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Temperature–composition phase diagram and gel properties of the gelatin–starch–water system

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The gelatin–starch–water system has been studied at different temperatures, at a total biopolymer concentration of 5.0 wt%. The weight ratios (W) of gelatin/starch used were 9:1, 8:2..... 2:8, 1:9, with pH values between 5.82 (at W = 9:1) and 6.50 (at W = 1:9). The systems were characterized rheologically and by turbidity measurements to construct a phase diagram in the temperature (T) and composition (W) variables. The T–W quadrant consists of three regions: a single-phase solutions region (A) and regions of complete and incomplete phase separation (B and C, respectively). The system in region C is a gel. Region B, lying between A and C, corresponds to two co-existing liquid phases. The transition from A to C (obtained by cooling the system at constant W) involves crossing region B. The properties of the resulting gels depend on the rate of this intersection. Gels formed on rapid cooling have an even distribution of turbidity, whereas slow cooling gives two gel layers of different turbidity. The gelation temperature and gel strength of the mixed systems are dominated by the gelatin component, with no indication of network formation by starch. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

Because of their wide-ranging industrial applications, notably in the food industry, the properties of gelatin (Ward & Courts, 1977; Ledward, 1986) and starch (Whistler & Paschall, 1967; Galliard, 1987) in aqueous systems have been studied extensively, and remain the focus of substantial current research (see for example Hudson, 1993; Ross-Murphy, 1992, 1994 for gelatin, and Morris, 1990; Autio & Poutanen, 1993; Keetels & van Vliet, 1994 for starch).

Aqueous mixtures of gelatin and starch have been characterized far less thoroughly. However, it has been known for almost a century that separation into two liquid layers occurs for various types of starch at concentrations above ~1 wt% (Beijerinck, 1896; Butschli, 1898; Ostwald & Hertel, 1929). More recently, it has been demonstrated (Grinberg *et al.*, 1971) that the concentrations of gelatin and starch in the individual phases are in good agreement with the main propositions of the thermodynamic approach to polymer incompatibility that has been applied by Tolstoguzov and co-workers to many other mixed biopolymer systems (for recent reviews see Tolstoguzov, 1986, 1988, 1993).

A central step in thermodynamic analysis of biopolymer–biopolymer–water systems is to obtain a diagram of phase states (or phase diagram). To characterize the system fully, the diagram must be constructed in f-space, where f is the number of independent variables determining the final state. In the simplest case $f = 3$, the variables being temperature (T) and the overall concentrations (C_1 and C_2) of the two constituent biopolymers (expressed as weight fractions of the total system). Alternatively, the independent variables can be defined as T, C and W, where C is the total polymer concentration ($C_1 + C_2$) and W is the weight ratio of the two components (C_1/C_2).

The phase diagrams constructed in most investigations, including the extensive work of Tolstoguzov and colleagues, however, are normally confined to two variables, C_1 and C_2 (i.e. they show the cross-section generated by intersection of the three-dimensional phase diagram with a plane of constant T). In the investigation of the gelatin–starch–water system by Grinberg *et al.* (1971), two such cross-sectional ‘slices’ were obtained, at T = 45°C and T = 35°C, with the conclusion that the amount of starch retained in the gelatin-rich phase increases as the temperature is decreased.

A fuller and more systematic description of the effect of temperature could, however, be achieved by sectioning the three-dimensional phase diagram in a different

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way, for example, by a plane of constant C, to obtain a two-dimensional diagram with T as one of the independent variables (the other being W). The aim of the present work was to construct such a T–W phase diagram for the gelatin–starch–water system.

MATERIALS AND METHODS

Commercial samples of food-grade gelatin and soluble potato starch were obtained from, respectively, the Kasansky gelatin plant and Zaspensky starch plant (Russia) and used without further purification. Individual stock solutions were prepared by dissolving gelatin and starch at, respectively, 80 and 100°C in distilled water, mixed at 80°C, and stirred for 15 min, to obtain gelatin–starch–water systems of the required composition. Most experiments were made at a fixed total polymer concentration of C = 5.0 wt%. The values of W (weight ratio of gelatin/starch) used were 9:1, 8:2,..., 2:8, 1:9, with pH values between 5.82 (at W = 9:1) and 6.50 (at W = 1:9).

Mixed solutions were quenched from 80°C to the required experimental temperature (typically within the range 65–45°C) and the development of turbidity was monitored at 400 nm, using a CФ-26 spectrophotometer. Samples for gel-strength measurements were placed in a refrigerator at 5°C, stored for 24 h, and characterized by a penetration testing using a Valenta device (Pernas *et al.*, 1967) with a hemispherical plunger of cross-sectional area 1.5 cm^2. Viscosity measurements were made at a constant shear stress of 4 Pa on a Heppler viscometer (from the former GDR).

RESULTS

Phase-separation temperature (T_s)

Mixed solutions prepared at high temperature (80°C) remained clear, indicating that at the total polymer concentration used (C = 5.0 wt%), the gelatin and starch components gave a homogeneous single-phase solution. On quenching to lower temperatures, however, the mixtures rapidly developed the turbidity characteristic of a phase-separated system with one liquid phase finely dispersed through a second, continuous, phase.

The time-course of turbidity increase at different quench temperatures is illustrated in Fig. 1 for W = 8:2 (i.e. 4.0 wt% gelatin; 1.0 wt% starch). The same general form of time dependence was observed at all other mixing ratios studied. In the first few minutes after quenching, turbidity increases linearly with time, yielding a constant initial rate (V_o) at each temperature.

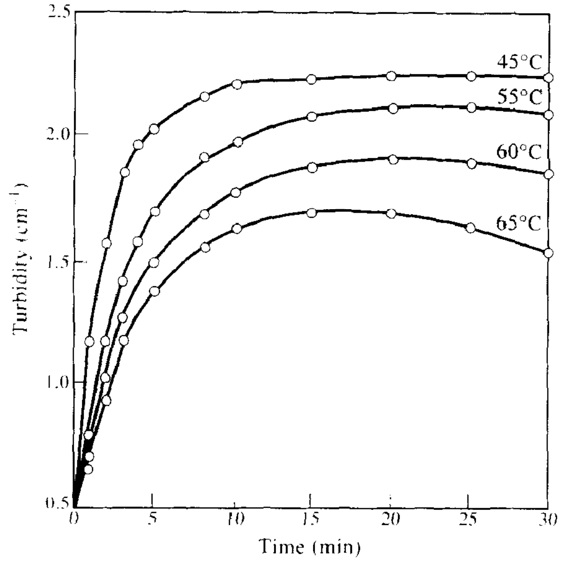

Fig. 1. Kinetics of turbidity increase in the gelatin–starch–water system, illustrated for a gelatin/starch weight ratio of W = 8:2 at a total polymer concentration of C = 5.0 wt%, after quenching from 80°C to the temperatures shown above the individual traces.

As illustrated in Fig. 2, the values of V_o observed at fixed C and W were found to decrease linearly with increasing temperature, and the temperature of phase separation (T_s) was obtained by extrapolation to zero rate (i.e. $T = T_s$ at V_o = 0).

Gelation temperature (T_g)

On further cooling, the gelatin–starch–water systems formed gels. The temperature at the onset of gelation (T_g) was determined from measurements of viscosity (η) on cooling at fixed intervals of time and temperature (2°C steps at 15 min intervals).

In the high-temperature solution state, a linear relationship was observed between ln η and $1/T$, as illu-

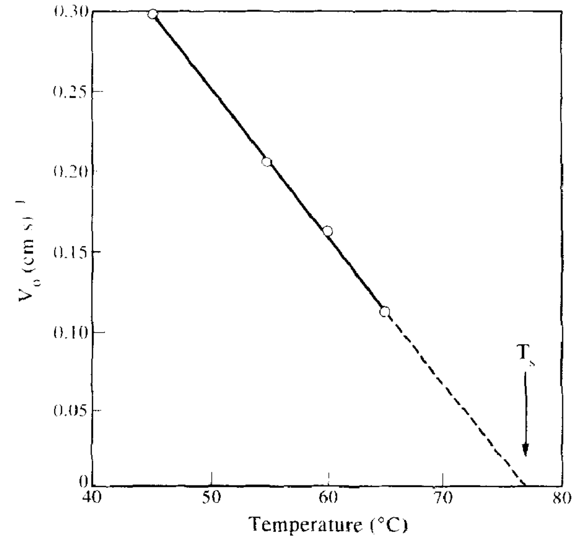

Fig. 2. Temperature dependence of the initial rate of turbidity increase (V_o) for the gelatin–starch–water system illustrated in Fig. 1 (C = 5.0 wt%; W = 8:2).

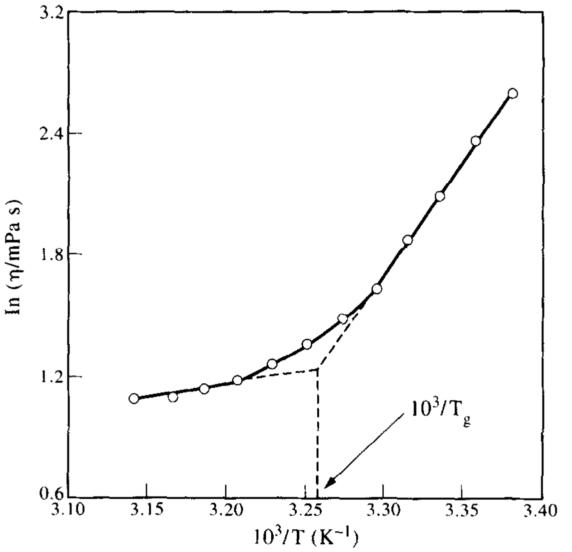

Fig. 3. Temperature-dependence of viscosity (measured at intervals of 15 min on cooling in steps of 2°C) for the gelatin-starch-water system (illustrated for C = 5.0 wt%; W = 8:2), showing the construction used for determination of the temperature (T_g) at the onset of gel-formation.

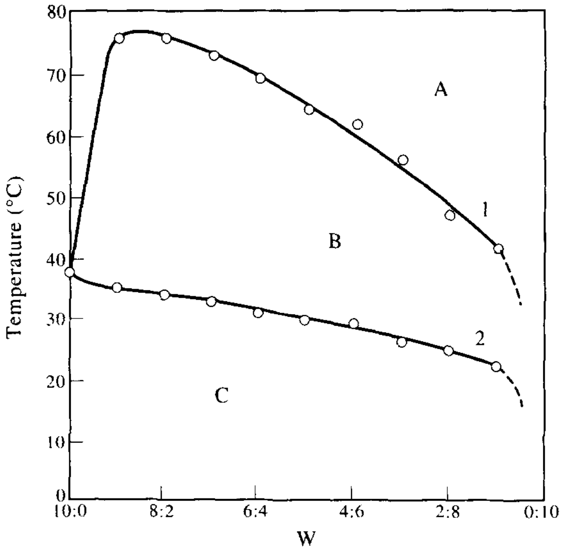

Fig. 4. Phase diagram for the gelatin-starch-water system at a total polymer concentration of C = 5.0 wt%. Curves 1 and 2 show the variation of, respectively, T_s and T_g with the weight-ratio of gelatin/starch (W). The three regions of the diagram correspond to: A - single-phase solution; B - two-phase liquid state; C - gel state.

strated in Fig. 3. The activation energy for viscous flow, obtained from the gradient of these linear Arrhenius plots, decreased systematically from 18.8 kJ mol^{-1} (at W = 9:1) to 15.6 kJ mol^{-1} (at W = 1:9). The corresponding values for 5.0 wt% solutions of gelatin and starch alone were 20.3 and 15.3 kJ mol^{-1}, respectively.

The onset of the cross-linking process leading to the formation of a gel network at lower temperature can be detected by a sharp increase in the temperature dependence of viscosity to a much steeper linear relationship between ln η and $1/T$. T_g was estimated (Fig. 3) by extrapolation of the two linear regions at high and low temperature to their point of intersection.

T–W phase diagram

Figure 4 shows the T–W phase diagram for the gelatin–starch–water system at a total polymer concentration of C = 5.0 wt%, defined by the variation of T_s and T_g (curves 1 and 2, respectively) with the weight ratio (W) of gelatin/starch. The diagram comprises three regions: A, B and C.

In region A, lying above the binodal (curve 1), the system remains stable as a single-phase solution. The solutions are transparent and show no thermal hysteresis in either turbidity or viscosity on heating and cooling.

Region B, lying below the binodal and above the gelation-temperature curve, corresponds to a two-phase state of the system. Visually, the mixed systems in this region have the turbid appearance of a fine dispersion of solid particles in a liquid medium. In reality they consist of two liquid phases which, over time, resolve into separate layers, the upper one rich in gelatin and the lower one rich in starch. The composition of these co-existing phases can be found from the points of intersection of a horizontal line (i.e. at constant T) with the left- and right-hand branches of the binodal (curve 1 in Fig. 4).

Region C corresponds to the gel state. The properties of the gels obtained by cooling from the single-phase solution state (transition from A to C) depend on the rate of temperature decrease. In particular, gels formed on rapid cooling (by placing hot solutions in a refrigerator at ~5°C) had an even distribution of turbidity, whereas slow cooling (hot solutions left to cool at an ambient temperature of ~20°C) gave two gel layers of different turbidity.

Figure 5 gives an indication of the effect of total polymer concentration (C) on the T–W phase diagram of the gelatin–starch–water system. It can be seen that increasing C displaces the binodal to higher temperature. The effect is greatest at low values of C, and becomes small at concentrations in the range 5–10 wt%. In this range, the dependence of gelation temperature (T_g) on total polymer concentration is also slight.

Composition dependence of gel properties

Figure 6 shows the variation of gel strength with weight ratio of gelatin/starch (W) for the gelatin–starch–water system at total polymer concentrations of C = 5.0 and 10.0 wt%. In both cases the resistance to penetration decreases monotonically with decreasing content of gelatin (i.e. decreasing W). Comparative measurements for gelatin alone at the same concentrations as in the

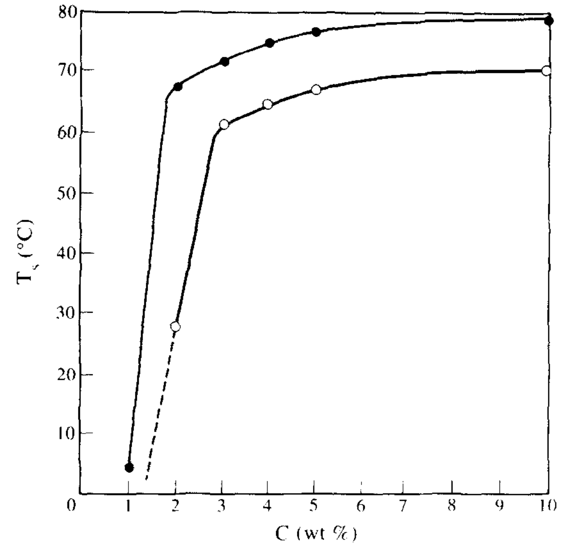

Fig. 5. Dependence of phase-separation temperature (T_s) on total polymer concentration (C) for the gelatin–starch–water system at gelatin/starch weight ratios of W = 8:2 (●) and W = 5:5 (○).

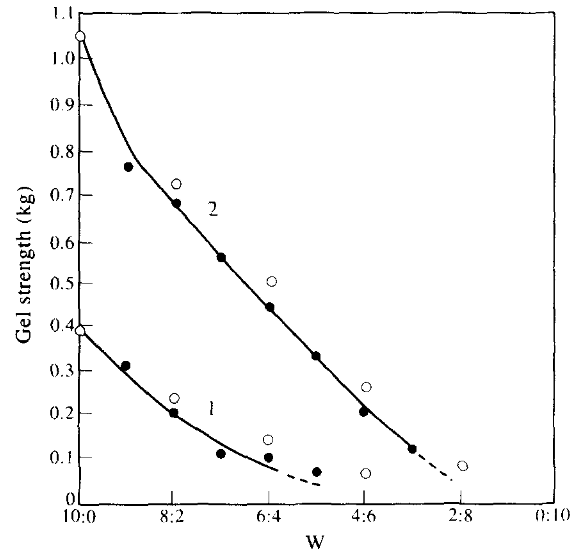

Fig. 6. Variation of gel strength (after 24 h at 5°C) with weight ratio of gelatin/starch (W) for the gelatin–starch–water system (●) at total polymer concentrations of: (1) C = 5.0 wt% and (2) C = 10.0 wt%, in comparison with gelatin alone (○) at the same concentrations as in the mixed systems.

mixed systems gave closely similar values (Fig. 6). The presence of starch was also found to have little effect on gelation temperature, with values of T_g (obtained by the procedure illustrated in Fig. 3) for gelatin alone lying close to those observed for the corresponding mixed systems (curve 2 in Fig. 4).

It seems clear from these observations that, at least for total polymer concentrations up to 10 wt%, the gel network of the gelatin–starch–water system is formed solely by gelatin macromolecules, with no contribution from the starch component.

DISCUSSION

In this investigation, we have used comparatively simple experimental procedures to explore thermodynamic incompatibility and gelation behaviour in the gelatin–starch–water system, yielding a phase diagram with temperature as an explicit variable. The same approaches could obviously be extended to characterization of other gelling biopolymer–biopolymer–water systems.

Extrapolation of the initial-slope kinetics of turbidity increase to zero rate (Fig. 2) seems a particularly convenient method of determining the temperature of de-mixing in the solution state. The viscometric approach used for estimation of T_g (Fig. 3) has the advantage of extreme sensitivity and precision. It does, however, detect the onset of intermolecular association rather than the formation of an infinite network, and will therefore give higher values of T_g than those obtained using more conventional criteria such as a rapid increase in G' from small-deformation oscillatory measurements or, more rigorously, the co-incidence of G' and G'' over a wide range of frequency (Durand *et al.*, 1987; te Nijenhuis & Winter, 1989). Nonetheless, the discrepancy is unlikely to be serious since physical gelation, once initiated, is normally an extremely sharp process.

The T–W phase diagram presented in Fig. 4 emphasizes the importance of temperature in determining the phase behaviour of aqueous biopolymer mixtures. At the total polymer concentration studied (C = 5.0 wt%), the binodal (curve 1 in Fig. 4) passes through a critical point at $T \approx 77$°C and W ≈ 9:1. Below this critical point the system resolves spontaneously into two co-existing liquid phases whose compositions progressively diverge with decreasing temperature. Increasing the total polymer concentration above 5.0 wt% has little effect (Fig. 5) on the position of the binodal, but decreasing C causes a substantial reduction in T_s, until eventually phase separation occurs only at temperatures below the helix–coil transition temperature of the gelatin component (~37°C).

As indicated in Fig. 1, phase-resolution within region B of the phase diagram (Fig. 4) occurs over a timescale of several minutes, followed by macroscopic separation into upper and lower layers. Rapid quenching through this region (i.e. from A to C in Fig. 4) gives gels with an even distribution of turbidity (i.e. macroscopically homogeneous). The samples used for the gel strength measurements presented in Fig. 6 were prepared in this way, by placing hot solutions (within region A of the phase diagram) into a refrigerator at ~5°C.

In all cases the gel strength, and gelation temperature, remained close to the values obtained for the same concentration of gelatin alone. It would therefore appear that when gelatin–starch–water mixtures (at total polymer concentrations within the range studied; up to 10 wt%) are cooled rapidly from the single-phase solution state (region A in Fig. 4) to the gel state (region C in Fig. 4), the gelatin component gels at its nominal concentration across the entire system, rather than at the higher concentrations anticipated for the gelatin-rich phase in region B (as defined by the left-hand branch of the binodal). Similar behaviour has been observed previously for other biopolymer–biopolymer–water systems where initial gelation is confined to one component (Clark *et al.*, 1982, 1993; McEvoy *et al.*, 1985).

Of particular relevance to the present investigation, mixed gels formed by rapid cooling of gelatin (2–5 wt%) in the presence of gelling maltodextrins (partially depolymerized potato starch) were found (Kasapis *et al.*, 1993) to give moduli (G') close to those for gelatin alone, until the total polymer concentration present was in excess of ~15 wt%. It has also been observed, by Fourier transform infrared microscopy (Durrani & Donald, 1994), that the distribution of the two polymeric components in mixed gels of gelatin and amylopectin depends strongly on the time for which the system is held in the two-phase solution state prior to quenching.

Thus the physical properties of the composite gels formed by aqueous mixtures of gelatin with starch polysaccharides appear to involve a subtle interplay between the rates of phase separation and gel formation, with the time-temperature course of the transition from region A to region C of the *T–W* phase diagram (Fig. 4) being of critical importance.

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