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Relative hydrophobicity of organic compounds measured by partitioning in aqueous two-phase systems

Nellie Gulyaeva, Alexander Zaslavsky, Pamela Lechner, Arnon Chait, Boris Zaslavsky*

Analiza, Inc., 4440 Warrensville Center Road, Cleveland, OH 44128, USA

Abstract

Partitioning of a variety of organic compounds, the majority of which represent therapeutic drugs, was examined in an aqueous dextran–polyethylene glycol (Dex–PEG) two-phase system containing 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer at pH 7.3 and in an octanol–buffer (0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.3) system. The possibility of introducing compounds to be partitioned in an aqueous two-phase system with dimethyl sulfoxide, and the effect of this solvent on the solute partitioning was explored. Relative hydrophobicity of the compounds was estimated and expressed in equivalent numbers of methylene units. Comparison of the results obtained for several subsets of compounds in the octanol–buffer and in aqueous Dex–PEG two-phase systems clearly demonstrates the advantage of aqueous two-phase partitioning for the hydrophobicity measurements over partitioning in octanol–buffer system.

Keywords: Partitioning; Aqueous two-phase systems; Hydrophobicity; Organic compounds

1. Introduction

Hydrophobicity of organic compounds is probably one of the most informative physicochemical properties in medicinal chemistry and is widely used in analysis of quantitative structure–activity relationships (QSARs) for pharmaceutical, environmental and biochemical applications [1–3]. Three different terms, hydrophilicity, hydrophobicity and lipophilicity are commonly used in the literature to describe the solute–solvent interactions. Hence, certain confusion exists in regard to which physicochemical methods provide what information, and how this information may be interpreted. The most satisfactory definition of the hydrophobicity and hydrophilicity terms, in our view, is the one offered by Rebinder [4]. That is, that hydrophobicity and hydrophilicity are the measures of the intensity of molecular interactions of the solute with water. Hydrophilicity is specified by the value of the free energy of hydration of a given compound. A hydrophobic compound should be regarded as one having very low hydrophilicity. This definition will be used here for the hydrophobicity term.

The above definition implies that the best measure of a solute hydrophobicity would be the value of the free energy of transfer of the solute from gas phase into aqueous media. Unfortunately, practical use of such a measure (or similar one as described by Wolfenden [5] and Radzieka and Wolfenden [6]) is limited being applicable only to volatile compounds. To overcome this difficulty, it was suggested to replace gas phase with organic solvent, such as hexane or octanol. Organic solvent was viewed as an

*Corresponding author. Tel.: +1-216-5864-595; fax: +1-216-5863-886.

E-mail address: bz@analiza.com (B. Zaslavsky)

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inert non-polar phase that does not interact with compounds being examined.

The partition coefficient, defined for dilute solutions as the molar concentration ratio ($P=C_o/C_w$) of a single species between the organic and aqueous phases at equilibrium, is a useful quantitative parameter for representing the hydrophobicity of the substance. The log *P* for the 1-octanol–water system is widely used as a structure descriptor in QSARs. Many methods for estimating log *P*, experimental as well as computational, are described in the literature. Most of the computational techniques are ''fragment constant'' methods, in which a structure is divided into previously defined fragments and the corresponding contributions are summed together to yield the final log *P* estimate. The contributions of the fragments used in these calculations are obviously only as good as the experimental data from which they are derived, and many correction factors are commonly used. Therefore, the experimental log *P* values are still viewed as the most reliable source of information about the hydrophobicity of organic compounds.

Accurate measurements of partition coefficients of solutes in the octanol–water system still remain a challenge in a modern analytical laboratory [7]. The main problem is to assay a solute whose concentrations in the phases may differ by several orders of magnitude. Additionally, because of widely varied molecular structures, different analytical techniques for assaying the solutes are often needed. Even if these issues are resolved, the possibility of comparison of the log *P* values for compounds with different molecular structures, in particular, for multifunctional compounds, remains an open question. The reason for this is the fundamentally incorrect assumption that the organic phase provides an inert non-aqueous environment which may be used as a reference state for different compounds. In reality, of course, the octanol phase is not inert, and octanol–solute interactions may be very different for compounds of different structure [8]. Nevertheless, the fact that log *P* values for different compounds within a limited series of structures may be used for QSAR analysis implies that the above assumption is applicable within certain limitations. Unfortunately, these limitations have not yet been systematically explored and defined.

The applicability of aqueous two-phase systems, with and without an organic solvent additive, to analysis of the relative hydrophobicity of organic compounds was explored in this work.

Aqueous two-phase systems are widely used for separation and analysis of biological cells, proteins, nucleic acids, etc. [9,10]. These systems arise in aqueous mixtures of different water-soluble polymers or a single polymer and a specific salt. When two certain polymers, e.g., dextran (Dex) and polyethylene glycol (PEG), are mixed in water above certain concentrations, the mixture separates into two immiscible aqueous phases. There is a clear interfacial boundary, and one phase is rich in one polymer and the other phase is rich in the other polymer. The aqueous solvent in both phases provides media suitable for biological products.

When a solute is introduced into such a system, it distributes between the two phases. Partitioning of a solute is characterized by the partition coefficient *K* defined as the ratio between the concentrations of the solute in the two phases. It was shown [9] that phase separation in aqueous polymer systems results from different effects of two polymers (or a single polymer and a salt) on the water structure. As the result, the solvent features of aqueous media in the coexisting phases are different as established by dielectric, solvatochromic, potentiometric and partition measurements (for review see Refs. [9,12]).

The basic rules of solute partitioning in aqueous two-phase systems were shown [9] to be similar to those in water–organic solvent systems. The differences between the properties of the two phases in aqueous polymer systems are very small relative to those observed in water–organic solvent systems, as should be expected for a pair of solvents of the same (aqueous) nature. The small difference between the solvent features of the phases in aqueous two-phase systems actually provides certain advantage from the viewpoint of enhanced sensitivity of solute partitioning toward modifications in the solute structure.

Partitioning of a variety of organic compounds in aqueous Dex–PEG two-phase systems with and without addition of dimethyl sulfoxide (DMSO) was examined here to estimate the relative hydrophobicity of organic compounds. The same compounds were also partitioned in the octanol–buffer system, and the partition coefficient values were compared

with the corresponding values in the aqueous Dex–PEG system.

2. Experimental

2.1. Materials

All compounds examined were purchased from Sigma–Aldrich (St. Louis, MO, USA) and used without further purification. Dextran-69 (relative molecular mass ~69 000 by light scattering, lot 106H0841) and PEG with relative molecular mass about 8000 (PEG-8000, lot 85H0654) were obtained from Sigma. All inorganic salts and reagents used were of analytical reagent grade.

2.2. Phase systems

For aqueous two-phase systems, a mixture of polymers was prepared by dispensing appropriate amounts of the aqueous stock ca. 35% (w/w) Dex-69 solution and 40.00% (w/w) PEG-8000 solution into a 1.2-ml microtube using a Hamilton MICROLAB 2200 single-probe liquid-handling sample processor. An appropriate amount of 0.6 *M* NaCl in 0.04 *M* Na-phosphate buffer, pH 7.3 was added so as to give the required ionic and polymer composition of: 6.00% (w/w) PEG-8000, 12.18% (w/w) Dex-69, and 0.15 *M* NaCl in 0.01 *M* Na-phosphate buffer, pH 7.3 in the final system whose total volume was 1.0 ml. 1-Octanol and a solution of 0.15 *M* NaCl in 0.01 *M* sodium phosphate buffer, pH 7.3 were mutually saturated by shaking, and separated in a separatory funnel. Compounds to be partitioned were dissolved at an appropriate concentration in a 0.2-ml aliquot of either buffer-saturated octanol, or octanol-saturated buffer. This solution was combined with aliquots of each phase so as to yield a system having two phases equal in volume. Systems were prepared in 1.2-ml microtubes using a Hamilton MICROLAB 2200 single-probe liquid-handling sample processor.

2.3. Partition experiments

Solutions of compounds to be partitioned in the aqueous Dex–PEG systems were prepared in water and/or DMSO at concentrations of ca. 1 to 15 mg/ml. A varied amount (30, 60, 90, 150 and 200 μl) of a given compound solution and the corresponding amount (170, 140, 110, 50 and 0 μl) of water or DMSO were added to a polymer–buffer mixture. The system was vigorously vortexed and centrifuged for 30 min at approximately 3000 rpm (1160 *g*) to speed the phase settling. Aliquots of 100 μl from the top and the bottom phases were withdrawn in duplicate for further analysis.

Concentrations of DMSO in the phases of the aqueous Dex–PEG systems were measured in the laboratory of ANTEK Instruments (Houston, TX, USA) with the ANTEK sulfur selective HPLC detection system (HPLC–SCD), Model 8040. The procedure in accordance with the ASTM method D5453 was used. Samples from both phases were diluted two-fold with water and injected into detector. The concentrations of DMSO in the phases were found to be 28.5% (v/v) in the upper PEG-rich phase, and 11.5% (v/v) in the bottom Dex-rich phase. The DMSO distribution coefficient amounts to 2.48.

Aliquots from the phases were appropriately diluted with water and assayed for the concentration of a compound being partitioned by measuring optical absorbance at the corresponding maximum wavelength using a HP-8453 diode-array UV–Vis spectrophotometer. In all instances the correspondingly diluted pure phases were used as blank solutions.

The partition coefficient, *K*, is defined as the ratio of the sample concentration (or absorbance) in the PEG-rich (upper) phase to the sample concentration (or absorbance) in the dextran-rich (bottom) phase. The *K* value for each solute was determined at 20°C as the slope of the plot of the concentration in the upper phase as a function of the concentration in the bottom phase from four to five partition experiments. The deviation from the average *K* value did not exceed 2% for any of the substances examined.

Solutions of compounds to be partitioned in the octanol–buffer systems were prepared in buffer-saturated octanol or in octanol-saturated buffer at concentrations of ca. 1 to 15 mg/ml. A varied amount (30, 60, 90, 150 and 200 μl) of a given compound solution and the corresponding amount (170, 140, 110, 50 and 0 μl) of the corresponding phase were added to an octanol–buffer system. The system was vigorously vortex mixed and centrifuged for 30 min

at approximately 3000 rpm (1160 *g*) to speed the phase settling. Aliquots of 100 μl from the top and the bottom phases were withdrawn in duplicate for further analysis.

Aliquots from the phases were appropriately diluted with DMSO and assayed for the concentration of the compound being partitioned by measuring optical absorbance at the corresponding maximum wavelength as indicated above. The correspondingly diluted pure phases were used as blank solutions in all cases.

The partition coefficient, *D*, is defined as the ratio of the sample concentration (or absorbance) in the buffer-saturated octanol phase to the sample concentration (or absorbance) in the octanol-saturated aqueous (bottom) phase. The *D* value for each solute was determined at 20°C as the slope of the plot of concentration in the upper phase as a function of the concentration in the bottom phase from four to five partition experiments. The deviation from the average *D* value did not exceed 3% for any of the substances examined.

3. Results and discussion

3.1. Calibration of aqueous Dex–PEG two-phase systems with and without DMSO additive

Sodium salts of DNP-derivatives of amino acids (Gly, Ala, nor-Val, nor-Leu and α-amino-*n*-caprylic acid) were partitioned in the system with and without DMSO additive as described above. The results obtained are plotted in Fig. 1 as logarithms of the partition coefficient, *K*, values vs. length of the aliphatic side-chain expressed in terms of equivalent number of CH_2 units (see below).

Partitioning of a homologous series of monofunctional aliphatic compounds in aqueous polymer two-phase systems is described as in [8]:

$$\ln K = A + E \cdot N_C \quad (1)$$

where *K* is the solute partition coefficient; N_C is the equivalent number of CH_2 groups in the aliphatic alkyl chain of the partitioned solute molecule; *A* and *E* are constants. The coefficient *E* in Eq. (1) represents an average ln *K* increment per CH_2 group.

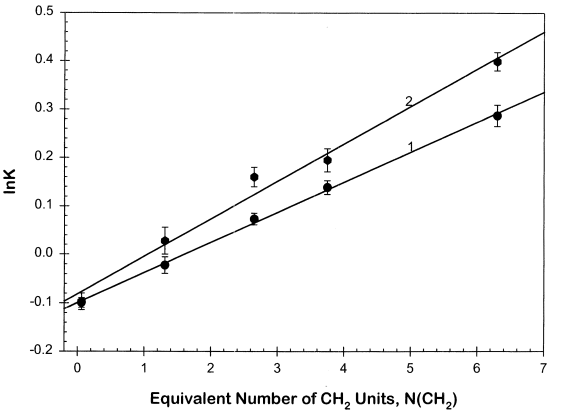

Fig. 1. Logarithm of the partition coefficient, ln *K*, value for sodium salts of DNP-amino acids (Gly, Ala, nor-Val, nor-Leu and α-amino-*n*-caprylic acid) in the aqueous Dex–PEG two-phase system as a function of the length of the aliphatic side-chain expressed in terms of equivalent number of CH_2 units. (1) Dex–PEG system without organic solvent additive; (2) Dex–PEG system with 20% (v/v) DMSO additive.

Coefficient *E* amounts to 0.062 ± 0.001 in the system without organic solvent additive and to 0.077 ± 0.005 in the system containing 20.0% (v/v) DMSO.

The coefficient *A* represents the total contribution of a polar moiety of the solute molecule. In the present case coefficient *A* amounts to -0.081 ± 0.019 in the presence of DMSO and to -0.099 ± 0.005 in the same system without organic solvent additive.

The coefficient *E* is related to the free energy of transfer of a CH_2 group from one to the other phase in a given two-phase system, $\Delta G(CH_2)$:

$$\Delta G(CH_2) = -RT \cdot E \quad (2)$$

It should be particularly noted that the methylene group increment, *E*, into the ln *K* value is independent of the nature of the aliphatic solutes being partitioned [8], and hence *E* or alternatively $\Delta G(CH_2)$ may be used as a measure of the difference between the affinities of the two phases for a CH_2 group, i.e., difference between the hydrophobic character of the two phases [8,11,12].

It has been demonstrated by various techniques (reviewed in Refs. [8,11]) that partitioning of a solute in an aqueous polymer two-phase system is governed by the difference between the intensities of the solute–solvent interactions in the two phases.

Hence the partition coefficient of a solute in such a system represents the free energy of transfer of the solute between two aqueous media of different solvent properties and therefore may be used as a measure of the relative hydrophobicity of the solute [8,12].

The ratio expressed as

$$\Delta G(\text{solute})_{tr}/\Delta G(CH_2) = n(CH_2) \quad (3)$$

or

$$\ln K/E = n(CH_2) \quad (3a)$$

has been defined as the equivalent quantity of methylene units and suggested [8,12] to be used as a measure of the relative hydrophobicity of a solute (or a moiety). A positive value of $n(CH_2)$ means that a given solute is hydrophobic and its relative hydrophobicity is equal to that of an n units of methylene groups. A negative value of $n(CH_2)$ means that the solute is hydrophilic and its relative hydrophobicity is the reverse of that of an n number of CH_2 units.

3.2. Partitioning of organic compounds in ATP

The K, and ln K values for the compounds studied are presented in Table 1. The ln K values in the two systems used – with and without DMSO additive, are plotted against each other in Fig. 2.

It can be seen from the data in Fig. 2 that there is a strong correlation between the partition coefficients for different compounds in the system with the DMSO additive, ln $K^{\text{Dex–PEG–DMSO}}$, and in the similar system without DMSO, $K^{\text{Dex–PEG}}$. This correlation may be described as:

$$\ln K^{\text{Dex–PEG–DMSO}} = b + a \cdot \ln K^{\text{Dex–PEG}};$$
$$N = 36; r^2 = 0.9727 \quad (4)$$

where N is the total number of compounds examined; r^2 is the correlation coefficient; a and b are constants. The values of the coefficients are: $b = 0.089 \pm 0.013$; and $a = 0.649 \pm 0.019$.

The correlation Eq. (4) indicates that water-insoluble compounds may be introduced into an aqueous two-phase system in DMSO, and the partition results recalculated for the system without DMSO. That clearly extends the possible range of applicability of the aqueous two-phase partition technique for a variety of organic compounds.

3.3. Partitioning of organic compounds in the octanol–buffer system

The logarithms of the partition coefficients log D values for the compounds studied are presented in Table 1. No general correlation may be found between the partition coefficients of the compounds in the octanol–buffer system and the partition coefficients for the same compounds in the aqueous Dex–PEG two-phase system. That might be expected, as the log D values obtained in the octanol–buffer system may be compared only for the compounds of similar nature/structure. However, for a series of similar compounds a relationship between the ln K and log D values might be expected.

Partition coefficients for the series of β-lactam antibiotics including nafcillin, dicloxacillin, cloxacillin, oxacillin, phenoxymethylpenicillin, benzylpenicillin, carbencillin, ampicillin, ceftriaxone, cefaclor, cefuroxime, cephalexin, cephalothin, cefamandole, cefuroxime, cefazolin cefaclor and cephradine, measured in the octanol–buffer and aqueous Dex–PEG systems are plotted against each other in Fig. 3, curve 1. The correlation observed is described as:

$$\ln K^{\text{Dex–PEG}} = b + a \cdot \log D^{\text{Octanol–buffer}};$$
$$N = 16; r^2 = 0.9826 \quad (5)$$

where N is the total number of compounds examined; r^2 is the correlation coefficient; a and b are constants. The values of the coefficients are: $b = 0.954 \pm 0.032$; and $a = 0.525 \pm 0.019$.

A similar relationship observed for sulfonphthalein dyes (phenol red, bromphenol red, cresol red, thymol blue and bromthymol blue) is also presented in Fig. 3, curve 2. This relationship is described as:

$$\ln K^{\text{Dex–PEG}} = b + a \cdot \log D^{\text{Octanol–buffer}};$$
$$N = 5; r^2 = 0.9990 \quad (6)$$

where N is the total number of compounds examined; r^2 is the correlation coefficient; a and b are constants. The values of the coefficients are: $b = 1.359 \pm 0.012$; and $a = 0.512 \pm 0.009$.

Partition coefficients for the series of β-adrenergic

Table 1
Partition coefficients of drugs in aqueous Dex–PEG[a] and Dex–PEG–DMSO[b], and in octanol–buffer[c] two-phase systems

#	Compound	$K^{Dex-PEG}$	ln $K^{Dex-PEG}$	$N(CH_2)^{Dex-PEG}$	K^{DMSO}	ln K^{DMSO}	D	log D
1	Alprenolol	2.052±0.075	0.719±0.035	11.6±0.6	1.672±0.032	0.514±0.019	1.679±0.028	0.225±0.007
2	Atenolol	1.874±0.076	0.628±0.041	10.1±0.7	1.626±0.011	0.486±0.016	1.053±0.023	0.022±0.009
3	Ceftriaxone	–[d]	(−0.680)[e]	(−11.0)[e]	0.713±0.021	−0.338±0.029	0.00104	−2.983
4	Cephalexin	0.945±0.035	−0.057±0.036	−0.9±0.6	–[d]		0.0093	−2.034
5	Chloramphenicol	1.209±0.042	0.190±0.034	3.1±0.6	1.232±0.028	0.209±0.022	1.764±0.044	0.246±0.011
6	Corticosterone	1.602±0.020	0.471±0.012	7.6±0.2	1.456±0.087	0.376±0.058		
7	Coumarin	1.791±0.064	0.583±0.033	9.4±0.5	1.590±0.021	0.464±0.013	27.42±1.4	1.438±0.023
8	Dexamethasone	–[d]	(1.070)[e]	(17.3)[e]	2.232±0.052	0.803±0.023	16.50	1.217
9	Diltiazem	1.775±0.012	0.574±0.007	9.3±0.1	1.600±0.061	0.470±0.035	0.538±0.033	−0.269±0.026
10	Hydrocortisone	1.697±0.023	0.529±0.014	8.5±0.2	1.568±0.009	0.450±0.005		
11	Imipramine	2.550±0.015	0.936±0.006	15.1±0.1	1.937±0.052	0.661±0.026	4.339±0.055	0.637±0.005
12	Metoprolol	–[d]	(0.225)[e]	(3.6)[e]	1.272±0.021	0.241±0.016	0.160±0.004	−0.795±0.010
13	Propranolol	2.250±0.020	0.811±0.009	13.1±0.2	1.917±0.007	0.651±0.004	3.649±0.070	0.562±0.008
14	Terbutaline	1.797±0.008	0.586±0.004	9.5±0.01	1.654±0.014	0.503±0.008	0.0094	−2.025
15	Theophylline	1.123±0.007	0.116±0.006	1.9±0.01	1.170±0.015	0.157±0.013	0.850±0.025	−0.071±0.013
16	Verapamil	3.394±0.078	1.222±0.023	19.7±0.4	2.479±0.081	0.908±0.030	3.592±0.042	0.555±0.007
17	Warfarin	–[d]	(1.038)[e]	(16.7)[e]	2.187±0.003	0.783±0.001	135.12±6.76	2.131±0.021
18	NitrophenylMannoside	1.160±0.011	0.148±0.010	2.4±0.2	1.301±0.020	0.263±0.015	0.858±0.003	−0.067±0.002
19	Gly–Gly	0.859±0.007	−0.152±0.008	−2.5±0.1	1.053±0.006	0.052±0.006		
20	Gly–Asp	0.678±0.008	−0.389±0.012	−6.3±0.19	0.563±0.002	−0.574±0.004		
21	Cresol Red Na	2.450±0.006	0.896±0.002	14.5±0.03	2.054±0.090	0.720±0.041	0.121±0.005	−0.919±0.018
22	Thymol Blue Na	5.930±0.220	1.780±0.038	28.7±0.6	3.831±0.051	1.343±0.013	7.568±0.222	0.879±0.013
23	Phenol Red Na	1.740±0.005	0.554±0.003	8.9±0.1	1.687±0.083	0.523±0.044	0.0251	−1.600
24	Bromthymol Blue Na	10.591±0.139	2.360±0.013	38.1±0.2	4.406±0.025	1.483±0.006	25.119	1.400
25	Bromphenol Red Na	2.075±0.030	0.730±0.015	11.8±0.2	1.733±0.064	0.550±0.038	0.063±0.007	−1.200±0.015
26	Cephalothin Na–salt	1.180±0.007	0.166±0.006	2.7±0.1	1.151±0.032	0.141±0.028	0.0201	−1.697
27	Cefamandole Na–salt	1.160±0.006	0.148±0.005	2.4±0.1	1.180±0.031	0.166±0.026	0.023±0.001	−1.637±0.007
28	Cefuroxime Na–salt	1.240±0.008	0.215±0.006	3.5±0.1	1.197±0.038	0.180±0.032	0.0398	−1.400
29	Cefazolin Na–salt	0.956±0.006	−0.045±0.006	−0.7±0.1	1.005±0.016	0.005±0.016	0.01296	−1.887
30	Cefachlor	0.950±0.018	−0.051±0.019	−0.8±0.3	–[d]		0.0120	−1.92
31	Cephradine	0.880±0.006	−0.128±0.007	−2.1±0.1	–[d]		0.008±0.004	−2.100±0.028
32	Nafcillin Na–salt	1.813±0.014	0.595±0.008	9.6±0.1	1.565±0.014	0.448±0.009	0.219±0.004	−0.660±0.009
33	Dicloxacillin Na–salt	1.877±0.040	0.630±0.021	10.2±0.3	1.677±0.025	0.517±0.015	0.274±0.005	−0.562±0.008
34	Cloxacillin Na–salt	1.562±0.005	0.446±0.003	7.2±0.1	1.504±0.005	0.408±0.003	0.109	−0.962
35	Oxacillin Na–salt	1.300±0.012	0.262±0.008	4.2±0.1	1.306±0.007	0.267±0.005	0.0532	−1.274
36	Phenoxymethylcillin-*K*	1.221±0.003	0.200±0.002	3.2±0.03	1.201±0.017	0.183±0.013	0.0398	−1.400
37	Benzylpenicillin-Na	1.070±0.011	0.068±0.047	1.1±0.8	1.197±0.037	0.180±0.029		−1.640
38	Carbenicillin Na–salt	1.064±0.014	0.062±0.013	1.0±0.2	1.143±0.008	0.134±0.007	0.0224	−1.65±0.011
39	Ampicillin	0.970±0.007	−0.030±0.007	−0.5±0.1	0.980±0.020	−0.020±0.022		−1.850
40	DNP-Glycine Na–salt	0.906±0.010	−0.099±0.011	−1.6±0.2	0.908±0.015	−0.097±0.017	0.025	−1.602
41	DNP-Alanine Na–salt	0.978±0.014	−0.022±0.014	−0.35±0.2	1.028±0.028	0.028±0.028	0.0469	−1.287
42	DNP-Norvaline Na–salt	1.077±0.024	0.074±0.022	1.2±0.4	1.174±0.024	0.160±0.020	0.225±0.006	−0.648±0.012
43	DNP-Norleucine Na–salt	1.149±0.023	0.139±0.020	2.2±0.3	1.215±0.030	0.195±0.024	0.866	−0.062
44	DNP-Aminocaprylic acid Na–salt	1.332±0.023	0.287±0.017	4.6±0.3	1.490±0.029	0.399±0.019	11.028	1.042

[a] System contained 0.15 *M* NaCl in 0.01 *M* Na–PB pH 7.3.
[b] System contained 20 % (v/v) DMSO and 0.15 *M* NaCl in 0.01 *M* Na–PB pH 7.3.
[c] Buffer composition – 0.15 *M* NaCl in 0.01 *M* Na–PB pH 7.3.
[d] Partitioning could not be measured due to low solubility and/or low extinction coefficient of the compound.
[e] Estimated from partition results in Dex–PEG–DMSO system and calculated with Eq. (4) (see text).

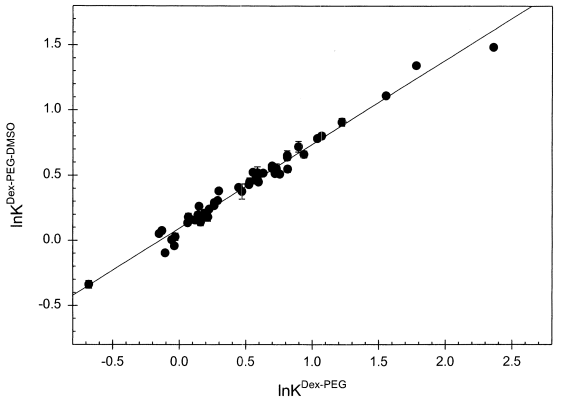

Fig. 2. Logarithm of partition coefficients of organic compounds in the aqueous Dex–PEG two-phase system with additive of 20% (v/v) DMSO, $K^{\text{Dex–PEG–DMSO}}$, vs. partition coefficients for the same compounds in the aqueous Dex–PEG two-phase system, $K^{\text{Dex–PEG}}$.

blockers – atenolol, alprenolol, metoprolol and propranolol in the aqueous Dex–PEG system are plotted versus those obtained in octanol–buffer system in Fig. 3, curve 3. The linear relationship observed is described as:

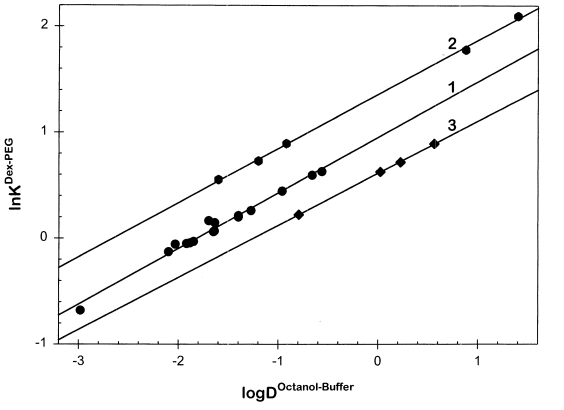

Fig. 3. Logarithm of partition coefficients for three different series of compounds in the aqueous Dex–PEG system, $K^{\text{Dex–PEG}}$, vs. logarithm of partition coefficients for the same compounds in the octanol–buffer system, $D^{\text{Octanol–buffer}}$: (1) β-lactam antibiotics (nafcillin, dicloxacillin, cloxacillin, oxacillin, phenoxymethylpenicillin, benzylpenicillin, carbenicillin, ampicillin, ceftriaxone, cefaclor, cefuroxime, cephalexin, cephalothin, cefamandole, cefuroxime, cefazolin, cefaclor and cephradine); (2) sulfonphthalein dyes (phenol red, bromphenol red, cresol red, thymol blue and bromthymol blue); (3) β-adrenergic blockers (atenolol, alprenolol, metoprolol and propranolol).

$$\ln K^{\text{Dex–PEG}} = b + a \cdot \log D^{\text{Octanol–buffer}};$$
$$N = 4; r^2 = 0.9997 \quad (7)$$

where N is the total number of compounds examined; r^2 is the correlation coefficient; a and b are constants. The values of the coefficients are: $b = 0.615 \pm 0.003$; and $a = 0.492 \pm 0.006$.

3.4. Advantages of ATP partitioning over octanol–buffer partitioning for QSAR analysis

Eqs. (5)–(7) indicate that the relationships between the partition coefficients in the aqueous Dex–PEG system, $K^{\text{Dex–PEG}}$, and those in the octanol–buffer system for the same compounds, $D^{\text{Octanol–buffer}}$, are parallel to each other. The average slope is the same (average coefficient a value is 0.510 ± 0.017). The intercepts, coefficient b values, however, vary significantly – from 0.615 to 1.359 depending on the general structure of the compounds examined. This result agrees qualitatively with the previous considerations [13] of the physical meaning of coefficients a and b in the so-called solvent regression equation (see Ref. [9], pp. 268–276). This observation also supports the assumption [9,12] that the octanol–buffer system may not be viewed as capable of providing the estimates of the relative hydrophobicity of compounds of different chemical nature/structure comparable on a universal scale. The obvious aforementioned reason is that water-saturated octanol does not provide an inert non-aqueous environment for compounds with functional groups. Since it is not known how the intensity of octanol–solute interactions differs for different compounds, the log P (or log D) values cannot be compared. Additionally, the distribution coefficient, D, depends on the ratio of ionic and non-ionic species of a compound being partitioned. The ratio in question may differ in octanol–buffer and in aqueous Dex–PEG system. The dissociation degree of the same compound may be viewed as essentially the same in the two phases of aqueous two-phase system as compared to that in the non-aqueous and aqueous phases of the octanol–buffer system. Aqueous two-phase partitioning occurring between phases of the same aqueous nature does provide comparable estimates of the relative hydrophobicity for chemically different compounds.

Finally, it should be mentioned that attempts to use aqueous two-phase systems formed by a single polymer and an inorganic salt, such as PEG–Mg_2SO_4, have been reported in the literature [14]. No systematic study of the applicability of such systems for the hydrophobicity measurements has been published, however, to our knowledge. Our results (to be published) indicate that the aqueous PEG–salt systems, such as PEG–sodium sulfate and PEG–sodium/potassium phosphate, in particular, may not be viewed as suitable for the relative hydrophobicity measurements, likely due to large differences between the electrostatic properties of the two phases in these systems. It is possible, however, that the information provided by partitioning in PEG–salt systems may be of importance for better understanding of the solute–solvent interactions in aqueous environment under conditions simulating certain situations occurring in vivo.

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