Rheological properties of O-carboxymethyl chitosan — gum Arabic coacervates as a function of coacervation pH

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ABSTRACT

The rheological properties of O-carboxymethyl chitosan (O-CMC) — gum Arabic (GA) coacervates varied greatly with the coacervation pH. At pH 3.0, the coacervate displayed the flow induced shear thickening property in shear rates below $100 \ s^{-1}$ and then behaved as a Newtonian fluid hereafter; at pH 4.5, the coacervate exhibited the characteristic shear thinning property of a pseudoplastic fluid; while at pH 6.0, the coacervate occurred as a Bingham fluid. The highest viscosity and modulus values of the coacervates were recorded not in the pH value with maximum electrostatic interaction. Zeta potential analysis revealed that the high proportion of O-CMC in the coacervates possibly contributed to the unique rheological properties of the coacervates. SEM observation imaged a layer-by-layer structure for the coacervates prepared at pH 3.0, 4.5, and 6.0. It was concluded that the composition of the O-CMC — GA coacervates greatly influenced their rheological properties and the desired property can be obtained by varying the coacervation pH.

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1. Introduction

Complex coacervation, which refers to the spontaneous liquid/liquid phase separation in colloidal systems driven by the electrostatic interaction between two oppositely charged colloids, is a unique and promising microencapsulation technology because of the very high payloads achievable (up to 99%) and the controlled release possibilities based on mechanical stress, temperature, or sustained release (Gouin, 2004). In addition to its wide applications in microencapsulation, the products of complex coacervation reactions, termed coacervates, have also found potential utilizations in many fields (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998).

Owning to the excellent safety and biodegradability of biopolymers (proteins and polysaccharides) involved in complex coacervation and the possible combination of their functional properties, coacervates are quite promising as novel food ingredients and biomaterials. For example, the whey protein isolate (WPI) — xanthan gum complexes can be used as fat replacer in neutral pH products to yield low-fat cake frostings and sandwich cookie fillings (Laneuville, Paquin, & Turgeon, 2005) and the WPI — low methoxyl pectin coacervate is able to substitute partial cream in Petit-Suisse cheeses without lowering the overall sensory acceptability (Ramírez-Santiago, Lobato-Calleros, Espinosa-Andrews, & Vernon-Carter, 2012).

The application of coacervates depends greatly on their rheological properties (Espinosa-Andrews, Sandoval-Castilla, Vázquez-Torres, Vernon-Carter, & Lobato-Calleros, 2010). Weinbreck, Wientjes, Nieuwenhuijse, Robijn, and de Kruif (2004) systematically explored the mechanism involved in the rheological properties of coacervates using the whey protein — gum Arabic (GA) pair as the model system and found that the high viscosity of the coacervates was mainly due to the attractive electrostatic interactions between the two biopolymers. This pioneering research provides valuable information for understanding the rheological properties of coacervates.

The electrostatic interaction involved in complex coacervation is mainly affected by ionic strength, initial polyelectrolyte ratio, and coacervation pH (Doublier, Garnier, Renard, & Sanchez, 2000). Generally, the addition of salt shields the electrostatic interaction between polyelectrolytes and thus weakens the rheological properties of coacervates. For instance, the storage moduli (G') of the bovine serum albumin — pectin coacervates are inversely proportional to NaCl concentration in the range 0.01—0.4 mol/L and the biopolymer ratio at which complete charge neutralization occurs produces the highest G' value (Ru, Wang, Lee, Ding, & Huang, 2012). The acidity at which coacervation occurs is another important influencing factor and the pH with maximum electrostatic interaction leads to the highest viscoelastic properties (Espinosa-

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Andrews et al., 2010). These findings support the hypothesis of Weinbreck, Wientjes, et al. (2004) very well.

Chitosan − alginate is the most intensively studied coacervation pair in addition to the gelatin − GA combination and has been broadly used in the microencapsulation and intestine-targeted delivery of probiotics and drugs (George & Abraham, 2006), but new complex coacervation systems based on chitosan are emerging in recent years. Among the systems, the chitosan − GA combination has attracted the most interests and the optimum conditions for their coacervation and the properties, composition, and application of the resultant coacervates have been reported by multiple authors (Coelho et al., 2011; Espinosa-Andrews, Baez-Gonzalez, Cruz-Sosa, & Vernon-Carter, 2007; Espinosa-Andrews et al., 2013, 2008, 2010; Malviya, Shukla, & Srivastava, 2009; Moschakis, Murray, & Biliaderis, 2010). The chitosan − GA coacervates formed in pH ranging from 2.5 to 7.0 display predominately the viscous property and the highest viscoelastic properties are observed in the pH value and biopolymer ratio at which maximum coacervation occurs (Espinosa-Andrews et al., 2013, 2010). These researches provide deep insights for designing tailor made chitosan − GA coacervates with desired functional properties.

Nevertheless, chitosan is soluble only in acidic solutions, which limits some of its applications. O-carboxymethyl chitosan (O-CMC) is an important derivative of chitosan and has many outstanding properties over native chitosan (Jayakumar et al., 2010). Our preliminary work found that the O-carboxymethylation modification markedly weakened the coacervation with GA compared with native chitosan. Nevertheless, the rheological properties of the resultant coacervates have not been concerned yet.

The purpose of this work is to investigate the effect of coacervation pH on the rheological properties of O-CMC − GA coacervates and explore the possible mechanism by zeta potential measurement, so that with this information specific applications of these coacervates can be achieved.

2. Materials and methods

2.1. Chemicals

Chitosan of viscosity 500 mPa·S and 95.2% degree of deacetylation was purchased from Shandong Lake Crustacean Products Co., Ltd (Qingdao, China). Gum Arabic (GA) was a gift from Guangzhou Darong Trading Company (Guangzhou, China). All other reagents were of analytical grade.

2.2. Preparation of O-CMC

O-CMC was prepared in strict compliance with the method of Chen and Park (2003). The degree of substitution of the resultant derivative was 0.35 according to potentiometric titration (Ge & Luo, 2005).

2.3. Preparation of O-CMC − GA coacervates

The O-CMC − GA coacervates were prepared by blending the stock solutions of O-CMC and GA in proper proportions followed by dilution with distilled water to produce a final reaction system of GA to O-CMC ratio of 4:1 and total biopolymer concentration of 2.5%. The initial pH values of the solutions were adjusted to 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, or 6.5 by adding either HCl (0.1 mol/L) or NaOH (0.1 mol/L) when required. The mixed solutions were allowed to react at 25 °C for 1 h under magnetic agitation. Afterward, the coacervate phases were separated by centrifugation at 1980 g for 10 min and collected for morphological observation, rheological property measurement, and/or SEM observation.

2.4. Coacervate yield measurement

The coacervate yield was calculated according to the following formula:

$$\text{Coacervate yield}(\%) = \frac{m_i}{m_0} \times 100$$

in which, m_0 is the total biopolymers weight used to make the biopolymers solutions and m_i is the weight of the freeze-dried coacervate phase.

2.5. Rheological measurement

The rheological measurements of the coacervates prepared at pH 3.0, 4.5, and 6.0 were performed using a MCR30 rheometer (Paar Physica, Messtechnik, Stuttgart, Germany) at 25 °C. Truncated cone−plate geometry (2°, 6 cm diameter) was used, in which the truncated cone had a gap of 0.047 mm between the flat surfaces of both elements. For each sample, flow curves were measured at increasing shear rate from 0.3 s^{-1} to 200 s^{-1} followed by a reverse flow at decreasing shear rate from 200 s^{-1} to 0.3 s^{-1}. The ramp mode was logarithmic and the time between two measurements was 30 s. Frequency sweeps (0.1–100 Hz) were carried out as well for a strain of 1.0%, which was in the linear regime as checked by strain sweep measurements (data not shown).

2.6. Zeta potential measurement

The electrical charge of the dilute phase was measured using a particle electrophoresis Nanoseries ZS instrument (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, United Kingdom) at 25 °C.

2.7. SEM observation

The freeze-dried powders of O-CMC − GA coacervates prepared at coacervation pH 3.0, 4.5, and 6.0 as well as those of O-CMC and GA were mounted on circular aluminum stubs with double-sided sticky tape, coated for 250 s with 15 nm gold, then examined and photographed in a scanning electron microscope (JSM-840 A, Jeol, Ltd., Tokyo, Japan) at an accelerating voltage of 15 kV.

2.8. Statistical analysis

All the experiments were performed on triplicate samples and values were expressed as mean values ± SD. Differences between mean values were conducted using the one-way analysis of variance (ANOVA) by SPSS 16.0 software. Differences were statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Effect of pH on O-CMC − GA coacervation

The medium pH strongly affects the charge density of biopolymers and in turn, the strength of coacervation. The pH value at which the maximum coacervate yield occurs corresponds to the strongest electrostatic interaction between the polyelectrolytes (Espinosa-Andrews et al., 2008). As can be seen in Fig. 1, the maximum coacervate yield was achieved at pH 3. When the acidity drifted from this value, the coacervate yield decreased significantly ($p < 0.05$). This value was lower than that of 4.5 for the chitosan − GA pair (Espinosa-Andrews et al., 2007). The pKa of the carboxyl group in O-CMC was reported in the range 2.0–4.0 (Feng et al.,