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PHYSICO-CHEMICAL FACTORS GOVERNING PARTITION BEHAVIOUR OF SOLUTES AND PARTICLES IN AQUEOUS POLYMERIC BIPHASIC SYSTEMS.

I. EFFECT OF IONIC COMPOSITION ON THE RELATIVE HYDROPHOBICITY OF THE PHASES

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SUMMARY

Partition coefficients for a homologous series of dinitrophenylated amino acids with aliphatic side-chains have been determined in aqueous polymeric Dextran 500–poly(ethylene glycol) 6000 and Ficoll 400–Dextran 70 biphasic systems of various ionic compositions, and in systems formed by *n*-octanol and the aqueous phases of the above systems. The free energy of transfer of a CH_2 group from one phase to the other in these systems was estimated and used as a measure of the hydrophobic character of an aqueous phase with respect to *n*-octanol. The results show that the hydrophobic properties of both phases of the aqueous polymeric biphasic systems are greatly affected by the ionic composition. It is demonstrated that solute partition behaviour is governed by the relative hydrophobicity of the phases, although this is not the only factor important for the partition of ionogenic solutes.

INTRODUCTION

Among the biphasic systems suitable for the partition of biological materials, the so-called aqueous polymeric systems predominate[1–4]. They are prepared by mixing aqueous solutions of polymers, *e.g.*, Dextran 500 and poly(ethylene glycol) 6000 or Ficoll 400 and Dextran 70. These biphasic systems can be buffered and rendered isotonic (if necessary) and have proved suitable both for separation purposes and for physico-chemical studies of biopolymers, biological membranes and cell organelles[1–4]. Partitioning of biological materials in these systems can be manipulated by the choice of polymer and ionic composition and concentration[1–4]. It is believed[1–4] that, depending on the composition and concentration of the polymers and of salts present, partitioning of substance depends either on its net charge or on its hydrophobic properties. However, the theory of partitioning is far from completely understood, due to the intricacy of the phase systems used and of the biological materials partitioned.

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It has been shown previously[5–8] that one of the important features of a given biphasic system is the relative hydrophobicity of the two phases. This characteristic can be estimated[6,8] in terms of the free energy of transfer of a CH_2 group from a given phase to a solution chosen as reference. The difference in the relative hydrophobicity of the two phases can be measured by the free energy of transfer of a CH_2 group from one phase to the other[5–8].

The effect of ionic composition on the above characteristics of aqueous dextran–poly(ethylene glycol) and Ficoll–dextran biphasic systems was studied in the present work.

MATERIALS AND METHODS

Materials

Ficoll 400 (Lot 11069) was obtained from Pharmacia (Sweden), Dextran 500 Loba (Austria), Dextran 70 (Lot 580870) under the trade name Polyglucinum from Minmedprom (U.S.S.R.) and poly(ethylene glycol) 6000 (PEG 6000) from Serva (G.F.R.).

Dinitrophenylated amino acids (DNP-Gly, DNP-L-Ala) were obtained from Serva, 2,4-dinitrofluorobenzene from Calbiochem, U.S.A., L-norleucine and DL-norvaline from Reanal (Hungary) and DL-2-amino-*n*-octanoic acid from BDH (Great Britain). The amino acids were dinitrophenylated as indicated in ref. 7, and their purities were checked by thin-layer chromatography. Their sodium salts were prepared by titration. Uridine and adenine were obtained from Calbiochem. 1-Octanol and other chemicals and salts were of analytical reagent grade and were used without further purification.

Partition experiments

Two aqueous polymeric phase systems were used. They were prepared as described elsewhere[5–8] and had the following compositions: 7% (w/w) Dextran 500, 4.4% (w/w) PEG 6000 containing NaCl and sodium phosphate buffer as indicated below; 12.5% (w/w) Ficoll 400, 10.8% (w/w) Dextran 70 and NaCl and sodium phosphate buffer as indicated below.

The partition experiments were carried out as described elsewhere[5–8]. The phases of the Ficoll–dextran system were allowed to settle at 25°C for 21–24 h, then aliquots of both phases were pipetted from the system and each was used for the solute concentration measurements and for subsequent partition experiments with *n*-octanol. The absorbance of each aliquot, appropriately diluted with water, was measured against a correspondingly diluted top or bottom phase blank.

The same partition technique was used with the dextran–PEG systems. After settling of the systems, aliquots of the phases were mixed with equal volumes of *n*-octanol saturated with the corresponding buffer. The biphasic systems formed by a given aqueous polymeric phase with *n*-octanol were centrifuged for 20–30 min at 400 × *g*. The same technique was used with the buffer–*n*-octanol systems. The solute concentrations in both phases of the system were measured as above.

The partition coefficient, K, in the aqueous polymeric biphasic systems is defined as the ratio of the sample concentration in the Ficoll-rich (PEG-rich) phase to that in the Dextran 70-rich (Dextran 500-rich) phase. The partition coefficient in the

aqueous solution–*n*-octanol systems is defined as the ratio of the sample concentration in the organic phase to that in the aqueous phase.

The partition coefficients were measured for each solute over approximately ten-fold concentration ranges and were found to be independent of the solute concentration in all the biphasic systems examined. The value for each solute was determined as the mean of two measurements on three dilutions carried out three or four times in a given biphasic system.

RESULTS

The approach used is based on the linear relationship between the logarithm of the partition coefficient and the number of C atoms in the aliphatic chain of homologous solutes partitioned in a given biphasic system[5–8]. Some of the relationships found for the homologous series of DNP-amino acids with aliphatic side-chains are shown in Fig. 1. These relationships can be described by the equation

$$\ln K = C + En \tag{1}$$

where n is the average equivalent quantity of CH_2 groups in the amino acid aliphatic side-chain, C and E are constants. It is known that parameter E is relatec to the free energy of transfer of a CH_2 group from one phase to the other of a given biphasic system, $\Delta G_{tr}^{CH_2}$, according to $\Delta G_{tr}^{CH_2} = -RTE$. The value of n is the number of C

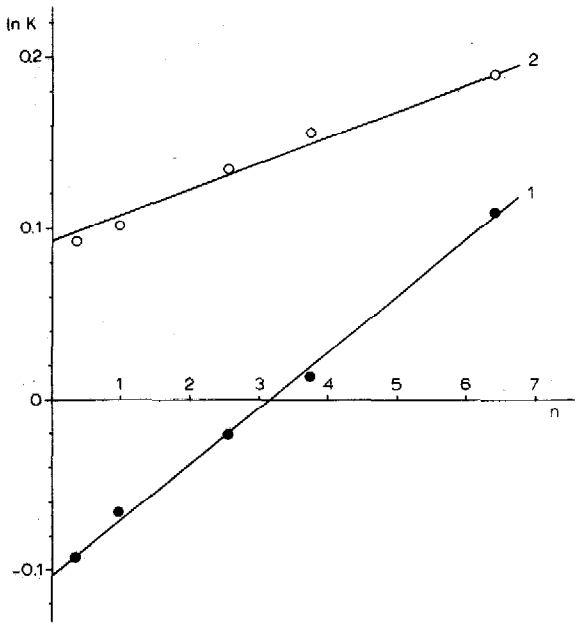

Fig. 1. Logarithm of the partition coefficient as a function of the aliphatic side-chain length, n, of dinitrophenylated amino acids: glycine, alanine, norvaline, norleucine and 2-amino-*n*-octanoic acid. 1. Dextran 500–PEG 6000 containing 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 6.15; 2, Ficoll 400–dextran 70 of the same ionic composition and pH.

atoms in the side-chain corrected for the non-linearity of the experimental relationship between the ln K and the number of C atoms caused by the effect of the polar fragment of the solute molecule on the hydrophobic properties of the side-chain[5–7,9].

A least-squares treatment of the experimental data obtained led to the C and E values listed in Tables I and II together with the corresponding $\Delta G_{tr}^{CH_2}$ values. The $\Delta G_{tr}^{CH_2}$ values characterize the differences in the relative hydrophobicity between the two phases of the aqueous polymeric biphasic systems. In order to estimate the hydrophobic chracter of each two phases relative to that of the reference solution, the above approach was applied to partitioning of the same series of DNP-amino acids in the biphasic systems formed by *n*-octanol with one of the aqueous phases of the polymeric biphasic systems. The C and E values determined for the dextran–PEG systems examined are listed ih Table III together with the corresponding values of the free energy of transfer of a CH_2 group from the aqueous phase in question to *n*-octanol. The same characteristics for the phases of the Ficoll–dextran systems are presented in Table IV.

TABLE I

PARTITION OF SODIUM SALTS OF DNP-AMINO ACIDS WITH ALIPHATIC SIDE-CHAINS IN AQUEOUS DEXTRAN–PEG BIPHASIC SYSTEM

Composition of the biphasic system: 7% (w/w) Dextran 500, 4.4% (w/w) PEG 6000, pH 6.8. Standard deviations: 0.001 in the E values, 0.8 cal/mol CH_2 in $\Delta G_{tr}^{CH_2}$ and 0.015 in C.

Phosphate buffer concentration (mol/kg)	*Concentration of NaCl (mol/kg)*	E	$-\Delta G_{tr}^{CH_2}$ *(cal/mol CH_2)*	C
0.01	–	0.0300	17.8	0.061
0.06	–	0.0317	18.8	0.090
0.085	–	0.0338	20.1	0.115
0.11	–	0.0374	22.2	0.140
0.01	0.0375	0.0234	13.8	−0.016
0.01	0.075	0.0234	13.9	−0.038
0.01	0.1125	0.0229	13.6	−0.044
0.01	0.15	0.0290	17.2	−0.053
0.01	0.20	0.0292	17.3	−0.053
0.01	0.30	0.0290	17.2	−0.053
0.01	0.50	0.0290	17.2	−0.051
0.01	1.00	0.0345	20.5	−0.030
0.06	0.075	0.0360	21.3	0.046
0.11	0.15	0.0366	21.7	0.060

DISCUSSION

Dextran–PEG biphasic systems

It has been shown previously[5] that the ionic composition of a dextran–PEG biphasic system affects the difference in the relative hydrophobicity of the phases. The results given in Table I indicate that the addition of small amounts of NaCl to a system containing 0.01 M sodium phosphate buffer, pH 6.8, reduces the difference between the relative hydrophobicity of the phases and that this difference remains

TABLE II

PARTITION OF SODIUM SALTS OF DNP-AMINO ACIDS WITH ALIPHATIC SIDE-CHAINS IN AQUEOUS DEXTRAN–PEG AND FICOLL–DEXTRAN BIPHASIC SYSTEMS AT VARIOUS IONIC COMPOSITIONS

The polymer compositions are given in the text. The standard deviations are as indicated in the footnote to Table I.

$HPO_4^{2-}/H_2PO_4^-$	*pH*	*Concentration of phosphate buffer (mol/kg)*	*Concentration of NaCl (mol/kg)*	*Dextran–PEG*			*Ficoll–dextran*		
				E	$-\Delta G_{tr}^{CH_2}$ *(cal/mol)*	*C*	*E*	$-\Delta G_{tr}^{CH_2}$ *(cal/mol)*	*C*
0.212	6.15	0.01	0.15	0.0330	19.6	−0.062	0.0142	8.4	0.093
0.212	6.15	0.11	–	0.0333	19.7	0.070	0.0142	8.4	0.235
0.360	6.40	0.01	0.15	0.0354	21.0	−0.072	0.0162	9.6	0.094
0.360	6.40	0.11	–	0.0339	20.1	0.087	0.0162	9.6	0.238
1.00	6.80	0.01	0.15	0.0290	17.3	−0.053	0.0199	11.8	0.096
1.00	6.80	0.11	–	0.0374	22.2	0.140	0.0199	11.8	0.260
4.26	7.40	0.01	0.15	0.0238	14.2	−0.059	0.0271	16.1	0.096
4.26	7.40	0.11	–	0.0386	22.9	0.152	0.0271	16.1	0.278
12.33	7.80	0.01	0.15	0.0242	14.3	−0.064	0.0300	17.8	0.102
12.33	7.80	0.11	–	0.0364	21.6	0.179	0.0300	17.8	0.301

TABLE III

PARTITION OF SODIUM SALTS OF DNP-AMINO ACIDS WITH ALIPHATIC SIDE-CHAINS IN AQUEOUS PHASE–*n*-OCTANOL BIPHASIC SYSTEMS

*Aqueous phase**	*pH***	*Concentration (M) of*		*E*	*C*	$-\Delta G_{tr}^{CH_2}$ *(cal/mol)*
		Buffer	*NaCl*			
PEG 6000	6.4	0.11	–	0.975	−3.461	578
PEG 6000	6.4	0.01	0.15	0.934	−3.086	554
Dextran 500	6.4	0.11	–	1.008	−3.324	598
Dextran 500	6.4	0.01	0.15	0.973	−3.127	577
PEG 6000	6.8	0.11	–	1.012	−3.897	600
PEG 6000	6.8	0.01	0.15	1.013	−3.676	601
Dextran 500	6.8	0.11	–	1.042	−3.771	618
Dextran 500	6.8	0.01	0.15	1.042	−3.771	618

* Enriched with the polymer indicated; polymer compositions and details of the preparation of the *n*-octanol–aqueous phase systems are given in the text.

** The $HPO_4^{2-}/H_2PO_4^-$ concentration ratios corresponding to the pH values are given in Table II.

constant up to *ca.* 0.12 *M* NaCl. Further increase of the NaCl concentration to 0.15 *M* increases the above difference which appears to be invariable in the range 0.15–0.50 *M* NaCl; a slight increase occurs at higher concentrations of the salt such as 1.0 *M*. These results are in accord with previous observations[5] for systems without sodium phosphate buffer.

As can be seen from the data in Table I, an increase in the concentration of the sodium phosphate buffer, pH 6.8, from 0.01 *M* to 0.11 *M* increases the difference between the hydrophobic character of the phases. The alterations in the hydrophobicity difference observed when the ratio of the concentrations of NaCl and buffer is changed agree with the aforementioned trends.

TABLE IV

PARTITION OF SODIUM SALTS OF DNP-AMINO ACIDS WITH ALIPHATIC SIDE-CHAINS IN AQUEOUS PHASE–*n*-OCTANOL BIPHASIC SYSTEMS

0.11 *M* sodium phosphate buffer was used in all the systems. For other details see Table III.

Aqueous phase	*pH*	*E*	*C*	$-\Delta G_{tr}^{CH_2}$ *(cal/mol)*
Ficoll	6.15	1.027	−3.249	609
Dextran 70	6.15	1.042	−3.025	618
Buffer	6.15	1.042	−3.396	618
Ficoll	6.40	1.008	−3.455	598
Dextran 70	6.40	1.025	−3.423	608
Buffer	6.40	1.042	−3.562	618
Ficoll	7.40	0.978	−3.920	580
Dextran 70	7.40	1.013	−3.825	601
Buffer	7.40	1.008	−3.686	598
Ficoll	7.80	0.951	−3.859	564
Dextran 70	7.80	0.980	−3.522	581
Buffer	7.80	1.042	−3.841	618

The results obtained seem to indicate that the difference in the relative hydrophobicity of the two phases of the Dextran–PEG biphasic system containing 0.11 *M* sodium phosphate buffer, pH 6.8, is dissimilar from that of the system containing 0.20 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 6.8. Furthermore the hydrophobic properties of the phases in the presence of 0.085 *M* sodium phosphate buffer, pH 6.8, would be unlike those of the phases containing 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 6.8. Therefore, a comparison of the partition results obtained in biphasic systems without due regard for the differences in the hydrophobic properties of the phases, as done by Walter and Anderson[10], seems to be inadequate.

It should be noted also that the difference in the relative hydrophobicity of the phases of the dextran–PEG biphasic system containing 0.11 *M* sodium phosphate buffer appears to be independent of the $HPO_4^{2-}/H_2PO_4^-$ concentration ratio. However, this ratio, seems to affect the hydrophobic properties of the phases in the presence of 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer. This fact can be explained by opposing influences of the phosphate ions' concentration ratio and of the ionic strength of the medium upon the difference in the hydrophobic character of the two phases.

From the data given in Table III, the effect of the $HPO_4^{2-}/H_2PO_4^-$ concentration ratio is incompletely characterized by the changes in the difference between the relative hydrophobicity of the phases. The hydrophobic character of each phase of system relative to that of *n*-octanol is also affected. This effect of the ionic composition, was studied in more detail with the Ficoll dextran biphasic systems.

Ficoll–dextran biphasic systems

From the data given in Table II and Fig. 2, the difference in the relative hydrophobicity of the two phases of the Ficoll–dextran system is influenced by the $HPO_4^{2-}/H_2PO_4^-$ concentration ratio but appears to be independent of the NaCl/sodium phosphate buffer concentration ratio over the concentration range used. The difference in the hydrophobic character of the phases of this biphasic system containing 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer appears to increase with increasing phosphate ions' concentration ratio, in contrast to the behaviour observed in the dextran–PEG system of the same salt composition. The data presented in Table IV and Fig. 2 show that the hydrophobic character of each phase of the Ficoll–dextrzn biphasic system relative to that of *n*-octanol is affected by the $HPO_4^{2-}/H_2PO_4^-$ concentration ratio in a similar way to the difference in the relative hydrophobicity of the two phases.

The mechanisms of these effects remain to be elucidated, but it seems possible to conclude that the hydrophobic properties of a given aqueous polymeric biphasic system are governed by the polymeric and ionic compositions and concentrations. These properties are reflected by the hydrophobic character of each phase of the system relative to that of *n*-octanol or any other reference solution. It seems probable that two different biphasic systems characterized by similar differences in the relative hydrophobicity of the phases of each system will display different overall hydrophobic properties due to the different hydrophobic characters of the individual phases relative to that of the reference solution.

In order to determine whether the partition coefficients of solutes do depend on the difference in the relative hydrophobicity of the two phases of a given biphasic

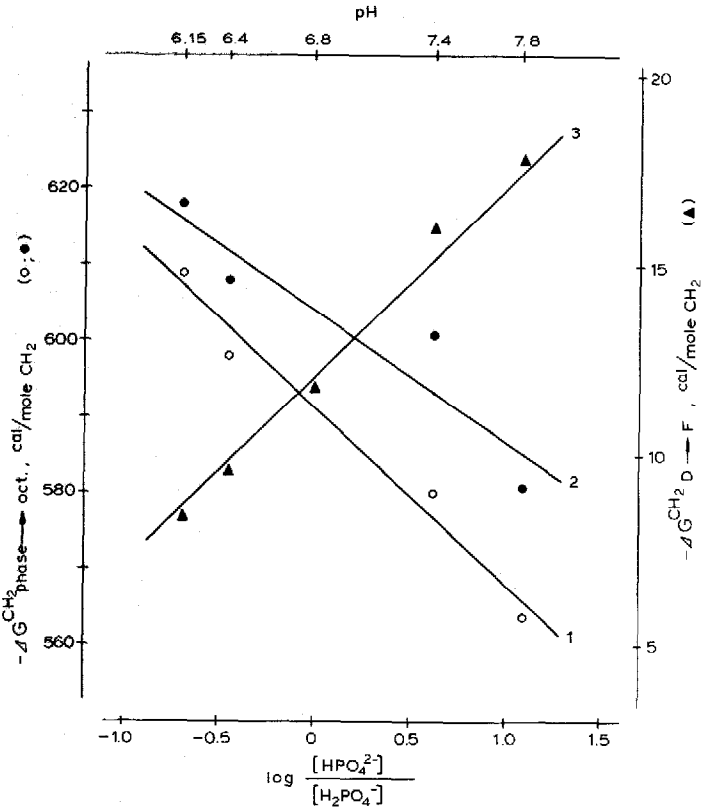

Fig. 2. Relative hydrophobicity of the phases of the aqueous Ficoll–dextran biphasic system containing 0.11 *M* sodium phosphate buffer as a function of the phosphate ions' concentration ratio. 1, Hydrophobicity of the Ficoll-rich phase relative to that of *n*-octanol; 2, hydrophobicity of the dextran-rich phase relative to that of *n*-octanol; 3, difference in the relative hydrophobicity of the two phases of the Ficoll–dextran biphasic system. The hydrophobic properties of the phases are expressed in terms of the free energy of transfer of a CH_2 group from the phase in question to *n*-octanol or from the dextran-rich phase to the Ficoll-rich phase of the system.

system, the partitioning of adenine and uridine in several systems was examined. The results in Table V indicate that the solute partition coefficients differ similarly in all the systems tested provided the difference in the relative hydrophobicity between the two phases of a given system is taken into consideration. These results confirm the importance of the calibration of biphasic systems prior to their use for physicochemical studies of biological materials[6,7].

In order to establish the effect of the hydrophobic properties of both phases of a given biphasic system on the partition behaviour of solutes, the *C* values in Table II should be examined. These values represent the corresponding ln *K* values for DNP-Gly. It has been shown previously[8,9] that, in order to take account of the difference in the relative hydrophobicity of the phases of a given biphasic system, the (ln *K*)/*E* ratio should be considered instead of the ln *K* values. From the curves in Fig. 3, the (ln *K*)/*E* ratio for DNP-Gly expressed in terms of the *C*/*E* ratio depends linearly on the relative hydrophobicity of the Ficoll-rich phase. It should be emphasized that par-

TABLE V

PARTITION OF ADENINE AND URIDINE IN SEVERAL AQUEOUS POLYMERIC BIPHASIC SYSTEMS

The polymer compositions are given in the text.

Biphasic system	*pH*	*Concentration of phosphate buffer (mol/kg)*	*Concentration of NaCl (mol/kg)*	*E*	*ln K*		*(ln K)/E**
					Adenine	*Uridine*	
Dextran–PEG	6.8	0.11	–	0.0374	1.208	1.036	4.12
	6.8	0.085	–	0.0338	1.189	1.030	4.23
	6.8	0.01	0.15	0.0290	1.168	1.027	4.41
	6.8	0.01	0.20	0.0292	1.162	1.025	4.28
Ficoll–dextran 70	6.15	0.11	–	0.0142	1.118	1.052	4.30
	7.40	0.11	–	0.0271	1.152	1.018	4.54
	7.80	0.11	–	0.0300	1.164	1.025	4.23
							av. 4.30 ± 0.14

* (ln K) is the difference between the ln K values for adenine and uridine.

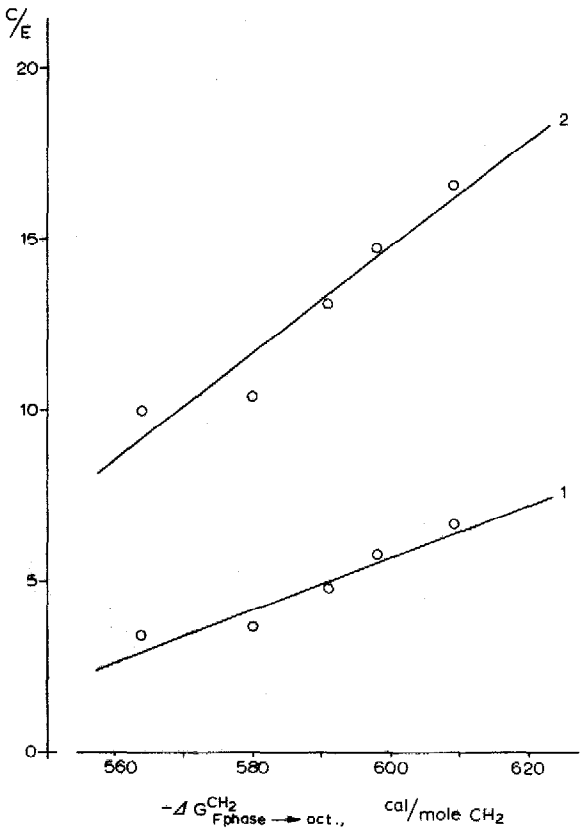

Fig. 3. The C/E ratio for DNP-Gly in aqueous Ficoll–dextran biphasic systems with different ionic compositions as a function of the relative hydrophobicity of the Ficoll-rich phase. Ionic compositions: 1, 0.15 M NaCl in 0.01 M sodium phosphate buffer; 2, 0.11 M sodium phosphate buffer. The pH is varied from 6.15 to 7.8.

because the state of the solute is constant in the pH range employed. Also, that the type of the relationships in Fig. 3 is independent of whether the relative hydrophobicity of the Ficoll-rich phase or that of the Dextran-rich phase is plotted. However, there is a difference in the slopes of the two curves in Fig. 3, which seems to indicate that the influence on the partition behaviour of the solute of the hydrophobic properties of the phases of the Ficoll–dextran biphasic system depends on the ionic composition of the system. This is reasonable since the hydrophobic properties of the phases are not the only important feature of the biphasic system for the partition behaviour of solutes.

It is generally accepted[1–4,10] that partitioning of an ionogenic substance in the systems under consideration is affected by the electrostatic interfacial potential difference created by the salts present in the systems. A study of this effect is reported in Part II.

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