Comparison of Lipid Membrane-Water Partitions with Various Organic Solvent-Water Partitions of Neutral Species and Ionic Species; Uniqueness of Cerasome as a Model for the Stratum Corneum in Partition Processes

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Abstract

Lipid membrane-water partitions (e.g., immobilized artificial membrane systems where the lipid membrane is a neutral phospholipid monolayer bound to gel beads) were compared to various organic solvent-water partitions using linear free energy relationships. To this end, we also measured the retention factors of 36 compounds (including neutral and ionic species) from water to liposomes made up of 3-sn-phosphatidylcholine and 3-sn-phosphatidyl-L-serine (80:20, mol/mol), employing liposome electrokinetic chromatography in this work. The results show that lipid membranes exhibit a considerably different chemical environment from those of organic solvents. For both neutral species and ionic species, partitions into the more polar hydroxylic solvents are chemically closer to partition into the lipid membrane as compared to partitions into the less polar hydroxylic solvents and into aprotic solvents. This means that solutes partition into the polar parts of lipid membranes, regardless of whether they are charged or not. In addition, cerasome (i.e., liposome composed mainly of stratum corneum lipids) was compared with regular phospholipid liposomes as a possible model for human stratum corneum in partitions. It was found that the cerasome-water partition exhibits a better chemical similarity to skin permeation. This is probably due to the unique structures of ceramides that occur in cerasome and in the stratum corneum lipid domain. We further show that membranes in membrane-water partitions exhibit very different properties.

KEY WORDS: Liposomes; LEKC; physicochemical properties; LFER; ionic species; partition

Introduction

The *n*-octanol-water partition coefficient (as log P_{oct}) is widely used as an estimate for partitions of chemicals into biological membranes, but it is now recognized that biological membranes vary so much in character that it is impossible for any given solvent-water system to be a useful model for all membranes (Abraham and Acree, 2012b). In addition, recent work on ionic partition into biological membranes suggests that that the *n*-octanol-water system is not satisfactory as a model for ionic partition (Abraham, 2011; Abraham and Austin, 2012). Consequently, the liposome-water partitioning system has been developed as a promising model for biological partition process because of the highly-ordered lipid bilayer microstructure of liposome. Workers have observed that ionic species partition significantly better into liposome than into *n*-octanol (Austin et al., 1995; Avdeef et al., 1998; Balon et al., 1999b; Fruttero et al., 1998; Mason et al., 1991), while the partition coefficients of neutral species were found to be comparable but not equal in liposome-water and n-octanol-water systems (Avdeef et al., 1998; Fruttero et al., 1998). Recently, Zhang et al. (2011) demonstrated that cerasome (i.e., liposome consisting mainly of stratum corneum (SC) lipids)-water system is chemically rather different from various organic solvent-water systems, using linear free energy relationships (LFERs) as a method of analysis. It was further shown that the cerasome-water partition is a reasonable chemical model for the SC-water partition, although this conclusion was deduced for neutral species only (Zhang et al., 2012).

Even so, it is of considerable interest to compare neutral liposomes with organic solvents, which are neutral, on solute interactions from a physicochemical point of view, given that cerasome is negatively charged. So far, there has been no easy-to-use way to measure a large number of neutral liposome-water partition parameters of neutral and ionic species, which are required for a rigorous physicochemical analysis. The current techniques used to characterize

lipid membrane-water partitions and their advantages and disadvantages are summarized in Table 1. The use of immobilized artificial membranes (IAMs) is a rapid reliable method to characterize neutral lipid membrane-water partitions for both neutral and ionic species. However, immobilized lipid monolayers in IAM are known to differ from lipid bilayers in liposomes in terms of the lateral mobility of lipids and the density of the polar phospholipid head-groups (Ong et al., 1996; Rand and Parsegian, 1989).

Table 1. Current techniques for analyzing the lipid membrane-water partitions and their respective characters

Techniques	Advantages	Disadvantages	Other Comments
Shake Equilibrium (Austin et al., 1995; Pauletti and Wunderli-Allenspach, 1994)	Standard approaches	Time-consuming; tedious; laborious; unwieldy	Traditional techniques (e. g. dialysis method, centrifugation method, ultrafiltration method)
Potentiometric Titration (Avdeef et al., 1998; Fruttero et al., 1998)	Relatively higher speed; log P_{lip} values for both ionic and neutral species[a]	Only suitable for ionizable solutes; time-consuming; tedious; laborious;	Based on a pH-metric titration technique
Immobilized Artificial Membrane (IAM) Chromatography (Ong et al., 1995; Pidgeon and Venkataram, 1989)	Speed; high-reproducibility; small sample amount; low purity requirement	Lipid monolayer with lack of lateral mobility of lipids and density of phospholipid head-groups	Set up on high-performance liquid chromatography (HPLC)
Immobilized Liposome Chromatography (ILC) (Beigi et al., 1995; Wiedmer et al., 2004)	Speed, high-reproducibility; small sample amount; low purity requirement	Unstable; irreproducible column preparation; unsuitable for lipophilic solutes (long retention times for many neutral molecules)	Set up on HPLC
Liposome Electrokinetic Chromatography (LEKC) (Carrozzino and Khaledi, 2004; Wiedmer and Shimmo, 2009)	Speed; high-reproducibility; small sample amount; low purity requirement	Unsuitable for neutral solutes with neutral liposomes used	Set up on capillary electrophoresis (CE)

[a] log P_{lip} represents the partition coefficient between liposome and water.

The focus of this work is to compare the physicochemical nature of neutral IAM systems and liposome-water partitions with organic solvent-water partitions using LFERs, as well as

some biological membrane systems, for which the ionic LFER equations are available. In addition, we also investigated what the crucial difference is between lipid monolayers in IAM and lipid bilayers in liposomes as regards interactions with solutes. Recently, Liu et al. (2008) have measured a large number of neutral and charged compounds in an IAM system, where phosphatidylcholines (PC) were immobilized to gel beads. In the present work, we measured the partitions of most of these compounds in liposomes made up of 3-*sn*-phosphatidylcholine (POPC) and 3-*sn*-Phosphatidyl-L-serine (PS) (80:20, mol/mol), using liposome electrokinetic chromatography (LEKC). We made use of these data to achieve the above aim.

Materials and Methods

Chemicals

A series of (4-methylbenzyl)alkylamines were synthesized according to known procedures (Meindl et al., 1984). The other compounds in Table 2 were purchased form Sigma-Aldrich (Steinheim, Germany), together with 3-*sn*-phosphatidylcholine (from synthetic) and 3-*sn*-phosphatidyl-L-serine (from bovine brain). Decanophenone was obtained from Alfa Aesar (Karlsruhe, Germany). Methanol and chloroform were purchased from Carl Roth (Karlsruhe, Germany).

Liposome Preparation

All liposomes were prepared once by the extrusion method. Briefly, the desired amounts of POPC and PS (80:20, mol/mol) were dissolved in chloroform/methanol mixture (9:1, v/v) in a 1L round bottom flask. A thin lipid film was formed by removing organic solvents under vacuum at 45°C with a rotary evaporator. The resulting film was hydrated and dispersed in 100 ml 10 mM phosphate buffer pH 7.4 by continuous shaking for 60 min at 45°C to yield multilamellar vesicles (MLVs) with a lipid concentration of 3 mM. Then the MLVs were extruded through a polycarbonate filter (100 nm pore size; Poretics, Livermore, USA) 20

times at room temperature using a EmulsiFlex C5 (Avestin, Ottawa, Canada) under an external pressure of 3.5 bar. The final product was a homogeneous liposome dispersion of large unilamellar vesicles (LUVs), for which the average particle size and zeta potential were 79.4 (±0.5) nm with a polydispersity index of 0.084 and -27.1 (±1.0) mV, respectively, measured using a Zetasizer Nano ZS (Malvern, Herrenberg, Germany). The liposome dispersion was very stable for at least two months at 4°C (unreported data), and thus used in the whole work.

LEKC

The LEKC experiments were carried out using a HPCE 1600AX (Agilent, Waldbronn, Germany) equipped with a diode array detector (DAD). 58.5/50 cm uncoated fused-silica capillaries of 50 µm id and 375 µm od (BGB Analytic, Schloßböckelheim, Germany) were employed throughout this study. A new capillary was rinsed with 1.0 M aqueous sodium hydroxide for 20 min, water for 5 min, 1.0 M hydrochloride acid for 20 min and water for 5min. The samples were analyzed at +20 kV, with a hydrodynamic injection at 50 mbar for 3s. The capillary was kept at 37°C and the detection wavelengths were set at 210 nm, 225 nm, and 245 nm.

The LEKC procedures were fully detailed in our previous work (Zhang et al., 2011). Briefly, neutral solutes were determined with the liposome dispersion used as the running solution, while charged solutes required measurements in the presence of the liposome dispersion and the buffer solution, respectively. The retention factors (k) of neutral and charged solutes can be calculated according to Eqs. 1 and 2, respectively,

$$k = \left(t_r - t_0\right) \Big/ t_0 \left(1 - t_r / t_{lip}\right) \quad (1)$$

$$k = \left(t_r - t_{eo}\right) \Big/ t_{eo} \left(1 - t_r / t_{lip}\right) \quad (2)$$

where t_r, t_{eo} and t_{lip} are the retention times of the solute, the electroosmotic flow marker (methanol) and the liposome marker (decanophenone) in the liposome dispersion,; t_0 is the

retention time of charged solutes in the buffer solution. The log k measurements of all the solutes were repeated 3 times. The compounds were dissolved in methanol to prepare stock solutions, which were diluted with the running solution before injection to approximately 2.0~3.0×10-4 mol/L. Before sample injection, the capillary was rinsed for 3 min with the running solution (buffer solution or liposome dispersion). Decanophenone dissolved in methanol was added where appropriate, as the liposome maker. All solutions were filtered (200 nm) prior to use.

LFER

An LFER equation proposed by Abraham (1993) has been successfully used to characterize numerous equilibrium systems, including *in vivo* and *in vitro* partition processes and transport processes, and to predict the corresponding equilibrium coefficients, including partition coefficients (e.g., log P_{oct} or log P_{lip}) and rate coefficients (e.g., skin permeability log K_p), see Eq. (3):

$$SP = c + eE + sS + aA + bB + vV \quad (3)$$

SP represents an equilibrium coefficient, such as log P, for a series of solutes in a given system. The independent variables are physicochemical properties or descriptors of the solutes as follows: E is the excess molar refraction in $(cm^3mol^{-1})/10$, S is the solute dipolarity/polarizability, A and B are the overall hydrogen bond acidity and basicity, respectively, and V is the McGowan characteristic molecular volume in $(cm^3mol^{-1})/100$. Eq. (3) was set up for processes involving neutral species only. Abraham and Acree (2010a, b, c, d) found that in order to apply Eq. (3) to ionic species it was necessary to introduce a new descriptor for cations, J^+, and a new descriptor for anions, J^-, leading to Eq. (4):

$$SP = c + eE + sS + aA + bB + vV + j^+J^+ + j^-J^- \quad (4)$$

J^+ is zero for anions, J^- is zero for cations, and both are zero for neutral species, in which case Eq. (4) reverts to Eq. (3). In addition it should be noted that the coefficients c, e, s, a, b and v, in Eq. (4) are the same as those in Eq. (3) for neutral solutes in the same system. All the solute descriptors can be calculated or estimated as detailed previously (Abraham, 2011; Abraham and Acree, 2010a, b, c). The descriptors for ions and ionic species are on the same scales as those for neutral molecules, so that Eq. (4) can include ions, ionic species and neutral molecules. Some values of the descriptors used in Eq. (4) are shown in Table 2. The coefficients in Eq. (4), that is, c, e, s, a, b, v, j^+ and j^- are obtained by multiple linear regression (MLR). They are not just fitting coefficients, but serve to characterize the given system.

Table 2. Compounds and species used in this work, their solute descriptors, and the corresponding equilibrium coefficients

NO.	Compounds	E	S	A	B	V	J^+	J^-	log P_{oct} [a]	log k_{LEKC} [b]	log k_{IAM} [c]	log k_s [d]	log k_{cer} [e]	log K_p [f]
1	Cortexolone	1.910	3.45	0.36	1.60	2.7389	0.0000	0.0000	2.52	-0.15	2.17	—	-1.11	—
2	Corticosterone	1.860	3.43	0.40	1.63	2.7389	0.0000	0.0000	1.94	-0.21	1.90	—	-1.27	-6.84
3	Estrone	1.730	2.05	0.50	1.08	2.1558	0.0000	0.0000	3.13	0.67	2.82	—	—	—
4	Estriol	1.970	1.74	1.06	1.63	2.2575	0.0000	0.0000	2.54	-0.45	2.57	—	-1.37	—
5	17-Hydroxyprogesterone	1.640	3.35	0.25	1.31	2.6802	0.0000	0.0000	3.17	0.33	2.62	—	-0.90	—
6	Testosterone	1.540	2.59	0.32	1.19	2.3827	0.0000	0.0000	3.29	0.11	2.59	—	-0.85	-5.54
7	Progesterone	1.450	3.29	0.00	1.14	2.6215	0.0000	0.0000	3.87	0.88	2.84	—	—	-4.90
8	Aniline	0.955	0.96	0.26	0.41	0.8162	0.0000	0.0000	0.90	-1.01	0.26	—	-1.73	-4.73
9	Nitrobenzene	0.871	1.11	0.00	0.28	0.8906	0.0000	0.0000	1.85	-0.60	0.99	—	-1.49	—
10	Resorcinol	0.980	1.11	1.09	0.52	0.8338	0.0000	0.0000	0.80	-0.89	0.36	—	-1.49	-6.70
11	Benzyl alcohol	0.803	0.87	0.39	0.56	0.9160	0.0000	0.0000	1.10	-1.28	0.28	—	-1.66	—
12	Phenol	0.805	0.89	0.60	0.30	0.7751	0.0000	0.0000	1.47	-0.77	0.62	—	-1.56	-5.27
13	4-Chlorophenol	0.915	1.08	0.67	0.20	0.8975	0.0000	0.0000	2.39	0.24	1.62	—	-1.01	-4.52
14	Styrene	0.849	0.65	0.00	0.16	0.9552	0.0000	0.0000	2.95	0.25	1.66	—	-0.62	—
15	Toluene	0.601	0.52	0.00	0.14	0.8573	0.0000	0.0000	2.73	-0.20	1.29	—	-0.83	-3.64
16	Ethylbenzene	0.613	0.51	0.00	0.15	0.9982	0.0000	0.0000	3.15	0.34	1.74	—	—	—
17	Aspirin, anion	0.931	3.91	0.04	3.03	1.2664	0.0000	2.1227	-3.69	-2.99	-0.15	—	-2.20	—
18	Flurbiprofen, anion	1.590	4.56	0.07	3.36	1.8174	0.0000	2.5383	-1.97	-0.78	1.78	2.08	-1.21	-6.20
19	Ketoprofen, anion	1.800	5.49	0.01	3.39	1.9564	0.0000	2.4851	-2.54	-1.47	1.26	1.38	-1.32	-5.71
20	Naproxen, anion	1.660	5.07	0.02	3.11	1.7606	0.0000	2.4260	-2.11	-1.14	1.35	1.56	-1.43	-6.73
21	Indomethacin, anion	2.390	5.62	0.10	4.38	2.5084	0.0000	2.9899	1.20	-0.64	2.37	2.48	—	-7.22
22	Mefenamic acid, anion	1.800	4.71	0.09	3.14	1.8996	0.0000	2.6427	-0.66	-0.24	2.35	2.93	-1.17	—
23	Ibuprofen, anion	0.880	3.50	0.08	3.31	1.7556	0.0000	2.4188	-1.62	-0.73	—	—	-1.19	-6.03

24	4-$MeC_6H_4CH_2NHMe$, cation	0.650	2.58	1.42	0.00	1.2604	1.2835	0.0000	-1.30	0.13	0.96	0.95	-0.63	-7.71
25	4-$MeC_6H_4CH_2NHEt$, cation	0.640	2.66	1.44	0.00	1.4013	1.2994	0.0000	-0.83	0.12	1.02	0.96	-0.63	-6.97
26	4-$MeC_6H_4CH_2NHPr$, cation	0.630	2.63	1.37	0.00	1.5422	1.3290	0.0000	-0.28	0.35	1.30	1.13	-0.56	-6.97
27	4-$MeC_6H_4CH_2NHBu$, cation	0.620	2.62	1.34	0.00	1.6831	1.3349	0.0000	0.30	0.72	1.87	1.41	-0.44	-6.72
28	4-$MeC_6H_4CH_2NH(CH_2)_4Me$, cation	0.610	2.60	1.34	0.00	1.8240	1.3136	0.0000	0.81	1.00	2.27	1.85	-0.08	-6.00
29	4-$MeC_6H_4CH_2NH(CH_2)_5Me$, cation	0.600	2.60	1.36	0.00	1.9649	1.2956	0.0000	1.63	1.30	2.77	2.34	0.26	-5.87
30	4-$MeC_6H_4CH_2NH(CH_2)_6Me$, cation	0.590	2.63	1.36	0.00	2.1058	1.2969	0.0000	2.43	1.68	2.92	2.88	0.95	-5.70
31	Metoprolol, cation	1.020	5.35	2.16	0.00	2.2819	2.3476	0.0000	-3.30	0.33	1.45	1.02	-0.79	—
32	Oxprenolol, cation	1.160	5.09	2.35	0.00	2.2389	2.2029	0.0000	-2.67	0.45	1.70	1.48	-0.50	—
33	Penbutolol, cation	0.775	4.66	1.98	0.00	2.6195	1.9630	0.0000	-0.26	2.70	3.70	3.45	0.74	—
34	Propranolol, cation	1.690	4.31	2.07	0.00	2.1695	2.4319	0.0000	-2.51	1.39	2.48	2.64	0.47	-7.90
35	Alprenolol, cation	1.100	4.46	1.78	0.00	2.1802	2.2574	0.0000	-2.44	1.08	2.08	2.19	0.06	-7.10
36	Acebutolol, cation	1.450	6.69	3.62	0.00	2.7771	2.2965	0.0000	-2.38	0.19	1.57	0.88	-1.01	—

[a] n-Octanol-water partition coefficient, taken from Bioloom Software (Version1.5; Biobyte Corporation, Claremont, U.S.A) for neutral species and calculated from the equation given by Abraham and Acree (2010d) for ionic species.

[b] Retention factors measured in LEKC where liposomes were made up of POPC and PS.

[c] Retention facors in IAM (IAM.PC.DD2 column), taken from Liu et al. (2010).

[d] Retention factors in ILC with liposomes composed of egg PC, taken from Liu et al. (2008) and our unpublished data.

[e] Retention factors in cerasome electrokinetic chromatography, taken from Zhang et al. (2011).

[f] Human skin permeability data from Zhang et al. (2012).

In order to set out descriptors for ions and ionic species, it is necessary to use some convention in order to obtain partition coefficients for single ions. Abraham and Zhao (2004) have explained this in considerable detail, and Abraham and Acree (2011) refer to the single ion problem as well. In short, all the descriptors for ions are based on the convention that for transfer from water to another phase, partition coefficients are assigned according to log $P(Ph_4As^+)$ or log $P(Ph_4P^+)$ = log $P(Ph_4B^-)$. Abraham (2011) has set out the classical diffusion analysis of a cation and an anion that diffuse independently and at different rates, yet still maintain electrical neutrality, and has shown specifically how this applies to diffusion from water into a membrane.

Results and Discussion

A set of 36 compounds (including neutral and ionic species) was analyzed by LEKC, where the liposome vesicles consist of neutral POPC and negatively charged PS. The retention factors (log k_{LEKC}) for these compounds are given in Table 2. log k_{LEKC} acts as a

partition index for the lipid membrane-water partition since k_{LEKC} is proportional to the corresponding partition coefficient, as is the case for other retention factors below. Liu et al. (2010) have reported the retention factors (log k_s) for 22 fully ionized solutes in ILC at pH 7.4, where unilamellar liposome vesicles composed of egg phosphatidylcholines (PC) were immobilized to gel beads. The values of log k_s for the solutes present in both databases can be used to compare interactions of ionic species with charged liposomes and neutral liposomes. A plot of log k_{LEKC} versus log k_s for ionic species is shown in Fig. 1. It is clear that the data points for cations and anions scatter over two almost parallel lines, with the line for the cations about two log units higher than the line for the anions. This is in agreement with a previous observation that electrostatic interaction influences the partition of ionic species into liposomes (Osterberg et al., 2001). The inclusion of negatively charged PS head-groups enhances the interaction with positively charged solutes and weakens the interaction with negatively charged solutes. Such electrostatic interactions exists in neutral phospholipid (e.g., PC) membrane as well (Avdeef et al., 1998).

The retention factors (log k_{IAM}) of a number of compounds were measured by Liu et al. (2008) on an IAM.PC.DD2 column at pH 7.0, of which some are shown in Table 2. Liu et al. (2008) used methanol as an organic modifier to accelerate the measurements of lipophilic compounds. However, log k values were determined at different concentrations of methanol and extrapolated to pure aqueous mobile phase, that is, log k_{IAM} here. Liu et al. (2008) showed that a plot of log k_s against log k_{IAM} for 22 charged solutes resulted in two parallel lines, depending on the solute charge. This was attributed to the different densities of the phospholipid head-groups of lipid membranes in the two systems (Liu et al., 2010). In contrast with log k_{LEKC}, log k_{IAM} exhibits a strikingly closer relationship with log k_s. It is expected that neutral membranes in IAM and ILC bring about no markedly different electrostatic interaction with ionic species. This also indicates that the effect of the charge of phospholipid head-groups is much greater than the effect of their density on ionic partition.

A plot of log k_{LEKC} versus log k_{IAM}, for the compounds in Table 2 except ibuprofen (anion) where we have no experimental value of log k_{IAM}, results in an overall poor correlation, see Fig. 2. This is as might be expected. Cations partition better and anions partition worse into negatively charged phospholipids than into neutral phospholipids, whereas the partitions of neutral species hardly vary with the charge of the phospholipids. As a result, the data points for neutral species locate below those for cations and above those for anions, see Fig. 2. This result reflects the importance of electrostatic interaction in the lipid membrane-water partitions of ionic species. Note that the incorporation of PS leads to a negligible change on the partitions of neutral species into PC liposomes, as seen from the work of Österberg et al. (2001).

It is noted that except for log k_{LEKC} and log k_{cer} (37 °C), the temperatures at which the retention factors in Table 2 were measured are room temperature, that is, close to 25°C. One could consider that the temperature dependence of partitions might affect the membrane system comparisons in this study. However, no significant partition differences in Soy-PC liposomes were found for the neutral and ionic species of two model drugs (propranolol and diclofenac) at standard laboratory 25 °C and physiologic temperature 37 °C (Balon et al., 1999a). Also, it has been known that the effect of temperature on partition coefficients is not great-usually on the order of 0.01 units per degree-and may be either positive or negative in solvent systems (Leo et al., 1971). Thus we suggest that the partitions into lipid membranes of solutes change very little over the temperature range (25 to 37) °C.

To compare the IAM system with organic solvent-water systems, we applied Eq. (4) to the 49 compounds studied by Liu et al. (2008), including 21 neutral solutes and 28 ionized solutes, together with an extra 9 compounds that we have recently studied. The resulting equation for 58 compounds is given as Eq. (5).

$$\log k_{IAM} = -0.812\ (\pm 0.141) + 0.629\ (\pm 0.125)\ E - 0.590\ (\pm 0.092)\ S + 0.195\ (\pm 0.144)\ A$$
$$- 2.448\ (\pm 0.188)\ B + 2.813\ (\pm 0.166)\ V - 0.829\ (\pm 0.178)\ J^{+} + 2.798\ (\pm 0.221)\ J^{-} \quad (5)$$

247 N = 58, R^2 = 0.912, SD = 0.292, F = 74, PRESS = 6.433, Q^2 = 0.868, PSD = 0.359

248 In this and the following equations, 95% confidence limits are given in parentheses; N is
249 the number of compounds or data points; R^2 is the squared correlation coefficient; SD is the
250 standard deviation, and F is the F-statistic. The leave-one-out statistics are PRESS, Q^2 and
251 PSD; the latter is the predicted standard deviation as defined before (Abraham et al., 2009). A
252 similar analysis of the LEKC system for all 36 compounds leads to Eq. (6).

253 $$\log k_{LEKC} = -1.768\ (\pm 0.201) + 0.538\ (\pm 0.221)\ E - 0.776(\pm 0.120)S - 0.199\ (\pm 0.172)\ A$$

254 $$-2.433\ (\pm 0.203)\ B + 2.646\ (\pm 0.227)\ V + 0.092\ (\pm 0.244)\ J^{+} + 2.698\ (\pm 0.332)\ J^{-} \quad (6)$$

255 N = 36, R^2 = 0.923, SD = 0.319, F = 48, PRESS = 6.661, Q^2 = 0.821, PSD = 0.488

256 Eq. (5) and Eq. (6) have been constructed using values of k for neutral compounds and
257 for fully ionized compounds, say k_N and k_I. For a partially ionized compound, with a fraction
258 ionized, *f*, the total value, k_T, can be calculated through $k_T = [k_N *(1-f) + k_I *f)]$. *f* depends on
259 the pKa of the compound and the experimental pH, so that calculated values of k_T amount to
260 a calculation of the dependence of k_T on pH. If descriptors are available for the neutral and
261 ionized forms of a compound that has not been studied experimentally, then k_N and k_I can be
262 predicted from the equation coefficients, and the dependence of k_T on pH predicted.

263 Abraham and Acree (2012b) have shown, for neutral compounds only, that permeation
264 through membranes is very varied, and that no one solvent-water partitioning system can be
265 used as a general model for all membranes. We can now compare membrane systems and
266 solvent-water systems using equations that include not just neutral compounds but ionic
267 species as well. We use a similar method to that of Abraham and Acree (2012b) in which a
268 principal component analysis, PCA, is carried out on the seven coefficients in Eq. (4), e, s, a,
269 b, v, j^+ and j^-, that characterize the given systems. The coefficients of the systems compared
270 are given in Table 3; systems # 1-6 are the membrane systems and systems # 7-32 are various
271 solvent-water partitioning systems. In the present case, the first two principal components

(PCs) account for 74% of the total variance in the dataset, the cumulative totals being 46, 74, 86, 95, 98, 100, 100%. The loadings of the first four principal components are given in Table 4, and show that PC1 is dominated by the b, v and j^+ coefficients and PC2 by the e, s and j^- coefficients. A plot of the scores of the second PC versus the first PC reveals how chemically close the systems are in terms of the distance between points in the two dimensional plot, see Fig. 3.

Table 3. Coefficients in Eq. (4) for a number of membrane systems (No 1-6) and solvent-water partitions (No 7-32)

System	No	SP	c	e	s	a	b	v	j^+	j^-	D(PC7)	Cos θ
Skin permeation[a]	1	log K_p	-5.420	-0.102	-0.457	-0.324	-2.680	2.066	-1.938	2.548	0.00	1.000
Cerasome[b]	2	log k_{cer}	-1.922	0.200	-0.629	-0.109	-1.451	1.757	0.334	1.958	2.61	0.837
Microsomal binding[c]	3	log k	-1.221	0.000	-0.763	0.437	-0.444	1.452	0.283	1.215	2.99	0.687
BBB-Permeation[d]	4	log PS	-1.268	-0.047	-0.876	-0.719	-1.571	1.767	0.469	1.663	2.48	0.803
$POPC_{80}/PS_{20}$[e]	5	log k_{LEKC}	-1.768	0.538	-0.776	-0.199	-2.433	2.646	0.092	2.698	3.52	0.883
PC[e]	6	log k_{IAM}	-0.812	0.629	-0.590	0.195	-2.448	2.813	-0.829	2.789	3.65	0.940
100%EtOH[f]	7	log P	0.222	0.471	-1.035	0.326	-3.596	3.857	-3.172	3.146	3.70	0.975
90% EtOH	8	log P	0.243	0.213	-0.575	0.262	-3.450	3.545	-2.794	2.705	2.33	0.978
80% EtOH	9	log P	0.172	0.175	-0.465	0.260	-3.212	3.323	-2.466	2.722	1.95	0.981
70% EtOH	10	log P	0.063	0.085	-0.368	0.311	-2.936	3.102	-2.203	2.550	1.50	0.979
60% EtOH	11	log P	-0.040	0.138	-0.335	0.293	-2.675	2.812	-1.858	2.394	1.48	0.797
50% EtOH	12	log P	-0.142	0.124	-0.252	0.251	-2.275	2.415	-1.569	2.051	1.43	0.978
40% EtOH	13	log P	-0.221	0.131	-0.159	0.171	-1.809	1.918	-1.271	1.676	1.75	0.979
30% EtOH	14	log P	-0.269	0.107	-0.098	0.133	-1.316	1.414	-0.941	1.290	2.22	0.979
20% EtOH	15	log P	-0.252	0.042	-0.040	0.096	-0.823	0.916	-0.677	0.851	2.77	0.978
10% EtOH	16	log P	-0.173	-0.023	-0.001	0.065	-0.372	0.454	-0.412	0.401	3.39	0.966
wet octanol [g]	17	log P	0.088	0.562	-1.054	0.034	-3.460	3.814	-3.023	2.580	3.90	0.967
Methanol	18	log P	0.276	0.334	-0.714	0.243	-3.320	3.549	-2.609	3.027	2.73	0.981
Hexan-1-ol	19	log P	0.115	0.492	-1.164	0.054	-3.971	4.131	-3.100	2.940	3.98	0.974
Formamide	20	log P	-0.171	0.070	0.308	0.589	-3.152	2.432	-3.152	2.432	2.24	0.956
Acetonitrile	21	log P	0.413	0.077	0.326	-1.566	-4.391	3.364	-2.243	0.101	3.49	0.816
N-Methylpyrrolidinone	22	log P	0.147	0.532	0.275	0.840	-4.794	3.674	-1.797	0.105	4.63	0.794
Dimethylsulfoxide	23	log P	-0.194	0.327	0.791	1.260	-4.540	3.361	-3.387	0.132	4.66	0.794
Propanone	24	log P	0.313	0.312	-0.121	-0.608	-4.753	3.942	-2.288	0.078	3.81	0.828
1,2-Dichloroethane	25	log P	0.183	0.294	-0.134	-2.801	-4.291	4.180	-3.429	-0.025	4.59	0.790
Dichloromethane	26	log P	0.319	0.102	-0.187	-3.058	-4.090	4.324	-3.984	0.086	4.55	0.790
NPOE	27	log P	0.121	0.600	-0.495	-2.246	-3.879	3.574	-2.314	0.350	4.52	0.821
Nitrobenzene	28	log P	-0.152	0.525	0.081	-2.332	-4.494	4.187	-3.373	0.777	4.79	0.855
Benzonitrile	29	log P	0.097	0.285	0.059	-1.605	-4.562	4.028	-2.729	0.136	4.00	0.827
Propylene carbonate	30	log P	0.004	0.168	0.504	-1.283	-4.407	3.421	-1.989	0.341	3.62	0.831
Sulfolane	31	log P	0.000	0.147	0.601	-0.318	-4.541	3.290	-1.200	-0.792	4.21	0.698

Ethylene glycol	32	log P	-0.270	0.578	-0.511	0.715	-2.619	2.729	-1.300	2.363	3.42	0.946

[a] From Zhang et al. (2012).
[b] From Zhang et al. (2011).
[c] From Abraham and Austin (2012) .
[d] Permeation from saline through the blood-brain barrier (Abraham, 2011).
[e] Obtained in this study.
[f] Partitions from water to vol % ethanol-water mixtures (Abraham and Acree, 2012a).
[g] Partitions from water to various solvents (Abraham and Acree, 2010a, c, d, 2012a; Abraham and Zhao, 2005).

Table 4. Loadings of the first four principal components

	PC1	PC2	PC3	PC4
e	0.251	-0.446	-0.067	0.749
s	0.188	0.572	-0.499	0.069
a	-0.316	-0.218	-0.821	0.039
b	-0.529	0.035	0.200	-0.004
v	0.512	-0.238	0.018	-0.056
j+	-0.454	0.136	0.159	0.543
j-	-0.232	-0.592	-0.087	-0.366

The points for the aprotic solvents, # 21- 31, cluster together and are far away from all the membrane systems. Wet octanol, # 17, is also far away from the membrane systems, so that its use as a membrane model is highly questionable. The very polar ethanol-water mixtures, # 12 and # 13 are good models for human skin permeation (# 1), and the slightly less polar mixtures, # 14 and # 15 are reasonable models for partition into cerasome (# 2) and for permeation from saline through the blood-brain barrier (# 4). The ethanol-water mixtures # 10 and # 11 are not quite as polar as # 14 and # 15, but are reasonable models for the LEKC and the IAM systems, # 5 and # 6, as is also the ethylene glycol-water system (# 32). Abraham and Acree (2012b) have obtained similar results for neutral compounds, so that we can now suggest that both neutral compounds and ionic species partition into the polar parts of lipid membrane. However on the whole, there is no “ideal” solvent that can be used as a general model for lipid membranes.

A number of workers have proposed that partitions in various water-solvent systems could be used as models for permeation. Collander and Bärlund (1933) used water-ether and water-olive oil as model systems for permeation through Chara cells, but this was criticised by Finkelstein (1976) who used water-hexadecane as a model system for a number of membranes. Xiang and Anderson (1994) noted that water-octanol had been used previously as a model system, but found that water-decadiene was the most suitable model for permeation through egg lecithin/decane bilayers. Abraham and Acree (2012b) applied principal component analysis to equations for permeation through various membranes, exactly as we have done, and reached the same conclusion as did Xiang and Anderson (1994), namely that different membranes required different model systems. Lukacova et al. (2013) have used Eq. (3) to compare partition into a diacetyl phosphatidylcholine/hexadecane phase with partitions in other systems such as water-octanol and water-hexadecane. Lukacova et al. (2013) studied only non-ionizable compounds, however. Various computational studies have been carried out on membrane permeation. For example, Tejwani et al. (2011) studied permeation across 1,2-dioleoyl-sn-glycero-3-phosphocoline bilayers using molecular dynamics simulation. They showed that their results were consistent with a barrier to permeation that was hydrocarbon-like. However, this does not impact on the possibility that other membranes have barriers that are not hydrocarbon-like. In any case, all this previous work has dealt with neutral solutes, whereas we have obtained results for both neutral and ionic solutes.

Zhang et al. (2011) have set up an equation for the cerasome electrokinetic chromatography in order to estimate SC-water partition (Table 3). From Fig. 3, it can be seen that the point for the cerasome-water partition (# 2) is closer to the point for permeation through the SC (# 1) than are the points for other lipid membrane-water partitions.

There are two main methods that have been used to assess quantitatively how near are LFERs of the form of Eq. (3) or Eq. (4). In the first method (Abraham and Acree, 2012b), the

actual seven-dimensional distances, D(PC7), between points from the scores for PC1-PC7 as given in Table 3 are calculated. Because the PC scores relate to the coefficients in Eq. (4) and because the coefficients relate to the various solute-phase interactions, the distances will themselves be an indication of how close equations are as regards the chemical properties of the corresponding phases. In the second method, due to Ishihama and Asakawa (1999), the seven coefficients in Eq. (4) are regarded as defining a line in seven-dimensional space. The angle, θ, between the two lines is a measure of how well the two corresponding equations are linearly related. Usually it is cos θ that is calculated, rather than θ. The nearer cos θ is to unity, the closer are the two equations in terms of correlation. It must be noted, however, that cos θ does equate to R^2. Both methods require some given equation to be selected as a 'standard', and we choose our equation for permeation through skin as the standard where D(PCA)7 = 0, and cos θ = 1.

Results are in Table 3. As regards the values of D(PCA)7 and cos θ the equation for cerasome (# 2) is nearer to that for permeation through the SC (# 1) than is the membrane system (# 5), but the membrane system (# 6) has cos θ appreciably closer to that for skin permeation. Cerasome is a unique liposome whose roles in modeling the SC cannot be replaced by regular phospholipid liposomes in partitions. This is probably due to the unusual structures of ceramides, the major type of lipids found in the SC. Ceramides consist of derivatives of sphingosines bases linked to a variety of fatty acids via amide bonds. Clearly, the polar head-groups in ceramides act as both acceptors and donors of hydrogen bonds by the hydroxyl and amino groups as compared to those in phospholipids, which act only as acceptors of hydrogen bonds (Moore et al., 1997). Ceramides therefore should generate strong hydrogen bonding with solutes that are hydrogen bond bases. Furthermore, the aliphatic chains in ceramides are mostly long-chain and saturated, and hence lead to high phase transition temperatures. Ceramides are thus mostly in a solid crystalline or gel state at

354 physiological temperature, which exhibits lower partition coefficients than the state of liquid
355 crystalline membranes present at higher temperatures (Bano, 2000; Sarmento et al., 1993).

356 Of the bulk solvents in Table 3, there are several solvents or solvent mixtures that have
357 cos θ that is near unity, and so might be expected to be good correlational models for
358 permeation through skin. Water-ethanol mixtures or polar alcohols such as methanol appear
359 to be much better models for permeation through skin than non-polar solvents such as
360 dichloroethane or dichloromethane. Although we have no LFER equations for distribution of
361 ionic species to hexadecane or to 1,9-decadiene, it seems rather clear that neither solvent
362 would be a suitable model. Wet octanol, interestingly, also seems a poor model for
363 permeation through skin.

364

365 # Conclusion

366 In this study, lipid membrane-water partitions have been compared to various organic
367 solvent-water partitions for both neutral and ionic species. It was found that partition into
368 lipid membranes is chemically markedly different from partitions into most organic solvents,
369 although partitions into aqueous ethanol can provide useful models for membrane partition.
370 Although the lipid membrane studied is actually the lipid monolayer bound to gel beads in
371 IAM, there are only small differences between such lipid monolayers and lipid bilayers in
372 liposome caused by the different densities of the phospholipid head-groups. In addition, our
373 results suggest that solutes, no matter whether they are charged or not, partition into the polar
374 parts of lipid membranes. Cerasome was compared with regular phospholipid liposomes as a
375 possible model for the SC in partitions. The results show that retention factors on an IAM
376 column provide a more useful model for skin permeation. However, cerasome differs
377 considerably from phospholipid liposomes, and so provides a different type of model
378 membrane due to the unique structure of ceramides that are present in cerasome.

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Abbreviations

A	Overall hydrogen bond acidity
B	Overall hydrogen bond basicity
BBB	Blood-brain barrier
CE	Capillary electrophoresis
EKC	Electrokinetic chromatography
E	Excess molar refraction in $(cm^3mol^{-1})/10$
HPLC	High-performance liquid chromatography
IAM	Immobilized artificial membrane
ILC	Immobilized liposome chromatography
k_{cer}	Retention factors in cerasome electrokinetic chromatography
k_{LEKC}	Retention factors in liposome electrokinetic chromatography
K_{ILC}	Retention factors in immobilized liposome chromatography
k_{IAM}	Retention factors in immobilized artificial membrane chromatography
K_p	Skin permeability
LEKC	Liposome electrokinetic chromatography
LFER	Linear free-energy relationship
P_{oct}	n-Octanol-water partition coefficient
P_{lip}	Partition coefficient between liposome and water
PC	Phosphatidylcholine or principal component in principal component analysis
PCA	Principal component analysis
POPC	3-sn-Phosphatidylcholine
PS	3-sn-Phosphatidyl-L-serine
S	Solute dipolarity/polarizability
V	McGowan characteristic molecular volume in $(cm^3mol^{-1})/100$

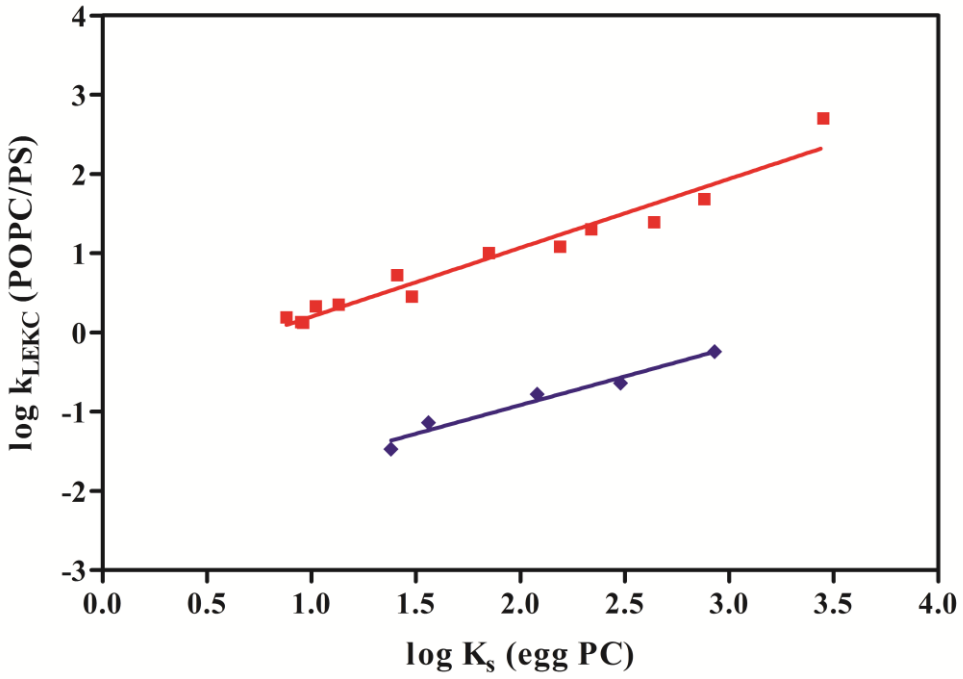
Legend to Figures

Figure 1. A plot of log k_{LEKC} ($POPC_{80}/PS_{20}$) in LEKC versus log k_s (egg PC) in ILC: ■ cations (protonated bases), the regression equation: $y = 0.8699x - 0.6710$, $R^2 = 0.9521$; ♦ anions (deprotonated acids), the regression equation: $y = 0.7228x - 2.362$, $R^2 = 0.9649$.

Figure 2. A plot of log k_{LEKC} (POPC/PS) in LEKC versus log k_{IAM} (IAM.PC.DD2 column) in IAM: ■ cations (protonated bases), the regression equation: $y = 0.9133x - 0.9529$, $R^2 = 0.9450$; ♦ anions (deprotonated acids), the regression equation: $y = 0.9783x - 2.654$, $R^2 = 0.9606$; ● smaller neutral molecules, the regression equation: $y = 1.001x - 1.412$, $R^2 = 0.9780$; ▲ larger neutral molecules (steroids), the regression equation: $y = 0.9876x - 2.302$, $R^2 = 0.4925$.

Figure 3. A plot of the scores of the second principal component (PC 2) against the first principal component (PC 1); ● membrane systems, ○ solvent-water partitions.

522 **Figure 1:**



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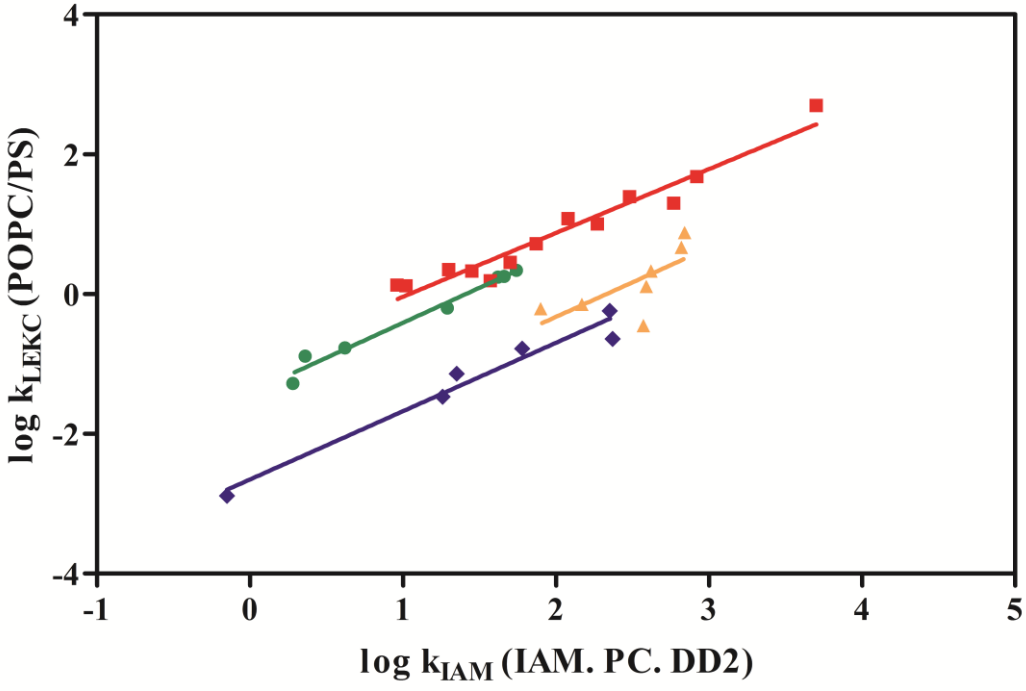
526 **Figure 2:**



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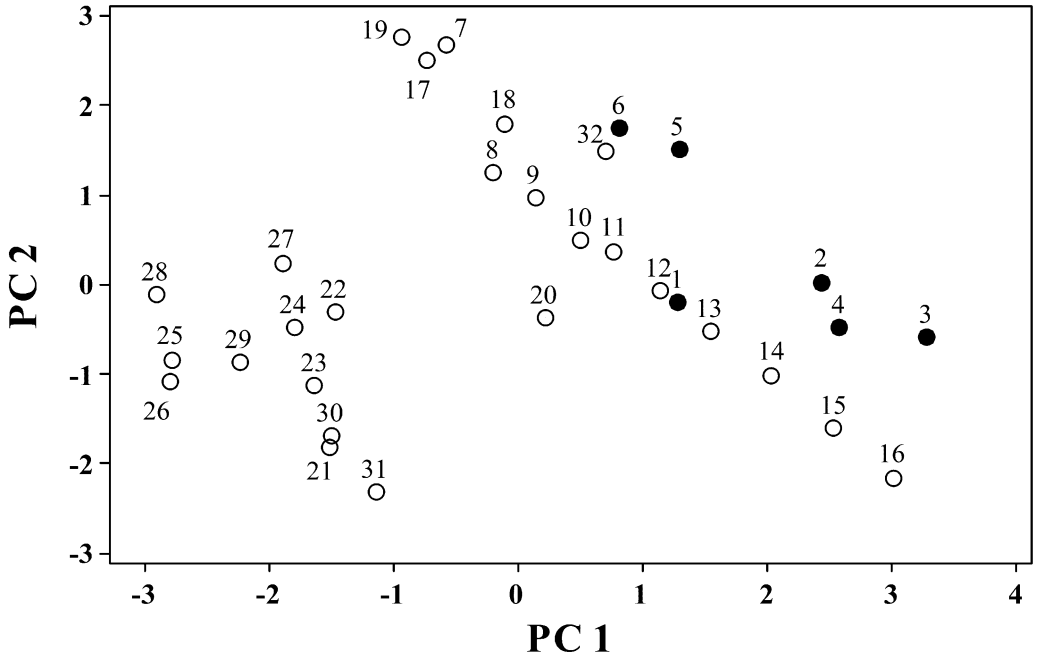
528 **Figure 3:**



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