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Phase Behavior of Aqueous Solutions of Bovine Serum Albumin in the Presence of Dextran, at Rest, and under Shear

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The demixing conditions for aqueous solutions of bovine serum albumin (BSA, fraction V) and for joint solutions of BSA plus dextran (DEX, $M_w = 2000$ kg/mol) were determined by turbidimetric measurements as a function of composition, temperature, and shear rate. Aqueous solutions of BSA phase separate upon heating. Within the region of BSA concentrations between 0.05 and 32 wt %, the demixing temperature, T_1, falls from ca. 65 °C to an almost constant value of 45 °C. Adding DEX to the BSA solutions reduces the homogeneous region of the mixture drastically where the amount of DEX required to lower T_1 to 25 °C decreases rapidly as the concentration of BSA is raised. Experiments concerning the influences of shear have been performed for the ternary system up to 500 s^{-1}. They demonstrate that the content of dextran determines the sign of the effect. At low DEX concentrations, the mechanical field favors the homogeneous state (shear-induced mixing), whereas the opposite effect (shear-induced demixing) is observed at high DEX concentrations. Possible reasons for this observation are discussed.

1. Introduction

The interaction between chemically different polymers of linear architecture is in the absence of shear by now studied experimentally in great detail and comparatively well understood theoretically. The same statement does, however, not hold true for combinations of proteins and chain molecules,[1−4] despite several experimental studies[1−6] and despite their practical importance.[5−7] Theoretical considerations predict homogeneity[8] for joint aqueous solutions of globular proteins and polysaccharides, whereas phase separation is observed in many cases.[4,7,9,10] To reach a better understanding of such systems, we have studied mixtures of a small globular protein (bovine serum albumin, BSA) and of a highly flexible chain polysaccharide (dextran, DEX) in aqueous solution. Dextran is able to form interpolymeric complexes with BSA in dilute aqueous solution, if the polysaccharide is in excess and if the protein exists in its associated state at the pH value (5.3)[11] close to the isoionic point of BSA (5.15).[12] Provided the total concentration of biopolymers in the mixture is high enough, the system phase separates,[10] and the stability of the protein molecules with respect to unfolding and thermoaggregation decreases significantly as a result of an increase in the protein hydrophobicity.[11] In the present work, we are particularly dealing with the questions regarding how the addition of DEX to aqueous solutions of BSA changes the demixing conditions and how the phase-separation conditions change as the system flows. Unlike mixtures of low molecular weight compounds, systems containing macromolecules are often very sensitive in their phase state against shear. The first observations of such phenomena date back more than half a century,[13,14] and it is interesting to note that they were made with the solutions of polymer blends in a common solvent. Recent measurements with the system water/gelatin/dextran[15] have demonstrated that the two-phase region shrinks upon the application of shear. No cases of the opposite effect, namely, shear-induced phase separation, have so far been reported for ternary systems of that kind, according to our knowledge. The results of the present study should not only be of theoretical importance, but also of practical importance: BSA solutions and the two-phase systems formed by the addition of dextran are widely used for biomedical and bioseparation purposes.[6,16−18] In many cases, like shear separation,[19] spinneretless spinning,[20] ultrafiltration, and extrusion, the effects of flow on the BSA/BSA interactions (protein fouling during ultrafiltration[20,21]) and on the phase-separation behavior[6,7] are of considerable importance.

2. Experimental Section

2.1. Materials. Bovine serum albumin (BSA), fraction V, pH 5 (lot A018080301), was obtained from Across Organics Chemical Company (New Jersey, U.S.A.); protein content ∼98−99%, trace analysis: Na $<$5000 ppm, Cl $<$3000 ppm, no fatty acids detected. The isoelectric point of the protein is about 4.8−5.0, [22] and the radius of gyration at pH 5.3 equals[22] 30.6 Å. The water used for solution preparation was distilled three times. Most measurements were performed at pH 5.3. The extinction of a 1 wt % solution of BSA at 279 nm was $A_{279}^{1cm} = 6.70$; that value is very close to the tabulated value[23] of 6.67. For the molecular weight of BSA, we use 69 kD (582 amino acid residues) in accordance with the data of amino acid analysis.[24]

The high molecular weight dextran T-2000 (DEX) was purchased from Amershan Pharmacia Biotech AB (Uppsala, Sweden). Its intrinsic viscosity in water at 293 K and the weight average molecular mass, reported by the manufacturer, are 0.9 dL/g and 2×10^6 Da, respectively.

To prepare BSA and DEX stock solutions, the biopolymer was gradually added to the thrice-distilled water and stirred at 298 K for 2 h. The solutions were centrifuged at 13.000 g for 1 h at 298 K to remove insoluble particles. Subsequently, the concentration of the biopolymer was determined by measuring the dry weight residue. In the case of BSA, the content of the protein nitrogen in the dry BSA sample was always taken into account in order to calculate the concentration of protein in solution. In some cases, the final protein concentration was also determined by spectrophotometric measurements. Both the BSA and DEX solutions show Newtonian behavior (at 298 K and at shear rates up to 30 s^{-1}) in the concentration range from 25 to 30 wt %, within which the hydrodynamic volumes of the different macromolecules already overlap.

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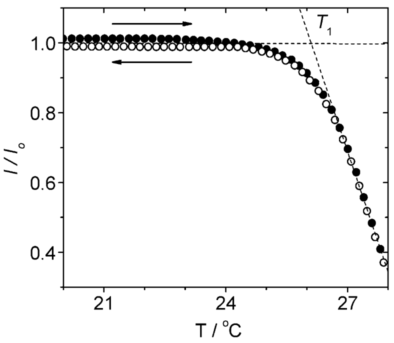

Figure 1. Example (BSA 5.58 wt %; DEX 5.85 wt %) for the determination of cloud point temperatures by means of turbidimetric measurements and for the reversibility of the effects observed within the depicted temperature range. *I* is the light intensity of a laser beam after passing the mixture, and I_o the value for the homogeneous mixture (i.e., at low temperatures). Heating = cooling rate = 0.1 K/min. The value T_1, determined as indicated in the graph, is defined as cloud point temperature

2.2. Procedures. *Turbidimetry at Rest.* Cloud points of aqueous solutions of BSA and of solution of BSA plus DEX were determined by measuring their light transmittance *I* as a function of either temperature or composition. A laser beam (5 mW, He−Ne laser, 632.8 nm) of intensity I_o passes through the solution, and the intensity, *I*, of the transmitted light is determined.

The apparatus and the procedures for the variation of temperature have already been described earlier in detail.[25] The demixing temperatures, T_1, are defined as the intercept of the tangent to the transmittance curves (I/I_o as a function of *T*) in the point of inflection, with $I/I_o = 1$ as shown in Figure 1. Mixtures containing large amounts of BSA are sometimes already somewhat turbid (typically, I/I_o is on the order of 0.9), despite the fact that they are homogeneous. T_1 was in these cases determined from the intersection of the tangent with the transparency value of the homogeneous system. In the present experiments, the samples were typically heated from 18 to 60 °C at a rate of 0.1 °C/min.

Isothermal measurements were performed by titrating solutions of BSA with solutions of DEX. Again, apparatus and procedures have already been described.[26] In this case, one determines the phase-separation conditions from plots of the transmittance as a function of the amount the DEX solution added. Characteristic demixing compositions were determined by analogy to T_1.

Turbidimetry Under Shear. Phase separation of the flowing polymer solutions was measured by means of a rheo-optical device described earlier.[27] The sample is subjected to shear in an annular gap of a concentric cylinder assembly made of optical glass. The radii of the inner and outer cylinders were 20.04 and 21.0 mm, respectively. The inner cylinder is driven by a motor whose speed can be regulated with constancy of ±1%. The entire assembly is immersed in a thermostat bath. For the photoelectric determination of the cloud points of the solution, the beam of He−Ne laser/1 mW) is split into two parts, one passing through both the cylinders perpendicular to the axis of rotation and the other providing the reference beam.

Most of the cloud points were determined in the usual manner by starting from homogeneous mixtures and varying either the temperature or the shear rate. In the latter case, one obtains a characteristic shear rate at which the transition from homogeneous to the coexistence of two phases takes place for a given temperature instead of T_1. In some cases, we have also determined the turbidity of mixtures, which are two phase at rest, as a function of $\dot{\gamma}$ and time. To establish reproducible starting points, the liquids were presheared before starting the measurements. The observation of the morphologies of the these mixtures under the microscope as a function of shear rate and time by means of the optical shear cell CSS 450 from Linkam Scientific, U.K., has demonstrated that a treatment at 1 s^{-1} for 8 min suffices to yield a reproducible basis.

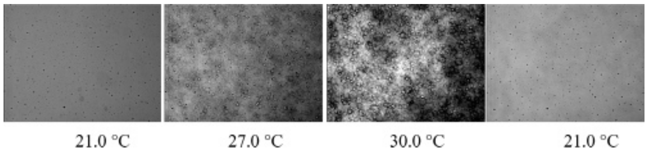

Figure 2. Micrographs taken during the heating and cooling experiments shown in Figure 1. The full length of each image corresponds to 640 μm.

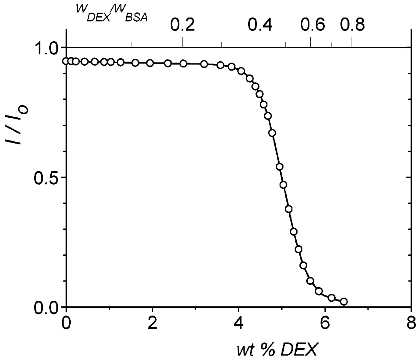

Figure 3. Example for the titrimetric determination of isothermal cloud points. Here, an aqueous solution of BSA (17.05 wt %) was titrated with an aqueous solution of DEX (12.89 wt %) at 298 K. The upper scale gives the ratio of the two polymers.

Microscopy. The morphology was investigated by means of the optical shear cell CSS 450 from Linkam Scientific, U.K. It consists of a plate/plate geometry made of glass. The cell is inserted into an optical microscope (BX50, Olympus, Japan). The microscope is equipped with a B/W CCD-camera M10 from JAI, Denmark.

3. Results and Discussion

3.1. Equilibrium Behavior. The change in the light transmittance of the solutions upon heating or cooling takes place reversibly if one does not penetrate too deeply into the two-phase area for too long a time. It is difficult to make a general statement regarding what “too deep” and “too long” mean, because these effects depend on the particular mixture and on the applied shear rates. Figure 1 presents a typical example of heating and cooling curves performed to determine T_1, the onset temperature of phase separation. In this case, the penetration was approximately 2°. A similar picture for a sheared solution is shown in Figure 12. With increasing time that the mixture spends inside the two-phase regime and with increasing distance from the cloud point, the extent of hysteresis rises, and sometimes, the homogeneous condition can no longer be reached within a reasonable time after return to the initial conditions.

An additional proof of reversibility of the present phase separation experiments is given in terms of micrographs of the mixtures taken at different temperatures (Figure 2). It is interesting to note that typical structures on the order of 10−15 μm are formed upon heating and disappear again upon cooling.

How the transmittance changes during titration experiments is exemplified in Figure 3. Instead of the quantity (milliliters) of the binary mixture H_2O/DEX that was added to the solution of BSA, initially free of DEX, this graph directly displays the DEX content of the ternary mixture. The composition at incipient phase separation is determined by analogy to the procedure demonstrated in Figure 1 by means of the tangent in the point of inflection.

The results of the titration experiments performed at 25 °C are displayed in Figure 4. For this representation, we have used

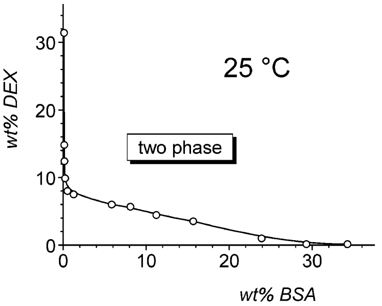

Figure 4. Cloud point curve of the system water/BSA/DEX at 298 K and at pH = 5.35.

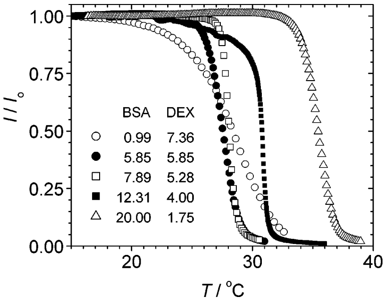

Figure 5. Light transmittance of aqueous solutions of BSA and DEX as a function of temperature measured at a heating rate of 0.1 s^{-1}. The concentrations (wt %) of each biopolymer in the ternary system are indicated in the graph.

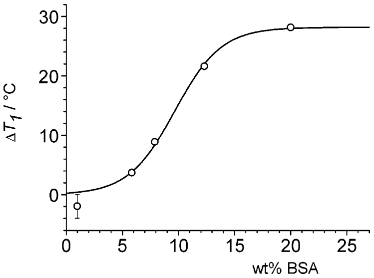

Figure 6. Augmentation of the demixing temperature caused by the *reduction* of the DEX concentration in the ternary mixture by 1 wt %. Along the curve, w_{DEX}/w_{BSA} rises from 0 to 6 (last data point).

Cartesian coordinates because of the small area the homogeneous region would occupy in the Gibbs phase triangle in the immediate vicinity of the water corner.

For arbitrarily selected BSA concentrations, the corresponding cloud point concentrations of DEX at 25 °C can be read from Figure 4. On the basis of this information, joint aqueous solutions of BSA and DEX were prepared such that the DEX concentration remains 1 wt % below the value required for incipient phase separation. These homogeneous mixtures were then heated until they segregate to form a second phase. In this manner, one can obtain information on the efficiency of DEX for changing the demixing conditions at different BSA concentrations. The corresponding cloud point measurements are depicted in Figure 5.

The elevation of T_1 (extension of the homogeneous region) caused by lowering the weight fraction of DEX in the mixture by 1 wt % (cf. Figure 6) is obtained by subtracting 25 °C from the demixing temperatures resulting from the curves of Figure 5. The sigmoidal dependence shown in this graph indicates that the sensitivity is largest at approximately 11 wt % BSA; below and above this value, the effect levels off. In the case of 20 wt % BSA, only approximately 0.05 wt % DEX suffices to reduce the demixing temperature by 1 °C, whereas about 0.2−0.3 wt % DEX is required to yield the same effect for ca. 6 wt % BSA.

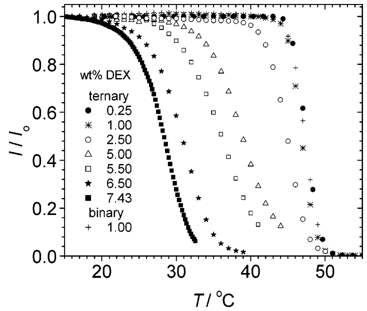

Figure 7. Light transmittance of joint solutions of BSA (always 1 wt %) and of DEX as a function of temperature for a heating rate of 0.1 °C/min. For comparison, we also show the curve for the binary solution containing 1 wt % DEX.

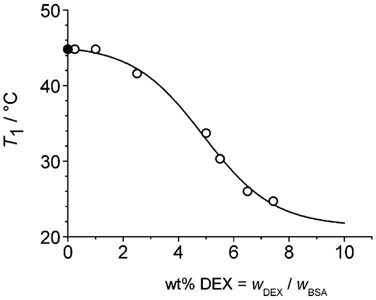

Figure 8. Demixing temperatures of 1 wt % aqueous solutions of BSA as a function of the concentration of DEX; the data point for the binary subsystem H_2O/BSA is shown as a full circle.

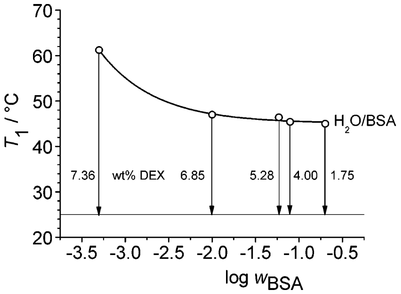

Figure 9. Cloud point temperature of the binary system water/BSA as a function of the logarithm of the weight fraction of BSA. The numbers listed at the arrows indicate how much DEX is required (as read from Figure 4) to lower the cloud point of the ternary system to 25 °C.

In the next set of turbidity measurements, we maintain the concentration of BSA at 1 wt % and increase the concentration of DEX systematically to see how the presence of the second polymer changes the phase separation temperature. From the primary data shown in Figure 7, it becomes immediately obvious that T_1 is lowered markedly (i.e., that the two-phase region increases).

As shown in Figure 8, the T_1 values determined from Figure 7 as a function of the DEX content fall on a sigmoidal dependence again; this time the point of inflection lies at approximately 4 wt % DEX; at this concentration, the sensitivity of a 1 wt % solution of BSA against the addition of DEX is largest.

According to Figure 8, the cloud point temperature of the ternary system extrapolates well to that of the binary subsystem water/BSA ($w_{BSA} = 0.01$). We use this feature in the following to gain additional information on the effect of DEX on the demixing temperature of aqueous BSA solutions. To this end, we plot in Figure 9 the T_1 values obtained for the binary subsystem H_2O/BSA as a function of the BSA concentration

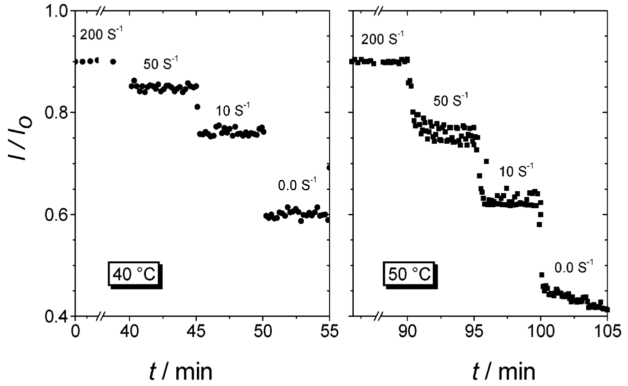

Figure 10. Isothermal light transmittance (34 wt % BSA, 0.12 wt % DEX) as a function of time at the indicated shear rates; for the homogeneous mixture $I/I_o = 0.9$. The stagnant solution demixes at 42 °C.

on a logarithmic scale. To reduce these T_1 values to 25 °C (cf. Figure 4), one requires the weight fractions of DEX indicated in this graph next to the arrows. This result demonstrates that the required amount decreases rapidly as the concentration of BSA rises.

3.2. Cloud Points of the Sheared Solutions. Shear can either lead to a homogenization of mixtures that are two-phase at rest (shear-induced mixing: SIM) or induce phase separation of homogeneous mixtures (shear-induced demixing: SID) depending on the particular system and the special experimental parameters. We applied two different experimental procedures to determine the onset of phase separation by means of turbidity measurements and kept either the temperature or the shear rate constant. With the *isothermal measurements*, the shear rate was altered stepwise such that the solutions become increasingly cloudy. To guarantee that steady states are reached in each case, $\dot{\gamma}$ is only changed as soon as the light transmittance no longer depends on time. In the case of SIM, we have started the measurements with mixtures that are at a given temperature only homogeneous because they are sheared at a sufficiently high rate, in contrast to the two-phase situation in the quiescent state. This homogeneity can either be reached by heating the solutions at a sufficiently high constant shear rate from a temperature below the phase-separation temperature of the system at rest to higher T values (e.g., Figure 10) or—if this is feasible—to start from an already phase-separated mixture and rise the shear rate until it becomes homogeneous and totally transparent (e.g., Figure 11). Alternatively, one can perform temperature sweeps at constant $\dot{\gamma}$ and a sufficiently low rate to determine the phase-separation temperatures of sheared solutions (e.g., Figure 12 for SIM or Figures 13 and 14 for SID). In the following two sections, we describe the different influences of shear rate on the demixing conditions. These findings will be compared and qualitatively interpreted in a separate section.

3.2.1. Shear-Induced Mixing. The phenomenon of shear-induced mixing was exclusively observed with ternary mixtures containing only a low fraction of DEX; Figure 10 shows some results of isothermal measurements. With these experiments, the homogeneous state at temperatures above the demixing temperature of the stagnant solution was reached by heating the sheared solution to the desired T value. Upon a reduction of $\dot{\gamma}$, phase separation sets in and the light transmittance falls. Steady states are normally reached very rapidly, as can be seen from this graph. To obtain the shear rate at which the solution demixes at constant temperature, one plots the transmittance as a function of $\dot{\gamma}$ and determines the intersect of the tangent in the point of inflection with the line $I/I_o = 1$.

An example for isothermal turbidity measurements starting from two-phase mixtures is shown in Figure 11. This graph demonstrates that homogeneous steady states are attainable within the temperature range of investigation, even if the solutions are initially macroscopically phase-separated. Shear rates of demixing are again determined from the measured transmittance as a function of $\dot{\gamma}$ as described above.

According to Figures 10 and 11, the light transmittance after a change in shear rate reaches a new steady-state value very rapidly, typically within less than a minute. To obtain some kinetic information, we have heated a homogeneous solution into the two-phase region and subsequently cooled it down again at a rate of 0.5 °C/min; the results are shown in Figure 12. That graph documents that the system reacts comparatively slowly under these conditions. This observation is not surprising, however, in view of the considerably longer time required for heat transfer as compared with that needed for the reorientation of polymer chains after a change in $\dot{\gamma}$. A similar experiment with comparable results has also been performed with a mixture containing 32.5 wt % BSA and 0.05 wt % DEX. In both cases, one observes a hysteresis in the curves I/I_o as a function of T, and the solutions become completely transparent again at the end of the temperature cycles.

3.2.2. Shear-Induced Demixing. According to the present findings, an expansion of the two-phase region is bound to the presence of comparable amounts of the two types of macromolecules. In other words, it is a changed proportion of the polymer components that causes the opposite effect of shear as compared with the situation described in the previous section. The experiments concerning SID were exclusively carried out by heating homogeneous mixture at constant shear rate until they become turbid. The graphs in Figures 13 and 14 show results of such measurements.

The curves of Figures 13 and 14 become increasingly flatter as the shear rate rises. This indicates that the transition from the homogeneous to the two-phase state takes place more gradually. According to a simple model, each value of the transmittance corresponds to an average steady-state size of droplets of the minor phase suspended in the matrix of the major phase. In this picture, homogeneity means that the “droplet radii” are on the order of the radii of gyration of the individual polymer chains;[28] at the demixing conditions monitored by T_1, the aggregation of the polymer molecules (preceding the formation of a second phase) surpasses a characteristic value. Within the frame of this consideration, the slower transition from homogeneity to the two-phase state at higher shear rates means that the temperature range within which the polymer aggregates become large enough to result in a certain turbidity increases as a result of the growing importance of the “droplet breakup” processes.

3.2.3. Comparison of Shear Effects. For binary systems that contain polymers, it is comparatively easy to understand the influences of shear on their demixing behavior.[28,29] All experimental observations can be rationalized in terms of a generalized Gibbs energy of mixing, defined as the usual Gibbs energy of mixing plus the mechanical (recoverable) energy the system stores during flow. The reason for the changes in phase separation lies in the fact that the stored energy, E_s, normally depends markedly on composition. For polymer solutions and low shear rates, it increases more than exponentially with polymer concentration via the increasing number of chain entanglements, which are the reason of energy storage. This feature leads to SIM, because the hump (more precisely, the points of inflection) in the Gibbs energy as a function of

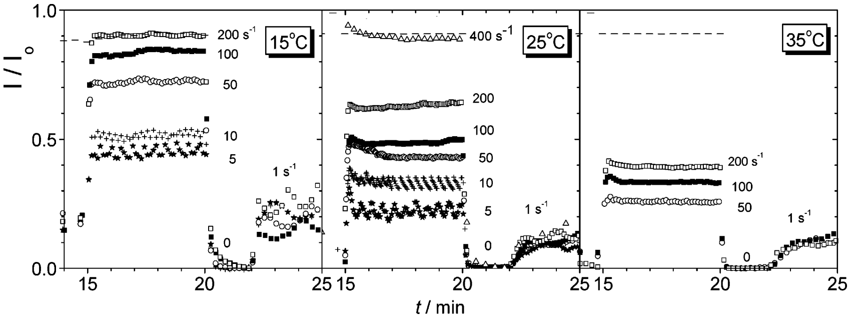

Figure 11. Isothermal turbidity measurements as a function of time at different $\dot{\gamma}$ for a ternary mixture (33.7 wt % BSA; 0.17 wt % DEX), which is phase-separated in the absence of shear; the stagnant solution demixes at 5 °C. The light transmittance of the corresponding binary subsystem (33.7 wt % BSA; no DEX) and of the homogeneous ternary mixture is indicated by a dotted line.

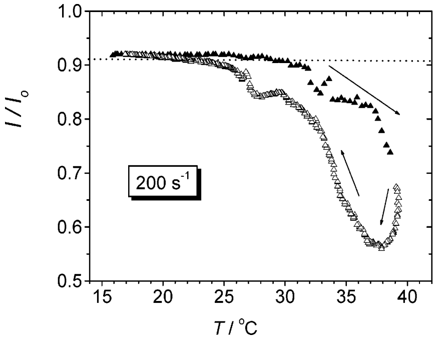

Figure 12. Light transmittance of a ternary mixture (33.7 wt % BSA; 0.18 wt % DEX) at $\dot{\gamma}$ = 200 s^{-1} as a function of *T*, changing the temperature at a rate of 0.5 °C/min.

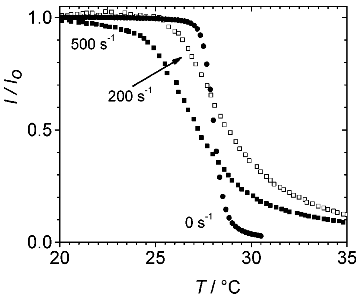

Figure 13. Light transmittance of a ternary mixture (7.89% BSA; 5.28% DEX) as a function of temperature for a heating rate of 0.1 °C/min and the different shear rates indicated in the graph.

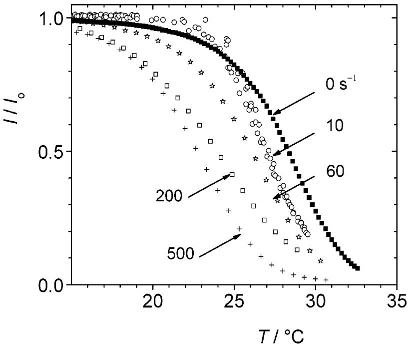

Figure 14. Same as Figure 13, but for 0.99% BSA + 7.36% DEX.

composition occurring for the stagnant solution and causing its phase separation is straightened out by the addition of the stored energy.

With polymer solutions and increasing shear rates, the situation may change due to disentanglement processes causing the phenomenon of shear thinning. Because the disentanglement effects become increasingly important as the viscosity of the solutions rises, E_s may decrease as the polymer concentration becomes larger. In this manner, it is possible to produce—out of a smooth composition dependence of the Gibbs energy (characteristic for homogeneous mixing)—the hump in the generalized Gibbs energy required for the phase separation of the flowing system. From what was stated above, it is obvious that the molecular reasons for the occurrence of SIM and SID lie in the possibility of temporary energy storage in flowing polymer solutions via chain entanglements. The basis of these phenomena lies in modified chain statistics, i.e., changes in the entropy of mixing. Generally speaking, there are, however, two principally different modes to store free energy, namely, the already mentioned entropy and an enthalpic possibility. According to experiments concerning shear influences on the phase separation of polymer blends, the first experimentally seizable effect consists of SID. This finding is unconceivable in terms of entropy effects but can be rationalized by assuming a destruction of a quasi-chemical equilibrium established in the quiescent state by shear.[30] In light of this approach, the equilibrium mixture is forceably randomized by the mechanical field such that energetically very unfavorable contacts between the two different types of monomeric units are formed. The energy required for this molecular homogenization is recovered as the system comes to a rest again.

The considerations presented above enable a rationalization of the effects observed with binary systems. Corresponding calculations describe the situation not only qualitatively but also quantitatively, if the thermodynamic information concerning the stagnant system and the rheological data concerning the energy stored in the flowing state are precise enough.[29,31−33] For the present ternary mixtures, this requirement is fulfilled neither for the thermodynamic nor for the rheological part for the time being. This is the reason the experimental observations shown in Figure 15 are discussed in a qualitative manner only.

For ternary systems of the present type, the stored energy depends on two kinds of polymers, and instead of the hump, we obtain a fold in the plain of the generalized Gibbs energy as a function of composition. Despite this intricate state of affairs, we are in the following speculating on the reasons for the present experimental observations. Now as before, the demixing of flowing mixtures takes place in such a manner that the generalized Gibbs energy of the entire system becomes minimum, but its calculation becomes much more complicated. Furthermore, we must keep in mind that the effects of chain entanglements (free energy storage via entropy) should only arise for DEX but not for BSA and that quasi-chemical equilibria concerning the contacts between the two types of macromolecules (free energy storage via enthalpy) may also play an important role.

According to the present results, the occurrence of SIM is bound to small amounts of DEX (0.1−0.2 wt % corresponding

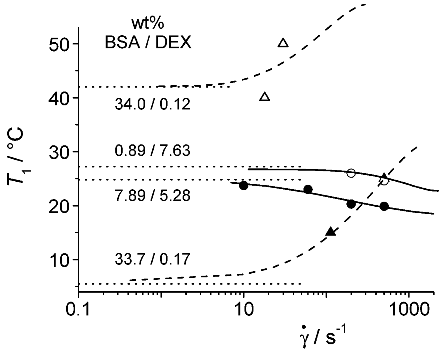

Figure 15. Cloud point temperatures as a function of shear rate. The T_1 values for the stagnant solutions are indicated by the dotted lines. At low DEX concentrations, one observed SIM (broken lines), but SID (full lines) at high DEX concentrations. The curves are only guides for the eye.

to concentration c of roughly 1−2 10^{-3} g/mL). These concentrations correspond to a coil overlap parameter, $\tilde{c} = c[\eta]$, on the order of 0.2. For a binary system that exhibits liquid/liquid demixing, this would not be large enough to store sufficient energy while flowing (the entanglement density does not suffice). However, with the present system, the situation may well be different due to the pronounced incompatibility of the components, which will probably lead to a kind of microphase separation. Due to the establishment of a quasi-chemical equilibrium, such mixtures can be looked at as dispersions of volume elements with high BSA and almost negligible DEX concentration (“drops” that have not yet developed a macroscopic interface) in a matrix of high DEX and almost negligible BSA concentration. From this consideration, it is obvious that the degree of coil overlap will be much larger within the continuous phase than that calculated for a homogeneous distribution of the components over the entire volume. For the reasons outlined above, the normal argumentation explaining SIM via a more than linear increase of the stored energy with rising concentration of linear macromolecules will probably remain valid.

The phenomenon of SID, on the other hand, requires large DEX concentrations, e.g., 8 wt % according to the present findings, which correspond to $\tilde{c} \approx 6$. Such solutions are practically free of volume elements, which contain almost pure solvent, i.e., can take up BSA molecules without considerable thermodynamic cost. In other words, the option to avoid adverse contacts between the two types of macromolecules by the formation of larger “drops” containing almost exclusively BSA no longer exists. This forces a locally much more homogeneous mixture, even if BSA/BSA and DEX/DEX contacts are highly favored over BSA/DEX contacts in the stagnant state. The scenario just outlined is to a large extent identical with that encountered with homopolymer/copolymer mixtures[30] for which SID was also observed. The molecular explanation proposed in the context of polymer blends should also hold true for the present ternary system: To avoid unfavorable contacts between the different kinds of polymers, the stagnant system establishes a quasi-chemical equilibrium to minimize its Gibbs energy. The special arrangements formed in this manner can be broken up at sufficiently large shear rates, and the mixture will forceably be randomized. Under this condition, energy is stored via additional adverse contacts between BSA and DEX molecules formed in this manner. Because of the special composition dependence of this stored energy, a homogeneous mixture may phase separate as it flows, i.e., it exhibits the phenomenon of SID.

It would of course be interesting to interpret the observed phenomena also in terms of the changes in the hydrophobic interactions and in the state of association of BSA that are induced by a shear field. Some effects of shear on these properties have already been studied, like the suppression of the aggregation of weakly associating proteins[34] or the reversible formation of aggregates due to hydrophobic interactions.[35]

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