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Recent advances in lipophilicity measurement by reversed-phase high-performance liquid chromatography

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ABSTRACT

Lipophilicity, quantified by the logarithm of the n-octanol/water partition coefficient (log*P*) or the distribution coefficient (log*D*), is a crucial parameter for modelling biological partition or distribution. As a maintream experimental method for lipophilicity measurement, reversed-phase high performance liquid chromatography (RP-HPLC) has attracted great interest and the attention of researchers throughout the world for its advantages including speed, reproducibility, insensitivity to impurities and degradation products, broad dynamic range, on-line detection, and reduced handling and sizes of samples. This review focuses on recent developments in lipophilicity measurement by RP-HPLC, both theoretical and experimental (mainly mobile and stationary phases).

Contents

Abbreviations: BMC, Biopartitioning micellar chromatography; CCC, Counter-current chromatography; CHI, Chromatographic hydrophobicity index; D, Distribution coefficient; DP-RTC, Dual-point retention time correction; HBC, Hydrogen-bond count; IAM, Immobilized artificial membrane; ILC, Immobilized liposome chromatography; MLC, Micellar liquid chromatography; MP-RTC, Multi-point retention-time correction; MS, Mass spectrometer; ODP, Octadecyl-poly(vinyl alcohol); OECD, Organization for Economic Co-operation and Development; P, n-octanol/water partition coefficient; PCB, Polychlorinated biphenyl; PBB, Polybrominated biphenyl; PBDE, Polybrominated diphenyl ether; POP, Persistent organic pollutant; PS-DVB, Polystyrene-divinylbenzene; QSAR, Quantitative structure-activity relationships; QSPR, Quantitative structure-activity relationship; RP-HPLC, Reversed-phase high-performance liquid chromatography; RP-TLC, Reversed-phase thin-layer chromatography; RTIL, Room-temperature ionic liquid; SFM, Shake-flask method; SP-RTC, Single-point retention-time correction; SSM, Slow-stirring method.

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1. Introduction

Lipophilicity, generally expressed by the logarithm of the n-octanol/water partition coefficient (log*P*), constitutes an important physicochemical parameter conventionally used in quantitative structure-activity relationships (QSARs) for bioactive compounds, including pharmaceuticals and natural products, and toxins, including environmental pollutants. It plays a key role in governing kinetic and dynamic aspects of drug or toxin actions and we should be aware of it early in the development of potential new drugs [1–3] and in evaluating the health risk of emerging environmental exposures [4,5]. Thus, reliable methods for deriving log*P* are very desirable.

In general, two classes of methods are developed for determination of log*P*, calculation and experimental. There are at least several tens of known computational approaches to predict this property, ranging from simple methods based on a small set of descriptors to sophisticated algorithms based on neural networks and involving thousands of correction factors [6]. Mannfold et al. [6], Tetko et al. [7] and Pallicer et al. [8] reviewed and compared these methods for calculating log*P*. Although convenient and efficient, methods for calculating log*P* commonly perform rather poorly, as expressed by rather large calculation errors, since the accuracy of these methods is inevitably influenced by the calculation models. The lack of enough reliable experimental log*P* data is another key reason affecting the accuracy in calculating log*P*. Although the prediction performance of models could be dramatically improved by using so-called local corrections without re-training, it is essential to develop reliable experimental methods [7].

Numerous experimental methods have been exploited for determination of log*P*. They could be classified into two categories: direct and indirect. In direct experimental methods, the n-octanol/water partition coefficients are often obtained directly from the concentration ratio of a neutral compound partitioned between n-octanol (C_o) and water (C_w) phases at equilibrium: $P = C_o/C_w$. The shake-flask method (SFM) [9] and slow-stirring method (SSM) [10] are two reliable methods; SFM is a standard method recommended by Organization for Economic Co-operation and Development (OECD). Other direct methods, such as the potentiometric titration method [11–14], the flow-based method [15–17], the water-plug aspiration/injection method [18], the use of 96-well microplates [18,19], and the use of magnetic nano-absorbent [20] have also been proposed. Though accurate in determining log*P* in the range -2 to 4, these direct methods take time, are labor consuming and require relatively large amounts of pure compounds. Besides, for compounds having a log*P* value larger than 4, these methods are often limited by the dynamic range of the detector (often an ultraviolet detector) or the minimum detection limit for the analyte in either phase.

Responding to the need for more speed and greater accuracy, workers have proposed many other separation-based approaches, such as reversed-phase high-performance liquid chromatography (RP-HPLC) [21], counter-current chromatography (CCC) [22], reversed-phase thin-layer chromatography (RP-TLC) [23], immobilized artificial membrane (IAM) chromatography [24,25], immobilized liposome chromatography (ILC) [26], micellar LC (MLC) [27–29] and biopartitioning micellar chromatography (BMC) [30,31], which were then adopted for log*P* screening. Many early reviews {e.g., Danielsson et al. [22], Finizio et al. [32], Poole et al. [33], Hartmann et al. [34] and Berthod et al. [35]} compared some of these experimental methods.

As the most classic and common indirect method for predicting log*P*, RP-HPLC has become a standard procedure recommended by OECD [21]. It offers several practical advantages, including speed, reproducibility, insensitivity to impurities or degradation products, broad dynamic range, on-line detection, and reduced handling and sizes of samples. Retention times (RTs) of compounds are usually the only parameters obtained in RP-HPLC methods [21]. These advantages attracted great interest and the attention of researchers throughout the world. Braumann [36] reviewed the determination of hydrophobic parameters by RP-HPLC for the first time. Later, Lambert [37] and Valkó [38] overviewed the development of the RP-HPLC method in determination of log*P*. Giaginis et al. [25] reviewed the lipophilicity assessment of basic drugs by HPLC.

This review focuses on recent advances in lipophilicity measurement based on the RP-HPLC method. As the most widely used classic indirect method, the traditional RP-HPLC method still has some defects. For example, log*P* values of strong acidic or basic compounds, metal complexes, surfactants and solutes interacted with mobile phases could not be measured well using traditional methods. Ion suppressors are often needed for log*P* measurement of weakly dissociable solutes. Recent research articles brought forward targeted improvement to solve problems caused by the defects in the classic RP-HPLC method, opening novel routes to fast, accurate determination of lipophilicity.

2. Theoretical basis

2.1. The Collander Equation

The lipophilicity index measured by RP-HPLC is the logarithm of the retention factor log*k*. It is often obtained according to Equation 1:

$$\log k = \log\left(\frac{t_R - t_0}{t_0}\right) \quad (1)$$

where t_R is the RT of the solute and t_0 the RT of an unretained solute, often methanol or sodium nitrate. log*k* could also be calculated using a more precise formula [39]:

$$\log k = \log\left(\frac{t_R - t_{delay} - (V_{ext}/F)}{t_0 - t_{delay} - (V_{ext}/F)} - 1\right) \quad (2)$$

where t_R and t_0 are the RTs of the solute and the unretained compound, respectively, t_{delay} is the injection delay, V_{ext} the extra-column volume and F the flow rate of the mobile phase.

Isocratic retention factors represent a relative scale of lipophilicity. In the past, there was significant interest in correlating the logarithm of P with the logarithm of retention factor k of the solute under a specific chromatographic condition [40]. However, the logarithms of retention factors corresponding to 100% water as mobile phase ($\log k_w$) were considered to be more representative lipophilicity indices. The linear correlation between log*P* values of neutral solutes and their $\log k_w$ values, known as the Collander Equation (Equation 3), has been illustrated both experimentally and theoretically [25,36]:

$$\log P = a \log k_w + b \quad (3)$$

where a and b are constants derived by linear regression analysis.

Since the neat aqueous fraction of mobile phase is hardly achieved for the limit of the reversed-phase column and quite long RTs of strongly hydrophobic solutes, the $\log k_w$ value is often derived by extrapolation. The Snyder-Soczewinski Equation (Equation 4) is the most common equation used for obtaining $\log k_w$ [41,42]:

$$\log k = \log k_w - S\varphi \quad (4)$$

where φ is the volume fraction of the organic modifier in the mobile phase and S a constant derived by linear regression analysis. In extrapolating $\log k_w$, at least four isocratic log*k* values should be employed. This relationship is not linear for the full range of organic-