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Study of interaction between water-soluble collagen and carboxymethyl cellulose in neutral aqueous solution

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ABSTRACT

The interactions of succinylated collagen (SC) and carboxymethyl cellulose (CMC) in neutral solutions (pH ~ 6.9) were investigated, which could provide information for the fields of tissue engineering and cosmetics. According to the results from viscometric measurements, the SC and CMC molecules were possibly compatible as CMC ≤ 50%. Ultrasensitive microcalorimetry reflected the increase in the values of transition temperature (T_m) and heat capacity change (ΔC_p) as CMC ≤ 50%, while both of them decreased as the CMC content further increased. Two-dimensional Fourier transform infrared spectroscopy analysis confirmed the hydrogen bonding and electrostatic interactions between SC and CMC, and the hydrogen bonding interaction played a key role as CMC ≤ 50%, while electrostatic interaction became predominant as more CMC was added. Atomic force morphology revealed the changes in the aggregation of SC induced by the addition of CMC.

1. Introduction

Collagen, the major structure component in connective tissues such as tendon, skin and bone, is widely used as biomaterials of different shapes including sponges, films, membranes and tubes due to its advantages such as readily availability, biocompatibility, low antigenicity and high mechanical strength (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011; Kołodziejska, Sikorski, & Niecikowska, 1999).

Collagen is a typical polyampholyte and has an isoelectric point near physiological pH, which means that it has a poor solubility under the neutral condition, resulting in the restriction in the application of collagen. In order to overcome this drawback, succinyl group can be introduced to collagen by the modification method called succinylation. The preparation and characterization of succinylated collagen has been performed by several researchers (Kim et al., 2000; Sripriya, Kumar, Balaji, Kumar, & Sehgal, 2011; Wallace & Rosenblatt, 2003; Zhang, Liu, Li, & Li, 2007). Succinylated collagen (SC) could dissolve easily to form a clear solution at neutral pH. The improved solubility under neutral condition makes collagen-based material to overcome precipitation before application, and thus makes them to be used for injectable biomaterials. Furthermore, succinylation of collagen would make the surface antibacterial and biocompatible due to the increased net negative charge on the molecules, which could minimize the bacterial adhesion without affecting the animal cell attachment (Kumar, Sripriya, Balaji, Senthil Kumar, & Sehgal, 2011). This modification of collagen will greatly help in designing collagen molecule for applications in medicine, pharmacy, foods as well as cosmetics (Sripriya et al., 2011; Zhang et al., 2007).

In the similar fields as stated above, it should be noted that carboxymethyl cellulose (CMC) also plays an important role. CMC is an anionic polysaccharide obtained from cellulose through the reaction of alkali cellulose with monochloroacetate or its sodium salt in organic medium. It is a highly water-soluble anionic polysaccharide widely used in pharmaceuticals, as an emulsifying agent, in cosmetics, and in foods due to its desirable qualities, such as filming, emulsification, suspension, water retentivity and thickening (Kono, 2014). The non-toxicity, biodegradability and biocompatibility of CMC make it one of the most important cellulose derivatives (Arinaitwe & Pawlik, 2014).

A variety of desirable features can usually be presented by mixing protein with polysaccharides in pharmacy, food and cosmetic system (Pei et al., 2013; Wang et al., 2014; Lima et al., 2006). Typically, the blends of SC and CMC have potential to be applied in the fields of tissue engineering and cosmetic. It is noteworthy to mention that the properties of co-blending system are not only determined by the property of the individual protein and polysaccharide themselves but also determined by the interactions among the protein and polysaccharides (Pan, Xu, Jiang, Chen, & Jin, 2016),

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so this brings us to the important question of how SC and CMC interact with each other, which is crucial to the design and processing of products containing the two molecules.

Therefore, the principal objective of this study was to clarify the compatibility and interactions between SC and CMC in aqueous solution, using a combination of viscometric measurements, calorimetric measurements, two-dimensional (2D) FTIR correlation analysis and microscopic techniques. Finally, the changes in thermal stability of SC as a result of CMC were discussed, combining with the compatibility and interactions between SC and CMC.

2. Experimental

2.1. Materials

Carboxymethyl cellulose (CMC) was purchased from Kelong Corporation (China) with a degree of substitution of 0.7 and had a viscosity of ~1200 mPa s for solution with concentration of 2% at 25 °C.

Collagen was prepared from bovine skins by the method described by Zhang, Li, and Shi (2006). Succinylated collagen (SC) was then prepared strictly according to the method described by Sripriya et al. (2011) and the degree of conversion from available epsilon amino groups of lysine residues to carboxy groups due to succinylation was 96.0 ± 0.4%, as determined using trinitrobenzene-sulfonic acid (TNBS) method (Bubnis & Ofner, 1992).

Pyrene was purchased from Sigma Chemicals (Sigma-Aldrich, Munich, Germany).

2.2. Preparation of blends

All the preparation processes were conducted at 4 °C. Both of the SC and CMC were added slowly into deionized water (pH ~ 6.9) to obtain the final concentration of 1.0 mg/mL, and then the two solutions were blended with the SC/CMC ratios of 10/0, 9/1, 7/3, 5/5, 3/7, 1/9 and 0/10 (v/v), respectively. After stirred gently for 4 h, the SC/CMC blends, all of which had a total mass concentration of 1.0 mg/mL, were obtained. According to the SC/CMC ratio, the samples were named as SC, B(9/1), B(7/3), B(5/5), B(3/7), B(1/9) and CMC, respectively. The prepared solutions were stored at 10 °C before tests.

2.3. Viscometric measurements

All viscometric measurements of SC, CMC and their blended solutions were performed at (25.0 ± 0.1) °C, using a wsn-1 Ubbelohde-type capillary viscometer. The solutions for this measurement were prepared by diluting the stock solutions (1 mg/mL) described in Section 2.2 with water and the final weight concentration were 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL, respectively. Measurements started after an equilibration time of 15 min. The elution time of each solution is then determined as the average of at least six readings, in which the difference of each reading was confined to less than 0.2 s.

Classic Huggins Equation (Huggins, 1941) was used for determining the compatibility between SC and CMC, and the used equations were shown as follows:

$$\frac{[\eta]_{sp,m}}{C_{scol}+C_{cmc}} = [\eta]_m^{exp} + b_m (C_{scol}+C_{cmc}) \quad (1)$$

where the subscript "m" denotes "mixture". $[\eta]_{sp,m}$ is the specific viscosity of the blends, and $[\eta]_m^{exp}$ is the intrinsic viscosity of the blends determined experimentally.

$$[\eta]_m^i = w_1[\eta]_1 + w_2[\eta]_2 \quad (2)$$

where $[\eta]_m^i$ is the ideal intrinsic viscosity of the blends.

$$\Delta[\eta] = [\eta]_m^{exp} - [\eta]_m^i \quad (3)$$

$$\alpha = k_m - \frac{k_1 w_1^2 [\eta]_1^2 + 2\sqrt{k_1 k_2} w_1 w_2 [\eta]_1 [\eta]_2 + k_2 w_2^2}{(w_1[\eta]_1 + w_2[\eta]_2)^2} \quad (4)$$

compatible if $\Delta[\eta]_m < 0, \alpha \geq 0$; incompatible if $\Delta[\eta]_m > 0, \alpha < 0$ (Jiang & Han, 1998).

2.4. Calorimetric measurements

Calorimetric measurements were performed on a microcalorimeter (Microcal VP-DSC, Malvern) with deionized water as the reference. All samples were stored for 24 h at 4 °C before tests. All of the scans were conducted from 20 to 60 °C at a constant heating rate of 1 °C/min. Baseline controls were obtained with deionized water in both sample and reference chambers, and subtracted from the sample runs. The endothermal curves after subtracting the scanned baseline control are then plotted. The difference in the specific heat capacities of the native and denatured states (ΔC_p) was determined directly from the thermograms as the difference in the position of the baseline before and after melting. The calorimetric enthalpy change (ΔH) was calculated from the area under each peak. The peak fitting was performed with the origin 7.0 software package (available in Micro-Cal) using a cubic interpolation to draw the baseline for calculating the enthalpy change and a non two-state model with cursor initiation for the peak deconvolution.

2.5. Fourier transform infrared spectroscopy (FTIR) measurements

The samples for FTIR measurements were produced by lyophilizing the solutions mentioned in Section 2.2. FTIR spectra were recorded with a resolution of 4 cm^{-1} in the range of 4000–800 cm^{-1} using a FTIR spectrophotometer (Thermo Scientific Nicolet IS10, USA) and 32 scans were performed for each spectrum.

2.6. Two-dimensional (2D) FTIR correlation analysis

The series of perturbation-dependent spectra were obtained by varying compositions of the blends of SC and CMC. The FTIR spectra had been divided into two sets: set A of low CMC content and set B of high CMC content. The calculation of the generalized 2D correlation spectra was based on the software 2D Pocha (developed by Daisuke Adachi, Kwansei-Gakuin University, Japan). Visualization of the 2D IR correlation contour maps was performed with the use of Origin 8.5 software, where the regions with solid and dashed lines indicated positive and negative correlation intensities, respectively.

2.7. Atomic force microscopy (AFM)

The samples (6 μL) were dropped quickly and evaporated on mica, dried at room temperature (~20 °C) for two days. The microstructure of the dried samples was observed by atomic force microscopy (Shimadzu SPM 9600, Japan) in dynamic mode at room temperature (~20 °C). Each sample was scanned with a scanning rate of 1 Hz.