It has been found that the closeness of fit, with the experimental values, of the log γ_{HCl} values calculated according to the different treatments at all four different temperatures are (a) larger for the Pitzer method as compared to the other methods, (b) almost comparable in magnitude in the case of the Scatchard and the Lim methods, and (c) slightly better from the "alternative method" as compared to the "original method" of Lim.

However, "It has been amply shown in the literature that any of the models mentioned give about equally good fits to aqueous electrolyte mixture data. Slight differences in the derived values of the activity coefficients of the components of the mixtures are due to differences in the weighting of the parameters in the various models" (comments of reviewer 4; authors concur). The fact that "the Pitzer approach gives different activity coefficient values for the salts at 1 *m* and substantially higher deviations between experimental and computed values of γ_{HCl} in comparison with the other schemes (may be due to the fact that) the procedure of estimating coefficients does not blank out the two-component contributions in the Pitzer treatment as well as with other approaches. In any case, the information gaps preclude inferences about the validity of the different approaches, which are fairly well established already over the limited concentration range here" (comments of reviewer 2; authors concur).

(v) The variation of the Friedman–Lim mixing coefficients g_0 and g_0' with increasing total molality, at all four temperatures (data not recorded here) for all three binary mixtures mentioned, show the same trend as found earlier in the case of hydrochloric acid–guanidinium chloride mixtures (which again is similar to that in the case of the HCl–KCl mixtures studied by Lim (*4*): g_0 decreases with increasing total molality, becoming increasingly more negative, either (*i*) from an initial positive value at 0.1 *m* or else (ii) after initially increasing from higher negative values. This limiting behavior for vanishingly low ionic strengths is in both cases (calculations by the original as also the alternative method) contradictory to that predicted by the theoretical calculations (*14*).

(vi) The Pitzer binary interaction term (θ_{MM}) obtained for all three alkylammonium chloride mixtures studied, at 25 °C (results not shown here), follow the order $\theta_{H^+-CH_3NH_3^+}$ (−0.028) > $\theta_{H^+-(CH_3)_2NH_2^+}$ (−0.058) > $\theta_{H^+-(CH_3)_3NH^+}$ (−0.081). Our earlier reported (*8*) $\theta_{H^+-(CH_3)_4N^+}$ value (−0.167) at the same temperature, and the value reported by Robinson, Ray, and Bates also at the same temperature (*15*), $\theta_{H^+-NH_4^+}$ = −0.0165, are consistent with the above values. These values clearly show that as the size of the cation in the series increases, together with a gradual decrease of the net surface charge density, the binary interaction term becomes increasingly more negative.

Acknowledgment

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Phase Equilibria in the System Poly(ethylene glycol) + Dextran + Water

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Tie lines in the system poly(ethylene glycol) 3000 + dextran 500 000 + water have been measured at 0, 20, and 40 °C. The concentration and molecular weight distributions of the polymers in coexisting liquid phases were determined by using size exclusion chromatography (SEC).

Introduction

Aqueous polymer–polymer two-phase systems are used for the separation of complex mixtures of biomolecules (*1*). The design of such separation processes requires accurate and reliable thermodynamic data of the basis phase equilibria. Phase compositions of some polymer–polymer systems have been determined in previous studies (*2*, *3*). But, for the consistent correlation of these phase equilibria, molecular weight distributions of the polymers in the coexsting phases have to be taken into account (*4*).

Experimental Section

Materials. The components were used in the highest purity commercially available without further purification. Poly(ethylene glycol) (PEG) was supplied by Hüls AG, Marl, Germany, and dextran by Pfeifer & Langen, Dormagen, Germany; water was triply distilled. The number- and weight-average molecular weights of the polymers were determined by using size exclusion chromatography (SEC) and compared with the data of the manufacturers, as given in Table I. The dextran molecular weight standards were supplied by Pharmacosmos, Viby Sj., Denmark; the PEG standards, by Polymer Laboratories, Church Stretton, Shropshire, U.K.

Analytical Methods. The PEG and dextran concentrations in each phase were measured by size exclusion chromatogra-

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Table I. Average Molecular Weights of Pure Components[a]

	lot no.	M_n/(g/mol)	M_w/(g/mol)
	Dextran 500		
manufacturer	500 86 22 00	116×10^3	430×10^3
this work		101×10^3	432×10^3
	PEG 3000		
manufacturer	P.2	3.0×10^3 (nominal)	3.3×10^3 (nominal)
this work		3.14×10^3	3.25×10^3

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

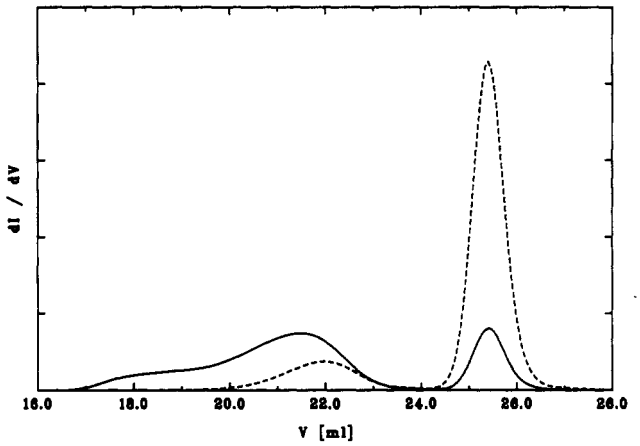

Figure 1. Chromatograms of tie line 17: (–) top phase; (—) bottom phase.

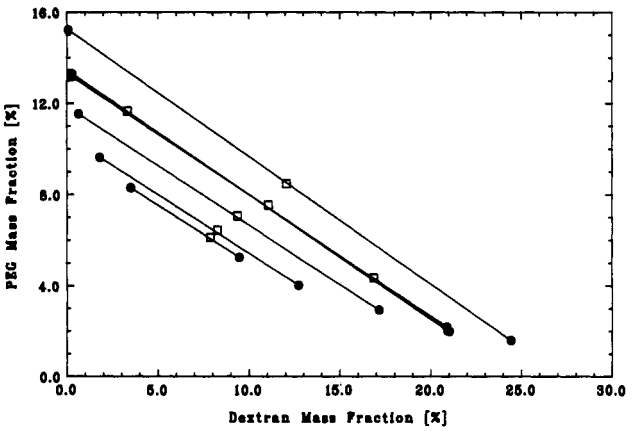

Figure 2. Tie lines for PEG + dextran + water at 40 °C: (□) total composition; (●) compositions of corresponding phases.

phy (SEC). The chromatographic equipment consists of a HPLC pump (ERC-64), a pulsation damper, a rotary valve (Rheodyne 7125), three 30-cm SEC columns in series (PSS HEMA 40, PSS HEMA 1000, TSK G6000PWXL), 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 eluent was distilled water, purified with a Millipore Milli-Q System, to which 200 ppm sodium azide was added (*5*).

The mixtures for the quantitative calibration were prepared by weight from the polymers and water. The samples of PEG and dextran for the calibration had been dried to constant weight in an evacuated oven at 20 and 60 °C, respectively. With use of measured densities the peak areas of the chromatograms were related to mass polymer per sample volume, resulting in an accuracy of better than ±0.15 wt%.

The molecular weight calibration was carried out with commercial dextran and PEG standards.

Phase Systems. The phase systems were generated from stock solutions with approximately 25 wt% polymer. The concentrations of the stock solutions were determined analytically with an accuracy of better than ±0.13 wt%. From the stock solutions and water, phase systems of about 9 g were weighed into sealed centrifuge tubes, thermostated for 2 h, repeatedly shaken, and finally centrifuged for 30 min at about 33 000 m/s^2. The employed centrifuge (Hettich Mikro Rapid/K) was modified to ensure a temperature stability of better than ±0.3 K in the centrifuge. Samples of about 1 g were pipetted directly from the PEG-rich top phase. To avoid a perturbation of the phase boundary and a contamination of the bottom-phase sample with top-phase droplets the dextran-rich bottom-phase sample of about 0.5 g was taken with a syringe directly through the wall of the plastic tube. Both samples were diluted with eluent to about 10–15 g. The diluted samples were filtered with a 0.2-μm filter (Chromafil A-20/25) and analyzed by SEC. The chromatograms corresponding to a single tie line are shown in Figure 1.

Results and Discussion

Tie lines with a mass ratio between top and bottom phases near unity were determined for three temperatures. Some systems with phase ratios unequal to unity were prepared to investigate the effect of the phase ratio on the phase compositions and the molecular weight distribution of dextran. The tie-line data are reported in Table II. The binodal curve and the tie lines at 40 °C are shown in Figure 2, where the compositions are plotted as mass fractions. The data compare well

Table II. Determination of the Tie Lines for PEG + Dextran + Water

		composition/(g/g)					
		total		top phase		bottom phase	
index	T/°C	PEG	dextran	PEG	dextran	PEG	dextran
1	0	0.0851	0.1217	0.1416	0.0012	0.0102	0.2903
2	0	0.0768	0.1082	0.1247	0.0011	0.0121	0.2564
3	0	0.0707	0.0954	0.1105	0.0022	0.0137	0.2254
4	0	0.0638	0.0819	0.0952	0.0056	0.0205	0.1812
5	0	0.0562	0.0741	0.0799	0.0137	0.0290	0.1391
6	0	0.0543	0.0702	0.0730	0.0206	0.0335	0.1194
7	20	0.0847	0.1208	0.1480	0.0006	0.0132	0.2588
8	20	0.0755	0.1084	0.1288	0.0014	0.0179	0.2192
9	20	0.0709	0.0915	0.1138	0.0052	0.0251	0.1822
10	20	0.0647	0.0807	0.0968	0.0136	0.0359	0.1431
11	20	0.0593	0.0765	0.0792	0.0347	0.0497	0.0986
12	20	0.0374	0.1900	0.1274	0.0031	0.0200	0.2233
13	20	0.1119	0.0324	0.1272	0.0016	0.0184	0.2201
14	40	0.0847	0.1207	0.1522	0.0008	0.0160	0.2444
15	40	0.0754	0.1109	0.1331	0.0027	0.0203	0.2094
16	40	0.0707	0.0937	0.1153	0.0065	0.0293	0.1717
17	40	0.0644	0.0827	0.0963	0.0181	0.0403	0.1274
18	40	0.0613	0.0789	0.0829	0.0350	0.0526	0.0948
19	40	0.1167	0.0334	0.1331	0.0022	0.0199	0.2104
20	40	0.0435	0.1687	0.1318	0.0027	0.0219	0.2088

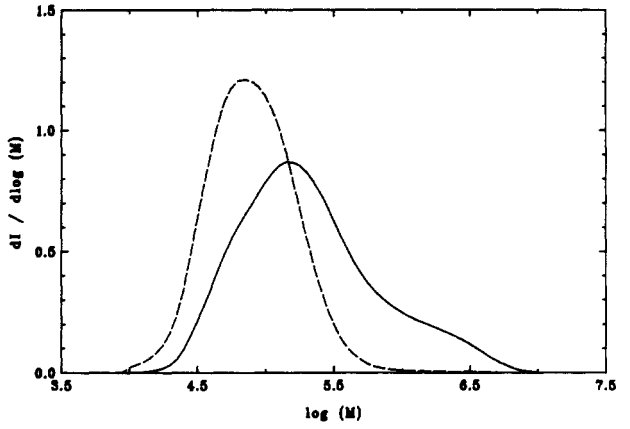

Figure 3. Molecular weight distribution of dextran in the corresponding phases of system 17: (–) top phase; (—) bottom phase.

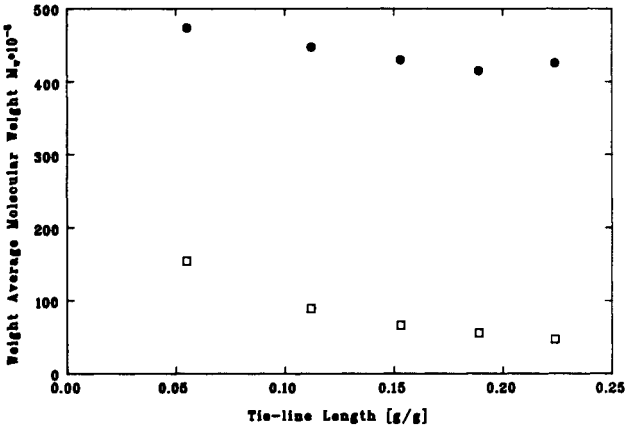

Figure 4. Weight-average molecular weight as a function of the tie-line length: (●) bottom phase, (□) top phase.

with those of previous studies (*2*, *3*), bearing in mind that the average molecular weights of the dextrans employed by the other authors differ slightly from our values. The complete data including the molecular weight distributions of dextran are given in the supplementary material. The average molecular weights are given in Table III. PEG has a very narrow molecular weight distribution, so that no difference in the molecular weight distribution of PEG in corresponding phases was detectable.

Figure 3 shows the different molecular weight distributions of dextran in the two coexisting phases. The distributions are significantly correlated with the tie-line length (Figure 4). The interpretation of this effect is that low molecular weight fractions of dextran dissolve favorably in the PEG-rich top phase. With decreasing tie-line length the dextran concentration in the PEG-rich phase increases. Thus more dextran molecules with a higher molecular weight have to dissolve in this phase, resulting in a higher average molecular weight of dextran. Correspondingly the average molecular weight of dextran in the dextran-rich phase increases, since the dextran fraction which dissolves in the PEG-rich phase has a lower molecular weight than the dextran-rich phase. The variation of the dextran molecular weight distribution when the phase ratio is shifted can be understood from the same arguments.

Table III. Average Molecular Weights of Dextran in the Phases

index	tie line length/ (g/g)	top phase M_n/ (g/mol)	top phase M_w/ (g/mol)	bottom phase M_n/ (g/mol)	bottom phase M_w/ (g/mol)
1	0.251	30758	51281	110148	439931
2	0.222	38251	62399	114351	435486
3	0.194	39384	60514	112625	432249
4	0.153	49206	70023	123445	460035
5	0.109	59550	89688	122235	458967
6	0.086	69080	114406	126831	490284
7	0.224	38811	47384	108530	425680
8	0.189	44898	55977	108171	414886
9	0.153	47373	66825	114007	430083
10	0.112	58989	89369	119205	447505
11	0.055	78495	154137	117580	473681
12	0.191	35864	66822	109393	429826
13	0.189	43584	57271	120991	444618
14	0.211	37473	50431	111950	433225
15	0.179	40139	62992	114965	432477
16	0.143	49976	71755	115741	438271
17	0.095	62026	110830	120856	457811
18	0.052	76350	154715	117566	459023
19	0.181	43369	62351	122336	448637
20	0.179	39799	53717	111907	419156

No effect of the phase ratio on the phase compositions was detectable for the system investigated. Theoretical studies (*6*) show that this influence is a general feature of polydisperse components but that the effect may be smaller than the experimental accuracy.

Glossary

M	molecular weight
dI	amount of polymer eluted between volume V and $V + dV$

Registry No. PEG, 25322-68-3; dextran, 9004-54-0.

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Supplementary Material Available: Tables of the complete data, including the molecular weight distributions of dextran (21 pages). Ordering information is given on any current masthead page.