SHORT COMMUNICATIONS

Further Evidence for the Multiphase Separation in Aqueous Solutions of Schizophyllan, a Triple-Helical Polysaccharide

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Thermodynamically it is probable that a ternary system separates into two or more phases under appropriate external conditions. Abe and Flory¹ were the first to show that this would occur in solutions of rodlike polymer bidisperse in molecular weight provided that the molecular weight ratio L_2/L_1 of the two component polymers is larger than about 5. According to this theory,^{1,2} a triangular phase diagram for a large L_2/L_1 is divided into five distinct regions, where the system is isotropic (region I), anisotropic (region A), or separates into an isotropic phase I and an anisotropic phase A (region IA), into an isotropic phase I_0 and two anisotropic phases A_1 and A_2 (region IAA), or into two anisotropic phases A_1 and A_2 (region AA).

We have examined experimentally phase behavior of schizophyllan, a triple helical polysaccharide, and found that aqueous solutions of this polysaccharide form a cholesteric liquid crystal above some critical concentration.²⁻⁶ The results for bidisperse polymer solutes are summarized briefly as follows.^{2,3} For $L_2/L_1 = 12.2$, the phase diagram contains all the five regions mentioned above. The region AA has been confirmed to be relatively wide but has not been determined accurately.

In contrast, the regions IAA and AA are missing in the diagram for $L_2/L_1 = 6.06$. Consequently all these results have been taken to be the first experimental evidence for the Abe-Flory prediction. Aside from the quantitative validity of the theoretical prediction, we have not yet elucidated the critical L_2/L_1 value below which the triphase separation does not occur nor determined the spread of the AA region.

The present study was undertaken to supplement the previous data with these respects using two samples with relatively narrow molecular weight distributions: sample J-22 ($\bar{M}_w = 111 \times 10^3$, $[\eta] = 45.8 \text{ cm}^3 \text{ g}^{-1}$), $\bar{M}_z/\bar{M}_w = 1.3$ and T-3 ($\bar{M}_w = 800 \times 10^3$, $[\eta] = 894 \text{ cm}^3 \text{ g}^{-1}$); $[\eta]$ stands for the intrinsic viscosity in water at 25°C.

EXPERIMENTAL

Following the procedure established before,^{2,3} a given mixture consisting of appropriate amounts of the two samples dissolved in water was separated macroscopically into the coexisting phases at 25°C and each separated phase was analyzed for the weight fraction w and $[\eta]$ of the polymer. The results

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Table I. Phase separation data for aqueous solutions of bidisperse schizophyllan at 25°C

Original mixture			Relative volumes of the coexisting phases		
	ξ	w	V_{I_0}	V_{A_2}	V_{A_1}
1 ^a	0.878	0.298	—	0.831	0.169
2 ^a	0.898	0.285	0.005	0.792	0.157
3	0.898	0.271	—	—	—
4 ^a	0.898	0.262	0.313	0.559	0.129
5 ^b	0.851	0.337	—	1.0	0
6 ^b	0.851	0.330	—	1.0	0
7 ^a	0.851	0.307	—	0.346	0.164
8	0.850	0.278	0.205	0.604	0.191
9	0.850	0.268	0.313	0.487	0.200
10	0.811	0.320	—	0.727	0.273
11	0.811	0.289	0.179	0.440	0.381
12	0.752	0.325	—	0.529	0.491

^a The volumes of phases A_1 and A_2 could not be determined accurately because a magnetic bar appeared at the interface between them.

^b The volume of phase A_1 was too small to be measured.

were used to evaluate the composition of each phase expressed in terms of w and the weight fraction ξ of J-22 in the polymer mixture.

RESULTS AND DISCUSSION

A total of twelve mixtures were investigated as listed in Table I, where the relative volumes V_{I_0} , V_{A_2} , and V_{A_1} of the coexisting phases are also included. Among them, mixtures 2, 3, 4, 8, 9, and 11 showed the triphase separation, whereas the rest showed only the anisotropic-anisotropic phase separation.

In all the cases examined, $[\eta]$ of the polymer recovered from the isotropic phase was smaller than that of J-22, though at most 18%, and ξ was taken to be unity for such cases. This is due to the molecular weight fractionation occurring on the phase separation. Table II summarizes all the numerical results obtained from the composition analyses. The accuracy of ξ is only moderate because of the fractionation effect mentioned above. It can be seen

Table II. Compositions of the coexisting phases at 25°C for aqueous schizophyllan

Mixture		I_0	A_2	A_1
4	w	0.231	0.291	0.319
	$[\eta]/\text{cm}^3 \text{g}^{-1}$	40.4	133	322
7	ξ	1.0 ^a	0.897	0.678
	w	—	0.308	0.323
9	$[\eta]/\text{cm}^3 \text{g}^{-1}$	—	125	326
	ξ	—	0.907	0.670
11	w	0.236	0.293	0.303
	$[\eta]/\text{cm}^3 \text{g}^{-1}$	37.6	200	276
12	ξ	1.0 ^a	0.818	0.729
	w	0.254 ^b	0.292	0.322
12	$[\eta]/\text{cm}^3 \text{g}^{-1}$	39	148	307
	ξ	1.0 ^b	0.880	0.692
12	w	0	0.316	0.346
	$[\eta]/\text{cm}^3 \text{g}^{-1}$	—	131	378
12	ξ	—	0.900	0.608

^a Only approximate because of fractionation effects (see the text).

^b Less reliable because of the limited amounts of the sample solutions available.

Table III. Composition of the three coexisting phases in the system J-22 + T-3 + water at 25°C.

	I_0	A_2	A_1
w	0.234	0.292	0.32 ₀
ξ	1.0 ^a	0.89	0.683

^a Assumed value (see the text).

in Table II that w and ξ of each phase are essentially the same among the three mixtures exhibiting the three phase separation, indicating that there is a single set of compositions for this equilibrium. However they do not agree precisely with one another. This is because of the experimental difficulty in determining the composition and also because of the fractionation effect mentioned above. Therefore, for the reason given below, we have taken the averages of the two closer sets of data among the three as the compositions of the coexisting phases and listed in Table III.

Figure 1 shows the triangular phase diagram constructed from these data; the axial ratio x was calculated from $x = \bar{M}_w/(M_L d)$, with the molecular weight per unit contour length M_L

Figure 1. Ternary phase diagram for the system J-22 + T-3 + water at 25°C. ●, conjugate phases for the corresponding binary systems; ○, mixtures exhibiting the triphase separation; ◐, ◑, ◒, solutions from the triphase separation; □, mixtures exhibiting the anisotropic-anisotropic phase separation; ◐, ◑, conjugate phases for this phase separation; $I_0A_1A_2$, region of the triphase separation.

and the diameter d , being 2150 nm^{-1} and 1.67 nm , respectively.^{7,8} Here the filled circles on the water-J-22 axis represent the phase boundary weight fractions for this binary system, whereas those on the water-T-3 axis represent the phase boundaries of aqueous T-3 previously determined. In this figure, open circles represent the compositions of the systems exhibiting the triphase separation, whereas half-filled circles refer to those of the separated phases. The solid triangle $I_0A_1A_2$

defines the region of the triphase separation based on the average compositions given in Table III. This determination of the triphase region may be justified by the fact that all the open circles exhibiting the triphase separation are now found in the triangle. Below the triphase region is seen the region for the anisotropic-anisotropic phase separation. Open and half-filled squares represent the corresponding compositions for this phase separation; those solutions separated from such original

Figure 2. Ternary phase diagram for the system U-110 + T-3 + water at 25°C reproduced from ref 2; $\bar{M}_w = 6.58 \times 10^4$ for sample U-110. Δ , solutions from the isotropic-anisotropic phase separation; the other symbols are the same as those in Figure 1.

mixtures are connected by the tie lines. Because of the limited amounts of the samples available, no attempt was made to determine the region for the isotropic-anisotropic phase separation.

Figure 2 shows the phase diagram of aqueous schizophyllan reproduced from the previous paper,² with the molecular weight ratio L_2/L_1 being 12.2 instead of 7.2 in the present case. Comparison of this figure with Figure 1 shows that although both polymer combinations exhibit the triphase and anisotropic-anisotropic phase separations, their regions are considerably narrower in the present combination than in the previous one. Thus it is expected that no triphase separation may be observed below a critical L_2/L_1 value between 7.2 and 6.06 because it has not been observed at $L_2/L_1 = 6.06$. Another feature of

the present phase diagram in Figure 1 is that the phase A_2 is shifted significantly toward the water-T-3 axis.

All these results manifest themselves the qualitative features of the Abe-Flory prediction. As discussed before with the previous data,^{2,3} however, the agreement between experiment and theory remained only qualitative, and there was a large quantitative difference between them. It can be shown that the same situation also holds for the present data. An important reason for this disagreement, among others, is the finite stiffness of the schizophyllan triple helix, which makes it difficult to approximate the helix by a straight cylinder; the triple helix is well described by a wormlike cylinder of a persistence length *ca.* 200 nm.^{7,8} Quite recently, Odijk⁹ has extended the theory of Khokhlov and Semenov¹⁰ of

binary solutions of wormlike cylinders to ternary systems containing a bidisperse polymer solute and indicated that his theory can interpret almost quantitatively our previous data for schizophyllan. All the schizophyllan data combined with similar ones obtained for poly (hexyl isocyanate) will be analyzed in more detail by the Odijk theory in a forthcoming publication.¹¹

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