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

5
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8
9 **Running heads:** Comparison of Membrane-Water and Solvent-Water Partitioning Systems of
10 Neutral and Ionic Species

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30 **Abstract**

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

48

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

51 **Introduction**

52 The *n*-octanol-water partition coefficient (as $\log P_{\text{oct}}$) is widely used as an estimate for
53 partitions of chemicals into biological membranes, but it is now recognized that biological
54 membranes vary so much in character that it is impossible for any given solvent-water system
55 to be a useful model for all membranes (Abraham and Acree, 2012b). In addition, recent
56 work on ionic partition into biological membranes suggests that that the *n*-octanol-water
57 system is not satisfactory as a model for ionic partition (Abraham, 2011; Abraham and Austin,
58 2012). Consequently, the liposome-water partitioning system has been developed as a
59 promising model for biological partition process because of the highly-ordered lipid bilayer
60 microstructure of liposome. Workers have observed that ionic species partition significantly
61 better into liposome than into *n*-octanol (Austin et al., 1995; Avdeef et al., 1998; Balon et al.,
62 1999b; Fruttero et al., 1998; Mason et al., 1991), while the partition coefficients of neutral
63 species were found to be comparable but not equal in liposome-water and *n*-octanol-water
64 systems (Avdeef et al., 1998; Fruttero et al., 1998). Recently, Zhang et al. (2011)
65 demonstrated that cerasome (i.e., liposome consisting mainly of stratum corneum (SC)
66 lipids)-water system is chemically rather different from various organic solvent-water
67 systems, using linear free energy relationships (LFERs) as a method of analysis. It was
68 further shown that the cerasome-water partition is a reasonable chemical model for the SC-
69 water partition, although this conclusion was deduced for neutral species only (Zhang et al.,
70 2012).

71 Even so, it is of considerable interest to compare neutral liposomes with organic solvents,
72 which are neutral, on solute interactions from a physicochemical point of view, given that
73 cerasome is negatively charged. So far, there has been no easy-to-use way to measure a large
74 number of neutral liposome-water partition parameters of neutral and ionic species, which are
75 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

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

97 **Materials and Methods**

98 **Chemicals**

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

105 **Liposome Preparation**

106 All liposomes were prepared once by the extrusion method. Briefly, the desired amounts
107 of POPC and PS (80:20, mol/mol) were dissolved in chloroform/methanol mixture (9:1, v/v)
108 in a 1L round bottom flask. A thin lipid film was formed by removing organic solvents under
109 vacuum at 45°C with a rotary evaporator. The resulting film was hydrated and dispersed in
110 100 ml 10 mM phosphate buffer pH 7.4 by continuous shaking for 60 min at 45°C to yield
111 multilamellar vesicles (MLVs) with a lipid concentration of 3 mM. Then the MLVs were
112 extruded through a polycarbonate filter (100 nm pore size; Poretics, Livermore, USA) 20

113 times at room temperature using a EmulsiFlex C5 (Avestin, Ottawa, Canada) under an
114 external pressure of 3.5 bar. The final product was a homogeneous liposome dispersion of
115 large unilamellar vesicles (LUVs), for which the average particle size and zeta potential were
116 79.4 (± 0.5) nm with a polydispersity index of 0.084 and -27.1 (± 1.0) mV, respectively,
117 measured using a Zetasizer Nano ZS (Malvern, Herrenberg, Germany). The liposome
118 dispersion was very stable for at least two months at 4°C (unreported data), and thus used in
119 the whole work.

120 **LEKC**

121 The LEKC experiments were carried out using a HPCE 1600AX (Agilent, Waldbronn,
122 Germany) equipped with a diode array detector (DAD). 58.5/50 cm uncoated fused-silica
123 capillaries of 50 μm id and 375 μm od (BGB Analytic, Schloßböckelheim, Germany) were
124 employed throughout this study. A new capillary was rinsed with 1.0 M aqueous sodium
125 hydroxide for 20 min, water for 5 min, 1.0 M hydrochloride acid for 20 min and water for
126 5min. The samples were analyzed at +20 kV, with a hydrodynamic injection at 50 mbar for
127 3s. The capillary was kept at 37°C and the detection wavelengths were set at 210 nm, 225 nm,
128 and 245 nm.

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

$$134 \quad k = (t_r - t_0) / t_0 \left(1 - t_r / t_{lip} \right) \quad (1)$$

$$135 \quad k = (t_r - t_{eo}) / t_{eo} \left(1 - t_r / t_{lip} \right) \quad (2)$$

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

138 retention time of charged solutes in the buffer solution. The log k measurements of all the
139 solutes were repeated 3 times. The compounds were dissolved in methanol to prepare stock
140 solutions, which were diluted with the running solution before injection to approximately
141 $2.0\sim 3.0\times 10^{-4}$ mol/L. Before sample injection, the capillary was rinsed for 3 min with the
142 running solution (buffer solution or liposome dispersion). Decanophenone dissolved in
143 methanol was added where appropriate, as the liposome maker. All solutions were filtered
144 (200 nm) prior to use.

145 **LFER**

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

$$151 \quad SP = c + eE + sS + aA + bB + vV \quad (3)$$

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

$$160 \quad SP = c + eE + sS + aA + bB + vV + j^+J^+ + j^-J^- \quad (4)$$

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

171

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

NO.	Compounds	E	S	A	B	V	J^+	J^-	$\log P_{\text{oct}}^a$	$\log k_{\text{LEKC}}^b$	$\log k_{\text{IAM}}^c$	$\log k_s^d$	$\log k_{\text{cer}}^e$	$\log K_p^f$
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 ₆ H ₄ CH ₂ NHMe, 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 ₆ H ₄ CH ₂ NHEt, 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 ₆ H ₄ CH ₂ NHPr, 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 ₆ H ₄ CH ₂ NHBu, 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 ₆ H ₄ CH ₂ NH(CH ₂) ₄ Me, 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 ₆ H ₄ CH ₂ NH(CH ₂) ₅ Me, 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 ₆ H ₄ CH ₂ NH(CH ₂) ₆ Me, 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	—

174 ^a n-Octanol-water partition coefficient, taken from Biolum Software (Version1.5; Biobyte Corporation, Claremont, U.S.A)

175 for neutral species and calculated from the equation given by Abraham and Acree (2010d) for ionic species.

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

177 ^c Retention factors in IAM (IAM.PC.DD2 column), taken from Liu et al. (2010).

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

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

180 ^f Human skin permeability data from Zhang et al. (2012).

181

182 In order to set out descriptors for ions and ionic species, it is necessary to use some
183 convention in order to obtain partition coefficients for single ions. Abraham and Zhao (2004)
184 have explained this in considerable detail, and Abraham and Acree (2011) refer to the single
185 ion problem as well. In short, all the descriptors for ions are based on the convention that for
186 transfer from water to another phase, partition coefficients are assigned according to log
187 $P(\text{Ph}_4\text{As}^+)$ or $\log P(\text{Ph}_4\text{P}^+) = \log P(\text{Ph}_4\text{B}^-)$. Abraham (2011) has set out the classical diffusion
188 analysis of a cation and an anion that diffuse independently and at different rates, yet still
189 maintain electrical neutrality, and has shown specifically how this applies to diffusion from
190 water into a membrane.

191 Results and Discussion

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

195 partition index for the lipid membrane-water partition since k_{LEKC} is proportional to the
196 corresponding partition coefficient, as is the case for other retention factors below. Liu et al.
197 (2010) have reported the retention factors ($\log k_s$) for 22 fully ionized solutes in ILC at pH
198 7.4, where unilamellar liposome vesicles composed of egg phosphatidylcholines (PC) were
199 immobilized to gel beads. The values of $\log k_s$ for the solutes present in both databases can be
200 used to compare interactions of ionic species with charged liposomes and neutral liposomes.
201 A plot of $\log k_{LEKC}$ versus $\log k_s$ for ionic species is shown in Fig. 1. It is clear that the data
202 points for cations and anions scatter over two almost parallel lines, with the line for the
203 cations about two log units higher than the line for the anions. This is in agreement with a
204 previous observation that electrostatic interaction influences the partition of ionic species into
205 liposomes (Osterberg et al., 2001). The inclusion of negatively charged PS head-groups
206 enhances the interaction with positively charged solutes and weakens the interaction with
207 negatively charged solutes. Such electrostatic interactions exists in neutral phospholipid (e.g.,
208 PC) membrane as well (Avdeef et al., 1998).

209 The retention factors ($\log k_{IAM}$) of a number of compounds were measured by Liu et al.
210 (2008) on an IAM.PC.DD2 column at pH 7.0, of which some are shown in Table 2. Liu et al.
211 (2008) used methanol as an organic modifier to accelerate the measurements of lipophilic
212 compounds. However, $\log k$ values were determined at different concentrations of methanol
213 and extrapolated to pure aqueous mobile phase, that is, $\log k_{IAM}$ here. Liu et al. (2008)
214 showed that a plot of $\log k_s$ against $\log k_{IAM}$ for 22 charged solutes resulted in two parallel
215 lines, depending on the solute charge. This was attributed to the different densities of the
216 phospholipid head-groups of lipid membranes in the two systems (Liu et al., 2010). In
217 contrast with $\log k_{LEKC}$, $\log k_{IAM}$ exhibits a strikingly closer relationship with $\log k_s$. It is
218 expected that neutral membranes in IAM and ILC bring about no markedly different
219 electrostatic interaction with ionic species. This also indicates that the effect of the charge of
220 phospholipid head-groups is much greater than the effect of their density on ionic partition.

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

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

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

$$\begin{aligned} 245 \quad \log k_{\text{IAM}} = & -0.812 (\pm 0.141) + 0.629 (\pm 0.125) E - 0.590 (\pm 0.092) S + 0.195 (\pm 0.144) A \\ 246 \quad & - 2.448 (\pm 0.188) B + 2.813 (\pm 0.166) V - 0.829 (\pm 0.178) J^+ + 2.798 (\pm 0.221) J^- \quad (5) \end{aligned}$$

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).

$$\begin{aligned} \log k_{LEKC} = & -1.768 (\pm 0.201) + 0.538 (\pm 0.221) E - 0.776 (\pm 0.120) S - 0.199 (\pm 0.172) A \\ & - 2.433 (\pm 0.203) B + 2.646 (\pm 0.227) V + 0.092 (\pm 0.244) J^+ + 2.698 (\pm 0.332) J^- \end{aligned} \quad (6)$$

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

272 (PCs) account for 74% of the total variance in the dataset, the cumulative totals being 46, 74,
 273 86, 95, 98, 100, 100%. The loadings of the first four principal components are given in Table
 274 4, and show that PC1 is dominated by the b , v and j^+ coefficients and PC2 by the e , s and j^-
 275 coefficients. A plot of the scores of the second PC versus the first PC reveals how chemically
 276 close the systems are in terms of the distance between points in the two dimensional plot, see
 277 Fig. 3.

278

279 **Table 3.** Coefficients in Eq. (4) for a number of membrane systems (No 1-6) and solvent-water
 280 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

281 ^a From Zhang et al. (2012).

282 ^b From Zhang et al. (2011).

283 ^c From Abraham and Austin (2012) .

284 ^d Permeation from saline through the blood-brain barrier (Abraham, 2011).

285 ^e Obtained in this study.

286 ^f Partitions from water to vol % ethanol-water mixtures (Abraham and Acree, 2012a).

287 ^g Partitions from water to various solvents (Abraham and Acree, 2010a, c, d, 2012a; Abraham and Zhao, 2005).

288

289

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

290

291 The points for the aprotic solvents, # 21- 31, cluster together and are far away from all the
292 membrane systems. Wet octanol, # 17, is also far away from the membrane systems, so that
293 its use as a membrane model is highly questionable. The very polar ethanol-water mixtures, #
294 12 and # 13 are good models for human skin permeation (# 1), and the slightly less polar
295 mixtures, # 14 and # 15 are reasonable models for partition into cerasome (# 2) and for
296 permeation from saline through the blood-brain barrier (# 4). The ethanol-water mixtures #
297 10 and # 11 are not quite as polar as # 14 and # 15, but are reasonable models for the LEKC
298 and the IAM systems, # 5 and # 6, as is also the ethylene glycol-water system (# 32).
299 Abraham and Acree (2012b) have obtained similar results for neutral compounds, so that we
300 can now suggest that both neutral compounds and ionic species partition into the polar parts
301 of lipid membrane. However on the whole, there is no “ideal” solvent that can be used as a
302 general model for lipid membranes.

303 A number of workers have proposed that partitions in various water-solvent systems
304 could be used as models for permeation. Collander and Bärlund (1933) used water-ether and
305 water-olive oil as model systems for permeation through Chara cells, but this was criticised
306 by Finkelstein (1976) who used water-hexadecane as a model system for a number of
307 membranes. Xiang and Anderson (1994) noted that water-octanol had been used previously
308 as a model system, but found that water-decadiene was the most suitable model for
309 permeation through egg lecithin/decane bilayers. Abraham and Acree (2012b) applied
310 principal component analysis to equations for permeation through various membranes,
311 exactly as we have done, and reached the same conclusion as did Xiang and Anderson
312 (1994), namely that different membranes required different model systems. Lukacova et al.
313 (2013) have used Eq. (3) to compare partition into a diacetyl phosphatidylcholine/hexadecane
314 phase with partitions in other systems such as water-octanol and water-hexadecane. Lukacova
315 et al. (2013) studied only non-ionizable compounds, however. Various computational studies
316 have been carried out on membrane permeation. For example, Tejwani et al. (2011) studied
317 permeation across 1,2-dioleoyl-sn-glycero-3-phosphocoline bilayers using molecular
318 dynamics simulation. They showed that their results were consistent with a barrier to
319 permeation that was hydrocarbon-like. However, this does not impact on the possibility that
320 other membranes have barriers that are not hydrocarbon-like. In any case, all this previous
321 work has dealt with neutral solutes, whereas we have obtained results for both neutral and
322 ionic solutes.

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

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

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

341 Results are in Table 3. As regards the values of $D(PCA)7$ and $\cos \theta$ the equation for
342 cerasome (# 2) is nearer to that for permeation through the SC (# 1) than is the membrane
343 system (# 5), but the membrane system (# 6) has $\cos \theta$ appreciably closer to that for skin
344 permeation. Cerasome is a unique liposome whose roles in modeling the SC cannot be
345 replaced by regular phospholipid liposomes in partitions. This is probably due to the unusual
346 structures of ceramides, the major type of lipids found in the SC. Ceramides consist of
347 derivatives of sphingosines bases linked to a variety of fatty acids via amide bonds. Clearly,
348 the polar head-groups in ceramides act as both acceptors and donors of hydrogen bonds by
349 the hydroxyl and amino groups as compared to those in phospholipids, which act only as
350 acceptors of hydrogen bonds (Moore et al., 1997). Ceramides therefore should generate
351 strong hydrogen bonding with solutes that are hydrogen bond bases. Furthermore, the
352 aliphatic chains in ceramides are mostly long-chain and saturated, and hence lead to high
353 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; Sarmiento et al., 1993).

356 Of the bulk solvents in Table 3, there are several solvents or solvent mixtures that have
357 $\cos \theta$ 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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495 **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 $(\text{cm}^3\text{mol}^{-1})/10$
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 $(\text{cm}^3\text{mol}^{-1})/100$

497 Legend to Figures

498 **Figure 1.** A plot of $\log k_{\text{LEKC}}$ (POPC₈₀/PS₂₀) in LEKC versus $\log k_s$ (egg PC) in ILC: ■
499 cations (protonated bases), the regression equation: $y = 0.8699x - 0.6710$, $R^2 = 0.9521$; ◆
500 anions (deprotonated acids), the regression equation: $y = 0.7228x - 2.362$, $R^2 = 0.9649$.

501

502 **Figure 2.** A plot of $\log k_{\text{LEKC}}$ (POPC/PS) in LEKC versus $\log k_{\text{IAM}}$ (IAM.PC.DD2 column) in
503 IAM: ■ cations (protonated bases), the regression equation: $y = 0.9133x - 0.9529$, $R^2 =$
504 0.9450 ; ◆ anions (deprotonated acids), the regression equation: $y = 0.9783x - 2.654$, $R^2 =$
505 0.9606 ; ● smaller neutral molecules, the regression equation: $y = 1.001x - 1.412$, $R^2 = 0.9780$;
506 ▲ larger neutral molecules (steroids), the regression equation: $y = 0.9876x - 2.302$, $R^2 =$
507 0.4925 .

508

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

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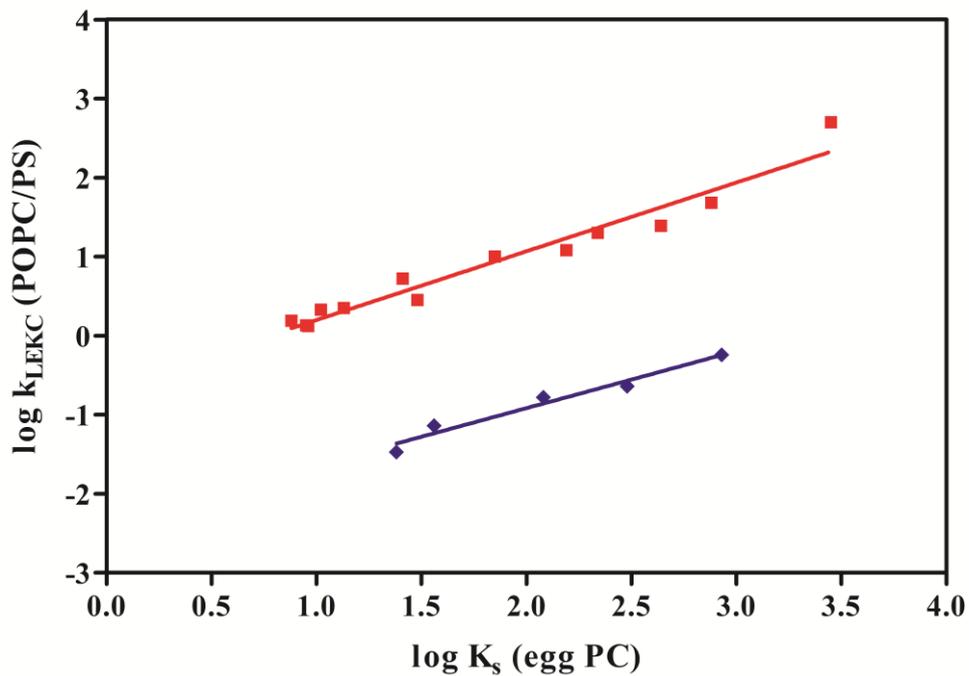
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522 **Figure 1:**

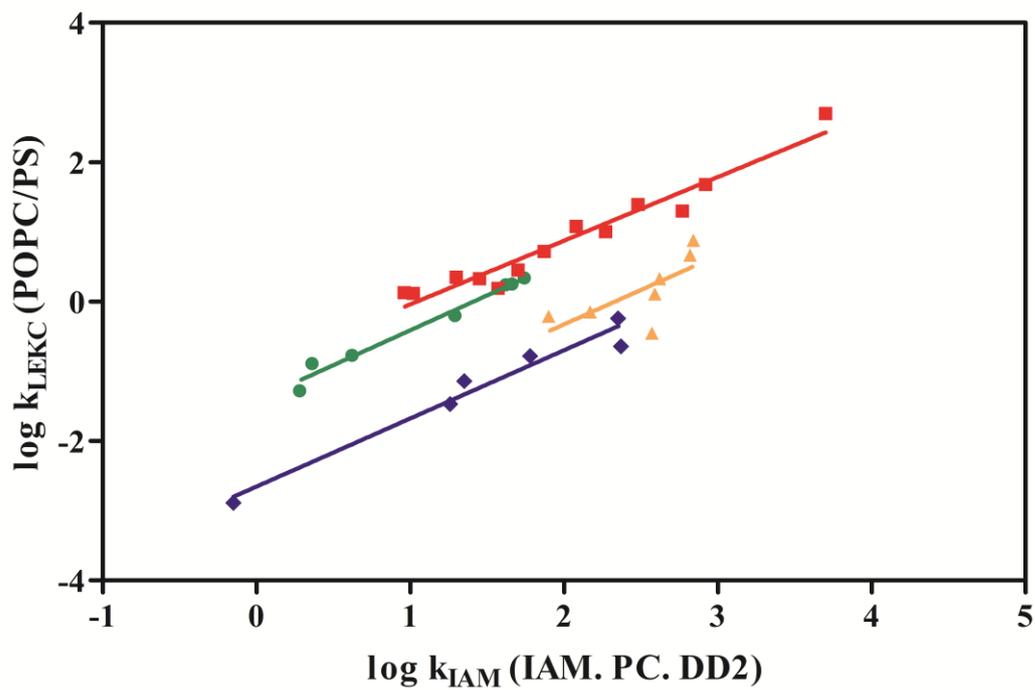


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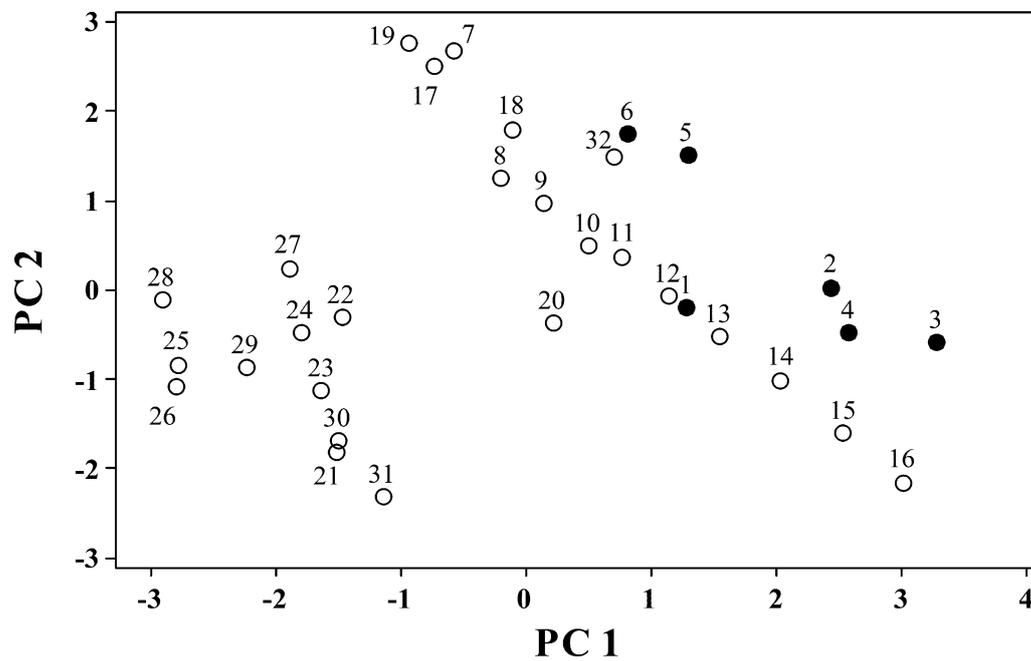
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526 **Figure 2:**



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528 **Figure 3:**



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