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Factors Affecting Solution Properties of Hemicelluloses

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Hemicelluloses are polysaccharide cell wall constituents of land plants composed of a branched β-1,4-xylan backbone with pendant L-arabinose and (usually) 4-O-methyl-D-glucuronic acid units, the latter giving the molecule the properties of a polyelectrolyte. In our work on structure and fractionation of hemicelluloses we have become very aware of the importance of such factors as temperature and trace quantities of metal ions on the solution properties of the hemicelluloses, and this paper describes a preliminary investigation of such effects.

Our hemicellulose sample was derived from sugar cane by chlorite delignification, extraction with 10% sodium hydroxide, neutralization of the extract, and precipitation of the resultant solution with ethanol. It would therefore be described as hemicellulose B in the original O'Dwyer classification [1]. The effects of "thermal history" on the precipitation behavior of a hemicellulose are shown in Fig. 1. All precipitations were

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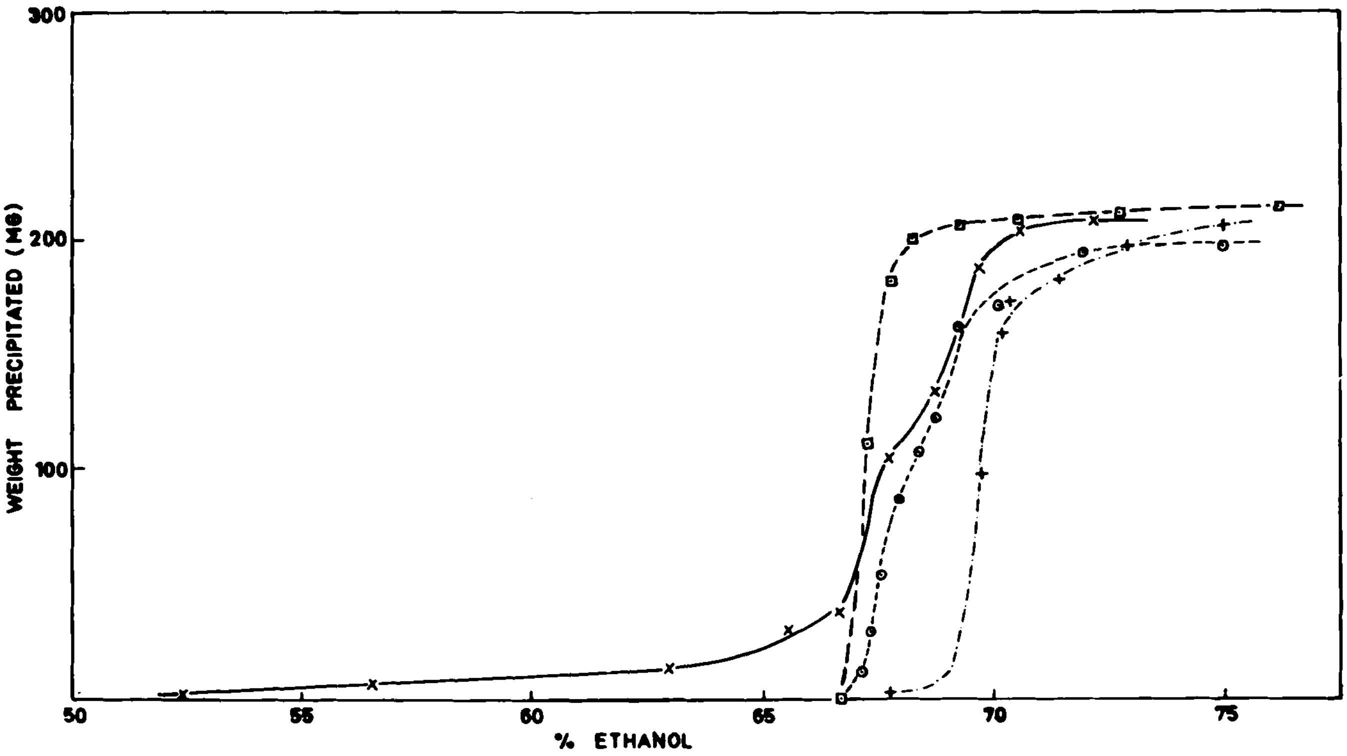

Fig. 1. The effect of "thermal history" on precipitation by ethanol of a hemicellulose. (X) Not heated. (+) Precipitated immediately after heating. (⧈) Precipitated 3 hr after heating. (⊙) Precipitated 3 days after heating.

carried out at room temperature (25 ± 4°) and were extremely reproducible in successive duplicate experiments on different days. It is concluded therefore that the precipitation steps are not sensitive to the temperature of precipitation within the range indicated. However, heating the solution in a boiling-water bath for 10 min and subsequent precipitation at room temperature caused a quite dramatic increase in the alcohol concentration at which precipitation occurred. This effect is tentatively ascribed to a thermal dispersion of molecular aggregates, which is slowly reversed on standing at room temperature.

The inflections in the precipitation curve for the unheated solution may indicate a selective aggregation of some components of the hemicellulose mixture (we have detected at least three different components by free-boundary electrophoresis) and this type of behavior has made possible the separation of chemically distinct species from hemicellulose mixtures [2]. In view of our present results however, it is evident that in such fractionation work, careful attention must be paid to the thermal history of the solution.

On investigation of the viscosity of aqueous solutions of the hemicellulose, it was noted that changes occurred after dissolution of the polymer. Figure 2 shows the change in viscosity after dissolving the freeze-dried polymer in water. The product dissolved rapidly to yield a milky, opalescent solution which showed no separation of phase on centrifuging at 5000 g. (20 min) or on filtration through a No. 4 glass sinter. The decrease in viscosity may be associated with the breakdown of residual and rudimentary hydrogen-bonded network polymer structures by slow hydration. The results are obviously very pertinent in studies of hemicellulose fractionation.

In further consideration of other factors influencing the viscosity of the solution, it became evident that, in common with other polyelectrolytes, the effect of electrolyte on the viscosity-concentration relationship would be considerable. This was verified (Fig. 3) and the results indicate a change in polymer configuration with change in uronic acid ionization under the influence of added electrolyte.

In subsequent viscosity studies therefore, electrolyte was added to stabilize the above effects. A solution of the hemicellulose was heated as in the above precipitation experiment and then kept in a viscometer at 30°, while the viscosity was measured at intervals. Results are shown in Fig. 4 and demonstrate an initial increase in viscosity after heating, followed by a slow decrease to a value below the original. The initial increase may confirm the previous suggestion that disaggregation occurs

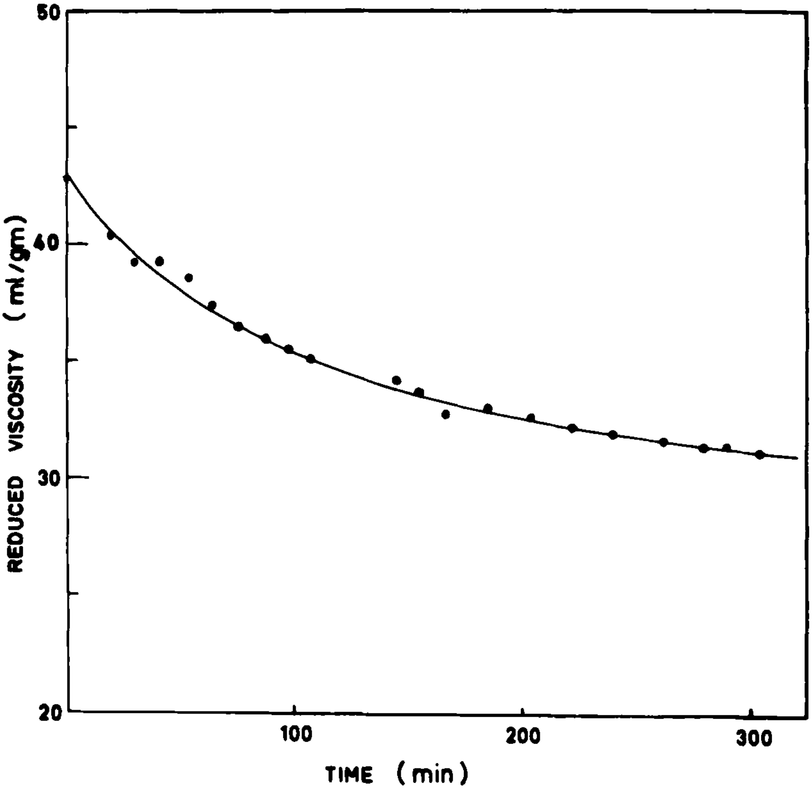

Fig. 2. The change in viscosity of a hemicellulose after dissolution in water at 35°. Reduced viscosity, $\eta_{sp}/c = (t - t_o)/t_oc$, where c is in g/ml.

on heating, but the subsequent decrease may also involve some unidentified effect in addition to the reaggregation already postulated.

The effect of various metal cations on precipitation of polyuronides such as alginic acid has been exhaustively studied by Smidsrød and Haug [3, 4], who obtained evidence of two distinct effects, viz., a decrease in solubility perhaps associated with intermolecular bonding by polyvalent cations (e.g., Mg^{2+}), and secondly a "salting-in" effect resulting in increased solubility at higher salt concentrations. Further work on pectates [5] has shown that in such polyuronides the decrease in solubility associated with polyvalent cations is not simply due to salt bridges through the carboxylic acid groups, but almost certainly also involves complexing with secondary alcohol groups in the polyuronide. It is quite possible that changes in solubility may occur by a change in molecular configuration following

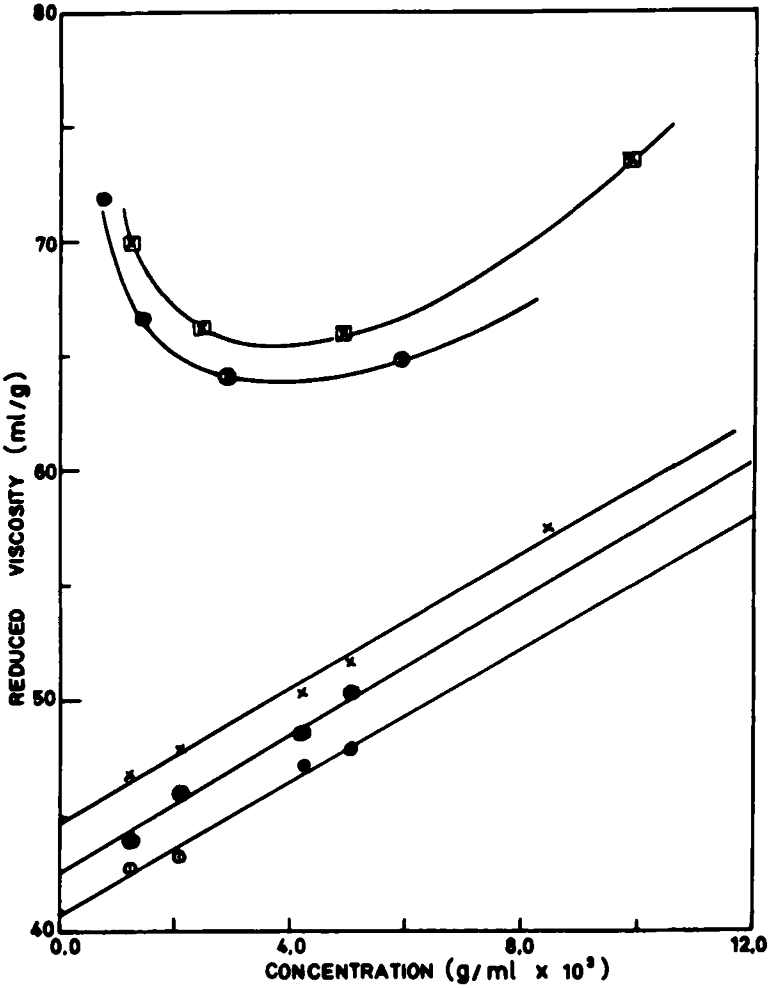

Fig. 3. Effect of added electrolyte on viscosity-concentration relationship for hemicellulose. (⊠) Viscosity in water after dissolution. (⊗) Viscosity in water after 24 hr. Viscosity in 0.2 M NaCl after dissolution (X), 24 hr (●), and 3 days (⊙).

intramolecular complexing of this type. In addition and especially at salt concentrations above 0.2 N, the changes in solubility of polysaccharides have been attributed to interaction of metal ions with the cluster structure of water as proposed by Frank and Wen [6].

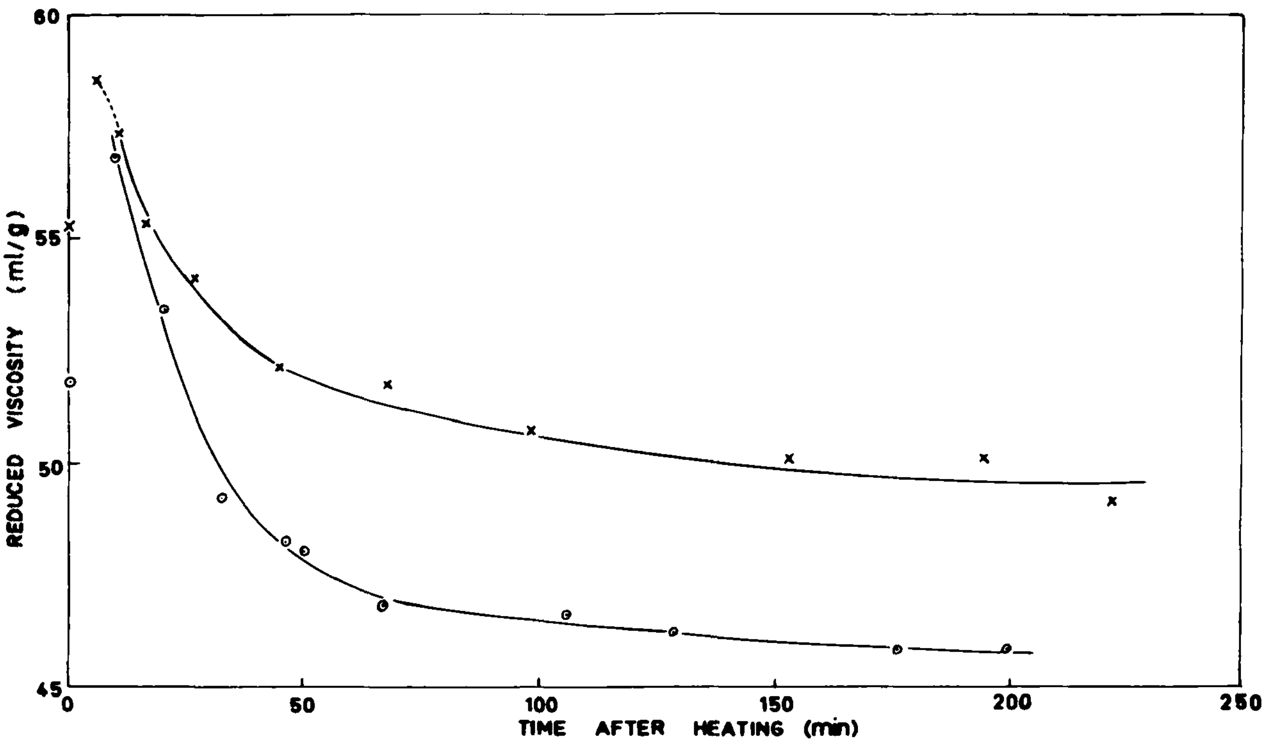

Fig. 4. The time-dependence of viscosity of hemicellulose after thermal dispersion. (X) 0.2 M NaCl solution. (⊙) 0.2 M $MgCl_2$ solution.

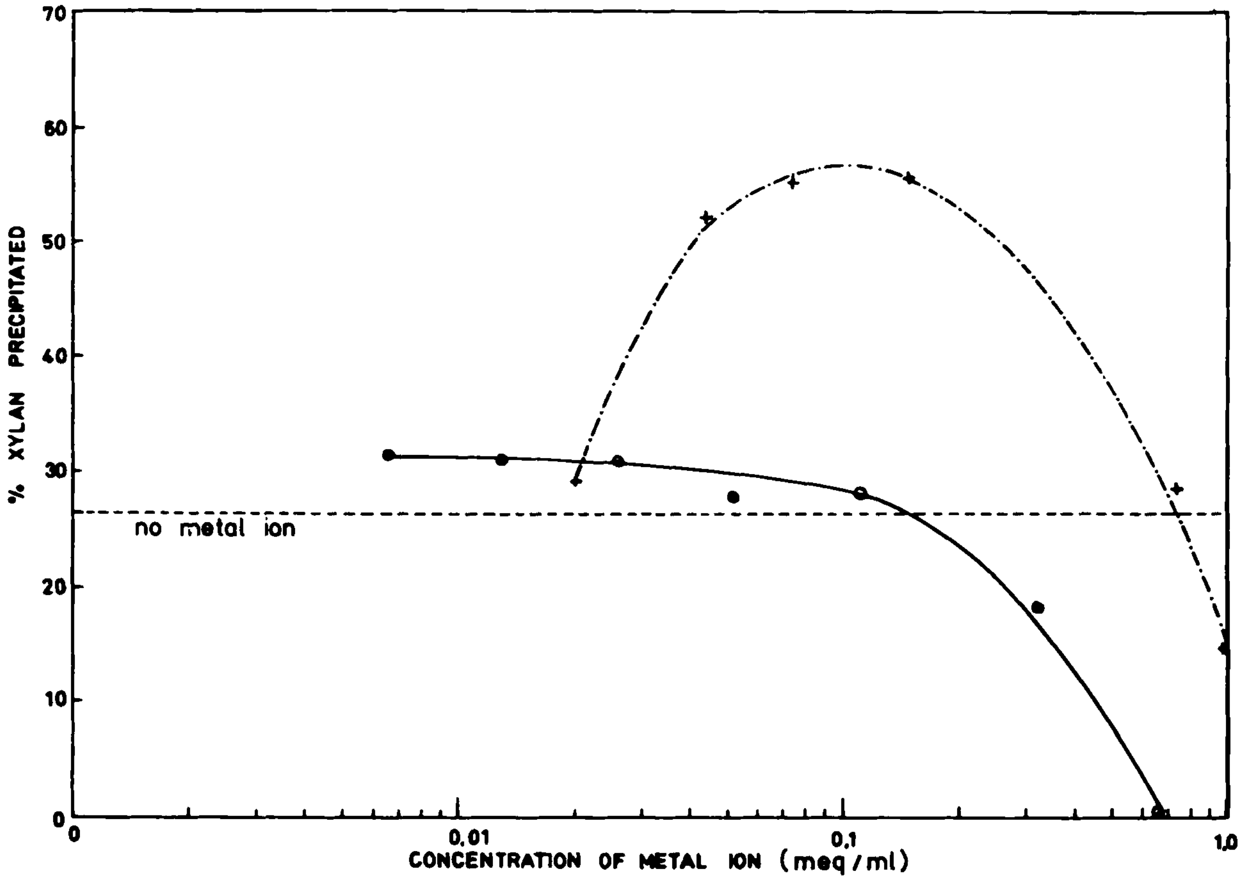

Fig. 5. The solubility of hemicellulose in aqueous ethanol solutions (20%) containing varying amounts of sodium and calcium nitrate. (X) Calcium ion. (⊙) Sodium ion.

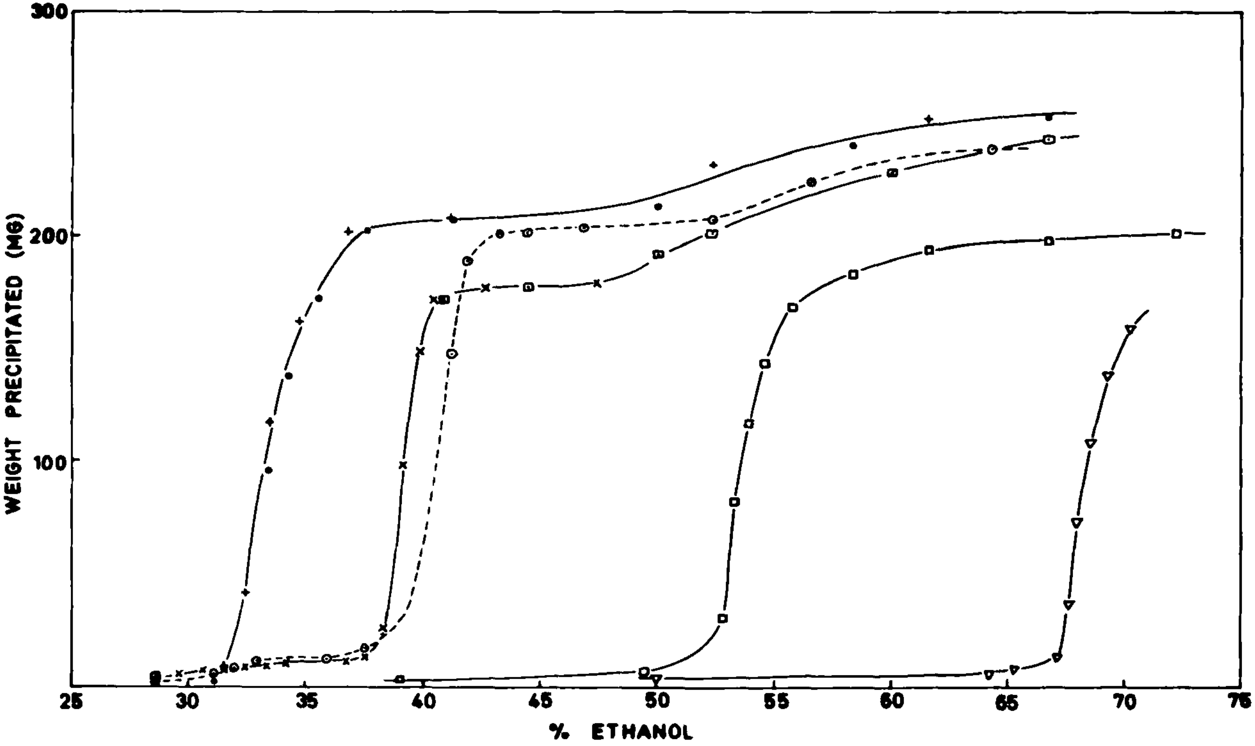

Fig. 6. Fractional precipitation by ethanol of hemicellulose in the presence of different concentrations of calcium chloride. (⊡,X) 1.0 M. (+,●) 0.1 M. (⊙) 0.01 M. (□) 0.005 M. (▽) 0.001 M.

In the hemicelluloses we have a very much lower content of carboxylic acid groups than in the polyuronides such as the alginates and pectates, but intermolecular bridges by either salt bridges or complex formation and also intramolecular complexing should still be possible. Indeed, the recent results of McGavin and co-workers [7] on metal ion complexes with pentoses indicated that such complexing was highly likely.

In view of the effects demonstrated above and in order to make possible subsequent studies of trace metal ion effects we have adopted the following procedure to ensure a hemicellulose solution of reproducible properties. All metal ions were removed by passing an aqueous solution of the hemicellulose through Amberlite IRC-50 (H) resin and the resultant solution was neutralized to pH 7.0 with sodium hydroxide and freeze-dried. The product was then dissolved in deionized water as required, heated on the boiling-water bath for 10 min, and then kept at 30° for at least 3 hr before beginning the study of any variable.

The results of our preliminary experiments on cation effects on solubility of the hemicellulose are shown in Fig. 5, using an experimental approach similar to Haug and Smidsrød [3], whereby the metal nitrate was added to a solution of the hemicellulose in 20% ethanol and the subsequent precipitate determined. The form of the curve is similar to that reported for alginate [3] and indicates increasing insolubilization by complex formation up to 0.2 N with "salting-in" at higher concentrations. These experiments will be extended in our subsequent work in an effort to determine the nature and site of the complex formation.

Obviously the formation of such complexes between metal ions and hemicelluloses will be of relevance in the fractionation of hemicelluloses and we have examined the effect of addition of calcium chloride on the fractional precipitation curve of our hemicellulose (Fig. 6). These curves are highly reproducible and show the dramatic effect of calcium ions at concentrations above 0.001 M on the fractionation curve. In addition, the "salting-in" effect at concentrations above 0.2 N was confirmed.

Quite obviously it is necessary in fractionation of hemicelluloses to pay much closer attention to both the thermal history of the solution than has been usual in the past and also to the trace ionic content.

EXPERIMENTAL

The methods used for the preparation of hemicelluloses from sugar cane bagasse were as described in *Methods in Carbohydrate Chemistry* [8, 9].

Fractional precipitation was effected by addition of ethanol to an 0.6% aqueous solution of hemicellulose. Fractions were centrifuged at 2000 g. for 5 min and the amount of each precipitate measured by dissolution in 1.O N sodium hydroxide and determination of its optical rotation.

Other precipitation experiments were carried out by mixing a 0.5% aqueous xylan solution (3 ml) with the appropriate metal salt solution (1 ml). Ethanol (1 ml) was then added slowly to the stirred mixture and after 1 hr at 30° separation was effected by centrifugation at 26,000 g. for 20 min. The amount of polysaccharide remaining in the resultant solution was determined using the phenol-sulfuric acid procedure. [10].

Viscosity was determined using a Ubbelohde viscometer. Measurements were carried out at 30 or 35° at different concentrations within a range of 2-8 g/ml.

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