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# Analysis of immobilized artificial membrane retention factors for both neutral and ionic species

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ABSTRACT

Retention data on an immobilised artificial membrane have been taken from the work of Li et al. and from Liu et al., and have been correlated with a set of descriptors that includes descriptors for ionized species, that is anions from deprotonated acids and cations from protonated bases. Log $k$(IAM) values can be predicted for acids or bases that are partially ionized at the experimental pH and log $k$(IAM) values for acids and bases can be predicted as a function of the fraction present as the ionized species, equivalent to prediction as a function of pH. It is shown that anions reduce the value of log $k$(IAM) by about 1.1 log units but that cations have almost no effect by comparison to the neutral species. By comparison to non-polar solvents, carboxylate anions and protonated base cations are considerably stabilized by both water and the IAM phase to about 6–8 log units and so the rather small influence of anions (−1.1 log unit) and cations (−0.1 log unit) on log $k$(IAM) is due to substantial cancellation of these stabilization effects. Indeed, the effect of change of phase from water to IAM on the neutral species is at least as large as the effect of change of phase on the ionic species.

## 1. Introduction

Although the water–octanol partition coefficient (as $\log P_{\text{oct}}$) is widely used as a model for partitions of chemicals into biological membranes, it is now recognized that biological membranes vary so much in character that it is impossible for any given water–solvent system to be a useful model for all membranes, especially when ionic species are considered [1–5]. Consequently, other phases such as immobilized artificial membrane (IAM) systems where the lipid membrane is a phospholipid monolayer bound to silica gel beads and water–liposome partitioning system have been developed as possible models for biological partition processes. The use of IAM systems as models is particularly attractive because retention factors, $k$(IAM), can be obtained by relatively straightforward chromatographic procedures. Several workers [6–10] have compared log $k$(IAM) values with biological data.

In a recent paper, Li et al. [11] have analyzed log $k$(IAM) values obtained on an IAM.PC.DD2 column filled with phosphatylcholine residues using the method that Abraham and Acree later used to correlate log $k$(IAM) capacity values of neutral species [12]. Li et al. [11] introduced a $\delta$-value calculated from Eqs. (1) and (2) to include ionic species. Although quite reasonable correlation equations were obtained, they yield no real mechanistic information.

$$\delta = \frac{1}{1 + 10^{(\mathrm{p}K_a - \mathrm{pH})}} \tag{1}$$

$$\delta = \frac{10^{(\mathrm{p}K_a - \mathrm{pH})}}{1 + 10^{(\mathrm{p}K_a - \mathrm{pH})}} \tag{2}$$

Subsequently, Li et al. [13] correlated another selection of log $k$(IAM) values with various solute descriptors and obtained Eq. (3) where $C \log P$ is the calculated logarithm of an octanol–water partition coefficient, RotB is the number of rotatable bonds, MW is the solute molecular weight, TSA is the total surface area and Rings is the number of rings in the solute. No account was taken of any ionization of the solute, and the mechanistic implications of Eq. (3) are not at all evident.

$$\begin{aligned}\text{Log}\, k(\text{IAM}) = {} & -1.177 + 0.308\, C \log P - 0.011\, \text{RotB} + 0.002\, \text{MW} \\ & + 0.003\, \text{TSA} + 0.213\, \text{Rings}\end{aligned} \tag{3}$$

The two sets of log $k$(IAM) values determined by Li et al. [11,13] include not only neutral species, but anionic species from the deprotonation of acids and cationic species from the protonation of

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bases. We have recently determined solute descriptors for a large number of anions and cations and have used these descriptors to analyze water–solvent partitions and a number of biological systems in order to elucidate the factors that influence the behavior of neutral and ionic species [5,14–21]. No such analysis of IAM systems has ever been reported and so we have taken advantage of the data obtained by Li et al. [11,13] to obtain this information.

## 2. Methods

We use the linear free energy relationship, LFER, Eq. (4) to correlate *SP* values for neutral solutes; *SP* can be log *P* where *P* is a set of water–solvent partitions in a given system, or it can be a biological end-point, or it can be as in the present case log *k*(IAM) for a series of solutes in a given system. The extension of Eq. (4) to include ionic solutes leads to Eq. (5).

$$\mathrm{SP} = c + e\,E + s\,S + a\,A + b\,B + v\,V \tag{4}$$

$$SP = c + e\,E + s\,S + a\,A + b\,B + v\,V + j^{+}J^{+} + j^{-}J^{-} \tag{5}$$

The independent variables, or descriptors, in Eqs. (4) and (5) are properties of solutes as follows [12,14,22–25]: *E* is an excess molar refraction in $\mathrm{cm}^3\,\mathrm{mol}^{-1}/10$, *S* is a combined dipolarity/polarizability descriptor, *A* is the overall solute hydrogen bond acidity, *B* is the overall solute hydrogen bond basicity, *V* is McGowan's [26] characteristic molecular volume in $\mathrm{cm}^3\,\mathrm{mol}^{-1}/100$, $J^+$ is a specific descriptor for cations and $J^-$ is a specific descriptor for anions. Cations have $J^- = 0$, anions have $J^+ = 0$ and neutral solutes have $J^+ = J^- = 0$, so that Eq. (5) then reverts to Eq. (4). For some solutes in certain particular systems, a modified hydrogen bond basicity, $B^0$, has to be used instead of *B* [27]. These solutes so far identified are aniline, the toluidines, pyridine and alkyl pyridines, and sulfoxides (but not sulfones), and the systems are invariably two-phase systems in which water is in contact with an organic phase that itself contains a substantial amount of water. Such systems include water–wet octanol and water–wet ether [27].

Although we can attribute physicochemical meanings to descriptors such as *A* and *B*, we have so far failed to relate $J^+$ and $J^-$ to any physicochemical property. We have a number of generalizations such as $J^-$ for alkylcarboxylate anions (1.9), alkylbenzoate anions (2.2), phenoxide anions (1.7) but no more. Since we are unable to relate $J^+$ and $J^-$ to any physicochemical property, the corresponding equation coefficients, $j^+$ and $j^-$, have to be regarded as empirical factors only.

The set of coefficients, *c*, *e*, *s*, *a*, *b*, *v*, $j^+$ and $j^-$ characterise the system and are determined by multiple linear regression analysis. These coefficients are not just fitting coefficients, but represent the complementary properties of the system: *e* gives the contribution in Eqs. (4) and (5) of the solute-system general dispersion interaction, *s* is the solvent dipolarity (plus some polarizability), *a* is the system hydrogen bond basicity (the complimentary property to solute hydrogen bond acidity) and *b* is the system hydrogen bond acidity (the complimentary property to solute hydrogen bond basicity). The coefficient *v* reflects general dispersion interactions that aid solubility of a solute and the solvent–solvent interactions in the system that oppose solubility.

## 3. Results

Li et al. [11] list capacity values as log *k*(IAM) at pH 7.0 and obtained an LFER equation for 53 compounds. Subsequently [13], they examined another set of log *k*(IAM) values for 55 compounds, many of which were the same compounds given before [11]. In order to obtain the most general equation, we have collected together all the compounds listed by Li et al. [11,13] and have used known p$K_a$ values to calculate the fraction of neutral, Fn, and ionic, Fi, species present at that pH. We give in Table 1 the compounds that are present at pH 7.0 almost exclusively as either the neutral form or as the ionized form, together with the required descriptors for Eq. (5). The descriptors for the ionic species were either those used previously [5,14–17] or were calculated using the equations given before [14]. For the 68 species listed in Table 1 we find Eq. (6).

$$\begin{aligned}\mathrm{Log}\,k(\mathrm{IAM}) = {} & -0.607 + 0.881\,E - 0.562\,S - 0.348\,A - 1.794\,B\\ & +2.250\,V - 0.245J^{+} + 2.010J^{-}\ N = 68,\ SD = 0.431,\\ & R^2 = 0.896,\ FS = 74.2,\ PRESS = 15.380,\\ & Q^2 = 0.857,\ PSD = 0.506\end{aligned} \tag{6}$$

Here and elsewhere *N* is the number of data points, *R* is the correlation coefficient, *FS* is the F-statistic, *PRESS* and $Q^2$ are the leave-one-out statistics and *PSD* is the predictive standard deviation [28]. There are a number of compounds that are partially ionized at pH 7.0; we treated these compounds separately and used Eq. (6) to predict values of log *k*(IAM) for the neutral and ionized species. We can then predict the overall (observed) log *k*(IAM) through Eq. (7). Some representative calculations are in Table 2.

$$k(\mathrm{obs}) = \mathrm{Fn}.k\mathrm{n} + \mathrm{Fi}.k\mathrm{i} \tag{7}$$

Liu et al. [29] have determined values of log *k*(IAM) chromatographically under conditions in which compounds were present entirely as the neutral form or entirely as the ionized form. The species, the corresponding values of log *k*(IAM) and the necessary descriptors are in Table 3.

Application of the LFER, Eq. (5), leads to Eq. (8) for all 49 species in Table 3. The coefficients in Eq. (8) are reasonably close to those in Eq. (6) bearing in mind the two different IAM systems, but Eq. (8) seems rather the better equation statistically, with SD only 0.302 log units.

$$\begin{aligned}\mathrm{Log}\,k(\mathrm{IAM}) = {} & -0.668 + 0.485\,E - 0.411\,S - 0.117\,A - 2.537\,B\\ & +2.707\,V - 0.791J^{+} + 2.696J^{-}\ N = 49,\ SD = 0.302,\\ & R^2 = 0.912,\ FS = 90.6,\ PRESS = 5.571,\ Q2 = 0.869,\\ & PSD = 0.369\end{aligned} \tag{8}$$

## 4. Discussion

A number of workers have used Eq. (4) for the correlation of log *k*(IAM) capacity values for neutral compounds [11,19] but Eqs. (6) and (8) represent the first equations in which specific descriptors for ionic species have been used. It is then possible to calculate and to predict log *k*(IAM) for partially ionized acids and bases, as shown for some representative compounds in Table 2. This can be done for any fraction of ionized species. As an example we show predicted values of log *k*(IAM) for hydrochlorothiazide as a function of the fraction of neutral form present, see Fig. 1. Since this fraction depends on the pH of the medium, this is equivalent to predicting log *k*(IAM) for hydrochlorothiazide as a function of pH. Such predictions can be made for any acid or base for which the neutral and ionic descriptors are known. It should be noted that the effects of ionized species do not just relate to the $j^+ J^+$ and $j^- J^-$ terms in Eqs. (6) and (8) but are a function also of the other terms in the equations. Thus cations have very large values of *S* and *A*, and anions have very large values of *S* and *B*. It is the resultant combination of all the terms in Eqs. (6) and (8) that lead to an overall effect on log *k*(IAM).