ELSEVIER

PII: S0144-8617(97)00096-9

Carbohydrate Polymers 34 (1997) 157–163

0144-8617/97/$17.00 + .00

Evidence of interaction between agarose and guar gum from changes in network response to solvent pertubation

Rosangela B. Garcia[1] and Cristina T. Andrade*

Instituto de Macromoléculas, Universidade Federal do Rio de Janeiro, P.O. Box 68525, 21945-970 Rio de Janeiro, Brazil

(Received 13 February 1997; revised version received 7 April 1997; accepted 10 April 1997)

Comparative investigation has been carried out on the gel setting temperatures and mechanical properties of agarose gels of several concentrations and of 1:1 agarose–guar gum gels of 1% total polymer concentration. In order to investigate the origin of the synergistic interactions observed, the 1% gels were submitted to immersion tests in ethyl alcohol and in dimethyl sulphoxide–water solutions. Weight changes were observed and the mechanical properties of the resulting gels revealed distinguishing characteristics between the two systems. The solid state ^{13}C NMR spectrum of 1:1 agarose–guar gum gel after immersion in pure dimethyl sulphoxide corroborates the role of guar gum in retaining the network, despite dissolution of a large quantity of agarose. Transmission electron microscopy (TEM) was used to image the supramolecular structure of agarose, guar gum and agarose–guar gum systems. Artefacts were observed for pure agarose and guar gum as a result of the procedure used for sample preparation. Nevertheless, the connectivity expected for a tridimensional network was observed in the agarose domains of the mixed gel and attributed to the stabilizing effect of guar gum. © 1997 Elsevier Science Ltd

INTRODUCTION

Interactions between xanthan or κ-carrageenan and locust bean gum (LBG) have attracted much attention, supported by the aim of obtaining viscous or gelling aqueous systems with low levels of additives. For xanthan gum, a non-gelling microbial polysaccharide, thermoreversible elastic gels are formed by admixture of LBG (Dea & Morrison, 1975; Dea *et al.*, 1977). The addition of this gum to κ-carrageenan, a gelling polysaccharide from certain red marine algae, is known to improve the rheological properties of the system and the mixture forms gels at concentrations below that required for κ-carrageenan alone. Also, more elastic gels without syneresis are obtained (Dea *et al.*, 1972).

LBG is the galactomannan extracted from the seeds of *Ceratonia siliqua*. In this galactomannan, the units of (1→4)-β-D-mannose that composes the linear backbone are present in 3.4 to 4.8 times the content of the (1→6)-α-D-galactose substituent. (Fernandes, 1994).

When compared to other galactomannans, LBG is unquestionably the most effective in producing an enhanced rheological response to xanthan or κ-carrageenan systems. Although some controversy still exists about the mechanistic aspects of the resulting synergistic mixtures, it is accepted that the unsubstituted regions of the mannan chain are responsible for the intermolecular associations.

In the present work, some experiments have been carried out on agarose and guar gum mixed systems. Agarose is the gelling fraction of agar, consisting of (1→3)-β-D-galactose and (1→4)-3,6-anhydro-α-L-galactose alternating repeat units. Guar gum is the galactomannan from *Cyamopsis tetragonolobus*, in which the D-mannose to D-galactose ratio is around 2.

Useful rheological properties have been reported on agarose and certain plant galactomannan mixtures. The strength of the interaction increases with the decrease in galactose content (Dea *et al.*, 1972). Optical rotation measurements have been carried out for

*Author to whom correspondence should be addressed.
[1]Present address: Departamento de Química, Universidade Federal do Rio Grande do Norte, Campus da Universidade, 59072-970 Natal, RN, Brazil.

mixtures of non-gelling agarose concentrations and galactomannans. The magnitude of the net positive contribution to optical rotation for the mixtures has been regarded as a measure of the ability of the galactomannan to interact with agarose. While LBG gave rise to a high positive contribution, little or no effect was observed for guar gum (Dea & Rees, 1987).

Recently, it has been found that mixed gels of κ-carrageenan and guar gum at 1% total polysaccharide concentration do not exhibit the synergistic features observed in viscoelastic measurements for κ-carrageenan with other galactomannans, such as LBG from different sources and commercial tara gum (Fernandes *et al.*, 1991).

Results of uniaxial compression experiments, gel setting temperatures and immersion tests carried out on mixed gels of agarose and guar gum are reported. Transmission electron microscopy (TEM) is used to observe the agarose and guar gum domains and their interface.

MATERIALS AND METHODS

Materials

Agarose was kindly supplied by CIALGAS, Companhia Industrial de Algas, and was used as received. The intrinsic viscosity, $[\eta] = 3.24$ dl/g, was determined in an Ostwald–Fenske viscometer in 0.75 M NaSCN at 35°C. The viscosity average molecular weight, $M_v = 1.23 \times 10^5$, was calculated according to the Mark–Houwink equation, taking $K = 0.07$ and $a = 0.72$ values from the literature (Rochas & Lahaye, 1989).

Guar gum was purchased from Dowell Schlumberger and purified by filtering 1 g/l solutions through 3.0 and 0.8 μm membranes. The product was recovered by addition of ethyl alcohol and dried under vacuum. The intrinsic viscosity, $[\eta] = 13.6$ dl/g, was determined in distilled water at 25°C, as stated previously. The viscosity average molecular weight, $M_v = 1.99 \times 10^6$ was obtained from $K = 3.8 \times 10^{-4}$ and $a = 0.723$ values (Baines & Morris, 1987).

Preparation of the solutions

Agarose solutions were prepared by dispersion in distilled water at ambient temperature for 3 h, followed by heating at 70°C for 30 min and under reflux for 15 min. Guar gum solutions were obtained by stirring the water dispersion at ambient temperature overnight. Mixed solutions of agarose and guar gum were made at 85°C from stock solutions of each component at 1.0% (w/w).

Determination of the guar gum mannose to galactose ratio

The D-mannose to D-galactose ratio, M/G = 1.5, was determined after total hydrolysis in 1 M H_2SO_4 under reflux for 16 h by HPLC. After neutralization with barium carbonate, 1 μl solution was injected onto a Waters chromatograph, equipped with a HPX-87P column at 85°C. Distilled water was used as the eluent, at 0.6 ml/min flow rate, and the result was obtained by a Waters R-401 refractive index detector and a Shimadzu C-R1B register.

Determination of the gel setting points

Gel setting points, T_s, were determined by measuring the apparent viscosity at 0.1 s^{-1} shear rate during the cooling of the solutions at 0.4°C/min. The solutions were placed in an SC4-31/13R Small Sample Adapter of an LVT Brookfield Synchro-Lectric viscometer.

Gel preparation

Agarose gels were prepared by pouring the hot solutions into glass moulds of 17 mm diameter and 150 mm height. After gelation, the gels were cut into five cylindric specimens, which were aged in water overnight. The same technique was followed to prepare agarose–guar gum mixed gels, from the required volumes of the two stock solutions.

Uniaxial compression experiments

Young's moduli, stress data and compression energies to break were obtained by compression experiments at 0.5 cm/min and 25°C, using a TM-M Instron Universal Testing Machine equipped with a CTM cell. The median value from a total of five results was obtained.

Immersion tests

Agarose gels of different concentrations and agarose–guar gum gels of several compositions were weighed and immersed in duplicate in ethyl alcohol at 25°C for 24 h. After being superficially dried, they were weighed again. The same procedure was followed with agarose gels at 1% (w/w) concentration and 1:1 agarose–guar gum gels at 1% total polysaccharide concentration, which were immersed in dimethyl sulphoxide (DMSO) solutions of different molar fractions. After being superficially dried, the specimens were submitted to compression tests under the conditions described above.

Solid state ^{13}C NMR

^{13}C CP/MAS NMR spectra were taken on a VXR300 Varian spectrometer with samples of 300 mg in double-air-bearing rotors of ZrO_2. Magic-angle spinning was carried out at 6.8 kHz spinning rate. The proton 90° pulse length was 5 μs and the repetition time was 2 s.

Transmission electron microscopy

Agarose, guar gum and (1:1) agarose–guar gum solutions of 0.5, 0.5 and 1% (total polymer concentration) were held at 50°C during 15 min and then cooled to 15°C at 0.4°C/min. When the solutions reached that temperature, they were quenched to −78°C in an ethyl alcohol–dry ice bath and freeze-dried. The samples for TEM analysis were prepared as described before (Garcia *et al.*, 1992).

RESULTS AND DISCUSSION

Table 1 shows some mechanical properties and the gel setting points of agarose and agarose–guar gum mixed gels. Considering that guar gum solutions of low concentrations are not capable of forming macroscopic gels, the mechanical and thermal properties of the mixed gels seem to be due to some kind of interaction between the two polymers. None of the composite gels showed any significant enhancement in mechanical properties when compared with pure agarose gels at the same total polymer concentration (1%). However, incorporation of guar gum does give gels that are substantially stronger and have consistently higher setting points than those formed by agarose alone at the same concentrations as in the mixed systems.

To investigate the origin of the enhancing effect caused by the addition of guar gum, agarose and agarose–guar gum gels were submitted to immersion tests in ethyl alcohol and in dimethyl sulphoxide solutions.

When a physical gel is brought into contact with a solvent, the changes observed will depend on the network stability, governed by the interactions between the molecular segments that constitute the junction zones, and on the interaction between the solvent and the non-elastically active segments. Both agarose and guar gum are insoluble in ethyl alcohol, and for agarose gels of 0.3 to 1% (w/w) concentrations, and agarose–guar gum gels of different compositions and at 1% total polymer concentration, weight losses were detected after immersion in ethyl alcohol for 24 h. Figure 1 shows the variation in weight observed for the gels. These changes may be attributed to exudated water from the samples. It can be seen that these losses decrease as the agarose concentration increases in the samples and this suggests that a higher number of agarose junction zones makes the retention of water by the interstices of the tridimensional gel structure easier. According to these results, the structure of agarose–guar gum gels is less capable of retaining water in the presence of ethyl alcohol.

Water–DMSO solutions of different DMSO molar fractions (X_{DMSO}) were used for immersion tests of agarose gels at 1% concentration and agarose–guar gum mixed gels at 1% total polymer concentration. DMSO is considered a good solvent for polysaccharides because of its polar character. Although DMSO dissolves agarose, the guar gum sample used in the present work was not solubilized in this solvent.

In Fig. 2, complete dissolution was noticed for the agarose gels immersed for 24 h in DMSO solutions of $X_{DMSO} > 0.60$. The results observed for agarose gels immersed in solutions of $X_{DMSO} < 0.60$ indicate the high degree of aggregation existing in the agarose junction zones. These gels became more transparent with increasing DMSO concentration and a maximum

Table 1. Young's modulus, E, compression stress at maximal load, σ_{max}, compression energy to break, E_n, and gel setting point, T_s, for agarose and agarose–guar gum gels

Agarose Concentration/Mixed gel composition (% w/w)	$E\times10^{-4}$ (Pa)	$\sigma_{max}\times10^{-4}$ (Pa)	$E_n\times10^{-4}$ (J×m^{-3})	T_s (°C)
0.3	0.40	0.45	1.10	—
0.3:0.7	0.75	1.70	4.49	36.0
0.4	0.60	0.75	1.90	34.0
0.4:0.6	1.00	2.25	6.08	37.0
0.5	1.00	1.05	2.80	35.0
0.5:0.5	1.75	2.65	8.79	39.0
0.6	1.50	1.40	3.80	35.0
0.6:0.4	2.44	2.98	9.65	38.0
0.7	1.70	1.90	5.30	36.0
0.7:0.3	2.99	3.44	8.80	39.0
0.8	2.40	2.15	6.20	38.0 (0.85%)
0.8:0.2	3.82	3.27	7.50	39.5
0.90	2.90	2.40	6.20	—
0.90:0.1	3.74	2.95	7.50	39.0
1.0	3.70	2.95	10.10	39.0

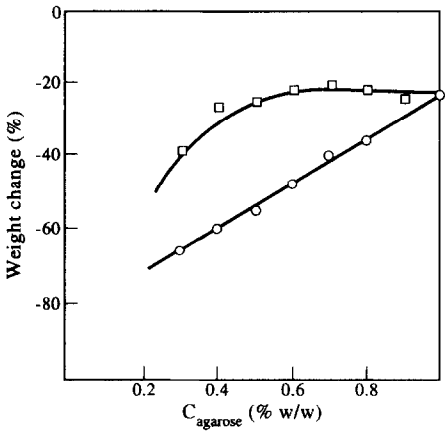

Fig. 1. Variation in weight with concentration for agarose gels (□) and with composition for agarose–guar gum gels (○) at 1% (w/w) total polymer concentration after immersion in EtOH for 24 h.

gain in weight of 5% was detected for the agarose gels that were immersed in the solution of $X_{DMSO} = 0.60$.

When 1:1 agarose–guar gum gels were immersed in DMSO solutions, weight losses were observed. Three distinct regions may be distinguished. In the first region, up to $X_{DMSO} \approx 0.28$, and in the interval $X_{DMSO} \approx 0.60$–1.00, weight losses increased markedly with DMSO concentration. In the interval $0.28 < X_{DMSO} < 0.60$, weight losses remained constant.

Studies on water–DMSO interactions have shown that the state of maximum interaction between these solvents occurs at $X_{DMSO} \approx 0.30$. In more dilute solutions, DMSO molecules would be completely solvated by water. Free DMSO molecules would be found in solutions of $X_{DMSO} > 0.30$ (Schichman & Amey, 1971).

According to the results above, the weight loss observed for the mixed gel in the range $X_{DMSO} < 0.28$ may be attributed to exudated water, probably due to stronger interactions between guar gum molecules and between guar gum and agarose molecules. In the range $0.28 < X_{DMSO} < 0.60$, in which no weight change occurred, it is thought that some conformational change has taken place, mainly of agarose molecules, as detected by the decreasing turbidity observed for the gels with increasing DMSO concentration. For $X_{DMSO} > 0.60$, the agarose–guar gum gels showed a significant decrease in weight and volume, which could be explained as resulting from dissolution of agarose molecules, although well-defined macroscopic gels were recovered.

Figure 3 is a photograph taken of the agarose and agarose–guar gum gels after immersion in aqueous DMSO solutions. Attention should be paid to the mixed gel recovered from pure DMSO, which has maintained its cylindrical form but shows a reduction in volume. Samples of this gel were dried and submitted to ^{13}C CP/MAS NMR. The spectrum obtained (curve III, Fig. 4) can be compared to the spectra of solid guar gum (curve I) and agarose (curve II), run at the same conditions. The similarity between curves I and III corroborates the hypothesis of agarose dissolution.

The mechanical properties of agarose and agarose–guar gum gels have been investigated after immersion in DMSO solutions for 24 h. Figure 5 shows the variation in Young's modulus with X_{DMSO}. For agarose gels, a gradual decrease was observed in the Young's modulus values with increasing DMSO concentration. Beyond $X_{DMSO} \approx 0.45$, a sharp drop occurred. As stated before, agarose gels are dissolved in water–DMSO solutions of $X_{DMSO} > 0.60$.

Recently, gelation temperatures determined by

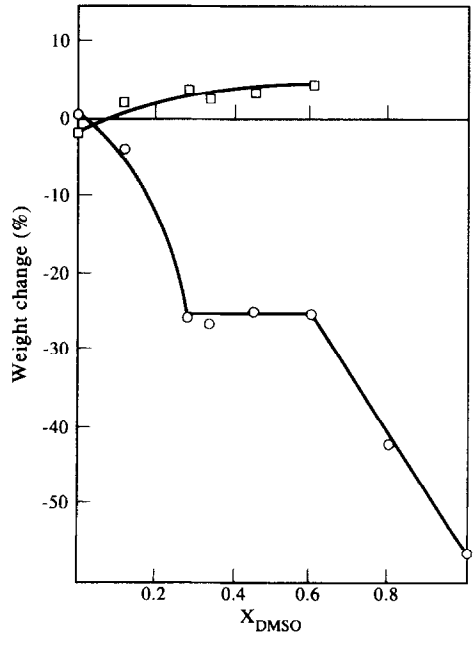

Fig. 2. Variation in weight with X_{DMSO} for 1% (w/w) agarose gel (□) and 1:1 agarose–guar gum gel (○) at 1% (w/w) total polymer concentration after immersion in aqueous DMSO solutions for 24 h.

Fig. 3. Photograph of agarose gels at 1% (w/w) polymer concentration (top) and 1:1 agarose–guar gum gels (bottom) at 1% (w/w) total polymer concentration after immersion in aqueous DMSO solutions of different DMSO molar fractions, X_{DMSO}, for 24 h.

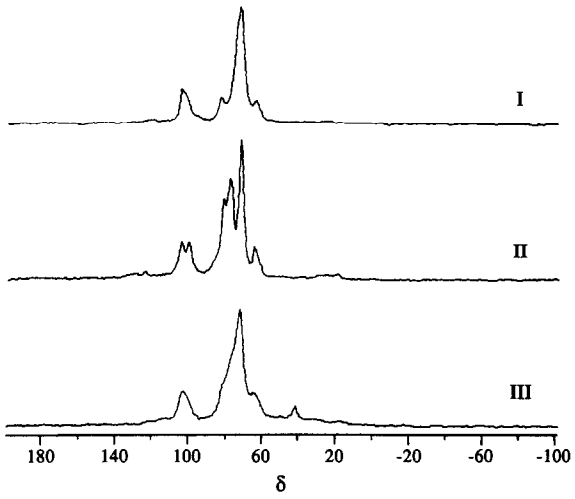

Fig. 4. CP/MAS ^{13}C NMR spectra of guar gum (curve I), agarose (curve II) and the resulting mixed gel after immersion in pure DMSO (curve III).

polarimetry on agarose solutions of different water/DMSO compositions have been reported (Rochas *et al.*, 1994) and showed a maximum for a DMSO content of 40% (v/v), which corresponds to $X_{DMSO}=0.28$. In the present work, the gels were prepared in water and the absorption of DMSO from the solutions of $X_{DMSO}<0.60$ seems to have partially solubilized the junction zones.

Different behaviour was observed for the variation in the Young's moduli of 1:1 agarose–guar gum gels, which pass through a discrete maximum at $X_{DMSO}\approx0.28$. As can be seen in Fig. 4, in the interval $0<X_{DMSO}<0.28$, a weight loss was detected after the immersion test, and the increase in the Young's modulus values in the same region supports the idea of loss of water from the mixed gels. Conversely, the Young's modulus suffered a decrease in the interval from $X_{DMSO}\approx0.45$ to $X_{DMSO}=0.80$, in which weight losses were also observed. In this case, the material that had been lost should have an effective participation in the junction zones.

The variation of the compression stress at maximal load, σ_{max}, with X_{DMSO} for the agarose and the mixed gels after immersion in aqueous DMSO solutions for 24 h is shown in Fig. 6. In both cases, a maximum at $X_{DMSO}=0.28$ is observed. For agarose, the solubilizing effect of DMSO acts first on the disordered segments between the junction zones. More concentrated DMSO solutions, $X_{DMSO}>0.28$, gradually disintegrate the network up to its complete dissolution. In the case of the mixed gels, a large increase in σ_{max} is observed for DMSO solutions in the range $0<X_{DMSO}<0.28$, which can be correlated to the the results of Fig. 5 and explained by the increasing number of junction zones. The solubilization of agarose molecules explains the reduction of σ_{max} for the mixed gels immersed in solutions of $X_{DMSO}>0.28$.

Figs 7–10 show TEM micrographs of agarose, guar gum and agarose–guar gum solutions that were cooled to 15°C and quenched to −78°C. A fiber-like structure can be observed for the agarose gel (Fig. 7). This artefact is the result of the collapse of the network into aggregates, as a consequence of water crystallization. Continuous and more flexible aggregates are observed in Fig. 8 for guar gum. Figures 9 and 10 show the supramolecular structure obtained for the agarose–guar gum gel after the same experimental procedure. Two types of domain can be identified; the one attributed to

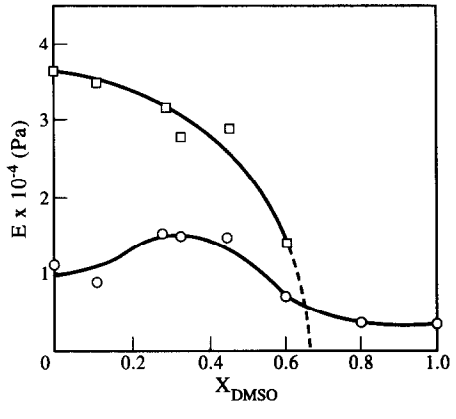

Fig. 5. Variation of Young's modulus with X_{DMSO} for pure agarose (□) and agarose–guar gum mixed gels (○) at 1% (w/w) total polymer concentration after immersion in aqueous DMSO solutions for 24 h.

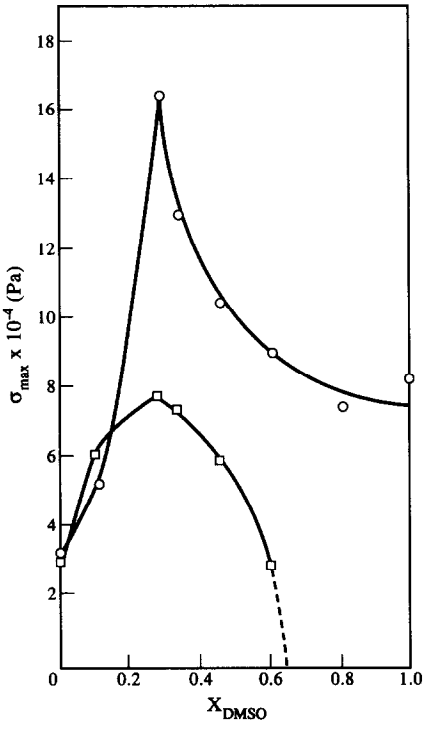

Fig. 6. Variation of the compression stress at maximal load, σ_{max}, for pure agarose (□) and agarose–guar gum mixed gels (○) at 1% (w/w) total polymer concentration after immersion in aqueous DMSO for 24 h.

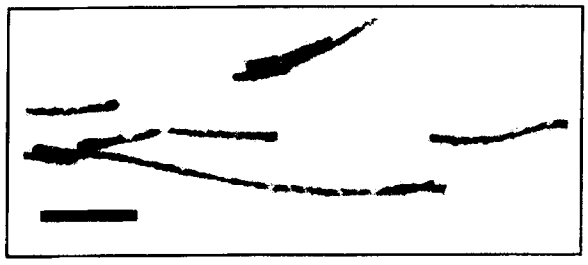

Fig. 7. Transmission electron micrograph of agarose gelling solution quenched from 15°C to −78°C (scale bar = 3 μm).

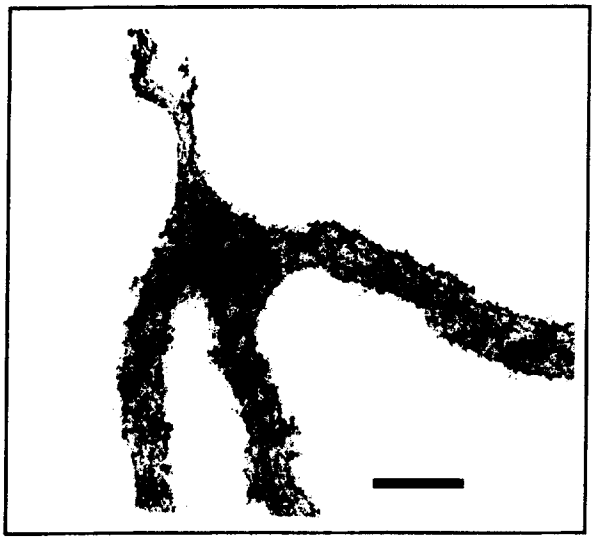

Fig. 8. Transmission electron micrograph of guar gum solution quenched from 15°C to −78°C (scale bar = 1 μm).

guar gum maintains the same characteristic feature observed before. Conversely, agarose domains in the mixed gel reveal the connectivity expected for a gelled system. This can be better observed in Fig. 10 and correlated to the role of guar gum in stabilizing the agarose junction zones (Garcia *et al.*, 1992).

CONCLUSION

A guar gum sample which had its M/G determined as 1.5 was used. As established by extensive research, there were not sufficient unsubstituted ('smooth') regions in the mannan backbone available for interaction with ordered agarose segments. However, the mechanical properties and the gel setting temperatures determined in the present work demonstrate the improvement in the stability of the mixed gels. The results of the immersion tests in ethyl alcohol and in aqueous DMSO solutions point to less aggregated junction zones and to more flexible segments between the junction zones in the mixed gel, when compared to pure agarose. The specimen resulting from the immersion of 1:1 agarose–guar gum gel in pure DMSO revealed that the network had been maintained, despite the agarose solubilization. This indicates that guar gum participates in the network formation by interacting with the external surface of the agarose helix.

ACKNOWLEDGEMENTS

The authors thank CAPES and CNPq for financial support, Dr A. Heyraud, Dr M.I. Bruno and M. De Bonis for the HPLC characterization of GG, NMR spectra and TEM micrographs.

REFERENCES

Baines, Z.V. and Morris, E.R. (1987) Flavour/taste perception in thickened systems. The effect of guar gum above and below c*. *Food Hydrocolloids* **1**, 197–205.

Dea, I.C.M. and Morrison, A. (1975) Chemistry and interactions of seed galactomannans. *Adv. Carbohydr. Chem. Biochem.* **31**, 241–312.

Dea, I.C.M. and Rees, D.A. (1987) Affinity interactions

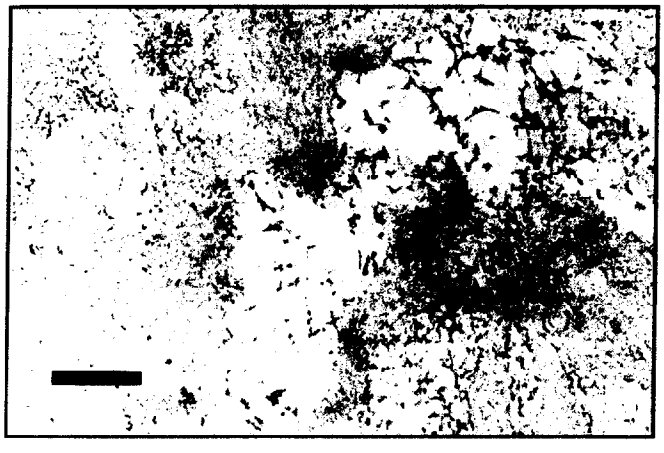

Fig. 9. Transmission electron micrograph of agarose–guar gum gelling solution quenched from 15°C to −78°C (scale bar = 3 μm).

Fig. 10. Transmission electron micrograph of agarose–guar gum gelling solution quenched from 15°C to −78°C (scale bar = 0.5 μm).

between agarose and β-1,4-glycans: a model for polysaccharide associations in algal cell walls. *Carbohydr. Polym.* **7**, 183–224.

Dea, I.C.M., McKinnon, A.A. and Rees, D.A. (1972) Tertiary and quaternary structure in aqueous polysaccharide systems which model cell wall cohesion: reversible changes in conformation and association of agarose, carrageenan and gaclactomannans. *J. Mol. Biol.* **68**, 153–172.

Dea, I.C.M., Morris, E.R., Rees, D.A., Welsh, E.J., Barnes, H.A. and Price, J. (1977) Association of like and unlike polysaccharides: mechanism and specificity in galactomannans, interacting bacterial polysaccharides and related systems. *Carbohydr. Res.* **57**, 249–272.

Fernandes, P.B. (1994) Determination of the physical functionality of galactomannans in kappa-carrageenan/galactomannan mixed systems by periodate oxidation. *Food Chem.* **49**, 367–371.

Fernandes, P.B., Gonçalves, M.P. and Doublier, J.L. (1991) A rheological characterization of kappa-carrageenan/galactomannan mixed gels: a comparison of locust bean gum samples. *Carbohydr. Polym.* **16**, 253–274.

Garcia, R.B., Lopes, L. and Andrade, C.T. (1992) Network formation from agarose–guar gum solutions. *Fresenius' J. Anal. Chem.* **344**, 510–513.

Rochas, C. and Lahaye, M. (1989) Average molecular weight and molecular weight distribution of agarose and agarose-type polysaccharides. *Carbohydr. Polym.* **10**, 289–298.

Rochas, C., Brûlet, A. and Guenet, J.-M. (1994) Thermoreversible gelation of agarose in water/dimethyl sulfoxide mixtures. *Macromolecules* **27**, 3830–3835.

Schichman, S.A. and Amey, R.L. (1971) Viscosity and local liquid structure in dimethyl sulfoxide–water mixtures. *J. Phys. Chem.* **75**, 98–102.