The Nature of Water in Tactic Poly(2-Hydroxyethyl Methacrylate) Hydrogels

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(Received February 6, 1981)

The hypothesis that three classes of water exist in hydrogels, namely X water (free water-like), Z water (bound water-like), and Y water (interfacial water-like), has been verified and generally accepted. To further check the validity of this hypothesis and to study the nature of X, Y, and Z water as conformation changes, several experiments have been done using Tactic Poly(2-hydroxyethyl methacrylate) (P-HEMA) gels.

Thermal expansivity data for tactic P-HEMA gel was obtained. In each case of isotactic and syndiotactic P-HEMA, the higher water content gels showed an extremely sharp valume change at 0 °C, indicating the presence of normal free water-like. Lower water content gels showed no anomalous change in thermal expansion, indicating that the water is bound water-like. The medium water content gels exhibited intermediate behavior. These results were also confirmed by bulk gel conductivity measurments. The differential scanning calorimeter(DSC) experiment was simply introduced to further verify the bound water-like quantities which was obtained by the method of dilatometry and specific conductivity. Observing the amounts of X, Y, and, Z water with the change of tacticity, the similar content of bound water-like may be due to the same primary structure of isotactic and syndiotactic polymer and the difference in free and interfacial water-like content may be due to the difference in secondary and tertiary structure of tactic polymer. Therefore, as the polymer conformation varies, the free and interfacial water-like content will be varied. In order to demonstrate these concepts, Russel *et al.*'s CPK® space-filling molecular models of isotacitic and syndiotactic P-HEMA was utilized.

Introduction

Water is the most common liquid on earth and is the very medium of life. The nature of water has long been received great interest and studied both in a pure state and in many other states.

Questions on water structure, water of hydration and similar problems are of biological significance *e.g.* for the structure of water in living cells, for the role of hydrated water around protein molecules in life processes etc., and water on such problems has been studied for many years.[1-9]

As the search continues for materials which will be stable and compatible with body fluids over long periods of contact, hydrogels stand out one of the most promizing classes of materials. These materials have been extensively discussed in the literature.[10,11] Most of the physical, physiological, and interfacial properties of such gels appear to be dependent on the organization of water within and on the surface of the hydrogels.[12] So the study on the nature of water in synthetic hydrogels is very important both in pure scientific interest and in practical aspect in order to develop the more biocomatible materials.

There is substantial evidence that a fraction of water in hydrogels may be significantly different from normal or bulk water. So it has been hypothesized that hydrogels may contain three classes of water[8,13,14]; X water(free water-like), Z water(bound water-like), and Y water(interfacial water-like).

Lee *et al.*[15] have checked the validity of the hypothesis from the various experimental works such as dilatometry, specific conductivity, and differential scanning calorimetry.

Recently many streoregular polymer has been made. Because the most molecules in living cells are stereoregular, it is important to study the nature of water in synthetic streoregular molecules. Gregonis *et al.*[16,17] has made the streoregular poly(2-hydroxyethyl methacrylate) (P-HEMA) polymers and hydrogels. This streoregular P-HEMA has also been made in our laboratoly to study the nature of water in it by the method of dilatometry, specific conductivity, and differential scanning calorimetry.

The object of this paper is to study the nature of water with the change of polymer conformation and to give more concrete concepts on the role of X, Y, and Z water. To do this, dilatometry and specific conductivity measurements has been carried out from −15 °C to room temperature for tactic P-HEMA and differential scanning calorimetry has been carried out from −40 to 10 °C.

Materials and Method

Commercial 2-hydroxyethyl methacrylate (HEMA) was obtained (Polysciences, Inc., Warrington). The impurity content is 0.2 % ethylene glycol dimethacrylate, 0.1 % methacrylic acid, and 0.075 % methyl hydroquinone ether. Highly syndiotactic P-HEMA is synthesized by 2537 A u.v. photo-polymerization at −40 °C.[16] This syndiotactic P-HEMA has more than 80 % tacticity which is taken from ^{13}C n.m.r. spectra. The production of isotactic P-HEMA[16] requires the use of a suitable blocking group on the free hydroxyl of HEMA. Substituting the benzoxyl group for hydroxyl of HEMA, benzoxyethyl methacrylate is produced. The anionic initiator for benzoxyethyl methacrylate polymerization is *n*-butyllithium and copper iodide. This reagents probably exist as a $Li^+(R_2)Cu^-$ complex. Using this reagent as initiator, isotactic poly(benzoxyethyl methacrylate) is produced. This isotactic poly(benzoxyethyl

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TABLE 1: Equilibrium Water Contents of Tactic P-HEMA at 150 °C

Isotactic P-HEMA	38 %
Syndiotactic P-HEMA	31 %

methacrylate) has more than 80 % tacticity which is taken from ^{13}C n.m.r. spectra. Selective hydrolysis of the benzoate ester occurs using aqueous KOH. Therefore isotactic P-HEMA is produced.

These P-HEMA are crosslinked with hexamethylene diisocyanate. The equilibrium water fraction is determined by placing the gels into distilled water for sufficient time in a water bath. The gels are dried at 60 °C for 24 hours in vaccum for constant weight.

$$\text{Water fraction} = \frac{\text{Wt. hydrated gel} - \text{Wt. dry gel}}{\text{Wt. hydrated gel}}$$

The equilibrium water contents of tactic P-HEMA is presented in Table 1.

To measure the thermal expansion and specific conductance, the dilatometer and conductance cell is immersed in a constant temperature bath (Lauda/Brinkmann Circulator Model Super K-2/R) to equilibrate to the desired temperature. Equilibrium temperature are obtained after equilibrating the gels for sufficient time for each measurement. To obtain the heat of fusion of water in the gel, the Differential Scanning Calorimetry(DSC) cell is placed under liquid nitrogen system. The measurement is started with after equilibrating the gels for sufficient time.

Dilatometric Measurements. The dilatometer[18] is filled with the sample and mercury. Every sample is measured for its volume change with a decrease and with an increase in the temperature from room temperature to −15 °C. The total volume change, $\Delta\Phi_{tot}$, is given by the following equation[19]:

$$\Delta\Phi_{tot} = \Delta\Phi_{gel} + \Delta\Phi_{Hg}$$
$$\Delta\Phi_{gel} = \Delta\Phi_{tot} - \Delta\Phi_{Hg}$$
$$= \frac{1}{M_s}\{S(h-h^0) - Q_{Hg}V^0_{Hg}(t-t)\} \quad (1)$$

where $\Delta\Phi_{Hg}$ is the specific volume change of the mercury and $\Delta\Phi_{gel}$ is the specific volume change of the gel; h and h^0 are the height of the mercury column at the temperature t °C and at the reference temperature, respectively; Q_{Hg} is the thermal expansion coefficient of mercury[20]; V^0_{Hg} is the volume of the mercury in the dilatometer at the reference temperature; t^0 is the reference temperature; M_s is the mass of the sample and S is the capillary cross sectional area (cm^2).

Specific Conductivity. Specific conductivity is measured with a conductivity bridge (Beckman Inst. Inc.), using a 1 KHz AC signal. The use of alternating current avoids polarization of the electrode in the conduction cell.[21] Platinum disc (99.98 %) is used for electrodes. Platinum electrodes are the best for alternating current work.[22] They permit the application of a fine porous coat of platinum black. The gel surface is painted with silver conductive paint (GC electronics, Rockford, Ill.) to eliminate contact resistance. Every sample is measured with a decrease in the temperature from room temperature to −15 °C.

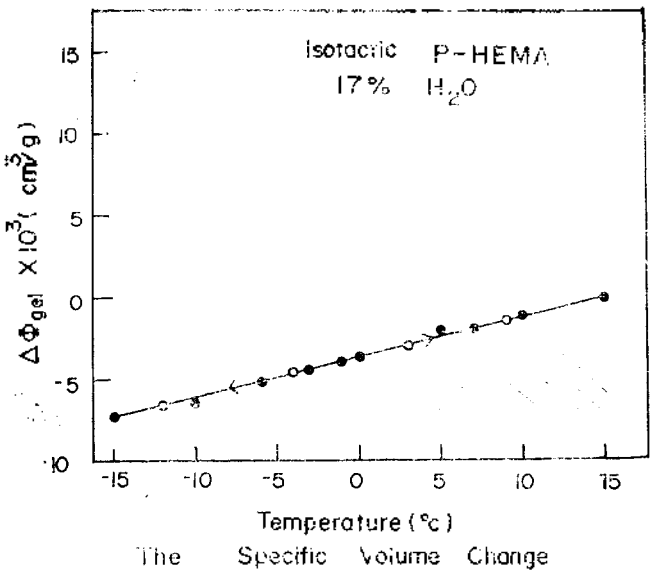
Differential Scanning Calorimetry. The differential scanning calorimeter(DSC) is a Perkin-Elmer DSC-1B. The instrument is calibrated by the melting transition of pure ammonium nitrate (125.2 and 169.6 °C) and triply distilled water (0 °C). The samples are sealed in an aluminum pan and weighed before and after the DSC runs. After cooling with liquid nitrogen and allowing to stand at −40 °C for sufficient time to stabilize, the temperature of the samples is raised at a programmed rate of 5 °C/min under pure nitrogen. Specific chart speed and DSC sensitivity range are 10 mm/min and 16, respectively. The heat of fusion of water in the gel is measured from the area of the transition peak. The relationship is

$$\Delta H_s = \Delta H_{ref}\frac{W_{ref}A_sR_sS_{ref}}{W_sA_{ref}R_{ref}S_s} \quad (2)$$

where the subscript s is refers to sample and ref refers to reference. ΔH, W, A, R, and S are the heat of transition, weight, area, range, and chart speed, respectively.

Results and Discussion

Dilatometric Measurements. The specific volume change of the gel, $\Delta\Phi_{gel}$ is obtained from the dilatometry measurements by equation 1. The results are presented in Figures 1 to 5 for isotactic P-HEMA and in Figures 6 to 8 for syndiotactic P-HEMA. The specific volume for each gel decreases upon cooling. First of all observing the isotactic P-HEMA data, one sees that the gels containing 24 % or more water (Figures 3 to 5) show a hysteresis in thermal expansion over the temperature range of −15 °C to room temperature. One sees that the 21 % and 17 % water gels show no transition (Figure 1 and 2). The 24 % water gel shows a slight hysteresis, the heating path discontinuously joining the cooling path in the vicinity of 0 °C(Figure 3). The hysteresis increases as the water content of the hydrogel increases (Figures 4 and 5). The hysteresis is most pronounced



The Specific Volume Change

Figure 1. The specific volume change *vs.* temperature for a 17 % water isotactic P-HEMA gel.

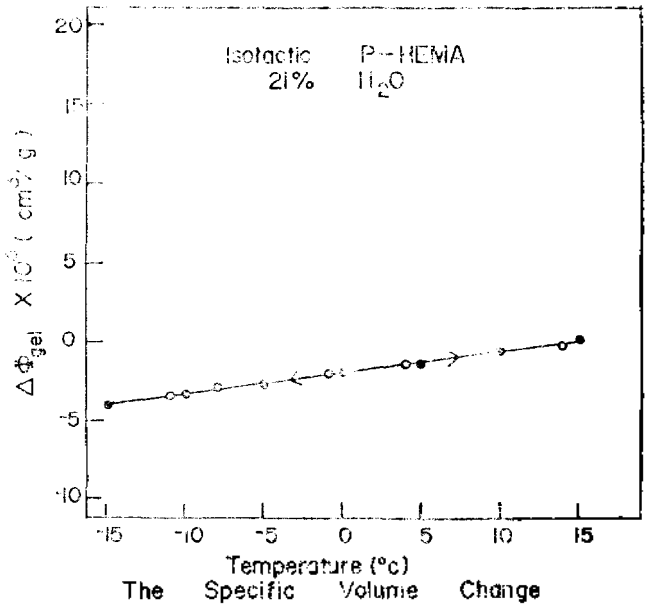


Figure 2. The specific volume change *vs.* temperature for a 21 % water isotactic P-HEMA gel.

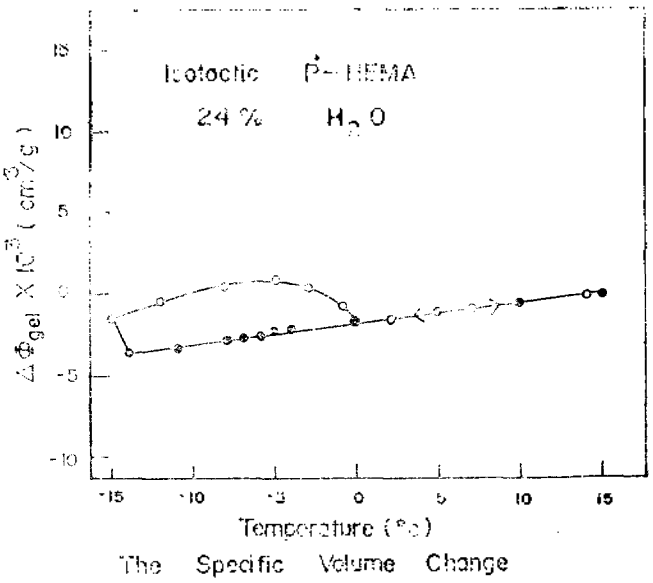


Figure 3. The specific volume change *vs.* temperature for a 24 % water isotactic P-HEMA gel.

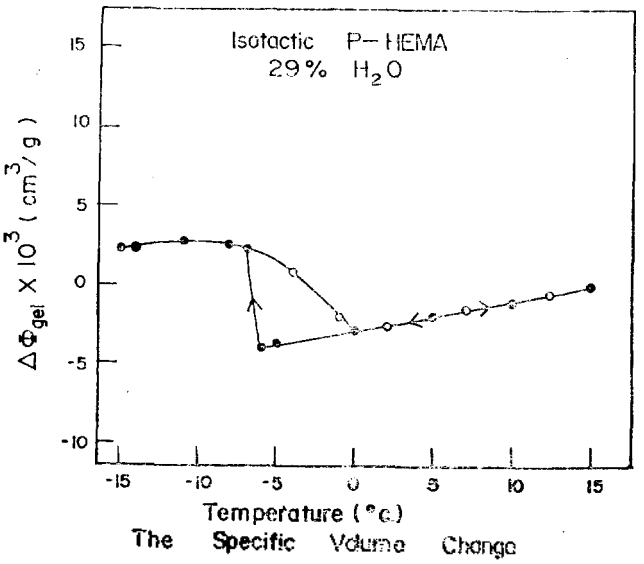


Figure 4. The specific volume change *vs.* temperature for a 29 % water isotactic P-HEMA gel.

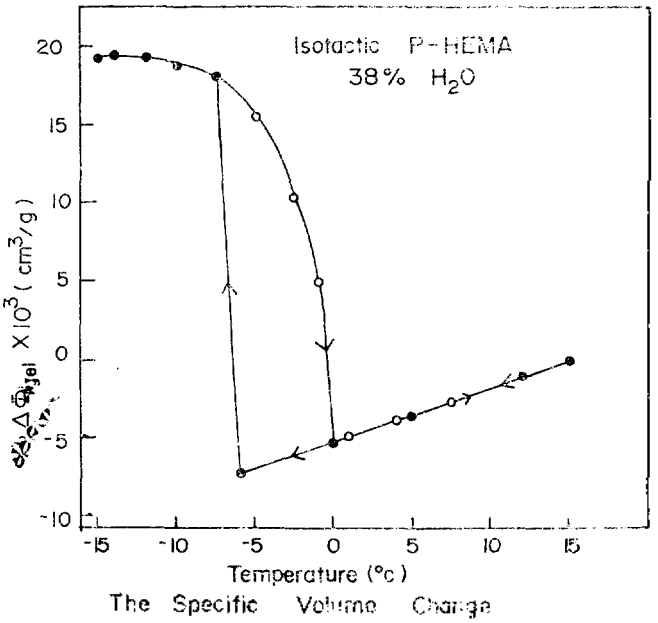


Figure 5. The Specific volume change *vs.* temperature for a 38 % water isotactic P-HEMA gel.

in the 38 % water content gel which shows extremely sharp specific volume change near 0 °C. subsequently observing the syndiotactic P-HEMA data, the 19 % water content gel shows no transition(Figure 6). The 21 % water content gel shows a slight hysteresis which is different from the isotactic P-HEMA result (Figure 7). Most pronounced hysteresis in syndiotactic P-HEMA is found at the 31 % water content. Since no anomalous change is detected for gels with lower water contents, the water in such gels is considered to show no transition in this temperature range. Most of the water in such lower water content gels is supposed to be Z water(bound water-like) because of Z water may have no transition over the range from −15 to 0° C. Judging from the extremly sharp change in the specific volume for higher water contents of the gel, the transition temperature of X water(free water-like) may be 0 °C, for X water is presumed to be the main component of the gel in higher water contents. The gradual decrease in the specific volume with increase in the temperature over the range from −15 to 0 °C is considered to indicate that the transition temperatures, perhaps for Y water(interfacial water-like), should be distributed over this temperature range.

From these results, we conclude that: (1) the transition temperature of X water may be 0 °C, (2) that of Y water may be distributed over the range from −15 to 0 °C, and (3) Z water may have no transition temperature in the range from −15 to 0 °C.

When one compares the degree of hysteresis of the isotactic and syndiotactic P-HEMA gels at the similar water content,

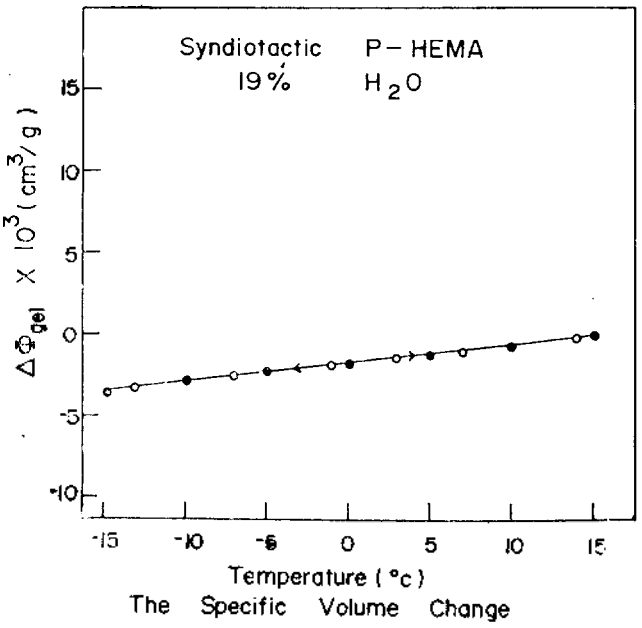


Figure 6. The specific volume change *vs.* temperature for a 19 % water syndiotactic P–HEMA gel.

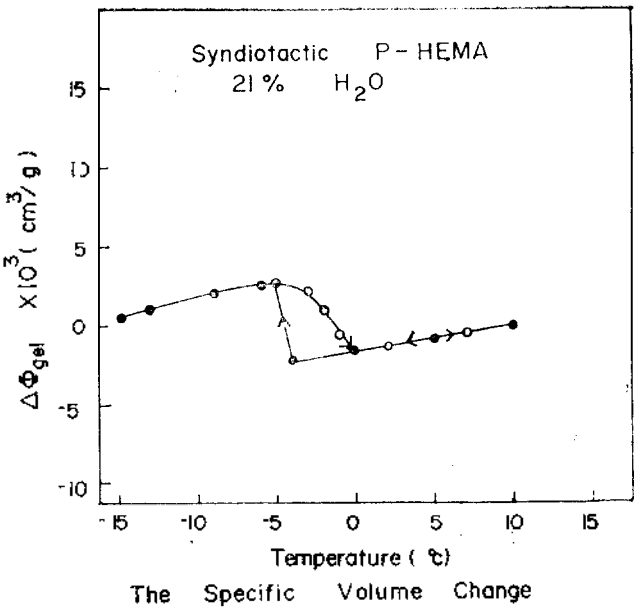


Figure 7. The specific volume change *vs.* temperature for a 21 % water syndiotactic P–HEMA gel.

one sees that syndiotactic P–HEMA shows greater hysteresis phenomena than isotactic P–HEMA. From this fact one may think that the syndiotactic P–HEMA gel has the higher percentage of X water than the isotactic P–HEMA gel while the isotactic P–HEMA gel has the higher quantity of Y water than the syndiotactic P–HEMA gel. This thought is verified by the following work. The assumption that the transition near 0 °C is mainly due to free water-like(X water), and the 19 % water gel in isotactic P–HEMA and the 21 % water gel in syndiotactic P–HEMA contain only bound water-like (Z water) lead us to determine the amount of X, Y, and Z water from equations 3 and 4:

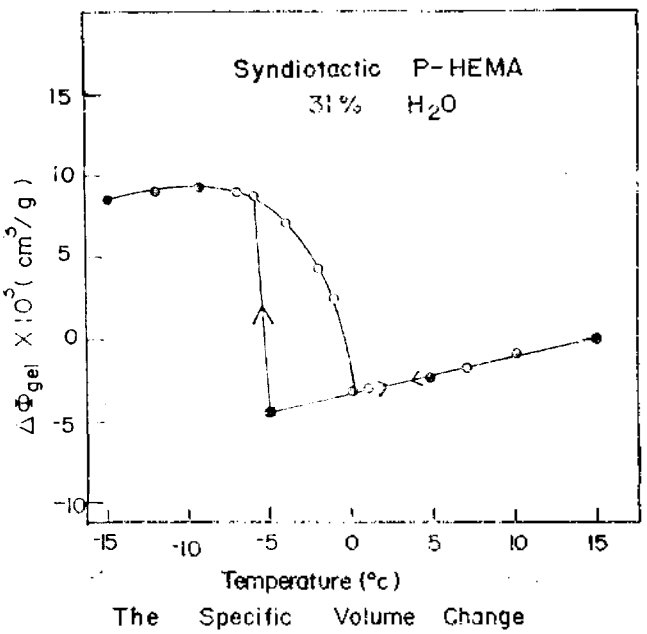


Figure 8. The specific volume change *vs.* temperature for a 31 % water syndiotactic P–HEMA gel.

TABLE 2: Approximate Contribution of "X, Y, and Z" Water to the Total Water Content (W) (All Values are Wt. %)

a. Isotactic p(HEMA)

W	17	21	24	29	38
X	0	0	0	0.8	7
Y	0	0	3	7.2	10
Z	17	21	21	21	21

b. Syndiotactic P(HEMA)

W	19	21	31
X	0	0.7	6.4
Y	0	1.3	5.6
Z	19	19	19

$$X = \frac{\Delta\Phi_{gel}}{\Delta\Phi_{w}} \times 100 \quad (3)$$

$$Y = W - X - Z \quad (4)$$

where X is the percentage of free water-like in the gel; $\Delta\Phi_w$ is the volume change of ice to water at 0 °C, 91.1×10^{-3}cm^3/g [23]; Y is the percentage of interfacial water-like in the gel; W is the total percentage of water in the gel; and Z is the percentage of bound water-like in the gel. The amount of free water-like in the gel is obtained using equation (3) with $\Delta\Phi_{gel}$ values taken from Figures 3, 4, 5, 6 and 8, and $\Delta\Phi_w$ value from reference 23. Also the amount of interfacial water-like is obtained using equation (4). The results are shown in Table 2. As shown in Table 2, the amount of X water in isotactic P–HEMA gel is higher than that in syndiotactic P–HEMA gel. This fact is probably due to the change of polymer conformations.

Specific Conductivity. Plots of specific conductivity versus temperature for isotactic P–HEMA gels are shown in Figure 9 and for syndiotactic P–HEMA gels are shown in Figure 10. Log K is linearly proportional to $1/T$ for temperatures higher than the transition temperature. A

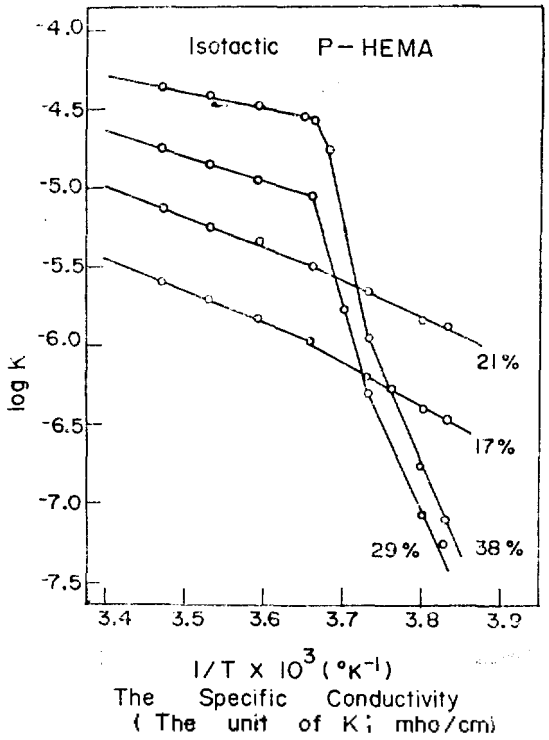


Figure 9. Specific conductivity *vs.* reciprocal temperature for isotactic P-HEMA gels.

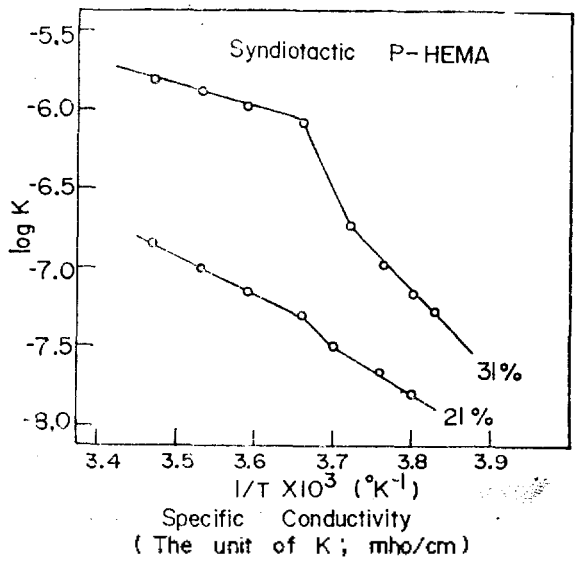


Figure 10. Specific conductivity *vs.* reciprocal temperature for syndiotactic P-HEMA gels.

sharp discontinuous change in the log K *vs.* $1/T$ curve near 0 °C indicates that the high water content gels have significant amounts of free water-like. As the water content becomes lower the change at the transition point becomes smaller, and finally 21 and 17% water content in the isotactic P-HEMA gel does not show sharp drop of specific conductivity at 0 °C (Figure 9). This is in good agreement with dilatometric results. On the other hand, in the case of syndiotactic P-HEMA gel the 21% water content gel shows slight discontinuity at 0 °C (Figure 10). This fact shows that the 21% water content gel in syndiotactic P-HEMA has slight amounts of X water and Y water. This is also in good agreement with dilatometric results. Because the gels under 21 % water content are hard and brittle we can't put these

TABLE 3: Activation Energy for Conductivity

a. Isotactic P(HEMA)

Water Content	ΔE_a (kcal/mol)
38%	4.6
29%	7.2
21%	8.6
17%	9.8

b. Syndiotactic P(HEMA)

Water content	ΔE_a (kcal/mol)
31%	6.4
21%	11.4

material between two platinum electrodes. Therefore we have only water content data between 21 and 31%.

According to absolute reaction rate theory, the specific conductivity, K, can be written as follows (24):

$$K = \text{const}\frac{Z(Ne)^2}{Nh}\exp\left(-\frac{E_a}{RT}\right) \quad (5)$$

where Z, N, e, R,ΔE_a, and h are ionic valence, Avogadro's numver, electronic charge, gas constant, activation energy for conduction, and Planck's constant, respectively. Equation (5) can be written as:

$$\log K = \text{const} - \frac{\Delta E_a}{2.303\,RT} \quad (6)$$

On the basis of equation (6), one obtains the apparent activation energy for conduction for each different gel from the slope of the straight line in Figures 9 and 10. This is shown in Table 3. When one compares the values in Table 3 with the other experimental values[15,25] at the similar water content our experimental value is higher than that. It is due to the specific character in our tactic P-HEMA. Our tactic P-HEMA is different in molecular weight and molecular conformation from nonstereoregular P-HEMA. In particular, because the molecular weight of syndiotactic P-HEMA is higher than that of isotactic P-HEMA, syndiotactic P-HEMA has higher activation energy value than isotactic P-HEMA. Nevertheless our activation energy value is much higher than any other experimental value[15,25]. This is probably because the water in stereoregular molecule is different in forms from the water in nonstereoregular molecule; *i.e.*, the water in stereoregular molecule is bound thightly in regular forms. It is hard to activate the tightly bounded water for conduction. So the higher activation energy value in our tactic P-HEMA is observed.

Differential Scanning Calorimetry (DSC). The integral heat of fusion of the water transition in gels are measured from the total peak of the DSC endotherms (equation 2). Here the DSC experiment is simply to prove the above-mentioned experimental results, *i.e.*, the amount of bound water-like. The lower water content (21 % H_2O) isotactic P-HEMA sample does not show any transition near 0 °C, but 21 % water content syndiotactic P-HEMA sample shows slight transition near 0 °C. Higher water content sample shows large transition and double peaks near 0 °C. At the similar water content the isotactic P-HEMA sample has

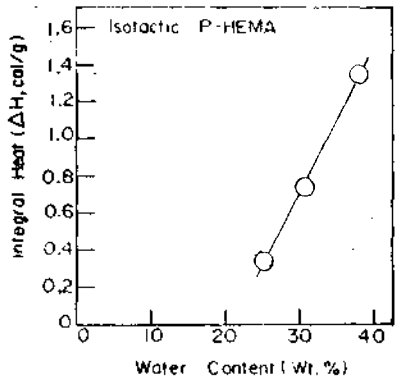


Figure 11. The integral heat of fusion of the water transition as a function of water content for isotactic P-HEMA gels.

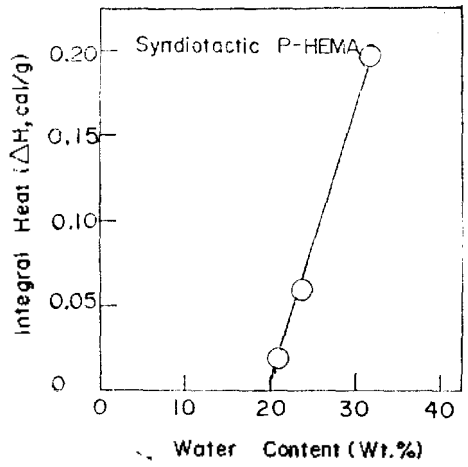


Figure 12. The integral heat of fusion of the water transition as a function of water content for syndiotactic P-HEMA gels.

higher and larger transition peak than the syndiotactic P-HEMA sample. This is probably due to the difference in molecular weight and molecular conformation for each polymer sample.

Figures 11 and 12 show the integral heats of fusion of the total water transition as a function of water content for each polymer sample. Extrapolation of the line $\Delta H=0$ intercepts the water content axis; this point is interpreted as the total bound water-like content of the sample. In Figures 11 and 12 the bound water content for the isotactic and syndiotactic P-HEMA gel is probably in good agreement with our other studies, *i.e.*, dilatometry and specific conductivity.

Conformational Differences in Stereoregular P-HEMA. In order to better understand the molecular structures, we have referred to the Russel *et al.*'s CPK® space-filling molecular models.[26] From these models, the isotactic and syndiotactic P-HEMA molecule have the following difference. The polar groups in the isotactic chain are all displaced outward from the helical backbone, giving rise to a helix which has a hydrophobic inner surface and hydrophilic outer surface. This is not the case for syndiotactic P-HEMA where polar and apolar groups are interspersed along the helix. This may account, in part, for the differences observed in the swelling behavior of isotoctic and syndiotactic P-HEMA (Table 1).

Conclusions

In this paper, we have dealt with the nature of water in tactic P-HEMA hydrogels on the basis of experimental results of dilatometry, conductivity, and differential scanning calorimetry measurements. Our data of dilatometry, conductivity, and differential scanning calorimetry are in good agreement with each other.

From the thermal volume expansion curve obtained by dilatometry, the specific conductivity curve obtained by conductivity measurements, and the integral heats curve obtained by DSC, we have firmly verified the hypothesis that three kinds of water, namely X water(free water-like), Y water(interfacial water-like), and Z water (bound water-like), may also exist in stereoregular polymer gels.

Major purpose of the present study is to see the nature of water with the conformational changes. From the Table 2, the bound water-like content in isotactic and syndiotactic P-HEMA gel is 21 % and 19 %, respectively. This difference is very slight as compared with the difference of Y and X water in isotactic and syndiotactic P-HEMA gel. This slight difference may be due to the same primary structure in isotactic and syndiotactic P-HEMA gel and the difference in Y and X water is probably due to the secondary and tertiary structure difference in isotactic and syndiotactic P-HEMA gel.

Varying the macromolecular conformation, the different amounts of interfacial and free water-like in macromolecules are observed mainly.

Acknowledgement. One of the authors (M. S. Jhon) expresses appreciation for financial support to Korea Science and Engineering Foundation and Korea Research Center for Theoretical Physics and Chemistry.

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Theoretical Study of the Effects of Cation on *t*RNA

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The effects of cation on *t*RNA have been theoretically investigated using the semiempirical potential enregy functions. The binding of Mg^{2+} to the model compound and the hydration scheme of the anticodon loop have been determined, and their stabilization energies produced by the introduction of magnesium pentahydrate and water molecules in the first hydration shell were calculated. The results indicate that magnesium pentahydrate is important for decreasing the flexibility of the anticodon loop and satisfying the large Y37 stereochemically during the protein synthesis. The effects of Mg^{2+} on the hydration scheme were also investigated.

Introduction

Cation is essential for many biological reactions. Especially, Mg^{2+} is important for the protein synthesis. Therefore much efforts, both theoretical and experimental, have gone into the study of cation binding to nucleic acids and components[1-5]

Recently, three dimensional structure of yeast phenylalanine *t*RNA(*t*RNA^{phe}) have been determined by X-ray crystallographic studies[6-8]. The ordered yeast *t*RNA^{phe} lattice has made possible the elucidation of the structure of this molecule in both orthorhombic and monoclinic crystal forms. These studies indicate the existence of at least four site-specifically bound hydrated Mg^{2+} ions in orthorhombic crystal of yeast *t*RNA^{phe}.

And very recently, Kim and Jhon[9] have calculated the hydration scheme of *t*RNA and stabilization energy due to the presence of water molecules in orthorhombic crystal of yeast *t*RNA^{phe} without considering Mg^{2+}.

Therefore it is important and more realistic that since Mg^{2+} is present in the anticodon loop, we consider the effects of Mg^{2+} on the stabilization and hydration of the anticodon loop.

In this study, using the semiempirical potential energy functions, it is found that Mg^{2+} in the anticodon loop is important for the conformation of P37, and the stabilization energy of Mg^{2+} is greater than that of the water molecules in the hydration shell.

The Method

The methods for the optimization of the Mg^{2+} cation binding, its hydration scheme, and the stabilization energy due to the hydration follow the general formula presented by Perahia *et al*[10]. The interaction energies have been obtained by using the potential energy functions by Caillet and Claverie[11,12] that are composed of three long-range contributions (electrostatic, polarization and dispersion) and a short range repulsive term. The nature of energy functions and their parameters are discussed elsewhere[9-12].