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Research Paper

Partition coefficient *vs.* binding constant: How best to assess molecular lipophilicity

Gregor Cevc *

The Advanced Treatments Institute, Tassilostr. 3, D-82131 Gauting, Germany

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ABSTRACT

Partition coefficient, *P*, is the preferred descriptor of molecular lipo- or hydrophilicity, and thus of relationships between a solute (*S*, e.g., a drug), a polar medium (*W*, e.g., an aqueous buffer), and an essentially apolar, organic, medium or a drug carrier (*O*). The coefficient is commonly identified with the *linear* ratio of solute quantities in the two media, $P = n_{SO}/n_{SW}$, even to characterise solute association with or binding to a surface (e.g., of a HPLC column or a drug carrier). To check the latter practice correctness—and credibility of the prevailing *P* definition—this paper compares an ideal solute distribution between two separate homogeneous fluid media (i.e., partitioning) to solute association with a uniform surface immersed in one such medium (i.e., binding). This reveals that solute partitioning and binding fundamentally differ and can only exceptionally be described, or analysed, with similar equations. Nonlinearised formulae that describe partitioning (Eq. (9)) and binding (Eq. (11)) can yield similar lipophilicity descriptor values only if solute preparation is relatively dilute; employing a large organic medium fraction is helpful in this respect. Additional prerequisites for partitioning and binding models match are: 1:1 stoichiometry at the association maximum *and* identical interfacial area of solute and organic medium molecules. If these requirements are not met, binding model yields different, potentially somewhat higher, but more often up to > 10 times lower results than partitioning model. The main reason is saturation of organic medium with solute molecules. Partitioning model excludes this phenomenon, which cannot always be prevented by focussing on dilute solute preparations. The current practice of using a linear model and approximate equations to study partitioning or binding can cause large errors ($> 10^3\times$), and is one possible reason for the notoriously high experimental $\log P$ values scattering. The latter makes $\log P$ predictions more difficult and less reliable than they could be if the measured data were evaluated with non-linearised partitioning or binding equations, as appropriate.

1. Introduction

Partition coefficient is a 'workhorse concept' in chemical industry [1,2] in general and in pharmaceutical R&D in particular [3]. Even the most succinct molecular descriptions include octanol–water partition coefficient, $P_{O/W}$, as a key characteristic (see e.g. http://pubchem.ncbi.nlm.nih.gov/). Rules of thumb used to gauge a compound drug-likeness also rely on octanol–water partition coefficient as relative lipophilicity indicator. Partition coefficient, *P* (or its log *P*), value is moreover regularly considered in molecular distribution studies, ADME analysis [3], drug carrier development [4], etc. Drug-carrier association studies thus typically invoke partition coefficient, especially when dealing with amphipatic or predominantly lipophilic drugs, or else with a carrier solubiliser [5–9].

According to Wikipedia, in the physical sciences, a partition coefficient is the ratio of concentrations of a compound in a mixture of two immiscible phases in equilibrium. In the chemical and pharmaceutical sciences, the two phases are typically restricted to mean two immiscible solvents. Partition coefficient is then identified with the ratio of compound concentrations in the two compartments formed by the solvents at equilibrium. Normally, one of the solvents is aqueous (herein index *W*) and the other is non-aqueous and lipophilic, e.g., 1-octanol (herein index *O*) [10]. The U.S. Environmental Protection Agency briefly defines octanol–water partition coefficient as "a coefficient representing the ratio of the solubility of a compound in octanol (a non-polar solvent) to its solubility in water (a polar solvent)" [11].

Researchers therefore devised a plethora of methods relying on octanol–water partition coefficient as a reference, to gauge relative molecular lipophilicity [2,12]. OECD Guidelines for the Testing of Chemicals presently recommend, as a means for measuring

* Tel.: +49 89 89 355 771.
E-mail address: cevc@advanced-treatments.org

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Nomenclature

a_S	solute activity
a_K	dimensionless, system specific, K_{SO} *vs.* $P'_{O/W}$ proportionality constant
a_X	dimensionless, system specific, X_{SO} *vs.* α_{SO} proportionality constant
b_K	dimensionless, system specific, constant in K_{SO} *vs.* $P'_{O/W}$ proportionality
b_X	dimensionless, system specific, constant in X_{SO} *vs.* α_{SO} proportionality
$c_i = n_{iW}/n_W$	relative aqueous concentration of the *i*-th component
$K_a = K_{SH}$	acidity constant, dimensionless
K_{SO}	solute–organic medium binding constant, dimensionless
K'_{SO}	solute–organic medium binding constant in conjunction with molar ratio
n_O	total organic medium concentration, in mol
n'_O	organic medium amount, in mol number (fraction)
$n_S = n_{SW} + n_{SO}$	total solute concentration, in mol
$n'_S = n'_{SW} + n'_{SO}$	solute amount, in mol number (fraction)
n_{SO}	solute concentration associated with organic medium, in mol
n'_{SO}	mol fraction of solute partitioned (dissolved) in organic medium
n_{SW}	solute concentration in water, in mol
n'_{SW}	mol fraction of solute dissolved in water
n_W	water concentration, in mol
n'_W	water amount, in mol number (fraction)
N_{SO}	bound solute/organic medium molecules ratio at saturation
$P_{O/W} = P'_{O/W} n_O$	solute partitioning coefficient organic medium/water (octanol/water)
$P'_{O/W}$	solute partitioning coefficient in organic medium
R_A	ratio of solute and organic molecules interfacial area
$X_{SO} = n_{SO}/n_S$	molar fraction of solute partitioned into organic medium
$X_{SO,b} = N_{SO} R_A \times \alpha_{SO} n_O/n_S$	molar fraction of solute bound to organic medium
$\alpha_{SO} = n_{SO}/n_O$	molar fraction of solute-occupied binding sites on organic medium; surface occupancy

partition coefficient: (*i*) shake flask method for solutes with $0 \leqslant \log P \leqslant 4$ [13] and (*ii*) HPLC method for solutes with $0 \leqslant \log P \leqslant 6(10)$ (testing solute binding to a reverse phase column followed by the results calibration) [14]. Further popular methods for P determination are: (*iii*) assessment of molecular binding to lipid vesicles (liposomes), micelles [15–18], nanoparticles, etc. [3]; (*iv*) acid-base titration [12]; and (*v*) electrochemical methods [19]. The models used to interpret such measurement results normally do not differentiate between partitioning and binding. Indeed, the OECD guidelines imply the use of similar mathematical formulae for analysing results of both most commendable P determination methods [13,14]. We have recently shown, however, that one must modify the common definition of partition coefficient to describe solute association with an organic–water interface. We moreover discovered that one cannot properly interpret liposome concentration effects on drug association with lipid vesicles without employing a suitable binding isotherm [16]. At least the methods (*ii*)–(*v*) hence can only afford drug binding constant, K, rather than partition coefficient, P.[1]

The question thus arises: 'May one use drug partition coefficient and drug binding constants interchangeably or at least treat them as being correlated?' Like other researchers in the field, I have accepted an affirmative answer—until the formal scrutiny described herein dictated that the right answer is: 'No'. The same analysis uncovered several likely reasons for the frustratingly large variability of partition coefficient data—but also a possible solution to the problem, which could moreover improve $\log P$ predictions accuracy. I therefore outline herein the, rarely fulfilled, conditions for validity of the postulated relationship $P = K$ (or $P \propto K$). I also identify the main limiting cases and specify the conditions under which the commonly used P formulae are valid. I moreover shed light on the preferable choice of either P or K for future molecular characterisation studies, their results description, and utility in drug carriers formulations. The conclusions should alert and enable scientists better to understand and interpret experimental findings on solute distribution between different media.

[1] The original paper uses the term partition coefficient for what is actually the constant characterising drug–liposome association, i.e., drug binding to lipid vesicles.

2. Solute distribution between two immiscible ideal solvents

2.1. Solute partitioning

The most common descriptor of a uniform distribution of a solute (index S) between an aqueous solvent (e.g., a buffer) and an organic solvent (e.g., octanol or some other lipophilic fluid) is partition coefficient. The prevailing definition of this descriptor postulates a linear relationship between the solute amounts in the equilibrated solvents:

$$P'_{O/W} \stackrel{\text{def}}{=} \frac{n'_{SO}}{n'_{SW}}.$$

Such popular definition, on the one hand, implies that solute concentration is negligibly low compared with both solvents concentrations. On the other hand, the definition excludes solute depletion from the bulk. This is evident from the broader definition of partition coefficient, which also quantifies effects on $P'_{O/W}$ of organic and aqueous solvent molar numbers, n'_O and n'_W, and of molar numbers of the solute partitioned into either of the solvents, n'_{SO} and n'_{SW}:

$$P'_{O/W} = \frac{n'_{SO}(n'_{SW} + n'_W)}{n'_{SW}(n'_{SO} + n'_O)}. \tag{1}$$

Mass conservation simultaneously dictates that the sum of solute molar numbers in the solvents must equal the total solute concentration.

The approximate, linear, relationship between partition coefficient,

$P'_{O/W} \approx X_{SO}/[(1 - X_{SO})n_O + X_{SO} n_S]$, molar fractions, $X_{..} = n'_{..}/n'_S$, and relative concentrations, $n_{..} = n'_{..}/n'_W$, may be used under the conditions listed in Appendix A. Being accurate to the first order of the solute molar fraction in an organic solvent, this approximation holds true merely for relatively dilute solute preparations. In the high dilution limit ($n'_{SO} \ll n'_O$ and $n'_S \ll n'_W$), the relationship simplifies to

$$P'_{O/W} \approx \frac{X_{SO}}{1 - X_{SO}} \frac{1}{n_O}. \tag{2}$$

Even for a dilute solute preparation, the common definition of partition coefficient, $P'_{O/W} = n'_{SO}/n'_{SW}$, is thus valid only if aqueous and organic medium are similarly abundant, $n_W = n_O$.