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Modeling water partition in composite gels of BSA with gelatin following thermal treatment

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ABSTRACT

A thorough experimental procedure of controlled cooling, isothermal at low temperature, heating, isothermal at high temperature and a second cooling run were carried out to record and rationalise the structural properties of BSA-gelatin mixtures over a wide range of temperature and polymer concentration. Research methodology included small-deformation dynamic oscillation in-shear, micro-differential scanning calorimetry, scanning electron microscopy and semi-theoretical modeling based on ideas of relating the elastic modulus of amorphous synthetic polymers to the topology of their binary blends. Cooling of solutions from ambient temperature produces gelatin continuous matrices supporting the BSA droplets, and modeling of the mechanical readings using an appropriate Lewis-Nielsen equation argues that the former is a highly hydrophilic molecule. Heating melts the gelatin network and leads to the formation of a continuous BSA structure, with dispersed inclusions of liquid gelatin, but produces a solvent partition factor in favour of the globular-protein gel. Subsequent cooling forms solid-like particles of gelatin within the interstices of the continuous BSA network. Modeling of steric exclusion phenomena in this filler composite using the isostrain blending law further supports the concept of increasing proportions of water being trapped within the continuous matrix of a biphasic gel.

1. Introduction

Several attempts have been made in the past to rationalise the structural properties of phase separated hydrocolloid gels using semi-theoretical approaches (Firoozmand & Rousseau, 2014). They have been based on a mixture of experimental evidence and physical theories from the "sophisticated synthetic polymer research" that dealt primarily with high-solid matrices in the absence of a molecular solvent (Rocco et al., 2016). In this respect, the pioneering work of Takayanagi and colleagues developed a set of blending laws to address the mechanical response for a composite of two amorphous polymers made from strips glued together in parallel or series (Takayanagi, Harima, & Iwata, 1963). These were polyvinyl chloride and nitrile-butadiene being examined at a temperature range where the former is a hard glass and the latter a soft rubber (Manson & Sperling, 1976). By rearranging ninety degrees the line of forces passing through each binary system, good agreement was found between modeling predictions and experimental results when the deformation of the weaker component was limited by the rigidity of the stronger material, with both deforming to the same extent (isostrain or parallel conditions). In the alternative isostress or series condition, the strength of the weaker component limits the force transmitted to the stronger material, with both being subjected to the same stress.

In biomaterials where macromolecules are dispersed with each other, the volume and composition of each phase will determine the overall textural properties of the mixture (Chronakis & Kasapis, 1995). This type of molecular dispersion has been investigated but it should be noted from the outset that work focused almost entirely on low-solid materials with, typically, up to 20% (w/w) solids (Oliver, Wieck, & Scholten, 2016). Therefore, there is a considerable amount of aqueous component in many food applications but, to date, it is rather difficult to record directly the water partition between two hydrocolloid phases. Progress has been made in treating biphasic gels by assuming extensive segregation between two incompatible hydrocolloids into separate phases, which is essentially a thermodynamic equilibrium treatment, and characterising the partition of aqueous component by their relative affinity for water in each phase (Clark, Richardson, Ross-Murphy, & Stubbs, 1983).

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The assumption of extensive segregation between two conformationally and/or electrostatically incompatible macromolecules, i.e. depleted of associative interactions leading to coupled networks, can be supported by the following: i) a massive increase in effective molecular weight at the onset of gelation, and (ii) migration of the minor component in each phase to join the growing network of the same polymer in the other phase (Morris, 1998). The blend of gelatin with potato maltodextrin provides experimental evidence for this, with slow cooling of solutions (1 °C/min) making the rate of phase separation faster than that of gelation resulting in reinforced matrices, whereas quenching (~33 °C/min) largely preserves the state of molecular mixing in solution yielding weakened gels (Alevisopoulos, Kasapis, & Abeysekera, 1996).

Due to space limitations, this section does not intend to be a comprehensive review of all published data on phase separated gels, but instead to provide an outline of the major attempts made to rationalise this type of phenomena. Given this, the aforementioned ideas of solvent partition between the phases of two incompatible hydrocolloids at equilibrium can be further treated with the Lewis and Nielsen approach (Nielsen, 1974). These researchers developed a theory which is versatile enough to cover in a single algorithm the complete range of mechanical measurements from the high values of the upper bound in the parallel model to the low values of the lower bound in the series model. In doing so, they introduced in the general equation parameters that take into account the different morphologies of rod and sphere-filled matrices at various extents of aggregation, and the maximum packing fraction of the filler phase in the biphasic dispersion (Hsieh, Kinloch, Masania, Taylor, & Sprenger, 2010). The current study will examine the application of this well developed body of theory on the elastic moduli of composite gels made of bovine serum albumin (BSA) and gelatin, and the effect of water partition on the structural properties of the blend following extensive thermal treatment.

2. Experimental protocol

2.1. Materials

The bovine serum albumin sample of this investigation was purified using a heat shock fractionation method by the supplier (Sigma-Aldrich, Sydney, Australia). It has a pH value of 6.9 and molecular weight of 66 kDa. Type B gelatin with a molecular weight of 283,600 kDa and bloom value of 189 g was donated by Sanofi Bio-Industries (Carentan, France). It has an isoelectric point of 4.5, hence being negatively charged at the almost neutral pH of this investigation.

2.2. Sample preparation

BSA powder (8, 9, 10, 11, 13, 14, 15, 17, 20, 23 and 25%, w/w) was dispersed in distilled water and stirred using a magnetic stirrer at room temperature for 2 h followed by overnight storage at 4 °C for improved hydration. On the following day, dispersions were stirred again at room temperature for at least 30 min prior to analysis. Gelatin powder (2, 3, 4, 5, 7, 10, 13, 15, 17 and 20%, w/w) was dispersed in distilled water at room temperature with gentle stirring using a magnetic stirrer and stored overnight for further hydration. On the next day, the samples were heated at 55 °C for 15 min until clear solutions were obtained; the hydration temperature of gelatin during preparation of single and mixed systems never exceeded 60 °C. Binary mixtures maintained the gelatin concentration at 2% (w/w) and varied that of BSA (6, 8, 10, 12, 14 and 16%, w/w) respectively. Samples were made by preparing initially solutions of the individual components, as described presently. Appropriate amounts of these stock preparations were combined at 40 °C, a temperature at which both components remain stable in solution, to yield the required mixtures.

2.3. Rheological measurements

Small-deformation dynamic oscillatory measurements in-shear were performed with a controlled strain rheometer (AR-G2, TA instruments, New Castle, DE, USA). The elastic (storage) modulus, G', and the viscous (loss) modulus, G'', of both BSA and gelatin samples as well as their binary mixtures were determined. A constant strain, angular frequency and a scan rate of 0.1%, 1 rad/s and 2 °C/min, respectively, and parallel plate geometry (40 mm) and 1 mm gap were used throughout the experimental routine. Samples were loaded onto the Peltier plate of the rheometer at 40 °C and silicon oil (dimethylpolysiloxane, 50 cP viscosity) was applied to the edges of the parallel-plate measuring geometry to minimize moisture loss. All samples were equilibrated at 40 °C for 5 min prior to subsequent experimentation.

Single gelatin preparations were control cooled from 40 to 10 °C, kept at that temperature for 60 min followed by a frequency sweep from 0.1 to 100 rad/s and a subsequent control heating run to 50 °C. BSA samples were control heated from 40 to 80 °C, kept there for 10 min followed by the implementation of a frequency sweep from 0.1 to 100 rad/s (about 10 min), control cooled to 10 °C followed by a similar frequency sweep. Binary mixtures were loaded onto the rheometer at 40 °C, cooled to 10 °C, kept there for 60 min followed by a frequency sweep from 0.1 to 100 rad/s. Afterwards, mixtures were heated to 80 °C, subjected to a ten-min isothermal step followed by a three-decade frequency sweep. Finally, mixed systems were cooled to 10 °C followed by an isothermal step (60 min) and a standard frequency sweep. All experiments were carried out in triplicate and consistent results are reported.

2.4. Differential scanning calorimetry

A Setaram VII micro-differential scanning calorimeter (Seturau, Caluire, France) was used. Samples of around 700 mg were weighed into aluminium pans and hermetically sealed with an equal weight of water in the reference pan. Single or binary mixtures were equilibrated at 40 °C for at least 30 min to eliminate the effect of thermal history. Experimental sequence for single BSA samples consisted of a heating run from 40 to 95 °C and cooling to 0 °C at a rate of 1 °C/min. The single gelatin samples were cooled from 40 to 0 °C at a rate of 1 °C/min, stayed at that temperature for 30 min and then heated to 90 °C at the same scan rate. Binary mixtures were cooled from 40 to 0 °C at 1 °C/min, held there for 30 min and heated to 95 °C followed by another cooling cycle to 0 °C at the same scan rate. Triplicate tests were carried out and essentially overlapping thermograms are reproduced in this communication.

2.5. Scanning electron microscopy

The network morphology of single BSA and gelatin preparations as well as the topology of their binary mixtures was visualized using Cryo-SEM. Gels were cooled rapidly to subzero temperatures by immersion in liquid nitrogen to avoid ice crystal formation and subsequent distortion of the polymer network encountered at low cooling rates. Subzero-temperature samples were gold coated before imaging by using FEI Quanta 200 ESEM (Hillsboro, Oregon, USA). An accelerating voltage of 30 kV under high vacuum mode, with spot size of 5.0 and magnifications of 1000 times were employed to characterize our systems.

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