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Contributed Paper

A Simple Method Based on One Phase Measurement for Determination of the Octanol-Water Partition Coefficient of Drugs

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ABSTRACT

The octanol-water partition coefficient (P_{ow}) is widely used to assessment of lipophilicity of drug. The P_{ow} can be measured by determining the ratio of the concentration of the drug in octanol to its concentration in water at equilibrium. In the standard method, two calibration curves are required in order to obtain the concentration of drug in the two phases. The procedure is thus tedious and time consuming. In this work, a simple approach for determining the P_{ow} of drug is described. Only the absorbance of one phase and volumes of octanol and water are used in the calculation; calibration curves are no longer required, making the procedure much more convenient. The method was applied to examples of hydrophobic and hydrophilic drugs. The P_{ow} values obtained by our method agreed well with literature values (R^2 = 0.998). This method is simple and capable for screening new drugs in a stage of drug discovery.

Keywords: partition coefficient, lipophilicity, drug screening

1. INTRODUCTION

Lipophilicity is an important physicochemical parameter in drug discovery and development. It shows the capability of a compound to diffuse across cell membrane. A compound, which has optimal lipophilicity, is considered to be a promising drug candidate [1]. Lipophilicity is generally measured in terms of the octanol-water partition coefficient (P_{ow}), which is defined as the concentration ratio of a compound partitioned between octanol (C_o) and water (C_w) phase at equilibrium: $P_{ow} = \frac{C_o}{C_w}$. Since the value of P_{ow} can vary in orders of magnitude, it is commonly expressed in the logarithmic form,

log (P_{ow}). The octanol-water system is widely used as a model in lipophilicity study [2], with water representing the liquid medium of the outer cell membrane. A buffer solution at pH 7.4 is often used in order to simulate the pH of human plasma. Octanol has a structure similar to the phospholipid layer of cell membrane, which has a hydrophilic head and a hydrophobic tail. Besides octanol, other solvents have been used, such as cyclohexane [3], poly (vinyl chloride) [4] and liposome [5].

The shake-flask procedure is a standard method recommended by the Organization for Economic Cooperation and Development (OECD) [6]. In this method, a compound is added into a separatory funnel containing a mixture of octanol and water. The funnel is shaken until the system reaches equilibrium. After the two phases has separated the concentrations of the compound in the octanol and water layers are measured. The advantage of this method is the direct measurement of P_{ow} with good precision and use of simple equipment. The P_{ow} value obtained by the shake-flask method provides effective prediction of a molecule passive transport across cell membrane, especially of the intestine system [7]. However, calculation of the P_{ow} requires use of calibration curves of the compound in the octanol and water phases, respectively. The construction of the two calibration curves for each compound is time-consuming and limits its use when there are very many drugs to be screened.

Indirect measurements using high performance liquid chromatography (HPLC), have been employed for the determination of P_{ow} value [8-10]. In this method, a series of reference compounds with molecular structures similar to the target compound is employed for constructing a graph of retention factors against P_{ow} values. The P_{ow} of the target compound can thus be determined from its retention factor from this graph. However the HPLC method may not be applicable if a sufficient number of reference compounds together with their P_{ow} values are not available. Moreover HPLC requires expensive equipment and expertise in chromatography.

There are other approaches for measuring P_{ow}; for example, potentiometric titration method [5], flow-based method [7,11-13], water-plug aspiration/injection method [14], use of 96-well microplate [4], and use of magnetic nano-absorbent [15]. There are also various computer programs for calculating P_{ow} [2,16]. Comparisons of the different methods have been reviewed [1,17].

In this work, a simple calculation based Beer's law equation [18] for evaluating P_{ow} is described. The method is based on the shake-flask procedure but with measurements of absorbance of only one of the liquid phase and the volumes of the octanol and water layers. This method is simpler than the standard method in which measurement of both phases must be employed.

2. MATERIALS AND METHODS

2.1 Solvent Preparation

1-octanol (96% v/v, Fluka, Switzerland) and 10 mM phosphate buffer saline (PBS) with pH at 7.4 were used as the solvents. PBS was prepared [19] by dissolving 1.74 g of Na_2HPO_4(anhydrous) (Fluka, Switzerland), 0.93 g of NaH_2PO_4(anhydrous) (Fluka, Switzerland) and 8.5 g of NaCl (Merck, Germany) in 1.0 L of deionized water. The pH was adjusted to 7.4 with HCl or NaOH. The octanol and PBS were saturated with each other by mixing and shaking at 120 rpm (IKA Labortechnik model HS250, Germany) for 24 hours. The immiscible layers were allowed to separate and used as the

solvents in the experiments.

2.2 Drug Sample Preparation

Acetaminophen (99.0%), aniline (99.5%) and riboflavin (98.0%) were purchased from Sigma-Aldrich, USA. Caffeine (99.0%) was purchased from Fluka, Switzerland. Salicylic acid (99.0%) was obtained from Carlo-Erba, Italy.

A drug sample was prepared by dissolving a suitable amount of the drug in 50 ml of water saturated octanol. This stock drug solution should have an absorbance ($A_{initial}$) in the range of 0.5-1.0 at the selected wavelength (usually the maximum absorption wavelength, λ_{max}). The drugs selected in this study have both positive and negative values of log P_{ow}, in order to show the capability of this procedure for measurement of P_{ow} for both hydrophobic and hydrophilic drugs.

2.3 Experimental Procedure

All experiments were carried out at controlled room temperature, 25 ± 1 °C. To determine the equilibration time required for partitioning, a set of 50 ml screw-topped test tubes containing 5 ml of the stock drug sample and 5 ml of PBS was prepared. The tubes were tightly capped and shaken at 120 rpm for various shaking times. At selected intervals, the absorbance of the drug in the octanol layer was measured on a spectrophotometer (Agilent Technologies model 8453, USA). The absorbance of drug in the octanol layer was monitored as a function of shaking time until the absorbance value was constant. This equilibration time (~ 10 min) was used in further experiments.

In the procedure for determining P_{ow}, 5 ml of PBS (V_w) was transferred into a series of 50 ml test tubes. Samples were prepared by adding various volumes (V_{drug}) of the stock drug to the PBS but keeping the total volume of the octanol layer (V_o) constant at 5 ml by addition of 1-octanol (Table 1). The tubes were tightly capped and shaken for 10 min. The two immiscible layers were allowed to separate and the top octanol layer was pipetted out and measured the absorbance (A_{final}).

In this work, the final volume of each octanol and aqueous layers was fixed at 5 ml, respectively. However, for strongly hydrophobic drug, which is not very soluble in the aqueous phase, the volume of the buffer may be increased in order to obtain a measurable change in absorbance of the drug in the octanol layer.

3. RESULTS AND DISCUSSION

3.1 Calculation of P_{ow}

The absorbance of the drug in octanol is first measured, denoted as $A_{initial}$. Using Beer's law ($A = \varepsilon bc$), the number of moles of the drug in a volume (V_{drug}) of octanol is given by $\frac{A_{initial}V_{drug}}{\varepsilon b}$, when ε is molar absorptivity and b is path length of the solution (1 cm). This volume (V_{drug}) of stock drug solution is added into a mixture of octanol and aqueous buffer. The mixture is shaken until the system reaches equilibrium and the final absorbance of drug in the octanol phase is measured and denoted as A_{final}. The concentration (C_o) of the drug in the octanol phase is given by $\frac{A_{final}}{\varepsilon b}$, with $\frac{A_{final}V_o}{\varepsilon b}$ as number of moles, where V_o is a final volume of the octanol phase. Thus, the number of moles of the drug partitioned into the aqueous phase can be calculated from $\frac{A_{initial}V_{drug} - A_{final}V_o}{\varepsilon b}$. The concentration ($C_w$) of the drug in the aqueous phase is $\frac{A_{initial}V_{drug} - A_{final}V_o}{\varepsilon b V_w}$, where V_w is a volume of aqueous buffer. Substituting for concentration of drugs in the two phases:

$$P_{ow} = \frac{C_o}{C_w}$$

$$P_{ow} = \frac{A_{final}V_w}{A_{initial}V_{drug} - A_{final}V_o} \quad (1)$$

Equation (1) can be rearranged to give a linear equation in V_{drug},

$$\frac{A_{final}}{A_{initial}} = \left[\frac{P_{ow}}{V_w + V_o P_{ow}}\right] V_{drug} \quad (2)$$

Substituting V_o and V_w as 5 ml, the equation is

$$\frac{A_{final}}{A_{initial}} = \left[\frac{P_{ow}}{5(1+P_{ow})}\right] V_{drug} \quad (3)$$

By fitting a straight line with zero intercept to the plot of $\frac{A_{final}}{A_{initial}}$ *vs* V_{drug}, the P_{ow} value can be calculated from the slope.

A similar procedure was first proposed by Edward *et al* [20]. However the equation for calculating P_{ow} was not explicitly given in the paper. Also the method employed measurements of the absorbance of the drug in the octanol phase, before and after partitioning with a single volume of the water phase. The procedure was only applied for determining P_{ow} of hydrazone analogs, which have similar positive log P_{ow} values.

Employing equation (3) for calculating P_{ow}, has some advantages. Firstly, the method does not require any calibration curve. Secondly, the stock drug solution can be prepared without the need to calculate the concentration. This makes the sample preparation easier. Lastly, the method requires only simple equipment.

3.2 Equilibration Time for Partitioning

When a compound distributes from octanol to the aqueous layer, the absorbance in the octanol layer decreases with time. Figure 1(a) shows the decrease in absorbance of acetaminophen (paracetamol) in the octanol layer after shaking for various times. The absorbance was monitored at 250 nm, the maximum absorption wavelength of acetaminophen. It was found that after 10 min, the absorbance was constant ($|\Delta A| <$ 0.01 abs. unit). Thus ten minutes was a suitable equilibration time for complete partitioning of acetaminophen. For other drug samples, similar equilibration times were found (data not shown). Thus ten minutes was selected as the time needed for attaining equilibrium state.

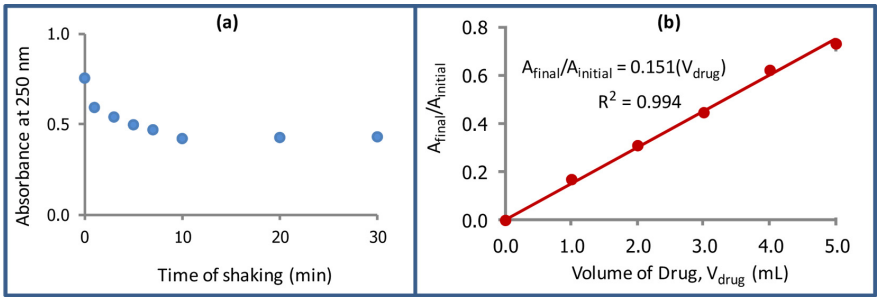

Figure 1. (a) The absorbance of acetaminophen in octanol layer as a function of shaking time (b) A plot of $A_{final}/A_{initial}$ *vs* V_{drug} for acetaminophen. The slope is given by $P_{ow}/5(1+P_{ow})$. By substituting the value of 0.151, the P_{ow} is calculated to be.

3.3 Speed of Shaking

The shaking speed was studied since this factor strongly influenced the time to reach equilibrium. Without shaking, equilibration time for partitioning may take several hours, since partitioning takes place only at the interface. However too vigorous mixing can cause emulsion formation, resulting in a long time required for complete phase separation. Emulsion can lead to serious error in measurement [17]. In this work, the speed of shaking was investigated over the range of 60 to 210 rpm. A speed of 120 rpm was found to be the optimal value. At this speed, formation of emulsion was minimized but with sufficient time for attaining equilibrium (approximately 10 minute).

3.4 Validation of Method

In order to test the reliability of the method, acetaminophen was used as the test compound. Using the procedure as described in the section of experimental procedure, absorbances of acetaminophen in the octanol phase were measured at 250 nm. The plot of $\frac{A_{final}}{A_{initial}}$ against V_{drug} gives a straight line with slope of 0.151, as shown in Figure 1(b). Using equation 3, P_{ow} was calculated from the slope to give log P_{ow} of 0.49. This result agrees well with a literature value of 0.48 [16]. This confirms the use of equation 3 for calculating P_{ow} values.

3.5 Application to Selected Drugs

The method was applied to the analysis of some generic drugs. Three replicate measurements were carried out for each drug. The results are shown in Table 2. The log P_{ow} values obtained by this method show good agreement with literature values. The Pearson's correlation coefficient (R^2) between the two data sets is 0.998. Analysis time for a measurement of P_{ow} by this method is about 25 min.

Table 1. The volume of solvents and stock drug solution used in determination of P_{ow}.

Tube no.	Total volume of octanol layer, V_o = 5 ml		Volume of aqueous buffer, V_w (ml)
	Volume of drug, V_{drug} (ml)	Volume of added octanol (ml)	
1	1.00	4.00	5.00
2	2.00	3.00	5.00
3	3.00	2.00	5.00
4	4.00	1.00	5.00
5	5.00	0.00	5.00

Table 2. Comparison of the measured partition coefficient (log P_{ow}) with literature values.

Drug	Phase for absorbance measurement	Partition coefficient (log P_{ow})		pK_a
		This method (n=3)	Literature	
Acetaminophen	Octanol	0.52 + 0.05	0.48 [16]	9.51
Aniline	Octanol	0.93 ± 0.07	0.90 [4]	9.42 (pK_b)
Caffeine	Octanol	0.07 ± 0.01	0.07 [21]	14.0
X	Octanol	1.32 ± 0.28	NA[a]	NA[a]
Riboflavin	Aqueous	-1.21 ± 0.24	-1.46 [21]	9.69
Salicylic acid	Aqueous	-1.22 ± 0.21[b]	-1.41 [11]	2.97

[a] NA: not available

[b] this number reported as log D

All preparations and measurements were performed in the octanol phase except for riboflavin and salicylic acid. These two drugs are hydrophilic compounds at given pH, with concentration in the aqueous layer greater than in octanol. Monitoring of the aqueous phase was thus employed. Measurements in aqueous phase can lead to poorer precision. The aqueous layer is denser and thus is the lower layer of the two immiscible layers. When pipetting the aqueous layer the tip of the pipette must pass through the upper octanol layer, leading to contamination. The same problem was also found in the water-plug aspiration/injection method [14]. However the precision obtained by our method satisfy the OECD criteria, which set that variation in measurement should be less than ± 0.3 log unit [6].

It should be noted that the log P_{ow} is usually determined for uncharged specie. In case of ionized specie, it should be described by term of the distribution coefficient (log D) [1]. Salicylic acid is an ionized compound at given pH. The value shown in Table 2 for this compound should be called log D. The conventional shake-flask method can be practically determined log P_{ow} in range of -2 to 4 [6]. Since the developed method is based on the shake-flask procedure, a range for determination of log P_{ow} by this method should thus be similar to the shake-flask procedure.

The method was applied to the measurement of P_{ow} of a new drug candidate (compound X in Table 2), an analog of the anti-malarial drug, pyracrine. The log P_{ow} of X was 1.32 ± 0.28. This shows that compound X may a potential drug candidate, according to criteria proposed by Hartmann and Schmitt [1]. Compounds which have log P_{ow} in range of 1 to 3 are considered to be potential orally active drugs, since such compounds show good penetration into cells of both nervous and non-nervous systems.

4. CONCLUSION

A new procedure for the determination of the P_{ow} of drug is proposed. The method uses only absorbances of one phase and volumes of the solutions in the calculation. By using this method, the tedious step of constructing calibration curves was eliminated. The P_{ow} obtained by the method agreed well with literature value. The precision of the method conforms to criteria set by the Organization for Economic Cooperation and Development (OECD). This method can measure P_{ow} of both hydrophobic and hydrophilic drug, and requires only general equipment available in all laboratory. The method should be suitable for

screening of lipophilicity of new drugs in the early stages of drug discovery. Further improvement in the method could include an automated liquid handling system for reduction of manual work [22].

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REFERENCES

[1] Hartmann T. and Schmitt J., Lipophilicity - beyond octanol/water: A short comparison of modern technologies, *Drug Discov. Today: Technologies*, 2004; **1**(4): 431-439.

[2] Tetko I.V., Jaroszewicz I., Platts J.A. and Kuduk-Jaworska J., Calculation of lipophilicity for Pt(II) complexes: Experimental comparison of several methods, *J. Inorg. Biochem.*, 2008; **102(7)**: 1424-1437.

[3] Johns J., Estimation of melatonin blood brain barrier permeability, *J. Bioanal. Biomed.*, 2011; **3(3)**: 64-69.

[4] Chen Z. and Weber S.G., High-throughput method for lipophilicity measurement, *Anal. Chem.*, 2007; **79(3)**: 1043-1049.

[5] Barzanti C., Evans R., Fouquet J., Gouzin L., Howarth N.M., Kean G., Levet E., Wang D., Wayemberg E., Yeboah A.A. and Kraft A., Potentiometric determination of octanol-water and liposome-water partition coefficients (log P) of ionizable organic compounds, *Tetrahedron Lett.*, 2007; **48(19)**: 3337-3341.

[6] Organisation for Economic Co-operation and Development (OECD), *OECD Guideline for the Testing of Chemicals No. 107, Partition Coefficient (n-octanol/water): Shake flask method*, Paris, 1995.

[7] Alimuddin M., Grant D., Bulloch D., Lee N., Peacock M. and Dahl R., Determination of log *D* via automated microfluidic liquid-liquid extraction, *J. Med. Chem.*, 2008; **51(16)**: 5410-5412.

[8] Valko K., Application of high-performance liquid chromatography based measurements of lipophilicity to model biological distribution, *J. Chromatogr. A*, 2004; **1037(1-2)**: 299-310.

[9] Giaginis C. and Tsantili-Kakoulidou A., Current state of the art in HPLC methodology for lipophilicity assessment of basic drug: A review, *J. Liq. Chromatogr. Relat. Technol.*, 2008; **31**: 79-96.

[10] Han S.Y. Qiao J.Q., Zhang Y.Y., Lian H.Z. and Ge X., Determination of n-octanol/water partition coefficients of weak ionizable solutes by RP-HPLC with neutral model compounds, *Talanta*, 2012; **97**: 355-361.

[11] Carlsson K. and Karlberg B., Determination of octanol-water partition coefficients using a micro-volume liquid-liquid flow extraction system, *Anal. Chim. Acta*, 2000; **423(1)**: 137-144.

[12] Marine N.A., Klein S.A. and Posner J.D., Partition coefficient measurements in picoliter drops using a segmented flow microfluidic device, *Anal. Chem.*, 2009; **81(4)**: 1471-1476.

[13] Stephan K., Saab J., Mokbel I., Goutaudier C. and Ferrigno R.,

Continuous-flow microfluidic method for octanol-water partition coefficient measurement, *Fluid Phase Equilibr.*, 2014; **380**: 116-120.

[14] Dohta Y., Yamashita T., Horiike S., Nakamura T. and Fukami T., A system for log D screening of 96-well plates using a water-plug aspiration/ injection method combined with high-performance liquid chromatography - mass spectrometry, *Anal. Chem.*, 2007; **79(21)**: 8312-8315.

[15] Gao X., Yu C.H., Tam K.Y. and Tsang S.C., New magnetic nano-absorbent for the determination of n-octanol/water partition coefficients, *J. Pharm. Biomed. Anal.*, 2005; **38**: 197-203.

[16] Machatha S.G. and Yalkowsky S.H., Comparison of the octanol/water partition coefficients calculated by ClogP, ACDlogP and KowWin to experimentally determined values, *Int. J. Pharm.*, 2005; **294**: 185-192.

[17] Danielsson L.G. and Zhang Y.H., Methods for determining n-octanol-water partition constants, *Trends Anal. Chem.*, 1996; **15(4)**: 188-195.

[18] Christian G.D., *Analytical Chemistry*, 6th Edn., John Wiley & Sons, USA, 2004.

[19] Mohan C., *Buffers: A Guide for the Preparation and Use of Buffers in Biological Systems*, EMD Biosciences Inc., Germany, 2003.

[20] Edward J.T., Chubb F.L. and Sangster J., Iron chelators of the pyridoxal isonicotinoyl hydrazone class. relationship of the lipophilicity of the apochelator to its ability to mobilize iron from reticulocytes *in vitro*: Reappraisal of reported partition coefficients, *Can. J. Physiol. Pharmacol.*, 1997; **75(12)**: 1362-1368.

[21] Leo A., Hansch C. and Elkins D., Partition coefficients and their uses, *Chem. Rev.*, 1971; **71(6)**: 525-616.

[22] Wattanasin P., Saetear P., Wilairat P., Nacapricha D. and Teerasong S., Zone fluidics for measurement of octanol-water partition coefficient of drugs, *Anal. Chim. Acta*, 2015; **860**: 1-7.