Carbohydrate Polymers **10** (1989) 129–141

Gel-Permeation and Optical Rotation Studies on Xanthan–Galactomannan Interactions

Norman W. H. Cheetham & Acharaporn Punruckvong*

School of Chemistry, The University of New South Wales, PO Box 1, Kensington, New South Wales 2033, Australia

(Received 30 May 1988; accepted 21 June 1988)

ABSTRACT

Native xanthan gum and native locust bean (carob) gum solutions gel when mixed, even at low concentrations. This makes study of the interaction mechanism difficult. Solutions of partly depolymerised xanthan and partly depolymerised locust bean gum did not form gels when mixed, and were amenable to study by optical rotation and gel-permeation chromatography. The depolymerised xanthan and locust bean samples interacted in a similar way to that of the respective native polymers, and so constituted a reliable model for conformational and interaction studies.

INTRODUCTION

Xanthan gum, the extracellular polysaccharide formed by *Xanthomonas campestris,* is an important food colloid, widely accepted in the industry because of its unique rheological properties. Aqueous dispersions exhibit high viscosities at low shear rates, and pronounced shear-thinning behaviour.

The use of synergistic interactions between different polysaccharides in order to manipulate rheological and textural properties is commercially attractive, and their technological exploitation is increasing (Glicksman, 1968; Dea *et al.,* 1986; Dea, 1987). The interaction between xanthan gum and certain D-galacto-D-mannans has been

*Present address: Thailand Institute of Scientific and Technical Research, 196 Phahonyothin Road, Bangkhen, Bankok, 10900, Thailand.

Carbohydrate Polymers 0144-8617/89/$03.50 —

of considerable interest both from the application and fundamental points of view (McCleary, 1979; Rees *et al.*, 1982; Dea, 1987).

Gel formation between xanthan gum and locust bean (carob) galactomannan (LBG) can occur at total polysaccharide concentrations as low as 0·2%. The β-1-4-linked D-mannan chains sparingly substituted at C_6 with α-D-galactopyranosyl units interact more extensively than those that are more highly substituted. In addition, the galactomannan fine structure has an important effect on the interaction with xanthan gum, i.e. the distribution of galactosyl units is involved. There appears to be a specific requirement for unsubstituted sequences (Dea *et al.*, 1977; Rees *et al.*, 1982) or faces (McCleary, 1979; Dea *et al.*, 1986). Enzymic studies have shown that galactomannan chains which contain a high proportion of regular alternating structures, leading to one unsubstituted and one substituted face, show stronger interactions with xanthan than do chains with less regular substitution, but comparable galactose levels. (Dea *et al.*, 1986). The precise nature of the xanthan–galactomannan interaction is unclear. The initial model proposed invokes interaction between the xanthan backbone in the ordered, helical conformation, and the mannan backbone (Dea *et al.*, 1977; Morris *et al.*, 1977). This model was slightly modified to account for the galactomannan fine structure effect (McCleary, 1979; Dea *et al.*, 1986). A different model for the interaction, involving the xanthan sidechains and the mannan backbone, has been proposed (Tako & Nakamura, 1984; Tako *et al.*, 1984; Tako & Nakamura, 1986). More recently, Cairns *et al.* (1986; 1987) have proposed a further model, based on X-ray fibre diffraction studies on stretched gels. They observed that a mixture of xanthan solution with galactomannan solution did not lead to gelation until heated above the helix–coil transition temperature (T_c) of xanthan, then cooled. The model for interaction involved the cellulose-like backbone of xanthan (in the disordered or non-helical form) and the mannan backbone of the galactomannan.

We have recently reported observations that the gel-forming interaction must be preceded by the conversion of at least some of the xanthan backbone to the disordered state (Cheetham & Mashimba, 1988).

Study of the xanthan–galactomannan interaction is restricted by the very property of the system which makes it interesting, i.e. gel formation. Such useful techniques as optical rotation and high resolution NMR spectroscopy on gels are largely excluded. We have overcome part of the problem by using partially depolymerised galactomannan, which does not gel with xanthan (Cheetham *et al.*, 1986). This allowed the isolation by gel-permeation chromatography (GPC), and characterisation, of

xanthan–galactomannan complexes. The work confirmed earlier observations that the least-substituted galactomannan chains interact most strongly with xanthan. However, high-resolution NMR could still be obtained only at temperatures above those at which the xanthan–LBG interactions took place.

Ultrasonic depolymerisation

Ultrasonic depolymerisation is a non-random process that produces fragments arising from rupture near the midpoint of the chain (Basedow & Ebert, 1977). Some investigators found a reversible reduction of the viscosity after sonication of several biopolymers such as gelatin, starch and agar, which was apparently caused by the dispersion of aggregates, rather than breakage of covalent bonds. For most polymers however, definite depolymerisation is observed, especially at high ultrasonic intensities (Bergmann, 1954), as is the case in this study. Xanthan is particularly difficult to depolymerise by ultrasound, but Paradossi and Brant (1982) succeeded in producing a series of xanthan samples ranging in M_w from 19×10^5 to $0{\cdot}8 \times 10^5$. The primary and secondary structures were apparently unaltered (Hacche *et al.*, 1987).

Oxidative-reductive depolymerisation

Some compounds and metallic ions are able to induce changes in carbohydrates in the presence of oxygen. These autoxidants form free radicals which have been found to cause degradation of starch, glycogen and other polysaccharides (Neuberg & Muira, 1911) and the reactions have been termed oxidative-reductive-depolymerisation (ORD). Hydrogen peroxide together with a ferrous or ferric salt is such an autoxidant (Fenton, 1894). The most effective organic autoxidants for the ORD reaction are enediols, thiols, and quinones. An efficient system for ORD is achieved by carrying out the reaction in the presence of micromolar amounts of metal ion catalyst (Harris *et al.*, 1972).

Ascorbic acid is an enediol whose autoxidation seems to yield free-radical species of the same type generated by the Fenton reagent (Robertson, 1961). In the presence of copper ions, the initial reaction is likely to be (Peloux *et al.*, 1962)

$$AH_2 + Cu^{2+} \rightarrow Cu^{+} + AH^{\circ} + H^{+}$$

AH_2 is reduced ascorbic acid; AH° is the organic free radical derived from ascorbic acid.

Factors which may influence the course of the ORD reaction are

autoxidant concentration, buffer components, pH, ionic strength, catalyst, temperature, and order of addition of various components (Herp, 1980). The limited data available in the literature do not permit definite conclusions concerning the relationship between linkage type and degree of degradation. The ORD reaction has, however, a low efficiency in terms of chemical changes (Pigman *et al.*, 1965). Composition analyses showed little changes, so the primary sequence of ORD xanthan was taken to be essentially unchanged. Here we report optical rotation and GPC studies on mixed polysaccharide systems consisting of xanthan partly depolymerised by ultrasonication or oxidative-reductive cleavage, and LBG depolymerised by β-mannanase treatment. Results indicate that the same intermolecular interactions are involved in the native system and in the system using the smaller-sized polysaccharides. The latter system thus forms the basis of a valid model for the more detailed study of xanthan–galactomannan interactions.

EXPERIMENTAL

Materials

Xanthan (Keltrol) samples were purified by the modified method of Holzwarth (1976) and the resulting sodium salt was freeze-dried.

Locust bean gum fractions A and E were those reported previously. LBG-A had mannose–galactose = 5·5 and M_w 350×10^3, LBG-E had mannose–galactose = 5·2 and M_w 144×10^3 (Cheetham *et al.*, 1986). Acetyl and pyruvyl levels in xanthan were determined using an HPLC technique (Cheetham & Punruckvong, 1985). Proton NMR spectra were obtained in D_2O at 300 MHz on a Brüker CXP-300 spectrometer operated in the Fourier-transform mode, at various temperatures.

Gel-permeation using HPLC

The HPLC system consisted of an M6000 pump (Waters Assoc.), a 7125 injector (Rheodyne), an ERC-7510 refractive index detector (Erma Optical Works, Tokyo), and a TSK-Gel G6000 PW column (Toyo Soda Co. Ltd, Tokyo), 500×7.5 mm. Flowrate was 0·6 ml min^{-1}, with water or 0·01 M Na_2SO_4 as solvent. Samples were filtered (Millipore 0·8 μm) before injection. Xanthan and LBG solutions used were 1% (w/v) unless stated otherwise.

Fractions eluting from the column were collected, freeze-dried, and analysed for constituent monosaccharides by gas-liquid chromatography.

Depolymerisation of xanthan

1. Sonication (Hacche et al., *1987)*
Xanthan (0·5 g) was dispersed in distilled water (100 ml). Nitrogen was bubbled through (5 min), 2–3 ml of acetone was added, and the solution was cooled in a refrigerated jacket (≈ 5°C). Sonication was carried out at ≈ 300 W with a Sonicor ultrasonic probe for 30 min. The solution was flushed with nitrogen, acetone (0·5 ml) was added, and sonication was carried out for a further 30 min. The procedure was repeated to a total of 12 h sonication. Sonicated solutions were centrifuged, and filtered (0·45 μm, Millipore type HA) to remove metal particles. It was then dialysed against 0·01 M disodium EDTA, distilled water, finally passed through a column of Chelex-100 resin (Bio-Rad) to replace divalent ions with Na^+, freeze-dried, and stored in a desiccator.

2. Oxidative-reductive depolymerisation: (ORD XANTHAN)
Xanthan (1 g) was dissolved in 0·3 M phosphate buffer (500 ml) pH 7·2. When the sample was homogeneous, 0·02 M L-ascorbic acid (1·76 g) and 0·002 M cupric sulphate solution (0·5 ml) were added. The reaction was maintained at 30°C. Toluene was added to inhibit microbial growth, and the reaction was allowed to proceed for periods up to 10 days. The solution was thoroughly dialysed against EDTA, distilled water, passed through chelex resin, and freeze-dried as described for sonicated xanthan.

Optical rotation vs temperature

Optical rotation measurements were taken at 589 nm on a Perkin–Elmer 141 Polarimeter, using a 10 cm pathlength cell (1 ml). Temperature over the range 20–95°C was controlled by a Haake circulating water bath. Cell temperature was monitored by a thermocouple thermometer. Measurements on water alone over the range 20–95°C showed no anomalous readings due to cell-strain. Polysaccharide samples (0·3% w/ v) were prepared by soaking in water overnight, autoclaving (20 min) at 120°C, and filtering (0·5 μm Millipore type HA). Samples were also prepared in 0·02 M KCl, 0·02 M KCl plus 4 M urea, and in 4 M urea alone. The KCl and urea were added after the autoclaving. Polysaccharide concentration was determined by a modified anthrone colori-

metric method (Fairbairn, 1953). Appropriate xanthan–LBG solutions were mixed, heated above 85°C, and introduced into the polarimeter cells.

Determination of the monosaccharide composition of polysaccharides

In studies of the xanthan–LBG interaction using gel-permeation chromatography, it was necessary to determine the monosaccharide composition of various polysaccharide fractions collected from the column. After hydrolysis with 2 M trifluoroacetic acid at 120° for 2·5 h, the monosaccharide composition was determined by the aldononitrile acetate method of Turner and Cherniak (1981). A Shimadzu GC-9A gas chromatography and an OV1701 fused silica capillary column (25 m) run isothermally at 220°C were used, together with a Hewlett–Packard 3390-A integrator.

RESULTS AND DISCUSSION

Table 1 shows the pyruvate and acetate levels of various xanthan samples as determined by HPLC. The only significant change caused by the treatments is the low value for acetate in sonicated xanthan. This could be reflected in the low T_c values for sonicated xanthan (Table 1) as acetate is known to stabilise the ordered conformation (Smith *et al.*, 1981; Tako & Nakamura, 1984).

Ultrasonic depolymerisation of xanthan

Sonication of xanthan for 12 h yielded a sample (SX) with much reduced viscosity. There was a rapid decrease in viscosity initially, but after

TABLE 1
Pyruvate and Acetate Values for Xanthan Samples Determined by HPLC

Sample	*Pyruvate % (w/w)*	*Acetate % (w/w)*
Native xanthan	4·4 (4·6)[c]	6·0 (6·3)[c]
Sonicated xanthan[a]	4·5 (4·6)	4·2 (6·3)
ORD xanthan[b]	4·6 (4·6)	5·9 (6·3)

[a]Sonicated 12 h.
[b]Treated for 10 days.
[c]Numbers in parentheses show the expected values assuming one substituent per xanthan repeat unit.

TABLE 2
Helix–Coil Transition Midpoints (T_c) for Various Xanthan Samples

Sample solvent	*Native xanthan (NX)*	*Sonicated xanthan (SX)*	*ORD xanthan*	*NX+ LBG-E*	*SX+ LBG-E*
Water	45	40	43	47	50
0·02 M KCl	62	55	57·5	67·5	62·5
4 M urea	55	43	48	60	51
0·02 M KCl + 4 M urea	64·5	46·5	54·5	68·5	50·5

several hours little further decrease occurred. A minimum molecular weight seems to exist, as found by El 'Piner (1964) and Schoon & Kretschmer (1966), for a given set of conditions. Chromatography in water gave misleadingly high values (> 758 000) because of the polyelectrolyte expansion and, probably, repulsion by carboxyl groups on the column. Use of 0.01 M sodium sulphate gave a more realistic result (≈ 400 000). Unfortunately, the lowest temperature which gave an NMR spectrum of reasonable resolution was 70°C, so interaction between sonicated xanthan and LBG could not be studied by this technique.

Oxidative-reductive depolymerisation of xanthan

Table 3 lists the retention times of various Shodex standards and xanthan samples. The ORD xanthan even after ten days, though the viscosity had dropped markedly, had an apparent M_w in excess of 758 000. This was reflected in a temperature of ≈ 85°C being needed before an NMR spectrum of reasonable quality was obtained.

Despite the exclusion of NMR as a technique to study xanthan–LBG interactions, in these studies optical rotation vs temperature and gel-permeation chromatography have been useful.

Gel-permeation results of xanthan–LBG interactions

Table 3 presents the results of gel-permeation chromatography of the xanthan and locust bean gum samples used, with 0·01 M sodium suphate as solvent. This was used instead of water for size characterisation, as polyelectrolyte expansion and anionic groups on the column had large effects in the latter solvent. As an example: sonicated xanthan had a retention time of 27·2 min in water, and 37·4 min in salt. The difference in retention times was much less for the neutral Shodex standards than for the anionic xanthan: the 758 000 standard eluted at 32·8 min in water

TABLE 3
GPC Retention Times[a] of Standards, Xanthan Samples, and Locust Bean Gum (LBG) Samples

Sample	*GPC retention time (min)*
Shodex standard M_w 758 000	33·6
Shodex standard M_w 358 000	37·0
Native xanthan	31·2
Sonicated xanthan	37·4
ORD xanthan (4 days)	31·4
ORD xanthan (10 days)	32
Locust bean gum (LBG-A)	32
Locust bean gum (LBG-E)	38

[a]At 0·6 ml min^{-1} in 0·01 M Na_2SO_4; column: TSK Gel G6000 PW.

and 33·6 min in salt. Interaction studies however were carried out with water as solvent, as this gave maximum resolution between the xanthan–LBG complex and the residual LBG, and allowed collection of these two species, in the case of LBG-E, without peak overlap. Results discussed hereafter are for sonicated xanthan, unless specified otherwise. ORD xanthan (10 days and 4 days) gave qualitatively the same results.

Figure 1 shows gel-permeation profiles of LBG-E, sonicated xanthan, and a 1:1 mixture (v/v) of sonicated xanthan and LBG-E. There is an increase in area of the xanthan peak, whereas the area of the LBG-E peak is reduced by about 15%. The retention time at the peak maximum of the xanthan is little changed, but the additional, small peak on the leading edge is substantially larger in 'molecular' size. We believe it consists of a xanthan–LBG complex, partly resolved from residual xanthan, eluting near the column void volume. Attempts to better resolve the complex from residual xanthan by chromatography in salt solutions were not successful. At least part of the LBG contributing to the complex is early eluting, aggregated material (Fig. 1(a)) which is in the sample before mixing. The unbound LBG-E in Fig. 1(c) had a Man/Gal of 4·06 and is termed the high-galactose fraction. LBG-E alone had a Man/Gal of 5·2. Thus the depolymerised xanthan behaved in the same way with LBG-E as the native xanthan had (Cheetham *et al.*, 1986), i.e. it preferentially bound those LBG-E chains which had the lower galactose levels. Less of the LBG-E bound to sonicated xanthan ($\approx$ 15%) compared to native xanthan ($\approx$ 56%, Cheetham *et al.*, 1986), as might be expected. As mentioned previously (Cheetham *et al.*, 1986), however, peak area measurements underestimate the amount of LBG bound, and monosaccharide ratios are more reliable indicators. A gel-permeation

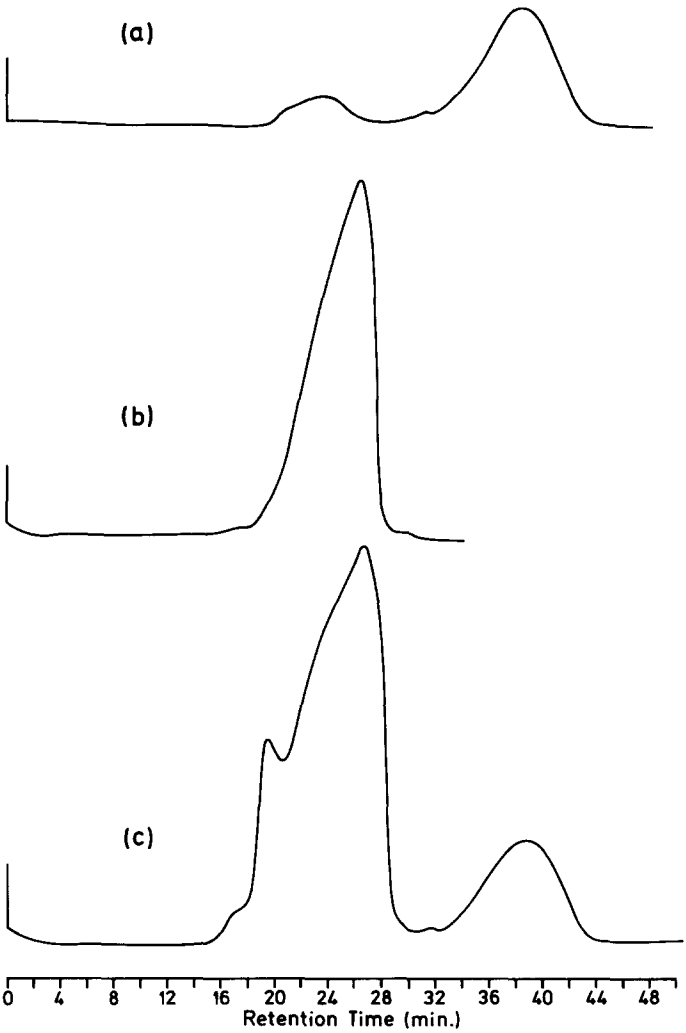

Fig. 1. Gel-permeation profiles of: (a) 1% LBG-E; (b) 1% sonicated xanthan; (c) a 1:1 mixture of samples in (a) and (b). Column, TSK Gel G6000 PW; Solvent, water; Flowrate, 0·7 ml min^{-1}.

experiment in which sonicated xanthan:LBG-E was 2:1 ≈ (v/v) was performed to ensure that enough xanthan was available to bind all the LBG-E present. The residual LBG-E peak was almost identical in area to that in the 1:1 experiment. Residual LBG-E was collected, hydrolysed and the monosaccharide ratios were determined by GLC. The value of 4·1 for Man/Gal showed, together with the peak area, that in the 2:1 experiment xanthan did not bind any additional LBG-E. Man/Gal ratios for the bound LBG-E in the complex were also the same for the

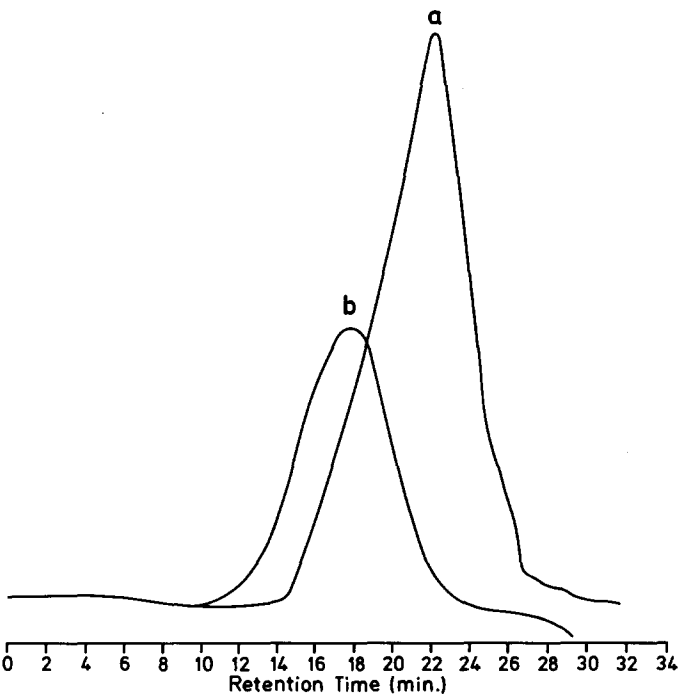

Fig. 2. Gel-permeation profiles of: (a) 1% sonicated xanthan plus 1% LBG-A (1/1, v/v); (b) 1% sonicated xanthan plus 1% LBG-A (1/2, v/v). Conditions as for Fig. 1.

1:1 (6·3) and 2:1 (6·35) experiments, as determined by GLC. Use of LBG-A instead of LBG-E (0·1% w/v in polysaccharide, as the viscosity was much higher than with LBG-E) resulted in a considerable increase in 'molecular' size of the complex, LBG-A having M_w 350 000. Gel-permeation profiles for sonicated xanthan and LBG-A mixed 1:1 and 1:2 v/v are shown in Fig. 2. The latter mixture resulted in a complex of larger 'molecular' size than the former, showing that all the xanthan binding sites had not been taken up in the 1:1 mixture. The same result was obtained with native xanthan and LBG-A (Cheetham *et al.*, 1986) though in that case the xanthan–LBG complexes were so close in size to the native xanthan that they appeared in GPC as a hump on its leading edge rather than an obviously larger species as in Fig. 2 above. It should be noted that unbound LBG-A is not resolved from the complex, so Man/Gal ratios cannot be determined. The present results provide further convincing evidence for the existence of a xanthan–LBG complex. They also show that complex formation occurs with the smaller molecular species of xanthan, presumably by the same mechanism as for the native xanthan, indicating strongly that a valid model system is being observed.

Optical rotation studies of xanthan–LBG interactions

The procedure for preparing the xanthan–LBG solutions for optical rotation studies, viz. heating above 85°C after mixing, ensured that the conditions of dissolution were similar to those used by other workers who studied optical rotation of xanthan (Morris *et al.*, 1977) and gelation (McCleary *et al.*, 1984; Dea *et al.*, 1986) of xanthan–galactomannan mixtures. Examination of the data in Table 2 shows that in both native and depolymerised xanthan, T_c in KCl solutions was approximately 15°C higher than that in water, so KCl stabilised the ordered conformation, as observed by others. Addition of urea alone increased T_c, but not as much as did KCl. Thus urea stabilised the ordered conformation also. Urea and KCl together in the depolymerised samples resulted in T_c values well below those in KCl, whereas in the case of native xanthan, T_cs were slightly enhanced relative to those in KCl. Apparently urea has a slight destabilising effect on the sidechains, but enhances backbone stability (Frangou *et al.*, 1982). The sidechains constitute some 65% by weight of the total xanthan molecule, and in the smaller samples the enhancement of backbone stability was outweighed by the side chain destabilisation, leading to a fall in T_c. This was especially noticeable in sonicated xanthan, which has lost approximately one third (Table 1) of its order-stabilising acetate. T_c values in the presence of locust bean gum (Table 2) for both native and sonicated xanthan were all enhanced, confirming other reports of this characteristic of xanthan–galactomannan interactions.

CONCLUSIONS

Both the gel-permeation and the optical rotation results confirm that the same types of interactions are taking place between native and depolymerised samples of xanthan and LBG. This indicates that the smaller fragments constitute a valid model for the study of the native system. Advantages of using the smaller fragments include (a) the opportunity to study xanthan–galactomannan interactions by polarimetry, at concentrations where the native molecules would form gels (and thus preclude the use of this useful technique); and (b) the ability to utilise gel-permeation chromatography, both analytical and preparative, to study the interactions. The possibility — if smaller fragments of xanthan can be produced, exists to study the interactions by NMR spectroscopy, a very powerful conformational probe.

ACKNOWLEDGEMENTS

We thank Professor D. A. Brant for helpful communications and advice on ultrasonic depolymerisation of xanthan, and Dr B. V. McCleary for samples of enzyme-depolymerised LBG. The work was supported by Colombo Plan Sponsorship of A. Punruckvong.

REFERENCES

Basedow, A. M. & Ebert, K. H. (1977). *Adv. Polym. Sci.*, **22**, 84.
Bergmann, L. (1954). *Der Ultraschall.* Hirzel Verlag.
Cairns, P., Miles, M. J. & Morris, V. J. (1986). *Nature (Lond.)*, **332**, 89.
Cairns, P., Miles, M. J., Morris, V. J. & Brownsey, G. J. (1987). *Carbohydr. Res.*, **160**, 410.
Cheetham, N. W. H. & Mashimba, E. (1988). *Carbohydr. Polym.*, **9**, 195–212.
Cheetham, N. W. H. & Punruckvong, A. (1985). *Carbohydr. Polym.*, **5**, 399.
Cheetham, N. W. H., McCleary, B. V., Teng, G., Lum, F. & Maryanto (1986). *Carbohydr. Polym.*, **6**, 257.
Dea, I. C. M. (1987). In *Industrial Polysaccharides, The Impact of Biotechnology & Advanced Methodologies,* ed. S. S. Stivala, V. Crescenzi & I. C. M. Dea. Gordon & Breach, New York, p. 367.
Dea, I. C. M., Morris, E. R., Rees, D. A., Welsh, E. J., Barnes, H. A. & Price, J. (1977). *Carbohydr. Res.*, **57**, 249.
Dea, I. C. M., Clark, A. H. & McCleary, B. V. (1986). *Carbohydr. Res.*, **147**, 275.
El'Piner (1964). *Ultrasound — Physical, Chemical & Biological Effects.* Consultants Bureau.
Fairbairn, N. J. (1953). *Chem. Ind., London,* January, p. 86.
Fenton, H. J. H. (1894). *J. Chem. Soc.*, **65**, 899.
Frangou, S. A., Morris, E. R., Rees, D. A., Richardson, R. K. & Ross-Murphy, S. B. (1982). *J. Polym. Sci. Polym. Lett.*, **20**, 531.
Glicksman, M. (1968). *Gum Technology in the Food Industry.* Academic Press, New York.
Hacche, L. S., Washington, G. E. & Brant, D. A. (1987). *Macromolecules,* **20**, 2179.
Harris, M. J., Herp, A. & Pigman, W. (1972). *J. Amer. Chem. Soc.*, **94**, 7579.
Herp, A. (1980). In *The Carbohydrates: Chemistry & Biochemistry,* ed. W. Pigman & D. Horton, 2nd edn, Vol. 1B, Academic Press, New York, p. 1279.
Holzwarth, G. (1976). *Biochemistry,* **15**, 4333.
McCleary, B. V. (1979). *Carbohydr. Res.*, **71**, 205.
McCleary, B. V., Dea, I. C. M. & Cooke, D. (1984). *Carbohydr. Polym.*, **4**, 253.
Morris, E. R., Rees, D. A., Young, G., Walkinshaw, M. D. & Darke, A. (1977). *J. Mol. Biol.*, **110**, 1.
Neuberg, C. & Muira, S. (1911). *Biochem. Z.*, **36**, 37.
Paradossi, G. & Brant, D. A. (1982). *Macromolecules,* **15**, 874.
Peloux, Y., Nofre, C., Cier, A. & Colobert, L. (1962). *Am. Inst. Pasteur,* **102**, 6.

Pigman, W., Matsumara, G. & Herp, A. (1965). *Proc. 4th Int. Congr. Rheology.* Wiley, New York, p. 505.
Rees, D. A., Morris, E. R., Thom, D. & Madden, J. K. (1982). In *The Polysaccharides, Vol. 1,* ed. G. O. Aspinall. Academic Press, New York, p. 195.
Robertson, W. B. (1961). *Ann. N.Y. Acad. Sci.,* **92,** 159.
Schoon, T. G. & Kretschmer, R. (1966). *Kolloid-Z,* **211** (1–2), 53.
Smith, I. H., Symes, K. C., Lawson, C. J. & Morris, E. R. (1981). *Int. J. Biol. Macromol.,* **3,** 129.
Tako, M. & Nakamura, S. (1984). *Agric. Biol. Chem.,* **48,** 2987.
Tako, M. & Nakamura, S. (1986). *F.E.B.S. Letters,* **204,** 33.
Tako, M., Asato, A. & Nakamura, S. (1984). *Agric. Biol. Chem.,* **48,** 2995.
Turner, H. S. & Cherniak, R. (1981). *Carbohydr. Res.,* **95,** 137.