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Research paper

Physical stability and moisture sorption of aqueous chitosan–amylose starch films plasticized with polyols

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Abstract

The short-term stability and the water sorption of films prepared from binary mixtures of chitosan and native amylose maize starch (*Hylon VII*) were evaluated using free films. The aqueous polymer solutions of the free films contained 2% (w/w) film formers, glycerol, or erythritol as a plasticizer, as well as acetic acid (1%) and purified water. Characterization of the present fresh and conditioned film formers and free films was done using X-ray diffraction analysis, determination of moisture sorption isotherms, and near infrared spectroscopy. The results indicated that clear changes in the crystallinity of the films are evident within a 3-month period of storage, and the changes in the solid state are dependent on the plasticizer and storage conditions. When stored at ambient conditions for 3 months, the aqueous chitosan–amylose starch films plasticized with erythritol exhibited a partly crystalline structure. This was as a result of sugar recrystallisation due to the high hydrogen bonding. The respective films plasticized with glycerol and stored at 25 °C/60% relative humidity (RH) or at 40 °C/75% RH remained flexible and amorphous for at least 3 months. The water sorption of the free films greatly increased as a function of storage time at 75 and 95% RH. The second derivative spectra of starting material and free films were capable of distinguishing the internal water from the free water after storage at different relative humidities. Free water resulted in a separate band at a lower wavelength (1903 nm) in comparison to the structured absorbed water band at 1920 nm, in the case of films the free water resulted in a band around 1900 nm.

Keywords: Amylose starch; Chitosan; Free films; Physical stability; Polyols; Water sorption

1. Introduction

Chitosan is a cationic natural polysaccharide derived by deacetylation of chitin. Chitosan meets the important requirements of excipients in drug delivery, i.e. biocompatibility and biodegradability. Due to these favorable properties the interest in chitosan and its derivatives as excipients in drug delivery has increased in recent years [1,2]. Higher molecular weight chitosans have been reported to have good film-forming properties as a result of intra- and intermolecular hydrogen bonding [3].

Recent studies on pharmaceutical and food chitosan film coatings have often examined the permeability to water vapor and moisture sorption of chitosan films [4–7]. The influence of molecular weight as well as degree of deacetylation on the moisture sorption behavior, swelling properties, and water vapor transmission rates have also been evaluated [5,7]. In most studies the films were produced from blends of chitosan and other polymers such as cellulose, polyvinyl alcohol, or polyvinyl pyrrolidone [4]. To date, only little attention has been paid to the effects of storage conditions on the stability of chitosan films.

The mechanism for the prediction of water transport through hydrophilic films like chitosan films is extremely complex. The complexity is due to nonlinear water sorption isotherms and water content dependent diffusivities [8]. The water vapor transmission of hydrophilic films varies nonlinearly with water vapor pressure. If the films are cationic and strongly hydrophilic, water interacts with

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the polymer matrix and increases the permeation for water vapor [9].

In our previous study, chitosan films prepared with amylose corn starch (*Hylon VII*) as a co-film-forming agent were evaluated using free films [10]. Erythritol was found as a plasticizer of choice for these composite films improving mechanical strength of the films. This study therefore aimed to investigate the physical stability and sorption behavior of the present chitosan films prepared with amylose corn starch (*Hylon VII*) as a co-film-forming agent. The effects of type and molecular weight of chitosan, type of plasticizer, and storage humidity conditions were evaluated.

2. Materials and methods

2.1. Materials

High, medium, and low molecular weight chitosans (Aldrich Chemical Company Inc., Milwaukee, WI, USA) were used in this study. Amylose-rich corn starch (*Hylon VII*) (National Starch and Chemical GmbH, Neustadt, Germany) was used as a co-film former, and glycerol (Ph.Eur) and i-erythritol (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) as plastizers. Acetic acid (Riedel-de Haën, Germany) and purified water were employed as solvents.

2.2. Free films and stability testing

The aqueous polymer solutions contained 2% (w/w) film former (mixture of high molecular weight chitosan and *Hylon VII*), glycerol, or erythritol as a plasticizer (20% w/w of the polymer weight), as well as acetic acid (1%) and purified water. The ratios of the chitosan–*Hylon VII* solutions plasticized with glycerol or erythritol were 80:20 and 60:40.

Hylon VII solutions were prepared in a high-pressure reactor equipped with a blade mixer (VTT Automation, Espoo, Finland). *Hylon* was first dispersed in cold water. Once loaded in the reactor chamber, the starch dispersion was gradually heated to 160 °C and the pressure in the vessel reached 3.0 bar. The starch solution obtained was cooled down to approximately 90 °C and filtered. The *Hylon* solution was mixed with the chitosan solution at approximately 60 °C, and the temperature of the solution for preparing free films was maintained above 50–60 °C. The plasticizer was added at a concentration of 20% (w/w) of the total dry weight. For preparing free films, the polymer solution was poured into polytetrafluoroethylene (Teflon®) molds, and the films were air dried for 4 h at 60 °C.

Films of binary mixtures of chitosan and *Hylon VII* were stored at 25 °C/60% RH (relative humidity) and 40 °C/75% RH for 3 months.

2.3. Characterization of materials and free films

2.3.1. XRD measurements

X-ray diffraction patterns of all the samples were measured using X-ray powder diffraction (XRPD) theta–theta diffractometry (Bruker AXC D8, Karlsruhe, Germany). The XRPD experiments were performed in symmetrical reflection mode using Cu K_α radiation (1.54 Å) and Göbel Mirror bent gradient multilayer optics. The scattered intensities were measured with a scintillation counter. The pure materials were measured at an angular range from 2 to 40° (2θ) with steps of 0.1° and a measuring time of 5 s/step. The X-ray diffraction patterns of high, medium, and low molecular weight chitosan, native amylose corn starch (*Hylon VII*), glycerol, and erythritol are shown in Fig. 1.

The diffraction pattern of erythritol included the strongest reflections at about 14.7, 20.2, 21, 24.6, 27.9, 29.7, 30.5, 31.3, and 32.9° (2θ). These reflections agreed with earlier reported reflections of erythritol [11]. The diffraction patterns of chitosan have the clearest reflections at about 10 and 20° and resemble closely the earlier presented chitosan form II [12]. The diffraction pattern of *Hylon VII* included weak reflections at about 17.2 and 20°, corresponding to the earlier reported structure of B-type starch [13]. No reflections are seen in the diffraction pattern of glycerol, indicating that the material is totally amorphous.

X-ray diffraction patterns of the film samples (15 days, 1, 2, and 3 months) were measured at angles ranging from 5 to 40° in 2θ with steps of 0.02° and a measuring time of 10 s for each angular step. The determination of crystallinity was based on the assumption that the experimental intensity curve is a linear combination of intensities of a crystalline and an amorphous component.

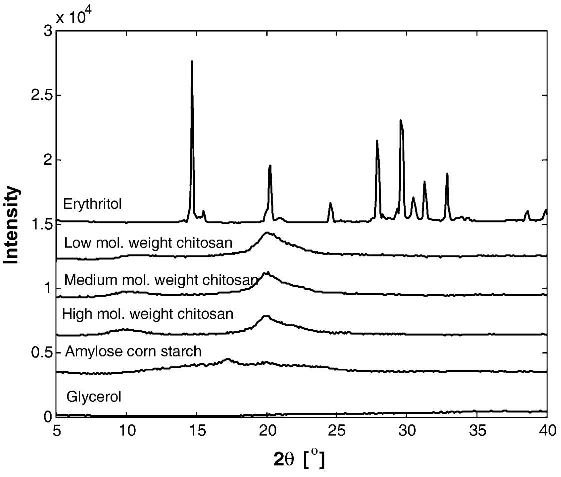

Fig. 1. X-ray diffraction patterns of pure film-forming polymers and plasticizers. From bottom to top: glycerol, native amylose corn starch (*Hylon VII*), high molecular weight chitosan, medium molecular weight chitosan, low molecular weight chitosan, and erythritol.

The crystallinity of the samples was estimated by fitting the intensity of the crystalline component and the intensity of the amorphous component to the experimental intensity curve. The crystallinity of the samples was obtained as the ratio of the integrals of the intensities of the crystalline component and the studied sample. The intensity curves of totally amorphous samples were used as the amorphous background of the samples. The experimental intensity curve, where the amorphous background was subtracted, was used as the crystalline model intensity curve.

2.3.2. Moisture sorption isotherms

Water sorption isotherms were determined by placing powders and films of binary mixtures of chitosan and *Hylon VII* into a controlled humidity environment at a constant temperature until equilibrium. After drying at 40 °C and 160 mbar for 24 h in a vacutherm (Heraeus VT 6025, Kendro Laboratory Products GmbH, 63450 Hanau, Germany), the dried powders and films were placed into environments of various relative humidities above salt solutions in desiccators. The relative humidities were 0% RH (silica gel), 11% RH (lithium chloride), 23% RH (potassium acetate), 33% RH (magnesium nitrate), 43% RH (potassium carbonate), 52% RH (magnesium nitrate), 59% RH (sodium bromide), 75% RH (sodium chloride), 85% RH (potassium chloride), and 95% RH (disodium hydrogen phosphate). All the salts were of reagent grade. Three types of chitosan powder, amylose-rich corn starch, and erythritol were placed into each condition while placing the films into 0, 23, 43, 75, and 95% of relative humidities.

Completely dry samples in weighing bottles were stored in desiccators for 9 days at 21 ± 2 °C. The bottles with samples were removed from the desiccator for quick weighing. After weighing, the bottles were replaced in the desiccators. The weight gain of the powders and the films were recorded at predetermined time intervals (2, 5, and 9 days). The measurement was made in triplicate. Moisture sorption isotherms were calculated as the equilibrium of moisture sorption (EMC), and experimental monolayer water values were determined from the adsorption isotherm using BET equations (Brunauer, Emett, and Teller's multilayer adsorption theory; Eq. (1)) [14] and GAB equations (Guggenheim, Andersen, and de Boer monolayer sorption theory; Eq. (2)) [15–17] for modelling moisture sorption isotherms.

$$a_w/(1 - a_w)m = 1/m_0C + a_w(C - 1)/m_0C \tag{1}$$

$$m = C_1km_0a_w/(1 - ka_w)(1 - ka_w + C_1ka_w) \tag{2}$$

where

m, moisture content
a_w, water activity
m_0, monolayer moisture content
C, constant related to excess enthalpy of sorption
C_1 and k are constants.

2.3.3. Near infrared spectroscopy

The near-infrared (NIR) spectra were measured with a Fourier Transform (FT)-NIR spectrometer (Bomem MD-160 DX, Hartmann and Braun, Que., Canada) using Bomem-GRAMS software (v. 4.04, Galactic Industries Inc., Salem, NH, USA) and Teflon as reference (99% reflective Spectralon, Labsphere me., North Sutton, NH, USA). The spectra were measured through the bottom of the glass vial containing the sample. The measurements were carried out in triplicate. The spectra were recorded over a range of 10,000–4000 cm^{-1} with a resolution of 16 cm^{-1} and averaged over 32 scans. Second derivative transformations of absorbance, log(1/*R*), were performed with 11-point Savitzky–Golay smoothing [18] using Matlab software (v. 5.3, MathWorks Inc., Natick, MA, USA).

3. Results and discussion

3.1. Moisture sorption properties of starting materials

The association of pharmaceutical solids with water can result in significant changes in, e.g. chemical stability, physical solid state properties, crystal growth and dissolution, dispersibility and wetting, caking, and flow.

The steady-state moisture in the starting materials was measured after 9 days of storage of the samples at different relative humidities. As seen in Fig. 2a, the moisture increase of low molecular weight chitosan was lower than that of high and medium molecular weight chitosan at a relative humidity of 95%. The moisture increase of *Hylon VII* was lower than that of other starting materials from relative humidities of 52–95%.

The storage of the erythritol at a high humidity resulted in a significant increase in water uptake, causing a liquefaction of the substance even higher than that of chitosans. Starch and chitosan are hydrophilic and retain a considerable amount of water. The amount depends on the relative humidity. At least in chitosan there exist three predominant absorption sites such as the hydroxyl group, the amino group, and the polymer chain end. The polymer chain end is supposed to be composed of a hydroxyl group or an aldehyde group [7]. Usually the amine content increases with increasing molecular weight. In the case of chitosan, the water is bound to the hydroxyl group more strongly than to the amine group. Therefore, the release of water molecules could preferentially occur via the amine group [19]. Modelling of the moisture sorption isotherms was done using the BET equation, and the experimental monolayer water values were 5.5 g water/100 g dry material (d.m.) for high molecular weight chitosan, 6.0 g water/100 g d.m. for medium molecular weight chitosan, and 5.0 g water/100 g d.m. for low molecular weight chitosan. Using the GAB equation, the monolayer values were 7.0, 7.4, and 6.2 g/100 g d.m. for high, medium, and low molecular weight chitosan, respectively. In the case of *Hylon VII*,

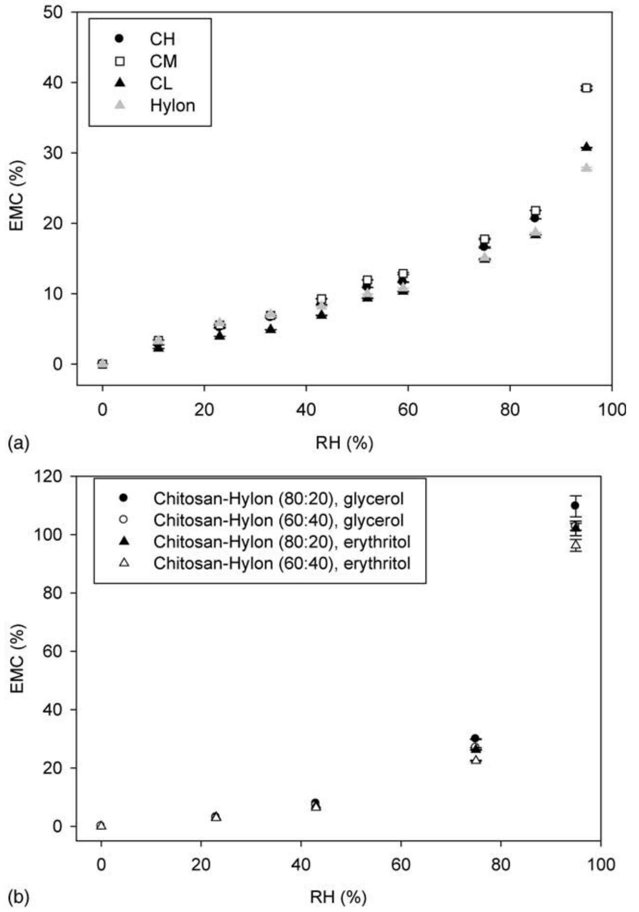

Fig. 2. Equilibrium moisture content (EMC%) of high molecular weight chitosan (CH), medium molecular weight chitosan (CM), low molecular weight chitosan (CL), amylose corn starch (*Hylon VII*) starting materials (a), and chitosan–*Hylon VII* films (b) stored at different relative humidities for 9 days.

the value by the BET equation was 4.9 g water/100 g d.m. and by the GAB equation 6.5 g water/100 g d.m.

A series of NIR spectra for high molecular weight chitosan and *Hylon VII* with increasing RH (following 9 days of storage with the saturated salt solution) are shown in Fig. 3a–d. With increasing moisture content the water bands of the starting material increased in size and shifted gradually from 1920 to 1903 nm. The second derivative spectra of the starting material were capable of distinguishing the absorbed water from the free water. The free water resulted in a separated band at a lower wavelength (1903 nm) in comparison to the structured absorbed water band at 1920 nm. The intensity of the water bands increased with increasing water content. The NIR spectra collected for the samples did not show any change that was induced during the storage at different humidities.

3.2. Effects of storage on solid-state properties of free films

X-ray diffraction patterns and calculated values for crystallinity of various film samples plasticized with erythritol are shown in Fig. 4a–c and Table 1, respectively. There are clear changes in the crystallinity of the films during storage, and the changes seem to be dependent on the storage conditions and the plasticizer. The yellow tint of all the films studied became more evident as the films were stored in higher temperature and relative humidity (40 °C/75% RH). Furthermore, the films became more flexible and less brittle when stored at a high relative humidity. When stored at 25 °C/60% RH, however, the aqueous chitosan–amylose starch films plasticized with erythritol exhibited white spots on the surface, and they were very brittle.

The diffraction patterns of the films plasticized with glycerol and stored at 25 °C/60% RH included only diffuse maxima with no reflections in the patterns. This means that the films are totally amorphous. The same films stored at 40 °C/75% RH were also amorphous. The effect of erythritol as a plasticizer was demonstrated by storing the films at 25 °C/60% RH and at 40 °C/75% RH. The reflections of crystalline erythritol were observed in the films stored for 2 months at 25 °C/60% RH (Fig. 4c). The reflections increased and became clearer at 3 months, but the amorphous background also increased, indicating that the films were partly crystalline.

Although all the 15-day-old samples were totally amorphous, the diffraction patterns of the films differed slightly. The diffraction pattern of the chitosan–*Hylon VII* (80:20) sample has a diffuse maximum at about 20° (2θ) (Fig. 4c), while the pattern of the chitosan–*Hylon VII* (60:40) (Fig. 4a and b) sample has two diffuse maxima at about 19.6 and 20° (2θ). This indicates that the macro-scructure of the samples was different.

The effect of water was demonstrated by storing the films at 75% RH (40 °C) (Fig. 4b). The films became mechanically very weak. As seen in Fig. 4a; the crystalline peaks appeared only in the films of chitosan–Hylon VII (60:40) plasticized with erythritol after 2 months of storage. The rest of the films analysed were amorphous.

As seen in Fig. 4a–c and Table 1, the crystallinity of the samples started to increase after 2 months. The crystallinities of the films stored at 25 °C/60% RH were higher than those of the respective films stored at 40 °C/75% RH. The diffraction pattern of the 40 °C/75% RH sample (Fig. 4b) after 2 months has a strong amorphous background and only two reflections of crystalline erythritol at about 24.6 and 28.32° (2 theta). Until 3 months, the diffraction pattern has a strong amorphous background and three reflections at about 19.6, 20.3, and 37.5° (2 theta). While the diffraction patterns of the 25 °C/60% RH samples after 2 and 3 months showed a slightly amorphous background and almost all of reflections of crystalline erythritol. The diffraction patterns differed due to the preferred orientation of the 40 °C/75% RH samples. So the storing has a tendency to increase the crystallinity of the samples, but the films stored at 25 °C/60% RH remain more isotropic.

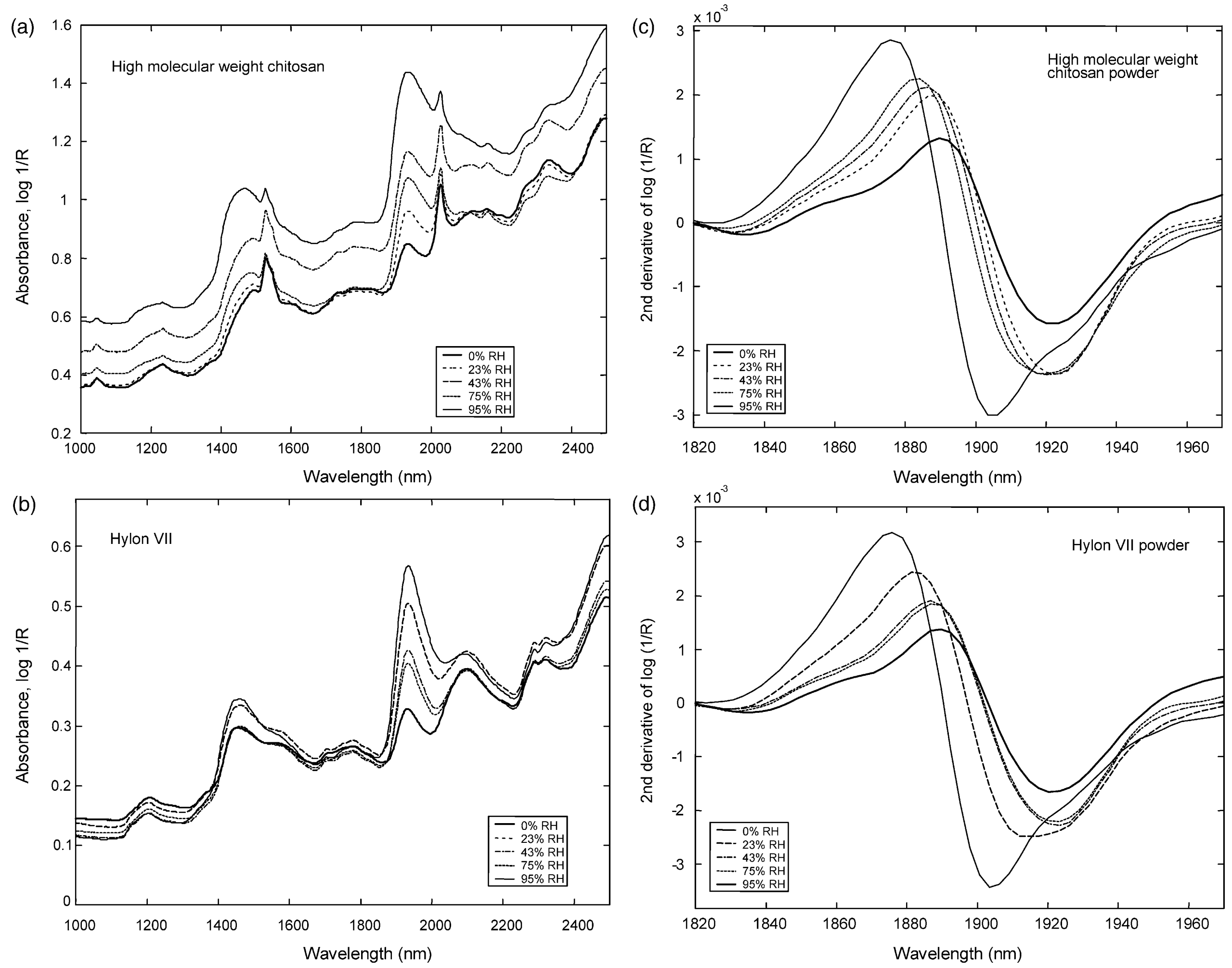

Fig. 3. NIR absorbance spectra (log(1/*R*)) and second derivative of log(1/*R*) of the starting materials studied. Absorbance 1000–2500 nm, (a) high molecular weight chitosan, (b) *Hylon VII*. Second derivative of log(1/*R*) 1820–1970 nm, (c) high molecular weight chitosan, (d) *Hylon VII*.

Independent of the ratio of chitosan and native starch in the films and the storage conditions, none of the films studied showed reflections specific to B-type starch (5.6, 15, 17, 22, and 24, 26) [20], indicating that *Hylon VII* remained amorphous.

3.3. Effects of storage on moisture sorption of free films

The equilibrium moisture contents (EMC %) of chitosan–*Hylon VII* films stored at different relative humidities for 9 days are shown in Fig. 2b. Aqueous chitosan–amylose

Table 1
Crystallinities of chitosan and *Hylon VII* films plasticized with erythritol

Storage time (days)	Storage conditions	Samples	Crystallinity ± 10%
15	25 °C/60% RH	Chitosan: *Hylon VII* (60:40)	0
		Chitosan: *Hylon VII* (80:20)	0
	40 °C/75% RH	Chitosan: *Hylon VII* (60:40)	0
30	25 °C/60% RH	Chitosan: *Hylon VII* (60:40)	0
		Chitosan: *Hylon VII* (80:20)	0
	40 °C/75% RH	Chitosan: *Hylon VII* (60:40)	0
60	25 °C/60% RH	Chitosan: *Hylon VII* (60:40)	35
		Chitosan: *Hylon VII* (80:20)	29
	40 °C/75% RH	Chitosan: *Hylon VII* (60:40)	12
90	25 °C/60% RH	Chitosan: *Hylon VII* (60:40)	35
		Chitosan: *Hylon VII* (80:20)	33
	40 °C/75% RH	Chitosan: *Hylon VII* (60:40)	20

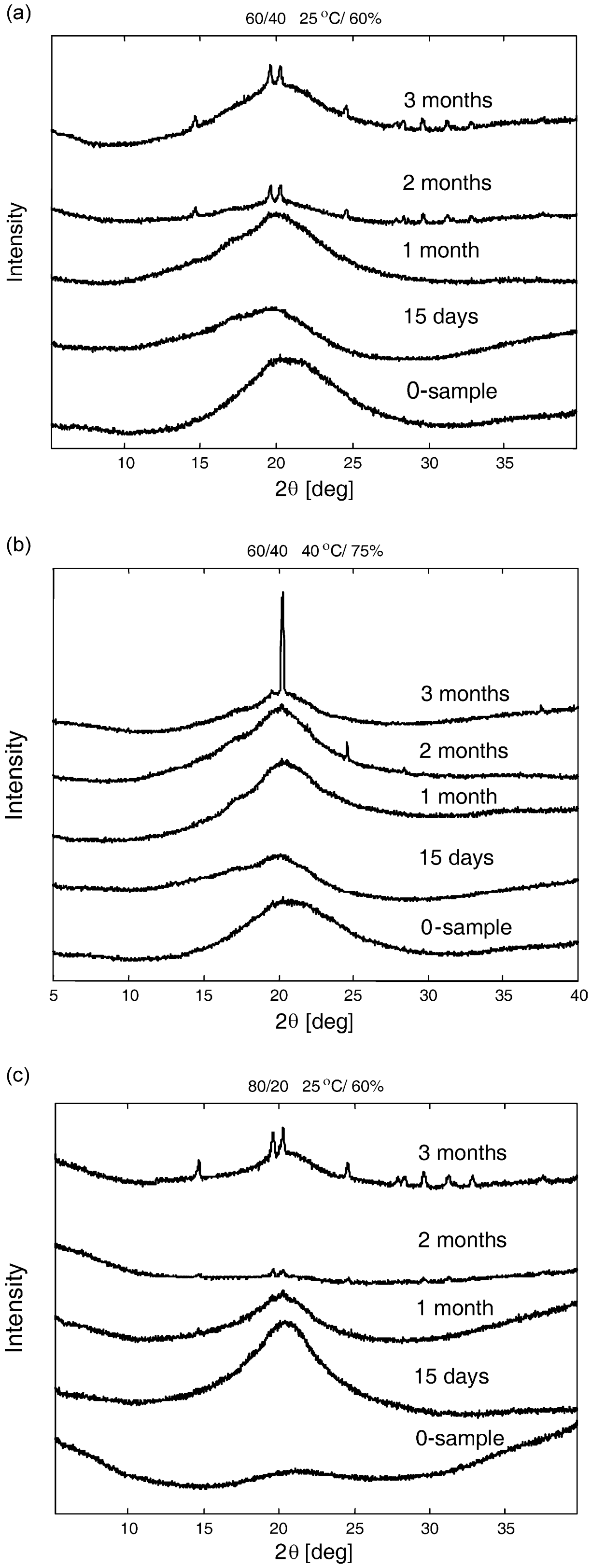

Fig. 4. X-ray diffraction patterns of fresh and aged chitosan–*Hylon VII* free films plasticized with erythritol. Key: (a) chitosan–*Hylon VII* (60:40) stored at 25 °C/60% RH, (b) chitosan–*Hylon VII* (60:40) stored at 40 °C/75% RH, and (c) chitosan–*Hylon VII* (80:20) stored at 25 °C/60% RH.

starch films stored at 0, 23, 43, 75, and 95% relative humidities for 9 days showed increasing moisture absorption with increasing relative humidity. These results are in good agreement with the results of Nunthanid and coworkers [6]. They found that the moisture sorption of higher molecular weight (H-type) chitosan films was higher than that of lower molecular weight (VL-type) chitosan films with increasing relative humidity.

The films of chitosan–Hylon VII plasticized with glycerol seem to absorb more moisture at a higher rate than the rest of the films studied; although the difference in the water uptake is very small, except at the higher relative humidities 75 and 95% RH (Fig. 2b). The water sorption of free films increased greatly as a function of storage time at 75 and 95% RH. With such a gain in moisture we would suspect swelling to occur at some point. Swelling would cause a conformational change in the microstructure of the film and open up the polymer structure to allow an increase in permeant flux. Changes in the polymer structure that occur in response to stresses generated within the film during sorption are a consequence of swelling [21].

Changes in crystallinity were evident by X-ray in the films plasticized with erythritol during the physical stability of 3 months. Therefore, additional studies of solid-state properties and swelling of the films are warranted.

The NIR spectrocopy absorption spectrum of pure water consists of five bands with maxima at 760, 970, 1190, 1450, and 1940 nm, but the intense absorption bands of water are found around 1450 and 1940 nm [22]. The frequencies and intensities of water bands alter in the NIR region with changes in the strength of hydrogen bonds and hydration. Since the first overtones of carbohydrates and the –OH groups of water overlap at the same wavelengths (around 1450 nm), the combination band (around 1940 nm) provides a better selectivity for water and, hence, is the most important absorption band in the NIR region [23].

The same water bands at the 1800–2100 nm region were identified by NIR from the chitosan films (Fig. 5a–d) as described before. The absorbed water was seen as a gradually increasing absorption maximum at 1920 nm. With increasing moisture content the water bands of chitosan films shifted gradually from 1920 to 1903 nm. Free water resulted in a separate band at around 1900 nm.

4. Conclusions

The changes in the physical stability of aqueous chitosan–amylose starch films are dependent on the storage conditions and the type of plasticizer. Until 3 months, storing had a tendency to increase the crystallinity of the films plasticized with erythritol in addition to the changes on their film surface. The respective films plasticized with glycerol remained amorphous. Although in the beginning the quality of the present films plasticized with erythritol was very good, stability of the films was poor. The films

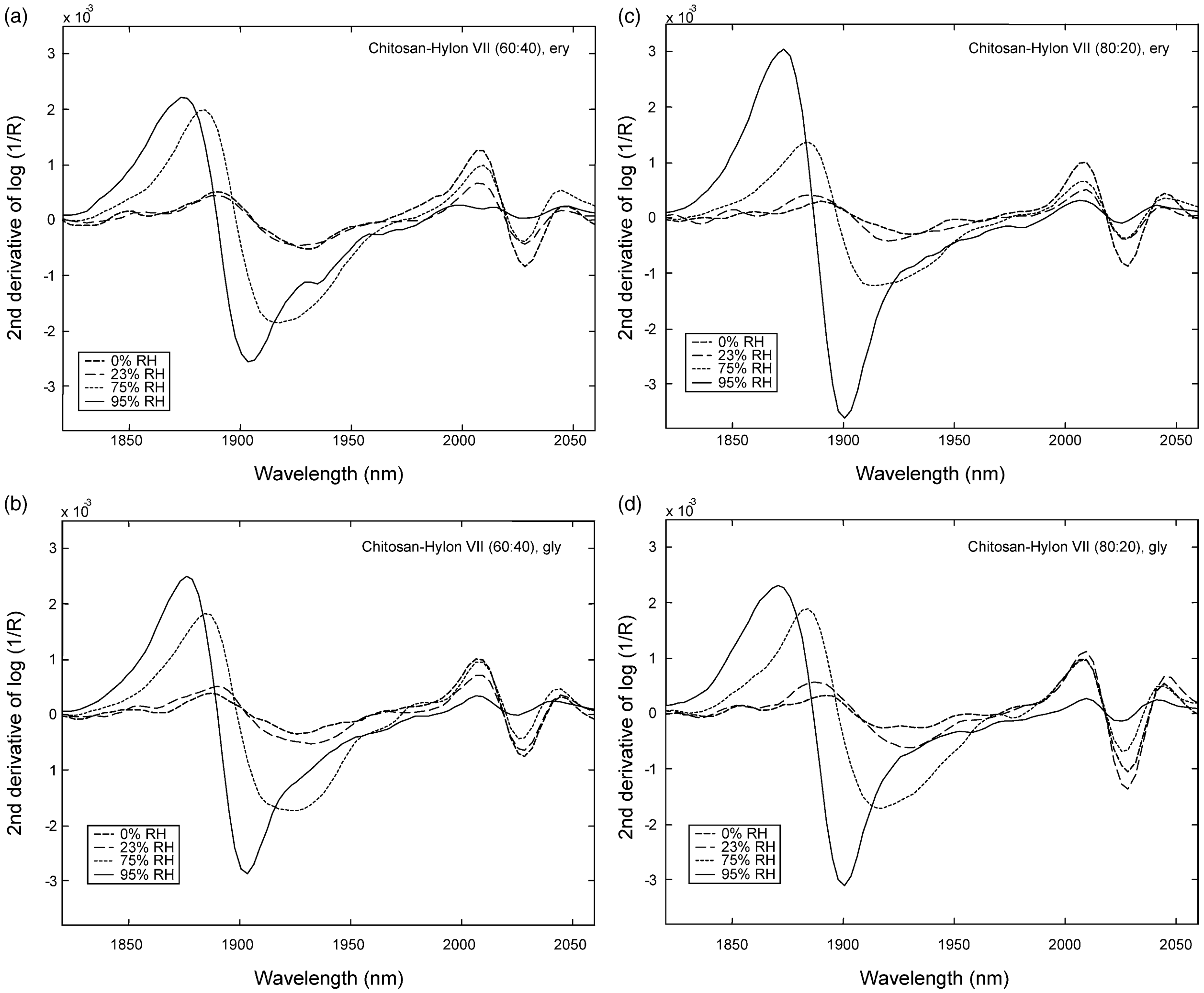

Fig. 5. Near infrared (NIR) reflectance spectra of (a) chitosan–*Hylon VII* (60:40) films with erythritol, (b) chitosan–*Hylon VII* (60:40) films with glycerol, (c) chitosan–*Hylon VII* (80:20) films with erythritol, and (d) chitosan–*Hylon VII* (80:20) films with glycerol. Second derivative of absorbance, log(1/*R*), at 1820–2060 nm.

prepared from binary mixtures of chitosan and *Hylon VII* plasticized with glycerol are a promising system for use in the coating process.

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