Carbohydrate Research, 162 (1987) 323–328
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Incompatibility of amylose and amylopectin in aqueous solution

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(Received March 21st, 1986; accepted for publication, December 1st, 1986)

The phenomenon of polymer immiscibility is well known and arises as a result of the generally unfavourable interactions between polymer species. Even a small positive free energy of interaction between different polymer species can result in limited miscibility, due to the small entropy gain on mixing these high-molecular-weight species. The miscibility of polymers in solution decreases with increasing polymer concentration and is rare at high concentrations[1]. If the segregation factor is strong, demixing is predicted when the polymer chains start to become entangled, above the coil-overlap threshold[2,3]. The phase behaviour of these ternary systems (polymer 1 + polymer 2 + solvent) is often strongly affected by polymer–solvent interactions[4,5], and immiscibility is generally increased when the affinity of one polymer for the solvent is significantly different from that of the other[4,5].

The phase behaviour of mixtures of synthetic polymers in organic solvents has been extensively studied. Biopolymers also exhibit immiscibility, with perhaps the best known example being the gelatin–gum arabic–water system[6]. In addition, incompatible mixtures of dextran and other biopolymers and synthetic polymers have been used to fractionate cells and organelles[7]. The incompatibility of dextran and amylose has also been demonstrated[8]. However, compared to synthetic polymer mixtures, there is relatively little information on the phase behaviour of polysaccharide mixtures.

Starch occurs as a mixture of mainly amylose and amylopectin, which are organised into a semi-crystalline granule. The behaviour of aqueous solutions of starch is of interest since, in many industrial applications, starch is processed by heating in the presence of water. Whereas amylose is essentially a (1→4)-α-D-glucan, amylopectin is a (1→4)-α-D-glucan with an average of one in every 20–25 residues branched at position 6. As these polysaccharides are so similar chemically, it might be expected that immiscibility, if it occurred at all, would only be observed at very high concentrations. However, even moderately concentrated aqueous solutions of amylose and amylopectin exhibit immiscibility and we now report on these findings.

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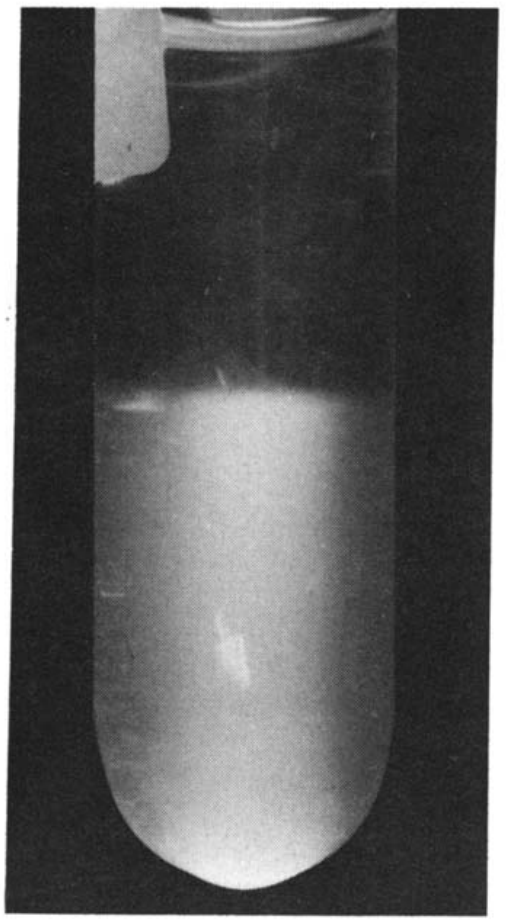

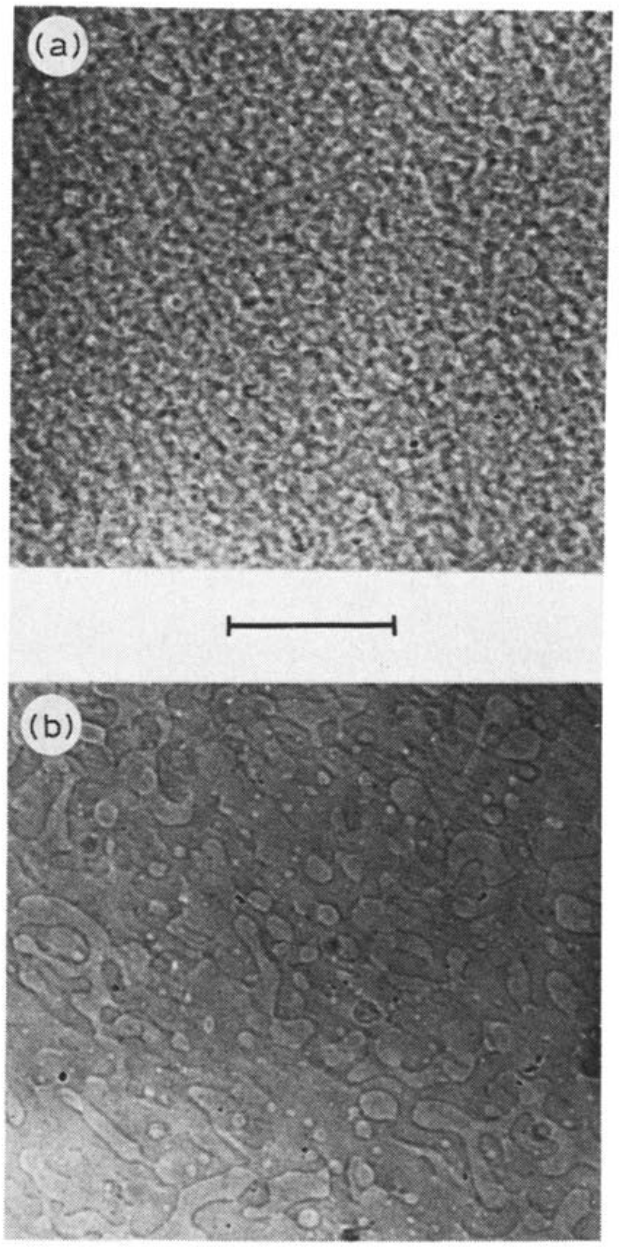

Fig. 1. Photomicrographs of aqueous 3% solutions of amylose and amylopectin: (a) 1 min and (b) 5 min after mixing. The scale bar indicates 100 μm.

Fig. 2. An aqueous mixture containing 6% each of amylose and amylopectin after storage for 48 h at 80°. Two separate layers are clearly distinguishable.

Amylose from smooth-seeded pea starch had an iodine-binding capacity of 19.5 ±0.5%, indicating that it was a pure sample. The samples, extracted by aqueous leaching at 70° and 70–90°, had intrinsic viscosities of 85 and 120 mL.g^{-1}, respectively, from which the weight-average molecular weights of these linear fractions[9] were calculated[10] to be 560,000 and 1,100,000, respectively. The potato amylose had an intrinsic viscosity of 68 mL.g^{-1}; however, its molecular weight cannot be directly compared with that of linear pea amylose by this method as it is lightly branched[11]. The waxy-maize amylopectin was >98% pure, as shown by its iodine-binding capacity of <0.2%, and had an intrinsic viscosity of 92 mL.g^{-1}. The intrinsic viscosity is comparable with that[10] of other amylopectins having molecular weights in the range 65–400 × 10^6.

On mixing concentrated aqueous solutions of amylose and amylopectin at

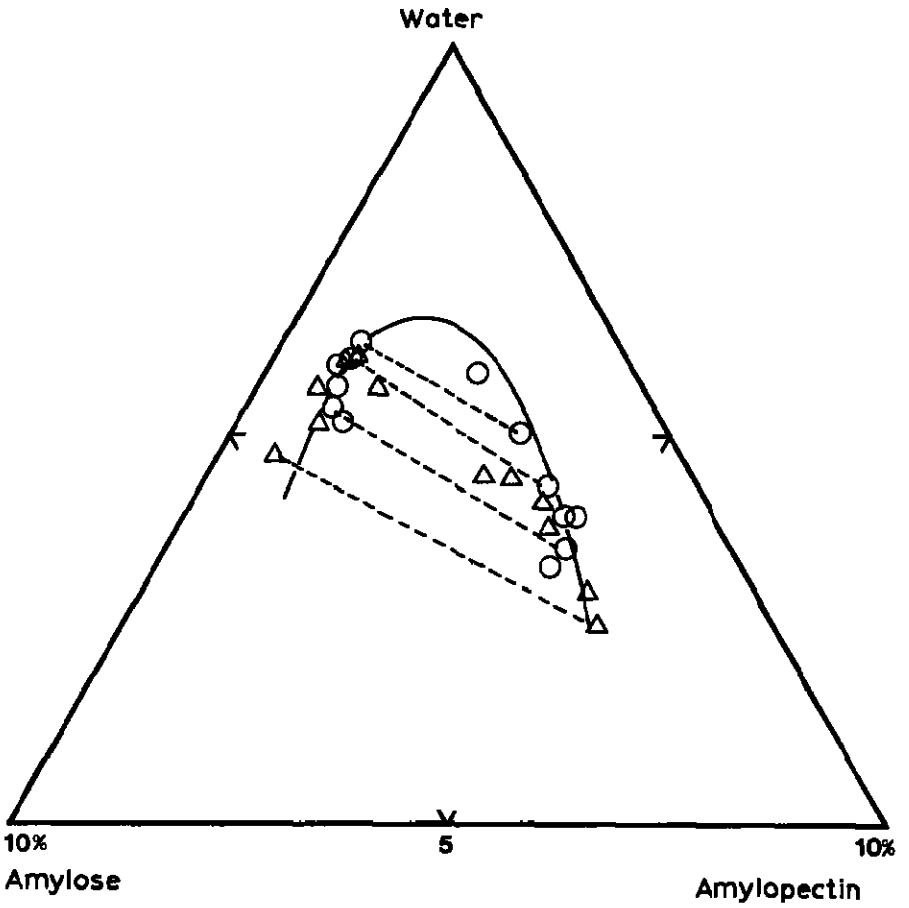

Fig. 3. Phase diagram for the amylose–amylopectin–water system (using pea amylose leached at 70°), obtained at 70° (○) and 90° (△).

80°, a granular texture was observed. Fig. 1a shows a photomicrograph taken 1 min after vigorous mixing of equal weights of the 6% solutions. With time, the granular structure coarsened until, after 5 min (Fig. 1b), droplets of one phase dispersed within the matrix of another were clearly recognisable. After 24 h, the mixture had separated into two phases (Fig. 2). The upper, clear phase was amylose-rich and the lower, opalescent phase was amylopectin-rich.

The composition of the phases was analysed, a phase diagram was obtained (Fig. 3), and the binodal was drawn through the experimental points. The concentration of onset of coil overlap is ~0.9% and ~1.2% for amylopectin[12] and pea amylose[8] (leached at 70°), respectively, as obtained by viscometry. From this, it is noted that phase separation occurs well within the entangled regime of both polymers.

The phase diagram (Fig. 3) was obtained for pea amylose (leached at 70°) and waxy-maize amylopectin at both 70° and 90°. As the same binodal can be drawn through all the points, within experimental error, the results show no observable temperature effect on the phase diagram. It was not possible to investigate a broader temperature range due to the high gelling temperature of amylose.

The influence of amylose source and molecular weight on the phase behaviour was also investigated using waxy-maize amylopectin at 80° and potato amylose or pea amylose leached between 70 and 90° (Fig. 4). It is apparent that this amylose of higher molecular weight and the potato amylose both show the same

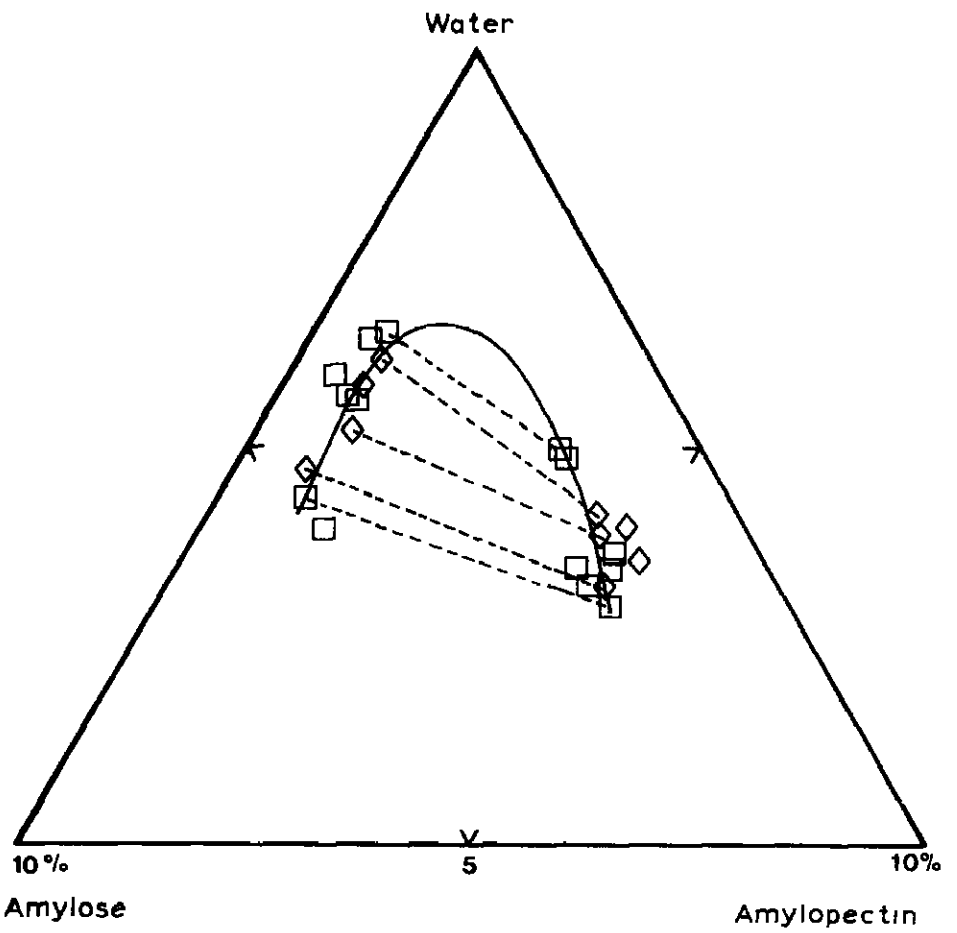

Fig. 4. Phase diagram for the amylose–amylopectin–water system at 80°, using potato amylose (□) and pea amylose leached at 70–90° (◇).

phase behaviour as observed previously, because the same binodal can be drawn in each case, within experimental error.

The binodal is not symmetrical, but is shifted towards the amylose-rich phase, which is consistent with the behaviour predicted for a mixture containing polymers of unequal molecular weight[4,5], the binodal being displaced towards the polymer of lower molecular weight, *i.e.*, amylose. The tie lines (between phases at equilibrium with each other) slope up to the amylose-rich phase, indicating that this phase has a higher affinity for the solvent, water.

Second virial coefficients give an indication of polymer–solvent interactions[13], but the second virial coefficients of dilute aqueous solutions of amylose and amylopectin at 20° are very similar[14], suggesting that the polymer–solvent interaction parameters are also similar. It has been shown[15] for polystyrene in cyclohexane, near the θ point, that osmotic pressure does not become independent of molecular weight until concentrations well above C^* are attained; hence, it is likely that, in the concentration regime studied here, molecular weight has some effect on osmotic pressure. It follows that amylose, which has a much lower molecular weight than amylopectin, is expected to have a higher osmotic pressure at a fixed concentration, as indicated by the sloping tie lines.

The investigation of the phase behaviour of amylose and amylopectin, using amylose of different molecular weights and structures as well as different temperatures (70–90°), shows that these factors do not strongly affect the unfavourable

interaction between these polysaccharides which gives rise to phase separation. It is noted that the differences in molecular weight between the amylose samples used are small relative to the large differences between amylose and amylopectin. From the observation that phase separation only occurs at concentrations well above C^*, it is apparent that the segregation factor is not very strong. It has been predicted[4,5] and observed[13] that immiscibility becomes greater with increasing molecular weight, so the high molecular weights of these polysaccharides, particularly the amylopectin, encourage phase separation.

The incompatibility of the linear and branched polymer fractions of starch in aqueous solution has important implications. This phenomenon should be considered in any account of the biosynthesis and assembly of the starch granule. Incompatibility may also affect the types of interactions which can occur during retrogradation of the starch polysaccharides and could throw some light on the gelatinisation behaviour of starch on heating in water, *i.e.*, the swelling of the starch granule with preferential solubilisation of the amylose.

EXPERIMENTAL

Amylose fractions were extracted from smooth-seeded pea starch as previously described[9], and purified by precipitation with 1-butanol. Amylose was similarly extracted from potato starch at 80°, in the presence of 0.1M NaCl which was later removed by re-dispersing the 1-butanol complex in aqueous 8% 1-butanol and re-centrifuging. Amylopectin was obtained from waxy-maize starch by dissolution of the granules in aqueous 90% methyl sulphoxide, followed by precipitation with ethanol. The iodine-binding capacity of the polysaccharides was determined by using a semi-micro differential potentiometric method[10]. Intrinsic viscosities were obtained at 25°, using Ubbelohde suspended level viscometers.

The phase diagrams were obtained by mixing solutions of amylose and amylopectin of various concentrations and allowing them to separate overnight in a water bath at the appropriate temperature. Each phase was removed, weighed, and then freeze-dried, and the amylose content of the remaining solid was determined by measuring the blue value[16], after dispersal in methyl sulphoxide and dilution with water.

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