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Hydrodynamic and other properties of poly(morpholino)alginamide (PMA)

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Some properties of poly(morpholino) alginamide (PMA) have been investigated. The pH–titration curve and the variation of viscosity with pH of PMA in solution suggest that the very high viscosity of PMA may be associated with the negative charges on the ionised carboxylic acid groups present on the polysaccharide chain. This is supported by the observation of a reversible salt effect which results in complete loss of viscosity in a 0.10% (w/v) sodium chloride solution.

These properties, in addition to a non-Newtonian variation of viscosity with shear rate, lead to the suggestion that the PMA consists of long, soluble, rigid chains interconnecting regions of morpholine-substituted polysaccharide.

INTRODUCTION

Alginic acid, an acidic polysaccharide from brown algae, contains β-D-mannuronic and α-L-guluronic acid units. The detailed structure, which may contain blocks of β-D-mannuronyl residues, blocks of α-L-guluronyl residues, or sections containing both types of hexuronic acid, may vary according to the source (that is, the different weed types) and the conditions of growth (Fischer & Dorfel, 1955; Haug *et al.*, 1967, 1968). Figure 1 illustrates features of the structure of alginic acid derivatives by showing a β-D-mannuronyl-α-L-guluronyl disaccharide portion.

Among the commercially important derivatives of alginic acid is propylene glycol alginate (PGA; 1, R or R' = $-O-CH_2-CH(CH_3)-OH$ or $-O-CH(CH_3)-CH_2-OH$; see Kennedy *et al.*, 1989) which finds considerable use in the food industry as a thickener and stabiliser (McNeely & Pettitt, 1973).

Recently (Gray & Philp, 1991), we reported that treatment of solutions of PGA with morpholine resulted in the formation of gels. It was shown that in this reaction many of the ester groups were converted into acyl morpholine amide species. For this reason we propose the name poly(morpholino)alginamide (PMA) for this material. It was suggested that gel formation was due to the existence in the structure of highly ordered ('insoluble') regions of polysaccharide, locally highly substituted by morpholine groups, interconnected by less highly ordered, 'soluble', segments, substituted by morpholine to a much lesser extent.

The setting of the PGA solutions to gels as a result of this reaction indicated that the product polymer (PMA) possesses greater intrinsic viscosity than the PGA itself, and therefore that the PMA would be a valuable addition to the range of alginate derivatives available commercially.

In this paper we report further studies on the properties of the PMA.

MATERIALS AND METHODS

Propylene glycol alginate (PGA) (E/RE; degree of substitution 80–85%), sodium alginate (DMB) and xanthan gum (XG) were kindly provided by Kelco International Ltd. Morpholine was purified by reduced pressure distillation.

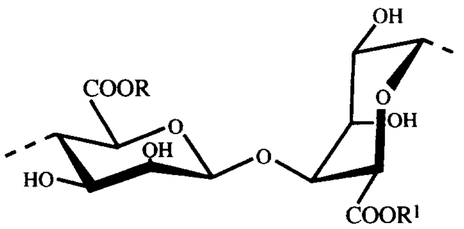

Fig. 1 β-1,4-D-Mannuronyl-α-L-guluronyl disaccharide unit.

General methods

pH Values were measured on a Radiometer Copenhagen pH Meter 28 calibrated with standard buffer solutions. Viscosity measurements were carried out with solutions at pH 7.0, except where stated otherwise, using either a Brookfield LVT Viscometer or a Shirley–Ferranti Cone and Plate Viscometer, as indicated. Values of $\log_{10}$ (viscosity) were calculated on the basis that viscosities are measured in c.p.s. All concentrations expressed as percentages refer to w/v.

Standard preparation of poly(morpholino)alginamide (PMA)

PGA (8.35 g) was dissolved in water (200 cm^3). Morpholine (33.5 cm^3) was added to water (100 cm^3) and the pH of this solution adjusted to 9.0 with conc. hydrochloric acid. The volume was then made up to 300 cm^3. This solution was then added, with rapid mixing, to the alginate solution and the resultant mixture was allowed to stand. A gel was produced within 5 min. After 18 h the mixture was broken up and the pH adjusted to 2.0. Propanone was then added to precipitate the product. The precipitate was then filtered off, washed twice with propanone and then dissolved in the minimum volume of water. The pH was checked to ensure that it remained at approximately 2.0 and the precipitation, filtration, washing and dissolution in water repeated twice more. The final precipitated product was washed with propanone and air dried. Yield: (from two batches as above) 13.56 g (80%).

Comparison of the viscosity of PMA with that of xanthan gum (XG)

The viscosities of a series of solutions of PMA (concentration range 0.25–0.5%) and of XG (concentration range 0.1–1.2%) were measured using a Brookfield LVT Viscometer. The results are shown in Fig. 2. Figure 3 shows the results of similar measurements for PGA and sodium alginate solutions (concentration range 0.7–5.0%). The results for PMA from the previous experiment are also shown for comparison.

pH Titration of poly(morpholino)alginamide (PMA)

PMA (0.20 g) was suspended in water (30 cm^3) with vigorous stirring and measured aliquots firstly of M/10 hydrochloric acid (up to 2.5 cm^3) and then of M/10 sodium hydroxide solution were added. The pH was measured after each addition. The curve of pH against sodium hydroxide solution added is shown in Fig. 4.

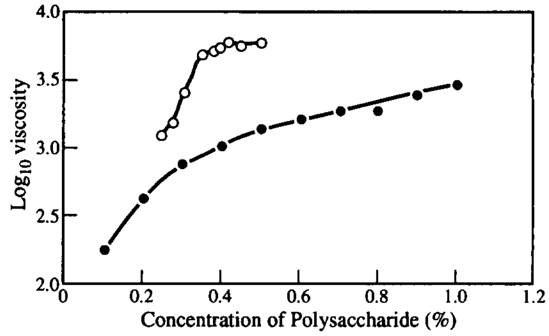

Fig. 2 Variation with concentration of the viscosities of PMA and xanthan gum. ○—PMA; ●—XG. Viscosities were measured as described in the Materials and Methods section and values for $\log_{10}$ viscosity are on the basis that viscosities are measured in c.p.s.

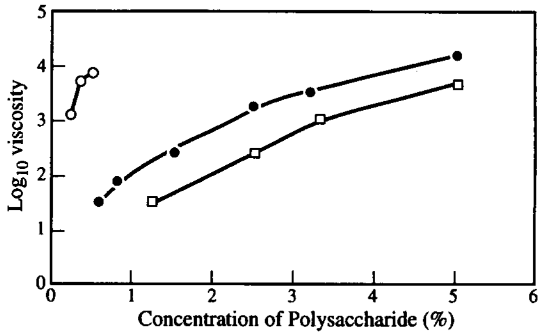

Fig. 3 Variation with concentration of the viscosities of sodium alginate and PGA. ●—sodium alginate; □—PGA; ○—PMA (values from Fig. 2 given for comparison).

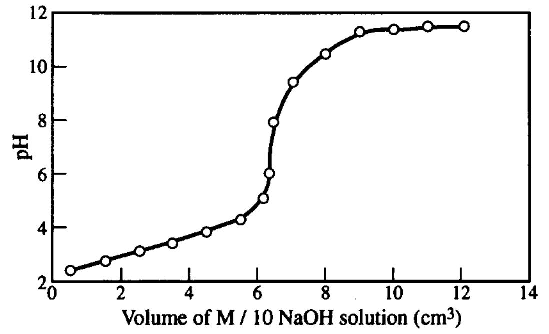

Fig. 4 Titration curve for PMA.

Variation of viscosity with pH

PMA (0.50 g) was suspended in water (150 cm^3) and the pH was shown to be 3.30. The pH was slowly raised by the addition of M or M/10 sodium hydroxide solution and the viscosity was measured at frequent intervals over the pH range using a Brookfield LVT Viscometer. At high pH it was found difficult to maintain the pH at steady values. The results are shown in Fig. 5.

Variation of viscosity of poly(morpholino)alginamide and other polysaccharides with temperature

A solution of PMA in water (200 cm^3; 0.32% w/v; at pH 7.0) was placed in a thermostatted water bath at 20°C and the viscosity measured using a Brookfield LVT Viscometer. The temperature of the water bath was then increased by a small interval and, after sufficient time to permit equilibration, the viscosity was again measured. The procedure was repeated at temperatures up to near boiling. This experiment was repeated using XG (0.3%), PGA (2.5%) and sodium alginate (1.7%). The results are shown in Fig. 6.

Variation of viscosity of PMA and of other polysaccharides with shear rate

The viscosities of various solutions of polysaccharides (at concentrations which gave values of $\log_{10}$ (viscosity) of between 3 and 4) were measured using a Shirley–Ferranti Cone and Plate Viscometer. For each polysaccharide, the variation of viscosity with shear rate was measured. The results are shown in Fig. 7.

Effect of salts on the viscosities of PMA and other polysaccharides

Sodium chloride Sodium chloride was added to aliquots of a solution of PMA to give a series of solutions with a sodium chloride concentration range of 0–1% and the viscosities were measured using a Shirley–Ferranti Cone and Plate Viscometer. Similarly the effects of various concentrations of sodium chloride on the viscosities of XG, PGA and sodium alginate were measured. The results are shown graphically in Fig. 8. The solution of PMA to which sodium chloride had been added was then dialysed exhaustively against distilled water and the viscosity measurement repeated.

Other salts The effects of two other sodium salts, sodium thiocyanate and sodium sulphate on the viscosity of PMA were examined in the same way. The results are shown in Fig. 9. The effects of ammonium sulphate, ammonium thiocyanate and lithium chloride were also examined in the same way. The results for these are shown in Fig. 10.

RESULTS

Viscosity of PMA and variation with concentration

Figure 2 shows the comparison between the viscosities of PMA at pH 7.0 and XG and their variations with concentration. Clearly, PMA had higher viscosity over the range 0.2–0.55%. For example at 0.5%, PMA had a visc-

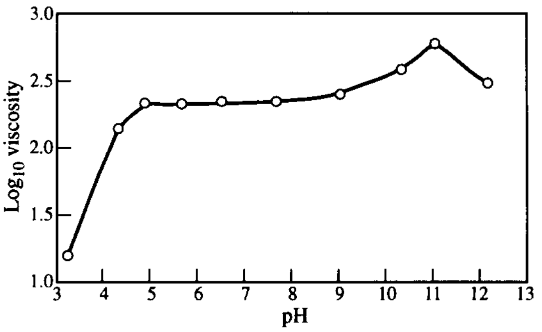

Fig. 5 Variation with pH of viscosity of PMA. See notes to Fig. 2.

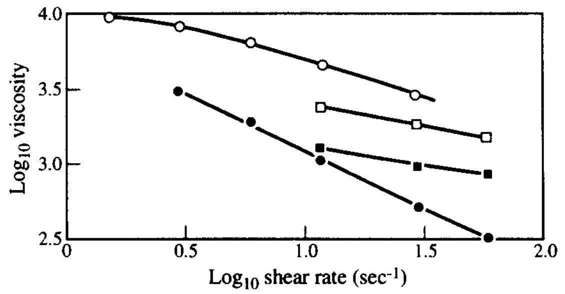

Fig. 7 Effect of shear rate on the viscosities of polysaccharide derivatives. ○—PMA; □—sodium alginate; ■—PGA; ●—XG. See notes to Fig. 2. Shear rates, s^{-1}.

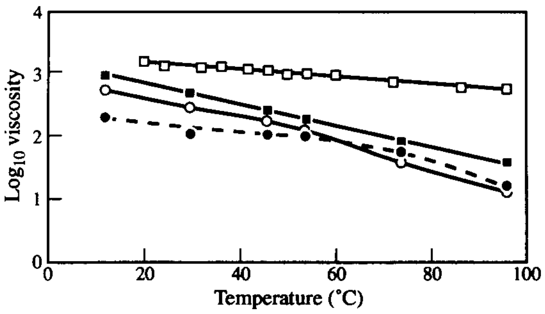

Fig. 6 Effect of temperature on the viscosities of polysaccharide derivatives. □—PMA; ■—sodium alginate; ○—PGA; ●—XG. See notes to Fig. 2.

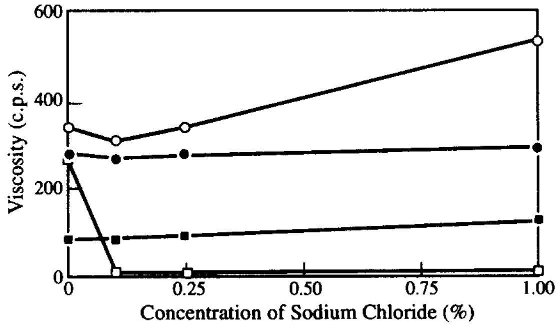

Fig. 8 Effect of sodium chloride on the viscosities of polysaccharide derivatives. □—PMA; ○—sodium alginate; ■—PGA; ●—XG. See notes to Fig. 2.

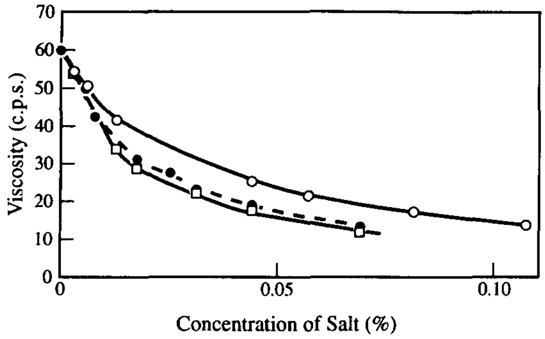

Fig. 9 Effect of sodium salts on the viscosity of PMA. ○—Sodium thiocyanate; ●—sodium chloride; □—sodium sulphate. See notes to Fig. 2.

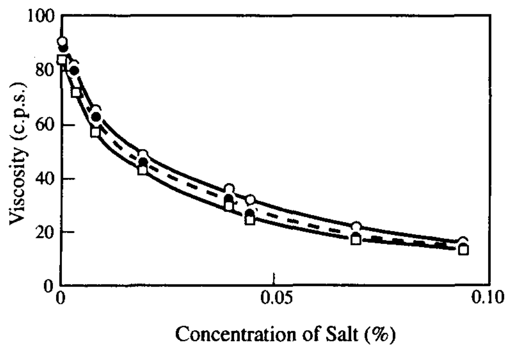

Fig. 10 Effect of salts on the viscosity of PMA. ○—Ammonium thiocyanate; ●—lithium chloride; □—ammonium sulphate. See notes to Fig. 2.

osity of 5600 c.p.s. ($\log_{10}$ viscosity = 3.75) while at the same concentration XG had viscosity of 1300 c.p.s. ($\log_{10}$ viscosity = 3.11). The viscosity of PMA appeared to reach a plateau at approximately 0.4% while for XG the value was still rising at 1.0%. The contrast with sodium alginate and with PGA (from which the PMA was prepared) is even greater, as shown in Fig. 3. Thus for example at 0.5% sodium alginate had a viscosity of 20 c.p.s. ($\log_{10}$ viscosity = 1.38) while that of PGA was even lower.

pH Titration of PMA

Figure 4 shows the titration curve for PMA. As described in the earlier paper (Gray & Philp, 1991), the PMA carries, in addition to morpholino–amide groups, many carboxylic acid groups and also retains a significant number of ester groups. It was noted that at high pH levels above 9–10, with PMA as well as with PGA, the pH dropped steadily if NaOH was not added. This is attributed to the hydrolysis of the ester groups at these pHs (Gray & Philp, 1991). Since there are no groups present with a pK_a of approximately 12, it appears therefore that the levelling off of pH on addition of sodium hydroxide after about 9 cm^3 in this experiment (Fig. 4) is due to the hydrolysis of the residual ester groups. The slow but progressive increase from pH 2 to approximately 4 represents the ionisation of the carboxyl groups. This ionisation is significant in view of the variation of viscosity with pH in this range.

Variation of viscosity with pH

At low pH the viscosity of the PMA was low, but on addition of the NaOH taking the pH to about 4, the viscosity rose also and levelled off from approximately pH 5 until pH 9 was reached. The increase in viscosity, corresponding as it does to the ionisation of the carboxyl groups, is likely therefore to be due to the negative charges on the alginate chains.

Effect of temperature on viscosity

The viscosity of sodium alginate decreased steadily with temperature at a nearly constant rate, approximately a 10-fold decrease over 58°C (Fig. 6). PGA showed a similar decrease up to about 60°C beyond which the rate of decrease became much greater. XG, whose absolute viscosity was much higher than these two and was more similar to that of PMA, lost viscosity more slowly than sodium alginate initially but above 60°C the rate of decrease accelerated somewhat.

By comparison with these, the PMA, at a concentration which gave a similar value for the viscosity, was far more stable in this respect, losing only about 25% for a 58° rise in temperature, and remaining highly viscous at 100°C.

Effect of shear rate on viscosity

Figure 7 shows the results obtained on examination of the effect on measured viscosity of varying the rate of shear using a Shirley–Ferranti Cone and Plate viscometer. Both PGA and sodium alginate showed relatively small changes in viscosity while XG showed a substantial and steady decrease with increasing shear rate. For PMA there was also a substantial change with increasing rate of shear, the slope of the curve changing at higher shear rates. High viscosity, along with non-Newtonian behaviours of this nature, have been attributed to the presence of long rigid chains in the molecular structure and it is interesting that here the PMA behaved in a similar manner to XG.

Effect of salts on the viscosity of PMA

Probably the most dramatic property of PMA is the fact that its viscosity is highly dependent on salt concentration. Figure 8 illustrates this phenomenon and shows how PMA compares in this respect with the other polysaccharide derivatives. Neither XG nor PGA showed significant change in viscosity with sodium chloride concentration even over a salt concentration range of 0–1.0%. The viscosity of sodium alginate dropped slightly on going from 0 to 0.1% salt and then increased as the salt concentration was increased to 1%. The viscosity of PMA however, dropped to virtually zero when only 0.1% sodium chloride was present (Fig. 8) and did not rise with increasing salt concentration.

This salt effect was not specific for the particular ions present, as shown by Figs 9 and 10. Each of the salts, sodium thiocyanate, sodium sulphate, ammonium thiocyanate, lithium chloride and ammonium sulphate caused reduction in the viscosity in a similar manner and to a similar degree. Also of interest, and of potential importance, is the observation that this salt effect is reversible. Thus a viscous solution of PMA which had lost its viscosity on addition of salt regained it completely on dialysis. The effect is therefore a true 'salt' effect.

DISCUSSION

The viscosity of PMA

From the outset, it is clear that the presence of the morpholine residues attached to the polysaccharide chain contributes in some significant way to the viscosity of the PMA. Thus, only morpholine (and its close analogue, thiamorpholine) were able to cause the gelling of the PGA in solution (Gray & Philp, 1991). Furthermore, Fig. 3 shows that unsubstituted alginate (sodium alginate) and alginate carrying some residues derivatised as propylene glycol ester groups (PGA), are far less viscous than the PMA obtained from them.

High viscosities in solutions of polymers are usually attributed to long, rigid chains in the molecular structures (Atkins *et al.*, 1970, 1971) (although Stokke *et al.* (1987) suggested that the chains of quaternary ammonium salts are actually quite flexible). Cross-linking between chains may also contribute (Hermans, 1949).

In addition, Morris (1986) suggested that gel-forming properties might be due to the existance of conformationally ordered regions (almost insoluble) interconnected by solubilising chains with random structures. In our earlier paper we reported that PMA consisted of a polysaccharide chain carrying three different types of groups; (a) residual ester groups, (b) morpholineamide groups, and (c) free carboxyl groups. We discussed the gel-forming properties of the polysaccharide in terms of insoluble regions of polysaccharide chain, locally highly substituted by morpholine, linked by soluble segments carrying only free carboxyl or propylene glycol ester groups.

The experimental results reported here allow us to extend this discussion in order to take further account of the carboxylic acid groups present in the polymer.

From the pH titration (Fig. 2), it is clear that a substantial number of free carboxyl groups are present in the structure. It is also clear that since there is a substantial increase in viscosity on increasing the pH up to approximately 5, and that the viscosity is essentially unchanged from pH 5 to 9, the viscosity is related to the ionisation of the carboxyl groups. (The slight increase at pH 11 may be due to the hydrolysis of some ester groups at this pH having more carboxylate ionic groups.

In a polyelectrolyte such as this, the high viscosity may be due to two phenomena. Firstly, the mutual repulsions of the negative charges along the chain will force the polysaccharide to take up a conformational arrangement in which the charges are as far apart as possible (that is, a long rigid rod-like structure will be formed).

Secondly, the electroviscous effect (McDowell, 1986) whereby the presence of the counterions retards the movement of the solvent past the polymer, may apply.

Either or both of these effects may be important in the case of PMA since they would both be affected by the presence of salts, and the viscosity of PMA is dramatically reduced by the presence of salts (Figs 8–10). It is interesting that this salt effect is non-specific, in that all the salts examined gave similar results, no matter what the position of the ions in the Hofmeister series (von Hippel & Schleich, 1969). It is also important that the salt effect is quite reversible.

Non-Newtonian behaviour, as shown by PMA, especially at high concentrations (Fig. 7), is also associated by many workers with long rigid chains (Milas & Rinaudo, 1986; Sikkema & Janssen, 1989; Westra, 1989).

In conclusion, therefore, poly(morpholino)alginamide (PMA), formed by the replacement of propylene glycol ester groups in PGA by morpholine amide groups, is a polymer of very high viscosity. Indeed, it shows a higher viscosity on a weight for weight basis than xanthan gum. The pH–titration data, the variation of viscosity with pH and the dramatic reduction of viscosity in the presence of salts all suggest that the viscosity is directly associated with the charged carboxylate groups in the polymer. This information, taken in conjunction with the non-Newtonian behaviour of PMA, leads to the suggestion that the PMA in solution above pH 4 consists of regions containing portions of polysaccharide chain carrying morpholine amide groups (these regions being almost insoluble) linked together by soluble but rigid sections carrying the ionised carboxylate groups.

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