

Investigation of Dispersion States and Rheological Properties of SPI in the DMSO/Urea

Ru Xiao, Duan Yin, Lixia Gu

The State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, Donghua University, Shanghai 200051, China

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ABSTRACT: Dimethylsulfoxide (DMSO)/urea were chosen as a hybrid solvent system to dissolve soy protein. The dispersion status and rheological properties of soy protein isolates (SPI) in the DMSO/urea were studied. The result showed that SPI has relatively high solubility in DMSO/urea blend system and urea served as a key factor of dissolving SPI in the system. SPI in the DMSO/urea hybrid system exhibited different dispersion statuses with increasing of SPI content. The SPI dispersions in the

DMSO/urea presented shear thinning or pseudoplastic behavior. The pseudoplastic behavior, the degree of non-Newtonian flow and the extent of structuralization of SPI dispersion system with increasing SPI content were discussed. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 110: 1961–1966, 2008

Key words: soy protein isolate; DMSO/urea; dispersions; rheology

INTRODUCTION

Petroleum-based polymeric materials have become increasingly important and popular because of their superior performance. However, the petroleum-based materials are nonbiodegradable and the disposal of the used materials has become a serious environmental problem. Moreover, petroleum resources are naturally nonrenewable and will be depleted in the future. Therefore, the abundant, renewable, and biodegradable natural macromolecules have become new resources to material scientist.

In exploring alternative raw materials to replace the traditional petroleum resources, soy proteins have been considered to have great potential because of their renewability, biodegradability, and large quantity. The use of soy proteins has recently expanded into the areas of plastics,^{1,2} adhesives,^{3,4} films,^{5,6} elastomers,⁷ and fibers.^{8,9}

In the processing of polymers, the dispersion states and rheological properties are very important. For the preponderance of polar and hydrophilic amino acids, soy protein usually presents good water solubility. Soy proteins have been extensively studied in alkaline solutions and alcohol denatured forms.^{10–12} Denatured soy proteins in a series of organic solvents revealed that soy proteins were not

soluble in nonpolar solvents such as benzene, ethane, carbon tetrachloromethane, and so on.¹³ The gel formation is one of the most important functions of soy protein. The gelation phenomena, which were induced by heat,^{14–16} protease,¹⁷ microbial transglutaminase,¹⁸ of soybean protein has been observed and investigated. There is extensive literature discussing about the rheological properties of soy protein dispersion recently, including suspension,¹⁹ emulsion,²⁰ and interfacial²¹ rheological properties. Moreover, the relations between rheological properties and formation as well as structure of soy protein gel, which would be effected by pressure,²² pH, and ionic,^{16,23} were investigated.

However, there was infrequency to investigate dispersion state and rheological properties of SPI in organic solvent. In this study, dimethylsulfoxide (DMSO)/urea was chosen as a hybrid solvent system to support the application of soy protein in chemical fiber, and the different dispersion states were observed, and the effect of urea concentration was studied. Consequently the rheological properties of SPI in the DMSO/urea hybrid solvent system were investigated.

MATERIALS AND METHODS

Materials and reagents

SPI was supplied by Shanghai Shenyuan Food Company. DMSO and urea were supplied by China Medicine (Group) Shanghai Chemical Reagent Corp.

Correspondence to: R. Xiao (xiaoru@dhu.edu.cn).

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Preparing SPI dispersion with different condition

Preparing SPI diluted solutions with different urea concentration

SPI was dissolved in DMSO/urea mixtures in a content of 0.1 wt % in DMSO. The urea concentrations were varied in 0, 1, 2, 3, 4, and 5M, respectively, while the treatment temperature was maintained at 90°C and treatment time was 2 h. The water bath was heated at a heating rate of 1°C/min to reach a desired temperature and maintained at the temperature for certain duration.

Calibration of SPI content in DMSO/urea versus UV absorbance at 280 nm

SPI powder was swollen in DMSO/urea blend solvent at room temperature, the content of SPI was 0.05 wt % in DMSO, the concentration of urea were 1, 2, and 3M, respectively, in DMSO. After swollen for 1 h, the mixture was heated to 90°C in a heating rate of 1°C/min. Then it was stirred for 2 h at a medium speed at 90°C. Solutions containing higher soy protein content, such as 0.1, 0.15, 0.2, 0.25, and 0.3 wt %, respectively, were prepared by the same method.

Preparation of saturated SPI solutions

Extra amount of SPI powder was swollen for about 1 h in DMSO/urea, in which the concentration of urea was 1M, then the mixture was stirred at 30°C for about 2 h and centrifuged at $12,000 \times g$ at 30°C for 1 h using CR22F centrifuge (Hitachi Koki, Japan), conditions that can reach thermodynamic phase separation equilibrium, according to reference. The top layer in the mixture after centrifugation was the saturated SPI solution. Solutions were prepared under treatment temperatures of 50, 60, 70, and 80°C, respectively, and the heating rate was 1°C/min. The saturated SPI solutions containing 2 and 3M urea were prepared following the above method.

Preparation SPI dispersion with different SPI or urea content

Compared with DMSO, 1 wt % SPI was added to DMSO/urea hybrid solvent containing 0, 1, 3, 5, 7M urea, respectively. After swollen for 1 h at room temperature, the SPI dispersion was heated to 90°C in a heating rate of 1°C/min. Then it was stirred for 2 h at a medium speed at 90°C. The dispersions containing 3, 5, 7, and 9 wt % SPI were prepared respectively, following the mentioned method.

Preparation of SPI dispersions for rheological properties measurement

SPI was added to DMSO/urea hybrid solvent containing 3M urea at room temperature, and was heated to the 90°C with the heating rate 1°C/min and then keeping constant 90°C for 2 h. The samples were debubbled under vacuum oven for 15 min at 70°C, and then were preserved hermetically.

Measurements

Intrinsic viscosities of soy proteins were measured using an Ubbelohde type of capillary viscometers at $30^\circ\text{C} \pm 0.02^\circ\text{C}$. UV spectra were obtained from a UV-VIS8500 spectrometer (Shanghai Tianmei Instrument, China). Blank DMSO and DMSO-urea solutions were used as control, the SPI solutions were put in the measured light line.

Laser light scattering and electrophoresis light scattering were recorded on a Malvern Zetasizer Nano-ZS (Malvern Instruments) at 27°C.

Thermal analysis was performed by differential scanning calorimetry (DSC) on a modulated DSC TA2910 (USA). The temperature was increased from room temperature to 120°C with a heating rate of 2°C/min. After the end of the first scan, the sample was quenched by liquid nitrogen with a cooling rate of 40°C/min. Then the temperature was increased from room temperature to 120°C with heating rate of 2°C/min again, after 1-min equilibrium at 20°C. All measurements were performed under nitrogen atmosphere.

Apparent viscosities of dispersion systems with different SPI concentrations were measured by using NDJ-72 revolving viscosity rheometer (Shanghai, China) at 85°C. The rheological properties of the dispersions were measured on a HAAKE RS150L rheometer (Hakke company, Germany) using a C35/1° cone-and-plate system. The realized region of shear rate $\dot{\gamma}$ was 0–800 s^{-1} , and the measurements were carried out at 60°C.

RESULTS AND DISCUSSION

Properties of SPI diluted solutions

Viscosity of polymer solution is an indicator of whether a solvent is suitable for dissolving a polymer. Thus, intrinsic viscosities of SPI solutions containing different urea concentrations were measured and the results are shown in the Figure 1. The values of intrinsic viscosities of SPI were low without urea in the system and then increased remarkably after urea was added. As the urea concentration was raised from 1 to 5M, the viscosities of SPI solutions increased almost linearly, indicating that urea molecules helped dissolution of SPI in DMSO. Most protein molecules are in globular form and possess

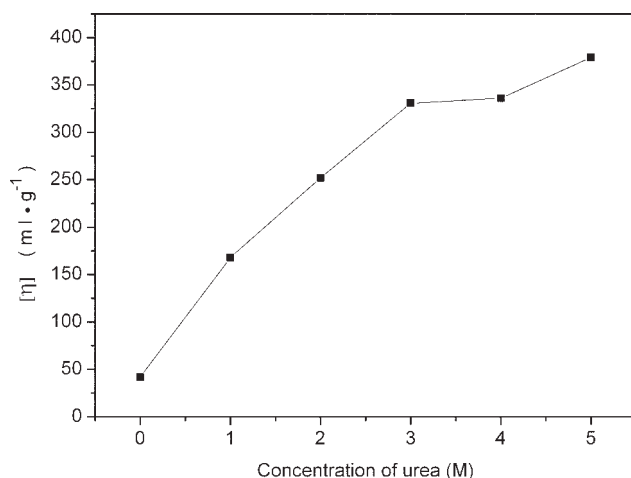


Figure 1 Intrinsic viscosity of SPI with different concentrations of urea.

strong intramolecular and intermolecular interactions, which inhibit dissolution of the protein in regular organic solvents. The viscosity increase reveals that the configurations of the globular protein molecules have changed and the protein molecules in the solutions become more extended or linear. As a result of configurational and conformational changes the proteins are denatured as well.

The linear form of the protein molecules should change dynamic sizes of the proteins in the solutions. Thus, particle size distributions of SPI in DMSO/urea systems with different concentrations of urea should vary. Laser light scattering results of the protein solutions, which proved the above hypothesis, are shown in the Figure 2. The average particle sizes of SPI were relatively small without existence of urea, and become increased gradually as the concentration of urea was increased in the solutions. The increase of molecular sizes were quite significant, from about 10–20 nm to 90–110 nm when the urea concentration was increased from 0 to 5M, representing 5–10 times of extension of molecular sizes.

The addition of urea in the solvent system improves dissolution and increases radius of gyration of the proteins. Urea molecules interact with protein molecules and form strong hydrogen bonds between the molecules. The hydrogen bonds between protein molecules could be replaced by the interactions between urea and polypeptide. Then, the polypeptide chain was destroyed and protein molecules become more in linear form rather than spiral or folded ones.

Studying about the maximum dissolution degree of SPI in the DMSO/urea blend solvent

The absorbance of protein at 280 nm was reported to measure concentration of protein. Therefore, a series

of solutions of different SPI contents with different urea concentrations were prepared and measured for UV absorbance at 280 nm. Calibration curves for SPI concentration versus UV absorbance at 280 nm were prepared, which are shown in the Figure 3. All of calibration curves displayed good linear relationship ($R > 0.998$), so the values of absorbance can reflect the concentrations of SPI in solutions. Consequently, the UV absorbance of saturated SPI solution was measured at 280 nm. Then from the calibration curves, the content of SPI in the saturated solution was determined. The dependence of dissolution degree of SPI in DMSO/urea blend solvent with different urea concentration is shown in the Figure 4. It was revealed that the dissolution degree of SPI in the DMSO/urea was small. The maximum value of dissolution was about 4.8%, occurring at a urea concentration of 3M at 90°C.

The dissolution degree of SPI in the distilled water under different pH, ranging from pH 2 to pH 8, was studied by Renkema et al.¹⁶ The maximum value was less than 1%. The minimum was in pH 4.5 which is the isoelectric point of SPI. The dissolution degree increased when pH less or higher than pH 4.5. Compared with the results in aqueous solution, the dissolution degree of SPI in the DMSO/urea was significantly increased with addition of urea.

Properties of SPI gel formation

DSC measurements

Figure 5 displays the DSC-thermograms of 8 wt % SPI in the DMSO/urea blend solvent. Two transitions were observed caused by denaturation of β -conglycinin at the lowest temperature and of glycinin at the highest temperature. The peak denaturation temperatures were 54.9°C for β -conglycinin and

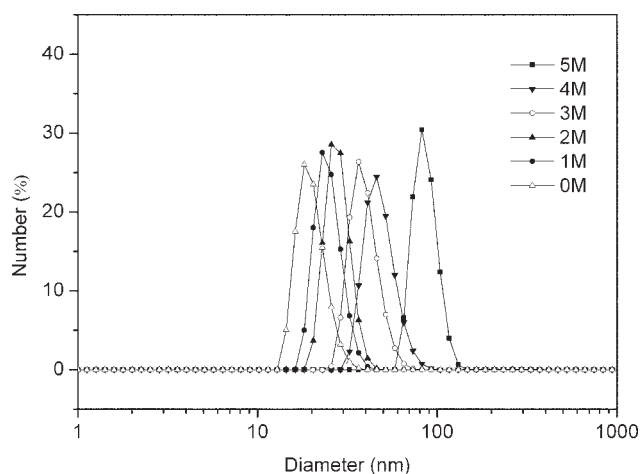


Figure 2 Particle size distributions of SPI with different urea concentration.

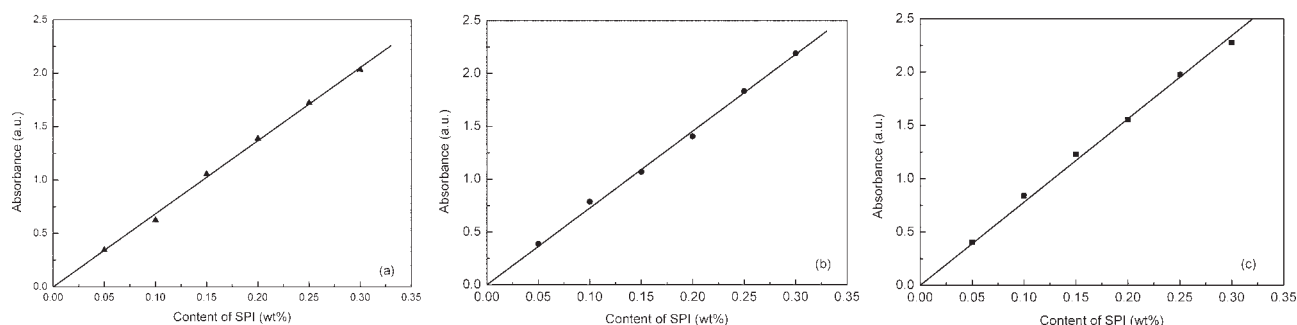


Figure 3 Calibration curves of SPI contents in DMSO/urea hybrid solvent systems with different urea concentration (a) 1M, (b) 2M, (c) 3M by UV absorbance at 280 nm.

88.9°C for glycinin. Renkema and Van Vliet studied the denaturation of SPI in the aqueous system at a heating rate of 1°C/min. The results show that the peak denaturation temperatures were 68°C for β -conglycinin and 88°C for glycinin.²⁴ The reason for that difference may be the influence of different solvent systems. Renkema indicated that the denaturation of SPI in the aqueous system may be induced by heat. The denaturation of SPI in the DMSO/urea may be induced by heat and chemicals. The solubility properties of SPI in the DMSO/urea were detected by measuring the apparent viscosity in the previous study. The results showed that the apparent viscosity increased significantly when the treatment temperature was higher than 50°C, and it reached a maximum when treatment temperature was about 85°C. So it was considered that the denaturation of SPI in the DMSO/urea began in the 50°C, and it was denatured completely when treatment temperature reached around 85°C. Therefore, the results are consistent with the DSC-thermograms. Heating the dispersion times showed a DSC-thermogram without endotherms indicating that denaturation of SPI was an irreversible process. So in this

study, 90°C was chosen as the treatment temperature in the process of SPI gel formation.

Effect of urea concentration

Figure 6 displays the apparent viscosity of dispersion of different SPI content in DMSO/urea with different urea concentration. A critical SPI content of gel formation can be defined at the turning point of each curve. It can be found that the critical SPI content at gel formation slightly increased with increasing urea concentration until 3M, and it few decreases with further increasing of concentration of urea. The gel formation process of protein means that more and more chains of protein becomes incorporated into network leading to a further built-up of the network structure.^{24,25} Apart from that, gel formation process of protein also means rearrangement of the protein chain in the network. Aforementioned, urea is main reason that soy protein can dissolve in the DMSO. It seems that the critical SPI content of gel formation will decreases with increasing of

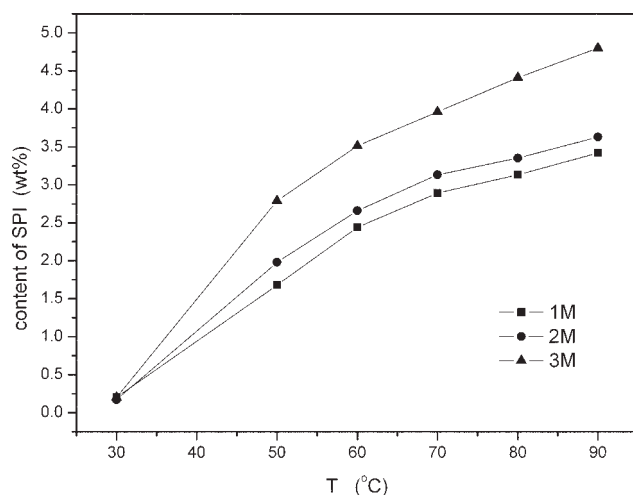


Figure 4 Dissolution degree of SPI in DMSO/urea hybrid solvent system with different urea concentrations.

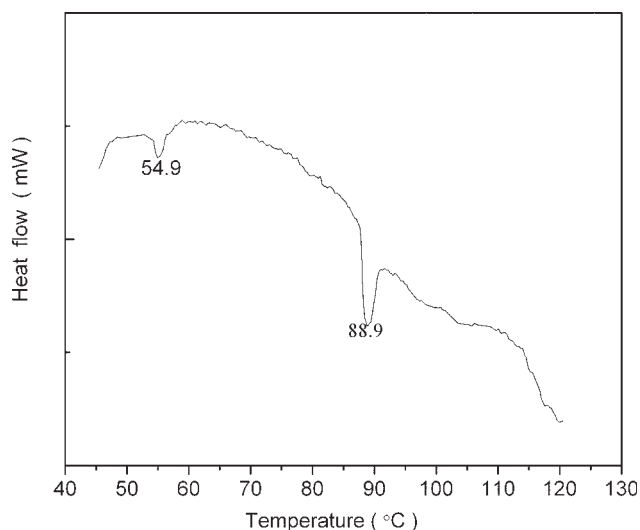


Figure 5 DSC curve of 8 wt % SPI in DMSO/urea hybrid solvent system.

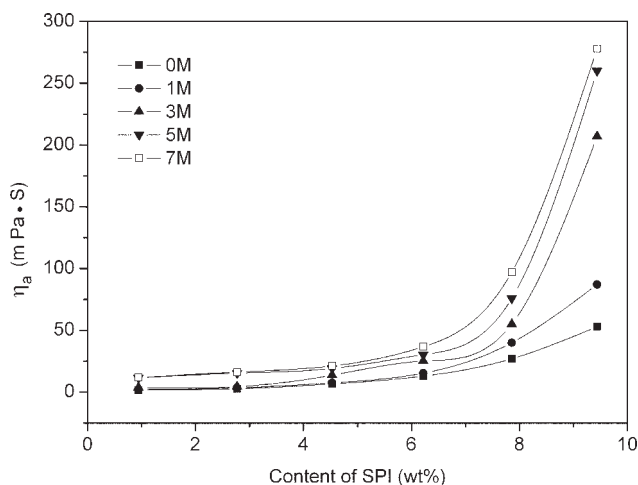


Figure 6 Apparent viscosity of different SPI contents in the DMSO/urea hybrid solvent with different urea concentrations at 85°C.

concentration of urea. On the other hand, the apparent viscosity of SPI dispersion with urea concentration of 0 and 1M is comparatively low owing to less activity of protein chains. Therefore, the weak gel network will be formed at relatively low of SPI content, and the critical SPI content of gel formation increases with increasing of urea content until 3M. However, the dissolution of protein chain in the urea/DMSO solvent increases, and the apparent viscosity also increases significantly with increasing of urea concentration further. Thus, the critical SPI content of gel formation few decreases after the concentration of urea reaches 3M.

It is mentioned that the dissolution degree of SPI in the DMSO/urea was about 4.8 wt % at 90°C with 3M urea concentration. Moreover, the critical SPI content of gel formation was about 7.1 wt % under the same condition (Fig. 6). According to the reference,²⁶ it stays sol state from solution transfer to gel state during gel formation process. Therefore, the different dispersion state, such as solution, sol and gel, were presented with increasing of SPI content in the DMSO/urea blend solvent system. Formation of gel phase in protein spinning solution will make fiber extrusion difficult. Therefore, it is very important to control the SPI content in the solution of wet spinning during fiber making process.

Rheological properties of SPI in DMSO/urea

Dependence of apparent viscosity on shear rate

The relation about apparent viscosity on shear rate of SPI dispersions with different SPI content at 60°C are shown in Figure 7. It can be observed that the systems of different SPI content in DMSO/urea are shear thinning or pseudoplastic behavior, a visible

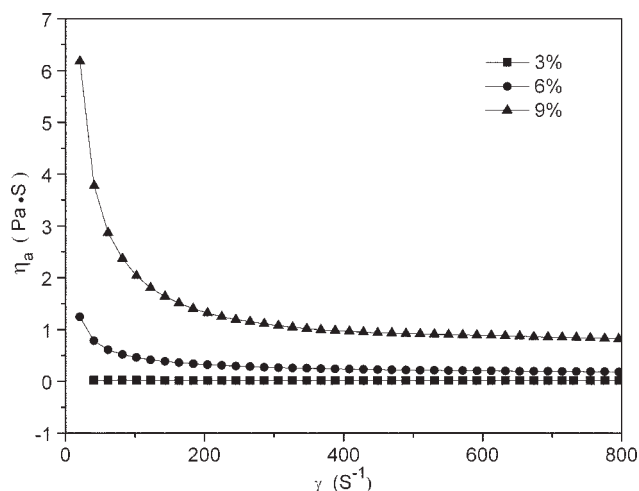


Figure 7 Dependence of apparent viscosity on shear rate of different SPI content in DMSO/urea hybrid solvent at 60°C.

decrease in viscosity with increasing shear rate. Moreover, the pseudoplastic behavior increases with increasing SPI content. The pseudoplastic behavior appears still at low shear rate, but the velocity decrease is slow with increasing shear rate. It is known that the protein has primary, secondary, tertiary, and quaternary structures because of space force. Thus, the macromolecular chain entanglement forms easily in the solution, which makes soy protein macromolecules difficult to move from each other. Moreover, it is mentioned that soy protein solution system gradually transferred to sol or gel state with increasing of soy protein content. Therefore the apparent viscosity increases with increasing SPI content. However, when shear rate increases, part of the entanglement and sol or gel structure may be rapidly be destroyed by increasing shear stress. On the

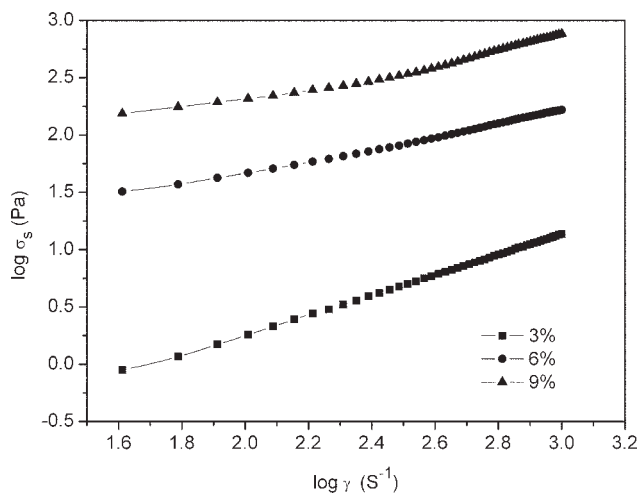


Figure 8 Flow curves of different SPI content in DMSO/urea hybrid solvent at 60°C.

TABLE I
Dependence of Non-Newtonian Index n and Structural Viscosity Index $\Delta\eta$ with Different SPI Contents in DMSO/Urea Hybrid Solvent System

Concentration of SPI (wt %)	3%	6%	9%
Non-Newtonian index n	0.88	0.56	0.55
Structural viscosity index $\Delta\eta$	1.88	29.3	61.6

other hand, molecular orientation may occur along with shear stress by the chain of soy protein, so the force of laminar flow decreases. Thus the dispersions of soy protein shows a decrease of apparent viscosity, and the extent of velocity decrease ascends with increasing of soy protein content.

Dependence of non-Newtonian index n on SPI content

Flow curves of the SPI dispersions with different SPI content at 60°C are given in Figure 8. It is evident that shear stress increases with increasing shear rate. Dependence of the non-Newtonian index n on the SPI content is shown in Table I. It is observed that the non-Newtonian index n is smaller than 1 and decreases sharply with increasing of SPI content. This may be due to that sol or gel states of soy protein were gradually formed in the DMSO/urea hybrid solvent with increasing of soy protein content. Thus the extent of non-Newtonian flow increases with increasing of SPI content.

Dependence of structural viscosity index $\Delta\eta$ on SPI content

The structural viscosity index $\Delta\eta$ indicates the extent of solution structuralization, $\Delta\eta = (-dl\eta_a/d\dot{\gamma}^{1/2}) \times 10^2$. A lot of polymers belong to pseudoplastic fluid, where $\Delta\eta$ is larger than 0. The structural viscosity index $\Delta\eta$ of SPI dispersions with different SPI content is showed in the Table I. It is evident that the structural viscosity index $\Delta\eta$ increases sharply with increasing SPI content. Comparing with 3 wt % SPI content, the structural viscosity index $\Delta\eta$ increases about 10 times when SPI content reach 6 wt %. Aforementioned, the states of SPI dispersion in the DMSO/urea mainly depend on the content of SPI. The sol or gel states of soy protein were formed in the DMSO/urea hybrid solvent with increasing of soy protein content. This means the network structure was formed gradually, and the space force increases with increasing of SPI content. Thus, the extent of system structuralization sharply increases. Generally, higher $\Delta\eta$ leads to lower spinnability of wet spinning solutions, worse fiber formation and lower mechanical property of the fiber. Therefore, it also proves the above result, it is very important to

control the SPI content in the solution of wet spinning during fiber making process.

CONCLUSION

SPI could be dissolved in a DMSO/urea blend solvent system to reach a relatively higher content. SPI in the DMSO/urea hybrid system exhibited different dispersion states, from solution transferring to sol and gel states with increasing of SPI content. The SPI dispersions in the DMSO/urea systems present shear thinning or pseudoplastic behavior. The pseudoplastic behavior, the degree of non-Newtonian flow and the extent of structuralization of SPI dispersions system increase with increasing SPI content. Therefore, carefully managing the difference may be helpful in preparation of soy protein fiber.

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