CHROM. 14,051

# COMPARISON OF CONVENTIONAL PARTITIONING SYSTEMS USED FOR STUDYING THE HYDROPHOBICITY OF POLAR ORGANIC COMPOUNDS

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(Received May 29th, 1981)

## SUMMARY

An analysis of published partition coefficients measured in various two-phase systems for homologous series of fatty acids, aliphatic alcohols and amines was performed in order to clarify the physical meaning of the constants in the so-called solvent regression equation: $\ln K_i = a_i \ln K_0 + b_i$, where $K_i$ and $K_0$ are the partition coefficients for a given solute determined in the partitioning system in question and in that chosen as the reference, respectively, and $a_i$ and $b_i$ are constants. The difference in the relative hydrophobicities of two phases of a given partitioning system is expressed in terms of the free energy of transfer of a $CH_2$ group from the non-aqueous to the aqueous phase of the system ($\Delta g_i^{CH_2}$). It is shown that $a_i$ is related to the $\Delta g_i^{CH_2}$ and $\Delta g_0^{CH_2}$ values by the equation $a_i = \Delta g_i^{CH_2}/\Delta g_0^{CH_2}$. The term $b_i$ appears to account for the specific solute–water (and/or solute–solvent) interactions characteristic of a polar group contained by the solute molecule.

The analysis of literature data indicates that the difference in the relative hydrophobicities between any two groups depends on the choice of partitioning system. The physical meaning of the relative hydrophobicity of a chemical compound is discussed, and it is concluded that an aqueous two-phase system should be used as the reference. The aqueous two-phase polymeric system Ficoll–dextran is proposed for this purpose and its advantages are briefly considered.

## INTRODUCTION

In structure–activity relationship (SAR) studies, preference is given to a partition coefficient that has either been determined or calculated in the octanol–water system, where hydrophobicity is considered to play a role in finding satisfactory interpretations[1–4]. The octanol–water system has both merits as well as its limitations, the latter appearing to be most noticeable with respect to highly hydrophilic biological solutes.

Recently, the aqueous two-phase polymeric system Ficoll–dextran has been proposed by us[5–10] for studying the relative hydrophobicities of biological solutes and particles. It has been shown[10] that the partition coefficients determined in the Ficoll–

dextran system can be correlated with those measured in the octanol–water system for the same solutes using the solvent regression equation[1-4,11,12]:

$$\ln K_i = a_i \ln K_0 + b_i \tag{1}$$

where $K_i$ and $K_0$ are the partition coefficients for a given solute in the $i$th two-phase system and in the system chosen as the reference, respectively, and $a_i$ and $b_i$ are constants characteristic of the systems compared.

It has been shown[9] that eqn. 1 can be used for the comparison of the partition coefficients determined for various solutes in Ficoll–dextran aqueous two-phase systems of different polymeric composition. It should be stressed that the $a_i$ and $b_i$ values were found to be independent of the nature of the solutes partitioned, which included proteins, DNP-amino acids, mono- and polynucleotides and nucleosides. The invariability of $a_i$ seems reasonable in view of the data reported earlier[9,10,13]. It has been previously propoxed[13] to use the free energy of transfer of a $CH_2$ group from one phase to the other of the two-phasic system examined ($\Delta g^{CH_2}$) as a measure of the difference in the relative hydrophobicity between the two phases. It has been shown experimentally[9,10] that the parameter $a_i$ in eqn. 1 is related to the $\Delta g_i^{CH_2}$ and $\Delta g_0^{CH_2}$ values by the equation

$$a_i = \Delta g_i^{CH_2}/\Delta g_0^{CH_2} \tag{2}$$

Eqn. 2 seems to make clear the physical meaning of the parameter $a_i$ in the solvent regression equation (eqn. 1). This meaning, on the one hand, agrees with the assumption[3,12] that $a_i$ reflects the relative hydrophogic character of the solvent pairs compared, but on the other hand appears to be at variance with the literature data[1-4,11,12]. It was particularly noted by Rekker[2] that the kind of solutes comprising a set for which the partition values are to be compared in different partitioning systems can have a considerable influence on $a_i$. Leo et al.[3,12] compared 20 different partitioning systems with the octanol–water system and derived correlations of the eqn. 1 type from the results obtained. In doing so, some differentiation had to be introduced among the solutes to be applied in a given regression equation. They divided the solutes into donor and acceptor compounds and found the $a_i$ and $b_i$ values to be different depending on this property of the solutes. According to Rekker[2], however, in designing a solvent regression equation there is no need for a differentiation between donor and acceptor solutes, provided that proper use is made of an increment value that reflects correctly the hydrogen bonding capacity of the various functional (polar) groups. It seems that an analysis of the partition coefficients for several homologous series of aliphatic monofunctional compounds determined in various partitioning systems could be use to clear the matter.

Such an analysis of the literature partition coefficients has been attempted by us with a two-fold purpose. First, it should answer the question of whether $a_i$ is really as invariable for all of the solutes partitioned in a given two-phase system as claimed by us earlier[9,10], and secondly, it should given more insight into the physical meaning of $b_i$ and be of help in the selection of the proper partitioning system for the study of the relative hydrophobicities of polar organic compounds. The results of the analysis are discussed in this paper.

EXPERIMENTAL

The values of the partition coefficients used in the regression analysis were taken from refs. 2, 3 and 14. When more than one partition coefficient was available for any given compound, they were averaged. Homologous series including not less than three members were considered in the analysis. The only homologous series available for the analysis were found to be those of aliphatic alcohols, amines, fatty acids[2,3] and N-acetyl ethyl esters of amino acids with an aliphatic side-chain[14].

The partition coefficients for each homologous series of solutes in a given two-phase system were treated according to the equation

$$\ln K_i = A_i + E_i n \qquad (3)$$

where $K_i$ is the partition coefficient of a given solute, $n$ is the number of $CH_2$ and $CH_3$ groups in the solute molecule alkyl chain, $A_i$ and $E_i$ are constants and the subscript $i$ denotes the two-phase system under consideration.

The constants $A_i$ and $E_i$ in eqn. 3 were calculated from the $K_i$ values using the least-squares method on a Hewlett-Packard 9815A calculator. The statistical significances of the values calculated were tested by the correlation coefficient, $r$, and the standard deviation, $s$. The same procedure was used for the regression analysis of all the relationships discussed below.

RESULTS AND DISCUSSION

The $A_i$ and $E_i$ values characteristic of the partition behaviour of fatty acids and aliphatic amines and alcohols in various partitioning systems are listed in Table I. The partition coefficient values for N-acetyl ethyl esters of glycine, alanine, norvaline and norleucine reported by Nandi[14] were treated as indicated above, and the following $A_i$ and $E_i$ values were obtained: $A_i = -4.071 \pm 0.036, 0.218 \pm 0.051, -1.128 \pm 0.087$ and $-0.123 \pm 0.127$ and $E_i = 1.322 \pm 0.014, 1.233 \pm 0.028, 1.014 \pm 0.034$ and $0.966 \pm 0.050$, for the systems carbon tetrachloride–water, chloroform–water, octanol–water and isoamyl alcohol–water, respectively.

As the logarithm of the partition coefficient of a given solute in the $i$th partitioning system is described by eqn. 3:

$$\ln K_i = A_i + E_i n$$

and combination of eqns. 1 and 2 gives the simple conclusion

$$b_i = E_i(A_i/E_i - A_0/E_0) \qquad (4)$$

or in an alternative form,

$$b_i = A_i - a_i A_0 \qquad (5)$$

The relationship between $a_i$ and $b_i$ described by eqn. 5 seems to clarify why both are considered[2,3,12] to be slightly different measures of the relative hydrophobicity of the organic solvent in a given partitioning system.

In order to determine $a_i$ and $b_i$ for the two-phase systems under consideration the octanol–water system was chosen as the reference. The $a_i$ and $b_i$ values were

TABLE I

CHARACTERISTICS OF THE PARTITION BEHAVIOUR OF ALIPHATIC ACIDS, ALCOHOLS AND AMINES IN WATER–ORGANIC SOLVENT TWO-PHASE SYSTEMS

The logarithm of the partition coefficient of a given solute (ln $K$) depends on the alkyl chain length ($n$) of the solute as ln $K = A + En$. Parameters $A$ and $E$ calculated from the literature $K$ values are presented as means $\pm$ standard deviations with the number of solutes examined in a given homologous series given in parentheses.

| Solvent (vs. H$_2$O) | Fatty acids | | Aliphatic alcohols | | Aliphatic amines | |
| --- | --- | --- | --- | --- | --- | --- |
| | $A$ | $E$ | $A$ | $E$ | $A$ | $E$ |
| Hexadecane | -7.674 ± 0.336 | 1.174 ± 0.066 (3) | -8.002 ± 0.326 | 1.393 ± 0.058 (4) | | |
| Dodecane | -7.810 ± 0.035 | 1.232 ± 0.007 (3) | -7.694 ± 0.268 | 1.379 ± 0.037 (4) | | |
| Octane | -7.817 ± 0.412 | 1.278 ± 0.089 (4) | -7.084 ± 0.304 | 1.310 ± 0.055 (4) | | |
| Heptane | -8.294 ± 0.205 | 1.394 ± 0.020 (4) | | | | |
| Cyclohexane | | | -9.214 ± 0.227 | 1.900 ± 0.073 (3) | | |
| Hexane | | | -8.581 ± 0.165 | 1.704 ± 0.053 (3) | | |
| Carbon tetrachloride | -7.206 ± 0.067 | 1.624 ± 0.036 (4) | -6.637 ± 0.047 | 1.485 ± 0.010 (5) | | |
| Xylene | -5.539 ± 0.103 | 1.241 ± 0.034 (6) | | | -3.540 ± 0.143 | 0.931 ± 0.030 (6) |
| Toluene | -5.960 ± 0.127 | 1.415 ± 0.042 (6) | | | -6.707 ± 0.667 | 1.819 ± 0.212 (3) |
| Benzene | -6.090 ± 0.242 | 1.531 ± 0.080 (6) | -6.056 ± 0.191 | 1.307 ± 0.052 (4) | | |
| Chloroform | -4.999 ± 0.124 | 1.457 ± 0.041 (6) | -4.628 ± 0.203 | 1.398 ± 0.041 (3) | | |
| Diisopropyl ether | -2.742 ± 0.475 | 1.140 ± 0.220 (3) | | | | |
| Diethyl ether | -1.532 ± 0.309 | 1.126 ± 0.102 (6) | -4.391 ± 0.010 | 1.431 ± 0.005 (3) | -4.794 ± 0.292 | 1.147 ± 0.078 (5) |
| Oleyl alcohol | -2.872 ± 0.050 | 1.329 ± 0.016 (4) | -4.058 ± 0.600 | 0.933 ± 0.193 (3) | | |
| Methyl isobutyl ketone | -1.955 ± 0.040 | 1.218 ± 0.010 (3) | | | | |
| Octanol | -1.618 ± 0.130 | 1.211 ± 0.029 (6) | -2.986 ± 0.092 | 1.250 ± 0.015 (8) | -2.658 ± 0.098 | 1.218 ± 0.020 (6) |
| n-Amyl alcohol | -1.167 ± 0.059 | 1.181 ± 0.018 (5) | | | | |
| Isoamyl alcohol | -0.691 ± 0.066 | 1.018 ± 0.020 (5) | | | | |
| n-Butanol | -0.836 ± 0.064 | 1.011 ± 0.019 (5) | | | -0.829 ± 0.534 | 0.815 ± 0.099 (4) |
| Methyl ethyl ketone | -0.555 ± 0.037 | 0.730 ± 0.011 (5) | | | | |

TABLE II

FACTORS FOR SCALING THE PARTITION COEFFICIENTS OBTAINED IN VARIOUS TWO-PHASE SYSTEMS ACCORDING TO THE SOLVENT REGRESSION EQUATION $\ln K_i = a_i \ln K_0 + b_i$ ($K$ PARTITION COEFFICIENT)

| Solvent (vs. $H_2O$) | Fatty acids | | Aliphatic alcohols | | Aliphatic amines | | Solubility of water in the solvent* ($mol/l \cdot 10^3$) |
|---|---|---|---|---|---|---|---|
| | a | b | a | b | a | b | |
| Hexadecane | 0.969 ± 0.059 | −6.106 ± 0.371 | 1.114 ± 0.048 | −4.676 ± 0.360 | | | 0.614** |
| Dodecane | 1.017 ± 0.025 | −6.164 ± 0.143 | 1.103 ± 0.033 | −4.400 ± 0.252 | | | 0.839** |
| Octane | 1.055 ± 0.078 | −6.110 ± 0.452 | 1.048 ± 0.045 | −3.955 ± 0.346 | | | 1.190** |
| Heptane | 1.151 ± 0.029 | −6.432 ± 0.212 | | | | | 3.30 |
| Cyclohexane | | | 1.520 ± 0.061 | −4.675 ± 0.323 | | | 2.50 |
| Carbon tetrachloride | 1.341 ± 0.080 | −5.036 ± 0.452 | 1.188 ± 0.016 | −3.09 ± 0.128 | | | 10.0 |
| Xylene | 1.025 ± 0.037 | −3.881 ± 0.179 | | | 0.764 ± 0.028 | −1.509 ± 0.148 | 18.8 |
| Toluene | 1.168 ± 0.044 | −4.070 ± 0.174 | | | 1.493 ± 0.176 | −2.739 ± 0.823 | 25.6 |
| Benzene | 1.264 ± 0.072 | −4.045 ± 0.328 | 1.046 ± 0.044 | −2.933 ± 0.251 | | | 26.0 |
| Chloroform | 1.203 ± 0.044 | −3.052 ± 0.212 | 1.118 ± 0.035 | −1.290 ± 0.250 | | | 68.4 |
| Diethyl ether | 0.930 ± 0.086 | −0.027 ± 0.360 | 1.145 ± 0.013 | −0.972 ± 0.113 | 0.942 ± 0.062 | −2.290 ± 0.383 | 530 |
| Oleyl alcohol | 1.097 ± 0.029 | −1.097 ± 0.158 | 0.746 ± 0.154 | −1.830 ± 0.759 | | | 712 |
| Methyl isobutyl ketone | 1.006 ± 0.010 | −0.328 ± 0.052 | | | | | 950 |
| Octanol | 1.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.000 | 2300 |
| n-Amyl alcohol | 0.975 ± 0.027 | 0.410 ± 0.140 | | | | | 4125 |
| Isoamyl alcohol | 0.841 ± 0.026 | 0.670 ± 0.134 | | | | | 5320 |
| n-Butanol | 0.835 ± 0.025 | 0.515 ± 0.132 | | | 0.669 ± 0.082 | 0.949 ± 0.437 | 9440 |
| Methyl ethyl ketone | 0.603 ± 0.017 | 0.421 ± 0.091 | | | | | 5320 |

* Values taken from ref. 12.
** Values estimated using eqn. 7 as indicated in the text.

calculated according to eqns. 2 and 5, respectively, from the corresponding $A_i$ and $E_i$ values and the $A_0$ and $E_0$ values for the octanol–water system listed in Table I. The values obtained are presented in Table II.

It can be seen that for a given partitioning system the $a_i$ value for all of the series of solutes under consideration appears to be constant, within the limits of the standard deviation. Some exceptions that are observed are probably due to the uncertainty of the relationship described by eqn. 3 when applied to homologous series including only compounds with very short alkyl chains. Thus, the $a_i$ values obtained seem to support the previous assumption[9,10] with regard to the physical meaning of $a_i$ in eqn. 1. It is clear that the $\Delta g_i^{CH_2}$ values can be calculated from the corresponding $E_i$ values according to the known equation

$$\Delta g_i^{CH_2} = RTE_i \tag{6}$$

The $\Delta g_i^{CH_2}$ values calculated from the averaged $E_i$ values for the corresponding partitioning systems are listed in Table III. The data obtained appear to be in line with the results reported by Gelles and Klapper[15] and Davis et al.[16], indicating that the calculated free energy of transfer of a $CH_2$ group depends on the organic solvent.

In establishing a scale for organic solvents, and for purposes where hydrogen bonding is not a dominant factor, a scale based on either dipole moment, dielectric constant or solubility parameter is known to be useful and informative. Leo and co-workers[3,12] have suggested the classification of various solvent partitioning systems according to the amount of water that the solvent contains in the saturated state. This scheme has its merits, but this parameter appears to describe the character of the solvent phase only. It seems to us that the free energy of transfer of a $CH_2$ group, $\Delta g^{CH_2}$, can be used as a general characteristic of both phases of a partitioning system. The reasons for this conclusion appear to be as follows.

The usual measure of the hydrophobic character of hydrocarbons is the free

TABLE III

FREE ENERGY OF TRANSFER OF A $CH_2$ GROUP FROM AQUEOUS TO ORGANIC PHASE OF WATER–ORGANIC SOLVENT TWO-PHASE SYSTEM

The $\Delta g^{CH_2}$ values calculated from the corresponding $E$ values (Table I) are averaged for all the homologous series of solutes examined in a given partitioning system (for details see text).

| Organic solvent | $-\Delta g^{CH_2}$ (cal/mole $CH_2$) | Organic solvent | $-\Delta g^{CH_2}$ (cal/mole $CH_2$) |
|---|---|---|---|
| Cyclohexane | 1127 ± 43 | Diethyl ether | 732 ± 101 |
| Hexane | 1010 ± 31 | Octanol | 727 ± 17 |
| Toluene | 959 ± 126 | Methyl isobutyl ketone | 722 ± 15 |
| Carbon tetrachloride | 922 ± 41 | n-Amyl alcohol | 700 ± 11 |
| Chloroform | 846 ± 24 | Oleyl alcohol | 670 ± 118 |
| Benzene | 842 ± 66 | Diisopropyl ether | 676 ± 130 |
| Heptane | 827 ± 12 | Xylene | 644 ± 92 |
| Dodecane | 774 ± 44 | Isoamyl alcohol | 604 ± 12 |
| Hexadecane | 761 ± 65 | n-Butanol | 542 ± 58 |
| Octane | 768 ± 53 | Methyl ethyl ketone | 433 ± 6 |

energy of transfer of a hydrocarbon molecule from water to a pure hydrocarbon. It has been shown[17-19] that the partition coefficient (or solubility in water) of a non-polar solute is directly related to the surface area (cavity area) of its molecule when the pure hydrocarbon standard state is chosen. When examining theoretical explanations for solution behaviour, it is found[20-22] that the solution process can be divided into three steps: the removal of the solute molecule from its environment, the formation of a cavity in water to which it is being transferred and the introduction of the solute molecule into the cavity.

According to Rekker[2], however, in achieving the partition equilibrium, solvent structuring would have to be given much more emphasis than solute volume (or its surface area), as partition cannot, in effect, be modelled by a single-cavity procedure as the solution process[20-22], as it is connected with a "cavity to cavity" transfer of the solute. The hypothesis appears to us to be sound and it agrees with the fact that we could not find any relationship between the $\Delta g_i^{CH_2}$ values calculated in this study and the solubility of water in the organic solvents used by Leo and co-workers[3,12]. The presence of water in a given solvent alters its structure, and hence the solubility of water can represent the character of the organic phase solely. As the aqueous phase of a given partitioning system contains a definite amount of the organic solvent, the structure of this phase is also altered. Thus, the $\Delta g_i^{CH_2}$ values seems to characterize both phases of a partitioning system.

Unlike the constant $a_i$, the parameter $b_i$ in the solvent regression equation (eqn. 1) appears to depend not only on the partitioning systems being compared but also on the nature of the solutes. According to Rekker[2], the essential role of the $b_i$ term in a solvent regression equation is to account for the difference between one functional group of a solute and another when transferred from the aqueous phase to the non-aqueous phase of the solvent system, and *vice versa*. For one functional group this transfer is much easier than for another, depending on local dehydration–solvation possibilities. Assuming that the solubility of water in a given solvent can govern the affinity of a functional group for this solvent phase, it is possible to correlate the $b_i$ values given in Table II with the water content of the non-aqueous phase at saturation. However, the ratio $b_i/E_i$ was used instead of $b_i$ as the proper allowance must be made for the difference in the relative hydrophobicities between both phases of the system. The correlations found are described by the following equations:

$$b_i/E_i = -0.525 \ (\pm \ 0.075) + 1.456 \ (\pm \ 0.058) \ \log S_{w_i} \tag{7}$$
$$n = 13, r = 0.992, s = 0.244$$

for fatty acids.

$$b_i/E_i = -0.316 \ (\pm \ 0.170) + 0.917 \ (\pm \ 0.077) \ \log S_{w_i} \tag{8}$$
$$n = 9, r = 0.976, s = 0.274$$

for aliphatic alcohols; and

$$b_i/E_i = 0.099 \ (\pm \ 0.245) + 0.881 \ (\pm \ 0.194) \ \log S_{w_i} \tag{9}$$
$$n = 5, r = 0.934, s = 0.474$$

for aliphatic amines, where $S_{w_i}$ is the solubility of water in a given organic solvent. The values for diethyl ether have been omitted in deriving eqns. 7 and 9 as those for oleyl alcohol in deriving eqn. 8 for the reasons discussed below. The solubility of water in hydrocarbons was estimated using eqn. 7 and the values obtained were employed in deriving eqn. 8.

It is evident that the correlation equations obtained differ markedly depending on the nature of solutes under consideration. The $b_i/E_i$ ratio is a relative parameter describing a given partitioning system with respect to the octanol–water system. According to eqn. 4 the $b_i/E_i$ ratio is determined by the $A/E$ values for the system in question and for the system chosen as the reference. In order to obtain an "absolute" scale we have attempted to use the $A_i/E_i$ ratio as a parameter specific for the chemical nature of the solutes partitioned and for the particular partitioning system. This parameter does not depend on the choice of any reference solvent system. We hoped that an examination of this parameter could provide additional insight into the "discriminating power" of the partitioning systems. The "discriminating power" term was proposed by Rekker[2] to denote the spread any given solvent system imparts to the partition values of a set of solutes presented to that system for partitioning.

The relationships between the $A_i/E_i$ values (calculated from the data in Table I) and the water content of the solvents at saturation, $S_{w_i}$, are presented in graphical form in Fig. 1. The relationships are described as:

$$A_i/E_i = -1.861 \ (\pm \ 0.067) + 1.456 \ (\pm \ 0.038) \log S_{w_i} \qquad (10)$$
$$n = 16, r = 0.995, s = 0.217$$

for fatty acids;

$$A_i/E_i = -2.662 \ (\pm \ 0.198) + 0.928 \ (\pm \ 0.091) \log S_{w_i} \qquad (11)$$
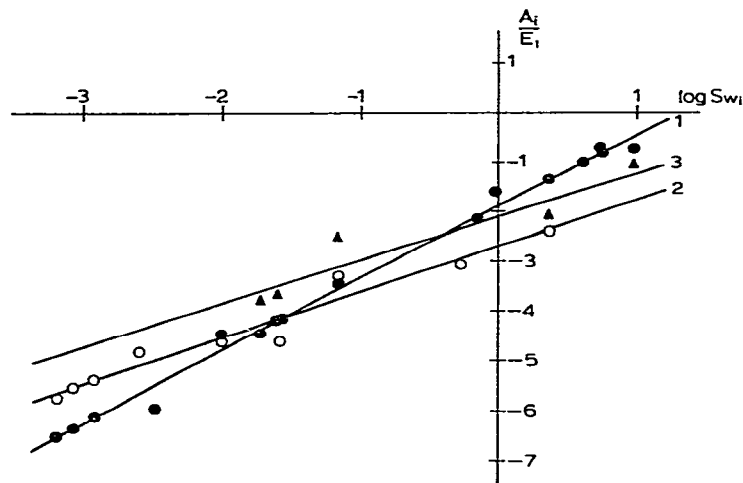$$n = 9, r = 0.968, s = 0.327$$



Fig. 1. Relationships between the ratio $A_i/E_i$ and the water content of the organic solvents at saturation, $S_{w_i}$, for: 1, fatty acids (●); 2, aliphatic alcohols (○); 3, aliphatic amines (▲).

for aliphatic alcohols; and

$$A_i/E_i = -2.082 \ (\pm \ 0.244) + 0.881 \ (\pm \ 0.194) \log S_{w_i} \qquad (12)$$
$$n = 5, r = 0.934, s = 0.474$$

for aliphatic amines.

As the $E_i$ value for a given solvent system is independent of the nature of the solutes being partitioned, and the $A_i$ value is specific of the hydrophilic group of the solute, it seems reasonable to assume that the $A_i/E_i$ ratio is characteristic of the specific interactions of the hydrophilic group with water. Such an assumption explains the observed correlations between the $A_i/E_i$ ratio and the $S_{w_i}$ value. This seems to be in line with the data reported by Harris et al.[23] and Huyskens and co-workers[24,25] that the contribution of a polar group to the free energy of transfer of a solute containing the group depends on the specific interactions of the group with water (and sometimes also with a given organic solvent). Harris et al.[23] have particularly shown that the free energy of transfer values for polar groups having the same relative surface areas but capable of undergoing specific solvation by aqueous or organic phases are markedly different and appear to be in direct proportion to the strength of the specific solute–solvent interactions involved.

The fact that the lines in Fig. 1 intersect means that the apparent hydrophobic character of one polar group with respect to the other may be reversed, depending on the particular partitioning system used. This situation implies that (i) the hydrophobic character of any functional group (or a solute containing such as group) is a relative property of the group (or the solute) and (ii) any comparison of the partition coefficients for a given set of solutes obtained in different solvent systems is valid only if the increment values specific for the functional groups which the solutes contain are known for the systems examined. Thus, the $\Delta g_i^{CH_2}$ values for the two systems to be compared must be determined in designing a solvent regression equation. Then $a_i$ can be calculated and its value is independent of the nature of the solutes considered. For every set of solutes containing a given polar group, the $b_i$ value must be found from the corresponding $K_i$ values and the solvent regression equation.

It should be emphasized that the difference in the relative hydrophobicities of two polar groups depends on the particular partitioning system chosen as a reference standard. The choice of the octanol–water system can be justified in view of the practical reasons given by Hansch and co-workers[1,3,12,26]. It seems necessary however, to consider the relative hydrophobic character as a physical property of any chemical substance and the factors governing the partition behaviour of a solute in any two-phase system before selection of any reference partitioning system.

According to Tanford[27], hydrophobic substances are defined as those readily soluble in many non-polar solvents, but only sparingly soluble in water. The origin of the hydrophobic effect was widely discussed in the literature owing to its biological importance[27–29]. It has been recognized that the hydrophobic effect which is the consequence of the hydrophobicity of some chemical substances is the result of the unique properties of water as the solvent. Water, however, represents only part of a conventional two-phase partitioning system. In addition to possible electronic effects, which are presumed to be small[30], the following factors seem to determine the magnitude of the partition coefficient of a solute in a given two-phase solvent

system[1,2,18,23]: (i) the surface area dependence of the ability of the functional group of a solute to interact with water, (ii) the size of the cavity formed in the aqueous phase, (iii) the surface area dependence of the ability of the functional group to interact with the solvent in the non-aqueous phase, (iv) the size of the cavity formed in the non-aqueous phase, (v) the solubility of water in the organic phase as a factor affecting the structuring properties of the phase and (vi) the solubility of the organic solvent in the aqueous phase as a factor affecting the structuring properties of the phase. With all these factors involved, it seems extremely difficult to make a proper choice of the reference solvent system.

As the hydrophobic character of a solute is a property related to the specific features of water, we believe that the best reference system would be one without any organic solvent. Systems of this type have been designed by Albertsson[31] and have been used for the analysis and separation of various solutes and cell particles. Aqueous two-phase systems are obtained by mixing aqueous solutions of two different polymers (e.g., dextran and polyethylene glycol or Ficoll and dextran) at certain concentrations. As each of the phases contains more than 70–90 % of water, they can be buffered and rendered isotonic (if necessary) and were found to be suitable for the partitioning of cells, viruses, membranes and biological macromolecules (for reviews, see refs. 32–34). It has been particularly shown that the Ficoll–dextran aqueous two-phase system can be used for evaluating the relative hydrophobicities of any solutes[5–10,13]. The estimates obtained can be expressed quantitatively in terms of the so-called equivalent number of $CH_2$ groups, $n^{CH_2}$. The equivalent $CH_2$ number, $n^{CH_2}$, is related to the partition coefficient of a solute, $K$, and to the $\Delta g^{CH_2}$ value characteristic of the two-phase system used by $n^{CH_2} = RT \ln K/\Delta g^{CH_2}$ (similar to the ratio $A_i/E_i$). The $n^{CH_2}$ values obtained for various non-polar amino acid side-chains[8] agreed well with those reported in the literature when scaled and expressed in the same $n^{CH_2}$ terms. The difference in the relative hydrophobicities of the two phases of Ficoll–dextran aqueous two-phase system as measured by the $\Delta g^{CH_2}$ value is 10–20 cal/mole of $CH_2$, and seems to be caused by different effects of the phase polymers on the structuring properties of water present in both phases[35]. From Rekker's point of view, this partitioning system should be classified as the hypodiscriminating one, but its main advantage appears to be the aqueous nature of both phases, which is similar to the biological medium.

It seems reasonable to recommend the Ficoll–dextran aqueous two-phase system as the reference in the study of the hydrophobicities of various substances. It should be noted the the partition coefficients obtained in the Ficoll–dextran system can be correlated with those found in the octanol-water system[10]. Therefore, the choice of the Ficoll–dextran aqueous two-phase system as the reference does not imply the rejection of employment of the octanol–water system.

The most promising aspect of the use of the Ficoll–dextran two-phase system in the study of hydrophobic character of biological materials seems to be the establishment of the correct scale of relative hydrophobicities for biological macromolecules, cell particles and synthetic drugs. We believe that such a scale would make it possible to gain more insight into the fundamentals of structure–activity correlations specific for drugs and natural solutes.

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