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Interrelationship between the zeta potential and viscoelastic properties in coacervates complexes

Hugo Espinosa-Andrews[a,*], Karina Esmeralda Enríquez-Ramírez[a], Eristeo García-Márquez[b], Cesar Ramírez-Santiago[c], Consuelo Lobato-Calleros[c], Jaime Vernon-Carter[b]

[a] Unidad de Tecnología Alimentaria, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. Av. Normalistas #800, Guadalajara, Jalisco 44270, México
[b] Departamento de Ingeniería de Procesos e Hidráulica, Universidad Autónoma Metropolitana – Iztapalapa, San Rafael Atlixco # 186, México, D.F. 09340, México
[c] Departamento de Preparatoria Agrícola and Departamento de Ingeniería Agroindustrial, Universidad Autónoma Chapingo, Km. 38.5 Carretera México – Texcoco, Texcoco, Edo. México 56230, México

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ABSTRACT

The formation of the complex coacervate (CC) phases between gum Arabic (GA) and low molecular weight chitosan (Ch) and the interrelationship between the zeta-potential and viscoelastic properties of the coacervate phase were investigated. The maximum charge difference of biopolymers stock dispersion was displayed in a range of pH between 4.0 and 5.5. Titration experiment between the oppositely charged biopolymers showed that the isoelectric point was found at a biopolymers mass ratio ($R_{[GA:Ch]}$) of $R_{[5.5:1]}$. Turbidity, size and ζ-potential of the soluble complexes (SC) showed an interrelation with the complex coacervate yield (CCY). Higher CCY values (82.2–88.1%) were obtained in the range from $R_{[3:1]}$ to $R_{[5.5:1]}$. Change the $R_{[GA:Ch]}$ in dispersion, make possible to produce CC's phases exhibiting cationic ($R_{[1:1]}$ and $R_{[3:1]}$), neutral ($R_{[5.5:1]}$) or anionic ($R_{[9:1]}$ and $R_{[7:1]}$) charged. All CC's exhibited liquid-viscoelastic behavior at lower frequencies and a crossover between G'' and G' at higher frequencies.

1. Introduction

Biopolymer–biopolymer interactions play an increasingly important role in modern-day researches in food science and biotechnology because they influence the microstructure formation of most biopolymer-containing systems, determining in great extent their texture, mechanical stability, consistency and, ultimately, appearance and taste (Semenova, 2007). In order to achieve a desired functionality (e.g., emulsifying, texture or film properties) in a product, a greater understanding of the factors affecting biopolymers interactions is required, particularly those of electrostatic origin. Attractive interactions between two biopolymers can become evident in various ways: (i) formation of small soluble complex (SC), manifesting itself in murky solutions, (ii) formation of a homogeneous weak gel, if interactions are weak, and (iii) precipitation of both biopolymers, if interactions are strong (Walstra, 2003). The nature of these interactions may lead to either segregative or associative phase behavior, depending on biopolymer characteristics (e.g., charge density, size, type and distribution of reactive groups), biopolymer concentration and ratio, and solvent conditions (e.g., pH, temperature, ionic strength) (Espinosa-Andrews, Báez-González, Cruz-Sosa, & Vernon-Carter, 2007; Espinosa-Andrews, Sandoval-Castilla, Vázquez-Torres, Vernon-Carter, & Lobato-Calleros, 2010; Liu, Low, & Nickerson, 2010; Schatz et al., 2004; Singh et al., 2007).

Complex coacervation involves spontaneous separation into coexisting solvent-rich and solvent-depleted phase, the latter consisting of a co-precipitate of both biopolymers (Dickinson, 1995). Complex coacervates (CC) have many applications in the fields of biotechnology, pharmaceutical and food industry and its specific application will depend on their structure and rheological properties (Espinosa-Andrews et al., 2010). For example, one of the main applications of the electrostatic complexes is in the stabilization of emulsions and in the formation of wall materials of microcapsules for obtaining enhanced functional properties (Pérez-Orozco, Barrios-Salgado, Román-Guerrero, & Pedroza-Islas, 2011). Recently, in the field of food science, Ramírez-Santiago, Lobato-Calleros, Espinosa-Andrews, & Vernon-Carter (2012) reported that it is possible to formulate reduced-fat

* Corresponding author. Tel.: +52 333345 5200; fax: +52 333345 5200 1001.
E-mail addresses: hespinosa@ciatej.net.mx, andrewshugo@hotmail.com (H. Espinosa-Andrews).

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Petit-Suisse cheeses where milk cream (i.e., milk fat globules) was partially substituted by a whey protein isolate–low-methoxyl pectin CC, displaying dynamic rheological properties and overall sensory acceptability similar to that of a full-fat Petit-Suisse cheese. Nevertheless, the key to moving further in the field of practical applications is to redouble efforts in the investigation of the structures and physicochemical characteristics of the CC.

The objective of this work was to determine the conditions leading to the formation of the complex coacervate between gum Arabic-low molecular weight chitosan and to establish an interrelationship between the zeta potential and viscoelastic properties of the coacervate phase.

2. Material and methods

2.1. Materials

Low molecular weight chitosan, (Ch; 92.2% degree of deacetylation, DDA) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Ch (1–300 kDa) is a cationic heterogeneous binary polysaccharide that consists primarily of 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose residues (Claesson & Ninhami, 1992); is produced by chemical, thermal, or biochemically degradation of high molecular weight chitosan (Niederhofer & Müller, 2004). The techno-functional applications of Ch are principally as a drug carrier for delivery systems (Park, Saravanakumar, Kim, & Kwon, 2010), emulsion stabilizer (Klinkesorn & Namatsila, 2009) or as a barrier material (Hernández-Ochoa, Gonzales-Gonzales, Gutiérrez-Mendez, Muñoz-Castellanos, & Quintero-Ramos, 2011). Gum Arabic (GA; *Acacia senegal*) tear drops was purchased from Natural Products (Yautepec, State of Morelos, Mexico). GA is a branched, neutral or slightly acidic, complex polysaccharide obtained as mixed calcium, magnesium and potassium salts (Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). Studies on the structure of GA indicate that the molecules consist of a β $(1\rightarrow3)$ linked galactopyranose backbone chain with numerous branches linked through β $(1\rightarrow6)$ galactopyranose residues and containing arabinofuranose, arabinopyranose, rhamnopyranose, glucuronic acid and 4-*O*-methyl-D-glucuronic acid, with small amount of proteinaceous material as an integral part of the structure (Espinosa-Andrews et al., 2008). Acetic acid (AA), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from J.T. Baker (Xalostoc, State of Mexico, Mexico).

2.2. Stock dispersions

Stock dispersions of both polysaccharides were prepared by dispersing Ch (3 wt%) in deionized water with acetic acid (1%, v/v) and the GA tear drops were pulverized, dissolved in deionized water and filtered for preparing a 10 wt% dispersion. Both stock dispersions were gently stirred for 12 h and stored overnight at 4 °C to ensure complete hydration of the biopolymers.

2.3. Zeta (ζ) potential of stock dispersions

The ζ-potential was determined using the Zetasizer Nano ZS90 equipment (Malvern Instruments, Worcestershire, UK). The stock dispersions were diluted to a concentration of approximately 0.01 wt% using deionized water prior to analysis. Diluted polysaccharides dispersions (10 mL) were transferred to an autotitrator MPT-2 (Malvern Instruments, Worcestershire, UK) to adjust the pH of the dispersions using either 0.1 N HCl or 0.1 N NaOH. Experiments were performed at pH ranging from 2 to 7 every 0.5 units with a pH resolution of $\pm$0.05 units. The ζ-potential was determined by measuring the direction and velocity of the biopolymers dispersion as they moved along the applied electric field. The equipment software converted the electrophoretic mobility measurements into ζ-potential values using the Smoluchowsky mathematical model.

Dilutions of 0.2 mg mL^{-1} of Ch and 5 mg mL^{-1} of GA were prepared in order to carry a titration experiment between the oppositely charged biopolymers. Ch (10 mL) was transferred to the autotitrator MPT-2 at pH of 4.5. Then, the GA was added above the isoelectric point of the mixture. At the same time the size distributions of the GA/Ch aggregates were determined using the Zetasizer Nano ZS90 by plotting the *z*-average radius (R_h) as a function of anionic polysaccharide (GA) addition. The R_h was calculated using the Stokes–Einstein equation: $R_h = k_B T/6\pi\eta_s D$; where k_B is the Boltzmann constant, T is the absolute temperature, η_s is the dynamic viscosity of the solvent and D is the *z*-average translational diffusion coefficient. The titration measurements are reported as the average $\pm$ standard deviation of measurements made on three independent samples with three measurements made per run at 20 °C.

2.4. Preparation of biopolymers mixtures

Based on the titration experiment between the oppositely charged biopolymers, five different weight biopolymers mass ratio ($R_{[GA:Ch]}$) at 5% (w/w) of total biopolymers weight concentrations were made ($R_{[1:1]}$, $R_{[3:1]}$, $R_{[5.5:1]}$, $R_{[7:1]}$ and $R_{[9:1]}$), by mixing the requisite amount of both stock dispersions at a constant pH value of 4.5 (Espinosa-Andrews et al., 2007). The dispersions were left to rest at 20 °C and turbidity and electrophoretic measurements were taken daily until two consecutive readings were equal (usually between 5 and 7 days). Complete separation of the precipitated CC from the SC was achieved by centrifuging at 8000 rpm for 10 min.

2.5. Characterization of the biopolymers complexes

2.5.1. Soluble complexes

The turbidity of the SC was characterized by measuring the optical density at λ = 600 nm using a Cintra 6 UV–vis spectrophotometer (GBC Scientific Equipment Pty. Ltd., Braeside, VIC, Australia). At this wavelength both individual polyelectrolytes do not absorb light. Deionized water was used to establish the baseline (Espinosa-Andrews et al., 2007).

The ζ-potential and the R_h of the SC were measured using the Zetasizer Nano ZS90 equipment at 20 °C.

2.5.2. Insoluble complexes

Complex coacervate yield (CCY) was determined using the following equation (Ramírez-Santiago et al., 2012).

$$\text{CCY} = \frac{\text{weight of CC in d.b.}}{\text{total weight of Ch} + \text{GA in the dispersion}} \times 100 \quad (1)$$

The ζ-potential of the CC was measured as a function of pH. Approximately 0.3 g of the CC were put into 10 mL HCl (0.02 N) in order to decrease the pH of the CC's, shaken vigorously and stored for 24 h. The pH of the redispersed CC was adjusted with 0.1 N NaOH from 2 to 7 every 0.5 units with a pH resolution of $\pm$0.1 units, and the ζ-potential was measured at the different pH values.

2.6. Rheological measurements

CC viscoelastic properties were determined with an AR1000 rheometer (TA Instruments, Newcastle, DE, USA) coupled to a truncated cone–plate geometry (1°, 60 mm diameter) with a gap of 0.029 mm between the flat surfaces of both elements at 5 °C (Espinosa-Andrews et al., 2010). This temperature was chosen for enhanced the sensibility of the measurements and to avoid sample evaporation. Amplitude strain sweeps (0.1–100%) were applied