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Phase separation in dextran/locust bean gum mixtures

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Dextran/locust bean gum (LBG) mixtures have been prepared and investigated with respect to their phase separation behaviour. These systems exhibited phase separation at 20°C, the upper phase, itself biphasic, being enriched with locust bean gum but also containing dextran, whereas the lower phase contained only dextran. This lower phase was a liquid. The upper phase, which did not flow, was characterized by means of rheological dynamic measurements. Clearly, its behaviour was typical of a gel, the three-dimensional structure of which can be ascribed to self-association of LBG chains owing to the very high concentration of the galactomannan in this upper phase. The self-association of the galactomannan was confirmed by fluorescence microscopy carried out on mixtures containing fluorescein isothiocyanate (FITC)-labelled dextran. The rheological behaviour of a concentrated LBG solution was also investigated as a function of time, clearly showing progressive formation of a weak gel structure.

INTRODUCTION

Locust bean gum (LBG) is a galactomannan consisting of a linear backbone of (1–4)-linked ß-D-mannose residues substituted with (1–6)-linked monosaccharide sidechains of α-D-galactose. It has a mannose-to-galactose ratio of approximately 4. This hydrocolloid is widely used as a thickener agent in the food industry. It can also yield gelling systems when mixed with other polysaccharides, such as xanthan or κ-carrageenan. The molecular origin of these well-known synergistic properties is not yet fully understood. It is generally assumed that the underlying mechanism is due to the formation of a coupled network through specific junction zones between each polysaccharide. However, alternative models based on the existence of a phase separated network have been proposed (Cairns *et al.*, 1987; Fernandes *et al.*, 1992). Recently, other models have been described assuming adsorption of glucomannan or galactomannan chains along aggregates of the other polysaccharide, κ-carrageenan or xanthan (Williams *et al.*, 1993; Lundin & Hermannson, 1995). However, no explanation is provided on the reasons for which such mechanisms should take place.

Dextran is also a polysaccharide consisting of a backbone of (1–6)-linked α-D-glucose with some (1–3)-linked sidechains of α-D-glucose, which appear to be relatively long and randomly distributed along the linear backbone (Smit *et al.*, 1992). Dextran has often been used in studies on aqueous polymer mixtures: for example, when mixed with polyvinylpyrrolidone, polyvinyl alcohol, ficoll, polyethyleneglycol, agarose or amylose, the system exhibits phase separation (Zaslavsky *et al.*, 1986; Sjöberg & Karlström, 1989; Kang & Sandler, 1988; Medin & Janson, 1993; Kalichevsky *et al.*, 1986). Incompatibility of dextran and LBG has also been reported by Dea *et al.* (1977), but only after freeze–thaw treatments.

Despite the fact that incompatibility between unlike polymers in mixtures is a well-known phenomenon, the thermodynamics of galactomannan-based mixtures have not been studied in this way. However, it has been shown that only small differences in the chemical structures of the polysaccharides can lead to incompatibility, such as the case of dextran/amylose or amylose/amylopectin mixtures in aqueous medium (Kalichevsky *et al.*, 1986; Kalichevsky & Ring, 1987). This mutual incompatibility generally leads to phase separation, each phase being enriched in one of the polymers.

This work is a part of a study dealing with the thermodynamics of biopolymer mixtures. We report here preliminary accounts of our studies on mixtures of

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LBG with dextran. This system was studied with the aim of investigating the influence of the addition of a polysaccharide on the behaviour of LBG at room temperature. For this purpose, high-pressure size exclusion chromatography (HPSEC), fluorescence microscopy and viscoelastic measurements were used. The rheological properties of LBG were also investigated as a function of time.

MATERIALS AND METHODS

Samples

The LBG sample was kindly provided from Meyhall Chemical (Switzerland). Its molecular weight was estimated as approximately 1.7×10^6 from the intrinsic viscosity value ($[\eta] = 14.5$ dl/g) and from the Mark–Houwink parameters obtained for guar gum (Robinson *et al.*, 1982), modified to take into account the mannose to galactose ratio (Fernandes *et al.*, 1991; Doublier & Launay, 1981). The dextran used for the experiment was a commercial sample (Dextran T-500, Lot IK 33693, Pharmacia Fine Chemicals AB, Sweden) with a reported molecular weight of approximately 5×10^5.

For fluorescence microscopy, a dextran sample labelled with fluoresceine isothiocyanate (FITC–dextran) was purchased from Sigma. Its reported molecular weight was 4.85×10^5 (Batch 121H5048).

Solutions of LBG (0.25–2% w/w) were prepared by cold dispersion of the powder for 15 min in distilled water containing 0.02% of sodium azide (NaN_3) as a bactericide, followed by heating at 80°C for 30 min under magnetic stirring. Solutions of dextran (0.5–20%) were prepared by dissolving the sample in distilled water containing 0.02% of NaN_3 under magnetic stirring at room temperature.

LBG solutions (3 ml) were mixed with dextran solutions (3 ml) in test tubes and the mixtures left at 20°C for 48 h before observation of the physical state (phase-separated or not). It was observed that centrifugation of the tubes did not influence the phase separation. In the case of phase separation, the two layers were carefully separated.

Methods

Fluorescence microscopy was performed with a Olympus Vanox microscope using a 400 nm exciter filter and a 530 nm emission filter on mixtures prepared with FITC–dextran. The samples were placed between a slide and a coverslip, and sealed to prevent dehydration.

The distribution of the polymers in the phases was determined by HPSEC. The SEC system consisted of a Waters 590 pump on-line to a Waters 717 autosampler, a guard column, three Shodex OHpak columns (30×0.8 cm each) thermostated at 40°C: a KB-806, a KB-805 and a KB-804 in the order of connection. This system was coupled on-line to an Erma Optical differential refractometer. The eluent (filtered through a 0.22 μm filter) was distilled water containing 0.02% NaN_3, at a flow rate of 1 ml/min. The samples (100 μl) were injected following dilutions and filtration through a 5 μm filter. The time required for each analysis was 33 min.

Rheological measurements were performed using a dynamic controlled strain rheometer (Rheometrics Fluid Spectrometer, RFSII), in oscillatory shear, with a cone-plate device (diameter 1.25 cm, cone angle 2.27°). Mechanical spectra of the samples were recorded between 10^{-2} and 100 rad/s at 20°C, at a strain of 1%.

RESULTS AND DISCUSSION

Immediately after mixing, the mixtures of dextran and LBG solutions were limpid. After 24 h at 20°C, macroscopic phase separation appeared for some of the mixtures, thus confirming the incompatibility between the two biopolymers previously suggested by Dea *et al.* (1977) after freeze–thaw treatments. The final observation was made after 48 h to ensure that phase separation was as complete as possible. Centrifugation of homogeneous samples did not promote phase separation, and centrifugation of samples showing phase separation did not change the phase volumes. The interfacial border was slightly clearer. Figure 1 shows a mixture containing 10% dextran and 1% LBG, observed after 48 h at 20°C without centrifugation. As can be seen in the figure, the two phases were visually distinguishable; for all the mixtures giving rise to phase separation, the lower phase appeared limpid and liquid, whereas the upper phase was opaque and did not flow. Furthermore, the lower phase always presented the larger volume.

The physical state of the blends after 48 h is shown in Fig. 2, where the LBG concentration in initial mixtures (i.e. before phase separation) is plotted as a function of the dextran concentration in the initial mixtures. The line separating the monophasic and biphasic areas represents an estimation of the binodal line. The monophasic area is very narrow; it can be seen that under our experimental conditions, no phase separation was observed at a dextran concentration of approximately 1%, and an LBG concentration of approximately 0.1%.

HPSEC experiments were performed on both phases in order to determine the distribution of the biopolymers inside the two phases. Figures 3a and b show the chromatograms obtained for the two phases of Fig. 1. It can be seen that the lower phase contained only dextran, whereas the upper phase contained both polysaccharides. From the peak area, the dextran concentration in the lower phase was determined as

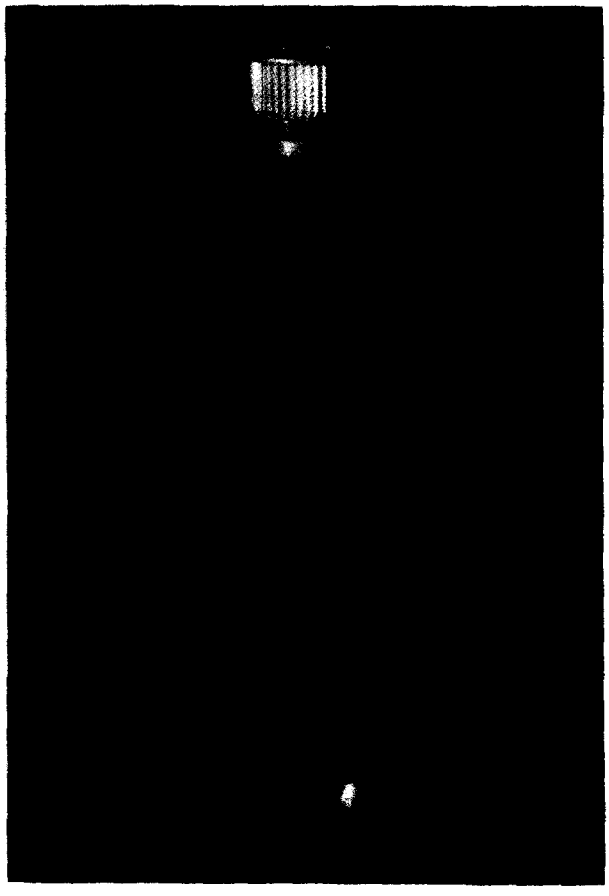

Fig. 1. Aqueous mixture of 10% dextran and 1% LBG, after 48 h at 20°C.

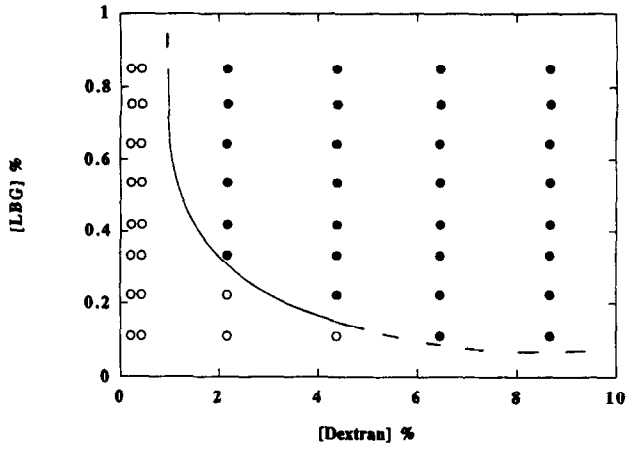

Fig. 2. Monophasic and biphasic areas for the dextran/LBG system. (○): mixtures not giving rise to phase separation; (●): mixtures leading to phase separation.

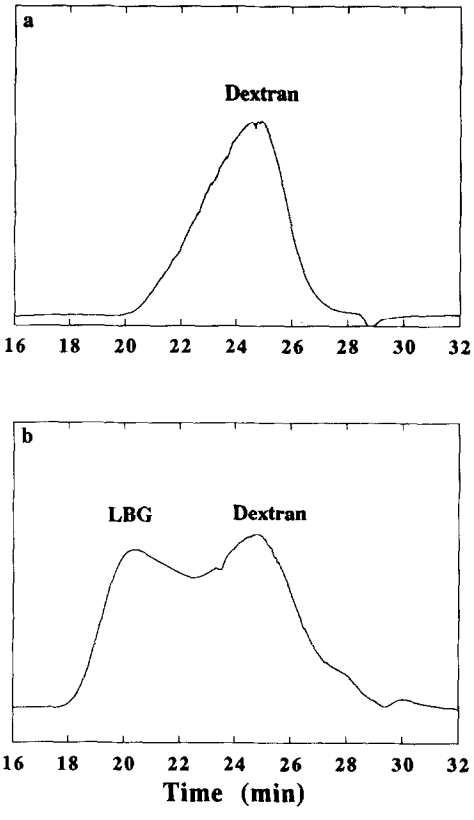

Fig. 3. Size exclusion chromatography of the two layers after phase separation of an initial mixture of 10% dextran and 1% LBG: (a) lower phase; (b) upper phase.

approximately 11%. It was not possible to determine the concentration of both polysaccharides in the upper phase directly from the corresponding chromatogram because of the overlapping of the two peaks, but a calculation yielded a dextran concentration of approximately 6% and an LBG content of approximately 4% in this phase. The phase separation phenomenon leads then to a total exclusion of LBG from the lower phase and to a slight concentration of dextran in this phase, whereas both polymers are present in the upper part of the test tube, LBG being very concentrated in this part.

In order to investigate the structure of the upper phase, mixtures were prepared using FITC–dextran. After phase separation, the opaque upper phase of the mixtures was examined by means of fluorescence microscopy. Figure 4 shows as an example the upper phase corresponding to an initial mixture containing 10% dextran and 1% LBG. It is clearly seen that LBG, in the dark in the picture, appeared to be strongly aggregated in clusters dispersed in a medium containing dextran, in the light in the picture. Thus, from Figs 3b and 4, it clearly appears that the upper phase is itself biphasic, i.e. that total phase separation was not completed. It can be assumed that, in the case of a total phase separation, the upper phase would consist of concentrated aggregated LBG. Moreover, the cluster size can be very high, which explains the opacity of the medium.

Rheological experiments were performed at 20°C on the phase containing both polysaccharides after separation of the two layers. Figure 5a shows a mechanical spectrum of this upper phase for an initial mixture containing 5% dextran and 1% LBG, leading to a phase composition of ~3% dextran and ~3%

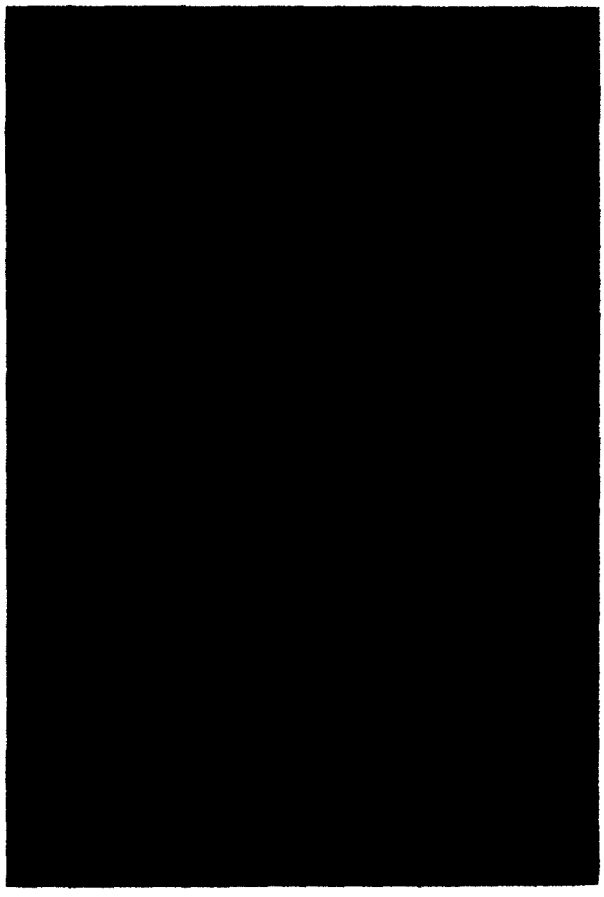

Fig. 4. Fluorescence microscopy of the upper phase of an initial mixture of 10% dextran and 1% LBG. Composition of the phase: ~4% dextran, ~6% LBG.

LBG. Figure 5b shows the mechanical spectrum corresponding to the upper phase of Fig. 1, i.e. containing ~6% dextran and ~4% LBG. Both spectra appeared very different from those expected for a macromolecular solution: indeed, G' was greater than G'' over the whole frequency range investigated and both moduli had a low frequency dependence. Moreover, G' showed a plateau at low frequency. This is clearly shown in Fig. 5a and would be more clearly seen below 10^{-2} rad/s in Fig. 5b. This indicates a weak gel structure in both cases. Dea *et al.* (1977) have already claimed that a firm gel can develop from a mixture of these two polysaccharides, but at a low storage temperature (2°C), and under low water activity conditions (60% w/v sucrose). The gelation of this phase could be the reason for the incomplete separation of dextran and LBG: indeed, total diffusion of the dextran towards the lower phase could be impeded by formation of the network.

The evolution of rheological behaviour as a function of time (up to 15 days) for a concentrated LBG solution (1.85%) stored at 20°C was also investigated (Figs 6a–c). It can be seen in Fig. 6a that the behaviour of the LBG solution, just after preparation, was typical of a macromolecular solution. It evolved steadily with time into a weak gel structure. This evolution can be attributed to interchain association of the LBG promoted by the high concentration of the polymer in the sample. This observation confirms the one made by Dea *et al.* (1977). However, no rheological data were shown. This result shows that the weak gel structure obtained for the upper phase of the mixtures is probably due to LBG auto-association promoted by the concentration of this polymer in definite areas because of its incompatibility with dextran, also present in this phase. This assumption is supported by the observations made by fluorescence microscopy.

CONCLUSION

The addition of a dextran solution to an LBG solution results in a macroscopic phase separation at room temperature, one of the phases containing only dextran while the other contains both polysaccharides, LBG and dextran being separated inside this phase as shown by fluorescence microscopy. Rheological experiments confirmed that LBG can self-associate if the concentration of the polymer is sufficiently great, this condition being fulfilled in the dextran/LBG mixtures

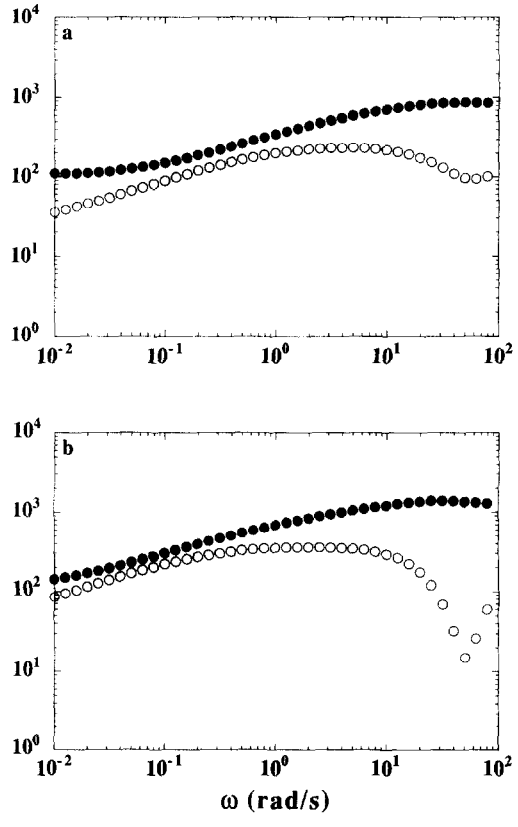

Fig. 5. Mechanical spectra of the upper phase of dextran/LBG mixtures at 20°C: G' (●), G'' (○). (a) Initial mixture: 5% dextran, 1% LBG. Composition of the phase: ~3% dextran, ~3% LBG. (b) Initial mixture: 10% dextran, 1% LBG. Composition of the phase: ~4% dextran, ~6% LBG.

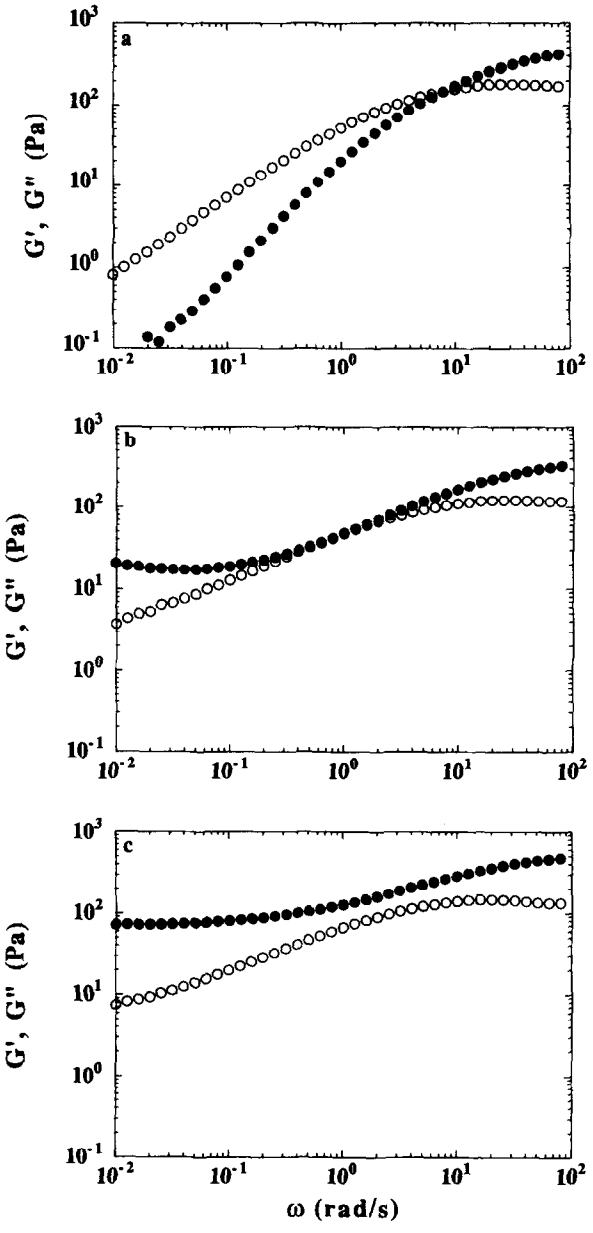

Fig. 6. Evolution of the mechanical spectra of a LBG solution with time (1.85% w/w, 20°C). G' (●), G'' (○). (a) Freshly prepared solution; (b) 8 days-aged solution; (c) 15 days-aged solution.

studied. Further experiments are in progress to determine the phase diagram of this mixed system and to characterize the rheological behaviour of the upper phase as a function of chemical composition (Garnier *et al.*, 1996), but these preliminary results can already provide new insights into the gelation mechanisms of galactomannan-based mixtures.

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