

Rheological Properties of Ca Salt of Xanthan in Aqueous Media[†]

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Received May 8, 1987

The non-Newtonian behavior and dynamic viscoelasticity of solutions of the Ca salt of xanthan were measured with a rheogoniometer. The Ca salt of xanthan showed pseudoplastic behavior <0.1% but was plastic >0.3%. Compared with native, Na, and K salts of xanthan, the Ca salt had higher dynamic viscoelasticity at high concentrations. The apparent viscosity of Ca salt of xanthan was very large at low temperatures and decreased with increasing temperature. These suggest very strong intermolecular association of the xanthan (Ca salt) molecules, probably due to the formation of ionic force between adjacent charged trisaccharide side-chains *via* Ca²⁺ on different molecules. Possible structures for quaternary association including intramolecular association of xanthan molecules (Ca and K salts) in aqueous solution were proposed.

Xanthan produced by *Xanthomonas campestris* is a commercially significant polysaccharide because of its unusual rheological properties.^{1~3)} These properties are exploited in foods, oil recovery, and textile printing.^{4,5)} We have proposed that xanthan molecules are associated in a quaternary structure through charged trisaccharide side chains²⁾ and the acetyl residue, which is attached to the inner mannose side chain,^{6,7)} contributes to its intramolecular association.⁸⁾ A new mode of interaction between xanthan and D-galacto-D-mannan, as in lock-and-key, involving side chains of the former and backbone of the latter molecules has been proposed.^{9~11)}

We report here the non-Newtonian behavior and dynamic viscoelasticity of Ca salt of xanthan, and its rheological properties are analyzed with respect to its association characteristics in comparison with those of native, deacetylated, and Na salt of xanthan, to propose a possible structure for quaternary association including intramolecular association in aqueous solution.

MATERIALS AND METHODS

Materials. The xanthan was identical with those used in our previous studies^{8~11)} and was obtained from Taiyo Kagaku Co., Ltd. A solution of 0.1% xanthan in distilled water was heated at 90°C for 30 min, and then cooled to room temperature, centrifuged at 12,000g for 1 hr, and filtered through Celite 545 (which had been treated with boiling 3M HCl for 30 min, and washed with distilled water until the pH was 6.5). In the presence of 0.1% KCl, ethanol (2 vols.) was added to the filtrate, and the precipitate was dried *in vacuo*. Purified xanthan was redissolved in water, deionized by passing through a column of Amberlite IR 120(H⁺), and neutralized with 100 mM Ca(OH)₂, NaOH, or KOH. Then ethanol (2 vols.) was added to the filtrate in the presence of 0.1% CaCl₂, NaCl, or KCl and the precipitate was dried *in vacuo*.

Specific rotation. The specific rotation was measured at 589 nm with an automatic digital polarimeter DIP-180 (Japan Spectroscopic Co., Ltd.) for solutions of 0.1% (w/v).

Viscosity and dynamic viscoelasticity measurements. Viscosity at different shear rates (1.188~95.030 sec⁻¹) and dynamic viscoelasticity at a steady angular velocity (3.77 rad/sec) were measured with a rheogoniometer consisting of a coaxial cylinder (1.8 cm diam.) with a rotating outer cylinder (2.2 cm diam.), 6.0 cm long (IR-103, Iwamoto

[†] Presented at the 184th Meeting of the Branch of Nishinippon, The Agricultural Chemical Society of Japan, Nagasaki, 1984.

Seisakusho Co., Ltd.). The temperature of the sample was controlled by circulating oil from a Thermo-cool (LCH-130F, Toyo Co., Ltd.) over the temperature range of 0~80°C and raised at a rate of 1°C/min by steps. Shear rate (D), shear stress (S), and apparent viscosity (η), were calculated with the equation of Margules.¹² Dynamic viscosity (η') and elasticity (G') were calculated by a modification of Markovitz's equation.¹³ The loss tangent was calculated from the following relationship

$$\tan \delta = G''/G'$$

where G'' was the loss modulus expressed as $\omega\eta'$, and ω was the angular velocity of the outer cylinder.

RESULTS

To compare the rheological behavior of the Ca salt of xanthan that of native and deacetylated xanthan, viscosity and dynamic viscoelasticity were measured under the same conditions as those of our previous studies.^{2,8} As reported previously,^{2,8} aqueous solutions of native xanthan showed plastic behavior, and flow indices were constant at a wide concentration range; deacetylated xanthan showed pseudoplastic behavior below 0.1% but plastic above 0.3%, and its flow indices were somewhat different at various concentrations.

The flow curve of the Ca salt of xanthan in a 0.1% solution approximated to pseudoplastic

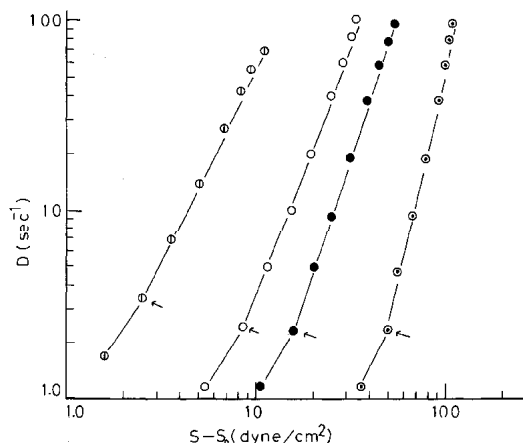


FIG. 1. Logarithmic Plots of Shear Rate and Shear Stress for the Ca Salt of Xanthan at Various Concentrations.

⊙, 0.1%; ○, 0.3%; ●, 0.5%; ⊖, 1.0%; ←, refraction point; S_0 , yield value.

behavior, but to plastic above 0.3%, and yield values were estimated to be 4, 25, and 100 dyn/cm² at 0.3, 0.5, and 1.0%, respectively. To estimate the non-Newtonian behavior quantitatively, the above results were adapted to the power law as illustrated in Fig. 1. The flow curves were divided into two straight lines, and refraction points were observed at various concentrations at which phenomenon agreed with those of native² and deacetylated⁸ xanthan. However, their flow indices were estimated to be 1.4, 1.5, 1.5, and 1.5 before the refraction point and 1.8, 2.3, 2.8, and 3.0 dyn/cm² after the refraction point at 0.1, 0.3, 0.5, and 1.0%, respectively. The tendency of the flow property of the Ca salt of xanthan was similar to that of the deacetylated one,⁸ indicating that the non-Newtonian behavior of the Ca salt of xanthan tends to differ some-

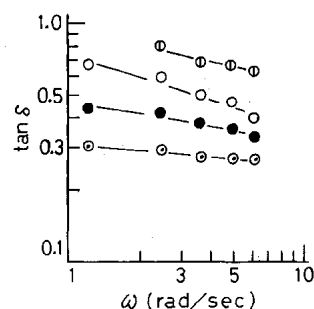


FIG. 2. Effects of Frequency on $\tan \delta$ of the Ca Salt of Xanthan at Various Concentrations.

⊙, 0.1%; ○, 0.3%; ●, 0.5%; ⊖, 1.0%.

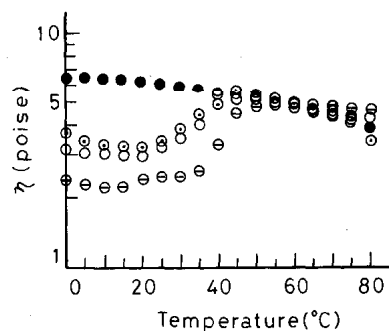


FIG. 3. Effects of Temperature on Viscosity of Various Forms of Xanthan at the Concentration of 0.5% at a Shear Rate of 9.503 sec.⁻¹.

⊖, native; ●, Ca salt; ⊙, K salt; ○, Na salt.

what with an increase in concentration from that of native xanthan.²⁾

The dynamic viscoelasticity–frequency relationship of the Ca salt of xanthan was measured. Dynamic viscosity decreased linearly with the increase in frequency, while the dynamic modulus increased linearly, and their slope varied with increasing concentration. The dependence of frequency on $\tan\delta$ of the Ca salt of xanthan is shown in Fig. 2. It decreased from 0.8 to 0.3 with increasing concentrations from 0.1 to 1.0% as in deacetylated xanthan,⁸⁾ however, the native xanthan was constant, showing 0.5~0.4 at a wide range of concentrations, 0.1~1.5%.²⁾ Thus, the result indicates that the quaternary association increases with the increase in concentration and the association characteristics of xanthan are changed after conversion into the Ca form.

The dynamic viscoelasticity–concentration relationship of the Ca salt of xanthan were measured. The dynamic viscoelasticity of the Ca salt of xanthan was lower than those of native and Na and K salts of xanthan below 0.3%, while they showed higher values above a 0.5% solution. Such phenomenon, showing the higher values at higher concentrations, may be due to the formation of much more intense intermolecular association than that of native, and Na and K salts of xanthan.

The apparent viscosity of the Ca salt of xanthan was high at low temperatures and decreased gradually with increasing temperature at concentrations below 0.5%, but at 1.0% solution, it was maximum at 50°C, then decreased gradually with further increases in temperature. As shown in Fig. 3, however, the viscosity of native, Na and K salts of xanthan (0.5%) showed a sigmoid curve. Compared with the Ca salt of xanthan, the viscosity of native, and Na and K salts of xanthan was low at low temperatures, but they increased gradually with increases in temperature and were maximum at 40~50°C, where all of samples, including the Ca salt of xanthan, kept almost the same values, then decreased gradually with further increases in temperature. The dynamic

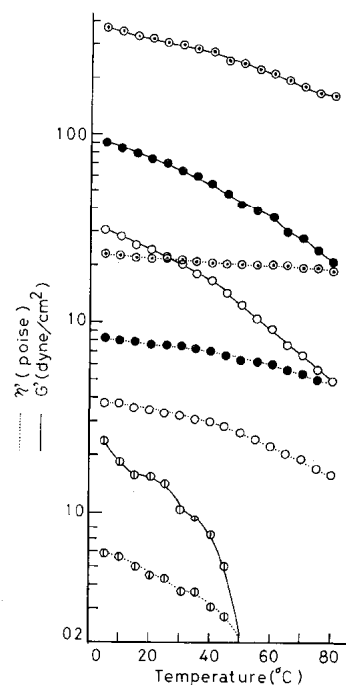


FIG. 4. Effects of Temperature on Dynamic Viscoelasticity of the Ca salt of Xanthan at Various Concentrations at Angular Velocity of 3.77 rad/sec.

⊙, 0.1%; ○, 0.3%; ●, 0.5%; ⊙, 1.0%; -----, dynamic viscosity; —, dynamic modulus.

viscoelasticity of the Ca salt of xanthan was very high at low temperatures, as shown in Fig. 4, and decreased gradually with increases in temperature even at 1.0% solution as in deacetylated xanthan.⁸⁾

The dynamic viscoelasticity of a solution of the Ca salt of xanthan was nearly independent of pH change between 5 to 11 where the tendency was agreed with that of native xanthan.⁸⁾ but not with the deacetylated one.⁸⁾ As reported previously,²⁾ the dynamic viscoelasticity of native xanthan increased greatly in the presence of salts. The Ca salt of xanthan solution (0.5%), however, showed only a little increase of the dynamic viscoelasticity even in the presence of CaCl_2 and MgCl_2 (0.1%). This may be due to the saturation of the intermolecular association before addition of the salt. The dynamic viscoelasticity was lower than that of the Ca salt of xanthan alone (0.5%) upon the addition of urea (4.0 M).

TABLE I. SPECIFIC ROTATION OF Ca-FORM XANTHAN AT 589 nm

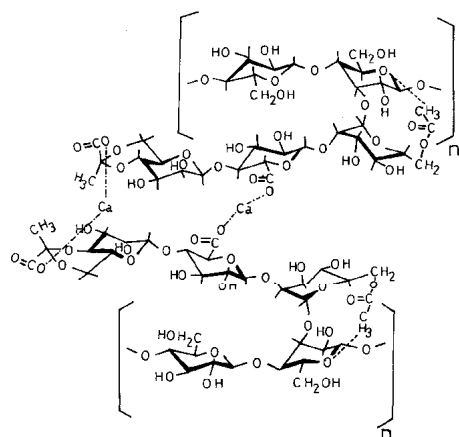
Xanthan	Temperature (°C)		
	5	28	60
Native	-12	-6	+12
Na-Form	-12	-2	+14
K-Form	-16	-6	+8
Ca-Form	-24	-2	+14

Concentration, 0.1%; water.

The specific rotation of the Ca salt of xanthan is summarized in comparison with that of native, Na and K salts of xanthan in Table I. It showed -24 , -2 , and $+14$ at 5, 28, and 60°C , respectively. The result indicates that the conformation of the Ca salt of xanthan is as variable in thermal change as in the other salts.

DISCUSSION

The flow indices of the Ca salt of xanthan varied with increasing concentration. The sigmoid curve of viscosity and dynamic viscoelasticity for the Ca salt of xanthan was not observed during increases in temperature. The much stronger dynamic viscoelasticity for the



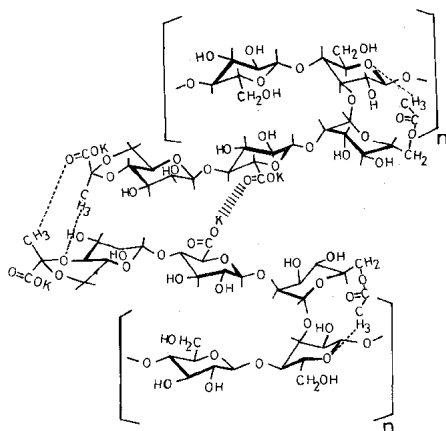
SCHEME 1. A Possible Structure of the Intermolecular Ca^{2+} Bridges of Xanthan Molecules (Ca Salt) Including Intramolecular Association in Aqueous Solution.

As the pyruvate residues are attached on two-thirds of outer *D*-mannose side chains, the Ca^{2+} bridges between carboxyl groups of the pyruvate residues may take place in part. ———, ionic force; ·····, hydrogen force.

Ca salt of xanthan could be observed than those of the native, and Na- and K-forms.

Thus, we propose a possible mode of intermolecular Ca^{2+} bridges on xanthan molecules as illustrated in Scheme 1. Intermolecular Ca^{2+} bridges may form between adjacent carboxyl groups of *D*-glucuronic acid residues, and of pyruvate residues,¹⁶⁾ which are attached on one-half of the outer *D*-mannose side chains with acetal-linked,^{6,7)} on different molecules with ionic force.^{17,18)} The intermolecular Ca^{2+} bridges proposed correspond to the smaller increase in dynamic modulus of the Ca salt of xanthan, in the presence of CaCl_2 , may be due to the saturation of the intermolecular Ca^{2+} bridges before additions of the salt as in *ι*-carrageenan molecules.¹⁸⁾ We have proposed that the acetate residues of xanthan may contribute to an intramolecular association,⁸⁾ and the ring oxygen groups of agarose molecules to an intermolecular hydrogen bonding.¹⁵⁾ Accordingly, we also propose a mechanism of intramolecular hydrogen bonding between the methyl group of the acetate residue and an adjacent hemiacetal oxygen atom¹⁷⁾ of the *D*-glucose residue at the branching backbone. The intramolecular hydrogen bonding, however, may not be rheologically responsible, probably due to the formation of strong intermolecular Ca^{2+} bridges.

The mechanism of the intramolecular hydrogen bonding provides an explanation for an intermolecular association of xanthan molecules which are substituted with univalent cations, such as K^+ and Na^+ . The intermolecular association in the K^+ and Na^+ salts of xanthan molecules is not as strong as that of the Ca salt of xanthan at lower temperatures ($<30^{\circ}\text{C}$), however, it increases with further increases in temperature, and the maximum degree of association is established at $45\sim 50^{\circ}\text{C}$, then it remains at almost the same degree as that of the Ca salt of xanthan. This suggests that the methyl groups of pyruvate residues, which are attached on two-thirds^{6,7)} of outer mannose side chains, may take part in the intermolecular association at higher tem-



SCHEME 2. A Possible Structure of the Intermolecular Hydrogen Bonding of Xanthan Molecules (K Salt) in Aqueous Solution.

An intermolecular hydrogen bonding may be built up at high temperature. As the pyruvate residues are attached on two-thirds of outer D-mannose side chains, the hydrogen bonding may take place in part.

-----, hydrogen force; \triangle , electrostatic force of attraction.

peratures as in methylcellulose molecules.¹⁹⁾ Thus, we also propose a possible intermolecular association between methyl groups of pyruvate residues¹⁶⁾ and acetal ring oxygen atoms of the side chains with hydrogen bonding on different molecules, as illustrated in Scheme 2. In the Ca salt of xanthan, the Ca^{2+} bridges may be built up in preference to the intermolecular hydrogen bonding at low temperatures $<45^\circ\text{C}$, but the bridges dissociate with increasing temperature, and the hydrogen bonding may be built up in place of the Ca^{2+} bridges. The unusual viscosity and dynamic viscoelasticity of xanthan,^{1,2)} showing a sigmoid curve with increasing temperature, may be attributed to the formation of the intermolecular hydrogen bonding which we are proposing.

The intermolecular hydrogen bonding, in

which the methyl groups of pyruvate residues contribute, will be developed into an interaction between xanthan and D-galacto-D-mannan.^{9~11)} A possible structure of association sites between xanthan and D-galacto-D-mannan molecules will be discussed elsewhere.

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