Partitioning of Some Amino Acids and Low Molecular Mass Peptides in Aqueous Two-Phase Systems of Poly(ethylene glycol) and Dextran in the Presence of Small Amounts of K_2HPO_4/KH_2PO_4-Buffer at 293 K: Experimental Results and Correlation

Ralf Tintinger,[†] Jiandung Zhu,[‡] Christoph Grossmann,[‡] and Gerd Maurer*

Lehrstuhl für Technische Thermodynamik, Fachbereich Maschinenbau und Verfahrenstechnik, Universität Kaiserslautern, D-67653 Kaiserslautern, Germany

Buffered aqueous two-phase systems are effective extraction systems for separating amphoteric hydrocarbons like, for example, polypeptides from aqueous phases. The design and basic engineering of such processes require the knowledge of the liquid−liquid equilibrium. Methods to calculate and predict the liquid−liquid phase equilibrium in those systems can only be developed when a large data base is available. The present contribution is aimed at that goal by presenting experimental results for the partitioning of small amounts (≈0.001 g/g solution) of model substances—amino acids glycine, L-glutamic acid, L-phenylalanine, and L-lysine as well as dipeptides and some higher, but still low, molecular peptides of those single amino acids—in aqueous two-phase systems of dextran and poly(ethylene glycol) in the presence of small amounts (about 0.05 mol/kg) of K_2HPO_4/KH_2PO_4-buffer at about 293 K. A group contribution model for the excess Gibbs energy is used to correlate/predict that liquid−liquid phase equilibrium.

Introduction

An extractive step as a means of product separation during bioprocesses is a feasible technology for enhancing the product yield as well as for reducing the cost of downstream processing. However, when biological materials have to be extracted from aqueous fermentation broths, the choice of a suitable extractant is very limited, as biological materials often denaturize in nonaqueous solvents and even in aqueous solvents beyond certain, often very small, ranges of temperature, ionic strength, and pH. For such extraction processes aqueous two-phase systems offer some interesting features, as ionic strength and pH can be adjusted conveniently. In aqueous liquids a phase split is observed when already small amounts (about 10 mass %) of two water soluble but incompatible substances are simultaneously dissolved. Polymers poly(ethylene glycol) (PEG) and dextran (DEX) are typical examples for such hydrophilic but incompatible substances. The phase equilibrium in aqueous two-phase systems and the partitioning of biological material on the coexisting phases have to be known for the basic engineering of such extraction processes. While there are many qualitative reports on the feasability of aqueous two-phase systems for extractive biotechnological separations in the literature (cf. Kula (1979); Albertsson (1986); Fisher and Sutherland (1989)), quantitative information on the phase equilibrium in such systems is still very limited. However, such information is required for the development of methods to correlate and predict the partitioning of biological materials onto aqueous two-phase systems. Experimental results on the partitioning of low molecular model compounds—like, for example, amino acids and low molecular peptides—in aqueous two-phase systems are especially suited for that purpose, as in contrary to high molecular substances like, e.g., many proteins and enzymes, the thermophysical properties of those low molecular substances are well-known.

* Author to whom correspondence should be addressed.
[†] Present address: DuPont de Nemours GmbH, 76677 Östringen, Germany.
[‡] Present address: BASF AG, 67056 Ludwigshafen, Germany.

The present publication reports on experimental results for the partitioning of small amounts (≈10^{-3} g substance/g solution) of four amino acids (glycine (gly), L-glutamic acid (glu), L-phenylalanine (phe), and L-lysine (lys)), four dipeptides (gly-gly, glu-glu, phe-phe, and lys-lys), and some peptides formed of a single amino acid (three tripeptides (3*gly, 3*glu, 3*lys) and three higher peptides (5*gly, 6*gly, 5*lys)) in aqueous two-phase systems of PEG 6000 (or PEG 35000) and DEX 500 at 293 K. Amino acids and peptides are amphoteric substances. They may be present as anions, cations, or neutral molecules (depending on the pH of the solution). The pH was adjusted to about 7 by adding small amounts (≈0.05 mol/kg solution) of an equimolar buffer of K_2HPO_4 and KH_2PO_4. A group contribution model for the excess Gibbs energy (Grossmann et al., 1995a,b) was applied to correlate/predict the phase equilibrium. The influence of the molecular mass of PEG on the partitioning of those solutes is small. Therefore it was sufficient to estimate model parameters from the experimental results for the partitioning in the systems with PEG 6000.

Experimental Section

Materials and Pure Component Properties. Table 1 gives information on the chemicals used in the present investigation as well as on some of their thermophysical properties needed for phase equilibrium calculations. Poly(ethylene glycol)s PEG 6000 and PEG 35000 are polydisperse materials. Their number averaged molecular mass $\bar{M}_n$ was obtained by combining gel permeation chromatography (GPC) and multiangle laser light scattering (MALLS) using a refractive index detector: $\bar{M}_n$(PEG 6000) = 6230 and $\bar{M}_n$(PEG 35000) = 34 100. The MALLS data of the present work were also used to determine the mass-averaged molecular mass $\bar{M}_w$. The ratio $P = \bar{M}_w/\bar{M}_n$ is often used to characterize the polydispersity of a polymer. For both PEGs that ratio is close to unity (1.04), thus indicating that both polymers are approximately monodisperse. Only a single dextran (DEX 500) was used. Number-averaged and mass-averaged molecular masses of the DEX 500-samples (determined by chemical end group analysis and

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Table 1. Chemicals

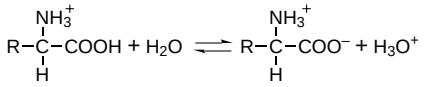
substance	supplier	$\bar{M}_n$	pK_I	pK_{II}	pK_{III}	pK_{IV}
PEG 6 000[a]	Hoechst, Frankfurt, Germany	6230				
PEG 35 000[b]		34100				
DEX 500	Pharmacia, Uppsala, Sweden	179000				
K_2HPO_4	Merck, Darmstadt, Germany	174.18	11.98[c]	7.21[d]	2.11[e]	
KH_2PO_4		136.09				
l-phenylalanine		165.19	1.83	9.13		
glycine	Carl Roth, Karlsruhe, Germany	75.07	2.34	9.60		
L-glutamic acid		147.13	2.19	9.67	4.25	
L-lysine	Serva, Heidelberg,Germany	146.19	2.20	8.90	10.28	
gly-gly		132.12	2.34	9.60		
glu-glu		276.25	2.19	9.67	4.25	4.40
phe-phe		312.37	1.83	9.13		
lys-lys		274.36	2.20	8.90	10.28	10.5
3*gly		189.15	2.34	9.60		
3*glu		405.36	2.19	9.67	4.25	4.40
3*lys		402.54	2.20	8.90	10.28	10.5
5*gly		303.27	2.34	9.60		
6*gly		360.33	2.34	9.60		
5*lys		658.88	2.20	8.90	10.28	10.5
water		18.02	14.17[f]			

[a] Lot number: 664762. [b] Lot number: E 06373013. [c] pK_V. [d] pK_{VI}. [e] pK_{VII}. [f] pK_{VIII}.

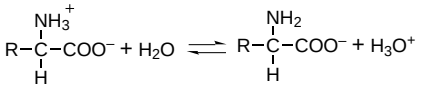
light scattering, respectively) were also provided by the supplier: $\bar{M}_n \approx 179\,000$; $\bar{M}_w \approx 507\,000$. The molecular masses of different DEX samples differed by a few percent. The arithmetic mean of the number-averaged molecular mass of the samples is given in Table 1. Although those samples are polydisperse, no effort was undertaken to investigate the influence of the polydispersity on the phase equilibrium.

Amino acids and peptides are amphoteric substances. Therefore the pH of the aqueous solution has an influence on the distribution of those biomolecules onto cationic, anionic, and amphoteric (i.e., neutral) species.

The dissociation reactions of an amino acid in pure water

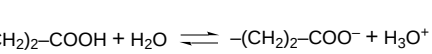


(I)



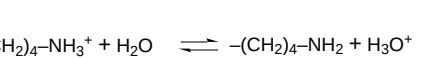
(II)

are described by the corresponding equilibrium constants p$K_I = -\log_{10} K_I$ and p$K_{II} = -\log_{10} K_{II}$. K_I and K_{II} are the chemical equilibrium constants defined on reference states as described in the paragraph on the Gibbs excess energy model. In Table 1 numbers are given for pK_I and pK_{II} at 293 K for the amino acids glycine, L-glutamic acid, L-phenylalanine, and L-lysine (cf. Yamamoto (1978)). While there is no dissociating group in the residue R, neither of glycine (R ≡ hydrogen atom) nor of phenylalanine (R ≡ $-(CH_2)-(C_6H_5)$), the residues of glutamic acid (R ≡ $-(CH_2)_2-(COOH)$) as well as of lysine (R ≡ $-(CH_2)_4-NH_2$) contain dissociating groups. The number for pK(293 K) for the dissociation of the carboxylic group in the residue of glutamic acid, i.e. for the reaction



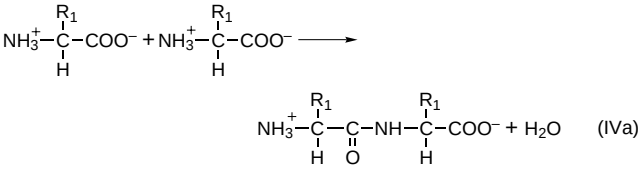
(IIIa)

and for the dissociation of the amino group in the residue of lysine, i.e. the reaction



(IIIb)

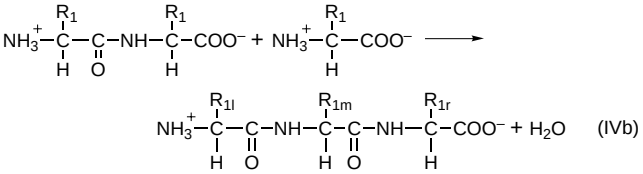
were also taken from Yamamoto (1978): pK_{IIIa} = p$K_{III,glutamic\ acid}$ = 4.25 and pK_{IIIb} = p$K_{III,lysine}$ = 10.28 (cf. pK_{III} in Table 1). The combination peptides result from a condensation reaction. For example, for a dipeptide formed by a reaction between the same amino acids, that reaction is



(IVa)

Numbers for pK of the dissociation of the amino group NH_3^+ (according to eq II) and of the carboxylic group −COOH (according to eq I) of the amino acid (cf. Table 1) were assumed to hold also for the dissociation of the left terminal amino group and the right terminal carboxylic group of the dipeptide (e.g. in gly-gly, p$K_I \approx$ p$K_{I,glycine}$ = 2.34 and p$K_{II} \approx$ p$K_{II,glycine}$ = 9.60.)

When a dipeptide reacts with an amino acid to a tripeptide



(IVb)

both functional groups of the amino acid in the middle of the molecule lose their dissociating properties. For the end groups in tripeptides the same approximations as for dipeptides were applied (e.g. in gly-gly-gly, p$K_I \approx$ p$K_{I,glycine}$ = 2.34 and p$K_{II} \approx$ p$K_{II,glycine}$ = 9.60). For the peptides they are given as pK_I and pK_{II} respectively in Table 1. The pK of a dissociating group in residue R of a peptide was adopted without any change from the corresponding single amino acid, when that dissociating group is in a left terminal residue (i.e., R_1 in eq IVa and R_{1l} of eq IVb)—it is given as pK_{III} in Table 1 (e.g., in glu-glu-glu, p$K_{III} \approx$ p$K_{III,glutamic\ acid}$ = 4.25, and in lys-lys-lys, p$K_{III} \approx$ p$K_{III,lysine}$ = 10.28)—while it was modified when it is in a middle group (i.e., in R_{1m} in eq IVb) or in a right terminal end group (i.e., in R_{1r} of eq IVb). For the carboxylic group in the residue R_{1m} of glutamic acid, pK = 4.40, and for the amino group in the residue R_{1m} of lysine, pK = 10.5. Numbers are given as pK_{IV} in Table 1. These modifications are based on recommendations by Horn and Heuck (1983) and Laussac and Sarkar (1985). However, as at pH ≈ 7 the dissociation of the carboxylic group in the residue of glutamic acid is nearly complete and the protonation of the

amino group in the residue of lysine can rather be neglected, these modifications have only a very small influence on the calculations.

Without any buffer the pH of aqueous solutions of PEG and DEX might vary depending, for example, on the amount of dissolved carbon dioxide. To avoid problems resulting from varying pH numbers, the pH was adjusted by adding small amounts of an equimolar buffer of K_2HPO_4 and KH_2PO_4. It was assumed that both salts dissociate completely in water and that the resulting phosphate ions undergo the following chemical reactions:

$$HPO_4^{2-} + H_2O \rightleftharpoons PO_4^{3-} + H_3O^+ \quad (V)$$

$$H_2PO_4^- + H_2O \rightleftharpoons HPO_4^{2-} + H_3O^+ \quad (VI)$$

$$H_3PO_4 + H_2O \rightleftharpoons H_2PO_4^- + H_3O^+ \quad (VII)$$

Furthermore the autoprotonation of water is taken into account: Chemical equilibrium constants (as p*K* numbers)

$$2H_2O \rightleftharpoons OH^- + H_3O^+ \quad (VIII)$$

for these reactions at 293 K are given in Table 1 (cf. also Grossmann et al. (1995a)).

Liquid−Liquid Phase Equilibrium Measurements. Details of the experiments have been described previously (Grossmann et al., 1995a,b, 1997; Tintinger et al., 1997) and therefore are not repeated here. The peptide concentration in the aqueous feed solution was approximately 1 mg/g in most experiments. However, in some measurement with 6*gly that concentration was only about 0.07 mg/g. In most experiments the buffer concentration in the aqueous feed solution was about 0.05 molal, but in a few experiments it was only about 0.01 molal. In some experiments both phases were analyzed for all solutes, while in others the phases were only analyzed for the peptides and the buffer, as in a large number of experiments it was shown that the presence of such small amounts of partitioning amino acids, peptides, and K_2HPO_4/KH_2PO_4 buffer does not change the composition of the phase-forming components beyond the limits of the experimental uncertainty. When the concentrations of PEG and DEX were not analyzed, they were taken from Grossmann et al. (1995b). Analyzing procedures for PEG, DEX, amino acids, and peptides have been described before (Grossmann et al., 1995a,b, 1997; Tintinger et al., 1997). The partitioning of the buffer was not determined exactly, as only the potassium ion concentration was measured in both phases. For that analysis a potassium-selective electrode (type 152213000, Ingold-Messtechnik AG, Urdorf, Switzerland) was used in combination with a reference electrode (type 373-90-WTE-ISE-S7, same supplier) and a high-precision voltmeter (type pH531, WTW, Weilheim, Germany). In preliminary experiments it was found that neither PEG and DEX nor any of the amino acids and peptides influence the experimental results beyond the overall uncertainty of the measurement of the potassium ion concentration of about 6%. The buffer concentration in both coexisting phases was calculated from the experimental results for the potassium concentration assuming that the molar ratio of (overall) $HPO_4^=$ to (overall) $H_2PO_4^-$ equals unity (as in the feed solution). The relative uncertainty of the experimental results for the concentration of PEG and DEX in the coexisting phases is about 3.5 and 1% respectively, whereas the relative uncertainty of the peptide concentration is about 3%. As amino acids and peptides are amphoteric substances, the pH was measured in both coexisting aqueous phases (absolute uncertainty: ±0.1 pH).

Experimental Results. The experimental results for the partitioning of four amino acids (glycine, L-glutamic acid, L-phenylanaline, and L-lysine) are given in Table 2a (for PEG 6000/DEX 500/water) and Table 3a (for PEG 35000/DEX 500/water). The experimental results for the partitioning of four dipeptides (gly-gly, glu-glu, phe-phe, and lys-lys) and six higher peptides (3*gly, 5*gly, 6*gly, 3*glu, 3*lys and 5 *lys) are presented in Table 2b,c (for PEG 6000/DEX 500/water) and Tables 3b,c (for PEG 35000/DEX 500/water) respectively. In most experiments the amount of the amino acid/peptide in the feed solution was about 0.7 to 1 mg/g. However, in the experiments with 5 *gly and 6*gly it was as low as 0.3 and 0.08 mg/g, respectively. The concentration of the K_2HPO_4 /KH_2PO_4 buffer in the feed was between 0.01 and 0.08 mol per kg of water.

The amino acids glycine, L-glutamic acid, and L-lysine prefer the DEX-rich lower phase over the PEG-rich upper phase, whereas L-phenylalanine shows the inverse preference. Peptides consisting only of either glycine-, glutamic acid-, or lysine-groups also prefer the DEX-rich over the PEG-rich phase while peptides of (only) phenylalanine-groups enrich in the PEG-rich phase. The potassium ion concentration is always larger in the DEX-rich phase than in the PEG-rich phase. The difference in the experimental results for the pH in the coexisting phases rarely exceeds the experimental uncertainty, i.e. 0.1 pH. Nevertheless it seems that the pH in the PEG-rich (i.e., upper) phase is somewhat above that in the DEX-rich (i.e., lower) phase. Neither the partitioning of the amino acids, the peptides, and the buffer nor the pH is essentialy affected by the molecular mass of PEG; i.e., increasing that molecular mass from about 6000 to about 35 000 has nearly no influence on those results.

The partition coefficients *K*, i.e., the ratio of mass fractions ξ in (PEG-rich) upper to (DEX-rich) lower phase, of the amino acids are rather close to unity. The deviation from unity increases with increasing difference in the concentration of one of the phase-forming polymers in the coexisting phases $\Delta\xi_i = \xi_{i,\text{upper phase}} - \xi_{i,\text{lower phase}}$ (i.e., with the tie-line length of the two-phase system), but it is at minimum 0.81, 0.85, and 0.60 for glycine, glutamic acid, and lysine, respectively, and at maximum 1.1 for phenylalanine. The difference in the peptide concentrations in the coexisting phases increases with the number of amino acid groups in a peptide. For example, the minimum number for the experimental results for the partition coefficient of glycine peptides decreases from about 0.76 for gly-gly to about 0.23 for 6*gly and from 0.33 for lys-lys to 0.13 for 5*lys.

The K_2HPO_4/KH_2PO_4-buffer prefers the DEX-rich (lower) phase over the PEG-rich (upper) phase. In the experiments of the present work, the partition coefficient of the buffer was at minimum about 0.5.

Correlation of Partition Coefficients and Comparison with Experimental Data

Model for the Excess Gibbs Energy G^E. The group contribution model for the Gibbs energy G^E applied in the present work has been described in detail recently (Grossmann et al., 1995a,b). Only the essential features are repeated here. The excess is defined using an asymmetric normalization. For water the reference state is the pure liquid, whereas for any solute it is a hypothetical liquid, one molal solution in water with interactions as at infinite dilution in pure water at the temperature of the mixture. G^E is assumed to be the sum of two contributions: a modified Debye−Hückel term (Pitzer, 1973) accounts for

Table 2. Partitioning of Some Amino Acids (AS), Dipeptides, and Higher Peptides in Aqueous Two-Phase Systems of PEG 6000 and DEX 500 at 293.15 K

(a) Amino Acids (AS)

feed					lower phase						upper phase					
water, g/g	PEG, g/g	DEX, g/g	AS, mg/g	buffer, mol/kg	water, g/g	PEG, g/g	DEX, g/g	AS, mg/g	buffer, mol/kg	pH	water, g/g	PEG, g/g	DEX, g/g	AS, mg/g	buffer, mol/kg	pH
							gly									
0.8841	0.0488	0.0571	1.04	0.058	0.8449	0.0195	0.1220	1.11	0.080	6.89	0.9115	0.0710	0.0060	1.01	0.068	6.89
0.8673	0.0518	0.0706	1.06	0.060	0.8183	0.0134	0.1548	1.17	0.080	6.85	0.9058	0.0808	0.0023	1.05	0.065	6.88
0.8409	0.0621	0.0873	1.05	0.056	0.7812	0.0078	0.1980	1.17	0.076	6.82	0.8862	0.1035	0.0005	1.01	0.057	6.86
0.8280	0.0689	0.0937	0.96	0.054	0.7761	0.0055	0.2075	1.09	0.063	6.82	0.8755	0.1163	0.0001	0.91	0.046	6.85
0.8060	0.0802	0.1038	0.99	0.058	0.7339	0.0020	0.2512	1.14	0.076	6.81	0.8560	0.1354	<0.0001	0.92	0.049	6.85
							glu									
0.8705	0.0494	0.0705	1.00	0.055	0.8198	0.0130	0.1524	0.99	0.089	6.64	0.9068	0.0788	0.0027	0.93	0.069	6.66
0.8449	0.0603	0.0853	1.01	0.055	0.7826	0.0075	0.1958	1.04	0.084	6.68	0.8871	0.1015	0.0001	0.93	0.067	6.64
0.8252	0.0700	0.0953	1.00	0.055	0.7581	0.0030	0.2238	1.00	0.091	6.61	0.8701	0.1190	0.0003	0.91	0.062	6.71
0.8053	0.0800	0.1052	1.01	0.055	0.7297	0.0020	0.2522	1.02	0.087	6.59	0.8541	0.1360	0.0002	0.89	0.057	6.62
0.8911	0.0468	0.0563	1.02	0.031	0.8582	0.0205	0.1160	1.05	0.027	6.48	0.9192	0.0675	0.0075	0.99	0.031	6.48
0.8708	0.0504	0.0731	1.00	0.030	0.8244	0.0150	0.1550	1.03	0.029	6.45	0.9085	0.0825	0.0040	0.94	0.026	6.49
0.8474	0.0627	0.0824	0.97	0.042	0.7861	0.0075	0.1970	1.02	0.054	6.57	0.8903	0.1020	0.0010	0.91	0.037	6.60
0.8297	0.0698	0.0947	1.00	0.031	0.7649	0.0040	0.2245	1.00	0.036	6.45	0.8752	0.1190	0.0005	0.91	0.028	6.47
0.8109	0.0783	0.1048	1.01	0.032	0.7409	0.0025	0.2490	1.04	0.042	6.47	0.8619	0.1325	<0.0001	0.89	0.031	6.50
							phe									
0.8963	0.0440	0.0570	1.24	0.010	0.8787	0.0245	0.0940	1.20		6.95	0.9224	0.0670	0.0080	1.21		6.98
0.8960	0.0451	0.0561	1.28	0.010	0.8761	0.0240	0.0970	1.26		6.92	0.9193	0.0690	0.0090	1.28		6.96
0.8837	0.0478	0.0657	1.30	0.010	0.8541	0.0160	0.1270	1.28		6.90	0.9135	0.0790	0.0048	1.32		6.96
0.8708	0.0552	0.0713	1.24	0.010	0.8287	0.0095	0.1590	1.22		6.88	0.9036	0.0915	0.0021	1.30		6.94
0.8518	0.0670	0.0784	1.25	0.010	0.7930	0.0066	0.1976	1.23		6.90	0.8904	0.1066	0.0003	1.30		6.94
0.8484	0.0697	0.0791	1.27	0.010	0.7927	0.0055	0.1990	1.20		6.90	0.8853	0.1115	0.0005	1.30		6.94
							lys									
0.8724	0.0497	0.0693	1.00	0.049	0.8365	0.0098	0.1447	1.12	0.051	7.08	0.9134	0.0756	0.0034	0.90	0.043	7.17
0.8467	0.0604	0.0845	1.00	0.047	0.7927	0.0057	0.1922	1.18	0.052	7.10	0.8735	0.1185	0.0009	0.82	0.040	7.18
0.8273	0.0697	0.0944	1.00	0.049	0.7656	0.0028	0.2213	1.27	0.059	7.07	0.8689	0.1235	0.0005	0.81	0.040	7.19
0.8061	0.0808	0.1046	1.00	0.049	0.7329	0.0012	0.2562	1.34	0.054	7.11	0.8564	0.1365	0.0003	0.77	0.039	7.19
0.8893	0.0455	0.0557	1.00	0.055	0.8657	0.0223	0.1026	1.11	0.053	7.05	0.9078	0.0768	0.0077	0.89	0.044	7.13
0.8704	0.0497	0.0701	0.99	0.056	0.8321	0.0100	0.1468	1.17	0.063	7.01	0.8846	0.1044	0.0027	0.85	0.047	7.01
0.8448	0.0599	0.0858	1.00	0.055	0.7955	0.0057	0.1884	1.27	0.059	7.01	0.8898	0.1016	0.0004	0.81	0.048	7.04
0.8288	0.0691	0.0927	0.98	0.054	0.7673	0.0043	0.2177	1.17	0.061	7.01	0.8531	0.1381	0.0005	0.76	0.049	7.05
0.8054	0.0793	0.1058	1.00	0.055	0.7395	0.0036	0.2467	1.22	0.058	7.00	0.8296	0.1618	<0.0001	0.73	0.050	7.06

(b) Dipeptides

feed					lower phase						upper phase					
water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH
							gly-gly[a]									
0.8827	0.0512	0.0555	1.06	0.061	0.8467	0.0180	0.1240	1.14	0.066	6.72	0.9102	0.0750	0.0050	1.02	0.056	6.75
0.8764	0.0524	0.0606	1.03	0.062	0.8365	0.0160	0.1360	1.11	0.067	6.73	0.9072	0.0790	0.0040	0.99	0.057	6.75
0.8589	0.0653	0.0651	1.00	0.062	0.8000	0.0080	0.1800	1.11	0.070	6.72	0.8905	0.0980	0.0020	0.92	0.055	6.74
0.8524	0.0647	0.0722	1.01	0.063	0.7908	0.0060	0.1910	1.11	0.071	6.75	0.8896	0.1010	<0.0001	0.92	0.055	6.79
0.8898	0.0441	0.0635	1.03	0.010	0.8733	0.0220	0.1020	1.05	0.011	6.61	0.9215	0.0680	0.0080	0.97	0.010	6.65
0.8731	0.0519	0.0725	1.02	0.010	0.8312	0.0130	0.1530	1.09	0.011	6.56	0.9096	0.0850	0.0030	0.96	0.009	6.55
0.8334	0.0723	0.0916	1.01	0.011	0.7659	0.0050	0.2260	1.11	0.013	6.46	0.8777	0.1200	<0.0001	0.91	0.009	6.48
0.8182	0.0796	0.0996	1.01	0.010	0.7450	0.0040	0.2480	1.15	0.012	6.40	0.8668	0.1310	<0.0001	0.88	0.008	6.46
							glu-glu									
0.8908	0.0455	0.0548	1.01	0.051	0.8584	0.0214	0.1112	1.01	0.052	6.64	0.9180	0.0666	0.0071	0.96	0.047	6.65
0.8679	0.0531	0.0701	1.00	0.050	0.8187	0.0122	0.1595	1.04	0.055	6.63	0.9005	0.0896	0.0021	0.94	0.045	6.66
0.8281	0.0697	0.0935	1.01	0.049	0.7592	0.0051	0.2256	1.10	0.058	6.61	0.8703	0.1211	0.0007	0.92	0.044	6.66
0.7843	0.0887	0.1173	1.12	0.055	0.7294	0.0019	0.2583	1.12	0.060	6.59	0.8524	0.1402	0.0004	0.87	0.040	6.68
							phe-phe[a]									
0.9031	0.0450	0.0500	0.419	0.010	0.8815	0.0255	0.0910	0.400		6.89	0.9227	0.0630	0.0125	0.439		6.84
0.8761	0.0520	0.0700	0.376	0.010	0.8390	0.0120	0.1470	0.358		6.82	0.9082	0.0870	0.0030	0.444		6.83
0.8552	0.0620	0.0810	0.350	0.010	0.8076	0.0070	0.1835	0.314		6.84	0.8937	0.1040	0.0005	0.420		6.81
0.8362	0.0720	0.0900	0.318	0.010	0.7771	0.0035	0.2175	0.270		6.78	0.8777	0.1205	<0.0001	0.385		6.85
							lys-lys[a]									
0.8895	0.0447	0.0552	0.99	0.062	0.8638	0.0220	0.1030	1.05	0.065	6.86	0.9141	0.0670	0.0090	0.91	0.058	6.89
0.8346	0.0597	0.0950	1.00	0.062	0.7798	0.0070	0.2010	1.22	0.071	6.85	0.8839	0.1070	<0.0001	0.69	0.054	6.90
0.8086	0.0699	0.1108	1.01	0.062	0.7329	0.0040	0.2500	1.44	0.075	6.85	0.8594	0.1320	<0.0001	0.62	0.052	6.91
0.7739	0.0851	0.1300	1.00	0.064	0.6924	0.0030	0.2900	2.15	0.080	6.85	0.8374	0.1530	<0.0001	0.71	0.057	6.92

(c) Higher Peptides

feed					lower phase						upper phase					
water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH
							3*gly									
0.8926	0.0428	0.0612	1.01	0.010	0.8696	0.0186	0.1092	1.04		6.84	0.9162	0.0702	0.0113	0.93		6.89
0.8718	0.0526	0.0730	1.01	0.010	0.8329	0.0048	0.1596	1.06		6.83	0.9084	0.0860	0.0033	0.91		6.88
0.8536	0.0619	0.0820	1.01	0.010	0.7999	0.0024	0.1950	1.07		6.81	0.8909	0.1052	0.0016	0.89		6.87
0.8276	0.0720	0.0979	1.00	0.010	0.7646	0.0014	0.2313	1.12		6.80	0.8068	0.0899	0.1008	1.00		6.86
0.8068	0.0899	0.1008	1.00	0.010	0.7262	0.0010	0.2700	1.22		6.80	0.8546	0.1430	<0.0001	0.96		6.88

Table 2. (Continued)

(c) Higher Peptides

feed					lower phase						upper phase					
water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH
								5*gly								
0.8706	0.0499	0.0697	0.502	0.060	0.8336	0.0111	0.1444	0.547	0.067	6.91	0.9035	0.0846	0.0026	0.478	0.057	6.96
0.8567	0.0543	0.0797	0.467	0.057	0.8129	0.0068	0.1693	0.536	0.067	6.91	0.8921	0.0982	0.0010	0.454	0.053	6.95
0.8503	0.0625	0.0802	0.459	0.042	0.7999	0.0036	0.1889	0.545	0.045	6.92	0.8885	0.1048	0.0009	0.443	0.034	6.99
0.8409	0.0649	0.0873	0.434	0.042	0.7872	0.0035	0.2022	0.511	0.043	6.92	0.8827	0.1117	0.0004	0.409	0.031	6.99
0.8899	0.0452	0.0549	0.499	0.062	0.8592	0.0202	0.1098	0.508	0.066	6.88	0.9186	0.0641	0.0080	0.480	0.056	6.88
								6*gly[a]								
0.8895	0.0450	0.0562	0.076	0.059	0.8651	0.0220	0.1030	0.112	0.063	7.06	0.9152	0.0670	0.0090	0.047	0.056	7.10
0.8606	0.0549	0.0751	0.077	0.060	0.8095	0.0100	0.1700	0.121	0.067	7.06	0.8986	0.0900	0.0030	0.033	0.054	7.12
0.8357	0.0602	0.0948	0.077	0.060	0.7813	0.0070	0.2010	0.131	0.068	7.05	0.8849	0.1070	<0.0001	0.030	0.052	7.13
0.8172	0.0701	0.1033	0.075	0.060	0.7539	0.0050	0.2300	0.117	0.071	7.03	0.8692	0.1230	<0.0001	0.027	0.050	7.15
								3*glu[a]								
0.9034	0.0449	0.0499	0.329	0.010	0.8816	0.0255	0.0910	0.345		6.44	0.9233	0.0625	0.0125	0.320		6.45
0.8831	0.0449	0.0702	0.329	0.010	0.8426	0.0125	0.1430	0.340		6.39	0.9108	0.0845	0.0030	0.306		6.43
0.8577	0.0603	0.0802	0.329	0.010	0.8096	0.0075	0.1810	0.346		6.36	0.8953	0.1020	0.0010	0.298		6.41
0.8380	0.0702	0.0900	0.329	0.010	0.7811	0.0040	0.2130	0.337		6.32	0.8803	0.1180	<0.0001	0.284		6.39
0.8068	0.0894	0.1020	0.294	0.010	0.7371	<0.0001	0.2610	0.338		6.32	0.8523	0.1460	<0.0001	0.266		6.40
								3*lys[a]								
0.8604	0.0549	0.0746	0.766	0.060	0.8100	0.0100	0.1690	0.781	0.066	6.98	0.8983	0.0900	0.0030	0.374	0.054	7.04
0.8090	0.0718	0.1091	0.784	0.060	0.7440	0.0050	0.2390	0.878	0.071	6.95	0.8649	0.1270	<0.0001	0.317	0.050	7.06
0.7788	0.0829	0.1286	0.762	0.058	0.6999	0.0040	0.2840	0.875	0.072	6.94	0.8426	0.1500	<0.0001	0.224	0.046	7.07
								5*lys[a]								
0.8600	0.0544	0.0740	1.00	0.068	0.8088	0.0100	0.1680	1.46	0.076	6.93	0.8978	0.0890	0.0030	0.682	0.061	6.99
0.8095	0.0709	0.1094	1.01	0.059	0.7332	0.0040	0.2500	1.70	0.072	6.92	0.8599	0.1320	<0.0001	0.401	0.049	7.02
0.7743	0.0849	0.1299	1.01	0.063	0.6927	0.0030	0.2900	1.86	0.080	6.91	0.8389	0.1530	<0.0001	0.294	0.051	7.01

[a] PEG and DEX not analyzed.

nonspecific electrostatic interactions, whereas all other interactions are summarized in an osmotic virial type of expression where concentrations are expressed through relative surface fractions.

$$\frac{G^{\mathrm{E}}}{n_{\mathrm{w}}RT} = -\frac{M_{\mathrm{w}}}{1000}A_{\varphi}\frac{4I}{b}\ln(1+b\sqrt{I}) + \left(\frac{1000}{M_{\mathrm{w}}}\right)^2 \sum_{i\neq \mathrm{w}}\sum_{j\neq \mathrm{w}} \frac{\Theta_i}{\Theta_{\mathrm{w}}}\frac{\Theta_j}{\Theta_{\mathrm{w}}}A_{i,j} \quad (1)$$

n_{w} is the number of moles of water, R is the universal gas constant, T is the absolute temperature; M_{w} is the molecular mass of water, and A_{φ} is the Debye−Hückel constant of water (A_{φ}(293 K) = 0.3882). I is ionic strength on a molality scale:

$$I = \frac{1}{2}\sum_{i=1}^{N} m_i z_i^2 \quad (2)$$

m_i and z_i are molality and charge number of species i. b = 1.2 is a parameter in the modified Debye-Hückel term. Θ_i is the surface fraction of species i

$$\Theta_i = \frac{m_i q_i}{\sum_{\text{all comp. } j} m_j q_j} \quad (3)$$

where q_i is the surface parameter of species i. $A_{i,j}$ is a binary parameter (i.e., second osmotic virial coefficient) characterizing interactions between surface sites of species i and j.

The surface parameter of species i, q_i, is calculated from the number of groups j in species i, $\nu_j^{(i)}$, and the surface parameter of group j, Q_j:

$$q_i = \sum_{\text{all groups } j} \nu_j^{(i)} Q_j \quad (4)$$

The virial coefficient for interactions between species i and j, $A_{i,j}$, is calculated from the surface fraction of group l in species i, $\Theta_l^{(i)}$, and those of group m in species j, $\Theta_m^{(j)}$, and binary group interaction parameters $a_{l,m}$:

$$A_{i,j} = \sum_{\text{all groups } l}\sum_{\text{all groups } m} \Theta_l^{(i)}\Theta_m^{(j)} a_{l,m} \quad (5)$$

$\Theta_l^{(i)}$ is the relative contribution of group l to the surface parameter of species i, q_i.

$$\Theta_l^{(i)} = \nu_l^{(i)} Q_l / q_i \quad (6)$$

In analogy to Pitzer's G^{E} model, the binary parameter for interactions between groups $a_{l,m}$ is written as the sum of one term that does not and another term that does depend on ionic strength:

$$a_{l,m} = a_{l,m}^{(0)} + a_{l,m}^{(1)}\frac{1}{2I}[1 - (1 + 2\sqrt{I})\exp(-2\sqrt{I})] \quad (7)$$

Parameters $a_{l,m}^{(0)}$ and $a_{l,m}^{(1)}$ are symmetric, i.e., $a_{l,m}^{(0)} = a_{m,l}^{(0)}$ and $a_{l,m}^{(1)} = a_{m,l}^{(1)}$ and are zero for m = l as well as for m or l representing water.

The resulting expressions for the activity of a solute species $i \neq$ w and for the activity of water are

$$\ln a_{i\neq \mathrm{w}} = \ln m_i - A_{\varphi} z_i^2\left[\frac{\sqrt{I}}{1+b\sqrt{I}} + \frac{2}{b}\ln(1+b\sqrt{I})\right] + 2\left(\frac{1000}{M_{\mathrm{w}}}\right)^2 \frac{q_i}{q_{\mathrm{w}}}\sum_{j\neq \mathrm{w}}\frac{\Theta_j}{\Theta_{\mathrm{w}}}[A_{i,j}^{(0)} + A_{i,j}^{(1)} f_2(I)] - z_i^2 f_3(I)\left(\frac{1000}{M_{\mathrm{w}}}\right)^3 \sum_{j\neq \mathrm{w}}\sum_{k\neq \mathrm{w}}\frac{\Theta_j}{\Theta_{\mathrm{w}}}\frac{\Theta_k}{\Theta_{\mathrm{w}}}A_{j,k}^{(1)} \quad (8)$$

Table 3. Partitioning of Some Amino Acids (AS), Dipeptides, and Higher Peptides in Aqueous Two-Phase Systems of PEG 35000 and DEX 500 at 293.15 K

(a) Amino Acids (AS)

feed					lower phase						upper phase					
water, g/g	PEG, g/g	DEX, g/g	AS, mg/g	buffer, mol/kg	water, g/g	PEG, g/g	DEX, g/g	AS, mg/g	buffer, mol/kg	pH	water, g/g	PEG, g/g	DEX, g/g	AS, mg/g	buffer, mol/kg	pH
							gly									
0.9301	0.0252	0.0330	1.00	0.069	0.9106	0.0039	0.0732	1.04	0.072	6.79	0.9453	0.0383	0.0051	0.99	0.066	6.80
0.9181	0.0300	0.0402	1.00	0.069	0.8870	0.0024	0.0981	1.05	0.074	6.78	0.9404	0.0464	0.0023	0.99	0.064	6.79
0.8733	0.0530	0.0621	1.00	0.069	0.8154	0.0006	0.1702	1.06	0.082	6.79	0.9064	0.0832	0.0004	0.95	0.059	6.79
0.8274	0.0703	0.0906	1.00	0.069	0.7354	0.0003	0.2504	1.05	0.083	6.76	0.8742	0.1158	0.0003	0.90	0.057	6.81
							glu[a]									
0.9139	0.0309	0.0450	0.99	0.059	0.8851	0.0030	0.1010	0.93	0.064	6.79	0.9385	0.0500	0.0020	0.89	0.055	6.83
0.8888	0.0406	0.0597	1.00	0.064	0.8481	0.0010	0.1390	0.86	0.071	6.79	0.9203	0.0690	0.0010	0.90	0.057	6.85
0.8304	0.0594	0.0998	1.00	0.060	0.7719	<0.0001	0.2160	0.74	0.073	6.77	0.8796	0.1120	<0.0001	0.81	0.049	6.87
0.7752	0.0854	0.1293	1.00	0.059	0.6950	<0.0001	0.2920	0.69	0.079	6.75	0.8405	0.1520	<0.0001	0.71	0.044	6.90
0.8899	0.0452	0.0554	1.00	0.055	0.8452	0.0002	0.1443		0.066	6.66	0.9154	0.0740	0.0005	0.95	0.059	6.72
0.8700	0.0503	0.0704	1.00	0.053	0.8202	0.0001	0.1667	1.03	0.077	6.66	0.9032	0.0870	0.0002	0.92	0.056	6.72
0.8453	0.0601	0.0849	1.02	0.056	0.7892	<0.0001	0.1979	1.03	0.077	6.66	0.8847	0.1060	0.0001	0.91	0.054	6.69
0.8256	0.0700	0.0949	1.00	0.055	0.7660	<0.0001	0.2237	0.99	0.060	6.64	0.8701	0.1220	0.0001	0.87	0.045	6.69
0.8059	0.0798	0.1048	1.00	0.055	0.7353	<0.0001	0.2509	0.90	0.083	6.65	0.8560	0.1355	0.0005	0.84	0.046	6.70
							phe									
0.9392	0.0249	0.0331	1.25	0.010	0.9207	0.0069	0.0696	1.27		7.00	0.9522	0.0370	0.0080	1.29		7.02
0.9185	0.0311	0.0477	1.16	0.010	0.8823	0.0020	0.1130	1.13		6.97	0.9443	0.0510	0.0020	1.18		7.02
0.8949	0.0430	0.0592	1.31	0.010	0.8555	0.0012	0.1405	1.29		6.99	0.9266	0.0700	0.0005	1.36		7.04
0.8897	0.0445	0.0646	1.19	0.010	0.8398	0.0005	0.1570	1.18		6.98	0.9217	0.0755	<0.0001	1.27		7.06
							lys									
0.9321	0.0250	0.0334	0.89	0.056	0.9191	0.0050	0.0756	1.01	0.060	7.12	0.9490	0.0367	0.0062	0.89	0.047	7.18
0.9199	0.0303	0.0403	0.89	0.055	0.8908	0.0021	0.0971	1.01	0.058	7.11	0.9415	0.0475	0.0026	0.86	0.048	7.13
0.8986	0.0410	0.0510	0.89	0.055	0.8625	0.0009	0.1256	1.05	0.064	7.11	0.9251	0.0659	0.0008	0.83	0.047	7.14
0.8750	0.0530	0.0625	0.89	0.055	0.8276	0.0003	0.1605	1.10	0.068	7.11	0.9083	0.0831	0.0004	0.79	0.048	7.14
0.8303	0.0699	0.0904	0.89	0.055	0.7746	0.0003	0.2130	1.18	0.071	7.10	0.8769	0.1153	0.0004	0.70	0.043	7.16
0.9279	0.0253	0.0328	1.00	0.084	0.8907	0.0042	0.0906	1.09	0.086	6.89	0.9434	0.0390	0.0048	0.96	0.076	6.99
0.9168	0.0298	0.0397	1.00	0.082	0.8892	0.0018	0.0942	1.11	0.087	6.99	0.9336	0.0515	0.0025	0.96	0.074	7.01
0.8947	0.0412	0.0505	1.00	0.081	0.8510	0.0010	0.1331	1.17	0.088	6.96	0.9194	0.0675	0.0007	0.93	0.073	7.02
0.8705	0.0529	0.0629	1.00	0.082	0.8225	0.0006	0.1611	1.25	0.094	6.97	0.9117	0.0757	0.0004	0.88	0.073	7.03
0.8252	0.0707	0.0903	1.00	0.082	0.7670	0.0005	0.2150	1.37	0.104	6.95	0.8686	0.1195	0.0006	0.80	0.068	7.05

(b) Dipeptides

feed					lower phase						upper phase					
water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH
							gly-gly									
0.9342	0.0262	0.0371	0.96	0.010	0.9084	0.0040	0.0850	1.01		6.83	0.9521	0.0400	0.0055	0.89		6.86
0.9150	0.0354	0.0470	1.00	0.010	0.8729	0.0015	0.1230	1.05		6.81	0.9400	0.0560	0.0015	0.90		6.86
0.9008	0.0462	0.0504	1.01	0.010	0.8503	0.0010	0.1460	1.12		6.82	0.9275	0.0695	0.0005	0.93		6.87
0.8890	0.0561	0.0523	1.00	0.010	0.8242	0.0001	0.1730	1.10		6.81	0.9169	0.0810	0.0007	0.85		6.88
							glu-glu									
0.9328	0.0253	0.0332	1.01	0.049	0.9055	0.0079	0.0779	0.89	0.050	6.60	0.9462	0.0397	0.0060	0.85	0.047	6.62
0.9209	0.0301	0.0402	1.01	0.050	0.8903	0.0030	0.0979	0.91	0.051	6.60	0.9369	0.0520	0.0028	0.85	0.048	6.61
0.9016	0.0399	0.0498	1.01	0.050	0.8632	0.0010	0.1266	0.93	0.053	6.60	0.9254	0.0654	0.0013	0.86	0.045	6.64
0.8759	0.0530	0.0622	1.01	0.051	0.8311	0.0006	0.1589	0.94	0.054	6.61	0.8746	0.1172	0.0009	0.81	0.042	6.66
0.8324	0.0703	0.0885	0.96	0.050	0.7769	0.0005	0.2129	0.95	0.057	6.60	0.8732	0.1189	0.0010	0.75	0.040	6.69
							lys-lys[b]									
0.9143	0.0295	0.0456	1.01	0.061	0.8847	0.0030	0.1010	1.03	0.066	7.00	0.9384	0.0500	0.0020	0.770	0.057	7.00
0.8900	0.0398	0.0597	1.00	0.061	0.8483	0.0010	0.1390	1.07	0.068	7.01	0.9207	0.0690	0.0010	0.732	0.055	7.02
0.8642	0.0502	0.0752	1.00	0.061	0.8169	0.0010	0.1700	1.15	0.071	7.06	0.9002	0.0900	0.0010	0.687	0.052	7.04
0.8307	0.0595	0.0994	0.992	0.061	0.7713	<0.0001	0.2160	1.15	0.074	6.98	0.8797	0.1120	<0.0001	0.578	0.050	7.06

(c) Higher Peptides

feed					lower phase						upper phase					
water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH
							5*gly[c]									
0.9326	0.0250	0.0328	0.698	0.057	0.9424	0.0043	0.0430	0.749	0.061	6.90	0.9458	0.0384	0.0058	0.698	0.060	6.90
0.9195	0.0305	0.0406	0.702	0.057	0.8901	0.0022	0.0976	0.772	0.060	6.87	0.9369	0.0516	0.0026	0.695	0.053	6.91
0.9012	0.0406	0.0490	0.692	0.055	0.8627	0.0013	0.1260	0.794	0.059	6.90	0.9242	0.0666	0.0006	0.702	0.050	6.91
0.8753	0.0531	0.0622	0.700	0.056	0.8297	0.0006	0.1590	0.805	0.064	6.86	0.9174	0.0734	0.0005	0.678	0.052	6.93
0.8501	0.0619	0.0798	0.619	0.049	0.7969	0.0002	0.1938	0.783	0.053	6.87	0.8885	0.1043	0.0003	0.625	0.041	6.96
0.8744	0.0530	0.0626	0.588	0.060	0.8272	0.0005	0.1603	0.661	0.073	6.85	0.9099	0.0805	0.0004	0.580	0.055	6.91
0.8877	0.0350	0.0670	0.401	0.064	0.8545	0.0020	0.1320	0.439	0.072	6.94	0.9207	0.0690	0.0010	0.391	0.057	7.02
0.8699	0.0439	0.0749	0.384	0.070	0.8300	0.0010	0.1560	0.430	0.081	6.94	0.9052	0.0840	0.0010	0.360	0.071	6.99
0.8453	0.0552	0.0899	0.396	0.060	0.7864	<0.0001	0.2020	0.465	0.072	6.94	0.8899	0.1020	<0.0001	0.342	0.050	7.04
0.8067	0.0679	0.1153	0.302	0.073	0.7433	<0.0001	0.2440	0.372	0.079	6.94	0.8641	0.1280	<0.0001	0.246	0.049	7.04

Table 3 (Continued)

(c) Higher Peptides

feed					lower phase						upper phase					
water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH	water, g/g	PEG, g/g	DEX, g/g	peptide, mg/g	buffer, mol/kg	pH
								6*gly[c]								
0.9084	0.0296	0.0526	0.085	0.060	0.8839	0.0030	0.1030	0.095	0.064	7.05	0.9384	0.0510	0.0020	0.040	0.055	7.08
0.8949	0.0395	0.0564	0.085	0.059	0.8607	0.0010	0.1280	0.107	0.065	7.01	0.9248	0.0660	0.0010	0.038	0.053	7.07
0.8652	0.0501	0.0751	0.085	0.061	0.8179	0.0010	0.1700	0.113	0.071	6.98	0.9009	0.0900	0.0010	0.040	0.052	7.07
0.8311	0.0601	0.0995	0.084	0.060	0.7725	<0.0001	0.2160	0.130	0.073	6.96	0.8803	0.1120	<0.0001	0.039	0.049	7.07
								3*glu								
0.9303	0.0263	0.0335	0.433	0.061	0.9060	0.0040	0.0800	0.461	0.061	6.77	0.9447	0.0400	0.0060	0.437	0.057	6.80
0.9182	0.0311	0.0396	0.427	0.069	0.8854	0.0030	0.1000	0.456	0.072	6.78	0.9367	0.0500	0.0030	0.424	0.064	6.82
0.9002	0.0408	0.0491	0.426	0.061	0.8637	0.0020	0.1240	0.463	0.063	6.78	0.9254	0.0640	0.0020	0.423	0.053	6.83
0.8731	0.0540	0.0620	0.427	0.068	0.8246	0.0010	0.1630	0.478	0.070	6.77	0.9039	0.0860	0.0010	0.403	0.056	6.84
0.8295	0.0711	0.0897	0.430	0.060	0.7641	<0.0001	0.2260	0.497	0.060	6.76	0.8757	0.1170	<0.0001	0.383	0.044	6.88
								3*lys[c]								
0.8953	0.0325	0.0622	0.779	0.060	0.8640	0.0010	0.1240	0.777	0.066	7.01	0.9272	0.0620	0.0020	0.466	0.054	7.06
0.8413	0.0473	0.1001	0.767	0.068	0.7937	0.0010	0.1920	0.751	0.081	6.98	0.8936	0.0970	<0.0001	0.343	0.058	7.07
0.7932	0.0669	0.1298	0.773	0.060	0.7272	<0.0001	0.2600	0.768	0.077	7.08	0.8576	0.1350	<0.0001	0.266	0.046	7.12
								5*lys[c]								
0.8891	0.0400	0.0606	1.01	0.059	0.8481	0.0010	0.1390	1.62	0.067	6.96	0.9209	0.0690	0.0010	0.770	0.054	7.09
0.8291	0.0595	0.1012	1.01	0.059	0.7710	<0.0001	0.2160	1.65	0.073	6.94	0.8800	0.1120	<0.0001	0.428	0.049	7.04
0.7742	0.0855	0.1300	1.01	0.060	0.6936	<0.0001	0.2920	1.88	0.081	6.92	0.8409	0.1520	<0.0001	0.238	0.044	7.07

[a] PEG and DEX not analyzed. [b] PEG, DEX, and buffer not analyzed. [c] Water, PEG, DEX, and buffer not analyzed.

PEG is assumed to consist of PEG middle and PEG end groups. Each PEG molecule contains two end groups. The number of PEG middle groups N_{middle} depends on the molecular mass. For PEG 6000 and PEG 35000, N_{middle} is 140 and 773, respectively. DEX 500 is assumed to consist of 1106 dextran groups. All parameters required for describing the aqueous two-phase equilibrium in systems with PEG and DEX were taken from the literature (Grossmann et al., 1995b). They are given in Table 4 (surface parameters Q) and Table 5 (interaction parameters). For incorporating the distribution of solute species, solutes are split into groups and group interaction parameters between all groups—with the exception of water—present in the aqueous two-phase system have to be known.

$$\ln a_w = -\frac{M_w}{1000}\left[\sum_{i\neq w} m_i - 2A_\varphi \frac{I^{1.5}}{1+b\sqrt{I}}\right] - \left(\frac{1000}{M_w}\right)^2 \sum_{i\neq w}\sum_{j\neq w} \frac{\Theta_i}{\Theta_w}\frac{\Theta_j}{\Theta_w}[A_{i,j}^{(0)} + A_{i,j}^{(1)} \exp(-2\sqrt{I})] \quad (9)$$

$$A_{i,j}^{(0)} = \sum_{\text{all groups } l}\ \sum_{\text{all groups } m} \Theta_l^{(i)}\Theta_m^{(j)} a_{l,m}^{(0)} \quad (10)$$

$$A_{i,j}^{(1)} = \sum_{\text{all groups } l}\ \sum_{\text{all groups } m} \Theta_l^{(i)}\Theta_m^{(j)} a_{l,m}^{(1)} \quad (11)$$

$$f_2(I) = \frac{1}{2I}[1 - (1 + 2\sqrt{I}) \exp(-2\sqrt{I})] \quad (12)$$

$$f_3(I) = \frac{1}{4I^2}[1 - (1 + 2\sqrt{I} + 2I) \exp(-2\sqrt{I})] \quad (13)$$

The amino acids are treated as single groups. Amino acids are amphoteric substances. They exist as neutral molecules (zwitterions), cations, and anions. Around the isoelectric point pH ≈ pI, where pI = (pK_I + pK_{II})/2, both functional groups are charged and the main chain carries no net charge. As the isoelectric point of all amino acids investigated here is close to the pH of the coexisting phases (pI ≈ 5.5/6.0; pH ≈ 7) the majority of the chains of an amino acid carries no net charge. For similar reasons, at pH ≈ 7, the carboxylic group in the side chain R of glutamic acid (pK = 4.25) is nearly completely dissociated and most amino groups in the side chain of lysine molecules (pK = 10.28) are protonated. Thus for modeling the partitioning of the amino acids in PEG/DEX/water, buffered to pH ≈ 7, glycine and phenylalanine exist mainly as neutral species, whereas glutamic acid and lysine exist mainly as anions and cations, respectively. Applying the same reasoning to the peptides investigated here yields that most peptide molecules of the single amino acids glycine and phenylalanine carry no net charge, whereas most molecules of peptides of glutamic acid and lysine are charged. Each peptide consisting of N_{AS} amino acid molecules is split into N_{AS} groups representing the single amino acid. However, there are different groups of the same amino acid depending on the position in the peptide: left terminal end group,

Table 4. Group Surface Parameters Q_k (Tintinger, 1995)

group	k	Q_k
water	1	1.40
H_3O^+	2	1.40
OH^-	3	1.40
PEG middle group: $-CH_2-O-CH_2-$	4	1.32
PEG end group: $-CH_2-OH$	5	1.74
DEX	6	5.76
K^+	7	1.40
PO_4^{3-}	8	1.40
HPO_4^{2-}	9	1.40
$H_2PO_4^-$	10	1.40
H_3PO_4	11	1.40
glycine	12	2.460
glycine: left terminal group	13	1.788
glycine: middle group	14	1.488
glycine right terminal group	15	2.160
glutamic acid[a]	16	4.452
glutamic acid: left terminal group[a]	17	3.780
glutamic acid: middle group[a]	18	3.480
glutamic acid: right terminal group[a]	19	4.152
phenylalanine	20	4.808
phenylalanine: left terminal group	21	4.136
phenylalanine: middle group	22	3.836
phenylalanine: right terminal group	23	4.508
lysine	24	5.004
lysine: left terminal group	25	4.332
lysine: middle group	26	4.032
lysine: right terminal group	27	4.704

[a] Charged side chain.

Table 5. Binary Interaction Parameters $a^{(0)}_{k,m}$ and $a^{(1)}_{k,m}$

k	m	$a^{(0)}_{k,m}$
4;5	4;5	0.00914
4;5	7	0.0450
4;5	9	0.0463
6	4;5	0.00478
6	6	0.00155
6	7	−0.0140
7	9	0.0662
7	8	0.425
7	8	6.568[a]
4;5	12;15	0.0315
4;5	13	0.0121
4;5	14	−0.00290
4;5	16;19	0.0122
4;5	17	−0.0133
4;5	18	−0.00869
4;5	20;23	0.00392
4;5	21	−0.0166
4;5	22	−0.0180
4,5	24;27	0.0214
4;5	25	0.00923
4;5	26	−0.00937

[a] $a^{(1)}_{k,m}$.

middle group, and right terminal end group. These groups have different surface parameters Q. Surface parameters were calculated using the method of Bondi (1964). They are given in Table 4. In principle that method should also be applied to calculate the surface parameters for ions resulting from the dissolved buffer molecules. However, for the sake of simplicity the surface parameter of water ($Q_w = 1.40$) was adopted for potassium and all phosphate ions as well as for H_3PO_4. This crude assumption is to be corrected when more experimental material for the properties of phosphate-buffered aqueous solutions becomes available.

Estimation of Interaction Parameters. For calculating the liquid−liquid equilibrium, besides chemical equilibrium constants and group surface parameters also binary parameters for interaction between solute groups m and l, $a^{(0)}_{l,m}$ and $a^{(1)}_{l,m}$, have to be known. Parameters for interactions between PEG and DEX groups were taken from Grossmann et al. (1995b); those for interactions between PEG groups and potassium ions as well as phosphate (ions and neutral molecules) were taken from Grossmann et al. (1995a). All other parameters were estimated using the experimental data presented here. As, in principle, the number of unknown interaction parameters is rather large, some simplifying assumptions were necessary. As the concentrations of the amino acids and peptides as well as that of the buffer are rather small, all parameters for interactions between these solutes were neglected (i.e., interaction parameters were set to zero). A single interaction parameter proved to be sufficient for correlating the uneven distribution of the buffer in the aqueous two-phase system. That parameter $a^{(0)}_{6,7}$ describes the influence of interactions between DEX groups (group number 6) and potassium ions (group number 7). It was adjusted to achieve a correlation that represents the experimental results for the partitioning of the buffer (i.e., for experimental results for the potassium concentrations in the coexisting phases) within experimental uncertainty: $a^{(0)}_{6,7} = -0.0140$. However, there are still six parameters: $a^{(0)}_{AS,m}$ and $a^{(1)}_{AS,m}$ for an amino acid group AS interacting with either one of the PEG groups ($m = 4$ or 5) or with the DEX group ($m = 6$). However, only a single parameter can be determined in a reasonable way from the experimental data on the partitioning of that amino acid in the two-phase systems discussed here. Therefore and in agreement with the results of a similar investigation on the partitioning of amino acids and peptides in the two-phase system PEG/K_2HPO_4/water (Grossmann et al., 1997; Tintinger et al., 1997); the following simplifications were applied:

(a) It was assumed that $a^{(1)}_{AS,m} = 0$ for all amino acid groups AS and m representing any PEG or DEX group (i.e., $m = 4$ or 5 or 6).

(b) It was not distinguished between PEG middle and PEG end groups (i.e., groups 4 and 5) as far as interactions with an amino acid group AS are concerned ($a^{(0)}_{AS,4} = a^{(0)}_{AS,5}$).

(c) The parameter for interactions between that amino acid group and a DEX group was set to zero (i.e., $a^{(0)}_{AS,6} = 0$).

Thus parameter $a^{(0)}_{AS,4} = a^{(0)}_{AS,5}$ indeed represents the effect of differences in the interactions of the amino acid group AS with PEG or DEX groups.

While the influence of a net charge on an amino group was neglected, the influence of the position of an amino acid group in a peptide was taken into account. This was done by assuming different numbers for interaction parameters between the PEG group on one side and amino acid groups on the other side, depending on the position of that amino acid group in a peptide (in left terminal, middle, or right terminal position). However, for the sake of simplicity, no difference was made between a group representing a pure amino acid molecule and that amino acid group in right terminal position in a peptide (e.g., $a^{(0)}_{12,4} = a^{(0)}_{15,4} \neq a^{(0)}_{13,4} \neq a^{(0)}_{14,4}$, where subscripts 12, 13, 14, and 15 stand for glycine as a pure substance, in left terminal, in middle, and in right terminal position, respectively).

All remaining, nonzero parameters for interactions with amino acid groups were fitted to the new experimental results for the partitioning of the amino acids and their di- and tripeptides in the aqueous two-phase system PEG 6000/DEX 500/water in the presence of small amounts of K_2HPO_4/KH_2PO_4 buffer at 293 K (i.e., the amino acid and peptide concentrations given in Table 2a−c). All nonneglected binary interaction parameters are given in Table 5. It is recommended to restrict the use of that set of parameters to about $6.5 \leq pH \leq 7.5$.

Comparison with Experimental Data. Phase equilibrium calculations were performed by minimization of the Gibbs energy of the experimental feed. Details of that procedure are available elsewhere (Grossmann, 1994). Thus the compositions of the coexisting phases were calculated. As a comparison between calculated and measured compositions in the two-phase system PEG/DEX/water is available in the literature (Grossmann et al., 1995b) and the presence of small amounts of amino acids, peptides, and K_2HPO_4/KH_2PO_4-buffer has only a very small influence on the polymer concentrations in the coexisting phases, the comparison between calculated and measured concentrations is mainly restricted to the partition coefficient of the amino acids and their peptides. The model correlates the experimental results for the partitioning of the amino acids and their di- and tripeptides within the experimental uncertainty. Typical examples are shown in Figures 1−4 where calculated partition coefficients are compared to the experimental results for glycine, L-glutamic acid, L-phenylalanine, and L-lysine as well as for the corresponding di- and tripeptides. In these figures the coexisting phases are characterized by the difference in the mass fraction of PEG in both phases: $\Delta\xi_{PEG} = \xi_{PEG,upper\ phase} - \xi_{PEG,lower\ phase}$. *Experimental* data for the partition coefficients are assigned to *experimental* data for $\Delta\xi_{PEG}$, while *calculated* partition coefficients are assigned to *calculated*

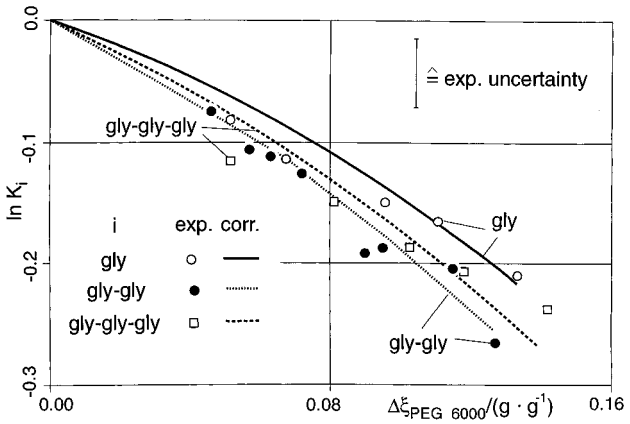


Figure 1. Comparison between measured and correlated partition coefficients of glycine and its di- and tripeptide in aqueous two-phase systems of PEG 6000 and DEX 500 (buffered with K_2HPO_4/KH_2PO_4) at 293.15 K.

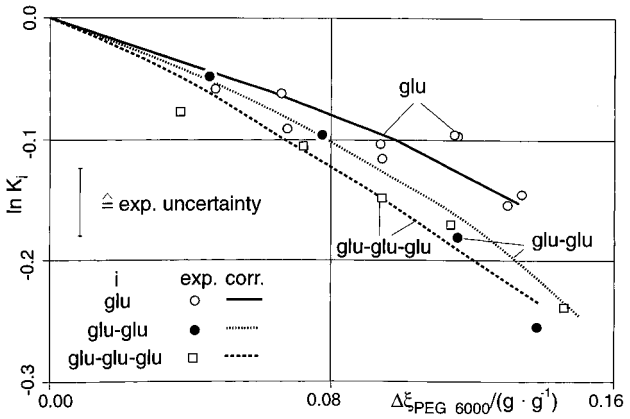


Figure 2. Comparison between measured and correlated partition coefficients of L-glutamic acid and its di- and tripeptide in aqueous two-phase systems of PEG 6000 and DEX 500 (buffered with K_2HPO_4/ KH_2PO_4) at 293.15 K.

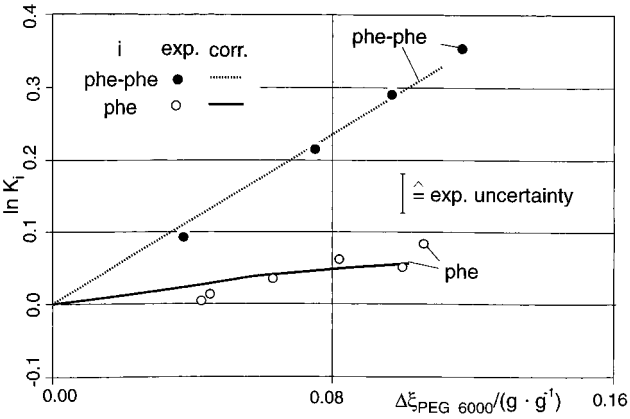


Figure 3. Comparison between measured and correlated partition coefficients of L-phenlyalanine and its dipeptide in aqueous two-phase systems of PEG 6000 and DEX 500 (buffered with K_2HPO_4/ KH_2PO_4) at 293.15 K.

numbers for $\Delta\xi_{PEG}$. The model does not distinguish between interactions of amino acid groups on one side and PEG middle or PEG end groups on the other side. Therefore it is not surprising that the model also correctly predicts that replacing PEG 6000 by PEG 35000 has nearly no influence on the partitioning of amino acids and peptides.

The set of interaction parameters was also used in model predictions of the partition coefficient of peptides 5*gly, 6*gly, and 5*lys. Predicted partition coefficients of 5*gly are only about 5% higher than the experimental results. However, that good agreement is not confirmed for either 5*lys or 6*gly. For 5*lys predicted partition coefficients are about 40% smaller than the experimental results—for example, $K_{5*lys,exp} \approx 0.25$ and $K_{5*lys,pred} \approx 0.15$ in the same two-phase system. In contrary to the experimental findings, the model predicts only a small preference of 6*gly for the DEX-rich lower phase—for example, $K_{6*gly,pred} \approx 0.9$ and $K_{6*gly,exp} \approx 0.37$ in the same two-phase system.

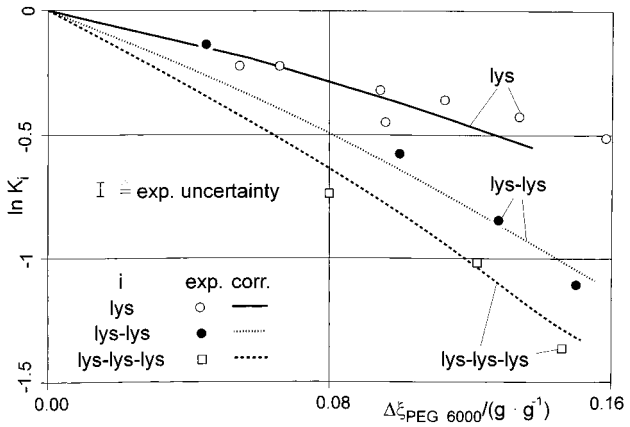


Figure 4. Comparison between measured and correlated partition coefficients of L-lysine and its di- and tripeptide in aqueous two-phase systems of PEG 6000 and DEX 500 (buffered with K_2HPO_4/ KH_2PO_4) at 293.15 K.

The model predicts that the addition of such small amounts of K_2HPO_4/KH_2PO_4 buffer at 293 K has only a very small influence on the liquid—liquid equilibrium. It correctly predicts that the miscibility gap is somewhat increased by small amounts of that buffer. The model also predicts a small pH difference between both aqueous phases. Although the predicted absolute number for that difference agrees well with the experimental results (ΔpH $\approx$ 0.01/0.05), the sign of the predicted difference does not agree with the experimental results. The pH difference is nearly 1 order of magnitude smaller than the uncertainty of a single pH measurement ($\approx$0.1). Therefore it remains an open question if that failure is due to the experimental uncertainty or to the model.

Aqueous two-phase systems are used in downstream processing polypeptides. There it is common practice to correlate the partitioning of polyelectrolytes by applying the concept of an electrical potential difference between the coexisting phases (cf., for example, Albertsson (1986)). From the Gibbs excess energy model presented before, that potential difference can be calculated (for details, see Grossmann and Maurer (1995)). Figure 5 shows the results of such a calculation for the system PEG 6000/DEX 500/water buffered with about 60 mmol K_2HPO_4/KH_2PO_4 per kg of water at 293 K. The electrical potential difference, $\Delta\Phi = \Phi_{upper\ phase} - \Phi_{lower\ phase}$, is negative. Its absolute number increases with increasing difference between the coexisting phases. This is in accordance with the observation that positively charged polyelectrolytes prefer the PEG-rich upper phase over the DEX-rich lower phase. Maximum absolute numbers for $\Delta\Phi$ reach about 2 mV, but as that property cannot be measured directly, no comparison with experimental data is possible.

Conclusion

Experimental results are reported for the partitioning of small amounts of amino acids glycine, L-glutamic acid, L-phenylalanine, and L-lysine, four dipetides, three trip-

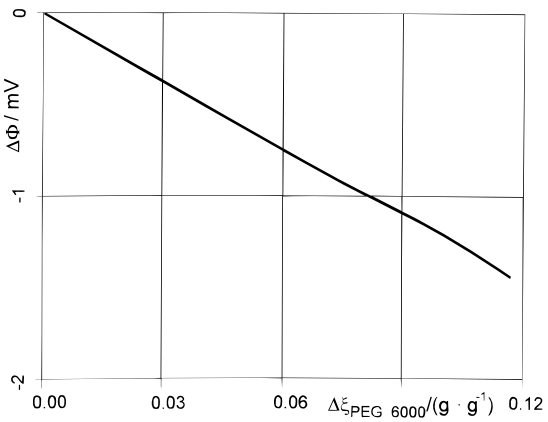


Figure 5. Calculated electrical potential difference: $\Delta\Phi = \Phi_{upper\ phase} - \Phi_{lower\ phase}$ in the aqueous two-phase system of PEG 6000 and DEX 500 (buffered with 0.06 mmol K_2HPO_4/KH_2PO_4 per kg solution) at 293.15 K.

eptides, and three higher peptides (peptides consisiting of a single amino acid only, i.e., no combination peptides) in aqueous two-phase systems of high molecular poly(ethylene glycol) (PEG 6000 or PEG 35000) and DEX 500 at 293 K in the presence of small amounts of K_2HPO_4/KH_2PO_4-buffer. The experimental results for the partition coefficient of the amino acids and peptides are correlated/predicted using a semiempirical group contribution model for the Gibbs excess energy. The model is an osmotic virial equation. It uses surface fractions to account for the probability of interactions between solutes. Some model parameters were taken from the literature, while others were estimated from the new experimental data. Calculated partition coefficients agree favorably with the experimental data.

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