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Conformation and Hydrolytic Stability of Polysaccharide from Xanthomonas campestris

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ABSTRACT

Xanthomonas campestris polysaccharide in the solid state is stable to 225°C in air and 250°C in inert atmosphere. In solution, even at moderate temperatures, the polymer undergoes hydrolytic degradation via the glycosidic linkages, and occurrence of main-chain scission results in lower solution viscosity. In solution, the polymer can exist in ordered and disordered conformations. In distilled water at temperatures > 50°C, the polymer exists in the disordered conformation. In the presence of salt, acid, or base the polymer exists in the ordered conformation. In the ordered conformation the polymer exhibits a far greater hydrolytic stability. The higher stability of the ordered conformation is especially demonstrated when the polymer is aged in acid or base solutions. Contrary to the expected lower stability of the glycosidic linkages in acid or base than in water, Xanthomonas campestris polysaccharide shows higher stability in these media.

INTRODUCTION

The polysaccharide produced by the microorganism Xanthomonas campestris (Xanthan) is used widely commercially as a viscosity-enhancing agent for aqueous solutions. It is a prime candidate as a mobility control agent in enhanced oil recovery. It maintains a high reduced viscosity (> 600 dl/g in water) over a broad range of temperature, ionic strength, shear, and pH. However, the polymer solution, aside from its many superior rheological properties, lacks stability even at moderately elevated temperatures. In the hope of stabilizing the polymer in solution so as to increase its useful temperature range we have investigated its mode of degradation.

The most recently proposed [1] primary structure of Xanthomonas campestris polysaccharide is shown in Fig. 1. The polymer can be considered as an anionic polyelectrolyte derivative of cellulose. The main chain contains β -(1-4)-linked D-glucose units, as in cellulose. The side chain is β -D-mannose-(1-4)- β -D-glucuronic acid-(1-2)- α -D-mannose-6-O-acetyl, and attached at C(3) to alternate glucose residue of the main chain. Pyruvate is attached on the average to about one-half of the terminal mannose residue; O-acetyl groups correspond to one residue for each pentasaccharide repeating unit. Because of this periodicity of covalent structure, the polymer, like proteins, also possesses secondary and tertiary structures and can exist in different

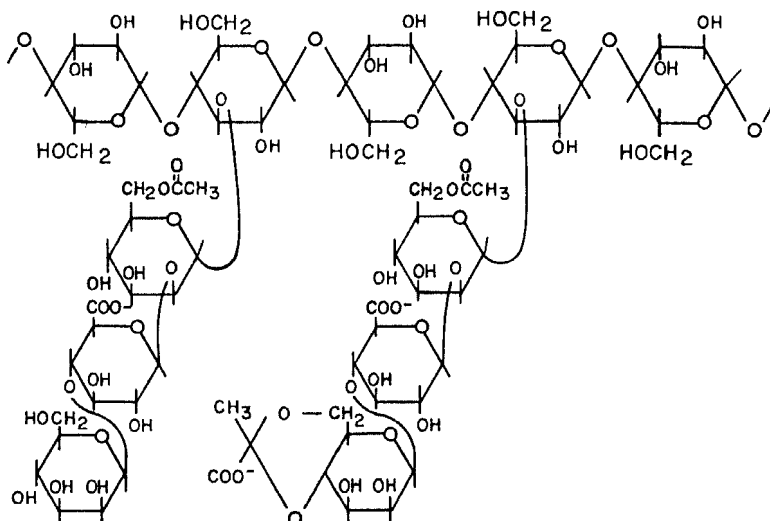


FIG. 1. Structure of Xanthomonas campestris polysaccharide.

conformations. The secondary structure is the shape of the single polysaccharide strand, the tertiary structure is the way the single strands fit together. Depending on the temperature and the presence of electrolytes [2-7] *Xanthomonas* polysaccharide can exist in ordered and disordered conformations. The structures of the ordered and the disordered conformations have been discussed by various investigators [4-6]. The polymer probably exists in a helical form in solution although the details of the conformations have not been clarified. The existence of the conformations and the concept of the order/disorder transition in solution, however, offer a satisfactory explanation of the outstanding rheological properties of *Xanthomonas* polysaccharide [2-7]. We have investigated the effect of conformation of the polymer on its stability. In a previous communication [8] we have reported that the ordered conformation has a greater stability towards shear stress. As the primary structure shows, the polymer contains many glycosidic linkages both in the main chain and the side chains. These glycosidic linkages are susceptible to hydrolysis which will result in degradation of the polymer and decrease in solution viscosity. We have found that the ordered conformation has higher stability towards thermal hydrolytic degradation. The results are discussed in this communication.

EXPERIMENTAL

Material and Preparation of *Xanthomonas campestris* Polysaccharide Solutions

Xanthomonas campestris polysaccharide used was Keltrol, which is the food grade polymer with the bacterial cells removed, manufactured by Kelco. Since the method of dissolution of this polymer affects the solution homogeneity and viscosity, a brief description is given here. A concentration of 0.1% (w/v) polymer in different concentrations of H^+ , OH^- and electrolytes was employed throughout this investigation. No bactericide or other additives were used in the solutions. A stock solution of 0.2% Keltrol was prepared by dissolving the polymer (the amount of which was corrected for the moisture content as determined by TGA) in distilled water with stirring at room temperature until a homogeneous solution was obtained. The stock solution was stored under refrigeration. For viscosity measurements, stock solutions could be stored in a refrigerator for three weeks without any change in viscosity. For measurements of optical rotations, solutions were prepared immediately prior to use. Dilutions were made with distilled water or the appropriate electrolyte, HCl or NaOH solutions to make the 0.1% polymer solutions of desired salt concentration or pH.

Detection of Polymer Conformation in Solutions

The change of conformation was detected by measuring the viscosity and optical rotation of a polymer solution under different conditions, such as temperature, pH and salt concentration. Following Rees' procedure [10], a sharp change in viscosity and a corresponding change in optical rotation with the change of conditions is taken as an indication of a change of polymer conformation.

Viscosity Measurements

Viscosities were measured in sizes 200, 150, and 100 Cannon-Fenske viscometers. A volume of 10 ml solution was used and the temperature was controlled to $\pm 0.1^\circ\text{C}$. Relative viscosity (η_{rel}) at 0.1% polymer concentration was measured as a function of temperature, of salt concentration, and of pH.

Optical Rotation Measurements

Optical rotation as a function of temperature, of pH, and of salt concentration for 0.1% polymer solutions was measured on a Bendix ETL-NPL type 143A automatic polarimeter with light at 5461 Å. The instrument operates on the Faraday magneto-optic principle and is capable of resolution to 0.0001° rotation. Measurements were made in a cell of 5.0 cm path length equipped with a circulating water jacket for temperature control to $\pm 0.02^\circ\text{C}$, and specific rotation $[\alpha]_{546}$ was calculated. Attempts to measure optical rotation at higher polymer concentrations were not successful because of increasing opaqueness of the solutions with concentration.

Determination of Hydrolytic Stability of Polymer Solutions

The hydrolytic stability of the polymer under a given set of conditions was followed by measuring the change of relative viscosity with time. An amount of 10 ml of polymer solution was pipetted into a size 150 Cannon-Fenske viscometer equipped with septum caps for both openings. The viscometer was mounted and immersed into a constant temperature bath. After the initial relative viscosity was recorded, the solution was aged for a total of ~ 1000 hr, and the relative viscosities were recorded at appropriate intervals. The change of viscosity with time was determined for 0.1% Ketrol solutions at

60°C and 75°C (1) in water, (2) in different concentration of NaCl and in 6% mixed brine (3.86% NaCl, 1.54% CaCl₂, and 0.6% MgCl₂), and (3) at different pH's.

RESULTS AND DISCUSSION

Stability in Solid State

While our primary interest was to investigate the stability in solution, some experiments were performed on the solid polymer to detect the intrinsic stability of the chemical bonds. Figures 2 and 3 show that the polymer as a solid is stable up to 250°C in N₂ and up to 225°C in air.

Conformation of *Xanthomonas campestris* Polysaccharide in Solution

In Distilled Water at Ambient pH = 5.5. Figure 4 shows plots of relative viscosities in distilled water as a function of temperature for two viscometer sizes, representing two shear rates. Size 100 corresponds to a shear rate of $1.27 \times 10^5/t$ (sec⁻¹) and size 200 to $2.96 \times 10^4/t$ (sec⁻¹), where t is the flow time in seconds [9]. In our experiments in H₂O, t ranges from 700 to 900 sec in size 100 viscometer, corresponding to a shear rate range of 140 to 180 sec⁻¹; t ranges from 50 to 70 sec in size 200, corresponding to a shear rate range of 420 to 590 sec⁻¹. In Fig. 4 higher viscosities were obtained at lower shear rates (upper curve). At both shear rates, η_{rel} increases from 30 to 60°C and decreases as temperature further increases to 75°C. Figure 5 is a plot of optical rotation as a function of temperature. The sharp increase in viscosity (Fig. 4) and the sharp decrease in optical rotation (Fig. 5) between 50 and 60°C are in agreement with the literature [10] and are attributed to a conformation transition.

In Salt Solutions at Ambient pH = 5.5. Figure 6 shows relative viscosity-temperature plots for 0.1% Keltrol in 10% NaCl and 6% mixed brine solutions. In both solutions, t ranges from 200 to 400 sec in a size 100 viscometer, corresponding to a shear rate range of 310 to 640 sec⁻¹; t ranges from 20 to 50 sec in the size 200 instrument, corresponding to a shear rate range of 590 to 1480 sec⁻¹. In salt solutions, distinctly different from what has been observed in distilled water (Fig. 4), viscosities at both shear rates decrease linearly with temperature. Figure 7 shows that only a small amount of NaCl (0.5%) decreases the viscosity drastically and

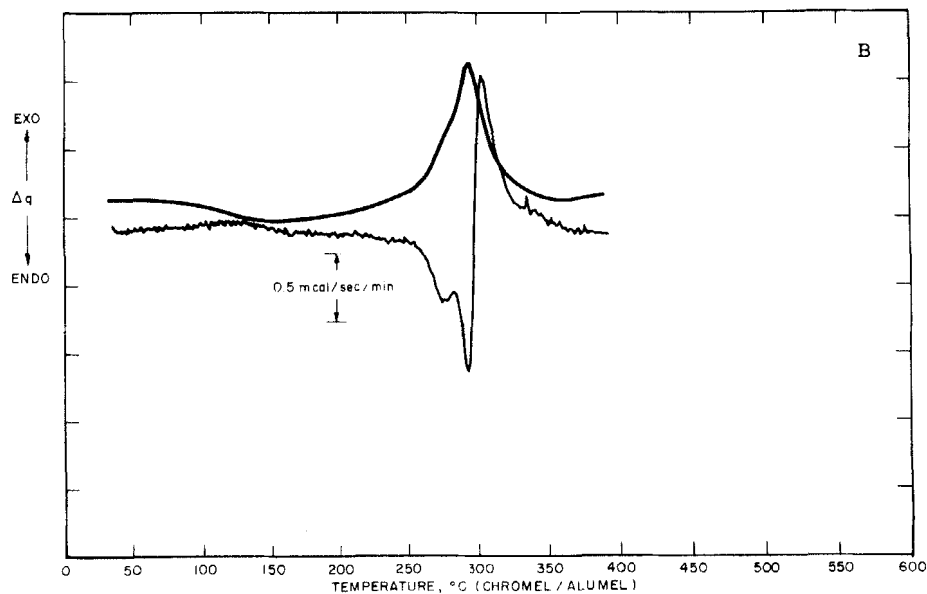
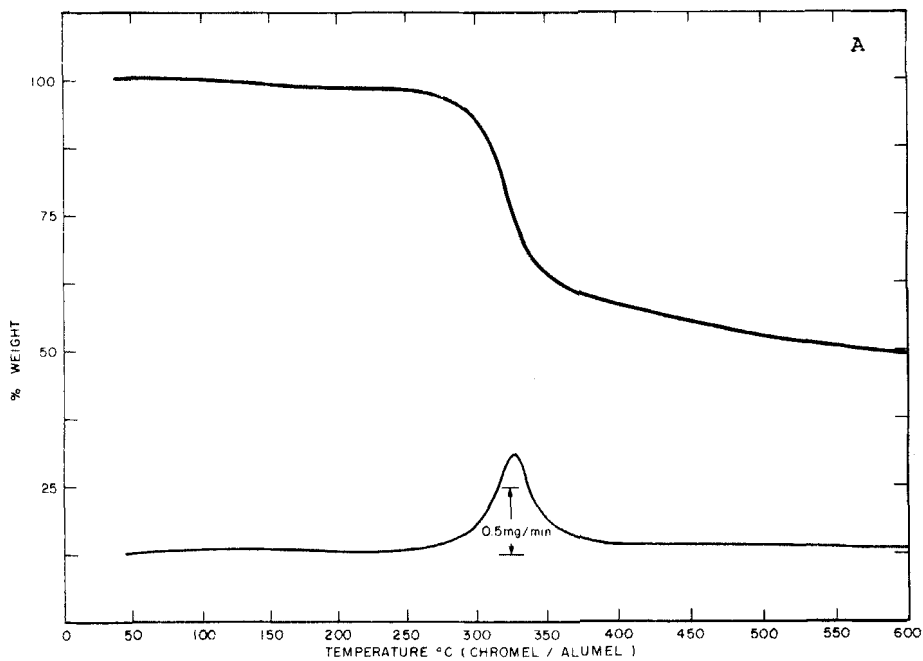


FIG. 2. Thermal stability of Keltrol after drying: (A) TGA in N_2 , sample weight 7.05 mg, heating rate $10^\circ C/min$; (B) DSC in N_2 , sample weight 2 mg, heating rate $20^\circ C/min$.

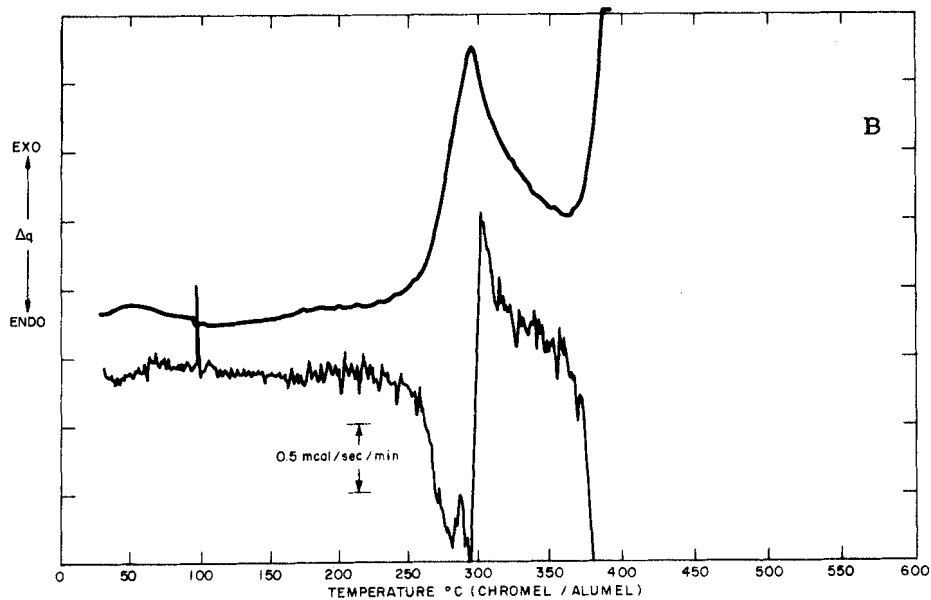
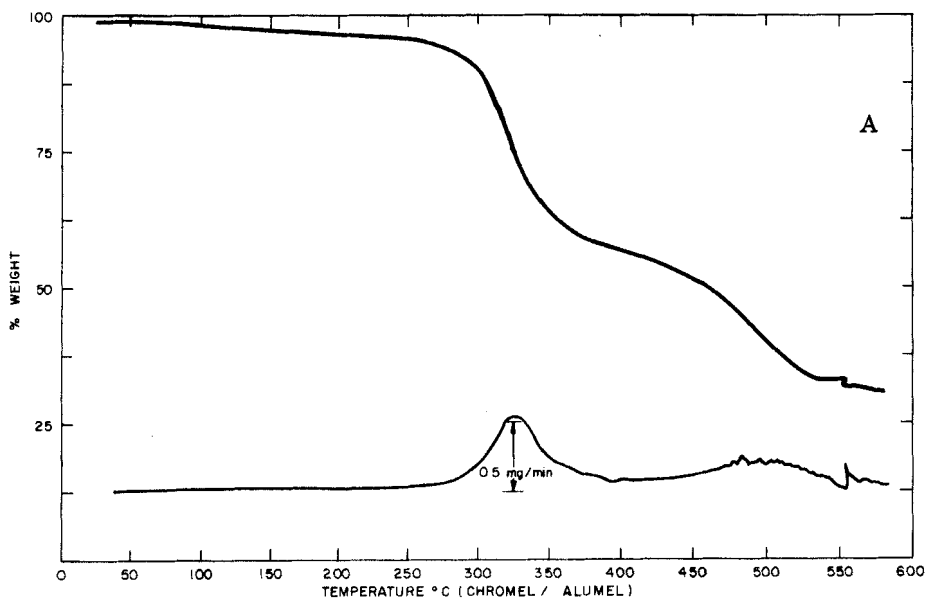


FIG. 3. Oxidative stability of dried Keltrol: (A) TGA in air, sample weight 9.3 mg, heating rate $10^{\circ}\text{C}/\text{min}$; (B) DSC in air, sample weight 2.0 mg, heating rate $20^{\circ}\text{C}/\text{min}$.

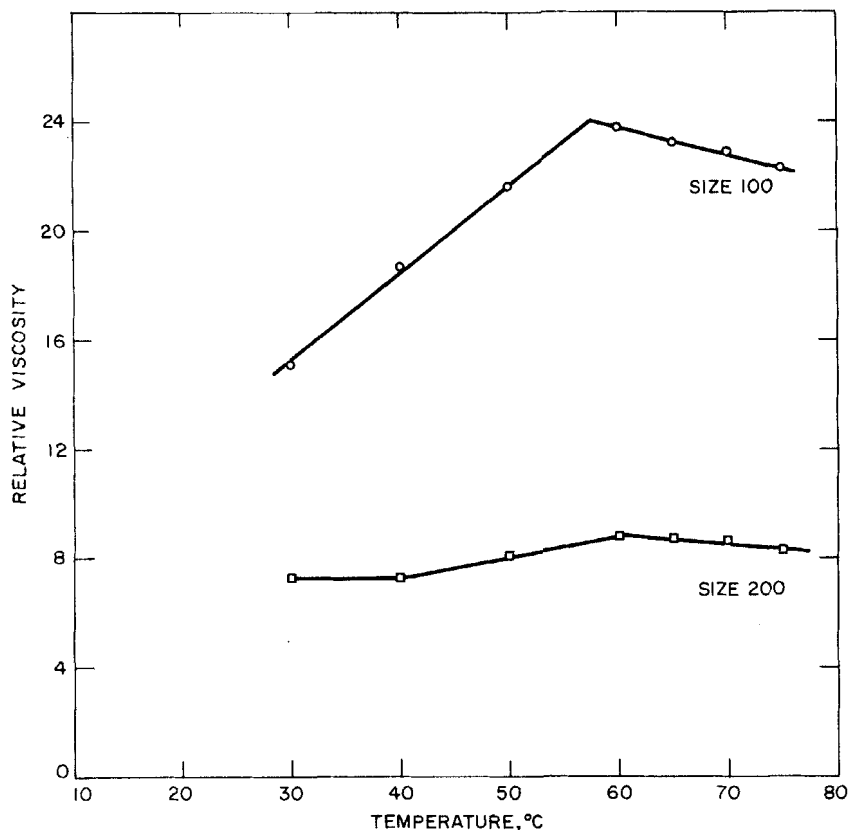


FIG. 4. Effect of temperature on relative viscosity of *Xanthomonas* biopolymer solution in H_2O . Keltrol, 0.1%; Cannon-Fenske viscometer, size 100 and size 200.

further increase in NaCl concentration does not decrease the viscosity further. This behavior has been observed at three temperatures: 30, 60, and 75°C. Figure 8 is a plot of optical rotation as a function of NaCl concentration. It is shown that optical rotation increases with increasing NaCl concentration, reaching a maximum at ~5% NaCl. Optical rotation is an interaction effect between atoms; a higher degree of rotation presumably represents a more ordered conformation of the molecule. Therefore, it is indicated that in the presence of NaCl *Xanthomonas* polysaccharide exists in solution in a more ordered

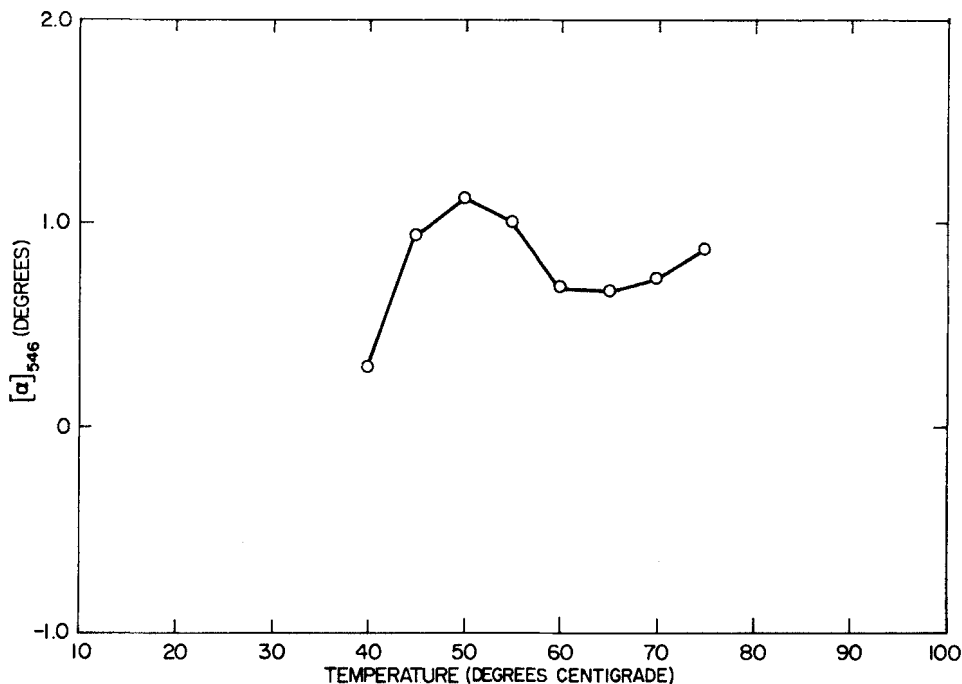


FIG. 5. Effect of temperature on optical rotation of Xanthomonas biopolymer in water. Keltrol, 0.1%; temperature, 30°C.

conformation (higher optical rotation) which also has a lower hydrodynamic volume (lower viscosity).

At Different pH's. Figure 9 depicts viscosity-pH profiles at 30, 60, and 75°C. A pronounced maximum viscosity is displayed at ambient pH = 5.5. Figures 10 and 11 show viscosity-temperature plots at different pH's. It is seen that at near neutral pH, e. g., 6.05 in Fig. 10 and 8.80 in Fig. 11, a similar viscosity-temperature relationship to that in H₂O at ambient pH = 5.5 (Fig. 4) is obtained, but at either high or low pH, linear relationships are observed. Figure 12 shows the change of optical rotation as a function of pH. Highest rotation is obtained at the lowest pH. A sharp drop is observed from pH 1 to 7 reaching a minimum at about 7, followed by a more gradual decrease from pH 7 to 13. These results in viscosity and optical rotation suggest that a more ordered conformation exists either at low pH's or at high pH's, especially at low pH's. In the

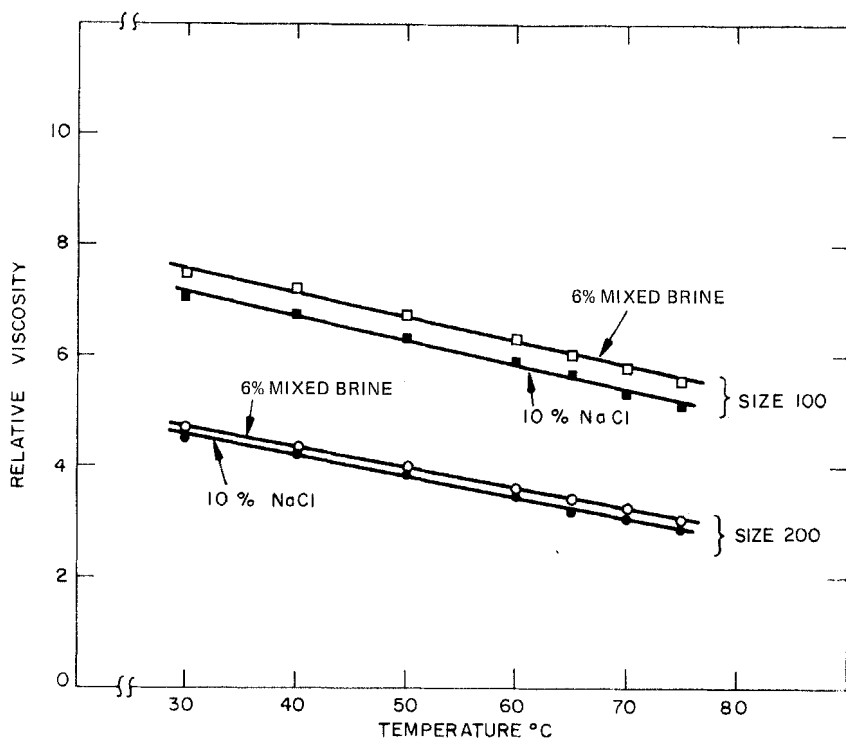


FIG. 6. Effect of temperature on relative viscosity of *Xanthomonas* biopolymer in 10% NaCl and 6% mixed brine. Keltrol, 0.1%; Cannon-Fenske viscometer, size 100 and size 200.

near neutral range, e. g., pH = 5.5, 6.05, or 8.80, the polymer assumes a more disordered conformation.

Chemical Stability of *Xanthomonas campestris* Polysaccharide in Solution

As described above, *Xanthomonas* polysaccharide showed considerable stability in the solid state. However, our experiments show that the solution viscosity decreases with time at elevated temperatures under most conditions. The decrease in viscosity is logically attributed to decrease in molecular weight due to hydrolysis of the glycosidic linkages in the polymer backbone and side chains. It is obvious that a break

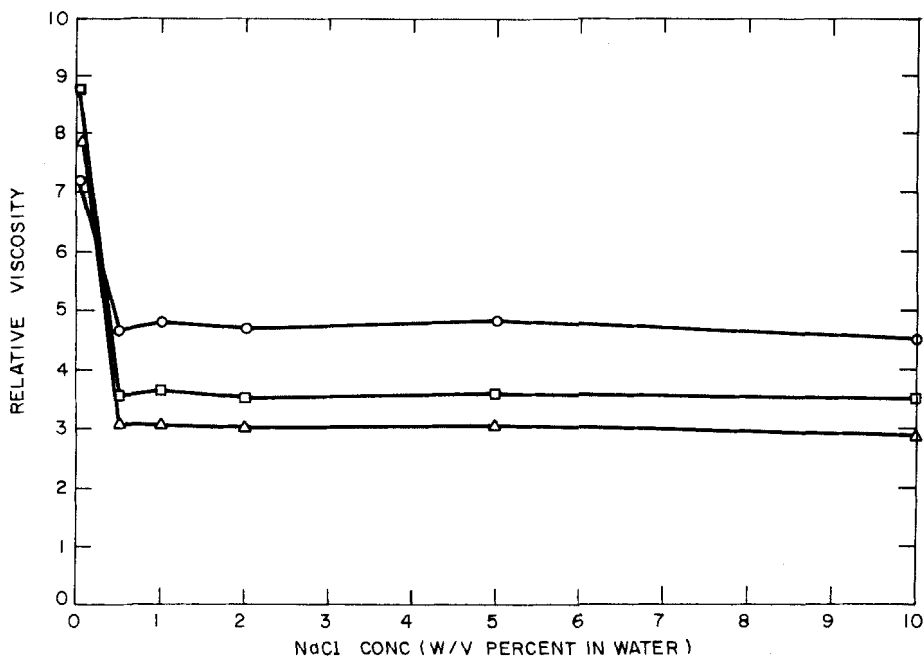


FIG. 7. Effect of NaCl concentration on viscosity of *Xanthomonas* biopolymer: (○) 30°C; (□) 60°C; (△) 75°C. Keltrol, 0.1%; Cannon-Fenske viscometer, size 200.

in the backbone will reduce the molecular weight more drastically than a break in the side chains, since the side chains contain only three sugar units (Fig. 1).

Effect of Conformation on Chemical Stability

We have discussed earlier that, depending on conditions, *Xanthomonas* polysaccharide exists either in a disordered or an ordered conformation, and we also have defined some conditions under which each conformation exists. In the following sections we shall discuss, in addition to the intrinsic stability of the chemical bonds, what effect the conformation of the molecule has on its stability.

Effect of Salt Concentration. Figures 13 and 14 depict comparisons of stability of 0.1% Keltrol solutions at 60°C and 75°C,

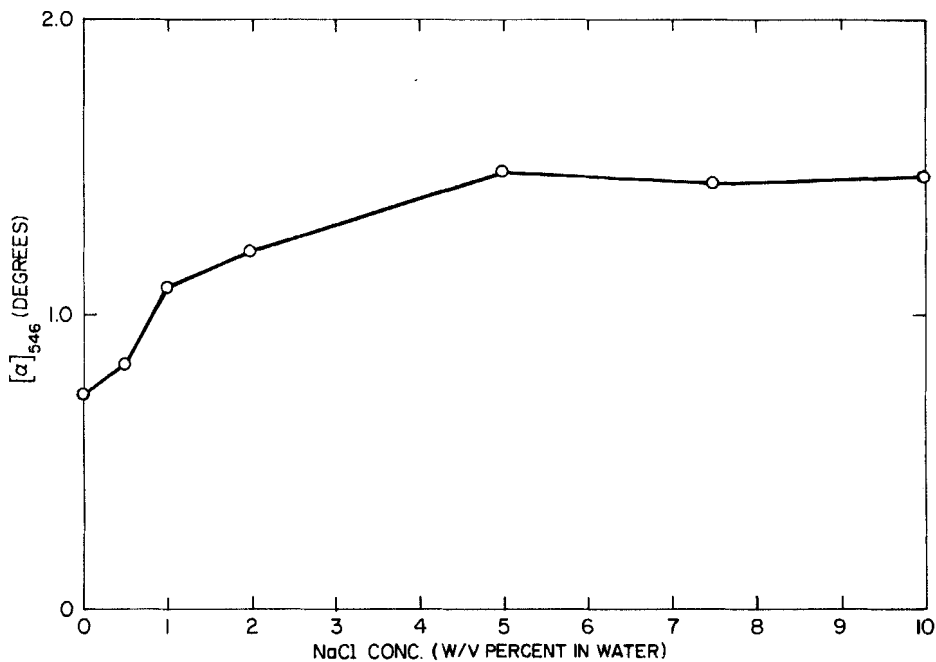


FIG. 8. Effect of NaCl concentration on optical rotation of Xanthomonas biopolymer. Keltrol, 0.1%; temperature, 30°C.

respectively, in water, where the polymer exists in a disordered conformation with relatively large hydrodynamic volume, in 10% NaCl, and in 6% mixed brine, where the polymer exists in an ordered conformation with smaller hydrodynamic volume. It is shown that in H₂O the high initial relative viscosity decreases rapidly with aging, especially at 75°C. In 10% NaCl or 6% mixed brine, the relative viscosities remain essentially constant with aging at 60°C and decrease gradually at 75°C. Since salts are not catalysts for hydrolytic degradation, the difference in stability is primarily attributed to the difference in conformation. The ordered conformation has higher stability.

Figure 15 displays relative viscosity-time plots at 60°C for 0.1% Keltrol solutions in increasing concentration of NaCl (0.5% to 10%). As previously shown in Fig. 8, the degree of optical rotation increases with increasing NaCl concentration indicating an increase in the order of conformation. Figure 15 shows corresponding increase in hydrolytic

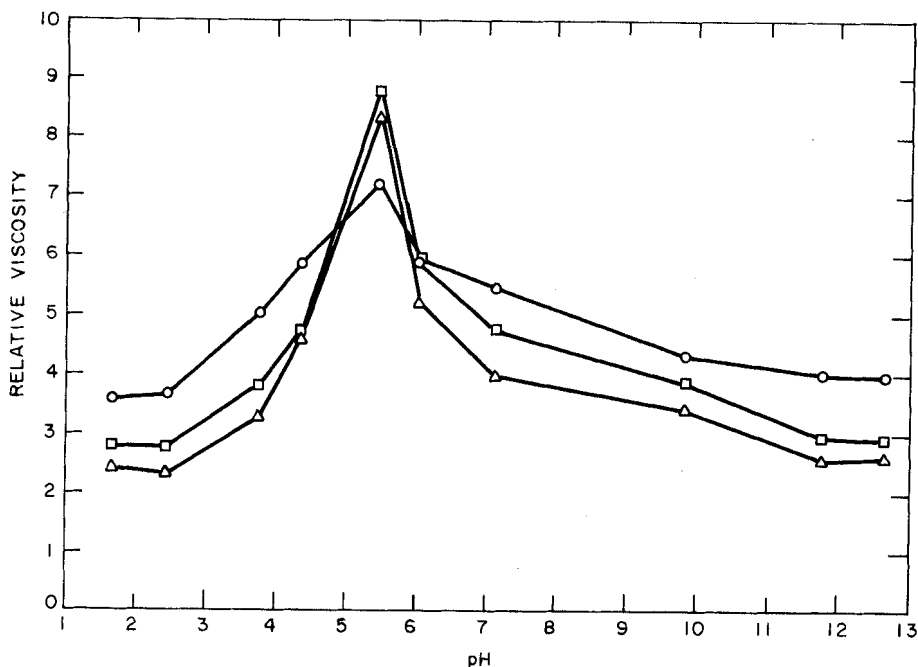


FIG. 9. Effect of pH on relative viscosity of *Xanthomonas* biopolymer in H₂O at different temperatures: (○) 30°C; (□) 60°C; (△) 75°C. Keltrol, 0.1%; Cannon-Fenske viscometer, size 200.

stability with NaCl concentration. The stability is in the order of 0.5% < 1.0% < 2.0% < 10%.

Effect of pH of Polymer Solution. Figures 16 and 17 show comparisons of hydrolytic stability of the polymer at 60°C in distilled water solution (ambient pH = 5.45) with those in acid solutions (pH = 2.45 and pH = 4.35) and in alkaline solutions (pH = 9.50 and pH = 11.35). It is observed that the polymer has the highest stability in either high or low pH regions, and the lowest stability in H₂O at ambient pH = 5.45. It is well established that hydrolysis of glycosidic linkages (acetal linkages) is catalyzed both by acid and by base, especially by acid. However, our results are the reverse of what is expected from the chemical stability of these linkages. As we have discussed above, at both high and low pH's the polymer exists in a more ordered conformation. Therefore, it is demonstrated that the conformation of the molecule has a pronounced effect

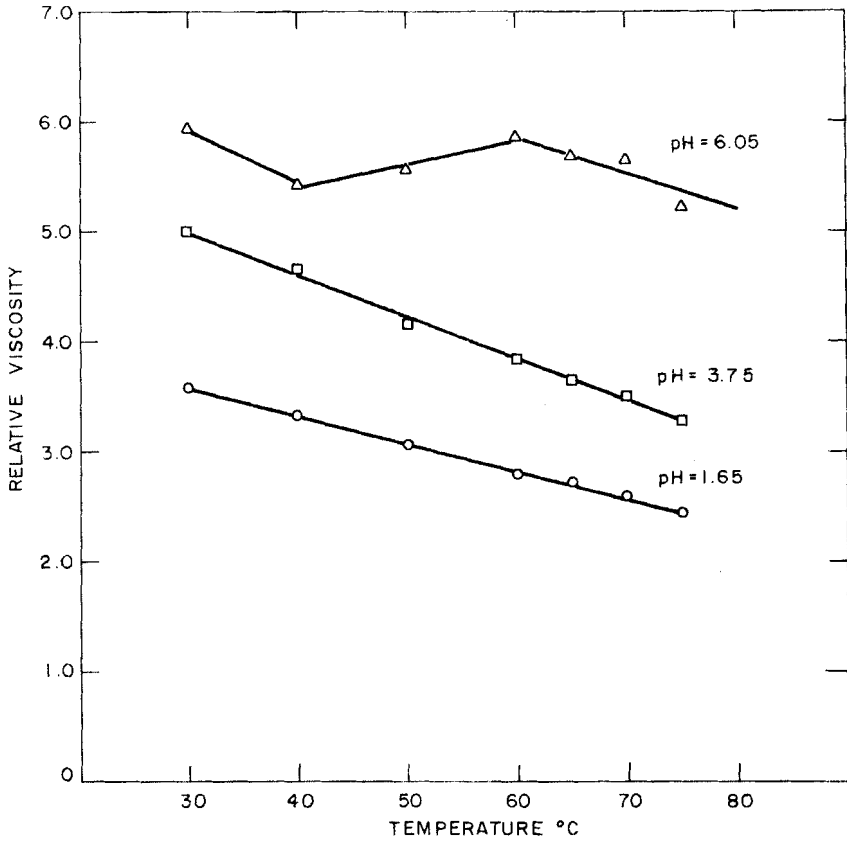


FIG. 10. Effect of pH on viscosity-temperature relationship of *Xanthomonas* biopolymer in acidic solutions in absence of NaCl. Keltrol, 0.1%; Cannon-Fenske viscometer, size 200.

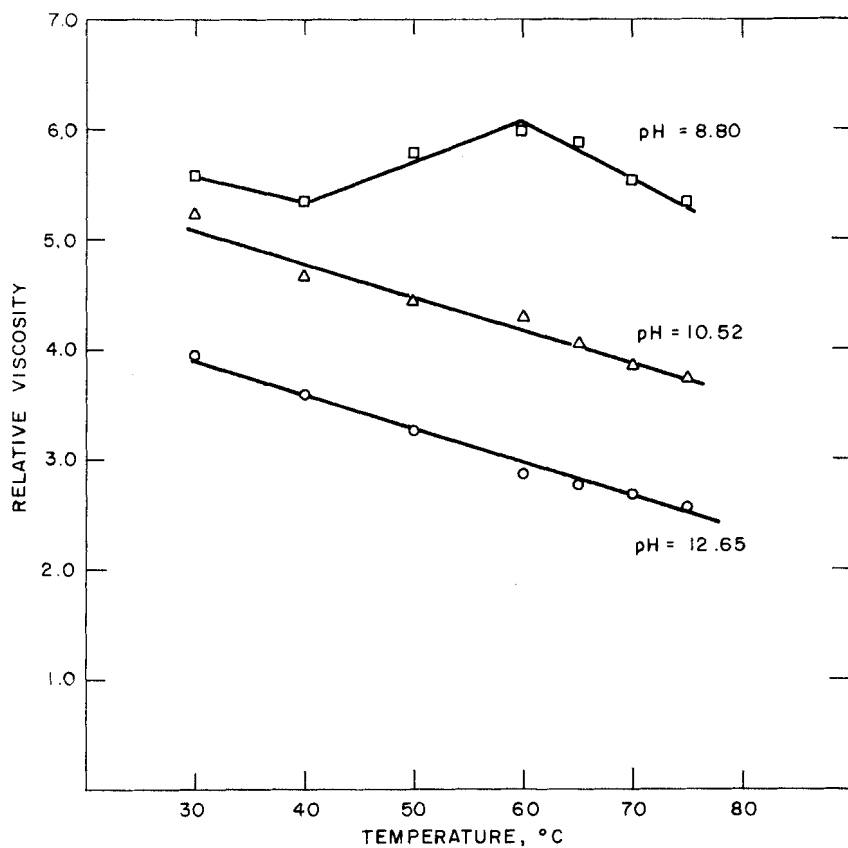


FIG. 11. Effect of pH on viscosity-temperature relationship of *Xanthomonas* biopolymer in alkaline solutions in absence of NaCl. Keltrol, 0.1%; Cannon-Fenske viscometer, size 200.

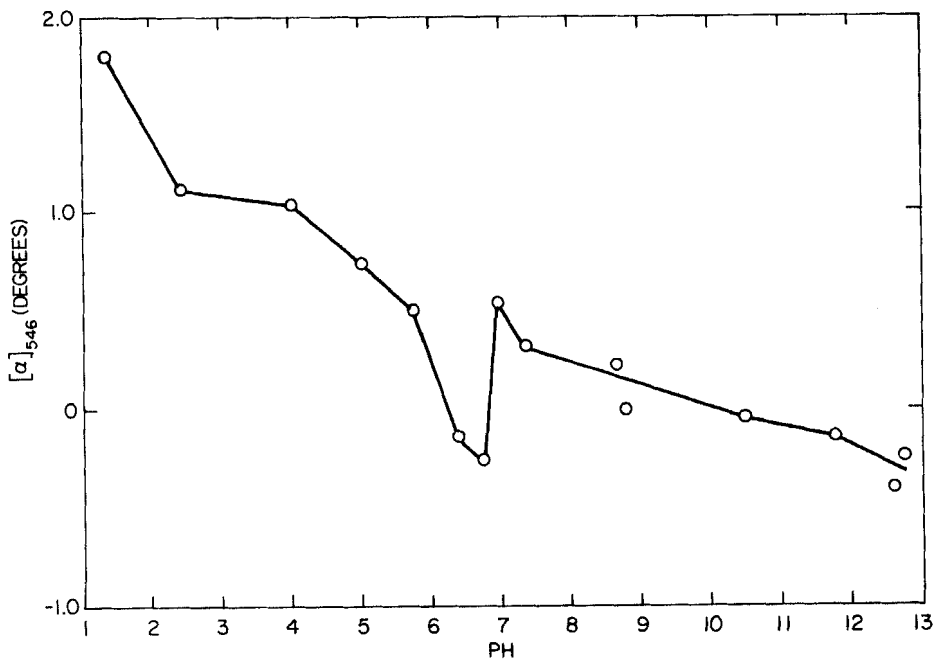


FIG. 12. Effect of pH on optical rotation of Xanthomonas biopolymer in water. Keltrol, 0.1%; temperature 30°C.

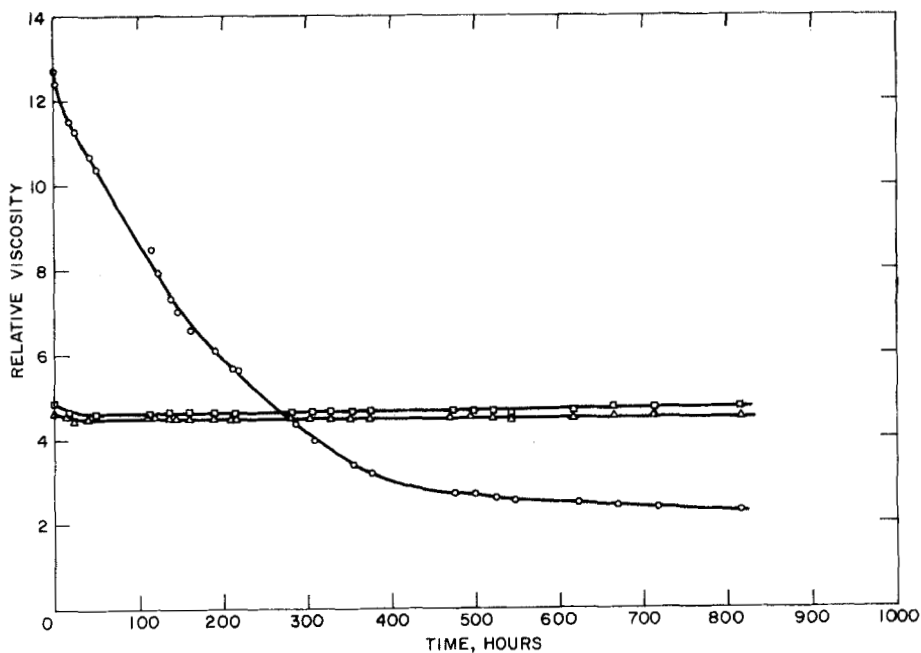


FIG. 13. Effect of conformation on viscosity and stability of *Xanthomonas* biopolymer at 60°C: (○) in H₂O (disordered conformation); (□) in 10% NaCl (ordered conformation); (△) in 6% mixed brine (ordered conformation). Keltrol, 0.1%; Cannon-Fenske viscometer, size 150.

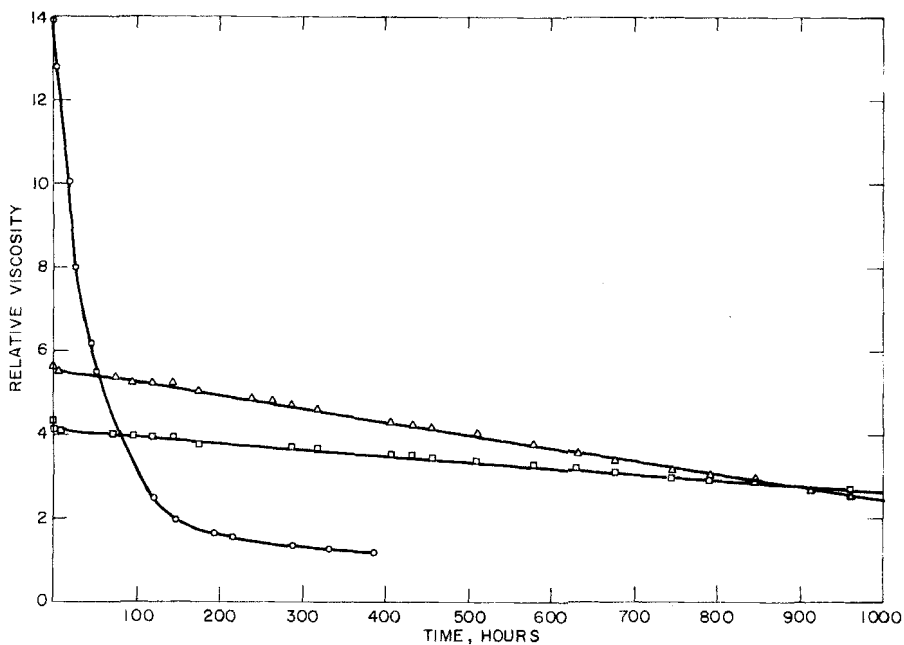


FIG. 14. Effect of conformation on viscosity and stability of *Xanthomonas* biopolymer at 75°C: (○) in H₂O (disordered conformation); (□) in 10% NaCl (ordered conformation); (Δ) in 6% mixed brine (ordered conformation). Keltrol, 0.1%; Cannon-Fenske viscometer, size 150.

on its stability. It appears that in the ordered conformation, the glycosidic linkages are more shielded from chemical attacks.

CONCLUSION

The outstanding solution rheology of *Xanthomonas campestris* polysaccharide has been related to the conformation of the polymers [2-5]. Rees and co-workers [2, 3] postulated that the polymer follows a conformational transition, ordered - disordered, when heated in aqueous solution based on the observation of a sharp rise in viscosity and a corresponding shift in optical rotation between 50° and 60°C. More recently Moorhouse et al. [4], proposed that the ordered conformation of xanthan in solid, and probably in

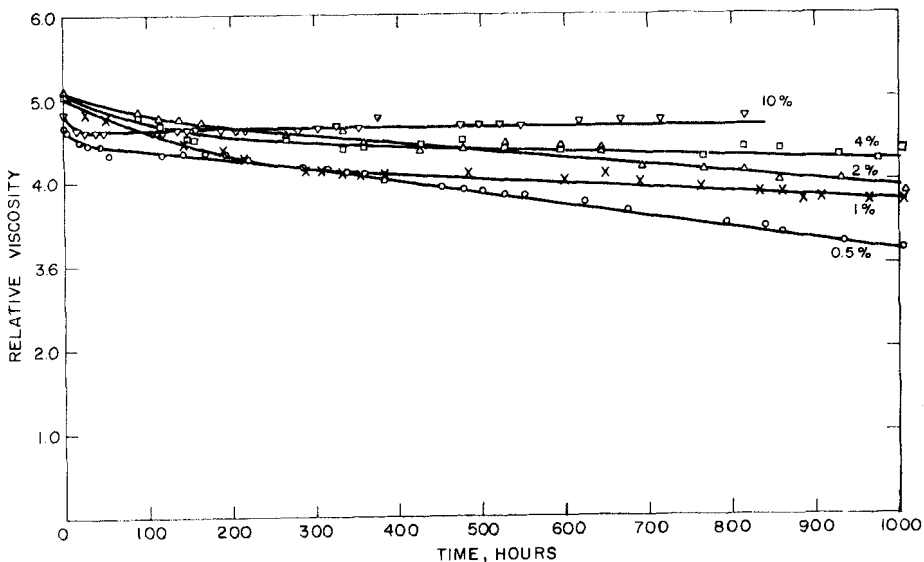


FIG. 15. Effect of NaCl concentration on viscosity stability of *Xanthomonas* biopolymer at 60°C: (○) 0.5%; (×) 1.0%; (△) 2.0%; (□) 4.0%; (▽) 10.0% NaCl. Keltrol, 0.1%; Cannon-Fenske viscometer, size 150; ambient pH = 5.5.

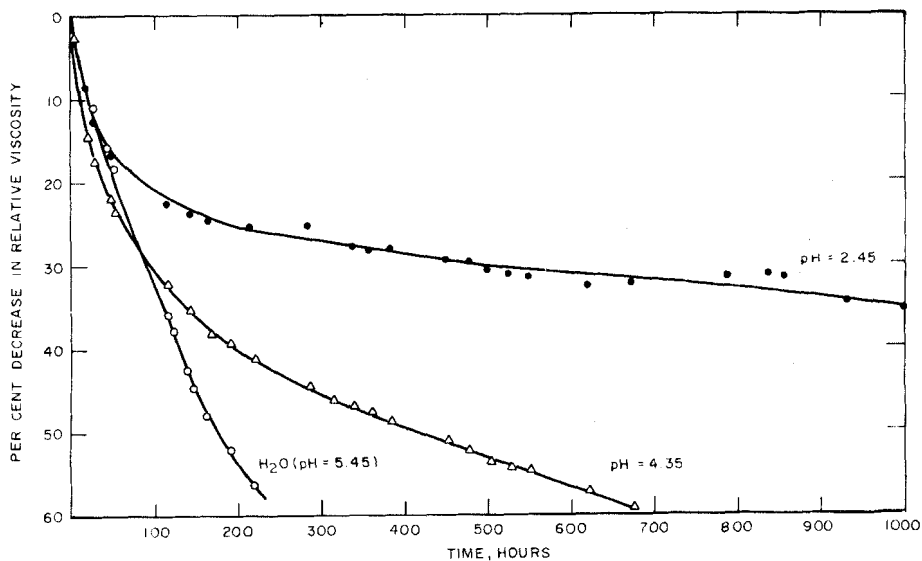


FIG. 16. Comparison of stability of *Xanthomonas* biopolymer in H₂O and acid solutions.

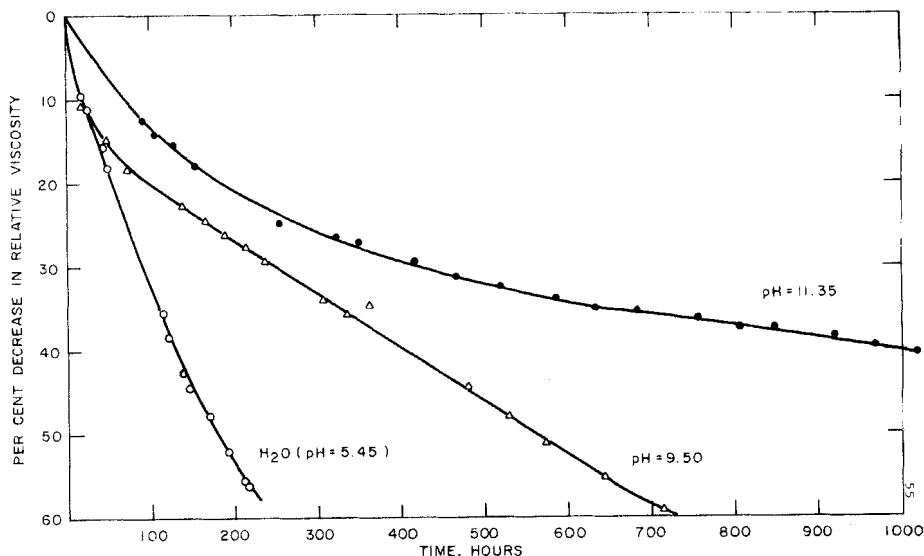


FIG. 17. Comparison of stability of *Xanthomonas* biopolymer in H₂O and alkaline solutions.

solution, is related to a 5/1 helix, with the side chains closely associated with the backbone. The authors suggested that the order/disorder transition seen with change of temperature in solution probably arises from conformational changes primarily within the side chain as it moves away from its close association with the ordered backbone. This extending of the side chains of the polysaccharide would cause an increase in hydrodynamic volume, and consequent increase in viscosity. Morris [5] similarly suggested that in the ordered conformation the charged trisaccharide side chains fold back around the cellulose backbone, to give a rigid, rod-like structure. The presence of salt stabilizes this conformation by minimizing electrostatic repulsions between the side chains. At the salt levels encountered in most industrial situations, the ordered form is stable to above 100°C; therefore, the viscosity is relatively insensitive to temperature. Holzwarth and Prestridge [6, 7] proposed that xanthan undergoes native-denatured-renatured transition and that native xanthan is composed of many subunit strands arranged in a right-handed double or triple helix. The conformational transition seen by optical activity and viscosity studies is accompanied by the dissociation of multistranded xanthan assembly (native - denatured).

In the presence of salt, the dissociated strands reassociate to a less perfect right-handed double or triple helix (denatured - renatured). Although the secondary and tertiary structures of xanthan are not yet clearly defined, the proposed structures for the ordered and disordered conformations are consistent not only with the rheological properties of xanthan polymer solution but also with the observed chemical stability in this investigation. In the ordered conformation the polymer main chain is more protected by the side chains. Although the side chains also contain hydrolyzable linkages, cleavage of a linkage will not cause nearly as much change in molecular weight and solution viscosity as in the main chain. In our investigation we also have found that xanthan exists in the ordered conformation in the presence of acid or base. The higher hydrolytic stability of the polymer in the presence of acid or base has demonstrated the significant magnitude of the effect of the conformation on the chemical stability of the polymer.

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