Colloid Polym Sci 275:1078–1082 (1997)
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SHORT COMMUNICATION

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# Effects of polyols and sugars on the structure of water in concentrated gelatin gels as studied by low temperature differential scanning calorimetry

Received: 9 January 1997
Accepted: 1 July 1997

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**Abstract** Phase-transition temperatures, glass-transition temperatures, melting temperatures, gel–sol transition temperatures in differential scanning calorimetry heating curves of gelatin solutions with and without various sugars and polyols quenched by liquid nitrogen were studied. Both sugars and polyols added to concentrated gelatin solutions shifted the glass-transition temperatures to lower temperatures, and it was attributed to the increase of unfreezable water which acts as a plasticizer. The mechanisms of the increase in unfreezable water, however, seem to be different for sugars and polyols; sugars increase unfreezable water by increasing the number of junction zones which hold unfreezable water, while polyols by themselves increase the amount of unfreezable water.

**Key words** Glass transition – gelatin – water – differential scanning calorimetry – sugars – polyols

## Introduction

It is important to study the state of water in gels to improve the quality of foods, since the structure of water is markedly changed when water molecules are restrained in biomaterials [1]. Many authors have found that there are at least two different states of water, i.e. bound water and free water in hydrogels, such as agarose gels [2–7], mucopolysaccharides [8], and gelatin gels [9, 10]. Aizawa et al. [2] studied the properties of water in macromolecular gels by dilatometry and nuclear magnetic resonance. They suggested that the states of water are closely related with rheological properties of hydrogels. Ikada et al. [8] studied the structure of water in mucopolysaccharides by low-temperature differential scanning calorimetry (DSC), and found three different states of water, and revealed that the amount of free water increased linearly with increasing total water content, whilst the other two species of bound water levelled off. Gelatin gels have been used as a model substance by many researchers [11–13]. It is suggested that sugars and polyols may interact directly with gelatin molecules and change the conformation and structure of junction zones [14]. It is also suggested that the mobility of water in gelatin gels may be changed by the addition of sugars and polyols. In the present work, the effects of various sugars and polyols on the phase-transition behavior of gelatin hydrogels were studied by differential scanning calorimetry at a low-temperature range.

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## Experimental

### Materials

Low molecular weight ($M_w = 11\,000$) gelatin with isoelectric point 6.7 was used in the present work. Ethylene glycol, propylene glycol, glycerin, deoxyribose, ribose, glucose, sucrose, raffinose, maltotriose, maltotetraose, α-cyclodextrin, maltohexaose of extra reagent grade (Wako Pure Chemical Industries Ltd., Osaka) were used without further purification.

### Measurements

Low-temperature DSC measurements were carried out using a sensitive Seiko DSC-10 equipped with low-temperature accessory. Alpha alumina was used as a reference material. Gel samples (12 mg) were sealed in 15 μl aluminium pans. The sample was quenched from room temperature to −150 °C by liquid nitrogen, and then raised at 5 °C/min.

## Results and discussion

DSC heating curves for gelatin gels of different concentrations from 23.2 to 57.7 wt%, quenched from room temperature to −150 °C using liquid nitrogen, are shown in Fig. 1. A step-like change in baseline observed at around −100 °C is attributed to glass transition. The glass-transition temperature $T_g$ is shown by an arrow in Fig. 1. $T_g$ shifted to higher temperatures with decreasing gelatin concentration beyond 55%. It is reported that various kinds of hydrogels form glassy state by quenching from a sol state to −150 °C, and a part of water molecules associated closely with matrix polymers solidify into an amorphous state [15]. By heating, amorphous ice associated with matrix becomes mobile. Gelatin gels with amorphous ice commence the free molecular motion which is detected as $T_g$ in heating DSC curves. A broad exothermic deviation is observed in samples containing a small amount of water (Fig. 1, curves a–c), which was attributed to the cold crystallization. Water molecules solidified in an amorphous state are mobilized at $T_g$ on heating, and molecular motion is enhanced by successive heating and reorganized as unstable ice just before melting. This is confirmed by the fact that the exotherm disappeared if the sample is annealed at a temperature lower than melting [15]. This suggests that cold crystallization can be observed for thermally unstable samples prepared by quenching [15]. Then, DSC curves deviated further to the endothermic side, followed by a sharp exothermic peak from 3.3 °C to −11.9 °C. As shown in curves a–c, starting deviation merged with broad exotherm of cold crystallization. On this account, it is difficult to define the exact starting temperature of melting. In spite of this, it is clear that starting temperature of endotherm shifted to higher temperatures with increasing water content. Unstable ice started to melt immediately after cold crystallization. When water content increased, a sharp exothermic peak appeared at around 3.3 °C to −11.9 °C, which is tentatively named as the pre-melt crystallization ($T_{pm}$). This exothermic peak seems to be apparent, since the melting of unstable ice overlaps with melting of hexagonal ice crystals which were formed by freezing of free water. $T_{pm}$ is observed clearly when the amount of unstable ice is large. The peak temperature of normal ice $T_m$ is observed around 0–8 °C. This is reasonable when the DSC scan rate is 5 °C/min. An endothermic peak at $T^*$ around 40 °C was attributed to the gel-to-sol transition, and it shifted to higher temperatures with increasing concentration of gelatin.

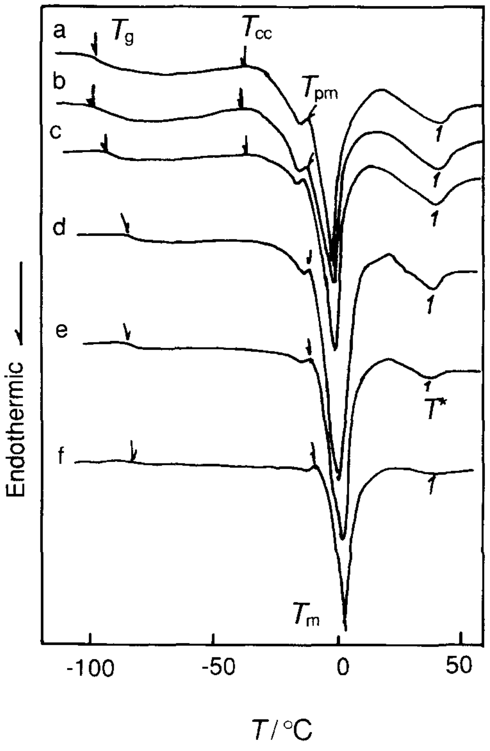

**Fig. 1** DSC heating curves for gelatin gels of different concentrations: (a) 57.7%; (b) 55.0%; (c) 51.2%; (d) 46.9%; (e) 35.2% (f) 23.2%; Heating rate, 5 °C/min

The phase transition temperatures of gel are shown as a function of water content $W_c$ in Fig. 2. The water content $W_c$ is defined as $W_c$ $(g/g) = W_w/W_s$ $(g/g)$, where $W_w$ is the

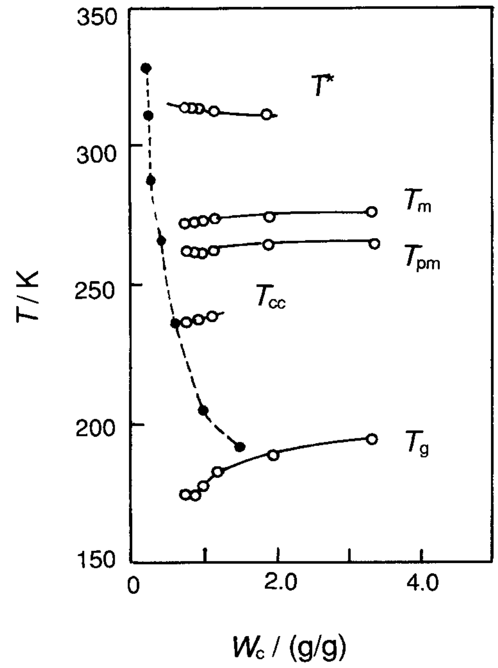

**Fig. 2** Relationship between phase-transition temperatures and water content $W_c(g/g)$ for gelatin–water system. $T_g$: glass-transition temperature, $T_{pm}$: pre-melt crystallization, $T_{\propto}$ : cold crystallization temperature, $T_m$: melting temperature, (●) $T_g$ taken from ref. [10]

weight of water in the system and $W_s$ is that of the completely dried sample. At water content range higher than $W_c = 0.78$ (a water content between curves a and b in Fig. 1), movement of gelatin molecules is restricted by the presence of ice, and on this account, $T_g$ shifted to higher temperatures with increasing water content. It was difficult to obtain a complete dissolution for gelatin solutions above 60%. However, $T_g$ seems to shift to higher temperatures with decreasing water content $W_c$ around $W_c = 0.78$. Borchard et al. [10] studied the glass transition in gelatin–water systems in the broad concentration range by differential scanning calorimetry. $T_g$ observed for gelatin water systems by Borchard et al. are also plotted in Fig. 2 (closed circles). $T_g$ observed at lower water content by Borchard et al. (closed circles) locate at the extension of our data (open circles) as shown in Fig. 2, however, a noticeable discrepancy appeared around $W_c = 0.7$–$1.5$. We cannot compare directly those results because their samples and the condition of measurement ($M_w = 50\,000$ and isoelectric point 7.5, scan rate 10 °C/min) are different, and the remarkable difference of $T_g$ at $W_c = 0.7$–$1.5$ should be attributed to this difference. Borchard et al. could achieve a complete quenching to the homogeneous glass, whilst the sample quantity of our experiment was much larger than theirs and the system might be separated into two phases, a phase of concentrated gelatin solution and a phase of almost pure water which exist as a homogeneous glass and ice, respectively, at liquid nitrogen temperature. However, if Borchard et al. added much water, they also might find the shift of $T_g$ to higher temperatures with increasing water content above a certain $W_c$. It is well known that $T_g$ of a polymer shifts to lower temperatures by the addition of a plasticizer [16]. $T_g$ shifted to lower temperatures with decreasing gelatin concentration, i.e. with increasing water content below $W_c = 0.78$, since water molecules play a role of plasticizer. It is also well known that $T_g$ shifts to higher temperatures in the presence of a large amount of water, because in such a case the whole system cannot be converted to glassy state, but some ice crystals are formed, and they inhibit the molecular motion of polymers chains [17]. The glass-transition temperature $T_g$ of gelatin gels as a function of gelatin concentration, therefore, showed a minimum around $C_{go}$ 56% ($W_c = 0.78$) (Fig. 2) as has been observed for many polysaccharide-water systems such as sodium cellulose sulfate–water [18], xanthan–water [19], carboxymethylcellulose–water [20]. Borchard et al. also observed the glass transition for gelatin–water systems from 20.1% to 61.3% annealed at 269 K for 1 h, and found that $T_g = 263$ K which was independent of the concentration of gelatin.

DSC heating curves for 40% gelatin gels containing ethylene glycol of various concentrations, and those containing glucose of various concentrations are shown in Figs. 3 and 4, respectively. $T_g$ of 40% gelatin gels containing polyols and sugars shifted to lower temperatures with increasing concentration of added polyols and sugars as was observed in 40% agarose gels [7]. This was explained by the decrease in freezable water, which could be estimated from the endothermic peak at $T_m$, with increasing concentration of added polyols and sugars; the decrease in freezable water is equivalent to the increase in unfreezable water which can act as a plasticizer, hence the shift of $T_g$ to lower temperatures.

The glass-transition temperature $T_g$ and the melting temperature $T_m$ of 40% gelatin gels containing sugars or polyols with different concentrations as a function of molecular weight of sugars or polyols is shown in Fig. 5. $T_g$ shifted to higher temperatures with increasing molecular weight of sugars or polyols added, and it ranged from 165 to 190 K. $T_m$ also shifted to higher temperatures with increasing molecular weight of sugars or polyols added, but it ranged from 268 to 273 K, and the shift was not so prospicuous with comparison to that for $T_g$.

The effects of sucrose on the rheological and thermal properties of gelatin gels were studied by dynamic viscoelastic measurements and DSC [14]. It was found that the gel-to-sol transition-temperature shifted to higher

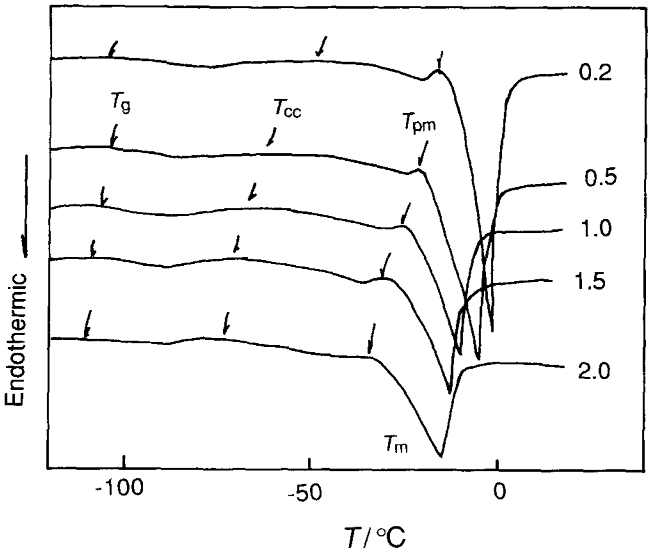

**Fig. 3** DSC heating curves for 40% gelatin gels containing ethylene glycol. Numerals in the figure represent the concentration of ethylene glycol in M

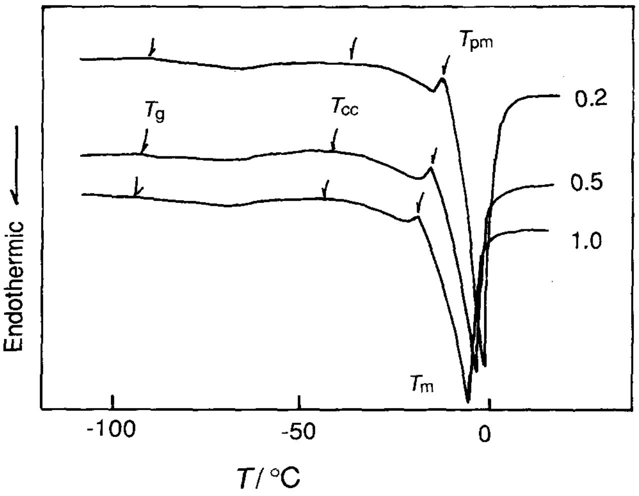

**Fig. 4** DSC heating curves for 40% gelatin gels containing glucose. Numerals in the figure represent the concentration of glucose in M

temperatures and the modulus of gels increased by the addition of sucrose. This should be attributed to the increase in the number of elastically active network chains. Based on the estimation by a modified theory of rubber elasticity [21], the size of junction zones in gelatin gels has been found to decrease but the number of junction zones should increase by the addition of sucrose [14]. This is equivalent to the decrease in the number of parallel links which constitute a single zipper [22], and at the same time the rotational freedom of parallel links decreased [14].

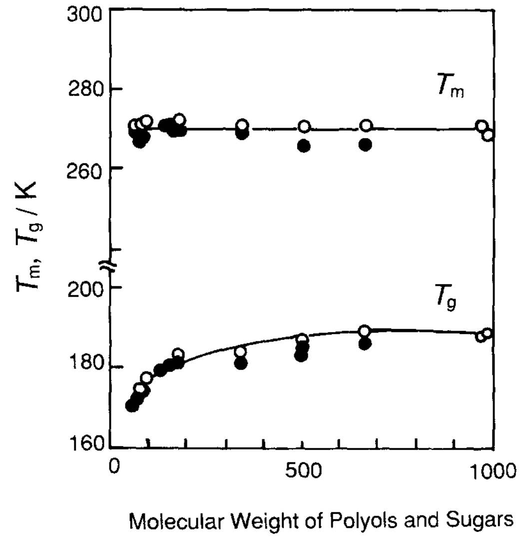

**Fig. 5** Glass-transition temperature $T_g$ and melting temperature $T_m$ of 40% geltain gels containing sugars or polyols of various concentrations as a functions of molecular weight of sugars or polyols added. (○) 0.2 M, (●) 0.5 M

The most part of unfreezable water was believed to exist in the junction zones from the low-temperature DSC studies of concentrated agarose gels [6]. Although the size of each junction zone becomes smaller in gelatin gels, the total amount of unfreezable water seems to increase by the addition of sugars because of the increase in the number of junction zones. The glass-transition temperature of 40% gelatin gels containing sugars shifted to higher temperatures with increasing unfreezable water ($g$ unfreezable water/mole solute) estimated for sugars alone [23]. Molecular weight $M_w$ and $g$ unfreezable water per mole solute for sugars UFW are as follows: deoxyribose, $M_w = 134.1$, UFW = 177.0; ribose, $M_w = 150.1$, UFW = 73.5; rhamnose, $M_w = 164.2$, UFW = 147.8; glucose, $M_w = 180.2$, UFW = 73.9; sucrose, $M_w = 342.3$, UFW = 191.7; raffinose, $M_w = 504.5$, UFW = 353.2, maltotriose, $M_w = 504.5$, UFW = 227.0; maltotetraose, $M_w = 666.6$, UFW = 366.6; maltohexaose, $M_w = 990.9$, UFW = 495.5, where values of UFW are taken from ref. [23]. The shift of the glass-transition temperature of 40% gelatin gels containing sugars to lower temperatures cannot be explained only by the increase of unfreezable water by sugars. The effect of sugars are not only to produce unfreezable water by itself, but sugars also interact directly with gelatin molecules, and to create new junction zones which can hold unfreezable water.

The effects of polyols seem to be different from those of sugars. Ethylne glycol shifts $T_g$ to lower temperatures than does propylene glycol or glycerin, and this order agrees with the amount of unfreezable water per mole polyol: molecular weight $M_w$ and $g$ unfreezable water per mole solute for polyols UFW are as follows, ethylene glycol, $M_w = 62.1$, UFW = 118.0; propylene glycol, $M_w = 76.1$, UFW = 97.4; glycerin, $M_w = 92.1$, UFW = 78.3, where values of UFW are taken from ref. [23]. It may be possible to explain the shift of $T_g$ to lower temperatures only by the amount of unfreezable water per polyol, but further study is required to confirm this using more than three polyols.

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