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The Incompatibility of Concentrated Aqueous Solutions of Dextran and Amylose and its Effect on Amylose Gelation

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SUMMARY

At 75 °C concentrated aqueous solutions of dextran and amylose exhibit immiscibility. The presence of dextran has a major effect on the microstructure and mechanical behaviour of amylose gels formed on cooling. At low dextran concentrations the modulus of the gel increases with increasing dextran concentration, whereas at higher dextran concentrations, polymer incompatibility and segregation of dextran-rich droplets reduces the firmness of the gel.

INTRODUCTION

Biopolymers are widely used as food additives to manipulate and control mechanical behaviour. In recent years polysaccharide gelation in single polymer systems has been extensively studied and models relating molecular interactions to gelation have been proposed. Two types of gel network can be broadly distinguished. In one, the network strands are individual polymer chains which are linked together at regions of local order or 'junction zones'. This type of network has been proposed for aqueous κ-carrageenan and alginate gels (Rees, 1969). In the other, the network strands consist of many polymer chains, an interconnected network arising as a result of a phase separation or precipitation, which produces polymer-rich and polymer-deficient regions. These gels are invariably turbid as the aggregates of polymer chains have a size of the order of the wave-

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length of light. Examples of this type of gel network are aqueous amylose (Miles *et al.*, 1985) and agarose gels (Wun *et al.*, 1974). In foods, polysaccharide gelling agents are usually present in association with other biopolymers. More information on their effect on polysaccharide gelation is needed.

For synthetic macromolecules, concentrated solutions of chemically dissimilar polymers (A and B) generally do not mix, but form two co-existing phases, each containing a preponderance of one polymer. Compatibility has only been demonstrated over a range of concentrations for a few polymer pairs (Dobry & Boyer-Kawenski, 1947). Compatibility can only occur if the free energy of interaction between the polymers is negative, whereas it is usually positive (De Gennes, 1979; Flory, 1953). If it is strongly positive, demixing occurs when the concentration exceeds the coil overlap concentration of the mixture. Biopolymer mixtures also exhibit immiscibility. The incompatibility of gelatin and gum arabic in concentrated aqueous solution is well known (Bungenberg de Jong & Lens, 1932) and there have been recent studies on agar and gelatin co-gels (Clark *et al.*, 1983). For polysaccharide mixtures it is known that the addition of some galactomannans can cause gelation in previously non-gelling concentrations of agar and κ-carrageenan (Dea & Morrison, 1975). A specific interaction between κ-carrgeenan chains and the galactomannan has been proposed to explain this phenomenon (Dea & Morrison, 1975). There have been surprisingly few reports of aqueous polysaccharide mixtures showing incompatibility.

Recently, in our laboratory, it was noted that hot concentrated aqueous solutions of the α-1,4-linked D-glucan, amylose and the α-1,6-linked D-glucan, dextran exhibited immiscibility. This system is an attractive one to study, since both polymers are neutral and based on the same monosaccharide, differing only in the mode of linkage.

This paper is a preliminary account of our findings. The effect of dextran on the gelling behaviour of amylose is described.

EXPERIMENTAL

Materials

Amylose was prepared by aqueous leaching of smooth-seeded pea starch granules at 70°C in an inert atmosphere as described by Miles

et al. (1985). The amylose was precipitated and collected as its 1-butanol complex. Amylose solutions were regenerated from the complex by heating to 95°C followed by removal of the 1-butanol in a heated nitrogen stream. The purified amylose had an iodine-binding capacity of 19·5 ± 0·5% w/w. The intrinsic viscosity of 95 ml g^{-1} indicated a weight-average molecular weight (Banks & Greenwood, 1975) of 700 000 g mol^{-1}. Fractions of pea amylose prepared in a similar way were found to have polydispersities of Mw/Mn ~ 2 (Ring *et al.*, 1985). The dextran used for the experiments was a commerical sample (Sigma D5251) produced by Leuconostoc mesenteroides, with a reported weight-average molecular weight of 472 000 g mol^{-1}, and polydispersity Mw/Mn ~ 3.

Determination of phase diagrams

Concentrated aqueous solutions of dextran and amylose were allowed to separate into two clear layers at 75°C. After two days the layers were carefully separated using a pipette, weighed and freeze-dried. The proportion of amylose present in the freeze-dried material was estimated from its blue value (Gilbert & Spragg, 1964), after dispersal in dimethyl sulphoxide and suitable dilution with water. Preliminary experiments with dilute amylose solutions of known concentrations indicated that the presence of dextran did not interfere with the determination of amylose concentration in the concentration range used. The dextran concentration in the freeze-dried material was then estimated from these data.

Viscometry

Ubbelohde suspended level viscometers were used to obtain the dependence of specific viscosity on concentration at 75°C. Using a variable head suspended level viscometer it was shown that at shear rates in the range 50–870 s^{-1}, the specific viscosity was independent of shear rate, both above and below C^*, the coil overlap concentration.

Measurements of shear modulus

The velocity, V, of a small amplitude shear wave (frequency 200 Hz) through the gel, was measured using a pulse shearometer (Rank Bros,

Cambridge) as described by Ring & Stainsby (1985). As the damping of the shear wave in the sample was small, the approximate relationship $G' = \rho V^2$, where ρ is the density of the gel, was used to calculate the shear modulus G'.

Preparation of mixed gels

Equal volumes of amylose and dextran solutions were thoroughly mixed at 75°C and rapidly quenched to 20°C. For the photomicrography, drops of the hot mixture were placed on a microscope slide and a coverslip added. The slide was then rapidly quenched to 20°C and microscopy was performed in the usual way.

RESULTS AND DISCUSSION

Mixtures of concentrated aqueous solutions of dextran and amylose become translucent immediately after mixing, and with time the mixtures separate into two visually distinguishable phases. Figure 1 shows a tube containing 5 ml of 7% w/w amylose and 5 ml of 7% w/w dextran, which has been left for 48 h at 75°C after mixing. The mixture has separated into two phases, the upper dextran-rich phase having the larger volume. Figure 2 presents the phase diagram for this ternary system at 75°C. The boundary of the two-phase region is not symmetrical, but is displaced towards the axis representing dextran concentration. Calculated phase diagrams (Hsu & Prausnitz, 1974) exhibit asymmetry when the two polymers have different molecular weights, the binodal shifting towards the lower molecular weight polymer, as is the case here. Additionally the preparations are polydisperse and the asymmetry may also arise as a result of the differences in the molecular weight distributions of the polymers. The most dilute system showing phase separation, contained 2·5% w/w dextran and 2·0% w/w amylose.

It is of interest to characterise the concentration regime in which phase separation occurs. The behaviour of the specific viscosity, η_{sp}, as a function of concentration at 75°C for each of the polymers, amylose and dextran, is shown in Figs 3(a) and (b). At low concentrations η_{sp} is linearly dependent on concentration, C, whereas at high concentrations the dependence changes to approximately C^2. The

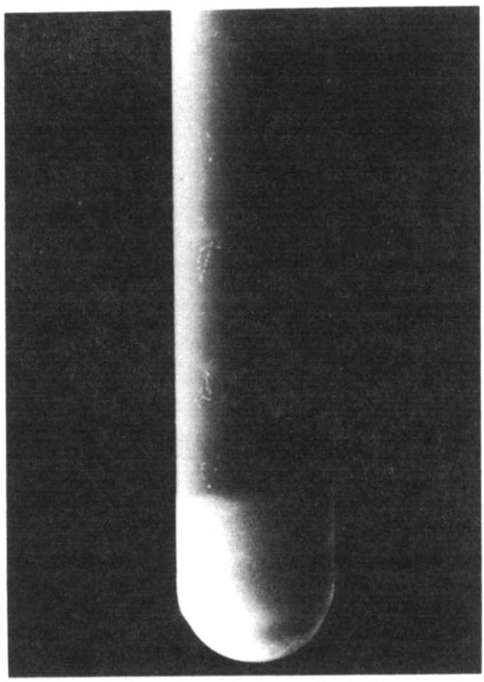

Fig. 1. An aqueous mixture of 3·5% w/w dextran and 3·5% w/w amylose, after 48 h at 75°C. Two separate layers are clearly distinguishble.

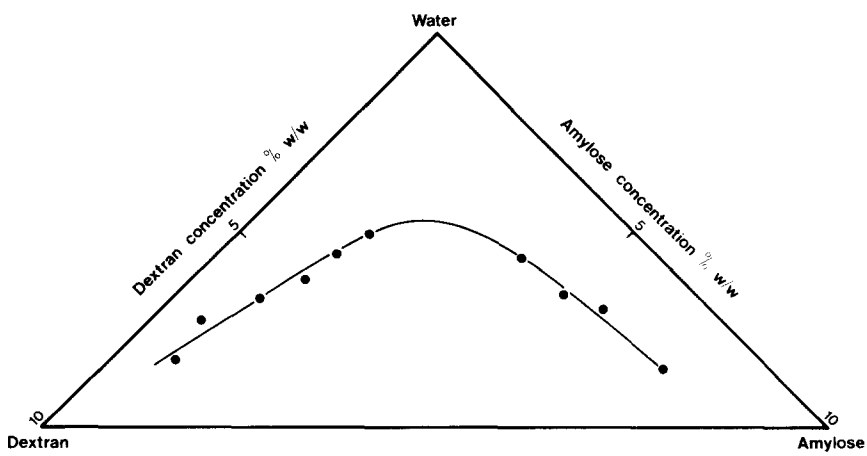

Fig. 2. Phase diagram for the amylose–dextran–water system.

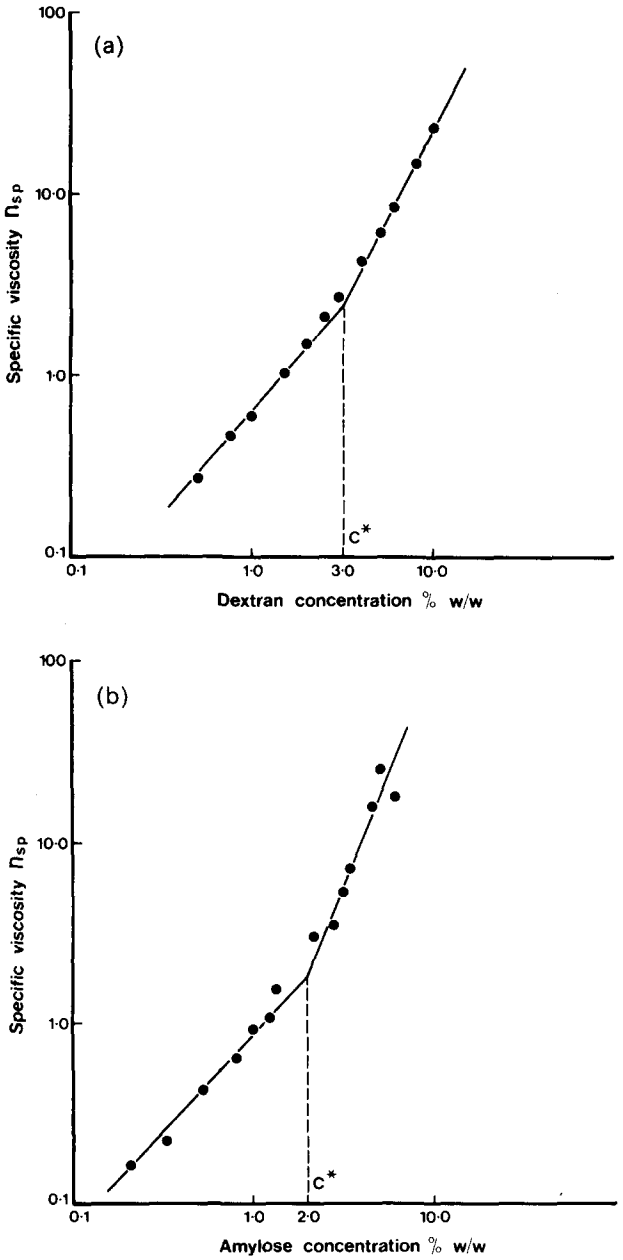

Fig. 3. Double logarithmic plots of specific viscosity, η_{sp}, versus polymer concentration for aqueous solutions of dextran (a) and amylose (b) at 75°C.

specific viscosity is then no longer simply dependent on the hydrodynamic volume of the polymer, but there is also a contribution due to entanglements. The threshold concentration, C^*, at which this change in behaviour occurs is 2·0% w/w for the amylose and 3·0% for the dextran fraction. Experimental results show that below C^*, η_{sp} for a mixture of dextran and amylose is equal to the sum of their individual specific viscosities. It is thus reasonable to assume that the hydrodynamic volume of each polymer in the solution is unaffected by the presence of the other. In this case, C^* for a mixture containing equal concentations of dextran and amylose can be predicted to occur when the amylose and dextran concentrations are each above approximately 1·3% w/w. As the most dilute system to show immiscibility has concentrations of both amylose and dextran well in excess of this figure, phase separation occurs well within the concentrated regime from entangled solutions. This indicates that the repulsive forces between amylose and dextran are relatively weak, not being strong enough to induce immediately separation on entanglement.

If concentrated solutions of amylose are rapidly quenched to room temperature, an opaque elastic gel quickly develops. The effect of dextran on this process of gelation is interesting. Immediately after mixing sufficiently concentrated solutions of amylose and dextran at 75°C, droplets of one phase, either dextran-rich or amylose-rich (depending on concentration), will be present in a matrix of the other phase. If such a mixture is quenched to room temperature, the amylose-rich phase gels. The material produced either contains amylose-rich gel droplets in a dextran-rich solution, or dextran-rich droplets in an amylose-rich gel matrix. By starting with different concentrations of amylose and dextran, and by varying thermal history, a range of non-equilibrium structures may be produced, the mechanical behaviour of these systems being correspondingly complex.

The effect of the addition of increasing concentrations of dextran on the stiffness of an amylose gel was investigated (Fig. 4). The final amylose concentration was 3·2% w/w in each case, which is well within the entangled regime. The shear modulus increases with increasing dextran concentration, reaching a peak at around 3% w/w and subsequently decreasing on further addition of dextran. However, when the same experiment was carried out using 0–8% w/w sucrose or D-glucose in place of dextran, the shear modulus remained at 7 500 $Nm^{-2} \pm 5\%$ for the same amylose concentration. Thus it appears that

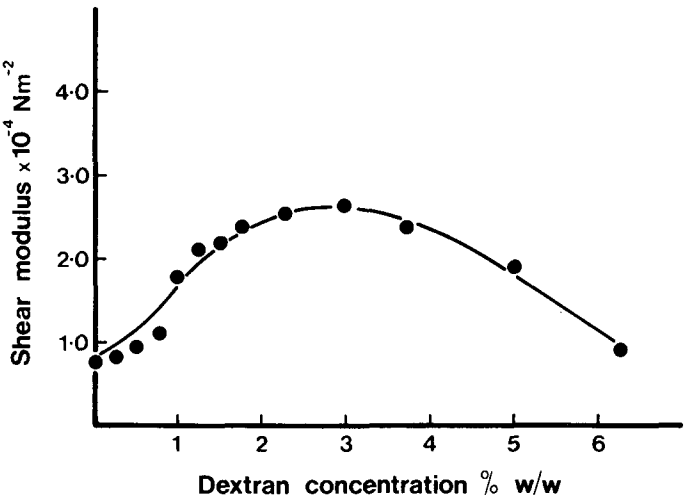

Fig. 4. Shear modulus of a 3·2% w/w amylose gel as a function of incresing dextran concentration.

these sugars act as low molecular weight diluents at these concentrations.

Figure 5 shows a series of photomicrographs of a 3·2% w/w amylose gel containing increasing amounts of dextran. At low dextran concentrations a 'coarse' amylose gel matrix with distinguishable aggregates of up to 2 μm in diameter is observed. As the dextran concentration is increased the gel network coarsens and above 1·5% w/w the appearance of the gel dramatically changes and dextran-rich inclusions of up to 20 μm in diameter can be observed. These materials are composites in which viscoelastic droplets modify the mechanical properties of an elastic matrix. As the dextran-rich droplets are fluid, they might be expected to weaken the gel structure. However, other effects must also be considered. First, on demixing, the concentration of amylose in the gel matrix will increase, resulting in an increase in shear modulus of the matrix. Secondly, on deformation of the composite, the surface area and hence the interfacial free energy of the droplets will increase, giving rise to a restoring force. The size distribution of the droplets may be important, as the droplets might have to exceed a certain size before they have a weakening effect on the gel structure. The magnitude of these effects is difficult to predict at

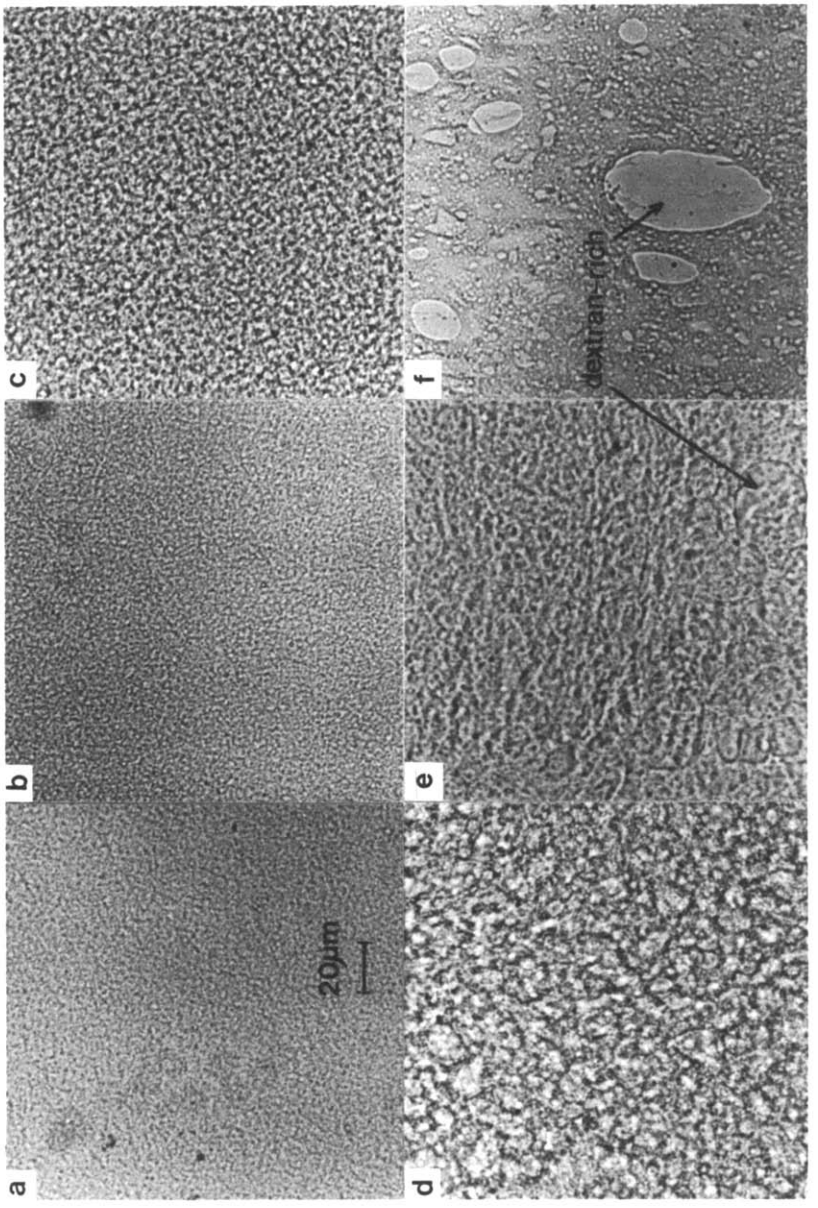

Fig. 5. Photomicrographs of a 3·2% w/w amylose gel with increasing dextran concentrations (a) 0, (b) 0·5%, (c) 1·0%, (d) 1·5%, (e) 2·5%, (f) 3·75% w/w.

present and further work is necessary.The effect of low concentrations of dextran on amylose gel rigidity is also difficult to account for, as there is no model at the present time which can adequately describe the relationship between amylose gel rigidity and network structure.

CONCLUSIONS

This study has shown that concentrated aqueous solutions of the chemically similar, neutral polysaccharides, amylose and dextran are immiscible. This immiscibility occurs well above the entanglement concentration, C^*, of the mixture. Dextran addition has a complex effect on the small deformation behaviour of amylose gels. At low concentrations of dextran, its presence increases the shear modulus of the gel. However, at dextran concentrations sufficient to produce immiscibility, dextran-rich droplets form in the amylose-rich matrix and weaken the structure of the gel.

REFERENCES

Banks, W. & Greenwood, C. T. (1975). *Starch and is components*, Edinburgh University Press, Edinburgh.

Bungenberg de Jong, H. G. & Lens, J. (1932). *Kolloid Zeitschrift* **58**, 209.

Clark, A. H., Richardson, R. K., Ross-Murphy, S. B. & Stubbs, J. M. (1983). *Macromolecules* **16**, 1367.

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

De Gennes, P. G. (1979). *Scaling concepts in polymer physics*, Cornell University Press, Ithaca, New York.

Dobry, A. & Boyer-Kawenoki, F. (1947). *J. Polym. Sci.* **2**, 90.

Flory, P. J. (1953). *Principles of polymer chemistry*, Cornell University press, Ithaca, New York.

Gilbert, G. A. & Spragg, S. P. (1964). In: *Methods in carbohydrate chemistry.* Vol. 4, eds R. L. Whistler and M. L. Wolfram, Academic Press, New York and London.

Hsu, C. C. & Prausnitz, J. M. (1974). *Macromolecules* **7**, 320.

Miles, M. J., Morris, V. J. & Ring, S. G. (1985). *Carbohydr. Res.* **135**, 271.

Rees, D. A. (1969). *Adv. Carbohydr. Chem. Biochem.* **24**, 267.

Ring, S. B. & Stainsby, G. (1985). *J. Sci. Food Agric.* **36**, 607.

Ring, S. G., I'Anson, K. J. & Morris, V. J. (1985). *Macromolecules* **18**, 182.

Wun, K. L., Feke, G. T. & Prins, W. (1974). *Faraday Discuss. Chem. Soc.* **57**, 146.