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REVIEW

LIPOPHILICITY – METHODS OF DETERMINATION AND ITS ROLE IN MEDICINAL CHEMISTRY

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Abstract: Lipophilicity is a physicochemical property of crucial importance in medicinal chemistry. On the molecular level it encodes information on the network of inter- and intramolecular forces affecting drug transport through lipid structures as well as drug's interactions with the target protein. In result, on the organism level, lipophilicity is an important factor defining pharmacokinetics and pharmacodynamics of a drug substance. Thus, it is a meaningful parameter that found innumerable applications in drug development, Quantitative Structure–Activity Relationships (QSARs) and Quantitative Structure-Pharmacokinetic Relationships (QSPkRs) analyses. This report reviews the importance of lipophilicity on each step of the presence of a medicinal substance in the organism and describes progress in experimental methods of its determination. It has been documented that the retention of a compound in reversed-phase liquid chromatography is governed by its lipophilicity and shows significant correlation with n-octanol/water partition coefficient. Hence, reversed phase chromatography may provide relevant information about the compounds' property. Elaboration of biomimetic stationary phases provides better insight into biological partition processes. Nowadays, there is an urgent need for both precise and quick procedures for quantification of molecular lipophilicity.

Keywords: lipophilicity, octanol/water partition coefficient, review, biological activity, QSAR

Lipophilicity is a physicochemical property of principal importance in drug discovery and development. It affects three phases of drug activity – its pharmaceutical, pharmacokinetic and pharmacodynamic action. In this paper, a complex nature of the lipophilicity, understanding of its importance for biological activity and experimental methods of measuring this property are overviewed. Special emphasis is placed on characterization of indirect methods of estimation of biological lipophilicity.

According to the "IUPAC Gold Book", lipophilicity represents the affinity of a molecule or a moiety for a lipophilic environment (1). The term lipophilicity should be distinguished from the term hydrophobicity although these expressions seem to be synonymous in the literature and often are used interchangeably. Hydrophobicity is the association of non-polar groups or molecules in an aqueous environment, which arises from the tendency of water to exclude non-polar molecules (1). Lipophilicity of the compound is commonly measured by its distribution behavior in a biphasic system, either liquid–liquid (e.g., partition coefficient in octanol/water) or solid-liquid (retention on reversed-phase high-performance liquid chromatography (RP-HPLC) or thin-layer chromatography (TLC) system. For comparison, aggregation of molecules of studied substance in aqueous environment can be considered as the exact measure of hydrophobicity (2).

Hydrophobicity is considered as an integral element of lipophilicity and deeper understanding of this two terms must shed light on the intermolecular interactions they can elicit. Lipophilicity is a physicochemical property which encodes two major structural contributions, namely a bulk term reflecting cavity formation, hydrophobic and dispersive forces, and a polar term reflecting more directional electrostatic interactions and hydrogen bonds. It is generally accepted that the first contribution is called hydrophobicity, and the second – polarity (3). Lipophilicity as an equilibrium property, describes the balance between these two contributions. It has to be noted, that depending on the considered sys-

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tem, both components may have different or even opposed contribution to the overall lipophilicity value observed. As it was mentioned above, lipophilicity is commonly described by the processes of partition between the two phases – a non-polar (organic phase) and a polar (mostly aqueous phase). The quantitative descriptor of lipophilicity, the partition coeficient P, is defined as the ratio of the concentrations of a neutral compound in organic (c_{org}) and aqueous (c_{aq}) phases under equilibrium conditions (Eq. 1.):

$$P = \frac{c_{org}}{c_{aq}} \qquad \text{(Eq. 1)}$$

Usually expressed in a logarithmic scale, log P refers to partitioning of unionized species. If a substance is ionizable another quantitative descriptor called the distribution coefficient (log D) is used. Log D expresses the contributions of all neutral and ionized species present at given pH. Distribution coefficient can be calculated according to the following equations (Eq. 2, 3):

$$\log D_{acids} = \log P + \log\left[\frac{1}{(1 + 10^{pH - pK_a})}\right] \qquad \text{(Eq. 2)}$$

$$\log D_{bases} = \log P + \log\left[\frac{1}{(1 + 10^{pK_a - pH})}\right] \qquad \text{(Eq. 3)}$$

where pK_a is dissociation equilibrium constant.

LIPOPHILICITY AND BIOLOGICAL ACTIVITY

Although lipophilicity is one of many factors involved in biological activity of a drug it is often one of the most influential. This property pertains to many stages of drug action. Prior to reaching a pharmacological target, lipophilicity determines solubility, reactivity and degradation of drugs, as well as formulation of pharmaceuticals. Moreover, compound lipophilicity is of principal importance for biological activity as the affinity for a lipophilic environment facilitates the transport of chemicals through membranes in a biological system and the formation of complexes between compounds and receptor binding site. Cell membrane, a selectively-permeable barrier, mostly consists of a phospholipid bilayer with embedded proteins. Amphiphilic phospholipids composed of fatty acid chains at one end and hydrophilic ionized head regions at the other arrange spontaneously in the lipid bilayer (Fig. 1). The drug interaction with lipid structures present in the organism is strongly related to its lipophilicity. Higher solubility in body fats than in an aqueous environment under equilibrium conditions reflects compound affinity for lipids.

Considering pharmacodynamic action of a drug, it has to be highlighted that the successful practice of drug development is crucially dependent on the principles of molecular recognition: the first and 'fundamental' requirement for a drug is to bind to its target. Drug-receptor interactions involve fundamentally the same intermolecular forces as those acting on the partitioning of a solute between water and an immiscible organic phase (4). It is generally accepted that binding of a drug to a protein passes through the replacement and release of ordered water molecules (cavity formation), and therefore, the recognition process is usually entropically driven. In addition, binding is usually accompanied by enthalpic contribution coming from favorable contacts between the lipophilic groups (dispersion interactions) (5). For lipophilic interactions it has been shown that the free enthalpy of binding is proportional to the lipophilic surface hidden from the solvent (6). Quantitative assessment of spatial lipophilic properties of drug and receptor molecules is nowadays part of efficient computational methods in drug design. Application of a concept of the 3-dimensional molecular hydrophobicity potential (MHP) permits detailed assessment of the hydrophobic and/or hydrophilic properties of various parts of molecules and has already been widely used in molecular modeling of ligand-receptor interactions, namely in docking (7).

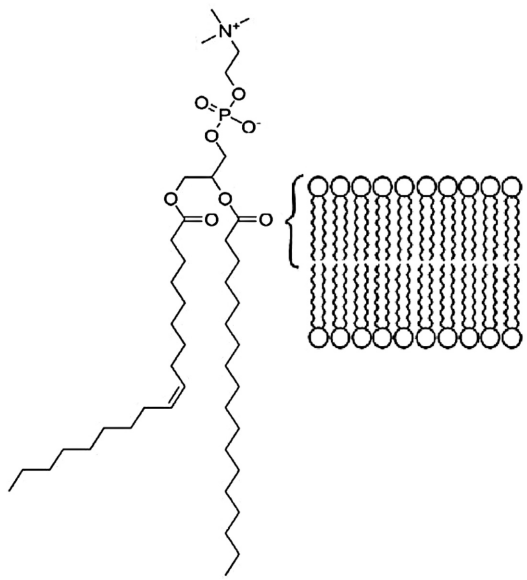

Figure 1. Chemical structure of the most common phospholipid (phosphatidylcholine) and its location within the bilayer

While lipophilicity affects pharmacodynamic profile of the drug, it seems to have the greatest impact on ADMET (absorption, distribution, metabolism, excretion, toxicity) properties. The partition coefficient determines where a drug is distributed after absorption and how rapidly is metabolized and eliminated. Withdrawal of a drug is often caused by drug toxicity which has to be estimated at the earliest possible stages of drug discovery, even before synthesis (8–11).

Absorption

It was early observed that lipophilicity is often closely correlated with permeability and solubility profile of a compound. Cross et al. in 2003 examined the relationship between skin permeability (human epidermal, full-thickness, dermal membranes) and lipophilicity of homologous alcohols (12). The study showed that the permeability coefficient improved with higher lipophilicity values of alcohols to octanol with no further increase for decanol. The concentrations of alcohols in all observed tissue stratums increased with increasing lipophilicity. The excessive increase of this parameter may contribute to the compound immobilization within e.g., a dermal membrane.

Oral delivery, the most recommended route of drug administration within the body enables to cross lipid bilayers in the intestinal epithelium. Lipophilic molecules can easily pass through the cell membrane and cytoplasm (transcellular transport) and reach blood stream and a target. On the other hand, insufficient absorption may result in reduced bioavailability. The intestinal permeability estimation is complicated due to several transport mechanisms and many factors should be taken into consideration (e.g., ionization). The prevalent models for intestinal absorption are Caco-2 cell system (13) and the PAMPA (parallel artificial membrane permeability assay) proposed by Kansy (14).

An immense challenge in neuropharmacology drug discovery is designing compounds crossing blood-brain barrier (BBB). This barrier consists of microvessel endothelial cells with tight intercellular junctions, therefore, it forms particularly selective layer permeable for molecules small in size.

Relationship between lipophilicity and BBB penetration was demonstrated by Waterhouse (15). The assay illustrates situations in which lipophilicity is a good predictor, as well as those where correlation is of low-grade. Consistent with results of the study, reduced brain barrier drug uptake of more lipophilic compounds is the consequence of increased non-specific plasma protein binding. Drugs with moderate lipophilicity values demonstrate highest uptake. Optimal log P/log D value for drug molecules acting on the central nervous system oscillates around 2.

Drug disposition

After absorption a drug is reversibly bound to plasma or serum proteins (human serum albumin, α_1-acid glycoprotein etc.) and transported in the bloodstream. Drug-protein complexes function as the drug reservoir, whilst the unbound drug fraction is available for ligand-target interactions and influences distribution and elimination. Human serum albumin (HSA) is composed of three homologous domains (I–III), each of which is divided into two subdomains (A and B). Two important drug-binding sites, also known as the warfarin (Sudlow's site I) and the indole-benzodiazepine binding sites (Sudlow's site II) are located in subdomains IIA and IIIA, respectively (16). Report on binding to both human plasma proteins and blood cells studied in a group of model anionic drugs by Lazicek and Laznickova (17) revealed that plasma protein binding and blood cell accumulation depend on lipophilicity. HAS binding sites are hydrophobic pockets with cationic amino acid residues near their entrances. In 2007, Soskic presented relative binding constants to human serum albumin for indole-3-acetic acid (IAA) and 34 of its derivatives with promising properties as prodrugs in cancer therapy (16).

Metabolism and excretion

A compound transported in a body is subjected to biotransformation and subsequently to hepatic elimination or renal excretion. The pathway of drug metabolism includes phase I (oxidation, reduction, hydrolysis) and phase II (conjunction). The phase I introduces or reveals polar groups (i.e., decreases lipophilicity) and facilitates elimination. For example, metabolism of propranolol depends on the delivery to liver (flow dependent hepatic elimination) and protein-bound drug fraction (which is unavailable to undergo glomerular excretion). The metabolism rate of free (unbound) drug concentration is controlled by chemical stability and ability to interact with site of an enzyme. Furthermore, more lipophilic compounds become more vulnerable to cytochrome P450 family enzymes (14, 18).

Toxicity

Yet in the 19th century, it was observed that lipophilic characteristic may affect toxicological

properties of chemicals. Cros et al. established that toxicity of homologous series of alcohols is directly proportional to the number of carbon atoms and inversely proportional to their water solubility (19). More recently, the role of lipophilicity of four mercury compounds has been demonstrated in cytotoxicity studies by Halbach (20). The differences in membrane permeability were responsible for varied accessibilities to functionally important thiol groups. The toxicity of mercurials investigated on isolated myocardial tissue increased with their lipid solubility. Moreover, Nava-Ocampo et al. examined the relationship between the octanol-water partition coefficient (log P) and the toxicity of some local anesthetic agents administered in experimental models (21). The toxicity was substantially increased at log P approximately 3.0 due to plasma availability of these compounds and excessively high transfer from plasma into the central nervous system (CNS), where the exposure of the nerve roots to the drug was prolonged. Recent investigation of new and known bile acids indicated that the relationship between cytotoxic properties and lipophilicity is complex, and probably indirect (22).

LIPOPHILICITY IN RATIONAL DESIGNING OF NEW DRUGS

At the turn of the 20th century, Meyer and Overton independently suggested that the narcotic (anesthetic) action of a group of organic compounds paralleled their olive oil/water partition coefficients (23, 24). This work is known as one of the first examples of Quantitative Structure-Activity Relationships analysis (QSARs). This method applies predictive models derived from application of statistical tools to correlate biological activity of chemicals with descriptors representative of molecular structure and/or properties:

Activity = f (physiochemical properties and/or structural properties)

QSAR is a powerful tool for drug development as it provides quantitative information relating properties of a compound to its activity and then enables a cost effective means of modifying drug molecules by *in silico* design and enhancement. By quantification of relationship between structure descriptors and activity, QSAR provides an understanding of the effect of structure on activity and can be used to help understand interactions between functional groups in the molecules of greatest activity with those of their target. Finally, this method gives the potential to make predictions leading to the synthesis of novel analogues.

Up to now, a great number of QSAR models have been published, the majority of which include lipophilicity as described by octanol–water partition coeficient. Lipophilicity is thus one of the most important factors in research aimed at rational design of new drugs, what is of great and still growing importance nowadays.

Lipophilicity is one of the factors included in the rule of five formulated by Lipinski in 1997, a mnemonic tool for medicinal chemists used for quick assessment of compounds during the drug discovery and optimization process with respect to the compounds' likelihood to display good solubility and permeability profiles (25).

To express the feasibility of a target to be effectively modulated by a ligand that has appropriate bio-physicochemical and ADME properties to be developed into a drug candidate, term 'drugability' is used. The rule of five, which was derived from a database of clinical candidates reaching Phase II trials or further, states that poor absorption or permeability of drug candidate are more likely when cLogP (the calculated octanol–water partition coefficient) is > 5; molecular mass is > 500 daltons (Da); the number of hydrogen bond donors (OH plus NH count) is > 5; and the number of hydrogen bond acceptors (O plus N atoms) is > 10.

Ten years after formulation of the rule of five, Leeson and Springthorpe showed in their study, that in oral drugs approved between 1983 and 2007, three of the rule-of-five properties (molecular mass; O plus N atom count; and OH plus NH count) sometimes exceeded that limits, whereas lipophilicity threshold of log P < 5 still held true (26). The fact that drug lipophilicity was changing less over time than other physical properties suggests that this is a particularly important drug-like property, control of which is crucial for ultimate success in drug development.

MEASUREMENT OF LIPOPHILICITY

Direct methods

A number of experimental protocols for log P/log D determination were suggested in the literature. The classical method for partition coefficient measurement called shake-flask procedure is a simple extraction in n-octan-1-ol/water system. However, the use of organic solvents to model complex biolipids is very simplistic. Fujita first proposed the octanol-water partition coefficient as a model of biological partition (27). The chemical structure of octanol is similar to molecules present in the cell membrane. In addition, hydroxyl groups

of both, water and octanol can participate in polar interactions with the solute molecule, and there is a considerable amount of water within the octanol phase (28). Octanol applied as organic solvent in the work of Leo (29), is characterized by its hydrogen bond donor and acceptor function of the hydroxyl group (amphiphilic property), however, some alternatives have been suggested by reason of different hydrogen bonding characteristics in membranes (or receptors) with diverse structures. Leahy with coworkers (30) have proposed the use of four solvents for modeling biological membranes. The critical quartet consisted of octanol, chloroform, cyclohexane and propylene glycol dipelargonate (PGDP). Log P values measured in these solvents demonstrated differences principally due to hydrogen bonding effects, forces that account for membrane partitioning (chloroform can donate hydrogen bonds, PGDP can only accept them, cyclohexane is inert). Experimental lipophilicity parameters for investigated solvent systems are to a certain extend dependent on the reference systems and vary from octanol/water partition coefficient. Amphiphilic properties of n-octan-1-ol that resemble interaction with phospholipids and correlation of lipophilicity values with biological activity make it as commonly accepted reference system in lipophilicity measurements. It should be noted, however, that for excessively hydrophilic or lipophilic solutes the octanol/water model no longer reflects drug partitioning in biological structures.

In the shake flask procedure after the equilibrium between all interacting components is attained, an appropriate analytical method (usually UV/VIS spectroscopy) is employed to determine concentrations of the substances dissolved in both phases. The standard shake-flask measurement is a time-consuming, labor-intensive process which allows log P determination in a narrow range of –3 to 3 (limitations of the analytical methods used for concentration determinations). The partition coefficient depends on the relative solubility of a substrate in a polar and non polar solvent and the log P has to be corrected for ionization. Difficulties arise with very hydrophilic or very hydrophobic compounds (usually because of solubility issues, emulsion formation, adsorption onto vessel walls). Another problem concerns compounds that can tautomerize or equilibrate between zwitterion and neutral form, chemicals with an amphiphilic property may behave as detergents. Furthermore, in shake-flask method high purity and relatively large amount of substances are necessary (31, 32). Modifications of this standard procedure handle with some of limitations mentioned above. First of all, the development of high throughput methods for screening of new drugs leads to miniaturization of standard shake-flask procedure. The literature shake-fiask method has trimmed down significantly in recent years from traditional greater than 100 mL volume in one of the phases to less than 1 mL and using 96-well shake-flask procedure (33). Recently, Alelyunas applied a rapid throughput octanol-water lipophilicity measurement based on 96-well shake-flask and LC/UV/APPI/MS. The method has been validated with satisfactory coeficient using 72 literature compounds with diverse ionization and log D values ranging from -2 to +6 (34).

Automated continuous flow (sampling) method can be used for lipophilicity determination of poorly soluble substances. The advantages of this technique called filter probe method include simplicity, degree of automation and time saving. Method ensures greater reproducibility and accuracy while a computer program monitors the system (35).

Another variation of the classical shake-flask method is the solid-phase microextraction (SPME). The SPME, developed by Pawliszyn's group in 1990 is an extraction method where a sample partitions between a fused silica fiber coated with a polymer and aqueous phase (urine, blood, water) (36, 37). Presaturation like in the shake-flask method is required. The introduction to a chromatographic system (coupled to LC, LC-MS, GC, GC-MS), enables determination of minute quantities highly non-polar compounds in the aqueous phase (38). As a result, process has application for higher lipophilicity values and reduces loss due to volatile compounds (39).

Direct experimental methods for lipophilicity determination include potentiometric titration method. This method considers the partition of neutral or ion-paired substances into the organic phase. The dissociation coefficients (pK_a) of analytes are measured by adding high precision titrators (recommended are minimum three determinations). To obtain log P values from the titrated aqueous pK_a and the apparent pK_a values (when different volumes of octanol are present) in a biphasic system a calculation program based on equation (e.g., pKaLOGP software) is available (40). The functional pH-metric log P determination is becoming more widespread, it was used for the determination of partition coefficients between liposomal membrane and water (41). The lipophilicity measurement of an ionizable drug at different pH values is necessary to

predict its passing through cellular barriers and interactions with the therapeutic targets.

Indirect methods

Major importance and practical application to determine compound lipophilicity value is focused on dynamic partitioning between two immiscible phases occurring in chromatography. Chromatographic retention in reversed phase format and n-octanol/water partitioning are energetically analogous. In purpose of lipophilicity measurement, reversed phases chromatography is the preferential system. Chemically bonded hydrocarbon-silica are stationary phases whereas silica, a hydrophilic 'end' is referred to as a 'head group' and hydrocarbon chains, which are a lipophilic 'end', may be compared to the structure of phospholipids. Furthermore, non-polar stationary phases are anisotropic thus more similar to partitioning into phospholipid bilayers than isotropic property pertained to bulk octanol phase.

The chromatographic methods, in contrast to time-consuming extraction, provide measurement of extended lipophilicity range. High performance liquid chromatography instrumentation enables to estimate log P values in the range of 0 to 6 (42). As a consequence of accessibility, precision, reproducibility of retention data, and partitioning automation HPLC has become a standard procedure. In comparison with direct methods impurities do not affect measurement as chromatography is the separation process. Moreover, broader range of chemicals may be analyzed injecting small quantities of sample.

An indirect approach of lipophilicity assessment involves series of reference compounds injected onto a C18 column. The retention factors of compounds with known log P values are used to create a calibration curve. The group of compounds with unknown log P values is then injected and their retention factors are used to predict log P from the calibration curve. It should be accentuated that chromatographic studies are valid for homologues or closely congeneric compounds (43).

Thin layer chromatography (TLC)

Chromatography is a powerful technique for the measurement of physicochemical parameters. Currently, high performance liquid chromatography is one of the major approaches in the chromatographic determination of lipophilicity, but many researches apply also reversed phase thin layer chromatography (RP-TLC).

The application of RP-TLC method in lipophilicity investigation was introduced by Biagi (44–46). The most popular lipophilicity descriptor estimated by this method is R_M and it is derived by the retention factor (R_f) according to the following formula (Eq. 4):

$$R_M = \log(\frac{1}{R_f} - 1) \qquad \text{(Eq. 4)}$$

where R_f is calculated on the basis of migration distances of a compound and the solvent front. As R_M value depends linearly on the concentration of the organic modifier in the mobile phase, the value is extrapolated to pure water as mobile phase according to Soczewiński-Wachtmeister equation (Eq. 5):

$$R_M = R_{MW} + S\varphi \qquad \text{(Eq. 5)}$$

where φ is volume percentage of organic modifier, S is the slope of the regression plot (indicates mechanism of retention) and R_{MW} is the value of R_M extrapolated to $\varphi = 0$.

Use of RP-TLC allows overcoming some of the difficulties associated with classical shake flask method. The advantages of TLC method are the speed of determination, better reproducibility, and less strict requirement for purity of the sample due to separation during the chromatographic process. The method is easy to perform and allows simultaneous analysis and comparison of several compounds with broad range of measurable lipophilicity values (2). Generally, reversed phase (RP) chromatography mode is used to simulate the octanol-water partitioning. From the wide range of available stationary phases, C-18 silanized silica gel is the recommended material for the estimation of drug lipophilicity (47). However, free silanol sites attribute to silanophilic interactions (hydrogen bonding, electrostatic interactions) and the stationary phase instability at pH above 8 are negative aspects. Determination of the retention values for pure water (0% modifier) is impossible (excessively long retention time, R_f close to zero), therefore, methanol-water and acetonitrile-water mixtures have been applied as developing solvents (48–50).

Sławik and Kowalski (51) studied lipophilicity and the effect of different mobile-phase solvents (acetone, acetonitrile, methanol) on the retention of seven derivatives of 1,2-benzisothiazol-3(2H)-one with high antimicrobial activity. High values of correlation coefficients between the volume fraction of the organic modifiers and the R_M values were established for each of organic solvents studied. Moreover, it was shown that the pH of the mobile phase within a pH range 2–11 did vary in R_M values, which confirmed that in case of weak acids there is no need for pK_a corrections for calculations of R_M values.

RP-TLC is a valuable method for estimation of the partition coefficient log P, when direct methods cannot be applied due to solubility problems. In this case, the calibration set of compounds is used to establish the relationship between the traditional log P and chromatographic R_M data. The approach was used, for example, by Mazak and coworkers (52) to predict log P values of several alkaloids of therapeutic interest.

Recently, a comparative study of several approaches of TLC as a tool for lipophilicity determination was presented by Komsta et al. (53). Study includes wide range of methods for TLC lipophilicity estimation: a single TLC run, extrapolation of a retention, principal component analysis of a retention matrix, parallel factor analysis (PARAFAC) on a three-way array and a partial least squares regression (PLS). The best results were obtained by authors for the single TLC runs. The advanced chemometric processing, such as PCA, PARAFAC and PLS did not show a visible advantage comparing to classical methods. Moreover, authors recommended methanol and dioxane as theoretically and practically most suitable organic modifiers for lipophilicity estimation, while acetonitrile did not show acceptable correlation of retention with lipophilicity.

High performance liquid chromatography (HPLC)
Chromatographic lipophilicity parameters

Analogously to RP-TLC, the retention behavior in RP-HPLC is often described by linear solvent strength (LSS) model where the logarithm of retention coefficient for an analyte log k is linearly related to the volume fraction of organic modifier in a binary eluent (Eq. 6):

$$\log k = \log k_W - S_\varphi \qquad \text{(Eq. 6)}$$

where φ is organic solvent concentration, S is a slope of the regression plot (specific to the organic modifier on the considered stationary phase). Log k_W is the mostly used lipophilicity parameter. The relationship between φ and the retention factor derived from data obtained in a series of isocratic measurements is not linear for the full range of organic solvent concentrations. The retention factor *k* is given by the expression (Eq. 7):

$$k = \frac{t_R - t_0}{t_0} \qquad \text{(Eq. 7)}$$

where t_R and t_0 are the retention times of the test chemical and of an unretained compound (dead time), respectively.

When the log k *vs.* φ plot is not linear in a broad range of the modifier concentration, quadratic model is used (Eq. 8):

$$\ln k = A\varphi^2 + B\varphi + C \qquad \text{(Eq. 8)}$$

A, B, and C are regression coefficients (54). The retention factor, k (or R_M) of analyte can be related to the partition coefficient (P) between two phases according to equation (Eq. 9):

$$\log k = \log P + \log \frac{V_S}{V_M} \qquad \text{(Eq. 9)}$$

V_S/V_M is the volume ratio of the stationary and mobile phases.

Log k_W, directly related to octanol-water log P, is regarded a representative index of lipophilicity. The octanol/water partition coefficient of a test substance can be calculated by experimental determination of retention factor k and then inputting k into the following formula (42) (Eq. 10):

$$\log P_{0W} = a + b \cdot \log k \qquad \text{(Eq. 10)}$$

where a and b are linear regression coefficients determined in experiments with a set of standard compounds.

Nowadays, chromatographic lipophilicity parameters are frequently regarded as independent descriptors that do not require the analysis in relation to log P. The aim of log k_W introduction was to reduce the influence of organic modifier addition into mobile phase on chromatographic process. S is a parameter indicating mechanism of retention: principally describing input of two components: the size of the solute (reflected by its volume or surface area) and the hydrogen-bonding capacity (27). Biagi proved that ratio S to log k_W is constant for structurally related compounds (55). Therefore, S may be considered as an alternative lipophilicity descriptor.

Valkó and Sleger introduced another parameter derived from polycratic method (56). φ_0 parameter is calculated organic solvent concentration in which quantity of analyte in mobile and stationary phase is equal ($\log k = 0$). This factor can be measured directly or estimated by interpolation of relation (Eq. 11):

$$\log k = f(\varphi) \qquad \text{(Eq. 11)}$$

Interpolation is more precise than extrapolation employed to obtain log k_W. Chromatographic parameter φ_0 demonstrated better correlation with the log P values than log k_W (57).

In gradient chromatography approach Valkó and coworkers combined two equations (Eq. 12 and 13):

$$\varphi_0 = \frac{\log k_W}{S} \qquad \text{(Eq. 12)}$$

$$t_R = (\frac{t_g}{S}) \log(2.3k_W S t_0 + \frac{1}{t_g} + t_0 + t_D \quad \text{(Eq. 13)}$$

where t_R denotes gradient retention time, t_D – dwell time, t_g – total time of gradient experiment. Second formula was reported by Quarry (58) in a gradient run. Investigators introduced a new lipophilicity scale – chromatographic hydrophobicity index (CHI). CHI is defined by the expression (Eq. 14):

$$CHI = At_R + B \quad \text{(Eq. 14)}$$

The CHI is obtained on the basis of t_R derived from fast gradient elution within less than 10 min per sample. A and B denote constants for the calibration set of analytes, where the gradient retention times, t_R are plotted against isocratically determined φ_0 values (illustrated above) of calibration compounds. The conversion of gradient retention times to the CHI index is appropriate for inter-laboratory collation and for enlarging a database. Additionally, the CHI values can also be projected to the logarithmic scale. Hence, relation assessment with log P or log D parameters is facilitated. The parameter allows comparison even of unrelated compounds.

Krass suggested another gradient HPLC parameter, k_g, expressed by the formula (59) (Eq. 15):

$$k_g = \frac{V_g - V_d - V_m}{V_m} \quad \text{(Eq. 15)}$$

V_g is the gradient volume, V_d is dwell volume, V_m denotes dead volume. Parameter k_g obtained for congeneric chemicals and the log k_W data derived from series of isocratic measurements showed a good correlation. The reliability of chromatographic parameters is not ascertained, hence requires further research.

There are two major approaches for investigation of lipophilicity measurement by use of HPLC: isocratic and gradient methods.

In isocratic method, previous estimation of probable compound lipophilicity is essential to prepare diverse compositions of mobile phase (organic modifier volume fraction) to cover lipophilicity range of determination (60). This approach, based on the extrapolation at 100% water plotting isocratic log k values as a function of the mobile phase composition is common and preferred lipophilicity parameter; however, some investigators have used isocratic log k values (measured at a given organic solvent concentration). In contrast to a gradient run several retention measurements at varied organic solvent concentrations (i.e., polycratic experiments) are needed, thus analyses take more time. It should be noted that a variety of HPLC stationary phases can be used for this purpose to achieve reliable and reproducible data. However, lipophilicity parameters obtained with different HPLC columns always need calibration using a standard set of compounds. The polycratic conditions were applied in the lipophilicity investigation of (*N*-phenyl-)-2,4-dihydroxybenzenecarbothioamide derivatives (61). From these compounds a subset of 30 derivatives was chosen for testing of biological activity. The lipophilicity of analyzed tioamides was the main factor responsible for fungistatic or bacteriostatic activity.

The gradient HPLC method consists in programmed increase during the chromatographic process of organic solvent in the aqueous mobile phase. During procedure all analytes are washed out of the column, impurities do not affect results and simultaneous analysis of several chemicals is possible (62). Linear solvent strength (LSS) model allows calculating log k_W from retention data obtained in two gradient HPLC experiments (63), in contrast, the isocratically measured log k is time-consuming and requires several chromatographic runs. The retention of an analyte can be presented by means of equation which Kaliszan and coworkers described in their paper (64). In another studies, the group applied gradient elution in HPLC to provide parameters of lipophilicity (log k_W) and acidity (pK_a) of analytes (65, 66). Good correlation was observed between log k_W obtained by reversed phase gradient run and value obtained by standard method of extrapolation. As concluded by Kaliszan, gradient HPLC is a convenient method of efficient screening of lipophilicity of drug candidates (65, 66).

Credible information about acidity and lipophilicity is of vital importance during the drug discovery process. One may use two appropriate gradient runs to determine both pK_a and log k_W. Another high throughput technique of pK_a and log k_W determination has been presented by Wiczling et al. in 2006 (67). The combined pH/organic technique is a method based on measuring times of retention of samples in a series of linear organic modifier gradients at different pH changes and different gradient intervals. These methods gain greater attention nowadays.

Stationary phases for lipophilicity determination by HLPC

Chromatographic separation system for lipophilicity estimation should model the octanol-water partition coefficient. Recently, Kaliszan has published a review on QSRRs, where he emphasized the most important studies on old and new

immobile phases for lipophilicity assessment (54). Octadecyl-bonded silica (ODS) and other alkyl ligands bonded to silica core are commonly used stationary phases for HPLC-based lipophilicity estimation. It must be noted, that silica-based materials are instable at higher pH, thus the investigation of basis analytes is unsatisfactory and lipophilicity determination of ionizable compounds on C-18 or C-8 columns encounters difficulties. The main problem associated with these stationary phases is the possibility of polar moieties interactions (of electrostatic or hydrogen bonding nature) with the residual silanol groups on the silica surface, which results in asymmetrical peaks. To limit interactions mentioned above, silica based stationary phases can be protected by polar groups. Alkylamide as well as carbamate phases are commercially available.

Giaginis and coworkers simulated octanol-water partitioning employing a base deactivated silica (BDS) column (68). In addition, in the case of basic drugs, n-decylamine was used as masking agent in the mobile phase. Moreover, Benhaim achieved minimization of the silicophilic effect using end-capped stationary phase, Ascentis RP-Amide column intended for lipophilicity estimation (69). Polar amide group is introduced on the bonded alkyl chain close to silica surface, therefore, access of the solute is prevented. Addition of n-octanol enhanced correlation between log P values and extrapolated log k_w.

The Gemini C18 column is a new generation hybrid silica-based column with an extended pH range capability utilized for lipophilicity parameters determination (70). The surface of stationary phase is particularly grafted silica-polymer hybrid that reduces direct contact with free acidic silanol groups.

Entirely devoid of reactive silanol groups is the polymer-based octadecylpolyvinyl (ODP) stationary phase which has been used for lipophilicity measurements by Donovan and Pescatore. The retention behavior from a fast methanol-water gradient employing short polyvinyl alcohol columns demonstrated higher correlation with the log P than silane based columns (71).

Modern monolithic silica stationary phases overcome disadvantages of conventional particle based supports. Predominantly, considering time of analysis for very lipophilic compounds, which are strongly retained. Mrkvickova (72) determined lipophilicity of potential antituberculotic agents using HPLC on monolithic stationary phase and calculated theoretical log P values for all compounds employing the chemical programs. Technique significantly reduced the time of analysis (higher stability of the columns allowed fast flow rate) and appropriate peak shapes were attained.

Biomimetic stationary phases

As it was presented above, the use of new stationary phases in HPLC system seem to be effective in prediction of octanol-water partition coefficient. However, it has to be underline that octanol is an isotropic phase and in this respect differs from natural membrane barriers, which are made of ordered and anisotropic lipid–protein membranes (73). It is widely emphasized in the literature that for more accurate description of compound distribution between various compartments *in vivo*, application of distinct types of lipophilicity is required (60). Recently, more and more research attention is paid to application in HPLC stationary phases that could directly mimic biologically important elements. These biomimetic stationary phases include immobilized drug-binding proteins, liposomes and membranes will be briefly characterized below.

Immobilized artificial membrane

Immobilized artificial membranes (IAMs) are stationary phases used in chromatography approaches to mimic the partitioning into phospholipid bilayers. The comparison of IAM and C-18 RP-HPLC phase is presented in Figure 2. IAM phases basically consist of phospholipids (mostly phosphatidylcholine) or phospholipid-like molecules covalently

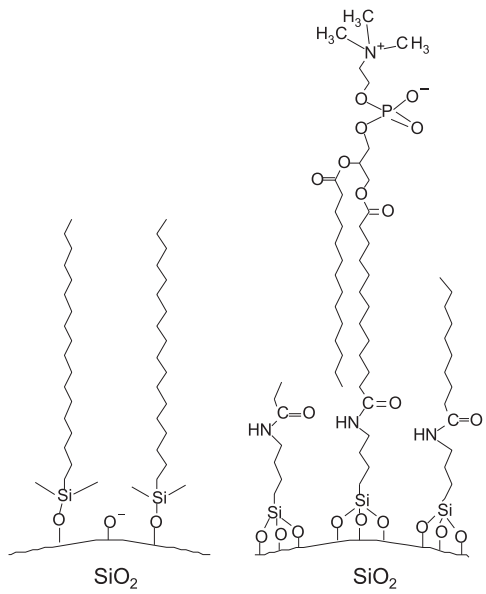

Figure 2. C-18 RP-HPLC phase (**A**) and IAM HPLC phase (**B**)

bonded to a propylamino-silica support material at monolayer densities and were developed nearly 20 years ago by Pidgeon et al. (74).

IAM columns are currently commercially available, which is a great advantage of this technique. Properties of the most frequently used IAM stationary phases were reviewed in details by Taillardat- Bertschinger et al. (75). Estimation of lipophilicity by IAM-HPLC is realized by isocratic and gradient methods. In comparison with RP-HPLC columns, IAM technique frequently permits the use of aqueous mobile phases without addition of organic modifier, which lead directly to log k_w value, which reduces considerably the time of analysis (41). Due to the capacity of IAM columns to permit the establishment of molecular interactions like those found in phospholipid membranes, this technique seems to be valuable in ADME properties estimation. IAM chromatography was proposed as screening tool for the prediction of blood-brain barrier (BBB) penetration (76). Our group used IAM column to determine lipophilicity of several groups of 2,4-dihydroxythiobenzanilide derivatives and found this parameter significantly correlated with bacteriostatic and fungistatic activity of these compounds (77). Recently, Reinter et al. determined and correlated several lipophilicity parameters for some GABAergic agents including octanol-water partition coefficient, retention data in HPLC by using C18 and IAM columns, and partition coefficients determined in phospholipid liposomes (78). The values obtained by HPLC using IAM column were quantitatively the closest to the partition coefficients determined in liposome systems. Barbato et al. compared data from IAM-HPLC with octanol-water partition coefficients and HSA/AGP affinity data for some zwitteronic quinolone antibacterial agents (79). Results showed that lipophilicity estimated by IAM chromatography is more consistent with pharmacokinetic behavior of these compounds than solvent/water partition coefficients.

Liposomes

Immobilization of liposomes into surface of support particles and their use as stationary phases for HPLC leads to technique called immobilized liposome chromatography (ILC). Liposomes as spherical vesicles form by aggregation of phospholipid molecules closely resemble natural cell membranes and are commonly used for modeling of their structure and functions. Structural properties of liposomes were summarized in review of Plember van Balen et al. (80) while the paper of Godard and Grushka gives the insight into different methods of immobilization of liposomes into the support particles (81). It has to be noted, that partitioning into liposomes involves electrostatic interactions as additional intermolecular recognition forces not encoded in traditional lipophilicity parameter octanol-water log P. This difference is of great importance when lipophilicity of ionizable drugs is considered, as their partition in membranes is considerably more effective than in octanol. Some authors use the term "phospholipophilicity" to describe partitioning into liposomes and distinguish this term from lipophilicity measured in liquid/liquid biphasic system (41). Lundahl and Beigi showed in their study good correlation between "phospholipophilicity" of several drugs estimated by ILC and drug permeability through Caco-2 model and absorption of orally administered doses in humans (82). Even if using liposomes as stationary phases for HPLC appears rational for ADME properties estimation, this technique is associated with technical difficulties due to limited stability of liposomes.

Proteins

Considering drug-binding proteins, retention values obtained on HPLC stationary phases supporting human serum albumin (HSA) and α_1-acid glycoprotein (AGP) correspond to lipophilicity values experimentally measured by shake flask method at neutral pH. Barbato and cooworkers, who observed this relationship for quinolone antibacterial agents, indicated that serum protein affinity of those substances depends on lipophilicity only for analytes with log P = 1.5. Above this value, no further increase of in protein affinity was observed (83). Similar correlation was shown by Chrysanthakopoulos et al. for approx. 40 structurally diverse drugs (84). To conclude, interaction mechanism with serum-protein is complex and only partially governed by lipophilicity. Although the octanol-water log P or HPLC based lipophilicity have been used in early years to correlate protein binding (85), nowadays it is generally accepted that lipophilicity alone is not sufficient to express this process. Therefore, HSA-HPLC provide potential to stimulate plasma protein binding, as retention data provided by this technique incorporate also other mechanism than lipophilicity, especially those of electrostatic nature.

To sum up, development of biomimetic stationary phases provided new scales of lipophilicity, which allow for including additional intermolecular recognition forces, not encoded in traditional lipophilicity parameters (see Table 1). From this point of view, these phases could provide more

Table 1. Comparison between recognition forces governing intermolecular interactions in biochemical and pharmacological process, and forces encoded in lipophilicity parameters obtained by different methods (69).

<table>
<tr><td colspan="2" rowspan="2">Recognition forces in biochemistry and molecular pharmacology</td><td colspan="2">Lipophilicity</td></tr>
<tr><td>Measured in liposomes/water systems or IAM-HPLC</td><td>Measured in liquid/liquid biphasic systems or RP-HPLC</td></tr>
<tr><td colspan="2">Charge transfer and aryl/aryl stacking interactions</td><td rowspan="2"></td><td rowspan="2"></td></tr>
<tr><td colspan="2">Ionic bonds</td><td rowspan="6">Polarity</td><td rowspan="6">Polarity</td></tr>
<tr><td colspan="2">Ion-dipol bonds (permanent, induced)</td></tr>
<tr><td colspan="2">Reinforced H-bonds</td></tr>
<tr><td colspan="2">Normal H-bonds</td></tr>
<tr><td rowspan="3">Van der Waals forces</td><td>Orientation forces (permanent dipole – permanent dipole)</td></tr>
<tr><td>Induction forces (permanent dipole – induces dipole)</td></tr>
<tr><td>Dispersion forces (instantaneous dipole – induced dipole)</td><td rowspan="2">Hydrophobicity</td><td rowspan="2">Hydrophobicity</td></tr>
<tr><td colspan="2">Hydrophobic interactions</td></tr>
</table>

appropriate model for *in vivo* biological partition processes.

Electrically driven separation methods

Lipophilicity estimation can be also realized by electrically driven separation methods. Capillary electrophoresis in all its aspects, including micellar chromatography and electrochromatography, is a rapidly expanding area of analytical chemistry. Application of electromigration techniques into lipophilicity determination could be useful, mainly in industrial field, to perform faster and less expensive analyses. Considering these techniques, the separation principle is based on both electrophoretic migration and differential partitioning of the compounds between an aqueous and a lipophilic pseudostationary phase.

Basically, in comparison with HPLC, electrically driven separation methods offer a shorter analysis time and considerably lower solvent consumption. However, the main advantage of use these methods is the higher peak efficiency in CE. In contrast to the HPLC, mobility of injected solutes is caused by a phenomenon known as electroosmosis, rather than by applied pressure. As the electrically driven flow rate is independent of the column length, so that, in principle, longer columns can be used. It follows that considerably higher efficiencies can be generated. A second consequence of employing electrodrive is that the plug-like flow-velocity profile in electroosmotic flow reduces dispersion of the band of the solute as it passes through the column, further increasing the column efficiency.

Capillary zone elecrophoresis (CZE), the simplest form of CE, cannot be considered as lipophilicity determination technique by itself, but it can be applied in direct determination of drugs previously included in liposomes. This method was proposed by Quaglia for estimation of lipophilicity of cardiovascular drugs (86). The solutions of drugs considered were placed in contact with liposomal vesicles at several incubation periods. During the contact time, the analytes cross, or not, the vesicle membrane according to their lipophilicity. The liposomal vesicles injected in the electrophoretic capillary allow the determination of the analyte inside the liposome. Knowing the volumes of liposomal vesicles and watery phases, it was possible to determine the drug/partition coeficient between the two phases. However, results of Quaglia showed good correlation with results from IAM-HPLC measurements, reproducibility of proposed method is negatively affected by long and laborious process of liposomes preparation as well as very scarce reproducibility of obtained liposomal vesicles. In comparison with CZE, micellar capillary electrophoretic technique (MEKC) includes forming phospholipidic micelles directly in the electrophoretic capillary. Quaglia et al. applied this technique for estimation of lipophilicity of cardiovascular drugs (87). Results of

the study showed that MEKC could be an alternative and less expensive to determine the partitioning of drugs in phosopholipidic phases than IAM-HPLC. One of the advantages of MECK is a possibility of modulation of the nature and composition of the micelles (the pseudo-stationary phase) by optimization due to selection of the surfactant. Sodium dodecyl sulfate (SDS) remains the most widely used surfactant for lipophilicity determination by MECK, but some authors proposed use of nanometer-sized oil droplets as pseudostationary phase for CE, which seems to mimic more closely phospholipidic membranes (88). This technique called microemulsion electrokinetic chromatography (MEEKC) allow to determine the lipophilicity of neutral, basic, and acidic compounds with log P ranging from 0 to 5 (89). Recently, Wan proposed MEEKC coupled with MS as a technique for prediction of biopartitioning of CNS drugs in brain tissue (90). However, although both MECK and MEECK demonstrated effective to measure partition in phospholipids, their reproducibility is negatively affected by the need of preparing micelles or microemulsion as stationary phase. Capillary electrochromatography (CEC), technique which combines the advantages of electrically driven separation methods and immobilized artificial membrane chromatography, was also applied with success to describe drug partition in membrane phospholipids. CEC experiments are carried out in a 100 µm (i.d.) fused-silica capillary, partially packed with IAM. In CEC, analogously to biochromatography, all the retention values have to be determined with a totally aqueous mobile phase, or to be extrapolated to 100% aqueous buffer. In comparison with IAM-HPLC, to assess drug afinity for membrane phospholipids by CEC, observed retention values have to be corrected by both the electroosmotic and electrophoretic mobility values according to equation (Eq. 16):

$$k_{CEC} = \frac{t_{m \cdot (1 + k_e) - t_0}}{t_0} \qquad \text{(Eq. 16)}$$

where k_{CEC} is an index of afinity for phospholipids, t_m and t_0 are the retention times of the analyte and a nonretained compound, respectively, measured in CEC; k_e is the velocity factor defined as: $k_e = \mu_p/\mu_0$ electrophoretic mobility; μ_0 = electroosmotic mobility. From a practical point of view, before lipophilicity determination by CEC, compound has to be analyzed in CE to measure the respective values of μ_p and μ_0.

Recently, Barbato and coworkers have provided an evaluation of the effectiveness of CEC data to describe partition in phospholipids by relating log k_{CEC} of 16 structurally unrelated compounds to both the logarithms of octanol-water partition coefficients and the retention data measured by immobilized artificial membrane chromatography (91). Phospholipid afinity scale by CEC related to that achieved by HPLC, but only if two different subclasses were considered separately, i.e., protonated and unprotonated analytes; indeed, all the compounds protonated at the experimental pH value (7.0) were retained stronger in CEC than in HPLC. This discrepancy may be due to the use of different buffers in CEC and HPLC, since, to avoid the occurrence of a high current, the eluent in CEC experiments was of different composition and lower ionic strength than in HPLC. Although for application of CEC in the determination of drug/phospholipid afinity a preliminary screening between protonated and unprotonated analytes is requested, this method seems to offer many advantages in comparison to artificial membrane chromatography: it is a faster technique (about 80% time saved for the most lipophilic analytes) and requires much less amount of analyte, eluent, and stationary phase to be performed. Finally, in Barbato study, CEC elution with 100% aqueous eluent was possible for the vast majority of analytes (approximately 80%), whereas it was possible for approximately 30% of compounds in HPLC.

PRACTICAL APPLICATIONS

To sum up, considerable progress regarding understanding of the role of lipophilicity for absorption, distribution, metabolism, excretion, as well as drug activity at the target has been achieved since the first studies correlating lipophilicity and biological activity were published by Meyer (23) and Overton (24). Simultaneously, this progress was associated with significant development in experimental methods of lipophilicity determination. Practical application of this knowledge and techniques in modern drug discovery include improving quality of drug candidates through physicochemical profiling (92) and will be briefly outlined in a few examples below.

Lipophilicity profiling was adopted to optimize the structure of doxorubicin derivatives with higher anticancer activity. Doxorubicin is a drug extensively used in anticancer therapy, but it has the highly hydrophilic character and its use is associated with severe side effects at high doses. To improve the lipophilicity of the drug, Chhikara et al. synthesized series of fatty acyl amide derivatives of doxorubicin (93). Obtained derivatives were

found to be more lipophilic when compared to doxorubicin and, as expected, they exhibited more antiproliferative activity in ovarian and colon cancer cell lines.

Similar strategy was applied by Saikia et al. to obtain analogues of phytol with antitubercular potential (94). Authors assumed in their study that moderate to high lipophilic derivatives would exhibit better antitubercular activity due to the lipophilic nature of the *Mycobacterium tuberculosis* cell wall, which in 60% consists of lipids. *In vitro* assays showed that three analogues with enhanced lipophilicity exhibited antitubercular activity better than phytol. The most potent analogue was chosen for *in vivo* evaluation.

Another example of lipophilicity optimization in drug discovery can be found in the group of tyrosinase inhibitors, which are targets for developing medicines to treat hypopigmentation-related problems, such as albinism and piebaldism. Compound called kojic acid–phenylalanine amide (KA-F-NH_2), which showed an excellent tyrosinase inhibitory activity, did not inhibit melanogenesis in melanocyte due to its low cell permeability. To enhance its cell permeability by increasing lipophilicity, Kwak et al. prepared metal coordination compounds of KA-F-NH_2 (95). Study showed that the zinc and copper complexes of KA-F-NH_2 inhibited tyrosinase activity as much as KA-F-NH_2 and reduced melanin contents in melanocyte efficiently. These results demonstrated that metal complex formation could be applied as a delivery system for hydrophilic molecules which have low cell permeability properties.

Lipophilicity is a critical parameter for central nervous system agents active *in vivo*. Enhancing lipophilicity was the approach adopted by Francotte et al. to design 'second generation' pyridothiadiazine dioxides (96). These compounds were expected to show activity in the potentiation of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate) receptors, which could be considered as innovative therapeutic strategy in the treatment of cognitive disorders, schizophrenia, depression and Parkinson's disease. As it was expected, lipophilicity optimization led to a compound, which showed cognition enhancing effects in memory tests in rats after intraperitoneal injection.

Recently, Jeong et al. developed new oxime reactivators of acetylcholinesterases (AChE) inhibited by organophosphorus agents (97). In their research emphasis was given to the finding that the lipophilic nature of fiuorinated compounds is responsible for their enhanced transport across the blood brain barrier. Authors used quantum mechanical calculations of lipophilicity to optimize the structures of fiuorinated pyridinium oximes. As the results of these theoretical treatments, series of new oximes with potentially increased BBB permeabilities were designed and synthesized.

Roleira et al. presented the rational design of new antioxidants drugs structurally based on caffeic, hydrocaffeic, ferulic and hydroferulic acids (98). Exogenous antioxidants are nowadays considered a promising therapeutic approach in neurodegenerative diseases since they could play an important role in preventing and/or minimizing neuronal oxidative damage. The aim of Roleira's work was to design compounds, which possess an amplified lipophilicity in relation to the precursor acids. The new set of lipophilic phenolic antioxidants of amide and ester type exhibited increased antioxidant activity. In addition, some compounds showed a proper lipophilicity to cross the blood-brain barrier.

Increasing of lipophilicity of drug candidates is frequently used approach to enhance their bioavailability, but it has to be mentioned, that modifying the chemical structure by adding lipid residues often results in changes in activity. As an example, the design of the series of novel balofloxacin ethylene isatin derivatives by Feng et al. could be presented (99). Authors synthesized derivatives with remarkable improvement in lipophilicity as compared to the parent compound balofloxacin, but simultaneously all of the synthesized compounds were less active than balofloxacin in *in vitro* antimycobacterial assays.

Potential changes in drug candidate activity cause that lipophilicity profiling is not only limited to analog development. Design of prodrugs is also frequently used strategy in this context. Formation of an inactive prodrug with optimized lipophilicity may be realized by attaching lipophilic promoiety that can be cleaved to the parent drug on entering the site of its action. Classical example of this approach is heroin (i.e., the diacetyl ester of morphine) that rapidly crosses the blood-brain barrier due to its higher lipophilicity. Once in the brain, it is presumed to be hydrolyzed to morphine (100). The same approach was employed with other therapeutic agents such as prodrugs of antiviral lamivudine (101), cytarabine (agent for the treatment of myeloblastic leukemias) (102), esmolol (anti-hypertensive and anti-arrhythmic agent) (103), or idebenone (antioxidant) (104). The advances and progress in the knowledge of current strategic approaches of prodrug design were recently reviewed by Jana et al. (105).

CONCLUSION

The exerted influence of lipophilicity on basic properties of chemicals results in incessant and intensive research in this field. Development of models to predict this parameter based on their retention behavior has attracted considerable attention. However, there is constant necessity for standardization of presented above methods for lipophilicity estimation. The ability to predict, as well as superiority of approach cannot be explicitly evaluated due to wide variety of chemical structures, methods (experimental, calculation), and partition systems. *In vivo* assessment remains invaluable, nevertheless *in vitro* studies enable to decrease number of these experiments, while *in silico* methods are deprived of analysis expenses. It should be noted that variety of log P calculation methods based on different algorithms are available (106).

Progress in the chromatographic assessments of data important for medicinal chemistry and molecular pharmacology is evident. Automated chromatographic procedure and optimization of its conditions enable to improve the reproducibility and precision of retention parameters. Stationary phases facilitate mimicking of biopartitioning, moreover, give better insight into permeability phenomenon and the role of molecular properties in the biological activity of similar and unrelated compounds. Widespread application of statistical (chemometric) approach allows extracting systematic information often dispersed over large sets of chromatographic data. Nowadays, there is an urgent need for both valid and quick procedures to quantify molecular lipophilicity.

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