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Polydispersity Effects in the System Poly(ethylene glycol) + Dextran + Water

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Tie lines in the system poly(ethylene glycol) (PEG) + dextran + water have been measured at 20 °C. The apparent polydispersity of PEG was varied by mixing two essentially monodisperse PEG fractions in different ratios. The overall phase composition for a given tie line was changed to obtain phase ratios different from unity. The concentrations and molecular-weight distributions of all polymers in the coexisting liquid phases were determined using size-exclusion chromatography (SEC). The results are compared to those of systems containing monodisperse PEG with different molecular weights.

Introduction

Aqueous polymer–polymer two-phase systems become increasingly attractive for the separation of complex mixtures of biomolecules (*1*). To design an industrial separation process, theoretical models for the prediction of the phase behavior are necessary. The development of such models requires accurate and reliable thermodynamic information on the phase compositions in the coexisting phases and on the polydispersity effects in the system. The polydispersity of the phase-forming polymer has a strong influence on some thermodynamic properties, especially the liquid–liquid equilibrium (*2*). The polymers in the coexisting phases have different molecular-weight distributions. Also the phase ratio for a given tie line has an influence on the coexistence curve. To describe these effects in modeling, the molecular-weight distribution has to be taken into account (*3*). For dextran the molecular-weight distributions in the top and bottom phases have been explored in a previous paper (*4*). In this study the polydisperse effects of PEG are investigated. Since the commercially available PEG is essentially monodisperse, the polydispersity was simulated by mixing two PEG's with different molecular weights.

Experimental Section

Materials. The components were used in the highest purity commercially available without further purification. PEG 600, 1550, and 3000 were supplied by the Hüls AG, Marl. Dextran 500 000 was purchased from Pfeifer & Langen, Dormagen. The water was triple distilled. The number- and mass-average molecular weights of the used PEG's, determined by size-exclusion chromatography, are presented in Table I. The dextran molecular-weight standards were supplied by Pharmacosmos, Denmark, the PEG standards by Polymer Laboratories, U.K.

Analytical Methods. The PEG and dextran concentrations in each phase were measured by size-exclusion chromatography (SEC). The chromatographic equipment consists of a degasser (ERC-3312), a HPLC pump (ERC-64), a pulsation damper, a rotary valve (Rheodyne 7125), three 30-cm SEC columns in series (TSK G6000PWXL, PSS HEMA 1000, TSK G3000PWXL) and a refractive-index detector (ERC-7512). The columns and the rotary valve with sample loop were placed in a column thermostat (Spark Holland 99). The refractive-index detector and the columns were thermostated at 35 °C. The eluent was distilled water, purified with a Millipore Milli-Q-System, to which 200 ppm sodium azide was added (*5*). The pumping rate of the HPLC pump was 1 cm^3/min. The calibration method was the same as that applied by Connemann et al. (*4*).

Table I. Average Molecular Weights of Stock Solutions[a]

	lot no.	$M_n/(g\ mol^{-1})$	$M_w/(g\ mol^{-1})$
PEG 600	P.2	585	600
PEG 1550	P.8	1 464	1 485
PEG 3000	P.2	2 810	3 026
PEG 600/3000 (M_n)		1 480	2 365
PEG 600/3000 (M_w)		862	1 560
dextran PL 500 VC	500 86 22	109 292	493 109

[a] M_n = number-average molecular weight; M_w = mass-average molecular weight.

Phase Systems. Six aqueous two-phase systems containing various PEG fractions, dextran 500 000, and water have been investigated. The generation and separation of the phase systems follow the same procedure as that described in ref 4. For identification the systems are marked with letters from A to G (Tables II and III).

Table II presents the tie-line data and average molecular weights of the systems A and B containing the PEG fractions 600 and 1550, respectively. Additionally a third ternary system prepared with PEG 3000 (system C) is included. The data for this system have been reported previously (*4*).

To explore the polydispersity effects of PEG, phase systems were generated using PEG stock solutions mixed from PEG 600 and PEG 3000. These two PEG fractions were chosen because they can be completely separated with SEC. The mixtures of PEG fractions can be adjusted to have a number-average or mass-average molecular weight close to that of PEG 1550. This could be achieved by mixing PEG 600 and PEG 3000 in the ratio of $w_{PEG\ 600}/w_{PEG\ 3000} = 0.348$ to obtain the polydisperse PEG with the number-average molecular weight close to 1550 g mol^{-1} and $w_{PEG\ 600}/w_{PEG\ 3000} = 1.525$ for that with the mass-average molecular weight close to 1550 g mol^{-1}. The exact molecular weights of the corresponding stock solutions are also given in Table I. The stock solution with an M_n close to that of PEG 1550 (index M_n) was used to prepare systems D, F, and G, the one with M_w similar to that of PEG 1550 (index M_w) for system E.

The systems D and E were generated with phase ratios near unity. For the systems F and G the overall composition was chosen close to the dextran-rich and PEG-rich ends of the tie lines, respectively. This experiments should reveal the effect of the phase ratio on the phase compositions and the molecular-weight distributions of both polymers for a

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Table II. Determination of the Tie Lines for the Ternary Systems PEG + Dextran + Water and Average Molecular Weights of Dextran

	composition/(mass %)							dextran average molecular weight/(g mol^{-1})			
	total		top phase		bottom phase		tie-line	top phase		bottom phase	
index	PEG	dextran	PEG	dextran	PEG	dextran	length/(g/g)	M_n	M_w	M_n	M_w
System A: PEG 600 + Dextran 500 000 + Water											
1	14.84	10.73	19.09	3.34	13.32	15.10	10.19	67 000	119 748	113 744	514 158
2	16.84	11.83	22.55	1.04	11.06	23.04	19.06	45 665	71 326	107 073	451 683
3	17.37	12.48	23.84	0.75	10.29	26.22	22.07	38 330	68 395	101 464	447 542
4	18.79	13.53	26.06	0.30	8.62	31.67	27.22	31 698	57 808	102 588	448 402
5	19.66	14.71	27.64	0.06	8.06	34.85	30.21	30 385	34 539	100 114	430 362
6	20.29	15.54	29.21	0.07	7.75	37.45	32.49	22 352	30 570	100 118	446 267
System B: PEG 1550 + Dextran 500 000 + Water											
7	9.05	8.08	11.15	3.18	7.28	11.93	7.59	77 794	138 494	129 136	486 174
8	9.06	8.49	11.69	2.64	6.94	13.29	9.24	70 185	115 761	118 567	462 465
9	9.58	10.09	13.86	0.88	5.20	19.34	16.00	50 536	70 517	119 028	441 921
10	9.85	11.80	15.47	0.41	4.25	23.23	19.76	37 870	49 985	101 766	393 164
11	8.95	14.95	16.19	0.31	3.80	25.10	21.47	34 212	42 852	106 709	399 612
12	11.08	13.99	18.16	0.14	3.07	29.48	25.41	27 860	34 021	105 530	400 696

Table III. Determination of the Tie Lines for the Quarternary Systems PEG 600/3000 + Dextran 500 000 + Water and Average Molecular Weights of PEG and Dextran

	composition/(mass %)										average molecular weight/(g mol^{-1})							
											top phase				bottom phase			
	total			top phase			bottom phase			tie-line length/	PEG		dextran		PEG		dextran	
index	PEG 600	PEG 3000	dextran	PEG 600	PEG 3000	dextran	PEG 600	PEG 3000	dextran	(g/g)	M_n	M_w	M_n	M_w	M_n	M_w	M_n	M_w
System D with PEG 600/3000 (M_n)																		
13	2.03	6.75	6.31	8.85	2.37	1.01	2.61	1.61	16.06	13.04	1549	2411	59 841	115 909	1090	1955	123 630	511 782
14	2.20	7.32	7.38	10.28	2.72	0.58	1.91	1.55	19.77	16.62	1369	2372	50 205	134 610	937	1775	115 613	471 896
15	2.36	7.85	8.93	11.60	2.90	0.18	1.32	1.54	23.43	20.14	1469	2414	34 081	139 665	826	1550	112 915	477 048
16	2.50	8.31	10.38	12.93	3.21	0.05	1.07	1.56	26.37	22.80	1487	2414	34 189	39 976	774	1440	111 116	480 602
17	2.61	8.69	11.75	14.12	3.52	0.10	0.84	1.57	28.81	24.88	1460	2409	37 775	68 868	667	1309	109 415	468 984
18	2.70	8.98	13.24	15.27	3.69	0.08	0.69	1.53	31.02	26.82	1502	2423	66 268	123 180	683	1228	108 017	460 696
System E with PEG 600/3000 (M_w)																		
19	4.16	4.88	7.81	5.51	5.74	3.34	4.60	3.33	10.16	5.92	906	1653	71 440	140 824	809	1463	111 000	479 326
20	4.38	5.15	9.08	6.35	7.16	1.25	3.73	2.70	15.49	12.34	936	1712	49 253	75 268	733	1267	105 901	441 200
21	5.16	6.05	10.53	7.85	8.85	0.36	2.96	2.14	21.89	18.66	935	1703	35 478	41 990	640	993	101 988	416 771
22	3.60	4.23	10.65	7.47	11.69	0.09	2.41	1.74	28.03	24.21	1068	1886	28 927	36 634	621	953	99 362	428 621
System F (Dextran-Rich)																		
23	3.70	1.10	14.92	1.73	9.69	0.67	1.14	2.34	17.66	14.73	1797	2561	47 576	67 225	1183	2063	109 187	471 143
24	3.06	0.91	17.77	1.60	10.58	0.38	1.01	1.91	19.80	16.82	1901	2609	41 064	57 452	1214	2072	110 979	478 258
25	2.65	0.79	22.28	1.70	12.82	0.11	0.78	1.17	24.63	21.24	1977	2630	37 856	59 561	1089	1906	110 594	473 940
26	2.52	0.75	25.18	1.73	14.70	0.25	0.79	0.84	27.91	23.98	2063	2694	23 308	32 140	984	1756	110 429	478 511
System G (PEG-Rich)																		
27	8.18	2.44	2.20	2.71	8.58	0.68	1.89	2.51	15.67	13.00	1487	2350	62 046	91 881	1057	1880	156 789	619 956
28	9.40	2.81	1.91	3.01	9.96	0.31	1.84	2.07	18.94	16.13	1482	2354	47 534	67 142	991	1782	138 075	542 165
29	11.33	3.38	1.60	3.55	11.80	0.13	1.92	1.03	24.71	21.29	1503	2379	38 062	56 423	783	1351	122 639	484 896
30	11.84	3.54	1.33	3.68	12.22	0.09	1.92	1.05	25.09	21.66	1490	2364	41 095	55 018	764	1351	122 098	503 323
31	12.91	3.86	1.21	3.98	13.23	0.03	2.00	0.89	26.67	23.09	1484	2356	35 051	39 940	794	1273	117 152	492 948

given tie line. The tie-line data and average molecular weights for these quarternary systems are given in Table III.

Results and Discussion

The molecular weight as well as the molecular-weight distribution of the phase-forming polymers have a considerable effect on the miscibility of the phase system. In Figure 1 the influence of the number- and mass-average molecular weights on the phase behavior is demonstrated by comparing the binodal curves of the various phase systems with a phase ratio close to unity. This figure illustrates that the miscibility of the components increases with decreasing PEG molecular weight. The systems B and E have miscibility gaps of similar size. The binodal curve of system D lies between the binodal curves of systems B and C. This indicates that it is the mass-average molecular weight which approximately determines the size of the region of immiscibility. Comparing the trend of the binodal curves of systems D and E at the dextran-rich end with those containing monodisperse PEG, it can be seen that the polydispersity of a polymer increases its solubility in the phase which is poor in this polymer.

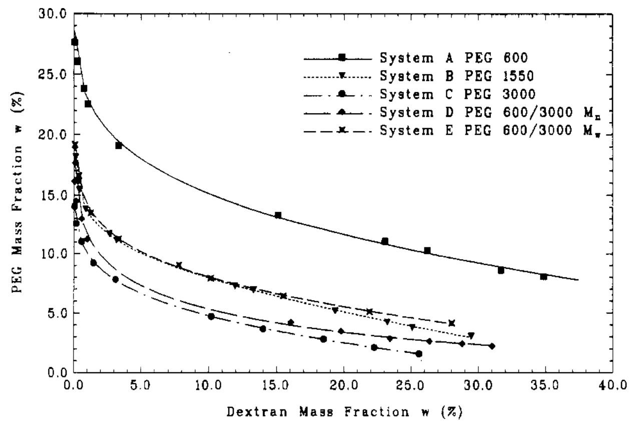

Figure 1. Tie lines of systems A–E at 20 °C.

Figure 2 presents chromatograms corresponding to the coexisting phases of tie line 15 to illustrate the different amounts of the three polymer fractions in these phases. From this figure it is apparent that the high-molecular-weight PEG

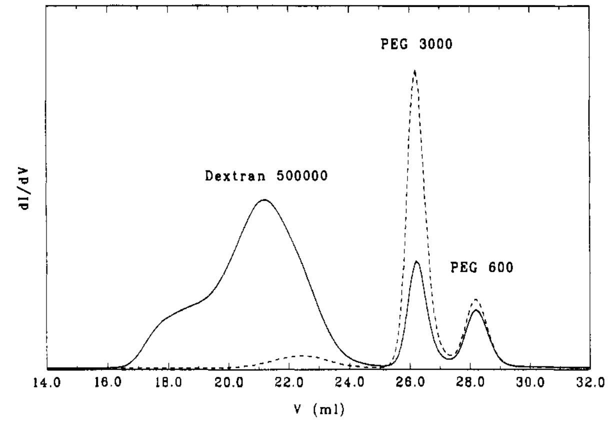

Figure 2. Chromatograms corresponding to tie line 15: (- - -) top phase; (—) bottom phase.

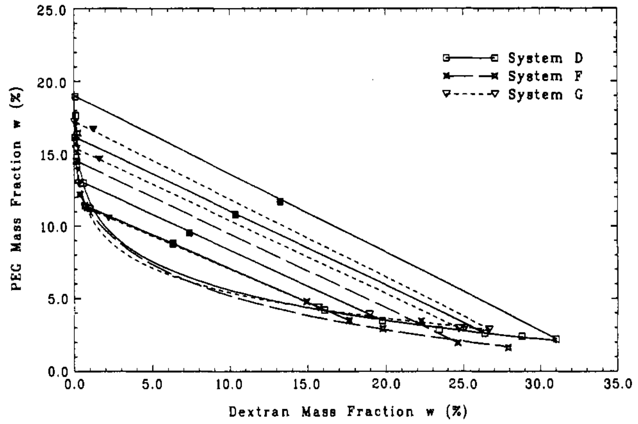

Figure 3. Tie lines of systems D, F, and G at 20 °C: (■, ▼, ×) total composition; (□, ▽, ⌧) composition of corresponding phases.

fraction remains preferably in the PEG-rich top phase while the small PEG molecules are driven into the dextran-rich bottom phase.

Figure 3 demonstrates the polydispersity effects which result from a phase ratio different from unity. For better readability, only every second tie line is shown. In the case of system F with the highest overall ratio of dextran/PEG the lowest miscibility of PEG in the dextran-rich bottom phase is bound. Furthermore, the tie lines of these system are longer than all others. With decreasing overall ratio of dextran/PEG the composition in the bottom phase is shifted to a higher dextran and a lower PEG concentration. For system G with a PEG-rich overall composition this trend is oppositely but only weakly affected. No effect of the phase ratio on the phase composition in the top phase was detectable for the system investigated. The effect may be smaller than the experimental accuracy.

To investigate the influence of the phase ratio on the molecular-weight distributions of dextran and PEG in the coexisting phases, the distributions corresponding to tie lines 23 (dextran rich) and 27 (PEG rich) are compared to that of tie line 13 in Figures 4 and 5.

Figure 4 demonstrates that preferably the low-molecular-weight dextran molecules dissolve in the top phase. In the case of a dextran-rich overall composition (tie line 23) the amount of the top phase is much smaller than that of the bottom phase. At the same time the equilibrium concentrations deviate only little from those in a system with a phase ratio near unity. Thus, the small amount of dextran in the top phase consists of the very short dextran molecules which are readily available due to the high overall dextran content of the system. Simultaneously the molecular-weight distribution of dextran in the top phase is narrow ($M_w/M_n = 1.74$), whereas the distribution in the bottom phase is essentially the same as that in the stock solution ($M_w/M_n = 4.32$).

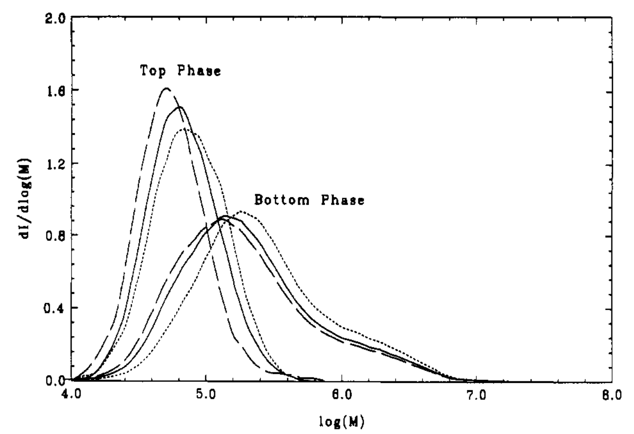

Figure 4. Molecular-weight distributions of dextran in the coexisting phases of tie lines 13 (—), 23 (- - -), and 27 (···).

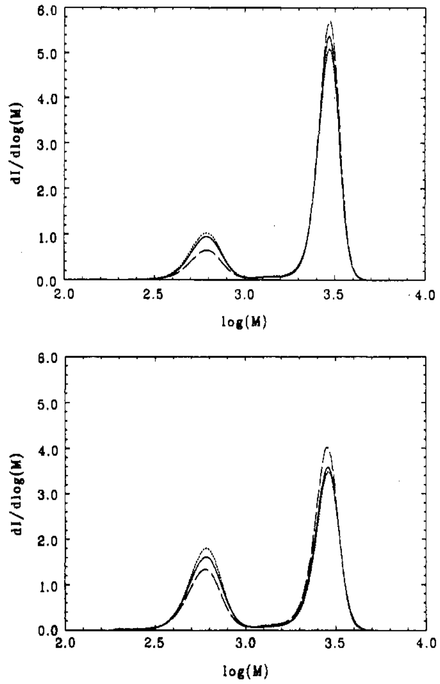

Figure 5. (Top) Molecular-weight distributions of polydisperse PEG in the top phase of tie lines 13 (—), 23 (- - -), and 27 (···). (Bottom) Molecular-weight distributions of polydisperse PEG in the bottom phase of tie lines 13 (—), 23 (- - -), and 27 (···).

If the overall concentration of PEG in the system is increased, more dextran has to dissolve in the increasing amount of the top phase to maintain the dextran concentration. The dextran molecules partitioning to the top phase now have to be of higher molecular weight since not enough short molecules are available. Thus, the molecular-weight distribution of dextran in the top phase broadens ($M_w/M_n = 1.78$) and is shifted to higher average values of molecular weight. Since the short dextran molecules are then missing in the bottom phase, the dextran molecular-weight distribution in the bottom phase becomes narrower ($M_w/M_n = 3.95$) and is also shifted to higher average values.

Exactly the same arguments hold for the distribution of PEG with different molecular weights between the phases. This can be seen in Figure 5 where the molecular-weight distributions of PEG in the top and bottom phases are shown again for the three tie lines 13, 23 (dextran rich), and 27 (PEG rich). Preferably the short PEG molecules partition to the dextran-rich bottom phase. Thus, the average molecular weight of PEG in the bottom phase is low for a PEG-rich overall composition. Simultaneously the molecular-weight distribution of PEG in the top phase is essentially the same as in the stock solution. The average molecular weight of PEG in the bottom phase as well as in the top phase increases as the phase ratio is shifted toward a dextran-rich overall composition of the system.

Registry Numbers Supplied by Author. Poly(ethylene glycol), 25322-68-3; dextran, 9004-54-0.

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