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Polyelectrolytes from polysaccharides: selective oxidation of guar gum — a revisited reaction

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The aim of this work was to study the properties of the carboxylated polyelectrolyte obtained from guar gum. The C-6 alcohol functions of galactose units side chains were oxidized first by GO-ase to aldehyde groups and then to carboxylic groups by halogen oxidation. The enzymic oxidation step was followed by the Dische method, by viscosity and light scattering measurements. With regard to previous reports, some changes were introduced in the two-step reaction, in order to prevent polymer degradation. Several characteristics of the carboxylated polyelectrolyte have been studied, such as molecular weight distribution, degree of substitution, viscosity, intrinsic viscosity determined by the isoionic method, radius of gyration, charge parameter and apparent intrinsic persistence length. The charged macromolecule formed from native guar showed all the typical characteristics of a polyelectrolyte. The viscometry results indicate that carboxylated guar has a much higher viscosity in low salt content than the native polymer, which improves its thickening properties.

INTRODUCTION

Guar gum is a high molecular weight natural carbohydrate polymer consisting of a polymannan backbone with single galactose unit side chains. D-mannose units are linked by $(1\rightarrow4)\beta$ linkages in the backbone and (1-6)α linkages with D-galactopyranosyl units. Guar gum can be used as a functional ingredient in the food and cosmetic industry and as dietary fibre (Garcia *et al.*, 1988; Sandford & Baird, 1983) etc. Gums as a class of material have a growing role in industry (Whistler, 1988).

The ability of these polysaccharides to change the flow properties of systems is the principal factor which governs their applicability. The modification of rheological properties of guar gum can improve and diversify its commercial applications. This can be achieved by derivatization of its functional groups (Yalpani & Hall, 1982) or by grafting with water-soluble non-toxic polymers (Bajpai & Jain, 1993).

The aim of this work was to modify guar by introducing charges in the chain, thus turning it into a polyelectrolyte. This can be achieved by oxidizing an alcohol function to a carboxylic acid in a highly specific way starting with an enzymic oxidation, which introduces an aldehyde group on galactose units followed by a chemical oxidation by halogen.

This derivatization to carboxylic acid was previously described in the literature (Yalpani & Hall, 1982; Dentini & Crescenzi, 1986). Many attempts have been made using the same procedures described there, but it has not been possible to isolate the product without degradation or by-products. Furthermore, up to now characterization of the polyelectrolytic properties of the product has been lacking.

MATERIALS AND METHODS

Guar gum was purified by dissolution in water (1 g/l) at room temperature followed by filtration. The solution was concentrated to 3 g/l by evaporation at 40°C under reduced pressure and then the polymer was isolated by ethanol precipitation (50% v/v) (Rinaudo, 1993). The dry polysaccharide was submitted to a total acid hydrolysis and the relative proportion of mannose and galactose was determined by liquid chromatography.

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Table 1. Evolution of the system during enzymic oxidation

Time reaction (h)	Absorbance (420 nm)	Relative viscosity	$(\overline{Mw})/(\overline{Mw})_0$
0	0·950	4·6	1·0
1	0·915	4·8	1·1
2	0·875	5·1	1·4
3	0·755	5·4	1·7
4	0·709	5·7	Aggregates
5	0·648	6·7	Aggregates
6	0·644	8·1	Aggregates

$\overline{Mw}_0$ is the initial weight average molecular weight. Concentration of reactants: 0·7 mg guar, 1·26 U GO-ase and 1·5 μl catalase per millilitre.

Enzymic oxidation

D-Galactose oxidase (GO-ase) was purchased from Sigma (lot 101H6812); catalase (which was always added on the basis of 1·2 μl of catalase per unit of GO-ase) was also obtained from Sigma. The reaction was followed by the Dische method (Dische, 1962) where the reduction in absorbance at 420 nm is proportional to the aldehyde formed.

In the first experiments, GO-ase and catalase were added to guar solution (0·7 mg/ml, 1·8 U GO-ase/mg polys) and the solution was adjusted to pH 7 by phosphate buffer addition, at room temperature. The reaction was also followed by viscosity and light scattering measurements (Frollini *et al.*, 1993), using a Contraves Low Shear 30 and a Wyatt Technology Dawn F, respectively. For light scattering experiments the starting solutions were filtered through 0·2 μm membrane filters.

The first results obtained, (Table 1) showed that the aldehyde group formation (indicated by the reduction of absorbance at 420 nm) was accompanied by a side reaction which caused a continuous increase in molecular weight, as attested by light scattering and viscosity measurements (Frollini *et al.*, 1993).

At this point, after ethanol addition, a precipitate was obtained, which remained partially insoluble when the material was redissolved in water. Several tests were performed in order to avoid the side reaction. It was found that its extent was dependent on the value of the initial guar concentration. The best result was achieved with a 0·25 mg/ml guar solution where the spectrophotometric measurements were similar to those described in Table 1 and the viscosity measurements remained constant.

In the literature it is always mentioned that when GO-ase is used the pH must be kept at 7 by addition of phosphate buffer, but in this case the buffer may coprecipitate with the polyelectrolyte in the second step. Hence, a reaction was performed without phosphate and it was observed that the pH remained around 6·8, and the absorbance measurements at 420 nm showed that the reaction continues to occur under these conditions. The solution was stirred overnight, at room temperature in a system open to the atmosphere. Under these conditions the absorbance no longer decreases with time which indicates that enzymic oxidation is maximal.

Chemical oxidation

The aldehyde oxidation reaction to the carboxylated derivative was first attempted using bromine in acid medium as described in the literature (Yalpani & Hall, 1982). The product was analysed by size exclusion chromatography and the results indicated extensive degradation. Viscometric measurements showed that the same degradation occurs for a guar solution submitted to bromine in acid medium. The oxidation was then attempted using I_2/KI at pH 9 (Dentini & Crescenzi, 1986) and the viscometric results indicated less degradation. After several tests it was confirmed that the degradation is prevented when the reaction is performed in the dark.

In this way, the aldehyde was oxidized directly at room temperature after the enzymic step, in the absence of light, adding 0·500 g I_2 and 0·1 g KI to an initial 0·25 mg/ml guar solution (ca. 660 ml). The pH was adjusted to 9 with sodium carbonate and the solution was stirred overnight instead of 4 h as described by Dentini & Crescenzi (1986) to ensure that the reaction was complete. The solution was then heated to 80°C and filtered through charcoal.

The polyelectrolyte was recovered by centrifugation, after NaCl (1 g) and ethanol addition. The product was exhaustively washed with ethanol up to 95% in order to eliminate the residual NaCl. The polyelectrolyte analysis started with molecular weight determination (size exclusion chromatography) in order to assess the degree of degradation occurring during the entire conversion process. In this preliminary work, kinetic studies with the aim of improving the yield of the two oxidation steps were not performed.

Determination of the degree of substitution (DS)

Potentiometric method

The acidic form of carboxylated guar was obtained by cation exchange on resin IR-120H and the DS was determined by potentiometric titration. Even though deionized and redistilled water was used in all solution preparations, an equal volume of water was passed through the resin and titrated under the same conditions as the solution, in order to correct the results for traces of acidic impurities obtained during the procedure used.

Colorimetric method

The galacturonic acid content was measured considering the absorbance at 520 nm, after polyelectrolyte hydrolysis and subsequent reaction with 3-hydroxybiphenyl (Blumenkrantz & Asboc-Hansen, 1973). A galacturonic standard curve (absorbance vs concentration) was used.

^{13}C *NMR*

The spectra of samples depolymerized by ultrasonication were obtained in D_2O (20 mg/ml), at 363 K. The relative areas of the signals C-6 (Gal/Man) were compared for native and oxidized guar.

Viscometry

The flow behavior of carboxylated guar was studied for a 10^{-3} g/ml solution in pure water and at 10^{-3} g/ml in 0·1 M NaCl solution over a range of shear rates (0·08–128·5 s^{-1}). The procedure was repeated for 10^{-3} g/ml aqueous guar solution. These and all other viscometric measurements were made at 25°C.

Intrinsic viscosity [η]

The value of $[\eta]$ for many polyelectrolytes often cannot be determined in pure water or in very low ionic strength solutions due to the peculiar behavior of the reduced viscosity (η_r); η_r increases with decreasing polyelectrolyte concentration and after passing a maximum it decreases. The intrinsic viscosity was determined at low shear rate ($\dot{\gamma} \leqslant 1{\cdot}7\ s^{-1}$) and at fairly high ionic strength (0·1 M NaCl), over the concentration range $0{\cdot}1 \times 10^{-3}$–1×10^{-3} g/ml. At this ionic strength and concentration the polyelectrolyte behaves like a neutral polymer i.e. a plot of the reduced viscosity (η_r) vs concentration is linear.

Isoionic dilution

The effect of charge on intrinsic viscosity can be evaluated using an isoionic dilution method, where the measurements are made at low NaCl concentration. A solution of initial polyelectrolyte concentration Cp_i (where Cp_i is in moles of COO^- per litre) in pure water was diluted serially with NaCl solutions of molar concentration C_s, such that $XCp_i = C_s$. XCp_i represents the equivalent salt concentration of the original solution of polyelectrolyte in pure water. The pure water solutions were prepared with deionized and redistilled water (conductivity $\simeq$ 0·7 μS) using polyethylene bottles in order to avoid ionic impurities. Mixing was performed while N_2 was being bubbled through the solution to prevent carbon dioxide from the air dissolving in the solution and increasing the ionic strength.

Light scattering and size exclusion chromatography

A Waters 150 C Size Exclusion Chromatography (SEC) apparatus coupled to a differential refractometer (RI), a single capillary viscometer and a Wyatt Technology Dawn-F Multi-Angle Laser Light Scattering detector (MALLS) was used for characterization of the original guar and the final polyelectrolyte product. Use of the MALLS detector in conjunction with the RI detector allows absolute determinations of polymer molar mass distributions and radii of gyration to be made without any calibration of the SEC columns. The mass distributions permit determination of the relevant molecular weight averages $\overline{Mn}$, $\overline{Mw}$ and $\overline{Mz}$, as well as the polydispersity indices usually defined in terms of $\overline{Mw}/\overline{Mn}$ and $\overline{Mz}/\overline{Mw}$. The intrinsic viscosities $[\eta]$ are also determined as a function of molar mass.

For this work, we used 2 Shodes OH pak B-805 and B-804 columns connected in series with 0·1 M NH_4NO_3 as eluent thermostated at 30° ± 0·1°C. Further data on the polyelectrolyte nature of the final product were obtained by using the MALLS in 'batch mode'; i.e. using unfractionated solutions of polymer in salt-free solution, and the addition of aliquots of NaCl solution allowed the decrease in second virial coefficient A_2 and radius of gyration $R_g = \langle S^2 \rangle^{1/2}$ (i.e. R_g is formally the root mean square radius of gyration) with increasing ionic strength to be monitored. These light scattering measurements were carried out with stock solutions of 0·10 mg/ml and 0·15 mg/ml aqueous solution of the native polysaccharide and the oxidized product which were then successively diluted with NaCl solution.

RESULTS AND DISCUSSION

The liquid chromatography analysis of hydrolysed guar gum gave a mannose/galactose ratio of 1·7.

Enzymic oxidation step

As shown in Table 1, when a guar solution with a higher initial concentration was used (0·7 mg/ml) a side reaction in the enzymic oxidation step occurred, as attested by the continuous increase in molecular weight. After 4 h reaction time, the molecular weight determination was no longer possible due to the presence of 'aggregates' (large particles).

Probably, the aldehyde groups of oxidized galactose units react with the closest hydroxyl groups on the adjacent chain, forming hemiacetal cross links which lead to an insoluble by-product. A similar result was observed during the oxidation of amylose (Painter & Larsen, 1970). The oxidation of galactose by GO-ase and its derivatives to galactose 6-aldehyde is used in analytical applications (Mazur, 1991) and it is obvious that this side reaction must be considered and prevented in order to quantify galactose. In a study with D-galactose-composed oligo and polysaccharides (Bretting & Jacobs, 1987) which aimed to relate the structural requirements for the interactions between GO-ase and its substrates, the results indicated a secondary reaction among the oxidation products. In the present work the conditions were changed as described previously and the results indicated that the aldehyde derivative was obtained with a minimal amount of by-products: the absorbance decreased in a similar way to that described in Table 1 and the relative viscosity remained around 1·18–2·00, which is nearly the same as the initial 0·25 mg/ml guar solution.

Chemical oxidation step

The initial results of the chemical oxidation step indicated a polysaccharide degradation by halogen. This degradation was observed by large reductions both in viscosity and light scattering intensity. When halogens are used as oxidizing agents, the concentrations of free halogen, hypohalous acid and hypohalite are very dependent on the acidity. For bromine in acidic solution, the free halogen molecule may be proposed as the active oxidant and in alkaline solution it is converted into hypobromite which can act as an oxidizing agent too, but can also break the carbon–carbon bonds. On the other hand, the hypoiodite present in an alkaline solution is an excellent oxidizing agent, whereas free iodine does not act as an oxidant (Green, 1980). In this work, the aldehyde oxidation with bromine was performed at pH ~7, where the free halogen and the hypobromite are present in solution and both may cause the observed degradation. The reaction with iodine at pH 9, where the hypoiodite is predominant, reduced the degradation. Although it is hard to determine exactly how the degradation occurs, it may be inferred that a radical reaction is responsible, because after several tests it was confirmed that the degradation is prevented when the reaction is performed in the dark.

SEC results

The size exclusion chromatography results are shown in Figs 1, 2 and in Table 2. Figure 1 shows that the mass distribution for the carboxylated guar is broader than that for the native guar, and the peak is at a lower molecular weight. Table 2 gives the decrease in $\overline{\mathrm{Mw}}$ and the increase in the polydispersity ratio $\overline{\mathrm{Mw}}/\overline{\mathrm{Mn}}$. While the data show that some degradation has occurred, it is clearly not a large effect. The intrinsic viscosity values $[\eta]$ in Table 2 show that at the relatively high ionic strength of 0·1 M, the behaviour of the charged and neutral polymer are similar; nonetheless the carboxylated guar has a somewhat higher $[\eta]$ even though its $\overline{\mathrm{Mw}}$ is lower, which probably reflects additional chain expansion of the charged polymer due to incomplete electrostatic screening at 0·1 M ionic strength. This is corroborated by Fig. 2, where R_g vs M for the two polymers is shown, and it is seen that, although they are quite close to each other, the charged polymer has a greater R_g at each mass. Similarly, in Table 2, the weight average radii of gyration R_{g_w} reflect the same trend; the carboxylated guar has a slightly higher R_{g_w} than the native guar, even though its $\overline{\mathrm{Mw}}$ is lower.

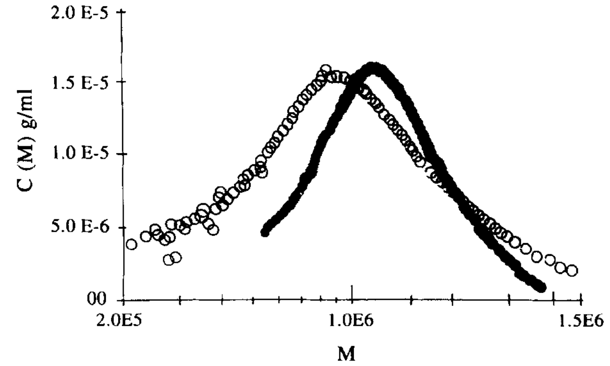

Fig. 1. Molecular weight distributions of carboxylated (○) and native (●) guar obtained from a SEC experiment using MALLS and differential refractometer detectors (eluent: 0·1 M NH_4NO_3 at 30°C).

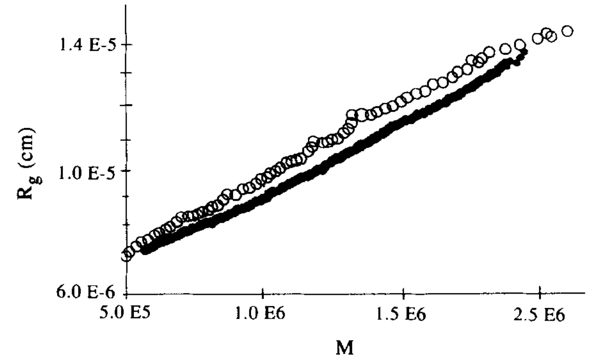

Fig. 2. Radius of gyration (R_g) as a function of molar mass (M) of carboxylated (○) and native (●) guar obtained from SEC experiments using MALLS and differential refractometers as detectors (eluent: 0·1 M NH_4NO_3 at 30°C).

Table 2. Average weight molecular weight, $\overline{\mathrm{Mw}}$, polydispersity index $\overline{\mathrm{Mw}}/\overline{\mathrm{Mn}}$, weight average radius of gyration, R_{g_w}, and intrinsic viscosity $[\eta]$ obtained from SEC experiment in 0·1 M NH_4NO_3 at 30°C for native and carboxylated guar

Sample	$\overline{\mathrm{Mw}}$	$\overline{\mathrm{Mw}}/\overline{\mathrm{Mn}}$	R_{g_w}(Å)	$[\eta]$(ml/g^{-1})
Guar	$1{\cdot}27 \times 10^6$	1·16	978	1169
Carboxylated guar	$0{\cdot}98 \times 10^6$	1·46	1005	1364

Chemical analysis of the carboxylated product

Assuming a mannose/galactose ratio of 1·7, the degree of substitution (DS) calculated from potentiometry and colorimetry results was 31 and 32%, respectively. Replication of the reactions under the same conditions led to the same results.

The C-6 carbons of the galactose and mannose residues of guar gum are readily identifiable by ^{13}C nuclear

Table 3. Relative areas of C-6 peaks in ^{13}C NMR spectra of native and carboxylated guar

Type of unit	Guar	Carboxylated guar
α-D-Galactopyranosyl	11·215[a]	13·398[a]
β-D-Mannopyranosyl unbranched	7·353[b]	12·266[b]
β-D-Mannopyranosyl branched	8·277[c]	14·444[c]

[a] Signal at 63·4 ppm.
[b] Signal at 62·9 ppm.
[c] Signal at 68·9 ppm.

magnetic resonance (NMR) (Grasdalen & Painter, 1980). The relative areas of these peaks (Table 3) were used to quantify the carboxylic groups formed by oxidation (assuming that only the intensity of the C-6 signal of guar gum is affected by oxidation).

From Table 3, the ratio of the C-6 peak area, (a)/(b)+(c), yields 0·72 for guar gum and 0·50 for the carboxylated derivative, which indicates that 30·6% of -OH_6 galactose units were converted to carboxylate. This result is in agreement with the other two values. From these data, it is concluded that the charge parameter of this polyelectrolyte is $\lambda \simeq 0{\cdot}28$ with $\lambda = \frac{e^2}{DkbT}$ with e the proton charge, b the average distance between the projection of the charge onto the chain axis, D the bulk dielectric constant of the solvent, k the Boltzmann constant and T the absolute temperature.

Viscometric and light scattering results; proof that the final product is a polyelectrolyte

The viscosity as a function of shear rate is shown in Fig. 3 for solutions of native and carboxylated guar. At low shear rates, the viscosity of a 10^{-3} g/ml aqueous solution of carboxylated polysaccharide is more than four times that of an equivalent guar solution. This is a well known effect of polyelectrolytes and relates to the increase in their dimensions as ionic strength decreases and allows electrostatic repulsions to expand the chain. In this case it is due to the expansion of the mannan backbone caused by electrostatic interactions among the COO- groups on the galactose side chains. When the solution of carboxylated guar is prepared in 0·1 M in NaCl, and the electrostatic repulsions are largely screened, the viscosity is nearly the same as the native guar gum solution in pure water or in 0·1 M NaCl, the viscosity of a guar gum solution being independent of the salt content.

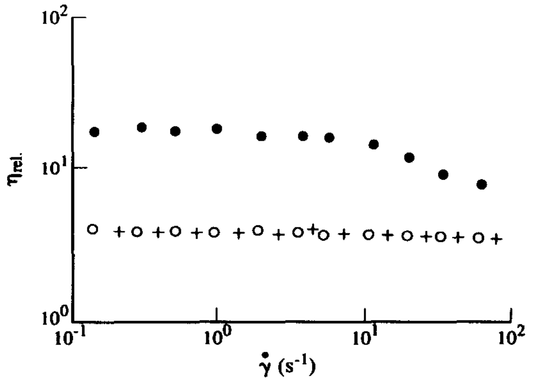

Fig. 3. Relative viscosity as a function of the shear rate ($\dot{\gamma}$) for 10^{-3} g/ml solutions of: carboxylated guar in water (●), in 0·1 M NaCl (○) and for native guar (+) in water and 0·1 M NaCl. $T = 25$°C.

The intrinsic viscosities for the two polymers were determined by viscometry in 0·1 M NaCl; the $[\eta]$ values obtained were 930 and 1275 ml/g for native and carboxylated polysaccharide, respectively, which is in reasonable agreement with the SEC values from Table 2.

In isoionic dilution, the intrinsic viscosity is determined at a low salt concentration C_s where $XCp_i = C_s$. *A priori* the value of X is unknown, but it is related to the degree of dissociation of the polyelectrolyte counterions. If X is chosen correctly an isoionic dilution will result, in which the polyelectrolyte feels the same ionic atmosphere as C_p decreases, as here a plot of η_r vs C will then be linear. If X is chosen too low then η_r vs C will be convex, and if X is too high the curve will be concave.

Values of $X = 1{\cdot}2$, $1{\cdot}0$, $0{\cdot}8$ were considered for carboxylated guar for η_r vs C where C is the polymer concentration in g/ml (Fig. 4). Isoionic dilution occurred for $X = 1{\cdot}0$ as predicted for a very low charge parameter with no counterion condensation. By extrapolation to $C \rightarrow 0$, it was found that $[\eta] = 6000$ ml/g. This is approximately five times higher than when the carboxylated guar is in 0·1 M NaCl solution which clearly shows the effects of polyelectrolyte chain expansion at extremely low ionic strength (i.e. 3.5×10^{-4} equiv. l^{-1}).

Figure 5 shows 'batch' static light scattering results for the carboxylated guar at different ionic strengths (controlled with NaCl). KC/I vs $\sin^2(\theta/2)$ is plotted, where C is the concentration of carboxylated guar, I is the measured absolute Rayleigh scattering ratio at observation angle θ, and K is an optical constant. The behavior of KC/I is exactly that expected for a good polyelectrolyte: as ionic strength increases both the slope and intercept of KC/I decrease. The decrease in intercept is due to the decrease in the second virial coefficient A_2 as interactions between polyelectrolyte chains are screened out, and the decrease in slope reflects the decrease in R_g of individual chains as intrachain electrostatic repulsions are screened. As expected, KC/I vs $\sin^2(\theta/2)$ for neutral guar is totally unaffected by ionic strength (not shown). The well known Zimm equation sums up these effects:

$$\frac{KC}{I} = \frac{1}{Mw}[1 + q^2\langle S^2\rangle/3] + 2A_2C,$$

where $q = (4\pi n/\lambda)\sin(\theta/2)$ is the scattering vector, λ being a vacuum wavelength of the laser (632 nm in this case)) and n is the index of refraction of the solvent

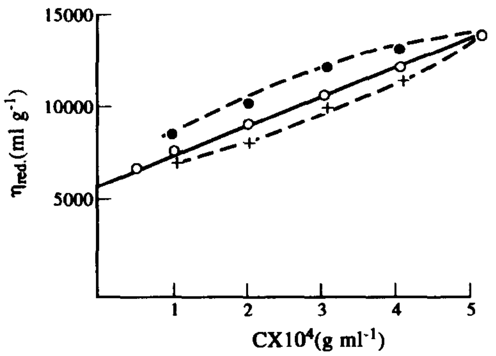

Fig. 4. Reduced viscosity of carboxylated guar as a function of polyelectrolyte concentration diluted with pure water ($C = 5{\cdot}0 \times 10^{-4}$ g/ml) and with different NaCl concentrations XCp_i with $X = 1{\cdot}2$ (●) $X = 1$ (○) and $X = 0{\cdot}8$ (+). $T = 25$°C.

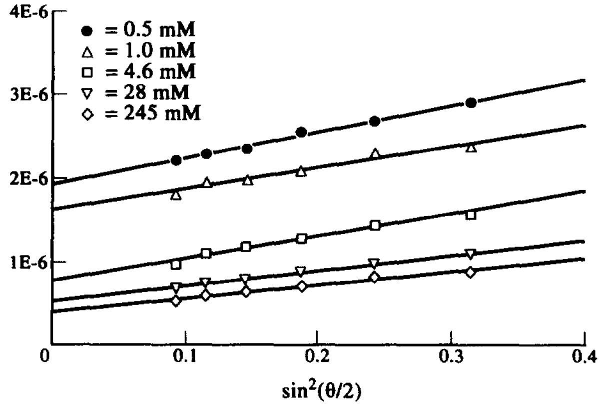

Fig. 5. Light scattering results: KC/I vs $\sin^2$ $(\theta/2)$ plot at different NaCl concentrations for the carboxylated guar using the Dawn MALLS in 'batch mode' at 25°C.

($n = 1{\cdot}33$ for water in this case). From these results an apparent intrinsic persistence length L_0 at high ionic strength of around 100 Å is obtained. This is quite comparable to semi-flexible, single chain, charged polysaccharides such as hyaluronate (Ghosh *et al.*, 1991; Fouissac *et al.*, 1992). The interpretation of curves such as Fig. 5, and the associated notions of apparent persistence lengths have been amply discussed elsewhere (Reed, 1994; Ghosh *et al.*, 1991; Reed & Reed, 1991). Then the polyelectrolytic behaviour of the carboxylated guar is classic and very similar to that obtained from acidic polysaccharides with comparable charge density.

CONCLUSIONS

The changes introduced in the two-step conversion of guar into a polyelectrolyte, although not drastically different from previous reports, were essential for producing the polyelectrolyte in a way which could be reproduced and which limited polymer degradation. The DS determinations indicated that nearly 31% of -OH on C-6 position of galactose units were converted to carboxylate under the conditions adopted in this work. No attempt to improve the degree of conversion was made, but experiments, in this field, are in progress in our laboratory. The charged macromolecule formed from native guar appears to be a random copolymer with a charge parameter $\lambda \simeq 0{\cdot}28$.

The viscometry results showed that the charges introduced on the galactose units yielded in low salt content a product with a much higher viscosity than the native guar, which substantially improves the thickening properties. The product was well characterized and the results indicated that a polyelectrolyte having good solubility compared with native guar was obtained with all the typical characteristics of a charged polymer.

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