



Phase transition kinetics and rheology of gelatin–alginate mixtures



Thomas B. Goudoulas, Natalie Germann*

Fluid Dynamics of Complex Biosystems, School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany

ARTICLE INFO

Article history:

Received 15 August 2016
 Received in revised form
 14 December 2016
 Accepted 19 December 2016
 Available online 20 December 2016

Keywords:

Binary gels
 Gelatin
 Alginate
 Gelation
 Thermal hysteresis
 Viscoelastic properties

ABSTRACT

The present study reports systematic rheological data of binary aqueous mixtures of gelatin and alginate up to a concentration ratio of 1:1. The mixtures were prepared and rheologically studied with respect to the gelation point, melting point, and kinetics. The effect of the polysaccharide concentration on the sol–gel and gel–sol transitions is presented for concentrations up to 5% (w/w). Under small amplitude oscillatory shearing conditions, both transitions were shifted to higher temperatures. The kinetic study showed that the storage modulus will continue to increase in time under isothermal conditions, even if the rate of increase is considerably reduced. Furthermore, all binary gels exhibited a higher storage modulus than the pure gelatin gels at 5 °C. The increase was 9 and 24% for 5 and 2.5% gelatin, respectively. Assuming that the storage modulus is directly related to the amount of the triple helices, all isothermal data could be successfully described by the kinetic gelation model proposed by Djabourov, Leblond, & Papon (1988a, 1988b). The excellent agreement between the rheological data and the gelation model confirm that this model can effectively predict the long-term elastic properties of the binary mixtures. Using isolated prototype modules, discrete gel specimens were formulated at 5 °C. Large amplitude oscillatory shear measurements (LAOS) showed that these specimens were still intact after the tests. The gels that were prepared directly on the rheometer plate exhibited strain hardening behavior during LAOS deformations. A nonlinear stress analysis of the Lissajous plots confirmed this finding.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Gels are materials that consist of a network of macromolecules in a continuous phase. If the network is affected by the temperature and can be reversibly created or destroyed, the gels are characterized as thermoreversible gels (Borchard & Lechtenfeld, 2001). Gelatin is most commonly used to create such gels. Gelatin is derived from collagen by hydrolytic degradation during which the regular triple helix structure is broken down to form random coils (Gornall & Terentjev, 2008). For temperatures below 40 °C, the gelatin chains undergo a progressive conformational change, known as the coil-to-helix transition (Gornall & Terentjev, 2008; Guo, Colby, Lusignan, & Howe, 2003). At high concentrations and for temperatures below 35 °C the thermodynamic conformation of the gelatin chains is essentially intermolecular (Gornall & Terentjev, 2008; Guo et al., 2003). At extremely low concentrations the renaturation process is completely intramolecular and gelation occurs for concentrations above 0.5–1% w/w (Guo et al.,

2003). For instance, Spandagos, Goudoulas, Luckham, and Matar (2012) reported storage and loss moduli of 0.1 and 0.04 Pa, respectively, for 0.7% gelatin specimens stored for 24 h at 4 °C. A direct correlation between the helix amount and the specific optical rotation of the gelatin mixtures were proposed by Djabourov, Leblond & Papon (1988a). In their study, the higher gelatin concentration resulted in a higher helix amount. Additionally, between heating and cooling of solutions, a hysteresis loop on the helix amount was reported. Finally, they proposed that the gelation kinetics consists of two processes; the first one (initial 5–7 min) is a fast exponential process, whereas the second one is based on a logarithmic rate of helices growth. The same concept of a fast nucleation stage and a second reorganization stage was adapted and improved by Guo et al. (2003).

The thermoreversibility of the gelatin gels is designated by the so-called sol–gel and gel–sol transitions. Each transition is related to a specific temperature, i.e. the parameters T_g and T_m refer to the gelation and melting temperatures, respectively. In a recent study on physically crosslinked ferrogels of 4% gelatin, strong changes of the magnetorelaxometric signals near 34 °C were reported (Wisotzki, Eberbeck, Kratz, & Mayr, 2016). This observation is related to the macroscopic gel–sol transition. Yet, a direct

* Corresponding author.

E-mail address: natalie.germann@tum.de (N. Germann).

comparison between different sets of gelation data is somehow difficult since the thermal history of the gelatin gels affects their mechanical and rheological properties. In addition, there are significant deviations between the experimental values and the ones calculated using homogeneous nucleation thermodynamics (Borchard, Bremer, & Keese, 1980).

Considerable differences between native and complex gelatin mixtures are sometimes expected because of the presence of additional components. Binary mixtures of gelatin and polysaccharides have received considerable research interest during the last twenty years (Bush & Hill, 1983; Guo et al., 2003; Saxena, Kaloti, & Bohidar, 2011). Picard, Giraudier, and Larreta-Garde (2009) performed rheological and optical measurements to examine the gelation of gelatin-hyaluronic acid mixtures. The T_g and G' were affected by the presence of the polysaccharide and an exponential dependence between G' and the polysaccharide concentration reported. Recently, gelatin solutions with and without milk proteins were studied (Pang, Deeth, Sopade, Sharma, & Bansal, 2014). The 1% gelatin samples did not gel. The gelation temperatures for 2.5% gelatin were found to be in the range of 15–18 °C; for 5% solutions the corresponding range was 20–22 °C. Wang, Natale, Virgilio, and Heuzey (2016) examined the effect of pH on the rheology of binary mixtures of gelatin and xanthan gum. They found that by adding a small amount of polysaccharide (0.2% w/v) and appropriately adjusting the pH value, the storage modulus was increased by almost two orders of magnitude. The thermoreversible behavior of the binary mixtures indicates that no covalent crosslinking occurred during the gelation.

Alginate is a linear polysaccharide composed of mannuronic and guluronic acids. It is extensively used in the food industry and bioengineering (Larsen, Bjørnstad, Pettersen, Tønnesen, & Melvik, 2015). The mixtures of proteins with polysaccharides can give a variety of phase behaviors, with synergistic or antagonistic action creating soluble or insoluble complexes (Tolstoguzov, 1995, 2003; Wang et al., 2016). Windhues and Borchard (2002) studied diluted solutions (up to 0.5%) of binary gelatin-alginate mixtures. Based on the light scattering results, they proposed quasi-binary behavior at high temperatures. The mixtures with high gelatin content and below 36 °C exhibited rod-like structures, which was considered as the initial stage for the helix-coil transition. Thermoreversible gels from binary gelatin-alginate mixtures were reported in the pioneer work on of Muchin, Streltsova, Vajnerman, and Tolstoguzov (1978). These gels were obtained for a ratio of gelatin to alginate equal to 2:1. For concentrations over 6%, thermoirreversible gels were obtained. Muchin et al. (1978) reported the importance of the pH value and the ionic strength and identified the limiting values for the two parameters to obtain thermoreversible gels. The ability to create permanent gels upon the presence of divalent cations (e.g. Ca^{++}) makes alginate an excellent candidate for binary gelatin mixtures (Boateng, Burgos-Amador, Okeke, & Pawar, 2015). Panouille and Larreta-Garde (2009) studied the sol-gel and gel-sol transitions of alginate/gelatin mixtures in the presence of Ca^{++} . To create the alginate gel, Ca^{++} ions were released at 27 °C. Subsequently, cycles of cooling and heating were applied. The solutions of 1.5% gelatin and 1% alginate after 30 min at 4 °C created gels. Finally, the presence of the alginate seems to favor the gelation at 20 °C. The pure alginate solutions did not show any temperature effect. This is consistent with other studies on alginate solutions, where the temperature only affects the viscosity and does not induces gel formation (Das et al., 2015).

The goal of this study was to rheologically characterize gelatin-alginate solutions and gels and to provide a reliable rheological protocol for sol-gel and gel-sol transitions. The contribution of each biopolymer to the gel's mechanical properties was investigated. A known kinetic gelation model successfully applied on the long-

term storage modulus under isothermal conditions. Finally, the linear and nonlinear viscoelastic properties of the gels were also studied.

2. Materials and methods

2.1. Materials

Two sources of gelatin and one alginate source were used in our study. All products were obtained by Sigma-Aldrich, Germany. Gelatin type A from porcine skin and of 50–100 kDa molecular weight ($pI\ 8.0 \pm 1.0$) (300 bloom, G2500, Sigma-Aldrich) was used in the majority of the experiments. Additionally, gelatin from cold water fish skin and of about 60 kDa molecular weight ($pI \approx 6.0$) (G7041, Sigma-Aldrich) was used for comparison reasons. The alginate source was an alginic acid sodium salt from brown algae (*Macrocystis pyrifera*) (A0682, Sigma-Aldrich). According to the material documentation, the guluronic and mannuronic acid contents were about 39 and 61%, respectively. The molecular weight was between 12 and 80 kDa. Sodium azide, provided by AppliChem GmbH (A1430,0010), was used as the biocide. Paraffin oil from Merck Chemicals GmbH (107,160) was applied at the rim of the geometry to prevent sample evaporation.

2.2. Preparation of binary mixtures and gels

Gelatin powder was dissolved in distilled water. A heating plate was utilized to heat the solution to 48 °C. Slow mechanical stirring was carried out using a magnetic stirrer. Once the gelatin was swollen, a stock solution of sodium azide (0.1% w/w) was used to adjust the gelatin concentrations to 5 and 10% (w/w). Typical times to completely dissolve the gelatin were between 1.5 and 3 h, depending on the gelatin concentration. The alginate solutions were prepared in the same fashion. To dissolve the polysaccharide, the temperature of the heating plate was significantly higher. Alginate solutions of 2, 5, and 10% (w/w) were finally prepared. The binary mixtures were subsequently prepared by mixing equal weights of gelatin and alginate solutions. Hence, the final weight of the binary mixture was twice of the initial pure material solution. In this way, the weight concentration of each compound in the binary mixture was half of the initial one. All binary liquid mixtures were gently agitated (by magnetic stirring) for half an hour at 48 °C before storage. Finally, two series of binary mixtures were obtained; the first one of 5% gelatin with 1, 2.5, and 5% alginate and the second one of 2.5% gelatin with 1 and 2.5% alginate. In addition, samples without alginate, i.e. pure gelatin gels, were also created with concentrations of 2.5% and 5% gelatin, by utilizing the stock solution of sodium azide. The final concentration of the biocide was 0.02% (w/w).

The prepared solutions were stored in a refrigerator at 5 °C. Whenever some liquid samples were needed for the measurements, small amounts of gelled samples were placed in a water bath at 45 °C for at least 15 min. To prepare the gel layers for the gel-sol experiments, a Gilson L Pipetman was utilized to place the liquid samples on the preparation module. The modules were designed in a fashion that uniform, undisturbed, easy-to-store and easy-to-handle gel specimens could be created. Each module comprises a holding stainless steel cylindrical body, an inner co-axial and detachable Teflon base, an overflow channel around the Teflon base, and a plastic cover to prevent evaporation of the liquid sample. The liquid mixture was poured on the top of the Teflon part where the gel layer was created afterwards. The diameter of the Teflon base was 25 mm, equal to the diameter of the two plates of the rheometer's geometry. The basic dimensions of the module are given in the SI file (Fig. S1 and Fig. S2). In the case of the gel-sol

Download English Version:

<https://daneshyari.com/en/article/4984041>

Download Persian Version:

<https://daneshyari.com/article/4984041>

[Daneshyari.com](https://daneshyari.com)