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## Rheological Properties of Aqueous Solutions of *Bacillus subtilis* FT-3 Polysaccharide

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### Abstract

Rheological properties of the aqueous solutions of FT-3 polysaccharide were studied by means of the creep measurement and steady flow viscosity. Salt-type FT-3 polysaccharide solutions (salt-type solutions) showed a shear thinning-type non-Newtonian flow over the range of measuring rates of shear in either case of the aqueous media with or without electrolytes. H-type FT-3 polysaccharide solutions (H-type solutions) exhibited the shear thinning flow in a range of low rates of shear, and the reduced viscosity of the H-type solutions was about one hundredth that of the salt-type solutions in the presence of electrolytes. No significant deformation due to the elastic property was observed in the creep compliance vs. time curve obtained with relatively concentrated salt-type solutions.

Factors affecting the rheological properties of the polysaccharide solutions are discussed from the view point of the conformational changes mainly due to ionized carboxylic groups of the polysaccharide molecules.

### Introduction

FT-3 polysaccharide, produced by *Bacillus subtilis* FT-3, is an acidic heteropolysaccharide composed of glucose, galactose, fucose, glucuronic acid, and *O*-acetyl residue in the approximate molar ratio of 2:2:1:1:1.5.<sup>1,2)</sup> All the component sugar residues are linked linearly except the galactose residue, which is bound as a branch to the glucose residue. This polysaccharide was reported to give a thermo-reversible gel when heated in a test tube placed in boiling water and cooled to room temperature.<sup>3,4)</sup> The rheological characteristics of the polysaccharide seem to be somewhat different from agar,<sup>5)</sup> curdlan,<sup>6,7)</sup> pectin,<sup>8-12)</sup> and other gels which have previously been reported.<sup>13,14)</sup> The aggregation of curdlan gel caused by heat-treatment is considered to be the result of an ordered form of the microfibril as a rigid rod molecule. The agar gel is thermo-reversible and the gel strength depends on the chain length, the amount of acidic residues (sulfuric acid and galacturonic acid), the amount of calcium, and, possibly, other factors.

The authors aim to study the relation between viscosity and structure of FT-3 polysaccharide and the effects of these factors on its gel-forming properties. For this purpose, the rheological properties of FT-3 polysaccharide were investigated.

### Experimental

#### 1. Materials

FT-3 polysaccharide produced by *Bacillus subtilis* FT-3 was purified as follows: after 5 days of culture on modified Czapeck medium containing 3% glucose, 0.3% NaNO<sub>3</sub>, and other salts, the culture broth was diluted 5-fold with deionized water, and centrifuged at 21,000 × g for 1 hr. Then NaCl was added to the supernatant to make a 0.5%

solution, and two volumes of ethanol were added to precipitate the polysaccharide. The precipitate was washed with acetone and ether. This was called partially purified FT-3 polysaccharide. For further purification, the specimen was dissolved in deionized water to make a 0.05% solution, then centrifuged at  $40,000 \times g$  for 1 hr to remove some impurities, mostly bacterial cells. The supernatant was dialyzed against deionized water for 3 days, with a change of water twice a day. After filtration through a glass filter, the filtrate was lyophilized, and a colorless filamentous specimen was obtained. This was called salt-type FT-3 polysaccharide.

For the preparation of the free form of the carboxylic acid of glucuronic acid in FT-3 polysaccharide, the partially purified specimen was dissolved in deionized water to make a 0.2% solution, and the solution was desalted with Amberlite IR-120 (H' form) with gentle shaking for 2 hr. After passage through a glass filter, the filtrate was centrifuged at  $21,000 \times g$  for 30 min. The supernatant was dialyzed against deionized water, then the dialyzate was lyophilized. This was called H-type FT-3 polysaccharide.

## 2. *Viscometry*

A cone-and-plate viscometer made by Tokyo Keiki, Ltd., was employed for measuring the steady flow viscosity of the aqueous diluted solutions of the polysaccharides. The principal characteristics of this viscometer are as follows: the values of the rotated cone ( $R_v$ ) is 2.4 cm, the cone angle to the plate ( $\varphi$ ) is 0.027 radian, and the angular velocity of the cone ( $N$ ) ranges from 0.052 to 10.47 radians  $\text{sec}^{-1}$ , and can be controlled by changing the position of a gear placed between the synchronous motor and the cone. When the torque of the torsion spring ( $M$ ) is detected at an angular velocity of the cone ( $N$ ), the rate of shear ( $d\gamma/dt$ ) and shearing stress ( $p$ ) are given by

$$d\gamma/dt = N/\varphi \quad (1)$$

and

$$p = 3M/2R_v^3 \quad (2)$$

From Eqs. (1) and (2), the viscosity of the sample ( $\eta$ ) can be calculated, as follows:

$$\eta = 3M\varphi/2 R_v^3 N \quad (3)$$

Temperature of the sample in the viscometer was kept at  $25 \pm 0.1^\circ\text{C}$  in all cases by means of a thermostatted water jacket surrounding the cone and plate.

## 3. *Creep measurement*

An attempt was made to measure the viscoelastic properties of the aqueous concentrated solutions of salt-type FT-3 polysaccharide by use of an apparatus which was basically the same as that used by Scott Blair and Burnett<sup>15)</sup> for measuring the creep behavior of weak milk gels. As illustrated schematically in Fig. 1, a U-tube with a diameter ( $R_u$ ) of about 0.8 cm contains about 10 ml of the sample solution and stands in a thermostatted water bath held at  $25^\circ\text{C}$ . The inside of the U-tube has been roughened with hydrofluoric acid so as to minimize trouble with syneresis occurring when the sample solutions are aged. One end of U-tube is connected to a source of air pressure and the other end is linked to a recorder consisting of a horizontal capillary tube with a diameter ( $R_c$ ) of 0.056 cm in which the movements of a small index of colored alcohol magnify

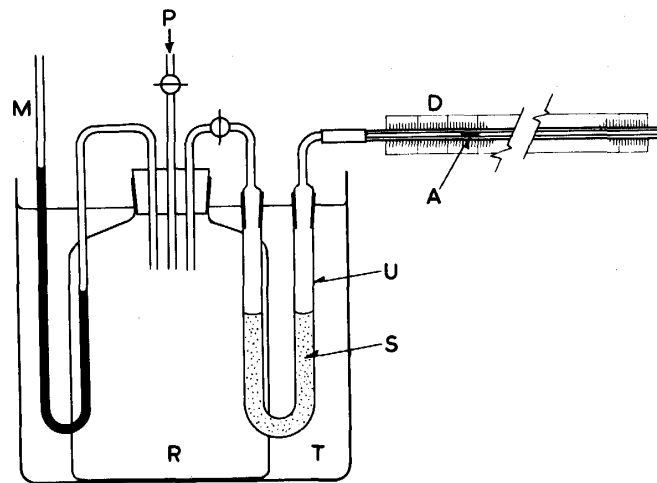


Fig. 1. Schematic diagram of the apparatus for measuring the creep behavior of concentrated polysaccharide solutions. U: U-tube, S: Sample, A: Colored alcohol, D: Strain detector. M: Kerosene manometer, P: Air pressure intake, R: Air reservoir, T: Thermostatted water bath

the displacements on the face of the sample being subjected to a change of air pressure. The pressure applied to each sample was registered on a kerosene manometer.

Assuming that there is no slip at the wall of the U-tube, and that the shear compliance depends only on time ( $t$ ), the volume flow  $A(t)$  of the sample is calculated as

$$Q(t) = \int_0^{R_u} 2\pi r \cdot x(r) dr \quad (4)$$

where  $r$  is the distance from the center of the U-tube and  $x(r)$  is the displacement of the sample at  $r$ . Integration by parts gives

$$Q(t) = \left[ \pi r^2 \cdot x(r) \right]_0^{R_u} - \int \pi r^2 \frac{dx}{dr} \cdot dr \quad (5)$$

Since  $x(r)=0$  at  $r=R_u$ , the first term on the right hand side of Eq. (5) is zero, and

$$-dx / dr = J(t) \cdot Pr / 2L \quad (6)$$

where  $J(t)$  is the creep compliance,  $P$  is the pressure applied, and  $L$  is the length of sample column in the U-tube. Substitution of Eq. (6) into Eq. (5) gives

$$Q(t) = \int_0^R \pi r^2 \cdot J(t) \frac{Pr}{2L} \cdot dr = \frac{\pi Pr^4}{8L} \cdot J(t) \quad (7)$$

since

$$Q(t) = \pi a^2 \cdot h(t) \quad (8)$$

$$\text{and } J(t) = 8La^2 \cdot h(t) / PR_u^4. \quad (9)$$

## Results and Discussion

### 1. Flow properties of aqueous diluted solutions of salt-type FT-3 polysaccharide

The salt-type FT-3 polysaccharide provides a shear-thinning flow fluid with aqueous media such as deionized water and aqueous solutions of electrolytes under shear rates ranging from  $1.92 \text{ sec}^{-1}$  to  $384 \text{ sec}^{-1}$  at  $25 \pm 0.1^\circ\text{C}$ . It seems that the flow properties of the solutions exhibit some feature of polyelectrolytes.

Figure 2 shows the plots of shear stress against rate of shear on a logarithmic scale for series of diluted solutions in deionized water. The linearity of these plots all with

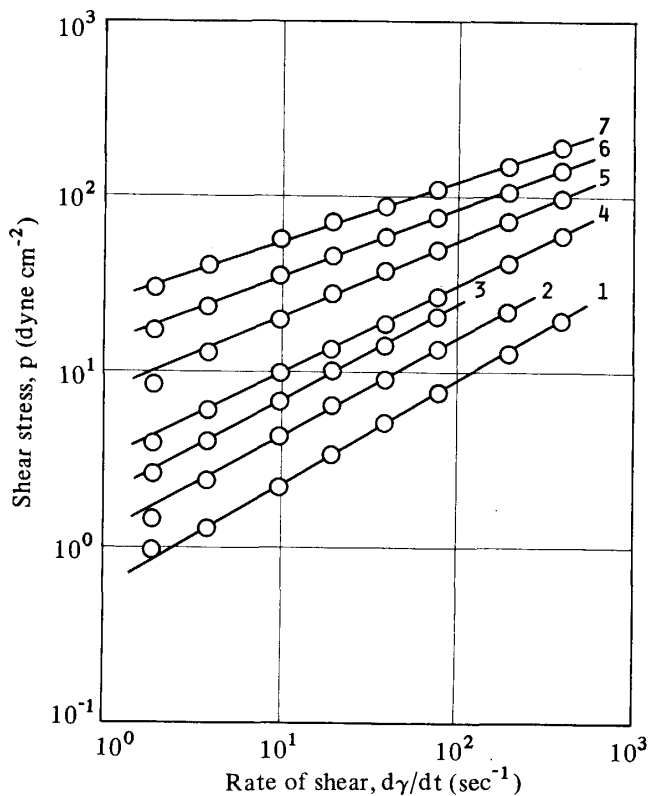


Fig. 2. Plots of shear stress against rate of shear for diluted salt-type FT-3 polysaccharide solutions (pH 7.3) in deionized water at  $25^\circ\text{C}$ .

Concentration of polysaccharide ( $\times 10^{-3} \text{ g ml}^{-1}$ ):

1, 0.5; 2, 1.0; 3, 1.5; 4, 2.0; 5, 3.0; 6, 4.0; 7, 5.0

slopes below unity indicates that the solutions can be classified as shear-thinning flow fluids, and that the correlation between shear stresses ( $p$ ) and rates of shear ( $d\gamma/dt$ ) will be expressed by the power law equation, as follows:

$$p = k (d\gamma/dt)^n \quad (10)$$

where  $k$  corresponds to the apparent viscosity of the solution and  $n$  is the slope of the  $\log p$  vs.  $\log d\gamma/dt$  plot. As summarized in Table I for the values of  $k$  and  $n$ , the apparent viscosity of the solutions increases markedly as the polysaccharide concentration increases up to  $5.0 \times 10^{-3} \text{ g ml}^{-1}$ , while the non-Newtonian property of the fluid is notable.

The addition of electrolytes affects the apparent viscosity of the salt-type polysaccharide solutions, although the power law equation (10) can be applied to the correlation between shear stresses and rates of shear except when organic acids exist in the

solution. As shown in Fig. 3 and Table I, the presence of  $0.1M$   $CaCl_2$  in the aqueous medium causes the apparent viscosity and non-Newtonian property of the solutions to decrease over the whole range of polysaccharide concentrations compared with these properties of solutions in deionized water. It is to be noted that when the pH of the solution is lowered to about 3 by adding acetic acid or citric acid, the solution exhibits thixotropy defined by an hysteresis loop to the changes in direction of the rate of shear in the viscometry.

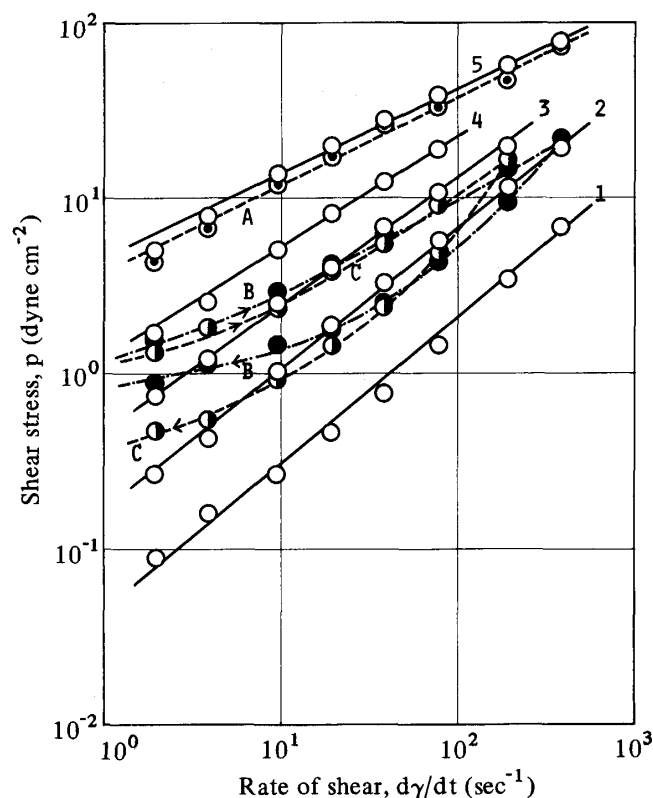


Fig. 3. Flow curves of diluted salt-type FT-3 polysaccharide solutions in aqueous electrolyte solutions.

Medium: 1–5 for  $0.1M$   $CaCl_2$  (pH 7.3), A is  $0.04N$  KOH (pH 12.1), B is  $0.04M$  citric acid (pH 3.08), C is  $0.08M$  acetic acid (pH 3.08)

Concentration of polysaccharide ( $\times 10^{-3}$  g ml $^{-1}$ )

1, 0.5; 2, 1.0; 3, 1.5; 4, 2.0; 5, 3.0; A, 3.0; B, 3.0; C, 3.0

Table I. The values of the constants  $n$  and  $k$  in Eq. [10] for aqueous solutions of salt-type FT-3 polysaccharide at  $25^\circ C$

Polysaccharide concentration ( $\times 10^{-3}$ g ml $^{-1}$ )	Deionized water		Aq. soln. of $0.1M$ $CaCl_2$	
	$n$	$k$	$n$	$k$
0.5	0.59	0.60	0.87	0.038
1.0	0.56	1.19	0.82	0.145
1.5	0.53	2.10	0.71	0.47
2.0	0.50	3.15	0.66	1.01
3.0	0.43	7.41	0.49	4.22
4.0	0.38	14.5		
5.0	0.34	25.4		

The results obtained suggest that the salt-type FT-3 polysaccharide molecules extend in deionized water in a randomly coiled configuration due to the good-solvent effect of water and the mutual repulsion of charged centers with the same sign. When the degree of ionization of the salt-type polysaccharide is decreased by the addition of electrolyte, the molecules assume a somewhat more coiled configuration which is effective in decreasing the steady flow viscosity of the solution<sup>16)</sup>. This arises from the increasing association of counter ions with the polysaccharide molecules and leads to a diminution in the mutual electrostatic repulsion of the charges on the polysaccharide chains within the molecules. Meanwhile, the molecular configuration of the salt-type sample in both the deionized water and aqueous electrolyte solution media may readily be changed to an elongated form in the direction of shear flow. Therefore, as shown in Fig. 4, the apparent reduced viscosity of the solutions in both media decreases with increasing rate of shear over the whole range of the polysaccharide concentrations tested. However, the values of reduced viscosity were remarkably high compared with previously known polysaccharide<sup>9)</sup> having molecular weights similar to that of FT-3.

## 2. Flow properties of the aqueous diluted solutions of H-type FT-3 polysaccharide

Since the degree of ionization of the carboxyl group in the H-type polysaccharide in aqueous media should be much less than that of the salt-type sample, the ionized

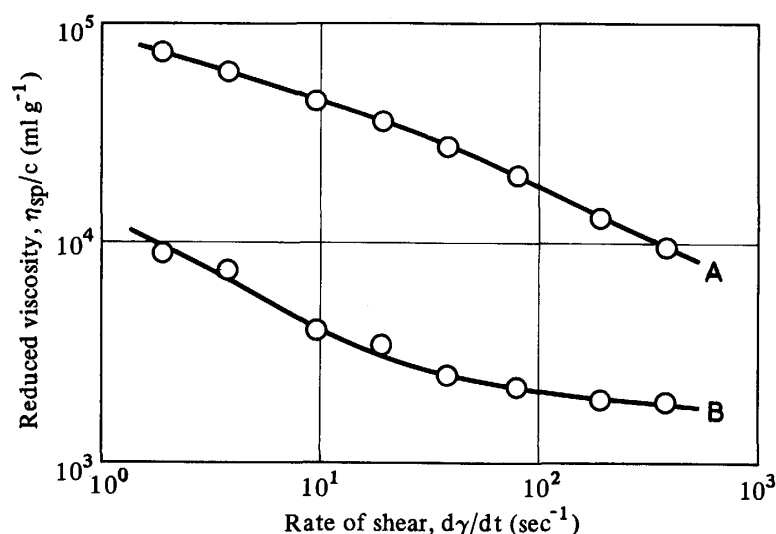


Fig. 4 Effect of rate of shear on the reduced viscosity of aqueous diluted solutions of salt-type FT-3 polysaccharide at 25°C.

A:  $0.5 \times 10^{-3}$  g polysaccharide/ml deionized water,

B:  $0.5 \times 10^{-3}$  g polysaccharide/ml aq. soln. of 0.1M  $\text{CaCl}_2$

carboxyls may reduce the extent of molecular configuration in the media caused by the decrease of mutual repulsion among the charged centers on the polysaccharide molecules.

Figure 5 shows a typical example of shear flow for the H-type polysaccharide solution with a concentration of  $3.0 \times 10^{-3}$  g  $\text{ml}^{-1}$  in various aqueous media. The results suggest that (1) the shear-thinning flow appears only in a range of low rates of shear, so that (2) it is possible to evaluate Newtonian viscosity of the solutions from the high-shear steady flow, and (3) the addition of electrolytes to the aqueous medium has only a small effect on the Newtonian viscosity of the solution. Furthermore, the concentration dependence of the viscosity is reduced over the whole range of concentration compared with that of the salt-type polysaccharide solution samples.

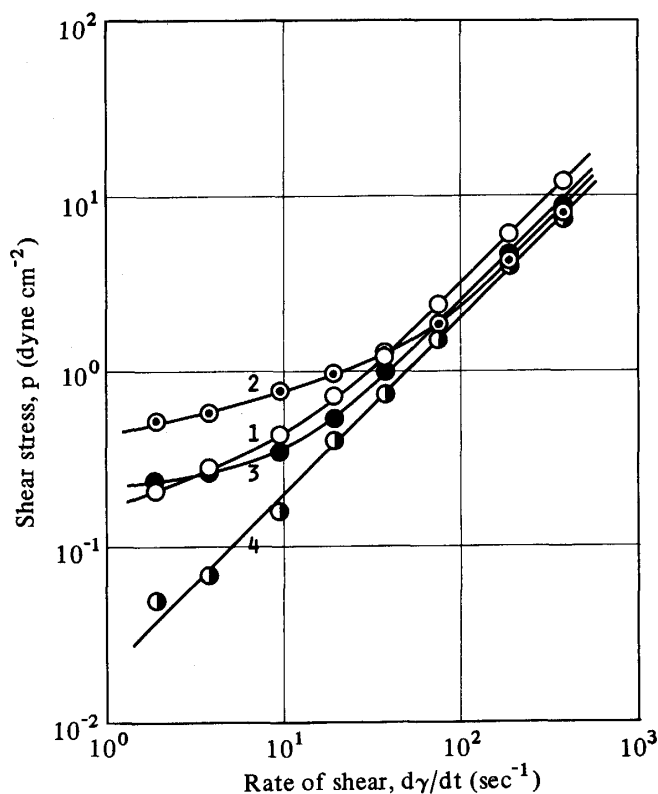


Fig. 5. Some examples of flow curves of diluted H-type FT-3 polysaccharide solutions in aqueous media at  $3.0 \times 10^{-3} \text{ g ml}^{-1}$  at  $25^\circ\text{C}$ .  
Medium: 1 for deionized water (pH 2.98), 2 for aq. soln. of  $0.1M \text{ CaCl}_2$  (pH 3.0), 3 for  $0.08M$  acetic acid (pH 2.63), 4 for  $0.04N$  KOH (pH 12.3)

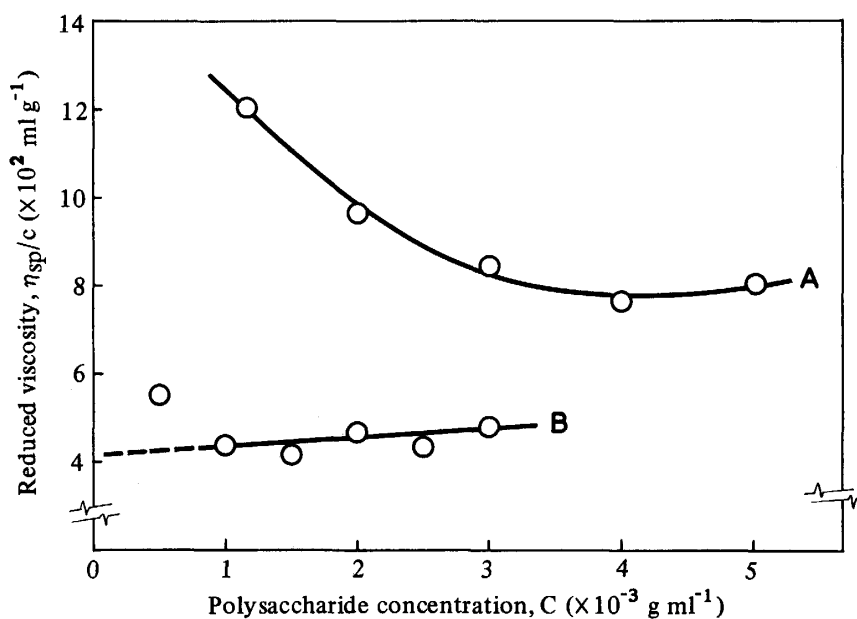


Fig. 6. Concentration dependence of reduced viscosity of aqueous solution of H-type FT-3 polysaccharide at a rate of shear of  $384 \text{ sec}^{-1}$  at  $25^\circ\text{C}$ .  
Medium: A is deionized water, B is aq. soln. of  $0.1M \text{ CaCl}_2$



Although these experimental results apparently support the presumptions described above, the H-type FT-3 polysaccharide still sustains the characteristics of polyelectrolytes.

As shown in Fig. 6, the reduced viscosity of the H-type polysaccharide solutions in deionized water decreases curvilinearly as the polysaccharide concentration increases up to  $4.0 \times 10^{-3} \text{ g ml}^{-1}$ . This means that the H-type polysaccharide still possesses some effective charges on the polyions for extending the coiled conformation of the molecules with continued dilution, due to a small degree of dissociation of carboxyl groups or to ionic impurities on the molecules. At any polysaccharide concentration, however, the reduced viscosity is greatly lowered by the addition of  $0.1M \text{ CaCl}_2$  to the medium, so that the intrinsic viscosity can be estimated by extrapolating the reduced viscosity onto the ordinate of Fig. 6. Thus, a value of about  $430 \text{ ml g}^{-1}$  was estimated at  $25^\circ\text{C}$ .

### 3. Creep compliance response for aqueous concentrated solutions of salt-type FT-3 polysaccharide

Some polysaccharides have a gel-forming ability in aqueous media, when entanglement and coupling between neighboring chains lead to the construction of junction zones of a network. One of the features of gels can generally be detected by the elastic or viscoelastic properties of the system.<sup>9)</sup> For salt-type FT-3 polysaccharide the apparent viscosity and non-Newtonian flow of the aqueous solutions are much pronounced as the concentration increases, so that one may expect to have a hydrogel with relatively concentrated systems of the salt-type polysaccharide.

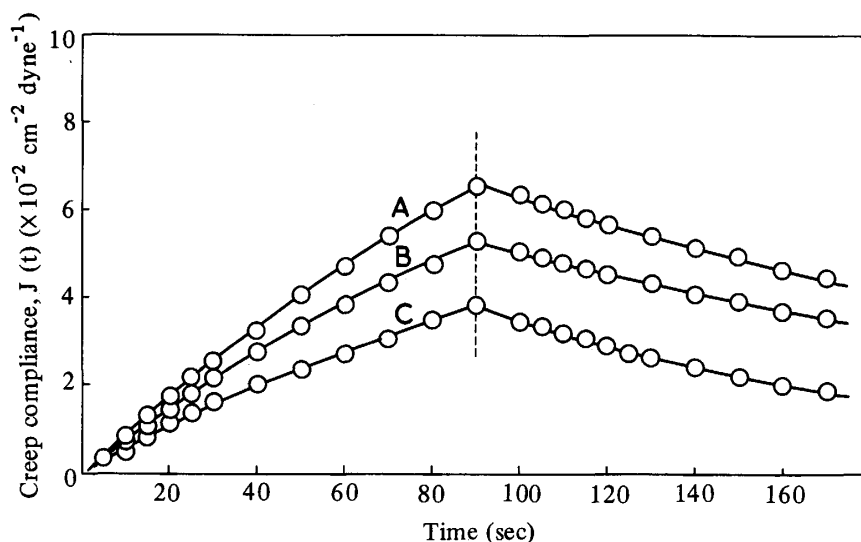


Fig. 7. Creep and creep recovery of aqueous solutions of salt-type FT-3 polysaccharide at  $1.5 \times 10^{-2} \text{ g ml}^{-1}$  in deionized water at  $25^\circ\text{C}$  without heat treatment  
Shear stress applied ( $\text{dyne cm}^{-2}$ ): A, 32.5; B, 24.4; C, 16.2

Figure 7 shows the creep and the creep recovery of a solution of 1.5% salt-type sample in deionized water under various small stresses. These curves suggest that the system still exhibits non-Newtonian viscous flow much more than elastic deformation. In fact, it is not possible to obtain elastic parameters, but one may estimate the apparent viscosity from the time dependence of the creep compliance, such as 1200 poise for  $32.5 \text{ dyne cm}^{-2}$  of stress, 1400 poise for  $24.4 \text{ dyne cm}^{-2}$ , and 1900 poise for  $16.2 \text{ dyne cm}^{-2}$ .

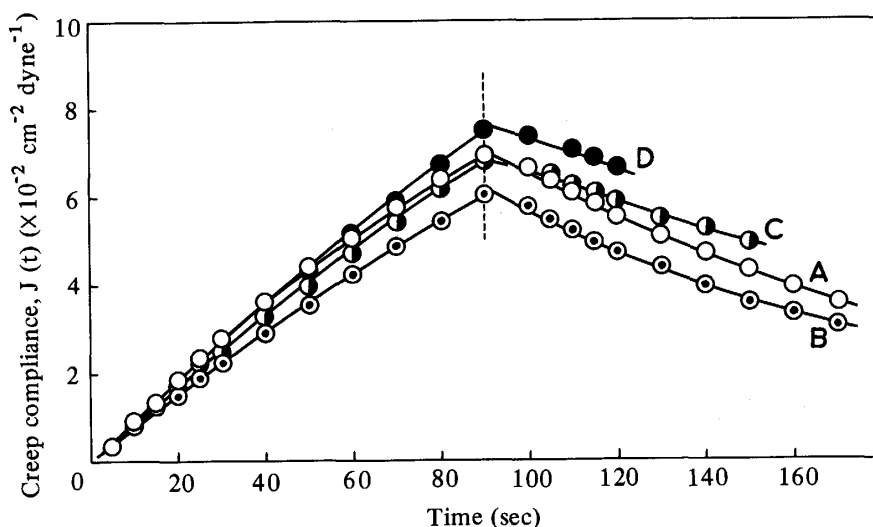


Fig. 8. Creep and creep recovery of aqueous solution of salt-type FT-3 polysaccharide at  $1.5 \times 10^{-2} \text{ g ml}^{-1}$  in deionized water at  $25^\circ\text{C}$ . The solution was treated at  $100^\circ\text{C}$  for 10 min. Shear stress applied ( $\text{dyne cm}^{-2}$ ): A, 34.8; B, 26.1; C, 17.4; D, 8.7

When this system is heated in boiling water for 10 min, the solution reversibly becomes slightly turbid, possibly caused by phase isolation of the solute in loosening the solvent effect of water. Figure 8 shows the creep and the creep recovery of the thermo-treated solution under various small stresses at  $25^\circ\text{C}$ . These results suggest that the treated solution behaves in a manner similar to the non-treated system with apparent viscosity scattered in a range from 1050 poise to 1300 poise depending on the stress. This behavior of the heat-treated solution is not influenced by the aging period at room temperature. Thus, heat treatment makes the salt-type FT-3 polysaccharide impossible to prepare a suitable elastic hydrogel.

The simplest explanation for the viscous flow of these samples is that the junction zones of the polymer chains move very readily when the sample is subjected to stress. It is not clear, however, whether the movement of the junction zones is brought about by the elastic repulsion of charged groups on the polysaccharide chains, or whether there is no entanglement coupling in the inner-chain interactions due to the good solvent effect of water.

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