A Study on Correlation Between Water State and Swelling Kinetics of Chitosan-Based Hydrogels

YUN LIN GUAN,[1,*] LEI SHAO,[1] and KANG DE YAO[2]

[1]Department of Applied Chemistry, Tianjin University, Tianjin 300072, People's Republic of China, and [2]Department of Materials Science and Engineering, Tianjin University, Tianjin 300072, People's Republic of China

SYNOPSIS

Four types chitosan-based hydrogels have been prepared: cross-linked chitosan (cr-CS), cross-linked chitosan interpenetrating polyether polymer network (cr-CS/PE semi-IPN), cocross-linked chitosan and gelatin hybrid polymer network (CS/Gel HPN), and Chitosan/Pectin (CS/Ptn) ionic complex hydrogels. Water was used as a probe to elucidate heterogeneous structures of these hydrogels using differential scanning calorimetry (DSC) to study the physical state of water in the hydrogels swollen in distilled water. Results show that three states of water exist in all these hydrogels, while there are variations in the amount of nonfreezing bound water for different hydrogels. The correlation between water state and swelling kinetics data is discussed. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Recently studied stimuli responsive hydrogels include cross-linking polymers, copolymers of hydrophilic monomers, interpenetrating polymer networks (IPNs), ionic complex networks, etc. In the meantime, the use of natural polymers, e.g., proteins and polysaccharides, for the preparation of responsive hydrogels for biomedical applications have attracted investigators. Generally, hydrogels have heterogeneous structure and are thought to be composed of statistically distributed microchannels or fluctuating pores created by the mobility of the polymer segments within networks in the presence of swelling agent.[1] However, very few physical means are available that are applicable to the identification of the microstructure of hydrogels. As the most dominant feature of hydrogels over other polymers is inclusion of water within network, it is conceivable that a study on the physical state of water in the hydrogels might provide useful information on their microstructure and behavior.[2] So we can use water as a probe to investigate water–polymer interactions in multicomponent heterogeneous hydrogels and get insight into their correlation with swelling kinetics.

The state of water in hydrogels has been studied by numerous workers. According to their results, the water in hydrogels can be generally classified into three species: nonfreezing bound, freezing intermediate, and freezing free water. At low concentrations, the water molecules in polymers representing the first stage of water up-taking and strongly bound type of absorbed water are unassociated; e.g., the water in nylon 6 is in isolated form up to a concentration of one water molecule per two amide groups.[3] Above a characteristic level of water for each polymer, a number of water molecules (greater than two) are attracted to a polar site of the polymer and thus form a water cluster $(X \cdots (H_2O)_n$, where $n = 2$–6).[4] With increased uptake of water, the second stage of water absorption of nylon 6 is three molecules per two amide groups in the noncrystalline regions.[3] As the concentration of water further increases, the newly absorbed water forms bridges between previously separated clusters. Notably, the water–absorption characteristics of gels are one of the major factors that affects the diffusive behavior of small molecules through gels, interfacial energetic of gels, and bulk properties, etc. In addition, it is supposed that the sorption of water can decrease the glass transition temperature of polymers because of the plasticization of water.

---

* To whom correspondence should be addressed.

**Table I Details of Synthesis of Chitosan-Based Hydrogels**

Samples	0.25N Acetic Acid Solution (g)	Chitosan CS (g)	The Second Component (g)	Crosslinker GA × 10³ (g)	CS/Polymer (g/g)
cr-CS	32	1	—	2.69	1
cr-CS/Polyether Semi-IPN	32	1	Polyether N330 0.94	2.69	0.52
CS/Gelatin HPN	32	1	Gelatin 0.94	2.69	0.52

Considerable research activities on intelligent polymeric gels are focused on synthetic polymers; therefore, we have studied natural polymers, especially chitosan. Chitosan is a poly(aminosaccharide), normally obtained by alkaline deacetylation of chitin. Chitosan with a repeating structural unit of 2-amino-2-deoxy-β-D-glucan has been widely used in biomedical and pharmaceutical fields. In our previous studies, we have prepared several pH-sensitive chitosan-based hydrogels, such as crosslinked chitosan (cr-CS) with glutaraldehyde (GA), crosslinked chitosan interpenetrating polyether polymer network (cr-CS/PE semi-IPN),[5-8] cocrosslinked chitosan and gelatin hybrid polymer network (CS/ Gel HPN),[9] and chitosan-pectin ionic complex network (CS/Ptn complex).[10] For the purpose of getting the information about the correlation between water state of hydrogels with their swelling kinetics, in this paper, we used water as a probe to elucidate complex structures of these four types of hydrogels

Table II The Assignments of Main Bands in IR Spectra of Chitosan-based Hydrogels and Materials Used for Preparation

Samples	Band Position (cm⁻¹)	Assignment
Chitosan	1155, 902	Saccharid structure[5]
	1597 (strong)	Amino characteristic[5]
	1661 (weak)	Amide 1 band[12]
Polyether	1111	C — O stretching
Gelatin	1656	C = O stretching[9]
	1544	Amino characteristic[9]
Pectin	1736	νC = O (as) of — COOH group[10]
	1146, 957	Saccharid structure
cr-CS	1638 (strong)	C = N formed due to imine reaction between amino groups and alderhyde groups[13]
	1551 (weak)	Amino groups unreacted
cr-CS/PE Semi-IPN	1636 (strong)	C = N formed due to imine reaction between amino groups and alderhyde groups[6,7,13]
	1107	The sum of C — O stretching from polyether and hydroxyl groups from chitosan[5]
	1456, 1300	Characteristic peaks from polyether[7]
CS/Gel HPN	1647, 1541	C = N formed due to amino groups from chitosan and gelatin reaction with aldehyde groups from glutaraldehyde[9]
CS/Ptn Complex	1615 (strong)	The salt of amino groups and carboxyl groups[14]
	1734 (weak)	νC = O (as) of — COOH group unreacted
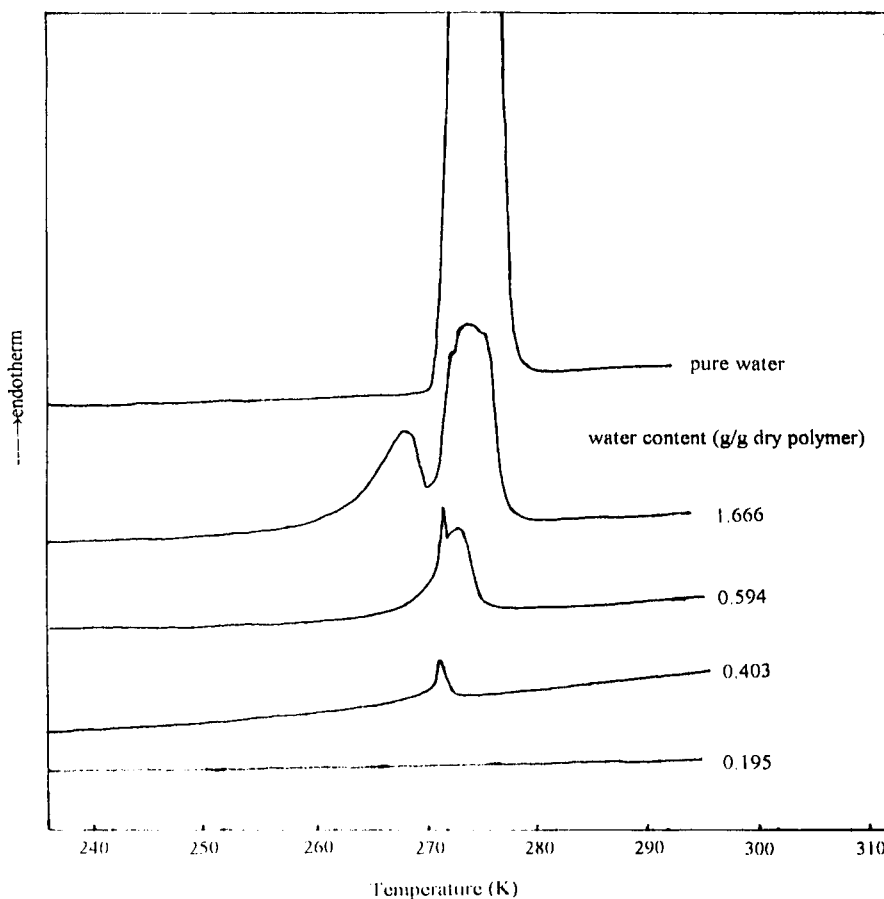
Figure 1 DSC melting thermograms of frozen waters in cr-CS hydrogel with different water contents swollen in distilled water.

through studying the state of water and discussed the interaction between it and the polymer in these inhomogeneous hydrogels.

EXPERIMENTAL

Materials

Chitosan was provided by Cheng Yu Co., Tianjin. Before use, it was purified by dissolution in acetic acid and separation with alkali. The viscosity-average molecular weight of the chitosan purified was 9.0×10^5, calculated by the Mark–Houwink equation:[11] $[\eta] = K_m M^\alpha$, where $K_m = 1.81 \times 10^{-3}$, $\alpha = 0.93$, and the N-deacetylation degree was 60%. Poly(oxypropylene glycol), polyether N330, was obtained from Tianjin No. 3 Petroleum Chemical Engineering Factory. It has three functional hydroxyl groups, and its average molecular weight was 3000 ± 100. Gelatin was a biochemical reagent manufactured by Tianjin No. 3 Chemical Reagent Factory.

Pectin with methoxy degree of 30% and average molecular weight 1×10^5 was provided from the Food Additive Co., Hebei Province, and used without further purification. Glutaraldehyde, acetic acid, and other reagents were chemical grade.

Synthesis of Chitosan-based Hydrogels

The cr-CS, cr-CS/PE semi-IPN, and CS/Gel HPN were prepared through similar methods.[5-9] The details of synthesis of these hydrogels were shown in Table I. Chitosan was dissolved in $0.25N$ acetic acid and was mixed with nothing, a certain amount of polyether N330, and gelatin for the three type samples mentioned above, respectively. Then the appropriate quantity of 0.5% glutaraldehyde solution was added with agitation. The mixture was poured into a frame mold and maintained at 45°C for film formation. The samples obtained were swollen in pH = 7.0 buffer solution of potassium dihydrogenphosphate and disodium hydrogenphosphate at
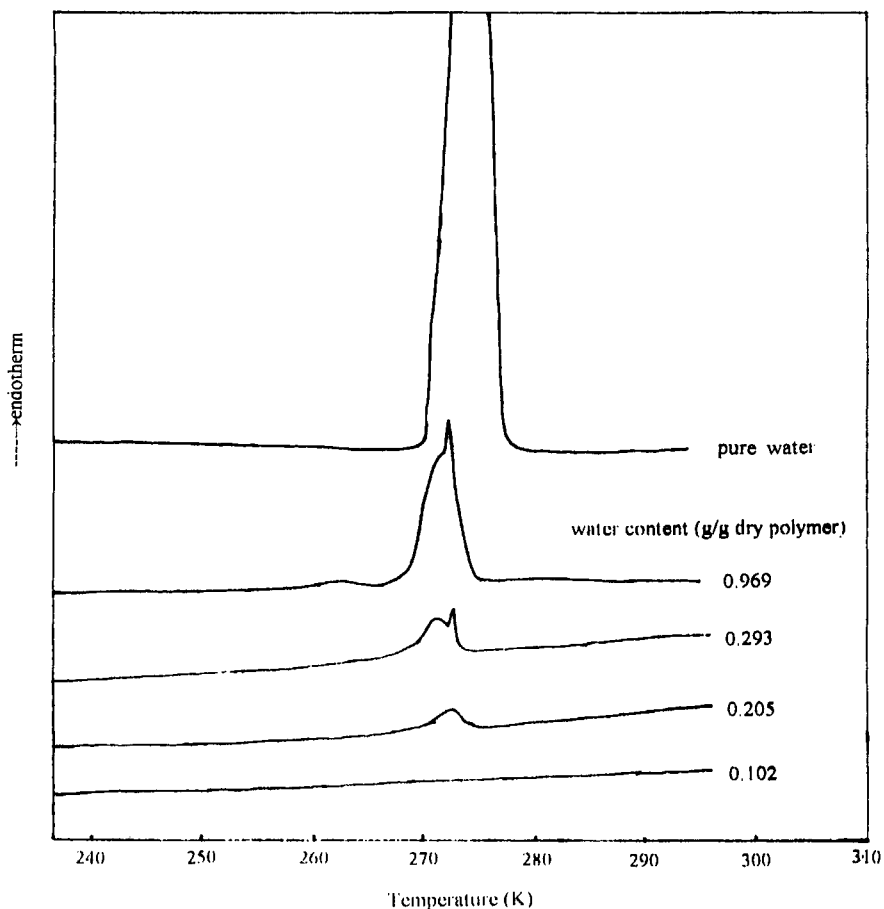
Figure 2 DSC melting thermograms of frozen waters in cr-CS/PE semi-IPN hydrogel with different water contents swollen in distilled water.

37°C for 4 h to reach equilibrium and then dried completely at 70°C.

For synthesizing CS/Ptn complex, 1 g chitosan was dissolved in 0.25N acetic acid and then diluted to the concentration of 0.1% by weight. 0.94 g pectin was dissolved in distilled water to the same concentration. Then, they were mixed under stirring to react for 1 h. The complex obtained was washed first with 0.25N acetic acid and then with distilled water until pH = 7. The complex was dried completely at 70°C.

Swelling Measurements

Swelling samples were cut into disks and swollen in distilled water at 25°C. The degree of swelling, W, is expressed as the amount of absorbed water per gram dry polymer during a definite time interval:

$$W = \frac{\text{weight of hydrogel} - \text{weight of xerogel}}{\text{weight of xerogel}}$$

$$\text{(g/g dry polymer)} \quad (1)$$

In the case of equilibrium swelling, W becomes the familiar equilibrium water content (EWC).

Infrared (IR) Spectra of Chitosan-based Hydrogels

The four types of chitosan-based hydrogels samples were first swollen in distilled water and were then ground to the suitable size of powder. After being dried completely in vacuum at 50°C, the powder could be used for IR analysis. Chitosan, polyether N330, gelatin, and pectin were ground when necessary and dried completely. IR spectra of all the samples were obtained with a Nicolet 5DX FT-IR spectrometer.
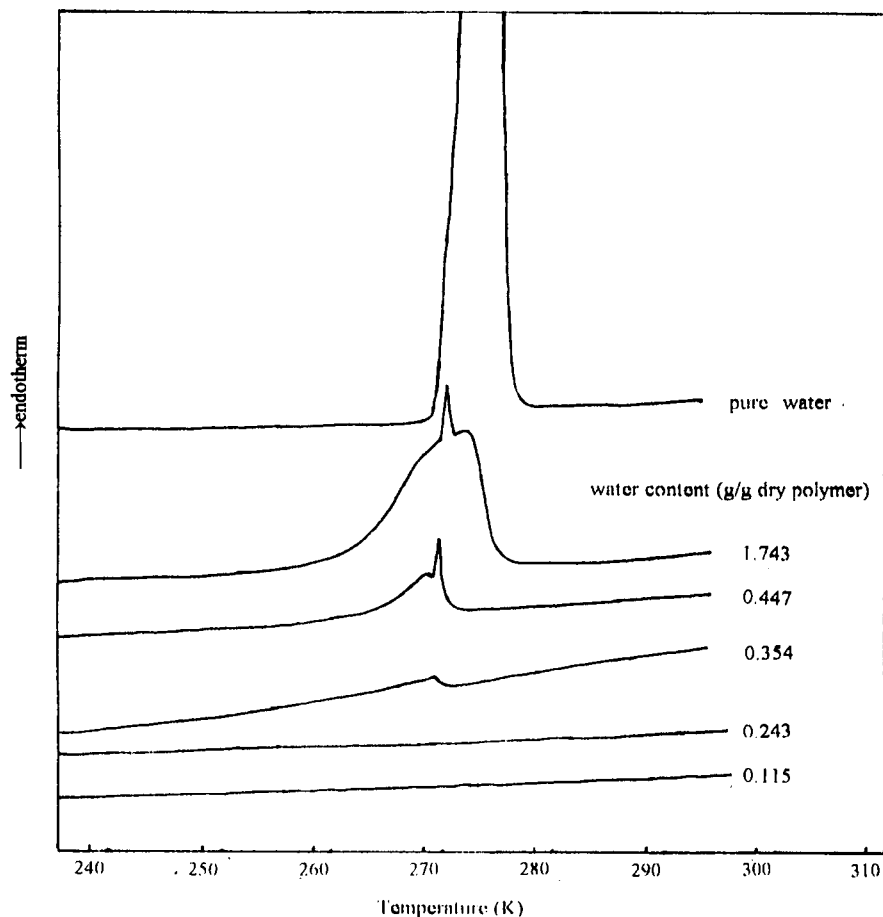
Figure 3 DSC melting thermograms of frozen waters in CS/Gel HPN hydrogel with different water contents swollen in distilled water.

Differential Scanning Calorimetry (DSC)

A Perkin-Elmer DSC-2C was used to measure the phase transition of water sorbed by the four types chitosan-based hydrogels. The xerogels of known dry weight were swollen in distilled water at 25°C, and the hydrogels with different swelling degree were surface-dried with a filter paper and transferred to aluminum pans. The pans were sealed to prevent water from evaporating and were weighed on a microbalance to calculate the total water content (W) of hydrogels. The weights of hydrogel samples were ca. 3–8 mg. Samples were cooled from room temperature down to 210K and then heated to 310K at a heating rate of 5K/min. The heat of fusion was evaluated from the thermogram area using pure water as a standard; therefore, the weight of freezing water in the hydrogel can be obtained. The percentage of nonfreezing water (W_{nf}) was estimated by subtracting the total fraction of freezing water (W_{tf}) from the total water content, W, in the hydrogel.

RESULT AND DISCUSSION

IR Analysis

The assignments of main bands in IR spectra of the four types chitosan-based hydrogels and materials used for preparation are listed in Table II. IR spectra have been investigated in our previous studies.[5–10]

DSC Analysis

The DSC thermograms for cr-CS, cr-CS/PE semi-IPN, CS/Gel HPN, and CS/Ptn complex hydrogels with different water content swollen in distilled water are shown in Figures 1–4, respectively. The absence of peaks below a certain water content in DSC curves indicated that this water is nonfreezing bound type, which has no detectable phase transition over the temperature range usually associated with the water freezing/melting transition from 200–300K.[15] It is supposed that in the initial swelling process, water molecules first disrupt the intermolecular hy-
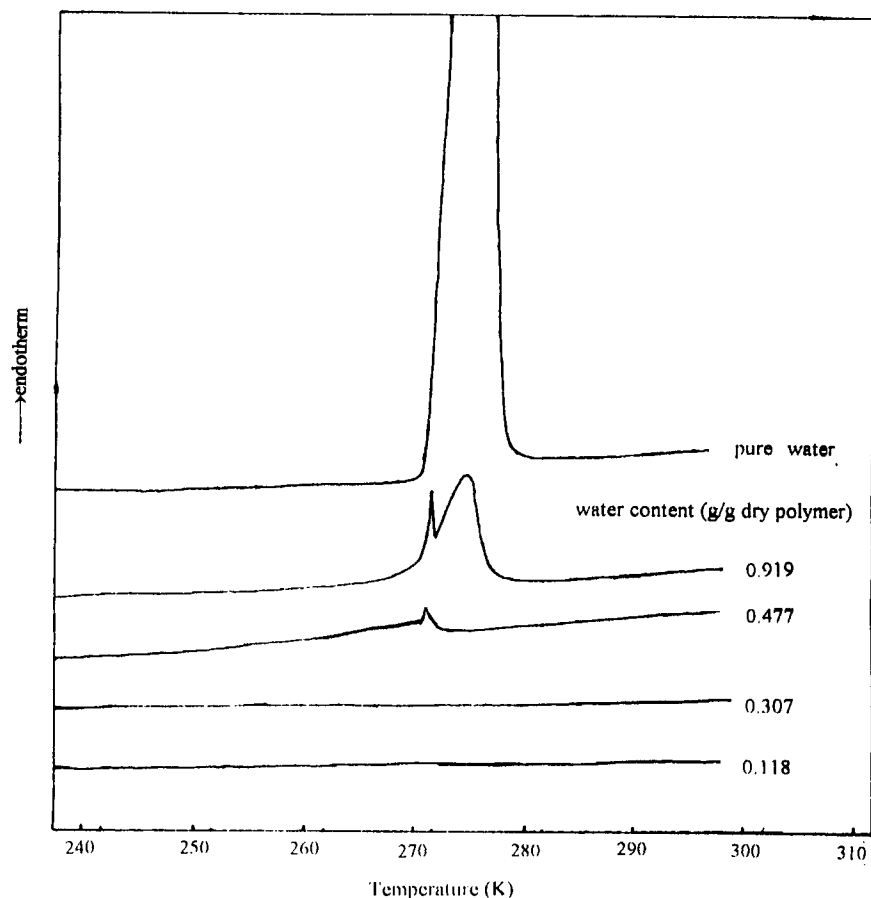
Figure 4   DSC melting thermograms of frozen waters in CS/Ptn complex hydrogel with different water contents swollen in distilled water.

drogen bonds that are not strong and then bond to the hydrophilic sites.[16] These water molecules are isolated and uniformly distributed throughout the polymer and exhibit greatly restricted mobility. Above a characteristic level of water for each polymer, there seems calorimetrically to be only one phase in the unbound water portion called freezing intermediate water. The additional water is preferentially oriented around the bound water and the polymer network structure as a secondary or tertiary hydration shell, which is in a form generally called clusters. These cage-like structures result from the tendency of water molecules to form the maximum amount of hydrogen bonds among them that space permits.[17] As the water content further increases, the splitting of the melting peak becomes more apparent in the DSC curves, suggesting the existence of two states of freezing water in the hydrogels. It is already known that the intermediate water in the freezing part exhibits lower melting temperature than the free water, as stated in the three-state water model.[18] And the free water portion becomes more

distinct thermodynamically as the hydrogels gradually approach the equilibrium state with bulk water.

The plots of the integrated change in enthalpy (per gram of dry polymer) of fusion of gels versus the nonequilibrated water content in the gels that did not reach equilibrium absorption with the water are shown in Figure 5. The content of nonfreezing bound water is the limiting value of the water content in a gel at zero enthalpy of fusion of the gel.[18] In Figure 5, the linear fit to the endothermic data of different samples yield different slopes and extrapolated values approximately representing the heats of fusion for the freezable water and the maximum amounts of nonfreezing bound water that polymers can support, respectively.[19,20] The corresponding results are shown in Table III. Ladbrooke and coworkers[21] proposed that the determination of different heats of fusion in this way ignored the possibility that the amount of nonfreezable water, coexisting with freezable water, might vary with total water content. Pouchly and coworkers[22] also remind us that the area under the endothermic peaks rep-
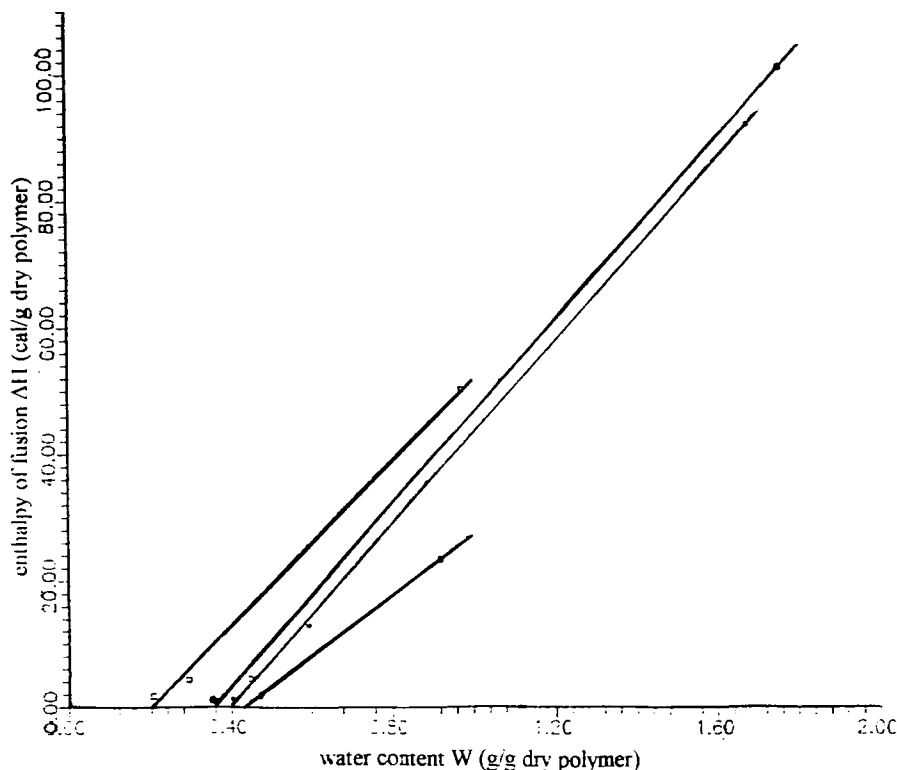
Figure 5 The DSC enthalpy of fusion of hydrogels versus the nonequilibrated water content in the gels: (*) cr-CS, $\Delta H = 72.74W - 29.14$; (□) cr-CS/PE semi-IPN, $\Delta H = 65.19W - 13.08$; (○) CS/Gel HPN, $\Delta H = 73.14W - 26.40$; and (●) CS/Ptn complex, $\Delta H = 48.14W - 20.98$.

resents the heat of melting, not of ice into pure water, but of the heat associated with the transformation of ice into water in the mixed phase. As can be seen from Table II, the heat of fusion for freezing water in these hydrogels are all lower than the heat of fusion for bulk water, $\Delta H_{fus} = 79.6$ cal/g; the lower heats of fusion imply the much more dispersed water phase,[19] which have much different properties from bulk water. This freezing includes the intermediate water, which has weak interactions with polymer chains, and bulk-like free water dispersed in micropores of hydrogels, which can be deduced from DSC curves in Figures 1–4. The result may give information of microstructures of hydrogels, i.e., the

cr-CS/PE semi-IPN and CS/Ptn complex, which have lower heats of fusion of freezable water, perhaps have more inhomogeneous structures than cr-CS and CS/Gel HPN and hence have more significant densification of structures, which leads to the lower equilibrated water absorption.[23]

The extrapolated values obtained from Figure 5 are also shown in Table III, which indicates the minimum total water contents at which the freezing water appears and below which the water in the hydrogels are all bound water. As mentioned above, these bound water molecules act initially to disrupt intermolecular hydrogen bonding and are attached to the hydrophilic sites in the network; thus, the

Table III   The Results of Linear Fit in Figure 5

| Samples	Linear-fit Equation	Slope ΔH_{fus} (cal/g)	Extrapolated Value W_{nf} (g/g Dry Polymer)
cr-CS	$\Delta H = 72.74W - 29.13$	72.74	0.401
cr-CS/Polyether Semi-IPN	$\Delta H = 65.19W - 13.08$	65.19	0.201
CS/Gelatin HPN	$\Delta H = 73.14W - 26.40$	73.14	0.361
CS/Pectin Complex	$\Delta H = 48.14W - 20.98$	48.14	0.436
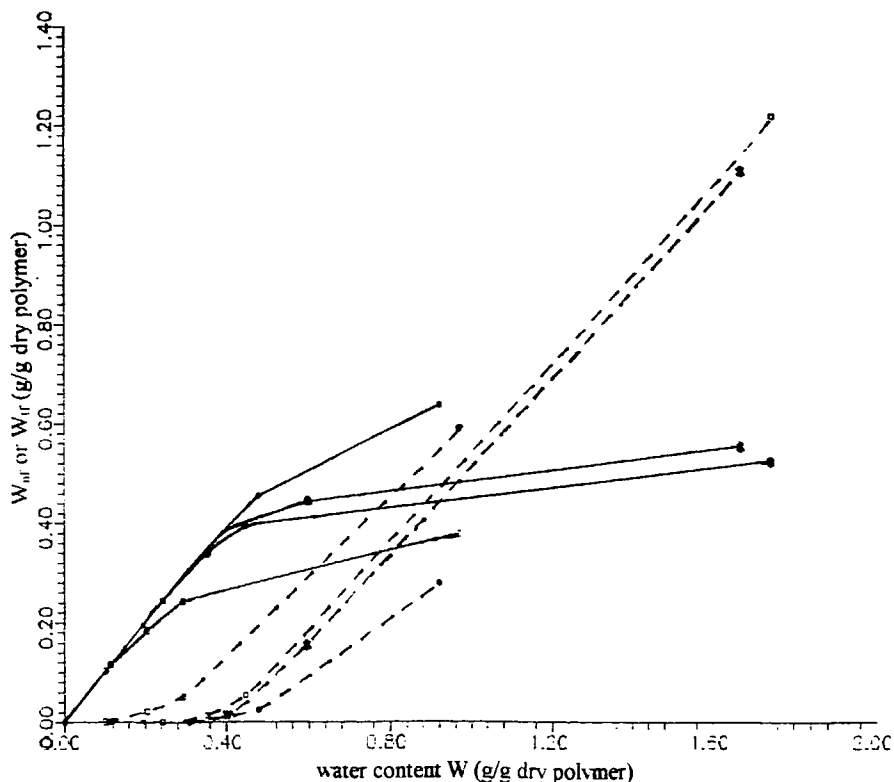
Figure 6 Calculated amounts of nonfreezing bound water W_{nf} (———) and total freezing water W_{tf} (– – –) as a function of water content W for four types of hydrogels: (∗) cr-CS; (○) cr-CS/PE semi-IPN; (□) CS/Gel HPN; and (●) CS/Pectin complex.

amount of these bound water is dependent on the hydrophilicity of polymer and the intensity of intermolecular hydrogen bonding within the network. W_{nf} in different hydrogels in Table III is listed as follows in the order of decreasing value: CS/Ptn complex > cr-CS > CS/Gel HPN > cr-CS/PE semi-IPN. The CS/Ptn complex was formed through the ionic bond of $—COO^-NH_3^+$ — between chitosan and pectin, but they cannot react completely. There must be some $—NH_2$ and $—COOH$ groups unreacted, and this polyampholyte containing a low charge content is known to associate intermolecularly through a clustering effect.[24] Because these two natural polyelectrolytes have high hydrophilicity and there are more hydrophilic groups, such as amino, carboxyl, hydroxyl, amide, ester groups, and ion clusters in the complex, the amount of bound water in the complex is the largest. In comparison to other three type chitosan-based hydrogels, the CS/PE semi-IPN has a hydrophobic component polyether N330. Amino groups of chitosan crosslinked form strong hydrogen-bonding with oxygen of polyether, so the hydrophilic sites which can interact with water molecules in per gram dry semi-IPN are fewer than that in two others, resulting in

the lowest value of W_{nf}. Chitosan and gelatin are both hydrophilic, but the value of W_{nf} for the cr-CS is higher than for the CS/Gel HPN, revealing that there may be more and stronger intermolecular hydrogen bonds in the HPN than that in the cross-linked chitosan network.

Figure 6 demonstrates calculated amounts of nonfreezing bound water (W_{nf}) and total freezing water (W_{tf}) as a function of water content (W) for the four type hydrogels. It is noticed that since the freezing water appeared in the gel during swelling, the contents of bound water have not kept constant. And as total water content (W) enhances, the content of bound water (W_{nf}) increases slowly and then reaches equilibrium, while that of freezing water (W_{tf}) rises gradually from zero at lower total water content and then fast at larger water content until it reaches the EWC. The plasticization of water for cr-CS, semi-IPN, and HPN have little differences because of variation in their hydrophilicity, intensities of hydrogen-bonding, and microstructures among them. But it is more obvious for the CS/Ptn complex, perhaps due to the interaction between water and polyelectrolyte network including ion-hydration in addition to hydrogen-bonding.
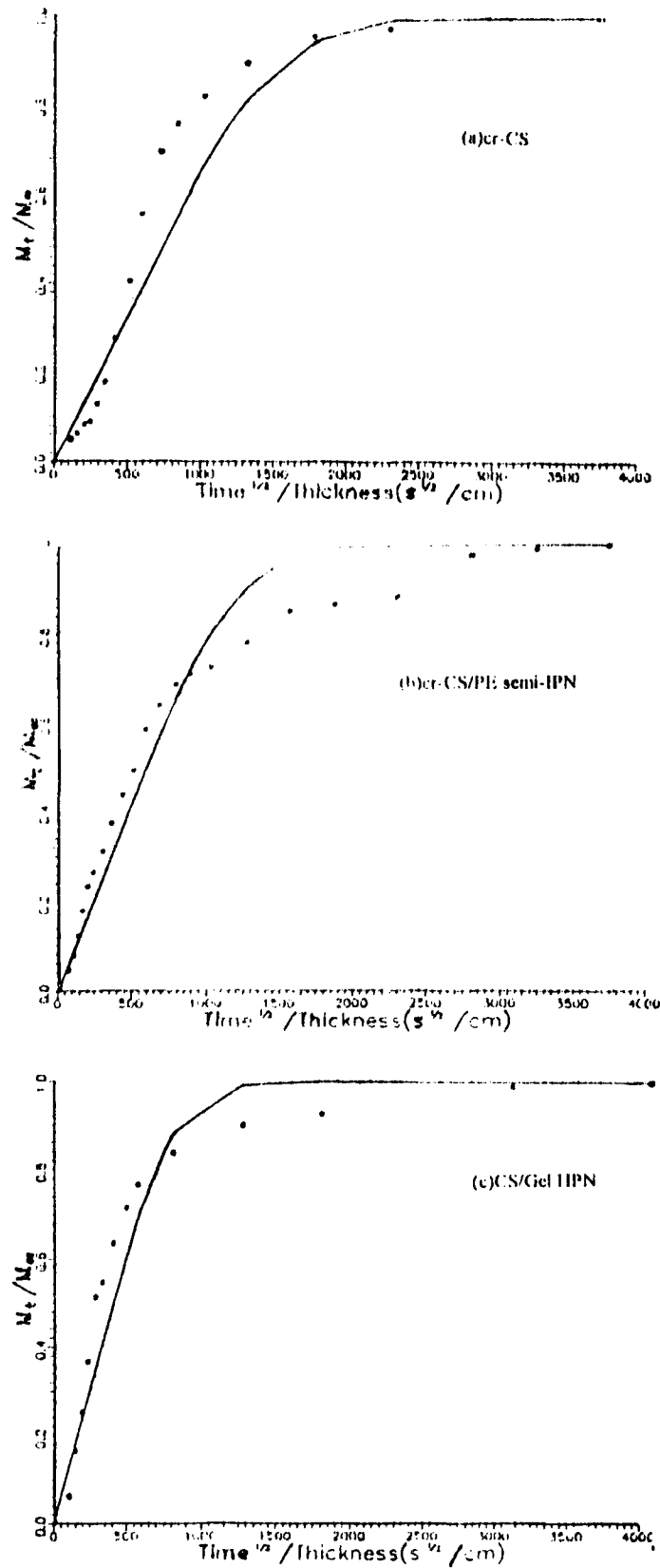
Figure 7   Swelling kinetics for cr-CS (a), cr-CS/PE semi-IPN (b), and CS/Gel HPN (c) hydrogels in distilled water at 25°C.

Swelling Kinetics

The weight changes of water sorption data are fitted to the Fickian equation,[25]

$$M_t/M_\infty = 1 - \sum [8/((2n + 1)^2\pi^2)]$$
$$\times \exp[-(2n + 1)^2\pi^2(D_p t/L^2)] \quad (2)$$

where M_t and M_∞ is the mass absorbed at time t and at equilibrium, D_p is the polymer-fixed reference diffusion coefficient, and L is the initial thickness of the sheet with aspect ratio greater than 10 : 1.

As shown in Figure 7, the swelling curves for cr-CS, cr-CS/PE semi-IPN, and CS/Gel HPN hydrogels are all sigmoidal when plotted against the square root of time and deviate with different extent from Fickian standard curves, indicating the swelling kinetics in distilled water for these three hydrogels are nonFickian. This could be due to an initially retarded swelling rate caused by the original low water content of the more compact network and the dominance of segments in the polymer network.

A best-fit diffusion coefficient can only provide a good basis for making the average swelling rate comparisons of different gels by ignoring deviations from eq. (2). The D_p (best-fit) values of cr-CS, cr-CS/PE semi-IPN, and CS/Gel HPN are 8.59×10^{-8}, 1.37×10^{-7}, and 3.00×10^{-7} cm$^2 \cdot$ s^{-1}, respectively, which indicate the magnitude of the average swelling rate. However, swelling curves changing from negative to positive deviation comparing with the corresponding Fickian standard curves need the longest time for cr-CS, the intermediate for CS/Gel HPN, and the shortest for cr-CS/PE semi-IPN. It is interesting to see from experimental data that at the time when the two above curves intersect, the amount of water that hydrogel contains is equal approximately to the amount of bound water shown in Table III; perhaps it is at this time the water-containing hydrogel transforms from the glassy state to the rubbery state. It is supposed that the more bound water that can be supported by a hydrogel, the longer time the needed for its swelling to change from negative to positive deviation from Fickian behavior. It suggests that the initial swelling process for a hydrogel is probably related to the amount of bound water that the polymer can support, which also affects other properties of the polymer, e.g., the glass transition temperature. These deductions deserve further investigation.

CONCLUSIONS

The enthalpy of the phase transition of the water in the four types of chitosan-based hydrogels confirmed that three states of water exist in these hydrogels, and the uptake of water into a polymer network was to be in the following order: nonfreezing bound, freezing intermediate, and freezing free water. The amount of water that is bound to the hydrophilic sites in the polymer through hydrogen-bonding and other interactions indicates the hydrophilicity of the polymer and intensity of intermolecular hydrogen bonding within the network. The heat of fusion of freezable water in hydrogels calculated and the characteristics of DSC curves indicate the heterogeneous microstructure of hydrogels. The CS/Ptn complex has the highest amount of bound water, while the cr-CS/PE semi-IPN has the lowest. And these two hydrogels have more inhomogeneous structure than others. The swelling kinetics behaviors for cr-CS, semi-IPN, and HPN hydrogels are nonFickian. The greater the amount of bound water that a hydrogel can support, the longer time probably needed for its swelling to change from negative to positive deviation from Fickian behavior.

REFERENCES

1. K. Hofer, E. Mayer, and G. P. Johari, *J. Phys. Chem.*, **94**, 2689 (1990).
2. W. I. Cha, S. H. Hyon, and Y. Ikada, *Makromol. Chem.*, **194**, 2433 (1993).
3. R. Puffr and J. Sebenda, *J. Polym. Sci., Part C*, **16**, 79 (1967).
4. H. Kusanagi and S. Yukawa, *Polymer*, **35**, 5637 (1994).
5. K. D. Yao, T. Peng, M. F. A. Goosen, J. M. Min, and Y. Y. He, *J. Appl. Polym. Sci.*, **48**, 343 (1993).
6. K. D. Yao, T. Peng, H. B. Feng, and Y. Y. He, *J. Polym. Sci., Polym. Chem.*, **32**, 1213 (1994).
7. T. Peng, K. D. Yao, C. Yuan, and M. F. A. Goosen, *J. Polym. Sci., Polym. Chem.*, **32**, 591 (1994).
8. K. D. Yao, T. Peng, M. X. Xu, C. Yuan, and M. F. A. Goosen, *Polym. Int.*, **34**, 213 (1994).
9. K. D. Yao, Y. J. Yin, M. X. Xu, and Y. F. Wang, *Polym. Int.*, **38**, 77 (1995).
10. K. D. Yao, J. Liu, G. X. Cheng, X. D. Lu, and H. L. Tu, *J. Appl. Polym. Sci.*, to appear.
11. G. A. F. Roberts and J. G. Domszy, *Int. J. Biol. Macromol.*, **4**, 374 (1982).

12. F. G. Pearson, R. H. Marchessault, and C. Y. Liang, *J. Polym. Sci.,* **43,** 101 (1960).

13. L. J. Bellamy, *The Infrared Spectra of Complex Molecules,* Chapman & Hall, London, New York, 1980, p. 52.

14. Japan Kokai Pat. 02-227464 (1990).

15. A. Higuchi, J. Komiyama, and T. Lijma, *Polym. Bull.,* **11,** 203 (1984).

16. K. Nakamura, T. Hatakeyama, and H. Hatakeyama, *Polymer,* **24,** 871 (1983).

17. E. G. Chatzi, H. Ishida, and J. L. Koening, *Appl. Spectrosc.,* **40,** 847 (1986).

18. H. B. Lee, M. S. Jhon, and J. D. Andrade, *J. Colloid Interface Sci.,* **51,** 255 (1975).

19. F. X. Quinn, E. Kampff, G. Smyth, and V. J. McBrievty, *Macromolecules,* **21,** 3191 (1988).

20. M. B. Ahmad, J. P. O'Mahony, M. B. Huglin, T. P. Davis, and A. G. Ricciardone, *J. Appl. Polym. Sci.,* **56,** 397 (1995).

21. B. D. Ladbrooke, T. J. Jenkinson, V. B. Kamat, and D. Chapman, *Biochim. Biophys. Acta,* **164,** 101 (1968).

22. J. Pouchly, J. Biros, and S. Benes, *Makromol. Chem.,* **180,** 745 (1979).

23. D. S. G. Hu and M. T. S. Lin, *Polymer,* **35,** 4416 (1994).

24. S. Wen and W. T. K. Sterenson, *Colloid. Polym. Sci.,* **271,** 38 (1993).

25. B. G. Kabra, M. K. Akhtar, and S. H. Gehrke, *Polymer,* **3,** 990 (1992).