Review

Separation methods for estimating octanol–water partition coefficients

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Abstract

Separation methods for the indirect estimation of the octanol–water partition coefficient ($\log P$) are reviewed with an emphasis on high throughput methods with a wide application range. The solvation parameter model is used to identify suitable separation systems for estimating $\log P$ in an efficient manner that negates the need for empirical trial and error experiments. With a few exceptions, systems based on reversed-phase chromatography employing chemically bonded phases are shown to be unsuitable for estimating $\log P$ for compounds of diverse structure. This is because the fundamental properties responsible for chromatographic retention tend to be different to those responsible for partition between octanol and water, especially the contribution from hydrogen bonding interactions. On the other hand, retention in several micellar and microemulsion electrokinetic chromatography systems is shown to be highly correlated with the octanol–water partition coefficient. These systems are suitable for the rapid, high throughput determination of $\log P$ for neutral, weakly acidic, and weakly basic compounds. For compounds with a permanent charge, electrophoretic migration and electrostatic interactions with the stationary phase results in inaccurate estimation of partition coefficients. The experimental determination of solute descriptors offers an alternative approach for estimating $\log P$, and other biopartitioning properties. A distinct advantage of this approach is that once the solute descriptors are known, solute properties can be estimated for any distribution or transport system for which a solvation parameter model has been established.
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Contents

1. Introduction

The development of high throughput in vitro biological screens and combinatorial chemical synthesis have changed dramatically the way biologically active compounds are identified and optimized for applications in medicine and agriculture [1–3]. As these approaches became routine in many industries, attention shifted to improving the efficiency of compound development by minimizing the attrition rate and shortening the development time. This resulted in an increasing demand for physicochemical property determinations to assess such factors as biopharmaceutical

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properties, toxicity, bioconcentration, environmental fate, etc. at an early stage of compound development. For example, a potential drug candidate has to cross several barriers, until it binds to the target and induces the desired response. These barriers are characterized as absorption, distribution, metabolism, and excretion or the "ADME" interface. Early prediction of ADME properties serves as an indication of the likely development success of a compound. Access to this valuable information can help identify candidates with inadequate properties as well as providing insights to guide structure optimization. In the same way, a small number of physicochemical properties are used in risk assessment to predict a compound's potential for bioconcentration and aquatic toxicity to arrive at an appropriate hazard category [4–6].

The core properties required to estimate absorption, distribution, and transport in biological systems are solubility, lipophilicity, stability, and acid–base character [2,7,8]. These properties are used directly or through structure activity relationships to help design active compounds and determine toxicity and membrane permeation. Solubility is usually expressed as the saturation solubility in a defined solvent at a specified temperature. Stability requires a working definition tailored to the environmental characteristics important for assessing survival rates. Lipophilicity is a complex property more often than not determined by the octanol–water partition coefficient. Acid–base character is determined by a compound's pK_a value. Each property is usually determined in a separate experiment. When these properties were determined mainly for registration purposes, traditional methods of measurement were adequate, since only a small number of measurements were required. It was more important that these measurements were accurate and speed and cost were secondary considerations. Traditional methods, however, are too slow, labor intensive, and expensive to employ alongside high throughput synthesis and screening methods. Combinatorial syntheses produce relatively small amounts of material that is often impure and frequently dissolved in a solvent, such as dimethyl sulfoxide, suitable for biological screens. Although accuracy remains a goal, of equal weight is that the methods selected for physicochemical measurements can accommodate the above sample characteristics as well as being fast and easily automated. If physicochemical property determinations are not to be a bottleneck in the discovery process, it must be at least as easy to measure a desired property as it is to synthesize the compound. In addition, the cost of making the measurement must not be an unreasonable burden on the discovery process. These requirements have challenged the analytical community to devise new strategies for rapid, automated, high throughput and rugged methods for physical property determinations that quite often differ significantly in principle and practice from traditional methods.

Although the concept of hydrophobicity and lipophilicity are widely used in relation to the sorption of organic chemicals from water, their exact meaning is somewhat vague or confused with their (alleged) consequences [9–11]. The hydrophobic effect refers to the tendency of non-polar compounds to prefer a non-aqueous environment over an aqueous environment. The driving force for this process is created by the preference of water to reform an ordered structure. Lipophilicity is an extension of the hydrophobic effect and includes the favorable solute–solvent interactions that contribute to the distribution of a solute between water and an organic solvent, or other solubilizing media, such as biomembranes. Hydrophobicity, therefore, is not synonymous with lipophilicity, but a mere component of it. Likewise, hydrophobicity and lipophilicity cannot be considered solute properties, but are a manifestation of the characteristic properties of the system or environment in which the solute finds itself. In this way, solute lipophilicity is at least, in part, a system property, with a driving force that has as much to do with the exclusion of the solute from water as its liking (or love) for the lipid media.

The concept of hydrophobicity or lipophilicity takes on a broader meaning in chemistry because of the importance of these processes in life sciences and the environment. The hydrophobic effect is assumed to be one of the driving forces for the passive transport of drugs through biological membranes and as a component of drug-receptor binding [1–3]. The biodistribution, protein binding, and metabolism of drugs may also be altered by their lipophilicity. It is generally held that lipophilic compounds are preferred targets for metabolism, often leading to high clearance rates and, frequently, lipophilicity correlates positively with a high protein binding. Non-specific toxicity is expected to correlate with a compound's propensity to accumulate in cell membranes and therefore, its lipophilicity [6,12]. Bioavailability and bioconcentration studies have attempted to determine the extent to which environmentally relevant chemicals enter and accumulate in the food chain through sorption from water and the ingestion of contaminated fish and plants by other animals. These factors are also commonly related to a compound's lipophilicity [11–13]. The distribution of compounds between soil or sediment and water is an important factor in risk assessment, the management of hazardous waste disposal, and the correct use of crop-protecting agents in agriculture [4,5,14,15]. These properties are strongly correlated with the compound's lipophilicity.

Oil–water partition coefficients in general, and the octanol–water partition coefficient in particular, are widely used as a measure of lipophilicity [8,11,16]. The octanol–water partition coefficient is one of the most commonly reported physicochemical properties of drugs and industrial chemicals and, if for no other reason, is the de facto scale most often used in establishing quantitative structure-activity relationships (QSARs). QSARs have been developed for all kinds of biological, pharmaceutical, and environmental property estimates based on the octanol–water partition coefficient as a general solute descriptor for the lipophilicity of organic compounds [4,5,8,9,11,16–18]. Given the large diversity in biological

systems and environmental compartments, it is unlikely that a single solute descriptor could adequately represent the range of expected system properties. Many correlation models are in fact restricted to a limited range of solute structures, because the factors responsible for membrane solubility are not the same as those responsible for solvation by wet octanol.

There is sometimes confusion over the definition and representation of the octanol–water partition coefficient. It is most often indicated as $\log P$, $\log K$, $\log D$, etc. frequently with a subscript O or W. $\log P$ is generally used to indicate any partition coefficient and $\log K$ any equilibrium constant. Since only the octanol–water partition coefficient is considered here, we will use $\log P$ for the octanol–water partition coefficient for a neutral substance or an ionizable substance in its neutral form. $\log P$ is then defined as

$$\log P = \log \left(\frac{C_{\mathrm{O}}}{C_{\mathrm{W}}} \right), \tag{1}$$

where C_{O} is the concentration of compound in the octanol phase and C_{W} its concentration in the aqueous phase when the system is at equilibrium. The use of $\log D$ is reserved for the partition coefficient in the octanol–water distribution system when the compound participates in a secondary chemical equilibrium in either or both phases (e.g. ionization, aggregation, ion pair formation, etc.). For the case of ionization in the aqueous phase, $\log P$ and $\log D$ are related by Eq. (2) for a weak monoprotic acid that is partially ionized in the aqueous phase and by Eq. (3) for a weak monoprotic base that is partially protonated in the aqueous phase.

$$\log D = \log P_{\mathrm{XH}} - \log(1 + 10^{(\mathrm{p}K_a - \mathrm{pH})}) \tag{2}$$

$$\log D = \log P_{\mathrm{X}} - \log(1 + 10^{(\mathrm{pH} - \mathrm{p}K_a)}), \tag{3}$$

where XH and X refer to the neutral form of the weak acid and base, respectively. If $\log P$ and the $\mathrm{p}K_a$ of a compound are known, $\log D$ can be calculated at any pH. For polyprotic compounds the equations become more complicated. Details can be found in [17].

2. Traditional and direct chromatographic methods

Standard procedures for the determination of octanol–water partition coefficients using the shake flask process are time consuming, tedious, prone to emulsion problems, and require relatively large amounts of pure compounds [16,19]. In addition, the following practical considerations are important: (a) complete separation of the layers since any droplets of octanol in the aqueous phase may contain large amounts of sample; (b) presaturation of the two phases is required; (c) the sample concentration must be less than the critical micelle concentration; (d) measurements need to be carried out at concentrations below the aqueous solubility limit; and (e) lipophilic basic compounds may adsorb onto surface of the apparatus. Stir-flask, generator column, potentiometric titra-

tion, and high-speed countercurrent chromatography provide improvements in some aspects of the measurement process but are unsuitable for high throughput methods. Emulsion problems can be minimized using a dialysis tube to separate the water and octanol phases and ultrasonic agitation to shorten the equilibration time [20,21]. If a separation method is used to determine the concentration of analyte in both phases then partition coefficients can be determined for impure compounds. This is still a relatively slow process, however, requiring several hours per sample. A micro-volume extraction system based on air segregation and sequential injection of aqueous sample and octanol in a capillary tube with on-column detection to determine sample concentration in both phases after a suitable delay time was recently described [22]. In order to create a closed system and to prevent axial sample dispersion in the capillary, air pockets are introduced before and after the sample/octanol plug. Equilibrium is reached within a short time due to the large contact area between the two phases (relative to their respective volumes) combined with rapid intrasegmental mixing. Equilibrium is monitored by reversing the flow direction in a cyclic fashion so that the aqueous buffer and octanol segments are passed continuously back and forth through the detector until the sample concentration stabilizes in each segment. This method shows reasonable promise for high throughput measurements with a typical analysis time of about 4 min and a sample requirement of less than 1 μl of solution. However, it is likely to be restricted to relatively pure compounds with favorable UV absorbance and a modest range of partition coefficient values. A micro-shake flask method has been demonstrated for the high throughput determination of octanol–water partition coefficients [7]. Equilibrium of the sample between octanol–water is performed in a standard sample vial using a vial-roller for mixing followed by injection from the aqueous phase only to determine sample concentrations by gradient elution liquid chromatography with UV detection. The sample solution is divided between four vials; the first contains the sample in aqueous buffer only and the other three different ratios of aqueous buffer containing sample and octanol. Different ratios are used to enhance the range of partition coefficients that can be determined. The partition coefficient is calculated from the ratio of the peak areas for the compound in the two phases and the phase ratio for each system. The throughput for this method is typically 24 samples per day per instrument. The main limitation is that the sample must have reasonable solubility ($>10\,\mu$g/ml) in the aqueous buffer. The range of $\log D$ values covered is about -1.5 to 3.5. Typical sample requirements are 0.5 mg.

To increase sample throughput, the traditional shake flask method has been automated and miniaturized by transfer to 96-well plate technology using robotic liquid handlers for sample preparation [23,24]. After equilibration, an aliquot is analyzed from each phase by gradient elution liquid chromatography with UV absorption [23] or single ion monitoring mass spectrometry [24]. High sample throughput is

obtained by use of fast gradient separations with a cycle time of about 5 min (10 min to analyze both layers). Both methods are limited by the dynamic range of the detector and the minimum detection limit for the analyte in either phase to $\log P$ values of about -2 to 4. The single-ion detection method used overnight shaking, centrifugation and phase separation as sample handing steps. The UV detector method used 30 min shaking, a rest period, and a programmed autosampler injector. The autosampler needle was made to sample from both the bottom of the well and higher up in the well allowing both layers for each sample to be injected consecutively. A solvent rinse step was programmed to wash the injector and transfer line between injections to minimize carryover, identified as a potential error source.

3. Indirect separation methods

Chromatographic and electrophoretic methods require very little material (which does not have to be pure), are fast compared to traditional methods, and are relatively easy to automate. These methods are, of course, indirect, based on the construction of a correlation model, Eq. (4), between a retention property characteristic of the solute and the separation system for a training set of solutes with known $\log P$ values. Then, further measurements of the retention property in the separation system can be used to estimate $\log P$ values for other compounds:

$$\log P = p + q \log k, \tag{4}$$

where k is the retention factor (or retention factor resulting from the extrapolation of $\log k$ values to zero organic solvent for binary mobile phase mixtures in liquid chromatography, k_W). For thin-layer chromatography, R_M or R_{MW} values are used in place of $\log k$ or $\log k_W$ where $R_M = \log[(1 - R_F)/R_F]$ and R_F is the retardation factor. In practice, it is important that the structures of the training set and samples are similar, otherwise incorrect estimates of the partition coefficient are obtained. This arises because the system properties that influence $\log k$ or $\log k_W$ are not usually quite the same as those expressed by the octanol–water partition coefficient. When structurally unrelated compounds are correlated via Eq. (4), the agreement is often poor. Failure to establish that the fundamental intermolecular interactions that control retention in the chromatographic system are correlated with the same properties responsible for partition in the octanol–water system is the main reason that so many published models have limited applicability.

3.1. Solvation parameter model

The solvation parameter model can be used to determine the contribution of individual intermolecular interactions responsible for the partition of neutral molecules in the octanol–water system. This model is also suitable for the determination of the contribution of the same interactions to retention in reversed-phase separation systems, which are most likely to provide surrogate models for the octanol–water partition system. Comparison of the system constants for the octanol–water and separation systems allows a decision to be made as to whether the separation model is correlated with the octanol–water partition system as indicated by Eq. (4).

The solvation parameter model in a general form suitable for establishing the factors responsible for partition in the octanol–water system and the retention properties of reversed-phase separation systems is set out below [9,17,25–27].

$$\log k \text{ or } \log P = c + eE + sS + aA + bB + vV \tag{5}$$

The model consists of product terms representing solute properties (descriptors), indicated by capital letters, and the complementary system properties, indicated by the lower case letters. Each product term describes the relative contribution of defined intermolecular interactions to the correlated solute property, in this case, either $\log k$ or $\log P$. The contribution from electron lone pair interactions is defined by eE, interactions of a dipole type by sS, hydrogen-bond interactions by aA and bB, and differences in cavity formation and dispersion interactions for transfer of the solute from one phase to the other by vV. The solute descriptors are formally defined as the excess molar refraction, E, dipolarity/polarizability, S, effective hydrogen-bond acidity, A, effective hydrogen-bond basicity, B, and McGowan's characteristic volume, V. A minor complication is that certain solutes (sulfoxides, anilines, pyridines) show variable hydrogen-bond basicity in partition systems where the non-aqueous phase absorbs appreciable amounts of water [17,28]. These solutes have two values for B. The appropriate value is selected based on system properties. For wet octanol, reversed-phase chromatography and micellar electrokinetic chromatography (MEKC), the separation systems of interest to this report, the same B descriptor appropriate for phases that absorb a significant amount of water is used in all cases. Solute descriptors are available for about 4000 compounds with others available through calculation and estimation methods [17,25,26,29]. Calculation methods that rely on a group contribution approach provide different degrees of success for complex molecules due to the difficulty of adjusting inter-group interactions for different molecular structures [30–32]. For the time being experimental methods for the determination of solute descriptors are more reliable [17,29,33].

The system constants reflect the difference in solvation properties in the two phases. Thus, both their sign and magnitude are important for interpretation of system properties. The system constants are defined as the difference in contributions from electron lone pair interactions, e, dipole-type interactions, s, hydrogen-bond basicity, a, hydrogen-bond acidity, b, and cohesion and dispersion interactions, v, for the two phases. The system constants are calculated by multiple linear regression analysis for a varied group of solutes

selected to satisfy the statistical and chemical requirements of the model [25,29,34–36].

The system constants for the octanol–water partition system are: $c = 0.09$ (± 0.02); $e = 0.56$ (± 0.01); $s = -1.05$ (± 0.02); $a = 0.03$ (± 0.02); $b = -3.46$ (± 0.03); and $v = 3.81$ (± 0.01) [28,37,38]. Water is quite soluble in octanol (0.27 mole fraction) and the solvation properties of wet octanol are not the same as those for dry octanol [39]. System constants with a positive sign favor transfer from the aqueous phase to wet octanol. Thus, wet octanol is more hydrophobic and polarizable than water but less dipolar and hydrogen-bond acidic. The hydrogen-bond basicity of water and wet octanol are about the same.

A separation system will emulate the octanol–water partition system if the system constants for both processes are (nearly) identical. It will correlate with the partition system through a relationship such as Eq. (4), if the ratios of the system constants (e.g. e/v, s/v, a/v, b/v) for both models are (nearly) identical [9,25,40–43]. As long as there is modest agreement in system constant ratios, a reasonable correlation model for homologs (which differ only in the v system constant) and other compounds with only a narrow range of descriptor properties is expected. There are many models of this kind described in the literature, but since they lack generality, they are unsuitable for estimating $\log P$ values for varied compounds. Ishihama and Asakawa [44] have described a vector methodology for comparing solvation models. In this case, the similarity between any two compared solvation equations is expressed as an angle, $\cos\theta$, that approaches one for highly correlated models. This approach has some merit but has been little used in practice [33].

3.2. Reversed-phase liquid chromatography

The use of reversed-phase liquid chromatography for the indirect determination of octanol–water partition coefficients was proposed in the early 1970s and has been reviewed many times since [7–9,11,16,17,45–49]. In spite of many attempts, success has been limited for compounds that exhibit structural diversity. The main facts seem to be (1) reversed-phase chromatographic systems provide a partial, but incomplete, model for the octanol–water partition system; (2) specific interactions can occur at sorbent surfaces that are absent in the octanol–water system (e.g. ion-exchange interactions with silanol groups); (3) pore size effects for sorbents, such as size or ion exclusion, have no parallel in the octanol–water partition system; (4) reproducibility of results on generic column types and different batches of the same column materials are often poor; (5) the elution range for isocratic systems is limited resulting in models with a small window of $\log P$ values and long measurement times in some cases; and (6) the pH operating range for silica-based column materials is limited (e.g. pH 2–7.5). The search for solutions to these problems includes a systematic search for separation systems capable of emulating the octanol–water partition system, solvent generated and coated stationary phases

designed to resemble the octanol–water partitioning system or lipophilic membranes, separations utilizing micellar mobile phases, and methods based on gradient elution separations. Before detailing these developments, there are some fundamental problems to discuss.

The chromatographic property used to correlate with $\log P$ is either the isocratic retention factor ($\log k$) or the retention factor for water as the mobile phase ($\log k_W$), usually obtained by extrapolation from isocratic measurements of $\log k$ for several different binary solvent mixtures. A decrease in polarity of the mobile phase, equivalent to increasing the volume fraction of organic solvent in an aqueous mixture, leads to a decrease in retention that can be described by Eq. (6)

$$\log k = \log k_W + a_1\phi + a_2\phi^2 \tag{6}$$

and if only a limited range of binary mobile phase compositions is considered, by Eq. (7)

$$\log k = \log k_W + S\phi, \tag{7}$$

where ϕ is the volume fraction of organic solvent (binary mobile phase), S the slope of the experimental data after fitting to a linear regression model (not to be mistaken for the solute descriptor S used in connection with the solvation parameter model), and a_1 and a_2 are regression constants for the second order model, which are not usually assigned any physical significance [9,27,50–55]. If it is assumed that the mobile phase composition range used to define the relationship for the extrapolation is unimportant, then $\log k_W$ values can be obtained for compounds with a wider range of retention properties than is possible for a single mobile phase composition. This simple picture may conform to contemporary practice, but is far from adequate and is difficult to justify. The majority of $\log k$ plots against mobile phase composition are curved when volume fractions of organic solvent close to zero are included in the plot. A large contribution to this curvature is the change in phase ratio associated with the significant change in the composition and structure of the stationary phase in contact with predominantly aqueous mobile phases. Within the intermediate mobile phase composition range, an approximate linear relationship between $\log k$ and the volume fraction of organic solvent can almost always be found, but the intercept obtained by linear extrapolation is generally not the same value as that obtained by curve fitting experimental data that includes measurements at a low volume fraction of organic solvent. Further, when different extrapolation ranges are used for the same compound, different values of $\log k_W$ are frequently obtained. As defined by Eqs. (6) and (7), $\log k_W$ should be independent of the organic solvent type. The available evidence demonstrates that the opposite is generally the case [9]. The problem seems to be that selective solvation of the stationary phase by the organic solvent results in an intercept value that is influenced by the properties of the solvent. In practice, general errors in estimating $\log k_W$ and its ambiguous definition make this parameter an unacceptable choice as a characteristic system property for use

in correlation models. The selection of the system variable has not received the critical attention it deserves in many proposed models for the estimation of $\log P$ and is a contributing factor to poor model performance in many studies.

The primary reason that reversed-phase chromatography fails to provide suitable models for estimating $\log P$ is that the intermolecular interactions that contribute to retention in reversed-phase chromatography are similar but not identical in character to those responsible for the octanol–water partition coefficient [9,56–59]. Differences in the contribution from hydrogen-bonding interactions are usually most notable. The solvation parameter model has been used to characterize several hundred reversed-phase chromatographic systems, and although no comprehensive databases are available, a number of large compilations have been reported [9,25,41,43]. These databases contain representative examples of all the common chemically bonded and porous polymer stationary phases as well as specialized phases such as immobilized artificial membranes and polymer encapsulated inorganic oxides with different mobile phase compositions. These databases can be searched using the system constant ratios to identify separation systems with properties similar to the octanol–water partition system. Only three separation systems are identified as possible models for the octanol–water partition system (see Table 1). This is a very small success rate and suggests that in general the widely used chemically bonded phases with common aqueous organic solvent mobile phases are not suitable for estimating $\log P$ for structurally diverse compounds. Two of the candidate systems are based on retention data determined as $\log k_W$, which is a poorly defined parameter subject to excessive experimental error (see above). We are skeptical of the robust nature of these models, although the Supelcosil LC-ABZ column did afford a reasonable correlation model for a varied group of compounds [59]. Recent results with methanol–water mobile phases containing less than 50% methanol indicate that $\log k$ values for the Supelocsil LC-ABZ column are suitable for estimating $\log P$ [59,60].

Du et al. [61] provided a representative example of the difficulty of estimating $\log P$ by reversed-phase liquid chromatography on chemically bonded phases. These authors used a Luna (C18) column and acetonitrile–water (40% (v/v)) containing 50 mM ammonium acetate adjusted to pH 7.4 for neutral compounds, pH 10.5 for weak bases (to suppress ionization) and pH 2 for weak acids as mobile phase and obtained the following model

$$\log k = -0.14 + 0.31E - 0.58S - 0.51A$$
$$- 1.58B + 1.79V \qquad (8)$$

with $e/v = 0.18$, $s/v = -0.32$, $a/v = -0.29$, and $b/v = -0.88$. There is reasonable agreement with the system constants for the octanol–water partition system except for the a/v ratio. The correlation model for $\log P$ based on the compounds used to construct Eq. (8) is (rms: root-mean-square

error)

$$\log P = 1.63\,(\pm 0.05)\log k + 1.10\,(\pm 0.05),$$
$$n = 111,\ r = 0.951,\ \text{rms} = 0.308 \qquad (9)$$

For the same data if a correction is made for the difference in hydrogen-bond basicity of the two systems by inclusion of the A solute descriptor a much better model is obtained

$$\log P = 2.07\,(\pm 0.04)\log k + 1.09\,(\pm 0.08)A$$
$$+ 0.52\,(\pm 0.05),$$
$$n = 111,\ r = 0.982,\ \text{rms} = 0.189 \qquad (10)$$

Thus, it can be concluded that the Luna column and acetonitrile–water separation system is a reasonable model for estimating $\log P$ for neutral compounds that lack significant hydrogen-bond acidity. It is not suitable, however, for estimating $\log P$ for a varied group of compounds, which includes compounds acting as hydrogen-bond acids.

Coated columns have received little attention for separations [27]. Problems associated with long-term stability can be tolerated for estimating $\log P$ if they provide a more realistic model for the octanol–water partition systems than the more widely used chemically bonded and porous polymer stationary phases. Extensive work has been done with octanol coated [62–64] and phosphatidylcholine coated [58,65] silica and chemically bonded phases. Reasonably stable octanol coated chemically bonded phases can be prepared by the solvent generated technique [62]. The mobile phase, phosphate buffer saturated with octanol is pumped through the column containing a conventional chemically bonded phase until equilibrium is reached. This can be a lengthy process requiring perhaps 1500 interparticle volumes of mobile phase. At equilibrium, the sorbent is loaded with the maximum amount of octanol that can be immobilized on the support without erosion of the stationary liquid. For maximum stability, the mobile phase and column should be thermostated at the same temperature. Reasonable models for predicting $\log P$ from $\log k$ were obtained for neutral and basic compounds with an accessible range of $0.5 \leq \log P \leq 4$ and standard error in the prediction of $\log P$ of about 0.07 log units. Discrepancies were higher for acidic compounds. Separation times are long for compounds with high $\log P$ values and would be a disadvantage for high throughput methods. Lombardo et al. [63,64] used octanol coated reversed-phase columns for the estimation of $\log P$ of neutral compounds (termed $E \log P$) [63] and basic and neutral compounds (termed $E \log D$) [64]. The mobile phase was a 20 mM MOPS buffer pH 7.4 containing 70 to 15% (v/v) methanol. The aqueous buffer was saturated with octanol and 0.25% (v/v) octanol was added to the methanol. For the estimation of $\log D$, 0.15% (v/v) n-decylamine was added to the octanol saturated aqueous buffer. A short column (50 mm × 4.6 mm) of Supelcosil LC-ABZ was used as support for the octanol liquid phase. $\log k_W$ was used as the parameter correlated with $\log P$ or $\log D$ according

Table 1

Reversed-phase chromatographic systems with similar system constant ratios to the octanol–water partition coefficient

Separation system	Dependent variable	System constant ratios				
		v	e/v	s/v	a/v	b/v
Octanol–water	$\log P$	3.81	0.15	−0.28	0.01	−0.91
Chemically bonded phases						
Stationary phase: Supelcosil LC-ABZ [58] Mobile phase: methanol–water (30–70% v/v)	$\log k_W$	3.63	0.09	−0.24	−0.08	−0.93
Stationary phase: Nucleosil RP-18 [9] Mobile phase: methanol–water (60–100% v/v)	$\log k_W$	3.66	0.07	−0.22	−0.10	−0.90
Stationary phase: Supelcosil LC-ABZ [59] Mobile phase: methanol–water (30:70)	$\log k$	2.60	0.15	−0.20	0	−0.91
Stationary phase: Supelcosil LC-ABZ [58] Mobile phase: methanol–water (40:60)	$\log k$	2.38	0.11	−0.18	0.08	−0.97
Stationary phase: Hypersil ODS [9] Mobile phase: methanol–water (45:55)	$\log k$	2.53	0.09	−0.24	−0.08	−0.93
Coated phases						
Stationary phase: Supelcosil LC-ABZ coated with octanol [63] Mobile phase: 20 mM MOPS pH 7.4 saturated with octanol and 15–70% (v/v) methanol containing 0.25% (v/v) octanol	$\log k_W$	3.48	0.12	−0.27	−0.01	−0.89
Stationary phase: silica gel coated with dipalmitoyl phosphatidylcholine [58] Mobile phase: 10% (v/v) acetonitrile–water	$\log k$	2.68	0.18	−0.16	0.01	−1.03
Thin-layer chromatography						
Stationary phase: Merck RP-18 F$_{245S}$ Mobile phase: methanol–aqueous buffer pH 7.4 [94]	R_{MW}	3.60	0.07	−0.18	−0.18	−0.83

to the scheme indicated in Table 2. Computer-calculated values of $\log P$ or $\log D$ were used to estimate the appropriate experimental conditions for the determination of $\log k_W$ using a three-point linear extrapolation requiring about 20 min per compound. It is possible that the phase ratio is reasonably constant over the mobile phase composition range employed for the extrapolation, and the estimation of $\log k_W$ is more accurate than observed for chemically bonded phases. The solvation parameter model was used to demonstrate that the factors contributing to $\log k_W$ on the coated column are virtually identical to the octanol–water partition coefficient (Table 1). The correlation models for neutral compounds ($\log P$) and neutral and basic compounds ($\log D$) have slopes close to 1.0 and are statistically sound

$$\log P = 1.10\ (\pm 0.03) \log k_W + 0.13\ (\pm 0.7),$$

$$n = 36,\ r^2 = 0.977,\ \text{S.E.} = 0.251,\ F = 1434 \tag{11}$$

Table 2

Experimental conditions for the estimation of $\log P$ by reversed-phase liquid chromatography using extrapolated values of $\log k_W$ on an octanol-coated Supelcosil LC-ABZ column

$\log P$ or $\log D$ value	Flow rate (ml/min)	Mobile phase composition (%) (v/v) methanol
−0.5 to 1.0	0.5	15, 20, and 25
1–3	1.0	40, 45, and 50
>3	2.0	60, 65, and 70

$$\log D = 1.13\ (\pm 0.02) \log k_W + 0.21\ (\pm 0.04),$$

$$n = 90,\ r^2 = 0.964,\ \text{S.E.} = 0.309,\ F = 2339 \tag{12}$$

This method should be suitable for the high throughput estimation of $\log P$ or $\log D$ of neutral and weakly basic compounds, but not acidic compounds (and possibly zwitterions as well). Method accuracy is acceptable (≈ 0.3 log units) with a throughput of about three compounds per instrument per hour. Stability of the coated liquid phase is a potential problem for unattended operation.

The system constant ratios for silica gel coated with dipalmitoyl phosphatidylcholine with a mobile phase containing 10, 20, or 30% (v/v) acetonitrile–water are similar to the octanol–water partition coefficient (see Table 1) [58]. There are small but significant differences in the s/v and b/v system constant ratios indicating that this is a useful but not exact system for estimation of $\log P$ [66]. In a later study, Hanna et al. [65] coated a reversed-phase column by equilibration in the recycle mode with a 1 mM solution of phosphatidylcholine in 80% (v/v) methanol–water. This column was then used with 40% (v/v) acetonitrile-aqueous buffer (pH 7.4) as mobile phase for the estimation of $\log P$. The fit of the correlation model for the retention factor and $\log P$ was only modest, but the compounds included in the model contained several that were partially ionized. Removal of acids from the correlation improved the fit. Neither the average error in the estimated $\log P$ values nor the typical time for a measurement is indi-

cated by the authors. The slope of the $\log P$ against $\log k$ plot for non-acidic compounds is significantly larger than one (≈ 2.38) indicating that the factors contributing to the chromatographic retention are different to those for the octanol–water partition coefficient. Improved results were obtained by combining the liquid chromatographic retention factor with the retention factor determined by MEKC. This approach, however, seems unnecessary complex and time consuming for general use in a high throughput laboratory.

Micellar liquid chromatography is a variant of reversed-phase chromatography, which uses a surfactant above its critical micelle concentration in an aqueous or aqueous-organic solvent as mobile phase [27,67]. Aqueous mobile phases with (sometimes) a small amount of organic solvent, such as 2-propanol, as an additive to improve the mass transfer properties of the system, were used predominantly for the estimation of $\log P$ [67–71]. In most cases, the surfactant was sodium dodecyl sulfate or tetradecyltrimethylammonium bromide. Retention in micellar liquid chromatography depends on the simultaneous distribution of sample between surfactant micelles and the surrounding aqueous solution occurring in the mobile phase and between the mobile phase and the surfactant modified stationary phase. The retention factor is described by

$$\frac{1}{k} = \frac{1}{k_{\mathrm{m}}} + \left(\frac{K_{\mathrm{AM}}}{k_{\mathrm{m}}}\right)[\mathrm{M}], \tag{13}$$

where k_{m} is the retention factor at zero micelle concentration (k at a surfactant monomer concentration equal to the critical micelle concentration), K_{AM} the solute-micelle binding constant and $[\mathrm{M}]$ the total concentration of surfactant in the mobile phase minus the critical micelle concentration. Plots of k or $\log k$ against $\log P$ are often curved, particularly if high values of $\log P$ are included. These plots are of limited use for estimating $\log P$. Values for k_{m} and K_{AM} can be determined from the slope and intercept of the plot of $1/k$ against $[\mathrm{M}]$. The values for K_{AM} and k_{m} are generally correlated but $\log k_{\mathrm{m}}$ often provides a better fit with $\log P$ than does $\log K_{\mathrm{AM}}$. To some extent, this may reflect the choice of surfactants commonly used in micellar liquid chromatography. From the more extensive data available for MEKC, it will be shown that some micellar systems are excellent models for the octanol–water partition coefficient (see Section 3.4), but these have been rarely used in micellar liquid chromatography. In addition, a number of separations with different values of $[\mathrm{M}]$ are required to establish k_{m}. This is time consuming and barely compatible with the requirements for high throughput methods. Sorption of the surfactant by the stationary phase results in the creation of a dynamic ion exchanger resulting in anomalous retention for ionized compounds with a poor fit to $\log P$ models containing neutral compounds. It is difficult to measure k_{m} for every hydrophobic compounds owing to the near-zero intercept in the $1/k$ against $[\mathrm{M}]$ plots. Although micellar liquid chromatography uses the same equipment

and columns as conventional reversed-phase chromatography, the presence of high surfactant concentrations in the mobile phase increases the requirement for system maintenance.

A disadvantage of isocratic separations by liquid chromatography for high throughput methods is that for some compounds retention will be either insufficient for an accurate determination of the retention factors or too long to allow measurement in an acceptable time. Columns of different length and/or variation of flow rates can afford a partial solution to this problem, but often require preliminary experiments or reliance on computation methods to predict which set of conditions to use for each sample. Although, it is possible to implement such procedures they are inconvenient for screening compound libraries generally provide in a multiwell plate format. For analysis, gradient elution is commonly specified for the separation of samples with a wide range of retention properties [27]. Generic gradient methods are also widely used in the pharmaceutical industry for screening compound libraries for purity determination [7]. The application of these methods for the high throughput estimation of $\log P$ is an obvious extension of what has become standard practice for many laboratories. For linear solvent strength gradients in reversed-phase chromatography the gradient retention time is related to isocratic separation conditions by Eq. (14) [27,72–77]

$$t_{\mathrm{g}} = C + \left(\frac{t_{\mathrm{M}}}{b}\right)\log k_{\mathrm{o}}, \tag{14}$$

where t_{g} is the gradient retention time, t_{M} the column hold-up time, b the gradient steepness parameter, $\log k_{\mathrm{o}}$ the isocratic retention factor for the mobile phase at the start of the gradient, and C is a rather complex system constant. The derivation of Eq. (14) assumes that $2.3bk_{\mathrm{o}} \gg 1$, a reasonable assumption for some but not all conditions, and the ratio (t_{M}/b) is compound independent, which is generally untrue. The second assumption is a source of compound-dependent retention dispersion compared with independent measurements of isocratic $\log k_{\mathrm{o}}$ values [74]. When the gradient starts from water $\log k_{\mathrm{o}}$ provides an estimate of $\log k_{\mathrm{w}}$ and t_{g} becomes a more conveniently determined surrogate parameter for $\log k_{\mathrm{W}}$.

Valko and coworkers [78–82] have described the use of short columns and fast gradients for the determination of a chromatographic hydrophobicity index (CHI), which can be correlated with $\log P$ or used as an independent measure of hydrophobicity. These authors assume that in a fast gradient separation each compound migrates as an unretained peak when the appropriate organic solvent concentration reaches the top of the column. Their approach is an extension of an isocratic hydrophobicity scale, ϕ_{o}, to gradient elution conditions. The parameter ϕ_{o} is defined as the percent by volume of acetonitrile required to achieve an equal distribution of compound between the mobile and stationary phases, corresponding to a retention factor of one in an isocratic mobile phase. It is assumed that Eq. (7) provides a realistic model of

the retention process, and for $\log k = 1$, $\phi_o = -\log k_W/S$. By determining the gradient elution time in a linear solvent gradient for a series of standards with experimentally determined isocratic values for ϕ_o, the gradient retention times and the CHI can be placed on the same scale by establishing the coefficients A and B in Eq. (15)

$$CHI = At_g + B \qquad (15)$$

Once the regression model is established for the standard compounds, the CHI for any compound can be calculated from its retention time in the same gradient system. The CHI is a system property and depends on the identity of the stationary phase, the type of organic modifier and for ionizable compounds the pH of the mobile phase. Thus, the CHI can be considered a high throughput method since typical gradient cycle times are 15 min for a 15 cm column [78] or 5 min for a 5 cm column [81]. Calibration of the separation system minimizes the effect of variations in gradient elution times on different columns of the same kind and for different instruments. The three hydrophobicity parameters for a separation system ($\log k_W$, ϕ_o, CHI) are significantly correlated with each other but are not identical [79]. Each parameter is also moderately correlated to $\log P$, but the fit to a linear model (e.g. Eq. (4)) depends on compound identity. One source of general disagreement is associated with differences between the real system and the assumptions used for the derivation of the linear solvent strength model. The second problem is that to be a successful model for the estimation of $\log P$ the fundamental characteristics of the separation system that explain retention must be strongly correlated with those responsible for partition in the octanol–water system. This requirement is no different to the situation for isocratic models and the failure of the CHI to model $\log P$ with the desired accuracy can be laid at the same door. A comparison of the system properties with those of the octanol–water partition coefficient can be made using the solvation parameter model (see Table 3) [79–81]. The system constant ratios for the systems employed by Valko and coworkers [78–82] are similar in character but different to the octanol–water partition coefficient. In particular, the a/v ratio for $\log P$ is close to zero so that $\log P$ is virtually insensitive to solute hydrogen-bond acidity whereas all the chromatographic systems are significantly influenced by solute hydrogen-bond acidity. Changing the organic solvent for the mobile phase from acetonitrile to methanol only resulted in a CHI scale further removed from the properties of the octanol–water partition coefficient. The best model for estimating $\log P$ from the CHI is obtained by including an additional term to account for the difference in solute hydrogen-bond acidity that requires computer estimated values for the A solute descriptor when these are unavailable from experimental sources [81].

$$\log P = 0.054\,(CHI) + 1.35A - 1.88,$$
$$n = 86,\ r^2 = 0.941,\ \text{S.E.} = 0.29,\ F = 655 \qquad (16)$$

This model allow $\log P$ for neutral compounds to be estimated to about 0.30 log units, but computational techniques for estimating solute descriptors are not well developed at present, and values for all structures may not be available, and in other cases some predictions will be inaccurate. An improvement in the general model for $\log P$ was obtained by including an index for the hydrogen-bond count, HBC (the number of hydrogens on the molecule that are able to form hydrogen bonds) [81]

$$\log P = 0.047\,(CHI) + 0.36\,(HBC) - 1.10,$$
$$n = 86,\ r^2 = 0.889,\ \text{S.E.} = 0.39,\ F = 336 \qquad (17)$$

Eq. (17) is certainly not as good as Eq. (16) for estimating $\log P$, but as long as the compound structure is known a value for the hydrogen-bond count would be available. Even so, with a typical uncertainty in estimated values of $\log P$ of 0.39 log units, there are better methods available for estimating $\log P$.

Donovan and Pescatore [83] proposed a high throughput gradient method for estimating $\log P$ using a short (2 cm × 4.6 mm i.d.) column packed with a porous polymer (poly(vinyl alcohol) esterified with octadecyl groups) and a gradient from 10 → 100% (v/v) methanol over 7 min. Aqueous buffers of pH 2, 10, or 13 were used to ensure compounds were separated in their neutral form. Toluene and triphenylene were added to each sample as internal standards to minimize variation in gradient retention times from run-to-run and instrument-to-instrument. The internal standards also facilitated scaling of the gradient retention times to force the slope of the model to one and intercept to zero. The method is described as providing fair accuracy and good precision for compounds with $\log P$ values between 2 and 6. There is good reason to believe that the results are no better than for other gradient methods. The system constants for the stationary phase ($\log k_W$ as the dependent variable) are significantly different to those for the octanol–water partition coefficient, particularly for compounds with significant hydrogen-bond acidity [9]. The descriptive statistics for the fit of the estimated $\log P$ values to their literature values is only modest ($n = 120$, S.E. = 0.43, $r^2 = 0.884$ and $F = 875$).

3.3. Reversed-phase thin-layer chromatography

The separation mechanism for thin-layer chromatography is the same as for liquid chromatography, and thus, it is reasonable to anticipate that the limitations identified in liquid chromatography apply equally to thin-layer chromatography. This is certainly true in part, except that the structure and bonding density of the stationary phases used for reversed-phase thin-layer chromatography are optimized in a different manner to those used in column liquid chromatography to promote acceptable flow of the mobile phase by capillary forces [27,84,85].

Table 3
Gradient elution reversed-phase chromatographic systems for estimating $\log P$

Separation system	Dependent variable	System constant ratios				
		v	e/v	s/v	a/v	b/v
Octanol–water	$\log P$	3.81	0.15	−0.28	0.01	−0.91
Chemically bonded phases						

Stationary phase: Inertsil ODS2-1K5 [79]
  Gradient: $0 \to 100$ acetonitrile over 9 min
  Aqueous buffer pH 2.6, 7.3, or 9.5
	CHI	68.4	0.05	−0.19	−0.42	−1.05

Stationary phase: IAM PC2 [80]
  Gradient: $0 \to 100$ acetonitrile over 9 min
  Aqueous buffer pH 2, 5.7, 6.1, or 7.4
	CHI	50.7	0.15	−0.17	0.14	−1.04

Stationary phase: Luna C-18(2) [81]
  Gradient: $0 \to 100$ acetonitrile over 2.5 min
  Aqueous buffer pH 2, 7.4, or 10.5
	CHI	67.7	0.07	−0.23	−0.35	−0.97

Stationary phase: Luna C-18(2) [81]
  Gradient: $0 \to 100$ methanol over 2.5 min
  Aqueous buffer pH 2, 7.4, or 10.5
	CHI	52.2	0.04	−0.17	−0.17	−0.85

Thin-layer chromatography has several attractive features for high throughput methods. Samples are separated in parallel and accessible for chemical reactions for convenient detection of compounds lacking a chromophore. Cost considerations are favorable and modern instrumentation affords a high level of automation and data processing capabilities. Although it should be noted that retention in thin-layer chromatography is affected by several parameters that need to be standardized to obtain reproducible results [27,85–87]. If separations are performed carelessly the data obtained can be unacceptable for modeling purposes. Early studies of the use of reversed-phase thin-layer chromatography for estimating $\log P$ are reviewed elsewhere [87–92]. In this section the role of the solvation parameter model for identifying suitable models for estimating $\log P$ is discussed as well as prospects for further use [9,93].

Many of the thin-layer chromatographic methods proposed for estimating $\log P$ are restricted to small data sets of compounds with similar properties. An important exception is the study by Dross et al. [87], which was re-evaluated by Abraham et al. [94] using the solvation parameter model. Dross proposed the use of the R_{MW} value obtained by extrapolation from methanol–water mobile phase compositions to estimate $\log P$. Abraham showed that the retention properties captured by the R_{MW} values was similar to those of the octanol–water partition coefficient but not identical to it (Table 1). Similar to the results presented for reversed-phase column chromatography, the hydrogen-bonding properties of the system show the largest deviation from those for the octanol–water partition system. This explains why the correlation between R_{MW} and $\log P$ for a varied group of compounds is only modest

$$R_{MW} = -0.040 + 0.974 \log P,$$
$$n = 78, \ r^2 = 0.963, \ \text{S.E.} = 0.267, \ F = 1992 \qquad (18)$$

Table 4
Reversed-phase thin-layer chromatographic systems in the database of system constants

Layers (Merck HPTLC)	Solvent system	Reference
RP-18 WF 254s	Methanol (0–90% v/v)	[95]
	2-Propnaol (0–70% v/v)	[95]
	2,2,2-Trifluoroethanol (0–50% v/v)	[95]
	Acetone (0–90% v/v)	[95]
	N,N-Dimethylformamide (0–60% v/v)	[95]
	Pyridine (0–50% v/v)	[98]
	Acetonitrile (0–80% v/v)	[95]
	Water	[95]
CN F 254s	Methanol (0–90% v/v)	[96]
	2-Propanol (0–80% v/v)	[96]
	2,2,2-Trifluoroethanol (0–80% v/v)	[96]
	Acetone (0–80% v/v)	[96]
	N,N-Dimethylformamide (0–80% v/v)	[96]
	Pyridine (0–50% v/v)	[98]
	Acetonitrile (0–80% v/v)	[96]
	Water	[96]
	Methanol + acetonitrile ternary mixtures	[97]
DIOL F 254s	Methanol (0–50% v/v)	[98]
	Acetone (0–60% v/v)	[98]
	Water	[98]

Aromatic and nitrogen heterocyclic bases were significant outliers and had to be excluded from the correlation model. Thus, Eq. (18) is restricted to neutral compounds with low proton basicity and cannot be considered a general model.

A large database of system constants for reversed-phase thin-layer chromatography with a wide range of mobile phases is available (see Table 4) [9,95–98]. This database can be searched for chromatographic systems that have properties correlated with $\log P$ (see Table 5). No system is an exact match for $\log P$. Several are similar to $\log P$ but have an incorrect contribution from solute dipolarity/polarizability

Table 5
System constant ratios for reversed-phase thin-layer separation systems with properties similar to the octanol–water partition coefficient

Stationary phase	Mobile phase (% v/v)	System constant ratios				
		v	e/v	s/v	a/v	b/v
Octanol	Water	3.81	0.15	−0.28	0.01	−0.91
Merck RP-18	20% Dimethylformamide	1.68	0.21	−0.11	−0.14	−0.86
	30% Dimethylformamide	1.44	0.16	−0.14	−0.15	−0.93
	60% Dimethylformamide	0.80	0.26	−0.18	0	−0.84
	15% Pyridine	1.41	0	−0.15	0	−0.87
Merck CN	30% Methanol	1.72	0.24	0	0	−0.92
	10% 2-Propanol	2.31	0.17	0	0	−0.87
	20% 2-Propanol	2.05	0.17	0	0	−0.95
	10% Acetonitrile	2.24	0.20	0	0	−0.90

Dependent variable, R_M.

(s/v) or hydrogen-bond acidity (a/v). Thus, several of these systems could be used to estimate $\log P$ for compounds that are either weakly dipolar/polarizable or hydrogen-bond acidic but none are suitable for estimating $\log P$ for a varied group of compounds.

3.4. Micellar, microemulsion and vesicle electrokinetic chromatography

The prospects for identifying suitable micellar or microemulsion electrokinetic separation models for esti-

Table 6
Surfactant system for micellar, microemulsion, and vesicle electrokinetic chromatography

Surfactant	Abbreviation	System constant ratios				
		v	e/v	s/v	a/v	b/v
Octanol–water partition coefficient		3.81	0.15	−0.28	0.01	−0.91
Alkane sulfates and sulfonates						
Sodium octyl sulfate	SOS	2.85	0.16	−0.11	−0.04	−0.66
Sodium decyl sulfate	SDecS	2.69	0.12	−0.09	0	−0.59
Sodium dodecyl sulfate	SDS	2.98	0.12	−0.14	−0.08	−0.64
Sodium dodecyl sulfonate (36 °C)	SDSu	2.84	0.12	−0.15	−0.01	−0.63
Sodium tetradecyl sulfate (35 °C)	STS	3.01	0.09	−0.11	−0.06	−0.60
Sodium N-lauroyl-N-methyltaurine	SLMT	2.88	0.18	−0.12	0.14	−0.82
Sodium N-dodecanoyl-N-methyltaurine	SDMT	3.07	0.23	−0.16	0.07	−0.84
Bile acids						
Sodium cholate	SC	2.45	0.26	−0.19	0	−0.93
Sodium deoxycholate	SDC	2.67	0.25	−0.18	0	−0.93
Sodium taurocholate	STC	2.43	0.25	−0.14	0	−0.85
Sodium taurodeoxycholate	STDC	2.62	0.26	−0.17	0	−0.83
Miscellaneous anionic surfactants						
Sodium N-lauroylsarcosinate	SLN	2.98	0.14	−0.12	0.15	−0.78
Sodium N-myristoylsarcosinate	SMN	2.99	0.16	−0.14	0.15	−0.82
Sodium dodecoxycarbonylvaline	SDCV	2.99	0.14	−0.19	0.05	−0.81
Sodium lauryl sulfoacetate (36 °C)	SLSA	2.97	0.16	−0.13	0.04	−0.82
Cationic surfactants						
Tetradecyltrimethylammonium bromide	TTAB	2.99	0.10	−0.07	0.29	−0.91
Hexadecyltrimethylammonium bromide	CTMAB	3.40	0.18	−0.16	0.17	−0.91
Microemulsion						
1.44% (w/w) SDS + 6.49% (w/w) butan-1-ol + 0.82% (w/w) heptane						
pH 7.0		3.05	0.09	−0.23	−0.02	−0.92
pH 10		2.24	0.16	−0.23	0	−0.88
pH 3		2.16	0.19	−0.23	0	−0.93
Vesicles						
Bis(2-ethylhexyl)sodium sulfosuccinate (40 mM) + 10% methanol	AOT	3.09	0.11	−0.14	0	−0.98
Hexadecyltrimethylammonium bromide-sodium octyl sulfate (30:70) total mass 1.8% (w/w)	CTAMB-SOS	2.85	0.19	−0.20	0.08	−1.14

mating $\log P$ are excellent. This is demonstrated by the system constant ratios for representative separation systems summarized in Table 6 [9,25]. Sodium dodecyl sulfate is the most popular surfactant for MEKC and was among the first surfactants used for estimating $\log P$ by MEKC [99–107]. Its modest success is explained by the large difference in hydrogen-bond basicity, and to a lesser extent, dipolarity/polarizability, for partition into sodium dodecyl sulfate micelles compared with the octanol–water partition system. A better model is provided by sodium N-dodeconyl-N-methyltaurine, although this surfactant has not been used to estimate $\log P$ [108]. As a group, the bile salts posses favorable properties for estimating $\log P$, and several of these surfactants have been used for this purpose [65,103,105,106,108,109]. Cationic surfactants studied so far possess the wrong blend of hydrogen-bond basicity and dipolarity/polarizability to provide suitable models for estimating $\log P$ [103,108,110]. The microemulsion prepared from sodium dodecyl sulfate, butan-1-ol and heptane has virtually identical system constant ratios to $\log P$ and is widely used as a separation model for estimating $\log P$ [109,111–117]. Mixed surfactant micelles and inclusion of organic solvents in the separation buffer allows fine tuning of the separation properties of ionic micelles, increasing the number of available separation systems suitable for estimating $\log P$ (see Table 7) [118–120].

Vesicles are self-assembling, organized structures with a continuous bilayer of monomers enclosing an aqueous core region used as potential synthetic membrane models. Synthetic vesicles can be formed from oppositely charged surfactants and double-chained anionic surfactants (see Table 6) [121,122]. Synthetic vesicles formed from bis(2-ethylhexyl)sodium sulfosuccinate in a phosphate buffer containing 10% (v/v) methanol and the mixed surfactant hexadecyltrimethylammonium bromide-sodium octyl sulfate provide suitable separation models for estimating $\log P$ [122]. The hydrogen bond acidity of the mixed surfactant vesicle is slightly different to the octanol–water partition system while the bis(2-ethylhexyl)sodium sulfosuccinate-aqueous methanol system is a better fit overall (see Table 6). Both systems, however, were suitable for estimating $\log P$ for a varied group of neutral compounds to about 0.23 log units.

To summarize, there are numerous micellar, microemulsion, and vesicle electrokinetic chromatographic systems that might be used to estimate $\log P$, and several provide (nearly) equivalent results. Consequently, there is little need to explore all possible systems. Several groups found the microemulsion discussed below suitable for estimating $\log P$. It is a stable, easy to prepare and use separation system with partitioning properties for neutral compounds almost identical to the octanol–water partition system.

The microemulsion containing sodium dodecyl sulfate 1.4% (w/w), n-butanol 6.49% (w/w), and n-heptane 0.82% (w/w) in an aqueous 0.05 M sodium phosphate–0.1 M sodium borate pH = 7 buffer was introduced by Ishihama and co-workers for the estimation of $\log P$ for neutral and weakly basic compounds [110,111]. Abraham and co-workers used the solvation parameter model to confirm that retention in the microemulsion system was strongly correlated with the octanol–water partition system [113].

Table 7
Affect of the concentration of non-ionic surfactant (Brij® 35) and organic solvent on system properties in MEKC

Concentration			System constant ratios				
Brij® 35 (mM)	Solvent	% v/v	v	e/v	s/v	a/v	b/v
Sodium N-dodecanoyl-N-methyltaurine (50 mM)							
0			3.07	0.23	−0.16	0.07	−0.84
5			3.07	0.25	−0.18	0.07	−0.89
12			3.20	0.23	−0.16	0.09	−0.90
20			3.08	0.23	−0.14	0.09	−0.92
30			3.17	0.22	−0.14	0.10	−0.92
40			3.00	0.19	−0.12	0.08	−0.94
50			3.09	0.19	−0.12	0.11	−0.96
20	Acetonitrile	5	3.21	0.21	−0.15	0.10	−0.90
20		10	2.80	0.25	−0.16	0.10	−1.01
20		15	2.57	0.19	−0.18	0	−0.89
20		20	2.20	0.16	−0.13	0	−0.97
20	Methanol	20	2.64	0.21	−0.13	0.11	−0.95
20	Propan-2-ol	20	2.40	0.20	−0.18	0	−0.92
20	Tetrahydrofuran	20	2.34	0.27	−0.15	0	−1.00
Sodium dodecyl sulfate (50 mM)							
10			3.00	0.21	−0.14	0	−0.88
15			2.67	0.25	−0.14	0	−0.98
20			2.80	0.28	−0.14	0	−0.96
25			3.06	0.23	−0.14	0.07	−0.94
35			3.08	0.24	−0.14	0.08	−0.96

The model obtained for 53 varied neutral and weakly basic compounds is given below

$$\log P = 1.28 \log k + 1.54,$$
$$n = 53, \ r^2 = 0.996, \ \text{S.E.} = 0.096, \ F = 5738 \qquad (19)$$

and allowed $\log P$ to be estimated to about 0.1 log units. Gluck and co-workers extended the method to a wider range of weakly acidic and basic compounds using electrolyte solutions buffered to pH 12 and 1.19 [114]. Separations with the pH 1.19 buffer required operation with a negative (reversed) potential resulting in loss of information for the electro-osmotic flow marker required for calculation of retention factors. This had to be determined in a separate experiment at pH 1.19 with a positive potential. This is not convenient for high throughput measurements. In addition, the high ionic strength of the pH 1.19 buffer resulted in poor peak shapes. Poole and coworkers introduced sulfonic acid coated capillary columns for the estimation of $\log P$ of weakly acidic compounds with a pH 3 buffer and overpressure at the inlet vial to reduce the separation time [109]. For a varied group of weakly acidic and neutral compounds at pH 3 and 30 °C the following correlation model was obtained

$$\log P = 1.46 \,(\pm 0.06) \log k + 1.46 \,(\pm 0.06),$$
$$n = 42, \ r = 0.971, \ \text{S.E.} = 0.28, \ F = 652 \qquad (20)$$

and for neutral and weakly basic compounds determined at pH 10 and 30 °C with an uncoated fused-silica capillary column

$$\log P = 1.60 \,(\pm 0.06) \log k + 1.35 \,(\pm 0.05),$$
$$n = 45, \ r = 0.979, \ \text{S.E.} = 0.27, \ F = 991 \qquad (21)$$

The difference in the slope and intercept for the two calibration models arises from the fact that the system constants for the two separation systems are slightly different at pH 3 and 10. A second set of 29 varied compounds not included in the calibration set was used to validate the model for neutral and weakly basic compounds (see Fig. 1). The compounds covered the $\log P$ range 0.3–5.8 and the difference between estimated $\log P$ values and accepted literature values was
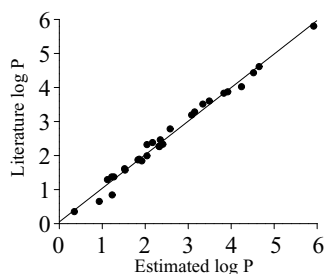

Fig. 1. Plot of literature $\log P$ against estimated $\log P$ for a group of varied neutral and basic compounds by microemulsion electrokinetic chromatography at pH 10. (With permission from Poole et al. [109].)

±0.12 log units with an average R.S.D. in estimated $\log P$ values of 4.3% ($n = 10$). This method is suitable for the high throughput estimation of $\log P$ to about 0.30 log units with a cycle time of about 30 min per sample. Unattended and overnight operation is fully supported.

Klotz and coworkers used a microemuslsion with a slightly different composition (2.16% (w/w) sodium dodecyl sulfate, 6.49% (w/w) *n*-butanol and 0.82% (w/w) *n*-heptane) to the system described above to estimate $\log P$ for neutral and weakly basic compounds at pH 7 [117]. The relative concentration of sodium dodecyl sulfate was increased to extend the migration window to obtain a higher peak capacity. This is of less interest for high throughput measurements since samples are typically single compounds or simple mixtures. This system might be useful, however, for estimating $\log P$ at either extreme of the $\log P$ range by improving the separation of the sample from the electro-osmotic flow and microemulsion phase marker compounds. Marker compounds are added to the separation system for calculation of retention factors. For a varied group of (mainly) neutral compounds including 80 pesticides with a range of $\log P$ values between −1 and 7 the correlation model obtained was

$$\log P = 1.90 \,(\pm 0.03) \log k + 1.18 \,(\pm 0.05),$$
$$n = 119, \ r^2 = 0.968, \ \text{S.E.} = 0.31 \qquad (22)$$

The separation efficiency of microemulsion electrokinetic chromatography (MEEKC) is significantly higher than for column liquid chromatography and the preparation of microemulsions is expected to be more reproducible than the synthesis of chemically bonded sorbents for liquid chromatography. In addition, all compounds elute in a fixed migration window in MEEKC and, therefore, gradient separation conditions or problem extrapolation methods are not required. This facilitates automated and unattended operation. MEEKC (and MEKC), however, is only suitable for estimating $\log P$ of neutral compounds or weak acids and bases after ion suppression. Ionized compounds are subject to additional electrophoretic migration and electrostatic interactions with the charged components of the separation system that affect retention but are unrelated to the partition mechanism for neutral compounds between the oily phase of the microemulsion and the aqueous buffer. In addition, retention factors for ionized compounds are not expected to correlate with $\log D$. Correction for the electrophoretic component of the retention factor is possible, but is not straightforward, or easily incorporated into high throughput methods [105,108,109]. The retention factor for partially ionized compounds is restated as

$$k = \frac{\mu_a - \mu_{\text{eff}}}{\mu_{\text{eff}} - \mu_M}, \qquad (23)$$

where μ_a and μ_M are the electrophoretic mobility of the solute in the aqueous phase and the oily phase of the microemulsion, respectively, and μ_{eff} is the effective mobility

of the solute in the microemulsion solution. Of these parameters, μ_{eff} and μ_M can be determined in a single MEEKC separation, but μ_a must be determined in a separate and independent capillary electrophoretic separation. The separation conditions for measurement of μ_a must be close to those used for the microemulsion separation without the presence of the oily phase. For the type of varied and complex structures encountered in industrial research calculation methods for μ_a are unfortunately unavailable.

4. Indirect methods using experimentally determined solute descriptors

In previous sections, we have shown that the solvation parameter model affords a useful method for identifying separation systems that provide suitable models for estimating $\log P$ without resorting to largely unproductive trial and error experiments. Since the system constants of the solvation parameter model for $\log P$ are reliably established, an alternative approach to estimate $\log P$ is to determine the solute descriptors for each compound for which an estimate of $\log P$ is required. Five solute descriptors for each compound are required for this purpose (see Eq. (5)). The descriptor V is calculated quite simply from the molecular formula and the number of rings in the molecule and the descriptor E either from the observed or calculated refractive index for the compound [17,25,26,29]. The remaining descriptors (S, A, and B), however, must be found from experiment. These descriptors are usually determined from a series of solute property models with established system constants in a process analogous to that used to determine system constants or by alternative mathematical models. Suitable solute properties include liquid–liquid partition, chromatographic retention, solubility, etc. [17,26,33]. A minimum of three systems with properties as different as possible are required to obtain reliable descriptors. For statistical evaluation and error protection, additional systems are beneficial. This is a disadvantage for high throughput methods, since it requires several different and independent experimental measurements to estimate the solute descriptors. Even if these tasks are performed in parallel, it represents increased work and capital outlay compared with methods that estimate $\log P$ from a single experiment. There are two factors, however, which suggest that these objections may be a lower barrier in the future than at present.

Although $\log P$ is widely used in many QSAR models to estimate critical properties for evaluation of activity, toxicity, environmental fate, transport properties, etc., most of these models exhibit only modest predictive ability and/or possess a narrow application range [8,9,16,42,123]. This is not too surprising given the chemical diversity of the systems modeled and it is somewhat illogical to expect a single descriptor to fit all situations. The solvation parameter model accommodates system diversity by changes in the system constants for each model, while all models use the same set of solute

descriptors. The greatest single advantage of the solute descriptor method is that once the descriptors are known a wide range of partition and related processes can be predicted directly. Contemporary applications include such diverse processes as the prediction of aqueous solubility [124], blood-brain distribution and permeation [125–127], human intestine absorption [127–129], skin permeation [126,130], uptake of organic compounds by cells in culture [131], nasal pungency and eye irritation thresholds [42], non-specific toxicity to fish [132] and microorganisms [133], and soil–water adsorption [14]. In virtually all these studies, $\log P$ was found to be either a poor or modest model for the estimated property compared with the solvation parameter model.

The objections to the use of solute descriptors for high throughput property estimations would disappear entirely if calculation methods based on structure for S, A, and B were available. Platts and co-workers used the database of known descriptor values to identify common substructures and relevant intramolecular interactions and evaluated their contribution to each descriptor [30]. The final model used 81 fragment values for E, S, and B with a separate set of 51 fragments for calculation of A. Errors of -0.5 to $0.15 \log$ units (for values covering a range of 2–6 log units) were found. This has to be compared with the typical error of 0.03 units in experimental values for A and B. The commercially available software *Absolv* for Windows PCs (Sirius Analytical Instruments; http://www.sirius-analytical.com) estimates solute descriptors by this approach. The generality of the method is limited, however, by the lack of experimental data for important fragments. If a fragment is not present in the database then no values can be assigned. Calculations employing quantum mechanics were explored with some success to estimate descriptor values for fragments not present in the database [134,135]. The error for these estimated descriptor fragments is still relatively large compared with experimental values. Platts and co-workers used software-derived descriptors to predict $\log P$ for a data set of over 8000 compounds [31]. The accuracy of the predictions was stated to be at about the middle for a number of software products available for calculation of $\log P$ from structure. This tends to confirm that current methods for calculating descriptors from molecular structure lack the accuracy and application range to render experimental methods redundant for the time being. In fact, one method of improving predictive methods is to assemble further experimental values for molecules with greater structural diversity than those represented at present in the descriptor database. Thus, in the near term, the experimental determination of solute descriptors for molecules of a wide structural diversity will be of increasing importance. Finally, it is hoped that calculation methods for descriptors can be improved to the point that they will replace experimental methods. This is important for lowering the cost of property estimations in a high throughput environment; for screening virtual compound libraries to eliminate poor synthetic targets; and to obtain values for highly reactive compounds otherwise dif-

ficult or impossible to study by conventional experimental techniques.

5. Conclusions

Separation methods have many favorable qualities for the high throughput estimation of the octanol–water partition coefficient. They are fast, robust, require little material that need not be pure, economical, and use automated equipment commonly found in analytical laboratories. The principal requirement for the development of robust methods for a wide range of compound types is that the fundamental properties of the separation system responsible for retention are highly correlated with the same properties responsible for partition in the octanol–water system. For neutral compounds the solvation parameter model provides a powerful tool to identify suitable separation systems conforming to this requirement. A significant outcome of this approach is that reversed-phase column and thin-layer chromatography provide a poor choice of separation systems because the system properties that control retention are quite different to those responsible for partitioning in the octanol–water system. By contrast, among the micellar and microemulsion systems used for electrokinetic chromatography many suitable separation systems with properties similar to the octanol–water partition system can be found. In particular, the fundamental properties responsible for partitioning in the electrolyte solution-microemulsion phase containing sodium dodecyl sulfate, *n*-butanol, and *n*-heptane fit the need almost exactly for a correlation model for the octanol–water partition system. Future developments will probably include less dependence on $\log P$ as a single descriptor for property estimations in favor of a suite of models tailored to individual distribution and transport properties based on the solvation parameter model. Emphasis will change to focus on the requirements for high throughput experimental and calculation methods to estimate solute descriptors for use in the solvation parameter model. The solvation parameter model itself could be made more useful by incorporating an additional descriptor or descriptors to allow the conjoint prediction of properties for neutral and ionized compounds.

6. Nomenclature

a	contribution of hydrogen-bond basicity to system properties
A	effective solute hydrogen-bond acidity
b	contribution of hydrogen-bond acidity to system properties
B	effective solute hydrogen-bond basicity
e	contribution of electron lone pair interactions to system properties
$\log k$	logarithm of the chromatographic retention factor in liquid chromatography and micellar electrokinetic chromatography
E	solute excess molar refraction
$\log D$	logarithm of the octanol–water partition coefficient when the compound participates in a secondary chemical equilibrium in either or both phases (e.g. ionization, aggregation, ion pair formation, etc.)
$\log k_{\mathrm{W}}$	logarithm of the chromatographic retention factor resulting from the extrapolation of $\log k$ values to zero organic solvent for binary mobile phase mixtures in liquid chromatography.
$\log P$	logarithm of the octanol–water partition coefficient for a neutral substance or an ionizable substance in its neutral form.
$\mathrm{p}K_{\mathrm{a}}$	negative logarithm of the acid–base dissociation constant
R_{F}	retardation factor for thin-layer chromatography
R_{M}	$\log\left[\frac{1-R_{\mathrm{F}}}{R_{\mathrm{F}}}\right]$
s	contribution of dipole-type interactions to system properties
S	solute dipolarity/polarizability
v	difference in cohesion and dispersion interactions between two condensed phases
V	McGowan's characteristic volume

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