Effect of trisodium citrate on swelling property and structure of cationic starch thin film

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A B S T R A C T

Effects of trisodium citrate (citrate), as an ionic crosslinker of cationic starch (CS), on swelling property of citrate-CS thin film immersing into various pH solutions were studied. The maximal turbidity of film forming solutions (FFS) indicates the most extensive ionic crosslinking of citrate to CS occurring at charge ratio (R) = 3 instead of the charge neutrality point (R = 1) due to the co-existence of electrostatic force and hydrogen bonding. The CS FFS is composed of leached amylose and unbursted swollen granules mainly occupied by amylopectin. Proved by the rheological properties of citrate-CS FFS and the microscopic images of citrate-CS thin films during swelling studies, citrate governs the pH-dependent swelling behavior within the unbursted swollen granule via ionic crosslinking either in solution or in thin films. Besides, the ionic crosslinking from citrate is able to decrease the surface hydrophilicity of CS thin film by hindering the exposure of cationic group. The small angle X-ray scattering (SAXS) patterns of swollen citrate-CS thin films are well fitted by employing the correlation length model. The rise of the exponent in low q region (n) is found to be correlated with the presence of deprotonated citrate induced aggregates, constructing a strong network against osmotic pressure, thus leading to a low water retention capacity of the thin film. Proposed nano-structure inside swollen granule from SAXS can explain the results of swelling test and microscopic images. The citrate-CS thin film can potentially serve as an active coating for pH-triggered releasing of active compounds.

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1. Introduction

In recent years there have been remarkable growth in the research and development on the pH regulated swelling/deswelling materials, regarding its diverse applications in drug delivery (Klinger & Landfester, 2012), sensing (Tokarev, Tokareva, Gopishetty, Katz, & Minko, 2010) and antimicrobial coating (Gensel et al., 2012). In general, pH-responsive materials are designed in the form of slab hydrogel (Chang, He, Zhou, & Zhang, 2011), microgel (Yao, Grishkewich, & Tam, 2013) and thin film. Compared to the macroscopic hydrogels, thin films have attracted more attentions due to their quick response to pH changes. The pH-responsive thin films are usually fabricated via coating processes on a solid support with advantages of the simple and effective way to prepare the switchable surfaces (Gensel et al., 2012; Kozlovskaya, Kharlampieva, Erel, & Sukhishvili, 2009). Synthetic polymers and

natural polyelectrolytes containing either weak acidic or basic groups have potentials for preparing pH-responsive materials, such as polyallyamine hydrocholoride, poly(acrylic acid) (Kozlovskaya et al., 2009), alginate, chitosan, gelatin and pectin (Farris et al., 2011; Sánchez-Díaz, Becerra-Bracamontes, González-Álvarez, Cruz-Barba, & Martínez-Ruvalcaba, 2010; Tokarev et al., 2010).

pH-responsive materials show a swelling/collapse phase transition across a critical pH that is correlated to the respective pK_a or pK_b values of the weak acidic groups (e.g. carboxylic) or basic groups (e.g. amino) on the polymer, respectively. As pH > pK_a or pH < pK_b where the deprotonation or the protonation occurs, the ionization of functional groups causes the increase of internal osmotic pressure in the network, resulting in a swelling behavior. On the contrary, the absence of charges while functional groups are protonated at pH < pK_a or deprotonated at pH > pK_b results in a deswelling behavior (Klinger & Landfester, 2012; Yao et al., 2013).

In order to keep the charged polymer intact after fully swelling, chemical or ionic crosslinking is usually required. The ionic crosslinking could be simply performed by introducing two oppositely charged polymers (Farris et al., 2011; Kozlovskaya et al., 2009) or

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directly mixing small polyions into polymer solutions (Sánchez-Díaz et al., 2010). The ionic crosslinking not only can strengthen the network against osmotic pressure but also can regulate a dual swelling/deswelling behavior as pH across pK_a and pK_b (Chang et al., 2011).

Starch, the 3rd abundant biopolymer in the world, is a neutral biopolymer with many good characters, including high purity, reasonable price, biodegradability and biocompatibility. As native starch is heated in excess water above its gelatinization temperature, usually 60–70 °C for cereal starches, the starch granule swells and amylose migrates out of the granule and the gelation or pasting of starch occurred when the temperature was lowered. The characteristics of starch gels and pastes are a function of the nature of continuous phase (a three-dimensional network of soluble amylose and amylopectin molecules joined by junction zones), the nature of discontinuous phase (granule ghosts and fragments), and interactions between the two phases. In order to have a good pH-regulated behavior of starch-based material, maleic acid (Xiao & Fang, 2009) and carboxylic acid (Wang, Li, Chen, Xin, & Yuan, 2013) as charged groups have been modified on a completely solubilized starch. Different from the previous studies, we tried to directly mix a small acidic salt into a partially gelatinized cationic starch to prepare a pH-responsive CS thin film.

The acidic salt, such as trisodium citrate, has been post-dipped on the chitosan film, which showed a high drug releasing rate at pH below 3.5 due to the weak interaction of protonated citrate and chitosan (Shu, Zhu, & Song, 2001). Actually, an adequate amount of salts in solution could be directly mixed into a polymer solution based on the charge ratio of salts and polymers (Bagaria & Wong, 2011; Sánchez-Díaz et al., 2010). It has been reported that the multivalent anions (tetrasodium EDTA and trisodium citrate) had better ability to crosslink ionically than the mono- and divalent anions (sodium acetate and disodium succinate) did on the polyallylamine (Bagaria & Wong, 2011). Sodium hexametaphosphate (3% based on chitosan) incorporated into a chitosan solution by direct-mixing, also gave chitosan film a swelling/deswelling behavior (Sánchez-Díaz et al., 2010).

In our preliminary tests, trisodium citrate demonstrated an effective ionic crosslinking toward CS thin film. Citrate-CS thin film showed a swelling/collapse transition across pH 3.0 after immersing into 0.01 M NaCl solutions with various pHs. This study provides a further investigation of the effect of citrate on the physical properties of CS as a function of charge ratio, pH and furthermore, tries to localize the distribution of citrate in CS. The purposes of this study are (1) to determine the optimal charge ratio of citrate to CS in FFS for the effective ionic crosslinking, (2) to examine the effect of citrate on the surface wettability and the swelling property of CS thin films prepared by direct mixing citrate into CS FFS, (3) to evaluate the pH-dependent swelling behavior of citrate-CS thin films, and (4) to examine the macro- and nano-scale structures of citrate-CS thin films by microscopy and SAXS. The results of this study suggest that the citrate ionically crosslinked CS thin film can be used as an active surface coating after carrying the hydrophilic active compounds, which has the pH-responsive releasing character.

2. Materials and methods

2.1. Materials

Native corn starch was received from GuTong Ltd. (Chiayi, Taiwan) with protein content of 0.06% (w/w) determined by Kjeldahl method. The cationic reagent (3-chloro-2-hydroxypropyl-trimethyl ammonium chloride, CHPTAC), trisodium citrate 2-hydrate (32320, Riedel-de Haën, Missouri, USA) which was abbreviated as

citrate in the text and fluorescein isothiocyanate (FITC)-dextran (molecular weight around 20,000 g/mol) were purchased from Sigma–Aldrich Co. (Missouri, USA). Chemicals used were of analytical grade.

2.2. Preparation of cationic starch (CS)

Cationic starch was prepared according to the method reported by Kuo and Lai (2007) with some modifications. Native corn starch (30 g) was suspended in 33 mL of 0.1 M NaOH, followed by adding 100 g CHPTAC with the pH 12 adjusted by using 5 N NaOH. The pH of the mixture was adjusted to 11 with 2 N NaOH, and then the mixture solution was diluted with 1 mM NaOH to the total volume of 333 mL. The cationization reaction of starch slurry was carried out in a 40 °C water bath for 24 h with continuously stirring (200 rpm). After reaction, an equivalent volume of 95% ethanol was added to precipitate the cationic corn starch. Ethanol precipitation has been widely used in recovering the granular cationic starch (Manelius, Nurmi, & Bertoft, 2000) and the gelatinized cationic starch (Khalil, Farag, & Hashem, 1993) from the reaction mixture. The precipitates were then collected by the centrifugation (10,000 × g, 10 min) at 4 °C. The precipitates were resuspended with 100 mL of 63% ethanol and neutralized with 4.8 N HCl. The neutralized cationic starch was repeatedly washed with 100 mL of 63% ethanol by either centrifugation or filtration until the conductivity of washing solution was below 10 μS/cm, at which most NaCl were removed. The absence of NaCl was confirmed by AgNO$_3$ test. The cationic starch was oven-dried at 40 °C for 1 day with the final moisture content of 13.35% before uses.

The number of cationic groups on CS was obtained by subtracting the contents of nitrogen in native corn starch from that in CS, in which the contents of nitrogen were determined by using Kjeldahl method (AACC 46-12). The degree of substitution (DS) of CHPTAC on starch was 0.12 that was calculated following the equation (1).

$$DS = \frac{(162.15 \times \% \text{ nitrogen})}{1401 - (151.64 \times \% \text{ nitrogen})} \tag{1}$$

where, 162.15 is the average molecular weight of anhydroglucose unit; 1401 is the 100 times of the atomic weight of nitrogen; and 151.64 indicates the molecular weight of the substituted, chloride free of CHPTAC group.

2.3. Preparation of film forming solution (FFS)

For FFS preparation, a stock CS slurry (0.4%, w/v) was partially gelatinized at 70 °C under continuously stirring (150 rpm) for 10 min, then stood at room temperature until bubbles escaped. The mixtures, containing well mixed CS stock solution (1 mL) and citrate stock solution (0–4 mM), were equilibrated at room temperature for 6 h at least to allow citrate diffusing among CS. For turbidity measurements, the FFS containing 0.2% (w/v) CS and 0–2 mM citrate were prepared. Only 0–0.6 mM citrate in 0.2% (w/v) CS FFS were prepared for film casting.

2.4. The constituents of CS FFS

After adding NaCl, the repulsive force among cationic groups of CS can be screened out and the CS FFS could be separated into supernatant and precipitate by centrifugation. Thus, the supernatant (1.7 mL) and precipitate (0.3 mL) were obtained by centrifuging 2 mL of CS FFS (0.2%, w/v) containing 0.05 M NaCl at 2180 × g for 10 min. The iodine-stained (0.2% I$_2$ in 2% KI) supernatant and precipitate were observed by using the optical microscopy (ECLIPSE