Log D analysis using dynamic approach

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Log D the logarithm (log_{10}) of the distribution coefficient (D), is one of the important parameters used in Lipinski's rule to assess the druggability of a molecule in pharmaceutical formulations. The distribution of a molecule between a hydrophobic organic phase and an aqueous buffer phase is influenced by the pH of the buffer system. In this work, we used both the conventional algebraic method and the generalized 'dynamic' approach to model the distribution coefficient of amphoteric, diamino-monoprotic molecule and monoprotic acid in the presence of salt or co-solvent. We have shown the equivalence of these methods by analysing the recently reported experimental data of amphoteric molecules such as naldixic acid, mebendazole, benazepril and telmisartan.

1. Introduction

Partition coefficient (P) is defined as the ratio of the concentration of a molecule, whether in ionized or unionized form, distributed between a hydrophobic phase and an aqueous phase [1–5]. Consider, a weak monoprotic acid, HA, which can exist in two forms such as, unionized (HA) and ionized (A−) species in an aqueous buffer system. If such an aqueous buffer system is equilibrated with a hydrophobic solvent (e.g. octanol), the unionized species and the ionized species in the aqueous phase will get partitioned into the hydrophobic phase with the partition coefficient defined by, \[ P_{H_A} = \frac{[H_A]}{[H_A]} \] and \[ P_{A^{-}} = \frac{[A^{-}]}{[A^{-}]} \], respectively. Since, it is less likely for a charged species like A−, to get partitioned into an octanol phase, prior to partitioning it forms a neutral ion pair with prevalently available cation in the aqueous solution. The distribution coefficient (D), on the other hand is dependent on the partition coefficient (P) and is defined as, \[ D = \frac{([H_A]+[A^{-}])}{([H_A]+[A^{-}])} \], the ratio of the sum of the concentrations of both ionized and unionized species of a molecule, distributed between the hydrophobic organic phase and the aqueous buffer phase. Since the dissociation of a weak monoprotic acid is dependent on the pH of the aqueous buffer system, the distribution coefficient also becomes dependent on pH. In an experiment designed to assess the lipophilicity of a molecule, the distribution coefficient (D), is measured at different pH conditions and the resultant profile of D, is fitted to a model, to obtain partition coefficients (P), \( p_K_a \) or \( p_K_a \) of all the species present in the system [1–5].

The mathematical model to predict the logD profile of simple cases such as monoprotic, diprotic, mono-alkaline and amphoteric can be easily derived using algebraic approach [6]. On the other hand, while studying the effect of salt or co-solvent on the distribution of monoprotic acid, dynamic approach is preferred because of its generality and simplicity in deriving the models [3,5,7–9]. In this article, we explicitly, derive the algebraic and dynamic models for amphoteric, di-amino-monoprotic, and monoprotic in the presence of salt or co-solvent [7–9]. Further, the logD profiles of recently reported amphoteric molecules such as naldixic acid, mebendazole, benazepril and telmisartan, were analysed to show the equivalence of dynamic approach and algebraic method [10].

2. Theory

A complex dynamic system can be modelled using several analogous kinetic mechanisms. If the experimental data points of the dynamic system is available prior to equilibrium, then the exact kinetic
mechanism can be delineated accurately. On the other-hand if the experimental
data is available only at equilibrium, then several analogous kinetic mechanisms can be used inter-changeably to determine the
equilibrium constants (SI 1 and 2). In logD analysis, since we deal with
systems that at equilibrium, several analogous kinetic mechanisms are available to model its data. Here we have considered previously
reported kinetic mechanisms for amphoteric, monoprotic acid in the
presence of salt (KCl) or co-solvent (DMSO) and diamino-monoprotic
amphoteric, to model the logD profile. Additionally, simple cases such as
monoprotic acid (SI3), diprotic acid (SI4), monoalkaline (SI5) are
detailed in the supplementary information for pedagogic purpose.

2.1. Equivalence of analogous kinetic mechanisms at equilibrium

Considering a simple system with four states/species (N = 4), A, B,
C, D; we show that several analogous kinetic mechanisms can be framed to model it (SI 2). Firstly, we define the ‘analogous kinetic mechanisms’ as a set of kinetic mechanisms whose equilibrium/steady state concentrations are the same for its species across mechanisms. In
other words, even though the members of the ‘analogous kinetic mechanisms’ remain distinguishable through their distinct time profiles for
A, B, C, and D, prior to steady-state or equilibrium, they are indi-
distinguishable at steady state or equilibrium. If the equilibrium constants
for one of the members of ‘analogous kinetic mechanisms’ is known
then we can easily derive the equilibrium constants for the rest of the
members of ‘analogous kinetic mechanisms’, which is stated here as the
equivalence of the ‘analogous kinetic mechanisms’ at equilibrium.

If we consider each species as a ‘node’ and the interconnecting equation reactions as bidirectional ‘edges’, then the graph theory
suggest a maximum of $E_{\text{max}} = N(N−1)/2$, edges or equilibrium re-
actions [11–13]. For a system with N = 4, species, there exist a
maximum of $E_{\text{max}} = 6$, equilibriums. On the other-hand, a minimum of
$E_{\text{min}} = (N−1) = 3$, edges or equilibrium would be required to
connect all the four species to obtain a non-disjointed or ‘connected graph’.
With a minimum of 3 and a maximum of 6 equilibriums, there exist 38
different analogous kinetic mechanisms for a 4 species system (SI 2).
Out of these 38 possibilities we will consider only two ‘analogous me-
chanisms’ to show their equivalence. Consider a simple linear me-
chanism (Fig. 1A) which minimally connects all the four species as
shown below (Eq. 1),

$$k_1 \quad k_2 \quad k_3 \quad k_4$$

$$A \rightleftharpoons B \rightleftharpoons C \rightleftharpoons D \quad (1)$$

In the above equilibrium, $k_1$, $k_2$, $k_3$, are the reverse rate constants for the reactions $A \rightleftharpoons B$, $B \rightleftharpoons C$, $C \rightleftharpoons D$, respectively. The three equilibrium constants $K_1$, $K_2$, $K_3$ are defined as $K_1 = \frac{k_1}{k_2}$, $K_2 = \frac{k_2}{k_3}$, $K_3 = \frac{k_3}{k_4}$, respectively. On the other-hand consider a complex mechanism (Fig. 1B) which not only
includes Eq. 1, but also three additional equilibriums Eqs. 2–4,

$$k_5$$

$$A \rightleftharpoons C \quad (2)$$

$$k_6$$

$$B \rightleftharpoons D \quad (3)$$

$$k_7$$

$$A \rightleftharpoons D \quad (4)$$

In the above equations (Eqs. 2–4), $k_5$, $k_6$, $k_7$, are the forward and $k_4$, $k_5$, $k_6$, are the reverse rate constants for the reactions $A \rightleftharpoons B$, $B \rightleftharpoons D$, $A \rightleftharpoons D$, respectively, and the corresponding equilibrium constants are defined as $K_4 = \frac{k_5}{k_6}$, $K_5 = \frac{k_6}{k_7}$, $K_6 = \frac{k_7}{k_4}$. If we assume both the
mechanisms to be analogous i.e, both lead to an identical ratios of $A$, $B$, $C$, $D$, at equilibrium, then the equilibrium constants $K_4$, $K_5$, $K_6$, are dependent on $K_1$, $K_2$, $K_3$, and can be easily derived by comparing a subset of (Eq. 1) and (Eq. 2) to write the following equation (Eq. 5),

$$A \rightleftharpoons B \rightleftharpoons C \rightleftharpoons D \quad (5)$$

The comparison clearly shows that $A \rightleftharpoons C$ is an abstraction of
$A \rightleftharpoons B \rightleftharpoons C$, hence we can combine the corresponding equilibrium constants and equate $K_4 = K_5 \times K_6$. Similarly, based on the comparisons of (Eq. 6) and (Eq. 7), we can write $K_5 = K_2 \times K_3$ and $K_6 = K_2 \times K_5$, respectively.

$$B \rightleftharpoons C \rightleftharpoons D \quad (6)$$

$$A \rightleftharpoons B \rightleftharpoons C \rightleftharpoons D \quad (7)$$

Thus, we can conclude that if we have a kinetic mechanism with $N$
species, we would require a minimal of ($N−1$) equilibriums that un-
quely connects these $N$ species, so as to determine the additional equilibrium constants existing in other ‘analogous mechanisms’. A
comparative simulation of both these kinetic mechanisms (Fig. 1A, B)
using dynamic approach is shown in Fig. 1C & D, to highlight, their
differences during pre-steady state phase and their equivalence during
the steady state phase. In the following sections, one of the ‘analogous kinetic mechanism’ that best represent the distribution of a molecule
between an aqueous buffer and octanol layer will be outlined. Based on
the proposed kinetic mechanism, the algebraic and the dynamic models
will be derived. The dynamic models proposed here make an assump-
tion that the mass transportation is instantaneously homogenous within
each liquid phases for all the species at all instance of time, i.e. perfectly
stirred system. The dynamic model for non-stirred systems, which will
not be discussed here, would require complex partial differential
equations that account for both the spatial and time dependence based
on Fick’s second law of diffusion.

2.2. Amphoteric model for amino acids

2.2.1. Kinetic model for simple amino acids

Consider an amino acid (NH$_2$–R–COOH or BAH or HAB) containing
a weak mono-protic acid (COOH or HA) and a weak basic/alkaline
group (NH$_2$ or B) distributed between an aqueous buffer and an or-
ganic hydrophobic solvent (octanol) (Fig. 2A) [5,6,14]. In the aqueous
phase, the amino acid, [NH$_2$–R–COOH] or [HAB], exists in an un-
ionized form [NH$_3$–R–COOH]$_a$ or [HAB]$_a$, and the ionized forms,
[NH$_3$–R–COO]$^{-}$ or [BA]$^−$, [NH$_3$–R–COOH]$^-$ or [HAB]$^-$, [NH$_3$–R–COO]$_a^{-}$ or [BAH]$^{a-}$]. The equilibrium among these four states can be written as (Eqs. 8–12),

$$k_8$$

$$\text{HABH}^+_a \rightleftharpoons \text{H}^+ + \text{BAH}^- \quad (8)$$

$$k_9$$

$$\text{HABH}^+_a \rightleftharpoons \text{H}^+ + \text{BAH}^- \quad (9)$$

$$k_{10}$$

$$\text{BA}^+ \rightleftharpoons \text{BAH}^- \quad (10)$$

$$k_{11}$$

$$\text{BA}^+ \rightleftharpoons \text{BAH}^- \quad (11)$$

$$k_{12}$$

$$\text{BA}^+ \rightleftharpoons \text{BAH}^- \quad (12)$$

$k_1$, $k_2$, $k_3$, $k_4$, are the forward and $k_{−1}$, $k_{−2}$, $k_{−3}$, $k_{−4}$, are the reverse kinetic rates for the dissociation of proton from the species,
\[
\begin{align*}
\text{HAB}^+ & \rightleftharpoons \text{HAB}^* \\
\text{HAB}^* & \rightleftharpoons \text{HAB}^+_0 \\
\text{HAB}^+_0 & \rightleftharpoons \text{HAB}^+_0^* \\
\text{HAB}^+_0^* & \rightleftharpoons \text{HAB}^+_0^+ \\
\text{HAB}^+_0^+ & \rightleftharpoons \text{HAB}^+_0^+^* \\
\text{HAB}^+_0^+^* & \rightleftharpoons \text{HAB}^+_0^+^+ \\
\text{HAB}^+_0^+^+ & \rightleftharpoons \text{HAB}^+_0^+^+^* \\
\text{HAB}^+_0^+^+^* & \rightleftharpoons \text{HAB}^+_0^+^+^+ \\
\end{align*}
\]

The four species, \([\text{HAB}^+_0^+^*]\), \([\text{HAB}^+_0^+^+^*]\), \([\text{HAB}^+_0^+^*]\), and \([\text{HAB}^+_0^+^+^*]\), can be partitioned into octanol layer as shown below (Eqs. 13–16).

Fig. 1. (A) A simple linear kinetic mechanism proposed for a four states/species system, A, B, C, D (\(N = 4\)). The system is ‘simple’ because, only a minimal of \((N – 1 = 3)\) equilibriums are considered. It is ‘connected’ because, all the species are connected to its neighbour at least once. (B) represents a complex ‘completely connected’ kinetic mechanism for the same system. This system has a maximum number of \((N(N – 1) = 6)\) equilibriums realizable for a 4 state system. (C) Shows the dynamic profile for [A], [B], [C], [D] using simple model (1 A), (SI. 2.1). (D) shows the dynamic profile for [A], [B], [C], [D] using complex model (1B), (SI. 2.2).

Prior to reaching the equilibrium (or pre-steady state phase) for simple and complex mechanisms at 1.5 s, \(8 \times 10^{-4}\) s, respectively, the time profiles for all 4 species remain distinct and distinguishable between mechanisms; but at equilibrium (steady state), the concentrations of all 4 species are equivalent despite their mechanistic differences. The simulation was carried out using \(k_i, k_{-i}, k_2, k_{-2}, k_3, k_{-3}, k_4, k_{-4}\), as 7, 1, 72, 1, 10584, 1, respectively. The initial concentrations of \([A]_0\), \([B]_0\), \([C]_0\), \([D]_0\) were set to 504, 1, 1512, 1, 10584, 1, respectively. The additional kinetic rates seen for (B), \(k_4, k_{-4}, k_6, k_{-6}, k_8, k_{-8}\), were set to 504, 1, 1512, 1, 10584, 1, respectively. The initial concentrations of \([A]_0\), \([B]_0\), \([C]_0\), \([D]_0\) were set to 1,0,0,0, respectively, for both these simulations.

\[
\begin{align*}
\text{HAB}^+_0 & \rightleftharpoons \text{HAB}^*_0 \\
\text{HAB}^*_0 & \rightleftharpoons \text{HAB}^+_0 \\
\text{HAB}^+_0 & \rightleftharpoons \text{HAB}^*_0 \\
\text{HAB}^*_0 & \rightleftharpoons \text{HAB}^+_0 \\
\text{HAB}^+_0 & \rightleftharpoons \text{HAB}^*_0 \\
\text{HAB}^*_0 & \rightleftharpoons \text{HAB}^+_0 \\
\end{align*}
\]
are the reverse kinetic rates, respectively, for the partitioning of HABH⁺, HAB⁻, AB⁺, AB⁻ from the aqueous phase into octanol phase, as HABH⁺, HAB⁻, AB⁺, AB⁻. The partition coefficients for the species HABH⁺, HAB⁻, AB⁺, AB⁻ are defined as, $P_{\text{HABH}^+} = \frac{P_{\text{HAB}^-}}{K_{\text{HAB}^+}}$, $P_{\text{HAB}^-} = \frac{P_{\text{AB}^+}}{K_{\text{HAB}^-}}$, $P_{\text{AB}^+} = \frac{P_{\text{AB}^-}}{K_{\text{AB}^+}}$, respectively.

The partition of singly charged species such as [HABH⁺], and [AB⁻] into the octanol layer is significantly influenced by ion pair formation (salt effect) in the aqueous phase. Whereas, the partitioning of zwitterions ~ABH⁺, is primarily influenced by its charge neutrality. The concentration of the zwitterions is prevalent at a particular pH called isoelectric point (pI) from its acidic group, $pK_a$ (COOH = COO⁻ + H⁺), and its basic group, $pK_b$ (NH₂ = NH₂⁺ + H⁺).

### 2.2.2. Algebraic method for simple amino acids

Since we have eight species ($N = 8$) in our proposed kinetic mechanism, we would require only a minimum of ($N - 1 = 7$) seven equilibriums, seven algebraic equations can be framed in terms of its species, [HABH⁺], [HAB⁻], [AB⁺], [AB⁻], [HABH⁺], [HAB⁻], [AB⁺], [AB⁻]. The distribution coefficient (D) for such a system can be defined as,

$$D = \left( \frac{[\text{HABH}^+] + [\text{HAB}^-] + [\text{AB}^+] + [\text{AB}^-]}{[\text{HABH}^+] + [\text{HAB}^-] + [\text{AB}^+] + [\text{AB}^-]} \right) \left( \frac{1}{7} \right)$$

In the above Eqn. 17, ‘r’ is the ratio of the volume of octanol to aqueous buffer (SI. 3.2). By re-expressing seven of the eight species [HABH⁺], [HAB⁻], [AB⁺], [AB⁻], [HABH⁺], [HAB⁻], [AB⁺], [AB⁻] from Eqs. 8, 9, 11, 13–16, in terms of the eight species, $[\text{HABH}^+]$, $[\text{HAB}^-]$, $[\text{AB}^+]$, $[\text{AB}^-]$ and substituting the resulting analytical expressions into Eq. 17, we obtain Eq. 18,

$$D = \left( \frac{P_{\text{HABH}^+} [H^+]^2 + P_{\text{HAB}} [H^+]^2 + P_{\text{AB}^+} [H^-]^2 + P_{\text{AB}} [H^-]^2}{[H^+]^2 + [H^-]^2} \right) \left( \frac{1}{7} \right)$$

Since, $K_i$ and $K_s$ are concerned with the dissociation of proton (H⁺) from its base unit (~BH⁻), (i.e. ~BH⁻ = ~B⁻ + H⁺); and $K_s$ and $K_a$ are concern with the dissociation of the proton from the acid unit, HAB⁻, (i.e. HAB⁻ = AB⁻ + H⁺), we can make a valid assumption that $K_i = K_s$, and $K_i = K_a$, in Eq. 18. Further, by substituting $K_i = 0$, i.e., excluding the neutral species [HAB] in Eq. 18, we can easily arrive at the expression for diprotic model (SI. 4).

#### 2.2.3. Dynamic method for simple amino acids

Based on the kinetic mechanism (Eqs. 8–16), the rate equation (SI.1) can be written for eight species, [HABH⁺], [HAB⁻], [AB⁺], [AB⁻], [HABH⁺], [HAB⁻], [AB⁺], [AB⁻], as follows,

$$\frac{d[HABH^+]}{dt} = -k_1[HABH^+] + k_{-1}[H^+] [HAB^-] - k_2[HABH^+]$$
$$+ k_{-2}[\text{AB}^+][H^-] - k_{p1}[HABH^+] + k_{-p1}[HAB^-]$$

$$\frac{d[HAB^-]}{dt} = +k_1[HABH^+] - k_{-1}[H^+] [HAB^-] - k_2[HABH^-]$$
$$+ k_{-2}[\text{AB}^+][H^-] - k_{p1}[HAB^-] + k_{-p1}[HAB^-]$$

$$\frac{d[\text{AB}^+]}{dt} = +k_1[HABH^-] - k_{-1}[H^-] [HAB^-] - k_2[HABH^-]$$
$$+ k_{-2}[\text{AB}^+][H^-] + k_{p1}[HAB^-] + k_{-p1}[HAB^-]$$

$$\frac{d[HABH^-]}{dt} = -k_1[HABH^-] + k_{-1}[H^+] [HAB^-] - k_2[HABH^-]$$
$$+ k_{-2}[\text{AB}^+][H^-] - k_{p1}[HABH^-] + k_{-p1}[HAB^-]$$

$$\frac{d[AB^-]}{dt} = +k_1[HABH^-] - k_{-1}[H^-] [HAB^-] - k_2[HABH^-]$$
$$+ k_{-2}[\text{AB}^+][H^-] - k_{p1}[HAB^-] + k_{-p1}[HAB^-]$$

$$\frac{d[HAB]}{dt} = +k_2[HAB^-] - k_{-2}[\text{H}][\text{AB}^-]$$
$$- k_{p1}[HAB^-] + k_{-p1}[HAB^-]$$

$$\frac{d[HAB^+]}{dt} = -k_2[HAB^-] + k_{-2}[\text{H}][\text{AB}^-] - k_{p1}[HAB^-]$$
$$+ k_{-p1}[HAB^-]$$
\[
\frac{d[ABH^+]}{dt} = +kp_4[ABH_4] - k_{p5}[ABH^+] \\
\frac{d[AB_4]}{dt} = +kp_4[AB_4] - k_{p5}[AB]_4
\]  
(25)

By numerically integrating the above set of coupled differential equations, Eqs. 19–26, we obtain the concentration of eight species, $[HABH^+]$, $[HAB_4]$, $[ABH^+]$, $[AB_4]$, $[HABH^+]$, $[HAB_4]$, $[ABH^+]$, and $[AB_4]$ at different time points. The resultant concentrations can be substituted into the Eq. 17, to obtain the distribution coefficient. In the above model, if we were to account for the dynamics of $[H_2^+]$, then we could write,

\[
\frac{d[H_2^+]}{dt} = \left( +k_1[HABH^+] - k_{-1}[HAB_4][H_2^+] + k_{4}[HAB_4] \\
- k_{-1}[ABH_4][H_2^+] + k_2[HABH^+] - k_{-2}[ABH^+] [H_2^+] \\
+ k_3[ABH^+] - k_{-3}[AB][H_2^+] \right)
\]  
+ Buffer terms

(27)

The “Buffer terms” as mentioned in the Eq. 27, are the terms that arise from the dissociation of the weak acid and weak base moieties that are specific to the buffer system [15]. In this work, we prefer to assume $[H_2^+]$ or $pH$, to be constant with respect to time, i.e. $\frac{d[H_2^+]}{dt} = 0$, due to fact that log$pH$D experiments are usually carried out in a controlled pH buffer system. To simulate the log$pH$D profile in Fig. 2B, the parameters such as $k_1$, $k_{-1}$, $k_2$, $k_{-2}$, $k_3$, $k_{-3}$, $k_4$, $k_{4}$, $k_{5}$, $k_{5}$, $k_{1}$, $k_{1}$, $k_{2}$, $k_{2}$, $k_{3}$, $k_{3}$, $k_{4}$, $k_{4}$, $k_{5}$, $k_{5}$, $k_{6}$, $k_{6}$, $k_{7}$, $k_{7}$, were set to $10^{-8}$, $1.0$, $10^{-3}$, $1.0$, $10^{-4}$, $1.0$, $10^{-5}$, $1.0$, $10^{-6}$, $1.0$, $10^{-7}$, $1.0$, respectively. The initial concentrations of all the eight species $[HABH^+]_0$, $[ABH^+]_0$, $[AB_4]$, $[HABH^+]_0$, $[ABH_4]_0$, $[ABH^+]_0$, $[AB]_4$, and $[AB]_4$, were set to $1.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0$, respectively and the $pH$, was varied linearly between 1 and 10.

### 2.3. Monoprotic acid with salt (KCl)

Consider the distribution of a weak mono-protic acid ($HA$) between an aqueous solvent and an organic hydrophobic solvent (octanol) in the presence of a salt such as potassium chloride (KCl) (Fig. 3A) [3,5,16,17]. In this mechanism, we first write the equilibrium constants in the aqueous phase, then the interface (aqueous-octanol partition) and finally the octanol phase.

In aqueous phase, the weak acid, $[HA]_0$, exists in the unionized form $[HA]$ and ionized form $[A^-]$. The equilibrium between these two states can be written as (Eq. 28),

\[
[k_1]_0
HA_{(aq)} \rightleftharpoons H^+ + A^-_aq
\]  
(28)

$k_1$, $k_{-1}$ are the forward and reverse kinetic rates for the dissociation of the weak acid, $[HA]_0$, respectively. Since the dissociation of the salt KCl into $[K^+]_1$ and $[Cl^-]$ (in aqueous buffer) is complete and irreversible we can write (Eq. 29) with, $k_p$, as its kinetic forward rate constant.

\[
K_{Cl\text{aq}} \rightleftharpoons k_p + Cl^-_{aq}
\]  
(29)

The unionized species, $[HA]_0$, gets partitioned into octanol layer with the forward and reverse kinetic rates, $k_{p3}$ and $k_{p5}$, respectively, and the corresponding partition equilibrium, $P_{HA} = \frac{k_{p3}}{k_{p5}}$ (Eq. 30),

\[
HA_{(aq)} \rightleftharpoons HA_{(o)}
\]  
(30)

The formation of the ionic complex, $K^+_3 : Cl^-_{aq}$, in the aqueous phase is highly probable at high salt concentration, whereas at low concentrations (saturation limit), it remains dissociated as $[K^+_3]$ and $[Cl^-]$. We represent the partitioning of $[K^+_3]$ and $[Cl^-]$ into octanol layer as Eq. 31, with the forward and reverse kinetic rates as $k_{p3}$ and $k_{p5}$, respectively; and the corresponding partition coefficient as, $P_{HA} = \frac{k_{p3}}{k_{p5}}$.

\[
K^+_3 + Cl^-_{aq} \rightleftharpoons K^+_3 Cl^-_{aq}
\]  
(31)

Even though the above Eq. 31, could have been written in a more explicit manner, $K^+_3 + Cl^-_{aq} \rightleftharpoons K^+_3 Cl^-_{aq}$, with the inclusion of an intermediate ionic complex ($K^+_3 Cl^-_{aq}$) in aqueous phase, the experimental determination of the association constant for such an ionic complex is practically difficult. Hence, we prefer to use an abstracted mechanism as proposed by Scherrer [18] for all the partition equilibria concerned with the ionic species.

The ionized species $[A^-]$, would require neutralization of its negative charge through the prevalent cation, $K^+_3$ from KCl, before partitioning into octanol layer. The forward and reverse kinetic rates of the partition of $[A^-] : [K^+_3]$ are $k_{p3}$, $k_{p5}$, respectively, with its partition equilibrium constants defined as, $P_{KA} = \frac{k_{p3}}{k_{p5}}$ (Eq. 32),

\[
A^-_aq \rightleftharpoons K^+_3 + A^-_aq
\]  
(32)

The $[H_2^+]$ ions (from $HA_0$, (Eq. 28)) and $[Cl^-]$ (from $[KCl]_aq$) gets partitioned into octanol with the forward and reverse kinetic rates as $k_{p3}$, $k_{p5}$, respectively, with its partition equilibrium constants defined as, $P_{Cl} = \frac{k_{p3}}{k_{p5}}$ (Eq. 33),

\[
H^+_aq + Cl^-_{aq} \rightleftharpoons HCl^-_{aq}
\]  
(33)

Additionally, as seen with the monoprotic acid (SI.3), it is also possible for a fraction of the ionized species $[A^-]$, to get directly partitioned into the octanol layer without ion-pair formation as $[A^-]_o$, whose forward and reverse kinetic rates are given by $k_{p3}$, $k_{p5}$, respectively, with its partition equilibrium constants defined as, $P_{A} = \frac{k_{p3}}{k_{p5}}$ (Eq. 34),

\[
A^-_aq \rightleftharpoons A^-_o
\]  
(34)

Finally, all the four species $HA_0$, $+KCl_\text{aq}$, $+KA^-_aq$, $+HCl^-_aq$, that got partitioned into the octanol layer undergo a dynamic equilibrium whose forward and reverse kinetic rates are given by $k_3$, $k_{-3}$, respectively, with its equilibrium constants defined as, $K_3 = \frac{k_3}{k_{-3}}$ (Eq. 35),

\[
HA_0 + +KCl_\text{aq} \rightleftharpoons +KA^-_aq + +HCl^-_aq
\]  
(35)

Based on the above kinetic mechanism Eqs., 28–35, the algebraic model and the dynamic models were derived and used for this analysis (SI 7).

### 2.4. Monoprotic acid in the presence of co-solvent

Consider the distribution of a weak mono-protic acid ($HA$) between an aqueous buffer and an organic hydrophobic solvent (octanol) in the presence of co-solvent (S) such as DMSO (Fig. 4A) [3,4].

In aqueous phase, the weak acid, $[HA]$, exists in unionized form $[HA]$ and ionized form $[A^-]$. The equilibrium between these two states can be written as (Eq. 36),
The equilibrium constant, $K_i$, is defined based on its forward and reverse kinetic rates, $k_i$, $k_{-i}$, respectively. The distribution of a cosolvent between an aqueous buffer and octanol is given by (Eq. 37),

$$k_i \frac{[HA]_u}{[A^-]_o} = k_{-i} \frac{[H^+]_o}{[H^-]_u}$$

The equilibrium constant, $K_i$, is defined based on its forward and reverse kinetic rates, $k_i$, $k_{-i}$, respectively. The distribution of a cosolvent between an aqueous buffer and octanol is given by (Eq. 37),

$$k_i \frac{[HA]_u}{[A^-]_o} = k_{-i} \frac{[H^+]_o}{[H^-]_u}$$

The logarithmic form of which are plotted against the logarithm of salt concentration in (C).
The equilibrium constant, $K_{s1} = \frac{k_{s1}}{k_{-s1}}$, is defined based on the forward and reverse kinetic rates, $k_{s1}$, $k_{-s1}$, respectively, for the co-solvation of $[HA_s]$ in the aqueous phase. $k_{s2}$, $k_{-s2}$ are the forward and reverse kinetic rates for the co-solvation of $[A^-]$ with its equilibrium constant defined as $K_{s2} = \frac{k_{s2}}{k_{-s2}}$. $n_1$, $n_2$, are the number of co-solvent molecules required to co-solvate $[HA_s]$, $[A^-]$, respectively. 

$[HA_s]$ and $[A^-]$ gets partitioned into octanol layer as shown below (Eqs. 40 and 41),

$$D = \left(\frac{[HA_s]^{n_1} + [A^-]^{n_2}}{[HA_o]^{n_1} + [A_o]^{n_2}}\right),$$

$$D = \left(\frac{[HA_o]^{n_1} + [A_o]^{n_2}}{[HA_s]^{n_1} + [A^-]^{n_2}}\right),$$

The data were fitted using an in-house written matlab code [31].

Fig. 4. (A) The kinetic model for a monoprotic acid in the presence of a co-solvent ([S]). In this mechanism, there exists a reversible equilibrium between $HA_s$, and $A^-$ in the aqueous phase, which also partitions into octanol as $HAS_a$, and $AS_a$, respectively. All four species, $HA_s$, $A^-$, $HAS_a$, $AS_a$ get co-solvated by the solvent, present in aqueous([S]), or octanol phase ([S]_o) with the stoichiometry of $n_1$, $n_2$, $n_3$, $n_4$, to form $HAS_a$, $A_s$, $HAS_o$, $AS_o$, respectively. (B) & (D) Shows the $log_{10}D$ profiles of the monoprotic acid when the ratio of monoprotic to solvent is varied between 1:0 and 1:1000, with the stoichiometry of $n_1$, $n_2$, $n_3$, $n_4$, to be 1:0:0:0 for (B) and 1:1:1:1 for (D), respectively. (C) & (E) shows how the $log_{10}P_{HA}$, $log_{10}P_A$, $pK_A$ of the monoprotic acid varies as the solvent concentration increases, from 0 to 1000 (shown here in $log_{10}$ scale). It is clear that the modulation of $log_{10}P_A$, $log_{10}P_{HA}$ and $pK_A$ is strongly dependent on the degree of the co-solvation of different species. In the simulation, the $P_{HA}$, $P_A$ and $K_A$ of the monoprotic acid were set to $10^3$, $10^{-3}$ and $10^{-2}$ respectively, the concentration of monoprotic acid was set to 1 and the solvent concentration was set to $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, 0.5, 1, 5, $10^1$, $10^2$, $10^3$ to simulate 12 different $log_{10}D$, profiles. Only five of the solvent profiles corresponding to 0, 1, 10, 100, 1000 were plotted in (B) and (D). The simulated profiles were fitted to the monoprotic model, $D = \left(\frac{[HA_s]^{n_1} + [A^-]^{n_2}}{[HA_o]^{n_1} + [A_o]^{n_2}}\right)$, to obtain the apparent $P_{HA}$’, $P_A$’ and $K_A$’, the logarithmic form of which are plotted against the logarithm of solvent concentrations.

Fig. 5. Experimental $log_{10}D$ analysis of (A) nalidixic acid, (B) mebendazole, (C) benazepril and (D) telmisartan using monoprotic, monoalkaline, simple amphoteric and diamino-monoprotic amphoteric models, respectively. The data were fitted using an in-house written matlab code [31].
\[ k_{p2} \]
\[ A_n^+ \rightleftharpoons A_n^- \]
\[ k_{n-p2} \]

The partition coefficient, \( P_{HA} = \frac{k_{pA}}{k_{n-pA}} \), is defined based on the forward and reverse kinetic rates, \( k_{p1}, k_{n-p1} \), respectively. Similarly, \( P_A = \frac{k_{pA}}{k_{n-pA}} \), is defined based on the forward and reverse kinetic rates, \( k_{p2}, k_{n-p2} \), respectively. The co-solvation of \([HA_n] \) and \([A_n^-] \) in octanol phase can be written as (Eqs. 42 and 43),

\[ k_{k1} \]
\[ HA_n + n_kS \rightleftharpoons HAS_n \]
\[ k_{n-k1} \]
\[ A_n^- + n_kS \rightleftharpoons AS_n \]
\[ k_{n-k4} \]

\( k_{k1}, k_{n-k1}, k_{k4}, k_{n-k4} \) are the forward and reverse kinetic rates for the co-solvation of \([HA_n] \) in the octanol phase, respectively, the corresponding equilibrium constant is defined as, \( K_{k1} = \frac{k_{k1}}{k_{n-k1}} \). Similarly, \( k_{k4}, k_{n-k4} \), are the forward and reverse kinetic rates for the co-solvation of \([A_n^-] \) in the octanol phase, respectively, the corresponding equilibrium constant is defined as, \( K_{k4} = \frac{k_{k4}}{k_{n-k4}} \). \( n_k \), \( n_s \) are the number of co-solvent molecules required to co-solvent \([HA_n] \), \([A_n^-] \), respectively.

In the above mechanism, we could have included additional partition equilibria such as, \( HAS_n \rightleftharpoons HA_n S \), and \( -AS_n \rightleftharpoons -AS_o \), but as explained in Section 2.1, these two paths can be achieved through already existing paths such as, \( HAS_n \rightleftharpoons HA_n \rightleftharpoons HA_o \rightleftharpoons HAS_o \) and \( -AS_n \rightleftharpoons -A_o \rightleftharpoons -AS_o \). Hence, we can easily derive and write the expression for \( P_{HAS} \) and \( P_{AS} \) as \( P_{HA \times K_{k1}} \) and \( P_{A \times K_{k4}} \), respectively. Based on the above kinetic mechanism Eqs.36-43, the algebraic model and the dynamic models were derived and used for this analysis (SI 8).

3. Results

The experimental data of naldixic acid, mebendazole, benzazepiril, telmisartan obtained from a recent study were used in our current analysis [10]. The \( \log_{10}D \) data of naldixic acid and mebendazole could be well represented through a monoprotic (SI 3, Eqn. S21) and mon-alkaline model (SI 5, Eqn. S65), respectively (Fig. 5A & B). The \( \log_{10}D \) profile of benzazepiril could be explained through a simple amino acid model (monoprotic-monalkaline) (Eq. 18, SI 6, Fig. 5C). Telmisartan, on the other-hand, required a complex monoprotic-dialkaline model to fit its experimental data (SI 9, Eqn. S168, Fig. 5D). The optimized \( pK_{as}, pK_{sa} \) and \( log_{10}D \) values were consistent with the previous studies and are summarised in Table 1 [10].

The quantitative relationship between the salt concentration and the distribution parameters such as \( P_{HA}, P_k, pK_n \) were derived for monoprotic acid (SI 7) and the simulation was carried out to assess the effect of salt on \( log_{10}D \) profile (Fig. 3B, C). The results show that the salt affects the \( P_k \) value in a linear manner (directly proportional), whereas, the, \( P_{HA} \) and \( pK_n \) remained unaffected [5]. Similar to salt, the effect of co-solvent (e.g. DMSO) on the \( log_{10}D \) profile of a monoprotic acid was assessed through another set of simulations (SI 8). The \( log_{10}D \) profile was primarily influenced by the stoichiometry (\( n_k, n_s, n_k, n_s \)) and the binding affinities (\( K_{k1}, K_{k2}, K_{k3}, K_{k4} \)) of the co-solvent towards the four species \([HA_n], [HA_n], [A_n^+] \) and \([A_n^-] \), present in the system. Keeping the binding affinities to be constant at 1:1:1:1, we studied the effect of different stoichiometry on \( log_{10}D \) profiles. In the first case, we assumed only \([HA_n] \) to interact with the co-solvent (\( S \)) with a stoichiometric ratio of \([HA_n]:[S] \) to be 1:1 or higher (2:1 or 3:1). This could be realized by setting the overall stoichiometric ratio of \( n_k \); \( n_s \); \( n_k \); \( n_s \) to be 1:0:0:0, respectively. In this case, the \( P_k \) remained constant (Fig. 4B, C), whereas, \( P_{HA} \) was decreasing non-linearly and \( pK_n \) was increasing non-linearly with the addition of co-solvent. On the other-hand, if we assume, \( n_k, n_s, n_k, n_s \) to be 1,1,1,1, all the parameters varied non-linearly, except, \( pK_k \) which remained constant (Fig. 4D, E) [3,5]. Finally, if we assume, a higher stoichiometric ratios, such as 2,1,1,1, or 3,1,1,1, all the parameters varied non-linearly with increase in co-solvent concentration (\( S_n \)). Thus, we observe that the effect of co-solvent on \( log_{10}D \) profile is non-linear, and is significantly dependent on the stoichiometry ratios that determine the degree of co-solvation of different monoprotic species.

4. Discussion

Among all the molecules considered in this study, only telmisartan required a complex di-alkaline-monoprotic model to explain its \( log_{10}D \) profile. The kinetic mechanism of telmisartan consisted of sixteen species with eight species in aqueous phase and another eight species in the octanol phase. Ignoring the eight species in the octanol phase, if we were to propose a kinetic mechanism for the rest of the eight species in the aqueous phase, the graph theory predicts a total of \( \sim 2.7 \times 10^8 \) possibilities (SI. 2) [11]. Based on the fact that the equilibrium constants are equivalent for ‘analogue kinetic mechanisms’, we choose a minimal of seven (i.e. \( N = 7 \)) equilibriums out of the maximum 28 equilibriums (\( N(N - 1)/2 \)) to explain an eight species system (\( N = 8 \)). With the inclusion of the law of conservation of mass for telmisartan (in both aqueous and octanol phase) and considering the partition equilibriums for the eight species (between aqueous and octanol layer), we arrive at 16 algebraic equations to solve for the equilibrium concentrations of 16 species. In contrast to algebraic method, in dynamic approach, the rate equations for sixteen species depends significantly on the connectivity seen among species as proposed in the kinetic mechanism. Though the concentrations of all the 16 species will vary significantly during the pre-steady state phase, the concentrations of all the species will remains invariably the same for ‘analogue kinetic mechanisms’ at equilibrium or steady state phase. The model-fit for benzazepiril and telmisartan (Fig. 5C & D) was carried out using algebraic

Table 1

| Naldixic acid | Mebendazole | Benzazepiril | Telmisartan |
|--------------|-------------|--------------|-------------|
| **Model**    | monoprotic  | monoprotic   | monoprotic-diamino amphoteric |
| **Parameters** | monoalkaline | simple amphoteric |                                  |
| **Partition coefficients (P)** | 1.71 ± 0.17 (P_{HA}) | -1.51 ± 0.26 (P_{pA}) | 0.46 ± 0.11 (P_{HAS}, P_{HAS}B, P_{AB}B) |
| \( pK_{as}/pK_k \) | 6.35 ± 0.13 (pK_{as}) | 10.25 ± 0.17 (pK_{As}) | 4.16 ± 0.27 (P_{HAS}, P_{HAS}B, P_{AB}B) |
| \( pK_{sa}/pK_k \) | 6.35 ± 0.13 (pK_{sa}) | 10.25 ± 0.17 (pK_{As}) | 4.16 ± 0.27 (P_{HAS}, P_{HAS}B, P_{AB}B) |
| **Fit parameters for naldixic acid, mebendazole, benzazepiril and telmisartan based on monoprotic, monoalkaline, simple amphoteric and diamino-monoprotic acid models, respectively. The parameters such as \( pK_k, pK_{sa} \) are equivalent to the conventional \( pK_n \) which is related to the dissociation of the \( H^+ \) ion from the acid moieties. The \( pK_{as}, pK_{sa} \) are not the conventional \( pK_n \) that is concerned with the dissociation of \( OH^- \) ion from the base moieties.** |
method (Eq. 18 & Eqn. S168) and the resultant optimized parameters were used to simulate \( \log_{10} D \) profiles through dynamic approach (Eqs. 19–26; Eqsns. S169–S184). The \( \log_{10} D \) values obtained through algebraic and dynamic approaches were comparable and a degree of two decimal points for most of the data points (Table 2).

The effect of salt on the \( \log_{10} D \) profile of monoprotic acid as assessed through simulation, suggests an apparent increase in \( P_2 \) value with increase in salt concentration (Ks) (SI 7, Fig. 3B & C). To explain this effect quantitatively, we compare Eqn. S186 with Eqn. S185 (i.e. the simple monoprotic model), and obtain the expression for apparent \( P_2 \) as

\[
P_2' = P_2 + \left( \frac{K_{PH} \cdot k_{a1} \cdot k_{a2}}{K_D} \right) K_s.
\]

The equation clearly shows that, \( P_2' \) and \( K_{PH} \) are linearly related to each other, with the slope and intercept, \( \left( P_2, \frac{K_{PH} \cdot k_{a1} \cdot k_{a2}}{K_D} \right) \) and \( P_2 \), respectively (SI10). When a salt (KCl) is added to a monoprotic acid, it tends to increase the formation of ion pair, \( K^+ : A^-_n \), in the aqueous phase. The neutral ion pair \( K^+ : A^-_n \) easily partitions into octanol in the form of \( [K^+A^-_n]^+ \), thereby, increasing the total concentration of \( A^-_n \) in the organic layer. Since, \( P_2' \) is defined as \([A^-_n]_{total} + 1/[A^-_n]_{total} \), where, \([A^-_n]_{total} = [A^-_n] + [K^+A^-_n] \), an increase in \([K^+A^-_n] \) due to addition of salt will proportionally increase the value of \( P_2' \), too [3,5].

The effect of co-solvent on a monoprotic acid is more complex compared to the effect of salt (SI 8, Fig. 4C, D). We compare Eqn. S191 with Eqn. S185 to obtain the relationship between the apparent \( P_{HA} \), \( P_{AH} \), and \( k_{PH} \) and the co-so solvent concentration (S_0 and S_1) as follows:

\[
P_{HA}' = P_{HA} \left( \frac{1 + k_{SHA}S_0}{1 + K_{S1}S_1} \right), \quad P_{AH}' = P_{AH} \left( \frac{1 + k_{SKA}S_0}{1 + K_{S2}S_1} \right) \quad \text{and} \quad K_{PH}' = K_{PH} \left( \frac{1 + k_{S3}S_0}{1 + K_{S4}S_1} \right)
\]

(SI10) [3]. In the above expressions, \( S_0 \) and \( S_1 \) are the concentrations of the co-solvent in the octanol and the aqueous phase, respectively. The degree of non-linearity in the above expressions are introduced by the stoichiometric ratios, \( n_1, n_2, n_3 \), and \( n_4 \). Previous studies have shown that the addition of co-solvent like DMSO will increase the \( k_{PH} \), of the monoprotic acid in the aqueous solutions (distribution into octanol was not considered) [19]. This can be easily realised by assuming, \( K_{S1}, K_{S2}, n_1, n_2 \) to be 1,0,1,0, respectively, and taking logarithm on both sides in the expression for \( K_{PH}' \), which yields, \( k_{PH}' = k_{PH} + \log_{10}(1+S_0) \). This expression clearly shows that, when the concentration of co-solvent is less (\( S_0 \leq 1 \)), the apparent \( k_{PH}' \) or \( k_{PH} \) for a monoprotic acid is constant and is equal to \( k_{PH} \). On the other hand, if the co-solvent concentration is high (\( S_0 > 1 \)), then the apparent \( k_{PH}' \) becomes linearly proportional to logrithm of co-solvent concentration in aqueous phase (\( \log_{10}(S_0) \)).

At high concentrations, the concentration of the analytes can differ significantly from its thermodynamic ‘activity’ (\( \gamma \)), under such circumstances, it is necessary to make appropriate corrections in the equilibrium constants (dissociation or partition) [20–22]. Consider a simple monoprotic case, where, expression for distribution coefficient can be written as \( D = \frac{P_{HA}([H^+_2] + P_{KH})}{|H^+_2| + K_s} \). As the concentrations of the species \([H^+_2], [H^+_4], [H^+_2A^+] \) or \([A^-_n] \) tend to increase significantly, then, we have to redefine, distribution coefficient as \( D = \frac{P_{HA}([H^+_2] + P_{KH})}{|H^+_2| + K_s} \), by replacing the concentrations of the species with its corresponding activities. For example, the activity of \([H^+_2] \) can be defined as \( [H^+_2]^* = y_{H^+_2}[H^+_2] = [H^+_2]^* \), where, \( y_{H^+_2} \) is called the ‘activity coefficient’. Further, activity coefficient itself is a variable that is dependent on ‘ionic strength’ (\( I \)), which, in turn is a function of the concentration (molarity or molality) of the species and its charge. Several theories (Debye-Huckel, Pieter, etc.) are available to calculate the activity coefficient of a given analyte at a given molarity or molality value [22,23]. Experimentally, \( \log_{10} D \) can be measured through direct methods such as electrochemistry at the interface of immiscible liquids (ITIES) [24–27], or indirect methods such as potentiometric, chromatography (HPLC, HPTLC, LCMS) [5]. The direct method has the advantage of innately taking into account the correction factors for the temperature and ionic concentrations, and also yields instantaneous time profile data suitable for dynamic analysis.

The \( \log_{10} D \) data analysis of multiple species system, becomes increasingly complex because of the inclusion of large number of parameters in the model. For such complex models it is often recommended to carry out ‘sensitivity analysis’ to identify the parameters of significance in order to reduce the complexity of the models [28–30]. A simple sensitivity analysis, could be based on Jacobian matrix obtained at global minimum of the model fit, which can be normalized to assess the significance of each parameter present in the model. On the other hand sophisticated, Monte-Carlo based approaches are available which additionally provide insights on the degree of interaction present among parameters in the model [30].

### Supporting Information

Supporting Information contains explicit derivation of \( \log_{10} D \) for mono-protic, di-protic acid, mono-alkaline, mono-protic acid with salt, mono-protic acid with solvent and monoprotic-dialkaline molecule. The expressions for apparent \( P_2 \), \( P_{HA} \), and \( K' \) for monoprotic acid in the presence of salt and co-solvent are provided. Matlab codes to derive algebraic model (mono-proptic, diprotic, monoalkaline, diamino monoprotic) and simulation of dynamic approach are provided (monoprotic

Table 2

| Benazepril | Calculated (log_{10}D) | Telmisartan | Calculated (log_{10}D) |
|-----------|---------------------|-------------|----------------------|
| pH        | Experimental (log_{10}D) | Algebraic | Dynamic |
| 1.95      | 0.38                | 0.39        | 0.39         |
| 2.42      | 0.74                | 0.74        | 0.74         |
| 3.00      | 1.06                | 1.06        | 1.07         |
| 3.75      | 1.29                | 1.24        | 1.25         |
| 4.19      | 1.28                | 1.26        | 1.27         |
| 4.75      | 1.25                | 1.23        | 1.23         |
| 5.30      | 0.99                | 1.09        | 1.09         |
| 6.04      | 0.60                | 0.68        | 0.67         |
| 6.65      | 0.34                | 0.22        | 0.21         |
| 7.22      | 0.12                | -0.15       | -0.15        |
| 7.65      | 0.36                | -0.32       | -0.32        |

| pH        | Experimental (log_{10}D) | Algebraic | Dynamic |
| 1.95      | 0.24                | 0.25        | 0.25         |
| 2.42      | 1.07                | 1.04        | 1.02         |
| 3.00      | 1.99                | 2.06        | 2.03         |
| 3.75      | 3.32                | 3.19        | 3.16         |
| 4.19      | 3.65                | 3.63        | 3.62         |
| 4.75      | 3.63                | 3.91        | 3.96         |
| 5.30      | 3.96                | 3.77        | 3.79         |
| 6.04      | 3.84                | 3.60        | 3.61         |
| 6.65      | 2.85                | 2.90        | 2.90         |
| 7.22      | 2.08                | 2.27        | 2.28         |
| 7.60      | 1.91                | 1.96        | 2.00         |
| 8.00      | 1.53                | 1.56        | 1.57         |
| 8.90      | 1.57                | 1.47        | 1.48         |
| 9.72      | 1.57                | 1.46        | 1.46         |
| 10.52     | 1.38                | 1.46        | 1.46         |
| 10.91     | 1.52                | 1.46        | 1.44         |
with co-solvent).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbrep.2018.07.006.

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