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Phase Model Interpretation of the Structural Properties of Two Molecular Soy Protein Fractions

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The structural properties of mixtures of two molecular soy fractions, 11S (glycinin) and 2S, were investigated in the presence of glucono-δ-lactone (GDL) during an isothermal run at 25 °C for 1500 min. Analytical methodology included small-deformation dynamic oscillation, visual observations, scanning electron microscopy, and blending-law modeling. The aim of the work was to identify the state of phase separation and the pattern of solvent distribution between the two constituent polymers. It was found that the high molecular weight distribution of 11S supported rapid kinetics of structure formation, with this continuous matrix being concentrated and, hence, further reinforced in the presence of small additions of 2S. Blending-law modeling suggested that 2S was able to retain disproportionate volumes of solvent within its phase. A consequence of such property was that high additions of 2S leading to equal concentrations of the two molecular fractions in the blend resulted in a catastrophic drop in the values of the overall network strength. This behavior is rather unexpected for the structural properties of a phase-separated system, and it has been rationalized on the basis of the high water-holding capacity of the small molecular weight fraction. 2S entraps in its phase the polymeric segments of glycinin, which are then unable to become structural knots of a cohesive three-dimensional morphology observed in single 11S preparations.

INTRODUCTION

Undenatured soy proteins are extracted at room temperature with dilute alkaline water (pH 7−9) from defatted soybean meal (*1*). They possess complex quaternary structures that require fractionation before detailed studies are made on them. To avoid further dissociation of the complex mixtures into their subunits, several purification techniques are employed at the same time based on different properties of the protein fractions (*2, 3*). Ultracentrifugation of the acid-precipitated proteins produces four distinct fractions with approximate sedimentation coefficients of 2S, 7S, 11S, and 15S. However, cellulose or Sephadex chromatography reveals that the centrifugation pattern is a complex mixture of two major storage globulins, namely, 11S (glycinin) and 7S (mainly β-conglycinin), and the "biologically active" proteins of 2S (Bowman−Birk trypsin inhibitor, cytochrome *c*, Kunitz trypsin inhibitor, etc.) (*4, 5*).

Through the years, fundamental research on the physicochemical, structural, and functional properties of soy focused largely on 11S and 7S, because these are the largest compositional fractions, being about 42 and 34% of the total protein solids (*6*). Thus, glycinin will become increasingly turbid at temperatures above 70 °C and precipitate at about 90 °C owing to the thermal activation of intermolecular associations via the 42 sulfydryl groups, which are located mainly in the acidic subunits of the molecule (*7, 8*). Heat-induced structure formation is enhanced in the presence of glucono-δ-lactone (GDL), which controls the acidity and electrostatic forces between adjacent macromolecules (*9*). Addition of GDL results in a 7S network with a fine pore size (0.5 μm) exhibiting lower yield stress, cohesiveness, and gumminess than the 11S counterpart (*10, 11*). Mixtures of 11S and 7S globulins are heterogeneous structures of minimum network strength at equal compositions of the two constituents, an outcome that indicates a transitory regime of microphase inversion from one continuous matrix to another (*12*).

The main reason behind the scarcity of studies dealing with the structure–function relationship in 2S is its composition in defatted soy meal being the smallest (≈15%) of the three major fractions (*2, 13*). There is a report by Burnett et al. on the emulsification properties of two variants of 2S from sunflower seed (*14*). Formulations of 11S with 2S from sesame flour were tried as a substitute for traditional uses of dairy and soybean proteins in beverages and were met with an acceptable response from a hedonic sensory evaluation panel (*15*). Furthermore, it was stated that a whey soy protein made of a mixture of 2S and 7S fractions exhibited good foaming capacity within the broad pH range of 2−10 (*6*).

A recent investigation by Tay et al. focused on the 2S soy fraction, which was shown to possess better foaming and emulsification properties than the higher molecular weight counterparts of 7S and 11S (*16*). Regarding gelation studies, results strongly argued that in the initial stages of structure formation, 2S fared better than 7S, with 11S exhibiting the

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highest rates of aggregation. Non-covalent interactions, as opposed to disulfide bridging, were found to be largely responsible for the changing functionality of the molecular fractions 2S and 7S throughout the experimentation from the formation of a vestigial structure to that of a mature gel. The present investigation addresses fundamental considerations of the phase behavior of 11S and 2S mixtures that may render weight to potential applications of these materials as functional ingredients in tailor-made composite gels.

MATERIALS AND METHODS

Source and Biochemical Characterization. Soybean belongs to the family Leguminosae (genus *Glycine L.*) (*17*). The material (product code 063-130; graded as 7-B defatted soy flour) used in this investigation to extract the protein fractions of 2S and 11S was purchased from Archer Daniels Midland Co. (Decatur, IL). The literature includes extensive data on the characterization of these fractions in terms of primary to quaternary structures, sulfhydryl content, composition and crystal structure of the molecular subunits, biological activity, and nutritional quality (*2, 5, 12, 18–20*).

Isolation of the 11S Molecular Soy Protein Fraction. This was obtained using the method of Nagano et al. with some modifications (*21*). In doing so, defatted soy flour was mixed with a 15-fold volume of deionized water and the pH was adjusted to 7.5 with 1 M NaOH. Water-extractable soybean protein was recovered by centrifugation at 9000*g* for 30 min at 20 °C. Sodium metabisulfite (0.98 g/L) was added to the supernatant, the pH was adjusted to 6.4 with 1 M HCl, and the preparation was kept in an ice bath overnight. This was then centrifuged at 6500*g* for 20 min at 4 °C, and the 11S obtained in the precipitate was dissolved in deionized water with pH adjustment to 7.5. Dialysis of the solution ensued against deionized water for 24 h at 4 °C. The resultant salt-free dialysate was freeze-dried and stored at 5 °C for subsequent experimentation. Eight replicates of the freeze-dried material were analyzed using the semi-micro Kjeldahl method, and a conversion factor of 6.25 was used to determine protein content of the 11S fraction, which was found to be 95.0 ± 1%.

Isolation of the 2S Molecular Soy Protein Fraction. This was obtained according to the method of Rao and Rao with some modifications (*1*). Defatted soybean flour was mixed with a 10-fold volume of deionized water containing 0.1% β-mercaptoethanol, and the preparation was stirred for 30 min. Water-extractable soybean protein was recovered by centrifugation at 6000*g* for 20 min at 20 °C. Magnesium chloride was added to the supernatant to produce a 0.1 M final solution, and this was kept at 4 °C for 6 h. The resultant suspension was centrifuged at 10000*g* for 30 min at 4 °C. Magnesium chloride was added to the supernatant to produce this time a 0.4 M final solution, and this was kept as before. The resultant fine suspension was centrifuged as for the first addition of $MgCl_2$. Ammonium sulfate was added to the supernatant to produce a 32% (w/v) final solution, thus inducing formation of a protein precipitate, which was obtained by centrifugation as described previously. The precipitate was dissolved in 1 M NaCl solution and dialyzed against the same solution for 24 h at 4 °C.

Following this, ethyl alcohol was added to the dialysate in the proportion of 1:1 (v/v), and the resultant suspension was stirred for 6 h at 4 °C. Centrifugation recovered the supernatant, which was adjusted to pH 7.5, dialyzed against deionized water for 24 h at 4 °C, and freeze-dried. Samples were dissolved in a phosphate buffer (pH 7.5) and introduced to Sephacryl S300 and Sephacryl S200 gel filtration columns (60 × 1.6 cm i.d. for both; GE Healthcare Bio-Sciences AB, Uppsala, Sweden) connected in tandem and equilibrated earlier with the same buffer solution. The columns were eluted with this buffer at a flow rate of 0.6 mL/min until the absorbance at 280 nm of the eluted fractions returned to baseline. High absorption fractions were collected, freeze-dried, and stored at 5 °C for subsequent use. The protein content of 2S fraction was about 92% by the Kjeldahl method (N × 6.25), and with that of 11S was used to prepare the specific concentrations required in single and mixed systems of the present investigation.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis. SDS-PAGE is an assay of protein purity and was employed in a mini-

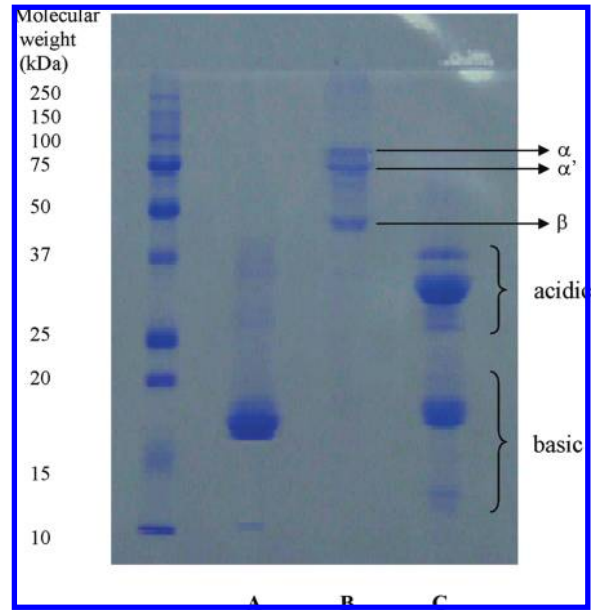

Figure 1. SDS-PAGE of soy protein: lane A, 2S protein fraction; lane B, 7S protein fraction; lane C, 11S protein fraction.

Protean 3 cell (Bio-Rad Laboratories, Hercules, CA). One hundred microliters of sample with a protein composition of 4 mg/mL was mixed with 95 μL of buffer made of deionized water, Tris-HCl (pH 6.8), glycerol, SDS, and bromophenol blue. Five microliters of mercaptoethanol was added to that, and the preparation was heated to 95 °C for 4 min. Then, 10 μL of the sample mixture was applied to the gel. Stacking and resolving gels were based on distinct formulations of deionized water, separating buffer, and 30% acrylamide/bis, with their concentrations being 4 and 12%, respectively. Precision Plus protein standards from Bio-Rad Laboratories were used as the molecular weight markers. Appropriate stacking, resolving, and electrolysis buffers were also prepared, and the voltage used for the migration was 125 V. Following migration, the protein fractions were stained with 0.1% Coomassie Brilliant Blue in water/methanol/acetic acid (4:1:5) and destained with water/methanol/acetic acid (85:7.5:7.5).

Figure 1 reproduces well resolved and according to experience SDS-PAGE patterns of the three soy protein fractions (2S, 7S, and 11S). In lane A, the molecular mass of 2S protein was about 17 kDa, which is identified to be the Bowman−Birk trypsin inhibitor. In lane B, the 7S protein fraction is made up of three subunits, α, α′, and β, having molecular masses of about 85−45 kDa. In lane C, the 11S protein fraction is made up of acidic and basic subunits of about 35 and 18 kDa, respectively. Molecular fractions of 11S and 2S were used for subsequent experimentation in this paper.

Small-Deformation Mechanical Spectroscopy on Shear. To evaluate the structural properties of our materials, 4.0 mL solutions were prepared as follows: For each solution, 3.68 mL was drawn from a separate 4.40 mL soy−protein preparation made in deionized water, pH adjusted to 7.5, and subjected to a heat treatment at 100 °C for 10 min. The remaining 0.32 mL was drawn from a freshly made 5% (w/v) GDL sample to keep the cosolute content constant at 0.4% (w/v) in the final solution. The amount of freeze-dried protein added to the 4.40 mL preparation was adjusted to yield the required protein concentration in the 4.0 mL solution used for mechanical analysis. Three milliliters was drawn from the latter and loaded onto the temperature-controlled platen of the rheometer (25 °C), with the exposed edges of the sample being covered with a silicone fluid from BDH (100 cs) to minimize water loss. The measuring geometry was a parallel plate with 40 mm diameter and a sample holding gap of 1.5 mm.

Measurements were performed with the Advanced Rheometrics Expansion System (ARES), which is a controlled strain rheometer (Rheometric Scientific, Piscataway, NJ). ARES has an air-lubricated and essentially noncompliant force rebalance transducer with a torque range between 0.02 and 2000 g cm. For precise control of sample temperature, an air convection oven was used, which has a dual element

heater/cooler with counter-rotating air flow covering a wide temperature range (between 130 and − 70 °C). The measuring geometry of parallel plates is placed in the oven, which supplies ultraclean and dry air at a controlled flow through the ARES oven heaters. Samples were subjected to an isothermal run (25 °C) for up to 24 h at the frequency of 1 rad/s. The time sweep was followed by a frequency sweep between 0.1 and 100 rad/s. In both cases, a strain within the linear viscoelastic region was used that varied from 0.005% for 11S to 0.1% for the 2S gels. The experimental protocol provides readings of the storage modulus (G'; elastic component of the network), loss modulus (G'' ; viscous component), and a measure of the "phase lag" δ (tan $\delta = G''/G'$) of the relative liquid-like and solid-like character of the material (*22*). Three replicates were analyzed for selected experimental concentrations of single and mixed preparations, with the process of gel formation being reproducible within a 3% error margin as a function of time scale of measurement.

Scanning Electron Microscopy (SEM). Single 2S and 11S gels containing 4.0% (w/v) protein in the presence of 0.4% (w/v) GDL and their mixtures prepared at a fixed 11S concentration (4.0%) with increasing amounts of 2S (from 0 to 4.0%) were left to age for 24 h at 25 °C prior to examination to imitate the experimental protocol utilized in the preceding section. Next, samples were deep frozen rapidly by submerging into liquid nitrogen followed by freeze-drying-induced dehydration. Specimens were cut into thin slices before being mounted onto a microscopy stub with a black double-sided tape and coated with platinum by means of a sputter coater. Networks were observed using SEM, which operated at an accelerating voltage of 15 kV (JEOL model JSM-5600LV, Tokyo, Japan) (*16, 23*).

RESULTS AND DISCUSSION

Small-Deformation Studies on the Gelation Properties of the Individual Components. Although scientific understanding of phase separation phenomena in binary biopolymer systems has made considerable advances in the past 20 years or so (*24, 25*), data on the structural properties of molecular soy fractions in composite gels are scarce and in the case of 2S nonexistent. The design of meaningful experiments to unveil features of the topology of 11S/2S mixtures requires a certain prior knowledge of the gelation behavior of the individual components. The transition from the sol to the gel state and the development of "mature" networks were monitored in the present investigation as a function of time scale of observation. Particular care was taken to ensure that results were obtained at the appropriate conditions of frequency of oscillation, strain amplitude and controlled temperature to reflect true small-deformation properties of a well-developed molecular structure.

Figure 2 illustrates the time course of gelation for representative samples of glycinin in the presence of 0.4% GDL, a preparation that imitates closely conditions of tofu making (*26*). Clearly, the polymeric segments of 11S are capable of developing rapidly a three-dimensional structure at 25 °C that reaches constant values for the "pseudo-equilibrium" storage modulus on shear within 500 min of the experimental routine. No further changes in the values of G' were observed at longer recording times (up to 1500 min), and the first part of the rheological characterization is depicted in **Figure 2** to contrast the rapid kinetics of gelation with those of 2S in the following discussion of this section.

It should be noted at this stage that GDL-containing 11S solutions undergo an aggregation process which produces contracted brittle gels with a notable degree of syneresis. This may lead to catastrophic slippage, but the present investigation was able to avoid breakdown of adhesion between the sample and the flat surface of the measuring plate by minimizing the disturbance of the gel during experimentation (applied strain of 0.005%). Besides 3% 11S, which is the smallest and most difficult concentration to work with, no artificial drop in the magnitude of the solid-like response was observed at such a small level of deformation.

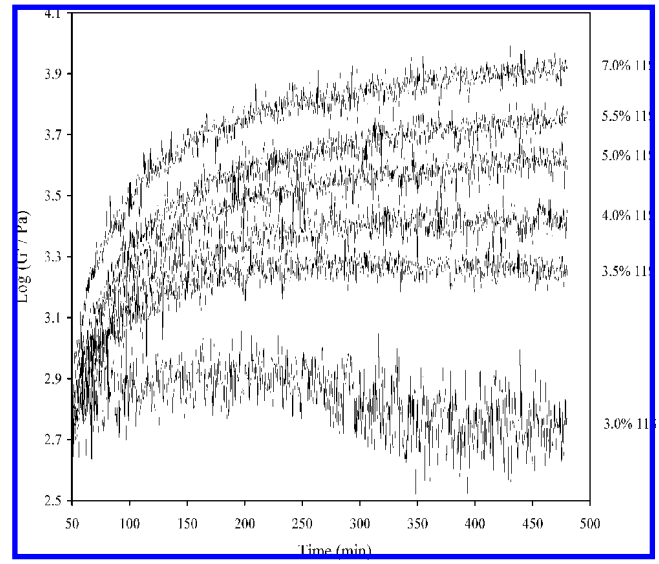

Figure 2. Time sweeps of storage modulus (G') obtained for 11S soy at the concentrations shown beside the experimental traces. Sample concentrations of 4.5, 6.0, and 6.5% are not shown to avoid clutter (0.4% GDL added; strain = 0.005%; frequency = 1 rad/s; temperature = 25 °C).

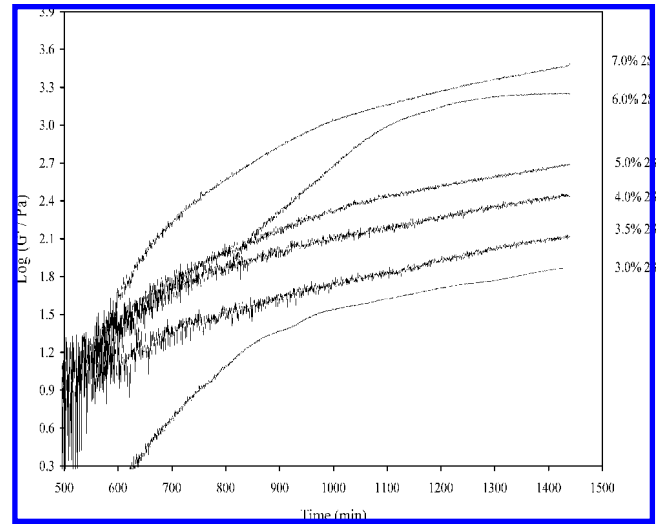

Figure 3. Time sweeps of storage modulus (G') obtained for 2S soy at the concentrations shown beside the experimental traces. Some experimental data and sample concentrations of 4.5 and 5.5% are not shown to avoid clutter (0.4% GDL added; strain = 0.1%; frequency = 1 rad/s; temperature = 25 °C).

Figure 3 shows the gelation profile of typical 2S preparations upon addition of 0.4% GDL at 25 °C for 1500 min. Variation in the isothermal development of G' values is rather limited and, overall, smooth traces are obtained in comparison to the 11S counterparts. In addition, the onset of gelation is delayed with structural development occurring in the second part of the experimental routine, that is, beyond the time frame of 500 min plotted in **Figure 3**. Further holding of the temperature at 25 °C yields a gradual rise in the magnitude of storage modulus, which approaches asymptotically an equilibrium value; hence, it is feasible to monitor this slow gelling process via the application of 0.1% strain to the system. The distinct pictorial rheology of the two soy molecular fractions is also manifest in the isothermal properties of their mixture, and it will be utilized

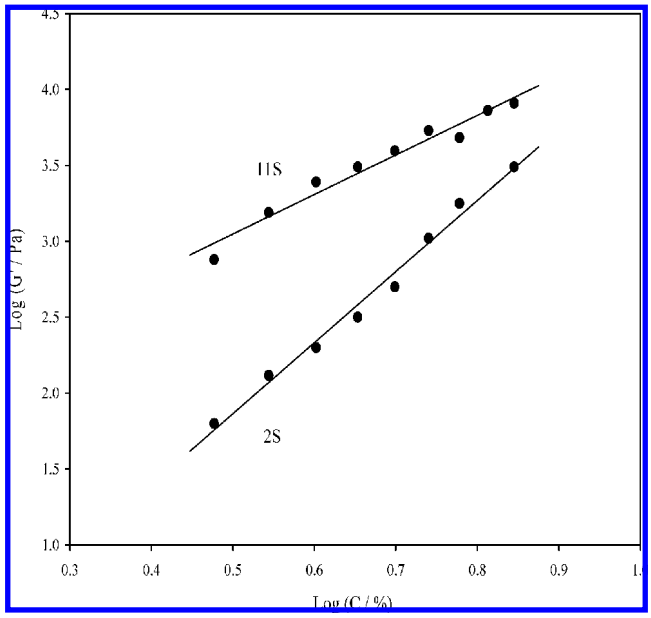

Figure 4. Concentration dependence of the values of storage modulus (*G*′) obtained for the two soy molecular fractions at the end of the time sweeps illustrated in **Figures 2** and **3**.

later in the framework of arguing for the dominant polymeric phase in the topology of the composite gel.

It is not within the scope of this investigation to elucidate on the type of interactions responsible for the stabilization of the glycinin or 2S protein gel, but rather to establish the mechanical properties of the individual components as a reference state for the subsequent mixed system analysis. Nevertheless, utilization of urea and *N*-ethylmaleimide in soy preparations demonstrated that 2S is largely stabilized via non-covalent interactions, whereas 11S will also draw on disulfide bridging as a means of achieving maximum network strength (*16, 27*). As alluded earlier, data on single soy fractions are needed to relate gel modulus to polymer concentrations for the modeling of the phase behavior in these blends. **Figure 4** reproduces good linear relationships obtained for this type of double-logarithmic plots incorporating data from the completion of the time sweeps in **Figures 2** and **3**. As expected, the high average molecular mass of 11S chains (≈300 kDa in ref *28*) facilitates formation of a functional network with dominant values of storage modulus in comparison to the firmness of the 2S (≈18 kDa) gels at a given polymer concentration. **Figure 4** suffices as a step of converting real (effective) polymer concentrations of their respective phases in the composite gel to shear moduli for "blending law" modeling. In studies of the physicochemical aspects of network structure, these data can be fit by an analytical expression (the "cascade" approach), which is a modification of the protocol proposed by Hermans, thus obtaining a set of parameters that relate to the theory of gelation (*29, 30*).

Observations on the Structural Behavior of the 11S/2S Mixed Gels. Following the analysis of single-component gels, a series of binary mixtures was prepared by keeping the concentration of glycinin constant at 4% (i.e., at a level leading to a stable gel in **Figure 2**) and varying the amount of 2S from 0 to 4% in 10 concentration steps. Upon mixing the two molecular fractions under conditions (temperature and polymer/GDL addition) where each soy constituent forms a stable solution in isolation, turbidity is observed due to microphase separation in the system. Implementation of the measuring routine generates **Figure 5**, which shows the time dependence

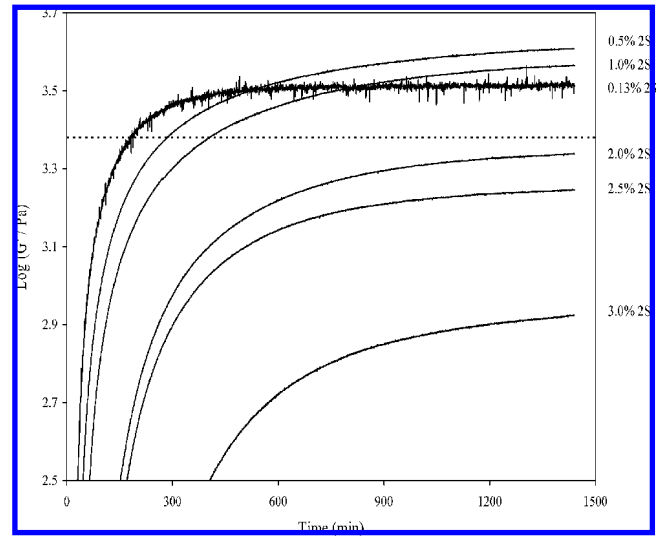

Figure 5. Time sweeps of storage modulus (*G*′) obtained for mixtures of 4% 11S in the presence of increasing concentrations of 2S shown beside the experimental traces. 2S concentrations of 0.25, 0.75, 1.5, and 4.0% are not shown to avoid clutter. Dashed line indicates the "pseudo equilibrium" *G*′ value of a single preparation of 4% 11S (0.4% GDL added; strain = 0.1%; frequency = 1 rad/s; temperature = 25 °C).

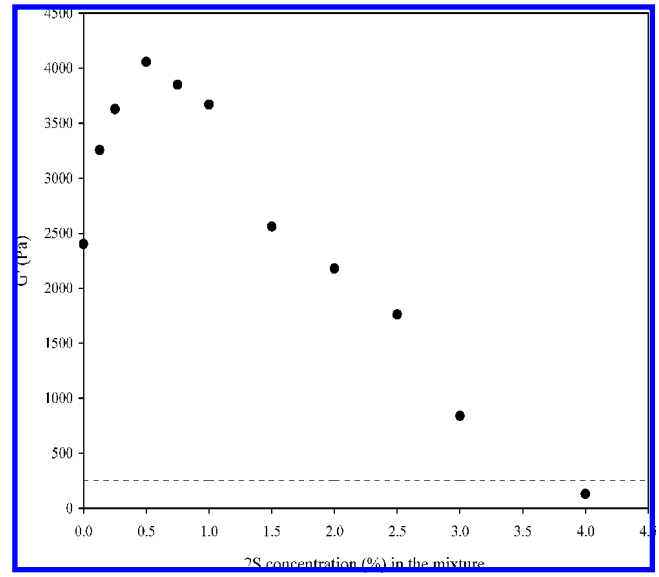

Figure 6. Experimental storage modulus (*G*′) values for 4% 11S with various concentrations of 2S at completion of the time sweeps illustrated in **Figure 5**. Dashed line indicates the "pseudo equilibrium" *G*′ value of a single preparation of 4% 2S. Remaining conditions were as in **Figure 5**.

of storage modulus (25 °C for 1500 min) for selected combinations of our mixtures. The figure incorporates the final *G*′ reading of 4% 11S from **Figure 2**, and it is clear that these experimental conditions allow gels to mature, approaching constant values of network strength. Furthermore, the dominance of the glycinin phase at low additions of 2S to the mixture (up to 0.5% solids) is discernible in the moderately spiky gelling process of 4% 11S plus 0.13% 2S.

The time course of observation in **Figure 5** unveils a dramatic variation in the gel-like consistency of 11S in the presence of increasing concentrations of 2S, and **Figure 6** focuses on this rather unexpected result. Previous explorations of the three-dimensional topology of biopolymer composites carried out in mixtures of hydrolyzed starch with gelatin, caseinate or whey protein, and denatured soy protein unveiled microphase separa-

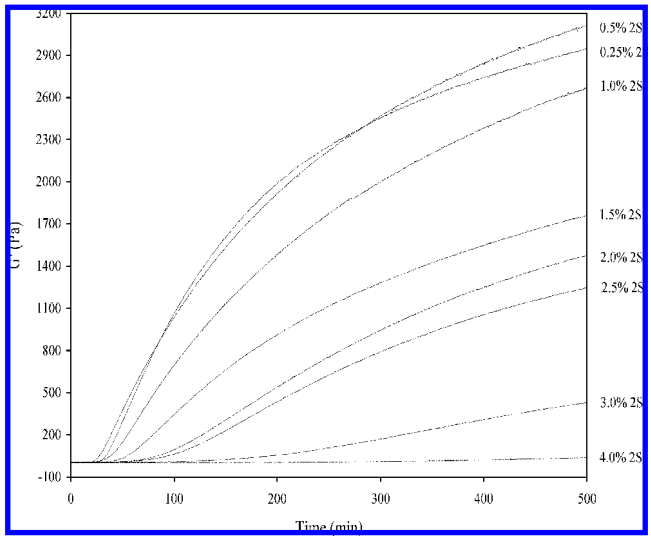

Figure 7. Time sweeps of storage modulus (G') emphasizing the initial stage of gelation obtained for mixtures of 4% 11S in the presence of increasing concentrations of 2S shown beside the experimental traces. 2S concentration of 0.75% is not shown to avoid clutter. Remaining conditions were as in **Figure 5**.

tion phenomena between the two constituents (*25, 31*). A clear phase inversion point from one dominant phase to another was also detected leading to reinforced networks with increasing levels of the second component (and total solids) in the blend. In other studies, interactions between nonstarchy polysaccharides were interpreted on the basis of either "molecularly" interpenetrating networks for agarose/deacylated gellan and agarose/κ-carrageenan gels (*32, 33*) or being segregative at appropriate additions of counterions: calcium ions in the case of gelatin/low-methoxy pectin and sodium ions for deacylated/high acyl gellan (*34, 35*). In the aforementioned mixtures, the development of modulus behavior was system specific, but it was found to follow a simple additivity rule or to possess a "synergistic" character that preserved or enhanced the contributions of the individual polymeric components.

In the event of a prolonged isothermal run on our molecular soy fractions, the familiar increase in the overall modulus of the composite gel is observed only at the lower range of 2S addition in **Figure 6**. At concentrations of 2S >0.5% in the system, there is an immediate diminishing effect on the composite strength, which at 1.5% 2S falls to levels comparable to those of the single glycinin preparation (4%). The catastrophic effect continues unabated at the upper range of solids and appears to be virtually complete at the 2S content of 4%. At this stage, the experimental modulus of the mixture is close to that recorded for the aforementioned concentration of the individual 2S gel (indicated as a dashed line in **Figure 6**).

The gelation profiles in **Figures 2** and **3** indicate that kinetic influences may determine the formation of composite gels at the isothermal regime of the present investigation. This is evident in **Figure 7**, which focuses on changes in storage modulus of the soy mixtures at the first 500 min of experimentation. There is a sharp and congruent increase in G' values of the 11S samples in mixture with 0.25 or 0.5% 2S at 25 °C. Beyond this point, a steadily declining rate of gelation is recorded with the systems being transformed at 4% 2S to match the reduced level of network strength upon completion of the experimental run in **Figure 6**. In conclusion from the rheological study, it is surmised that high levels of 2S increasingly entrap polymeric segments of glycinin in the 2S phase, thus "freezing" them in the system and preventing them from contributing to functional connections

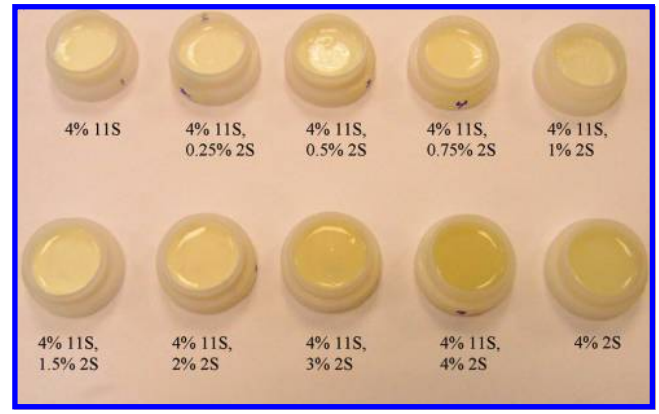

Figure 8. Visual observations of changes in consistency of 11S gels in the presence of increasing amounts of 2S, as compared to single preparations of the two molecular fractions.

within the phase/polymeric network of the high molecular weight soy fraction.

Figure 8 constitutes a straightforward but indicative demonstration of the changing nature of soy mixtures left to form structures within the normal course of experimentation (1500 min; 25 °C). Systematic addition of 2S alters the consistency from firm and pale yellow gels to relatively heterogeneous and readily yielding soft gels with an olive yellow color. Typically, a commercial soy isolate contains approximately 42% 11S and 15% 2S. According to **Figure 6**, and the visual observations in **Figure 8**, an 11S/2S ratio in the isolate of near 3 would have no discernible effect on the structural properties of the glycinin gel. These could be enhanced upon "removal" of two-thirds of the 2S content from the commercial preparation.

The severe disruption of glycinin aggregation discussed on the basis of rheological and visual observations can be further documented using SEM. **Figure 9** illustrates SEM micrographs obtained for single and mixed preparations at 1500× magnification. At 4% solids and the processing temperature of 25 °C, the high molecular weight soy fraction forms well-developed "honeycomb" structures following addition of GDL. Networks appear to have an approximate pore size of 10 μm. By comparison, extensive structural defects are noted in the three-dimensional image of the low molecular weight soy fraction, which includes several pores and cavities with a broad size distribution. Progressive inclusion of 2S in the system does not bring about the formation of a highly organized blend in which constituents maintain their macromolecular definition within the corresponding phase-separated domain. Such an image would have been according to experience from conventional studies of binary biopolymer gels (*24, 36, 37*).

In the present study, the three-dimensional form of 11S is progressively lost but the organization of the resultant composite gel does not transform to something with increasing 2S parentage. Instead, a "new type of network" is observed at this level of magnification being almost ruptured in the inner structure. This exhibits an open surface with large cavities of 20 μm or more in size (e.g., 4% 11S plus 4% 2S). These images are in qualitative agreement with the notion of the polymeric segments of 2S being in close proximity to those of glycinin, thus preventing the latter from forming a cohesive morphology of high network strength and characteristic molecular arrangement.

"Blending-Law" Based Quantitative Analysis in Support of the Molecular Soy Fraction Phase Model. The unexpected and rather unique phase behavior of our mixture at high levels

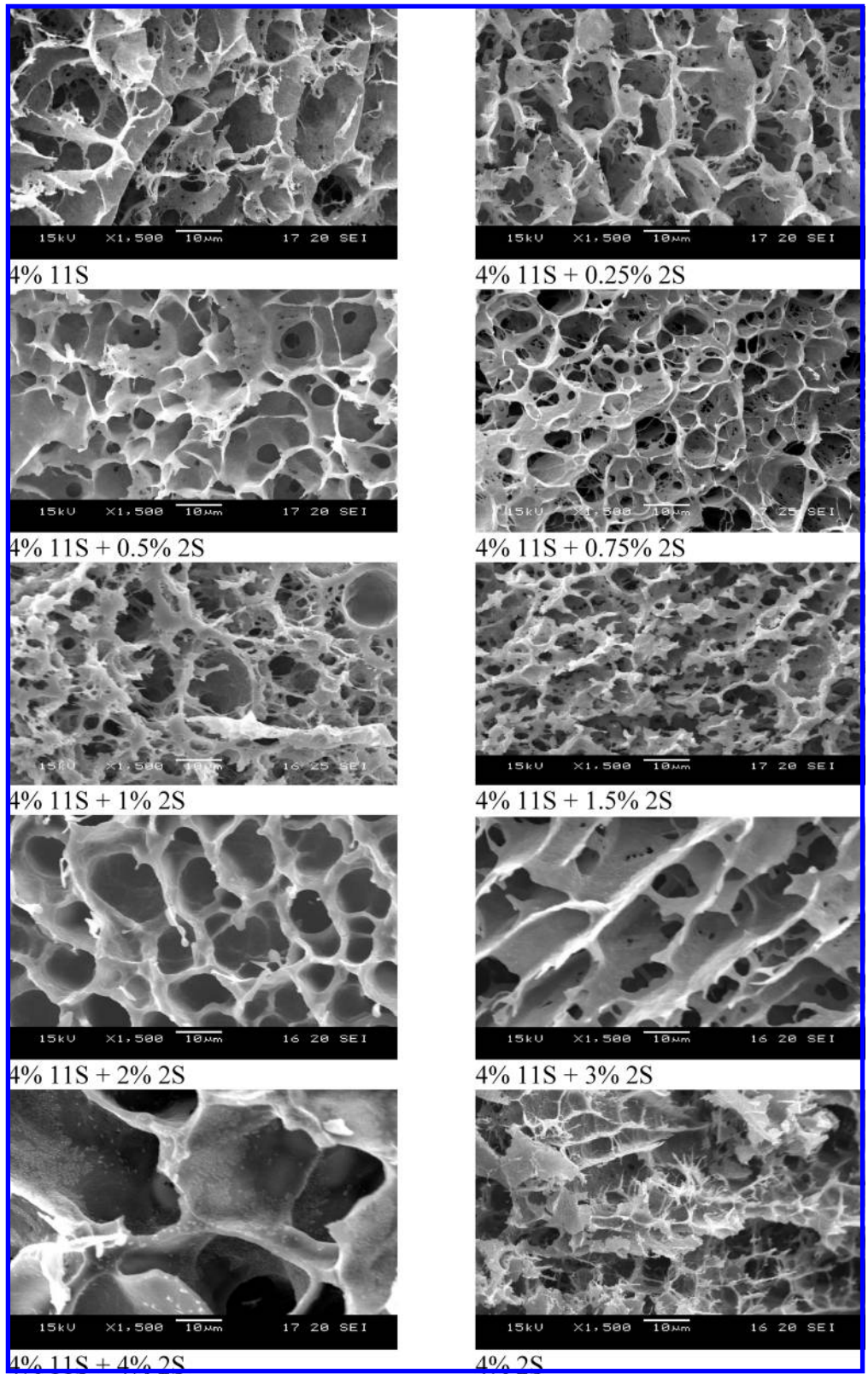

Figure 9. Scanning electron microscopy of single and mixed gels of 11S and 2S at concentrations indicated at the bottom of the micrographs. Magnification is 1500×; bar is 10 μm.

of 2S poses the question as to its origin. Previous investigations of the topology of bipolymeric gels made evident that the ability to hold solvent within one's phase is the ultimate determinant of the relative predominance of this constituent over its counterpart. Because at the moment it is rather difficult to identify an experimental technique for reliable measurements of phase volume in the gel state, a computerized algorithm was devised to consider all possible distributions of solvent between the two polymeric components (*38*). The methodology was put to the test in systems of direct practical relevance to added-value food products, and it was able to rationalize phase separation phenomena based on the combined framework of gelation and steric exclusion of the two macromolecules (*25, 37, 39*).

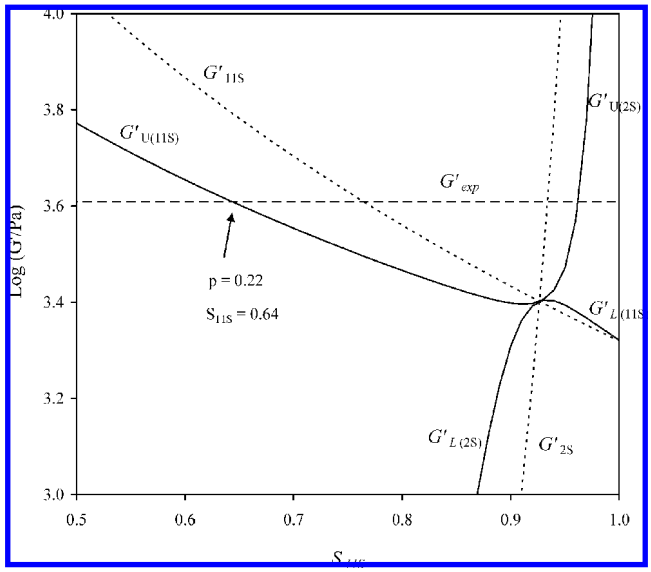

Figure 10. Analysis of the effect of solvent partition on the calculated storage modulus (G') of single 11S and 2S preparations and the calculated upper/lower bounds of 4% 11S plus 0.5% 2S mixed gel, with the experimental modulus of the mixture shown as a dashed line.

Nevertheless, its utility is confined to regimes of a positive relationship between "performance" characteristics (e.g., enhanced strength of the composite gel) and increasing content of solids in the system. A hybrid approach was also developed to address the case when the overall modulus of the composite gel remains close to or falls moderately below the modulus of the two constituents at their nominal (original) concentrations (*40*). This pattern of behavior was treated on the basis of deswelling ideas, assuming that the faster gelling species does so at its original concentration throughout the system, followed by ordering of the slower gelling species that claims some of the solvent from the earlier formed network.

Application of this line of attack to soy is illustrated in **Figure 10** for the mixture of 4% 11S and 0.5% 2S. Blending laws in the following mathematical format were used to relate the storage modulus values of the two component phases to the overall network strength of the binary gel (*30*)

$$G'_{U} = \varphi G'_{11S} + (1-\varphi)G'_{2S} \quad (1)$$

$$1/G'_{L} = \varphi/G'_{11S} + (1-\varphi)/G'_{2S} \quad (2)$$

where G'_{U} and G'_{L} are the shear moduli of the upper and lower bound of the composite, G'_{11S} and G'_{2S} are the shear moduli of the 11S and 2S phase, and φ is the volume of the 11S phase, which in this case is approximated to the amount of solvent held in the phase. The analysis affords a straightforward calculation of the relative amount of solvent held at each polymeric phase using the so-called p factor (*29*)

$$p = (S_{11S}/X_{11S})/(S_{2S}/X_{2S}) \quad (3)$$

where, S_{11S} and S_{2S} refer, respectively, to the amount of solvent in the 11S and 2S phase ($S_{11S} + S_{2S}) = 1$, with X_{11S} and X_{2S} being the original concentrations of the two soy fractions (i.e., 4% 11S and 0.5% 2S in our example). Interested readers are referred to a recent discussion on the ins-and-outs of this semitheoretical framework including the requirement for complete phase separation in the gel (*39*).

Figure 10 depicts the variation in modulus values (G'_{11S} and G'_{2S}) as a function of the hypothetical fraction of solvent in the glycinin phase ($0.5 < S_{11S} < 1.0$). These were calculated for every possible distribution of solvent yielding each time the effective (final) concentrations of the two soy proteins in the calibration curves of **Figure 4**. Moduli and phase volumes of the individual phases are then estimated and utilized in eqs 1 and 2 to produce corresponding values of network strength for the blend. As the value of S_{11S} increases from 0.5 to 1.0, the calculated values of G'_{11S} decrease, whereas those of G'_{2S} increase. For $G'_{11S} > G'_{2S}$ and glycinin forming the supporting matrix, the above approach gives an upper bound behavior [$G'_{U(11S)}$], whereas $G'_{11S} < G'_{2S}$ with glycinin maintaining phase continuity results in the lower bound model [$G'_{L(11S)}$].

During isothermal setting, 11S develops its continuous network prior to structuring of 2S owing to its rapid kinetics of gelation seen, for example, in **Figures 2** and **3**. A similar picture in relation to the kinetic interplay of the state of gelation and phase separation phenomena has emerged for other protein–protein and protein−polysaccharide mixtures (*40, 41*). Therefore, the continuous-phase prediction for 11S descending from the top left corner (upper bound) to the bottom right one (lower bound) of the diagram appears to possess physical meaning. Gratifyingly, the experimental value of the composite gel crosses the upper bound prediction of the glycinin continuous matrix. This relates to a solvent content in the phase of about 0.64. Using eq 3, the p estimate on that is 0.22, making the solvent affinity of 2S several times higher than for 11S, with the effective concentration of the polymeric components in each phase being 1.4 and 6.3%, respectively.

Concluding Remarks. This paper elucidates the state of phase separation between 11S and 2S at isothermal conditions of gelation and reports on the ability of the low molecular weight, hence, slow gelling soy fraction to dictate the structural characteristics of the mixture at comparable concentrations of the two components. As far as we are aware, this type of behavior is unseen in standard investigations of exclusion phenomena in bipolymeric systems. Blending-law modeling indicates that 2S possesses a high affinity for solvent as compared to 11S. The quantitative interpretation of the experimental evidence from rheology is congruent with the proposal put forward presently of a disproportionate 2S phase entrapping the polymeric segments of 11S, which are increasingly unable to participate in the formation of a continuous glycinin assembly. According to the scanning electron micrographs in **Figure 9**, the high water-holding capacity of the 2S phase leads to a distinct three-dimensional topology in the mixture where noticeable remnants of the 11S network eventually are nonexistent.

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