



## The effect of citric acid on the structural properties and cytotoxicity of the polyvinyl alcohol/starch films when molding at high temperature

Rui Shi<sup>a</sup>, Jingliang Bi<sup>a</sup>, Zizheng Zhang<sup>a</sup>, Aichen Zhu<sup>a</sup>, Dafu Chen<sup>b</sup>, Xinhua Zhou<sup>b</sup>,  
Liquan Zhang<sup>a,\*</sup>, Wei Tian<sup>c,\*</sup>

<sup>a</sup>The Key Laboratory of Beijing City on Preparation and Processing of Novel Polymer Materials, Beijing University of Chemical Technology, Box 57, Beijing 100029, People's Republic of China

<sup>b</sup>Laboratory of Bone Tissue Engineering of Beijing, Research Institute of Traumatology and Orthopaedics, Beijing 100035, People's Republic of China

<sup>c</sup>Department of Spine Surgery of Beijing JiShuiTan Hospital, The Fourth Clinical Medical College of Peking University, Beijing 100035, People's Republic of China

### ARTICLE INFO

#### Article history:

Received 17 June 2007

Received in revised form 4 March 2008

Accepted 25 April 2008

Available online 11 May 2008

#### Keywords:

PVA

Starch

Citric acid

Crosslink

Cytotoxicity

### ABSTRACT

A series of starch/polyvinyl alcohol (PVA) films, denoted SP films, with varying concentrations (5–30 wt%) of citric acid (CA) were solvent cast at 140 °C. The effects of CA on the chemical structure, thermal properties, swelling degree, mechanical properties, crystallinity, and cytotoxicity were investigated. Fourier-transform infrared (FT-IR) spectroscopy showed that an esterification took place between CA and starch (or PVA) during molding at 140 °C. This esterification and the multi-carboxyl structure of CA resulted in a chemical cross-linking of the blended system. Furthermore, the esterification occurred more easily between starch and CA as opposed to between the PVA and CA. The residual-free CA acted as a plasticizer for the starch and PVA. As compared to the hydroxyl groups on glycerol, the carboxyl groups on CA were capable of forming stronger hydrogen bonds between CA and other components, and this cross-linking and strong hydrogen bonding enhanced the thermal stability of the SP films. Consequently, the water absorbance decreased from 33% to 20% as the CA percentage increased from 5 to 30 wt%. When 5 wt% CA was added, the tensile strength of the sample increased from 39 to 48 MPa, but when even more CA was added (from 5 to 30 wt%), the tensile strength decreased from 48 to 42 MPa and the elongation at break increased from 102% to 208%. This was caused by the plasticizing effect of the residual-free CA in the blend. The cell relative growth rates of samples with varying CA concentrations exceeded 80% after 7 days of incubation, and this demonstrated that there was no significant toxicity on the cells' growth when the CA content was less than 20 wt%.

© 2008 Published by Elsevier Ltd.

### 1. Introduction

Biodegradable polymers with controllable lifetimes have been considered as promising alternatives to non-degradable ones (Albertson, 1980; Albertson, Barenstedt, & Karlsson, 1994), and have played important roles in the environment and in the biomedical fields (Chiellini, Cinelli, Chiellini, & Imam, 2004; Giusti et al., 1994; Giusti, Lazzeri, & Lelli, 1993). Blends of polysaccharides and synthetic polymers have been extensively studied since these blends can be prepared with the biodegradable properties (Arvanitoyannis, 1999; Graaf & Janssen, 2000, 2001; Kaplan, 1998). Polysaccharides such as starch and dextran are typical examples of

natural biodegradable hydrophilic polymers with relatively good biocompatibility. However, as polysaccharides dissolve easily in water, they do not present mechanical and shape stabilities in fluids. An effective method for overcoming this issue is to blend them with synthesized polymer gel networks in order to form natural and synthesized polymer blend hydrogels. Among the existing synthesized polymers, PVA possesses many useful properties, such as excellent chemical resistance, optical and physical properties (Nakayama, Takatsuka, & Matsuda, 1999), good film-forming capability, water solubility (Cinelli, Chiellini, Gordon, & Chiellini, 2005), and an excellent biocompatibility (Jiang, Campbell, Boughner, Wan, & Quantz, 2004; Wan, Campbell, Zhang, Hui, & Boughner, 2002). The strength, flexibility, and water resistance of starch productions improved when PVA aqueous solution was added (Cinelli, Chiellini, Lawton, & Imam, 2006). Furthermore, the polarity of PVA has been found to help the blend in accelerating the hydrolytic attack by the atmospheric moisture, thus resulting in the degradation of the sugar molecules in natural polymers (Raj, Siddaramaiah, & Somashekar, 2004).

\* Corresponding authors. Address: Center of Advanced Elastomer Materials, School of Materials Science and Engineering, Beijing University of Chemical Technology, Box 57, Beijing, 100029, PR China (L. Zhang). Tel.: +86 10 6442 1186; fax: +86 10 6443 3964 (L. Zhang), +86 010 58516782 (W. Tian).

E-mail addresses: [zhanglq@mail.buct.edu.cn](mailto:zhanglq@mail.buct.edu.cn) (L. Zhang), [tianweia@163bj.com](mailto:tianweia@163bj.com) (W. Tian).

However, since starch and PVA molecules a large number of hydroxyl groups, SP films display a hydrophilic nature. Moreover, in the PVA/starch composites, starch is only partially compatible with PVA. The addition of starch to PVA does not improve its physical properties because of the poor compatibility between PVA and starch (Siddaramaiah, Raj, & Somashekar, 2004). Thus, the mechanical properties and water resistance of the SP films need to be improved for it to be used in the environment and biomedical fields.

A number of plasticizers, e.g. water and polyols (Arvanitoyannis, Kolokuris, Nakayama, Yamamoto, & Aiba, 1997; Yoon, Chough, & Park, 2007), have been essayed in order to decrease the glass-transition temperature ( $T_g$ ) and increase the flexibility and workability of starch and PVA (Arvanitoyannis et al., 1997; Jayasekara, Harding, Bowater, Christie, & Lonergan, 2003; Raj et al., 2004; Sreedhar, Sairam, Chattopadhyay, Syamala Rathnam, & Mohan Rao, 2005). However, results have shown that the elongation at break could be increased at the sacrifice of the elongation at break, and that the materials consistently displayed a poor water resistance (Zou, Qu, & Zou, 2007). Other effective means commonly used to modify the PVA/starch composites in order to improve their mechanical and water resistance properties include: (1) chemically modifying PVA or starch (Nabar, Draybuck, & Narayan, 2006), e.g. esterification, oxidation, etherification, cross-linking and methylation (Dermigöz et al., 2000; Kiatkamjornwong, Thakeow, & Sonsuk, 2001; Kim, 2003; Kim & Lee, 2002; Thakore, Desai, Sarawade, & Devi, 2001; Xu, Miladinov, & Hanna, 2004; Zhao et al., 2006), (2) physically modifying the SP composites, e.g. by adding corn fibers to the blends (Cinelli et al., 2006), or (3) chemically modifying the SP composites during or after the blending process, e.g. cross-linking reactions, grafting (Beliakova, Aly, & Abdel-Mohdy, 2004), and surface modifications (Jayasekara, Harding, Bowater, Christie, & Lonergan, 2004). Examples of cross-linking reagents include glutaraldehyde (Ramaraj, 2007), boric acid (Yin, Li, Liu, & Li, 2005), and epichlorohydrin (Sreedhar, Chattopadhyay, Karunakar, & Sastri, 2006), and those of methods include radiation (Zhai, Yoshii, & Kume, 2003), and photo-cross-linking (Follain, Joly, Dole, & Bliard, 2005). However, cross-linking agents such as those mentioned above always display toxicity and thus their potential applications as biomaterials are limited. When compared to the modification methods in the blending process, those requiring a pre-treatment of starch or PVA, and a post-treatment of SP films are complicated. To overcome these disadvantages, certain non-toxic functional additives and simple modification techniques are required to improve the mechanical properties and water resistibility of the SP films.

CA with one hydroxyl and three carboxyl groups exists widely in citrus fruits and pineapples, where it is the main organic acid. CA was chosen as the additive for the following reasons. First of all, as a result of its multi-carboxylic structure, esterification could take place between the carboxyl groups on CA and the hydroxyl groups on the PVA or starch. Such an esterification would improve the water resistibility (Borredon, Bikiaris, Prinos, & Panayiotou, 1997). Furthermore, because of the multi-carboxyl structure, CA may serve as a cross-linking agent. Cross-linking of a blend reinforces the intermolecular binding by introducing covalent bonds that supplement natural intermolecular hydrogen bonds (Krumova, López, Benavente, Mijangos, & Pereña, 2000) so as to improve the mechanical properties and water resistibility. Second, the residual-free CA in the PS blends may act as a plasticizer. As compared to the hydroxyl groups on glycerol, the carboxyl groups on CA can thus form stronger hydrogen bonds with the hydroxyl groups on PVA/starch blends, thus improving the interactions between the molecules (Shi et al., 2007a; Yu, Wang, & Ma, 2005). And as a third point, CA is rated as nutritionally harmless since it is a nontoxic metabolic product of the body (Krebs or citric acid cy-

cle). Consequently, it has already been approved by FDA for use in humans (Yang, Webb, & Ameer, 2004). These nontoxic properties will benefit the incorporation of CA in PS films (PSC) and such films would have potential applications in the food packing industry and in the biomedical field.

Although Yoon and co-workers also used CA as the additive to prepare PS films as the plasticizers (Yoon, Chough, & Park, 2006a, 2006b), they selected 50 °C as the molding temperature. The esterification will not occur in the blending system under such a low temperature. A series of structural and physical properties changes result from the esterification which need a high temperature.

It is known that a saccharide is a very important ingredient, and the main energy resource of an organism. It has been reported that there is a saccharide protein layer outside the cell of the most animal's cell (Lamba, Baumgartner, & Cooper, 1998). Both the monosaccharide and polysaccharide have an excellent compatibility with the peptide and the protein, which provide starch with an excellent cell compatibility. (Dermigöz et al., 2000; Lamba et al., 1998) PVA is also reported to have biocompatibility and excellent mechanical properties. This material eventually blended with its copolymers is used in several drug delivery applications, especially in the preparation of environment-sensitive hydrogels for 'intelligent' insulin release. (Hisamitsu, Kataoka, Okano, & Sakurai, 1997; Kitano, Koyama, Kataoka, Okano, & Sakurai, 1992; Shiino et al., 1994) Therefore, the application of SP films in biomedical and clinical field (such as drug control release carrier and biomembrane) will have a specific advantage and important research value. (Autori et al., 2006; Cascone, Barbani, Cristallini, Ciardelli, & Lazzeri, 2001; Pal, Banthia, & Majumdar, 2006) However, the cell compatibility of the PS films was rarely reported. Moreover, there was no report about the cytotoxicity of the PS films, which contained CA. The effect of CA percentage in SP film on cytotoxicity is very important for the potential application in biomedical field. The cytotoxicity test can help us to determine the safe CA concentration range.

In this work, we investigated the effect of CA percentages on the structure and physical properties of the SP films. The effect of CA concentration on cytotoxicity was also studied. This work will pave the way for the application of SPC films in biomedical field.

## 2. Experimental

### 2.1. Materials

Corn starch was supplied by J&K Chemical Ltd. (Japan), which is composed of 25% amylose and 75% amylopectin, and the original moisture content was 12 wt%. The glycerol (AR) was obtained from Century Star Chemical Products, Inc. (Beijing, China). Citric acid (AR) was supplied by Xu Dong Chemical Plant (Beijing, China), PVA was obtained from Shanghai Reagent Company (Shanghai, China), which was 99% hydrolyzed with an average polymerization degree of  $1750 \pm 50$ . The water used to prepare starch/PVA blend films was redistilled after deionization.

### 2.2. Preparation of PVA/starch blend films

Films were obtained by the casting method. First, the solution was prepared by dissolving the PVA, starch and glycerol in hot water. The mixture was blended for 4 h with a mechanical stirrer (1000 rpm) at 95 °C to form a homogeneous gel-like solution. In order to avoid the acidolysis, CA solution was added into the mixture with a mechanical stirrer for 10 min (1200 rpm) at 30 °C. Bubbles were removed with an aspirator. The mixing composition is shown in Table 1. A certain amount of the gel-like solution thus prepared was poured on a pre-warmed (140 °C) glass plate. Water was evap-

**Table 1**  
Materials' abbreviations and corresponding sample compositions

Abbreviations	Samples (weight proportions)		
	PVA:starch (w/w)	Glycerol (wt% of the dry weight)	CA (wt% of the dry weight)
PSG	3:1	20	–
PSGC5	3:1	20	5
PSGC10	3:1	20	10
PSGC20	3:1	20	20
PSGC30	3:1	20	30
PSC20	3:1	–	20

orated from the molds in a ventilated oven at 140 °C for 1 h. The dried films were sealed in polyethylene bags and stored at 20 °C and at 50% RH for 1 week prior to performing the measurements.

### 2.3. Characterizations

#### 2.3.1. Fourier-transform infrared (FT-IR) spectroscopy

The IR spectra were measured with a Nicolet-210 spectrophotometer (Nicolet Co., USA). The samples were prepared by mixing the fine powder with KBr and pressing. The spectra were obtained at a resolution of 4 cm<sup>-1</sup> in the range 4000 to 500 cm<sup>-1</sup>.

#### 2.3.2. X-ray diffraction (XRD)

The wide-angle XRD patterns of the products were recorded with a Rigaku model D/Max2500VB2+/PC X-ray diffractometer (Japan) with nickel filtered Cu-K $\alpha$  radiation. The samples were 0.1-mm-thick with a smooth surface. The scattering angles ranged from 3° to 60° at 5° min<sup>-1</sup>.

#### 2.3.3. Differential scanning calorimeter (DSC)

Differential scanning calorimetry (DSC) thermograms were recorded by a NETZSCH DSC 204 F1 (Germany) instrument. The sample (3–10 mg) was placed in an aluminum pan and was first cooled from room temperature to –150 °C and held there for 3 min. Subsequently, a heating scan was conducted from –120 to 150 °C at a heating rate of 20 °C/min. The glass-transition temperature was taken as the midpoint of the heat capacity change.

#### 2.3.4. Thermogravimetric (TG) analysis

Thermogravimetry analyses were carried out by a NETZSCH TG 209C (Germany) instrument. About 10 mg sample was positioned in silica pans, and the samples were heated at 10 °C/min from ambient temperature to 600 °C. Thermal analyses were performed under the nitrogen flow (10 mL/min).

#### 2.3.5. Swelling degree

The swelling degree of the samples was characterized at 37 °C, for which rounded samples with 1 cm diameter were placed in de-ionized water. The experiments were carried out by measuring the weight gain as a function of immersion time in 20 ml water. The swelling degree was calculated by using the following equation,

$$\text{Swelling degree (\%)} = \frac{W_t - W_o}{W_o} \times 100 \quad (1)$$

where  $W_t$  is the wet weight after degrading for a predetermined time,  $W_o$  is the original weight of the sample.

#### 2.3.6. Mechanical testing

Specimens, with the shape of a Dumb-bell according to ISO/DIS 37-1990 type 3 specifications (0.1 mm thickness and 2 mm width), were prepared. Tensile tests were performed in a universal tensile testing machine (CMT4104) equipped with 500 N load cell and operated at a cross-head speed of 10 mm/min. The specimen's

elongation was derived from the extensometer separation of 18 mm. Five samples of each product were tested at 21 °C and the average value of each quantity was reported.

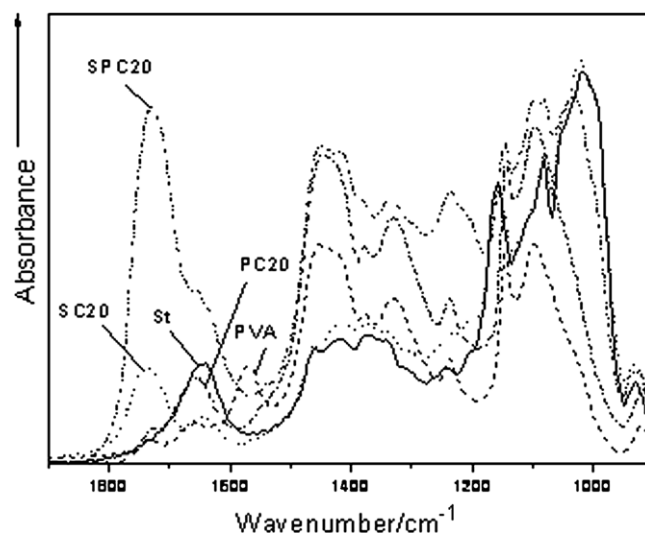
#### 2.3.7. Cytotoxicity test

The samples were incubated in phosphate-buffered saline (PBS) solution for 8 h to decrease the effects of impurity on cells. The 0.1-mm-thick slices were sterilized by washing with a 75% (v/v) ethanol solution in sterilized water, followed by CO<sub>60</sub> exposure for 15 min, and then incubated in Dulbecco's modified Eagle's medium (DMEM) at a proportion of 3 cm<sup>2</sup> ml<sup>-1</sup> for 24 h at 37 °C. The extract solution was then filtered (0.22  $\mu$ m pore size) to eliminate the possible presence of solid particles of the material. L929 cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) at a density of 4.0  $\times$  10<sup>4</sup> cells/ml and plated into 96-well micrometer plates. The plates were incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> in air. After that, the medium was replaced by the previously prepared extracted dilutions (50%, volume proportion) and was used as a culture medium and by itself as a control. After 2, 4 and 7 days of incubation, the cell culture was treated with MTT. 50 ml/well of MTT (1 mg/ml in medium 199 without phenol red, Sigma, St. Louis, USA) treated samples were incubated for further 4 h at 37 °C in a humidified atmosphere of 5% of CO<sub>2</sub> in air. At this stage the MTT was removed and 100 ml/well of isopropanol (BDH, Poole, England) was added in order to dissolve the formazan crystals. The optical density (OD) was read on a multiwell microplate reader (EL 312e Biokinetics Reader, Biotek Instruments) at 570 nm. All materials extracts were tested for a minimum of three separate experiments with comparable results.

## 3. Results and discussion

### 3.1. The effects of CA on chemical structure (FT-IR)

The effects of CA on the FT-IR spectra of starch, PVA and PVA/starch blends are shown in Fig. 1. Because it is unlikely that all the carboxyl groups are esterified, so the broad peak at 1729 cm<sup>-1</sup> is the C=O stretching vibration peak, and it is probably a coalescence peak which is caused by the ester bond and carboxyl C=O groups in CA. Fig. 1 showed that the peak at 1729 cm<sup>-1</sup> didn't exist on the IR spectra of starch. A small peak at 1729 cm<sup>-1</sup> was observed which may be caused by the impurity from PVA because a



**Fig. 1.** The effects of CA on FT-IR spectra of starch, PVA and PVA/starch blends when molding at 140 °C.

similar peak was observed in PVA spectra. However, on the IR spectra of PVA/CA (PC) and starch/CA (SC), the peak at  $1729\text{ cm}^{-1}$  was exhibited. This phenomenon demonstrates that the esterification occurred between CA and starch as well as between CA and PVA. Before the test, all the samples were immersed into the de-ionized water to erase the effects of free CA in the blend, so that the height of the peak could reflect the esterification degree. Compared to SC, the peak at  $1729\text{ cm}^{-1}$  of PC was lower. This phenomenon illustrates that the esterification occurred more easily between the starch and CA than that between PVA and CA.

It can be seen obviously that the peak height increased at  $1729\text{ cm}^{-1}$  as the CA concentration increased (see Fig. 2(a)). Because the samples were pre-immersed in the de-ionized water to erase the effects of free CA molecules, the increase of the peak's height is an excellent evidence that the esterification degree increased as the CA concentration increased. Fig. 2(b) shows that the peak at  $3340\text{ cm}^{-1}$  of PSC20 without glycerol was higher than that of PSGC20 with glycerol content. The reason may be caused by that a part of CA was reacted with glycerol to obtain some glycerol citrates which diffused into the water in the process of immersion. The presence of glycerol prevented the reaction between CA and starch or PVA.

The peak around  $3340\text{ cm}^{-1}$  is assigned to the stretching vibration of the hydroxyl groups. The contrast of peaks heights at

$3340\text{ cm}^{-1}$  is shown in Fig. 2(b). The peak's height was measured from the baseline, which passed through the lowest point of the spectra and parallel to the  $x$ -axis. The peak at  $758\text{ cm}^{-1}$  is assigned to C—O—C ring vibrations on starch (Jayasekara et al., 2004). It is due to the starch only and the starch concentration of the samples is same so that this is an ideal reference frequency to measure other peaks height.

It can be seen from Fig. 2(b) that the peak's height at  $3340\text{ cm}^{-1}$  increased as the CA concentration increased. This is because the number of the hydroxyl and carboxyl groups increased as the CA concentration increased.

We also noticed that the peak at  $3340\text{ cm}^{-1}$  of PSC20 was higher than that of PSG, and this may be because fact that CA can form stronger hydrogen bonds with starch or PVA when compared with glycerol. The peak of PSC20 at  $3340\text{ cm}^{-1}$  was also higher than that of PSGC20. It can be explained by that a part of CA was reacted with glycerol when glycerol and CA were added together, so few hydrogen bonds were produced.

### 3.2. The effects of CA on thermal properties

#### 3.2.1. Differential scanning calorimeter (DSC)

The effects of CA, glycerol and CA/glycerol mixture on DSC curve of PVA were investigated and the results are shown in Fig. 3. Two peaks were observed on the DSC curve of the pure PVA. The peak at  $230\text{ }^\circ\text{C}$  is assigned to the melting point-related peak of PVA and the peak starting from  $240\text{ }^\circ\text{C}$  is due to the decomposition of the PVA. When glycerol or CA was added, the melting temperature decreased and the height of the melting peak decreased. The melting enthalpy ( $\Delta H$ ) and crystallinity also decreased when glycerol or CA was added. This is because a part of the crystallinity was destroyed as the function of glycerol or CA.

As shown in Fig. 3, the  $T_g$  of the pure PVA is around  $54.8\text{ }^\circ\text{C}$ , which is smaller than that reported in other works (Sreedhar et al., 2005). This is because the PVA film had already contained some water as the plasticizer. When glycerol was added (PG), the  $T_g$  decreased to as low as  $11.7\text{ }^\circ\text{C}$ . The reduction in  $T_g$  and  $H$  was attributed to the reduction in the cohesive forces of attraction between the polymer chains. The plasticizer molecules penetrated the polymer matrix because they were smaller than the polymer molecule. As a result, polar attractive forces were established between the plasticizer and chain segments, which were responsible for the reduction of the cohesive forces and, therefore, a reduction of  $T_g$ .

However, when the CA was added to PGC, and molded at less than  $140\text{ }^\circ\text{C}$  for 1 h, a small increase in  $T_g$  was detected. This phe-

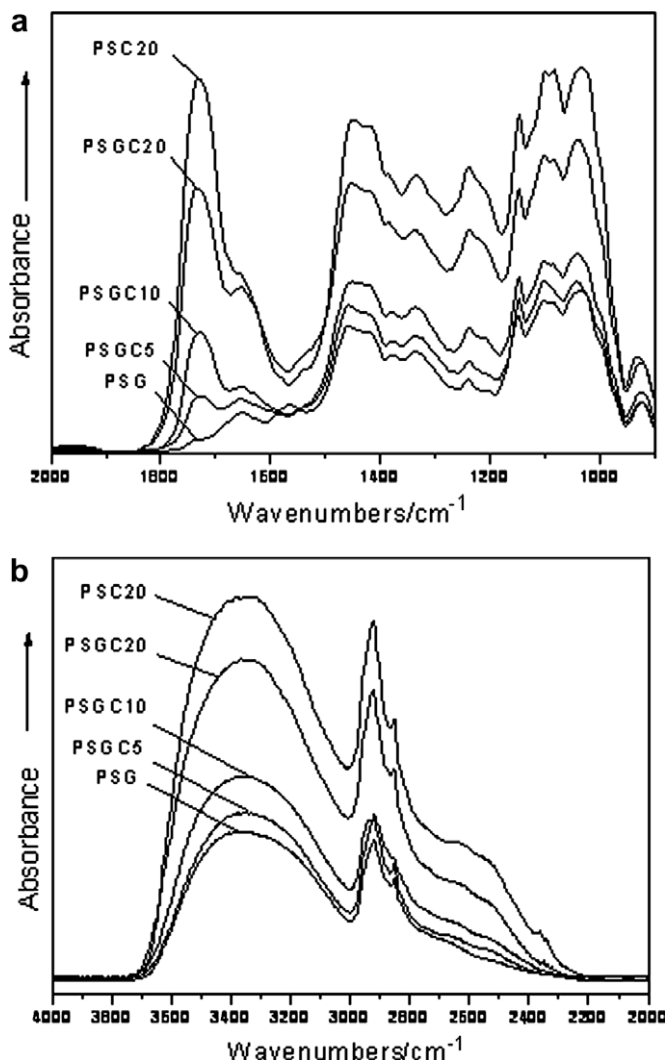


Fig. 2. The effects of CA concentrations on the peak's height at  $1729\text{ cm}^{-1}$  (a) and  $3340\text{ cm}^{-1}$  (b).

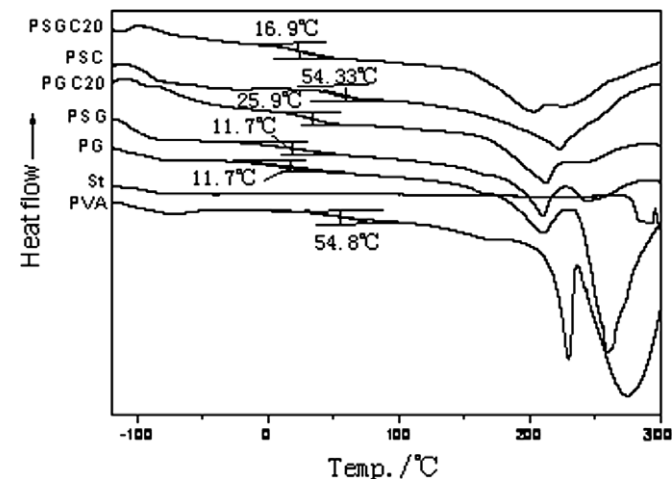


Fig. 3. The effects of glycerol and CA on DSC curve of PVA and PVA/starch blends.



nomenon provided another evidence for the cross-linking occurred in the presence of CA. The cross-linking will limit the movement of the molecular segments, which resulted in the increase of  $T_g$ . The DSC curve of PG was compared with that of the PSG and it was observed that the addition of starch did not affect the  $T_g$ , and this is because the  $T_g$  of pure corn starch always cannot be seen always on the DSC curve. The absence of a clear  $T_g$  for starch may be due to the following factors: amorphous chains surrounded by crystalline domains, the presence of moisture, physical crosslinks inhibiting the movements of the amorphous chain segments, and the presence of inter-crystalline phases not showing normal thermal behavior (Sreedhar et al., 2005).

We also investigated the influences of glycerol and CA on the DSC curves of PVA/starch mixture. Compared to the DSC curves of pure starch, the curve of PSG was similar to that of the PVA. The differences are that the plasticizing effect of glycerol enhanced the segment mobility, so that the  $T_g$  decreased. Furthermore, the decrease of the molecular interactions resulted in the decrease of the  $T_m$ , as well as the  $\Delta H$  and crystallinity. Compared with the PSG, the  $T_g$  of PSC20 was higher when the same percentage of CA or glycerol was added, and this may be caused by two reasons: one is that the partial cross-linking limited the molecular segment mobility and another is that the strong hydrogen bonding between the CA/starch or CA/PVA resisted the movement of the molecules. Compared to the PSC20, the  $T_g$  and  $T_m$  of PSGC20 decreased, and this was because the molecular interactions among the macromolecules decreased in the presence of glycerol.

The effects of CA concentration on DSC curves of PSG films are shown in Fig. 4. Generally, with increasing amounts of CA,  $T_g$  or  $T_m$  did not shift much, to either lower or higher temperatures. However, the melting enthalpy ( $\Delta H$ ) decreased as the CA increased, and it could be attributed to the reduction in the intermolecular forces in the presence of CA-cross linked glycerol networks.

### 3.2.2. Thermogravimetric (TG) analysis

Fig. 5 shows the effect of the additive type and concentration of glycerol and CA on TG curves of PVA. Pure PVA curve showed a two-step decomposition pattern. The first step began around 200 °C, and the second one began around 382 °C. The final temperature of the decomposition was around 460 °C. The first step of weight loss could be attributed to the loss of loosely bound water, accompanied by the formation of volatile disintegrated products. The second step was mainly caused by the heat decomposition of

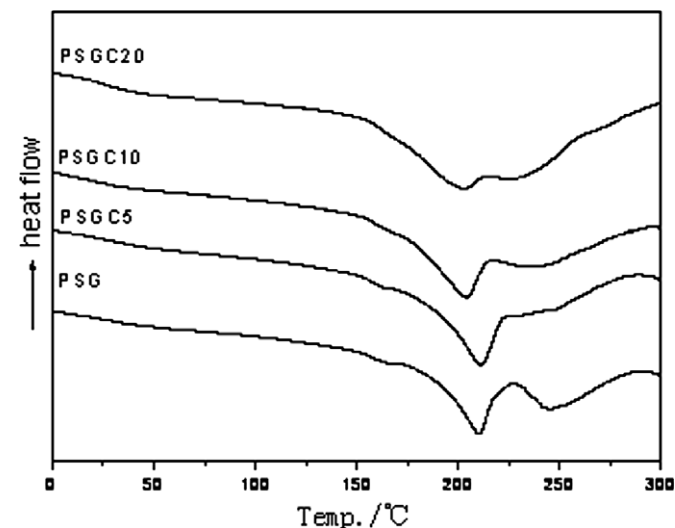


Fig. 4. The effects of CA concentration on DSC curves of PSG films.

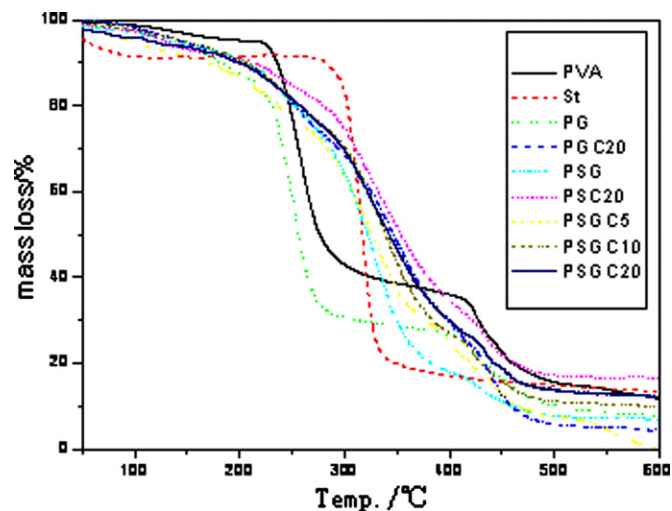


Fig. 5. The effect of additive type and concentration on TG curves.

the molecules, and the products were composed of small molecular carbon and hydrocarbon. The shape of the TG curve of PG did not change when glycerol was added. However, after CA was added, the shape of the TG curve of PGC20 changed as compared to that of the PSG. The CA cross-linked SP film showed a three-step decomposition pattern as shown in Fig. 3. The first step was attributed to the starch and the elimination of water from PVA. The second step was same as that of PVA. And the third step was attributed to the crosslinked component. This result is similar to that of the boric acid crosslinked SP composites (Zou et al., 2007).

The addition of CA obviously reduced the weight loss rate and increased the residual weight percentage at 600 °C. The cross-linking and strong hydrogen bonds as the function of CA enhanced the bonding among the molecules, so that the thermal stability of the PVA increased.

It can be seen that the TG curve of the corn starch exhibited a one-step decomposition pattern with the onset temperature ( $T_{ON}$ ) at 274 °C and the end-set temperature ( $T_{EN}$ ) at 334 °C. Pure PVA exhibited a two-step decomposition pattern as mentioned above. The TG curve of PSG showed a three-step decomposition patterns: 218–240 °C, 298–357 °C, 415–456 °C, which is similar to the classical TG curve of the PVA/starch blend. When compared to the PVA or starch, the residual weight percentage of PSG at 600 °C decreased. This could be attributed to the volatilization of the glycerol.

Fig. 5 also shows that the TG curves of the PSC films treated with CA (post-treated and without the glycerol) showed a two-step decomposition pattern. The weight residue for PSC20 at 600 °C was higher than that of PSG, and this demonstrated that the thermal stability of the CA added films was higher than those without CA. Partial cross-linking was one of the reasons, which improved the thermal stability. Moreover, compared to glycerol, stronger hydrogen bonds formed between CA and PVA/starch may be another reason to which improved the initial decomposition temperature.

It can also be seen that the weight loss rate and residual weight percentage at 600 °C of PSGC20 were higher than those of PSG and lower than those of PSC20. This maybe because to the cross-linking was prevented in the presence of glycerol. Compared with the PSC20, a part of CA reacted with the addition of glycerol, so the cross-linking degree decreased and the thermal stability of the PSGC20 also decreased.

The thermal stability of the PSGC films increased with the increase in CA concentration, as observed from a comparison of the residual mass percentage at 600 °C and the weight loss rate. This

phenomenon can be attributed to the cross-linking degree and the hydrogen bonds increased as the CA concentration increased.

It seems like the addition of the CA enhanced the incarbonization. When the CA concentration increased, then more and more CA molecules were chemically bonded with the starch chains, so that the initial carbon content increased as the esterification occurred. Therefore, the residual weight increased as the CA concentration increased.

### 3.3. The effects of citric acid concentrations on the swelling degree

The water absorption capacity and the degradability are the most important properties for biodegradable materials. It was reported that the water absorption acquired equilibrium in 12 h (Zou et al., 2007), so we selected 24 h, which is longer than equilibrium time as the observing time. CA is an hydroxyl carboxylic acid. There are three carboxylic groups and one hydroxyl per monomer. It was believed that CA could crosslink starch and PVA, and decrease the water absorption of SP composites. So CA acted not only as a plasticizer, but also as a cross linker and an esterification agent. It has been found that the swelling degree is related to the esterification degree and crosslink degree. Generally, the swelling degree decreased as the esterification and crosslink degree increased (Borredon et al., 1997; Zou et al., 2007).

Table 2 shows the swelling degree of the samples which have been dipped in the de-ionized water for 24 h. After 24 h soaking, the swelling degree of PSGC5 was similar to that of the PSG. The small amount of CA did not make significant effect on the swelling degree. However, the swelling degree decreased as the CA concentration increased. This phenomenon reflected indirectly, that crosslink degree and the esterification degree increased as the CA concentration increased. The swelling degree results are well consistent with that of the previous result reported by Zou and his co-workers. They also found that the swelling degree decreased as the crosslink agent (CA and borax) increased (Zou et al., 2007).

### 3.4. The effect of citric acid on tensile properties

The mechanical properties are not only related with the cross linker but are also related with the plasticizer. The cross linker and the plasticizer always have the contrary effects on the tensile properties. Generally, the tensile strength increased and the elongation at break decreased as the percentage of cross linker increased. The results are opposite when the plasticizers increased (Sreedhar et al., 2005). The CA acted both as the cross linker and the plasticizer in the SP composites. So that different functions of CA would be exhibited when different amounts of CA were added.

The tensile strength and elongation at break as the function of CA concentration are shown in Table 3. The tensile strength of PSGC5 was higher than that of the PSG without CA. This could be attributed to the cross-linking caused by the small quantity of

**Table 2**  
The effect of CA concentration on the swelling degree at 24 h

Samples	PSG	PSGC5	PSGC10	PSGC20	PSGC20
SD (%)	31.2 ± 1.7	32.6 ± 2.0	30.5 ± 1.1	22 ± 1.1	19.6 ± 1.3

**Table 3**  
The effect of CA percentage on tensile strength and elongation at break

	PSG	PSGC5	PSGC10	PSGC20	PSGC20
Tensile strength (MPa)	39	48	46	44	42
Elongation at break (%)	81.2	101.8	126.7	178.9	207.8

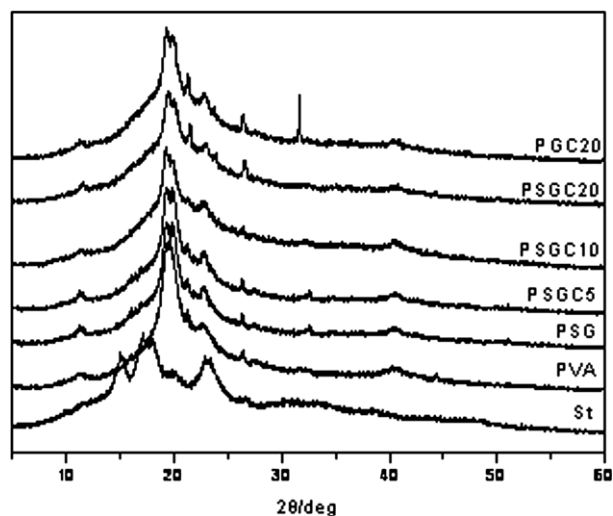
CA. As the CA percentage was increased, the residual CA in the blends played a role as the plasticizer, which reduced the interactions among the macromolecules, which resulted in the decrease of the tensile strength and increase of the elongation at break. We also noticed that with the increase of the CA concentration (from 5% to 30%), the tensile strength did not reduce significantly (from 48 to 42 Mpa), however, the elongation at break increased noticeably (from 101.8% to 207.8%). It is a good method to improve the flexibility without sacrificing the strength so much. The ordinary cross linkers (ex. borax) (Sreedhar et al., 2005) and plasticizers (polyols) (Arvanitoyannis et al., 1997) do not have the diplex function.

### 3.5. The effect of CA on crystal properties

XRD curves showed that PVA has a high percentage of crystallinity ( $X_c \sim 54\%$ , see Fig. 6). XRD patterns of raw materials and hydrogen membranes are shown in Fig. 6. It is observed that corn starch has peaks at 15, 17, 18, 23 and 26.5  $2\theta$ , where the peak at 18° was most intense (Shi et al., 2007b), while PVA showed an obvious diffraction peak at 19.3 (García-Cerda, Escarenó-Castro, & Salazar-Zertuche, 2007). In the blend of SPG films, most of the diffraction peaks are similar to those of the PVA compared to the corn starch diffraction peaks. This is due to the destruction of the crystalline structure of starch granules as a result of gelatinization during the blending process with PVA above 100 °C. No obvious retrogradation of starch was observed from the XRD patterns, indicating that the diffraction pattern of PVA dominated in the blends and that the crystalline structure of starch has been strongly inhibited by PVA and glycerol molecules.

In the XRD pattern, the intensity of the peak at 19.3° decreased due to the addition of glycerol and CA. The peak at 19.3° is caused by the crystallinity of the PVA. From Fig. 6, we can see that the addition of CA greatly decreased the crystal peak around 19.3°. Moreover, the peak's height at 19.3° decreased as CA concentration increased. The plasticizing and cross-linking effect of CA will reduce the crystallinity of the PVA.

A new peak around 31.5° appeared on the curve of PSC. This peak was caused by the residual CA in the blends. However, the peak did not appear on the DSC curve of PSGC20. The reason maybe that glycerol resisted the CA assemble, and the CA attributed in the blends in the molecular lever.



**Fig. 6.** The effect of the additive types and CA percentage on XRD curves.

### 3.6. The effect of CA on the cytotoxicity test

Apart from favorable physico-chemical and mechanical properties, the most important requirement for a biodegradable polymer to be used in medical applications is its biocompatibility in a specific environment. Cytotoxicity testing represents the initial step in testing biocompatibility of potential biomaterials and medical devices.

The OD values of control, PSG and PSGC with different CA concentrations are shown in Fig. 7(a). The OD value reflected the number of the live cells directly. The cell Relative Growth Rates (RGR) of these samples was shown in Fig. 7(b). The RGR was calculated according to the Eq. (2). The RGR was classified to six grades as shown in Table 4. Grades 0 and 1 were accepted as qualified. Grade 2 should be considered by combining with the cell's morphology. Greater than grade 3 was considered as unqualified.

$$\text{RGR} (\%) = \frac{\text{Absorbance of the material} - \text{Absorbance of the blank}}{\text{Absorbance of the negative} - \text{Absorbance of the blank}} \quad (2)$$

It can be seen that the RGR of all the SP films with or without CA was greater than 80%. The cytotoxicity test results of all the SP films reached grade 1 according to the GB/T16175-1996 standard which are listed in Table 4. The result of the cytotoxicity test demonstrated that when the CA concentration was less than 20%, there was no significant toxicity effect on the cell's proliferation.

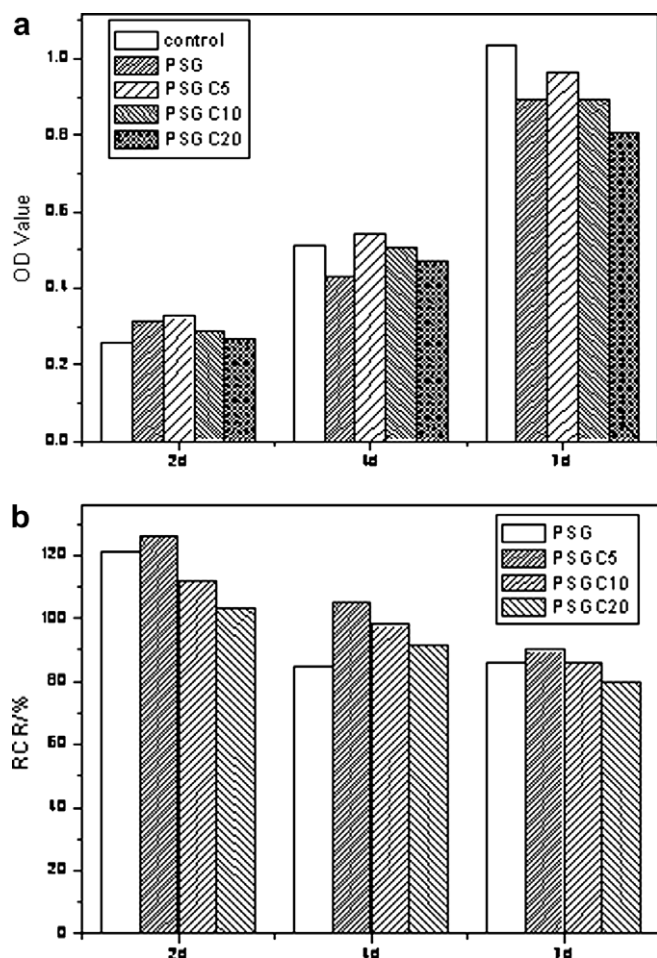


Fig. 7. The optical density (OD) value (a) and relative growth rate (RGR/%) (b) of PSGC with different CA concentrations.

Table 4

The standard of the response grades

Grades	RGR (%)
0	≥ 100
1	75–99
2	50–74
3	25–49
4	1–24
5	0

It seems that the OD value and RGR of the PSG without CA did not exhibit better cell compatibility than that of the PSGCs. This result indicated that the CA in blend did not have any significant cell toxicity. However, the OD value and RGR decreased as the CA concentration increased. This illustrated that the CA still has some negative effects on the cell growth. The result also showed that when the CA percentage was less than 20%, the cells still exhibited good growing conditions.

### 4. Conclusions

The PS films were prepared under 140 °C in the presence of glycerol and CA. The esterification occurred between CA/starch and CA/PVA, and it occurred more easily between the CA and starch than between CA and PVA. The esterification and multi-carboxyl structure of the CA resulted in the cross-linking. The structural changes gave rise to a series of property changes, including thermal properties, swelling degrees, mechanical properties, and crystal properties. The thermal stability improved as a function of cross-linking and it improved molecular interactions. The swelling degree decreased from 33% to 20% as the CA percentage increased from 5 to 30 wt%. When 5 wt% CA was added, the tensile strength of the sample increased from 39 to 48 MPa, but when even more CA was added (from 5 to 30 wt%), the tensile strength decreased from 48 to 42 MPa and the elongation at break increased from 102% to 208%. This was caused by the plasticizing effect of the residual-free CA in the blend. The cytotoxicity result showed that the cell Relative Growth Rates (RGR) of all samples with different CA concentration exceeded 80% after 7 days of incubation. No significant negative effects of CA were observed on the cell's proliferation when the CA content was less than 20 wt%. The in vitro degradation behavior of the PSC films is under investigation.

### Acknowledgement

This work was supported by key project of Beijing Natural Science Foundation Major Project 2061002.

### References

- Arvanitoyannis, I., Kolokuris, I., Nakayama, A., Yamamoto, N., & Aiba, S. (1997). Physico-chemical studies of chitosan–poly (vinyl alcohol) blends plasticized with sorbitol and sucrose. *Carbohydrate Polymers*, 34, 9–19.
- Albertson, A. C. (1980). Microbial and oxidative effect in degradation of polyethylene. *Journal of Polymer Science*, 25, 1665–1671.
- Albertson, A. C., Barenstedt, C., & Karlsson, S. (1994). Degradation of enhanced environmental degradable polyethylene in biological aqueous media mechanism during first stages. *Journal of Polymer Science*, 51, 1097–1105.
- Arvanitoyannis, I. S. (1999). Totally and partially biodegradable blend based on natural and synthetic macromolecules: Preparation, physical properties, and potential as food packaging material. *Journal of Macromolecular Science. Part C: Reviews in Macromolecular Chemistry and Physics*, C39, 205–271.
- Autori Ciardelli, G., Silvestri, D., Barbani, N., & Ionita, M. (2006). Bioartificial polymer membranes as innovative systems for biomedical or biotechnological uses. *Desalination*, 200, 493–495.
- Beliakova, M. K., Aly, A. A., & Abdel-Mohdy, F. A. (2004). Grafting of poly(methacrylic acid) on starch and poly(vinyl alcohol). *Starch/Stärke*, 56(9), 407–412.

- Borredon, E., Bikiaris, D., Prinos, J., & Panayiotou, C. (1997). Properties of fatty-acid esters of starch and their blends with LDPE. *Journal of Applied Polymer Science*, 65, 705–721.
- Cascone, M. G., Barbani, N., Cristallini, G. C., Ciardelli, G., & Lazzeri, L. (2001). Bioartificial polymeric materials based on polysaccharides. *Journal of Biomaterials Science Polymer Edition*, 12, 267–281.
- Chiellini, E., Cinelli, P., Chiellini, F., & Imam, S. H. (2004). Environmentally degradable bio-based polymeric blends and composites. *Macromolecular Bioscience*, 4, 218–231.
- Cinelli, P., Chiellini, E., Gordon, L. S. H., & Chiellini, L. E. (2005). Characterization of biodegradable composite films prepared from blends of poly(vinyl alcohol), cornstarch, and lignocellulosic fiber. *Journal of Polymer Environment*, 13, 47–59.
- Cinelli, P., Chiellini, L. E. J., Lawton, N. W., Imam, M. G. (2006). Foamed articles based on potato starch, corn fibers and poly(vinyl alcohol). *Polymer Degradation and Stability*, 91, 1147–1155.
- Dermigöz, P. D., Elvira, C., Mano, E. J. F., Cunha, S. H. A. M., Piskin, E., & Reis, R. L. (2000). Chemical modification of starch based biodegradable polymeric blends: Effects on water uptake, degradation behaviour and mechanical properties. *Polymer Degradation and Stability*, 70, 161.
- Follain, N., Joly, C., Dole, W. P., & Bliard, S. H. C. (2005). Properties of starch based blends. Part 2: Influence of poly(vinyl alcohol) addition and photocrosslinking on starch based materials mechanical properties. *Carbohydrate Polymers*, 60, 185–192.
- Giusti, D. P., Lazzeri, C. L., Barbani, J. F. N., Lelli, A. M. L., De-Petris, E. S., & Cascone, R. L. M. G. (1994). Collagen based new bioartificial polymeric materials. *Biomaterials*, 15, 1229–1233.
- Giusti, N. P., Lazzeri, C. L., & Lelli, P. L. (1993). Bioartificial polymeric materials: A new method to design biomaterials by using both biological and synthetic polymers. *Trends in Polymer Science*, 1, 261.
- Graaf, R. A., & Janssen, L. P. B. M. (2000). The production of a new partially biodegradable starches plastic by reactive extrusion. *Polymer Engineering and Science*, 34, 2–10.
- Graaf, R. A., & Janssen, L. P. B. M. (2001). Properties and manufacturing of new starch plastic. *Polymer Engineering and Science*, 41, 3–12.
- García-Cerda, L. A., Escarenó-Castro, M. U., & Salazar-Zertuche, M. (2007). Preparation and characterization of poly(vinyl alcohol)-cobalt ferrite nanocomposites. *Journal of Non-Crystalline Solids*, 353, 808–810.
- Hisamitsu, I., Kataoka, K., Okano, T., & Sakurai, Y. (1997). Glucose responsive gel from phenylborate polymer and poly(vinyl alcohol): Prompt response at physiological pH through the interaction of borate with amino group in the gel. *Pharmaceutical Research*, 14, 289.
- Jayasekara, R., Harding, I., Bowater, I., Christie, G. B. Y., & Lonergan, G. T. (2003). Biodegradation by composting of surface modified starch and PVA blended films. *Journal of Polymers and the Environment*, 11, 49–56.
- Jayasekara, R., Harding, I., Bowater, I., Christie, G. B. Y., & Lonergan, G. T. (2004). Preparation, surface modification and characterisation of solution cast starch PVA blended films. *Polymer Testing*, 23, 17–27.
- Jiang, H. J., Campbell, G., Boughner, D., Wan, W. K., & Quantz, M. (2004). Design and manufacture of a poly(vinyl alcohol) (PVA) cryogel tri-leaflet heart valve prosthesis. *Medical Engineering and Physics*, 26, 269–277.
- Kaplan, D. L. (1998). *Biopolymers from renewable resources*. Berlin: Springer. (pp. 30–43).
- Kiatkamjornwong, S., Thakeow, P., & Sonsuk, M. (2001). Chemical modification of cassava starch for degradable polyethylene sheets. *Polymer Degradation and Stability*, 73, 363–375.
- Kim, M., & Lee, S.-J. (2002). Characteristics of crosslinked potato starch and starch-filled linear low-density polyethylene films. *Carbohydrate Polymers*, 50, 331–337.
- Kim, M. (2003). Evaluation of degradability of hydroxypropylated potato starch/polyethylene blend films. *Carbohydrate Polymers*, 54, 173–181.
- Kitano, S., Koyama, Y., Kataoka, K., Okano, T., & Sakurai, Y. (1992). A novel drug delivery system utilizing a glucose responsive polymer complex between poly(vinyl alcohol) and poly(N-vinyl-2-pyrrolidone) with a phenylboronic acid moiety. *Journal of Controlled Release*, 19, 162.
- Krumova, M., López, D., Benavente, R., Mijangos, C., & Pereña, J. M. (2000). Effect of crosslinking on the mechanical and thermal properties of poly(vinyl alcohol). *Polymer*, 41, 9265.
- Lamba, N. M. K., Baumgartner, J. A., & Cooper, S. L. (1998). *Frontiers in tissue engineering*. New York: Elsevier. (pp. 121–137).
- Nabar, Y. U., Draybuck, D., & Narayan, R. (2006). Physicomechanical and hydrophobic properties of starch foams extruded with different biodegradable polymers. *Journal of Applied Polymer Science*, 102, 58–68.
- Nakayama, Y., Takatsuka, M., & Matsuda, T. (1999). Surface hydrogelation using photolysis of dithiocarbamate or xanthate: Hydrogelation, surface fixation, and bioactive substance immobilization. *Langmuir*, 15, 1667–1672.
- Pal, K., Banthia, A. K., & Majumdar, D. K. (2006). Starch based hydrogel with potential biomedical application as artificial skin. *African Journal of Biomedical Research*, 9, 23–29.
- Raj, B. S., Siddaramaiah & Somashekar, R. (2004). Structure–property relation in poly(vinyl alcohol)/starch composites. *Journal of Applied Polymer Science*, 91, 630–635.
- Ramaraj, B. (2007). Crosslinked poly(vinyl alcohol) and starch composite films. II. Physicomechanical, thermal properties and swelling studies. *Journal of Applied Polymer Science*, 103, 909–916.
- Shi, R., Zhang, Z. Z., Liu, Q. Y., Han, Y. M., Zhang, L. Q., & Chen, D. F., et al. (2007a). Characterization of citric acid/glycerol co-plasticized thermoplastic starch prepared by melt blending. *Carbohydrate Polymers*, 69, 748–755.
- Shi, R., Liu, Q. Y., Ding, T., Han, Y. M., Zhang, L. Q., & Chen, D. F., et al. (2007b). Ageing of soft thermoplastic starch with high glycerol content. *Journal of Applied Polymer Science*, 103, 574–586.
- Shiino, D., Murata, Y., Kataoka, K., Koyama, Y., Yokoyama, M., & Okano, T., et al. (1994). Preparation and characterization of a glucose-responsive insulin releasing polymer device. *Biomaterials*, 15, 121.
- Siddaramaiah, S., Raj, B., & Somashekar, R. (2004). Structure–property relation in poly(vinyl alcohol)/starch composites. *Journal of Applied Polymer Science*, 91, 630–635.
- Sreedhar, B., Sairam, M., Chattopadhyay, D. K., Syamala Rathnam, P. A., & Mohan Rao, D. V. (2005). Thermal, mechanical, and surface characterization of starch-poly(vinyl alcohol) blends and borax-crosslinked films. *Journal of Applied Polymer Science*, 96, 1313–1322.
- Sreedhar, B., Chattopadhyay, D. K., Karunakar, M. S. H., & Sastry, A. R. K. (2006). Thermal and surface characterization of plasticized starch poly(vinyl alcohol) blends crosslinked with epichlorohydrin. *Journal of Applied Polymer Science*, 101, 25–34.
- Thakore, I. M., Desai, S., Sarawade, B. D., & Devi, S. (2001). Studies on biodegradability, morphology and thermomechanical properties of LDPE/modified starch blends. *European Polymer Journal*, 37, 151–160.
- Wan, W. K., Campbell, G., Zhang, Z. F., Hui, A. J., & Boughner, D. R. (2002). Optimizing the tensile properties of poly(vinyl alcohol) hydrogel for the of a bioprosthetic heart valve stent. *Journal of Biomedical Material Research*, 63, 854.
- Xu, Y. X., Miladinov, V., & Hanna, M. A. (2004). Synthesis and characterization of starch acetates with high degree of substitution. *Cereal Chemistry*, 81, 735–740.
- Yang, J., Webb, A., & Ameer, G. (2004). Novel citric acid-based biodegradable elastomers for tissue engineering. *Advanced Materials*, 16, 511–516.
- Yin, Y., Li, J., Liu, Y., & Li, Z. (2005). Starch crosslinked with poly(vinyl alcohol) by boric acid. *Journal of Applied Polymer Science*, 96, 1394–1397.
- Yoon, S. D., Chough, S. H., & Park, H. R. (2006a). Properties of starch-based blend films using citric acid as additive. *Journal of Applied Polymer Science*, 100, 2554–2560.
- Yoon, S. D., Chough, S. H., & Park, H. R. (2006b). Effects of additives with different functional groups on the physical properties of starch/PVA blend film. *Journal of Applied Polymer Science*, 100, 3733–3740.
- Yoon, S. D., Chough, S. H., & Park, H. R. (2007). Preparation of resistant starch/poly(vinyl alcohol) blend films with added plasticizer and crosslinking agents. *Journal of Applied Polymer Science*, 106, 2485–2493.
- Yu, J. G., Wang, N., & Ma, X. F. (2005). The effects of citric acid on the properties of thermoplastic starch plasticized by glycerol. *Starch/Stärke*, 57, 494–504.
- Zhai, M., Yoshii, F., & Kume, T. (2003). Radiation modification of starch-based plastic sheets. *Carbohydrate Polymers*, 52, 311–317.
- Zhao, G. H., Liu, Y., Fang, C. L., Zhang, M., Zhou, C. Q., & Chen, Z. D. (2006). Water resistance, mechanical properties and biodegradability of methylated-cornstarch/poly(vinyl alcohol) blend film. *Polymer Degradation and Stability*, 91, 703–711.
- Zou, G. X., Qu, J. P., & Zou, X. L. (2007). Optimization of water absorption of starch/PVA composites. *Polymer Composites*, 28, 674–679.