An attempt was made to use thiolated polyvinylalcohol (SH-PVA) for studying the role of SS linkage on the physical properties of foods and proteins. An aqueous solution of SH-PVA ranging in concentrations of 3% and above gelated on treatment with bromate within 10 min. The gel was analyzed by the creep test on the basis of a mechanical four-element model and was found to exhibit the property of non-linear viscoelasticity. The mechanical parameters of the gel were comparable to those of the agar gel. Change in the solubility of SH-PVA during oxidation were not parallel to the extent of the formation of SS linkages. Molecular behavior of the polymer was investigated in relationship to oxidation conditions. Oxidation at a concentration of 0.4% at pH 5.0 brought about a slight decrease in viscosity, while there was no change in the gel filtration pattern as compared with control. At the concentration of 2% the oxidation resulted in 1.5 times-increment in viscosity. This agreed with the data of gel filtration. This study provides more definitive evidence on the role of SS linkage on the gel structure.

Many investigators have reported the possible roles of the hydrogen bond, electrostatic force between ionic groups, hydrophobic interaction, and disulfide linkage on the physical properties of food (1). Disulfide linkage is a particularly important factor. Rheological properties of flour dough (2), rice (3), and soybean products (4) have been studied from this viewpoint. Foodstuffs are, however, multiple systems in which there are several complicated constituents, and their elucidation has not yet been satisfactory.

It was found that polyvinylalcohol was partially esterified with thioglycolic acid gelates on treatment with an oxidant. This polymer, which has been shown by the present authors to have a character behavior similar to that of proteins

1 This paper was presented at the Annual Meeting of the Agricultural and Chemical Society of Japan, Sendai, April, 1972.
2 国則博代, 西山宗子, 松本 博
in polarography (5), seems to be a convenient model compound for clarifying the role of SS linkage in the rheological properties of food.

The experiment was made by oxidizing thiolated polyvinylalcohol (SH-PVA) with potassium bromate. Bromate is considered appropriate because it is known to be a mild oxidant that stoichiometrically oxidizes sulfhydryl to disulfide without further oxidation in dough (6).

**MATERIALS AND METHODS**

*Chemicals.* The polyvinylalcohol used, Gosenol AL 02 (polymerization degree 270±30, 99.8% saponificated), was a product of the Nippon Synthetic Chemical Industry Co., Ltd., Amagasaki. Thioglycolic acid, purchased from Wako Pure Chemicals Ltd., Osaka, was freshly distilled under reduced pressure and its distillate at 108°C was used in this experiment. Agar, special Agar-Noble, was a product of Difco Laboratories, Detroit, Michigan.

*Preparation of thiolated polyvinylalcohol.* SH-PVA was prepared by esterifying polyvinylalcohol with thioglycolic acid in the presence of hydrochloric acid at 80°C for 1 hr. Details were described in a previous paper (5). Sulfhydryl contents of the products were found to be 5.3×10⁻⁴ to 8.4×10⁻⁴ eq per gram as shown in each section.

*Treatment of thiolated polyvinylalcohol with bromate.* A stock solution of approximately 8% SH-PVA was prepared. Its exact concentration was determined by evaporating it to dryness at 105°C. It was diluted with buffer and water to a desired concentration. Oxidation was carried out by adding potassium bromate in amounts of 0.5, 1.0, or 2.0 equivalents of the sulfhydryl of the sample; the solution was then allowed to react at 30°C. In creep test and turbidimetry, reactions were carried out in a U tube and cuvette, respectively.

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**Fig. 1.** Apparatus for the creep test. A: U tube for sample, B: Air reservoir, C: Rubber bulb for pressure, D: Kerosene manometer, E: Capillary containing Brodie liquid, a, b, c, d: Glass stopcocks.
Agar gel. Agar gel for the creep test was prepared in a U tube by dissolving 100 mg of agar in 25 ml of water in a boiling water bath and allowing it to stand for 3 hr at 30°C until analysis.

Creep test. The creep test was undertaken by using the apparatus shown in Fig. 1. Sample gel in the U tube (A) was pushed by a constant pressure (P) which was controlled by means of the reservoir (B) and a kerosene manometer (D). Instantaneous gel deformation was accompanied by a slow and time-dependent one. Successive readings of the movement of the Brodie liquid in a capillary tube (E) were taken at approximately 5 sec intervals for 120 sec. Creep compliance, $J_t$, was expressed as shown in Eq. (1)

$$J_t = \frac{\gamma}{F} = \frac{4Q}{\pi R^3} \cdot \frac{2L}{PR}$$

Eq. (1)

in which $F$=shearing stress, $\gamma$=strain, $L$=length of the sample gel in the U tube, $R$=inner radius of the U tube, $P$=net pressure given on the gel, and $Q$=volume of the displaced gel.

Further, if the cell constant, $K$, is expressed in the form of Eq. (2), Eq. (1) is replaced by Eq. (3) and therefore $J_t$ can be calculated by substituting the estimated values, $L$, $h$, and $\varepsilon$.

$$K = \frac{8a^2}{\rho g R^4}$$

Eq. (2)

in which $a$=radius of the capillary, $\rho$=density of kerosene, and $g$=gravity.

$$J_t = K \cdot \frac{L\varepsilon}{h}$$

Eq. (3)

in which $\varepsilon$=movement of Brodie liquid in the capillary and $h$=reading of the kerosene manometer.

A model creep curve is shown in Fig. 2A. From the figure, mechanical parameters are evaluated according to Eq. (4) and Eq. (5) on the assumption of

![Fig. 2. Model creep curve (A) and mechanical four-element model (B).](image-url)
the mechanical four-element model shown in Fig. 2B.

\[ J_t = J_0 + J_1(1 - e^{-t/\tau}) + \frac{dJ}{dt}.t = \frac{1}{G_1} + \frac{1}{G_2}(1 - e^{-t/\tau}) + \frac{1}{\eta_3}t \]  
Eq. (4)

\[ \tau = \frac{\eta_3}{G_2} \]  
Eq. (5)

in which \( J_t \) = total compliance, \( J_0 \) = instantaneous elastic compliance, \( J_1 \) = retarded elastic compliance, \( G_1 \) = instantaneous modulus, \( G_2 \) = retarded elasticity, \( \eta_3 \) = retarded viscosity, \( \eta_s \) = Newtonian viscosity, and \( \tau \) = retardation time.

**Measurement of turbidity.** Turbidity of the gel was determined by measuring absorbance at 570 nm with an Hitachi Spectrophotometer, type 102.

**Determination of sulfhydryl and disulfide groups.** Sulfhydryl and disulfide were determined by argentometry in Tris-HNO₃ buffer (pH 7.4) (7) containing 8 M urea.

**Measurement of viscosity.** Viscosity was measured with an Ostwald viscometer at 30°C. As a control experiment, SH-PVA to which had been previously added NEM was used.

**Gel filtration.** Four milliliters was put on a column (25 mm × 40 cm) which was prepared by equilibrating Bio-Gel P-300 (50–100 mesh) in 1/15 M acetate buffer (pH 5.0). The same buffer was used as an eluent at a flow rate of 12 ml per hr. A portion of each 5 ml-fraction was previously tested by the lead acetate reaction for detecting sulfur compound and the rest was titrated with silver nitrate.

**RESULTS**

**Gelation of SH-PVA by oxidation**

The solution of SH-PVA remains homogeneous, clear and fluid even at a concentration of 8%. Changes in solubility and/or viscosity, however, take place

![Gels of oxidized SH-PVA.](image)

4% SH-PVA 4% SH-PVA 4% SH-PVA 3% SH-PVA
1 eq KBrO₃ 2 eq KBrO₃ 2 eq KBrO₃ 2 eq KBrO₃
pH 5.0 pH 5.0 pH 7.0 pH 5.0

Fig. 3. Gels of oxidized SH-PVA. Aqueous SH-PVA (6.7 × 10⁻⁴ eq SH/g), 4% (w/v) (A, B, C) and 3% (D), were treated with 1 eq (A) or 2 eq (B, C, D) of potassium bromate in 1/15 M acetate buffer (pH 5.0) (A, B, D) or 1/15 M phosphate buffer (pH 7.0) (C) for 60 min at 30°C.
by addition of oxidants such as bromate or hydrogen peroxide. Colloidal precipitation or an increase in viscosity occurs in diluted SH-PVA and gelation takes place in SH-PVA of concentrations of more than 3%. Examples of the gels are shown in Fig. 3.

Properties of the gels are likely to be dependent on pH, and amount of oxidant. Thus, an acidic gel is sticky and soft and an neutral one is turbid and fragile. Concentration of SH-PVA also affected the property of the gels to a great extent.

Change in the turbidity of the gels prepared under various conditions are summarized in Fig. 4.

It is interesting to note that the turbidity increased more rapidly in a neutral medium than in an acidic one. The same results were obtained in more diluted solutions.

**Rheological analysis of the gels**

An attempt was carried out to characterize the gels in rheological parameters. Sample gels were prepared by treating 3 or 4% SH-PVA solution with...
Fig. 5. Creep curves for the gels of oxidized SH-PVA. SH-PVA (5.3 × 10^{-4} eq SH/g) was treated with 1 or 2 eq of potassium bromate in 25 ml of 1/15 M acetate buffer (pH 5.0) or 1/15 M phosphate buffer (pH 7.0) at 30°C for 60 min. The reaction was carried out in the U tube. Gel were tested under the shearing stresses between 12.5 and 100 dyne/cm². A: 4% SH-PVA in 1/15 M acetate buffer (pH 5.0), B: 3% SH-PVA in 1/15 M phosphate buffer (pH 7.0). Open symbols: 1.0 eq of bromate to SH of the sample, closed symbols: 2.0 eq of bromate.
1 or 2 eq of bromate to the SH of the sample. The creep curves are shown in Fig. 5. In the case of 3% SH-PVA at pH 5.0, an abnormal deformation of the gel precluded the test and in the case of 4% SH-PVA at pH 7.0 a slip of the gel caused it.

The creep curves were analysed by means of the mechanical four-element model (8). Table 1 summarizes the parameters. Although no definitive indication was found in the parameters it is clear that most of the parameters increase with increasing stress. This dependence of the parameters upon shearing stress indicates that the gels have the property of non-linear viscoelasticity.

Table 1. Rheological parameters for gels of oxidized SH-PVA. Creep curves for each sample partly shown in Fig. 5 were analyzed on the assumption of four-element model.

| Experiment | Sample gels | Shearing stress | Parameters |
|------------|-------------|----------------|------------|
| A          | SH-PVA 1 eq/SH | 12.5 dyne/cm² | 2.2 dyne ×10⁵/cm | 1.5 dyne ×10⁵/cm | 2.3 poise ×10⁴ | 5.7 poise ×10⁴ | 14.0 sec |
| A          | SH-PVA 4% | 25.0          | 2.9 dyne ×10⁵/cm | 4.1 poise | 5.8 poise | 7.0 sec | 15.0 |
| A          | SH-PVA 4% (pH 5.0) | 50.0 dyne/cm² | 5.3 dyne ×10⁵/cm | 5.1 poise | 11.9 poise | 7.9 sec | 23.5 |
| A          | SH-PVA 4% (pH 5.0) | 100.0         | 5.9 dyne ×10⁵/cm | 6.7 poise | 16.0 poise | 43.5 sec | 24.0 |
| B          | SH-PVA 1 eq/SH | 12.5 dyne/cm² | 2.9 dyne ×10⁵/cm | 5.9 poise | 11.7 poise | 4.0 sec | 20.0 |
| B          | SH-PVA 3% | 25.0          | 5.9 dyne ×10⁵/cm | 14.7 poise | 24.2 poise | 9.9 sec | 16.5 |
| B          | SH-PVA 3% (pH 7.0) | 50.0 dyne/cm² | 9.3 dyne ×10⁵/cm | 50.0 poise | 55.0 poise | 10.5 sec | 11.0 |
| B          | SH-PVA 3% (pH 7.0) | 100.0         | 14.5 dyne ×10⁵/cm | 71.5 poise | 111.7 poise | 25.8 sec | 9.5 |

It is noteworthy to compare the gel with an agar gel on the parameter. The gel prepared with 0.4% agar was found to have 8×10⁴ dyne/cm² of G₁ and G₂, 2×10⁴ poise of γ₂, 9×10⁴ poise of γ₃ and 26 sec of τ at the shearing stress of 50 dyne/cm², which are comparable to those of SH-PVA. The result suggests that SS linkage is able to play a predominant role in supporting the gel structure. Actually the gels were redissolved by the addition of sodium sulfite to a final concentration of 0.1 M. No gelation appeared with bromate when NEM was previously added.

Change in sulfhydryl during oxidation of SH-PVA

Since gelation precludes titration of SH-PVA, ranging in concentration from...
0.4 to 2\%, was used in this experiment. In a late stage of the reaction at pH 7.0 precipitation disturbed titration. Sulfhydryl plus half disulfide was expressed as total sulfhydryl. Thus it remained constant all through the experimental period, 60 min, without further oxidation even in the presence of excess bromate. Percent of the free sulfhydryl to total sulfhydryl was plotted against the reaction time as shown in Fig. 6.

From a comparison of Fig. 6 and Fig. 4 it was found that the oxidation of sulfhydryl and increase in turbidity were not parallel. While the difference in oxidation between pH 5.0 and pH 7.0 is little, solubility was influenced by pH to a great extent. The fact that the precipitate was redissolved with excess sulfite must be an evidence that the solubility change was also brought about by the SS linkages. Further, the pH dependence of solubility may indicate that the formation of SS linkage changes the hydrated environment of the SH-PVA molecule in a manner dependent on pH, and that turbidity is brought about by a subsequent rearrangement of the SH-PVA molecule into a compact shape.

**Effect of oxidation on the molecular behavior of SH-PVA**

The molecular behavior of oxidized SH-PVA was investigated by measuring the viscosity and gel filtration pattern.

a. **Viscosity.** Change in viscosity was studied by using 0.4, 1 and 2\% of
SH-PVA treated with 2 eq of bromate to SH of the sample at pH 5.0. Reaction was stopped at 30 min by addition of excess NEM to SH in the original sample when the precipitate had scarcely appeared and all of the SH groups had been oxidized, as seen in Fig. 6.

Figure 7 was drawn by plotting the reduced viscosities $\eta_{sp}/c$ vs. concentration of SH-PVA ($c$).

![Graph](image)

Fig. 7. Reduced viscosities of oxidized SH-PVA under various concentrations. 0.4% (●), 1.0% (○), and 2.0% (◇) of SH-PVA ($6.7 \times 10^{-4}$ eq SH/g) were treated with 2 eq of bromate to SH of the original sample in 1/15 M acetate buffer (pH 5.0) for 30 min at 30°C. After reaction they were diluted with 1/15 M acetate buffer (pH 5.0) to each concentration seen in the figure and their viscosities were determined. Control experiment (●) was carried out by using the sample previously added NEM.

The reduced viscosity is more or less dependent on the concentration of oxidized SH-PVA under the conditions employed. Intrinsic viscosity was obtained by the extrapolation of the linear $\eta_{sp}/c$ vs. $c$ plot. The intrinsic viscosity of the untreated SH-PVA (control) was 28 ml/g and that of the oxidized product varied with the concentration at which the oxidation was carried out.

The intrinsic viscosity of 1% SH-PVA oxidized was close to that of control. Two following possibilities are proposed. The first is that the reaction between intramolecular SH groups proceeded without change in molecular size, and the second is that both the intramolecular and intermolecular disulfides were formed at the same time, being available for increasing molecular weight and for rearranging molecule to a oriented configuration, respectively. The second is more likely because an increase in the molecular size was demonstrated in the following experiment.
In the case of 2% SH-PVA, the intrinsic viscosity of the oxidized product was 45.8 ml/g. This finding may imply that the formation of intermolecular SS linkages mainly occurred at this concentration.

When oxidation was carried out at a concentration of 0.4% decrease in the intrinsic viscosity was observed. It may be reasonable to consider that the molecule was changed to an oriented configuration by the resulting intramolecular SS linkages.

b. Gel filtration. Figure 8 represents the gel filtration patterns of bromate-treated and untreated SH-PVA.

![Gel filtration pattern](image)

All of the sulfhydryls in the original sample were recovered as half disulfides in the oxidized products as seen in the figure.

Untreated SH-PVA (control) was eluted ranging from 30 to 200 ml without any particular peak. This feature might be due to a poly-dispersion of the polymer.

In the cases of 1% and 2% SH-PVA, the samples were eluted as a single peak at a position prior to that of control, whereas in the case of 0.4% SH-PVA, the elution pattern nearly overlapped that of the control.

These results might indicate that oxidation at low concentrations causes intramolecular disulfide and that at high concentration causes intermolecular disulfide. A similar feature has been reported in flour proteins (9, 10).
DISCUSSION

While it has been generally accepted that electrostatic force plays an important role in the gel structure, the participation of SS linkages has been also proposed. HUGGINS et al. (11) showed that an aqueous solution of bovine plasma albumin gelated in the presence of a small amount of simple thiol. LEVITT (12) suggested that the three-dimensional network through SS linkages fortified the gel structure of gelation from the consideration of the results obtained by using the thiolated gelatin.

Although the creep test used in this study failed to provide a quantitative explanation for the physical properties of the gels the results may lend some support to the important contribution of SS linkages to the physical properties of proteins and foodstuffs. This was also evidenced by viscosity measurement and gel filtration.

Disulfide linkage has been employed as a primary factor in insolubilizing denatured protein (9, 13). It may not, however, be the sole factor but the ratio of intermolecular disulfide to the intramolecular one and the subsequent change in the hydration of the molecule may determine the solubility.

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