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A gelation mechanism for gelatin/polysaccharide aqueous mixtures

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ABSTRACT

Gelatin/xanthan gum (XG, an anionic polysaccharide) and gelatin/chitosan (CHI, a cationic polysaccharide) aqueous mixtures exhibit enhanced gelation properties compared to the neat components solutions. The gelation properties are governed by the extent of complexation, and therefore are affected by pH and protein to polysaccharide ratio. Inhibition or low extent of complexation results in a slow increase of the elastic modulus (G'), whereas too strong complexation leads to phase separation by precipitation. A maximum G' is obtained near a pH where phase separation occurs, and the microstructure at this optimal pH is a network structure characterized by biopolymer-rich and biopolymer-poor domains. Based on this rheology-microstructure relationship, a general gelation mechanism on a molecular level for gelatin/polysaccharide aqueous mixtures is proposed. This mechanism is further supported by the results of micro-differential scanning calorimetry.

1. Introduction

The interactions between proteins and polysaccharides in solution have received increasing interest in recent years since they can be utilized to control the functional properties of food products (Le, Rioux, & Turgeon, 2017; Schmitt & Turgeon, 2011; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998; Turgeon & Laneuville, 2009), separate proteins (Wang Y. F. et al., 1996), and design delivery matrices for bioactive molecules (Le et al., 2017; Turgeon and Laneuville, 2009; Zhu et al., 2007). Two types of interactions occur depending on environmental factors such as pH and ionic strength: segregative phase separation (thermodynamic incompatibility) and associative phase separation (thermodynamic compatibility) (Turgeon and Laneuville, 2009; van der Wielen, van de Heijning, & Brouwer). Segregative phase separation leads to the formation of protein-rich and polysaccharide-rich phases due to electrostatic repulsion, whereas associative phase separation results in solvent-rich and biopolymer-rich phases caused by electrostatic complexation. Three different mixed protein/polysaccharide networks can form through the two types of interactions, namely interpenetrating gels, phase-separated gels and coupled gels (Stokes, 2012). Mixed gels can also display a combination of those features, and have greater flexibility in terms of mechanical properties compared to those of individual components. For example, enhanced and tunable gelation properties are obtained for some mixtures by controlling the pH, ionic strength and protein-to-polysaccharide ratio, such as low bloom index gelatin B/xanthan gum (L-GB/XG) (Wang C.-S. et al., 2016) and β-lactoglobulin/XG systems (Bertrand & Turgeon, 2007; Le & Turgeon, 2013; Sanchez, Schmitt, Babak, & Hardy, 1997). For the moment, the majority of protein/polysaccharide mixed gel studies have focused on proteins and anionic polysaccharides, with fewer works on proteins and cationic polysaccharides.

Chitosan (CHI), as the only naturally derived cationic polysaccharide, has been of great interest in various areas, such as in food technology, biomedical and pharmaceutical industries due to its good biodegradability, high biocompatibility, low toxicity and excellent antibacterial activity (Chen R.-H. et al., 2011; Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003; Riva et al., 2011). CHI has a pKa around 6.3–6.5, thus is soluble only in acidic solutions (Ravi Kumar, 2000; Rinaudo, 2006). Recently, it was reported that GB and CHI can form a gel in a pH range where the two biopolymers are oppositely-charged, although a long storage time is required (Wang X.-Y. et al., 2016). More detailed studies are needed to understand this gelation mechanism.

Recently, we have proposed a specific gelation mechanism for

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GB/XG aqueous mixtures displaying enhanced (synergistic) viscoelastic properties (Wang, Virgilio, Wood-Adams & Heuzey, 2017). In this work, the gelation behavior of mixed gels of gelatin (Type A and B) and 2 types of oppositely charged polysaccharides (XG and CHI) was investigated in order to propose a more general mechanism behind the gelation of protein/polysaccharide aqueous mixtures.

2. Materials and methods

2.1. Materials

Four grades of gelatin, namely: 48,720, (type A, Bloom index = 50–75, $c_{crit} \approx 2.5\%$ w/v, L-GA, purity > 95%); G1890 (type A, Bloom index = 300, $c_{crit} \approx 1.0\%$ w/v, H-GA, purity > 95%); G6650 (type B, Bloom index = 75, $c_{crit} \approx 4.0\%$ w/v, L-GB, purity > 95%) and G9382 (type B, Bloom index = 225, $c_{crit} \approx 2.0\%$ w/v, H-GB, purity > 95%), were purchased from Sigma-Aldrich, Canada. Xanthan gum (XG) (G1253, MW: ~2–4 x 10^6 kDa, purity > 85%) was also purchased from Sigma Aldrich, while chitosan (CHI) (degree of deacetylation 90%, dynamic viscosity 1000 mPa·s, MW: 200–300 kDa, purity > 90%) was supplied by BioLog Biotechnologie und Logistik GmbH (Landsberg, Germany). Other chemicals (HCl, NaOH, Nile Blue A and 5-(4,6-dichlorotriazinyl) aminofluorescein, Fluorescein 5 (6)-isothiocyanate) were of analytical grade (Sigma Aldrich, Canada), and used as received.

2.2. Sample preparation

Gelatin solutions (0.4–4.0% w/v) were prepared by allowing gelatin powder to swell in Milli-Q water (18.2 Ω) for 15–20 min at room temperature, followed by stirring at a speed of ~300 rpm at 60 °C for 15 min. XG solutions (0.2 and 0.4% w/v) were prepared by dissolving the powder into Milli-Q water at a stirring speed of 600–700 rpm for at least 12 h at room temperature. CHI solutions (0.4 and 0.8%, w/v) were obtained by dissolving CHI powder into 50 mM acetic acid at a stirring speed of 600–700 rpm for at least 6 h at room temperature. Mixed gelatin/XG and gelatin/CHI solutions with fixed XG concentration (0.2% w/v) and CHI concentration (0.4% w/v), and different gelatin concentrations (0.2–1.6% w/v), were prepared by mixing equal volumes of gelatin and XG or gelatin and CHI primary solutions while stirring at 60 °C for approximately 30 min. The pH of the mixtures was adjusted using 1M HCl or NaOH to the desired values.

2.3. Zeta potential measurements

Zeta potential values of gelatin, XG and CHI solutions were determined by laser doppler velocimetry and phase analysis light scattering (M3-PALS) using a Malvern Zetasizer Nano ZSP instrument (Malvern Instruments Ltd., Malvern, Worcestershire, UK). The zeta potential was determined from the direction and velocity of the molecules in the applied electric field. The Smoluchowski model was used by the software to convert the electrophoretic mobility measurements into zeta potential values. All the samples were diluted to about 0.05% (w/v) and then put into a disposable folded capillary cell (DTS1060) to measure the zeta potential. The temperature of the cell was maintained at 25 °C. The data presented are the average values of three individual measurements.

2.4. Gel point determination

Small volumes (7–8 mL) of freshly prepared gelatin/XG or gelatin/CHI mixed solutions were transferred into 20 mL vials (Fisherbrand, O.D. × H (with cap): 28 × 61 mm) and kept at room temperature for 24 h. The vials were then inverted for 1 min to qualitatively assess gel formation and strength, and to determine the critical gelling concentration c_{crit} over which no flow is observed.

2.5. Time-resolved small amplitude oscillatory shear

Freshly prepared neat or mixed solutions were directly poured into a rough surface Couette flow geometry (cup and bob diameters of 18.066 mm and 16.66 mm, respectively) and measurements were performed using a stress-controlled Physica MCR 501 rheometer (Anton Paar, Graz, Austria). Before the time sweep tests, all systems were heated at a rate of 5 °C/min up to 60 °C. The samples were kept at this temperature for 10 min to erase the previous thermal histories and were subsequently cooled down to 20 °C at a rate of 5 °C/min. Dynamic time sweep measurements were performed at 1 rad/s and 20 °C in the LVE regime ($\gamma_0 = 3\%$) for 8 h. The storage modulus (G'), loss modulus (G''), and related complex viscosity ($|\eta^*|$) were recorded as functions of time. Samples were covered with a thin film of low viscosity mineral oil to prevent water evaporation. The oil was shown not to affect the rheological measurements. The experiments were performed at least twice with good reproducibility (<5%).

Time-resolved dynamic rheological temperature sweeps were performed at 1 rad/s in the LVE regime ($\gamma_0 = 3\%$). Samples were first heated up to 60 °C to a homogeneous state for 30 min. The temperature was then rapidly lowered (10 °C/min) to the desired value, and G', G'' and $|\eta^*|$ were recorded as a function of time during 60 min. This procedure was repeated until the temperature reached 20 °C.

2.6. Confocal laser scanning microscopy

CLSM observations of the gelatin/XG and gelatin/CHI solutions were performed with an Olympus IX 81 inverted confocal microscope (Olympus Canada Inc., Richmond Hill, ON, Canada). Gelatin (A and B) was stained with Nile Blue A (N0766, Sigma) in solution under magnetic stirring for 30 min before mixing with XG or CHI solutions. On the other hand, XG was covalently labelled with 5-(4,6-dichlorotriazinyl) aminofluorescein (DTAF) (D0531, Sigma) using a method described previously (Wang C.-S. et al., 2016). CHI was covalently labelled with Fluorescein 5 (6)-isothiocyanate (FITC) using a modified method of Qaqish et al. (Qaqish & Amiji, 1999). CHI and FITC were first dissolved in 200 mL 0.1 M HCl and 200 mL dehydrated methanol, respectively. The two solutions were then mixed together and kept in the dark at room temperature for 3 h with gentle stirring for chemical grafting. The pH was adjusted to 10.0 by adding 0.1 M NaOH to precipitate FITC-labelled CHI. The precipitate was obtained by centrifugation (10,000 g, 30 min) and was then dissolved in 0.1 M HCl. The FITC-labelled CHI solution was exhaustively dialyzed against Milli-Q water in the dark for 3 days to remove any unreacted FITC and the counterions. Sodium azide (0.02 wt%) was added to inhibit bacteria growth, and the Milli-Q water was changed every 2 h during dialysis. After freeze-drying, FITC-labelled CHI (yellow powder) was obtained (yield = 98%). Preliminary experiments showed that labeling does not change the rheological behavior of the solutions. After mixing, solution samples were poured in Petri dishes (P35G-1.5–14-C, MatTek), closed with cover slips and hermetically sealed with oil. Observation of XG and CHI was made by excitation of DTAF and FITC at 488 nm, the emission being recorded between 510 and 550 nm. Observation of gelatin was made by excitation of Nile Blue A at 633 nm, the