MICROSCOPY OF XANTHAN/GALACTOMANNAN SYSTEMS

C. SCHORSCH, C. GARNIER and J.-L. DOUBLIER

Laboratoire de Physico-Chimie des Macromolécules, Institut National de Recherche Agronomique, Rue de la Géraudière, BP 527, 44026 Nantes Cedex 03, France

Xanthan gum/galactomannan (guar gum and locust bean gum) mixtures are widely used in the food industry due to the synergistic properties they develop. Despite the molecular mechanisms underlying these synergistic phenomena still being a matter of debate, it is generally considered that these properties arise from specific interactions between xanthan and galactomannan chains. In the present work, light microscopic techniques were used to gain more insight into the structure of such mixtures. 50/50 xanthan/galactomannan mixtures were prepared in aqueous solution (no salt added) and the total concentration ranged from 2 to 4%.

By the use of polarized light microscopy the formation of cholesteric mesophases in xanthan gum was clearly seen as already reported by several authors. In xanthan/galactomannan mixtures, we observed birefringent areas suggesting a concentration of xanthan inside these zones. Moreover these mesophases in the blend appeared more anisotropic than in xanthan gum alone. The same blends were prepared using a fluorescein-labelled galactomannan and were observed using fluorescence microscopy. This labelled-galactomannan also appeared concentrated in definite areas. Clearly, these results indicate that phase separation has occurred in these systems yielding xanthan-enriched and galactomannan-enriched phases. Moreover, xanthan molecules organize themselves as liquid-crystalline mesophases.

SUPERMOLECULAR ASPECTS OF XANTHAN–LOCUST BEAN GUM GELS BASED ON RHEOLOGY AND ELECTRON MICROSCOPY

L. LUNDIN and A.-M. HERMANSSON

SIK – The Swedish Institute for Food Research, PO Box 5401, S-402 29 Göteborg, Sweden

The viscoelastic properties and supermolecular structure of synergistic gels formed by xanthan and locust bean gum (LBG) of two different mannose:galactose ratios (M:G), have been investigated by small deformation viscoelastic measurements and by low angle rotary shadowing transmission for electron microscopy.

The rheological properties at 20°C for mixtures subjected to heating and cooling cycles in the temperature range 30–80°C were found to be dependent on M:G ratio. Mixtures of xanthan and LBG mixed at temperature $\leqslant$40°C were found to form true gels with low phase angles. Blends of xanthan and LBG with low M:G ratio showed no increased synergistic effects as the temperature was increased, whilst the mixture of xanthan and LBG with high M:G ratio showed a strong increase in synergistic effects as the temperature was raised above 60°C. A difference in gelation temperature (T_g) of ~13°C was observed between the mixtures of xanthan and the two LBG fractions. (T_g) for xanthan with high M:G ratio was ~53°C, whilst T_g for mixtures of xanthan and LBG with low M:G ratio was ~40°C.

Results obtained using electron microscopy showed that the xanthan–LBG network was formed from xanthan supermolecular strands, and addition of LBG did not influence the xanthan structure. The observed structural features of the gels were independent of heat treatment and LBG fraction. The structural similarities and the rheological differences observed between xanthan and the LBG fractions are discussed in comparison with existing interaction models at the molecular level. Based on these results a speculative network model at the supermolecular level is presented.

SCREENING FOR SYNERGISTIC INTERACTIONS IN DILUTE POLYSACCHARIDE SOLUTIONS

F.M. GOYCOOLEA, E.R. MORRIS and M.J. GIDLEY[a]

Department of Food Research and Technology, Cranfield University, Silsoe College, Silsoe, Bedford MK45 4DT, UK

[a]Unilever Research, Colworth Laboratory, Sharnbrook, Bedford MK44 1LQ, UK

A simple viscometric approach has been used to screen for binding interactions between different polysaccharides in very dilute solution where exclusion effects should be negligible. The method involves preparing stock solutions to approximately the same, low, viscosity ($\eta_{sp} \approx 1$), dialysing to identical ionic conditions, mixing in various proportions, and looking for departures from the initial common viscosity.

Mixtures of xanthan or deacetylated xanthan with locust bean gum (LBG) or konjac mannan (KM) show massive enhancement of viscosity, as anticipated from formation of synergistic gels at higher concentrations. However, no viscosity changes on mixing with LBG or KM were observed for other conformationally ordered bacterial polysaccharides (welan and rhamsan) or for alginate and pectin with sufficient Ca^{2+} to induce almost complete conversion to the dimeric 'egg box' form, demonstrating that conformational rigidity is not, in itself, sufficient for other polysaccharides to form heterotypic junctions with mannan or glucomannan chains.

Interactions of carrageenans with LBG appear to depend on both conformation and extent of aggregation. Mixtures of LBG with K^+ kappa carrageenan in 100 mM KCl (which is known to promote extensive aggregation of double helices) gave erratic values for rotational viscosity and showed typical gel-like mechanical spectra under low amplitude oscillation. Disordered carrageenans (K^+ kappa in water and lambda in 100 mM KCl) showed no evidence of interaction with LBG. Negative results were also obtained for iota carrageenan under ionic conditions believed to promote ordering without significant aggregation (100 mM KCl). However, under conditions where limited aggregation might be expected (iota carrageenan in 90 mM $CaCl_2$; Me_4N^+ kappa carrageenan in 150 mM Me_4NI) significant reductions in viscosity were observed on mixing with LBG, which may indicate some intermolecular association but without formation of an extended network structure.

DETERMINATION OF DIFFUSION COEFFICIENTS OF POLYMERS IN GELS USING THE CONFOCAL LASER SCANNING MICROSCOPE

L.S. CUTTS, P.A. ROBERTS, J. ADLER, M.C. DAVIES and C.D. MELIA

Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK

The development of a photobleaching technique, CFMM (continuous fluorescence multipoint microphotolysis) to

measure diffusion coefficients in gel systems using a confocal laser scanning microscope is described. Diffusion coefficients (D), were determined for fluorescently labelled dextrans of varying molecular weight in agarose gels. The results were compared with the 'repeated line scans' method developed from studies using epi-fluorescence microscopy by Henry *et al.* (1992) and the classical method based on the 'double diffusion cell' e.g. Bain *et al.* (1992).

Good agreement was achieved between the latter method and CFMM for all the dextrans studied. The repeated line scan method gave higher diffusion coefficients for the lower molecular weight dextrans possibly because of interfacial tension and swelling effects which aggravate the curved meniscus surface.

The CFMM technique was experimentally simple, involved only a single image and enabled diffusion coefficients to be determined rapidly at defined microscopic locations within gel systems.

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COMPOSITIONAL MAPPING OF MIXED GELS USING FTIR MICROSPECTROSCOPY

C.M. DURRANI and A.M. DONALD

Polymer & Colloids Group, Cavendish Laboratory, University of Cambridge, Madingley Road, Cambridge, CB3 0HE, UK

We have developed a technique to product compositional maps of phase-separated protein/polysaccharide mixed gels using Fourier transform infrared (FTIR) microspectroscopy. The maps plot out the composition of either the protein, the polysaccharide or the water as a function of position in the sample. The maps can be presented in the form of two-dimensional contour plots or three-dimensional surface plots.

The technique is automated and uses an FTIR microscope interfaced to a motorised stage. The stage drives a sample in stepwise fashion to cover a two dimensional grid of points across the sample. At each position in the grid, the FTIR microscope records the infrared spectrum over a cross-sectional area of sample, the size of which is specified by apertures in the microscope.

We have used the technique to produce compositional maps of the amylopectin/gelatin and dextran/gelatin systems using 40 μm × 40 μm apertures with a grid step size of 40 μm. Compositional maps were generated in the first instance by simply plotting the area of an infrared absorption peak from one of the components in the sample. Fully quantitative compositional maps in terms of actual concentration were also produced by analysing the spectra with the method of partial least-squares (PLS).

We recently showed how PLS analysis can be used in conjunction with FTIR spectroscopy to plot the phase diagram of bulk phase-separated solutions above the gel temperature of both components. Our mapping technique therefore allows the compositions in a gel to be directly compared with those reached at equilibrium in the absence of gelation, using the same molecular probe, namely, infrared radiation. Furthermore, our technique can be applied to any other protein/polysaccharide mixture – provided that phase separation takes place on a scale larger than the diffraction limit of infrared radiation (~20 μm).

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PHASE SEPARATION IN AQUEOUS PROTEIN–POLYSACCHARIDE SYSTEMS

A.R. HOSKINS, I.D. ROBB and P.A. WILLIAMS

Centre for Water Soluble Polymers, The North East Wales Institute, Connah's Quay, Deeside, Clwyd CH5 4BR, UK

Solutions containing two different polymers frequently exhibit incompatibility resulting in the formation of two liquid layers, with each layer enriched in one or other of the polymers. We have observed such behaviour upon mixing aqueous solutions of globular proteins such as bovine serum albumin (BSA) with neutral polysaccharides notably dextran and hydroxyethyl cellulose (HEC).

Several factors have been found to affect the phase separation of such protein–polysaccharide systems; pH, polysaccharide structure and molecular mass and addition of salt.

Phase separation occurs most readily at the pH corresponding to the protein isoelectric point. As the pH moves away from the isoelectric point phase separation is suppressed and a one phase system results. Phase diagrams have been constructed which show that HEC produces phase separation at lower concentrations than dextran of similar molecular mass. An explanation may derive from the fact that HEC is a fairly rigid linear polymer in solution, whereas dextran is a more flexible and compact molecule. The effect of polysaccharide molecular mass on the phase behaviour is also more pronounced for the systems containing HEC than dextran. The phase diagrams of the BSA – dextran 250 and BSA – dextran 2000 systems are almost identical, whereas phase separation was found to occur at progressively lower HEC concentrations as the molecular weight increased from 64,000 to 450,000.

Addition of salt to a phase separated system at the protein isoelectric point has been found to result in a one-phase system. This is reported to be as a result of shielding of the dipolar attractions between protein molecules. At a pH above or below the isoelectric point the protein molecules are charged and electrostatic repulsive forces exist preventing phase separation. Salt ions would be expected to shield these forces resulting in a two-phase system. However, this was not observed in the BSA – dextran mixture at pH 6·0. This may be due to binding of the anions to the protein which in effect shifts the isoelectric point to a more acidic pH.

The dependence of phase separation on a number of factors opens up the possibility of selective protein concentration or isolation by addition of polysaccharide to mixtures of