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Effect of sodium carboxymethyl cellulose on complex coacervates formation with gelatin: Coacervates characterization, stabilization and formation mechanism

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ABSTRACT

The complexation mechanism of gelatin (G) and carboxymethyl cellulose (CMC) and their coacervates formation process were studied as a function of pH and protein (Pr) to polysaccharide (Ps) mixing ratio (Pr:Ps). Three different CMCs were chosen (FL9, FH9 and FVH6) and five mixing ratio of 1:1, 6:1, 7:1, 8:1 and 9:1 (w/w), were studied to disclose their individual coacervates transition pattern for zeta potential, turbidity, morphology, size distribution and coacervates yield. The coacervates formation mechanism and stability of formed coacervates were examined using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR), Circular Dichroism (CD), Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). Coacervates with better morphology, good size distribution and highest yield were observed with G-FL9 at mixing ratio of 7:1 and pH 4.40. The evaluation of coacervates formation mechanism showed that G molecules experienced a conformational change in its secondary structure from a flexible pattern to an ordered poly-proline II (PPII) helix. The vibrations of O–H and N–H bonds spectra at 3437 and 3449 cm^{-1}, respectively for G/CMC coacervates, shifted to lower wave numbers due to the conformational changes of gelatin and CMC, during the coacervates formation. G/CMC complex coacervates were more thermally stable than individual gelatin. Therefore, these complex coacervates will be able to protect and deliver heat sensitive bioactives and food ingredients.

1. Introduction

Complex coacervation process occurs due to electrostatic attraction of oppositely charged biopolymers (Devi, Sarmah, Khatun, & Maji, 2016; Lv, Zhang, Zhang, Abbas, & Karangwa, 2013) that induce the formation of biopolymer soluble or insoluble complexes. The formation of insoluble complexes results in macroscopic phase separation into the coacervate phase and solvent rich phase (Anema & de Kruif, 2013; Kaushik, Dowling, Barrow, & Adhikari, 2015; Wee et al., 2014; Wu & McClements, 2015). Beside, other weak energy interactions especially hydrogen bonding, hydrophobic interactions can also contribute to the formation of complexes and coacervates (Turgeon, Schmitt, & Sanchez, 2007). The complexation process and the resultant coacervate types formed are driven and influenced by various parameters; pH, protein to polysaccharide ratio (Pr:Ps), total biopolymer concentration, molecular conformation and flexibility among others (Schmitt & Turgeon, 2011; Timilsena, Wang, Adhikari, & Adhikari, 2016; Wee et al., 2014; Wu et al., 2011). Generally, coacervates are formed when biopolymers with a low charge density and/or very flexible backbone are utilized, implying that charge density is critical in the formation of liquid coacervates (Lv, Zhang, Abbas, & Karangwa, 2012; Turgeon & Laneuville, 2009). The complex coacervation method has been used to develop effective delivery systems to encapsulate, protect, and release bioactives and flavors and has several benefits, such as high pay load, encapsulation efficiency (Prata & Grosso, 2015; Santos, Bozza, Thomazini, & Favaro-Trindade, 2015; Aziz, Gill, Dutilleul, Neufeld, & Kermasha, 2014) and controlled release of encapsulated materials (Dong et al., 2011).

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Proteins and polysaccharides have been extensively utilized in recent years as functional ingredients to improve texture, structure and shelf-life of most food products and for bioactives coating and delivery (Anvari, Pan, Yoon, & Chung, 2015; Niu et al., 2015; Qiu, Zhao, & McClements, 2015; Wu & McClements, 2015). The interactions between proteins and polysaccharides are either attractive or repulsive. They can be manipulated to form a variety of biopolymer complexes, such as soluble complexes, coacervates or precipitates (Anvari et al., 2015; Jones, Lesmes, Dubin, & McClements, 2010; Souza, Garcia Rojas, Melo, Gaspar, & Lins, 2013). Protein and polysaccharide complex coacervates exhibit new functional properties by combining advantages of both biopolymers (Yan & Zhang, 2014).

Gelatin is a natural non-toxic water-soluble protein derived from collagen. Polypeptide structure of gelatin molecule facilitates its interactions with other oppositely charged ingredients (Dai, Wu, Li, Zhou, Li, & Chen., 2010; Milanović, Petrović, Sovilj, & Katona, 2014), which makes it a very important wall material used for microcapsules production by complex coacervation (Dai et al., 2010; Wu & McClements, 2015; Zhang, Zhang, Hu, Bao, & Huang, 2012).

Sodium carboxymethyl cellulose (CMC) is one of the most important anionic nontoxic water-soluble polysaccharides widely used in food, pharmaceutical and medical industry (Koupantsis, Pavlidou, & Paraskevopoulou, 2016; Nur Hazirah, Isa, & Sarbon, 2016). Structurally, CMC (degree of substitution, DS, viscosity and molecular weight, Mw) influences the stability of protein in acid environment (Du et al., 2009). The DS, usually in the range of 0.4–1.5 is an important characteristic of CMC owning to its charge distribution, which defines its electrostatic interactions. CMCs with higher DS have stronger negative charges that induce more electrostatic attraction with protein (Iwasaki, Shioi, & Kouno, 1977; Li et al., 2017). For those with DS above 1.5, it is hard to produce unclustered coacervates and microcapsules, whereas that of DS below 0.4 exhibits a very low solubility (Du et al., 2009; Iwasaki, Shioi, & Kouno, 1977).

The combination of gelatin and gum Arabic is the most common system used in studies of complex coacervation (Anvari et al., 2015; Lv et al., 2013). For food systems, CMC is the most widely used material compared to GA due to its biodegradability, biocompatibility and relatively low cost (Carpineti, Martinez, Pilosof, & Pérez, 2014; Devi et al., 2016). However, the few studies, which reported the use of gelatin and CMC to prepare microcapsules (Devi & Maji, 2011; Wu et al., 2011), surfactants were added to achieve protein/CMC microcapsules by complex coacervation method. This stems from the fact that CMC has a weak surface-activity and difficult to form protein/CMC microcapsules when used alone in complex coacervation technique (Dai et al., 2010; Wu et al., 2011). Additionally, the use of CMC is limited by the narrow pH range of protein/CMC coacervation compared to GA. To the best of our knowledge, gelatin and CMC complex coacervation and its formation mechanism still have not been well documented.

The aim of this work was therefore to study the G-CMC complex coacervate formation process and its mechanism. We systematically studied the complex coacervate formation process between G and different CMC types and investigated their conformational transition and the nature of interaction at the molecular level. The complex coacervates formation process as a function of pH and protein to polysaccharide mixing ratio (G/CMC MR) was carried out. Optical microscopy was used to evaluate the coacervates morphology during complex formation. ATR-FTIR and Far-UV CD were used to evaluate the nature of interaction between G and CMC and their conformational transitions during the complex formation. The thermal stability of the resultant product was investigated based on TGA and DSC measurements.

2. Materials and methods

2.1. Materials

Gelatin (G, type B, bloom 225), sodium hydroxide and acetic acid were purchased from Shanghai Chemical Reagent Corporation (Shanghai, China). Three types of food grade sodium carboxymethyl cellulose (CMC) FL9, FH9 and FVH6 with different degree of substitution (DS) 1.03, 0.95 and 0.86 respectively, were purchased from Yixing Tongda chemical corporation (Wuxi China). All materials were used without any further purification. All aqueous solutions were prepared with deionized water (Milli-Q water).

2.2. Preparation of stock solutions

Stock dispersions of individual G, CMC or G/CMC with desired ratio were prepared in percentage by weight (% wt.) with determined biopolymer concentration. Biopolymer powders were dissolved in deionized water under gentle stirring at 60 °C for 2 h. Then, the stock dispersions were centrifuged at 5000 rpm for 30 min at room temperature to remove insoluble matter or air bubbles. The homogeneous mixtures were used for the further processes and tests. The pH of the G, CMC and G-CMC mixture stock solutions were 6.5, 6.6 and 6.3 respectively.

2.3. Determination of CMC properties

2.3.1. Analysis of molecular weight

The molecular weight (MW) distribution of CMC samples were determined using high performance gel-filtration chromatography (HPGFC). Waters 1525 liquid chromatography system (Waters Co., Milford, MA, USA) equipped with 2410 refractive index (RI) detector and Empower work station was used for this experiment. The Ultrahydrogel^{TM}Linear (300 mm × 7.8 mmi.d × 2, Japan) column was used while the mobile phase (0.1 M $NaNO_3$) was delivered at a flow rate of 0.9 ml/min and the column temperature was set at 30 °C. CMC samples 0.5% (w/v) were dissolved in 0.1 M $NaNO_3$ solution and filtered through 0.22 μm nylon syringe filters, then injected into the HPLC system. A molecular weight calibration curve was obtained from the following Dextran standards from Sigma: Dextran T-2000 (2,000,000 Da), Dextran T-150 (133,850 Da), Dextran T-40 (36,800 Da), Dextran T-10 (9750 Da) and Dextran T-5 (2700 Da).

2.3.2. Viscosity measurement

The CMC (1% w/v) viscosity was measured at 25 °C by using a Brookfield viscometer (LVDV2T model; Brookfield Engineering Laboratories. Inc., Middleboro, MA, USA) with spindle speeds at 100 rpm and a shear rate of 60 s^{-1}. Spindle LV-3 was used to get readings within the scale. Sample was filled into the cylindrical vessel and allowed to equilibrate at desired temperature. In order to control the temperature, the water-jacketed stainless steel cylindrical vessel was connected to a constant temperature bath which was able to maintain temperature uniformity within 25 ± 0.1 °C. The viscosity reading was taken after 60 s in each sample.

2.4. Determination of optimum pH and G/CMC ratio for complex coacervation

2.4.1. Zeta potential measurement

The Zeta potential was determined against pH change at 45 °C of individual biopolymer dispersions G, CMC and their mixture formulations at a concentration of 0.1%, w/v. Zeta potential measurements were performed at 25 °C using a Zetasizer Nano-ZS (Malvern Instruments, Westborough, MA UK) capable of electrophoresis