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Review

Application of high-performance liquid chromatography based measurements of lipophilicity to model biological distribution

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Abstract

Octanol–water partition coefficients are the most widely used measure of lipophilicity in modelling biological partition/distribution. It has long been recognised that the retention of a compound in reversed-phase liquid chromatography is governed by its lipophilicity/hydrophobicity, and thus shows correlation with an octanol–water partition coefficient. A great number of publications have reported the efforts made to adjust HPLC conditions to measure surrogate octanol–water partition coefficients. However, there is no general consensus in this field. HPLC provides a platform to measure various types of lipophilicity that can provide relevant information about the compounds' property. In this way HPLC can be more valuable than just a surrogate for octanol–water partition. Chromatography using biomimetic stationary phases may provide better insight for biological partition/distribution processes. The research in this field is still ongoing and a large variety of HPLC conditions have been suggested. This review will outline approaches to overcoming the difficulties of standardisation and describe different theoretical approaches for comparison of HPLC lipophilicity data obtained under various conditions, along with the relation of these results to biological partition/distribution.

Keywords: Reviews; Octanol–water partition coefficients; Lipophilicity; Chromatographic hydrophobicity index; Immobilised artificial membrane chromatography; Volume of distribution

Contents

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1. Introduction

Since the introduction of the Hansch approach [1] to drug design, the lipophilicity of drug molecules, agrochemicals and general chemicals gained great importance. Fujita et al. [2] first proposed the octanol–water partition coefficient (P) as a good model for biological partition. Since then, the logarithm of the octanol–water partition coefficient ($\log P$) has become the most widely used lipophilicity parameter. Octanol–water partition coefficients have a prominent role in various physicochemical models that describe for example: oral absorption [3], permeability [4], CNS penetration [5], solubility [6], and serum albumin binding [7]. Various in silico calculation software packages such as $c\log P$, Prolog P, ACD $\log P/D$ have made possible the use of octanol–water partition coefficients in predictive models for absorption, distribution, excretion and metabolism (ADME) properties. The lipophilicity of various compounds is also important from an environmental protection point of view, as it can be related to soil absorption, concentration in fish and in other animals. Therefore, fast reliable measurements of lipophilicity are gaining popularity in the pharmaceutical and agrochemical industries.

High-performance liquid chromatography (HPLC) provides an easy, reliable and accurate way to determine the concentration of a compound in solvents used for the measurements of partition coefficient, thus it can be used as a method of concentration determination to obtain octanol–water partition coefficient.

However, the technique has greater potential to determine the partition properties of compounds based on their chromatographic retention times. The chromatographic retention time directly relates to the compound's distribution between the mobile and the stationary phases. The retention factor (k) determined from the retention time (t_R) and dead time (t_0) as $(t_R - t_0)/t_0$ is equal to the ratio of the average number of analyte molecules in the stationary phase to the average number of molecules in the mobile phase (Eq. (1)) during the elution process.

$$k = \frac{n_s}{n} = \frac{t_R - t_0}{t_0} \tag{1}$$

where k is the retention factor, n_s is the average number of molecules in the stationary phase, n_m stands for the average number of molecules in the mobile phase, t_R is the retention time, and t_0 is the dead time.

The retention factor, k can be related to the partition coefficient of the compound (K) between the mobile and the stationary phase, according to Eq. (2).

$$\log k = \log K + \log\left(\frac{V_s}{V_m}\right) \tag{2}$$

As can be seen, we need to know the value of the volume ratio of the stationary and mobile phases (V_s/V_m) to be able to obtain the absolute value of the chromatographic partition coefficient.

Eqs. (1) and (2) form the theoretical bases of deriving partition data from chromatographic retention. Thus, partition coefficients can be obtained from time measurements instead of concentration determination. The retention time is independent of the compound concentration/amount injected into the chromatographic system, and it is the main indicator of a true partition process. Impurities do not affect the measurements as they are usually separated from the main component. The solvent used to dissolve the compound also is separated, and thus low solubility does not affect the measurements. The above advantages are very important in the early drug discovery stage, when compounds for further optimisation have to be selected from combinatorial libraries containing thousands of compounds. The HPLC technique is widely used at this stage to check compound integrity and quality.

Various stationary phases can be used such as normal paraffin hydrocarbons, immobilised octanol, and biomimetic phases such as immobilised artificial membrane (IAM), human serum albumin (HSA), α-acid glycoprotein (AGP), etc. and the pH and polarity of the mobile phase can also be altered. The technique, therefore, is easily applicable for measurement of more than just octanol–water partitions. Since the early publications [8–10] in the beginning of the 1970s there have been several hundreds of papers published about the applicability of reversed-phase liquid chromatography for the determination of lipophilicity. From time to time several review papers summarised the major approaches [11–15].

Dorsey and Khaledi [16] have discussed the theoretical differences between a chromatographic lipophilicity measure and partitioning between bulk solvents. They have also discussed the thermodynamic basis for possible failures in comparing partitioning of small molecules between bulk solvents and chromatographic mobile and stationary phases, as well as partitioning of solutes into lipid layers and biological membranes. Biological partition of compounds in aqueous bi-phasic systems, such as blood and various tissues, certainly happens in close to equal volume ratios involving a large area of contact surfaces between the partitioning phases, and this is similar to the situation in the chromatographic partition process. Significant differences between bulk solvent partition and partition involving large surface may be observed mainly for surface-active compounds, such as amphiphilic and charged molecules with large hydrophobic molecular surfaces.

In spite of the obvious advantage of the HPLC technique, and the possibility of using biomimetic phases that may prove to be better models for biological partitions, the collation of HPLC based lipophilicity data is not widespread. The major problem in providing a more easily measured alternative to the industry standard octanol–water partition measurements lies in the variety of chromatographic stationary phases available and the lack of standardisation which make inter-laboratory comparison of the data very difficult. To overcome these difficulties, the chromatographic system

needs to be calibrated using known standards that also allow the chromatographic retention data to be converted into biomimetic partition coefficients. The reproducibility of current commercially available stationary phases and the automation and reliability of modern HPLC instrumentation now make it possible to fully exploit the advantages of the technique to collate a large amount of lipophilicity data in a reproducible way. However, it also requires a consensus for standardisation.

2. Direct measurement of octanol–water partition coefficients by chromatographic methods

HPLC is a powerful technique for separation and quantification and it provides a generally applicable approach for the determination of compound concentration. A recent review [17] explains the various ways HPLC can be applied as an end point for the determination of octanol–water partition coefficients. The equilibration of the compound in octanol and water can take place in an auto-sampler vial, 96-well plates or specially designed devices. By applying a generic fast gradient method [18] the equilibrium concentration of the compound in the two phases can be determined in minutes without the need for special method development. The proportion of the peak areas obtained from the aqueous and the octanol phases can directly provide partition coefficients by taking into account the volumes of the two partitioning solvents. The biggest advantage of this approach is that it provides a true thermodynamic octanol–water partition coefficient. The HPLC technique is used for automatic determination of the concentration of the compound in the two partitioning solvents. In this respect, the result is independent of the chromatographic conditions used.

3. Measurements of chromatographic lipophilicity

3.1. Adjusting the chromatographic lipophilicity to model octanol–water partition

There are several early publications that describe various methods for using octanol as the stationary phase and octanol–saturated water as the mobile phase [19,20]. The difficulty of this approach is the immobilisation of octanol on the stationary phase surface, and keeping its volume constant during the elution process. The dynamic range of the precisely measurable octanol–water partition coefficients is small, as the octanol saturated mobile phase has low elution strength. In order to cover a wide range of octanol–water partition coefficients ($-1 < \log P < 5$) the volume ratio of the octanol phase and water has to be changed. The chromatographic system is calibrated by measuring the $\log k$ values for a set of compounds with known $\log P$ values. Recently Sirius Analytical (http://www.sirius-analytical.com) has used this approach as the basis of a commercially available instrument. The instrument is a dedicated HPLC system in which a proprietary stationary phase is covered with octanol. The octanol saturated aqueous mobile phase is re-circulated to maintain octanol in the stationary phase constant. In order to increase the measurable range of $\log P$ values, they apply three different conditions by changing the column dimensions and adding organic modifier to the mobile phase. The preliminary calculation of the expected $\log P$ values can help to select the appropriate method. In this way the instrument is able to measure the $\log P$ range from -1 to 5.5. A set of molecules with known $\log P$ values (that are analysed at the same time as the samples) is used to calibrate the system and convert the chromatographic retention times into octanol–water partition coefficients. This approach has the advantage that a small amount of sample is needed for the $\log P$ determination, there is no need for quantitative analysis, and the $\log P$ values are derived directly from retention time measurements. However, these chromatographic conditions are usually much less efficient in terms of theoretical plate numbers, and thus provide limited separations of impurities from the main component.

Several attempts have been made to apply commercially available stationary phases and search for mobile phase additives that make the chromatographic partition system similar to the octanol–water partition system [21,22]. These approaches have the advantage of using commercially available efficient stationary phases.

However, it is not certain that these systems behave exactly like octanol–water, especially in the case of ionised solutes. Although good agreement has been found between the chromatographic and equilibrium octanol–water partition data, deviations can be expected, as in chromatography there is a large surface between the two partitioning phases and surface properties (surface activity, shape) that can influence the interactions. For example, it was found [23] that negatively charged compounds did not show good correlation with the octanol–water distribution coefficients, even if the chromatographic partition was carefully tuned to model octanol–water partition.

4. Measurement of partition coefficients other than octanol–water using HPLC

4.1. Isocratic methods

HPLC is usually performed with high efficiency bonded reversed-phase columns and today the commercially available columns are both robust and reproducible. Partitioning from aqueous/organic mobile phases into the standard reversed-phase (often C_{18}) HPLC stationary phases can be used as a direct measure of lipophilicity. In order to cover a wide range of lipophilicity various concentrations of the organic solvent in the mobile phase must be used. Thus, it requires a preliminary estimate of the expected lipophilicity in order to choose the appropriate mobile phase composition.

Several measurements at different mobile phase concentrations are needed for each compound and this slows down the process and complicates data processing. To compare retention using different organic phase concentrations they are extrapolated to the same condition, which is normally to zero organic solvent concentration. Eq. (3) is used the most often for such extrapolation.

$$\log k = S\varphi + \log k_w \quad (3)$$

Eq. (3) shows the linear relationship between the organic solvent concentration (φ) and the logarithmic retention factor. The relationship is not linear for the full range of organic solvent concentrations, but is a good approximation within the working limits of $-0.5 < \log k < 1.5$. A linear plot of measured $\log k$ versus φ provides the intercept ($\log k_w$), which is the extrapolated retention factor to zero organic phase concentration. In most cases, unless all the measurements are made on an identical HPLC column, it is also necessary to measure a standard set of compounds in order to calibrate the chromatographic system. Standardisation as suggested by the OECD guidelines [24] for example, is based on calibration using octanol–water $\log P$ values and the extrapolated $\log k_w$ values. However, this is not a rigorously correct approach for two reasons. Firstly, the $\log k$ versus organic phase concentration plot is non linear, therefore different $\log k_w$ values can be obtained for the same compound, column, and instrument, when the $\log k_w$ are derived using a different set of mobile phase compositions, or a different type of modifier (acetonitrile or methanol). Secondly, the reversed-phase chromatographic system does not model well the octanol–water system for structurally diverse compounds.

It has been demonstrated [25] that the straight lines obtained by Eq. (3) can cross each other hence different lipophilicity rankings are obtained at different organic solvent concentrations. The best correlation with the octanol–water partition coefficients is not necessarily achieved at the zero organic phase concentration, therefore the S value in Eq. (3) can be used as a second independent variable together with the $\log k_w$ values (Eq. (4)),

$$\log P = aS + b\log k_w + c \quad (4)$$

where a, b and c are regression coefficients. The ratio of a to b gives organic modifier concentration values, to which the $\log k$ values should be extrapolated to give the best statistical correlation to the $\log P$ values. In this way the chromatographic partition can be tuned to better model the octanol–water partition.

The quotient of the slope and intercept in Eq. (3) ($-\log k_w/S = \varphi_0$) also showed a better correlation with the $\log P$ values than $\log k_w$ [26] as is shown by Eq. (5).

$$\log P = d\varphi_0 + e \quad (5)$$

where d and e are regression coefficients.

The quotient (φ_0) is equivalent to the organic solvent concentration in the mobile phase that is required to get the compound retention time exactly twice that of the dead time, i.e. $\log k = 0$.

However, it has to be pointed out that good correlation between reversed-phase chromatographic $\log k$ values and $\log P$ values for a set of compounds does not necessarily prove that the octanol–water system and the HPLC partition system are comparable.

4.2. Gradient methods

A linear gradient increase of the organic solvent concentration in the mobile phase during a chromatographic run helps to overcome the difficulties inherent in the choice of suitable isocratic conditions. In order to maintain the resolution of the separation, slow organic phase gradient methods are used. Mutton [18] has pointed out that by increasing the flow rate and using short columns it is possible to reduce the gradient time and thus, the analysis time with a minimum loss of resolution. Such fast generic methods are now widely applied by the chemists in drug research for rapid identification and analysis of compounds using reversed-phase HPLC coupled with mass spectrometry. Usually an acetonitrile gradient is used and the analysis time per compound can be reduced to 5 min. That inspired us to investigate the possibilities of using fast gradient reversed-phase chromatographic retention times as a measure of lipophilicity of the compounds. During a gradient run any point of the run time is equivalent to a particular organic solvent concentration and by knowing the dead volume and dwell volume of the HPLC system it is possible to estimate the organic phase concentration as the compound elutes from the column. During a fast organic phase gradient the solvent slope parameter S (see Eq. (3)) has a negligible influence on the gradient retention time. When the appropriate organic phase concentration reaches the column each compound will be carried through the column at approximately the mobile phase velocity. To a first approximation the retention time in a fast gradient run should be linearly related to the isocratic φ_0 values (see Eq. (5)). It has been shown that the gradient retention times are in good correlation to the isocratically obtained φ_0 values using experimental data from 76 diverse drug molecules obtained from both isocratic and gradient elution [27]. The gradient retention times of a calibration set of compounds give a straight line when plotted against the φ_0 values. The slope and the intercept of the calibration line can be used to convert the gradient retention times to a chromatographic hydrophobicity index (CHI) that is suitable for inter-laboratory comparison and for building a database. The CHI index measurement takes only 5 min using standard HPLC conditions and is a platform widely used for quality control by research chemists. The conditions used cover a 6–7$\log P$ unit range of lipophilicity and simple data processing can be used to convert the gradient retention times to CHI values. The CHI values can also be projected to the logarithmic scale that is more appropriate for free energy related comparisons with the usual $\log P$ and $\log D$ parameters

used by chemists (Eq. (6)):

$$\text{CHI}\log D = 0.054\text{CHI} - 1.467 \quad (6)$$

The constants of Eq. (6) were obtained by correlation of measured octanol–water $\log D$ values at pH 7.4 and with CHI obtained at pH 7.4 for the data of 80 drug molecules comprising neutral, acidic and basic compounds. It should be noted that there are significant differences between CHI $\log D$ values and the octanol–water $\log D$ values. For neutral molecules H-bond donor compounds generally show a lower CHI $\log D$ than octanol–water $\log D$. Charged molecules, however, tend to give higher CHI $\log D$ values than octanol–water $\log D$ values (as will be discussed later). So the conversion is pragmatic and it is used only for expressing the CHI scale (normally 0–100) as a $\log P$ scale (normally –1 to 5). CHI values of more than 25 000 research compounds have been collected in the corporate database at GlaxoSmithKline that proved to be useful in the lead optimisation.

Camurri and Zaramella [28] have adapted the methodology for mass spectrometry detection of the compounds during the gradient run. The MS detection makes it possible to determine CHI values for mixtures of compounds and in this way reduce the analysis time further. Companies such as Comgenex (Budapest, Hungary) have adopted this method. From a single gradient run during their standard LC–MS based quality control procedure the CHI index can be derived easily by using a common calibration set.

Equivalent selectivity for the fast gradient and the isocratic systems has been demonstrated by Du et al. [29]. The connection between the gradient and isocratic retention times has been discussed extensively by Snyder and utilised in DryLab software [30], that computes isocratic method development conditions based on a few carefully selected gradient measurements. Kaliszan et al. [31] have reported a method to estimate the isocratic extrapolated $\log k_w$ values (and the acid dissociation constant, pK_a) using two gradient retention times (one is an organic phase, the other is a pH gradient) of a compound.

Donovan and Pescatore [32] have reported the use of gradient methods to obtain a wide range of measured lipophilicity values from gradient retention times, but they use ODP column and a methanol gradient and claim their values to be close to octanol–water partition coefficients. Kerns et al. [33] recently published a similar gradient reversed-phase HPLC method for pharmaceutical profiling for lipophilicity and compound integrity. They calibrated the gradient retention times directly with the octanol–water $\log D$ values of six compounds. The gradient retention times correlated approximately with the $\log D$ values using a Polaris C_{18} column and acetonitrile gradient. The starting mobile phase pH was 7.4. The biggest advantage of this methodology is the provision of a lipophilicity parameter directly from the LC–MS compound integrity/purity measurements. In general using gradient HPLC and C_{18} phases reproducible lipophilicity values can be obtained in a high throughput way while the perfect match with the traditionally used octanol–water partition coefficients is lost.

5. Comparing partition coefficients obtained in different partitioning systems

5.1. Solvation equation based approach

Partition coefficients obtained from different partitioning systems can show good correlation for a set of structurally related compounds based on the Collander equation (Eq. (7)).

$$\log P_1 = f \log P_2 + g \quad (7)$$

However, when a large number of structurally diverse compounds are compared the correlation weakens, especially when the H-bond donor/acceptor properties and the dipolarity/polarisability of the partitioning solvents are different from the octanol. The Abraham solvation equation model [34] can be used for the investigation of the similarities or differences between various organic solvent/water partitions. The solvation equation model suggests that molecular size, and a small number of polarity descriptors (H-bond donor/acceptor, dipolarity) are sufficient to describe such similarities and differences. By measuring $\log k$ (or the extrapolated $\log k_w$ values for a set of probe molecules (minimum 25 compounds, preferably 40–60) with known molecular descriptors (excess molar refraction, E; dipolarity/polarisability, S, H-bond acidity, 0; H-bond basicity, B, and molecular size, V) the solvation equation characteristic for the chromatographic partitioning system can be constructed using multiple linear regression (Eq. (8)).

$$\log k = eE + sS + aA + bB + vV \quad (8)$$

The regression coefficients of the molecular descriptors (e, e, a, b, and v) are characteristic for the chromatographic partition system. Numerous equations have been published [35–39] for the characterisation of various reversed-phase types of columns (C_{18}, perfluorinated hydrocarbons, cyclodextrin, CN) using acetonitrile and methanol as organic modifier in the mobile phase. Table 1 contains the coefficients of the solvation equations obtained for various chromatographic systems. The generally good statistical fit of the chromatographic retention data and the molecular descriptors derived from bulk organic solvent/water partition coefficients support the comparability of the two types of partition data. However, it should be noted, that the parameters of the solvation equation are often based on the retention data of relatively small, mono- or bi-functional molecules in unionised form.

Du et al. [40] compared the linear solvation equations obtained for isocratic HPLC retention factors (using C_{18} stationary phases and acetonitrile as organic modifier in the mobile phase) and for the octanol water partition coefficients. The major difference between the two partition systems is their sensitivity toward H-bond acidity of the

Table 1
Relative coefficients of the solvation equations for HPLC systems with different selectivity [38]

		c/v	r/v	s/v	a/v	b/v	v	N	S.D.	R
1	In ODS–MeOH	1.40 ± 0.23	0.11 ± 0.25	−0.20 ± 0.24	−0.21 ± 0.26	−0.91 ± 0.28	5.15 ± 0.27	69	0.67	0.954
3	In ODS–TFE	1.22 ± 0.25	0.12 ± 0.33	−0.35 ± 0.25	−0.55 ± 0.28	−0.69 ± 0.31	5.67 ± 0.28	68	0.67	0.965
4	In ODS–HFIP	1.76 ± 0.31	0.14 ± 0.37	−0.31 ± 0.31	−1.06 ± 0.34	−0.89 ± 0.38	4.47 ± 0.36	55	0.77	0.953
5	In ODS–AcN	1.48 ± 0.14	0.09 ± 0.15	−0.22 ± 0.15	−0.33 ± 0.15	−1.02 ± 0.17	4.80 ± 0.16	68	0.38	0.984
16	FO–TFE	2.40 ± 0.23	−0.04 ± 0.24	−0.18 ± 0.25	−1.18 ± 0.26	−0.61 ± 0.30	3.11 ± 0.28	65	0.64	0.950
20	DCN–AcN	1.54 ± 0.23	0.05 ± 0.22	−0.08 ± 0.21	−0.15 ± 0.26	−1.13 ± 0.26	3.68 ± 0.25	60	0.57	0.936
21	DCN–MeOH	0.73 ± 0.29	0.15 ± 0.30	−0.19 ± 0.29	−0.13 ± 0.32	−0.83 ± 0.35	5.42 ± 0.33	69	0.82	0.924
23	PLRP–AcN	1.94 ± 0.20	−0.15 ± 0.35	−0.10 ± 0.21	−0.57 ± 0.25	−1.29 ± 0.28	4.38 ± 0.23	66	0.58	0.969

molecules. While the water saturated octanol phase easily accommodates compounds with H-bond donor groups, the chromatographic C_{18} stationary phase has no polar functionality. Therefore, H-bond donor functional groups decrease chromatographic retention and consequently chromatographic partition coefficients. The correlation between isocratic retention data (with C_{18} stationary phases and aqueous acetonitrile mobile phases) and octanol $\log P$ can be improved significantly by adding into the equation a simple H-bond donor count or the Abraham H-bond acidity descriptor A as is shown by Eqs. (9) and (10).

$$\log P = 1.91 \log k + 0.37\,\mathrm{HBC} + 0.72 \quad (9)$$

where $N = 111$, $r = 0.962$, S.D. $= 0.272$.

$$\log P = 2.07 \log k + 1.09A + 0.52 \quad (10)$$

where $N = 111$, $r = 0.982$, S.D. $= 0.189$.

The gradient chromatographic CHI values obtained for the unionised forms of the molecules (CHIN) also have shown an acceptable correlation with the octanol–water $\log P$ values when the H-bond donor count (HBC) or the Abraham H-bond acidity (A) descriptor are included [41]. Eqs. (11) and (12) show the relationships obtained for a training set of 86 known drug molecules.

$$\log P_{\mathrm{oct}} = 0.054\,\mathrm{CHIN} + 1.32A - 1.88 \quad (11)$$

Table 2
Relative coefficients of the solvation equations obtained for various organic solvent partitions, C_{18} and biomimetic chromatographic partition data and selected biological partition

Distribution	e/v	s/v	a/v	b/v
Octanol–water [45]	0.15	−0.28	0.01	−0.91
Isobutanol–water [46]	0.17	−0.23	−0.02	−0.83
Pentanol–water [46]	0.18	−0.24	0.00	−0.87
Alkane–water [46]	0.15	−0.39	−0.82	−1.13
Cyclohexane–water [46]	0.18	−0.37	−0.81	−1.06
CHI (ODS, ACN) [38]	0.16	−0.24	−0.29	−1.01
CHI MeOH [38]	0.10	−0.20	−0.15	−0.85
CHI IAM [61]	0.16	−0.17	0.12	−1.03
$\log K$(HSA) [55]	0.51	−0.22	0.11	−1.22
Blood/brain barrier [42]	0.19	−0.69	−0.72	−1.28
Oral absorption [43]	0.14	0.11	−1.39	−1.5

where $n = 86$, $r = 0.970$, $s = 0.29$, $F = 655$.

$$\log P_{\mathrm{oct}} = 0.047\,\mathrm{CHIN} + 0.36\mathrm{HBC} - 1.10 \quad (12)$$

where $n = 86$, $r = 0.943$, $s = 0.39$, $F = 336$.

Solvation equations have been derived for various biological partition/distribution processes, like blood/brain barrier distribution [42], oral absorption [43], skin-penetration [44]. The solvation equations obtained for biological partition processes can be compared with the solvation equations obtained for organic solvent/water partition coefficients [45,46]. The system coefficients are listed in Table 2. It has been noticed that some of the biological partition systems are also sensitive to H-bond donor functionality. For example, compounds with strong H-bond acidity do not partition well into brain tissues.

Cimpean and Poole [47] suggested using the solvation equation approach in a systematic search for surrogate chromatographic models of the bio-partitioning process. The wide variety of available stationary phases and mobile phase additives provides the flexibility of using chromatography to adjust the properties of the partitioning phases to model directly biological partition instead of mimicking octanol–water partition. The regression coefficients of the molecular descriptors in the solvation equation obtained in various chromatographic and biological partition processes can be compared to find the best chromatographic model system. However, it is important to mention that the solvation equation and the Abraham descriptors are valid only for distribution of compounds in their unionised form. Appropriate pH should be used for HPLC measurements on ionisable compounds to suppress the ionisation and determine retention parameters for the neutral form of the molecule. The solvation equation approach does not describe the effects of ionisation on biological partition.

6. Effect of ionisation on reversed-phase retention

As in octanol–water partition, reversed-phase retention depends on the pH of the aqueous mobile phase and the presence of charge on a compound decreases its reversed-phase retention [48]. From the change of reversed-phase retention caused by the change of the mobile phase pH, it is possible

to derive the pK_a values of the compound [31]. As it was mentioned earlier, the presence of charge in a large lipophilic molecule makes it amphiphilic and thus it affects surface activity. This can cause significant discrepancies between bulk solvent partition and the chromatographic partition where a large interface is involved. The pH dependence of gradient retention times has been extensively studied by Roses et al. [49,51]. The major discrepancies (further discussed in [17]) are the smaller differences between the lipophilicity of the ionised and unionised molecule and the distortion of the typical log D–pH curves for weak acids and bases.

7. Biomimetic chromatographic partition systems

7.1. Immobilised protein stationary phases (human serum albumin, α-acid glycoprotein)

One of the great advantages of using HPLC technology for lipophilicity determination of compounds is that various types of stationary phases can be applied including ones that mimic bio logically important constituents such as membranes and proteins. A frequently used type of biomimetic HPLC stationary phase contains plasma proteins immobilised by attachment to silica support materials. These include human and rat serum albumin (HSA and RSA, respectively) and α-acid glycoprotein (AGP). These phases can be used to measure the binding affinity of compounds to these proteins. It is known that high plasma protein binding can reduce brain penetration [51] as it affects the unbound (free) drug concentration that is available to diffuse from the blood and reach the target tissue. Several authors (Noctor et al. [52], Tiller at el. [53], Colmenarejo et al. [7]) have demonstrated the applications of chemically bonded serum albumin stationary phases. The theory and practice of using immobilised serum albumin has been reviewed by Hage and Austin [54]. These methods are based on the assumption that the chemically bonded HSA retains the binding specificity and conformational mobility of the native serum albumin. These published methods are based on isocratic retention time/factor (log k) measurements that can be converted to % bound values by Eq. (13).

$$\%\,\mathrm{HSA} = 100\left(\frac{k}{k+1}\right) \tag{13}$$

The retention factor, k equals the ratio of the number of moles in the stationary and mobile phases. However, this equation is valid only for non-specific binding, when compound retention is independent of the injected amount and the overall binding can be modelled by partition. The chromatographic retention factor, k is equivalent to the albumin partition coefficient only if the V_s/V_m is unity. The good agreement between % binding data obtained by HPLC and the plasma protein binding data obtained by ultrafiltration, or dialysis methods suggest, that the volume ratio of the aqueous biphase plasma also should be close to 1:1. This partition model is valid as long as the % plasma bound data, and the HPLC retention data are not dose dependent. The HPLC methods are faster to measure and more precise in ranking compounds binding (especially at a high binding region) than the traditional ultrafiltration or equilibrium dialysis methods. In order to reduce analysis time a few volume per cent (up to 10–15%) iso propanol in the mobile phase can be used to elute strongly bound compounds. A fast, generic HPLC method based on an iso propanol gradient and a chemically bonded HSA HPLC column has been published [55]. For the estimation of the protein binding the system is calibrated using literature plasma protein binding % data. The % binding values are converted to linear free energy related log K values that are in linear correlation with the logarithmic gradient retention times (log gt_R). Using the same methodology a set of compounds with known molecular descriptors have been analysed and a solvation equation has been derived. The system coefficients characteristic for the non-specific binding properties of the serum albumin are also listed in Table 2. It can be seen that the albumin-based partition is very similar to the octanol–water partition for neutral compounds. So the octanol–water system in this respect appears to be an appropriate model for describing how compounds partition in aqueous protein bi-phasic systems. Based on the solvation equation a good correlation with octanol–water partition data can be expected. Figs. 1 and 2 shows the correlation obtained for 70 known drug molecules [17] Fig. 1 shows the correlation between the HPLC based HSA partition data (log K) and the octanol–water partition coefficients (log P) of the neutral forms of the molecules and Fig. 2 the correlation with the octanol–water distribution coefficients (log D) at pH 7.4.

Figs. 1 and 2 show that the ionisation of the compounds does not reduce the HSA binding ability as much as it reduces the octanol–water log D values. This finding is very similar to that of described by Van de. Waterbeemd et al. [56] and Davis and Riley [57]. However, when the lipophilicity of the uncharged molecules is considered (c log P) no significant separation of acids and bases could be observed. These results clearly suggest that the octanol–water system does not model well the partition of charged molecules into albumin phase.

7.2. Immobilised artificial membrane chromatography

Pidgeon and Venkataram [58] patented a method for immobilising phospholipids on HPLC grade silica stationary phases. The immobilised phospholipids mimics the lipid environment of a fluid cell membrane on a solid matrix. Nowadays various immobilised artificial membrane (IAM) HPLC stationary phases are commercially available from Regis Technologies. The recent review by Taillardat-Bertschinger et al. [59] describes in detail the properties of the commercially available IAM HPLC columns and their applications to predict compound's interaction with biological membranes. Stewart and Chan [60] published an excellent

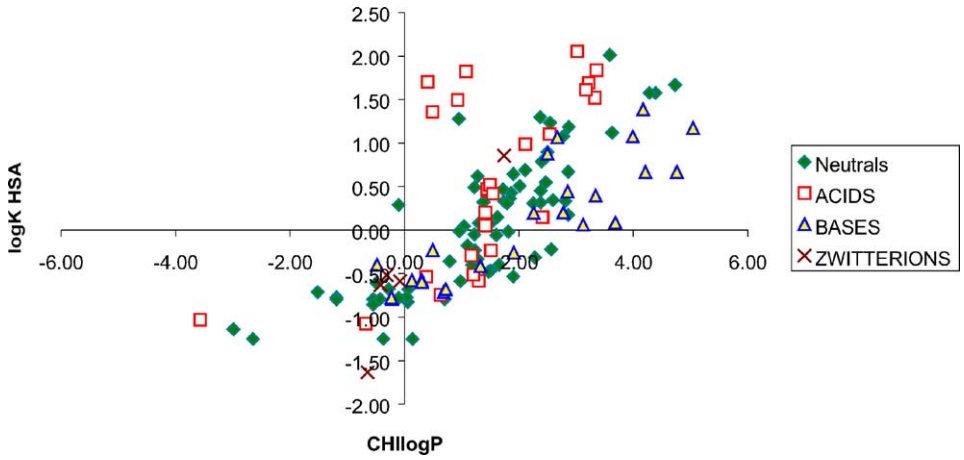

Fig. 1. Correlation of octanol–water partition coefficients (log *P*) and HSA binding [log *K*(HSA)] for neutral, negatively and positively charged compounds (from ref. [17]).

review about the use of immobilised artificial membrane chromatography for modelling drug transport. Usually isocratic methods were suggested incorporating a very low volume percent (15%) of acetonitrile in the mobile phase. Eq. (2) is suggested for derivation of membrane partition data from isocratic retention factors. Eq. (3) can be applied for the extrapolation of retention factors (log *k*) obtained with various concentrations of organic modifiers to pure aqueous mobile phase. A gradient method has been developed and published [61] that applies acetonitrile gradient on IAM HPLC column and provides a chromatographic hydrophobicity index (CHI IAM). In which the gradient retention times are calibrated with data obtained from isocratic measurements. The solvation equation approach [34] was used to demonstrate the similar selectivity of the isocratic and gradient systems. Calibration of the column each day helps overcome the problem of column-to-column, and day to day reproducibility. The CHI IAM values have been collected for more than 1000 research compounds in GSK and they are extensively used for various project specific purposes (e.g. in brain penetration models and hepatotoxicity models [62]. To convert the CHI IAM values into the linear free energy related log *k* values Eq. (14) can be used. The equation was obtained by plotting isocratic log k_0 and CHI IAM data published earlier [61].

$$\log k_{0\,\mathrm{IAM}} = 0.046\mathrm{CHI\,IAM} + 0.42 \qquad (14)$$

where $n = 48$, $r = 0.93$.

The coefficients of the solvation equation obtained using IAM stationary phase [61] are also shown in Table 2. Again, the coefficients in the equation are very similar to those obtained for the octanol–water system. The immobilised artificial membrane partition is also insensitive to the H-bond acidity of the compounds, so in this respect again the octanol–water partition system seems to be an appropriate model. However, investigation of the relationship between log *P* and log *D* values and the CHI IAM values (see Figs. 3 and 4) revealed that the effect of ionisation is very

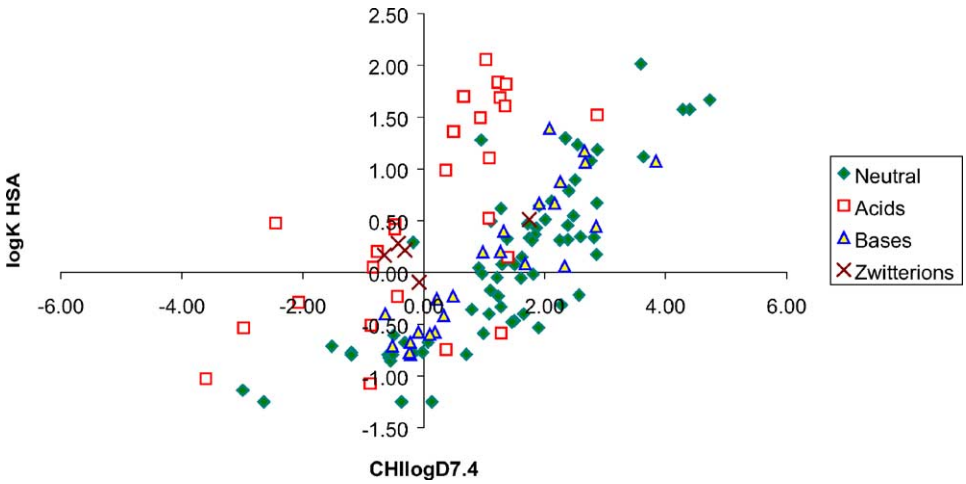

Fig. 2. Correlation of octanol–water distribution coefficients (log *D*) and HSA binding [log *K*(HSA)] for neutral, negatively and positively charged compounds (from ref. [17]).

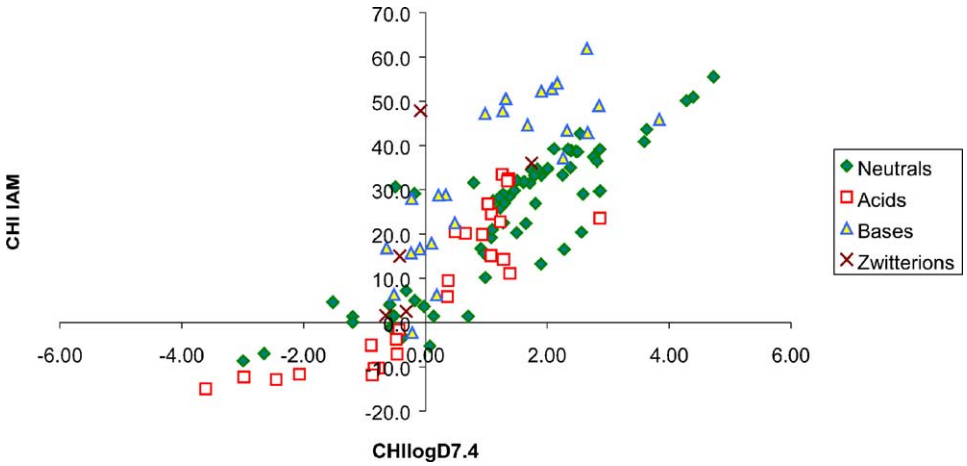

Fig. 3. The correlation of chromatographic hydrophobicity index (CHI) obtained on IAM stationary phase with octanol–water distribution coefficients ($\log D$) referring to pH 7.4 for acidic, basic and neutral drug molecules [17].

different in the two partition systems. Interestingly, in IAM partition positively charged compounds showed higher partition to the membrane and this is opposite to the effect in HSA partition.

These examples show clearly that the octanol–water system is in general a good model for protein binding and membrane partition, regarding its selectivity for neutral compounds. However, the effect of positive and negative charge is remarkably different in the octanol–water bulk solvent partition, membrane partition and HSA partition. Fig. 5 demonstrates the different effects of the positive and negative charge to membrane and HSA partition. We can assume that the biological distribution depends on the compound partition coefficients related to the aqueous compartments (free drug solution) and to various protein, and or lipid rich compartments. Knowing the relative volumes of the free and bound compartments we can model volume of distribution [63], or various blood/tissue partition of the compounds. Based on our preliminary data [17] for example, good correlation was found between the logarithmic value of the volume of distribution ($\log V_d$) and the $\log K$(IAM) and $\log K$(HSA) values of 44 drug molecules. When terms for the typical permeability limiting negative charge and size parameters (CMR) were included excellent agreement was found (Fig. 6) between the measured and calculated $\log V_d$ values based on Eq. (15).

$$\log V_d = 1.36 - 0.33 \log K(\text{HSA}) + 0.56 \log K(\text{IAM}) - 0.033\text{CMR} - 0.37\,\text{negative charge} \quad (15)$$

where $N = 42$, $r = 0.92$, $s = 0.20$, $F = 52$.

Poor correlation was obtained when $c \log P$ was introduced into the equation as the measure of compound lipophilicity. As $\log K$(IAM) values are sensitive to the positive charge (i.e. positively charged compounds partition to the membrane very strongly), an indicator variable for

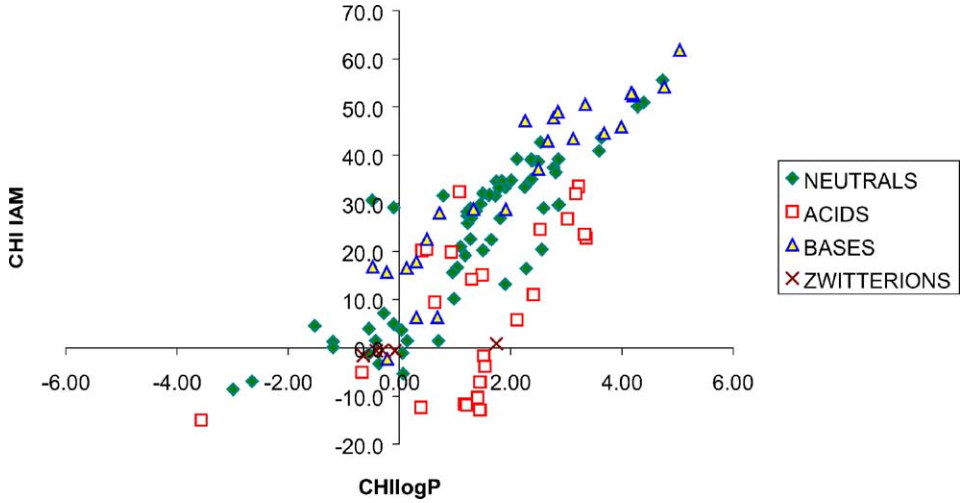

Fig. 4. The correlation of chromatographic hydrophobicity index (CHI) obtained on IAM stationary phase with octanol–water partition coefficients ($\log P$) referring to the partition of the unionised form of acidic, basic and neutral drug molecules [17].

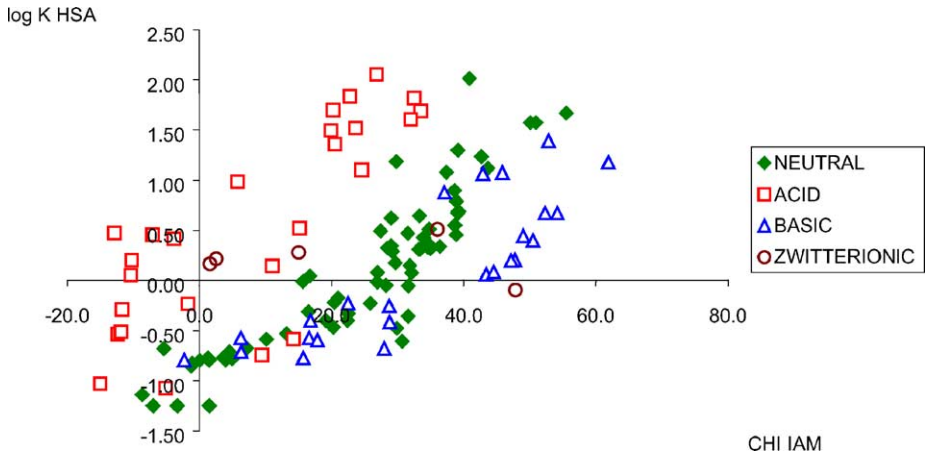

Fig. 5. The correlation of membrane partition (CHI IAM) and HSA partition [log K(HSA)] measured at pH 7.4 for neutral, positively charged and negatively charged compounds (from ref. [17]).

the positive charge was also introduced into the model and Eq. (16) was obtained. The plot of the measured versus calculated values can be seen in Fig. 7.

$$\log V_d = 1.95 + 0.052c \log P - 0.03\text{CMR} - 0.62\,\text{negative charge} + 0.45\,\text{positive charge} \quad (16)$$

where $N = 42$, $r = 0.75$, $s = 0.34$, $F = 12$.

These models are based on data from only 42 drug molecules extracted from the literature. Nevertheless, the major differences between the octanol–water log P and the biomimetic chromatographic partition data can be demonstrated. Although, the log K(IAM) and the log K(HSA) values show a trend with the c log P values (the solvation equation also supports these similarities), the three partition systems show remarkably different sensitivities towards the ionisation state of the molecules.

A pharmacokinetic simulation software package, PK-SIM, has been developed at Bayer [64] based on IAM partition and HSA binding data and they make their partition measurements using the Nimbus technology [65] for immobilisation of phospholipids and proteins on silica particles. Lombardo et al. [63] published a model for the volume of distribution, using the chromatographically determined E log D values for neutral and basic compounds. The effect of ionisation was also taken into the model. Unfortunately, their model is not applicable for acidic compounds.

It is worth mentioning that bio-micellar chromatography has been also suggested [66–68] for directly modelling biological partition processes. The authors claim that the retention obtained using the suggested bio-micellar mobile phase with the applied stationary phase provides a better model for biological partition processes than octanol–water partition or reversed-phase partition. Several correlation studies have been shown to support this postulate. However, it is very difficult to prove such similarities based solely on a correlation of structurally closely related compounds. The comparison of the solvation equations of the bio-micellar

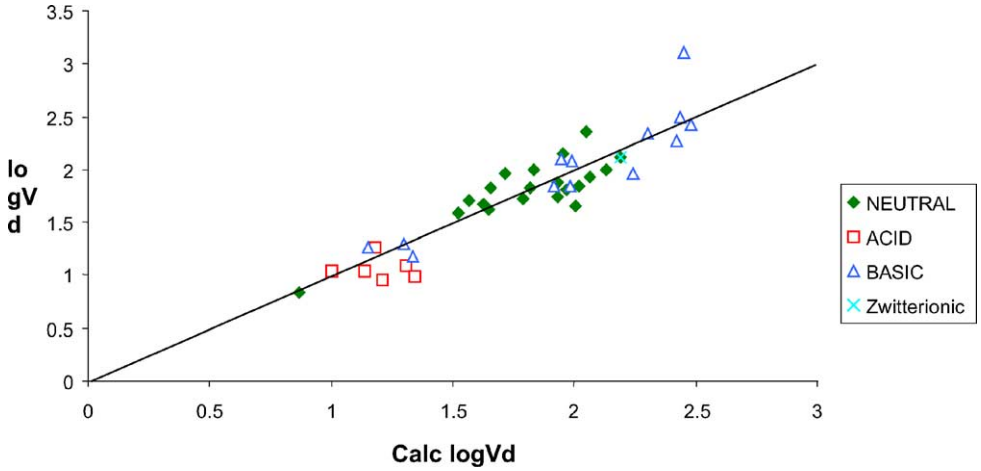

Fig. 6. The plot of the literature (measured) and calculated (by Eq. (15)) volume of distribution (log V_d) for 42 known drug molecules (data taken from ref. [17]). The model based on HPLC based membrane partition and albumin binding data, including the molecular size and the presence of negative charge.

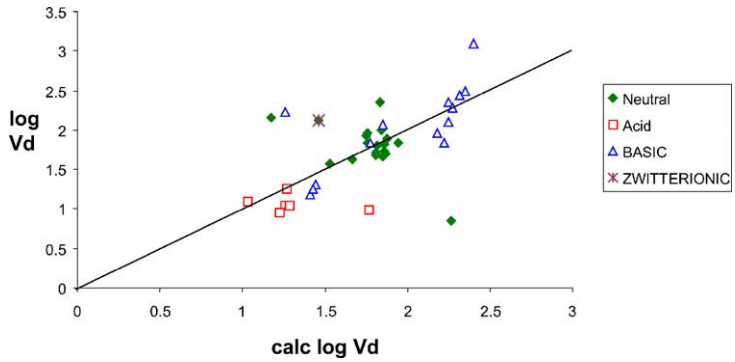

Fig. 7. The plot of the literature (measured) and calculated (by Eq. (15)) volume of distribution ($\log V_d$) for 42 known drug molecules (data taken from ref. [17]). The model calculated octanol–water partition coefficients ($c\log P$), including the molecular size and the presence of negative charge.

system with the modelled biological partition system would be a more reliable tool to reveal such similarities. Also, it would be worthwhile to investigate the effect of ionisation on bio-micellar partition systems.

8. Conclusion

Based on the presented examples from recent publications, it is evident that octanol–water partition/distribution is not always the most appropriate model for biological partition processes. The biomimetic chromatographic partition systems may prove to be better models. For a more accurate description of compound distribution between various compartments in vivo, we require a better understanding of the differences between various types of lipophilicity and therefore we need more than one lipophilicity scale to properly describe molecular behaviour. This suggestion is not new, as Leahy et al. [69] proposed measuring four different partitions ('critical solvent quartet') for a better description of the lipophilicity of molecules in terms of their in vivo distribution. Nasal et al. [70] also suggested measuring several chromatographic lipophilicities, and using the plots of the first two principal components to distinguish compounds according to pharmacological activity. Chromatographic measurements of lipophilicity using biomimetic stationary phases can improve our insight into in vivo partition processes.

Besides these theoretical advantages, HPLC technology is well developed, and provides a high throughput, robust platform to measure properties of a large number of compounds. However, we need some sort of standardisation to collect data that can be compared by different investigators. It seems that both isocratic and gradient retention data obtained in C_{18} systems with proper calibration can serve as a basis for providing octanol–water like partition data for neutral compounds ($\log P$) by incorporating a simple correction based on an H-bond acidity term. However, this system provides only an approximate model for describing the distribution of ionised compounds between bulk solvents [17,49,50]. The effect of ionisation, however, is different in biomimetic partitions and very probably we need several appropriate biomimetic chromatographic systems to be able to characterise the wide variety of biological absorption and distribution processes.

A recent publication [71] suggests that chromatographic band broadening observed at high flow rates could also provide information about the rate of partition, that can be used to model compound's permeability. A similar chromatographic approach has also been published for measurements of the onset and offset rate in albumin binding [72].

Modern HPLC instrumentation is robust and able to provide accurate, high throughput data in an automated environment using small quantities of research compounds that are sometimes impure. What we need is proper selection of the most useful chromatographic conditions, and standardisation to fully appreciate the true potential of the chromatographic technique.

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