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# Interactions in N-[(2-hydroxyl)-propyl-3-trimethyl ammonium] chitosan chloride/sodium carboxymethyl cellulose based films

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**Abstract:** To illuminate the interactions in N-[(2-Hydroxyl)-propyl-3-trimethyl ammonium] chitosan chloride (HTCC)/sodium carboxymethyl cellulose (CMC) based films, the film-forming solutions were studied by rheology, and HTCC/CMC based films were characterized by Fourier transform infrared spectroscopy, scanning electron microscopy and thermogravimetric analysis. The HTCC/CMC based film-forming solutions with glycerol (polyvinyl alcohol (PVA) and anthocyanin) showed shear-thinning behavior. The apparent viscosity of the HTCC/CMC/glycerol (PVA and anthocyanin) film-forming solutions decreased slower than that of HTCC/CMC film-forming solution, due to the electrostatic interaction between HTCC and CMC, and hydrogen bonding interaction between the two biomacromolecules and glycerol, PVA and anthocyanin, which resulted in the formation of three-dimensional networks. The anthocyanin with rigid molecular structure formed different three-dimensional matrix with HTCC/CMC, resulted in viscosity dominated film-forming solution. The pH affected the electrostatic interaction between HTCC, CMC and anthocyanin through neutralizing  $-\text{COO}^-$  groups, and temperature showed a synergistic effect on both electrostatic interaction and hydrogen bonding. The inter-molecular interactions resulted in the red-shift of typical absorption peaks, and the increase of initial decomposition temperature and decomposition enthalpy of HTCC/CMC based films. The homogenous and smooth surface, and the compact texture of HTCC/CMC based films confirmed the intermolecular interaction between HTCC, CMC and PVA (glycerol and anthocyanin).

**Keywords:** N-[(2-Hydroxyl)-propyl-3-trimethyl ammonium] chitosan chloride; sodium carboxymethyl cellulose; interaction; rheological property; film

## 1. Introduction

Biopolymers including cellulose[1-3], chitosan/chitin[4, 5], starch[6, 7], collagen/gelatin [8, 9] have attracted increasing attention for their biodegradability, biocompatibility, and potential utilization

as suitable alternatives to certain synthetic polymers. Among these biopolymers, cellulose is the most abundant and the most widely studied polysaccharide. However, the cellulose films are disadvantaged by their unsatisfactory mechanical properties, brittleness, and low vapor permeability due to strong inter- and intra-molecular hydrogen bonds [2].

To improve the properties of cellulose films and promote their applications, chemical modification or introduction of nanoparticles has been done. Rachtanapun and coworkers [10] extracted cellulose from durian rind and converted it to carboxymethyl cellulose. The carboxymethyl cellulose films showed the highest tensile strength of 140.77 MPa and water vapor transmission rate of 220.85 g/day·m<sup>2</sup>. Pola et al. [11] found that cellulose acetate-based active films presented high antifungal activity in vapor phase, which might be used as package for fruits and vegetables. Esaki et al. [12] revealed that lactose-modified cellulose films showed stronger adhesion to rat hepatocyte compared to original cellulose cell, this suggested that the lactose-modified cellulose films are bioactive and can be utilized in cell culture engineering. Nanofiltration membranes prepared with cellulose acetate showed good properties for removal of ibuprofen and sulfamethazine removals[13], and those prepared by regeneration of trimethylsilyl cellulose showed zero NaCl rejection[14]. Introduction of the nanoparticles, such as SnO<sub>2</sub> [15], ZnO [16], graphene oxide [17, 18] and reduced graphene oxide [17, 19] into the film were found to improve its mechanical properties, thermal stability, glass transition temperature, Young's modulus and surface roughness. The improvements were ascribed to the effective attachment of CMC to the nanoparticles' surface through strong hydrogen bonding interactions, constraining the segmental motion of the CMC chains[15, 17]. However, the potential risks of the synthetic nanoparticles to human health must be taken into further consideration[20]. Biopolymers, such as chitosan/chitin[21, 22], cellulose (including nanofibrillated cellulose, nanocrystalline cellulose and hemicellulose)[23], starch [24, 25] and protein[26] could improve the mechanical properties, flexibility, thermal stability, and barrier property of cellulose-based films. Blend systems that composed of two oppositely charged polyelectrolytes have excellent functional properties with wide applications[23]. Based on the good biocompatibility and strong electrostatic interaction between the oppositely charged biomacromolecules, N-(2-hydroxyl)-propyl-3-trimethyl ammonium chitosan (HTCC) could increase the tensile strength and elongation at break of CMC-based films are increased by 139% and 84.5% at  $m_{\text{HTCC}}/m_{\text{CMC}}=10\%$ [37], respectively.

Bio-based intelligent packaging materials have attractive attentions for their antioxidant, antimicrobial and biocatalytic activity, and detective, sensitive, recordable properties[28]. Among various intelligent devices, pH indicators have been studied widely for the food corruption process is usually accompanied by a pH change. Anthocyanin, exists extensively in saturated fruits, vegetables and flowers, and its color changes from purple to green clearly with the pH of solution increasing from 2 to 10 [29-31], is often used in food packaging film as an indicator[32-34]. Meanwhile, glycerol, polyvinyl chloride and polyvinyl alcohol are used as plasticizers to improve the mechanical properties of films [23, 35]. However, the mechanism of anthocyanin and plasticizers affecting mechanical properties of bio-based films are seldom reported.

In the current work, the effects of HTCC/CMC film-forming solution preparation method, presence of anthocyanin, plasticizer type (glycerol and polyvinyl alcohol), pH of solution as well as temperature on the rheological properties of HTCC/CMC film-forming solutions were investigated. Subsequently, HTCC/CMC bio-based films were prepared, and their structure and physical properties were investigated by Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and thermogravimetric analysis (TGA), respectively. Based on the analyzed results, we revealed the affecting mechanism of anthocyanin and plasticizers (glycerol and polyvinyl alcohol) on HTCC/CMC bio-based films. The present study will contribute to the development of intelligent packaging films.

## 2. Materials and Methods

### 2.1 Materials

Chitosan was purchased from Xiya Reagent (Chengdu, China) and dried in vacuum at 80 °C for 24 h before the experiments. The degree of deacetylation (determined by elemental analysis), viscosity average molecular weight (determined by Ubbelohde viscosity), and viscosity (provided by the vendor) were 91.2%,  $M_v=5.2 \times 10^5$ , and  $<200 \text{ mPa}\cdot\text{s}$ , respectively. 2,3-Epoxypropyltrimethyl ammonium chloride (EPTAC) was purchased from Adamas Reagent Co., Ltd. (Shanghai, China). The content of epoxy was 95.0% (determined by the hydrogen bromide-acetic acid non-aqueous titration method). Sodium carboxymethyl cellulose (CMC, UPS grade), with viscosity of 1000-1400  $\text{mPa}\cdot\text{s}$  (provided by the vendor), was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. The degree of substitution, determined by elemental analysis method [36], was 1.59. Polyvinyl alcohol (PVA-1799, degree of polymerization 1700, degree of hydrolysis 99%) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Hydrochloric acid (A.R.), sodium hydroxide (A.R.) and glycerol (A.R.) were all purchased from Sinopharm Chemical Reagent Co., Ltd., China.

N-[(2-hydroxyl)-propyl-3-trimethyl ammonium] chitosan chloride (HTCC) with degree of substitution (DS) of 0.96 was synthesized according to our previous work[37]. The DS was calculated according to the integral of  $^1\text{H}$  NMR spectra[38].

### 2.2 Extraction of anthocyanin

The extraction of anthocyanin was carried out according to the method in Ref.[39] with appropriate modifications. The fresh purple cabbage was freeze dried for 24 h at  $-60 \text{ }^\circ\text{C}$ , and the dried purple cabbage was powdered. About 30 g dried purple cabbage powder was added into an 1 L flask with 400 mL ethanol/deionized water mixed solution, of which the pH value was adjusted to 2 with 1.0 mol/L hydrochloric acid solution. The volume ratio of ethanol to water was 7/3. After being placed in the  $4 \text{ }^\circ\text{C}$  refrigerator for 48 h, the blend solution was centrifuged for 10 min on a frozen centrifuge at the speed of 10000 rpm. The pH of the supernatant was adjusted to 7.0, and the anthocyanin powder was obtained after the supernatant was freeze-dried.

The molecular structures of HTCC, CMC, anthocyanin and glycerol are listed in Schematic 1.

Schematic 1

### **2.3 Preparation of HTCC, CMC and HTCC/CMC solutions**

Certain amounts of HTCC and CMC powders were weighted into a series of 50.0 mL bottles with caps, respectively. The total weight of each solution was fixed at 25.0 g. Certain amounts of anthocyanin, glycerol and PVA were added into HTCC/CMC blend solutions. The concentration of anthocyanin was fixed at 1.0 g/L. To improve the dissolution rate of each component, the composite systems were placed in a water bath at 60 °C and electromagnetically stirred for 4 h. The solutions were then stood for 24 h at 25 °C.

### **2.4 Rheological measurements**

The rheological properties of HTCC, CMC and blend solutions were performed on a DHR-2 rheometer (TA Instrument, USA) with a parallel-plate geometry (45.0 mm in diameter and 1.0 mm in gap). In oscillatory measurements, an amplitude sweep at a fixed angular frequency of 10.0 rad/s was performed prior to the following angular frequency sweep in order to ensure the selected strain was in the linear viscoelastic region (the region in which the storage and loss moduli were independent of the strain). Then the storage modulus ( $G'$ ), and the loss modulus ( $G''$ ) were measured in the angular frequency range of 0.1-100.0 rad/s at a set temperature. In the steady shear experiments, the shear rate was typically increased from 0.1 to 1000  $s^{-1}$  with 15 min.

### **2.5 Preparation and characterization of HTCC doped CMC-based films**

#### **2.5.1 Preparation of composite films**

The series of HTCC/CMC blend solutions (30.0 g) with different additives were casted into Teflon plates with inner diameter of 80 mm, which were dried at 40 °C for 24 h in a ventilated oven (DHG-9023A, Shanghai Yiheng Scientific Instrument Co., China).

#### **2.5.2 Thermal properties**

Thermogravimetric analysis of the HTCC/CMC-based films were measured on an SDT Q600 simultaneous thermal analyzer (TA Instruments, USA) from room temperature to 800 °C under nitrogen atmosphere (100.0 mL/min). The heating rate was 10.0 °C/min.

#### **2.5.3 FTIR spectra**

FTIR spectra of the HTCC/CMC-based films were recorded on a Bruker TENSOR27 infrared spectrometer (Shimadzu, Japan), with an attenuated total reflectance (ATR), from 4000 to 400  $cm^{-1}$  at room temperature. The samples were scanned 32 times and the peak resolution ratio was 1.0  $cm^{-1}$ . It was corrected against the background spectrum of the solvent at ambient temperature.

#### **2.5.4 Morphologies**

The morphologies of the HTCC/CMC-based films were observed on an EVO18 field-emission scanning electron microscope (FE-SEM, Carl Zeiss, AG, Germany), fitted with a field emission source and operated at an accelerating voltage of 5.0 kV. The samples were freeze-dried and sputtered with gold before the SEM observation.

## **3. Results and discussion**

### **3.1. Effect of heating during its preparing solutions on rheological properties**

To investigate the effect of heating on the interaction between HTCC and CMC molecules during the preparation of HTCC/CMC film-forming solutions, two samples with mass ratios of HTCC to CMC of 16% were prepared. One sample was prepared under stirring for 4 h in a 60 °C water bath (**sample 1**), and the other was stirred for 4 h at 25 °C (**sample 2**). The concentrations of HTCC and CMC were 0.16 g/L and 1.0 g/L, respectively. The apparent viscosity and the storage and loss moduli of **sample 1**, measured after the film-forming solutions were stood for 24 h at 25 °C, are all lower than those of **sample 2** (Fig. 1A and 1B). At a high temperature (such as 60 °C during preparation of HTCC/CMC film-forming solution), the intermolecular electrostatic interaction, hydrogen binding, and hydrophobic interactions, as well as Van der Waals interactions decrease, accompanied by the increase of thermal activities of HTCC and CMC molecules[40, 41]. This results in the reorganization of the two oppositely charged biopolymers into an optimum structure, as a result, the viscosity, storage and loss moduli of **sample 1** is lower than that of **sample 2**[27]. More importantly, the linear viscoelastic region of **sample 1** is much larger than that of **sample 2** (Fig. 1C), which confirms the formation of the optimum structure between the two biomacromolecules. Therefore, in the following studies, the HTCC/CMC film-forming solutions were prepared according to the preparation process of **sample 1**.

Fig. 1

### 3.2 Effect of plasticizer types on rheological properties

The plots of apparent viscosity for HTCC/CMC film-forming solutions with different plasticizer (PVA or glycerol) versus shear rate at 25 °C are shown in Fig. 2A. The concentration of PVA or glycerol is 1.0 wt%, respectively. The apparent viscosities decreased with the increase of shear rate, indicating the solutions are all non-Newton fluids (namely, pseudoplastic fluids). The shear thinning behavior is caused by the breakage of the network formed by HTCC, CMC and plasticizer, and their molecules align in the direction of flow during the shearing process[42]. The apparent viscosity of HTCC/CMC/glycerol film-forming solution is the highest, followed by those of HTCC/CMC/PVA and HTCC/CMC film-forming solutions (control experiment). The result is consistent with what Ayala et al. [43] obtained, namely, glycerol in cassava starch solution promoted the generation of massive hydrogen bonds, increasing the apparent viscosity. Both glycerol and PVA form a three-dimensional matrix with the two biomacromolecules by hydrogen bonding interaction, resists the shear-thinning behavior. The glycerol, with lower molecular weight than that of PVA, is more prone to form the three-dimensional matrix with the two biomacromolecules[44]. Therefore, the apparent viscosity of HTCC/CMC/glycerol film-forming solution is higher than that of HTCC/CMC/PVA system.

The relationship between the apparent viscosity and shear rate could be fitted by Ostwald de Waele model (Eq. (1)) and Cross-model (Eq.(2))[45].

$$\eta = k \cdot \dot{\gamma}^{n-1} \quad (1)$$

$$\eta = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{1 + (\lambda \cdot \dot{\gamma}_b)^m} \quad (2)$$

where,  $k$  and  $n$  are consistency coefficient and flow behavior index, respectively;  $\eta_0$ ,  $\eta_\infty$ ,  $\lambda$  and  $m$  are zero-shear viscosity, infinite-shear viscosity, critical shear rate and time constant, respectively. The value of  $\lambda$  represents the magnitude the structure could be maintained to the shear, a high value of  $\lambda$  means a shear-thinning behavior occurred at a low shear rate. Constant  $m$  reflects the variation of structure with shear rates; the solution shows Newtonian behavior when  $m$  is zero, and turns to shear-thinning behavior when  $m$  tends to 1.

The parameters of HTCC/CMC film-forming solutions with different plasticizers are listed in Table 1. The correlated coefficients are all larger than 0.99, attesting the three solutions fit Ostwald de Waele model. The values of  $n$ , less than 1, decrease in order of control sample < sample with PVA < sample with glycerol, indicating a more pronounced shear-thinning behavior of HTCC/CMC film-forming solutions. However, that of  $k$  shows an opposite trend. These  $n$  values are much smaller than that of pure HTCC (15 g/L)[45]. This could be attributed to the presence of glycerol that induced the formation of large amount of hydrogen bonds with the two biomacromolecules and glycerol, and the formation of three-dimensional network[46]. The  $\eta_0$  and  $\eta_\infty$  values of HTCC/CMC film-forming solutions increase with the addition of PVA or glycerol. Compared with that of pure HTCC solution[27], larger values of  $\lambda$  and  $m$  indicate more shear-thinning behavior of HTCC/CMC/PVA (or glycerol) blend solutions. These results agree with that of apparent viscosity.

Table 1

The linear viscoelastic regions for HTCC/CMC film-forming solutions with different plasticizers were also measured (Fig. S1). The values of critical oscillation strain (the value corresponding to storage modulus decrease initially) decrease in order of control sample (16.26 %), sample with PVA (6.14 %), and sample with glycerol (3.94 %). This can be ascribed to the hydrogen bonding interaction and the formation of network structure[45].

Fig. 2

Fig.2B shows the variation of  $G'$  and  $G''$  for HTCC/CMC film-forming solution with different plasticizers as a function of the frequency at 25 °C. For all samples, the  $G'$  is larger than corresponding  $G''$ , and both values increase slightly with a rise in angular frequency. This indicates that the three blended solutions are dominated by elastic property and display typical gel-like properties[47, 48]. Small ratios of  $G''/G'$  (Table 2) also demonstrate the prominent elastic behavior of HTCC/CMC film-forming solutions with different plasticizers. The viscoelasticity of HTCC/CMC film-forming solution studied is different from our previous work[27], in which  $G''$  is higher than  $G'$ . This can be ascribed to the higher CMC concentration used in the previous work(20 g/L) than that used in the current work (1.0 g/L); therefore, this film-forming solution was dominated by elastic property. The result is consistent with those reported in Ref.[49, 50], namely, the  $G''$  was higher than  $G'$  when the CMC concentrations were 1 wt% and 3 wt%, and  $G'$  was higher than  $G''$  when CMC concentration was high than 5 wt%.

Table 2

### 3.3. Effect of anthocyanin and pH on rheological properties

The plots of apparent viscosity of HTCC/CMC film-forming solutions with or without (control) anthocyanin at different pH versus shear rate in the range of 0.1-1000 s<sup>-1</sup> at 25 °C are shown in Fig. 3A. The viscosity profiles show distinct characteristics: as the shear rate is lower than 0.4 rad/s, the viscosity of the HTCC/CMC/anthocyanin (pH=7) film-forming solution is equal to that of the control one. Then the viscosity of HTCC/CMC film-forming solution decrease sharply with increasing shear rate, whereas, that of HTCC/CMC/anthocyanin(pH=7) film-forming solution decrease slightly. This phenomenon is caused by the specific structure of anthocyanin[51], and massive –OH groups in anthocyanin interacting with those in HTCC and CMC, forming stable three-dimensional networks. Therefore, the apparent viscosity decreases slightly compared with those of control samples[43].

With decreasing pH of HTCC/CMC/anthocyanin film-forming solution, the apparent viscosity at low shear rate decreases obviously, implying that the stability of the three-dimensional network is affected (Fig. 3A). With the addition of H<sup>+</sup>, the molecular structural form of anthocyanin changes[52, 53], and parts of –COO<sup>-</sup> in CMC are neutralized. Both changes weaken the interaction among CMC, HTCC, and anthocyanin[54], resulting in the decrease of apparent viscosity.

Fig. 3

The rheological parameters were calculated and listed in Table 1. The  $k$  and  $n$  values of HTCC/CMC/anthocyanin film-forming solutions are much higher than those of control one; in contrast, those of  $\lambda$  and  $m$  are much smaller than those of control one. This means that the shearing-thinning behavior of HTCC/CMC/anthocyanin film-forming solution is slightly weakened by shear rate, and the HTCC/CMC/anthocyanin film-forming solution has higher onset shear rate of shear thinning behavior than control one. The  $k$  and  $n$  values decrease with the decrease of pH; namely, the shear-thinning behavior increases. These results are caused by the variation of interaction between anthocyanin and the two biomacromolecules.

The variation of  $G'$  and  $G''$  for HTCC/CMC/anthocyanin film-forming solution at different pH with angular frequency at 25 °C are shown in Fig.3B. For HTCC/CMC/anthocyanin film-forming solution, both  $G'$  and  $G''$  increase with frequency, indicating the dependence of viscoelastic response on frequency. The HTCC/CMC/anthocyanin film-forming solutions show predominantly viscous behavior (the  $G''$  is higher than  $G'$ ) (Table 2) under certain angular frequency, similar to the rheological properties of high CMC concentration[49, 50]. It is due to the hydrogen bonding and electrostatic interactions between anthocyanin and the two biomacromolecules, as well as the formation of stable three-dimensional network. The crossover frequencies, where  $G'$  is equal to  $G''$ , are 51 (pH=7), 75 (pH=6) and 77(pH=5) rad/s, respectively, showing a close pH dependence. This behavior is attributed to the decrease in the intermolecular interaction and the destruction of their network. After the crossover frequency, the samples are dominated by elasticity.

### 3.4. Effect of temperature on viscoelasticity

The variation of viscoelastic properties of HTCC/CMC film-forming solutions as a function of temperature is shown in Fig.4. The temperature increasing rate of 2 °C/min and a frequency of 1.0 rad/s were applied. From Fig.4, we could find that both the storage ( $G'$ ) and loss ( $G''$ ) moduli almost keep constant with the temperature rising from 15 to 25 °C, and increase slightly with temperature raising from 25 to 40 °C. With rising temperature, the hydrogen bonding, van der Waals force between HTCC and CMC, as well as the degree of hydration of ionic groups on the biomacromolecules are weakened[56]. Meanwhile, the electrostatic interaction between HTCC and CMC molecules is strengthened. From the changes of HTCC/CMC film-forming solution viscoelastic properties, we could conclude that a synergistic effect of electrostatic interaction and hydrogen bonding is present in the aspect of inter-molecular interaction between HTCC and CMC within 15-25 °C; and the electrostatic interaction dominates the HTCC/CMC composite structure within 25-40 °C[55].

Fig. 4

### 3.5. FTIR spectra of HTCC/CMC composite films

To test the interaction between the additives and HTCC and CMC molecules, HTCC/CMC films with or without (control) glycerol, PVA and anthocyanin films were prepared, and characterized by attenuated total reflection-FTIR spectroscopy (Fig. 5). The concentrations of CMC, PVA, glycerol and anthocyanin in the film-forming solution are all 1.0 wt%, and mass ratio of HTCC/CMC is 16 %. Compared to the flat peaks of control film, the broad peaks of HTCC/CMC/glycerol (PVA, anthocyanin) films at 3290  $\text{cm}^{-1}$  are ascribed to the formation of hydrogen bonds between O-H, N-H and C-H groups[57, 58]. The peaks at 1576  $\text{cm}^{-1}$  are assigned to the tightly bounded water[59], and the asymmetric stretching vibration of C=O in HTCC, which is red-shifted from 1592  $\text{cm}^{-1}$ [37]. The peaks at 1405  $\text{cm}^{-1}$  and 1320  $\text{cm}^{-1}$  belong to the symmetric stretching vibrations of C-H in  $-\text{N}(\text{CH}_3)_3^+$  and C-O, respectively, which are red-shifted from 1419  $\text{cm}^{-1}$  and 1325  $\text{cm}^{-1}$ , respectively. This is ascribed to the electrostatic interaction between  $-\text{N}(\text{CH}_3)_3^+$  and  $-\text{COO}^-$  groups, and hydrogen bonding between -OH,  $-\text{NH}_2$  and C-H groups[60].

Fig. 5

### 3.6. Morphologies of HTCC/CMC composite films

Typical SEM images of HTCC/CMC bio-based films for upper surfaces and cross sections are observed and shown in Fig.6. The upper surface images show that there are many cracks on pure CMC (Fig.S2) and HTCC/CMC films (Fig.6A), with the addition of PVA (glycerol and anthocyanin (pH=7)) the surfaces of HTCC/CMC/PVA (glycerol and anthocyanin (pH=7)) films become continuous, homogenous and smooth with only occasional cracks. The surface of HTCC/CMC/glycerol is the smoothest, followed by those of HTCC/CMC/anthocyanin (pH=7) and HTCC/CMC/PVA in sequence. This is because of the generation of massive hydrogen bonds between glycerol and the two biomacromolecules, and the formation of HTCC/CMC/glycerol three-dimensional matrix[61]. The interaction between PVA (and anthocyanin) and the two biomacromolecules is weaker than that of

HTCC/CMC/glycerol due to their molecular characteristics. With the decrease of pH, the electrostatic interaction between anthocyanin and CMC is destroyed due to the neutralization of  $-\text{COO}^-$  [62]. Therefore, the surfaces of HTCC/CMC/anthocyanin (pH=5 and 6) are coarser than that of HTCC/CMC/anthocyanin (pH=7). The results are consistent with those obtained from rheological properties (Fig.2 and 3).

The cross-section images (Fig.6B) show the surfaces of HTCC/CMC/glycerol (and anthocyanin (pH=7)) are very exquisite, compared with those of pure CMC (Fig.S2), HTCC/CMC and HTCC/CMC/PVA films, which are defected, coarse or wrinkled structures. That is, cross-section images show that glycerol and anthocyanin can be excellently dispersed in HTCC/CMC matrix through intermolecular interactions. With the decrease of pH, the cross-section images of HTCC/CMC/anthocyanin become coarser than that of HTCC/CMC/anthocyanin (pH=7), and many holes appeared in HTCC/CMC/anthocyanin (pH=5) cross-section (Fig.6B7). All these changes are ascribed to the destruction of intermolecular interaction between the two biomacromolecules and anthocyanin with decreasing pH.

Fig. 6

### 3.7. Thermal properties of THCC/CMC based films

The TGA curves of HTCC/CMC bio-based films with glycerol, PVA and anthocyanin (pH=7) show two significant weight-loss stages (Fig.7). The first one initiates from ca. 50 °C and terminates at ca. 265 °C, which is caused by the loss of adsorbed and bounded water. The first weight losses of HTCC/CMC films with glycerol, PVA and anthocyanin are 14.73%, 14.42% and 13.38%, respectively, which are larger than that of the control film (12.52%). It is because of the massive  $-\text{OH}$  groups in glycerol, PVA and anthocyanin molecules that could adsorb (or bound) water [46]. The first weight loss of HTCC/CMC/glycerol (PVA) films is larger than that of HTCC/CMC/anthocyanin film, which might be caused by the difference of the three molecules. The second weight loss, corresponding to the decomposition of the bio-based films, is divided into two processes. The first process terminates at ca. 300 °C, accompanied by a very fast weight loss; the second process terminates at ca. 480 °C, accompanied by a slow weight loss. The order of the decomposition temperature is glycerol > anthocyanin (pH=7) > PVA > control sample. The decomposition temperatures ranges are 206 °C (control), 212 °C (PVA), 223 °C (glycerol) and 221 °C (anthocyanin), respectively. The weight losses from ca. 265 °C to ca. 480 °C are 49% (control), 53% (PVA), 56% (glycerol) and 45% (anthocyanin), respectively. The TGA results indicate that the order of the intermolecular interaction is glycerol  $\approx$  anthocyanin > PVA, which agrees with the SEM images.

Fig. 7

## 4. Conclusions

Electrostatic interaction and hydrogen bonding in HTCC/CMC film-forming solutions and HTCC/CMC based films have been studied. The results provided very detailed information about characteristics and changes caused by the inter-molecular interactions between HTCC and CMC and glycerol (PVA and anthocyanin). Intermolecular interactions were confirmed by the resistance of

shear-thinning behavior, synergistic effect on viscoelasticity of HTCC/CMC film-forming solution, red-shift of characteristic absorption peaks, increase of initial decomposition temperature and decomposition enthalpy of the bio-based films. Rheological properties of HTCC/CMC film-forming solutions and HTCC/CMC based films were enhanced by addition of glycerol, PVA or anthocyanin. The inter-molecular interaction between the two biomacromolecules and glycerol was stronger than that between the two biomacromolecules and PVA due to the smaller molecules of glycerol, as massive -OH groups therein. The inter-molecular interaction between the two biomacromolecules and anthocyanin was strongly dependent on the pH of the film-forming solution, as the -COO<sup>-</sup> groups were partially neutralized at low pH and the electrostatic interaction between anthocyanin and the two biomacromolecules was weakened. In the preparation process of the HTCC/CMC/glycerol (anthocyanin) bio-based films, only the inter-molecular interaction induced the formation of films with homogenous and smooth surfaces, and a compact texture. The preparation process of the HTCC/CMC based film was simple and non-toxic, which would be interesting for the preparation of pH sensitive packaging materials.

**Supplementary Materials:** The following are available online at [www.mdpi.com/link](http://www.mdpi.com/link), Figure S1: Plots of  $G'$  versus oscillation strain for HTCC/CMC blend solutions with different plasticizer types at angular frequency of 10 rad/s; Figure S2: TGA curves of HTCC/CMC composite films with glycerol, PVA and anthocyanin (pH=7).

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**Conflicts of Interest:** The authors declare no conflict of interest.

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