Morphology and Phase Separation of Hydrophobic Clusters of Soy Globular Protein Polymers

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Protein hydrophobic interaction has been considered the most important factor dominating protein folding, aggregation, gelling, self-assembly, adhesion, and cohesion properties. In this paper, morphology and phase separation of hydrophobic clusters, networks, and aggregates of soy globular protein polymers, induced by using a reducing agent (NaHSO₃), are studied using microscopic instruments. The morphology and phase separation of these hydrophobic clusters are sensitive to protein structure and composition, pH, and ionic-strength \( (I_m) \). Most of the clusters are in spherical-shape architecture and mainly consist of hydrophobic polypeptides. Rod-shape clusters were also observed at higher ionic strength, and mainly consist of hydrophilic polypeptides. The ratio of hydrophobic/hydrophilic (HB/HL) polypeptides is important to facilitate the formation of clusters in an environment with a certain pH value and ionic strength. At HB/HL < 0.8, uniform spherical clusters were observed and diameters ranged from 30 to 70 nm. At HB/HL > 0.8, large spherical clusters were formed with diameters ranging from 100 to 1000 nm, and at HB/HL ≥ 1.8, large hydrophobic aggregates formed, and size of aggregates can be up to 2500 nm. When solid content increased from 3% to 38%, at \( I_m \leq 0.058 \text{ mol} \cdot \text{L}^{-1} \), pH = 4.8, HB/HL ratio < 0.8, the networks turned into a continuous substance that has strong adhesion to various surfaces; at \( I_m \geq 0.115 \text{ mol} \cdot \text{L}^{-1} \), HB/HL ratio ≥ 1.8, the large aggregates became very cohesive and viscoelastic. Clear phase separation was observed during curing between hydrophobic and hydrophilic protein polymers. Phase-separation degree increased as HB/HL ratio increased.

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Introduction

It has long been known that the driving force for protein folding is provided by water, so that the hydrophobic clusters exclude water and are tightly packed together and buried inside of the protein. Therefore, the surface of most proteins in water is hydrophilic, and it is impossible for such macro proteins with a hydrophilic surface to interact with each other, forming a hydrophobic cluster. Once the protein is unfolded or denatured and turned inside out, the buried hydrophobic groups are exposed toward the surface of the protein, which would promote protein-protein interactions and form hydrophobic clusters.

Milk-protein, casein-colloid, aggregates can be induced by ionic strength, pH, and temperature. They have been studied using various techniques including FT-IR spectroscopy,[1] thermodynamic linkage analysis,[2] scattering and turbidimetric techniques,[3] and transmission electron microscopy and scanning electron microscopy.[4] Spherical micelles (up to 300 nm) form at low ionic strength.[2,4] Caseins are heterogeneous proteins containing mainly \( \alpha S1, \alpha S2, \beta, \) and \( \kappa \) polypeptides consisting of both hydrophobic and hydrophilic amino acids. Caseins have been widely used as emulsifiers in food products by adding sodium caseinate as an emulsifying agent at neutral pH. As pH approaches the isoelectric point, caseins become an aggregated emulsion gel.[5] Micelle structure was also observed in a model monomeric protein with 101 amino acids, a mixture of \( \alpha \)-helix and \( \beta \)-sheet from a ribosomal subunit of prokaryote.[6] The monomeric protein was unfolded using detergent sodium dodecyl sulfate and stimulated by NaCl. The protein formed a spherical micelle structure at NaCl below the critical pH and a cylindrical structure at NaCl above the critical pH. Temperature also promoted protein micelle structure formation.[4,6]

Soy proteins have been recently considered as potential alternative polymers for environmentally friendly adhesives.[7] Like many globular proteins, soy proteins are made up of the 20 various amino acids forming primary, secondary, tertiary, and quaternary structures. Soy proteins consist of many polypeptide chains. Glycinin and conglycinin proteins are major subunits of soy proteins.[8,9]

**Scheme 1.** A schematic description of hydrophobic clusters and aggregates of soy globular proteins. (A) Native soy protein, conglycinin contains \( \alpha \) and \( \alpha' \) hydrophilic polypeptides and \( \beta \) hydrophobic polypeptide, which is mainly associated by hydrogen and hydrophobic bonding; glycinin contains acidic (A) hydrophilic and basic (B) hydrophobic polypeptides, which are associated by disulfide bonds as well as hydrogen and hydrophobic bonding; and all these polypeptides are arranged based on their structure and biological function during synthesis in the soy seed. Once reducing agent is introduced, those bonds are broken forming individual polypeptides. Several scenario could occur depending on the force difference (\( \Delta F \)) between hydrophobic force (HBF) and electrostatic force (ESF\_repell or ESF\_attract). (B) HBF < ESF\_repell; (C) HBF = ESF\_repell; (D) HBF > ESF\_repell; (E) HBF > ESF\_repell and (F) ESF\_repell > 0, and ESF\_attract > 0.
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Glycinin, with a molecular mass of 300–380 kDa, has six major polypeptides, and each containing a pair of acidic and basic polypeptides that are alternatively linked by a disulfide bond, forming a hexamer.\[10\] Conglycinin protein, with a molecular mass of 150–200 kDa, contains three major polypeptides, including α, α', and β.\[11,12\] The polypeptides of conglycinin protein are held together primarily by hydrophobic forces and hydrogen bonding\[23\] (Scheme 1A). Structure, surface properties, and conformation of soy globular protein are sensitively influenced by environmental conditions such as pH, ionic strength, and temperature.\[14–23\] Analogues of globular protein aggregates could exist but may require a different stimulation environment. The resulting proteins may present different properties because of their differences in structure, composition, and molecular size.

This paper reports the morphology and phase separation of hydrophobic clusters caused by hydrophobic globular polypeptides of soy protein induced by ionic strength in an aqueous system. The hydrophobic cluster is defined, in this paper, as the cluster formed by interprotein-protein interactions.

Experimental Part

Materials

Protein samples were isolated from defatted soy flour using the acidic precipitation method. Soy flour with a protein dispersion index of 90 was provided by Cargill (Cedar Rapids, Iowa, USA), containing about 50% protein and 10% moisture, and 98% particle size of the soy flour was <150 μm. The soy flour was dissolved in distilled water at a 1:16 (soy flour: water) ratio at room temperature. pH of the slurry was adjusted to 9.5, and the slurry was stirred for about 1 h at room temperature, then centrifuged at 12 000 × g force for 4 °C for 10 min. The precipitates were mainly carbohydrate-based materials and were discarded; the supernatant was saved as the starting material named as soy protein isolate (SPI) for protein sample preparation. The protein content of the supernatant was adjusted to 4.8 and collected as another set of samples. The leftover SPI solution at 5.4 pH, at each pH level, was centrifuged at 10 000 × g force for 4 °C for 10 min to remove some remaining soy globulin proteins (precipitates) that were not reduced. The supernatant at pH 5.4 was collected as sample, and then the pH of the supernatant was adjusted to 4.8 and collected as another set of samples. These samples were studied as comparisons with SPI samples. The supernatant at pH 4.8, at each pH level, was centrifuged at 10 000 × g force for 4 °C for 10 min. The precipitate of the centrifuged was collected as the protein sample with 38% solid content.

Ionic-Strength Estimation

Ionic strength was estimated using the equation (\(I_m = 1/2 \sum M Z^2\)) based on a molality basis\[10\] where \(\Sigma\) is the sum of all B ions used in the system. \(Z\) is the charge number of ion B, and M is the molar concentration of ion B. In this paper, NaHSO\(_3\) was used to prepare protein-water solutions in three different ionic strengths (0.029, 0.058, and 0.115 mol·L\(^{-1}\)). The ionic strength was adjusted by changing the pH of the system, using either sodium hydroxide (NaOH) or hydrochloride (HCl), and was stabilized by adjusting the pH of the system.

Transmission Electron Microscopy (TEM)

A model CM 100 (FEI Company, Hillsboro, Oregon, USA) was operated at 100 kV. Protein and polypeptide samples were diluted to 1% with distilled water and sonicated for 3 min in an E&P 320 ultrasonic stirrer (E&P Manufacturing Company, Keary, N.J., USA). Samples were adsorbed for approximately 30 s at room temperature onto Formvar/carbon-coated 200-mesh copper grids (Electron Microscopy Sciences, Fort Washington, Penn., USA), and stained with 2% (w/v) uranyl acetate (Ladd Research Industries, Inc., Burlington, V.T., USA) for 60 s at room temperature before being viewed by TEM.

Light Microscopy (LM)

Images were taken on an Axioplan 2 MOT research microscope (Carl Zeiss, Inc., Thornwood, N.Y., USA) equipped with a Zeiss Axioscam HR digital camera, a fully motorized stage with mark-and-find software, plan neofluor objectives (1.25×/0.35, 10×/0.3, 20×/0.5, 40×/0.75, 40×/1.3 oil), plan apochromat objectives (63×/1.4 oil, 100×/1.4 oil), an achroplan objective (4×/0.1), differential contrast interference (DIC), phase contrast (ph), dark field, bright field, and Axiovision 3.1 software with interactive measurements and D deconvolution modules.

Protein Samples Preparation

NaHSO\(_3\) was added to the SPI solution at various amounts to produce four ionic strengths \(I_m\): 0.000, 0.029, 0.058, and 0.115 mol·L\(^{-1}\). Then the pH of the solution was adjusted to 5.4 and 4.8, and protein samples were collected at three pH levels, respectively.

The leftover SPI solution at 5.4 pH, at each pH level, was centrifuged at 10 000 × g force and 4 °C for 10 min to remove some remaining soy globulin proteins (precipitates) that were not reduced. The supernatant at pH 5.4 was collected as sample, and then the pH of the supernatant was adjusted to 4.8 and collected as another set of samples. These samples were studied as comparisons with SPI samples. The supernatant at pH 4.8, at each pH level, was centrifuged at 10 000 × g force and 4 °C for 10 min. The precipitate of the centrifuged was collected as the protein sample with 38% solid content.
Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

To estimate the polypeptide composition, SDS-PAGE was performed using a discontinuous buffer system on a 12% separating gel and 4% stacking gel, as described by Laemmli. Protein samples were mixed with SDS-PAGE sample buffer solution containing 5% β-mercaptoethanol, 2% SDS, 25% glycerol, and 0.01% bromphenol blue. Approximately 5 μg of protein sample were loaded per well. The gel electrophoresis was carried out at a 100 V constant voltage. The gel was stained with 0.25% Coomassie Brilliant Blue-R250 and destained with solution containing 10% acetic acid and 40% methanol. Molecular weight-marker proteins were run along with the samples. The percentage of polypeptide component was estimated by analyzing the gel image with Kodak 1D Image Analysis software, version 4.6 (Eastman Kodak Company, Rochester N.Y., USA).

X-Ray Diffraction

An X-ray scattering method was used to determine crystal and amorphous phases of the protein samples. A Philips APD 3520, wide-angle X-ray diffractometer was used. A voltage of 35 kV, current of 20 mA, and a curved crystal graphite monochromator (\(\lambda = 0.154 \text{ nm}\)) were employed. The protein samples were freeze-dried and ground into powder with particle size less than 100 mesh. The powder sample was continuously scanned from \(10^\circ\) to \(35^\circ\) (2\(\theta\)) with a speed of 2\(^\circ\) (2\(\theta\)) per min.

Results

TEM Images of Soy Protein Polypeptides in Aqueous System

SPI Solution with 3% Solid Concentration

For the control sample (SPI) without NaHSO₃ treatment, irregular clusters were observed, formed by a mixture of smaller spherical clusters and rod-shape clusters (Figure 1 A). Diameter of the clusters was approximately 50 to 70 nm. At low ionic strength, \(l_m = 0.029 \text{ mol} \cdot \text{L}^{-1}\), treated with NaHSO₃ reducing agent, small uniform spherical clusters were observed at pH = 9.5. These clusters had an average diameter of nearly 30 nm (Figure 1B). As pH decreased, the number of clusters decreased, but diameters became larger. For example, at pH = 5.4, the average diameter of the cluster was about 45 nm, and while at pH = 4.8, these clusters became aggregated into huge irregular spherical shape clusters with diameters ranging from 200 nm to 2 000 nm (Figure 1D). In the case of pH = 5.4, “Free-walking” polypeptides (Figure 1C arrow) existed, mainly the hydrophilic polypeptides. Distance between the cluster lines was from \(25 \text{ nm}\) to \(150 \text{ nm}\). However, at pH = 4.8, these free-walking polypeptides disappeared, which attracted to the large hydrophobic clusters. The smaller, dark-color spherical spots observed on the surface of the large clusters, with an average diameter of about 50 nm (Figure 1D, arrow), could be those hydrophilic polypeptides.

At medium ionic strength, \(l_m = 0.058 \text{ mol} \cdot \text{L}^{-1}\), treated with NaHSO₃ reducing agent, no cluster was formed at pH = 9.5 (Figure 1E). As pH decreased, for example at
pH = 5.4, a uniform chain network-like structure was observed (Figure 1F). While at pH = 4.8, uniform spherical clusters were formed with an average diameter of about 25 nm. Distance between clusters was from 10 to 40 nm.

At strong ionic strength, \( I_m = 0.115 \text{ mol} \cdot \text{L}^{-1} \), treated with NaHSO3 reducing agent, uniform spherical clusters were observed at pH = 9.5 (Figure 1H), and rod-shape clusters were in between these spherical clusters (Figure 1F arrow). The rod-shape clusters were mainly hydrophilic polypeptides. Such morphology remained the same at pH = 5.4 (Figure 1I), except that the rod-shape clusters were not as clear as those at pH = 9.5. At pH = 4.8, large, non-uniform aggregates were observed again (Figure 1J) similar to those aggregates shown in Figure 1D. However, the diameter of these aggregates was larger than those in Figure 1D, ranging from \( \approx 500 \text{ nm} \) to \( \approx 5 \mu \text{m} \).

### SPI Solution Centrifuged at pH 5.4

The protein (3% solid content) in the supernatant, pH remained 5.4, formed uniform spherical clusters in the presence of a reducing agent (NaHSO3). As \( I_m \) increased, the number and diameter of the clusters increased. At \( I_m > 0.058 \text{ mol} \cdot \text{L}^{-1} \), large-diameter clusters aggregated. All clusters had dark centers and light rings surrounded by dark rings (Figure 2 A), which indicated the spherical clusters collapsed after coating onto the copper grids. Diameter of samples treated at \( I_m = 0.029 \text{ mol} \cdot \text{L}^{-1} \) was from 70 to 500 nm, and diameter was from 100 to 1500 nm at \( I_m = 0.058 \text{ mol} \cdot \text{L}^{-1} \). Clusters at \( I_m = 0.029 \text{ mol} \cdot \text{L}^{-1} \) had a clear boundary with their environment. At \( I_m = 0.058 \text{ mol} \cdot \text{L}^{-1} \), although the clusters presented a clear line of the sphere (arrows) (Figure 2B), numerous polypeptides were tightly attached to the cluster, forming a continuous boundary surrounding the cluster (arrows) (Figure 2 C). More interesting, at \( I_m = 0.115 \text{ mol} \cdot \text{L}^{-1} \), these large clusters formed large aggregates (arrows) (Figure 2 D), and many small spherical clusters (arrow I) and irregular aggregates (arrow II) were attached to the surface of the large aggregates (Figure 2 E). Size of the large aggregates was up to 2500 nm.

At 3% solid, pH = 4.8, network-clusters formed. Figure 3 A (arrow) is a typical network structure observed. The degree of the network increased as \( I_m \) increased from 0.029 to 0.058 \text{ mol} \cdot \text{L}^{-1} \), and the network structure was uniform. But at \( I_m > 0.058 \text{ mol} \cdot \text{L}^{-1} \), aggregates of large clusters were favored over a network of small clusters. The complex structure was not uniform at \( I_m = 0.115 \text{ mol} \cdot \text{L}^{-1} \).

Some spherical clusters remained intact (Figure 3 B) or coupled with other clusters.

As solid content increased, for example at 38% solids, pH = 4.8, with NaHSO3 used as a reducing agent, chains of clusters formed at \( I_m < 0.115 \text{ mol} \cdot \text{L}^{-1} \) (Figure 3 C, arrow). The chain was made up of numerous polypeptides clusters with diameters ranging from 5 to 25 nm. But aggregate formation was still favored over chain formation at \( I_m \geq 0.115 \text{ mol} \cdot \text{L}^{-1} \) (Figure 3 D). Many large spherical clusters remained as tightly aggregated balls with diameters ranging from 100 to 600 nm (Figure 3 D, arrow I), and these aggregated balls were connected with chain-like network-structures (Figure 3 D, arrow II).

### LM Images and Phase Separation of Cured Proteins

At pH = 4.8, treated with NaHSO3, solid content was increased from 38% to 90% by curing at room condition. Phase separation between hydrophobic and hydrophilic polypeptides was clearly observed (Figure 4). At \( I_m = 0.0 \text{ mol} \cdot \text{L}^{-1} \), protein molecules were individually uniformly packed next to each other, forming a typical uniform brittle structure, and some cavities were also present, due to local protein aggregates, but no clear phase separation occurred. At \( I_m \geq 0.115 \text{ mol} \cdot \text{L}^{-1} \), spider-web-ordered structure was observed (Figure 4A). The degree of ordered spider-web structures became stronger as \( I_m \) increased. The average diameter of the spider-web structures also increased as \( I_m \) increased.

![Figure 2. TEM images of soy protein samples at 3% solid content treated with NaHSO3 and centrifuged at A) pH = 5.4 at \( I_m = 0.058 \text{ mol} \cdot \text{L}^{-1} \); B and C are enlarged images of A); D) at \( I_m = 0.115 \text{ mol} \cdot \text{L}^{-1} \), and E) is an enlarged image of D).](image-url)
When the protein sample was spread into a thin film, the spider-web pattern was totally destroyed and turned into a randomly oriented net pattern (Figure 4B). According to theories of hydrophobic interactions, phase separation is caused by hydrophobic aggregation during protein curing upon water removal, and spherical clusters are formed by mainly hydrophobic polypeptides. Therefore, the line phase should contain mainly hydrophilic polypeptides, which are squeezed out due to hydrophobic aggregation during curing. To confirm this conclusion, the cured protein gel was soaked in distilled water at room conditions for 48 h; the protein gel remaining on the glass surface was examined by using LM, and was then collected for SDS-PAGE analysis. A LM image showed that the majority of the line phase was dissolved into water, leaving cavities (Figure 4C). SDS-PAGE results confirmed that proteins in the confined area contained both hydrophobic and hydrophilic amino acids (Figure 5), but the percentage of the hydrophilic component was significantly reduced (Table 1). α' and α polypeptides are considered hydrophilic polypeptides; their contents were reduced from 13.3% and 9.6% to 4.1% and 4.6%, respectively. The acidic polypeptide is also considered a hydrophilic polypeptide, and its content was reduced from 25.7% to 7.3%. These results confirmed that the line phase was mainly constructed by hydrophilic polypeptides.

**X-Ray Diffraction**

Like most globular proteins, soy proteins are also, in most instances, in amorphous form. X-Ray diffraction patterns of both the control and sodium bisulfite-treated samples were almost identical, and no obvious crystal peaks were observed. Both control and treated protein samples were a mixture of polypeptides and were tested in powder form. This result indicates that none of the treated polypeptide formed crystal-like structures during curing.
Discussion

Sodium bisulfite, as a reducing agent, breaks down the sulfur-sulfur covalent bonds in the protein. When this happens, protein conformation changes, and the hydrogen bonding structure would also be changed. When there are more ions than needed for reduction, electrostatic bonding is interrupted. All these changes induce conformation change; in consequence, hydrophobic bonding is also interrupted among those polypeptides, resulting in a mixture of free polypeptides in the solution, such as $\alpha$, $\alpha'$, $\beta$, and acidic and basic polypeptides. Each polypeptide has its own isoelectric point (pI) (Table 2).\(^{25,26}\) $\alpha$, $\alpha'$, and acidic polypeptides are considered hydrophilic proteins, and $\beta$ and basic polypeptides are considered hydrophobic proteins.\(^{27–30}\)

Hydrophobic force (HBF), related to protein structure and composition, is a major driving force to form clusters;\(^{3,4}\) and electrostatic force (ESF) favored (ESF\(_{\text{attract}}\))\(^{31,32}\) or disfavored (ESF\(_{\text{repell}}\)) hydrophobic interaction depending on protein structure, composition, pH, and ion contents in the environment. We propose a model (Scheme 1) that would help in understanding the phenomena observed in this study. At a given protein structure and composition, when HBF $\leq$ ESF\(_{\text{repell}}\), a uniform mixture of polypeptides exists (Scheme 1B). The untreated SPI presented in Figure 1A belongs to this scenario. At HBF $>$ ESF\(_{\text{repell}}\), clusters would form as shown in Scheme 1D, and the cluster size increased as the force difference ($\Delta F$) ($\Delta F = \text{HBF} - \text{ESF}_{\text{repell}}$) increased. Meanwhile, the number of clusters decreased (Scheme 1E). This phenomenon was also observed in this study. The soy protein treated with NaHSO\(_3\) formed uniform hydrophobic clusters (Figure 1B and C; Figure 2A), and the number and size of these clusters are significantly affected by $I_m$ and pH as well as hydrophobic polypeptides content.

At HBF $\leq$ ESF\(_{\text{repell}}\), a chain-like network would form as proposed in Scheme 1C. The network structure presented in Figure 3A is considered to fit this model. As pH approached to 4.8, which is close to the isoelectric point of SPI (pI $= 4.5$), positive charge on the surface of protein molecules increased that would make the ESF\(_{\text{repell}}\) equal or close to HBF, resulting in a chain-like network. However, when ESF becomes an attractive force, ESF\(_{\text{attract}}\) favors hydrophobic interaction. In this case, protein aggregates formed as proposed in Scheme 1F, which was observed in this study as shown in Figure 2D.

The native protein structure described in Scheme 1A follows the theory proposed by Van Holde.\(^{33}\) The cluster formation phenomena described in Scheme 1C and D are in agreement with the thermodynamic theory proposed by Anfinsen.\(^{23}\) The model proposed in Scheme 1 can be a generic model for globular proteins. Proteins with both hydrophobic and hydrophilic components, as well as charged amino acids, have to be first unfolded and disassociated if the protein contains more than one polypeptide. The unfolding method should include physical, chemical, and enzymatic methods. At a given protein
structure and composition, hydrophobic clusters, aggregates, or a network can be achieved by manipulating pH and \( I_m \) to change the \( \Delta F \) between HBF and either ESF\(_{\text{repell}} \) or ESF\(_{\text{attract}} \). Properties of the clusters and network depend on protein structure, composition, pH, and \( I_m \). At a higher protein concentration (i.e., 38% solid shown in Figure 3C), unfolded polypeptides can become associated into a continuous liquid, and in another case shown in Scheme 1F, would form into viscous cohesive substances (i.e., 38% solid shown in Figure 3D). Proteins at each stage described in the proposed model have their unique properties that would be good for a specific application.

The ratio of hydrophobic and hydrophilic (HB/HL) polypeptides is critical in controlling cluster size and aggregation. As HB/HL increases from 0.16 to 0.22, corresponding to \( I_m \) increases from 0.029 to 0.058 mol \( \cdot L^{-1} \), cluster size increases (Table 2). At HB/HL=0.8, uniform and small-size clusters can be obtained regardless of \( I_m \) range used in this study, though occurring at different pH values (Figure 1). At HB/HL=1.8, proteins became large aggregates (Figure 2D). There should be a critical HB/HL value \( H_c \) around 0.8 at which the average cluster size reaches its lowest peak. When HB/HL\( > H_c \), protein becomes aggregated, and aggregation degree increases as HB/HL value increases. While, when HB/HL\( < H_c \), proteins form uniform clusters, and cluster size increased as HB/HL ratio increases up to a certain value, then decreases as HB/HL ratio approaches to \( H_c \).

**Conclusion**

Soy protein can be unfolded by sodium bisulfite, resulting in five major hydrophilic (acidic, \( \alpha \), \( \alpha' \)) and hydrophobic (\( \beta \) and basic) polypeptides. The hydrophobic polypeptides formed clusters and networks. The number and diameter of these clusters and network structure can be manipulated by changing pH and ionic strength. At higher protein concentration, these clusters turned into chain-like continuous liquid at pH \( \approx 4.8 \) and \( I_m < 0.058 \) mol \( \cdot L^{-1} \), which has strong adhesion to various surfaces, while at \( I_m \geq 0.115 \) mol \( \cdot L^{-1} \), a viscous and cohesive substance formed, which has potential for fabricating fibers, films, and plastics. Upon curing, hydrophobic clusters aggregated and phase separation between hydrophobic and hydrophilic polypeptides occurred.

Hydrophobic interactions follow the global, minimum-free-energy theory when proteins are not biologically functional, which is the major reason causing cluster formation. While Van der Waals forces become important at higher protein concentration to form continuous chain like structure.

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Table 2. Isoelectric point of soy polypeptides, diameters of clusters, and SDS-PAGE estimated contents (%) of polypeptides of soy protein isolates (SPI) treated with NaHSO\(_3\) at \( I_m = 0.029, 0.058 \) mol \( \cdot L^{-1} \), and 0.115 levels; where 7S stands for conglycinin, 11S for glycinin, HB for hydrophobic polypeptides, and HL hydrophilic polypeptides. Polypeptides contents were estimated based on the percentage of 11S and 7S.

<table>
<thead>
<tr>
<th>Polypeptides</th>
<th>SPI centrifuged at pH 5.4</th>
<th>SPI without centrifuge</th>
<th>Isoelectric point (^{a)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I_m ) [mol ( \cdot L^{-1} )]</td>
<td>0.029</td>
<td>0.058</td>
<td>0.115</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>43.6</td>
<td>31.2</td>
<td>11.0</td>
</tr>
<tr>
<td>( \alpha' )</td>
<td>33.5</td>
<td>34.8</td>
<td>8.6</td>
</tr>
<tr>
<td>( \beta )</td>
<td>14.0</td>
<td>18.1</td>
<td>7.9</td>
</tr>
<tr>
<td>Acidic</td>
<td>8.9</td>
<td>15.9</td>
<td>16.1</td>
</tr>
<tr>
<td>Basic</td>
<td>0.0</td>
<td>0.0</td>
<td>56.4</td>
</tr>
<tr>
<td>11S</td>
<td>8.9</td>
<td>15.9</td>
<td>72.5</td>
</tr>
<tr>
<td>7S</td>
<td>91.1</td>
<td>84.1</td>
<td>27.5</td>
</tr>
<tr>
<td>11S/7S ratio</td>
<td>0.1</td>
<td>0.2</td>
<td>2.4</td>
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<tr>
<td>Total HB</td>
<td>14.0</td>
<td>18.1</td>
<td>64.3</td>
</tr>
<tr>
<td>Total HL</td>
<td>86.0</td>
<td>82.0</td>
<td>35.7</td>
</tr>
<tr>
<td>HB/HL ratio</td>
<td>0.16</td>
<td>0.22</td>
<td>1.8</td>
</tr>
<tr>
<td>D of cluster (^{b)} ) [nm]</td>
<td>70–500</td>
<td>100–1500</td>
<td>500–2500</td>
</tr>
</tbody>
</table>

\(^{a)}\) Data from ref.[24,25]; \(^{b)}\) D = diameter.


