Some physical measurements in the one-phase region of a water–dextran–poly(ethylene glycol) system

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(Received 14 September 1990; accepted 26 November 1990)

Concentration-dependent phase separation into two polymer-containing liquid phases has been studied for the polymer pair poly(ethylene glycol) and dextran. Below the point of phase separation, the light scattering of polymer mixtures was considerably greater than for single polymers in solution. Also the viscosity of polymer mixtures was larger than the sum of the viscosity increments of polymer solutions. On the other hand, both heat capacity and density were additive in the one-phase region. The possible solvent structure in mixed polymer solutions is discussed.

(**Keywords: phase transition; aqueous liquid–liquid system; light scattering; viscosity; heat capacity; density**)

INTRODUCTION

Phase separation is commonly observed for mixtures of polymers in certain concentration intervals in a solvent[1]. When water is used as the solvent, the liquid phases constitute a two-phase system suitable for extraction of sensitive hydrophilic biological cell constituents like proteins, nucleic acids and membrane particles[1–3]. In contrast to the large amount of empirical knowledge concerning such systems, there are only a few studies of their basic physical characteristics. Some theoretical models that account for their fundamental properties, e.g. the phase transition, have been proposed[4–10].

In the present work the behaviour of polymer mixtures of dextran and poly(ethylene glycol) (PEG) in water has been studied with light scattering and viscosity at concentrations below the phase separation. The observation of an increase in light scattering was first announced in an oral presentation at the Second International Conference on Partitioning, in Sheffield, 1981. Also the heat capacity and the density of the polymer mixtures were compared with the corresponding ones for solutions of the single polymers.

MATERIAL AND METHODS

Chemicals

Dextran T-500 (mol. wt 500000) was purchased from Pharmacia (Uppsala, Sweden), and PEG 8000 (mol. wt 7000–9000) from Union Carbide (New York, USA). Hydroxypropyl dextran 500 (with 1.5 moles of hydroxypropyl groups per mole of glucose) was a gift from Professor P.-Å. Albertsson. All other chemicals were of analytical grade.

Phase transition

The phase transition from two phases to one was determined by dropwise dilution of 20 g of the mixed two-phase system of known composition with water containing the same concentration of salt until the turbid mixture became clear for at least 1 min with a path length of light of 2.5 cm. Systems and salt solutions were thermostated at 22°C.

Light scattering

The turbidity of polymer solutions and their mixtures was measured as the apparent absorbance at 310 nm using 1 cm cuvettes at 22°C in a Hitachi 100-60 spectrophotometer.

Viscosity

The viscosity was determined with an Ubbelohde capillary viscosimeter using 2.5 ml of liquid. The flow time for water was 9.8 s. The temperature was 22°C.

Heat capacity

The heat capacity measurements were performed in a drop heat capacity microcalorimeter, developed by Suurkuusk and Wadsö[11]. The instrument consists mainly of a furnace and the receiving calorimeter. The temperature of the furnace was kept constant within $\pm 2 \times 10^{-4}$°C by means of a Eurotherm proportional controller. The receiving calorimeter was immersed in a water thermostat, whose temperature was kept constant to about $\pm 5 \times 10^{-4}$°C.

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0032-3861/92/010152–04

The sample, held in stainless-steel ampoules (maximum sample volume of 0.7 cm^3), was first kept in the furnace at temperature t_1 and then dropped to the receiving calorimeter, at temperature t_2. The heat evolved was measured, and by suitable calibration the heat capacity of the solution was obtained. The working temperatures were 24.00°C for the furnace and 20.00°C for the calorimeter. The heat capacities reported thus refer to a mean temperature of 22.00°C. The instrument was calibrated with degassed water. For each mixture, one series of measurements was performed, consisting of eight determinations. In all cases, the uncertainty of the measured heat capacity of the solution was ± 0.001 J K^{-1} g^{-1}.

Density

For the density measurements an Anton Paar DMA 601 oscillating tube densitometer was used. Temperature control was achieved by use of a water thermostat, which was kept at 22.34 ± 0.01°C. The sample temperature was measured by a thermistor fitted in the oscillating tube. For each sample, three to six determinations were made. The instrument was calibrated with degassed water and nitrogen.

RESULTS AND DISCUSSION

Light scattering and phase transition

Solutions containing both dextran and PEG in water showed considerable light scattering in the u.v. region (*Figure 1*) at concentrations below the two-phase area. The apparent absorbance was proportional to $\lambda^{3.3}$ (λ is the wavelength of the light). When equal amounts of the two polymers were used, the excess in light scattering ($\lambda = 310$ nm) started at 1% of each polymer (dextran 500, PEG 8000) and increased in an exponential way when the polymer concentrations approached the transition point, which occurred at 3.75%. The light scattering probably reflects the formation of microregions that differ in polymer composition. The size of these regions increases with polymer concentration until they collapse into bulk phases. This is in line with the model of spinodal phase decomposition[12].

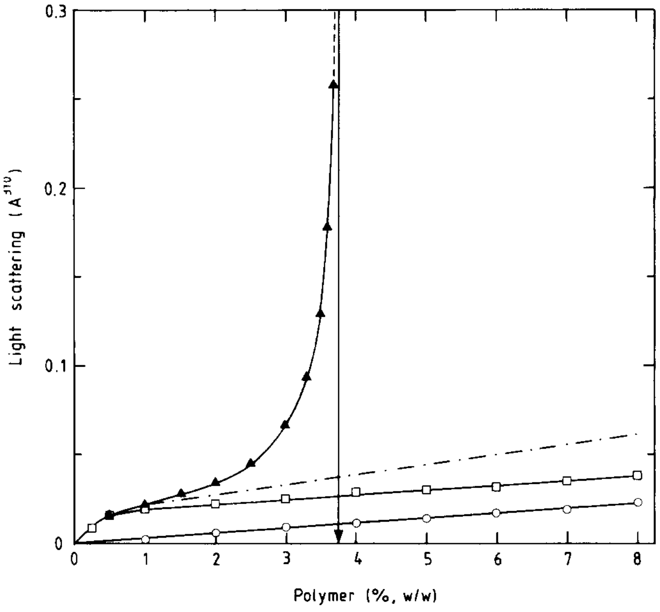

Figure 1 Structural organization in mixtures of dextran 500 and PEG 8000 in water, demonstrated by light scattering measured at the apparent absorbance at 310 nm: (○) PEG solution; (□) dextran solution; (▲) mixture of equal concentrations of the two polymers (total polymer concentration is twice that indicated); (–·–·–) sum of light scattering for each polymer. The maximal deviation in the light scattering was ± 0.004 absorbance units when A^{310} was below 0.2. The phase transition point is indicated with the arrow. Temperature, 22 ± 0.2°C

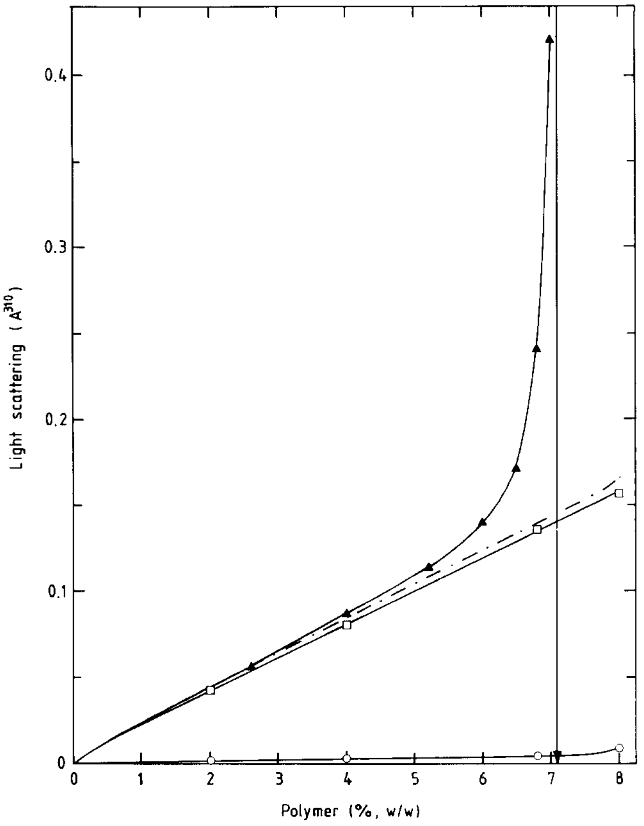

Figure 2 Structural organization in mixture of hydroxypropyl dextran 500 and PEG 8000 in *N*,*N*-dimethylformamide, demonstrated by light scattering at 310 nm; (○) PEG solution; (□) hydroxypropyl dextran solution; (▲) mixture of equal concentrations of the two polymers (total polymer concentration is twice that indicated); (–·–·–) sum of light scattering for each polymer. The maximal deviation in the light scattering was ± 0.004 absorbance units when A^{310} was below 0.25. The phase transition point is indicated with the arrow. Temperature, 22 ± 0.2°C

Two-phase systems can also be obtained by dissolving two polymers in an organic solvent. One such system is composed of PEG and hydroxypropyl dextran in *N*,*N*-dimethylformamide[13]. Also in this case the same light scattering phenomenon was observed below the transition point (7.1% w/w of each polymer) (*Figure 2*). The excess of the light scattering at the same distance from the transition point (measured on the percentage polymer scale) was practically identical for the aqueous and non-aqueous systems.

Effect of salts on phase decomposition

The critical concentrations of dextran and PEG required to obtain two phases depended on the kind of salt included in the system (*Table 1*). Potassium sulphate lowered the phase transition point while other salts like lithium or tetrabutylammonium phosphates increased the necessary polymer concentrations.

The shift in transition point towards higher polymer concentration can be explained by interactions between

Table 1 Phase transition point for systems containing equal amounts of dextran 500 and PEG 8000 and salts (50 mmol kg^{-1}). Temperature, 22°C

Salt	Dextran (%)	PEG (%)
None	3.75	3.75
K_2SO_4	3.71	3.71
Tetrabutylammonium phosphate (pH 6.8)	4.03	4.03

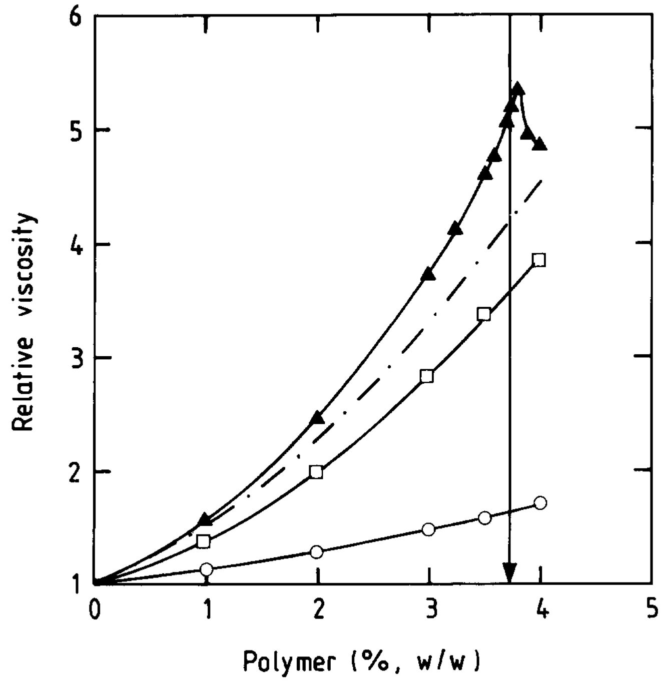

Figure 3 Viscosity, relative to water, of: (○) PEG 8000; (□) dextran 500; and (▲) a mixture of equal concentrations of dextran 500 and PEG 8000 in water. Maximal deviation of relative viscosity ($n = 5$) was ±0.01 units. Temperature, 22 ± 0.2°C

polymer and ions forming pseudo-polyelectrolytes[14,15]. It has been demonstrated[16] that covalent binding of one or two charged groups per PEG molecule strongly reduces the phase formation with dextran in water by as much as 4 percentage points of each polymer. The light scattering (data not shown) was practically the same with or without salt in the system. When 50 mmol kg^{-1} potassium sulphate was included, the light scattering curve was shifted by no more than 0.05 percentage points *towards* the transition point. In the case of 50 mmol kg^{-1} tetrabutylammonium phosphate buffer (pH 6.8) the curve shifted less than 0.05 percentage points (compared with the salt-free system) *away from* the transition point.

Viscosity and phase transition

The relative viscosity of mixtures of dextran and PEG is higher than the sum of the increment of the separate polymers (*Figure 3*). Because of the very slow settling of the phases in the two-phase region (close to the binodal curve), it was possible to measure the viscosity of mixed phase systems. The phase transition gave rise to a marked drop in the viscosity.

Heat capacity

The results for the heat capacity measurements can be seen in *Table 2*. From the measurements performed in the one-polymer systems, the apparent heat capacity, $C_{p,\phi,2}$, of the polymer at the desired concentration was calculated according to:

$$C_{p,\phi,2} = \frac{C_p - w_1 C_{p,1}}{w_2} \qquad (1)$$

where w_1 and w_2 are the mass ratios of water and the polymer, respectively, C_p is the heat capacity of the solution and $C_{p,1}$ is the heat capacity of pure water. From those values, the heat capacities of the three-component system (1 = water; 2 and 3 = polymers) were calculated according to:

$$C_p(\text{solution}) = w_1 C_{p,1} + w_2 C_{p,\phi,2} + w_3 C_{p,\phi,3} \qquad (2)$$

Comparison of the two last columns in *Table 2* shows that calculated and experimental values agree with each other, within uncertainty limits. So, in terms of heat capacity, the three-component systems show additive effects from the two polymers.

This does not seem to contradict the result obtained by light scattering and viscosity. We were working in a dilute region, where both polymers can be fully hydrated. Thus, the possible formation of 'micro-aggregates' of the polymers might not be reflected in any noticeable heat capacity change for the system. Earlier calorimetric measurements on PEG 600[17] showed that the change in heat capacity for the dissolution of water into water–PEG mixtures, $\Delta_{sol}C_{p,H_2O}$, was zero at high water content (more than five water molecules per ethylene oxide group). From determinations of the heat capacity of aqueous solutions of PEG 8000[18] over the whole polymer composition range, it was found that the apparent specific heat capacity of the polymer reached a constant value in the dilute region. Recent measurements of enthalpies of mixing of PEG and dextran solutions presented no thermal discontinuity at the transition from one to two phases[19].

Density

The density of mixtures of PEG and dextran in water were found to be only marginally higher than the calculated values from single-polymer solutions (*Table 3*).

GENERAL DISCUSSION

Light scattering and viscosity measurements show that certain structural arrangements occur in the one-phase region of the ternary mixture of water, dextran and PEG. This structure-related behaviour increases as the two-phase area is approached. For the same reason the heat capacity and density, which mainly reflect the properties of the water present in excess of the two other components, showed no deviations when compared with solutions of a single polymer.

Table 2 Heat capacity, C_p, measurements on solutions of PEG 8000 (PEG) and dextran 500 (Dx) in water at 22°C

Concentration of each polymer (% w/w)	C_p (J K^{-1} g^{-1})			
	PEG	Dx	PEG + Dx	PEG + Dx, calc.
3.50	4.141	4.090	4.048	4.050
3.70	4.134	4.087	4.037	4.040

Table 3 Density of polymer solutions at 22.34 ± 0.01°C. The errors of the density measurements were within ±0.0001 g ml^{-1}. Dx = dextran 500, PEG = PEG 8000

Concentration of each polymer (% w/w)	Density (g ml^{-1})			
	PEG	Dx	PEG + Dx	PEG + Dx, calc.
2.50	1.0018	1.0076	1.0117	1.0117
3.00	1.0026	1.0095	1.0148	1.0144
3.50	1.0035	1.0117	1.0177	1.0175
3.70	1.0038	1.0125	1.0188	1.0186

The most probable explanation is that already in the one-phase region preferential interactions exist between the polymers which do not lead to separation into bulk phases. These interactions between polymer segments of the same kind may favour the formation of microstructures rich in either PEG or dextran. These structures can be seen as concentration fluctuations, whose lifetime would be short enough to avoid phase separation. Correlating phenomena have been observed in systems based on two polymers in an organic solvent by dynamic and static light scattering as well as neutron scattering[20]. Borsali *et al.*[21] raised the question of whether this phenomenon is due to formation of aggregates. The existence of microstructures in the one-phase region close to the critical point of water–dextran–PEG systems has also been suggested by Treffry *et al.*[19].

The formation of these structures does not involve changes in volume or solvation, which should be reflected in density and heat capacity, respectively. Thus, no change in the structure of water can be detected. It has been suggested by Zaslavsky *et al.*[22] that phase separation should be due to formation of specifically structured water surrounding each kind of polymer. This assumption is contradicted by our findings. Also, the fact that we observe the same light scattering phenomenon in both *N,N*-dimethylformamide and water strongly indicates that phase separation is not due to any unique and strong structuring effect of the solvent.

ACKNOWLEDGEMENTS

We thank Professor Ingemar Wadsö for valuable discussions and Mrs Eva Qvarnström for assistance with the densitometer. This work was supported by grants from the Technical Research Council of the National Swedish Board for Technical Development (STUF) and by a grant from Junta Nacional de Investigação Cientifica (M.B.).

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