

# Water in Dextran Hydrogels

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Received 18 October 2009; accepted 28 December 2009

DOI 10.1002/app.32066

Published online 29 March 2010 in Wiley InterScience (www.interscience.wiley.com).

**ABSTRACT:** A series of dextran hydrogels were synthesized with potassium chloride as a crosslinker.  $^{13}\text{C}$ -NMR and  $^{17}\text{O}$ -NMR were used to study the crosslinking role of potassium chloride. Differential scanning calorimetry and thermogravimetric analysis were used to study the state and properties of water in the dextran hydrogels. We found there were three kinds of water in the physically crosslinked dextran hydrogels: free water, freezing bound water, and nonfreezing water. The maximum amount of

nonfreezing water in the 50% dextran hydrogel was about 8%. Both  $^{13}\text{C}$ -NMR and  $^{17}\text{O}$ -NMR spectra proved that KCl played the crosslinking role and it interacted with dextran via the oxygen atoms linked to  $\text{C}_4$  and  $\text{C}_6$  and, also, the oxygen atom linked to  $\text{C}_1$  on the dextran ring. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 117: 1631–1637, 2010

**Key words:** hydrogels; NMR

## INTRODUCTION

Polysaccharides play an important role in biochemical processes. For example, polysaccharides on the cell's surface help the cell easily recognize other cells, and charged polysaccharides can prevent tumor cells from aggregating.<sup>1</sup> In addition, polysaccharides are used as column packing for liquid chromatography.<sup>2</sup> These materials contain a substantial amount of water in the polymer networks, and their characteristic physicochemical properties depend not only on the water content but also on the state of water.

Polysaccharide hydrogels are three-dimensional networks that swell in water and aqueous solutions. Therefore, the physicochemical properties of polysaccharide hydrogels not only depend on the polysaccharide but also on the water. Numerous workers<sup>3–6</sup> have studied the states of water in chemically crosslinked hydrogels using differential scanning calorimetry (DSC),  $^{13}\text{C}$ -NMR,<sup>4</sup> and other simulation techniques.<sup>7–9</sup> Some scientists have proposed that the water in chemically crosslinked hydrogels can be classified into three species according to the strength of the interactions between water and polymers in the hydrogels: free water, freezing bound water, and nonfreezing water, but supercooled water was pro-

ven to exist in some hydrogel systems, and it is regarded as another type of water.<sup>3–5</sup> Furthermore, most of these studies have focused on the irreversible process between the solution and the gel due to strong interactions in the network. Few studies on physically crosslinked polysaccharide hydrogels have been reported. Does supercooled water exist in these systems, which are reversible between sol and gel because of weak interactions?

It was reported<sup>10–12</sup> that physical hydrogels of certain polysaccharides are usually formed by the presence of ions. For instance, alginate beads are formed from alginate solutions in the presence of cations such as  $\text{Ca}^{2+}$  or  $\text{Ba}^{2+}$ .<sup>10</sup> It was also reported that the hydrogel of dextran is formed in the presence of a large amount of potassium ions, whereas the viscous dextran is unable to form gels in a pure aqueous solution.<sup>11,12</sup> However, the role of cations and how the dextran chains interact with KCl in the formation process of dextran hydrogels are still unknown. In this study,  $^{13}\text{C}$ -NMR and  $^{17}\text{O}$ -NMR techniques were used to obtain information on dextran in the sol state. Furthermore, DSC and thermogravimetric analysis (TGA) were used to study the state and properties of water in the dextran hydrogels. These studies will provide very important information about the structure and properties of dextran hydrogels.

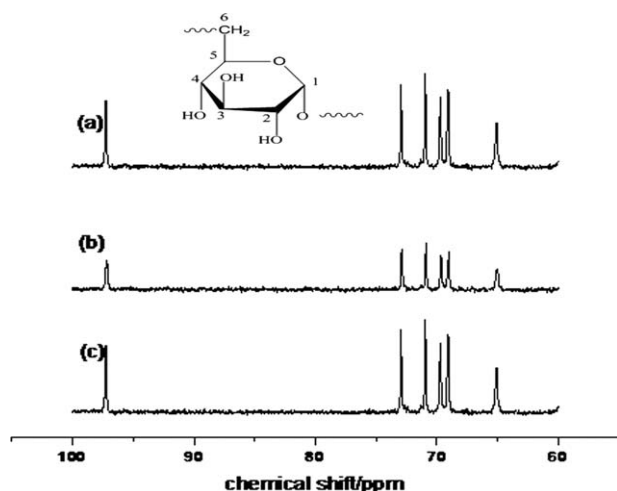
## EXPERIMENTAL

### Chemicals

Potassium chloride (Guangzhou Chemical Reagent Factory, Guangzhou, P.R.China analytical-reagent

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Contract grant sponsor: National Natural Science Foundation of China (to J.-Z.Y.); contract grant number: 20704044.



**Figure 1**  $^{13}\text{C}$ -NMR spectra of three kinds of dry dextran gels dissolved in 0.7 mL of  $\text{D}_2\text{O}$ : (a) Dex0.05, (b) Dex0.2, and (c) Dex0.5.

grade) and dextran (weight-average molecular weight = 20,000, Sinopharm Chemical Reagent Co.) were used as received.

### Sample preparation

We prepared dextran gels by dissolving calculated amounts of dextran in 25 wt % potassium chloride aqueous solution at  $90^\circ\text{C}$  with continuous stirring until a homogeneous solution was formed. The homogeneous solution was cooled to room temperature for 24 h, and wet dextran gels with different mass percentages (5, 10, 20, 30, and 50%) of dextran were formed. The five wet dextran gel samples were put in a vacuum oven at  $50^\circ\text{C}$  for several weeks until they reached a constant weight, and five dry dextran gel samples were obtained. In addition, six wet dextran gel samples with 30% dextran were put in a vacuum oven and then dried for different durations to obtain six dextran hydrogel samples with different water contents. The water contents of the six dextran hydrogel samples were 9.5, 15.2, 26.1, 31.4, 38.1, and 46.5%.

### $^{13}\text{C}$ -NMR and $^{17}\text{O}$ -NMR spectra

Quantitative, proton-decoupled, high-resolution  $^{13}\text{C}$ -NMR and  $^{17}\text{O}$ -NMR spectra were obtained with a

Varian INOVA 500-MHz (USA) spectrometer. The spectra were performed on hydrogels swollen in  $\text{D}_2\text{O}$ .

### DSC measurements

DSC measurements were conducted with a TA Instruments (USA) covering the temperature range of  $-80$  to  $20^\circ\text{C}$  at a scanning rate of  $10^\circ\text{C}/\text{min}$ .

### TGA measurements

TGA was carried out with a TA Instruments 2910 in a dynamic nitrogen atmosphere at a heating rate of  $20^\circ\text{C}/\text{min}$  and a temperature interval between 30 and  $600^\circ\text{C}$ .

## RESULTS AND DISCUSSION

### $^{13}\text{C}$ -NMR and $^{17}\text{O}$ -NMR spectra

The  $^{13}\text{C}$ -NMR and  $^{17}\text{O}$ -NMR techniques were used to study the interactions between dextran and KCl in the hydrogel system. The high-resolution  $^{13}\text{C}$ -NMR spectra of 5, 20, and 50% dry dextran gels are shown in Figure 1. Six resonance peaks appeared for all three samples, which corresponded to the six carbon atoms of the repeat unit of dextran. The  $^{13}\text{C}$ -NMR chemical shift assignments of dextran are shown in Table I. The  $^{13}\text{C}$ -NMR chemical shifts of the three samples with different dextran and KCl concentrations were very similar. However, as shown in Table II, the half-widths of the six carbon peaks of dextran increased with increasing weight ratio of potassium chloride to dextran repeat units. In particular, the  $\text{C}_6$  and  $\text{C}_4$  peaks showed maximum half-width increase values of 7.703 and 9.537 Hz, respectively, when the weight ratio of KCl to dextran repeat units increased from 1 : 10.3 to 1 : 0.54. These half-widths changes demonstrated that the crosslinking interactions between dextran and KCl were strengthened with increasing KCl concentration and proved that KCl played the role of crosslinking agent and that the crosslinking mainly took place through the oxygen atoms linked to  $\text{C}_4$  and  $\text{C}_6$ . The  $\text{C}_1$  peak showed a maximum half-width increase value of 17.156 Hz when the weight ratio of KCl to

**TABLE I**  
 $^{13}\text{C}$  Chemical Shift Assignments of Dextran

Sample	Dextran (wt %)	$\text{C}_6$ (ppm)	$\text{C}_5$ (ppm)	$\text{C}_4$ (ppm)	$\text{C}_3$ (ppm)	$\text{C}_2$ (ppm)	$\text{C}_1$ (ppm)	$W_{\text{Dex}}/W_{\text{KCl}}^{\text{a}}$
Dex0.05	5	65.102	69.069	69.690	72.942	70.923	97.271	10.3 : 1
Dex0.2	20	65.057	69.071	69.715	72.936	70.933	97.266	2.2 : 1
Dex0.5	50	65.096	69.087	69.723	72.952	70.956	97.266	0.54 : 1

<sup>a</sup> Weight ratio of repeat units of dextran to potassium chloride.

TABLE II  
 $^{13}\text{C}$  Half-Width of Peak for the Dextran Repeat Units of Different Samples

Sample	C <sub>6</sub> half-width (Hz)	C <sub>5</sub> half-width (Hz)	C <sub>4</sub> half-width (Hz)	C <sub>3</sub> half-width (Hz)	C <sub>2</sub> half-width (Hz)	C <sub>1</sub> half-width (Hz)	$W_{\text{Dex}}/W_{\text{KCl}}^{\text{a}}$
Dex0.05	34.277	22.173	25.195	19.671	15.285	36.189	10.3 : 1
Dex0.2	33.737	20.986	19.299	15.449	14.251	30.296	2.2 : 1
Dex0.5	26.574	16.668	15.658	12.793	12.295	19.033	0.54 : 1

<sup>a</sup> Weight ratio of repeat units of dextran to potassium chloride.

dextran repeat units increased from 1 : 10.3 to 1 : 0.54; this indicated that one or two neighboring oxygen atoms linked to C<sub>1</sub> interacted intensely with KCl. This was confirmed by the following  $^{17}\text{O}$ -NMR spectra, shown in Figure 2.

The high-resolution  $^{17}\text{O}$ -NMR spectra of the 5 and 20% dry dextran gels dissolved in D<sub>2</sub>O are shown in Figure 3. Similar to the  $^{13}\text{C}$ -NMR spectra, the  $^{17}\text{O}$ -NMR spectra of the two dry dextran gel samples resembled each other, and their chemical shifts did not show drastic changes with dextran and KCl concentrations. There were two resonances peaks (peaks 1 and 2), which corresponded to the oxygen atom linked to C<sub>1</sub> on the dextran ring plane (peak 2) and the other oxygen atoms above or below the dextran ring. The half-widths of the two peaks, as shown in Table III, demonstrated that the crosslinking interactions between dextran and KCl were strengthened with increasing KCl concentration, which was in agreement with the  $^{13}\text{C}$ -NMR results. The half-width of peak 2 showed an increase of 51.9 Hz when the weight ratio of KCl to dextran repeat units increased from 1 : 10.3 to 1 : 2.2; this indicated that only the oxygen atom on the dextran ring linked to C<sub>1</sub> interacted strongly with KCl. Therefore,

both the  $^{13}\text{C}$ -NMR and  $^{17}\text{O}$ -NMR spectra proved that KCl played the crosslinking role and interacted with dextran via the oxygen atoms linked to C<sub>6</sub> and C<sub>4</sub> and also one oxygen atom linked to C<sub>1</sub> on the dextran ring.

### DSC

The DSC technique was used to study the states and properties of water in the hydrogel systems. Figure

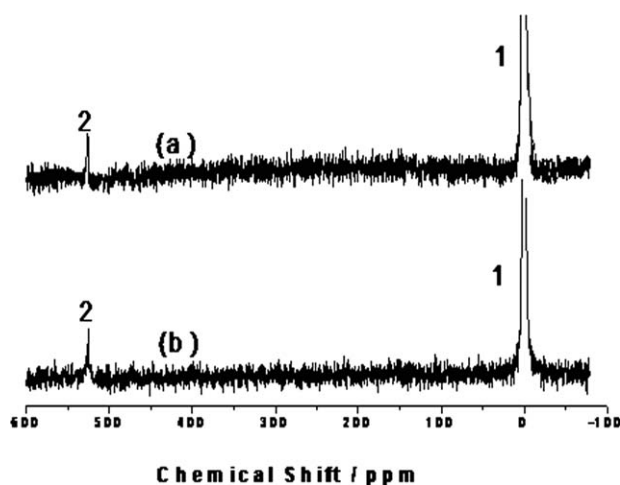


Figure 2 DSC curves of different dextran gels in the (a) heating process and (b) cooling process with dextran contents of 5, 10, 20, 30, and 50%.

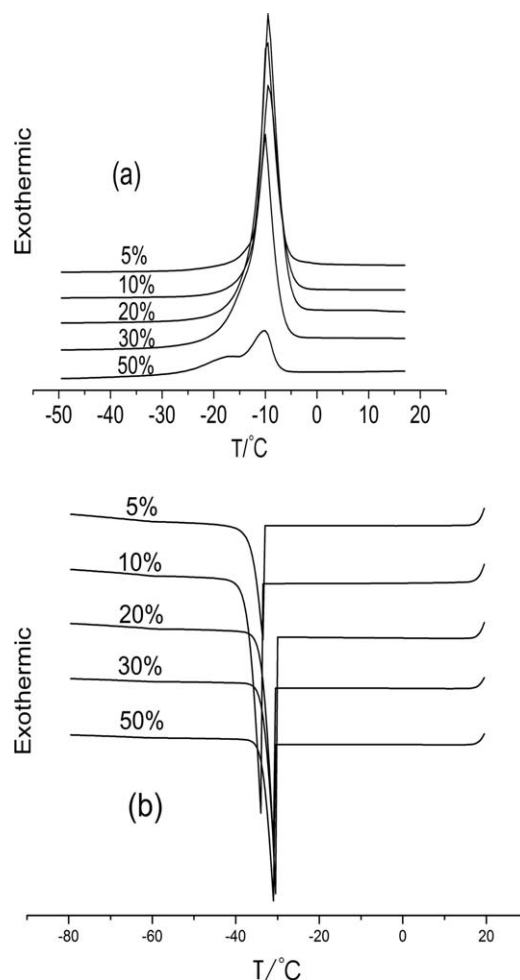


Figure 3  $^{17}\text{O}$ -NMR spectra of two kinds of dry dextran gels dissolved in 0.7 mL of D<sub>2</sub>O: (a) Dex0.05 and (b) Dex0.2 (T = temperature).

**TABLE III**  
<sup>17</sup>O Half-Width of Peaks for the Dextran Repeat Units of Different Samples

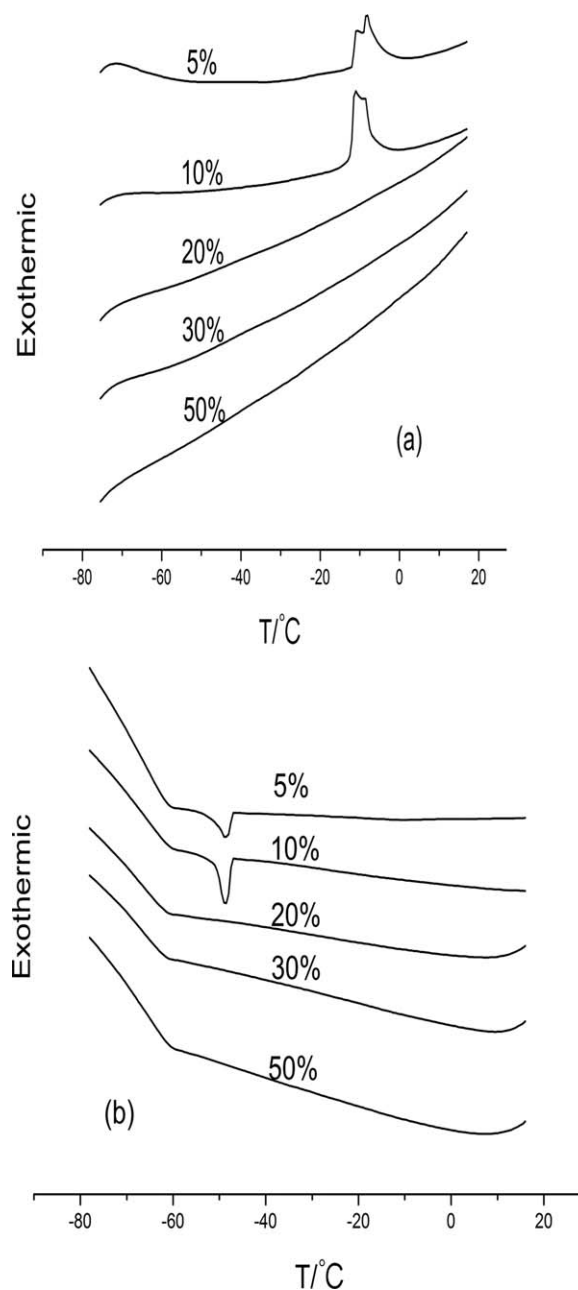
Sample	Half-width of peak 1 (Hz)	Half-width of peak 2 (Hz)	$W_{\text{Dex}}/W_{\text{KCl}}^a$
Dex0.05	81.5	154.8	10.3 : 1
Dex0.2	80.4	102.9	2.2 : 1

<sup>a</sup> Weight ratio of repeat units of dextran to potassium chloride.

2 shows the DSC curves of the dextran wet gels. With increasing dextran concentration, the endothermic melting peak in the heating curves gradually split into two peaks at about  $-20$  and  $-10^\circ\text{C}$ . Two distinct melting peaks were observed in the curve of the 50% dextran gel sample, as shown in Figure 2(a); this suggested the existence of two states of water. The peak at  $-20^\circ\text{C}$  was assigned as the melting point of freezing bound water, and the peak at  $-10^\circ\text{C}$  was the melting point of free water. The peak split indicated that free water emerged when the freezing bound water reached a maximum content. It is also shown in Figure 2 that the peak at  $-20^\circ\text{C}$  became more obvious and broader with increasing dextran content; this indicates that the interactions between water and dextran increased with increasing dextran content. The cooling curves of the five wet samples showed similar asymmetric freezing peaks due to the existence of free water and freezing bound water at about  $-30^\circ\text{C}$ , as shown in Figure 2(b). Peaks for nonfreezing water and super-cooled water were not present.

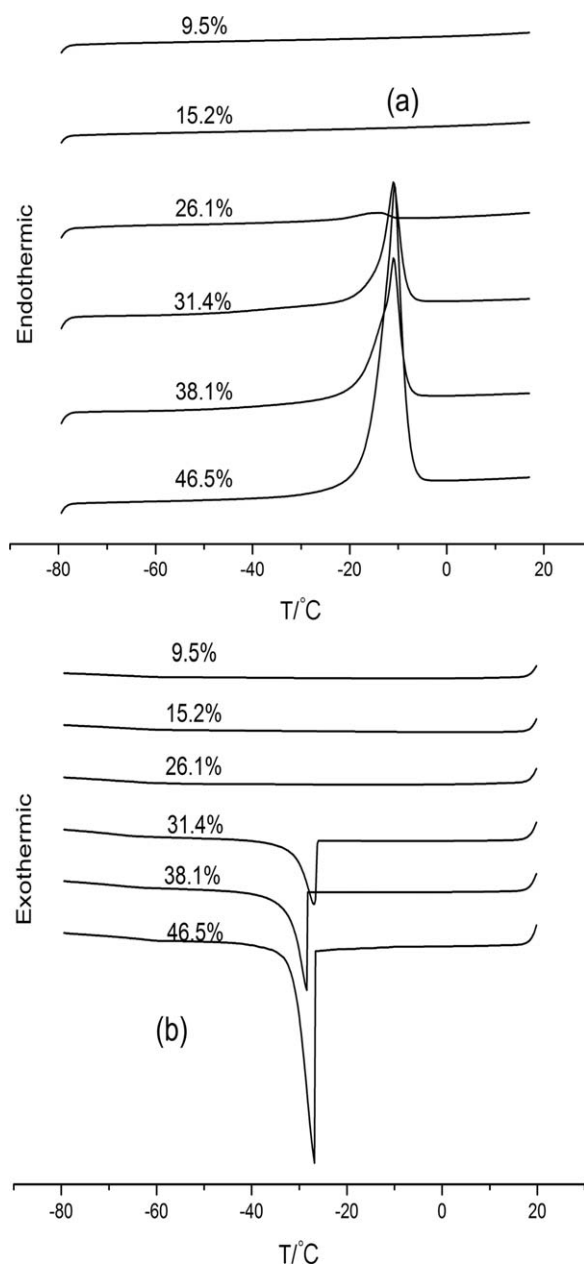
Figure 4 shows the DSC curves of the five dry dextran gel samples. All five samples were dried for 1 week in a vacuum oven, and we supposed that there was no free water in the samples. There was one peak in the heating and cooling curves for the 5 and 10% dry dextran gel samples, but there was no peak in the other three samples; this indicated that there was no freezable water (freezing bound water or free water) in the 20, 30, and 50% dry dextran gels. Also, the melting peaks in the heating curves for the 5 and 10% dry dextran gels gradually split into two peaks at  $-10$  and  $-5^\circ\text{C}$ ; these temperatures were different from the melting points of freezing bound water and free water at  $-20$  and  $-10^\circ\text{C}$  for the wet dextran samples, as shown in Figure 3(a). For the cooling curves, the freezing peaks for the 5 and 10% dry gels appeared at about  $-50^\circ\text{C}$ ; this was also different from the cooling peak at  $-30^\circ\text{C}$  for the wet dextran gel, as shown in Figure 3. These phenomena could be explained by the facts that the three kinds of water can be transformed to each other and the transformations depend on the structure of hydrogels, which is affected by the dextran

content, KCl content, crosslinking networks, and hydrophilic properties of polymer chains. For the 5 and 10% dextran dry gel samples, the crosslinking networks were loose, and the interactions between the dextran chains and water were weak. Even the nonfreezing water could be transformed into freezing bound water, and the freezing bound water could be transformed into free water. For the dextran gels with dextran contents greater than 20%, the crosslinking networks were dense, and the interactions between the dextran chains and water were



**Figure 4** DSC curves of different dry dextran gels in the (a) heating process and (b) cooling process with dextran contents of 5, 10, 20, 30, and 50% ( $T$  = temperature).





**Figure 5** DSC curves of Dex0.3 in the (a) heating process and (b) cooling process with water contents was 9.5, 15.2, 26.1, 31.4, 38.1, and 46.5% ( $T$  = temperature).

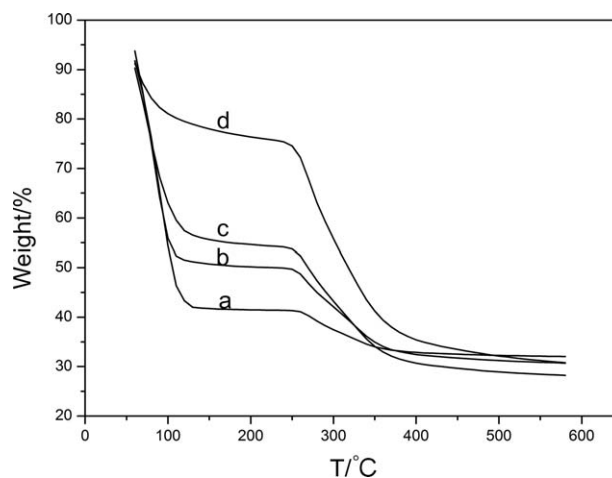
very strong, so the nonfreezing water and freezing bound water could not be transformed easily. These results are in agreement with those for the chitosan hydrogel system.<sup>5</sup>

To investigate the relationship between the water amount and peaks appearing in the DSC curves in the dextran hydrogels, six dextran hydrogel samples with known water contents were examined with DSC. First, six Dex0.3 samples, whose original dextran contents were 30%, were put in a vacuum oven and then dried for different durations to obtain six dextran hydrogel samples with different water con-

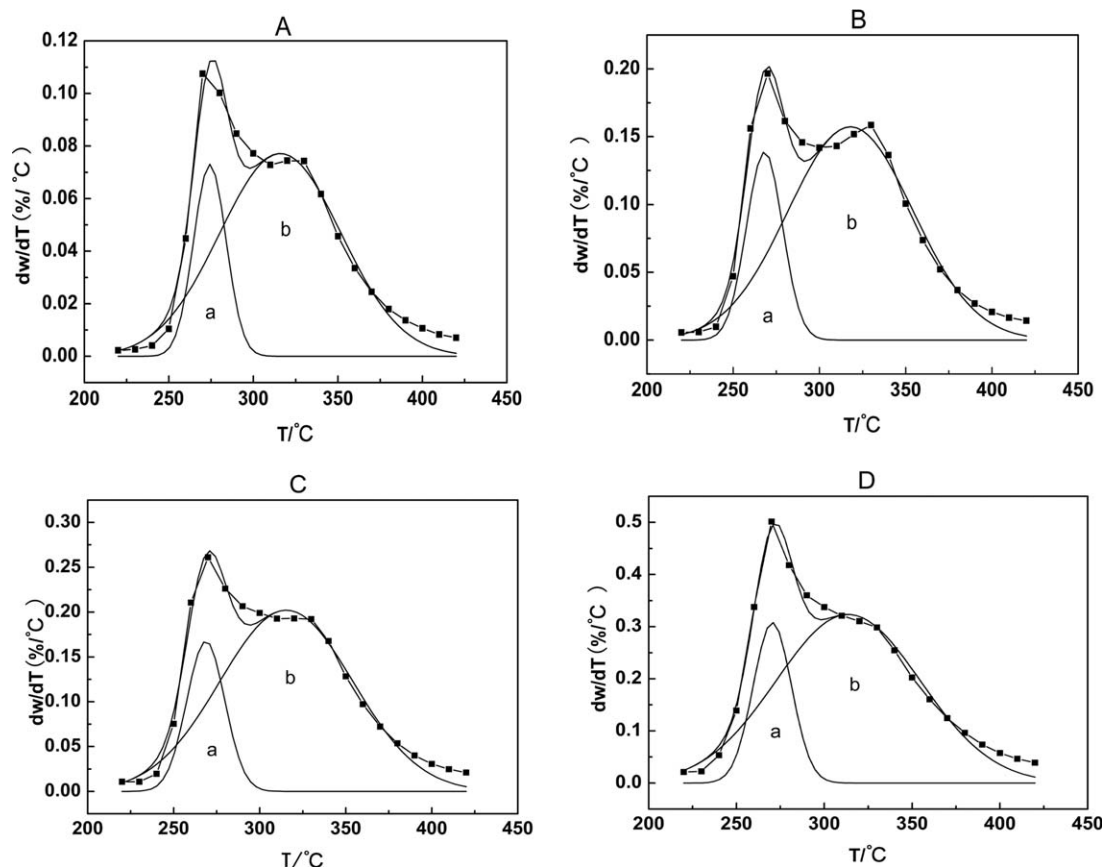
tents. The water contents of the six dextran hydrogel samples were 9.5, 15.2, 26.1, 31.4, 38.1, and 46.5%, respectively. Their DSC curves are shown in Figure 5. We observed that there were one melting peak and one freezing peak in the DSC heating and cooling curves, respectively, when the water contents of the dextran gel samples were 31.4, 38.1, and 46.5%; this indicated that the frozen water included free water and freezing bound water. However, there were no peaks in the other three samples, in which the water contents were 9.5, 15.2, and 26.1%, respectively; this indicated that only nonfreezing water was present in these three samples. These results illustrate that only when the water content of the dextran hydrogel sample was greater than 26.1% did the melting or freezing peak of the frozen water appear in the DSC curves. That is, the freezing bound water and free water emerged when the nonfreezing water reached the final maximum content. Therefore, the formation of different states of water within the dextran network took place in the following order: nonfreezing water, freezing bound water, and free water; that is, the free water was the last type of water to emerge after the nonfreezing and freezing bound waters reached their final maximum contents. Our observations were in accordance with the proposal of other scientists.<sup>13,14</sup>

### Thermogravimetry (TG) and differential thermogravimetry (DTG)

TG and DTG techniques were used to further study the content of the three kinds of water in the hydrogel systems. Figures 6 and 7 show the TG and DTG curves of the 10, 20, 30, and 50% dextran gels. There were two weight loss regions in all curves, as shown



**Figure 6** TG curves of dextran gels with different dextran contents: (a) 10, (b) 20, (c) 30, and (d) 50% ( $T$  = temperature).



**Figure 7** DTG curves of dextran gels with different dextran contents: (A) 10, (B) 20, (C) 30, and (D) 50% ( $T$  = temperature,  $w$  = weight loss,  $dw/dT$  = weight loss per temperature).

in Figure 6. The first weight loss region appeared below  $120^{\circ}\text{C}$ , in which free water and freezing bound water were lost. The second weight loss region appeared in the range  $220$ – $420^{\circ}\text{C}$ . In this region, the DTG curves of the four samples in Figure 6 split into two peaks (peaks a and b). The temperature of peak a was about  $270^{\circ}\text{C}$ , which corresponded to the loss temperature of nonfreezing water. The temperature of peak b was about  $315^{\circ}\text{C}$ , which corresponded to the decomposition temperature of dextran. Therefore, by the integration of peak a, the amount of nonfreezing water was evaluated. The integrating areas of peak a, as shown in Table IV, suggest that the amount of nonfreezing water increased with increasing dextran content. The maxi-

imum amount of nonfreezing water in the 50% dextran hydrogel was about 8%.

## CONCLUSIONS

A dextran solution was transformed into a dextran gel in the presence of potassium chloride. Both  $^{13}\text{C}$ -NMR and  $^{17}\text{O}$ -NMR spectra proved that KCl played the crosslinking role and that it interacted with dextran via oxygen atoms linked to  $\text{C}_6$  and  $\text{C}_4$  and also the oxygen atom on the dextran ring. Three kinds of water, including free water, freezing bound water, and nonfreezing water, were proven to exist in the dextran hydrogels. Free water was the last type of

**TABLE IV**  
DTG Peak Split Results

Sample	Dex0.1	Dex0.2	Dex0.3	Dex0.4
Dextran content (%)	10	20	30	50
Peak split range ( $^{\circ}\text{C}$ )	220–420	220–420	220–420	220–420
Temperatures of the split peaks ( $^{\circ}\text{C}$ )	274 and 316	268 and 318	267 and 315	270 and 314
Integral area percentage of peak a (%)	1.76	3.64	4.64	8.40

water to emerge after the nonfreezing and freezing bound waters reached their final maximum contents in the dextran hydrogels. The amount of nonfreezing water in the dextran hydrogels increased with increasing dextran content. The maximum amount of nonfreezing water in the 50% dextran hydrogel was about 8%.

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