EVIDENCE FOR MOLECULAR AGGREGATION IN HEMICELLULOSES

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ABSTRACT

Aqueous solutions of hemicelluloses from a number of different plant sources have been examined for time- and temperature-dependent effects by using viscometry, ultracentrifugation, gel filtration, light transmission, and optical rotation. The results indicate a dynamic system of aggregating polysaccharide molecules. There is some evidence to suggest that the structure of the hemicellulose influences the observed aggregation.

INTRODUCTION

When determining the viscosity of a hemicellulose solution from sugar-cane bagasse, a time-dependent effect was noted as shown in Fig. 1. We report in this paper the results of an investigation of the causes of this effect by a range of physical methods.

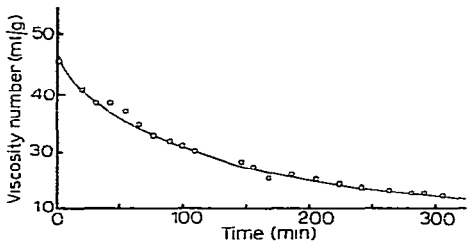

Fig. 1. Change in viscosity number of a 1% aqueous solution of hemicellulose from sugar-cane bagasse at 34.8°.

EXPERIMENTAL AND RESULTS

Materials. — Hemicellulose samples were derived from sugar-cane bagasse, guinea grass (*Panicum maximum*), and Townsville lucerne (*Stylosanthes humilis*) by extraction with 10% aqueous sodium hydroxide from chlorite holocelluloses. All

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samples had previously been extracted with cold and hot water, and 1% aqueous ammonium oxalate prior to delignification.

Hemicelluloses were recovered by neutralisation of the alkaline extract with acetic acid and precipitation with three volumes of ethanol. According to the original classification of O' Dwyer[1], they are therefore described as hemicellulose *B* components.

The hemicellulose from Townsville lucerne was further fractionated into linear and branched fractions by precipitation using iodine[2,3]. Otherwise, samples were not further fractionated.

Chemical compositions of the hemicelluloses were determined after hydrolysis, by gas–liquid chromatography of alditol acetates[4]. The results are summarised in Table I, where the neutral sugar composition is reported as an absolute percentage in the first two examples, and as relative percentage composition for the lucerne fractions.

TABLE I

MONOSACCHARIDE COMPOSITION OF HEMICELLULOSES

Plant source	*Neutral sugars*[a]					*Uronic acids* (%)	*Equivalent wt. of hemicellulose*
	Rha	*Ara*	*Xyl*	*Gal*	*Glc*		
Guinea grass	—	12.7	66.4	2.4	10.6	8	875
Bagasse	—	5 4	72.2	—	5.6	7.1	—
Townsville lucerne							
(*i*) linear	1.9	2.7	87.8	3.1	4.5	2.3	1463
(*ii*) branched	14.8	21.0	24.7	39.5	—	2.2	861

[a]See text.

All hemicellulose solutions used for physical measurements were made up with deionised water, unless otherwise indicated, and adjusted to pH 7.0 with aqueous sodium hydroxide.

Viscometry. — Viscosities were determined for 1% solutions by using an Ubbelohde viscometer. A supplementary heater was used for rapid changes in thermostat temperature.

Fig. 2 shows the effect of heating a solution of guinea-grass hemicellulose in a closed flask on a boiling–water bath for 10 min ("standard heating conditions") and immediately equilibrating the solution to thermostat temperature (39.5°) for time-dependence studies. Figs. 3 and 4 show results for similar studies on the linear and branched polymers, respectively, from Townsville lucerne.

Fig. 5 reflects a variation in procedure, in that, after being heated for 10 min on a boiling-water bath, the solution from guinea grass was cooled to room temperature (25°) for 20 min before being equilibrated to thermostat temperature (39.5°) for time-dependence viscometry. After 3 h, the solution was re-heated under standard conditions and returned directly to the thermostat for equilibration and further time-dependence study.

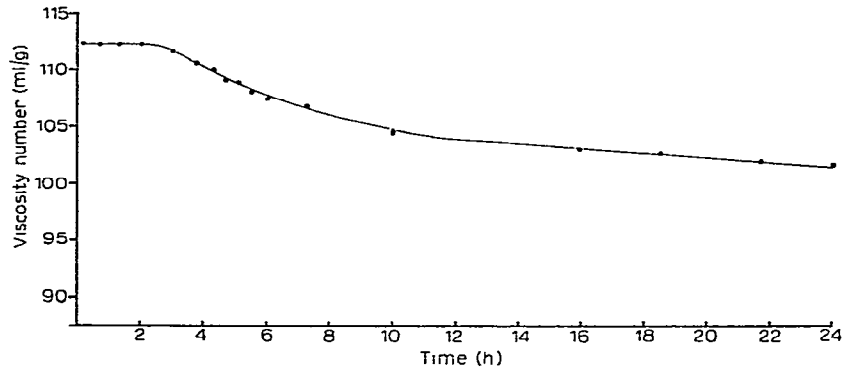
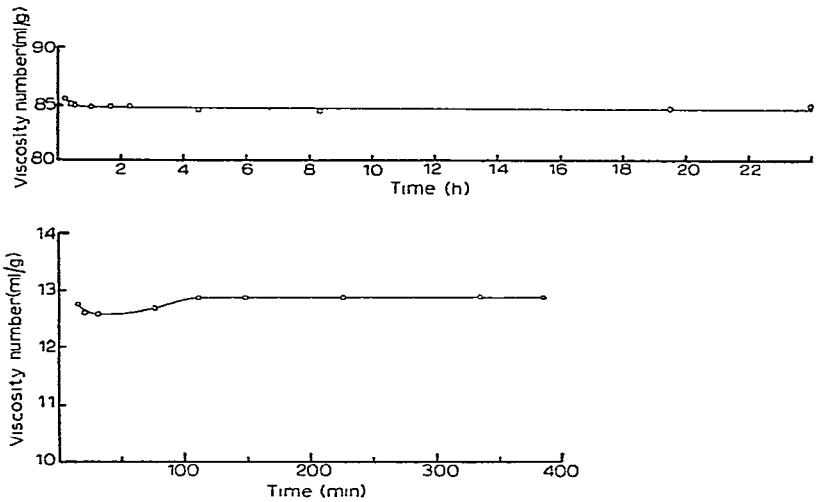


Fig. 2. Change in viscosity for guinea-grass hemicellulose after standard thermal treatment and immediate equilibration to 39 5°.



Figs. 3 and 4. Changes in viscosity number for linear and branched hemicelluloses, respectively, from Townsville lucerne, treated as in Fig. 2

A solution of guinea-grass hemicellulose which had been heated at 100° for 10 min and then stored for 5 days at 5° was equilibrated to 31°, and the changes in viscosity number were noted as the temperature of the solution was raised stepwise. An equilibration time of 10 min was allowed after each temperature increment. Solvent flow-times were determined in a separate experiment where the temperature was similarly varied, but no shear corrections have been made. At 67°, the solution was removed from the bath and heated for 10 min on a boiling-water bath before re-equilibration to 67°. Viscosity numbers were then determined as the temperature of the bath was decreased stepwise to a temperature where the viscosity began to fall rapidly. The results are shown in Fig. 6. When the analysis was repeated without removal from the bath for boiling-water treatment, the hysteresis effect shown in Fig. 7 was obtained.

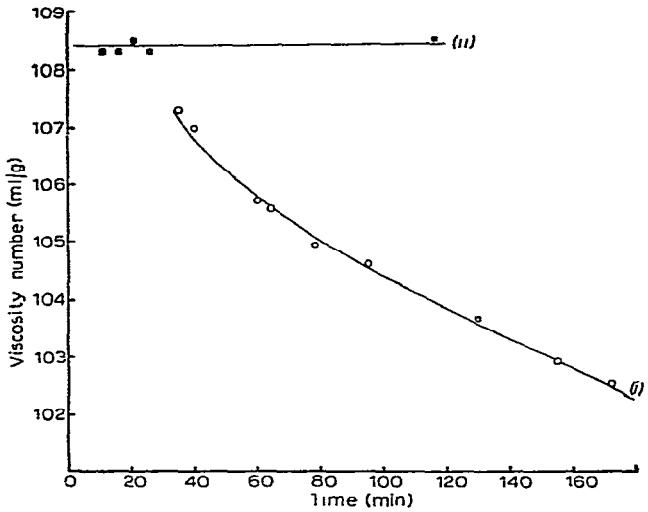


Fig. 5. (*i*) Change in viscosity of guinea-grass hemicellulose after standard thermal treatment and cooling to room temperature (25°) for 25 min before equilibration to 39.5° for viscosity determinations. (*ii*) Change in viscosity in the same solution on re-heating and equilibration directly to 39.5°.

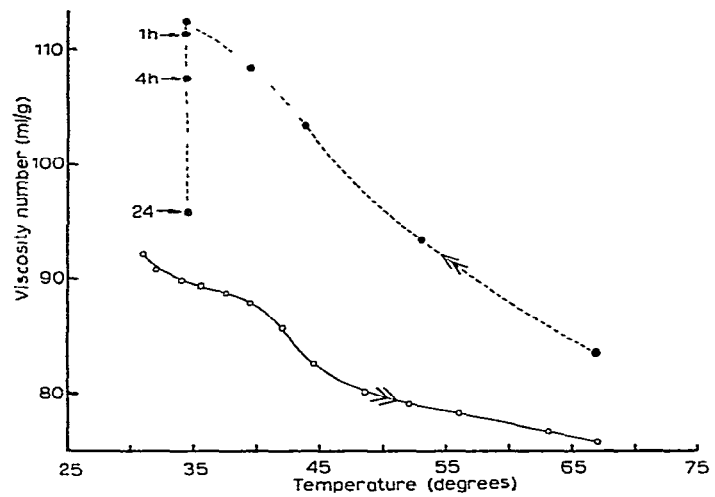


Fig. 6. Hysteresis in viscosity for guinea-grass hemicellulose heated (O——O) in steps to boiling-water bath temperature and then cooled (●---●).

Optical rotation. — A 1% solution of guinea-grass hemicellulose was heated under standard conditions and transferred to a jacketed cell of a Perkin–Elmer Model 141 Polarimeter. The temperature was decreased stepwise and readings were made at intervals of 5–7 min after reaching control conditions. At 34.1°, the temperature was held constant, and variation in optical rotation with time was observed for 1 h. In a 24-h period, there was little further change. The results are shown in Fig. 8.

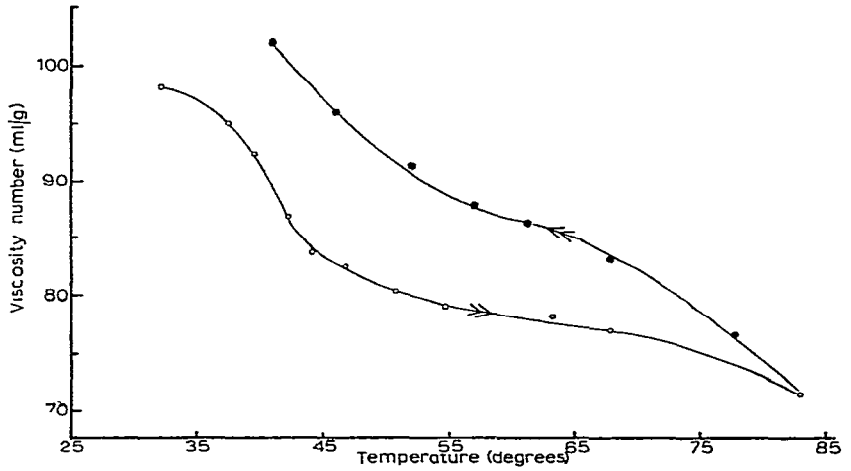


Fig. 7. Hysteresis in viscosity for guinea-grass hemicellulose heated (O——O) in steps to 85° and then cooled (●——●).

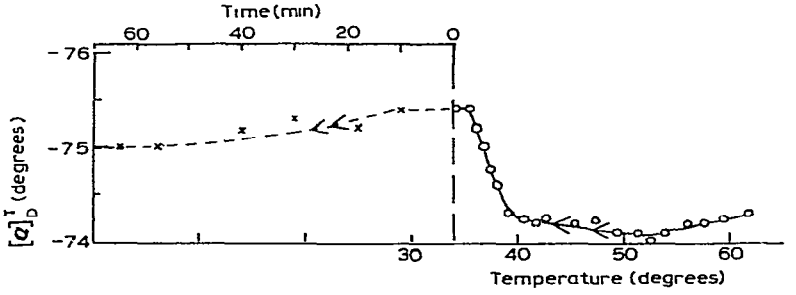


Fig. 8. Change in optical rotation on stepwise cooling to 34.1° (O——O) of guinea-grass hemicellulose after standard thermal treatment. At 34.1°, the temperature was held constant and further variation with time recorded (×—•—×)

Turbidity changes. — In the analysis shown in Fig. 9, the absorbance of the hemicellulose solution from guinea grass was recorded at 587 nm, as a function of temperature, by using a Unicam SP-800 recording spectrophotometer fitted with a variable-temperature cell-carrier. Solutions at concentrations of 0.92% and 0.46% were heated under standard conditions before equilibration to 48.5° and progressive decreases to 30.1°. Thereafter, changes in turbidity were recorded as a function of time as shown in Fig. 10.

Ultracentrifugation. — Studies on the sedimentation behaviour of guinea-grass hemicellulose were made by using a Spinco Model E ultracentrifuge. Both a Kegeles synthetic boundary cell and a standard 12-mm cell were used at 59,780 r.p.m. by using Schlieren optics at a diaphragm angle of 70°. The temperature was controlled at 25° during centrifugation.

Analyses using synthetic boundaries revealed hyperfine peaks which sedimented rapidly to the bottom as the centrifuge ran to "speed". Movement of rapidly sedi-

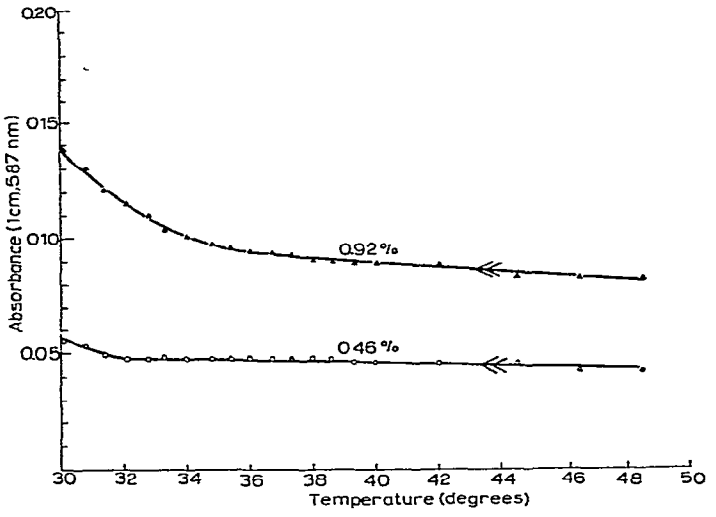


Fig. 9. Change in turbidity at 587 nm of gunea-grass hemicellulose solutions of 0 92 and 0.46% concentration during stepwise cooling after standard thermal treatment.

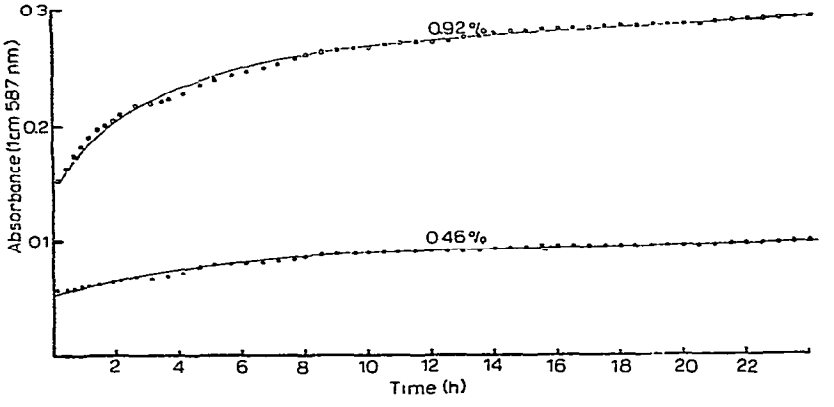


Fig. 10. Increase in turbidity of solutions from Fig. 9 at 30.1°.

menting components coincided with clarification of the solution, and it was noted that the number of these peaks increased as the solution "aged" after thermal treatment. The results are illustrated in Fig. 11.

Sedimentation coefficients were determined for the two components observed by using the standard cell at 25°, 10 min and 27 h after standard, hot-water treatment. Relative areas were determined by weighing excised peaks, and the results are shown in Table II.

Murphy[5] has recorded components having sedimentation coefficients of 45.5 and 1.00 in hemicellulose from bagasse in 33mM sodium tetraborate. Blake[6] noted two components from spear grass having S values of 15.92 and 0.98 in the same buffer. This same solution revealed only a single component (S = 31.1), after standing for

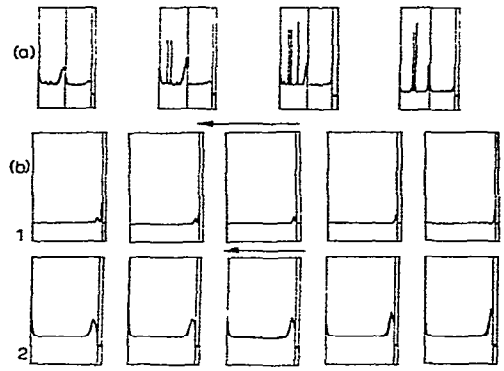


Fig. 11. Ultracentrifugal analysis of 1% aqueous solutions of guinea-grass hemicellulose after thermal treatment. (*a*) Synthetic boundary analysis (during "run-up" to 59.780 r.p.m.) after ageing 20 h at 0–2°. (*b*) Standard cell analysis of the two major components 27 h at 25° after thermal treatment; 1, "heavy" component; 2, "light" component.

TABLE II

ULTRACENTRIFUGATION OF THE HEMICELLULOSE COMPONENTS OF GUINEA GRASS AT 25° IN AQUEOUS SOLUTION AFTER HEATING

Time after heating	*Sedimentation coefficient*		*Relative areas of slow/fast component*
	Slow	*Fast*	
10 min	0.98	30.8	8.5
27 h	1 22	62.9	4.1

3 days at 0°, when examined after a preliminary clarification at 37,000 g. Obviously, however, the S values measured in borate solution are not directly comparable with those measured in water, since intermolecular borate linkages may be present.

Gel filtration. — Columns (30 × 1.2 cm) of Sepharose 2B and Sega 2F at 25° were used to study the components of guinea-grass hemicellulose after thermal treatment. 50 µl of a 1% solution were loaded at convenient time-intervals after heating, and the effluent was monitored continuously at 0.1 ml/min by using a Technicon Autoanalyser, adapted to the phenol–sulphuric acid procedure of colorimetric analysis[7].

The fractionation pattern showed little difference in both systems, and the results from the Sega 2F column, 5 min and 16 h after heating, are shown in Fig. 12. All systems were equilibrated to deionised water. The ratio of the areas of the two major components to that of the trailing component, obtained by weighing excised peaks, is shown in Table III.

DISCUSSION

Our results suggest that temperature- and time-dependent molecular association and, possibly, changes in conformation occur in aqueous solutions of xylan hemicellu-

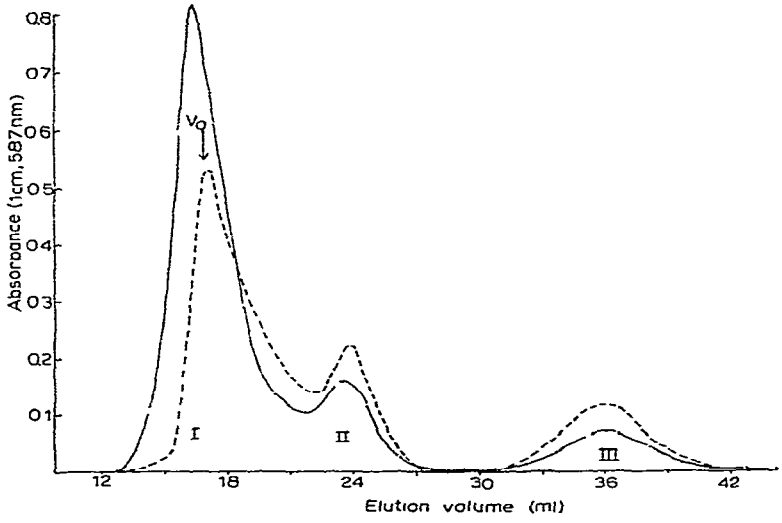


Fig. 12. Gel filtration on Sega 2F (30 × 1.2 cm), 5 min (– – –) and 16 h (——) after standard thermal treatment.

TABLE III

COMPONENT DISTRIBUTION IN GUINEA-GRASS HEMICELLULOSE AS MEASURED BY GEL FILTRATION (SEE FIG. 12)

Time after heating	*Area of components I+II* / *Area of component III*
5 min	4.3
13 min	4 5
7 h	8.5
16 h	8.7

loses and emphasise the importance of a complete definition of the thermal history of the solution in any description of molecular properties of such polymers[8].

The effect was first demonstrated in the experiment shown in Fig. 1, in which it was found that the viscosity of a hemicellulose solution at constant temperature continued to fall for several hours. The thermal history of the hemicellulose solution appeared to have an important influence on time-dependent viscosity changes, and these effects are demonstrated in Figs. 2 and 5. There is a period of stability in the viscosity of the solution if it is not cooled below 40° after heating, but this is time dependent and a change occurs after three hours. This same change can be triggered by cooling to ambient temperature directly after heating and cannot be reversed by heating to 40°. In our experience, this effect is induced by cooling after heat treatment of the solution. In studies of solution viscosity before heating, not reported here, the initial viscosity numbers varied randomly and, in the case of bagasse hemicellulose, the observed change (Fig. 1) was difficult to reproduce. This behaviour, in the absence

of heat treatment, may reflect the condition of the polymeric constituents in the dry state before dissolution. In this respect, Anderson and co-workers[9] have detected artefacts in acidic polysaccharides, which they claim arise from aggregation in freeze-dried materials during storage.

Figs. 2, 3, and 4 indicate differences arising from structural variation in the hemicelluloses. Pronounced effects are observed in the hemicelluloses from monocotyledons, where the preponderant components are arabino-4-*O*-methylglucuronoxylans, whereas little change is noted in the hemicellulose components of the legume studied. This latter group are mainly 4-*O*-methylglucuronoxylans. Since, however, our results have all been obtained with unpurified hemicelluloses, known to contain mixtures of related molecular types, we prefer not to speculate on any possible involvement of the single L-arabinofuranose side-groups in intermolecular aggregation or conformational effects.

Turbidity measurements indicate some dependence of aggregation on concentration, as shown (Fig. 9) by the observation that, in the cooling curve, the rapid increase in turbidity occurs at a higher temperature at the higher concentration.

Changes in sedimentation coefficients (Table II) and in the relative proportions of different components observed on ultracentrifugation confirm the occurrence of a time-dependent molecular aggregation to particles of increasing size. The abnormally sharp peaks noted (Fig. 11) in the synthetic boundary analysis are reminiscent of those previously claimed to indicate aggregation of acidic polysaccharides[10]. This behaviour could be attributed to progressive separation of a system of loosely bound clusters from an agglomerate, under an increasing centrifugal field, to leave a residue of relatively low molecular weight. Such a component (of low molecular weight) would be in keeping with previously reported molecular weights of xylans[11]. However, the absence of ideality in the solution, derived particularly from the absence of stabilization of the polyelectrolyte by a supporting ionic medium, requires caution in interpretation of these peaks.

At first inspection, the results of gel filtration (Figs. 12 and 13) and ultracentrifugation do not appear compatible. The latter implies the existence of a major component of relatively low molecular weight which undergoes aggregation to larger molecular species. However, the results from gel systems that have effective, molecular-weight, fractionation ranges for proteins of 2×10^6–25×10^6 (Sepharose 2B) and 0.5×10^6–150×10^6 (Sega 2F) suggest that the aggregated molecules are in the majority. It is noted, however, that the changing proportion between the peaks for higher and lower molecular weights is again indicative of progressive aggregation.

The apparent discrepancy between gel-filtration and ultracentrifuge results may be interpreted as follows. The xylan molecules are relatively stiff and extensively hydrated, and therefore present an abnormally large hydrodynamic volume (compared with proteins) in gel filtration. This might result in exclusion of (and hence failure to fractionate) a wide range of molecular aggregates of the hemicelluloses, whereas in the ultracentrifuge, some of these aggregates may separate, thus changing the apparent ratio of highly aggregated to less-aggregated molecules.

Applegarth and Dutton[12] have reported aggregation in the chromatography of hemicelluloses, noting changes in elution patterns for fractionation on DEAE-cellulose brought about by 7M urea. Such a reagent is known to be effective in breaking non-covalent associations within and between macromolecules[13].

Rees and co-workers[14] have studies the sol–gel–sol transformations of aqueous κ-carrageenan, noting complex changes in optical rotation. They interpreted these, qualitatively, in terms of a double-helix model involved in the intermolecular junction zones of the gel. Similar shifts in optical rotation have been observed in ι-carrageenan after its modification to shorter chain-lengths incapable of gelation[15]. This is interpreted in terms of a coil to double-helix transition in the polysaccharide, whereby cooperative stabilisation through intermolecular cohesion between chain residues produces an ordered conformation in aqueous solution large enough to over-ride solvation and solution conformational entropy in the polymer.

We note that the change in optical rotation observed on cooling a hot solution of hemicellulose (Fig. 8) is analogous to that observed with the carrageenans by Rees and co-workers, although our experiment covered a narrower range of temperature and gave a smaller effect. These changes represent an increase in the magnitude of the optical rotation and are therefore not due to the separation of material from solution. By analogy with the work of Rees and co-workers[15], Fig. 8 gives a clear indication of a cooperative change of some kind, and it is possible that the changes may be related to helix formation in the xylan molecule, in addition to the aggregation demonstrated by other methods. This will be the subject of more-detailed examination, using more highly purified xylans, in collaboration with Dr. D. A. Rees.

The complex changes in viscosity of the hemicellulose solutions suggest that several different effects may operate simultaneously. The viscosity changes may be associated with changes in molecular conformation and also with various types of aggregation. The latter might conceivably range from parallel association of relatively linear xylans (which would probably decrease the viscosity) to random, non-ordered interchain-bonding (which would probably increase the viscosity). The possible changes in molecular conformation might range from random coil to helix or double-helix formations. Our evidence does not enable us to distinguish between such effects; however, Fig. 2 shows that a "lag phase" may exist before the viscosity begins to fall at constant temperature, thus indicating the possible occurrence of "nucleation" of aggregation or conformational change. Figs. 6 and 7 show the presence of a definite transition temperature at about 40° in the heating curve, but it is not possible to say whether this transition is associated with dissociation of aggregates or a change in molecular conformation.

In conclusion, we have demonstrated that extensive and time-dependent molecular aggregation of hemicelluloses occurs in aqueous solution, that this effect is most apparent after heating the solution, and that there is very tentative evidence that conformational changes may also occur. All of the effects demonstrated are of potential importance in fractionation of hemicelluloses.

ACKNOWLEDGMENTS

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