# DSC and TGA Studies of the Behavior of Water in Native and Crosslinked Gelatin

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Received 9 March 1998; accepted 24 June 1998

ABSTRACT: Water molecules absorbed into gelatin are found to be only partially crystallizable. The fraction of noncrystallizable water depends on whether the gelatin is native or crosslinked, and on the crosslinking conditions as well. This dependence is explained by the  $T_g$ -regulation effect newly proposed by Rault and coworkers for water-swollen gelatin cooled below 0°C. According to this effect, a part of the frozen water cannot crystallize because during the cooling the amorphous gelatin–water phase becomes glassy before the water crystallization temperature is reached. During the heating of water-plasticized gelatin samples in a TGA cell, the crystallizable water separates from the gelatin, mainly in the temperature interval 50–100°C, whereas the noncrystallizable water leaves the gelatin gradually over the entire temperature interval investigated, up to 300°C. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 71: 465–470, 1999

Key words: gelatin; water absorption; thermal analysis

# **INTRODUCTION**

It is a common observation that water in hydrophilic polymers crystallizes only partially upon cooling below 0°C, with this crystallizable form frequently called "unbound water." The remaining part is considered as noncrystallizable or "bound water." Recently Rault and coworkers published several articles dealing with water in various hydrophilic polymers such as poly(vinyl alcohol), poly(vinyl pyrrolidone), polyacrylamide, and polyether block copolymer amide.<sup>1-5</sup> They do not use the concept of bound and unbound water, but designate the system "polymer–water," in which the water acts as a plasticizer. Later, Rault et al. define a critical weight concentration  $C^*$  of the water in the system: below  $C^*$  water does not crystallize, whereas above  $C^*$  it crystallizes partially, forming ice crystals. Because the noncrystalline water acts as plasticizer, the glass transition temperature of the system follows the Fox-Flory equation<sup>6</sup>

$$(1/T_g) = (C_w/T_g^w) + [(1 - C_w)/T_g^g]$$
(1)

where  $T_g$ ,  $T_g^w$ , and  $T_g^g$  are the glass transition temperatures of the system, water, and dry gelatin, respectively, and  $C_w$  is the concentration of water. The critical concentration  $C^*$  is determined as the abscissa of the intersection of the Fox-Flory curve and the melting curve of the ice.

Gelatin is a very interesting polymer because it is highly hydrophilic; its glass transition temperature in the dry state is 217°C, but strongly decreases to approximately 0°C according to the Fox-Flory equation for additions of water up to 25 wt %.<sup>7</sup> It is well known that the glass transition

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Contract grant sponsor: National Science Foundation; contract grant number INT-9514149

Contract grant sponsor: Bulgarian Ministry of Education, Science and Technology; contract grant number: TH-714

Journal of Applied Polymer Science, Vol. 71, 465-470 (1999)

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temperature also depends on whether the polymer is uncrosslinked or crosslinked and, in the latter case, depends also on crosslink density. It is the aim of the present work to investigate the behavior of the system gelatin-water, namely the crystallization of water upon cooling and its evaporation upon heating. This is done as a function of the overall water concentration, for both uncrosslinked and crosslinked gelatin samples. The method of Rault and coworkers for investigation of hydrophilic polymers was closely followed.<sup>1-5</sup> In addition, some thermogravimetric analysis (TGA) measurements were also done to clarify the process of evaporation of water from the gelatin upon heating, and in this way to look for additional differences between the two types of water.

## **EXPERIMENTAL**

#### **Materials**

Gelatin powder type A (bloom value 300) was purchased from Sigma-Aldrich and used as received. The crosslinking reagent was a water solution of 25 wt % glutaric aldehyde (GA) obtained from Roanal, Hungary. Potassium thiocyanate (KCNS) was purchased from Reachim, USSR.

#### **Sample Preparation**

The gelatin was soaked overnight at 5°C and then dispersed at 50°C in a water bath in the presence of a phosphate buffer. The solution thus obtained was poured into an aluminum dish and consequently dried at room temperature. In this way a sample of "native" gelatin was obtained.

Two crosslinked samples differing in gelatin concentration were obtained as follows: A 2-*M* water solution of KCNS was used as solvent to obtain two solutions with 5.5 and 11 wt % gelatin. The crosslinking was performed by adding an appropriate amount of a 25 wt % water solution of GA so that in the final solutions its concentration was 0.00165 *M*. Another sample, crosslinked in a 0.0033 *M* solution of GA, was also obtained from the 5.5 wt % solution. In both cases the crosslinking agent was added with thorough and continuous mixing to obtain uniform networks.

Room-conditioned gelatin fibers typically contain 15-17 wt % water, as demonstrated by comparing their weight with those of completely dry samples. Samples with higher water contents were prepared by swelling the samples for several minutes to an hour between moistened sheets of filter paper. They were thereafter immediately tested in a Polymer Laboratories differential scanning calorimetry (PL-DSC) instrument. Samples with water less than 15–17 wt % were prepared by drying samples at various temperatures for the required durations.

## Techniques

The thermal behavior of the system gelatin-water was investigated by the DSC technique, using sample weights between 2 and 15 mg. The heating started from between -50 and -125°C, depending on the water content, and went to 160°C at a heating rate of 10°C/min in a nitrogen atmosphere. All the DSC traces were normalized and redrawn according to sample weight and heatflow sensitivity. In this way the curves reflect only the effect of the water concentration, in every set of traces for every sample (native and two samples crosslinked in 5.5 and 11 wt % solutions of gelatin, respectively). The amount of crystallizable water was calculated from the enthalpy of the ice-melting peak, which was taken to be 5.99 kJ/mol.<sup>8</sup>

The TGA traces were obtained using a Perkin-Elmer 7 thermal analyzer in a heating mode from 25 to 260 or 300°C and a heating rate of 10°C/min in a nitrogen atmosphere. All water concentrations were specified by weight.

# **RESULTS AND DISCUSSION**

Figure 1 shows DSC traces for samples of native gelatin, differing in water concentration  $C_w$  as indicated. For water concentrations  $C_w$  of 0.11 or less there is one large endothermic downward peak, reflecting the evaporation of the noncrystal-lizable water, together with another downward peak at a high temperature (about 150 and 100°C, respectively). For a water concentration of 0.24, a low-temperature downward peak at about 0°C appears in addition to the large downward peak at about 30°C and a higher temperature downward peak at about 30°C. For a  $C_w$  of 0.33, the two downward peaks at about 0 and 30°C remain, and for higher water concentrations up to 0.67, another downward peak develops at 110°C.

It seems obvious that the low-temperature downward peak, situated around 0°C, is due to the melting of ice (Fig. 1,  $0.24 \leq C_w \leq 0.67$ ). This downward peak appears at about 0.24 water concentration and its area, which reflects the melting enthalpy of the ice, increases with water



**Figure 1** DSC traces of samples of native gelatin, differing in  $C_w$ , the concentration of water immediately before the DSC run.

concentration. The downward peak for water concentrations of 0.24 and higher, situated in the region 50–75°C, represents the evaporation of both crystallizable and noncrystallizable water. The reason for the splitting of this downward peak for a water concentration of 0.44, and especially of 0.58 and 0.67, is unclear. It may be due to the melting of small and imperfect gelatin crystallites. Due to the so-called "glass transition regulation effect,"<sup>5</sup> no glass transition temperature could be detected because it was hidden by the ice-melting peak.

The nature of the observed jump in  $c_p$  at about 25°C in the DSC trace of the dry sample (Fig. 1,  $C_w = 0$ ) is unclear. Although we think the sample was dried completely, its relatively large thickness may have made total removal of water unlikely. In this case, the jump in  $c_p$  would reflect the onset of the liberation of this remaining water; also see the other DSC traces (Fig. 1,  $C_w \geq 0.11$ ).

Figures 2 and 3 show DSC traces for the gelatin samples crosslinked in 5.5 and 11 wt % solutions and differing in the water concentration as indicated. These figures are quite similar to Figure 1 except that the ice melting peak appears at higher water concentration. It is worth mentioning that a high-temperature downward peak at about 130°C again appears in Figure 3.



**Figure 2** DSC traces of gelatin samples, crosslinked in 5.5 wt % gelatin solution in water-dissolved KCNS and 0.00165 M solution of glutaric aldehyde, for various values of the concentration of water  $C_w$  immediately before the DSC run.

As may be seen from Figures 1–3, the ice melting temperature is around 0°C and becomes slightly positive with increase in the water con-



**Figure 3** DSC traces of gelatin samples, crosslinked in 11 wt % gelatin solution in water-dissolved KCNS and 0.00165 M solution of glutaric aldehyde, for various values of  $C_w$ .



**Figure 4** Ice melting enthalpy  $\Delta H$  vs. water concentration for the sample of native gelatin.

centration, whereas in the case of water in PVA it is about  $-25^{\circ}$ C for water concentration  $0.3^{1}$ Rault et al. explain this low temperature by dissolution effects.<sup>1</sup> The ice melting temperature changes (Figs. 1-3) with water concentration, as is usually the case.<sup>9,10</sup> This temperature is the highest for the highest concentration (about 12°C in Figs. 1 and 3, and 0°C in Fig. 2) and decreases with concentration. This dependence is clearly pronounced for the sample of native gelatin and the sample crosslinked in 11 wt % solution (Fig. 1,  $0.24 \leq C_w \leq 0.67$ ; Fig. 3,  $0.38 \leq C_w \leq 0.60$ , respectively) and only very weakly expressed for the sample crosslinked in 5.5 wt % solution (Fig. 2, 0.34  $\leq C_w \leq$  0.60). The increase of the ice melting temperature with  $C_w$  could possibly be explained by an overheating effect due to the crystallizable water, the amount of which increases with  $C_w$ , as may be concluded from the ice peak melting area. The mean size of the domains formed by this kind of water also increases with  $C_w$ ; hence, the effect of overheating is more clearly pronounced, which can be seen in Figures 1–3: the higher the  $C_w$ , the higher the ice melting temperature.

Figure 4 shows the ice melting enthalpy  $\Delta H$  vs. water concentration for the sample of native gel-



**Figure 5** Concentration of noncrystalline  $C_{nc}$  and crystalline  $C_{cr}$  water vs. overall water concentration.

atin. A straight line was drawn through the data according to the least-squares method. After extrapolation to  $\Delta H = 0$  this line gives the critical water concentration  $C^* = 0.30$ . Below this concentration the water exists only in the noncrystalline state, whereas part of it is crystallizable for concentrations higher than  $C^*$ .

Extrapolations similar to those in Figure 4 were obtained for the samples crosslinked in 5.5 and 11 wt % solutions of gelatin. The respective values of  $C^*$  are shown in Table I together with the correlation coefficients for the straight-line representations.

Figure 5 shows the concentration of both noncrystallizable  $C_{nc}$  and crystallizable  $C_{cr}$  water vs. overall water concentration. The concentration of the crystallizable water was obtained from the melting enthalpy of the ice<sup>8</sup> and the concentration of the noncrystallizable water was obtained as a difference between the overall water concentration and that of the crystalline water (if any). As already stated,  $C_{cr} = 0$  up to the critical concentration  $C^*$  and increases linearly above it. Quite opposite behavior was observed for the noncrystallizable water-it increases linearly from zero up to  $C^*$  for  $C_w \leq C^*$  and remains constant  $C_{nc}$ =  $C^*$  for  $C_w > C^*$ . Figures 5 and 6 are very similar to the data of Rault and coworkers for various other water-plasticized polymers.<sup>1-4</sup>

Table I Critical Concentration  $C^*$  and Correlation Coefficient R for Three Gelatin Samples

Gelatin Sample	Critical Concentration $C^*$	Correlation Coefficient $R$
Native	0.30	0.98
Crosslinked in 5.5% solution of gelatin	0.23	0.96
Crosslinked in 11% solution of gelatin	0.35	0.99



**Figure 6** TGA traces of samples of native gelatin (a); gelatin crosslinked in 5.5 wt % gelatin solution in water with KCNS and a 0.00165 M solution of glutaric aldehyde (b); and gelatin crosslinked in 11 wt % solution in KCNS and 0.00165 M solution of glutaric aldehyde (c).

Figure 6 shows the TGA traces for the sample of native gelatin and the two crosslinked samples. For all three samples the water content decreases continuously with temperature up to 260°C. Although all three samples are room conditioned, the sample crosslinked in 5.5% solution has the lowest water content. It seems that water leaves the room-conditioned samples gradually over the entire temperature interval up to 260°C. In this connection it is interesting to investigate the process of liberation of the water from the system water-gelatin (water concentration taken as a parameter) and to evaluate the weight losses during heating. For this purpose the TGA results for water concentrations varying from 0.0 to 0.75 are shown in Figure 7. A sample having a relatively dense network structure (from the 0.0033 M GA) was used in this set of TGA measurements. As is clearly seen from Figure 7, for low and moderate concentrations up to 0.37 the water leaves the system gradually in the temperature range 25-300°C, whereas for the higher concentrations of 0.64 and 0.75 the majority of the water leaves between 25 and 100°C. Because for low overall water concentrations most of the water is in the noncrystalline state, it may be concluded that the crystallized water leaves the system at relatively low temperatures of 25–100°C, whereas the noncrystallized water leaves the system gradually with an increase in temperature over the entire temperature interval 25-300°C.

A thorough inspection of Figure 7 shows that there are some differences between the weights of the samples taken as the initial water concentration and the water concentration estimated as unity minus the value read from the ordinate. The most pronounced discrepancy of 0.07 is for the sample having an initial water concentration of 0.37 (Fig. 7). It cannot be explained as a drift of the baseline, which is usually  $\pm 0.025$  mg.<sup>11</sup> On the other hand, the initial point (20°C, 100%) in Figure 7 is not very well defined for the particular thermal analyzer used, i.e., for a fixed value of 100% there is some drift of the temperature before it starts to increase linearly, and during this drift the weight also drifts from 100%. In addition, for the same samples the starting sample weight is only a few mg in order to have water dispersed uniformly into them. Also, some of them are significantly thicker, and very probably the total amount of water is not removed in the relatively short period of continuous heating (approximately 28 min). These considerations could account for the 7% discrepancy for the sample with 0.37 overall water content, namely: (a) there is a large systematic error in this case, and (b) possibly there is several percent water left even at 300°C.

# CONCLUSIONS

It is clear that crystallization of water in gelatin is possible only if the glass transition temperature of the system is lower than the ice crystallization temperature. Water, on the other hand, strongly affects the system glass transition temperature because  $T_g$  of water is  $-125^{\circ}$ C<sup>12</sup> and that of dry gelatin is  $217^{\circ}$ C.<sup>7</sup> For this reason, increase of the water concentration leads to a drastic drop of  $T_g$  of the system to 0°C, at which point some water starts to crystallize and the



Figure 7 TGA traces of the gelatin sample crosslinked in 5.5 wt % gelatin solution in KCNS and 0.0033 *M* solution of glutaric aldehyde.

amount of noncrystalline water remains constant (Fig. 4). Thus, as in the case of many hydrophilic polymers,<sup>1-4</sup> it seems more appropriate to consider water in gelatin being crystallizable and noncrystallizable, instead of unbound and bound.

Using the Fox-Flory equation and the ice melting temperature,  $C^*$  can be predicted as the abscissa of the intersection of the Fox-Flory curve and the melting curve of the ice. However, it is hard to predict  $C^*$  for crosslinked gelatin. Generally speaking,  $T_g$  increases with the degree of crosslinking.<sup>13</sup> In our case, for crosslinked gelatin the Fox-Flory curve shifts to the right and crosses the ice melting line at higher  $C^*$ . This explains the value of  $C^* = 0.35$  for the gelatin crosslinked with 11% GA, but fails to explain the lower value of  $C^* = 0.26$  for the crosslinked sample obtained from the 5.5% solution. In this case, it is better not to use the Fox-Flory equation but to obtain  $T_{\boldsymbol{g}}$  experimentally by dynamic mechanical thermal analysis; DSC cannot be used because  $T_g$  is hidden by the melting peak of the ice.

It is a pleasure to acknowledge the financial support provided by the National Science Foundation through Grant INT-9514149, and by the Bulgarian Ministry of Education, Science and Technology under contract TH-714. A. A. Apostolov appreciates the hospitality of the Department of Chemistry of the University of Cincinnati, where part of this work was carried out.

# REFERENCES

- Rault, J.; Gref, R.; Ping, Z. H.; Nguyen, Q. T.; Néel, J. Polymer 1995, 36, 1655.
- Rault, J.; Ping, Z. H.; Nguyen, Q. T. Polym Bull 1995, 35, 649.
- Rault, J.; Ping, Z. H.; Nguyen, Q. T. J Non-Cryst Solids 1994, 172, 733.
- Ponomariova, T.; Melnichenko, Yu.; Albouy, P. A.; Rault, J. Polymer 1997, 38, 3561.
- Rault, J. in Hydrogen Bond Networks; Bellissent-Funel, M. C.; Dore, J. C., Eds.; Kluwer Academic Publishers: The Netherlands, 1994; p. 441.
- 6. Fox, T. G.; Flory, P. J. J Polym Sci 1954, 14, 315.
- Rose, P. I. in The Theory of the Photographic Process; 4th ed.; James, T. H., Ed.; Macmillan Publishing Co.; New York, 1977; p. 67.
- Encyclopedia of Chemistry, Vol. 1; Sovetskaya Enciklopedia: Moscow, p. 394.
- Pappas, N. A.; Merrill, E. W. J Appl Polym Sci 1976, 20, 1457.
- Hatakeyama, T.; Hirose, S.; Hatakeyama, H. Makromol Chem 1987, 188, 1975; Makromol Chem 1989, 184, 1265.
- Thomas, L. C. in Thermal Analysis Application for Materials Characterization, Western Kentucky University: Bowling Green, KY, 1998.
- Rasmussen, D. H.; MacKenzie, A. P. J Phys Chem 1971, 75, 967.
- Plazek, D. J.; Ngai, K. L. in Physical Properties of Polymers; Mark, J. E., Ed.; Springer: New York, NY, 1996; p. 144.