

# Effect of Glass Transition and Cross-Linking on Rheological Properties of Zein: Development of a Preliminary State Diagram

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## ABSTRACT

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Rheological properties and thermal properties are used to characterize the physical states of zein. In this research, the transition temperature from glassy to rubbery state of Sigma zein and purified zein was measured with differential scanning calorimetry (DSC) at various moisture contents. The temperature and frequency dependence of storage ( $G'$ ) and loss ( $G''$ ) moduli were obtained for Sigma zein with 15–35% moisture.

Zein showed a networking reaction in the temperature range of 65–160°C for moistures  $\geq 25\%$ . Below 65°C, zein behaved like an entangled polymeric fluid. As the temperature exceeded 160°C, the cross-linked/aggregated zein softened. A preliminary state diagram of zein as a function of temperature and moisture content was developed.

An improved understanding of structure-function relationships for food biopolymers necessitates defining the physical and chemical states shown by the polymer. The physical state of food components affects the transport properties (viscosity, density, mass, and thermal diffusivity) as well as the reactivity of the material, which influences its functionality during processing, storage, and consumption. A state diagram represents various states in which a material can exist as a function of temperature, concentration, time, pressure, etc. (Slade et al 1989).

This article concerns the effect of phase transitions on reactivity of food polymers and the resulting changes in rheological properties. Nonreactive synthetic polymers have displayed five regions of viscoelastic behavior (Sperling 1986). At low temperatures, the polymer is glassy and brittle. In this state, the mobility is restricted to vibration and short-range rotational motions. Young's modulus for a glassy polymer just below the glass transition is  $\approx 3 \times 10^9$ .

The glassy region is followed by a drop of the modulus by a factor up to five orders of magnitude in a 20–30°C range. The polymer is described as leathery, and the stiffness of the polymer is affected by a change of a few degrees in temperature. The glass transition temperature ( $T_g$ ) varies widely from polymer to polymer and is affected by the presence of plasticizers (Ferry 1980, Slade et al 1989, Roos and Karel 1991a).

After a sharp drop during the glass transition, the modulus becomes constant again in the rubbery plateau. The polymer exhibits long-range rubber elasticity and can be stretched often several hundred percent and then snap back when released to substantially its original length. If the polymer is linear, then the modulus will drop again with an increase in temperature and ultimately become free flowing. If the polymer is cross-linked, the network formed suppresses flow.

Mobility of a polymer increases drastically above the  $T_g$  and, as a result, the reactivity of the polymer is enhanced (White and Cakebread 1966, Levine and Slade 1990). The  $T_g$  depends on molecular weight, side branches, steric hindrance, covalent and noncovalent bonding (disulfide bonding, hydrogen bonding, hydrophobic interactions) within the molecular structure, ratio of amorphous and crystalline regions, presence of a plasticizer, copolymers, and other physical conditions such as pressure (Sperling 1986).

Mobility of amorphous food polymers such as cereal proteins is affected by the presence of water. Hosoney et al (1986), Cocero and Kokini (1991), and deGraaf et al (1993) showed that gluten, glutenin, and gliadin are glassy, amorphous polymers and demonstrated that the  $T_g$  of these proteins is reduced by adding water.

The change in  $T_g$  due to the plasticizing effect of water has been successfully modeled with the Gordon and Taylor equation (1952) (Roos and Karel 1991a, Cocero and Kokini 1991, deGraaf et al 1993):

$$T_g = w_1 T_{g1} + k w_2 T_{g2} / w_1 + k w_2$$

where  $w_1$  and  $w_2$  are weight fractions of the polymer and the plasticizer;  $T_{g1}$  and  $T_{g2}$  are the glass transition temperatures of the polymer and the plasticizer, respectively, in degrees Kelvin; and  $k$  is a constant. The  $T_g$  of water is reported to be  $-135^\circ\text{C}$  (Johari et al 1987).

Many researchers have shown that cereal proteins may undergo aggregation/cross-linking. For example, Remsen and Clark (1978) postulated that soy proteins can come in contact with the neighboring molecules resulting in possible association. The association of these chains will form a loose network changing the rheological properties. They demonstrated increasing viscosity of soy protein isolate with increasing temperature.

Schofield et al (1983) suggested that glutenin proteins are unfolded on heating to 75°C, and this facilitates sulfhydryl-disulfide (SH-SS) interchange between exposed groups. The protein is then "locked" into the denatured state due to this disulfide interchange. At temperatures  $>75^\circ\text{C}$ , gliadin was also affected by a similar mechanism. Davies (1986) observed an increase in fluidity with increasing temperature of gluten. A sharp increase in storage modulus ( $G'$ ) occurred at  $\approx 80^\circ\text{C}$ . He suggested protein-protein aggregation and formation of a network structure due to thermosetting of gluten  $\approx 80^\circ\text{C}$ .

Slade et al (1989) hypothesized that gluten protein molecules gain sufficient mobility, due to thermal and water plasticization above the  $T_g$ , to form a thermoset network by SS cross-linking. This thermally irreversible thermosetting reaction would be analogous to curing of epoxy resin or vulcanization of rubber.

Chan (1991) showed that there was no significant change in the level of SH-SS ratio in corn protein subjected to extrusion conditions. However, the solubility of corn protein was adversely affected.

While a significant amount of exploratory work has been done in relation to soy and wheat proteins to understand phase changes and their effect on functionality, and in particular their rheological properties, little has been done in relation to corn proteins. The

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first objective of this research was to study the plasticizing effect of water on the  $T_g$  of zein using differential scanning calorimetry (DSC). The second objective was to construct a state diagram for this protein by determining the association and reaction (cross-linking) region and the subsequent flow region using rheological measurements under pressure to allow measurements above the boiling point of water, where the transitions occur.

## MATERIALS AND METHODS

### Materials

Zein, lot #97F0568, was obtained from Sigma Chemical Co., St. Louis, MO. The protein content of Sigma zein was 95% ( $N \times 6.25$ ) as given by Sigma.

To obtain samples with well-defined moisture contents for the measurement of the effect of water or glass transition, zein was exposed to saturated salt solutions giving water activities of 0.12, 0.23, 0.44, 0.64, 0.85, and 0.97. The samples were exposed for one week at room temperature when the water activity was  $<0.84$ . A one week period was selected as the time when the sample reached equilibrium as determined by successive weighings. At the water activity  $\geq 0.84$ , samples were exposed for three days. It was also exposed to phosphorous pentoxide to get a "bone-dry" sample. The time of exposure was determined by weighing the sample over time to get a negligible change in weight. Gain or loss in weight was determined, and the water content on total weight was calculated at the respective water activity. The pans were sealed and then subjected to DSC.

### DSC

$T_g$  was determined using DSC (Mettler TA 4000 system DSC 30S). The protein samples were scanned over the glass transition region at  $5^\circ\text{C}/\text{min}$ , and the  $T_g$  was measured at the midpoint of the transition. The results presented were averages of triplicate runs or as mentioned.

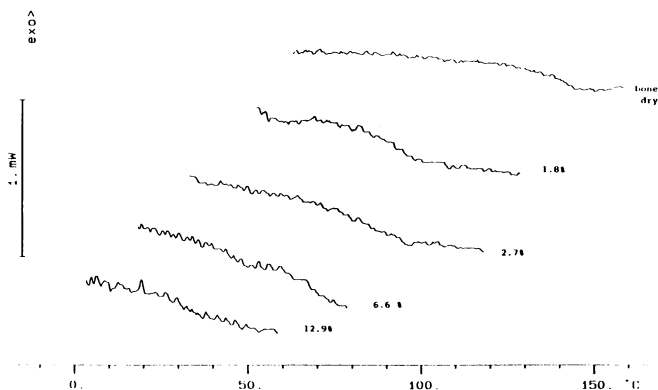


Fig. 1. Differential scanning calorimetry (DSC) thermograms of Sigma zein at various moistures

TABLE I  
Glass Transition of Sigma Zein as a Function of Moisture Content and Corresponding Water Activity

Water Activity	Equilibrium Water Content (%)	Midpoint of Glass Transition ( $^\circ\text{C}$ )
0	0	139
0.23	1.8	96
0.44	2.7	85
0.64	6.6	47
0.84	12.9	28
0.97	30.2	-15

### Pressure Rheometry

A pressure rheometer (RPR, Rheometrics, Inc., Piscataway, NJ) was used to conduct amplitude oscillatory experiments to measure the storage modulus ( $G'$ ) and the loss modulus ( $G''$ ). The RPR allows measurements to be conducted under pressure, thus preventing loss of solvent (water) at higher temperatures.

The necessary quantity of water was added to zein, and the mixture was vigorously mixed to obtain the required moisture contents of 15, 20, 25, 30, and 35%. The samples were then allowed to equilibrate overnight at a refrigeration temperature of  $8^\circ\text{C}$ .

These samples were subjected to small amplitude oscillatory measurements at  $50$ – $160^\circ\text{C}$ . Temperature of the heating bath was raised at  $6^\circ\text{C}/\text{min}$ . The strain applied was 0.5%. The parallel plate geometry used had a plate diameter of 20 mm and a gap of 2 mm. The frequency used was 6.28 rad/sec, which corresponds to 1 Hz. The pressure applied in the measuring cell was 200 psi., which was kept constant for all experiments. This pressure was selected to avoid moisture loss as the temperature was increased  $>100^\circ\text{C}$ .

## RESULTS AND DISCUSSION

### Effect of Moisture on Zein Glass Transition

The transition from glass to rubber was measured with DSC. Typical thermograms of Sigma zein at various moisture contents are shown in Figure 1. The figure shows the change in  $\Delta H$  as a function of temperature, which was increased at a rate of  $5^\circ\text{C}/\text{min}$ . The  $T_g$  corresponded to the midpoint of the sigmoidal shift in the  $\Delta H$ . The transition in bone-dry Sigma zein at  $134^\circ\text{C}$  and the end point was at  $144^\circ\text{C}$ , giving the midpoint of the transition as  $139^\circ\text{C}$  (Fig. 1). The midpoint of glass transitions at various moisture contents were tabulated (Table I).

The  $T_g$  of bone-dry Sigma zein was  $139^\circ\text{C}$ , which was lower than the  $T_g$  of gluten (Hoseney et al 1986) and glutenin (Cocero and Kokini 1991). One possible reason for this is the much lower molecular weight of zein as compared to gluten and glutenin.

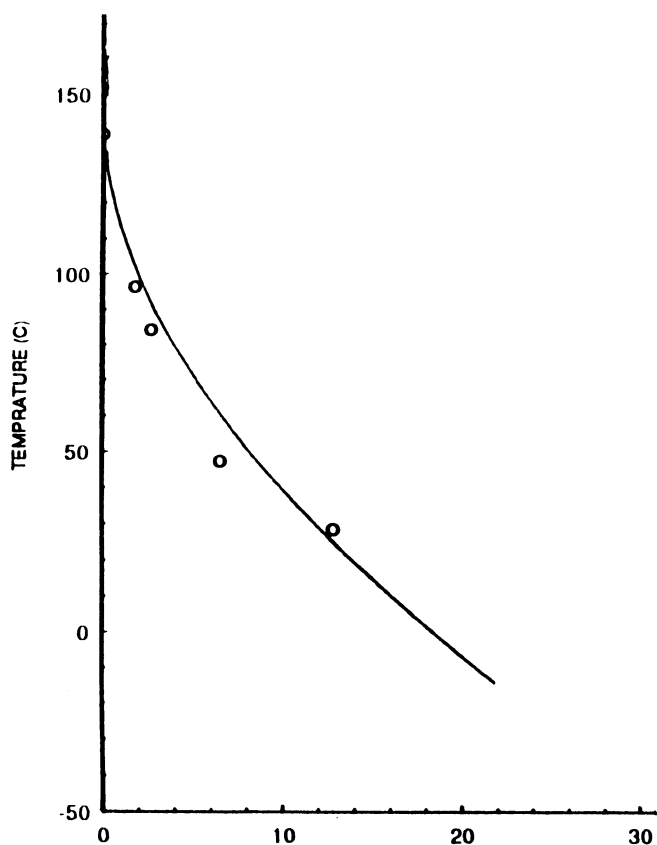


Fig. 2. Glass transition of Sigma zein as a function of moisture content.

Figure 2 shows the  $T_g$  of zein plotted as a function of moisture content. Zein was significantly plasticized by water, especially in the first 6% water where the drop in the  $T_g$  was  $\approx 80^\circ\text{C}$ . The plasticizing effect of water decreased significantly once the moisture content increased to  $\approx 10\%$ .

#### Simulation of $T_g$ using the Gordon and Taylor Equation

The plasticizing effect of water on gliadin followed the Gordon and Taylor equation as reported by deGraaf et al (1993). This equation also predicts the  $T_g$  of zein very well in low- to intermediate-moisture levels. The  $k$  value obtained was 6.24 for zein; 3.6 for gliadin (de Graaf et al 1993); 6.14 for glutenin, ranging from 3 to 7 for simple sugars; and 5.2 for starch (Roos and Karel 1991a). The physical significance of the  $k$  value is not clear, but in some cases it has been shown to relate to the ratio of the specific heat of two components, the polymer and the plasticizer.

At sufficiently high moisture levels, the  $T_g$  is below the freezing point, and the hydrated proteins are freeze-concentrated due to ice formation. In such cases, a  $T_g'$  of freeze-concentrated protein is obtained (Roos and Karel 1991b). At a moisture content of  $\approx 30\%$ , the  $T_g$  is below the freezing point of water and, therefore, it was not possible to obtain a true  $T_g$  due to formation of ice during the cooling of the zein. Approximately 10–13% water was frozen. A minimum on the order of 200 water molecules must associate with a domain of  $\approx 40\text{\AA}$  to form a critical nucleus that will grow spontaneously into an ice crystal. Thus, zein with high moisture forms spontaneous crystals of ice and, hence, all the water is not involved in formation of a single matrix.

#### Characterization of Complexation in Zein

The rheological properties,  $G'$ , and  $G''$  measured using pressure rheometry for zein with 15, 20, 25, 20, and 35% moisture contents as a function of temperature were measured. Figure 3 depicts a typical temperature sweep of zein with 15% moisture. The magnitudes of  $G'$  and  $G''$  were relatively high and almost identical within the temperature range of 50–115°C. The high magnitude of  $G'$  and  $G''$  was expected due to low moisture content. A small decrease in the magnitude of  $G'$  and  $G''$  was observed as the temperature increased from 50 to 120°C. This region was then characterized as the region of entangled polymer flow. This flow region begins  $\approx 100^\circ\text{C}$  above the glass transition temperature. In the intermediate region  $T_g + 100^\circ\text{C}$ , rubbery flow is expected to occur (Ferry 1980).

As the temperature exceeded 122°C, zein experienced a sharp increase in  $G'$  and a simultaneous decrease in  $G''$  similar to that observed with gliadin (Madeka and Kokini 1994). This was due to cross-linking reaction occurring once the mobility of zein mole-

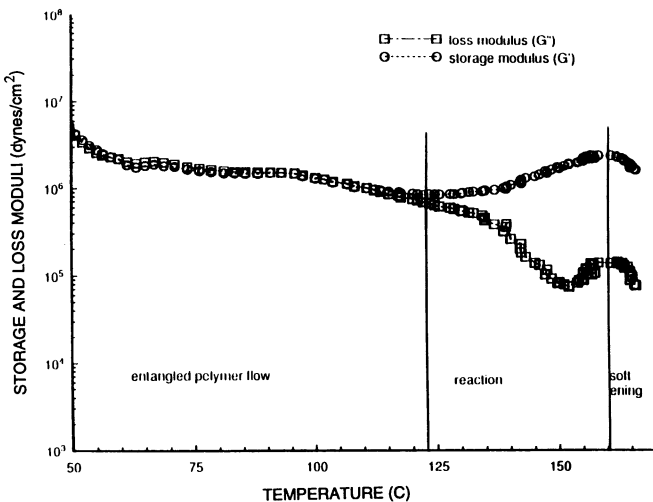


Fig. 3. Temperature sweep of zein with 15% moisture.

cule was high enough. It is interesting to note that up to a temperature of 85–100°C above  $T_g$  no significant reaction is observed. Once the temperature is high enough, individual zein molecules gain enough mobility to come together and cross-link. At a moisture content of 15%, cross-linking occurred when the temperature was  $\approx 100^\circ\text{C}$  above its  $T_g$ . The aggregation observed in zein was similar to that observed in gliadin. However, in gliadin containing 20–40% moisture, the reaction occurred at 70°C. Chan (1991) demonstrated that extrusion lowered the solubility of corn meal. He also showed that there was no significant change in the ratio of SS to SH linkages. This leads to the conclusion that SS interchange may play some role in the formation of cross-links, which would make zein a thermosetting material, causing a decrease in solubility.

An increase in the elastic component and a simultaneous decrease in the viscous component of zein suggested that the reaction causes the formation of cross-links. A conversion is observed from an entangled polymer to a cross-link network structure. The  $G''$  exhibited a minimum of 150°C followed by a maximum in magnitude at 160°C, while  $G'$  went on increasing up to 160°C. At 160°C, the polymer appears to have reached a maximum cross-link density. The magnitude of  $G'$  at its maximum at 160°C was  $10^6$  dynes/cm<sup>2</sup> and  $G''$  at its maximum was  $10^5$  dynes/cm<sup>2</sup>. The maximum in  $G''$  would suggest melting of the cross-linked structure allowing for increasing mobility necessary for temperature softening. When the temperature was further increased to 166°C, the  $G'$  started declining once the temperature

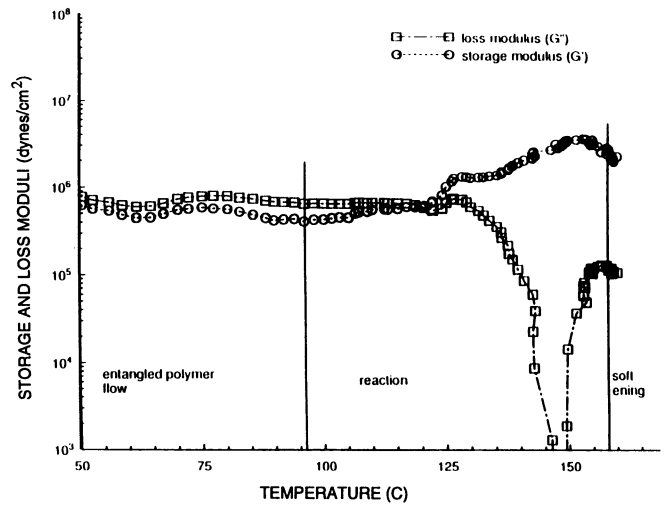


Fig. 4. Temperature sweep of zein with 20% moisture.

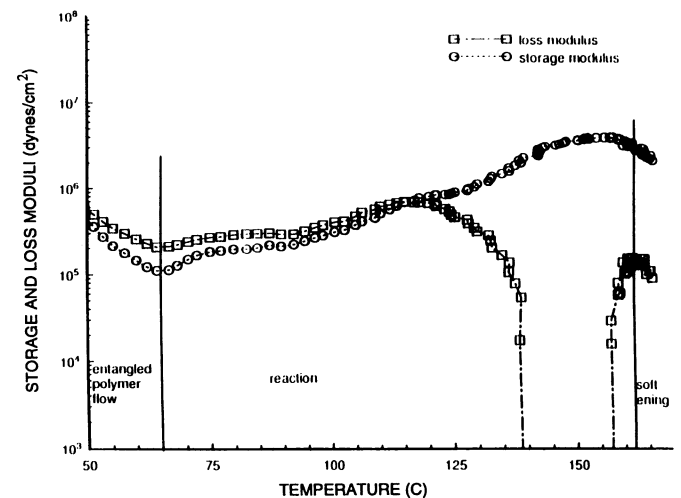


Fig. 5. Temperature sweep of zein with 25% moisture.

exceeded 160°C. The  $G''$  also went on decreasing from its peak value, thus zein was experiencing a softening.

The temperature sweep of zein with 15% moisture also shows three distinct regions as in the case of gliadin (Madeka and Kokini 1994): entangled polymer flow in the range through 120°C; networking reaction in the range of 122–160°C, including a maximally cross-linked structure from 150–160°C; and finally softening from 160°C and above. When the molecular weight between cross-links was estimated using the equation  $M_c = \rho RT/G'$ , it was found that  $M_c \approx 22,000$ . Considering that the average molecular weight for zein is on the order of 33,000 (18,000 to 44,000 range) (Laszity 1984), the number of cross-links would be expected to be equal to 0.75 because two molecules are participating in cross-link formation, and this gives an average number of cross-links equal to 0.75.

The  $G'$  and  $G''$  for zein with 20% moisture as a function of temperature are shown in Figure 4. In this case, the  $G''$  were slightly higher than the  $G'$  in the temperature range of 50–90°C and were largely independent of the temperature. The  $G'$  started increasing with temperature at 96°C, while the  $G''$  showed a plateau up to 120°C. The temperature of 96°C, at which the networking reaction began, was slightly lower than 122°C compared to zein with 15% moisture.

Moisture played an important role in lowering the temperature at which the networking reaction occurs. The plasticizing effect of water is known to enhance the mobility of the amorphous mole-

cules. It appears that the networking temperature in this case was also  $\approx 100^\circ\text{C}$  higher than its  $T_g$ . The reaction continued to occur up to 155°C, when  $G'$  had reached its maximum of  $10^6$  dynes/cm<sup>2</sup>. When the molecular weight between cross-links was estimated, a value of  $\approx 20,000$  Da, similar to that of zein with 15% moisture was obtained. It appears that moisture facilitates the initiation of the reaction at a given temperature, but once the reaction occurs, the degree of cross-linking is independent of moisture content.

The  $G''$  on the other hand started declining after showing a small peak at 125°C. The  $G''$  approached a zero value in the temperature range of 145–150°C, suggesting formation of a pronounced three dimensional network structure; zein became a highly elastic material in this small temperature range. The increase in moisture increased the reaction potential for zein. The magnitude of  $G''$  approached zero as compared to  $10^4$  dynes/cm<sup>2</sup> for zein with 15% moisture. The  $G''$  again goes through a maximum at 157°C, suggesting that the networking reaction is completed and the network structure now begins to increase in mobility through thermal motion.

This softening through thermal motions occurred at 157°C. Once this temperature was exceeded,  $G'$  and  $G''$  both started declining with the increasing temperature. The softening temperature was  $\approx 5^\circ\text{C}$  lower than in zein with 15% moisture.

The temperature sweeps for zein with 25, 30, and 35% moistures are shown in Figures 5–7. The increasing moisture content shifted the initiation of the networking reaction to lower tempera-

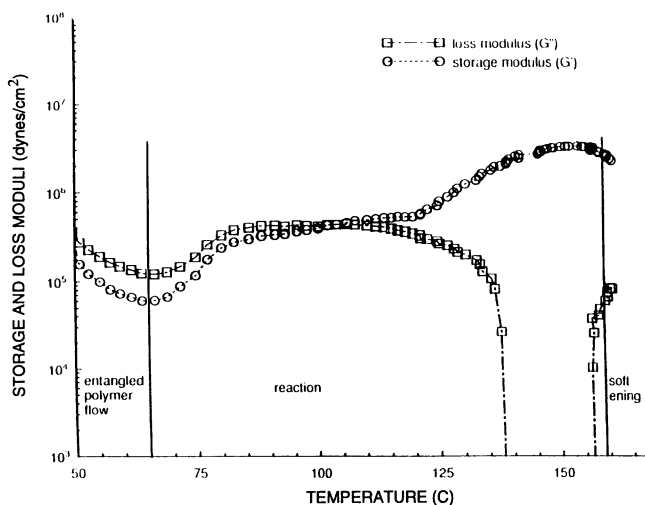


Fig. 6. Temperature sweep of zein with 30% moisture.

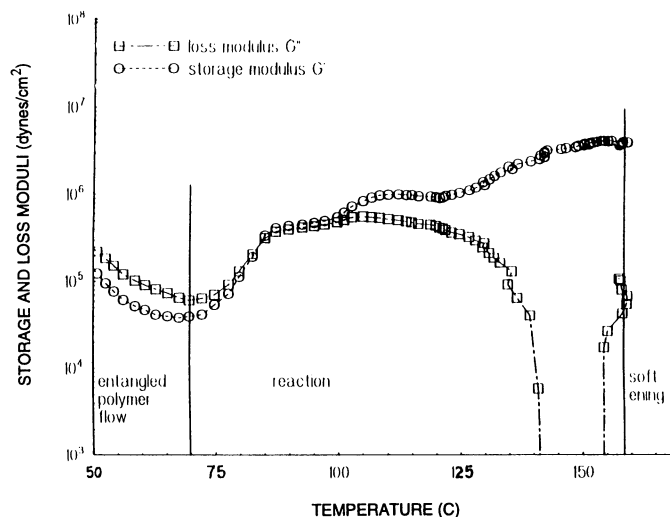


Fig. 7. Temperature sweep of zein with 35% moisture.

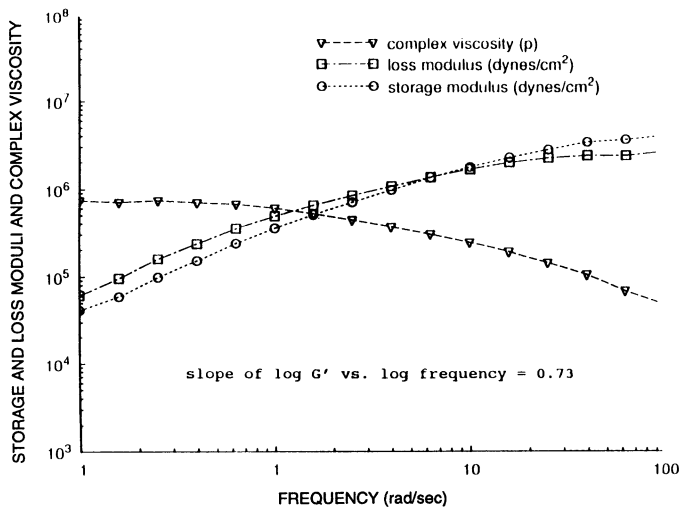


Fig. 8. Frequency sweep of zein with 25% moisture at 50°C.

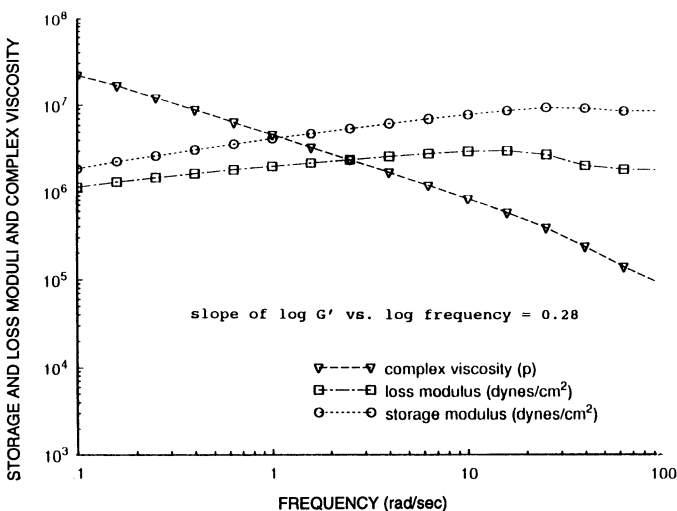


Fig. 9. Frequency sweep of zein with 25% moisture at 70°C.

tures. However, beyond 25% moisture, no further shifts were observed. The initiation of the networking reaction was at 65°C for zein with 25, 30, and 35% moisture. It confirms the earlier observation that water played an important role in increasing the mobility of zein, facilitating the reaction of individual peptides for the SS exchange reaction to occur.

The initiation temperature of networking reaction did not go below 65°C although the water content was increased from 25 to 35%. Increased moisture >25% resulted in the reduction of the magnitude of  $G'$  and  $G''$  in the initial stages of the reaction up to 100°C. Above 100°C the magnitude of  $G'$  and  $G''$  did not vary with increasing moisture content.

The magnitude of peak in  $G'$ , minimum in  $G''$ , and softening temperatures were almost identical. Again, this results in the same molecular weight between cross-links at 25, 30, and 35% moisture content as with 15 and 20% moisture, clearly suggesting that moisture does not affect the extent of the cross-linking reaction.

Frequency sweeps of zein with 25% moisture content were conducted isothermally under a 200 psi pressure at 50, 70, 100, 140, and 160°C. The sweep at 50°C is shown in Figure 8. Zein at 50°C, showed a constant viscosity in the frequency range up to 1 rad/sec, where  $G'$  and  $G''$  both increased linearly on  $G''$  log-log scale with a slope of 1. In the higher frequency range, zein showed pseudoplastic behavior. The flow behavior index ( $n$ ) was

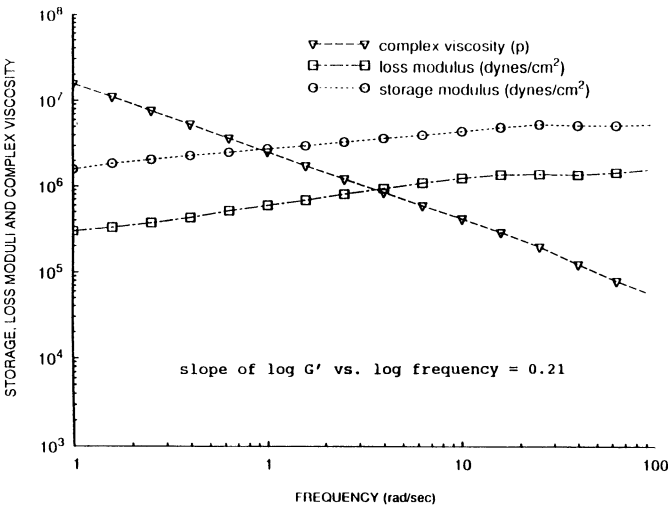


Fig. 10. Frequency sweep of zein with 25% moisture at 100°C.

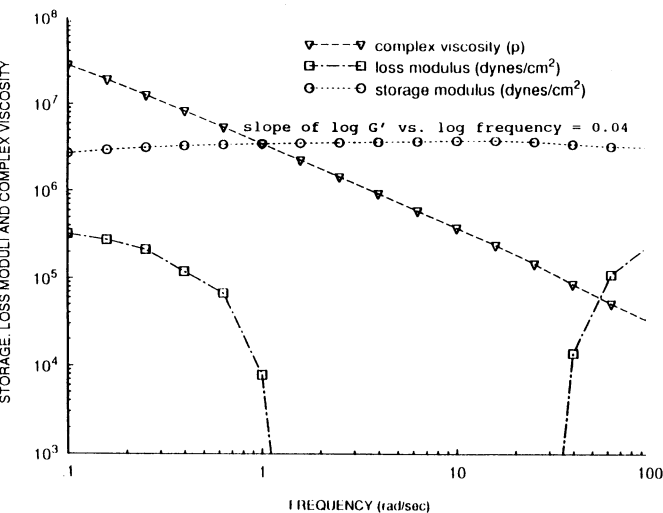


Fig. 11. Frequency sweep of zein with 25% moisture at 140°C.

0.59, as expected from most entangled polymer flow, and the slope of  $\log G'$  vs.  $\log \omega$  was 0.73 in the frequency range of 0.1 to 10 rad/sec. The magnitude of  $G'$  and  $G''$  were very close to each other. This was indicative of entangled polymer flow.

The data at 70°C (Fig. 9) exhibited a dramatic increase in the magnitude of  $G'$  relative to the magnitude of  $G''$  at 50°C. The increase in both  $G'$  and  $G''$  at low frequencies ( $\approx 1$  rad/sec) was about tenfold. The slope of  $\log G'(\omega)$  vs.  $\log \omega$  fell to 0.28, clearly suggesting network formation. The increase in  $G'$ , reduction in the slope and flow behavior index was indicative of an entangled polymer undergoing cross-linking reaction possibly through SS exchange reactions as suggested by Chan (1991).

Increasing temperature to 100°C (Fig. 10), resulted in a slope for  $G'(\omega)$  vs.  $\omega$  of 0.21 and a flow behavior index to 0.20 respectively, clearly showing a significant reduction from 70°C. The difference in magnitude between  $G'$  and  $G''$  widened to half a decade suggesting the development of a network due to SS exchange reaction.

The frequency sweep of zein at 140°C (Fig. 11) showed no significant dependence of the  $G'$  on frequency and no sharp minimum in the  $G''$ . This suggested that a 3-D network was clearly formed and zein was in the cross-linked state as suggested by the behavior of  $G''$ .

At 160°C (Fig. 12), there was a minor reduction in the slope  $G''(\omega)$  and in the flow behavior index (0.03 in both cases). The

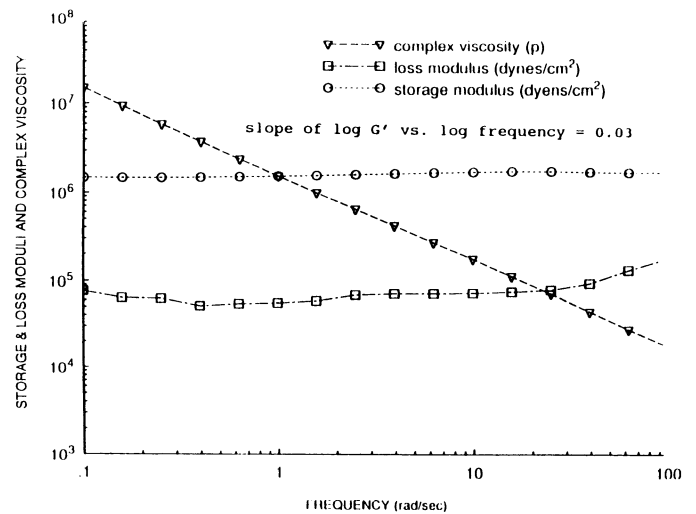


Fig. 12. Frequency sweep of zein with 25% moisture at 160°C.

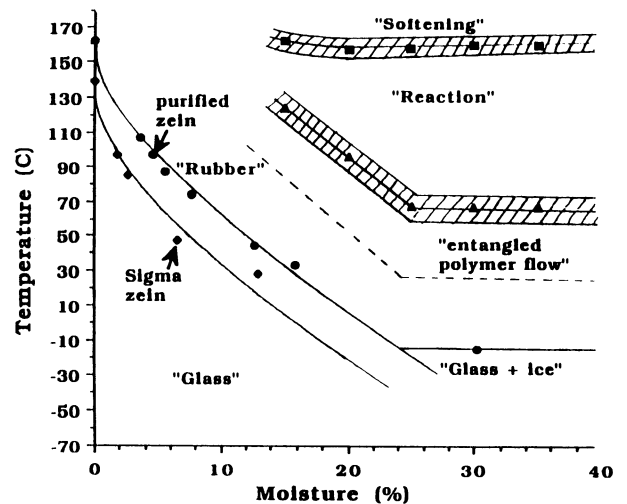


Fig. 13. State diagram for zein.

magnitude of  $G'$  dropped very slightly, and the dip in  $G''$  disappeared completely. It appears that at this temperature, the  $G'$  and  $G''$  both were only slightly dependent of frequency and gelled/aggregated zein was softening very slowly.

Thus frequency sweeps for zein with 25% moisture characterized the entangled polymer region  $\approx 50^\circ\text{C}$ . At  $70^\circ\text{C}$ , the material started the formation of a 3-D network, most probably due to SS exchange reactions. At  $140^\circ\text{C}$ , zein had reached its maximum structure, and at  $160^\circ\text{C}$ , zein began to soften.

#### Development of Preliminary State Diagrams from DSC and Rheological Data

A preliminary state diagram for Sigma zein was developed from the glass transition data as a function of moisture content in combination with the regions obtained with the temperature and frequency sweeps. Figure 13 depicts the state of zein as a function of temperature and moisture content up to 35%.

The glass transition in Sigma zein showed very pronounced plasticization with water. The state diagram shows a glassy region below the glass transition line with a rubbery region above the glass transition line. Above  $\approx 24\%$ , moisture cannot participate in the plasticizing function because it is in "excess" and freezes out at  $-15^\circ\text{C}$ .

The diagram also shows a dashed line which may exist and represents a transition from rubber to the entangled polymer flow. The evidence for this transition came from frequency sweeps with a higher slope value of  $\log G'$  vs.  $\log \omega$ . Also  $G'$  and  $G''$  are almost identical in magnitude, which is characteristically indicative of this state. The mobility of zein in the entangled polymer region enabled zein molecules to cross-link, possibly through SS exchange reactions. As seen in the figure, the temperature at which the networking reaction began was a function of moisture content. This reaction region was in a smaller temperature range at lower moisture content ( $\approx 40^\circ\text{C}$  with 15% moisture as compared to  $\approx 100^\circ\text{C}$  with 30% moisture). This may be due to the fact that higher moisture increased the mobility of the system, and the cross-linking began at a lower temperature. On the other hand, the initial temperature of softening was not significantly affected by the moisture content. The temperatures of  $65$  and  $160^\circ\text{C}$  may correlate with the gelling temperature of the nonreacted material  $T_{\text{gel}}$  and the melting temperature of the highly cross-linked polymer  $T_{\text{mg}}$  as suggested by Slade et al (1989) in the time-temperature transformation diagram for gluten.

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