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Rheological Properties of Hydrogels of Agar-agar. III. Stress Relaxation of Agarose Gels

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A stress relaxation experiment on hydrogels of agarose isolated from agar-agar was made by means of a chainomatic balance relaxometer for the purpose of investigating the mechanism of gelation. The measurements were made on the agarose gels of the concentration range from 1.2 to 2.0 wt%, and also on agar-agar gels for the comparison, at various temperatures from 25 to 55°C. The relaxation curves obtained were represented approximately by a mechanical model consisting of three Maxwell models in parallel. The apparent activation energy, ΔH, which was calculated from the temperature dependence of the longest relaxation time, τ_1, was about 5 kcal/mol for all gels treated in this study, which was nearly the same magnitude as that for agar-agar gels. It was observed in the curves obtained that the relaxation curves for agarose gels had quite similar characteristics to those for agar-agar gels of the same concentration and was observed that the total instantaneous modulus of the former gels amounted to about 80% of that of the latter gels. From these results it may be concluded that agarose is a main constituent in the network structure in agar-agar gels and that the hydrogen bonding plays a major role in the agar-agar gels as well as in the agarose gels.

In the preceding papers[1,2] the authors reported the results of stress relaxation experiments made for hydrogels of agar-agar. The hydrogels of agar-agar is well-known as a typical example of thermoreversible gels, and has long been the object of rheological and colloid-chemical studies. It is believed at present that a dominant factor governing the mechanism of gelation of agar-agar gels is the balance between the hydrophilic and the hydrophobic properties of the molecule of agar-agar, or the balance between the affinity to water and the tendency of formation of microcystalline structures in agar-agar molecules.[3-6] The detailed mechanism of gelation has not yet been cleared.

According to a series of studies made by Araki *et al.*[7] agar-agar is composed of two polysaccharides, agarose and agaropectin. Agarose is the major component. In the present paper, an attemt is made to measure the stress relaxation of the hydrogels of agarose isolated from agar-agar, and the results are compared with the data of agar-agar gels themselves.

Experimental

A sample used was the powder of agar-agar obtained from Nihon Kaiso Kogyo, Co., Ltd. The ash content of the sample is 0.95 wt%, and the intrinsic viscosity in the aqueous solution obtained with Ostwald viscometer,[*1] $[\eta]_{25^\circ C}$, is 15.6 (100 cc/g). The molecular weight of the sample calculated from the intrinsic viscosity was 4.2×10^4.[5]

Agarose was isolated from agar-agar as follows:[8] agar-ggar, 1% by weight, was dissolved into water, and then acrynol[*2] was added to make the solution of 0.1 mol/*l*. Agarose was separated from the solution by centrifuge (6000 rpm). Agarose gel separated was heated to melt, mixed with ethyl alcohol,

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[1] K. Arakawa, This Bulletin, **34**, 1233 (1961).

[2] M. Watase and K. Arakawa, *ibid.*, **40**, 472 (1967).

[3] T. Nakagawa "Rheology," Iwanami Pub., Tokyo (1960), p. 68.

[4] I. Kagawa, *Kagaku* (*Science*), **26**, 243 (1956).

[5] N. Hirai and N. Nishimura, *Nippon Kagaku Zasshi* (*J. Chem. Soc. Japan, Pure Chem. Sect.*), **75**, 248 (1954).

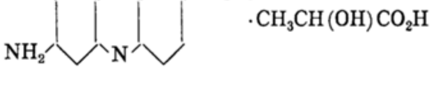
[6] M. Watase and K. Arakawa, *ibid.*, to be published.

[7] C. Araki, This Bulletin, **29**, 543 (1950); C. Araki and K. Arai, *ibid.*, **30**, 287 (1957); S. Hirase, *ibid.*, **30**, 68, 70, 75 (1957).

[*1] The flow time of pure water through the viscometer is 194.3 sec at 25°C.

[8] T. Akabane and K. Katsuura, The 13th Annual Symposium on Polymers, Kobe, July 8, 1967.

[*2]



and centrifuged again to separate agarose. This purification with alcohol was repeated until the yellow color due to acrynol vanished. The intrinsic viscosity of purified agarose in aqueous solution is 15 (100 cc/g) at 25°C.

The test piece of agarose gels was prepared by the same procedure as that of agar-agar gels reported previously.[1] The concentrations of agarose gels prepared was 1.25, 1.43, 1.67 and 2.0 wt% respectively. The concentration of agar-agar gels was 2.0 wt%.

Stress relaxation curves up to 4 hr were obtained with a chainomatic balance relaxometer. The details of the apparatus and the experimental procedures were reported previously.[9] The measurements were made at temperatures from 25 to 55°C.

Results

Stress Relaxation Curves. The relaxation Young's modulus of hydrogels of agarose and agar-agar was obtained. The results are shown in Figs. 1 (a)—(e). These relaxation curves are approximately represented by a six-element mechanical model composed of three Maxwell models in parallel. In this model the relaxation Young's modulus, $E_r(t)$, is expressed as:

$$E_r(t) = \sum_{i=1}^{3} E_i \exp(-t/\tau_i) \qquad (1)$$

where E_i and $\tau_i (\tau_1 > \tau_2 > \tau_3)$ are the elastic constant and the relaxation time for the ith Maxwell element respectively, and t is the time. All relaxation curves obtained were fitted with Eq. (1), and the parameters in it are shown in Table 1.

Concentration Dependence of the Elastic

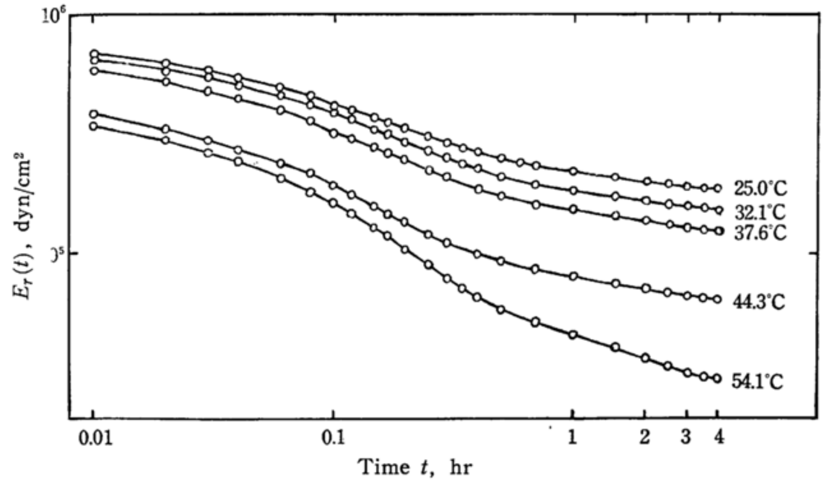


Fig. 1 (a). Relaxation curves for agarose gels. 2.0 wt%

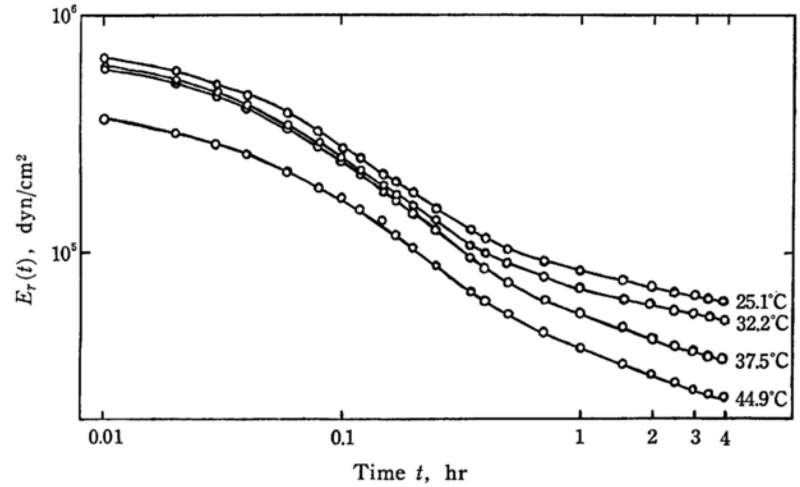


Fig. 1 (b). Relaxation curves for agarose gels. 1.6_7 wt%

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9) K. Arakawa, This Bulletin, **33**, 1568 (1960).

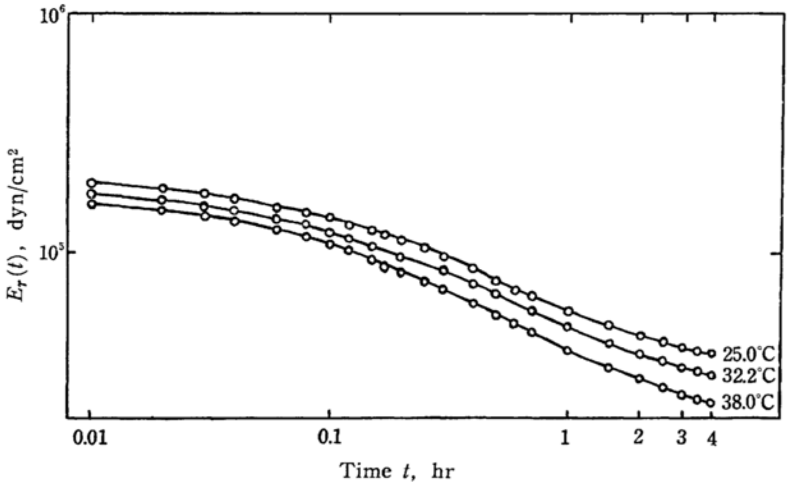

Fig. 1(c). Relaxation curves for agarose gels. 1.4_3 wt%

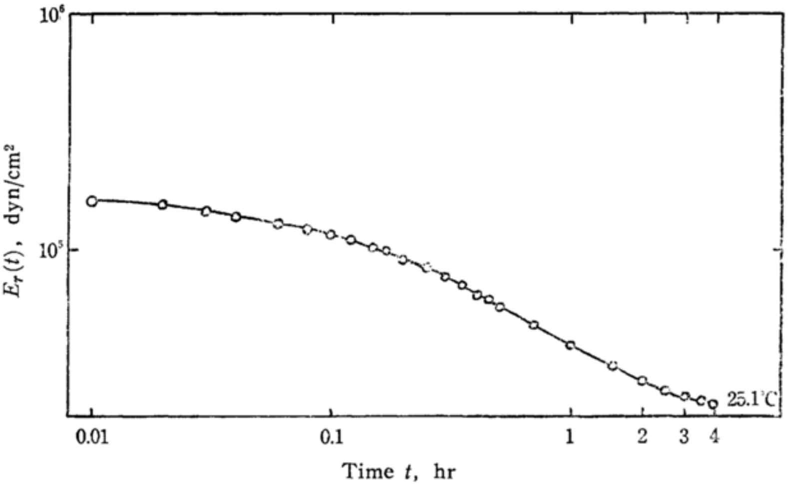

Fig. 1(d). Relaxation curves for agarose gels. 1.2_5 wt%

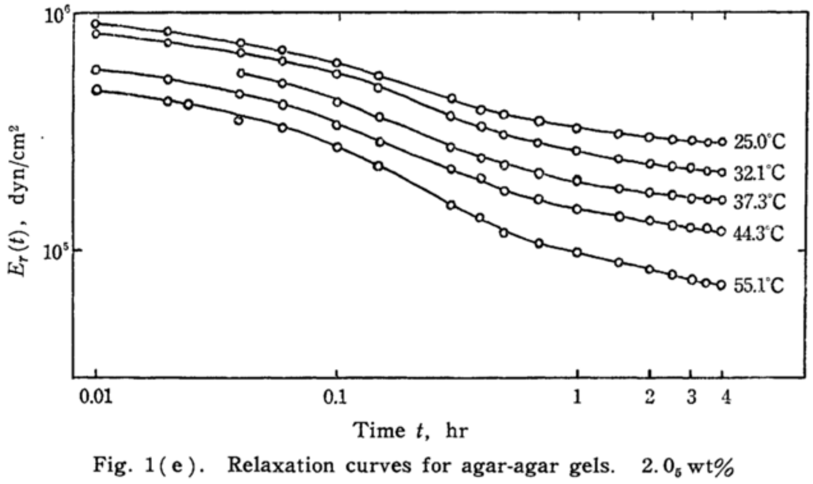

Fig. 1(e). Relaxation curves for agar-agar gels. 2.0_5 wt%

TABLE 1. CONSTANTS OF MECHANICAL MODEL

Temp.	E_1	E_2	E_3	E_0	τ_1	τ_2	τ_3
°C	(10^5 dyn/cm^2)				hr	min	min
(a) Agarose gel, 2.0 wt%							
25.0	2.1	2.0	3.8	7.9	27.2	24	3.7
32.1	1.7_5	1.9	3.5_5	7.2	27.2	19_5	3.9
37.6	1.4_5	1.6	3.5_5	6.6	22.6	19	3.9
44.3	0.7_5	0.8_7	3.0	4.6	20.5	19	3.7
54.1	0.4	0.8	2.7	3.9	12.1	22	4.4
(b) Agarose gel, 1.6_7 wt%							
25.1	0.8	1.5_5	4.8	7.1_5	16.5	17_5	3.8
32.2	0.7	1.2	5.0	6.9	15.3	19_5	3.7_5
37.5	0.5	1.3_5	5.1_5	6.8_5	13.4	19	3.8
44.9	0.3_5	0.8_7	2.9	4.1	10.6	23_5	3.7_5
(c) Agarose gel, 1.4_3 wt%							
25.0	0.5	1.0	0.6_7	2.2	13.7	23	2.8
32.2	0.4_2	0.8_7	0.7_1	2.0	12.3	26	3.3
38.0	0.3_3	0.7_6	0.8	1.9	8.4	25	3.9
(d) Agarose gel, 1.25 wt%							
25.1	0.3_2	0.9_2	0.4_7	1.7	10.5	25	4.1
(e) Agar-agar gel, 2.0 wt%							
25.0	3.2	2.9	3.3	9.4	28.6	18	4.4
32.1	2.5	2.9	3.6	9.0	21.3	19	4.2
37.3	1.9	1.9	3.4	7.2	20.1	18_5	4.7
44.3	1.4	1.8_7	3.2_5	6.5	18.1	21	3.8
55.1	0.9_2	1.8	3.1	5.8	12.8	18	3.3

Constant, E_1. The values of E_1 at 25°C, thus obtained for agarose gels, are plotted against the concentrations in Fig. 2.

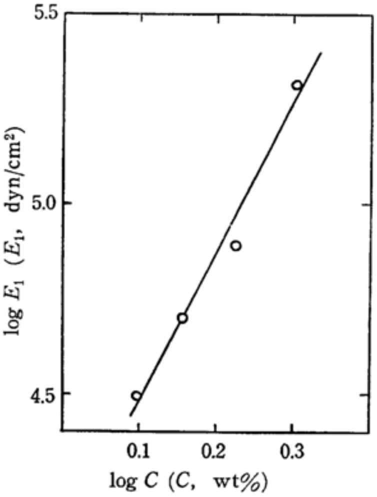

Fig. 2. Concentration dependence of the elastic constant, E_1, for agarose gels at 25°C.

The concentration dependence of E_1, which is the elastic constant of the Maxwell element with the longest relaxation time, τ_1, is approximately represented by an equation

$$E_1 = k_1 C^4 \tag{2}$$

where k_1 is a constant and C the concentration. According to N. Hirai, who made similar studies on various kinds of gels,[10] the elastic modulus of gels, E_0, which corresponds to $\sum_{i=1}^{3} E_i$ in our case, was found to be proportional to about 4th power of concentration below a certain critical concentration, C_0, and to be proportional to about 2nd power above C_0. The critical concentration C_0 was found to be 0.85% for the hydrogels of agar-agar.[10] Our results are proportional to the 4th power of concentration as is shown by Eq. (2), and therefore the critical concentration C_0 of agarose gels should be higher than that of agar-agar gels.

Relaxation Times and Activation Energy. The values of $\log \tau_1$ have been found

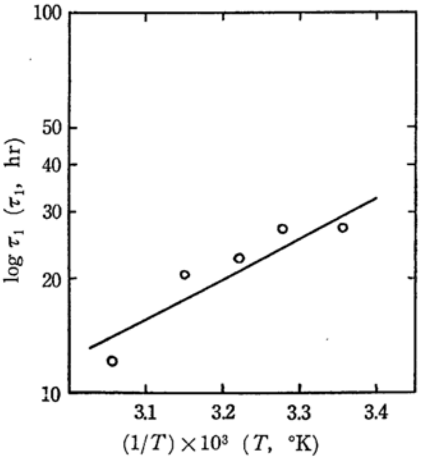

Fig. 3 (a). Temperature dependence of the relaxation time, τ_1, for the 2.0 wt% agarose gels.

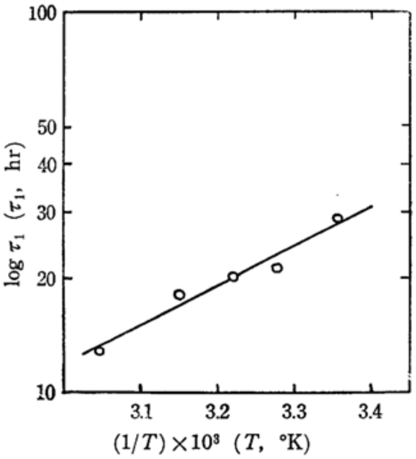

Fig. 3 (b). Temperature dependence of the relaxation time, τ_1, for the 2.0 wt% agar-agar gels.

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[10] N. Hirai, *Nippon Kagaku Zasshi* (*J. Chem. Soc. Japan, Pure Chem. Sect.*), **72**, 837 (1951).

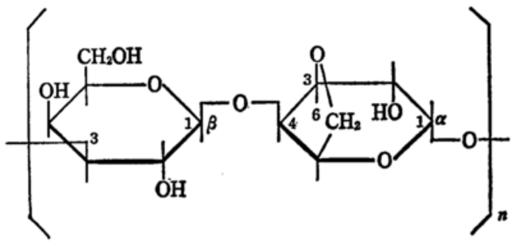
TABLE 2. ACTIVATION ENERGY ΔH

	Concn. wt%	ΔH kcal/mol
Agarose gel	2.0	5.1
	1.6_7	4.5
	1.4_3	4.8
Agar-agar gel	2.0	5.0

to change approximately linearly with $1/T$. The $\log \tau_1$ is plotted against $1/T$ for the 2.0 wt% agarose gel and 2.0 wt% agar-agar gel in Fig. 3 (a) and (b) as illustrative examples. The apparent activation energy ΔH obtained from the plots is given in Table 2. The activation energy is about 5 kcal/mol for hydrogels of agarose, which is nearly equal to that of agar-agar gels reported in the preceding paper.[2)]

Discussion

According to Araki and his coworkers[7)] agar-agar is a mixture of agarose and agaropectin. Agarose, which is a major component of agar-agar, is composed of residues of β-D-galactopyranose and 3,6-anhydro-α-L-galactopyranose arrayed alternatively. The molecular constitution of agaropectin is not clear. The most of uronic acid and sulfuric acid ester in agar-agar are known to be contained in agaropectin as its components. The possible chemical structure of agarose proposed by Araki is shown in Fig. 4.



D-Galactose 3,6-Anhydro-L-Galactose

Fig. 4. Possible structure of agarose.[7)]

The stress relaxation curves of 2 wt% agarose shown in Fig. 1 (a) are quite similar to those of agar-agar gels of the same concentration given in Fig. 1 (e). The similarity is also seen in the calculated values of relaxation times given in Table 1, where the relaxation times of agarose are nearly equal to that of agar-agar gels. The magnitude of ΔH of the main network structure in gels is about 5 kcal/mol for both gels. As is seen in Table 1, the magnitude of instantaneous elastic modulus, $E_0=\sum_{i=1}^{3}E_i$ for agarose gels is about 80% of E_0 for the agar-agar gels of the same concentration. These facts show that agarose plays a major role in the gel formation of agar-agar gels.

In conclusion, the mechanical behavior of hydrogels of agarose is quite similar in its character to that of hydrogels of agar-agar, and agarose is a component of agar-agar gels which plays a major role in the gel formation.

As has been shown by Araki *et al.*, agarose contains only a small amount of ionic groups,[11)] and in the gelation mechanism of aqueous solutions of agarose the electrostatic interaction is of little importance. The magnitude of ΔH for agarose gels obtained in this experiment is about 5 kcal/mol, which is of the order of magnitude of that for the breaking of hydrogen bond. From these facts as well as from the possible chemical structure of agarose shown in Fig. 4 it may be concluded that the hydrogen bonding plays a major role in the gelation mechanism of agarose gels, and also concluded that it is the case in agar-agar gels since agarose plays a major role in the gel formation of agar-agar gels.

The authors wish to express their heartiful thanks to Miss Yuko Endo for her cooperation in performing this study.

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11) C. Araki, *ibid.*, **58**, 1338 (1937).