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'Green' crosslinking of native starches with malonic acid and their properties

Trina Ghosh Dastidar[a], Anil N. Netravali[b,*]

[a] Department of Materials Science and Engineering, Cornell University, Ithaca, NY 14853-4401, USA
[b] Department of Fiber Science & Apparel Design, Cornell University, Ithaca, NY 14853-4401, USA

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ABSTRACT

Starch is a highly hydrophilic biomaterial with weak mechanical properties rendering it useless for commercial applications. A fully 'green' water based process is presented to crosslink corn (cereal) and potato (tuber) starch to enhance mechanical properties as well as lower hydrophilicity. In addition, malonic acid, a green, plant based water soluble and relatively inexpensive polycarboxylic acid, was used as the crosslinker. The reactivity of potato starch toward esterification and crosslinking was found to be higher than that of corn starch owing to the inherent differences in the granule morphology and internal structure of the two starches. It was observed that potato starch granules had a higher degree of substitution (DS) of 0.19 than corn starch granules (DS = 0.1) under similar reaction conditions. Chemical, thermal and mechanical test results confirmed the crosslinking as well as reduced moisture sensitivity.

1. Introduction

Starches, proteins and cellulose together form an important and a large part of the available biomass and have received considerable attention because of their low cost, biodegradability, annually renewal and abundant supply throughout the world (Arvanitoyannis, Kalichevsky, Blanshard, & Psomiadou, 1994; vanSoest & Essers, 1997). These biomaterials can be used as suitable substitutes for petroleum based products, particularly if their properties can be matched with those of petroleum based polymers. Starch is composed of two polymers of D-glucopyranose; amylose and amylopectin. Amylose is formed by glucose units joined by 1,4-glycosidic linkages and amylopectin is formed by glucose units joined by 1,4- as well as 1,6-glycosidic linkages. While amylose is a low molecular weight polymer consisting of 1000–10,000 glucose units and is linear, amylopectin is a larger branched macromolecule with degree of polymerization (DP) sometimes exceeding one million (Du, Jia, Xu, & Zhou, 2007). Starch based materials and composites have been used in highly sophisticated applications including biomaterials for tissue engineering (Lan et al., 2010; Pashkuleva, Azevedo, & Reis, 2008; Pashkuleva, Marques, Vaz, & Reis, 2005; Reddy & Yang, 2009; Santos et al., 2009).

Native starches have been crosslinked using polycarboxylic acids such as citric acid, polyphosphates such as sodium trimetaphosphate, sodium tripolyphosphate as well as epichlorohydrin, phosphorus oxychloride and 1,2,3,4-diepoxybutane (Ayoub & Rizvi, 2009; Chen & Wang, 2006; Jyothi, Moorthy, & Rajasekharan, 2006; Mao, Wang, Meng, Zhang, & Zheng, 2006; Passauer, Liebner, & Fischer, 2009; Reddy & Yang, 2010). Carboxymethylated starch with a DS of 0.45 has been further crosslinked using malic, tartaric, citric, malonic, succinic, glutaric and adipic acid to synthesize crosslinked hydrogels (Seidel et al., 2001). Crosslinking is a thermosetting modification that interconnects the starch molecules by covalent bonding, thus it not only increases the molecular weight but also enhances the mechanical properties. Water stability of starches is improved by crosslinking while at the same time the swelling is reduced (Reddy & Yang, 2010). Further, crosslinked films also show higher thermal stability and resistance to degradation than non-crosslinked films (Reddy & Yang, 2010).

The present paper describes esterification and crosslinking of native corn starch (CS) and potato starch (PS) using malonic acid (MA) and the effects of chemical modification on their thermal, mechanical properties and swelling abilities under aqueous conditions. Corn, a cereal starch containing 27% amylose, and potato, a tuber starch containing 24% amylose, show many differences in functional properties under similar processing conditions owing to their different origins (Mishra & Rai, 2006). The degree of amylose content influences the reactivity of corn and potato starches. Among other constituents lipid contents of the two starches are different; 0.32% in PS and 1.22% in CS (Mishra & Rai, 2006). Lipids form complex with the amylose in CS which leads to more rigid structure and high turbidity. PS also has higher content of phosphate ester groups bound to the native starch, which has been claimed

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* Corresponding author. Tel.: +1 607 255 1875; fax: +1 607 255 1093.
E-mail address: ann2@cornell.edu (A.N. Netravali).

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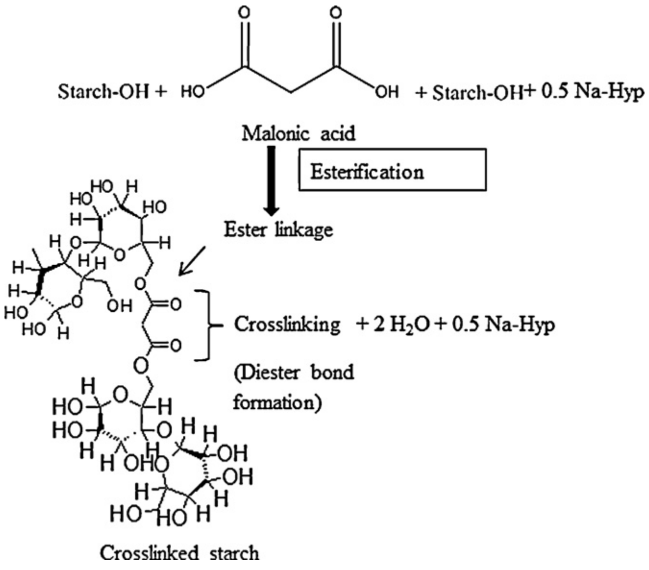

Fig. 1. Proposed scheme for crosslinking starch with MA.

to cause lower pasting temperature, higher viscosity and improved clarity (Mishra & Rai, 2006). PS granules are also fragile in nature and have a different crystal structure (B-type) as compared to CS (A-type crystal structure) (Mishra & Rai, 2006).

MA is a plant derived green dicarboxylic acid with a molecular weight of 104 g/mol that can react with the hydroxyl groups on the D-glucopyranose ring present in starch by forming ester bonds. MA is soluble in water at room temperature and was chosen as a crosslinker for starch since it is non-toxic and relatively inexpensive. In general, esterification reactions are carried out in organic solvents, require the use of toxic reagents and repeated washing of the end product by water and alcohol to remove excess organic solvents. An example is the acetylation of starch carried out by Garg and Jana (2011) in the presence of acetic anhydride and pyridine. The esterification of starch with MA, shown in Fig. 1, carried out in aqueous and non-toxic conditions, using simple lab based techniques, is a benign, economical and facile way of crosslinked starch–ester synthesis.

2. Experimental procedures

2.1. Materials

Native CS (73% amylopectin and 27% amylose) and PS (76% amylopectin and 24% amylose) powders were obtained from Sigma–Aldrich (St. Louis, MO). Analytical grade MA and sodium hypophosphite monohydrate (Nahyp) were also purchased from Sigma–Aldrich.

2.2. Preparation of precured and cured starch films

To process CS and PS into a crosslinked resin, starch was at first gelatinized by adding 20 g starch to 500 ml water and heating at 90 °C for 45 min with constant stirring. Theoretical calculations based on stoichiometry showed that ≈31.5% MA is required for complete crosslinking of starch with MA. For the precuring of starch resins 3, 5, 7.5 and 10 g MA (or 15%, 25%, 37.5% and 50% MA based on the initial dry weight of starch) were added, separately, to the gelatinized starch followed by addition of sodium hypophosphite (50% by weight of MA) as catalyst. Nahyp is proven to accelerate the process of esterification with polycarboxylic acids by increasing the speed of the formation of the cyclic anhydride intermediate (Sauperl & Stana-Kleinschek, 2010).

The mixture was stirred continuously for 60 min at 90 °C with a magnetic stirrer. After cooling the precured starch was cast to form thin films of approximately 0.3 mm thickness on a Teflon® coated glass plate and dried in an oven for 48 h at 40 °C. The dried films were peeled off from the Teflon® coated plates and stored in sealed polythene packets. The precured PS and CS films were further heated in Carver Hydraulic hot press (model 3891-4PROA00) for complete crosslinking (curing) at 120 °C under 0.1 MPa pressure for 20 min. Precured PS films (precured with 37.5% MA at 90 °C for 60 min) were also cured at 100 °C and 140 °C at 0.1 MPa pressure for 20 min to understand the effect of temperature on crosslinking. The cured CS and PS films were then thoroughly washed in water to remove excess MA and Nahyp. The starch films were completely soaked in DI water for 8–10 h, the water was changed a couple of times, and it was expected that the unreacted MA and Nahyp molecules which were highly soluble in water leached out into the water (also confirmed by FTIR of the washed water containing traces of MA). The washed films were either air-dried in an oven at 70 °C for 2 days or conditioned at 21 °C and 65% relative humidity for 4.5 days for further characterization.

2.3. Determination of degree of substitution (DS)

The DS of precured PS and CS films (as a function of concentration of MA) and cured PS films (as a function of temperature) were calculated using the titration method (Mathew & Abraham, 2008). Precured or cured starch specimens, 0.5 g, were accurately weighed into a 100 ml vial and 50 ml deionized (DI) water was added. The sealed vial with water and starch was agitated in a shaker bath at 200 rpm for 4.5 days. The excess unreacted MA that leached out into the water was carefully neutralized with standard sodium hydroxide solution using phenolphthalein indicator. Excess standard NaOH (1 N, 10 ml) was added and shaken on a shaker bath for 1 h at 200 rpm to achieve homogeneous mixing. The entire set up was stored at 50 °C for 3 days with occasional shaking for complete hydrolysis. At the end of 3 days the excess alkali was back-titrated with standard HCl (0.4 M) solution. A blank (control) was simultaneously titrated with native starch instead of precured or cured starch. Degree of substitution was calculated using following formulae (Mathew & Abraham, 2007):

$$\%\,\text{malonate} = \frac{([\text{ml}\,(\text{blank}) - \text{ml}\,(\text{sample})]) \times \text{normality of acid} \times 0.104 \times 100}{\text{sample weight in grams}\,(\text{dry basis})}$$

$$\text{degree of substitution}\,(\text{DS}) = \frac{162 \times \%\,\text{malonate}}{104 \times 1000 - (103 \times \%\,\text{malonate})}$$

2.4. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy

ATR-FTIR spectra were collected using a Nicolet Magna 560 FTIR spectrometer with a split pea accessory for ATR. Each scan was an average of 150 scans recorded from 4000 cm^{-1} to 550 cm^{-1} wavenumbers obtained at a resolution of 4 cm^{-1}. The spectra of pure starches, pure MA and precured and cured starches were obtained and compared.

In order to construct a calibration curve from the ATR-FTIR spectra, the absorption peak at 1725 cm^{-1} due to the ester carbonyl (C=O) stretching vibrations was chosen as the analyte peak. It monitored the concentration of ester, which is related to the concentration of MA in the specimen. Since the absorption due to aliphatic –C–H stretching vibration, at 2929 cm^{-1}, remains unchanged after the crosslinking reactions, it was chosen as the reference peak for the internal standard. The ratio of absorbance by the analyte peak and the reference peak was plotted against concentration of MA. This method was similar to the calibration method used by Coma, Sebti, Pardon, Pichavant, and Deschamps

(2003), to assess the degree of crosslinking of cellulose with citric acid.

2.5. Thermogravimetric analysis (TGA)

Native and crosslinked starch specimens (both PS and CS) were scanned from 25 °C to 600 °C using a thermogravimetric analyzer (TGA-2050, TA Instruments, Inc., New Castle, DE) at a rate of 10 °C/min in nitrogen atmosphere to characterize their thermal stability and degradation behavior. Malonic acid was scanned from 25 °C to 400 °C at 10 °C/min.

2.6. Differential scanning calorimetry (DSC)

In order to test the specimen in DSC the precured and cured specimen were conditioned for 3 days at 21 °C and 65% relative humidity. Specimens weighing about 12 mg (MA, native PS as well as precured and cured PS) were accurately weighed and scanned on the DSC (model-2920, TA Instruments, Inc., New Castle, DE) from 25 °C to 400 °C at a ramp rate of 25 °C/min to obtain thermograms (Reddy & Yang, 2010). The DSC was calibrated using ASTM 968-99 and E967-97 procedures. The melting, decomposition and crystallization temperatures were obtained using ASTM 794-98 procedure.

2.7. Wide angle X-ray diffraction (WXRD) study

Scintag θ–θ powder wide angle diffractometer (PADX, Scintag, Inc., Cupertino, CA) with a solid-state intrinsic germanium detector was used at 40 kV and 40 mA to study the X-ray diffraction (WXRD) patterns in MA, native and crosslinked (cured) PS and CS specimens. The specimens were scanned from 5 °C to 40 °C at the speed of 1 °C/min using the Cu Kα X-ray radiation (1.5405 Å) at 45 kV and 40 mA. The crosslinked resin specimens were used in the form of films (Imberty & Perez, 1988).

2.8. Swelling power and gel fraction of films

The swelling power of crosslinked PS (cured at 120 °C and 0.1 MPa for 20 min) films with 15%, 25% and 37.5% initial concentration of MA, were determined according to the method given by Yun and Yoon (Yun, Wee, Byun, & Yoon, 2008). Accurately weighed dry crosslinked (cured) PS films (0.5 g) were immersed in distilled water at room temperature (25 °C) for 3 days. At the end of soaking period the films were taken out, the moisture on the surface was removed and the weight of the films was measured.

The swelling power was calculated as follows:

$$\text{Swelling power} = \frac{w_e - w_o}{w_o}$$

where w_o is the dry weight of the starch films and w_e is the weight of the film after being immersed in water for 3 days.

The swelling power of starch in DMSO was found by using the modification of a method demonstrated by Zhou, Zhang, Ma, and Tong (2008). The crosslinked starch films were conditioned in 21 °C and 65% relative humidity for 2 days prior to soaking in 20 mL DMSO at room temperature for 24 h. The films were accurately weighed (m_d) before immersing in DMSO. The swollen films were filtered, washed with water and ethanol, wiped lightly and weighed (m_s).

The swelling power was calculated as follows:

$$\text{swelling power} = \frac{m_s - m_d}{m_d}$$

To calculate the gel fraction the insoluble part of the film was washed thoroughly with water and ethanol and vacuum dried at 80 °C. The films were conditioned at 21 °C at 65% relative humidity and weighed (m_g).

The gel fraction was calculated as follows:

$$\text{gel fraction} = \frac{m_g}{m_b} \times 100\%$$

2.9. Mechanical properties

The resin films were cut into rectangular pieces of 50 mm × 10 mm dimensions to test their tensile properties. The film thickness was approximately 0.30 mm. The films were thoroughly washed with water, conditioned for 4.5 days at 65% relative humidity and at a temperature of 21 °C prior to testing. The tensile properties of gelatinized and crosslinked films were characterized using Instron, model 5566 (Instron Co., Canton, MA), according to ASTM D882-02. A gauge length of 30 mm and a strain rate of 0.6 were used for all specimens. At least 5 specimens were tested to obtain the average values.

3. Results and discussions

3.1. Infrared spectroscopy

The ATR-FTIR spectra of gelatinized PS, MA, blend of native PS and MA and PS crosslinked (cured at 120 °C at 2000 lb for 20 min) with 37.5% MA are presented in Fig. 2A. From the ATR-FTIR spectra it can be observed that gelatinized starch has three C–O stretching absorption peaks (C–O–C and C–O–H) between 923 and 1162 cm^{-1}, the fingerprint region (Fang, Fowler, Tomkinson, & Hill, 2002). The peaks at 1083 and 1023 cm^{-1} are attributed to the glucopyranose ring O–C stretching vibrations. The peak at 1640 cm^{-1} is assigned to the water adsorbed by starch molecules. The hydrogen bonded hydroxyl group appears as a very broad peak at 3403 cm^{-1} while the aliphatic C–H stretch is observed as a sharp peak at 2629 cm^{-1} (Fang et al., 2002). ATR-FTIR spectrum of MA shows a sharp peak at 1695 cm^{-1} for the carbonyl stretching. The broad peak at 2890 cm^{-1} is attributed to the stretching of hydrogen bonded hydroxyl groups. Hydroxyl, O–H, deformations and C–O stretching modes show peaks at 1433 cm^{-1} and 1293 cm^{-1}, respectively. On comparing the spectra of gelatinized PS, MA and PS esterified with MA shown in Fig. 2A, the presence of ester bond can be confirmed by the presence of carbonyl (C=O) peak in cured (crosslinked) PS that is observed at 1725 cm^{-1}. A similar peak at 1725 cm^{-1} was also observed for CS esterified with MA. The ATR-FTIR spectra obtained for CS and PS esterified with MA were identical and hence the spectrum for CS is not presented. It was further noted that the carbonyl stretching peak for MA appeared at 1695 cm^{-1} and that for the mixture of PS and MA appeared at 1682 cm^{-1}. These results are similar to those observed by Sauperl and Stana-Kleinschek (2010) in which the carbonyl peak for unreacted 1,2,3,4-butanetetracarboxylic acid appeared at 1701 cm^{-1} and shifted to 1725 cm^{-1} after ester formation (Sauperl & Stana-Kleinschek, 2010; Yang, Lu, & Lickfield, 2002). Mathew and Abraham (2007) reported the esterification of native PS with ferulic acid which showed the presence of carbonyl peak in the FTIR spectrum at around 1726 cm^{-1} which was distinct from the carbonyl peak obtained for ferulic acid at 1692 cm^{-1}.

Fig. 2B shows the extent of esterification of PS and CS as a function of initial MA content, using the internal calibration curve. A gradual increase in the A_{1725}/A_{2929} ratio was observed as the concentration of MA increased which leveled off as the concentration of MA reached 50%. This implies that with the increase in MA concentration there was an increase in the number of ester bonds formed (Coma et al., 2003). The absorbance of the standard peak (A_{2929}) did not vary with crosslinking. The absorbance at 1725 cm^{-1} becomes saturated at a higher concentration of MA (50%) which may indicate

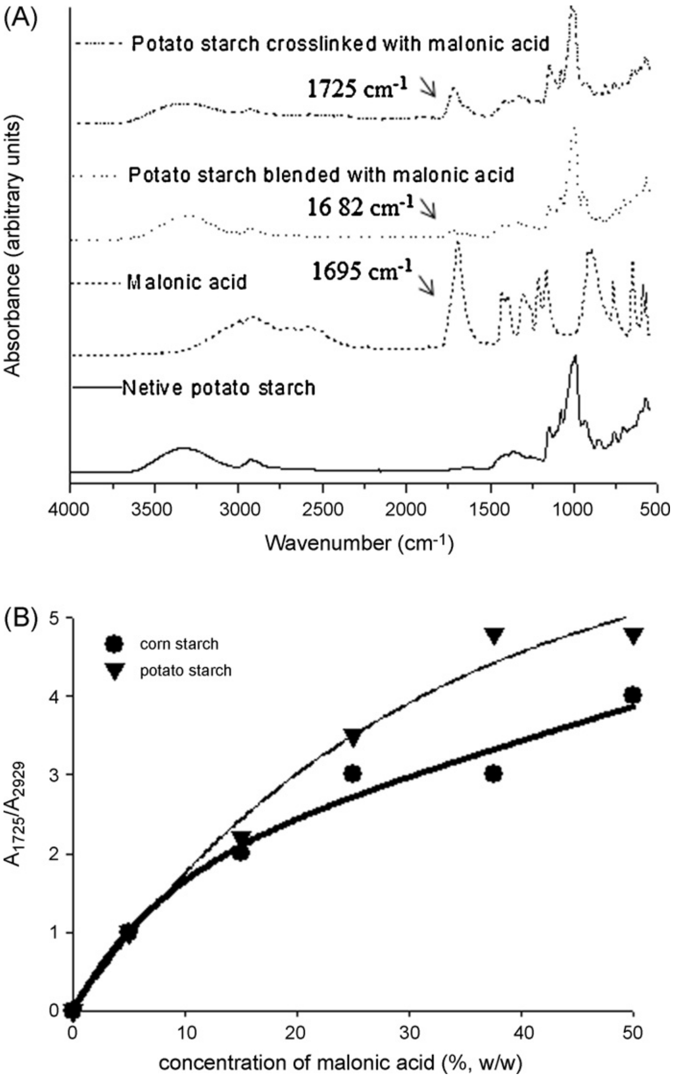

Fig. 2. (A) The ATR-FTIR spectra of gelatinized PS, MA, blend of native PS and MA and PS crosslinked (cured at 120 °C at 2000 lb for 20 min) with 37.5% MA. (B) Extent of esterification of PS and CS as a function of initial MA content, using the internal calibration curve.

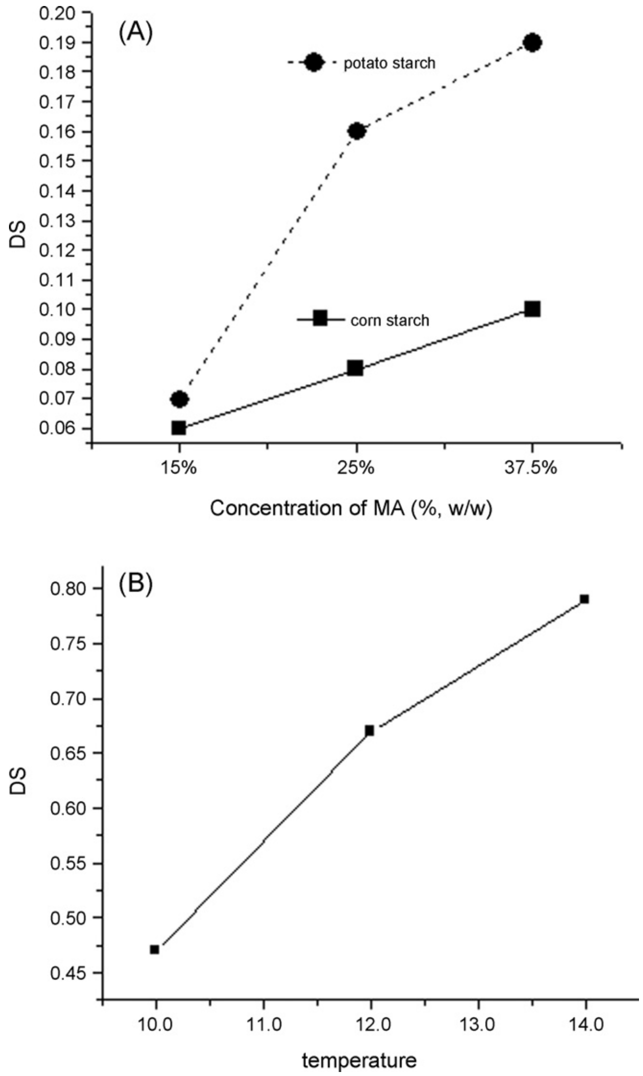

Fig. 3. DS of (A) precured PS and CS as a function of MA concentration (B) PS (precured with 37.5% MA at 90 °C for 60 min) as a function of curing temperature.

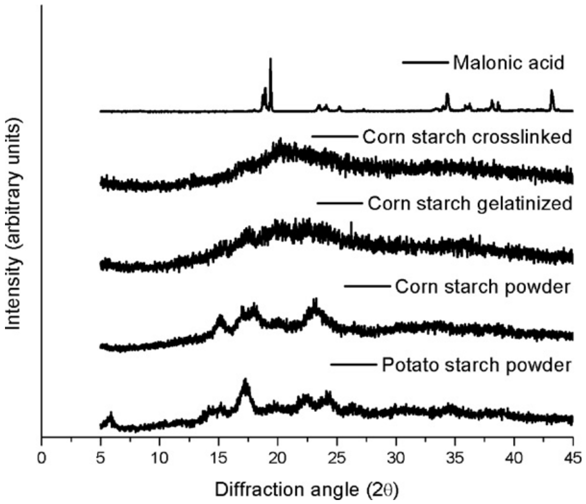

Fig. 4. WXRD patterns of native PS and CS powders, MA and gelatinized and crosslinked CS.

a possible saturation in terms of crosslinking percentage or saturation of the infrared signals (Coma et al., 2003). On comparing the internal calibration curves for precured PS and CS it was evident that at any concentration of MA, PS had a higher extent of esterification. This can be related to the more flexible granular microstructure of PS. This observation was also confirmed by the DS values of precured PS and CS determined by titration method as discussed later. It was also found that the ester carbonyl peak absorbance increased with the decrease in pH the highest being at pH of 1.5.

3.2. Degree of substitution by titration

The DS of a starch derivative is defined as the number of hydroxyl groups substituted per D-glucopyranosyl ring (Mathew & Abraham, 2007). Since each ring possesses three hydroxyl groups, the maximum DS possible is 3. However, the primary hydroxyl group (C-6) is much more reactive than the two secondary hydroxyl groups (C-2 and C-3) due to steric hindrance (Mathew & Abraham, 2007). DS is affected by various factors like source of starch, amylose and amylopectin content, reactant concentration, reaction time and temperature (Mathew & Abraham, 2007; Xu, Miladinov, & Hanna, 2004; Zhu, Zhang, & Lai, 2007). Fig. 3A shows

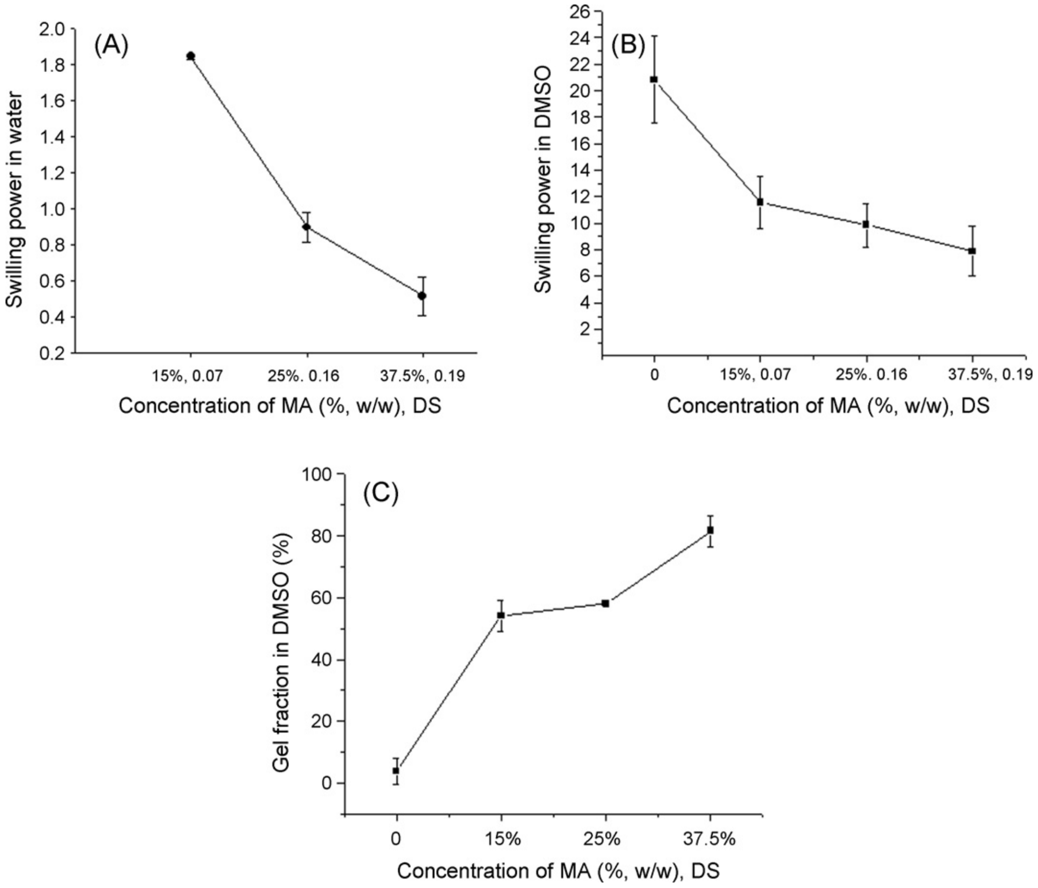

Fig. 5. (A) The swelling power of crosslinked PS (cured at 120 °C and 0.1 MPa for 20 min) with 15%, 25% and 37.5% initial MA concentration, in water (B) the swelling power of crosslinked PS (cured at 120 °C and 0.1 MPa for 20 min) with 15%, 25% and 37.5% initial MA concentration, in DMSO and (C) the gel fraction of crosslinked PS (cured at 120 °C and 0.1 MPa for 20 min) with 15%, 25% and 37.5% initial MA concentration, DMSO.

DS of precured PS and CS as a function of MA concentration while Fig. 3B shows the DS of PS (precured with 37.5% MA at 90 °C for 60 min) as a function of curing temperature. The DS values were calculated by the titration method. It is clear that the DS values increased with increase in the MA concentration (Fig. 3A). This is because high MA concentration results in greater availability of MA in the vicinity of the OH groups on the starch molecules (Xu et al., 2004). The DS values were 0.07, 0.16 and 0.19 for PS precured with 15%, 25% and 37.5% MA for 60 min, respectively; the corresponding DS values for CS were 0.06, 0.08 and 0.1 (Fig. 3A). Since the internal calibration curve in Fig. 2B already showed that crosslinking reached a saturation at higher concentrations of MA, DS was not calculated for MA concentrations above 37.5%. The data in Fig. 3A indicate that the DS for CS at any concentration was lower than that of PS. This is expected since PS has been shown to be more reactive than CS (Singh, Chawla, & Singh, 2004). This is further attributed to the more compact structure and presence of lipids in CS granules. Lipids form complex with the amylose and leads to the formation of a rigid structure which inhibits penetration of external reactants and prevents substitution. PS on the other hand has a more flexible structure and the hydroxyl groups are more exposed for reaction (Mishra & Rai, 2006; Singh et al., 2004). The DS values obtained by the titration method (Fig. 3A) confirm the earlier discussed results of the extent of esterification reaction obtained by the internal calibration curves (Fig. 2B) that higher esterification is obtained with increase in the concentration of MA. This trend is consistent with the results of crosslinking cellulose with a polycarboxylic acid like citric acid reported by Coma et al. (2003). As mentioned earlier, the DS values obtained for PS were higher than those of CS at any concentration of MA. Since the DS of the precured PS specimen, with 37.5% MA, was the highest, this specimen was chosen for curing in a hot press. The DS of cured specimens were significantly higher than DS of precured specimens as could be expected. DS values for the crosslinked PS specimens (with 37.5% MA) cured at 100, 120 and 140 °C were found to be 0.47, 0.67 and 0.79, respectively, as shown in Fig. 3B. It was also observed that films of PS precured with 37.5% MA for 60 min, showed a significant reduction in the ester carbonyl peak absorbance in ATR-FTIR after soaking in deionized water (pH = 7) for 2 days and drying thereafter. This was possibly due to hydrolysis of the ester bond in presence of water as well as washing away of unreacted malonic acid. However, in the case of PS specimens precured with 37.5% MA for 60 min followed by curing for 120 min at 0.1 MPa and 20 min, the ester carbonyl peak was much more stable and soaking in water for 1 day to 4 days did not produce any significant reduction in the ATR-FTIR absorbance of the ester carbonyl peak. From these experiments it was concluded that after curing, the number of ester bonds formed was significantly higher and the number of ester bonds hydrolyzed, after soaking in water, was negligible compared to the total number of ester bonds formed. This confirms that high temperature (120 °C) curing increases the extent of esterification. Also, there was much less unreacted MA in the system after curing which is indicative of higher crosslinking that leads to higher ester peak intensity both before and after the soaking in DI water

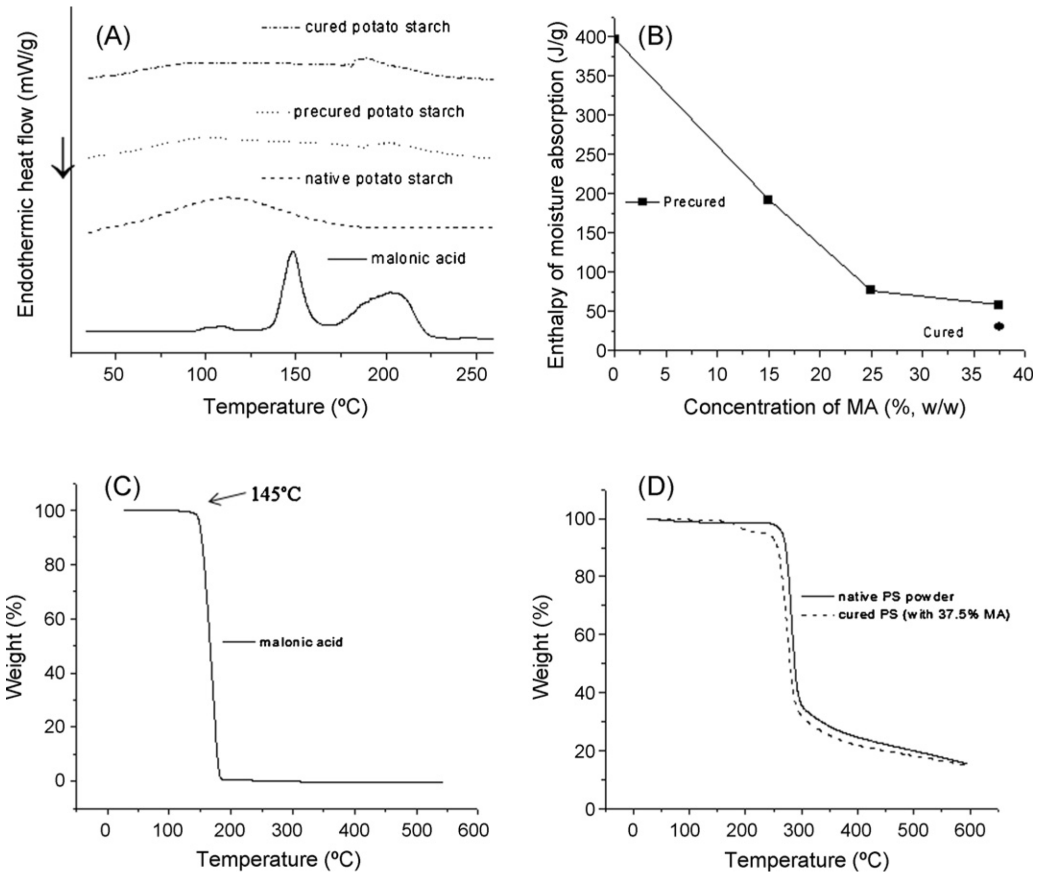

Fig. 6. (A) DSC thermograms of MA and native, precured and cured PS heated from 25 °C to 260 °C at 25 °C/min, (B) DSC enthalpy values for moisture absorption for PS specimens precured with different MA concentrations, (C) thermal degradation behavior of MA and (D) TGA of PS powder and cured PS (with 37.5% MA).

(pH = 7). These results combined with the increase in DS values with increase in curing temperature led us to conclude that the DS of starch specimens increased with increase in curing temperature due to higher extent of esterification at higher temperature. While in the hot press MA and starch also react at a higher temperature in the absence of water that leads to higher esterification and crosslinking than precuring the specimens in aqueous conditions. Carrying out esterification reaction in the presence of minimum water is crucial due to reversibility and hydrolysis of the esterification reaction, as explained earlier. The DS of the starch ester prepared with malonic acid in aqueous conditions were higher than the DS values reported (maximum DS of 0.02) for starch (waxy maize and amaranth) ester formation in aqueous conditions with n-octenyl succinic anhydride (Bhosale & Singhal, 2006).

3.3. Wide angle X-ray diffraction (WXRD) studies

Fig. 4 shows WXRD patterns for native PS and CS powders, MA and gelatinized and crosslinked CS. Native CS shows A-type crystal structure typical for cereal starches whereas native PS shows B-type crystal structure typical for root or tuber starch (Zobel, 1988). It was observed that native CS powder showed crystal peaks at 2θ of 15°, 17°, 18° and 23° and native PS powder showed crystal peaks at 2θ of 5.6°, 15°, 17° and 22° which is consistent with previously reported values (Zobel, 1988). The WXRD patterns for gelatinized and crosslinked PS were identical to those of crosslinked CS, and therefore, not shown. WXRD patterns in Fig. 4 indicate that the crystal structures of CS and PS as well as MA were completely destroyed after gelatinization and crosslinking. The destruction of crystallization due to gelatinization helped in exposing the hydroxyl groups of the starch molecules which ensured better reaction with MA molecules. In addition, the substitution of the hydroxyl groups on the starch molecules with the ester groups prohibited the inter- and intra-molecular hydrogen bonding, completely disrupting the crystal structures. This is in agreement with the DSC results which confirmed that the MA crosslinked starch showed no crystal melting endotherm and are discussed in details later. It is also expected that as the crosslinked molecules cannot reorganize in 3D crystal structures this prevented the recrystallization of the starch molecules after crosslinking.

3.4. Swelling power and gel fraction studies

Fig. 5 shows (A) the swelling power of crosslinked PS (cured at 120 °C and 0.1 MPa for 20 min) with 15%, 25% and 37.5% initial MA concentration, in water (B) the swelling power of crosslinked PS (cured at 120 °C and 0.1 MPa for 20 min) with 15%, 25% and 37.5% initial MA concentration, in DMSO and (C) the gel fraction of crosslinked PS (cured at 120 °C and 0.1 MPa for 20 min) with 15%, 25% and 37.5% initial MA concentration, in DMSO. The swelling power of starch depends on the nature of the polymer network like presence of hydrophilic groups, crosslink density, elasticity of polymer network, pH and the swelling medium and its temperature (Pang, Sun, Tay, & Tchong, 2011).

The inherent property of starch to absorb a lot of moisture affects the mechanical properties of starch and any improvement in reducing the moisture sensitivity is important for industrial application of starch. It has been shown in Fig. 5A the swelling power decreases as the initial concentration of MA increases. As the concentration of MA initially used to crosslink starch increases, there is an increase in the degree of substitution as shown in Fig. 3A. The decrease in the swelling power with the increase in initial MA concentration was clearly due to the increase in the crosslinking density which hindered the penetration of water into the starch molecular network. The destruction of crystallization due to gelatinization helped in exposing the hydroxyl groups of the starch molecules which ensured better reaction with MA molecules. This led to higher degree of substitution as well as crosslinking. The formation of a network structure with crosslinking prevents absorption of water.

The wet and swollen crosslinked (cured) PS specimens curled up and folded but remained intact in water and the water was completely transparent indicating that the specimens did not disintegrate. The gelatinized PS (without MA) specimen on the other hand disintegrated in water. Swelling powers of CS for different concentrations of MA were not calculated because of the difficulty in handling the swollen CS films which led to inconsistent values. The swelling power of 1.15 obtained for crosslinked (cured) CS with 37.5% MA after swelling for 3 days was, higher than that of PS (0.6), under similar conditions, because of the lower reactivity resulting in lower crosslinking density of the CS films. As explained earlier, crosslinking strengthens the polymer network reducing the absorption of water and does not allow it to swell.

Fig. 5B and C shows that the swelling power decrease and the gel fraction of starch films increase with increase in the initial concentration of MA used to crosslink the starch films. As shown earlier in Fig. 3A the DS of starch increases with increase in the initial MA concentration. The decrease in swelling power and increase in gel fraction was due to the increase in crosslinking or increase in the DS with increasing initial MA concentration. Gelatinized starch (without MA) is almost completely soluble in DMSO and the gel fraction increases with increase in crosslinking as expected.

3.5. DSC and TGA studies

DSC thermograms of MA and native, precured and cured PS specimens heated from 25 °C to 260 °C at 25 °C/min are shown in Fig. 6A. A broad endothermic peak was observed for native, precured and cured PS specimens at 100 °C corresponding to the evaporation of the absorbed moisture. The DSC thermogram for native, precured and cured specimens indicated no glass transition or crystal melting peak before 260 °C (Zhang et al., 2010). After 260 °C the starch decomposes, as was indicated by TGA studies that are discussed later. After crosslinking, the remaining dispersed MA remains completely amorphous as indicated by the absence of crystal melting peak in the DSC thermograms and was confirmed by WXRD results discussed earlier.

Fig. 6B indicates the DSC enthalpy values of moisture absorption for PS specimens precured with different MA concentrations. The data indicate that as the concentration of MA increased the enthalpy for moisture absorption of precured PS specimens decreased. This is expected since higher concentration of MA leads to higher crosslinking lowering the moisture content in the crosslinked starch. This trend is similar to the decrease in swelling power for PS with increase in concentration of MA, as explained before. The plot also shows that DSC enthalpy value for moisture absorption for cured PS specimen (with 37.5% MA) is lower than the precured specimen with the same initial concentration of MA due to higher extent of crosslinking with curing.

Fig. 6C shows the TGA thermogram of MA while Fig. 6D shows the thermogram of PS powder and cured PS (with 37.5% MA). These thermograms show that MA starts to degrade at 145 °C (Fig. 6C) while native PS and CS powder as well as gelatinized PS and CS powder start to degrade at 260 °C (Fig. 6D). It was observed that after crosslinking there was no change in the thermal properties of PS and CS. In the studies done by Zhang et al. (2010) it was observed that the initial thermal degradation temperature was 278 °C for gelatinized CS which decreased to 204 °C for starch oxalate ester (DS = 0.87, obtained by titration). This was believed to be due the presence of half reacted carboxylic acid groups (starch oxalate half ester formation) which resulted in lower thermal stability of the starch resin. Since reaction of starch with malonic acid did not lead to lowering of the thermal degradation temperature it can be expected that both the carboxylic groups of MA reacted with starch to form a complete ester.

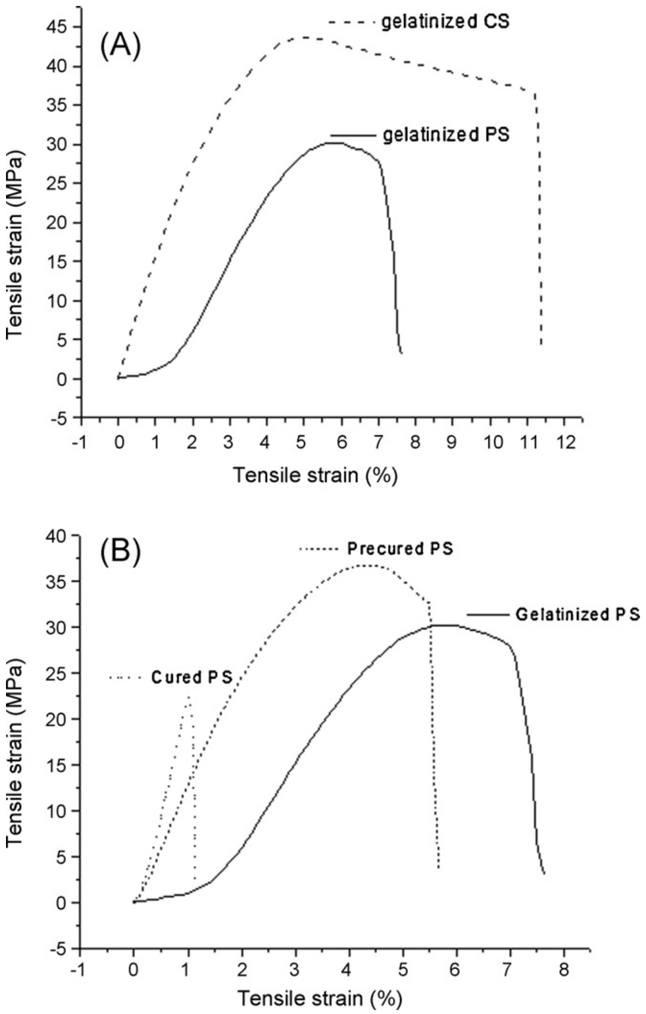

Fig. 7. Tensile stress–strain curves of (A) gelatinized CS and PS and (B) gelatinized, precured and cured PS.

3.6. Tensile properties

The tensile properties such as Young's modulus, tensile stress at maximum load and strain (%) at maximum load of gelatinized, precured and cured PS films are summarized in Table 1. The tensile stress–strain curves for gelatinized PS (without MA) presented in Fig. 7A show classic yielding prior to fracturing.

The stress–strain curves of gelatinized, precured and cured PS are presented in Fig. 7B. The Young's modulus for gelatinized PS was 923 MPa. After precuring and curing the Young's modulus increased significantly to 1359.8 and 2701 MPa, respectively (Fig. 7B). This increase in modulus is attributed to higher crosslinking of the PS after curing as compared to precured starch and gelatinized starch. Crosslinking forms a rigid network structure and thus increases the Young's modulus. However, part of the increased Young's modulus is also due to the lower moisture absorption after crosslinking. The

Table 1
The tensile properties such as Young's modulus, tensile stress at maximum load, tensile strain (%) at maximum load and tensile strain (%) at break of gelatinized, precured and cured PS films.

Sample	Modulus (MPa)	Tensile stress at maximum load (MPa)	Tensile strain at maximum load (%)	Tensile strain at break (%)
Gelatinized PS	923 (12.54)	33.45 (17.67)	5.85 (32.5)	7.6 (45.72)
Precured PS	1359.8 (22.68)	35.5 (2.14)	4.68 (15.8)	6.3 (17.76)
Cured PS	2701 (18.79)	23.17 (27.74)	1.58 (34.48)	2.08 (46.19)

tensile stress at maximum load decreased from 35.5 MPa for precured PS and to 23.17 MPa for cured PS and strain at break (%) at maximum load decreased from 4.68% to 1.58% for precured and cured starches, respectively. The lower value of strain at break (%) at maximum load is attributed to the brittle nature of the cured PS compared to the precured PS and native PS both of which show yielding prior to fracture. The decrease in stress at maximum load for the cured PS is primarily due to its significantly lower fracture strain. It was also evident from Table 1 that there was a significant increase in strain at break as compared to the strain at maximum load for both gelatinized and precured starch indicating yielding. The strain at break (2.08%) in case of cured PS was not much higher than strain at maximum load (1.58%) indicating a brittle fracture; there was no yielding prior to fracture. The typical stress–strain curve for a highly crosslinked specimen as in the case of cured PS (Fig. 7B) in absence of a plasticizer show brittle fracture, the specimen attains the highest load and then fails catastrophically. This is attributed to the decrease in molecular mobility as a result of crosslinking and formation of a thermosetting polymer (Chabba & Netravali, 2005).

4. Conclusions

A completely green, easy to scale-up water based process was developed for crosslinking of starches using malonic acid, a plant based, non-toxic, sustainable crosslinker. Malonic acid esterification of corn starch and potato starch indicated that potato starch is more reactive compared to corn starch owing to the inherent structural differences in these two starches. The differences in reactivity in the starches were evident from the degree of substitution, calculated using a chemical titration method, which showed that PS has higher degree of substitution than CS. The same results were confirmed from the internal calibration curve plotted using the ATR-FTIR spectra. Curing was found to increase the degree of substitution and hence crosslinking. The extent of esterification and crosslinking also increased with increase in concentration of malonic acid. Crosslinking potato starch with malonic acid also made the starch films brittle but led to an increase in Young's modulus. The hydrophilicity of crosslinked potato starch also decreased as a result of crosslinking which may have contributed to the higher Young's modulus. These increases in Young's modulus and hydrophobicity are advantageous for industrial application of starch as films and resins for developing green composites.

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