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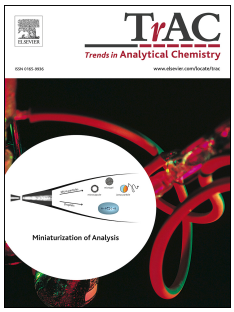

State of the art and prospects of methods for determination of lipophilicity of chemical compounds

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State of the art and prospects of methods for determination of lipophilicity of chemical compounds

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Abstract

Lipophilicity of the compounds is useful to (i) explain their distribution in biological systems, which is different in plant and in animal organisms, (ii) predict the possible pathways of pollutant transport in the environment, and (iii) support drug discovery process and select optimal composition in terms of bioactivity and bioavailability. The lipophilic properties can be determined by two main approaches, experimental, which apply instrumental techniques or computational, which is based on the complex algorithms. This review focuses primarily on various analytical methods that are used in the lipophilicity measurements. The classical methods and others based on chromatographic, electroanalytical and electroseparation approaches are compared and described in details. Modern solutions with chromatographic systems and their practical applications in the measurements of lipophilic and biomimetic properties of compounds have been included. However, there is an urgent need to standardize the high-throughput and reliable analytical procedure of the evaluation of lipophilic properties.

Abbreviations:

ACN, Acetonitrile; AGP, α_1-Acid glycoprotein; CD, Conductivity detector; CE, Capillary electrophoresis; CHI, Chromatographic hydrophobicity index; CMC, Critical micelle concentration; CTAB, Cetyltrimethylammonium bromide; CV, Cyclic voltammetry; DDT, Dichlorodiphenyltrichloroethane; DMSO, Dimethyl sulfoxide; ECD, Electron capture detector; ED, Electrochemical detector; EDC,1,2-Dichloroethane; EKC, Electrokinetic chromatography; ELSD, Evaporative light scattering detection; EOF, Electroosmotic flow; ESI-TOF-MS, Electrospray ionization time-of-flight mass spectrometry; FID, Flame ionization detector; FLD, Fluorescence detector; GC,

Gas chromatography; HSA, Human serum albumin; IAM, Immobilized artificial membrane; IPA, Isopropyl alcohol; IP-HPLC, Ion-pair high performance liquid chromatography; LI, Lipophilicity Index; IUPAC, International Union of Pure and Applied Chemistry; MeOH, Methanol; MEKC, Micellar electrokinetic chromatography; MEEKC, Microemulsion electrokinetic chromatography; MOPS, 4-Morpholinepropanesulfonic acid; MP, Mobile phase; MS, Mass spectrometry; NMR, Nuclear magnetic resonance; NP, Normal phase; OD-PVA, Octadecyl-poly(vinyl alcohol); ODS, Octadecyl-bonded silica; OECD, Organization for Economic Co-operation and Development; PBS, Phosphate buffer saline; PDMS, Polydimethylsiloxane; PGDP, Propylene glycol dipelargonate; PBS, phosphate buffer saline; PS, polystyrene; PS-DVB, Polystyrene-divinylbenzene; RID**,** Refractive index detector; RP-HPLC, Reversed-phase high performance liquid chromatography; RP-TLC, Reversed-phase thin-layer chromatography; RT, Retention time; RTILs, Room-temperature ionic liquids; SDS, Sodium dodecyl sulfate; SFM, Shake-flask method; SP, Stationary phase; SPME, Solid phase microextraction; SSM, slow stirring method TBAB, Tetrabutylammonium bromide; TEA, Triethylamine; THF, Tetrahydrofuran; TMAC, Tetramethylammonium chloride; UV–Vis, ultraviolet-visible spectrophotometry; VEKC, Vesicular electrokinetic chromatography.

D, Distribution coefficient; k, Retention factor (liquid chromatography); k', Retention factor (electroseparation methods); K, Distribution constant; K_{oa}, n-octanol-air partition coefficient; K_{oc}, organic carbon-water partition coefficient; P (K_{ow}), n-octanol/water partition coefficient; pKa, acid dissociation constant.

Keywords:
Lipophilicity; Shake-flask method; Potentiometric titration; High-performance liquid chromatography; Thin-layer chromatography; Capillary electrophoresis; Electrokinetic chromatography; Cyclic voltammetry

Contents

1. Introduction

According to International Union of Pure and Applied Chemistry (IUPAC) lipophilicity is the affinity of a molecule or a moiety for a lipophilic environment. It is commonly measured by the distribution behavior in a biphasic system [1], either liquid-liquid or solid-liquid system. This physicochemical property of a compound describes the balance between two major contributions: hydrophobicity and polarity. Hydrophobic effect relates to the tendency of non-polar compounds to prefer a non-aqueous environment to an aqueous one. Lipophilicity extends the hydrophobicity because of the polar term related to electrostatic interactions and hydrogen bonds. Therefore, lipophilicity comprises the favorable solute-solvent interactions that contribute to the distribution of a solute between two media: water and organic solvents. Other specific solubilizing media as biomembranes are also considered [2].

Different methods enable the measurement of solute-solvent interactions and based on them the lipophilicity of a compound can be determined. Many various lipophilicity descriptors have already beenproposed, however the background point is the n-octanol-water partition coefficient P expressed as logP or $\log K_{ow}$ [3]. It is defined as the ratio of the concentrations of a neutral compound or an ionizable compound in its neutral form in n-octanol (C_O) and aqueous (water; C_W) phases under equilibrium conditions (Eq. 1) [1,4–6].

$$\log P = \log(C_O/C_W) \quad (1)$$

Moreover, if a compound undergoes ionization in the aqueous phase another lipophilicity descriptor called distribution coefficient (logD) is used. It takes into account all forms of a compound, neutral and ionized, present at a given pH. For weak monoprotic acids or bases that are partially ionized in the

aqueous phase, distribution and partition coefficients are related through Eq. 2 and Eq. 3, respectively:

$$\log D_{acids} = \log P - \log(1 + 10^{pH - pKa}) \quad (2)$$

$$\log D_{bases} = \log P - \log(1 + 10^{pKa - pH}) \quad (3)$$

where pK_a is acid dissociation constant. According to these equations (Eq. 2, 3) the solubility of an ionizable compound increases exponentially with the difference between pH and pKa [7].

The lipophilic properties of the compounds allow to characterize chemicals according to several aspects. First, they help to explain distribution of the compounds and to predict their transport in different biological systems considering the fact that the relations between lipophilic/hydrophilic properties are different in plant and animal organism. The second aspect considers the prognosis of the pollutant pathway in the environment. This knowledge allows to determine compound ability to be transported in and between the environment compartments or the possible place of its accumulation [3,8]. Partition coefficient K_{ow} is a useful index of the potential for bioaccumulation, bioconcentration and biomagnification of environmental pollutants. It has been assumed that bioaccumulation does not occur for substances with K_{ow} value lower than 2000 ($\log K_{ow}$ <3.3), whereas easy bioaccumulated substances with tendency to biomagnification through food chain exhibit $\log K_{ow}$ higher than 5 [9], which can be determined by some of the methods listed in Table 1. This coefficient is also related with n-octanol-air partition coefficient (K_{oa}) that describes partitioning between atmosphere and terrestrial environment or the organic carbon-water partition coefficient (K_{oc}) that is useful in the prediction of the mobility of organic soil contaminants [10–13]. The lipophilic properties are also useful for chemicals in respect to their optimal attribute for specific tasks in the industry [2,4].

The pathway of drugs and food components to reach their target site is complex since adsorption depends on solubility and permeability. Both properties are influenced by the lipophilicity of the compounds, however, in a different way. Moreover, the degree of ionization affects compound's lipophilicity and it influences its solubility and permeability thus absorption too [5,14–17]. The absorbed compounds undergo metabolic transformations, which can result in the changes of physicochemical properties such as the molecule size or mass, charge and lipophilicity [18,19]. Due to the complex nature of bioactive compounds in drugs and food as well as their different mechanism

of absorption and metabolism [7,20] the lipophilic characteristics performed on biomimetic stationary phases (SPs) are useful tools in the assessment of biological effects of these components. Consequently, the specific partition coefficients determined with the application of immobilized artificial membrane (K_{IAM}), human serum albumin (K_{HSA}) and α_1-acid glycoprotein (K_{AGP}), have been proposed [21].

Therefore, the lipophilic characteristics of environmental pollutants, food-derived bioactive compounds and drug ingredients can help to better understand their fate outside and inside the living organisms and to build models for biological absorption and partition processes trying to predict *in vivo* distribution of potential bioactive molecules (i.e. nutraceuticals and pharmaceuticals) [22,23].

Considering above, logP value of the solute allows to propose the fate of the chemical in the body as it describes compound ability to reach its intended target. Moreover, the lipophilicity of the compounds can be used as input parameters to design models for prediction of environmental transport of pollutants between water phase and natural organic matter in soil and sediments including the transport by biological membranes. In addition, the distribution between water and soil sediments relates also to chemicals of food chain and those undergoing the ingestion [3,13,16].

In recent years, the studies of lipophilic properties of bioactive compounds, including pharmaceuticals and natural products, as well as toxins, including environmental pollutants, have become a topic of increasing interest. In fact, a literature search performed on the Web of Science and Scopus databases revealed that the number of publications on lipophilicity and partition coefficient has quadrupled or even increased fivefold (Fig. 1) in the past two decades (28084 or 71601 until 1997, while between 1998-2018 around 153805 or 274422 scientific papers have been published according to Web of Science and Scopus search engine, respectively).

This tendency has provided development of different approaches in lipophilicity determination (see Table 1). We propose to divide them into classical methods and a set of methods based on different backgrounds: chromatographic, electroseparation, potentiostatic or voltamperometric (Fig.2). The well recognized classical methods are shake-flask technique or stir-flask technique, whereas a variety of chromatographic methods (reversed-phase thin-layer chromatography (RP-TLC) and reversed-phase high-performance liquid chromatography (RP-HPLC)) are today the most popular ones [24,25]. Main advantages and limitations of the lipophilicity determination techniques are shown in Table 1 and will

be discussed in details further. The logP values can be also calculated using various computer softwares or Internet available modules that applied different algorithms based on structural, atomistic, topological, electrotopological, or other considerations on a drawn chemical structure [26].

149 **Table 1.** Comparison of methods for determination of lipophilicity based on their usefulness, main advantages and limitations.

	SFM	**SSM**	**Potentiometric titration**	**TLC**	**HPLC**	**CE**	**EKC methods**	**CV**
Measurement	Direct	direct	direct	indirect	indirect	indirect	indirect	indirect
Log*P* range	Classic: -3 < logP < 4 Miniaturized: -2 < logP < 6 SPME-based: 0 < logP < 5	logP > 5	-1 < logP < 8	4 < logP	-3 < logP < 8	0 < logP < 5	-1 < logP < 7	-8 < logP < 1
Sample amount required	Classic: 10-50 mg Miniaturized: 1-10 mg SPME-based: <2 mg	10-50 mg	2-50 mg	2-10 mg	<1 mg	<1 mg	1-2 mg	1-10 mg
Partition solvent	n-octanol, aqueous buffer systems	water, n-octanol	n-octanol, KCl	water and organic solvent (e.g. MeOH, ACN, THF, dioxane)	aqueous buffer, ACN or MeOH	buffers	buffers, surfactants	1,2-dichloro-ethane or nitrobenzene, buffers
Consumption of organic solvent	Classic: high Miniaturized: low SPME-based: solventless	high	low	very low	Isocratic: low to medium Gradient: very low	low	low	medium
pH range	0 < pH < 14	0 < pH < 14	1.8 < pH < 12.2	2 < pH < 11	Isocratic: 1.5 < pH < 10.5 (13*) Gradient: 1 < pH < 12 (13*)	2 < pH < 12	2 < pH < 12	0 < pH < 14
Apparatus required	Classic: basic glassware and labware, UV-Vis spectrophotometer or LC system (LC-UV, LC-MS) or NMR detection Miniaturized: multi-well plates, liquid handling robot, shaker and centrifuge for plates, highly sensitive quantification system	basic glassware and labware, GC or LC system with appropriate detection (e.g. FID, ECD, MS, UV)	potentiometric titrator with a set of electrodes	manual or fully automated TLC system with detection unit (UV, FLD); fast, reproducible, high-throughput, but more expensive	LC system with various detection (UV, FLD, MS, MS/MS)	CE system with CD, ED, FLD, UV or MS detection	CE system with CD, ED, FLD, UV or MS detection	typical apparatus for electrochemical measurements

	(LC-UV/MS, LC-MS/MS, NMR); high-throughout, but more expensive approach SPME-based: SPME device, GC or LC system with appropriate detection (e.g. FID, ECD, MS, UV)			approach				
Time consumption	Classic: ~1 day Miniaturized: 10-30 min; up to 200 compounds/day SPME-based: up to 2 h (depending on type of SPME fiber used	2-4 days	up to 30 min; determination of lipophilicity profile of one compound	30-60 min; simultaneous analysis of several to several dozen compounds	Isocratic: 30-120 min (depends on the dimension of the column used); several compounds in single run Gradient: 5-20 min; up to tens of compounds per run	up to 60 min; simultaneous analysis of several to several dozen compounds	15-30 min; 20-30 compounds per run (2 h) in multi-well plate format	up to 30 min; single measurements
Lipophilicity index determined	Classic, miniaturized: logP SPME-based: logK	logP	logD, $logP^N$, $logP^I$	R_M, R_{M0}	Isocratic: logk, $logk_w$, S, CHI, logP Gradient: CHI, k_g, logP, logD	logP	logk', logP	$logP^{o,i}$
Application	Neutral and ionizable compounds (only in their neutral form), limited to highly hydrophobic and sparingly soluble compounds	Highly hydrophobic compounds in neutral form	Ionizable compounds with acid-base properties	Neutral compounds, ionizable compounds only in their unionized form (appropriate mobile phase pH and composition is required)	Neutral compounds, ionizable compounds only in their unionized form (appropriate mobile phase pH and composition is required)	Cations and anions of inorganic and organic salts	Neutral compounds	Ionizable compounds

* pH limit for polymer-based stationary phases of HPLC columns; CD – conductivity detector; ECD – electron capture detector; ED – electrochemical detector; FID – flame ionization detector; FLD – fluorescence detector; SPME – solid-phase microextraction; CHI – chromatographic hydrophobicity index; D – distribution coefficient; k – retention factor in LC methods; k' – retention factor in EKC methods; k_g – apparent gradient capacity factor; k_w – retention factor extrapolated to zero organic phase concentration; K – distribution constant; P – n-octanol/water partition coefficient; P^I – partition coefficient of ionized forms; P^N – partition coefficient of neutral forms; R_M - retardation parameter; S – slope of the linear relationship between the organic solvent concentration (φ) and the logk

2. Classical methods for lipophilicity determination

The oldest and the most known method of lipophilicity measurement, shake-flask method (SFM), is based on liquid-liquid extraction using n-octanol/water system [24]. The analyzed substance is dissolved in two flasks, the first one filled with aqueous buffer solution and the second one containing an organic solvent (n-octanol). Both solutions should be saturated before the compound introduction, buffer with n-octanol, and n-octanol with water. These solutions are introduced to the laboratory separator and then shaken to achieve equilibration of the solute between the aqueous and organic phases as shown in Fig.3a [27]. After the equilibrium is reached, UV-Vis spectrophotometry or other appropriate analytical method is employed to determine the concentration of the compound dissolved in each phase [23].

Due to the simplicity and evident correlation with partitioning phenomenon, SFM is recommended as a benchmark procedure for the other methods. However, reliable measurement of lipophilicity by this method is only achievable in the logP range from -3 to 4 [27–29]. Furthermore, the procedure is tedious and time-consuming and requires relatively large amount of pure solutes. In addition, there is a possibility of formation of stable emulsions after the shaking step [28,30–33]. Emulsion in n-octanol/water system can be a serious problem, particularly in the case of the hydrophobic compounds. The $\log K_{ow}$ depends on relative solubility of the compound in water and organic solvents and it has to be corrected for ionization. Moreover, due to amphiphilic properties, some compounds may behave as detergents [23,30,34]. Modifications of this classical method (Fig.3) enable to handle with some of limitations described above. In case of model of biological partition, different solutions are used to determine the partition coefficient. Aside from n-octanol/water set, which is characterized by the properties of hydroxyl group connected with function of hydrogen-bond donor and acceptor, some other solvents that allow to imitate different physiological cell barriers have been proposed. These systems include chloroform and water (a set with largely proton donors) or alkane (e.g. cyclohexane or dodecane) and water (a set without hydrogen acceptors and donors) or propylene glycol dipelargonate (PGDP) and water (a set with largely proton acceptors as in phospholipid membranes). PGDP, chloroform, n-octanol and cyclohexane are known as solvents that encoding important hydrogen bonding properties. Due to hydrogen bonding effects, the values of partition coefficient measured in these four solvents are different but their forces account for membrane partitioning. Lipophilicity parameters measured experimentally for these solvent systems are partially

dependent on the reference system and vary from classical n-octanol/water partition coefficient. The n-octanol/water model does not reflect the drug partitioning in biological structures anymore, thus these critical quartet (chloroform, cyclohexane, n-octanol and PGDP) has been proposed to be used instead of one organic solvent [35,36].

Miniaturization of SFM has led to the development of the high-throughput methods for screening of new targets with pharmacological effects. In traditional procedure even hundreds milliliters of each phase was used during the extraction. Nowadays, the volume of organic and aqueous solvents has trimmed down to less than 1 mL and the 96-well plate format of SFM has been presented [37]. The miniaturized SFM may be coupled with sensitive detection technique, such as mass spectrometry (MS). The LC-UV/MS systems have been successfully applied for the final determination step and the obtained results were in good correlation with literature values. The biggest advantages of the miniaturized method are rapidity, great flexibility, the use of small amount of solutes, the extended range of measured logP (from -2 to 6) and the potential to be fully automated. However, the miniaturization does not exclude the emulsion problem, especially for hydrophobic compounds [23,37–39].

In case of poorly water soluble substances, the lipophilicity may be determined by automated continuous sampling method, called filter probe method. This method is simple, partially automated and time-saving. Furthermore, computer program monitoring showed that filter probe method ensured greater accuracy and reproducibility [40]. However, during the analysis of the partition coefficient of highly hydrophobic compound, the concentration of this compound in organic phase is much higher than that in aqueous phase. In such case, contamination of aqueous phase by n-octanol layer is usually occurring during sampling. To solve this problem, the water-plug aspiration/injection method has been developed. In this method, the sample is taken by a small syringe with needle filled with a few microliters of water as a plug. This is expected to prevent contamination while the needle is passing through the n-octanol layer to reach sample in the aqueous layer, because the water plug stops the entrance of the n-octanol into the needle [38].

Another modification of classical methods applied for the determination of logP is the procedure based on nuclear magnetic resonance spectroscopy (NMR). The extraction step takes places in NMR tubes, content of which is vigorously mixed for 20 minutes. The concentration of analyte in water

phase is measured twice: before addition of the n-octanol and after the extraction. Then, P value is calculated from the following equation (Eq. 4):

$$P = (I_w - I_{wo})/I_w \quad (4)$$

where I_w and I_{wo} are the intensity of the signal of analyte in pure water and in water with n-octanol added, respectively. This method was proposed by Cumming and Rücker and so far it has been used to determine the partition coefficient of some common solvents, such as acetone and tetrahydrofuran (THF) [41]. Moreover, it is well-suited for fast and easy measurement of n-octanol-water partition coefficient of the compounds with sufficient water solubility and logP in the range from -1 to 1.

Another variation of the classical methods is the solid phase microextraction (SPME) developed by Pawliszyn [42]. During the solventless extraction, the sample partitions between aqueous phase (blood, urine or environmental water) and a fused silica fiber coated with a polymer. In SPME, the equilibrium is established as the partition of analytes between the stationary and aqueous phase, and it depends on distribution coefficients (D), the temperature of the sample, stirring rate, the ionic strength of the solvent and the thickness of the fiber coating [43–45]. The amount of analyte adsorbed by the fiber (N) at the equilibrium is defined by the following equation (Eq. 5):

$$N = K \cdot V_s \cdot C_0 \quad (5)$$

where V_s is the volume of the SP, C_0 is the initial concentration of analyte in aqueous phase, and K is the distribution constant of analyte partitioning between the aqueous and stationary phase. However, when the value of distribution constant is high and the volume of sample is small, there is a possibility that analytes are mainly presented on the SP. In that case, the Eq. 6. should be used. According to this equation, the distribution coefficient is defined as:

$$K = (N \cdot V_{aq})/(V_s \cdot (V_{aq} \cdot C_0 - N)) \quad (6)$$

where V_{aq} is the volume of aqueous phase [46,47].

In 1996 Dean et al. proposed the use of the fused-silica fiber coated with polyacrylate for determination of the K of phenols. They confirmed that K values determined by SPME method can be correlated with logP values. Their results indicated that SPME was an appropriate method for estimating logP. Currently, the non-polar fiber coatings (i.e. polydimethylsiloxane (PDMS)) are commonly used in this technique. Studies have shown that distribution coefficients obtained by SPME with PDMS coating also correlate well with $\log K_{ow}$ values [47,48]. SPME coupled with gas chromatography, and less often with LC, and MS detection enables the determination of minimal

quantities of highly non-polar compounds in aqueous phase and minimizes the loss of volatile compounds [49].

Slow stirring method (SSM) follows the same principle as SFM. However, the emulsion formation is limited. Although this method allows to determine the logP of neutral compounds, it is time-consuming, rather expensive and requires a large amount of sample [50–52].

3. Separation and electroanalytical methods for f lipophilicity determination

Currently, classical methods of lipophilicity determination are almost totally replaced by indirect methods that include chromatographic, electrochemical and electroseparation methods. In contrast to time-consuming equilibration, chromatography and other techniques provide measurement of extended range of lipophilicity during rapid analysis, in which the sample impurities usually do not affect the measurements [24,53].

3.1. Chromatographic methods

Chromatographic methods have been applied for the lipophilicity determination since 1970s [54,55]. Due to their many advantages and relatively few limitations, nowadays they are the most popular experimental indirect methods for estimating logP values.

3.1.1. Thin-layer chromatography based methods

TLC is a chromatographic technique used for separation of non-volatile mixtures. The first application of TLC was the determination of impurities in pharmaceutical preparations and since 1938 this technique has been applied in diverse fields of chemistry. The adaptability of TLC may offer lots of new possibilities to evaluate lipophilic character [24]. In this case, different reversed-phase modes (see Table 2) are commonly used where the stationary phase is a foil coated with thin layer of silica or aluminum derivatives, both modified with hydrophobic ligand bounded covalently or by absorption, and the mixture of water and water-soluble organic solvent works as the mobile phase (MP) [36]. The retention of analytes can vary during changing the content of organic solvent in the MP and the activity of SP is based on the contribution of many specific parameters influencing the chromatographic behavior. The most important ones are the chemical structures of the sorbents, the surface area, the density of the free active centers per unit of sorbent surface area and the energy of intermolecular interactions between a molecule and a type of sorbent active centers. These

parameters affect the data obtained for measured lipophilicity of molecules, so the standardization of the TLC procedure in relation with biological impact is needed [24,56].

The most popular chromatographic lipophilicity descriptor is the retardation parameter (R_M) defined by Bate-Smith and Westall through the following formula (Eq. 7) [57]:

$$R_M = \log((1/R_f) - 1) \quad (7)$$

where the R_f is the retention factor, which is calculated as the ratio of the migration distances of solute and the solvent front. The R_M value depends linearly on the concentration of organic modifier in MP and this relationship is described by a TLC adapted Soczewiński-Wachtmeister equation [58,59] (Eq. 8):

$$R_M = - S \cdot \varphi + R_{Mw} \quad (8)$$

The value of R_{Mw} is extrapolated to pure water as a MP. The regression slope (S) is directly linked to specific surface area of SP and is considered also as an alternative lipophilicity descriptor. The last factor, φ, represents the volume of organic solvent in the MP [24,58,59].

The RP-TLC is easy to perform and rapid. It requires small amount of the samples and allows the analysis of several compounds simultaneously. Moreover, there is no need to test pure compounds or to perform the problematic quantitative analysis. This technique is especially suitable for the investigation of compounds with low water solubility (logP > 4). Furthermore, the use of TLC plates coated with RP material allows to overcome some of the disadvantages associated with SFM, because the reversed-phase mode simulates the process of n-octanol-water partitioning [23,25].

Recently, new approaches in the lipophilicity determination using TLC have been reported [60–62]. Janicka et al. have used micellar TLC and over-pressured-layer chromatography (OPLC) [60,63], where surfactants (anionic sodium dodecyl sulfate (SDS), cationic cetyltrimethylammonium bromide (CTAB) and non-ionic Brij-35) are used as the modifiers of MP. Due to amphiphilic character of micelles formed, both non-polar and polar interactions between them and solutes take place during the analysis. As a consequence, these systems are supposed to be more similar to biomembranes than in classical TLC [64]. Another method applied for the lipophilicity evaluation is normal-phase TLC (NP-TLC), where MP contains two organic solvents, polar (e.g. acetone, ethanol, ethyl acetate) and non-polar (e.g. benzene, cyclohexane, carbon tetrachloride, toluene). An excellent review of these TLC approaches for lipophilicity studies has been published lately [59].

3.1.2. Liquid chromatography based methods

The use of RP-HPLC as indirect method for the determination of lipophilic properties has been the subject of several reviews [6,23,65–68]. Nowadays it has become one of the most commonly used procedures in the lipophilicity studies recommended by Organization for Economic Co-operation and Development (OECD). In general, this method is based on dynamic partitioning of a compound between two immiscible phases, solid and liquid (SP of the column and MP) (Fig. 4), which is consistent with the IUPAC definition of partition coefficient [69]. Based on the solvophobic theory the interaction between the solute and the SP is considered as a reversible association of the solute molecules with the SP moiety. The distribution of the compound between the SP and MP is directly related to the chromatographic retention time (RT, t_R). The solute retention factor (k) is proportional to the ratio of the average number of analyte molecules in the SP (n_s) to the average number of molecules in the MP (n_m) during the chromatographic elution (Fig. 4). This lipophilicity index (LI) is usually expressed using a logarithmic scale and can be related to the distribution constant (K) of the compound between the MP and SP, as shown in Figure 4. Therefore, the retention of dissolved compound is governed by this equilibrium constant [70].

Both equations shown in Figure 3 provide the theoretical basis for the partition data obtained from retention of the compound in the selected chromatographic system. In contrast to the determination of compound concentration required within the classical methods, only RT measurements are necessary to determine the LIs by RP-HPLC procedures and thus it is the main indicator of a real partition process. This chromatographic separation-based approach also provides other practical advantages: speed up the experimental work, good reproducibility, process automation, broad dynamic range, on-line detection (mainly UV-Vis or refractive index detector (RID)), small amount of sample required, independence of measurements from low compound solubility as well as impurities or degradation products. However, some limitations of the RP-HPLC method have also been noted, including (i) insufficient modeling of the n-octanol-water system for structurally diverse compounds, (ii) pore size effects for sorbents filling chromatographic column have no counterpart in the n-octanol-water partition system, (iii) possible interactions with the surface of the SP that not occur in the n-octanol-water system, (iv) time-consuming isocratic measurements in some cases, (v) limited pH working range for most of the SPs (2.0-7.5). In order to overcome these drawbacks, some solutions have been recently introduced, just to mention novel types of columns [21,71,72] that are designed to

mimic the n-octanol-water system or biological membranes and let to operate over a wide pH range (1.0-12.0). Moreover, application of short columns with smaller inner diameter results in acceleration of experiments and reduction of costs, especially reduced consumption of organic solvents. Separations using micellar MPs, and gradient elution procedures have been proposed lately [60,70,73,74].

3.1.2.1. Chromatographic partition systems for measuring lipophilic properties

One of the most important aspects in the lipophilicity assessment by HPLC is to develop a chromatographic system that mimics the standard n-octanol-water partition system as closely as possible. Therefore, various SPs as well as MP modifying agents have been introduced and tested for this purpose. Improved or newly-developed SPs for the lipophilicity studies were summarized by Kaliszan [75] and Giaginis et al. [76] and they include mainly silica-based, polymer-based and biomimetic phases as shown in Table 2. Octadecyl-bonded silica (ODS) and other alkyl groups bonded to silica core are one of the most commonly used SPs for HPLC-based lipophilicity measurements. However, the possibility of polar moieties interactions, including hydrogen bonding or electrostatic attraction, with the remaining free-silanol groups on the silica surface may affect the partitioning mechanism of RP-HPLC and thus results in increases in RTs and peaks asymmetry [67,75]. Therefore, the studies of lipophilic character of ionizable basic compounds on silica-based columns can be difficult and give overestimated results [77,78].

Some improvements of silica-based SPs have been recently proposed. End-capping of the free-silanol sites by short alkyl groups (i.e. trimethylsilyl group (TMS)) is usually performed in order to provide higher degree of silanization [79] and thus make the column packaging material more suitable for analysis of strong hydrogen-bonding and ionized compounds [80]. Another solution for reducing effect of residual silanols is embedding or end-capping polar groups (i.e. amide, carbamate, ether, sulfonamide or ammonium) in the alkyl chains [72]. Due to accurate mimicking of biopartitioning and good correlation with K_{ow}, alkylamide-silica HPLC columns are one of the most frequently applied phases of this type [71,81]. The possibility of use of MP with a high water content or even pure water without the risk of hydrophobicity collapse of these type of SPs is an additional advantage. However, the polar moieties incorporated into the silica backbone may interact with some analytes, i.e. polyphenols and thus addition of masking agents is also required [67,82]. Octanol-coated SPs were

successfully applied for the estimation of logP of neutral or basic compounds [83,84]. On the other hand, the problems associated with long-term stability of these columns may occur [6].

In order to overcome pH limitation of ODS columns and to make the lipophilicity assessment of basic analytes in their neutral form possible, new generation of SPs have been developed. They include: grafted polymer-silica hybrid columns [74,85,86], columns with hybrid organic-inorganic silica in which hydroxyl groups are replaced by methyl ones, and columns based on the bidentate technology that include a propylene bridge [71,72]. These types of column are protected from silanol interactions and have an extended pH range capability up to 12 [82,86].

More recently, the polymer-based SPs, including octadecyl-poly(vinyl alcohol) (OD-PVA), and polystyrene-divinylbenzene (PS-DVB) based columns, have also been successfully applied for the lipophilicity measurements [87–90]. In contrast to silica-based SPs, the polymer-based resins are rigid, macroporous, cross-linked polymers completely free of silanol groups and other polar sites, and hence irreversible binding of polar compounds is eliminated [77]. Furthermore, these columns are chemically inert in most organic solvents and stable over a wide pH range (1-13). However, their retention mechanism is governed by a different balance of structural properties (mainly dipolarity/polarizability parameter) as determined by linear solvation/free-energy relationships and thus obtained results may correlate better to alkane-water than to n-octanol-water partitions, which mimics the blood-brain partitioning more reliably [88,90]. Further evaluation of retention behavior on polymer-based SPs is needed for better use in the lipophilicity studies.

As an alternative choice for a more accurate description of compound distribution between various compartments *in vivo*, the SPs that could directly mimic biologically important elements and provide biomimetic characteristic are increasingly used in recent years. These biomimetic SPs include IAMs, liposomes and plasma proteins (i.e. HSA, AGP). The theoretical and practical aspects of using biomimetic columns and their detailed characterization have been the subject of several reviews [21,68,76,91–94]. The retention factors of compounds obtained using protein-based SPs can be easily converted to binding parameters such as %HSA = 100×k/(k+1). These columns provide potential to simulate plasma protein binding, as retention mechanism incorporates other interactions than in n-octanol-water partitioning, especially those of electrostatic nature. It should be noted that since protein binding occurs naturally only at physiological conditions, there is no need to use MPs

with different pHs. Verification of protein SPs stability is also essential and can be easily ensured by HPLC analysis of racemic mixture of warfarin that should reveled the separation of enantiomers [21,68].

IAM columns introduced and patented by Pidgeon et al. [95] to model the lipid bilayers of the cells are prepared by covalent binding of phospholipids monolayers (i.e. phosphatidylcholine) to amine-modified silica support. IAM columns are highly stable with little phospholipid loss during analyses or storage [96] and commercially available, including the single chain and double chain SPs, which differ in the end-capping of free propylamine residues [97]. It is reported that double chain IAM phases better simulates the structure of natural phospholipids and hence the resulting lipophilicity indices correlate better with permeability data [98,99]. The amphiphilic character of phospholipid functional groups play an important role in IAM retention especially if charged molecules are analyzed. Thus, electrostatic interactions also affect the retention mechanism, which is mainly governed by hydrophobic/solvophobic interactions [81,97]. It should be emphasized that pure water can be used as a MP in IAM chromatography, which allows fast and direct determination of logk value extrapolated to zero organic phase concentration referred as $\log k_w$. The addition of acetonitrile as organic modifier is recommended, when compounds with high affinity for the IAM SP are analyzed and then $\log k_w$ values require extrapolation. A novel SPs that may simulate cell membrane partitioning in the similar way as the IAM phases were introduced. They are *N,O*-dialakylphosporamidate-based materials having in their structure amine groups, phosphate groups and hydrophobic long alkyl chains [100]. Summarizing, the future trends in column development for the lipophilicity studies could be immobilization of other important proteins, enzymes or membrane lipids on the SP or introduction of similar functional groups to the silica surface and measure compound's interactions with them.

The mixtures of water and organic modifiers with some additives are commonly applied as MPs of chromatographic partitioning systems for the lipophilicity measurements. To speed up RP-HPLC analyses, especially of highly lipophilic compounds, methanol and acetonitrile are the most widely used modifiers. Methanol appears to be the most suitable organic solvent for the lipophilicity studies because it does not disturb the hydrogen-bonding network of water. On the other hand, acetonitrile, which generates the most asymmetrical peaks for basic analytes, proves to better simulate the 'organic phase' [67,76]. The correlations between chromatographic hydrophobicity index (CHI, φ_0) and logP values reported by Valko et al. using fast gradient RP-HPLC procedures showed that acetonitrile

serves as a better organic modifier than methanol for both ODS and IAM columns [70,101]. According to the solvation equation, this results from the significant difference between the CHI_{MeOH} lipophilicity scale and the logP scale in terms of H-bond acidity, H-bond basicity, size and dipolarity/polarizability. In order to match the CHI_{ACN} scale with logP scale only H-bond acidity term should be considered thus acetonitrile was suggested as the preferred organic modifier by Valko et al. [70]. THF and isopropyl alcohol (IPA) are also used in some lipophilicity assays [73,102]. Due to the dissociation of most analytes in aqueous MPs, some chemical additives are required. These MP additives have been extensively discussed in many reviews [21,67,76,81,82] and therefore, only some important will be highlighted here. They can be divided into two main groups: ion suppressors and masking agents. In order to suppress dissociation of ionizable analytes and keep them in a neutral form different buffers have been applied, including morpholinepropanesulfonic acid, phosphate buffer and phosphate-buffered saline [82,84]. Ammonium acetate buffers are also used because they exhibit good compatibility with mass spectrometry. Acetic or perchloric acid and ammonia or triethylamine (TEA) were employed as ion suppressors in the lipophilicity assessment of weak acidic and weakly basic compounds, respectively [103,104]. Masking agents including hydrophobic amines (i.e. TEA, *n*-decylamine, *N,N*-dimethyloctylamine) and room-temperature ionic liquids (RTILs) are often used as MP additives to reduce or even to suppress silanophilic interactions. A small addition of amines (0.15-0.20%) is considered as the most suitable masking agents in combination with methanol as organic modifier [82,84]. Unlike amines, RTILs have no effect on the pH of MP. However, the use of RTILs complicates MS detection and may add noise or a background signal to UV detection [105,106]. In recent years, the addition of small amount of n-octanol to the methanolic MP has improved the HPLC determination of LIs. Almost 1:1 correlation between $\log k_w$ and logP or logD was obtained with n-octanol-modified MP [86,107].

437 **Table 2. Chromatographic partition systems used in lipophilicity studies and their applications.**

Type of stationary phase	Mobile phase composition	Other important conditions	Type of analytes/samples	Measured LI	Ref.
THIN-LAYER CHROMATOGRAPHY SYSTEMS					
Silica gel TLC plate modified with: *(1)* cyanopropyl groups (CN), *(2)* octadecyl carbon chain (C_{18}), both with F_{254} fluorescence indicator	*(1)* *(a)* Aqueous phase: CTAB Organic phase: ACN *(b)* Aqueous phase: SDS Organic phase: ACN *(2)* Aqueous phase: H_2O Organic phase: MeOH	Saturation: 20 min Visualization: mixture of MeOH and sulfuric acid Detection: UV	25 Aromatic compounds (e.g. ethylbenzene, eugenol, fenitrotion, nabumeton, phenol, vanillin)	R_M, R_{Mw}	[62]
Silica gel TLC plate modified with: *(1)* cyanopropyl groups (CN), *(2)* octadecyl carbon chain (C_{18}), *(3)* diol groups (DIOL), *(4)* octyl carbon chain (C_8), *(5)* dimethyl groups (C_2), all with F_{254} fluorescence indicator	*(a)* Aqueous phase: H_2O Organic phase: MeOH *(b)* Aqueous phase: H_2O Organic phase: dioxane	Saturation: 20 min Detection: densitometric scanning	Naproxen	R_{Mw}	[108]
Silica gel TLC plate modified with: *(1)* cyanopropyl groups (CN), *(2)* octadecyl carbon chain (C_{18}), *(3)* diol groups (DIOL), *(4)* octyl carbon chain (C_8), *(5)* dimethyl groups (C_2), all with F_{254} fluorescence indicator	Aqueous phase: H_2O Organic phase: ACN or MeOH	Saturation: 20 min Detection: UV	8 Cephalosporins	R_{Mw}, R_M, $PC1/R_M$	[109]
Silica gel TLC plate modified with octadecyl carbon chain (C_{18}) / F_{254} fluorescence indicator	Aqueous phase: H_2O Organic phase: ACN or MeOH or acetone	Saturation: 20 min Detection: UV	6 Statin drugs	R_{Mw}, C_0	[110]
Silica gel TLC plate modified with: *(1)* cyanopropyl groups (CN), *(2)* octadecyl carbon chain (C_{18}), *(3)* octyl carbon chain (C_8), *(4)* amino groups (NH_2), all with F_{254} fluorescence indicator	Aqueous phase: H_2O Organic phase: CAN	Saturation: 10 min Visualization: ethanolic solution of bromcresol green treated with NaOH Detection: UV	4 Artificial and 13 natural sweeteners	R_{Mw}, R_M	[24]

Silica gel TLC plate modified with: *(1)* cyanopropyl groups (CN), *(2)* octadecyl carbon chain (C_{18}), *(3)* diol groups (DIOL), *(4)* octyl carbon chain (C_8), *(5)* dimethyl groups (C_2), all with F_{254} fluorescence indicator	Aqueous phase: phosphate buffer Organic phase: MeOH	Visualization: fluorescamine/ 2,2-diphenyl-1-picrylhydrazyl Detection: UV	3 Amine neurotransmitters and 18 derivatives	R_M, R_{Mw}, $PC1/R_M$	[111]
Silica gel TLC plate modified with: *(1)* octadecyl carbon chain (C_{18}), *(2)* cyanopropyl groups (CN), both with F_{254} fluorescence indicator	*(1)* Aqueous phase: H_2O Organic phase: acetone, dioxane or MeOH *(2)* Aqueous phase: SDS Organic phase: THF	Saturation: 15 min Detection: UV	1,2,4-Triazoles (21 compounds)	R_{Mw}	[60]
Silica gel TLC plate modified with octadecyl carbon chain (C_{18}) / F_{254} fluorescence indicator	Aqueous phase: H_2O Organic phase: acetone or ACN or MeOH or THF or IPA	Detection: UV	15 Fluoroquinolones	R_{Mw}	[112]
Silica gel TLC plate modified with: *(1)* octadecyl carbon chain (C_{18}), *(2)* cyanopropyl groups (CN), both with F_{254} fluorescence indicator	Aqueous phase: H_2O Organic phase: MeOH	Saturation: 15 min Visualization: manganese chloride in sulfuric acid Detection: UV	Bile acids and their derivatives (27 compounds)	R_{Mw}, $PC1/R_M$	[113]
Silica gel TLC plate modified with cyanopropyl groups (CN) / F_{254} fluorescence indicator	*(1)* Aqueous phase: Brij (35) Organic phase: ACN *(2)* Aqueous phase: CTAB Organic phase: ACN *(3)* Aqueous phase: SDS Organic phase: acetone or dioxane or THF	Drying of plates: iodine vapor	13 Fatty acids and 4 polyphenols	P_{SW}, K_{ma}	[61]
Silica gel TLC plate modified with octadecyl carbon chain (C_{18}) / F_{254} fluorescence indicator	Aqueous phase: H_2O Organic phase: acetone or DMSO	Saturation: 15 min Detection: UV	4-Amino-7-chloroquinoline based compounds (18 compounds)	R_{Mw}, R_M	[114]
HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY SYSTEMS					
SILICA-BASED COLUMNS					
Kromasil C18 (250 mm × 4.6 mm, 5 µm particle size); Akzo Nobel / Eka Chemicals Inc.	Aqueous phase: H_2O (10%) Organic phase: MeOH (90%)	HPLC mode: isocratic Detection: RID	Non-ionic surfactants (alcohol ethoxylates) including highly hydrophobic substances (logP > 6)	logk, logP	[115]
Spherex C18 (250 mm × 4.6 mm, 5 µm particle size); Phenomenex Inc.	Aqueous phase: H_2O (35-90%) Organic phase: MeOH (10-65%) Masking agent: 10 mM TMAC	HPLC mode: isocratic Detection: UV (220 nm) Dead time marker: KBr	22 Penicilin drugs	logk, $logk_w$	[116]
C18 column (250 mm × 4 mm, 5 µm	Aqueous phase: H_2O (15%)	HPLC mode: isocratic	16 Polycyclic aromatic hydrocarbons	logk, logP	[117]

particle size)	Organic phase: MeOH (85%)	Detection: UV (254 nm)	(3.3 < logP < 6.3)		
Altima C18 (150 mm × 4.6 mm, 5 µm particle size); Hichrom Ltd.	Aqueous phase: 0.02 M MOPS buffer, pH 7.2 (25-70%) Organic phase: MeOH (30-75%)	HPLC mode: isocratic Detection: UV-Vis (215 and 500 nm) or RID	57 Terpenoids including monoterpene hydrocarbons and oxygenated terpenes: alcohols, aldehydes, ketones, acetates (1.81 < logP < 4.48)	logk, $logk_w$, logP	[118]
Gemini C18 hybrid silica-based columns (150 mm × 4.6 mm and 50 mm × 4.6 mm, 5 µm particle size); Phenomenex Inc.	Aqueous phase: *(1)* 20 mM Na_2HPO_4, pH 3.0 *(2)* 20 mM Na_2HPO_4, pH 7.0 *(3)* 20 mM $Na_2B_4O_7$, pH 10.0 Organic phase: MeOH (40-55%)	HPLC mode: isocratic Detection: UV (254 nm)	28 Pharmaceuticals including: basic (local anesthetics, β-blockers), acidic (non-steroidal anti-inflammatory drugs) and neutral (steroid hormones) drugs.	$logk_w$, S	[86]
Kromasil C18 (250 mm × 4.6 mm, 5 µm particle size); Akzo Nobel / Eka Chemicals Inc.	Aqueous phase: H_2O (20%) Organic phase: MeOH (80%)	HPLC mode: isocratic Detection: UV-Vis (λ_{max} for each compound) Dead time marker: $NaNO_3$	21 Persistent organic pollutants (POPs; 2.0 < logP < 7.0) including model compounds and synthetic organochlorine pesticides (DDT and DDT-related compounds)	$logk_w$, logP	[119]
XBridge-C18 column packed with bridged ethylene hybrid (BEH) particles (50 mm × 3 mm, 2.5 µm particle size); Waters Corporation	Aqueous phase: *(1)* 10 mM $HCOONH_4$ (pH: 2.5, 3.3, 4.1, 8.9, 9.7) *(2)* 10 mM CH_3COONH_4 (pH: 4.9, 5.8) *(3)* 10 mM NH_4HCO_3 (pH: 6.8, 10.5) Organic phase: MeOH	HPLC mode: two pH/organic modifier gradient sets Detection: ESI-TOF-MS (range 50-1200 m/z) Dead volume marker: citric acid	40 drugs including antibiotics, antidepressant, β-blockers, anti-arrhythmic agents, anticoagulants, antipsychotics, hypertensive drugs, anesthetic drugs, antispasmodic drugs, anti-inflammatory drugs, antifungal drugs, analgesic and antipyretic drugs	$logk_w$, logP, logD, pK_a*	[120]
LiChroCART Purosphere RP-18e (125 mm × 3 mm, 5 µm particle size), Zorbax Eclipse XDBC8 (150 mm × 4.6 mm, 5 µm particle size), Discovery RP-Amide C16 (150 mm × 4.6 mm, 5 µm particle size), LiChrospher 100 CN (250 mm × 4 mm, 5 µm particle size), and Kinetex PFP (150 mm × 2.1 mm, 2.6 µm particle size)	Aqueous phase: 0.1% HCOOH Organic phase: MeOH (50-70%)	HPLC mode: isocratic Detection: UV (254 nm) Dead time marker: uracil	22 antioxidant compounds including phenolic acids, flavonoids, anthocyanins, xantonoids, proanthocyanidins	logk, $logk_w$,	[121]
Luna C18(2) (150 mm × 4.6 mm, 5 µm particle size), Candeza CD-C18 (150 mm × 4.6 mm, 3 µm particle size), TSK-gel ODS-80TS (150 mm × 4.6 mm, 5 µm	Aqueous phase: 0.1% HCOOH Organic phase: ACN with 0.1% HCOOH	HPLC mode: gradient Detection: UV (254 nm)	21 antitumor acridinone (imidazoacridinone and triazoloacridinone) derivatives	$logk_w$, logP	[122]

particle size), Ascentis C18 (150 mm × 4.6 mm, 5 µm particle size), Unison UK-C18 (150 mm × 4.6 mm, 3 µm particle size), and Zorbax SB-C8 (75 mm × 4.6 mm, 3.5 µm particle size)					
LiChroCART LiChrospher RP-18e (250 mm × 4 mm, 5 µm particle size), LiChroCART Purosphere RP-18e (125 mm × 3 mm, 5 µm particle size), Zorbax Eclipse XDBC8 (150 mm × 4.6 mm, 5 µm particle size)	Aqueous phase: *(1)* H_2O with NH_3aq, pH 9.6 *(2)* H_2O with HCOOH, pH 2.8 Organic phase: MeOH	HPLC mode: isocratic Detection: UV (230 nm), MS Dead time marker: acetone	10 compounds with increased toxicity (mycotoxins and alkaloids) and 12 amines with important biological activity	k, k_0, S, PC1/k	[123]
POLYMER-BASED COLUMNS					
PRP-1column (150 mm × 4.1 mm, 10 µm particle size); Hamilton company and ACT-1 (150 mm × 4.6 mm, 10 µm particle size); Interaction chemicals	Aqueous phase: 0.1 M CH_3COONH_4, pH 4.6 (35 or 30%) Organic phase: ACN (65 or 70%)	HPLC mode: isocratic Detection: UV (210, 230 or 254 nm) Dead time marker: $NaNO_3$	40 Compounds from different classes: non-H bonders, single amphiprotics (with 1 hydroxyl or amide substituent), and double amphiprotics	logk	[124]
Asahipak ODP 50-4D (150 mm × 4.6 mm, 5 µm particle size); Shodex Group, Showa Denko K.K.	<u>HPLC experiments</u> Aqueous phase: *(1)* H_2O *(2)* 20 mM $NH_4H_2PO_4$, pH 2.0 *(3)* 20 mM $NH_4H_2PO_4$, pH 10.0 Organic phase: MeOH and ACN for experiments with each of the aqueous phases <u>IP-HPLC experiments</u> Aqueous phase: *(1)* 20 mM $NH_4H_2PO_4$, pH 7.0 *(2)* 20 mM $NH_4H_2PO_4$, pH 7.0 + 10 mm TBAB Organic phase: MeOH	HPLC mode: isocratic and gradient IP-HPLC mode: isocratic Detection: UV-Vis (λ_{max} for each compound)	72 Chemical compounds including 24 neutral compounds, 20 week acid compounds, 14 strong acid compounds, 14 basic compounds	$logk_w$, CHI, k_g	[87]
C18-derivatized PS-DVB column (Act-I, 50 mm length); Interaction chemicals	Aqueous phase: H_2O (40%) Organic phase: MeOH (60%)	HPLC mode: isocratic Detection: UV (230 nm) Dead time marker: $NaNO_3$	50 Different chemical compounds including non-hydrogen bonding compounds, acids/alcohols, bases, and hydrogen bonding acceptors (-0.20 < logP < 3.88)	logk, logP	[125]
ODP-50 column (20 mm × 4 mm, 5 µm particle size); Supelco	Aqueous phase: *(1)* 26 mM CF_3COOH, pH 2.0 *(2)* 10 mM Na_3PO_4, pH 7.0 *(3)* 10 mM Na_3PO_4, pH 10.0	HPLC mode: gradient Detection: UV (260, 285 nm)	16 Compounds with antimicrobial activity from group of 3(*2H*)-isothiazolones	logP	[126]

	Organic phase: MeOH				
PRP-1 column (150 mm × 4.6 mm, 5 µm particle size); Hamilton company	Aqueous phase: 25 mM CH_3COONH_4 buffer of pH *(1)* 4.5, *(2)* 7.2, *(3)* 9.8 Organic phase: ACN	HPLC mode: isocratic and gradient Detection: UV or ELSD	21 Commercially available drugs and 24 biologically active marine natural products	$logk_w$, CHI, logP	[77]
ODP-50 column (20 mm × 4 mm, 5 µm particle size); Supelco	Aqueous phase:10 mM Na_3PO_4 adjusted to pH *(1)* 2, *(2)* 7, *(3)* 10 Organic phase: MeOH	HPLC mode: gradient Detection: UV (260-285 nm)	120 Different chemical compounds including 26 pharmaceuticals, 24 fungicides, 25 herbicides, 25 insecticides and 20 miscellaneous	logP	[90]
Supelguard ODP-50 column (20 mm × 4 mm); Supelco	Aqueous phase: 15 mM potassium phosphate buffer adjusted to pH *(1)* 2.0 (H_3PO_4), *(2)* 7.4, *(3)* 11.0 (KOH) Organic phase: MeOH	HPLC mode: gradient Detection: UV (230, 260 nm)	15 β-blockers and structurally related compounds	logD	[89]
Asahipak ODP-50-4B column (50 mm × 4.6 mm , 5 µm particle size); Asahi Chemicals	Aqueous phase: 20 mM phosphate buffer prepared using *n*-octanol - saturated H_2O and adjusted to pH *(1)* 3.0, *(2)* 4.0, *(3)* 7.0 Organic phase: MeOH with addition of 0.25% *n*-octanol	HPLC mode: isocratic Detection: UV-Vis (λ_{max} for each compound) Dead time marker: uracil	Set of 41 compounds including model solutes (13 acids, 8 neutrals, 5 bases) and 15 drugs (-0.69 < logP < 4.80)	logk, $logk_w$, S	[88]
PLRP-S column (50 mm × 4.6 mm, 5 µm particle size), Agilent	Aqueous phase: 20 mM CH_3COONH_4, pH 7.0 Organic phase: ACN (10, 15 and 20%)	HPLC mode: isocratic Detection: UV-Vis	Amyloid β-peptides: $Aβ_{12–28}$ and $Aβ_{25–35}$	logk	[127]
PLRP-S column (50 mm × 4.6 mm, 5 µm particle size), Agilent	Aqueous phase (pH range 2-11): *(1)* 0.1% formate buffer *(2)* 0.1% CH_3COONH_4 *(3)* 10 mM triethylamine Organic phase: ACN (80%)	HPLC mode: isocratic Detection: UV (215, 254, 280, 310 nm) and MS Dead time marker: astemizole (pH ~ 2) valsartan (pH ~ 10)	3 Sets of compounds: *(1)* 44 neutral compounds (simple organic molecules, commercial drugs), *(2)* 10 compounds (5 pairs) capable and incapable to form intramolecular hydrogen bonds, *(3)* 76 commercial drugs	logk	[128]
BIOMIMETIC COLUMNS					
IAM.PC.DD2 column (150 mm × 4.6 mm, 10 µm particle size); Regis Technologies Inc.	Aqueous phase: *(1)* 0.1% (v/v) HCOOH, pH 2.8 *(2)* 10 mM CH_3COONH_4, pH 7.0 Organic phase: MeOH	HPLC mode: gradient Detection: UV (230, 254 nm)	32 Analogs of 4-hydroxycoumarin (biologically active compounds)	CHI	[129]

IAM.PC.MG (150 mm × 4.6 mm, 10 µm particle size) and IAM.PC.DD2 (100 mm × 4.6 mm, 10 µm particle size); Regis Technologies Inc.	Aqueous phase: 0.1 M phosphate buffer of pH *(1)* 7.0, *(2)* 5.5 Organic phase: ACN (15-30% if required)	HPLC mode: isocratic Detection: UV (λ_{max} for each compound)	10 Quinolone antibacterial agents, including both acidic and zwitterionic congeners	logk, $logk_w$, logP	[130]
IAM.PC.MG (150 mm × 4.6 mm, 10 µm particle size) and IAM.PC.DD2 (30 mm × 4.6 mm, 12 µm particle size) columns; Regis Technologies Inc., ChromTech chiral-HSA and ChromTech chiral-AGP (50 mm × 4 mm, 5 µm particle size) columns; Supelco	<u>For IAM columns:</u> *(1)* High purity water *(2)* 20 mM MOPS with pH of 4.2 and 7.4 *(3)* PBS with pH of 4.2 and 7.4 *(4)* 50 mM CH_3COONH_4, pH 7.4 <u>For the HSA and AGP columns:</u> *(1)* High purity water *(2)* PBS with pH of 4.2 (only AGP), 5.0 (only HSA) and 7.0 *(3)* 50 mM CH_3COONH_4, pH 7.0	HPLC mode: isocratic Detection: UV-Vis (205 nm)	11 Selenium species including methylseleninic acid, methylselenocysteine, dimethyl-selenourea, selenites Se(IV), selenates Se(VI), seleno-DL-methionine, L-selenocystine, selenocystamine, selenourea, dimethyl selenide, dimethyl diselenide	logk, $logk_w$, logD	[5,131]
IAM.PC.DD2 (100 mm × 3 mm), Chiralpack-HSA and Chiralpack -AGP (50 mm × 3 mm); Regis Technologies Inc.	Aqueous phase: 50 mM CH_3COONH_4, pH 7.4 Organic phase: ACN (IAM column), IPA (HSA and AGP columns)	HPLC mode: gradient Detection: UV (254 nm)	117 Marketed drugs	logk, logP	[102]
Chiral-HSA and Chiral-AGP (100 mm × 4 mm); Chrom Tech Ltd.	<u>For HSA column:</u> Aqueous phase: 10 mM phosphate buffer, pH 7.0 Organic phase: IPA (20% required only for nebivolol) <u>For AGP column:</u> Aqueous phase: 10 mM phosphate buffer, pH 7.0 (75% or 90%) Organic phase: MeOH	HPLC mode: isocratic Detection: UV (λ_{max} for each compound)	13 β-blockers (enantiomers) including acebutolol, alprenolol, atenolol, betaxolol, labetalol, metoprolol, nadolol, nebivolol, oxprenolol, pindolol, propranolol, sotalol, and timolol	logk, $logk_w$, logP, logD	[91]
Chiral-HSA (50 mm × 3 mm); Chrom Tech Ltd., and RexChrom IAM PC2 $(CH_2)_{12}$ (150 mm × 4.6 mm); Fisher Scientific	Aqueous phase: 50 mM CH_3COONH_4, pH 7.4 Organic phase: IPA (HSA column), ACN (IAM column)	HPLC mode: gradient Detection: UV (230, 254 nm)	68 Drug molcules	$logK_{HSA}$, CHI_{IAM}	[73,101]

Chiral-HSA (50 mm × 3 mm); Chrom Tech Ltd.	Aqueous phase: PBS, 0.157 M K^+/Na^+, pH 7.0 Organic phase: ACN and IPA (5-20%)	HPLC mode: isocratic Detection: UV (220, 254 nm)	63 Structurally diverse basic, acidic and neutral drugs	logk, S, $logk_w$	[132]
IAM.PC.MG (150 mm × 4.6 mm) and IAM.PC.DD2 (100 mm × 4.6 mm); Regis Technologies Inc.	Aqueous phase: 0.1 M phosphate buffer, pH 7.0 Organic phase: ACN (10-30% if required)	HPLC mode: isocratic Detection: UV (λ_{max} for each compound)	14 Basic drugs spanning a wide lipophilicity range	logk, $logk_w$	[133]
IAM.PC.MG (150 mm × 4.6 mm); Regis Technologies Inc.	Aqueous phase: 16 mM phosphate buffer, pH 7.4 Organic phase: ACN (4-27%)	HPLC mode: isocratic Detection: UV (λ_{max} for each compound; 225-290 nm)	11 Arylpropionic non-steroidal anti-inflammatory drugs	logk, $logk_w$	[134]
IAM.PC.DD2 (100 mm × 4.6 mm); Regis Technologies Inc.	Aqueous phase: 10 mM phosphate buffer of pH *(1)* 3.0, *(2)* 5.0, *(3)* 7.4 Organic phase: ACN (≤ 30% if required)	HPLC mode: isocratic Detection: UV or MS/MS	86 Monoprotic positively charged amines including monopolar amines, dipolar amines, amines with polar N moieties, polycyclic amine structures, and complex multifunctional amine structures	logk, $logk_w$	[135]

ACN – acetonitrile; AGP – α_1-acid glycoprotein; Brij 35 – polioxyethylene lauryl ether; CTAB – cetyltrimethylammonium bromide; DDT – dichlorodiphenyl-trichloroethane; DMSO – dimethyl sulfoxide; DVB – divinylbenzene; ELSD – evaporative light scattering detection; ESI-TOF-MS – electrospray ionization time-of-flight mass spectrometry; HSA – human serum albumin; IPA – isopropyl alcohol; IP-HPLC – ion-pair high performance liquid chromatography; LI – lipophilicity index; MeOH – methanol; MOPS – 4-morpholinepropanesulfonic acid; MS – mass spectrometry; PBS – phosphate buffer saline; PS – polystyrene; RID – refractive index detector; SDS – sodium dodecyl sulfate; TBAB – tetrabutylammonium bromide; THF – tetrahydrofuran; TMAC – tetramethylammonium chloride; * possibility to determine acid dissociation constant (pK_a) in the same HPLC run

3.1.2.2. HPLC procedures for lipophilicity assessment

As summarized in details in recent reviews [21,23,67,68,76], the HPLC-based procedures in isocratic and gradient elution mode have been widely used for determination of various LIs, mainly $logk_w$, φ_0 and S (slope of the linear relationship between the organic solvent concentration (φ) and the logk). Therefore, here only the main principles of most commonly applied HPLC approaches will be discussed.

Most of the reported procedures are based on isocratic measurements of compounds retention that is normalized by column dead time and expressed as retention factor (see Fig.4). In isocratic method, several HPLC analyses at different MP compositions (min. 4 various concentrations of organic modifier) must be carried out for each compound, which requires preliminary experiments and is more time-consuming. The obtained results should be extrapolated to the same MP composition, normally to pure aqueous phase. The Snyder-Soczewinski equation (Eq. 9) is the most commonly used for such extrapolation [136]:

$$logk = -S \cdot \varphi + logk_w \quad (9)$$

Although this relationship in non-linear in the full range of organic modifier concentration, a good approximation within the working limits of $-0.5 < logk < 1.5$ is ensured [68]. If necessary, a quadratic model (Eq. 10) can be applied:

$$logk = A \cdot \varphi^2 + B \cdot \varphi + logk_w \quad (10)$$

where A and B are regression coefficients. $Logk_w$ is regarded as the most representative LI, since its value is of the same order of magnitude as logP or logD. Both isocratic retention factors (logk or $logk_w$) are directly correlated to n-octanol-water logP/logD via Collander equation (Eq. 11):

$$logP/logD = a \cdot logk_w + b \quad (11)$$

where a and b are linear regression coefficients determined by analyzing a set of standard compounds according to OECD guidelines [69].

Assessment of LIs of highly lipophilic compounds using standard polycratic approach is difficult or rather impossible due to RTs being too long and thus analysis becomes very time-consuming and labor-intensive. Therefore, a new approach to determine $logk_w$ through the correlation with logk was introduced [137]. As the S and $logk_w$ parameters indicating mechanism of retention give a linear relationship for structurally related compounds (see Eq. 12) [28], the $logk_w$ value can be evaluated

based on logk measured only for the corresponding organic modifier concentration according to the following formula (Eq. 13):

$$S = a \cdot \log k_w + b \quad (12)$$

$$\log k_w = (b \cdot \varphi + \log k)/(1 - a \cdot \varphi) \quad (13)$$

where a and b correspond to the slope and the intercept, respectively.

In order to speed up the lipophilicity evaluation, various gradient HPLC methods have been proposed, and as a result a new LI namely CHI was introduced by Valkó et al. [138,139]. It links the isocratic and gradient retention together and represents the volume of organic modifier in MP for which the amounts of solute distributed between two phases are equal (k = 1, logk = 0). The CHI is computed as follows:

$$\varphi_0 = -\log k_w/S \quad (14)$$

It has been shown that gradient RTs (t_{Rg}) correlate well with isocratically determined CHI values and thus give a straight line when plotted against the CHIs obtained for given set of standards [70,138]. The slope (a) and the intercept (b) of the calibration equation (Eq. 15) are used to convert t_{Rg} of analytes to CHI scale (range of 0-100) that is useful for inter-laboratory study and database creation:

$$\varphi_0\,(\mathrm{CHI}) = a \cdot t_{Rg} + b \quad (15)$$

The CHI values can also be converted to logP scale. However, the best correlation was observed for neutral form of compounds. Considering the H-bond acidity or the H-bond count terms, the relationship between logP/logD and CHI can be improved as reported by Valkó et al. [70].

As an alternative LI, an apparent capacity factor (k_g) was introduced by Krass et al. [140] and defined as follows:

$$k_g = (V_g - V_d - V_m)/V_m \quad (16)$$

where V_g is gradient retention volume, V_d is system delay volume and V_m is column dead volume. A good correlation between k_g and $\log k_w$ obtained in a series of isocratic HPLC runs was reported.

Nowadays, the rapid-gradient RP-HPLC methods using high flow rate and short columns to reduce the analysis time (up to ~5 min) with a negligible loss of resolution have been widely applied for the lipophilicity assessment. The use of MS detection allows to determine CHIs for mixture of compounds, which further accelerates the experimental work [141]. Another improvement has been proposed by Wiczling et al. [120,142], who employed time-of-flight MS to enlarge the number of analytes in

individual HPLC run and to facilitate their identification in complex mixture. They have developed a double pH/methanol gradient procedure for the simultaneous determination of $logk_w$ and pK_a values based on two gradient RTs of a compound (one is an organic phase, the other is a pH gradient), with a screening rate of about 100 compounds/day.

Summarizing, using gradient RP-HPLC methods, reproducible LIs can be obtained in a high-throughput way. The current trend is also focused on the use of chemometric tools, such as principal component analysis, to extract the meaningful and interpretable features from the multivariate HPLC raw data on lipophilicity and then provide new highly descriptive LIs (i.e. principal components and their derivatives) [26,121].

3.2. Electroseparation methods

Electroseparation techniques used to determine the lipophilic properties are based generally on capillary electrophoresis (CE). In this method, analytes migrate through electrolyte solutions (buffers with pH adjusted to certain values) under the influence of electric field. They can be separated in two different ways: according to ionic mobility or/and partitioning into an alternate phase via non-covalent interactions [143,144]. Migration of the analytes is confined by the sum of two factors: electroosmotic mobility (μ_{eo}) and intrinsic electrophoretic mobility (μ_{ep}). This limitation is caused by the action of electroosmotic flow (EOF) of the buffer solution, which is defined as a bulk liquid motion that results when the external electric field interacts with the net surplus of charged ions in the diffuse part of the electrical double layer [145,146]. The velocity of the EOF (u_{eo}) can be defined as:

$$u_{eo} = \mu_{eo} \cdot E \quad (17)$$

where E is the intensity of the applied electric field (V/m). The electroosmotic mobility (μ_{eo}) may be experimentally determined by measuring the migration time of a neutral analyte.

The use of CE has few advantages such as small amount of compounds required, speed and relatively easy automation and low consumption of solvents. In many cases CE is used as an alternative or complementary technique to HPLC due to its high separation efficiency [6,53,147]. Hence, CE remains to play an important role in separation of medium size molecules (e.g. peptides, oligosaccharides and oligonucleotides), as well as in analysis of large macromolecules, such as polysaccharides, nucleic acids and synthetic polyelectrolytes. [143,148,149].

For analysis of both charged and neutral compounds electrokinetic chromatography (EKC) methods, including micellar electrokinetic chromatography (MEKC), microemulsion electrokinetic chromatography (MEEKC) and vesicular electrokinetic chromatography (VEKC) are applied [150]. MEKC has gained in popularity for the indirect estimation of logP values of small molecules. The main difference between MEKC and CE is that the solution contains a surfactant, which concentration is greater than critical micelle concentration (CMC), so the concentration of monomers reaches a critical value when only micelles are present. These micelles are the pseudo-SP and the analytes are separated by differential partitioning between them and surrounding buffer solution (MP) [151]. Usually, MEKC is performed in open capillaries under the alkaline conditions to generate a strong EOF. Anionic micelles of SDS is the most common surfactant for MEKC systems and it was the first surfactant used for logP determination [152]. In comparison with n-octanol/water partition system, the advantage of MEKC results from the large difference in hydrogen-bond basicity, and to a lesser extent, dipolarity/polarizability, for partition into SDS micelles. Although, a better partition model is provided by sodium N-dodeconyl-N-methyltaurine (SDMT), this surfactant has not been used for estimating logP [6]. However, Ibrahim et al.[153] investigated the use of bile salts as a pseudo-SP in MEKC. The results showed that sodium deoxycholate (SDC) was the most appropriate surfactant used to estimate lipophilic properties of fungicides. Due to the fact that bile salts can be found in biological systems, the SDC-MEKC method is considered to be environmentally friendly. More detailed information on the MEKC principle and the reagents used are summarized by Terabe [151] and Silva[152] . As in the case of LC, determination of logP is based on the relationship between this coefficient and the retention factor [154]. This factor in MEKC technique may be defined as (Eq. 18):

$$k' = (t_R - t_0)/(t_0 \cdot (1 - (t_R/t_{mc}))) \quad (18)$$

where t_R, t_0 and t_{mc} are the migration times of the solute, the EOF marker (e.g. methanol), and the micelle marker (e.g. Sudan III or Sudan IV), respectively [151]. Compared to SFM, MECK has the advantages of speed, wide dynamic range, high sample throughput and small sample size [150,151].

For indirect determination of logP values MEEKC can also be applied. This technique can be seen as extension of MEKC, however in this case the microemulsion is used as migrating pseudo-SP. Microemuslions are immiscible oils droplets in water (usually heptane or octane droplets) that are stabilized by co-surfactants and surfactants situated at the surface of the droplet [147,155,156]. The most commonly used surfactant and co-surfactant in MEEKC are SDS and 1-butanol, respectively

[6,156]. The mechanism of separation in MEEKC is based on differential partitioning of analyte into migrating microemulsion, which is related to analyte hydrophobicity. Therefore, the migration time of analyte is proportional to its hydrophobicity, so its retention factor (k') is related to values of logP. To determine the k' value it is necessary to precisely measure the migration time of EOF marker (t_0) and microemulsion marker (t_{me}), such as octanophenone, along with analyte (t_R) and then to calculate the retention factor from the following formula [150] (Eq. 19):

$$k' = (t_R - t_0)/(t_0 \cdot (1 - (t_R/t_{me}))) \quad (19)$$

In order to determine logP values, Xia et al. [157]developed novel MEEKC approach based on peak-shift assay. In comparison to conventional approach, this method provides the logP values of the compounds without the use of reference substances with known logP values reported in the literature. Fernández- Pumarega et al. [158] presented the applicability of MEEKC in estimation of logD of acidic drugs at several ionization degrees. They reported that the overestimation of logD was observed only at degree of ionization higher than 99.5%. However, further research is needed to check if this behavior can occur with other compounds. This paper showed that MEEKC can be an alternative to other approaches used in lipophilicity assessment. Compared to MEKC, in MEEKC better solubilization properties for water insoluble compounds has been observed [156,159]. Furthermore, the hydrogen bond effects that affect the partitioning behavior of some solutes during lipophilicity determination are minimal in MEEKC compared to RP-HPLC or MEKC. This may be due to the addition of co-surfactant (1-butanol) that minimizes electrostatic interactions as reported by Ishihama et al. [160]. They also showed that MEEKC provides better correlation of retention factor with logP determined by SFM than MEKC and RP-HPLC methods for which the hydrogen acceptor and hydrogen donor effects must be considered. In addition, some limitations of SFM (e.g. large amount of high purity sample, lack of automation) and chromatographic methods (e.g. limited pH range, lifetime of SP) are overcome in the MEEKC[155].

Another EKC technique applied for logP determination is VEKC. Vesicles are self-assembling, organized structures that contain continuous bilayer of monomers and enclose an aqueous core region used as potential models of synthetic membranes. In nature, this formation occurs through the aggregation of phospholipids to form liposomes [6]. Hence, vesicles synthesized from phospholipid aggregates may be used as pseudo-SP in VEKC. These vesicles are inherently physiological, but they are stable only in multilamellar form. Due to the fact that in EKC analysis the monolamellar forms

are preferred, the use of liposomes requires some additional laborious preparation procedures allowing the production of monolayer vesicles [161]. However, the vesicles can also be synthesized using oppositely charged surfactants and double ionic surfactants, such as bis(2-ethylhexyl)sodium sulfosuccinate in a phosphate buffer containing 10% (v/v) methanol (an anionic, double chain surfactant known as Aerosol OT or AOT) or hexadecyltrimethylammonium bromide-sodium octyl sulfate (double ionic surfactant, known as CTAMB-SOS) [6,150]. Jiang et al. [162] investigated the influence of the molar ratio of cationic to anionic surfactants on the vesicle properties and the performance of VEKC. It is well known that due to the electrostatic attractions among oppositely charged head groups of ionic surfactants and the hydrophobic interaction of their hydrocarbon tails the strong synergistic effects may occur. Some of physical properties of vesicles can be modified by changing the ratio of ionic surfactants. These changes affect the performance of VEKC. It has been reported that the molar ratio of 3:7 and 5:5 systems used as pseudo-SP had similar selectivity. However, the second system showed rapid and efficient separation for nonpolar substances [162]. Because of the similarity of surfactant vesicles and liposomes, both VEKC systems may be used not only as separation models for estimation of logP of various neutral and charged compounds, but also as a model for environmental and biological partitioning (e.g. permeability, diffusion or membrane transport) [6,163–165]. To obtain logP values, the retention factor (k') determined by VEKC technique should be calculated according to following formula (Eq. 20):

$$k' = (t_R - t_0)/(t_0 \cdot (1 - (t_R/t_v))) \quad (20)$$

where t_v, t_R and t_0 are the migration times of vesicle marker (e.g. dodecanophenone or octylbenzene), the solute and the EOF marker, respectively [163]. The use of VEKC for determination of n-octanol-water partition coefficients has similar advantages to MEKC. However, it is difficult to obtain reproducible results using this technique [150].

3.3. Electroanalytical methods

A variety of electrochemical methods have been developed, but the most frequently chosen methods for lipophilicity assessment are potentiometric and voltammetric ones [166]. Potentiometric methods were described for the first time in 1952 and since that time, they have been applied in different branches of science and industry. The determination of lipophilic profile of compound directly from a single acid-base titration in dual-phase partition solvent system is fast, however, it is necessary to know the acid dissociation constant (pK_a) of tested compounds in case of using some of these

methods. Potentiometric methods create a good alternative to SFM especially in the case of acidic or basic compounds. Furthermore, the use of these methods enable to determine the logP in range of -1 to 8. Unfortunately, they require a relatively large amount of samples [27,32].

Classical potentiometric titration can be used for the evaluation of the partition of ionizable compounds or ion-pared substances into the organic phase. Simple titration procedure, together with calculation of pK_a values involved the ionization of water in n-octanol, has been proposed by Scherrer and Donovan [167]. During the measurement, the pK_a of analytes is determined by adding high precision titrators. A potentiometric titration procedure in KCl/water-saturated n-octanol provides a link to logP through the thermodynamic cycle of ionization and partitioning (Fig. 5). The use of this titration method in order to apply it for the determination of substituent ion pair stabilization values (IPS) may give more accurate data concerning logD calculations.

The greatest advantage of potentiometric titration over other methods is the independence of measurements from the magnitude of logP value. In the same time, a reproducibility of a few hundredths of a logP in the calculated difference between $logP^N$ (neutral forms) and $logP^I$ (ionized forms) is maintained.

Titrations in KCl/water-saturated n-octanol provide essential data to advance our understanding of ion pair partitioning in n-octanol, but the ultimate goal of the lipophilicity studies is the application of this knowledge to the anisotropic world of cells, membranes, proteins, and nucleic acids [23,167].

Cyclic voltammetry (CV) has been used for the indirect determination of the lipophilicity of organic salts. Generally, typical CV system consists of four-electrode potentiostat, the aqueous phase and nitrobenzene or 1,2- dichloroethane (EDC) as the organic phase [50,168]. Bouchard et. al. proposed to use CV for examination of the lipophilicity of quaternary ammonium drugs and proved that the partition of some ions is influenced by Galvani potential difference, not ion-pairing phenomenon. The obtained values of standard water-EDC partition coefficient of ions correlated well (R^2 = 0.94) with logP values of neutral molecular structures closest to these ions that were calculated by computer algorithms [168]. In contrast to traditional SFM, the partition coefficient determined by CV does not depend on experimental conditions. Furthermore, the use of this technique enables to estimate very low logP values ranging from - 8 to 1. The main disadvantages of CV are the limited number of

solvent systems that can be applied for lipophilicity determination and the large amount of sample required (1-10 mg) [50,53,168,169].

4. Conclusion

Lipophilicity is an essential physicochemical property, which affects biological activities, assimilation and environmental fate of compounds. Despite the importance of lipophilicity, its evaluation is still problematic and constantly attracts researchers' attention. Nowadays, classical methods are almost completely replaced by instrumental ones, in particular separation techniques. Compared with the classical approach, the use of chromatographic or EKC methods does not require a long analysis time and a large amount of high purity samples. The purity of the sample may be problematic for unstable analytes and may lead to false results. Since only retention factors must be determined using separation techniques, the analytical quantification is not required. This drawback of classical liquid-liquid system (i.e. SFM) is really serious in the case of highly lipophilic or sparingly soluble compounds due to the need for a highly sensitive quantification system. In turn, potentiometric titration as fully automated and time-saving instrumental technique is only applicable to acidic and basic compounds of high purity and may not be suitable for highly hydrophobic molecules due to solubility problems. Chromatographic methods, especially TLC and fast HPLC, considered as economical and environmentally friendly solutions, can be used to determine lipophilicity of both neutral and dissociable compounds. However, the pH and composition of the mobile phase should be carefully selected in order to ensure that the compound is in its neutral form. Furthermore, new solutions have been introduced to reduce the limitations of indirect separation-based methods, including employment of new HPLC columns, addition of masking agents to mobile phase, modifications of CE, application of surfactants in TLC experiments, and development of new lipophilicity indices. The gradient HPLC methods proposed as alternative to isocratic ones give other additional advantages, particularly speed up experimental process and reduce costs. However, there is still an urgent need for valid, standardized and high-throughput procedures to determine the lipophilicity of chemical compounds.

Future trends may also include (i) design of new biomimetic SPs through immobilization of important biomolecules or binding similar functional groups to the silica surface in order to mimic partitioning in different biological and environmental systems thus help to better understand these phenomena, (ii)

development of new biological partition/distribution models using well designed HPLC retention properties, (iii) application of chemometric approaches to provide new highly descriptive LIs and build large data bases for comparison purposes, (iv) estimation of potential kinetic aspects of partitioning based on changes in the symmetry and width of chromatographic peak as a function of the MP flow rate.

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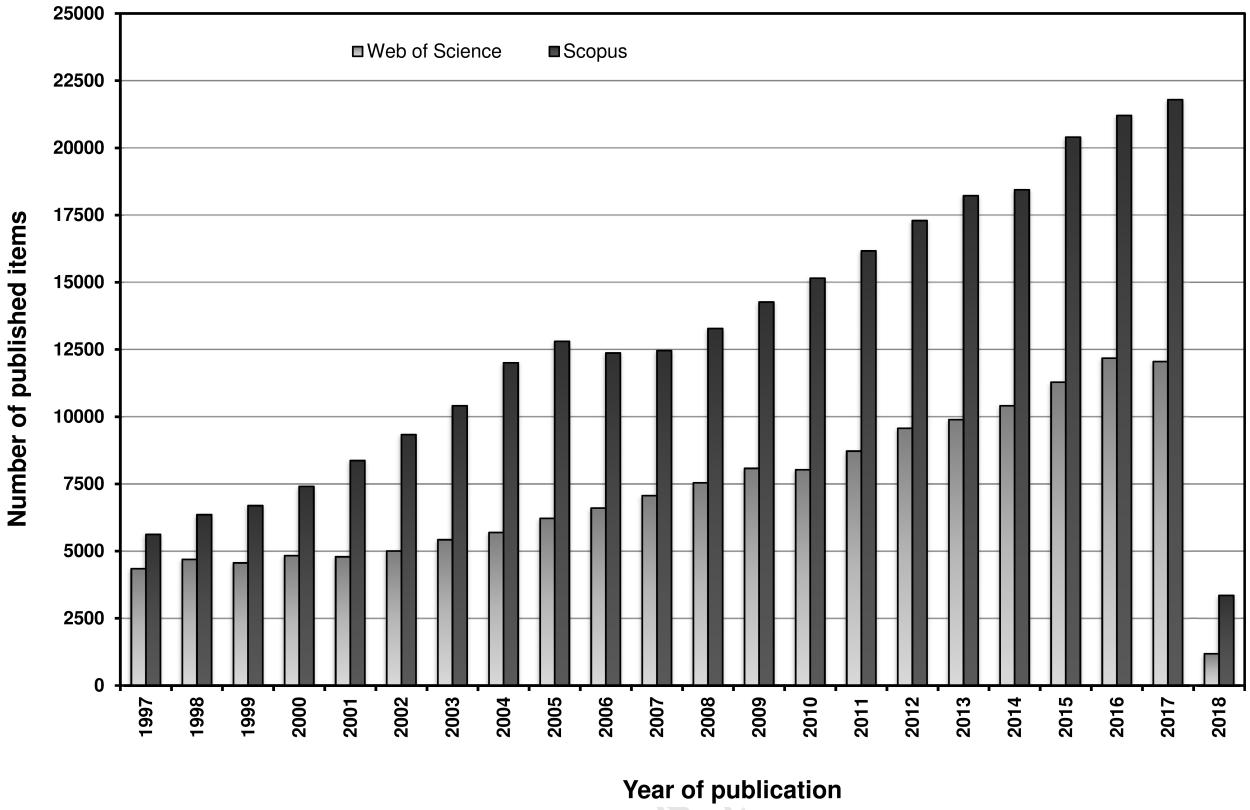
Fig. 1. Charts that shows the total number of publications by year that include the phrases "lipophilicity" or "lipophilic properties" or "partition coefficient" or "distribution coefficient" or "hydrophobicity" in the title, abstract, keywords or text, as searched through the Web of Science and Scopus databases. The difference in search results may be due to the type of published items that are considered by these search engines (i.e. original papers, reviews, book chapters, conference papers, short survey, etc.).

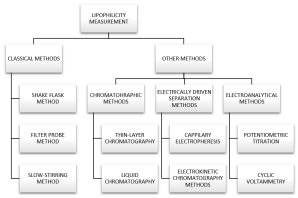
Fig. 2. Proposed classification of methods of lipophilicity assessment.

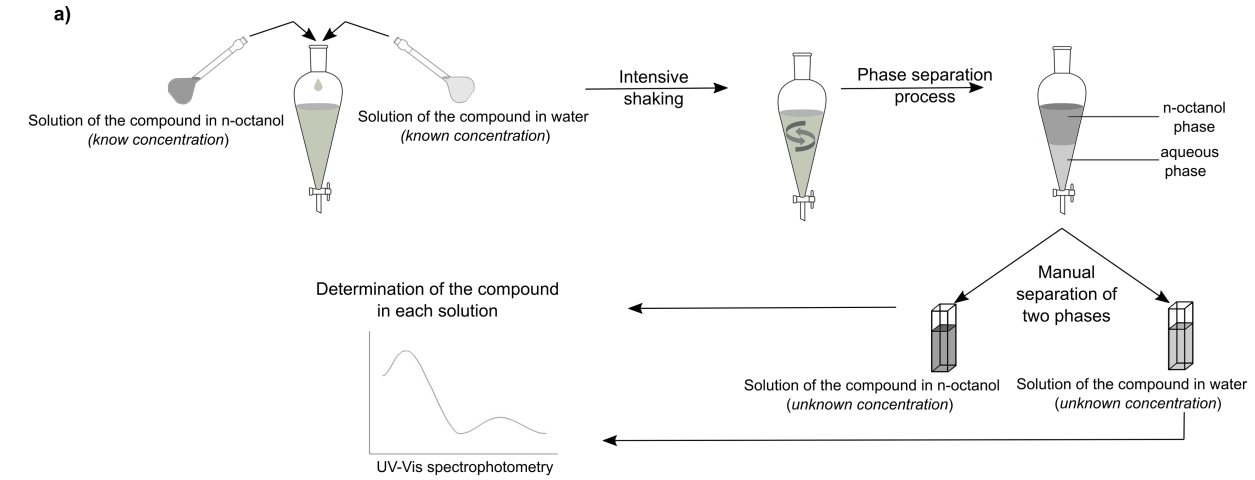
Fig. 3. Schematic presentation of direct experimental approaches of lipophilicity determination: a) procedure of the shake-flask method (SFM), b) the water-plug aspiration/injection method, c) modification of SFM based on NMR measurement, d) modification of SFM based on solid phase microextraction [26, 27, 38, 41]

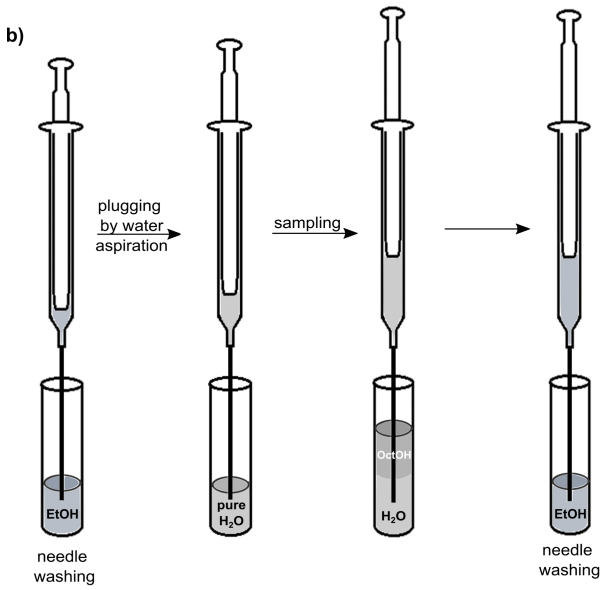
Fig. 4. Partition process of less (white particles) and higher lipophilic (grey particles) compounds between stationary and mobile phases during chromatographic run. The retention factor (k) of compound obtained on the HPLC stationary phase is proportional to its affinity (partition or distribution coefficient; K) to the bonded groups presented on the surface of the column packing material, as depicted in the equations, where n_s and n_m stands for average numbers of molecules in the stationary and mobile phase, respectively, C_s and C_m are the molar concentrations of analyte in the stationary and mobile phase, respectively, V_s and V_m are the volumes of stationary and mobile phase, respectively t_R and t_0 are the retention times of the analyte and the unretained compound, i.e. uracil, thiourea (known as column dead time).

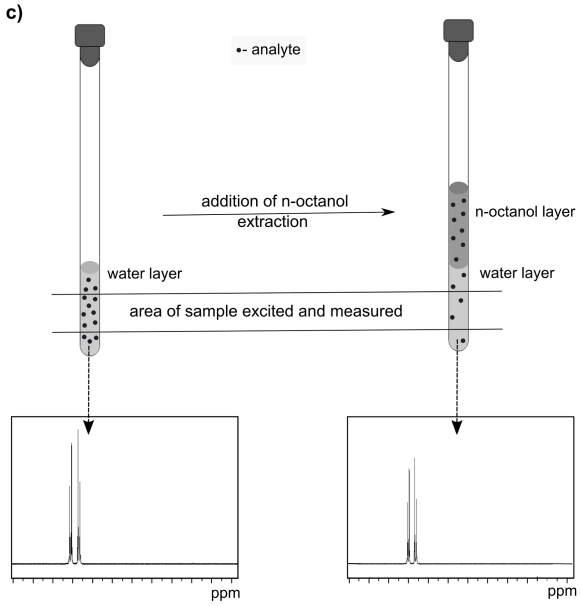
Fig. 5. The thermodynamic cycle of the ionization and partitioning of acid (HA) between n-octanol and aqueous phases based on Scherer and Donovan [167]. The pKa in n-octanol (pKa_0) is defined to be equal to the pH of the aqueous phase (0.15M KCl) which is in equilibrium with the partition system when the concentration of acid (HA_0) and anions (A_0^-) in n-octanol phase are equal. Hence, the knowledge of pKa_0, partition coefficient of neutral forms of a compound ($logP^N$) and pKa in aqueous phase allows to calculate the partition coefficient of the ionized forms ($logP^I$). When the circle of equilibria is present, the difference between $logP^N$ and $logP^I$ is the same as the difference between pKa values in aqueous and n-octanol phases.

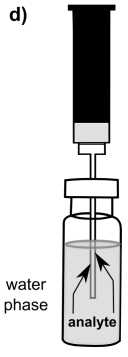


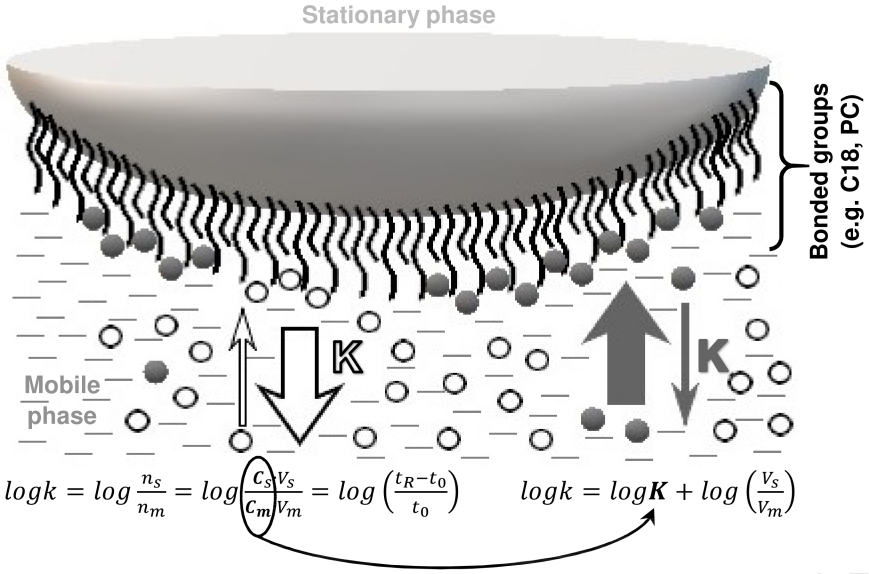


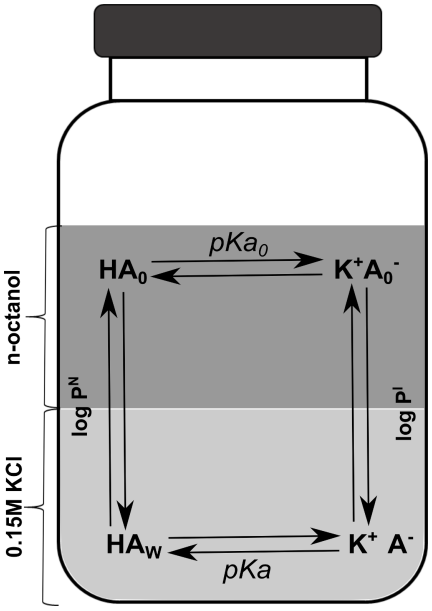












- The role of lipophilicity in biological, environmental and technical science.
- Lipophilicity assessment using classical and modern approaches.
- Advantages and limitations of methods of lipophilicity determination.
- Novel solutions in chromatographic methods used for lipophilicity determination.