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Properties of Water in Macromoleular Gels. III. Dilatometric Studies of the Properties of Water in Macromolecular Gels

Masuo AIZAWA and Shuichi SUZUKI
Research Laboratory of Resources Utilization, Tokyo Institute of Technology, Meguro-ku, Tokyo
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The dilatometric properties of W_1, W_2, and W_3, which were proposed by the present authors, for the classification of water in macromolecular gels, are studied over the range from −30 to 60°C. From the results of the thermal expansion of agarose gels and Sephadex (cross-linked dextrans), higher water contents of gel were found to show an extremely sharp volume change at 0°C; on the contrary, lower water contents of gel exhibited no anomalous change in the specific volume in the range from −30 to 0°C, and a gradual decrease in the specific volume was detected with an increase in the temperature in the range from −20 to 0°C for medium water contents of the gel, though the gel was expected to increase in its volume in that temperature range. Thus, the new classification for the states of water in macromolecular gels was shown to be reasonable, at the same time, the transition temperatures of W_1, W_2, and W_3 were evaluated on the basis of the above results.

The thermal expansion of water in macromolecular gels, even of W_1, was found to be extremely depressed by the polymers of such gels.

In contrast with the conventional classification of water in macromolecular gels, namely, bound water and free water, the present authors previously proposed that water in gels be classified into three groups, W_1, W_2, and W_3, according to its electrochemical properties, where both W_1 and W_2 correspond to conventional free water, and W_3, to bound water.[1,2]

Most physicochemical properties of ordinary water

1) J. Mizuguchi, M. Takahashi, and M. Aizawa, *Nippon Kagaku Zasshi* **91**, 723 (1970).
2) J. Mizuguchi, M. Takahashi, and M. Aizawa, *ibid.*, **91**, 961 (1970).

are well known to change markedly at 0°C. For example, the electric conductivity for high water contents of agarose gel was found to change greatly at 0°C and to decrease gradually with a decrease in the temperature below 0°C, while that for lower water contents of gel shows no marked change at 0°C, but decreases linearly with a decrease in the temperature. Therefore, various states of water with different transition temperatures are speculated to coexist in gels.[1]

Since W_1, W_2, and W_3 are all supposed to have different transition temperatures, the thermal expansion for varied water contents of agarose gels is expected to be quite different from case to case. In addition, the above W_2 and W_3 were found to have different properties from those of ordinary water, but no difference from ordinary water was detected in W_1 throughout the electrochemical investigations. In W_1, however, the gels are prevented from flowing out. Therefore, the thermal expansion of water is considered to be obstructed in macromolecular gels, which are constructed of a network of macromolecules.

In view of the above facts, the dilatometric properties, especially those of water at lower temperatures, for typical polysaccharide gels, such as agarose gels and cross-linked dextran gels have been investigated over the range from −30 to 60°C, and this has been with the thermal expansion of ordinary water.

Experimental

Materials. The agarose for immuno-electrophoresis, Sephadex G-10, G-50, and G-200 (cross-linked dextrans purchased from Pharmacia) were prepared for gels with various water contents.

The gels were assayed for their water contents by weighing them after drying at 105°C for 24 hr.

Glossary of Principal Symbols. The definition of the principal symbols used in the report are as follows: s, the capillary sectional area of the dilatometer (cm²); v_{Hg25}, the volume of mercury at 25°C (cm³); α_{Hg}, the thermal expansion coefficient of mercury (1.34×10^{-5} degree^{-1}); t, the temperature (°C); m_s, the weight of the sample (g); p, and w, the weight fractions of polymer and water respectively in the sample, $p+w=1$; h, the mercury height difference in the dilatometer between t and 25°C (cm); $\Delta\Phi_g$, $\Delta\Phi_{wg}$, and $\Delta\Phi_w$, the specific volume changes in the gel, in the water in the gel, and in pure water respectively, as compared with the corresponding specific volume at 25°C (cm³/g).

Methods. In the thermal-expansion measurements, the above gels were determined for their volume over the range from 25 to −30°C by means of a dilatometer filled with the sample and mercury. The apparent specific volume change, $\Delta\Phi_g$ of the gel was calculated in accordance with the following equation.[3]

$$\Delta\Phi_g = \{h-(1/s)\alpha_{Hg}V_{Hg25}(t-25)\}s/m_s \qquad (1)$$

Results and Discussion

Hysteresis in the Thermal Expansion of Macromolecular Gels. To profile the general features of the thermal expansion of macromolecular gels, Sephadex G-200, G-50, and G-10 at the same water fraction (w=0.50) were measured for their volume changes with an increase and with a decrease in the temperature over the range from 25 to −30°C. The results are presented in Figs. 1, 2, and 3. The specific volume change is de-

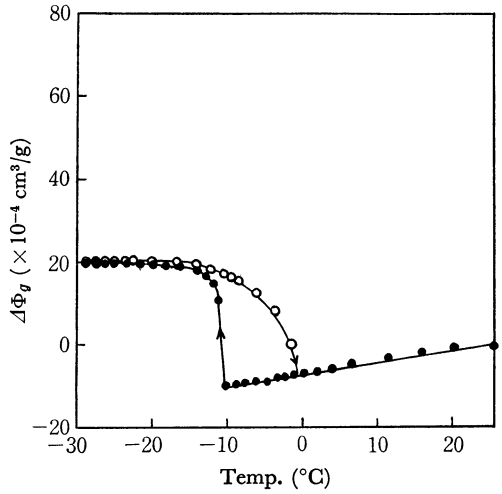

Fig. 1. Hysteresis in thermal expansion of Sephadex G-200 gel (w=0.50)

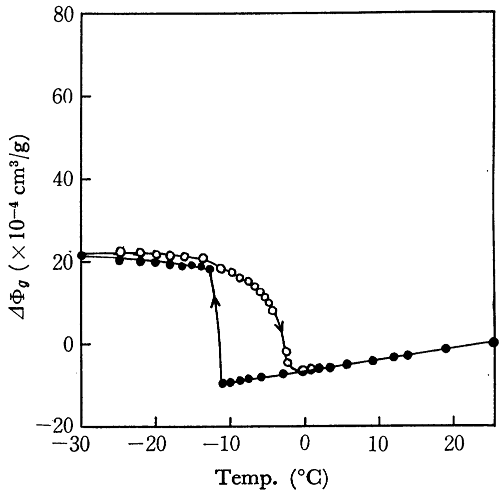

Fig. 2. Hysteresis in thermal expansion of Sephadex G-50 gel (w=0.500)

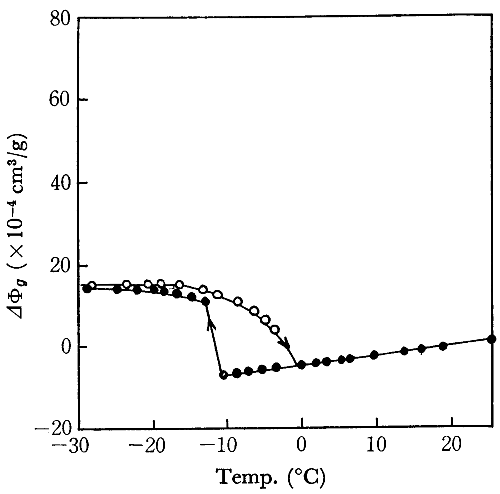

Fig. 3. Hysteresis in thermal expansion of Sephadex G-10 gel (w=0.50)

3) Soc. Polymer Sci. Japan, ed., "Experimental Polymer Science. IV", Kyoritsu, Tokyo (1961) p. 43.

noted by the coordinate, taking 25°C as the standard temperature. The specific volume for each gel decreases upon cooling, there is an abrupt increase at approximately −10°C and a gradual decrease with a decrease in the temperature to −30°C. The specific volume of the above gel cooled at −30°C shows a gradual increase with an elevation in the temperatures, even around −10°C, though it decreases markedly near 0°C.

Sephadex gels were found to show hysteresis in thermal expansion over the range of approximately −10 to 0°C.

Relationship between Water Content and the Thermal Expansion of Macromolecular Gels. The thermal expansion of gels is expected to be extremely dependent on the water content as has been described above.

The specific volume changes for agarose are presented in Figs. 4, 5, and 6. The curve with closed circles shows the specific volume change with a decrease in the temperature, while the curve with open circles indicates that with an increase in the temperature. In agarose gel with a higher water content (w=0.974), the specific volume changes sharply at about −10°C with a decrease in the temperature and at 0°C with a decrease in the temperature. As for the gel whose water fraction is 0.974, it is notable that the specific volume decreases gradually with an increase in the temperature over the range from −20 to 0°C, though it was expected to increase in this temperature range. On the contrary, the agarose gel with a lower water content (w=0.290) shows neither hysteresis in thermal expansion nor any anomalous change in the specific volume around 0°C.

In Fig. 7, the thermal expansions of Sephadex G-50 at various water contents (w=0.833, 0.500, and 0.288) with an increase in the temperature from −30°C up to 25°C are shown. The gel with a lower water content (w=0.288) shows no marked change around 0°C, much like the agarose gel with a lower water content. On the other hand, the specific volumes for the other two gels (w=0.500 and 0.833) decrease gradually over the range from −20 to 0°C.

Since no anomalous change was detected for gels with lower water contents, the water in such gels is considered to show no transition in this temperature range. Most

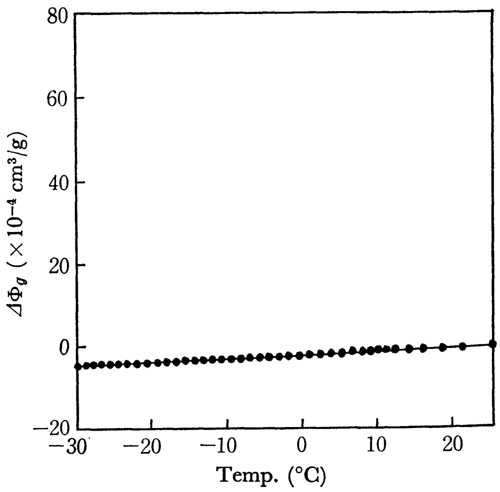

Fig. 6. Hysteresis in thermal expansion of agarose gel (w=0.290)

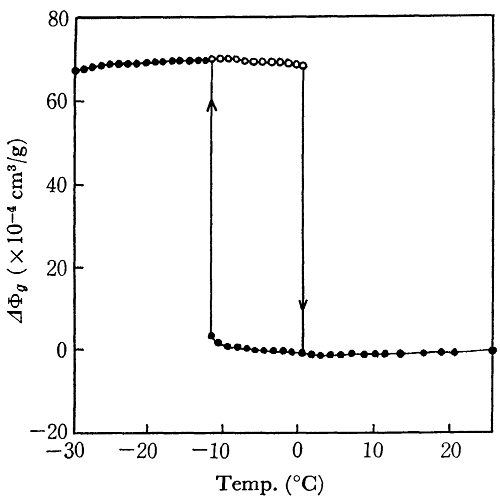

Fig. 4. Hysteresis in thermal expansion of agarose gel (w=0.974)

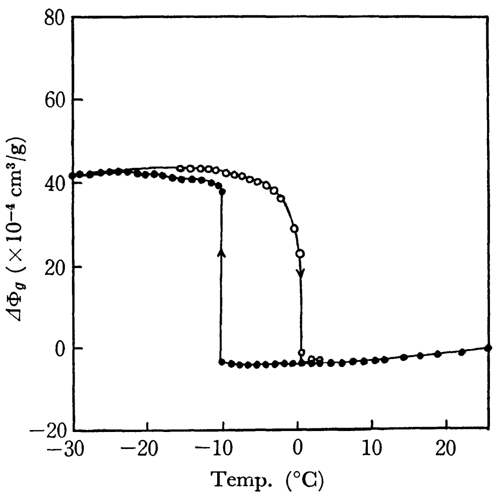

Fig. 5. Hysteresis in thermal expnsion of agarose gel (w=0.772)

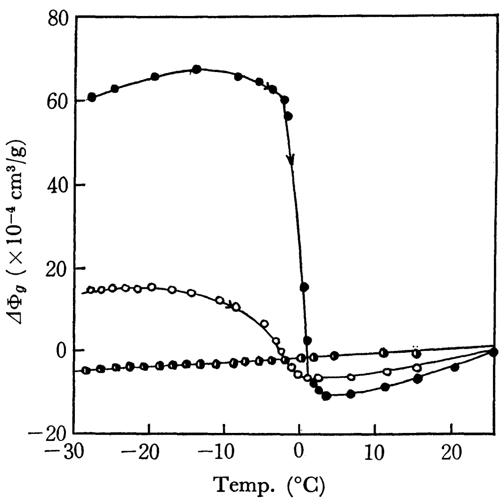

Fig. 7. Thermal expansion of Sephadex G-50 gels
-●- w=0.833, -○- w=0.500, -◑- w=0.288

of the water in such lower-water-content gels is supposed to be w_3, as is indicated previously. Therefore, w_3 may have no transition over the range from −30 to 0°C.

Judging from the extremely sharp change in the specific volume for higher water contents of the gel, the transition temperature of w_1 may be 0°C, for w_1 is presumed to be the main component of the gel in higher water contents.

The gradual decrease in the specific volume with an increase in the temperature over the range from −20 to 0°C is considered to indicate that the transition temperatures, perhaps for w_2, should be distributed over this temperature range.

These results lead us to conclude that: (1) the transition temperature of w_1 may be 0°C, (2) that of w_2 may be distributed over the range from −20 to 0°C, and (3) w_3 may have no transition temperature in the range from −30 to 0°C. The above conclusions quite agree with those of electrochemical studies.

Thermal Expansion of Water in Macromolecular Gels. The apparent specific volume changes for agarose gels are shown in Fig. 8. The dotted curve in Fig. 8 presents the specific volume change for pure water. The thermal expansion of agarose gels was found to depend greatly on their water contents and to be extremely retarded as compared with that of ordinary water. In order to evaluate the thermal expansion of water in gels, the apparent specific volume changes, $\Delta\Phi_{w_g}$ were calculated on the basis of the following equation;[4)]

$$\Delta\Phi_{wg} = (\Delta\Phi_g - p\Delta\Phi_p)/w \qquad (2)$$

Where the symbols are as defined in the glossary. The specific volume change, $\Delta\Phi_w$, for ordinary water was determined from its specific volume,[5)] Φ_w, at the temperature t, which was expressed by

$$\Phi_w = (1+b_1 t)/(\sum_{n=0}^{5} a_n t^n) \qquad (3)$$

$a_0 = 0.9998396$ $10^3 a_1 = 78.224944$ $10^6 a_2 = -7.922210$
$10^9 a_3 = -55.44846$ $10^{12} a_4 = 149.7562$ $10^{15} a_5 = -393.2952$
$10^3 b_1 = 18.159725$,

$\Delta\Phi_p$ was measured for dried agarose or Sephadex.

The calculated specific volume changes in the water in agarose gels are listed in Tables 1, 2, and 3. The data in the fifth column in each table concern the apparent specific volume change in the water in the gel relative to that of ordinary water at the temperature t.

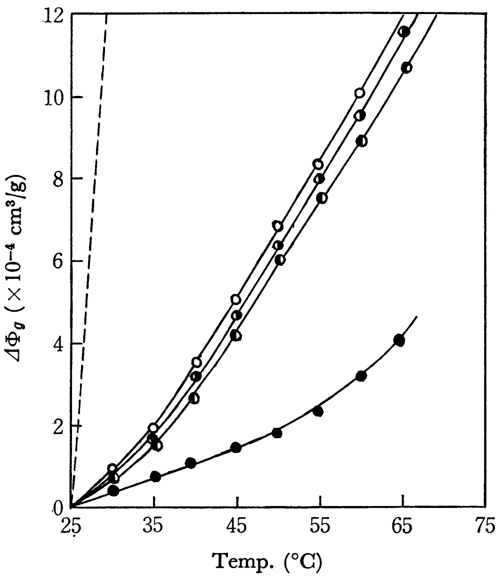

Fig. 8. Thermal expansion of agarose gels
------ ordinary water,
-○- agarose gel (w=0.974)
-◑- agarose gel (w=0.952)
-◐- agarose gel (w=0.772)
-●- agarose gel (w=0.290)

TABLE 1. THERMAL EXPANSION OF WATER IN AGAROSE GEL (w=0.974)

t(C°)	$\Delta\Phi_g$ ($\times 10^{-4}$ cm^3/g)	$p\Delta\Phi_p$ ($\times 10^{-4}$ cm^3/g)	$\Delta\Phi_{w_g}$ ($\times 10^{-4}$ cm^3/g)	$\Delta\Phi_{w_g}/\Delta\Phi_w$
25.0	0	—	—	—
30.0	0.97	0.01	0.99	0.07
35.0	1.90	0.02	1.94	0.07
40.0	3.51	0.02	3.58	0.07
45.0	4.93	0.03	5.03	0.07
50.0	6.83	0.04	6.99	0.07
55.0	8.02	0.05	8.18	0.07
60.0	10.30	0.06	10.50	0.07

TABLE 2. THERMAL EXPANSION OF WATER IN AGAROSE GEL (w=0.772)

t(°C)	$\Delta\Phi_g$ ($\times 10^{-3}$ cm^3/g)	$p\Delta\Phi_p$ ($\times 10^{-4}$ cm^3/g)	$\Delta\Phi_{w_g}$ ($\times 10^{-4}$ cm^3/g)	$\Delta\Phi_{w_g}/\Delta\Phi_w$
25.0	0	—	—	—
30.0	0.83	0.05	1.01	0.07
35.0	1.76	0.13	2.12	0.07
40.0	2.70	0.18	3.28	0.07
45.0	4.22	0.25	5.12	0.07
50.0	5.98	0.32	7.35	0.07
55.0	7.57	0.41	9.28	0.07
60.0	8.75	0.56	10.60	0.07

TABLE 3. THERMAL EXPANDSION OF WATER IN AGAROSE GEL (w=0.290)

t(°C)	$\Delta\Phi_g$ ($\times 10^{-4}$ cm^3/g)	$p\Delta\Phi_p$ ($\times 10^{-4}$ cm^3/g)	$\Delta\Phi_{w_g}$ ($\times 10^{-4}$ cm^3/g)	$\Delta\Phi_{w_g}/\Delta\Phi_w$
25.0	0	—	—	—
30.0	0.35	0.16	0.66	0.05
35.0	0.70	0.40	1.04	0.03
40.0	1.05	0.55	1.75	0.03
45.0	1.40	0.78	2.14	0.03
50.0	1.75	1.00	2.59	0.03
55.0	2.30	1.26	3.60	0.03
60.0	2.14	1.74	4.85	0.03

For agarose gels, the $\Delta\Phi_{w_g}/\Delta\Phi_w$ ratios in all cases deviate greatly from 1.0. The thermal expansions of water in gels with high water contents may be retarded to approximately 7%, and that in lower-water-content gels, to approximately 3%, as compared with that in ordinary water.

4) S. Pocsik, *Acta Biochim. Biophys. Acad. Sci. Hung.*, **4**, 395 (1969).
5) G. S. Kell, *J. Chem. Eng. Data*, **12**, 66 (1967).

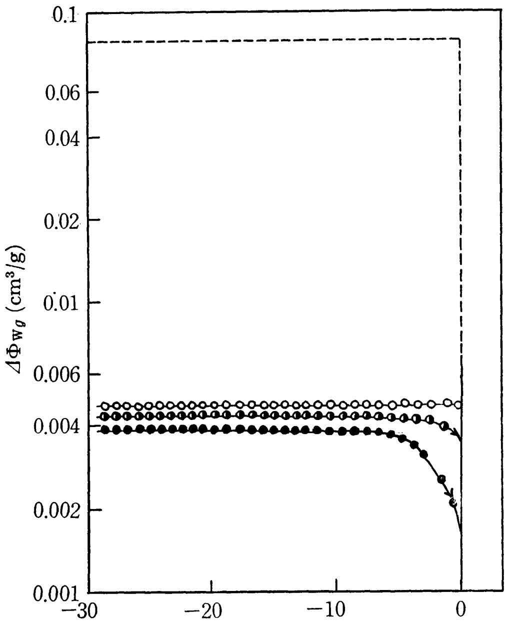

Fig. 9. Thermal expansion of water in agarose gels on the comparison with that of ordinary water.
------ ordinary water
-○- agarose gel (w=0.974)
-◑- agarose gel (w=0.867)
-●- agarose gel (w=0.772)

Since most of the water in lower-water-content gels (w=0.290) may be W_3, thermal expansion coefficient for W_3 can be evaluated as about 3% of that for ordinary water. On the other hand, high-water-content gels (w=0.974 and 0.772) may be occupied much by W_1, and W_2 and little by W_3. Thus, it may be reasonable to conclude that the thermal expansion of most water, even of W_1, is extremely depressed in agarose gels, as was expected.

Volume Change in the Transition of Water in Gels as Compared with That of Ordinary Water. The apparent specific volume change for water in agarose gels is shown in Fig. 9 and compared with that of ordinary water. As is indicated in the figure, the apparent specific volume change in the transition at 0°C for water in gels is extremely retarded to less than 10% of that for ordinary water. Therefore, most of the water in gels may be considered to be obstructed, even in thermal expansion at the transition, by the network of the polymer.

Summary

The dilatometric properties of water in macromolecular gels indicate that our proposed new classification for the states of water, namely, W_1, W_2, and W_3, is reasonable, and that the transition temperature of W_1 may be 0°C, that of W_2 may be distributed over the range from −20 to 0°C, and W_3 may have no transition temperature in the range from −30 to 0°C.

The thermal expansion of most water, even W_1, was found to be extremely depressed in macromolecular gels.