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Log D analysis using dynamic approach

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ABSTRACT

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 nalidixic 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_a) and ionized (A_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, $P_{HA} = [HA_o]/[HA_a]$ and $P_{A^-} = [A_o^-]/[A_a^-]$, 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, $D = ([HA_o]+[A_o^-])/([HA_a]+[A_a^-])$, 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), pK_a or pK_b 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 nalidixic 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

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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 are 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 (SI.3), diprotic acid (SI.4), monoalkaline (SI.5) 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 ($\boldsymbol{N = 4}$), $\boldsymbol{A}$, $\boldsymbol{B}$, $\boldsymbol{C}$, $\boldsymbol{D}$; 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 $\boldsymbol{A}$, $\boldsymbol{B}$, $\boldsymbol{C}$, and $\boldsymbol{D}$, prior to steady-state or equilibrium, they are indistinguishable 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 contants 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 equilibrium reactions as bidirectional 'edges', then the graph theory suggest a maximum of $\boldsymbol{E_{max} = N(N-1)/2}$, edges or equilibrium reactions [11–13]. For a system with $\boldsymbol{N = 4}$, species, there exist a maximum of $\boldsymbol{E_{max} = 6}$, equilibriums. On the other-hand, a minimum of $\boldsymbol{E_{min} = (N-1) = 3}$, edges or equilibriums 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 equilibrums, 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 mechanisms' to show their equivalence. Consider a simple linear mechanism (Fig. 1A) which minimally connects all the four species as shown below (Eq. 1),

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} D \tag{1}$$

In the above equilibrium, $\boldsymbol{k_1}$, $\boldsymbol{k_2}$, $\boldsymbol{k_3}$, are the forward and $\boldsymbol{k_{-1}}$, $\boldsymbol{k_{-2}}$, $\boldsymbol{k_{-3}}$, are the reverse rate constants for the reactions $\boldsymbol{A \rightleftharpoons B}$, $\boldsymbol{B \rightleftharpoons C}$, $\boldsymbol{C \rightleftharpoons D}$, respectively. The three equilibrium constants $\boldsymbol{K_1}$, $\boldsymbol{K_2}$, $\boldsymbol{K_3}$ are defined as $\boldsymbol{K_1 = \frac{k_1}{k_{-1}}}$, $\boldsymbol{K_2 = \frac{k_2}{k_{-2}}}$, $\boldsymbol{K_3 = \frac{k_3}{k_{-3}}}$, 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,

$$A \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} C \tag{2}$$

$$B \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} D \tag{3}$$

$$A \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} D \tag{4}$$

In the above equations (Eqs. 2–4), k_4, k_5, k_6, are the forward and k_{-4}, k_{-5}, k_{-6}, are the reverse rate constants for the reactions $A \rightleftharpoons C$, $B \rightleftharpoons D$, $A \rightleftharpoons D$, respectively, and the corresponding equilibrum constants are defined as $K_4 = \frac{k_4}{k_{-4}}$, $K_5 = \frac{k_5}{k_{-5}}$, $K_6 = \frac{k_6}{k_{-6}}$. 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),

$$A \overset{K_1}{\rightleftharpoons} B \overset{K_2}{\rightleftharpoons} C \equiv A \overset{K_4}{\rightleftharpoons} C \tag{5}$$

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 $K_4 = K_1 \times K_2$. Similarly, based on the comparisons of (Eq. 6) and (Eq. 7), we can write $K_5 = K_2 \times K_3$ and $K_6 = K_1 \times K_2 \times K_3$, repsectively.

$$B \overset{K_2}{\rightleftharpoons} C \overset{K_3}{\rightleftharpoons} D \equiv B \overset{K_5}{\rightleftharpoons} D \tag{6}$$

$$A \overset{K_1}{\rightleftharpoons} B \overset{K_2}{\rightleftharpoons} C \overset{K_3}{\rightleftharpoons} D \equiv A \overset{K_6}{\rightleftharpoons} D \tag{7}$$

Thus, we can conclude that if we have a kinetic mechanism with $\boldsymbol{N}$ species, we would require a minimal of $(\boldsymbol{N-1})$ equilibriums that uniquely connects these $\boldsymbol{N}$ species, so as to determine the additional equilibrium constants existing in other 'analogous mechanisms'. A comparitive 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 assumption 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. Amphotheric 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 organic hydrophobic solvent (octanol) (Fig. 2A) [5,6,14]. In the aqueous phase, the amino acid, $[NH_2 - R - COOH]$or $[HAB]$, exists in an unionized form $[NH_2{-}R - COOH_a]$ or $[HAB_a]$, and the ionized forms, $[NH_2{-}R{-}COO^-{}_a]$ or $[^-AB_a]$, $[NH_3^+{-}R - COOH_a]$ or $[HABH_a^+]$, $[NH_3^+{-}R{-}COO^-{}_a]$ or $[^-ABH_a^+]$. The equilibrium among these four states can be written as (Eqs. 8–12),

$$HABH_a^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} H_a^+ + HAB_a \tag{8}$$

$$HABH_a^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} H_a^+ + {}^-ABH_a^+ \tag{9}$$

$$HAB_a \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} {}^-ABH_a^+ \tag{10}$$

$$HAB_a \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} H_a^+ + {}^-AB_a \tag{11}$$

$${}^-ABH_a^+ \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} H_a^+ + {}^-AB_a \tag{12}$$

k_1, k_2, k_4, k_5, are the forward kinetic rates and k_{-1}, k_{-2}, k_{-4}, k_{-5}, are the reverse kinetic rates for the dissociation of proton from the species,