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PARAMETERIZATION OF HYDROPHOBIC PROPERTIES OF AQUEOUS POLYMERIC BIPHASIC SYSTEMS AND WATER–ORGANIC SOLVENT SYSTEMS

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SUMMARY

Partition coefficients for a homologous series of dinitrophenylated amino acids with aliphatic side-chains have been determined in two aqueous polymeric Ficoll–dextran 70 and dextran 500–polyethylene glycol 6000 biphasic systems and in the systems formed by *n*-octanol and the aqueous phases of the above systems. The results afford an estimation of the free energy of transfer of a CH_2 group from one to the other phase of the systems examined. This parameter ($\Delta g_{tr}^{CH_2}$) was taken as a measure of the hydrophobic character of an aqueous phase with respect to *n*-octanol. It was shown that when the partition of a set of homologues in two biphasic systems is correlated according to the known equation $\ln K_i = a \ln K_0 + b$, where K_i and K_0 are the partition coefficients for a given solute in the *i*th system and in the system chosen for reference, respectively, the parameter a is related to the $\Delta g_{tr_i}^{CH_2}$ and $\Delta g_{tr_0}^{CH_2}$ by $a = \Delta g_{tr_i}^{CH_2}/\Delta g_{tr_0}^{CH_2}$.

This equation was used to determine the hydrophobic character of various organic solvents and that of the aqueous polymeric phases of the aqueous biphasic systems studied, and was found to be valid for comparison of the partition values determined in an aqueous polymeric biphasic system and in the water–*n*-octanol system. This seems to extend the possibilities of structure–activity relationships studies as the Ficoll–dextran aqueous biphasic system provides as promising a means for their study in biological chemistry as the water–*n*-octanol system in drug research.

INTRODUCTION

It has been shown[1–8] that the aqueous biphasic polymeric systems described by Albertsson[9] provide a means for estimating the relative hydrophobicities of biological solutes and particles. We developed[2–8] a new Ficoll–dextran biphasic system that has several advantages over the common dextran–polyethylene glycol system, particularly for analytical studies of both macromolecule and cell surface properties.

It should be noted, however, that the Ficoll–dextran system cannot be used for partitioning non-polar compounds because of their low solubility in water. The

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hydrophobic character of drugs is generally measured by partition coefficients determined using *n*-octanol–water as the distribution system[10–12]. Various solvent systems can be used for this purpose but measurements of the relative hydrophobicities of ionizable compounds always suffer from non-identity of the solute species in the two phases of a given water–organic solvent biphasic system[10–12]. Obviously the conventional biphasic systems containing an organic solvent cannot be applied to biopolymers because of the denaturing effects of organic solvents.

Therefore, it seems that the best way to formulate a general hydrophobicity scale for both biological macromolecules and low-molecular-weight polar and nonpolar solutes is to establish an extra-thermodynamic relationship between the partition values determined in the above aqueous polymeric biphasic system and in the *n*-octanol–water system similar to those reported for various pairs of partition systems[10–13].

In this study we have attempted to establish such a relationship and to measure the hydrophobic character specific for the phases of two different aqueous polymeric biphasic systems relative to *n*-octanol.

EXPERIMENTAL

Materials

Ficoll-400 (lot 11069) and dextran T-500 (lot 9307, $\bar{M}_w = 484 \cdot 10^3$, $\bar{M}_n = 165.5 \cdot 10^3$) were obtained from Pharmacia (Uppsala, Sweden). Dextran 70 (lot 580870, $\bar{M}_w = 64.5 \cdot 10^3$, $\bar{M}_n = 22.7 \cdot 10^3$) was obtained under the trade-name Polyglucinum from Minmedprom (Moscow, U.S.S.R.). Polyethylene glycol 6000 was purchased from Serva (Heidelberg, G.F.R.).

Dinitrophenylated amino acids (DNP-glycine and DNP-L-alanine) were supplied by Serva. 2,4-Dinitrofluorobenzene was obtained from Calbiochem (Los Angeles, CA, U.S.A.). L-Norleucine and DL-norvaline were supplied by Reanal (Budapest, Hungary), and DL-2-amino-*n*-octanoic acid was purchased from BDH (Poole, Great Britain). The amino acids were dinitrophenylated as described in ref. 14. All dinitrophenylated amino acids were checked for purity by thin-layer chromatography, and their sodium salts were prepared by titration.

1-Octanol and other chemicals and salts were analytical-reagent grade materials and were used without further purification.

Methods

Buffered Ficoll–dextran 70 and Dextran 500–polyethylene glycol 6000 biphasic systems were prepared by weighing appropriate amounts of the stock polymer solutions as described previously[1]. The Ficoll–dextran biphasic systems contained 12.5% (w/w) Ficoll-400, 10.8% (w/w) dextran 70 and either 0.15 *M* sodium chloride in 0.01 *M* sodium phosphate buffer (pH 7.4) or 0.11 *M* sodium phosphate buffer (pH 7.4). The dextran–polyethylene glycol biphasic systems contained 7% (w/w) dextran 500 and 4.4% (w/w) polyethylene glycol 6000 (salt and buffer concentrations as indicated above).

The partition experiments were carried out as described elsewhere[2,5,6]. The phases of the Ficoll–dextran system were allowed to settle at 23°C for 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 at 360 nm against a correspondingly diluted top or bottom phase blank.

The same partition technique was used with the dextran–polyethylene glycol systems except that the systems were centrifuged for 20 min at 1200 *g* to speed phase settling.

After settling of the aqueous biphasic systems, aliquots of the phases were mixed with equal volumes of *n*-octanol. The biphasic systems formed by a given aqueous polymeric phase with *n*-octanol were centrifuged for 5 min at 1200 *g*. The same technique was used with the buffer–*n*-octanol systems. The concentrations of a solute in both phases of the systems were measured as above, except those in the *n*-octanol phases, which were measured at 350 nm.

The partition coefficient, *K*, in the aqueous polymeric biphasic systems is defined as the ratio of the sample concentration in the Ficoll-rich (polyethylene glycol-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 *n*-octanol phase to that in the aqueous phase.

The partition coefficients were measured for each solute over approximately 10-fold concentration ranges and were found to be independent of the solute concentration in the aqueous polymeric biphasic systems and also in the aqueous solution–*n*-octanol systems.

The partition coefficient for each solute was determined as the mean of two measurements on three dilutions from each partition experiment carried out 2–4 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 CH_2 groups in the aliphatic chain of the compounds distributed in a given biphasic system[1,2,7]. Some of the relationships found are shown in Fig. 1a and b. These relationships can be described by the equation

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

where n is the number of CH_2 groups in the amino acid aliphatic side-chain, C is the ln K value for DNP-glycine and E represents an average ln K increment per CH_2 group. It is clear that E is related to the free energy of transfer of a CH_2 group from one to the other phase of a given biphasic system ($\Delta g_{tr}^{CH_2}$) according to the equation $\Delta g_{tr}^{CH_2} = RTE$.

A least-squares treatment of the data obtained led to the C and E values listed in Table I, together with the corresponding $\Delta g_{tr}^{CH_2}$ values.

Linear relationships are known to exist between the logarithms of partition coefficients of substances determined separately in a pair of partition systems[10–13]. These relationships are described by the equation

$$\ln K_i = a \ln K_0 + b \tag{2}$$

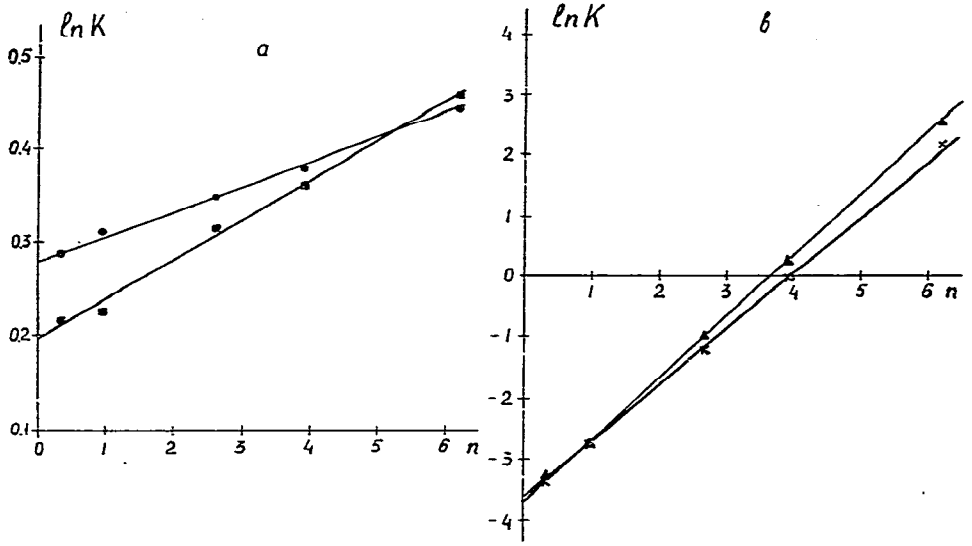

Fig. 1. Logarithm of the partition coefficient as a function of the aliphatic side-chain length (n) of the dinitrophenylated amino acids glycine, alanine, norvaline, norleucine and 2-amino-n-octanoic acid in the following biphasic systems: (a) 1, aqueous polymeric biphasic system containing 12.5% (w/w) Ficoll-400, 10.8% (w/w) dextran 70, 0.11 M sodium phosphate buffer (pH 7.4); 2, aqueous polymeric biphasic system including 7.0% (w/w) dextran 500, 4.4% (w/w) polyethylene glycol 6000, 0.11 M sodium phosphate buffer (pH 7.4); (b) 1, n-octanol–aqueous solution of 0.11 M sodium phosphate buffer (pH 7.4); 2, n-octanol–polyethylene glycol 6000-rich phase taken from the above aqueous polymeric system including 0.11 M sodium phosphate buffer (pH 7.4).

where K_i and K_0 are, respectively, the partition coefficients for a given solute in the ith phase system under study and in the system chosen for reference, and a and b are constants.

The values of a and b (which were calculated using the experimental K values with the corresponding buffer–n-octanol biphasic system as the reference) are given in Table II.

DISCUSSION

It has been suggested[1,15] that the free energy of the interface transfer of a CH_2 group specific for a given biphasic system ($\Delta g_{tr}^{CH_2}$) should be used as a measure of the difference in the relative hydrophobicities between the two phases of the system. When this criterion is used for comparison of different aqueous phases with a given organic phase it can be taken as a measure of the relative hydrophobicity of these phases. The same parameter can be applied for comparison of different organic solvents with water or with any salt solution. n-Octanol was chosen here as the reference organic solvent for the reasons given above. As the $\Delta g_{tr}^{CH_2}$ value specific for the water–n-octanol system is known[10–12], it is possible to calculate the $\Delta g_{tr}^{CH_2}$ values for the hypothetical polymer solution–water biphasic systems from the data given in Table I.

The relative hydrophobicities of the polymeric aqueous phases examined are shown in Fig. 2 and are described quantitatively (in terms of the $\Delta g_{tr}^{CH_2}$ values) in Table III. The hydrophobic character of different organic solvents was evaluated

TABLE I

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

Partition of a homologous series of solutes in a given phase system is described by the equation $\ln K = C + En$ (see text). Parameter E is related to the free energy of interface transfer of a CH_2 group ($\Delta g_{tr}^{CH_2}$) by $\Delta g_{tr}^{CH_2} = RTE$.

*Biphasic system**	*Salt composition***	*C*	*E*	$\Delta g_{tr}^{CH_2}$ *(cal/mole)*
n-Octanol–buffer	I	−3.686 ± 0.018	1.008 ± 0.005	598 ± 8
	II	−3.886 ± 0.024	1.008 ± 0.006	598 ± 8
Ficoll–dextran 70	I	0.281 ± 0.006	0.027 ± 0.002	16 ± 1
	II	0.099 ± 0.006	0.027 ± 0.002	16 ± 1
Polyethylene glycol–dextran 500	I	0.196 ± 0.011	0.042 ± 0.003	25 ± 2
	II	−0.073 ± 0.008	0.037 ± 0.002	22 ± 1
n-Octanol–dextran 70	I	−3.77 ± 0.11	1.014 ± 0.027	601 ± 16
	II	−3.95 ± 0.05	1.007 ± 0.013	597 ± 8
n-Octanol–Ficoll	I	−3.92 ± 0.09	0.979 ± 0.022	580 ± 13
	II	−4.03 ± 0.10	0.984 ± 0.025	584 ± 15
n-Octanol–dextran 500	I	−3.47 ± 0.02	0.963 ± 0.007	571 ± 4
	II	−3.57 ± 0.10	0.928 ± 0.030	550 ± 18
n-Octanol–polyethylene glycol	I	−3.63 ± 0.07	0.921 ± 0.021	546 ± 12
	II	−3.50 ± 0.12	0.890 ± 0.035	528 ± 21

* Polymer compositions of the aqueous biphasic systems are given in the text. For details of the preparation of the *n*-octanol–aqueous phase systems see text.

** Salt composition: I, 0.11 *M* sodium phosphate buffer (pH 7.4); II, 0.15 *M* sodium chloride in 0.01 *M* sodium phosphate buffer (pH 7.4).

TABLE II

FACTORS FOR CALCULATING THE PARTITION VALUES DETERMINED IN VARIOUS BIPHASIC SYSTEMS

The relationship between the partition values for a solute determined in two different biphasic systems is described by the equation $\ln K_i = a \ln K_0 + b$, where K_i and K_0 are the partition coefficients of a solute in the ith phase system under study and in the system chosen for reference, respectively, and a and b are constants. The system buffer–*n*-octanol was chosen for reference.

Biphasic system	*Salt composition**	*a*	*b*
Ficoll–dextran 70	I	0.027 ± 0.002	0.380 ± 0.02
	II	0.027 ± 0.002	0.204 ± 0.02
Polyethylene glycol–dextran 500	I	0.042 ± 0.003	0.351 ± 0.02
	II	0.037 ± 0.002	0.071 ± 0.02
n-Octanol–dextran 70	I	1.006 ± 0.025	−0.062 ± 0.11
	II	0.999 ± 0.013	−0.068 ± 0.06
n-Octanol–Ficoll	I	0.971 ± 0.020	−0.341 ± 0.09
	II	0.976 ± 0.023	−0.237 ± 0.10
n-Octanol–dextran 500	I	0.955 ± 0.008	0.050 ± 0.03
	II	0.921 ± 0.028	0.009 ± 0.10
n-Octanol–polyethylene glycol	I	0.914 ± 0.019	−0.261 ± 0.07
	II	0.883 ± 0.020	−0.069 ± 0.12

* Salt composition: I, 0.11 *M* sodium phosphate buffer (pH 7.4); II, 0.15 *M* sodium chloride in 0.01 *M* sodium phosphate buffer (pH 7.4).

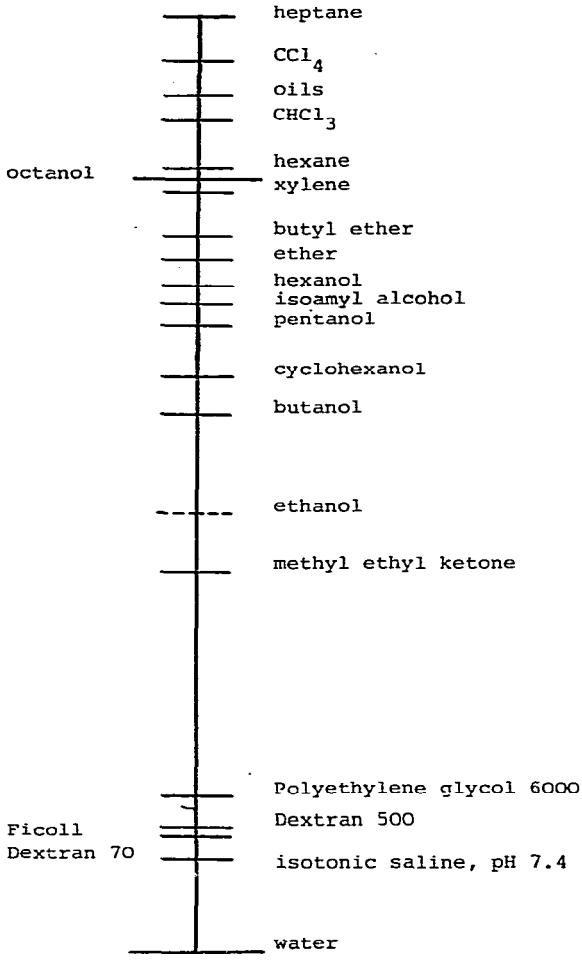

Fig. 2. Hydrophobicity scale for organic solvents and aqueous polymeric phases. Hydrophobicity is shown according to the $\Delta g_{tr}^{CH_2}$ values given in Table III. Polyethylene glycol 6000–polyethylene glycol 6000-rich phase, dextran 500–dextran 500-rich phase, Ficoll–Ficoll-rich phase and dextran 70–dextran 70-rich phase —all the phases from the corresponding aqueous polymeric biphasic systems containing 0.11 *M* sodium phosphate buffer (pH 7.4). Ethanol is scaled according to the extrapolated value of $\Delta g_{tr}^{CH_2}$ as indicated in the text.

from the literature data[10,12] as follows. It was shown earlier[8] that parameter a in eqn. 2 represents the difference in the relative hydrophobicities between the two phases of a given biphasic system and is related to the corresponding $\Delta g_{tr_i}^{CH_2}$ and $\Delta g_{tr_0}^{CH_2}$ by the equation

$$a_i = \Delta g_{tr_i}^{CH_2} / \Delta g_{tr_0}^{CH_2} \quad (3)$$

where the subscripts i and 0 denote the biphasic system under study and the reference, respectively.

All of the data given in Tables I and II are consistent with eqn. 3. Thus, the a_i

TABLE III

RELATIVE HYDROPHOBICITY OF ORGANIC SOLVENTS AND AQUEOUS SOLUTIONS EXPRESSED IN TERMS OF THE FREE ENERGY OF TRANSFER OF A CH_2 GROUP FROM WATER TO A GIVEN MEDIUM ($\Delta g_{tr}^{CH_2}$)

Solvent	$-\Delta g_{tr}^{CH_2}$ *(cal/mole)*	*Calculated from the data given in ref.*
n-Heptane	825 ± 10	Taken as reported in ref. 17
CCl_4	784 ± 8	16[a]
Oils	757 ± 16	10[b]
$CHCl_3$	731 ± 17	16[a]
n-Hexane	692 ± 60	19[c]
n-Octanol	683 ± 12	10, 12[b]
Oleyl alcohol	682 ± 43	10[b]
Xylene	672 ± 43	10[b]
Di-*n*-butyl ether	631 ± 34	16[a]
Diethyl ether	612 ± 74	12[b]
n-Hexanol	587 ± 24	16[a]
Isoamyl alcohol	573 ± 30	16[a]
Primary pentanols	552 ± 49	10[b]
Cyclohexanol	509 ± 62	10[b]
Primary butanols	476 ± 16	10[b]
Ethanol	388 ± 27	Extrapolated value from data in Fig. 3
Methyl ethyl ketone	337 ± 48	10[b]
Polyethylene glycol 6000[d]	155 ± 24	This work, Table I
Polyethylene glycol 6000[e]	137 ± 17	This work, Table I
Dextran 500[f]	133 ± 22	This work, Table I
Dextran 500[g]	112 ± 13	This work, Table I
Ficoll-400[h]	101 ± 18	This work, Table I
Dextran 70[i]	84 ± 22	This work, Table I
Isotonic saline (pH 7.4)[j]	85 ± 14	This work, Table I

[a] The $\Delta g_{tr}^{CH_2}$ values are calculated using the linear relationship between the logarithm of the partition coefficient and the number of CH_2 groups in the aliphatic side-chains of the amino acid derivatives studied in ref. 16.

[b] The $\Delta g_{tr}^{CH_2}$ values are calculated from the *a* values for the corresponding solvent regression equations reported in refs. 10 and 12 and the $\Delta g_{tr}^{CH_2}$ value of 683 cal/mole as reported for the *n*-octanol–water biphasic system[10–12].

[c] The $\Delta g_{tr}^{CH_2}$ value was calculated from the solubility data for amino acids reported in ref. 19.

[d] Polyethylene glycol-rich phase of the aqueous biphasic system polyethylene glycol 6000–dextran 500 in the presence of 0.15 *M* sodium chloride in 0.01 *M* sodium phosphate buffer (pH 7.4) (polymer composition is indicated in the text).

[e] As in [d] but in the presence of 0.11 *M* sodium phosphate buffer (pH 7.4).

[f] Dextran 500-rich phase of the system indicated in [d].

[g] As in [f] but in the presence of 0.11 *M* sodium phosphate buffer (pH 7.4).

[h] Ficoll-rich phase of the aqueous biphasic system Ficoll–dextran 70 in the presence of 0.11 *M* sodium phosphate buffer (pH 7.4) or in the presence of 0.15 *M* sodium chloride in 0.01 *M* sodium phosphate buffer (pH 7.4) (polymer composition is indicated in the text).

[i] Dextran 70-rich phase of the system indicated in [h].

[j] Isotonic saline containing either 0.11 *M* sodium phosphate buffer (pH 7.4) or 0.15 *M* sodium chloride in 0.01 *M* sodium phosphate buffer (pH 7.4).

values reported by Hansch[12] and Leo *et al.*[10] were treated according to eqn. 3 using the $\Delta g_{tr}^{CH_2}$ value of 683 ± 12 cal/mole for the *n*-octanol–water system[10–12]. The $\Delta g_{tr}^{CH_2}$ values for isoamyl alcohol–water, di-*n*-butyl ether–water, *n*-hexanol–water, chloroform–water and carbon tetrachloride–water systems were calculated from the experimental data reported by Nandi[16] using the approach applied in this study. The $\Delta g_{tr}^{CH_2}$ value for the *n*-heptane–water system was taken from ref. 17.

It should be noted that the $\Delta g_{tr}^{CH_2}$ values obtained for various *n*-alcohols appear to depend on the alcohol chain length, as shown in Fig. 3, except for the value for ethanol calculated from the data reported in ref. 18. However, the latter data are the only ones obtained not by the partition technique but by solubility measurements[18]. The extrapolated value of 388 ± 27 cal/mole seems to represent the hydrophobic character of ethanol much better than 589 ± 83 cal/mole estimated from the solubility data reported in ref. 18, as ethanol is the only alcohol under consideration that is completely miscible with water. Hence, the relative hydrophobicity of ethanol is shown in Fig. 2 according to the extrapolated value of $\Delta g_{tr}^{CH_2}$.

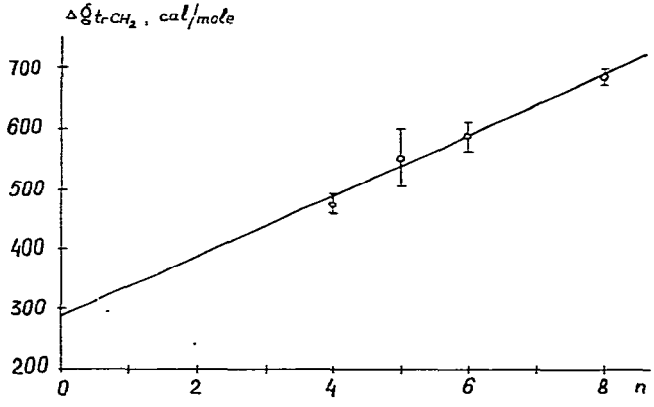

Fig. 3. $\Delta g_{tr}^{CH_2}$ values for *n*-alcohols as a function of the alcohol chain length (*n*).

The approach described above affords an evaluation of the relative hydrophobicities of aqueous polymer solutions, which appears to open up a promising line of research on polymer–solvent interactions. It is surprising that, to our knowledge, no use has been made of this approach previously.

It is necessary to emphasize that although $\Delta g_{tr}^{CH_2}$ appears to be completely satisfactory as a measure of the hydrophobic character of a given organic solvent or aqueous solution, it undoubtedly has some limitations. These limitations seem to be concerned with the different characters of the interactions between different moieties of a solute molecule and water or various organic solvents. A methylene group has a simple geometry and seems to be inert towards any aqueous or organic solvent. However, the affinity of polar, ionizable and many complex non-polar moieties for a solvent may vary widely. These variations are reflected by differences in the extent of solvation specific for different molecules in various solvents and they may affect the corresponding partition values. It seems reasonable to assume that the parameter *b* in

eqn. 2 represents the effects of the specific solute–solvent interactions on the solute partition coefficient. If this is so, b (not a, which is constant for a given pair of biphasic systems and does not depend on the chemical nature of the solute being partitioned) must be considered as a measure of the specific interactions of a given solute moiety with a particular solvent.

The above meaning of both parameters a and b in eqn. 2 seems to be consistent with the data reported in the literature[10–12]. A more comprehensive discussion of the a and b values reported for various water–organic solvent pairs[10–12], however, is beyond the scope of the present study and will be given in a subsequent publication.

The data given in Tables I and II allow one to compare the aqueous binary Ficoll–dextran and dextran–polyethylene glycol systems. The relative hydrophobicities of the two phases of the dextran–polyethylene glycol system appear to exceed those characteristic of the phases of the Ficoll–dextran system. It was shown[8], however, that the relative hydrophobicities of the phases of the Ficoll–dextran system depend particularly on the molecular weight of dextran and on the polymer composition of the system. Comparison of the data given in Table I for the dextran–polyethylene glycol system with those reported earlier for the same system with another polymer composition[1] indicates that the polymer composition of an aqueous polymeric biphasic system does affect its relative hydrophobicity. That seems to be in line with Albertson's view[9] with respect to the position of aqueous polymer solutions in a range of solvents with different hydrophobicities.

The data obtained in this study indicate that it is possible to correlate the partition values obtained in the aqueous polymeric biphasic system Ficoll–dextran with those determined in the water–*n*-octanol (or any water–organic solvent) system. The Ficoll–dextran biphasic system appears to provide a new means for studying the hydrophobic character of biological particles and any polar and ionizable solutes. This biphasic system can be considered as promising a tool for the study of structure–activity relations in biological chemistry as the water–*n*-octanol system was proved to be in drug research.

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