Carbohydrate Polymers 4 (1984) 253–270

Interaction Properties of D-Galactose-Depleted Guar Galactomannan Samples

B.V. McCleary

Biological and Chemical Research Institute, NSW Department of Agriculture, Rydalmere 2116, New South Wales, Australia

I.C.M. Dea, J. Windust and D. Cooke

Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ, UK

(Received: 25 July 1983)

SUMMARY

The interaction of galactose-depleted guar galactomannan with agarose has been studied by optical rotation, and with xanthan using an Instron Materials Tester and a Rheometrics Mechanical Spectrometer. Samples were prepared by treatment of guar galactomannan with highly purified α-D-galactosidase. Modified guar galactomannans with a D-galactose content of 19–25%, in admixture with agarose, showed similar optical rotation changes on heating and cooling as did mixtures of agarose and carob galactomannan (23% D-galactose content). Modified samples with 13–16% D-galactose, in the presence of agarose, showed more pronounced optical rotation changes on heating and cooling, but samples with less than 13% D-galactose were only sparingly soluble even on autoclaving. The degree of interaction of galactose-depleted guar galactomannan samples with xanthan, as measured rheologically, increased as the D-galactose content decreased, paralleling the optical rotation changes with galactomannan/agarose mixtures. In the presence of xanthan, samples with a D-galactose content of 25% or less formed firm rubbery gels.

INTRODUCTION

The strength of the interaction of galactomannans with xanthan, agarose and κ-carrageenan increases with decreasing D-galactose content

Carbohydrate Polymers 0144-8617/84/$03.00 –

(Hui & Neukom, 1964; Dea *et al.*, 1972; Dea & Morrison, 1975; Dea *et al.*, 1977; McCleary, 1979*a*). The possibility that fine-structural differences in the distribution of D-galactosyl residues along the D-mannan backbone of galactomannans are also important has been substantiated in only one instance (McCleary, 1979*a*). It has been shown that although galactomannans from seeds of *Leucaena leucocephala* and *Cyamopsis tetragonolobus* (guar) have similar D-galactose contents, the degree of interaction of xanthan with the *Leucaena* galactomannan is much greater than with guar. This has been related to fine-structural differences between the two galactomannans. Viscosity measurements indicate that the *Leucaena* galactomannan has a lower molecular weight, indicating that molecular size is not the cause of the differences in properties of these two galactomannans. For guar and carob (*Ceratonia siliqua*) galactomannans, in which the D-galactosyl residues are distributed in neither a uniform nor a block-type pattern (Hoffman & Svensson, 1978; Painter *et al.*, 1979; McCleary, 1979*a*), the striking difference in the degree of interaction with other polysaccharides appears to be due simply to the different D-galactose contents (McCleary, 1979*a*). Molecular size differences are not the cause of the different interaction properties of guar and carob galactomannans since from comparative intrinsic viscosity values (McCleary *et al.*, 1976; McCleary *et al.*, in preparation) guar galactomannan would appear to have a slightly higher molecular weight (Robinson *et al.*, 1982) (see Table 1).

Treatment of guar galactomannan with highly purified α-D-galactosidase (EC 3.2.1.22), devoid of the chain splitting enzyme β-D-mannanase (EC 3.2.1.78), yields galactomannans which interact strongly with xanthan (McCleary *et al.*, 1981). In the present investigations the behaviour of these materials in mixed polysaccharide systems has been studied spectroscopically and using destructive and non-destructive rheological techniques.

MATERIALS AND METHODS

(a) Polysaccharide samples

(i) Galactomannans

Guar (*Cyamopsis tetragonolobus*) and carob (*Ceratonia siliqua*) galactomannan were extracted and purified from whole seed or commercial flours as previously described (McCleary *et al.*, 1983).

TABLE 1
Properties of Carob Galactomannan and D-Galactose-depleted Guar Galactomannan Samples

Galactomannan sample	*Galactose content of galactomannan samples*			*Intrinsic viscosity, dl g^{-1}*		*Degree of hydrolysis by* A. niger *β-mannanase, %*
	Enzymic	*Spectroscopic*	*GLC*	*I*[a]	*II*[b]	
A (native)	39·0	40·0	38·4	16·0	14·1	5
B	33·6	34·0	32·7	19·3	14·4	14
C	28·7	28·0	26·5	16·6	17·8	20
D	24·6	22·5	21·9	19·6	18·0	23
E	19·2	18·7	18·9	21·8	19·5	29
F	16·4	13·7	14·3	21·4	19·6	30
G	13·7	12·2	11·9	21·2	19·6	31
H	10·0	–[c]	9·8	–[c]	–[c]	32
Carob	23·0	23·0	22·6	13·4	10·9	22

[a] Determined using a Contraves low shear viscometer.
[b] Determined using an Ubbelohde suspended level viscometer.
[c] Not determined due to polymer solubility problems.

(ii) Agarose
The agarose used was a production batch (Batch No. 202) obtained from Seravac Labs., Pty., Maidenhead, Berkshire, UK. The sample is essentially free of substituents.

(iii) Xanthan
The extracellular polysaccharide from *Xanthomonas campestris* was a commercial sample of xanthan obtained from Kelco Co. Inc., New Jersey, USA. It was the sample used in earlier investigations (Dea *et al.*, 1977).

(b) Enzymes

α-Galactosidase II from germinating guar seed (McCleary, 1983) was purified to homogeneity by affinity chromatography on *N*-ε-amino-

caproyl-α-D-galactopyranosylamine linked to Sepharose 4B (Harpaz *et al.*, 1974). β-D-Mannanase present in the commercially available *A. niger* preparation 'Myles Hemicellulase 100,000' (Myles Laboratories Inc., Elkhart, Indiana, USA) was prepared by the procedure previously described for the purification of β-mannanase from another *A. niger* preparation (Sigma Cellulase, Cat. No. 0705) (McCleary, 1979*b*). The β-mannanase from these two *A. niger* preparations had identical action patterns. The enzyme purified from Myles Hemicellulase preparation had a specific activity of 2·2 μkat mg^{-1} protein at 40°C and pH 4·5 with carob galactomannan (0·2% w/v) as substrate.

(c) Preparation of D-galactose depleted guar galactomannan samples

To aliquots (800 ml) of guar galactomannan solution (0·5% w/v) in 50 mM sodium acetate buffer (pH 4·5) was added guar-seed α-D-galactosidase II (McCleary, 1983) (0–200 nkat on this substrate). The solutions were overlaid with a few drops of toluene, sealed, and incubated at 35°C for 24 h. The reaction was then terminated, and free galactose was removed by precipitation of the polymer with ethanol (2 vol). The galactomannans were washed with alcohol and acetone and dried *in vacuo*.

(d) Hydrolysis of galactomannans by *A. niger* β-mannanase

To a solution of galactomannan (0·4% w/v) in 20 mM acetate buffer (pH 4·5) was added β-mannanase (400 nkat g^{-1} on galactomannan) (McCleary, 1979*b*; McCleary & Matheson, 1983) and the solution incubated at 40°C for 20 h. The reaction was terminated by incubation at 100°C for 10 min and the solution centrifuged (20 000 *g*, 30 min), concentrated at reduced pressure (below 40°C), adjusted to 4% w/v carbohydrate and aliquots (2–5 ml) fractionated by Bio-Gel P-2 chromatography at 60°C (Kainuma *et al.*, 1976; McCleary *et al.*, 1983). Aliquots were also removed for the determination of total carbohydrate by the anthrone procedure (Loewus, 1959) and reducing sugar level using the Nelson/Somogyi procedure (Somogyi, 1952).

(e) Galactose/mannose ratios

(i) Enzyme technique

Solutions were adjusted to a concentration of approximately 1 mg ml^{-1} and aliquots removed for determination of total carbohydrate (Loewus, 1959) and D-galactose content by the α-galactosidase/β-galactose dehydrogenase procedure (McCleary *et al.*, 1983).

(ii) Spectroscopic technique

This involved dispersing the sample (15 mg) in distilled water (10 g) in a screw-cap bottle using a magnetic stirrer. The sample was autoclaved for 30 min in a pressure cooker at 120°C and inspected for dissolution. In practically all cases the sample had to be autoclaved a second time. The solution was then transferred, while still hot, to a jacketed 5 or 10 cm polarimeter cell at 90°C, cooled to 25°C and left to equilibrate for 1 h. Optical rotation was read at four wavelengths (365, 436, 546 and 578 nm) using a Perkin Elmer type 241 Polarimeter and a Drude plot of $(\text{rotation})^{-1}$ versus (wavelength) checked for linearity. The molar rotation was then determined using the value at 365 nm and this was converted to % galactose via a calibration graph which had been constructed using the galactomannans from guar, *Caesalpinia spinosa* and carob, of known galactose contents (Buffington *et al.*, 1980).

(iii) Gas-liquid chromatography

This involved gas chromatography of the mixture of galactitol and mannitol hexa-acetates that resulted from hydrolysis of galactomannan followed by reduction and acetylation (Albersheim *et al.*, 1967).

(f) Optical rotation measurements

Optical rotation measurements were carried out on agarose-galactomannan mixtures with the Perkin Elmer 241 polarimeter using 10 cm cells. Measurements were made at the wavelength 436 nm. The experimental procedures and precautions have been described earlier (Dea *et al.*, 1972).

(g) Rheological characterization of the interaction between galactomannan and xanthan

Gels were prepared to give a concentration of 1% galactomannan and 0·5% xanthan. The constituents were dispersed in water (15 ml) in a screw-top bottle using a top-drive Atomix, autoclaved for 5 min at 120°C, mixed with the Atomix then centrifuged (3000 rpm, 2 min) to remove bubbles, warmed in a water bath to remelt and then poured into perspex moulds 0·5 in diameter by 0·48 in deep. Yield stress (the force required to rupture the gel) was measured on the gel plugs at room temperature, after ageing for 24 h, using the Instron Materials Tester. Samples were compressed between parallel plates using a cross-head speed of 20 mm min^{-1} (chart speed 200 mm min^{-1}), and the yield stress was obtained from the height of the first peak.

The remains of each of the above samples were remelted in a hot-water bath and stirred to ensure homogeneity. Subsequently, measurements were made on a Rheometrics Mechanical Spectrometer. Samples were poured onto the plate hot and the cone lowered before the gel set.

Measurements of the storage modulus (G^1) were made at 25°C. The temperature was then raised and G^1 measured with increasing temperature up to about 70°C which was well past the melting temperature.

(h) Intrinsic viscosity measurements

(i) Using a Contraves Low Shear 30 viscometer

Samples (*c.* 100 mg) were wetted with ethanol (0·5 ml) to aid dispersion and urea solution (50 ml, 0·5 M) added with shaking. The suspension was stored at 4°C for 20 h and then heated at 100°C for 10 min. A further aliquot (50 ml) of urea was added and the solution homogenized using a Polytron blender. Carbohydrate concentration was determined (Loewus, 1959) and adjusted to values of 0·005–0·03% w/v. Viscosities were measured at 25°C using the Contraves Low Shear 30 viscometer and the intrinsic viscosity calculated.

(ii) Using an Ubbelohde suspended-level viscometer

Samples were suspended and dissolved as above but with 0·5 M KCl as solvent. Solution concentrations employed were 0·005–0·05% w/v carbohydrate. No allowance was made for shear thinning.

RESULTS

Galactose-depleted guar galactomannan samples were prepared by treating the polymer with highly purified α-D-galactosidase II from guar-seed. In the presence of dilute guar galactomannan solutions (0·1% w/v) this enzyme could reduce the D-galactose content of the polymer from 38 to 15% with no significant decrease in solution viscosity, indicating that the enzyme was completely devoid of the chain splitting enzyme, β-D-mannanase (McCleary *et al.*, 1981).

Some properties of galactose-depleted guar galactomannan samples are shown in Table 1. The D-galactose content ranges from 39 to 10%. As the D-galactose content decreased the intrinsic viscosity of the remaining polysaccharide increased approximately proportional to the increase in the amount of D-mannose in the galactomannan. This result is consistent with the previous conclusions (McCleary *et al.*, 1981) that in dilute solutions the viscosity of galactomannans is totally dependent on the nature of the D-mannan backbone (assuming, of course, that the D-galactose content is sufficient to maintain solubility).

Information on the fine-structures of the galactose-depleted guar galactomannan samples was obtained by treating the polysaccharides with a highly purified β-D-mannanase from *A. niger*. Details of the subsite binding requirements and action pattern of this enzyme have been discussed (McCleary & Matheson, 1983). The patterns of amounts of oligosaccharides released on hydrolysis of each of the galactomannans (Table 2) is consistent with a non-regular distribution of D-galactosyl residues in the original polymer. Galactomannans with a block-type D-galactose distribution would be expected to yield β-D-mannobiose, β-D-mannotriose and a high degree of polymerization (*DP*) fraction with a D-galactose content approaching 50%, as the major reaction products. Galactomannans with a uniform pattern of D-galactose distribution similar to that proposed for guar galactomannan by Baker & Whistler (1975) (i.e. a D-galactosyl residue on every second D-mannosyl residue) would yield the trisaccharide 6^1-α-D-galactosyl(1 → 4)β-D-mannobiose as the major reaction product.

The D-galactose distribution in carob galactomannan is also non-regular, as shown by detailed computer studies on the products of hydrolysis of this polymer by β-mannanases with different subsite binding requirements (McCleary *et al.*, in preparation). However, from the data in Table 2 it is evident that the pattern of non-regularity of

TABLE 2

Oligosaccharides Produced on Hydrolysis of Carob Galactomannan and of D-Galactose Depleted Guar Galactomannan by *A. niger* β-Mannanase

Oligosaccharide		*Galactomannans, galactose contents and amounts of oligosaccharides*[b]					
DP	*Structure*[a]	*Carob, 23%*[c]	*Galactose-depleted guar galactomannans*				
			39%	*33·6%*	*28·7%*	*24·6%*	*19·2%*	*13·7%*
1	Man	1[b]	0	0·5	1	1·5	2	2·5
2	Man_2	20	1	4·5	10·5	16·5	20·5	28·5
3	Man_3	13	0·5	5	7·5	12	21·5	37
3	Gal^1Man_2	12	2·5	13	23	27	24	16
4	Gal^1Man_3	7	0·5	5	9	11	13	9
7	$Gal^{3,4}Man_5$	15	1	5	5	6·5	5·5	1·5
>7	–	32	94·5	67	44	25·5	13·5	5·5

[a] Man, mannose; Man_2, (1→4)β-D-mannobiose; Man_3, (1→4)β-D-mannotriose; Gal^1Man_2, 6^1-α-D-galactosyl(1→4)β-D-mannobiose; Gal^1Man_3, 6^1-α-D-galactosyl (1→4)β-D-mannotriose; $Gal^{3,4}Man_5$, $6^3,6^4$-di-α-D-galactosyl(1→4)β-D-manno-pentaose.

[b] Amounts of oligosaccharides, wt %.

[c] Galactose contents of galactomannan samples as determined using the enzymic procedure.

D-galactose distribution in this polysaccharide is different from that in α-galactosidase modified guar galactomannans of similar D-galactose contents. This fine-structure difference is reflected in the slightly different interaction properties of these polysaccharides (see Table 3 and Figs 1–3). We have previously reported (Dea *et al.*, 1972) that the addition of carob galactomannan to non-gelling 0·05% agarose results in gelation, and changes the form of the optical trace; the cooling curve is now a composite of the usual negative contribution from the agarose coil to helix conversion and a new, positive contribution from a galactomannan transition which does not occur in the absence of agarose. The hysteresis now shows a complex butterfly form instead of the usual loop, which was interpreted as showing that although the

TABLE 3
Interaction Properties of Carob Galactomannan and Modified Guar Galactomannan Samples with Agarose and Xanthan

Galactomannan sample	*Positive contribution to optical rotation change from the galactomannan at 436 nm*[a] *(degrees)*	*Average yield stress*[b] *(N)*	*Storage modulus*[b] *(d cm^{-2}) x 10^{-3}*	*Gel melting point*[b] *(°C)*
A (native guar)	0·003	–[c]	0·57	–[c]
B	0·007	–[c]	1·45	–[c]
C	0·008	–[c]	2·63	35–36
D	0·015	2·6	4·25	37–39
E	0·018	4·4	5·86	40–41
F	0·030	10·9	6·55	44–46
G	0·040	12·9	6·74	45–47
H	–[d]	9·8	5·79	47–49
Carob	0·021	3·4	2·80	38–40

[a] Obtained from the temperature dependence of optical rotation traces for mixtures of agarose (0·05%) and galactomannan (0·1%). (See Figs 1 and 2.)
[b] Obtained from examination of the mixed gels of xanthan (0·5%) and galactomannan (1·0%). (See Figs 3 and 4.) The yield stress values are the average of three separate measurements.
[c] Either gels did not form, or were too weak to allow measurement of properties.
[d] Not determined due to polymer solubility problems.

disorder–order conversions for the two polysaccharides occur together on the cooling curve, on re-heating, the order–disorder transition for the galactomannan can be caused to occur first. The temperature dependence of optical rotation for agarose alone and an agarose/carob galactomannan mixture are shown in Fig. 1, together with that of a mixture of agarose with guar galactomannan. The gelling interaction of guar galactomannan with agarose is very much weaker than that with carob galactomannan (Dea *et al.*, 1972), and this lower degree of interaction is also apparent from the optical rotation data. Thus agarose (0·05%) alone shows a negative optical rotation transition at 436 nm, on cooling, of 0·016°, while a mixture of agarose (0·05%) and guar

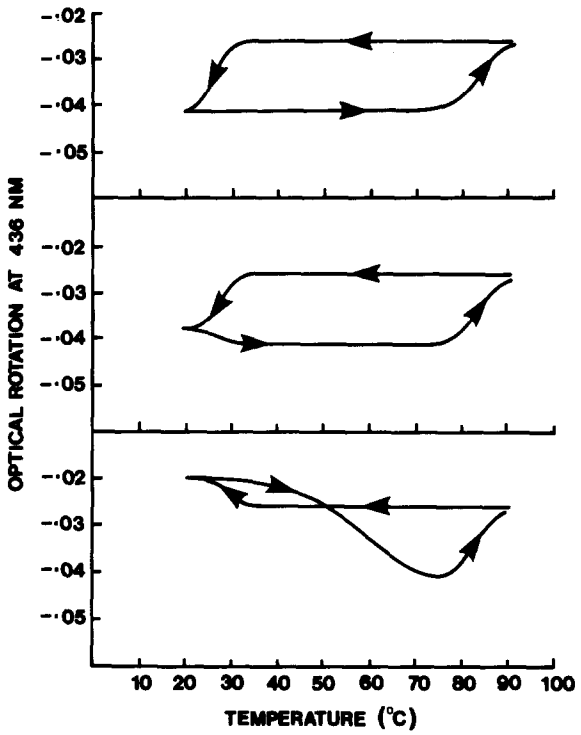

Fig. 1. Comparison of the optical rotation variations with temperature for (top) agarose (0·05%), (middle) a mixture of agarose (0·05%) and guar galactomannan (0·1%), and (bottom) a mixture of agarose (0·05%) and carob galactomannan (0·1%). The middle and bottom curves were normalized to the curve of agarose.

galactomannan (0·1%) shows a reduced negative optical rotation transition on cooling of 0·013°. This indicates a positive contribution to the optical rotation change from guar galactomannan of 0·003°. In contrast, a mixture of agarose (0·05%) and carob galactomannan (0·1%) shows a net positive optical rotation transition on cooling of 0·005°, indicating a positive contribution to the optical rotation change from carob galactomannan of 0·021°. (See Table 3.)

The temperature dependence of optical rotation for agarose/modified guar galactomannan mixtures was determined for samples A to G. Data could not be obtained for sample H because the limited solubility of this low galactose-containing galactomannan resulted in a turbid mixed

gel which prevented reproducible optical rotation measurements. As outlined above, the degree of interaction between the various galactomannans and agarose can be quantified by calculating the positive contribution to the change in optical rotation from the galactomannan on cooling the mixed gels. This data is listed in Table 3, and indicates a general trend for the positive contribution to the change in optical rotation to increase with decrease in the galactose content of the galactomannan. On this basis, samples F and G (galactose contents 16% and 13%) interact with agarose much more strongly than carob galactomannan (galactose content 23%). However, sample E (galactose content 19%) interacts with agarose to the same extent as carob galactomannan, indicating that galactose content is not the only criterion for determining the degree of interaction between agarose and galactomannans.

A selection of the temperature dependences of optical rotation traces for agarose/galactomannan mixtures are shown in Fig. 2. This indicates that there is a gradual change in optical rotation behaviour from that of agarose/guar galactomannan, through that of agarose/sample E which closely resembles agarose/carob galactomannan, to the exaggerated optical rotation profiles for mixtures of agarose with the highly galactose-depleted samples.

On admixture of xanthan (0·5% w/v) with native guar gum (sample A) and the least modified sample B (1·0% w/v) no evidence of gel formation on cooling to 20°C was observed. Mixtures of xanthan with samples C to H formed gels under these conditions. The gel formed using sample C was, however, only just self-supporting, and was too weak to permit accurate measurement of yield stress using the Instron Materials Tester. The yield stress values for the gels formed with the other modified guar samples and with carob gum are listed in Table 3. It can be seen that as the galactose content of the modified guar samples decreased from 24·6% (sample D) to 13·7% (sample G), the yield stress value for the mixed xanthan/galactomannan gel increased markedly. Because of its low galactose content (10%) sample H had only limited solubility. When dissolved alone in water it showed a tendency to partially precipitate on standing at room temperature. This also occurred in mixtures with xanthan and resulted in the formation of an uncharacteristic turbid mixed gel. The lower value of yield stress for the gel obtained from xanthan and sample H is considered to be due to this limited solubility. The variation of yield stress for the mixed gels with galactose content of the modified guar samples is shown in Fig. 3.

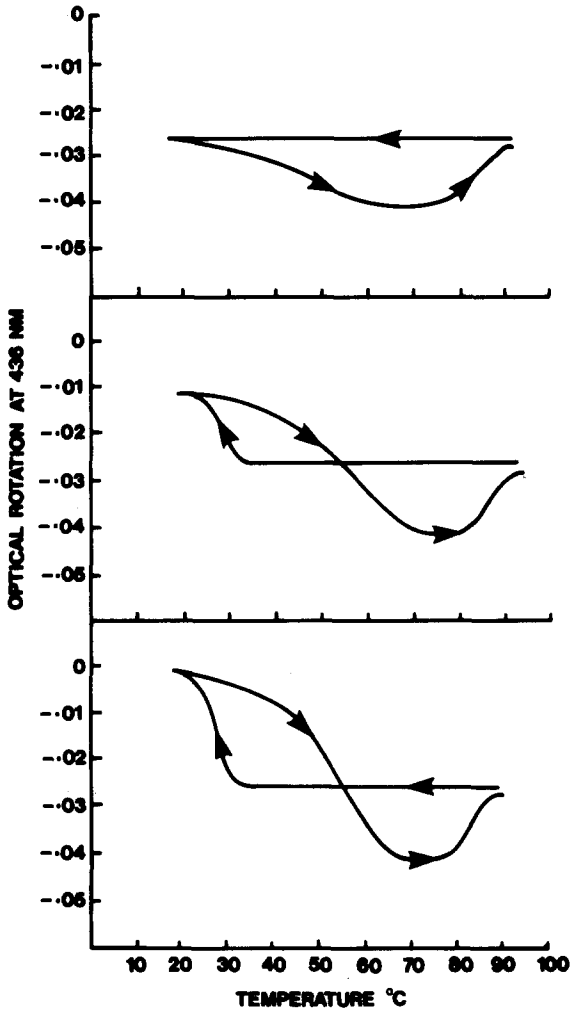

Fig. 2. Comparison of the optical rotation variations with temperature for (top) a mixture of agarose (0·05%) and modified guar sample D (0·1%), (middle) a mixture of agarose (0·05%) and modified guar sample F (0·1%), and (bottom) a mixture of agarose (0·05%) and modified guar sample G (0·1%). All curves have been normalized to the curve for agarose.

Examination of the same polysaccharide mixture using a Rheometrics Mechanical Spectrometer gave the storage modulus (G^1) values listed in Table 3 and graphically represented against D-galactose content of the modified guar samples in Fig. 3. The G^1 values show a similar trend to

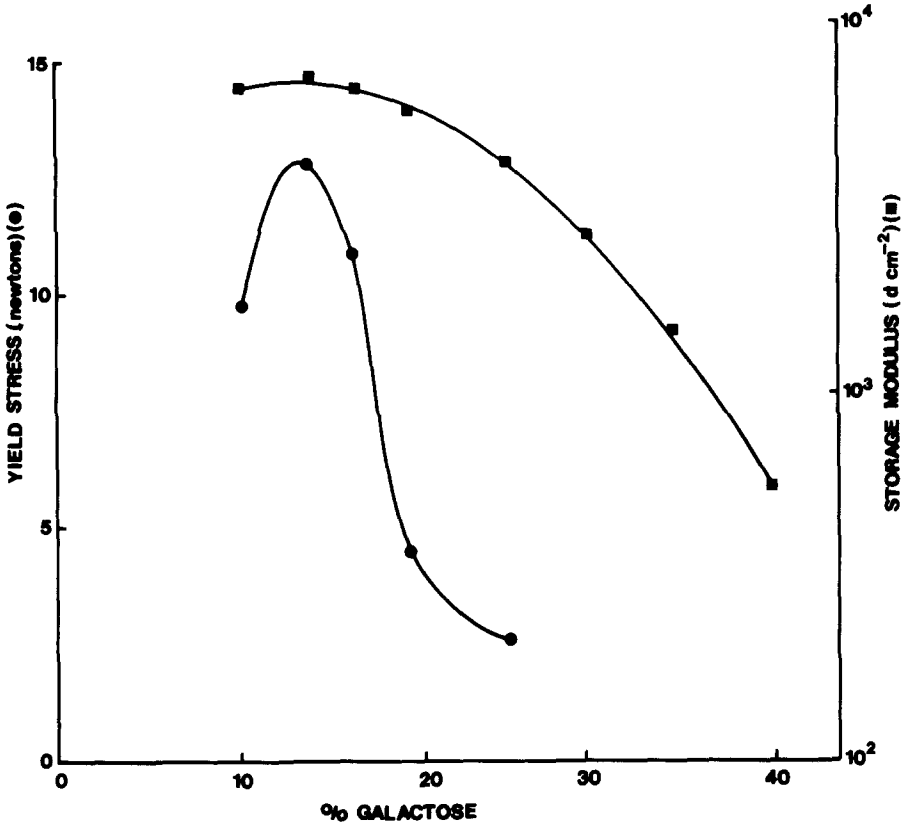

Fig. 3. Variation in the yield stress and storage modulus for mixed gels of xanthan (0·5%) and modified guar galactomannans (1·0%) with galactose content of galactomannan.

that of yield stress. The G^1 data is however more sensitive, and indicates that although sample B (34% galactose) does not cause xanthan to gel, the two polymers do show a significant increase in rheological interaction over that with guar gum. The results again demonstrate that over-modification of the galactomannan, to give a product with limited solubility, results in a weaker galactomannan/xanthan gelled system. The variation of storage modulus with temperature is shown in Fig. 4. The inflection point of these curves can be taken as a measure of the melting points of the interaction between the galactomannans and xanthan. These melting points are listed in Table 3, and it can be seen

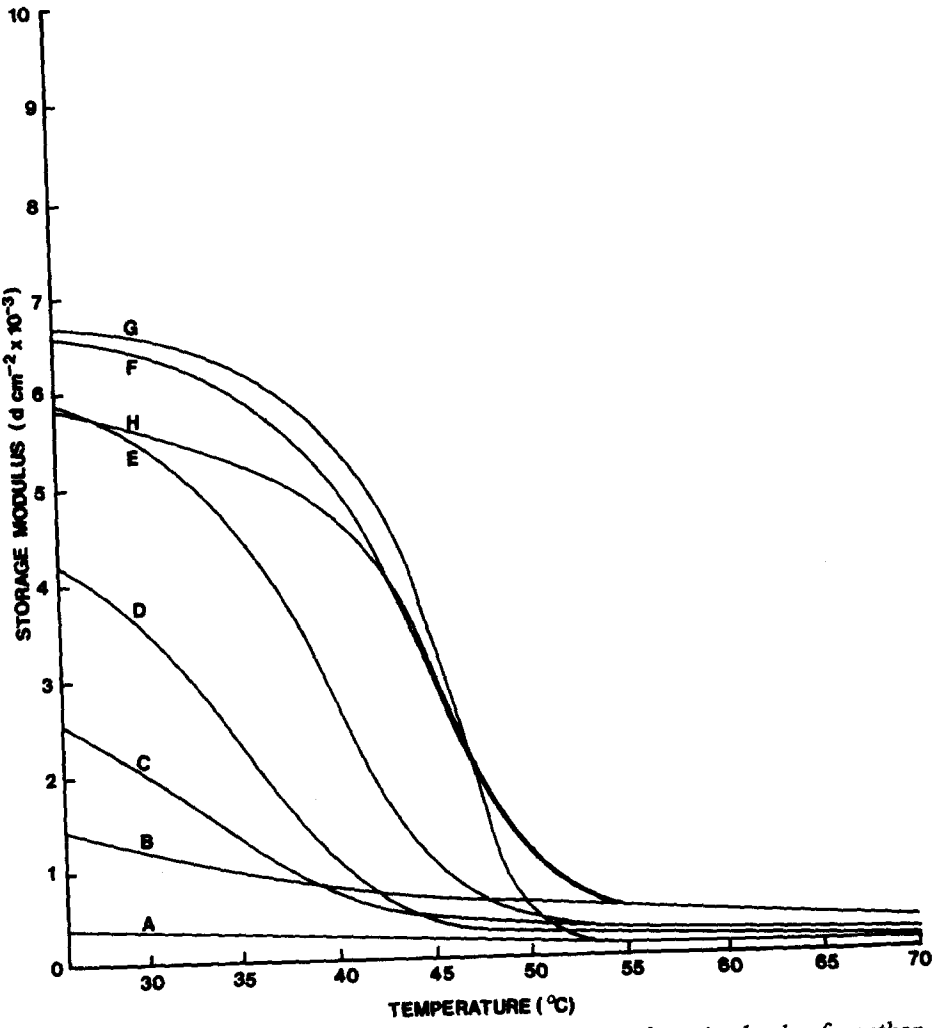

Fig. 4. Variation of storage modulus with temperature for mixed gels of xanthan (0·5%) and modified guar galactomannans (1·0%).

that they increase with decrease in galactose content of the galactomannan.

From the rheological data it is apparent that the more highly galactose-depleted guar galactomannan samples interact better with xanthan than carob galactomannan. However, as in the case of the agarose/galactomannan interactions, there is evidence that the galactose

content is not the only criterion for determining the strength of interaction, since carob galactomannan (D-galactose, 23%) interacts rheologically with xanthan to the same extent as Sample E (D-galactose, 19%).

DISCUSSION

The results presented above are consistent with previous reports (Hui & Neukom, 1964; Dea *et al.*, 1972; Dea & Morrison, 1975; Dea *et al.*, 1977; McCleary, 1979*a*; McCleary *et al.*, 1981) that, in general, the most effective galactomannans in the co-gelling interactions with agarose and xanthan are those in which the mannan backbone is least substituted by D-galactose stubs. This supports the suggestion that those regions of the galactomannan chain which are sparsely substituted or unsubstituted by D-galactose are primarily involved in these polysaccharide interactions.

In this study, some interaction properties of a range of enzyme modified guar galactomannan samples have been examined. These modified galactomannans are D-galactose-depleted samples obtained by the action of highly purified α-galactosidase II from germinating guar seeds. The α-galactosidase used was entirely free of β-mannanase activity, and intrinsic viscosity measurements clearly indicate that no main-chain cleavage had occurred in the preparation of these samples. Molecular size differences are therefore not a factor in the varying abilities of the modified samples to interact with agarose and xanthan.

The modified guar galactomannans studied ranged in D-galactose content from 39% (native guar) to 10%. Samples F and G (16% and 13% D-galactose respectively) interacted more strongly with both agarose, as indicated by optical rotation measurements, and xanthan, as indicated rheologically, than did carob galactomannan (23% D-galactose). Sample H, which was more substantially depleted in D-galactose (10% D-galactose) had only limited solubility in water and rapidly precipitated from aqueous solution on cooling. On admixture of sample H with either agarose or xanthan, there is therefore a competition between self-association of the galactomannan and mixed polysaccharide associations. The net result is that for samples with very low levels of D-galactose substitution, weaker and more turbid mixed gels are obtained with agarose and xanthan.

Of the modified guar galactomannans studied, samples D and E (25% and 19% D-galactose) showed similar, but not identical, interaction properties to carob galactomannan (23% D-galactose). The relevant data is listed in Table 3. From the optical rotation data with agarose and the rheological data with xanthan, there is a clear indication that carob galactomannan shows slightly stronger interaction properties than would be expected from its galactose content. These enhanced interaction properties cannot be accounted for on molecular size grounds since intrinsic viscosity data indicate that, if anything, carob galactomannan has a lower molecular weight than the modified guar samples. Examination of the array of oligosaccharides produced by *A. niger* β-mannanase degradation (Table 2) indicates that the modified guar galactomannan samples D and E differ from carob galactomannan in the distribution of D-galactose substituents. The significantly higher levels of the trisaccharide $Gal^{1}Man_{2}$ and the significantly lower levels of the heptasaccharide $Gal^{3,4}Man_{5}$ in the β-mannanase digest of samples D and E compared with carob galactomannan suggests that in carob galactomannan there is a higher proportion of unsubstituted regions of sufficient length for interaction, than there is in the two modified samples, even though all three galactomannans have a similar D-galactose content.

It has been previously reported (McCleary, 1979*a*) that the fine structure of distribution of D-galactose substituents along the D-mannan backbone of galactomannans can have a major effect on interaction properties. In this earlier study, guar galactomannan and *Leucaena leucocephala* galactomannan, both having D-galactose contents of *c.* 38%, were compared and it was found that the rheological interaction between xanthan and the *Leucaena* galactomannan was much stronger than that with guar galactomannan. Examination of the two galactomannans by β-mannanase degradation indicated major differences in the distribution of D-galactose substituents along the polymer backbone. Thus the *Leucaena* galactomannan has a significant proportion of the chain in which alternate D-mannosyl residues are substituted by D-galactose, while the guar galactomannan has a much more irregular distribution of D-galactose substituents along the molecule. The indications from the present study are that fine-structural differences in the distribution of D-galactose substituents in galactomannans can also influence the interaction properties of galactomannans with lower D-galactose contents.

Modified guar samples with D-galactose contents of 19% and 25% and carob galactomannan all appear to have a non-regular distribution of D-galactose substituents, but there are differences as shown by the patterns of amounts of oligosaccharides produced on β-mannanase treatment. Computer analysis (McCleary *et al.*, in preparation) of these results indicates that guar α-galactosidase II preferentially removes D-galactose residues from one face of the galactomannan molecule leaving a disproportionate amount of the repeating unit -[Man-Man(Gal)]$_n$ which yields 6^1-α-D-galactosyl(1 → 4)-β-D-mannobiose on β-mannanase degradation of the polymer (McCleary & Matheson, 1983; McCleary *et al.*, 1983). It would be of interest to establish whether this action pattern is typical of all α-galactosidases by studying the pattern of degradation of galactomannan by a range of enzymes of both plant and microbial origin, and in addition to consider analogous enzyme systems such as the action of α-L-arabinofuranosidase on arabinoxylans.

ACKNOWLEDGEMENTS

Part of this work was performed by B.McC. as visiting scientist, Unilever Research, Colworth Laboratory. He expresses thanks to Unilever Research for financial support and to the Nuffield Foundation for a Travelling Fellowship.

REFERENCES

Albersheim, P., Nevins, D. J., English, P. D. & Karr, A. (1967). *Carbohydr. Res.* **5**, 340.

Baker, C. W. & Whistler, R. L. (1975). *Carbohydr. Res.* **45**, 237.

Buffington, L. A., Stevens, E. S., Morris, E. R. & Rees, D. A. (1980). *Int. J. Biol. Macromol.* **2**, 199.

Dea, I. C. M. & Morrison, A. (1975). *Adv. Carbohydr. Chem. Biochem.* **31**, 241.

Dea, I. C. M., McKinnon, A. A. & Rees, D. A. (1972). *J. Mol. Biol.* **68**, 153.

Dea, I. C. M., Morris, E. R., Rees, D. A. Welsh, E. J., Barnes, H. A. & Price, J. (1977). *Carbohydr. Res.* **57**, 249.

Harpaz, N., Flowers, H. M. & Sharon, N. (1974). *Biochim. Biophys. Acta* **341**, 213.

Hoffman, J. & Svensson, S. (1978). *Carbohydr. Res.* **65**, 65.

Hui, P. A. & Neukom, H. (1964). *Tappi* **47**, 39.

Kainuma, K., Nogami, A. & Mercier, C. (1976). *J. Chromatogr.* **121**, 361.

Loewus, F. A. (1959). *Anal. Chem.* **24**, 219.
McCleary, B. V. (1979*a*). *Carbohydr. Res.* **71**, 205.
McCleary, B. V. (1979*b*). *Phytochemistry* **18**, 757.
McCleary, B. V. (1983). *Phytochemistry* **22**, 649.
McCleary, B. V. & Matheson, N. K. (1983). *Carbohydr. Res.* **119**, 191.
McCleary, B. V., Matheson, N. K. & Small, D. M. (1976). *Phytochemistry* **15**, 1111.
McCleary, B. V., Amado, R., Waibel, R. & Neukom, H. (1981). *Carbohydr. Res.* **92**, 269.
McCleary, B. V., Nurthen, E., Taravel, F. R. & Joseleau, J.-P. (1983). *Carbohydr. Res.* **118**, 91.
McCleary, B. V., Clark, A. H. & Dea, I. C. M., in preparation.
Painter, T. J., Gonzalez, J. J. & Hemmer, P. C. (1979). *Carbohydr. Res.* **69**, 217.
Robinson, C., Ross-Murphy, S. B. & Morris, E. R. (1982). *Carbohydr. Res.* **107**, 17.
Somogyi, M. (1952). *J. Biol. Chem.* **195**, 19.