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Physical properties of pectin–high amylose starch mixtures cross-linked with sodium trimetaphosphate

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ABSTRACT

Pectin–high amylose starch mixtures (1:4; 1:1; 4:1) were cross-linked at different degrees and characterized by rheological, thermal, X-ray diffraction and NMR analyses. For comparison, samples without cross-linker addition were also prepared and characterized. Although all samples behaved as gels, the results evidenced that the phosphorylation reaction promotes the network strengthening, resulting in covalent gels (highest critical stress, G' and recovery %). Likewise, cross-linked samples presented the highest thermal stability. However, alkaline treatment without cross-linker allowed a structural reorganization of samples, as they also behaved as covalent gels, but weaker than those gels from cross-linked samples, and presented higher thermal stability than the physical mixtures. X-ray diffractograms also evidenced the occurrence of physical and chemical modifications due to the cross-linking process and indicated that samples without cross-linker underwent some structural reorganization, resulting in a decrease of crystallinity. The chemical shift of resonance signals corroborates the occurrence of structural modifications by both alkaline treatment and cross-linking reaction.

1. Introduction

Polysaccharides are natural polymers well known for their biocompatibility and biodegradability, which make them raw materials of great interest for the design of controlled drug delivery systems. Starch is one of the most abundant available polymers and can be obtained from a variety of sources. It is constituted by amylose, representing the linear fraction of the macromolecule, while amylopectin is the highly branched fraction. Among various commercially available starches, high amylose starch (modified starch containing 70% of amylose) has been reported as possessing improved properties for controlled drug delivery purposes when compared to conventional starch (Rioux et al., 2002; Onofre et al., 2009).

Additionally, the modification of high amylose starch can be can be done by reaction of its hydroxyl groups (esterification, etherification and oxidation) and its properties, such as solubility, swelling rate, rheological behavior, gel and film formation, and biodegradation rate, can be modified (Rioux et al., 2002). Cross-linking reaction has been shown to be a key technique to change the properties of starches and can be achieved by adding intra- and inter-molecular bonds randomly distributed in the starch granules (Singh et al., 2007).

Sodium trimetaphosphate (STMP), monosodium phosphate, sodium tripolyphosphate, epichlorohydrin, phosphoryl chloride and vinyl chloride are the main agents used to cross-link food grade starches (Woo and Seib, 1997; Wattanchant et al., 2003).

A high content of amylose combined to physical and chemical modifications of this material results, for example, in products with higher viscosity and in granules that are more resistant against swelling (Richardson et al., 2000; Van Hung et al., 2006).

Many researches have demonstrated the successful use of high amylose starches cross-linked by different chemicals, such as epichlorohydrin and STMP, in the development of controlled drug delivery systems (Lenaerts et al., 1991; Fang et al., 2008; Cury et al., 2009a, 2009b; Li et al., 2009; O'Brien et al., 2009).

Pectins are a family of complex polysaccharides constituted mainly by linearly linked α-(1–4)-D-galacturonic acid residues partially esterified with methanol. The degree of methoxylation (DM) is used to classify pectins as high methoxyl pectins (DM > 50) and low methoxyl pectins (DM < 50) (Sakai et al., 1993; Thakur et al., 1997; Ghaffari et al., 2007; Lutz et al., 2009). They are widely used in the pharmaceutical industry to compose hydrophilic matrices in

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oral controlled release dosage forms (Sungthongieen et al., 2004; Wei et al., 2006).

Recently, mixtures of conventional starch and pectin cross-linked by STMP were evaluated for food applications and exhibited superior mechanical properties in comparison to the isolated polymers (Khondkar et al., 2007).

Considering that the cross-linked mixtures of high amylose starch/pectin represent a promising material for use in the food and pharmaceutical field, the aim of this work is to contribute to the understanding of the structural characteristics of this material. For this purpose, pectin and high amylose starch mixed at different ratios were cross-linked to different degrees, by varying the cross-linking reaction conditions. The products were characterized by rheological, thermal, X-ray diffraction and NMR analyses.

2. Materials and methods

2.1. Materials

Pectin (type LM-506CS, batch:S74431) was provided by CP Kelko (Copenhagen, Denmark), high amylose starch (Hylon VII, batch:HA9140) was obtained from National Starch & Chemical (New Jersey, EUA), sodium trimetaphosphate (batch:112K1365) was purchased from Sigma–Aldrich Co. (St. Louis, USA), sodium hydroxide (batch: 6 11648) was supplied by Grupo Química (Rio de Janeiro, Brazil), 37% hydrochloric acid (batch: 29957) was provided by Quimis (Diadema, Brazil), ethyl alcohol (batch:127698) was obtained from Synth (Diadema, Brazil), and nimesulide (batch:NM/3680308) was provided by Henrifarma (São Paulo, Brazil).

2.2. Cross-linking of polymers

Cross-linking of polymers was performed by the method described by Cury et al. (2008), with some adaptations. High amylose starch and pectin mixed at different mass ratios (1:4, 1:1, and 4:1) were cross-linked with STMP (30% of the polymer mass) at room temperature. Different degrees of cross-linking were achieved by varying the base (NaOH) concentration (2% and 4%) and the pectin/high amylose starch/NaOH/STMP contact time (1, 2 and 4 h). After the desired mixing time, all samples were treated with the adequate amount of 1 mol L^{-1} HCl in order to set the pH at 6. The solids were separated by vacuum filtration and washed repeatedly with ethanol of different concentrations (85 °GL, 65 °GL and 96 °GL). The final product was dried at room temperature, pulverized and sieved (sieve opening = 0.97 mm). The samples were labeled according to the polymer mixture ratio (pectin:high amylose)–base strength–cross-linking reaction time. The physical mixtures were indicated by the prefix PMP-HA and the samples without cross-linker by the suffix W. For instance, 14-4-2 means 1:4 polymer ratio, 4% NaOH and 2 h of cross-linking reaction.

2.3. Rheological measurements

The polymers aqueous dispersions (5%) were prepared under stirring for 48 h and their dynamic viscoelastic properties were measured by using a controlled stress rheometer (Haake Rheostress 1, Gebruder Haake, Germany) equipped with two parallel-plates of 35 mm diameter and a gap of 200 μm. A circulating water bath (Haake C25P, Germany) for sample temperature control and a software (Rheowin 2.94) for data acquisition were also used. All measurements were carried out in triplicate, within the linear viscoelastic range, at 37 °C.

Small deformation oscillatory experiments were conducted by using three steps of rheological measurements: (1) stress sweeps between 0.1 and 100 Pa at constant frequency (1 Hz) to determine the linear viscoelastic region and the maximum deformation attainable by a sample, (2) frequency sweeps (0.6–623 rad/s) at a constant stress (5 Pa) to obtain mechanical spectra by recording the dynamic moduli G' and G'' as a function of frequency, (3) creep/recovery tests at constant stress (5 Pa). In this assay the stress was applied instantly and kept by 300 s. After removing stress, compliance was measured during further 300 s.

2.4. Thermal analysis

The thermogravimetric analysis (TG), differential thermogravimetric analysis (DTG) and differential thermal analysis (DTA) of samples 11-4-2, 14-4-2, 11-4-2W, 14-4-2W, PMP-HA 11 and PMP-HA 14, were performed on TA Instruments (SDT 600) (New Castle, DE, USA). The instrument was calibrated with indium and an empty pan was used as reference. Samples (10 mg) were accurately weighed in coated alumina pans and heated from 25 to 1200 °C at 10 °C/min under nitrogen atmosphere.

2.5. X-ray diffraction measurements

The X-ray diffraction analysis of pectin, high amylose starch, PMP-HA 11 and of samples 11-4-2, 14-4-2, 11-4-2W and 14-4-2W were performed on a X-ray diffractometer (Siemens® – Model D5000; Germany), using nickel-filtered Cu K_α radiation (tube operating at 40 kV and 30 mA). The scanning regions were collected from 4 to 60° (2θ) in step size of 0.05 (2θ).

2.6. NMR analysis

The solid state NMR analyses were carried out on a Bruker Avance III (Germany) spectrometer at 400.13 MHz to ^{1}H, and 100.62 MHz to ^{13}C. All the chemical shifts were referenced to TSP-4 (sodium trimethylsilyl propionate), the contact time for CP/MAS (cross-polarization/magic angle spinning) spectrum was 4 ms, and the samples were measured at 4 mm ZrO rotor spinning at 10 kHz.

3. Results and discussion

3.1. Rheological measurements

Dynamic testing is commonly used to provide a more explicit and detailed characterization of a gel structure (O'Brien et al., 2009). Results of stress sweep are shown in Fig. 1. The samples containing the highest pectin proportion presented the lowest critical stress values (6.4–22.10 Pa), indicating that they present the weakest gel structures. The fact that samples 1:1 and 1:4 (pectin:high amylose starch ratio) have supported higher critical stress (100 Pa) before they begin to flow suggests that these samples are systems with a more organized structure and a more rigid network (Durairaj et al., 2009; O'Brien et al., 2009). Besides, the highest values for critical stress presented by cross-linked samples indicate that the phosphorylation reaction of the cross-linking process strengthened the gel network, as suggested by O'Brien et al. (2009) in a study about starch phosphates. The frequency sweep (0.6–623 rad/s) at constant stress (5 Pa) was performed in order to obtain a mechanical spectrum of the samples and to analyze the frequency dependence of G' and G'' moduli. Such assay can provide useful information about the gel structure and it can be used to determine the difference between entangled networks, covalently cross-linked materials and physical gels (Clark and Ross-Murphy, 1987; Doucet et al., 2001; Khondkar et al., 2007).

The mechanical spectra of samples 11-4-1, 11-4-2 and 11-4-4 are shown in Fig. 2, which demonstrates the behavioral trend followed by all the samples studied, indicating a viscoelastic nature with prevalence of elastic behavior because $G' \gg G''$ within the

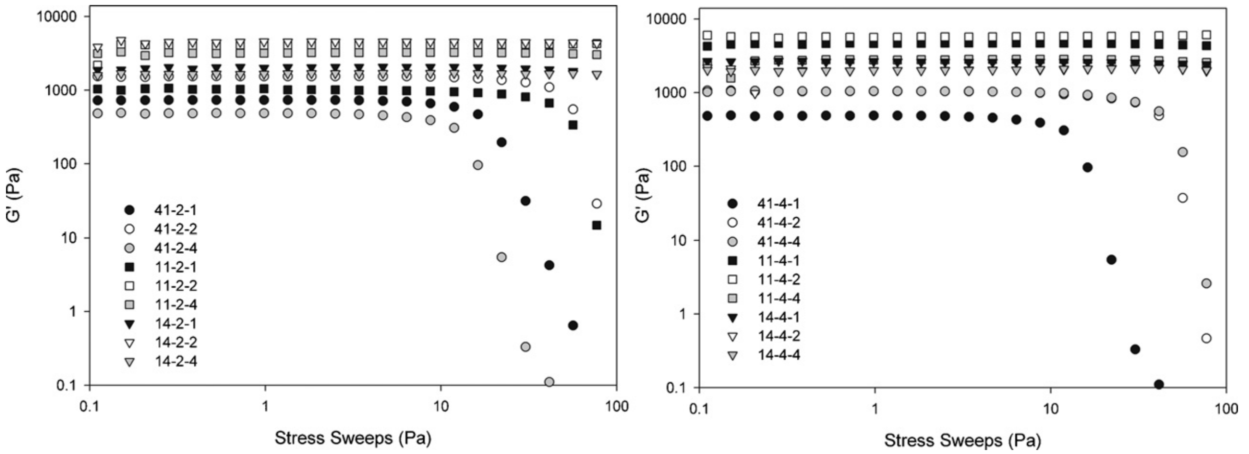

Fig. 1. Stress sweeps of samples (1 Hz, 37 °C).

whole frequency range and G' is almost frequency independent (O'Brien et al., 2009). A more detailed data analysis by linear regression of the frequency sweep results allows to observe that the samples 1:1 and 1:4 were more frequency independent ($r^2 = 0.999$ and 0.994, respectively) and presented higher G' values than sample 4:1 ($r^2 = 0.948$). These findings are in agreement with critical stress values previously presented. Besides, the reaction conditions 2 h and 4% of base strength seem to contribute to building systems structurally more organized and to enhance the elastic character of the systems.

The degree of frequency dependence can also be evaluated by parameters established by a "power law" (Ramkumar and Bhattacharya, 1996; Khondkar et al., 2007):

$$G' = A\omega^B \quad (1)$$

where G' is the storage modulus, ω is the oscillation frequency and A is a constant.

The B value can be related to both the strength and the structure of the gel, $B = 0$ indicating a covalent gel while $B > 0$ suggesting a physical gel (Hsu et al., 2000; Yoneya et al., 2003; Khondkar et al., 2007). The low values of B (Table 1) for all samples should be related to strong gels structures.

Concerning to samples W, synthesized without adding cross-linking agent, both the critical stress, G', and B values were invariably lower than those for samples cross-linked with SMTP, evidencing the network strengthening resulted from the phosphorylation reaction.

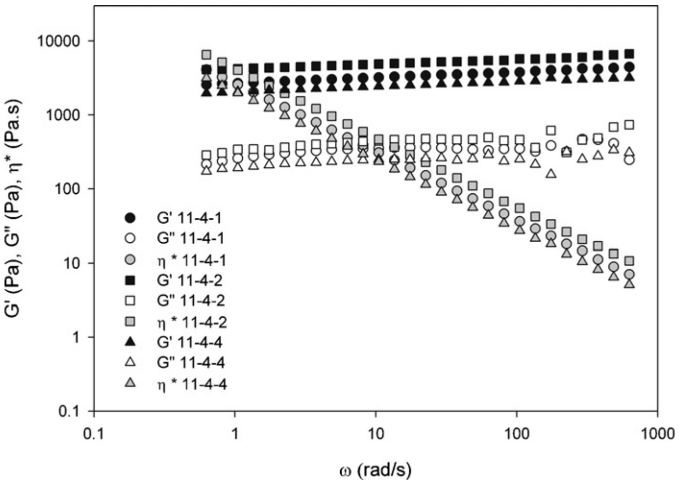

Fig. 2. Mechanical spectra (5 Pa; 37 °C) of samples 11-4-1, 11-4-2 and 11-4-4.

In the "creep and recovery" tests the samples were submitted to constant stress (5 Pa) during 300 s to evaluate the material deformation. Subsequently, the stress was removed to determine the recovery at the same time (300 s). All samples studied followed the same trend presented by samples 11-4-1, 11-4-2 and 11-4-4 (Fig. 3), evidencing their viscoelastic characteristic. The % recovery of all samples was calculated by Eq. (2) and it can be noted that samples containing higher proportions of pectin (4:1 pectin:high amylose starch ratio) showed lower recovery ability (47–68% recovery) than those prepared with higher proportions of high amylose starch; 1:1 and 1:4 pectin:high amylose starch ratio; that presented 67–102% and 56–107%, respectively, indicating, again, that the former presents a weaker gel structure.

$$\%R = \left[\frac{J_{\max} - J_{\min}}{J_{\max}}\right] \times 100 \quad (2)$$

where $\%R$ is the recovery percentage, $J_{\max}$ is the last point on the fluency phase and $J_{\min}$ is the last point of the recovery phase (Ghannam, 2004).

Table 1
Values of *B* exponent of the samples (5 Pa; 37 °C).

Samples	*B* exponent
41-2-1	0.0260
41-2-2	0.0137
41-2-4	0.0253
41-4-1	0.0195
41-4-2	0.0137
41-4-4	0.0131
11-2-1	0.0145
11-2-2	0.0133
11-2-4	0.0138
11-4-1	0.0142
11-4-2	0.0116
11-4-4	0.0132
14-2-1	0.0140
14-2-2	0.0122
14-2-4	0.0126
14-4-1	0.0117
14-4-2	0.0102
14-4-4	0.0119
41-4-4W	0.0211
11-4-4W	0.0178
14-4-4W	0.0161

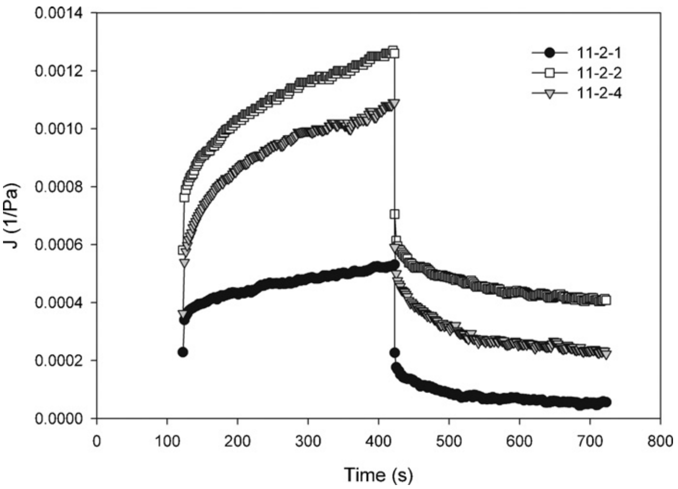

Fig. 3. Compliance *versus* time in creep and recovery tests at 37 °C for samples 11-4-1, 11-4-2, 11-4-4.

3.2. Thermal analysis

The TG/DTG profiles presented in Fig. 4 show a first degradation stage between 40 and 110 °C, which is related to moisture evaporation, according to previous studies on other polysaccharides (Einhorn-Stoll et al., 2007; Ghaffari et al., 2007; Shi and Gunasekaran, 2008). For cross-linked samples, after this initial peak, an additional sharp peak between 110 °C and 150 °C can be observed, which is related to chemically bonded water (Godeck et al., 2001; Shi and Gunasekaran, 2008).

The second and main decomposition stage of all samples can be observed between 200 °C and 400 °C, which corresponds to 28–37%, 51–56% and 60–67% of mass loss (cross-linked sample, sample without cross-linker and physical mixture, respectively). These results evidence the higher thermal stability of samples submitted to cross-linking by phosphorylation and also demonstrate that the alkaline treatment of the polymer results in a structural reorganization, which promotes some increasing of thermal stability in relation to untreated samples.

A more detailed analysis of the results allows observing that physical mixtures exhibit two peaks in this main stage. The first one, occurring at temperatures above 210 °C, can be attributed to depolymerization of pectin chains (Einhorn-Stoll et al., 2007; Ghaffari et al., 2007; Shi and Gunasekaran, 2008) and the second, at temperatures higher than 244 °C, is related to high amylose starch decomposition (Massicote et al., 2008), since it prevails in samples containing a higher proportion of this polymer (PMP-HA 14).

Confirming previous results from rheological analysis, the presence of only one peak at 200–400 °C indicates that the alkaline treatment caused structural reorganization. However, only cross-linked samples presented a third degradation stage, in which about 30% of residual mass is degraded at temperatures above 900 °C, evidencing the higher thermal stability of these samples in relation to the others.

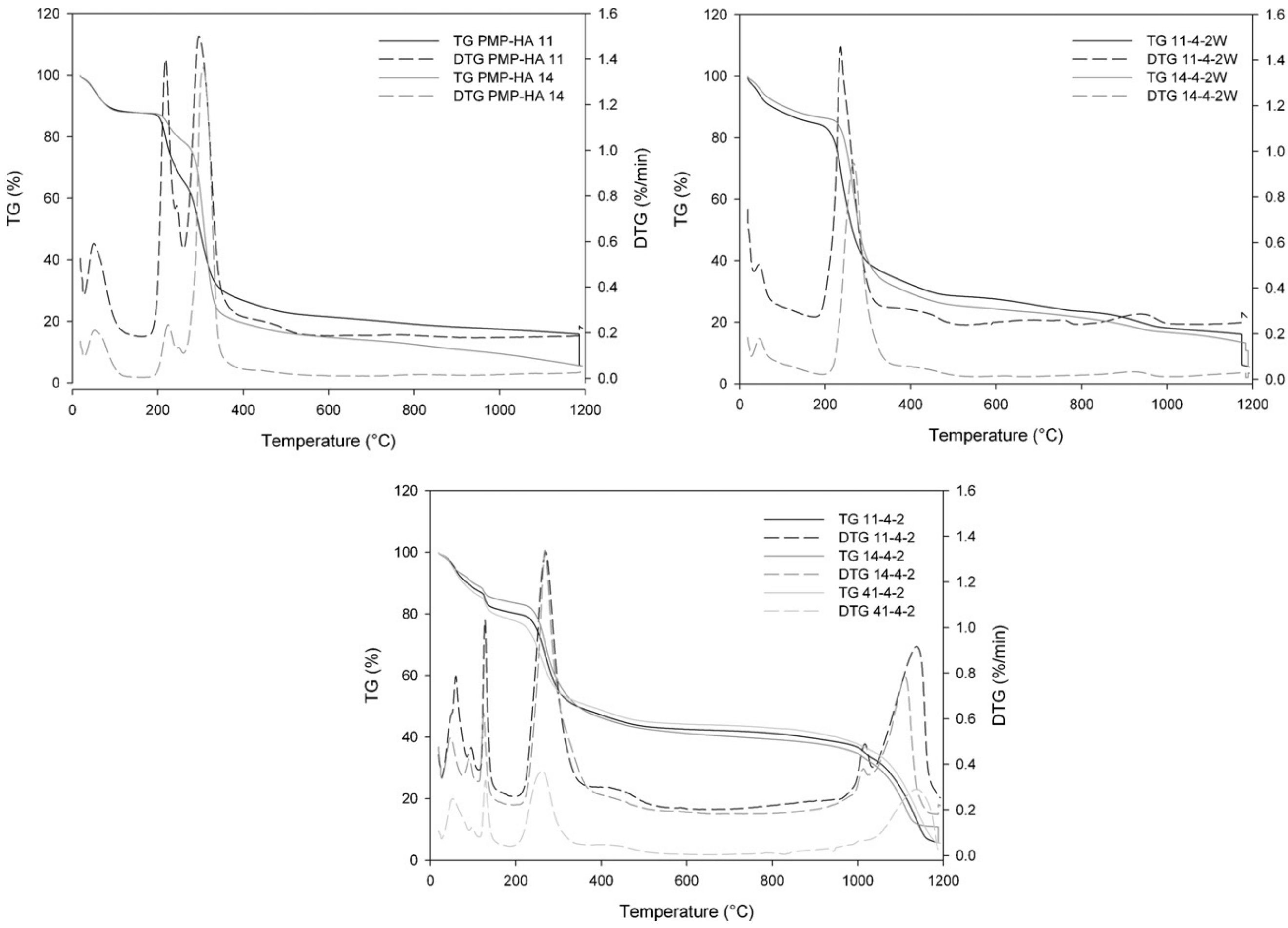

Fig. 4. Thermogravimetric (TG/DTG) curves.

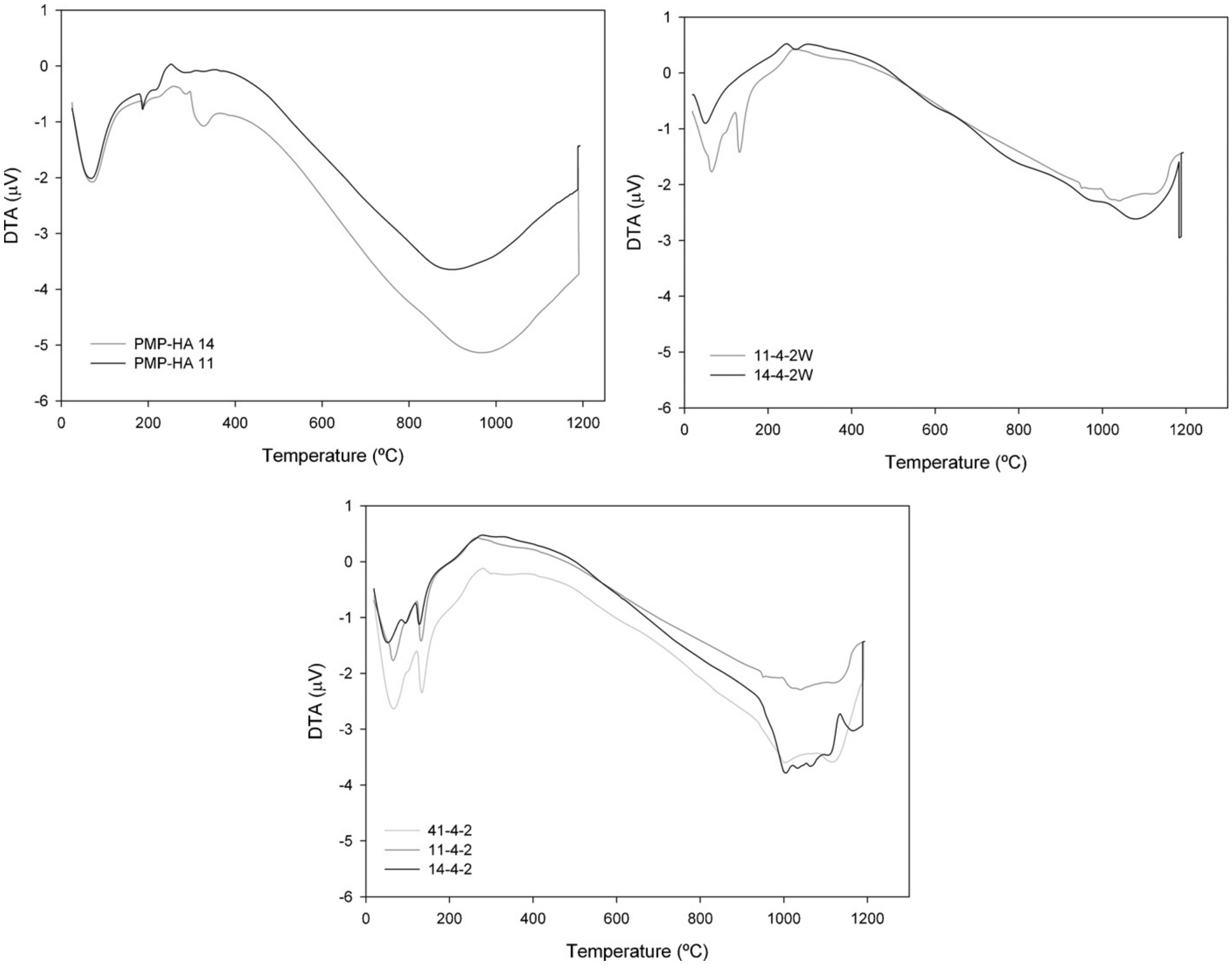

Fig. 5. DTA curves of samples.

DTA profiles (Fig. 5) show an initial endothermic peak at temperatures below 200 °C, corresponding to loss of adsorbed moisture. Large exothermic peaks can be observed at temperatures between 200 and 450 °C, which must be related to thermal depolymerization of pectin chains (Shi and Gunasekaran, 2008). These findings are in agreement with TG/DTG results.

3.3. X-ray diffraction measurements

The X-ray diffraction patterns of all samples are presented in Fig. 6. High amylose starch is a semi-crystalline polymer that can adopt different crystalline structures (A, B, C and V). The A, B and C structures exhibit a packed double helix conformation while the V structure is related to a single helix conformation, as result of amylose complexation with others components, such as water, iodine, butanol and fatty acids (Rioux et al., 2002).

The X-ray diffractogram of the high amylose starch sample (HYLON VII) shows characteristic peaks of B structure near to 17°, 19°, 23° and 25° (2θ). The pectin X-ray diffractogram presents a series of intense peaks at 12.7°, 18.42°, 28.22° and 40.14° (2θ), demonstrating the crystalline behavior of this polymer (Mishra et al., 2008). The physical mixture exhibit crystalline peaks typical of pectin combined with an amorphous halo peak of high amylose starch.

From the X-ray diffractogram of samples treated in alkaline medium (Fig. 6), one can observe that the peaks at 19° (2θ) and about 28° (2θ), which are characteristic of B structure of high amylose starch and pectin, respectively, are not present. Instead, a new broader region between 16 and 24° (2θ) is observed, which can be attributed to a decrease of the crystallinity degree (Rioux et al., 2002). This behavior suggests that the alkaline treatment of such samples promotes a structural reorganization that results in a decay of the degree of crystallinity.

The cross-linked samples show (Fig. 6) new predominant peaks at 29° and 30° (2θ), as well as reduction of intensity or even the disappearance of specific peaks of the original polymers. In this way, one characteristic peak of high amylose starch at 19° (2θ) almost disappears, while another sharp peak at about 24° (2θ) becomes evident and a new peak at about 15° (2θ) can be observed (Ispas-Szabo et al., 2000).

The changes observed in X-ray diffraction of cross-linked samples demonstrate the occurrence of physical and chemical modifications, indicating alterations on the tridimensional network attributed to the cross-linking process.

3.4. NMR analysis

^{13}C NMR spectra of pectin, high amylose starch, 11-4-2W and 11-4-2 showed some structural differences between the samples

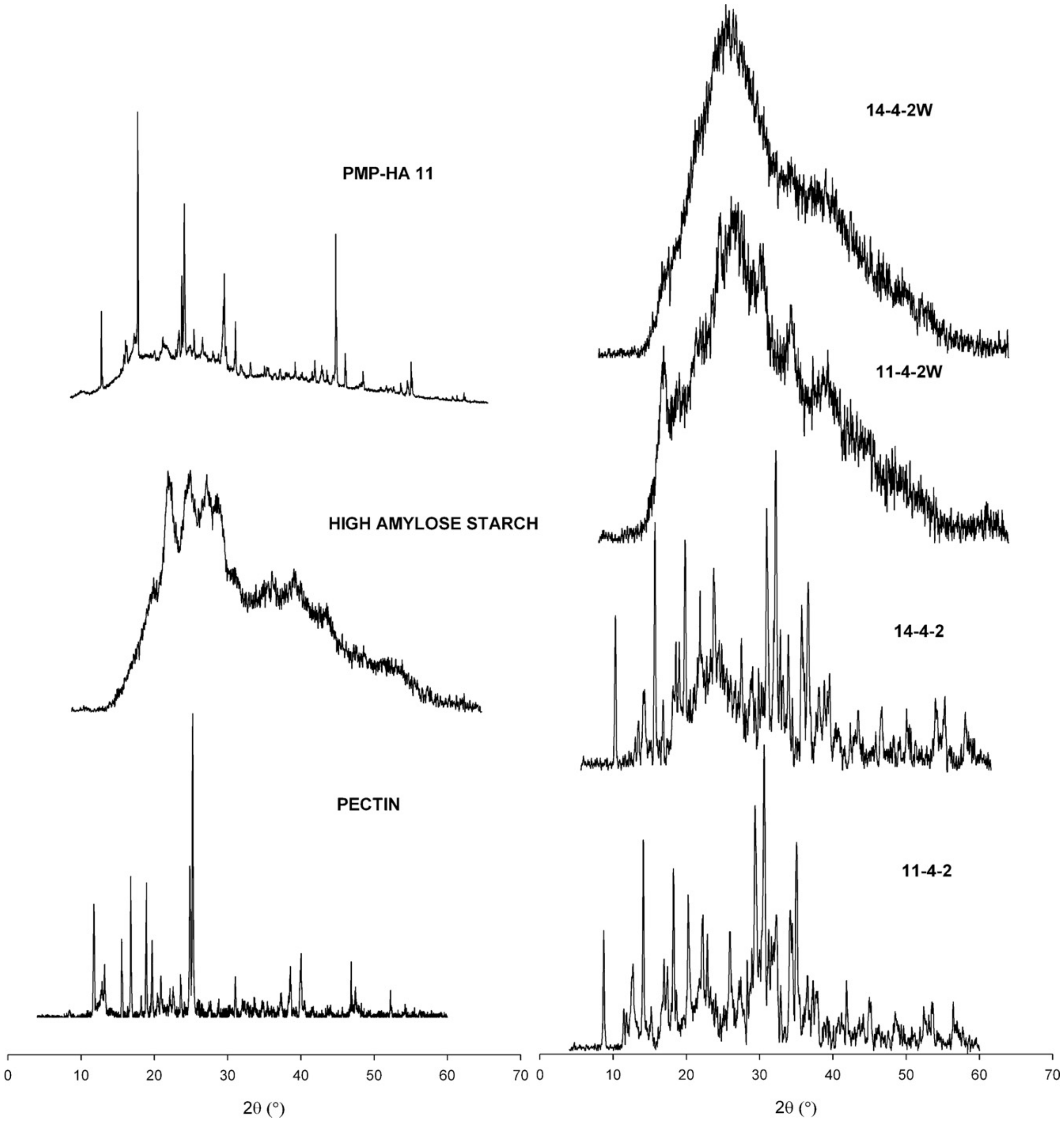

Fig. 6. X-ray diffraction patterns of samples.

synthesized with STMP and those prepared without this reagent (Fig. 7).

The spectrum of pectin presents predominant signals around 70–82 ppm, which, according to Westerlund et al. (1991), can be attributed to galacturonic ring carbons (C_2–C_5) and the same peaks were displaced or even absent in the spectra of samples 11-4-2W and 11-4-2. A signal in 53.27 ppm appears only in pectin spectrum and it can be related to methyl-ester group close to carboxyl groups of pectin (Westereng et al., 2007).

The major signal presented in high amylose starch and 11-4-2W spectra (Fig. 7) occurs around 71.5 ppm, which must include the resonance signals of C_2 (73.8 ppm), C_3 (75.4 ppm) and C_5 (72.6 ppm) (Gil and Geraldes, 1987; Thérien-Aubin et al., 2007).

The spectra of samples 11-4-2 and 11-4-2W exhibit interesting differences in the region of resonance of carbonyl group (carboxyl and ester carbonyl) around 173–177 ppm, which must be related to DE of pectin (Westerlund et al., 1991).

While for pectin the resonance of carbonyl group occurred between 170.3 and 175.4 ppm, the sample 11-4-2W presents decreased intensity of these signals and their displacement to the left side. Besides, only two signals were observed at 174.8 and 176 ppm, a feature that can be related to a reduction on DE (Westerlund et al., 1991). On the other hand, in spectra of sample 11-4-2, no characteristic peaks of carbonyl groups were observed, indicating that STMP caused a different structural rearrangement due to the presence of phosphate linkages.

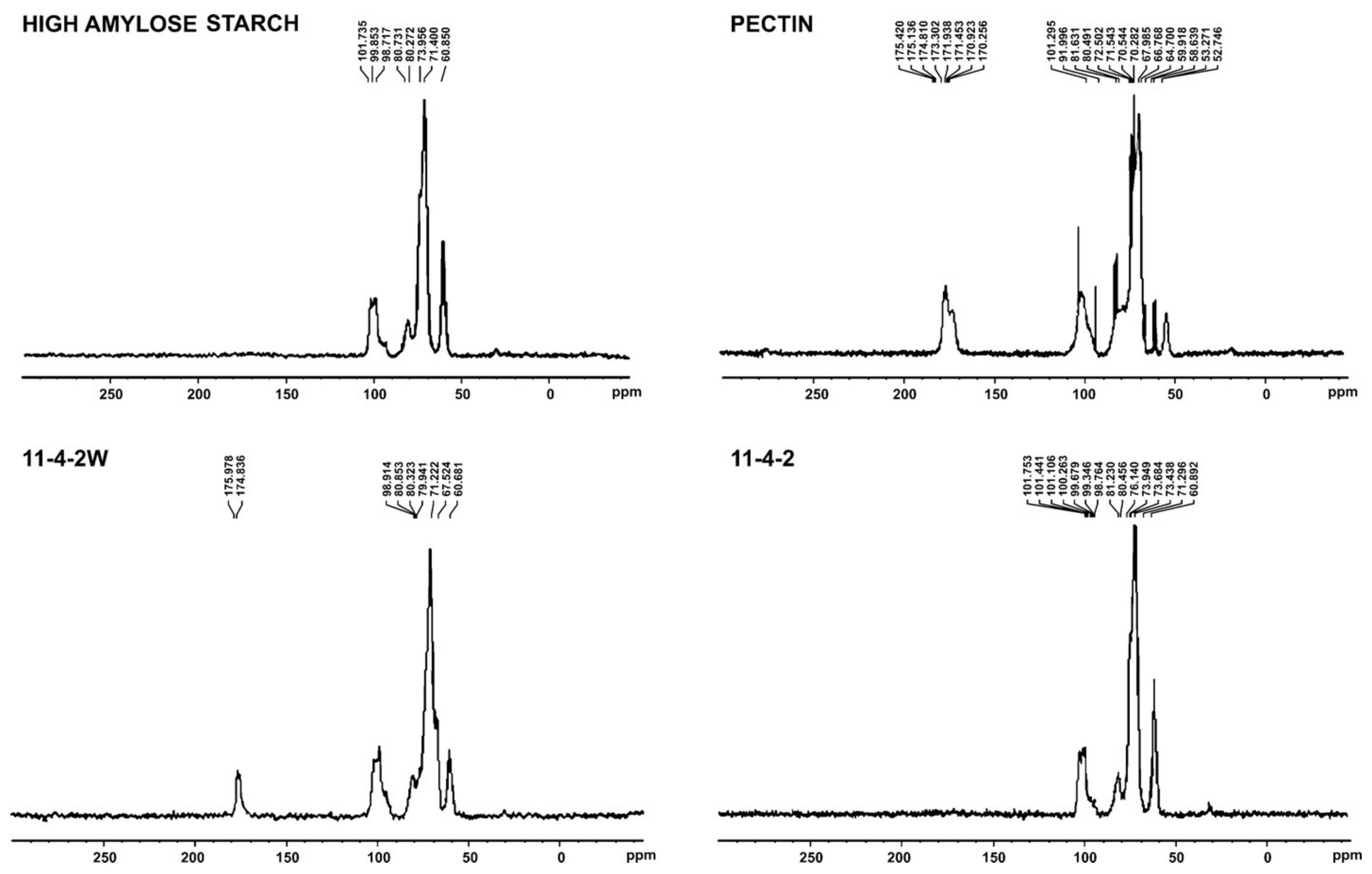

Fig. 7. ^{13}C NMR spectra of samples.

4. Conclusions

All samples studied behaved as covalent gels, since they presented G' higher than G'' within the whole frequency range and these parameters were almost frequency independent. However, cross-linked samples showed properties of the strongest gels, indicating that the phosphorylation reaction promotes the strengthening of the network, resulting in higher and more frequency independent G' values, as well as higher critical stress and recovery %. Likewise, the increase of high amylose starch ratio in the polymer mixtures contributes for the gel strengthening. Thermal stability of cross-linked samples was higher than that of the samples without cross-linker, which, in turn, was higher than of physical mixtures. X-ray diffractograms evidenced that physical and chemical modifications took place along the tridimensional network due not only to cross-linking process. When compared to physical mixtures, structural changes are observed also in samples submitted to the reaction conditions, but without added cross-linker. This behavior indicates a structural reorganization due to the alkaline treatment alone, suggesting the possibility that some cross-linking is resulting from the interaction between the polymers.

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References

Clark, A.H., Ross-Murphy, S.B., 1987. Structural and mechanical properties of biopolymers gels. Adv. Polym. Sci. 83, 57–192.

Cury, B.S.F., Klein, S.I., Evangelista, R.C., 2008. Modeling a system of phosphated cross-linked high amylose for controlled drug release. Part 1: synthesis and polymer characterization. React. Funct. Polym. 68, 1200–1206.

Cury, B.S.F., Castro, A.D., Klein, S.I., Evangelista, R.C., 2009a. Influence of phosphated cross-linked high amylose on in vitro release of different drugs. Carbohydr. Polym. 78, 789–793.

Cury, B.S.F., Castro, A.D., Klein, S.I., Evangelista, R.C., 2009b. Modeling a system of phosphated cross-linked high amylose for controlled drug release. Part 2: physical parameters, cross-linking degrees and drug delivery relationships. Int. J. Pharm. 371, 8–15.

Doucet, D., Gauthier, S.F., Foegeding, E.A., 2001. Rheological characterization of a gel formed during extensive enzymatic hydrolysis. J. Food Sci. 66, 711–715.

Durairaj, R., Ramesh, S., Mallik, S., Seman, A., Ekere, N., 2009. Rheological characterisation and printing performance of Sn/Ag/Cu solder pastes. Mater. Des. 30, 3812–3818.

Einhorn-Stoll, U., Kunzek, H., Dongowski, G., 2007. Thermal analysis of chemically and mechanically modified pectins. Food Hydrocolloid 21, 1101–1112.

Fang, Y.-Y., Wang, L.-J., Li, D., Li, B.-Z., Bhandari, B., Chen, X.D., M.A.O.A., Z.-H., 2008. Preparation of crosslinked starch microspheres and their drug loading and releasing properties. Carbohydr. Polym. 74, 379–384.

Ghaffari, A., Navaee, K., Oskoui, M., Bayatil., K., Rafiee-Tehrani, M., 2007. Preparation and characterization of free mixed-film of pectin/chitosan/Eudragit® RS intended for sigmoidal drug delivery. Eur. J. Pharm. Biopharm. 67, 175–186.

Ghannam, M.T., 2004. Creep-recovery experimental investigation of crude oil-polymer emulsions. J. Appl. Polym. Sci. 92, 226–237.

Gil, V.M.S., Geraldes, C.F.G., 1987. Ressonância magnética nuclear: fundamentos, métodos e aplicações, second ed. Fundação Galouste Gulbenkian, Lisboa.

Godeck, R., Kunzek, H., Kabbert, R., 2001. Thermal analysis of plant cell wall materials depending on the chemical structure and pre-treatment prior to drying. Eur. Food Res. Technol. 213, 395–404.

Hsu, S., Lu, S., Huang, C., 2000. Viscoelastic changes of rice starch suspensions during gelatinization. J. Food Sci. 65, 215–220.

Ispas-Szabo, P., Ravenelle, F., Hassan, I., Preda, M., Mateescu, M.A., 2000. Structure–properties relationship in cross-linked high-amylose starch for use in controlled drug release. Carbohydr. Res. 323, 163–175.

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

Lenaerts, V., Dumoulin, Y., Mateescu, M.A., 1991. Controlled release of theophylline from cross-linked amylose tablets. J. Control. Release 15, 39–45.

Li, B.-Z., Wang, L.-J., Li, D., Chiu, Y.L., Zhang, Z.-J., Shi, J., Chen, D., Maoa, Z.-H., 2009. Physical properties and loading capacity of starch-based microparticles crosslinked with trisodium trimetaphosphate. J. Food Eng. 92, 255–260.

Lutz, R., Aserin, A., Wicker, L., Garti, N., 2009. Structure and physical properties of pectins with block-wise distribution of carboxylic acid groups. Food Hydrocolloid 23, 786–794.

Massicote, L.P., Baile, W.E., Mateescu, M.A., 2008. Carboxylated high amylose starch as pharmaceutical excipient. Structural insights and formulation of pancreatic enzymes. Int. J. Pharm. 356, 212–223.

Mishra, R.K., Datt, M., Banthia, A.K., 2008. Synthesis and characterization of Pectin/PVP hydrogel membranes for drug delivery system. AAPS PharmSciTech. 9, 395–403.

O'Brien, S., Wang, Y., Vervaet, C., Remon, J.P., 2009. Starch phosphates prepared by reactive extrusion as a sustained release agent. Carbohydr. Polym. 76, 557–566.

Onofre, F., Wanga, Y.J., Mauromoustkos, A., 2009. Effects of structure and modification on sustained release properties of starches. Carbohydr. Polym. 76, 541–547.

Ramkumar, D.H.S., Bhattacharya, M., 1996. Relaxation behaviour and the application of integral constitutive equations to wheat dough. J. Texture Stud. 27, 517–544.

Richardson, P.H., Jeffcoat, R., Shi, Y., 2000. High-amylose starches: from biosynthesis to their use as food ingredients. MRS Bull., 20–24.

Rioux, B., Ispas-Szabo, P., Aït-Kadi, A., Mateescu, M., Juhász, J., 2002. Structure–properties relationship in cross-linked high amylose starch cast film. Carbohydr. Polym. 50, 371–378.

Sakai, T., Sakamoto, T., Hallert, J., Vandamme, E.J., 1993. Pectin, pectinase, and protopectinase-production, properties, and applications. Adv. Appl. Microbiol. 39, 213–294.

Shi, L., Gunasekaran, S., 2008. Preparation of pectin–ZnO nanocomposite. Nano Express 3, 491–495.

Singh, J., Kaur, L., McCarthy, O.J., 2007. Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications – a review. Food Hydrocolloid 21, 1–22.

Sungthongieen, S., Sriamornsak, P., Pitaksuteepong, T., Somsiri, A., Puttipipatkhachorn, S., 2004. Effect of degree of esterification of pectin and calcium amount on drug release from pectin-based matrix tablets. AAPS PharmSciTech. 5, 18.

Thakur, B.R., Singh, R.K., Handa, A.K., 1997. Chemistry and uses of pectin review. Crit. Rev. Food Sci. Nutr. 37, 47–73.

Thérien-Aubin, H., Janvier, F., Baille, W.E., Zhu, X.X., Marchessault, R.H., 2007. Study of hydration of cross-linked high amylose starch by solid state ^{13}C NMR spectroscopy. Carbohydr. Res. 342, 1525–1529.

Van Hung, P., Maeda, T., Morita, N., 2006. Waxy and high-amylose wheat starches and flours characteristics, functionality and application. Trends Food Sci. Technol. 17, 448–456.

Wattanchant, S., Muhammad, K., Hashim, D., Rahman, R.A., 2003. Effect of crosslinking reagents and hydroxypropylation levels on dual-modified sago starch properties. Food Chem. 80, 463–471.

Wei, X., Sun, N., Wu, B., Yin, C., Wu, W., 2006. Sigmoidal release of indomethacin from pectin matrix tablets: effect of in situ crosslinking by calcium cations. Int. J. Pharm. 318, 132–138.

Westereng, B., Michaelsen, T.E., Samuelsen, A.B., Knutsen, S.H., 2007. Effects of extraction conditions on the chemical structure and biological activity of white cabbage pectin. Carbohydr. Polym. 72, 32–42.

Westerlund, E., Aman, P., Andersson, R.E., Andersson, R., 1991. Investigation of the distribution of methyl ester groups in pectin by high-field ^{13}C NMR. Carbohydr. Polym. 14, 179–187.

Woo, K., Seib, P.A., 1997. Cross-linking of wheat starch and hydroxypropylated wheat starch in alkaline slurry with sodium trimetaphosphate. Carbohydr. Polym. 33, 263–271.

Yoneya, T., Ishibashi, K., Hironaka, K., Yamamoto, K., 2003. Influence of cross-linked potato starch treated with POCl3 on DSC, rheological properties and granule size. Carbohydr. Polym. 53, 447–457.