

Thermal and Mechanical Properties and Water Absorption of Guanidine Hydrochloride-Modified Soy Protein (11S)

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ABSTRACT: Thermal and mechanical properties and water absorption of guanidine hydrochloride (GuHCl)-modified 11S soy protein and molded plastics made from it were studied using differential scanning calorimetry (DSC) dynamic mechanical analysis (DMA) mechanical tests and scanning electron microscopy (SEM). The DSC results showed that the denaturation temperature of GuHCl-modified 11S solutions was higher than that of the control sample and the high concentration GuHCl completely denatured 11S. Nonfreezing water of the modified 11S solution exhibited a minimum value at 0.9 M GuHCl. Both DSC and DMA results showed that GuHCl was a plasticizer of 11S and the glass transition temperature of modified 11S plastics decreased with increasing GuHCl concentration. Both the stress and strain of modified 11S plastics reached their highest values at a 0.9 M GuHCl concentration. The SEM observations supported these results. A water-absorption test showed an improvement in the water resistance of 11S plastics with GuHCl modification. The water absorption had a minimum value at 0.9 M GuHCl. The interaction between GuHCl molecules and 11S protein was found to have important effects on the thermal and mechanical properties and the water absorption of 11S plastics. © 2000 John Wiley & Sons, Inc. *J Appl Polym Sci* 78: 1063–1070, 2000

Key words: 11S soy protein; guanidine hydrochloride; thermal properties; mechanical properties; water absorption

INTRODUCTION

Synthetic petroleum polymer materials have achieved great success since the beginning of this century. They have been used widely in many fields because of their high strength, light weight, low price, easy processability and energy efficiency. However, the environmental impact of persistent plastic wastes from disposable items has grown more acute worldwide in recent decades. The development of biodegradable plastics,

which degrade in the environment by means of humidity and the action of microorganisms is needed as an alternative to help solve these environmental problems.

Soybean proteins have been considered recently as petroleum polymer alternatives in the manufacture of adhesives, plastics and various binders. Soybean protein, the major component of the soybean (30–45%),¹ is readily available from renewable resources and agricultural processing by-products. Plastics made from soy protein have high strength; for example, 40 MPa of tensile strength for the plastics made from soy protein powder with 11.7% moisture content,² and good biodegradable performance. Therefore, more and more attention has been focused on these materials.

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Much research on soy protein plastics was conducted in the 1930s and 1940s, and soybean products were incorporated into phenolic resins mainly as fillers or extenders.^{3,4} Then, little was done on developing soy protein plastics, because petroleum-based plastics dominated the market. In the 1990s, soy protein plastics gained researchers' attention again for their environmentally friendly properties. Most of the studies focused on the mechanical properties and water absorption of soy protein plastics and showed that both were affected greatly by the composition of soybean protein and the processing conditions.^{2,5-7} Soy protein plastics were rigid, brittle, and water-sensitive. Therefore, many efforts have been made to modify their brittleness and to improve their water resistance. Incorporating plasticizers, such as glycerol⁸⁻¹⁰ and polyols,^{11,12} was a widely used method to improve processability and toughness. Polysaccharides also were used to improve the properties of soy protein materials.^{13,14} Starch is a polymeric material that was used widely to modify soy protein plastics.¹⁵⁻²³ These soy protein-starch plastics could be extruded and injection-molded into articles of various shapes and sizes, and the products had good tensile properties and water resistance. Soy protein plastics treated with a crosslinking agent or incorporated with cellulose filler also showed decreased water absorption.²⁴ Recently, blending with bioabsorbable polyphosphate fillers was found to improve significantly the intrinsic poor water resistance of biodegradable soy protein plastic.^{19,20,25,26} All these studies showed that soy protein plastics have a great potential for practical use.

The goal of this study was to investigate the thermal and mechanical properties and water absorption of guanidine hydrochloride (GuHCl)-modified 11S soy protein and molded plastics made from it. Soy proteins contain two major components: glycinins (11S-rich globulin, 52%) and conglycinins (7S-rich globulin, 35%). We chose the 11S component to reduce the factors that potentially can affect properties of the products.

EXPERIMENTAL

Materials and Specimen Preparation

Soybean protein fraction 11S was extracted from defatted soybean flour (Cargill, Cedar Rapids, IA) following the procedures described by Sun et al.²⁷

The 11S fraction had a purity of 90% and moisture content of about 6 wt % as measured by an air-oven method.²⁸ The GuHCl (99%) was purchased from the Sigma Chemical Co. (St. Louis, MO).

To prepare GuHCl-modified 11S, 25 g of 11S protein was dissolved in 250 mL of a solution with GuHCl concentrations of 0.5, 0.9, and 2.4M. The control sample was prepared by dissolving 25 g of 11S in 250 mL distilled water. The mixtures were stirred continuously for 6 h and then freeze-dried. The freeze-dried mixtures were powdered and contained approximately 10% moisture.

To prepare specimens for the tensile test, the control and GuHCl-modified 11S powders were placed in a dumbbell-shaped tensile bar mold (type IV) and compression-molded using a Carver hot press (Model, 3890 Auto "M," Carver Inc., Wabash, IN) according to ASTM standard D686-92.²⁹ The specimen was molded at 120°C for 4 min and then cooled to 50°C before removal from the mold. Flash was removed carefully by sanding the edges of the specimen with grade 180 abrasive sandpaper. The moisture content of these specimens was about 2.6%.

Thermal Analysis

Thermal properties of the GuHCl-modified 11S solution and the molded GuHCl-modified 11S were measured using a Perkin-Elmer Pyris-1 differential scanning calorimeter (DSC) (Perkin-Elmer, Norwalk, CT). The instrument was calibrated with indium and zinc standards before official measurements, and all measurements were conducted under a nitrogen atmosphere. The heating rate was 10°C/min.

Dynamic Mechanical Analysis (DMA)

Dynamic mechanical analysis was carried out using a DMA-7e dynamic mechanical analyzer (Perkin-Elmer, Norwalk, CT) with the 3-point bending-rectangle method at 1 Hz. The heating rate was 3°C/min. The samples for DMA testing were cut from the narrow part of the molded bar, and the size was about 15 × 6 × 2 mm³.

Mechanical Property Tests and Morphology

Mechanical properties were measured using an Instron testing system (Model 4466, Canton, MA) according to ASTM standard D638-92.³⁰ Each specimen was preconditioned at 23°C and 50% relative humidity for 48 h and tested at a 5 mm/

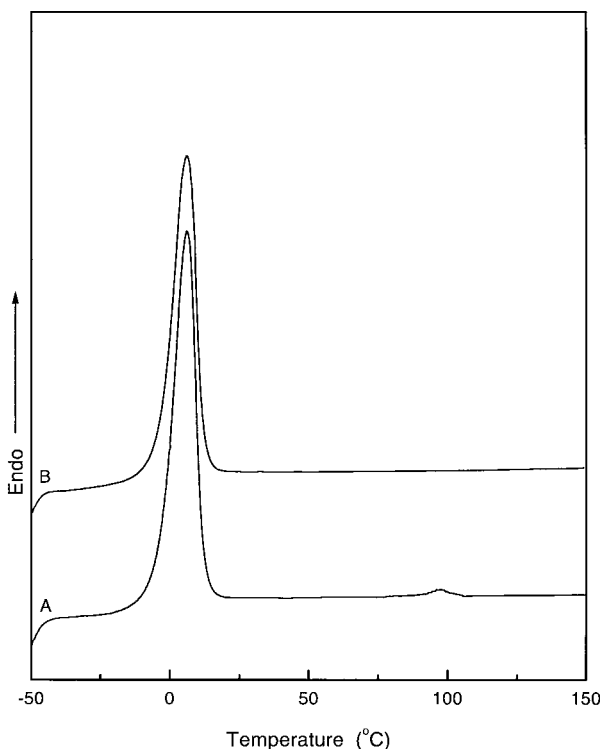


Figure 1 DSC thermograms of 0.5M GuHCl-modified 11S solution: (A) first scan; (B) second scan.

min crosshead speed. The stress, strain at maximum stress, and Young's modulus were obtained from the tests. The values presented are averages of five specimens.

The fracture surface of the tensile test specimens was observed using a scanning electron microscope (SEM; AutoScan, ETEC Corp., Highlands, TX) at an accelerated voltage of 20 kV. The specimens were coated with thin layers of gold of 200 Å before observation.

Water Absorption

Water absorption was measured using the ASTM standard D570-81.³¹ The specimens were preconditioned by drying in an oven at 50°C for 24 h. Then they were cooled in a desiccator for a few minutes, weighed, and submerged in distilled water at 25°C for 2 and 26 h. The specimens were removed from the water, dried with a paper towel, and weighed again. Dry matter from the plastics left in the water during soaking also was included in the water-absorption calculation. The values presented are averages of three specimens.

RESULTS AND DISCUSSION

Thermal Properties

Typical thermograms of a low-concentration GuHCl-modified 11S solution showed an ice-melting transition at around 0°C and a thermal denaturation transition at around 90°C [Fig. 1(A)]. After the solution was heated to 150°C and then quenched, however, the thermograms showed only an ice-melting transition [Fig. 1(B)]. This means that, under the experimental conditions, the thermal-denatured 11S structure was not recovered after quenching. The 11S protein was denatured by the addition of GuHCl, and the thermal denaturation temperature (T_d) of the 11S protein increased after being modified by 0.5 and 0.9M GuHCl (Fig. 2). For the 2.4M GuHCl-modified 11S protein solution, no thermal denaturation transition was observed because the 11S protein was completely denatured during modification.

Most proteins with an ordered native structure undergo a marked denaturation transition upon addition of GuHCl because of its preferential interaction with them.^{32,33} After complete denaturation, these proteins are random coils, without a residual noncovalent structure. At low concentration, the number of GuHCl molecules was not enough for a complete denaturation of the 11S-

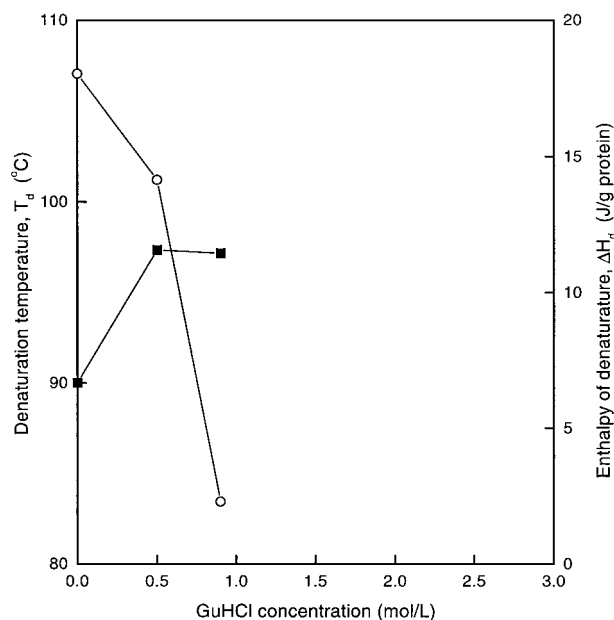


Figure 2 (■) Denaturation temperature (T_d) and (○) denaturation enthalpy (ΔH_d) of 11S solutions as affected by GuHCl concentration.

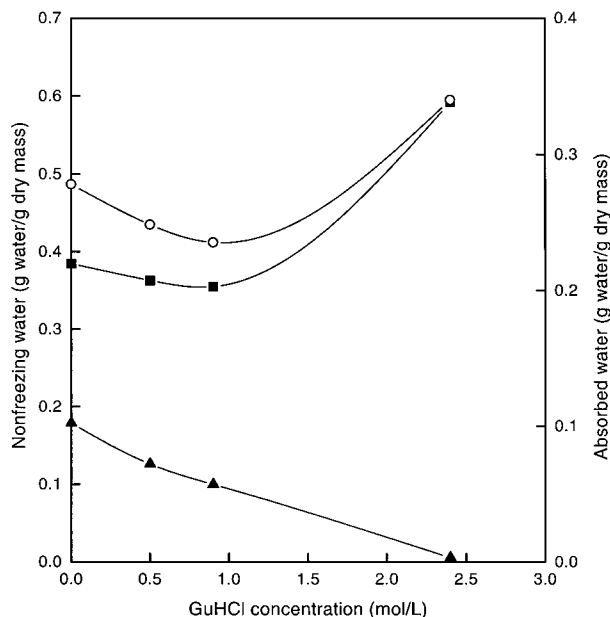


Figure 3 Nonfreezing water of 11S solutions (■) before and (○) after denaturation and (▲) absorbed water as affected by GuHCl concentration.

protein structure. Therefore, the remaining 11S structure continued to be denatured during DSC heating, and, hence, the thermograms showed a thermal denaturation transition (Fig. 2). However, at higher GuHCl concentration, for example, at 2.4M, most ordered structures of 11S were destroyed during mixing, and no thermal denaturation transition was observed during DSC heating. The less stable structure of 11S was denatured first and, then, the more stable structure. Therefore, the protein structure of the partly denatured 11S was more stable to heat, and, hence, the T_d increased. In other words, the thermal stability of 11S increased after partial modification by GuHCl.

The enthalpy of denaturation (ΔH_d) decreased as the GuHCl concentration increased (Fig. 2). With increasing GuHCl concentration, more and more 11S structures were denatured during mixing. Therefore, less energy was needed to further denature 11S during DSC heating.

Nonfreezing water (or bound water) reflects the interaction between protein and water molecules and relates closely to the water-holding capacity of protein. The study of nonfreezing water also can provide important information on the structure and functional properties of proteins.^{34–39} The nonfreezing water of the GuHCl-modified 11S solution was calculated from the

ice-melting transition^{37–39} and plotted against the GuHCl concentration (Fig. 3). Nonfreezing water of the modified 11S solution both before and after denaturation decreased first and then increased greatly as the GuHCl concentration increased and exhibited a minimum value at about 0.9M GuHCl. The decrease of nonfreezing water can be attributed to the interaction between the protein and the GuHCl molecules, which reduced the number of protein groups that could interact with the water molecules. Additionally, exposed hydrophobic groups during GuHCl modification could have contributed to the decrease of the nonfreezing water. However, at high concentration, GuHCl molecules would be excessive and also absorb water. Also, the denatured 11S protein with a more random coil structure could have more hydrophilic groups exposed to water, which could improve the interaction between protein and water. The nonfreezing water of 11S was always larger after denaturation than before denaturation (Fig. 3). The absorbed water of the modified 11S solution during thermal denaturation also was calculated,³⁹ and it decreased with increasing GuHCl concentration (Fig. 3). This

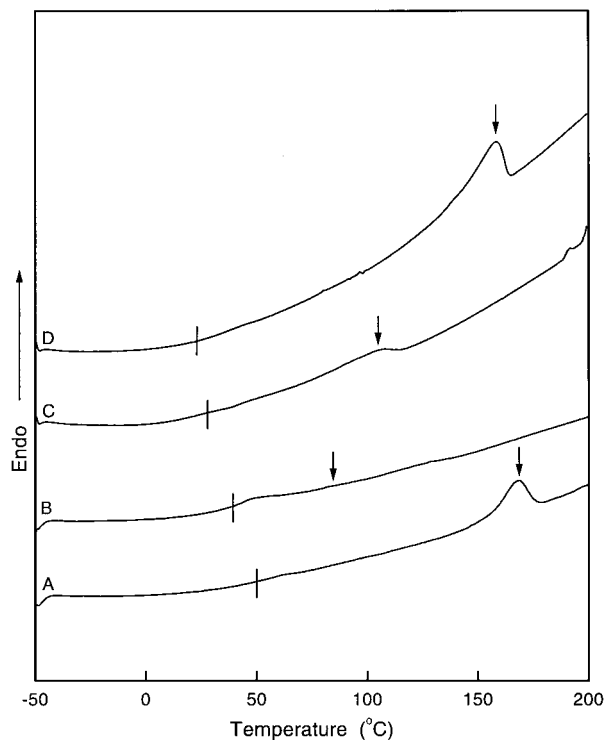


Figure 4 DSC thermograms of 11S plastics: (A) control; (B) 0.5M GuHCl-modified; (C) 0.9M GuHCl-modified; (D) 2.4M GuHCl-modified.

Table I DMA and DSC Results for Molded Plastics Made from GuHCl-modified 11S

Treatment	DMA Results		DSC Results		
	T_g (°C)	T_g (°C)	T_d (°C)	T_m (°C)	
Control	74.2	46.5	167.4	—	
0.5M GH	35.0	34.3	—	89.3	
0.9M GH	22.1	25.6	—	104.5	
2.4M GH	18.5	24.3	—	157.6	

Moisture content: 2.6%.

was understandable, because the absorbed water was determined mainly by exposure of hydrophilic groups to water during the thermal denaturation,³⁹ and more and more hydrophilic groups had already been exposed to water during modification as the GuHCl concentration increased.

The molded plastics made from the GuHCl-modified 11S exhibited a glass transition temperature (T_g) (Fig. 4). The T_g decreased from 46.5°C for the control to 24.3°C for 2.4M GuHCl-modified 11S (Table I). The control 11S plastic still showed a thermal denaturation transition at 167.4°C, but the GuHCl-modified 11S plastics did not. This was probably because GuHCl had already partially or completely denatured the 11S protein before the hot-pressing process and this helped to achieve the complete denaturation during the

pressing process. Instead, those GuHCl-modified 11S plastics seemed to show a GuHCl melting transition (Fig. 4). With increasing GuHCl concentration, the melting transition became more pronounced, and the peak temperature also increased (Fig. 4 and Table I).

Dynamic Mechanical Properties

The control 11S plastic had two sharp decreases for the storage modulus (E') [Fig. 5(a)]. The first one was due to the glass transition of 11S. At T_g , mechanical energy absorbed by the 11S molecule reached its maximum value and, hence, showed a peak in the loss modulus (E'') curve [Fig. 5(b)]. The second decrease in the E' curve could be attributed to the denaturation transition of 11S.

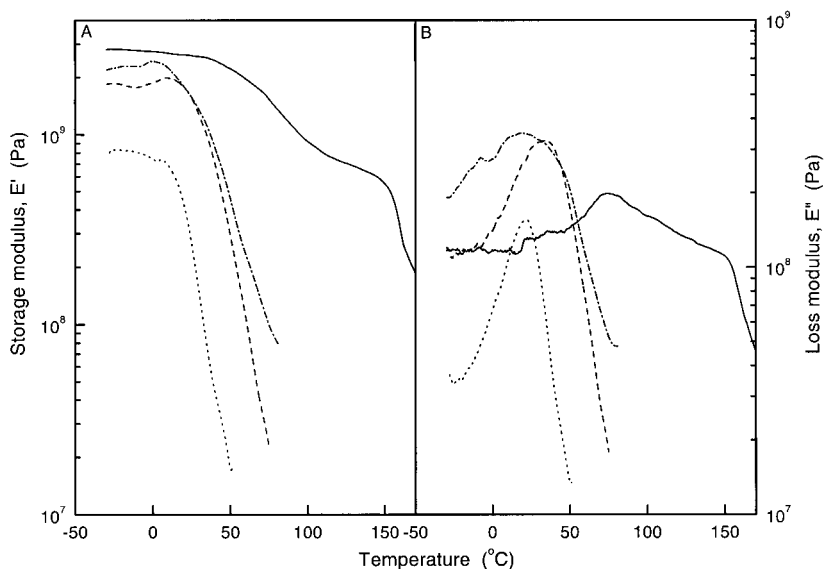


Figure 5 Dynamic mechanical properties: (A) storage modulus E' and (B) loss modulus E'' , of 11S plastics: (—) control; (---) 0.5M GuHCl-modified; (····) GuHCl-modified; (- · - · - ·) 2.4M GuHCl-modified.

Table II Mechanical Properties of Molded Plastics Made from GuHCl-modified 11S

Treatment	Stress at Break (σ_B) (MPa)	Strain at Break (ε_B) (%)	Modulus (E) (GPa)
Control	19.3 \pm 0.8	1.40 \pm 0.12	1.43 \pm 0.06
0.5M GH	16.4 \pm 1.1	1.26 \pm 0.14	1.38 \pm 0.12
0.9M GH	20.6 \pm 1.3	2.74 \pm 0.12	0.83 \pm 0.06
2.4M GH	3.9 \pm 1.1	1.35 \pm 0.09	0.44 \pm 0.08

Moisture content: 2.6%.

After denaturation, the rigid and ordered structure of protein was unfolded to a relatively soft and random structure; therefore, E' decreased sharply.

However, for these plastics made from GuHCl-modified 11S, no data were recorded beyond 80°C because the specimens became too soft to test. These samples exhibited only one decrease in the E' curves and one peak in the E'' curves. The peak temperatures of the E'' curves for both the control and modified 11S plastics were taken as the T_g , and the results are summarized in Table I. Similar to the T_g results from DSC, the T_g obtained from DMA also decreased with increasing GuHCl concentration. Therefore, both DSC and DMA results showed that GuHCl could be considered as a plasticizer for 11S.

Mechanical Properties and Morphology

Without a plasticizer, 11S plastics are rigid and brittle. The control plastic had a Young's modulus (E) of 1.43 GPa, a stress at break (σ_B) of 19.3 MPa, and a strain at break (ε_B) of 1.40% under the

experimental conditions (Table II). After being modified by GuHCl, E decreased with increasing concentration because of the plasticization effect of GuHCl. However, σ_B and ε_B had maximum values at 0.9M GuHCl. The increase of ε_B apparently was due to the plasticization effect of GuHCl. In the modified-11S plastics, GuHCl interacted well with the protein molecules through hydrogen bonds. At a proper concentration, GuHCl improved interactions among 11S protein molecules. The denatured structure of GuHCl-modified 11S was favorable to these interactions, because it brought them closer together and increased the contact area among them. However, at the higher concentration of 2.4M, GuHCl increased the distance between 11S protein molecules, reduced the interactions among them, and hence, decreased both σ_B and ε_B of the 11S plastics.

The fracture surface of the control 11S plastics was smooth and had some cracks [Fig. 6(a)], which are typical characteristics of brittle fracture. For the 0.9M GuHCl-modified 11S plastics

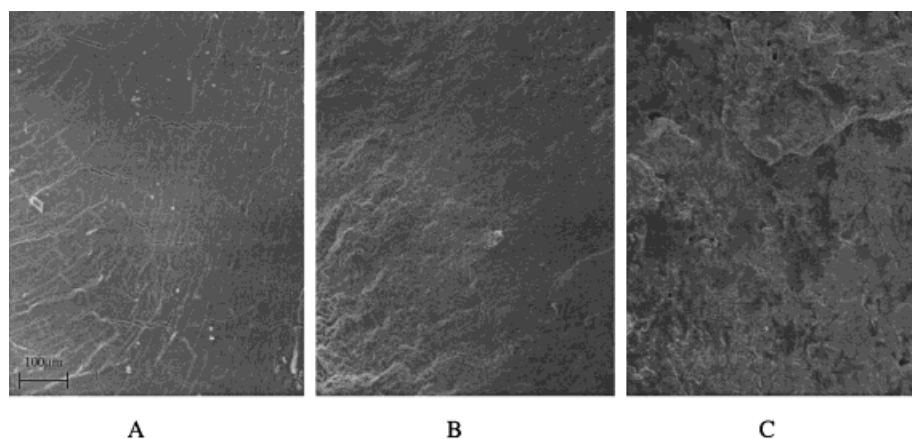


Figure 6 SEM photographs of 11S plastics: (A) control; (B) 0.9M GuHCl-modified; (c) 2.4M GuHCl-modified.

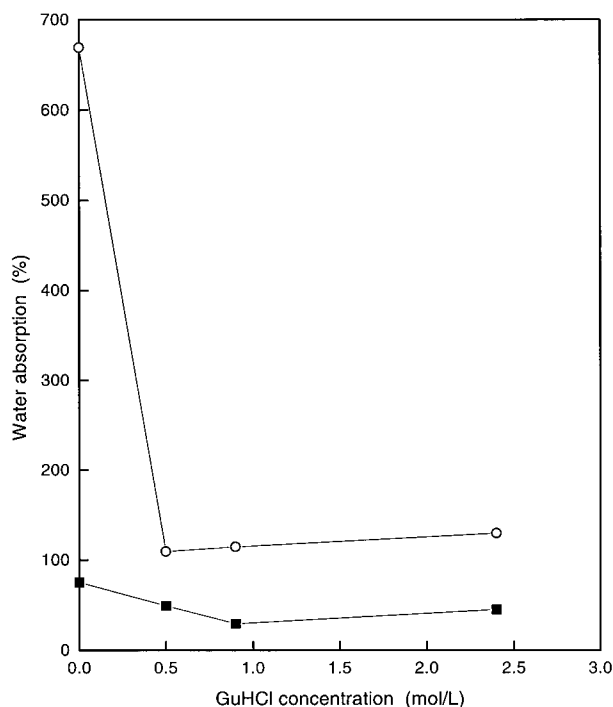


Figure 7 Water absorption of 11S plastics after (■) 2 h and (○) 26 h as affected by GuHCl concentration.

[Fig. 6(b)], the fracture surface was rather coarse and fluctuant, displaying some characteristics of tough fracture. However, the 2.4M GuHCl-modified 11S plastic showed dark protein domains dispersed in a bright GuHCl matrix [Fig. 6(c)]. These results further explained why 0.9M GuHCl-modified 11S plastics had higher σ_B and ϵ_B than those of the other samples.

Water Absorption

GuHCl modification had marked effects on the water absorption of 11S plastics. The water absorption of modified plastics was much lower than that of the control 11S plastic (Fig. 7), especially after 26 h of water soaking, when it reached a minimum value of about 0.9M. However, the water absorption of the modified 11S plastics increased as the GuHCl concentration increased. This variable trend was similar to that of non-freezing water (Fig. 3). The decrease in water absorption at the low GuHCl concentration was due mainly to the interaction between the 11S protein and the GuHCl molecules, which reduced the number of protein groups that can interact with the water molecules. The increase of water absorption at higher concentrations presumably was due to a superabundance of GuHCl, which

increased the distance between protein molecules and made the structure of 11S plastics looser. The excessive GuHCl itself also absorbed the water molecules, which contributed directly to the water absorption of the plastics.

CONCLUSIONS

The properties of plastics made from GuHCl-modified 11S protein were dependent on the GuHCl concentration. Modification of the 11S with 0.9M GuHCl resulted in plastics with a coarse and tough fracture surface structure, the highest tensile strength and elongation, and the lowest water absorption.

The GuHCl in 11S protein plastics acted not only as a denaturant but also as a plasticizer, resulting in a decrease in T_g as the concentration increased. The interaction between the GuHCl molecules and the 11S protein played an important role in determining the structure and properties of the 11S plastics.

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