurs between water and polar functional groups of hydrophilic films by hydrogen bonds during the process of water vapor permeability, which can lead to plasticization of films, changing the mobility of chains and facilitating the diffusion process, since the water has the same mechanism of structural change of plasticizers (Gennadios, Brandenburg, Park, Weller, & Testin, 1994; Ghanbarzadeh et al., 2007).

The slope of mass loss *versus* time curves was used to calculate the WVP of the free films (Table 3). The higher values of permeability presented by 1014 films are an indication that the thicker films can absorb more water molecules from the environment due to higher polymeric mass and demonstrates that the porous structures observed in SEM analysis should contribute for

Table 3
Mechanical properties, WVP (g mm m^{-2} h^{-1} Pa^{-1}) and RS content of free films.

Films	(P_s) (MPa)	(E_b) (%)	(E_p) (kJ m^{-3})	WVP ($\times 10^{-4}$ g mm m^{-2} h^{-1} Pa^{-1})	RS (%)
511G	24.329 ± 2.355	1.071 ± 0.025	145.725 ± 21.202	1.91 ± 0.41	96.10 ± 0.17
511P	20.881 ± 8.676	0.999 ± 0.005	64.049 ± 24.870	0.91 ± 0.33	96.68 ± 0.24
511W	16.259 ± 5.031	0.997 ± 0.009	48.617 ± 18.749	2.33 ± 0.22	73.33 ± 0.64
514G	20.721 ± 2.211	1.069 ± 0.010	126.938 ± 9.083	1.87 ± 0.04	70.77 ± 2.41
514P	26.441 ± 1.358	1.107 ± 0.041	122.034 ± 48.481	1.58 ± 0.31	65.80 ± 1.07
514W	29.354 ± 2.659	1.045 ± 0.046	134.870 ± 14.445	2.04 ± 0.48	68.67 ± 2.05
1014G	10.584 ± 6.609	1.066 ± 0.053	37.252 ± 6.791	3.93 ± 0.80	96.41 ± 2.05
1014P	10.793 ± 1.898	1.101 ± 0.051	27.662 ± 3.392	4.83 ± 0.39	72.03 ± 4.30
1014W	8.896 ± 0.974	1.021 ± 0.011	13.549 ± 1.288	5.46 ± 0.35	70.58 ± 6.18

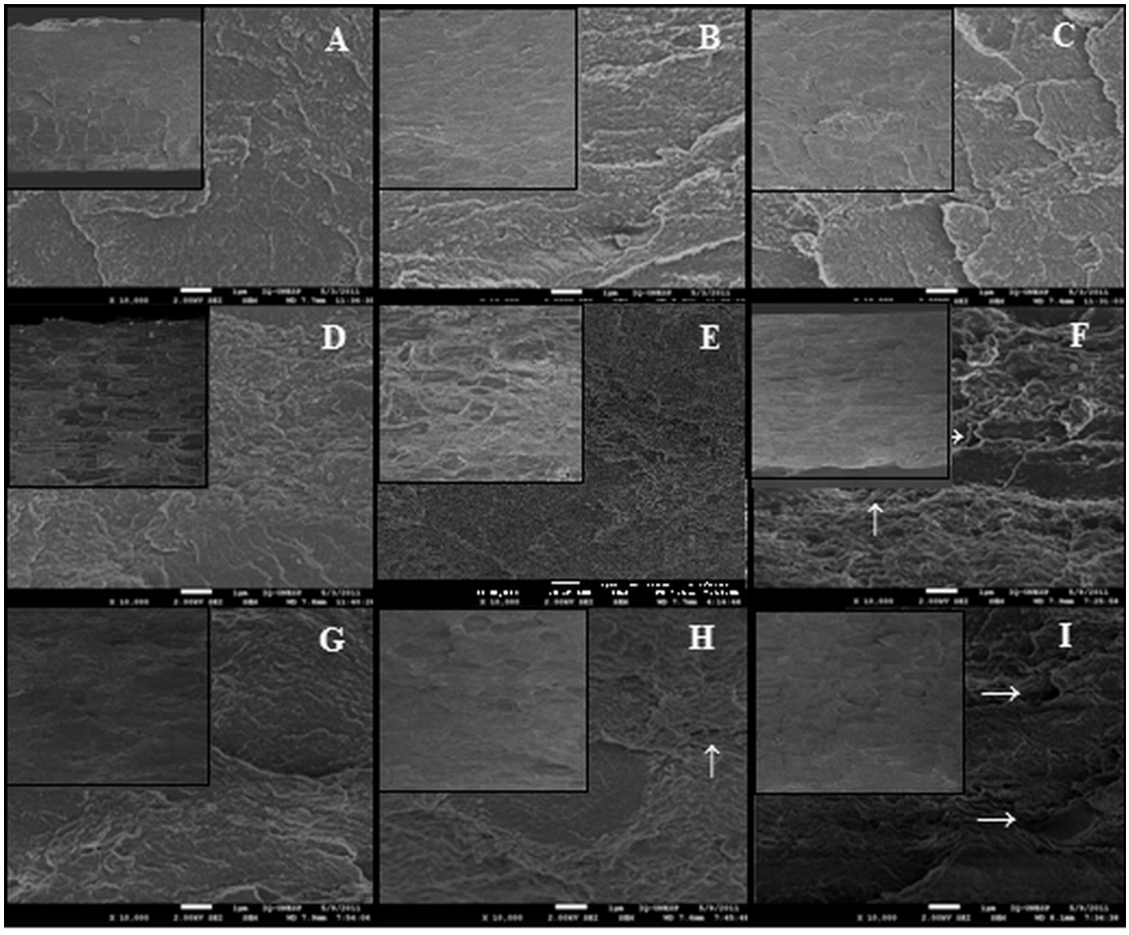

Fig. 3. Scanning electron micrographs (SEM) of free film surfaces and transversal sections (embedded into the left-hand corner of the surface images), recorded at 10,000× magnification. 511G (a), 511P (b), 511 W (c), 514G (d), 514P (e), 514 W (f), 1014G (g), 1014P (h), 1014 W (i). Arrows indicates the pores presence.

their higher permeability. Despite the high hydrophilicity of plasticizers, films containing these compounds showed lower values of WVP than their counterpart W samples, demonstrating that the integrity of the films was the determining factor for their barrier properties. Furthermore, the observed behavior can be explained by the free-volume theory for polymers, wherein a plasticizer at low concentration is intercalated in the polymeric chain without leading to an increase in free volume, unlike when it is in high concentrations (López et al., 2008).

3.9. X-ray diffraction analysis

The granules of starch are composed of amorphous and crystalline arrangements, so starch can present A, B or C patterns, consisting of double helices, and V pattern, composed of a simple helix (Mutungi, Passauer, Onyango, Jaros, & Rohm, 2012). HAS shows characteristic peaks of B polymorph at 17.02°, 19.8°, 23° and 25° (2θ) while pectin exhibited well defined peaks at 12.7°, 16.72°, 18.42°, 25.32° and 40.14° (2θ) related to its crystallinity (Fig. 4). The physical mixture exhibited crystalline peaks typical of pectin combined with an amorphous halo peak of high amylose starch. In films diffractograms can be observed the disappearance of the majority of characteristic peaks of the isolated polymers, a fact that is not necessarily related to the amorphization of the samples since the new peaks appeared at 13° and 21° (2θ). These important peaks are related to the V crystalline structure built due to the cooling of gelatinized starch, in which the structure is changed from B to V (Htoon et al., 2010). Besides, these changes

Table 4
Liquid uptake (%) of free films at equilibrium (120 min) in different media and dissolution (%) of free films in acidic medium (2 h) and phosphate medium (3 h).

FILMS	Liquid uptake (%)			Dissolution (%)	
	Acidic medium (0.1N HCl, pH 2.0)	Phosphate buffer pH 7.4	Phosphate buffer pH 6.0	Acidic medium (0.1N HCl, pH 1.6)	Phosphate buffer pH 7.4
511G	508.027 ± 11.284	453.165 ± 50.678	702.372 ± 19.912	24.693 ± 1.555	45.614 ± 3.470
511P	461.211 ± 56.714	1004.428 ± 74.537	76.926 ± 25.603	22.774 ± 5.385	55.040 ± 3.733
511W	429.505 ± 42.788	742.621 ± 117.281	468.574 ± 32.129	20.872 ± 4.865	36.312 ± 4.390
514G	413.198 ± 54.631	704.979 ± 17.162	497.928 ± 36.548	37.173 ± 2.820	79.933 ± 3.543
514P	523.311 ± 57.835	882.767 ± 29.596	455.167 ± 10.584	44.587 ± 2.343	83.659 ± 6.783
514W	332.017 ± 8.851	855.774 ± 83.572	717.877 ± 66.555	35.690 ± 0.695	72.327 ± 5.921
1014G	396.771 ± 19.740	771.352 ± 90.175	465.637 ± 14.889	45.533 ± 1.025	85.955 ± 3.877
1014P	345.665 ± 27.369	571.117 ± 43.157	480.466 ± 11.923	38.560 ± 0.292	84.514 ± 2.187
1014W	385.871 ± 26.481	488.473 ± 66.103	389.715 ± 40.000	42.286 ± 4.468	81.898 ± 3.569

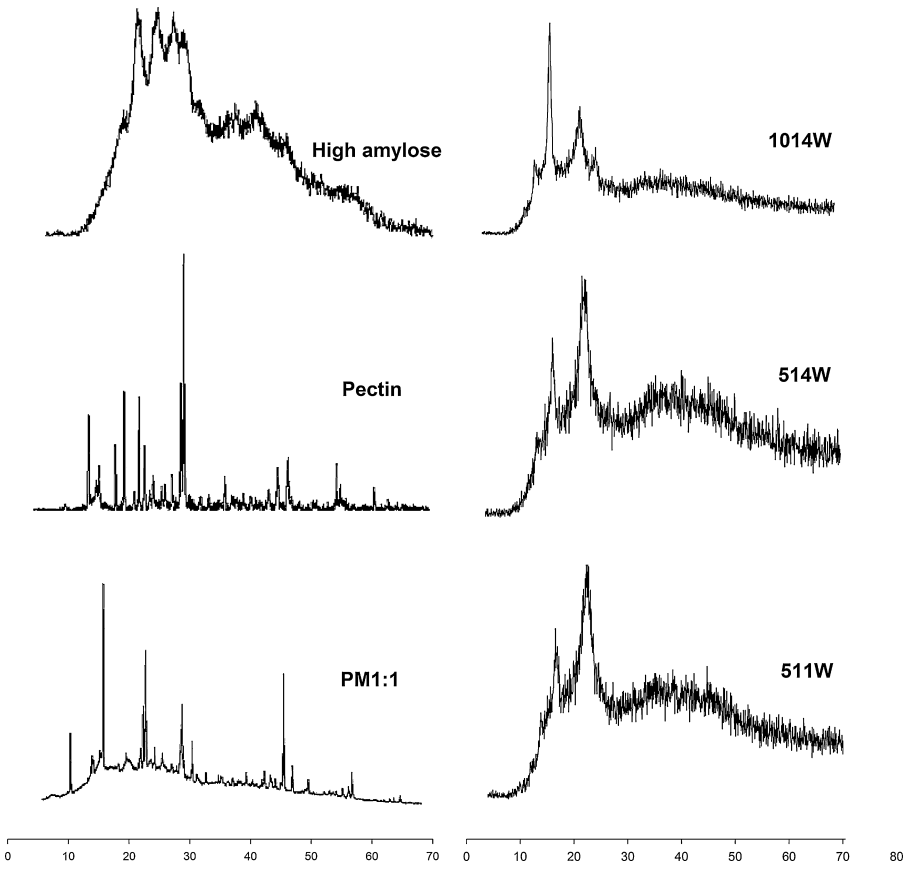

Fig. 4. X-ray diffraction patterns of samples.

in structural conformation are indicative of presence of resistant starch.

3.10. "In vitro" dissolution of free films

Dissolution tests of free films in different media that simulate the GI tract were performed in order to predict the protection that the coating could offer to the dosage form. 511 films (Table 4) showed the lowest dissolution values in both gastric and enteric media, probably due to the low proportion of pectin, which has high solubility and high liquid uptake ability (Guimarães et al., 2008; Maior et al., 2008). Dissolution properties were not affected by polymer concentration, but in simulated enteric medium, 511P film presented higher dissolution than 511 W (about 1.5 times), behavior that may be attributed to the hydrophilic nature of propylene glycol, which leads to increased mobility of the polymer chains (Bando & Mcginity, 2006). Besides, the hydrophilic plasticizers, when in contact with the dissolution medium, tend to be leached from polymeric films, decreasing their mechanical strength and facilitating pore formation, which are enhancers of the dissolution rate (Lecomte, Siepmann, Walther, Macrae, & Bodmeier, 2004). In acid media, the presence and kind of plasticizers did not affect significantly the dissolution properties ($p > 0.05$).

From these results it can be concluded that the films obtained with the same proportion of polymer provide a higher resistance to the conditions of the upper GI tract, but future investigations are necessary, for example, the impact of pectin crosslinking, since this technique modifies the properties of polysaccharides by adding intra and intermolecular bonds randomly distributed, reducing pectin solubility. This technique has been used successfully by our research group in other types of systems (Carbinatto et al., 2012; Cury, Castro, & Evangelista, 2009; Prezotti et al., 2012; Soares, Castro, Cury, & Evangelista, 2013).

3.11. "In vitro" enzymatic digestion test

The access of α-amylase to the glycosidic linkages of retrograded starch, mainly RS 3, is restricted due to the tight packing of high amylose double helices (Flores-Morales, Jiménez-Estrada, & Mora-Escobedo, 2012) and this feature is a key factor to prevent the premature drug release in the upper segments of GIT.

The results of the resistance against enzymatic digestion of free films were investigated in α-amylase pancreatic solution. Table 3 shows that the 511G, 511P and 1014G films presented lower susceptibility to enzymatic digestion, because of their high RS contents of 96.10%, 96.68% and 96.41%, respectively. All of these samples contain plasticizer in their composition, indicating that the higher chains mobility promoted by these compounds allow a higher level of both reorganization of the network and the crystallization, factors that restrict the access of enzymes. Indeed, the results indicate that the retrogradation process continues during preparation/drying of films, since the plasticizers were added only after the thermal cycles of the retrogradation process. The lower values of RS presented by 514 samples indicate that the pectin disadvantaged the retrogradation process of starch.

4. Conclusions

Retrogradation process used in this work was efficient and advantageous, since it produced material with high contents of resistant starch, but the presence of pectin, generally did not favor this. The association of resistant starch and pectin resulted in a material with appropriate film forming properties, which are

absent in dispersions of resistant starch. Variables such as addition and type of plasticizer, concentration and proportion of polymers, allowed us to modulate free films properties. All films presented high resistance against digestion by pancreatic enzymes and films containing lower proportion of pectin have lowest dissolution in acid media. These results indicate that these films can be a promising technological strategy for the coating of solid dosage forms intended for colon-specific drug delivery.

Acknowledgements

The financial support provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) is acknowledged.

References

Ahmad, F. B., & Willians, P. A. (2001). Effects of galactomannans on the thermal e rheological properties of sago starch. *Journal of Agricultural and Food Chemistry*, *49*, 1578–1586.

Ahmed, I. S., & Ayres, J. W. (2011). Comparison of in vitro and in vivo performance of a colonic delivery system. *International Journal of Pharmaceutics*, *409*, 169–177.

Akhagari, A., Farahmand, F., Garekani, H. A., Sadeghi, F., & Vandamme, T. F. (2006). Permeability and swelling studies on free films containing insulin in combination with different polymethacrylates aimed for colonic drug delivery. *European Journal of Pharmaceutical Science*, *28*, 307–314.

Al-Hassan, A. A., & Norziah, M. H. (2012). Starch-gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids*, *26*, 108–117.

Alves, V. D., Mali, S., Beléia, A., & Grossmann, M. V. E. (2007). Effect of glycerol and amylose enrichment on cassava starch film properties. *Journal of Food Engineering*, *78*, 941–946.

Ambigaipalan, P., Hoover, R., Donner, E., Liu, Q., Jaiswal, S., Chibbar, R., Nantanga, K. K. M., & Seetharaman, K. (2011). Structure of faba bean, black bean and pinto bean starches at different levels of granule organization and their physicochemical properties. *Food Research International*, *44*, 2962–2974.

Babic, J., Subaric, D., Ackar, D., Pilizota, V., Kopjar, M., & Tiban, N. N. (2006). Effects of pectin and carrageenan on thermophysical and rheological properties of tapioca starch. *Czech Journal of Food Science*, *24*, 275–282.

Bando, H., & Mcginity, J. W. (2006). Relationship between drug dissolution and leaching of plasticizer for pellets coated with an aqueous Eudragit® S100:L100 dispersion. *International Journal of Pharmaceutics*, *323*, 11–17.

Bernfeld, P. (1955). *Methods in enzymology* (1st ed.). New York: Academic Press.

Bertuzzi, M. A., Vidaurre, E. F. C., Armada, M., & Gottifredi, J. C. (2007). Water vapor permeability of edible starch based films. *Journal of Food Engineering*, *80*, 972–978.

Carbinatto, F. M., Castro, A. D., Cury, B. S. F., Magalhães, A., & Evangelista, R. C. (2012). Physical properties of pectin-high-amylose starch mixtures cross-linked with sodium trimetaphosphate. *International Journal of Pharmaceutics*, *423*, 281–288.

Chung, H. J., Lim, H. S., & Lim, S. T. (2006). Effect of partial gelatinization and retrogradation on the enzymatic digestion of waxy rice starch. *Journal of Cereal Science*, *43*, 353–359.

Cui, R., & Oates, C. G. (1997). The effect of retrogradation on enzyme susceptibility of sago starch. *Carbohydrate Polymers*, *32*, 65–72.

Cury, B. S. F., Castro, A. D., & Evangelista, R. C. (2009). Modeling a system of phosphated cross-linked high amylose for controlled drug release. Part 2: Physical parameters, cross-linking degrees and drug delivery relationship. *International Journal of Pharmaceutics*, *371*, 8–15.

Das, S., & Ng, K. Y. (2010). Colon-specific delivery of resveratrol: Optimization of multi-particulate calcium-pectinate carrier. *International Journal of Pharmaceutics*, *385*, 20–28.

Dev, R. K., Bali, V., & Pathak, K. (2011). Novel microbially triggered colon specific delivery system of 5-Fluorouracil: Statistical optimization, in vitro, in vivo, cytotoxic and stability assessment. *International Journal of Pharmaceutics*, *411*, 142–151.

Dimantov, A., Greeberg, M., Kesselman, E., & Shimoni, E. (2003). Study of high amylase corn starch as food grade enteric coating in a microcapsule model system. *Innovative Food Science Emerging Technologies*, *5*, 93–100.

Ebude, N. K., & Jones, A. B. (2004). Sustained release of acetaminophen from a heterogeneous mixture of two hydrophilic non-ionic cellulose ether polymers. *International Journal of Pharmaceutics*, *272*, 19–27.

Englyst, H. N., Wiggins, H. S., & Cummings, J. H. (1982). Determination of the non-starch polysaccharides in plant foods by gas liquid chromatography of constituent sugars as alditol acetates. *Analyst*, *107*, 307–318.

Farris, S., Schaich, K. M., Liu, L., Cooke, P. H., Piergovanni, L., & Yam, K. L. (2011). Gelatin pectin composite films from polyion-complex hydrogels. *Food Hydrocolloids*, *25*, 61–70.

Felton, L. A. (2007). Characterization of coating systems. *AAPS PharmSciTech*, *8*, E1–E9.

Fishman, M. L., Coffin, D. R., Unruh, J. J., & Ly, T. (1996). Pectin, starch, glycerol films: blends or composites. *Journal of Macromolecular Science Pure*, *33*, 639–654.

Flores-Morales, A., Jiménez-Estrada, M., & Mora-Escobedo, R. (2012). Determination of the structural changes by FT-IR, Raman, and CP/MAS ^{13}C NMR spectroscopy on retrograded starch of maize tortillas. *Carbohydrate Polymers*, *87*, 61–68.

Friend, D. R. (2005). New oral delivery systems for treatment of inflammatory bowel disease. *Advanced Drug Delivery Reviews*, *57*, 247–265.

Gennadios, A., Brandenburg, A. H., Park, J. W., Weller, C. L., & Testin, R. F. (1994). Water vapor permeability of wheat gluten and soy protein isolate films. *Industrial Crops and Products*, *2*, 189–195.

Gennadios, A., Weller, C. L., & Gooding, C. H. (1994). Measurement errors in water vapor permeability of highly permeable, hydrophilic edible films. *Journal of Food Engineering*, *21*, 395–409.

Ghanbarzadeh, B., Musavi, M., Oromiehie, A. R., Rezayi, K., Rad, E. R., & Milani, J. (2007). Effect of plasticizing sugars on water vapor permeability, surface energy and microstructure properties of zein films. *LWT–Food Science and Technology*, *40*, 1191–1197.

Guimarães, F., Oliveira, C., Sequeiros, E., Torres, M., Susano, M., Henriques, M., Oliveira, R., Galindo, R. E., Carvalho, S., Parreira, N. M. G., Vaz, F., & Cavaleiro, A. (2008). Structural and mechanical properties of Ti–Si–C–ON for biomedical applications. *Surface and Coatings Technology Journal*, *202*, 2403–2407.

Gunaratne, A., Ranaweera, S., & Corke, H. (2007). Thermal, pasting, and gelling properties of wheat and potato starches in the presence of sucrose, glucose, glycerol, and hydroxypropyl β-cyclodextrin. *Carbohydrate Polymers*, *70*, 112–122.

Haralampu, S. G. (2000). Resistant starch—A review of the physical properties and biological impact of RS3. *Carbohydrate Polymers*, *41*, 285–292.

He, W., Du, Q., Cao, D. Y., Xiang, B., & Fan, L. F. (2008). Study on colon-specific pectin/ethylcellulose film-coates 5-fluorouracil pellets in rats. *International Journal of Pharmaceutics*, *348*, 35–45.

Htoon, A. K., Shrestha, A. K., Flanagan, B. M., Lopez-Rubio, A., Bird, A. R., Gilbert, E. P., & Gidley, M. J. (2009). Effects of processing high amylose maize starches under controlled conditions on structural organization and amylase digestibility. *Carbohydrate Polymers*, *75*, 236–245.

Htoon, A. K., Uthayakumaran, S., Piyasiri, U., Appelqvist, A. M., López-Rubio, A., Gilbert, E. P., & Mulder, R. J. (2010). The effect of acid dextrinisation on enzyme-resistant starch content in extruded maize starch. *Food Chemistry*, *120*, 140–149.

Kakoulides, E. P., Smart, J. D., & Tsibouklis, J. (1998). Azocrosslinked poly(acrylic acid) for colonic delivery and adhesion specificity: In vitro degradation and preliminary ex vivo bioadhesion studies. *Journal of Controlled Release*, *54*, 95–109.

Khondkar, D., Tester, R. F., Hudson, N., Karkalas, J., & Morrow, J. (2007). Rheological behaviour of uncross-linked and cross-linked gelatinised waxy maize starch with pectin gels. *Food Hydrocolloids*, *21*, 1296–1301.

Kohyama, K., Matsuki, J., Yasui, T., & Sasaki, T. (2004). A differential thermal analysis of the gelatinization and retrogradation of wheat starches with different amylopectin chain lengths. *Carbohydrate Polymers*, *58*, 71–77.

Lawal, L. S., Lapasin, R., Bellich, B., Olayiwola, T. O., Cesàrio, A., Yoshimura, M., & Nishinari, K. (2011). Rheology and functional properties of starches isolated from five improved rice varieties from West Africa. *Food Hydrocolloids*, *25*, 1785–1792.

Lecomte, F., Siepmann, J., Walther, M., Macrae, R. J., & Bodmeier, R. (2004). Polymer blends used for the aqueous coating of solid dosage forms: importance of the type of plasticizer. *Journal of Controlled Release*, *99*, 1–13.

Lecomte, F., Siepmann, J., Walther, M., Macrae, R. J., & Bodmeier, R. (2005). pH-sensitive polymer blends used as coating materials to control drug release from spherical beads: Elucidation of the underlying mass transport mechanisms. *Pharmaceutical Research*, *22*, 1129–2114.

Lee, M. H., Baek, M. H., Cha, D. S., Park, H. J., & Lim, S. T. (2002). Freeze–thaw stabilization of sweet potato starch gel by polysaccharide gums. *Food Hydrocolloids*, *16*, 345–352.

Lii, C. Y., Lai, M. F., & Liu, K. F. (1998). Factors influencing the retrogradation of two rice starches in low-molecular-weight saccharide solutions. *Journal of Cereal Science*, *28*, 175–185.

Limmatvapirat, S., Limmatvapirat, C., Puttipipatkhachorn, S., Nuntanid, J., & Luangtana-Anan, M. (2007). Enhanced enteric properties and stability of shellac films through composite salts formation. *European Journal of Pharmaceutical and Biopharmaceutics*, *67*, 690–698.

Lin, S. Y., Chen, K. S., & Run-Chu, L. (2000). Organic esters of plasticizers affecting the water absorption, adhesive property, glass transition temperature and plasticizer permanence of Eudragit acrylic films. *Journal of Controlled Release*, *68*, 343–350.

Liu, L. S., Fishman, M. L., Kost, J., & Hicks, K. B. (2003). Pectin-based systems for colon-specific drug delivery via oral route. *Biomaterials*, *24*, 3333–3343.

Liu, H., Yu, L., Chen, L., & Li, L. (2007). Retrogradation of corn starch after thermal treatment at different temperatures. *Carbohydrate Polymers*, *69*, 756–762.

López, O. V., García, M. A., & Zaritzky, N. E. (2008). Film forming capacity of chemically modified corn starches. *Carbohydrate Polymers*, *73*, 573–581.

Maior, J., Reis, A. V., Muniz, E. C., & Cavalcanti, A. O. (2008). Reaction of pectil and glycidyl methacrilate and ulterior formation of free films by reticulation. *International Journal of Pharmaceutics*, *355*, 184–194.

Mandala, I. G., Palogou, E. D., & Kostaropoulos, A. E. (2002). Influence of preparation and storage conditions on texture of xanthan–starch mixtures. *Journal of Food Engineering*, *53*, 27–38.

Marinich, J. A., Ferrero, C., & Jiménez-Castellanos, M. R. (2012). Graft copolymers of ethil methacrylate on waxy maize starch derivatives as novel excipients for matrix tablets: Drug release and fronts movement kinetics. *European Journal of Pharmaceutics and Biopharmaceutics*, *80*, 674–681.

Maroni, A., Zema, L., Del Curto, M. D., Foppoli, A., & Gazzaniga, A. (2012). Oral colon delivery of insulin with the aid of functional adjuvants. *Advanced Drug Delivery Reviews*, *64*, 540–556.

Mennini, N., Furlanetto, S., Cirri, M., & Mura, P. (2012). Quality by design approach for developing chitosan-Ca-alginate microspheres for colon delivery of celecoxib-hydroxypropyl-b-cyclodextrin-PVP complex. *European Journal of Pharmaceutical and Biopharmaceutics*, *80*, 67–75.

Mulhbacher, J., Ispas-Szabo, P., & Mateescu, M. A. (2004). Cross-linked high amylose starch derivatives for drug release: II. Swelling properties and mechanistic study. *International Journal of Pharmaceutics*, *278*, 231–238.

Murillo-Martínes, M. M., Pedroza-Islas, R., Lobato-Calleros, C., Martínez-Ferez, A., & Vernon-Cartier, E. J. (2011). Designing W1/O/W2 double emulsions stabilized by protein-polysaccharide complexes for producing edible films: Rheological, mechanical and water vapor properties. *Food Hydrocolloids*, *25*, 577–585.

Muschert, S., Siepmann, F., Cuppok, Y., Leclercq, B., Carlin, B., & Siepmann, J. (2009). Improved long termstability of aqueous ethylcellulose film coatings: importance of the type of drug and starter core. *International Journal of Pharmaceutics*, *368*, 138–145.

Mutungi, C., Passauer, L., Onyango, C., Jaros, D., & Rohm, H. (2012). Debranched cassava starch crystallinity determination by Raman spectroscopy: Correlation of features in Raman spectra with X-ray diffraction and 13C CP/MAS NMR spectroscopy. *Carbohydrate Polymers*, *87*, 598–606.

Park, E. Y., Baik, B. K., & Lim, S. T. (2009). Influences of temperature-cycled storage on retrogradation and in vitro digestibility of waxy maize starch gel. *Journal of Cereal Science*, *50*, 43–48.

Patel, V. F., & Patel, N. M. (2007). Statistical evaluation of influence of xanthan gum and guar gum blends on dipyridamole release from floating matrix tablets. *Drug Development and Industrial Pharmacy*, *33*, 327–334.

Prezotti, F. G., Meneguin, A. B., Evangelista, R. C., & Cury, B. S. F. (2012). Preparation and characterization of free films of high amylose/pectin mixtures cross-linked with sodium trimetaphosphate. *Drug Development and Industrial Pharmacy*, *38*, 1–6.

Qiao, X., Tang, Z., & Sun, K. (2011). Plasticization of corn starch by polyol mixtures. *Carbohydrate Polymers*, *83*, 659–664.

Qin, F., Man, J., Cai, C., Xu, B., Gu, M., Zhu, L., Shi, Y. C., Liu, Q., & Wei, C. (2012). Physicochemical properties of high-amylose rice starches during kernel Development. *Carbohydrate Polymers*, *88*, 690–698.

Rashid, I., Al-Remawy, M., Leharne, S. A., Chowdhry, B. Z., & Badwan, A. (2011). A novel multifunctional pharmaceutical excipient: Modification of the permeability of starch by processing with magnesium silicate. *International Journal of Pharmaceutics*, *411*, 18–26.

Saxena, A., Kaloti, M., & Bohidar, H. B. (2011). Rheological properties of binary and ternary protein–polysaccharide co-hydrogels and comparative release kinetics of salbutamol sulphate from their matrices. *International Journal of Biological Macromolecules*, *48*, 263–270.

Silva, M. A., Bierhalz, A. C. K., & Kieckbusch, T. G. (2009). Alginate and pectin composite films crosslinked with Ca^{2+} ions: Effect of the plasticizer concentration. *Carbohydrate Polymers*, *77*, 736–742.

Sinha, V. R., & Kumria, R. (2001). Polysaccharides in colon-specific drug delivery. *International Journal of Pharmaceutics*, *224*, 19–38.

Soares, G. A., Castro, A. D., Cury, B. S. F., & Evangelista, R. C. (2013). Blends of cross-linked high amylose starch/pectin loaded with diclofenac. *Carbohydrate Polymers*, *91*, 125–142.

Sriamornsak, P. (2002). Analysis of selected physico-chemical properties of pectin and alginate gels intended for drug delivery. Ph.D. Thesis, Charles Sturt University, Australia.

Sriamornsak, P., & Wattanakorn, N. (2008). Rheological synergy in aqueous mixtures of pectin and mucin. *Carbohydrate Polymers*, *74*, 474–481.

Sungthongjeen, S., Puttipipatkhachorn, S., Paeratakul, O., Dashevsky, A., & Bodmeier, R. (2004). Development of pulsatile release tablets with swelling and rupture layers. *Journal of Controlled Release*, *95*, 147–159.

Swarbrick, J. (2006). *Encyclopedia of pharmaceutical technology* (3rd ed.). New York: Informa Healthcare.

Tako, M. (1996). Molecular origin for the thermal stability of waxy-rice (Kogane) starch. *Starch/Starke*, *48*, 414–416.

Teacă, C. A., Bodîrlău, R., & Spiridon, J. (2013). Effect of cellulose reinforcement on the properties of organic acid modified starch microparticles/plasticized starch bio-composite films. *Carbohydrate Polymers*, *93*, 307–315.

Temsiripong, T., Pongsawatmanit, R., Ikeda, S., & Nishinari, K. (2005). Influence of xyloglucan on gelatinization and retrogradation of tapioca starch. *Food Hydrocolloids*, *19*, 1054–1063.

Teng, L. Y., Chin, N. L., & Yusof, Y. A. (2011). Rheological and textural studies of fresh and freeze-thawed native sago starch sugar gels. I. Optimization using response surface methodology. *Food Hydrocolloids*, *25*, 1530–1537.

Thompson, D. B. (2000). Strategies for the manufacture of resistant starch. *Trends in Food Science & Technology*, *11*, 245–253.

Tuovinen, L., Peltonen, S., Liikola, M., Hotakainen, M., Lahtela-Kakkonen, M., Poso, A., & Järvinen, K. (2004). Drug release from starch-acetate microparticles and films with and without incorporated a-amylase. *Biomaterials*, *25*, 4355–4362.

Tuovinen, L., Ruhanena, E., Kinnarinenb, T., Rönkkö, S., Pelkonenc, J., Urttia, A., Peltonend, S., & Järvinen, K. (2004). Starch acetate microparticles for drug delivery into retinal pigment epithelium-in vitro study. *Journal of Controlled Release*, *98*, 407–413.

United States Pharmacopeia & National Formulary. (2007). (30th ed.). United States: Pharmacopeial Convention.

Vieira, M. G. A., Silva, M. A., Santos, L. O., & Beppu, M. M. (2011). Natural-based plasticizers and biopolymer films: A review. *European Polymer Journal*, *47*, 254–263.

Voigt, R. (2000). *Pharmazeutische technologie: für Studium und Beruf* (9th ed.). Stuttgart: Apotheker.

Wang, Q., Hu, X., Du, Y., & Kennedy, J. F. (2010). Alginate/starch blend fibers and their properties for drug controlled release. *Carbohydrate Polymers*, *82*, 842–847.

Xie, X. X., Liu, Q., & Cui, S. W. (2006). Studies on the granular structure of resistant starches (type 4) from normal, high amylose and waxy corn starch citrates. *Food Research International Journal*, *39*, 332–341.

Xie, F. A., Halley, P. J. A., & Avérous, L. B. (2011). Rheology to understand and optimize processibility, structures and properties of starch polymeric materials. *Chemical Engineering Science Journal*, *66*, 3460–3470.

Yang, L., Chu, J. S., & Fix, J. A. (2002). Colon-specific drug delivery: New approaches and in vitro/in vivo evaluation. *International Journal of Pharmaceutics*, *235*, 1–15.

Yang, L. (2008). Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. *Journal of Controlled Release*, *125*, 77–86.

Yang, W., Flament, M. P., Siepmann, F., Busignies, V., Leclerc, B., Herry, C., Tchoreloff, P., & Siepmann, J. (2010). Curing of aqueous polymeric film coatings: Importance of the coating level and type of plasticizer. *European Journal of Pharmaceutical and Biopharmaceutics*, *74*, 362–370.

Yoshimura, M., Takaya, T., & Nishinari, K. (1999). Effects of xyloglucan on the gelatinization and retrogradation of corn starch as studied by rheology and differential scanning calorimetry. *Food Hydrocolloids*, *13*, 101–111.

Yuan, R. C., Thompson, D. B., & Boyer, C. D. (1993). Fine-structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from 3 wx-containing genotypes in 2 inbred lines. *Cereal Chemistry*, *70*, 81–89.

Zavareze, E. R., & Dias, A. R. G. (2011). Impact of heat-moisture treatment and annealing in starches: A review. *Carbohydrate Polymers*, *83*, 317–328.

Zhang, J., & Wang, Z. U. (2009). Optimization of reaction conditions for resistant Canna edulis Ker starch phosphorylation and its structural characterization. *Industrial Crops and Products Journal*, *30*, 113.

Zhou, Y., Wang, D., Zhang, L., Du, X., & Zhou, X. (2008). Effect of polysaccharides on gelatinization and retrogradation of wheat starch. *Food Hydrocolloids*, *22*, 505–512.