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Review

Lipophilicity study of some non-steroidal anti-inflammatory agents and cephalosporin antibiotics: A review

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ABSTRACT

Lipophilicity properties have long been considered a vital component of drug discovery and development, providing insight into the role of molecular properties in the biological activity of known and new compounds. An extensive survey of the literature published in analytical and pharmaceutical chemistry journals has been conducted. Separation, optical, electrochemical and calculation methods which were developed and used for determination of lipophilicity non-steroidal anti-inflammatory agents and cephalosporin antibiotics in drugs and biological materials, have been reviewed. This review covers over 100 miscellaneous methods. Presented review highlighted some recent developments and new techniques that have been used in the lipophilicity detection of two different kinds of drugs.

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Contents

Abbreviations: ^{13}C NMR, carbon nuclear magnetic resonance; ^{1}H NMR, proton nuclear magnetic resonance; ADME, absorption distribution metabolism elimination; AID, adjuvant induced disease; AUC, plasma–time curve; BBB, blood–brain barrier; BMC, biopartitioning micellar chromatography; BSA, bovine serum albumin; CHI, chromatographic hydrophobicity index; COX, cyclooxygenase; CSF, cerebrospinal fluid; D, distribution coefficient; DCE, 1,2-dichloroethane; Dex-PEG, dextran-polyethylene glycol; DMPC, dimyristoylphosphatidylcholine; DMSO, dimethyl sulfoxide; E_f, enhancement factor; f_u, unbound fraction; HOMO, highest occupied molecular orbital; HPLC, high performance liquid chromatography; HSA, human serum albumin; IAM, immobilized artificial membrane; ILC, immobilized liposome chromatography; K, the association constant; $\log k_w$, logarithmic chromatographic retention factor; $\log k_{IAM}$, chromatographic retention factor; LSER, linear solvation/free energy relationship; LSS, linear solvent strength; LUMO, lowest unoccupied molecular orbital; LUV, large unilamellar vesicle; M, concentration [mol L^{-1}]; MEs, microemulsions; MI, migration index; MOPS, morpholinepropanesulfonic acid; MS, mass spectroscopy; MW, molecular weight; NSAIDs, non-steroidal anti-inflammatory drugs; o/w, octanol–water; o-NPOE, o-nitrophenyl octyl ether; P P_{ow}, octanol–water partition coefficient; P_{app}, apparent partition coefficient (permeability coefficient); PG, 1,2-propanediol; pK_a, dissociation constant; PSA, prostate specific antigen; QMAR, quantitative migration-activity relationship; QRAR, quantitative retention activity relationship; QSAR, quantitative structure activity relationship; QSPR, quantitative statistical property relationship; QSRR, quantitative structure retention relationship; QSTR, quantitative structure toxicity relationship; r, correlation coefficient; RBCs, red blood cells; R_M, R_{MW} retention values; RP-HPLC, reversed phase high performance liquid chromatography; RP-LC, reversed phase liquid chromatography; RP-TLC, reversed phase thin layer chromatography; S, the slope; SUV, small unilamellar vesicle; TEM, transmission electron microscopy; TLC, thin layer chromatography; UV, ultraviolet; VIS, visible.

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1. Introduction

The distribution of a solute between two phases in which it is soluble has been an important subject for experimentation and study for many years. In one form or another, this technique has been used since earliest times to isolate natural products such as the essences of flowers. The first systematic study of distribution between two immiscible liquids which led to a theory with predictive capabilities was carried out by Berthelot and Jungfleisch [1]. These investigators accurately measured the amounts present at equilibrium of both I_2 and Br_2 when distributed between CS_2 and water. They also measured the amounts of various organic acids; H_2SO_4, HCl, and NH_3 when distributed between ethyl ether and water. From these early investigations came the first appreciation of the basic fact that the ratio of the concentrations of solute distributed between two immiscible solvents was a constant and did not depend on the relative volumes of used solutions. It was concluded from these early observations that there was a small variation in partition coefficient with temperature, with the more volatile solvent being favored by a temperature decrease. In 1891, Nernst made the next significant contribution to the subject [2]. He stressed the fact that the partition coefficient would be constant only if a single molecular species were being considered as partitioned between the two phases. This association and dissociation of solutes in different phases remains the most vexing problem in studying partition coefficients. During the early years of the twentieth century a great number of careful partition experiments were reported in the literature, most of which were carried out with the objective of determining the ionization constant in an aqueous medium of moderately ionized acids and bases. As early as 1909, Herz published formulas which related the partition coefficient (P) to the number of extractions necessary to remove a given weight of solute from solution [3]. From 1940s the mechanical technique of multiple extraction was vastly improved, and countercurrent distribution became an established tool for both the separation and characterization of complex mixtures [4]. During the two decades bracketing the turn of the century, while the partition coefficient was being studied by physical chemists as an end in itself, pharmacologists became quite interested in the partition coefficient through the work of Mayer and Overton who showed that the relative narcotic activities of drugs often paralleled their oil–water partition coefficient. However, the correlation of so-called nonspecific narcotic activity with partition coefficients did not lead to any really useful generalizations in understanding the mechanism of drug action in the broad sense. Consequently, the interest of both groups in partition coefficients declined greatly.

Molecular lipophilicity is a major physicochemical property which affects the oral absorption, permeability, cell uptake, protein binding, blood–brain penetration, and metabolism of bioactive substances. The ability to predict drug absorption through the gastro-intestinal barrier is a key issue in the selection of new drug candidates for oral delivery. Passive diffusion, driven by a concentration gradient, is the main mechanism of drug uptake through the intestinal epithelium. It can occur between cell junctions (paracellular transport) or through the cytoplasm (transcellular transport). Lipophilic compounds cross the plasma membrane easily and are, therefore, mainly transported transcellularly. Cell membranes are relatively impermeable to hydrophilic compounds, so these are transported predominantly via the paracellular route. Excessive lipophilicity is also a common cause of poor solubility of substances and can lead to incomplete absorption after oral administration. It is also generally believed that very lipophilic compounds have greater affinity for plasma–protein binding and are easily transported across the blood–brain barrier (BBB).

Lipophilicity is one of the parameters of chemical substances which influence their biological activities. It is a prime parameter in describing both pharmacodynamic and pharmacokinetic aspects of drug action. In biological systems lipophilicity largely determines the solubility of drugs in biological fluids, penetration through the biological membranes, rate of absorption, affinity to plasma and tissue proteins, distribution into the specific body compartments or in organism. Lipophilicity is defined by the partitioning of a compound between a non-aqueous and an aqueous phase.

Lipophilicity, widely expressed by the logarithm of n-octanol–water partition coefficient ($\log P$) or distribution coefficient ($\log D$) for ionizable compounds, plays an important role of several ADME (absorption, distribution, metabolism and elimination) aspects, as well as in the pharmacodynamic and toxicological profile of drugs [5]. The logarithm of n-octanol–water partition coefficient (P_{ow}) is generally accepted as a useful parameter in structure activity relationship studies for the prediction of biological or pharmacological activity compounds. The partition coefficient is a ratio of concentrations of un-ionized compound between the two solutions. To measure the partition coefficient of ionizable solutes, the pH of the aqueous phase is adjusted such that the predominant form of the compound is un-ionized. The logarithm of the ratio of the concentrations of the un-ionized solute in the solvents is called $\log P$.

$$\log P_{\text{octanol/water}} = \frac{\log\,[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{un-ionized water}}}$$

The distribution coefficient (D) is defined as the ratio of the concentration of compound in the lipid phase to the concentration of all species in the aqueous phase at a given pH. Estimation of $\log D$ from $\log P$ and $\mathrm{p}K_{\mathrm{a}}$ describes equation:

$$\log D_{\text{acids}} = \log P + \log\left[\frac{1}{1 + 10^{\mathrm{pH}-\mathrm{p}K_{\mathrm{a}}}}\right]$$

$$\log D_{\text{bases}} = \log P + \log\left[\tfrac{1}{1+10^{\mathrm{p}K_{\mathrm{a}}-\mathrm{pH}}}\right]$$

Approximations when the compound is largely ionized: For acids with $(\mathrm{pH} - \mathrm{p}K_{\mathrm{a}}) > 1$, $\log D_{\text{acids}} \approx \log P + \mathrm{p}K_{\mathrm{a}} - \mathrm{pH}$
For bases with $(\mathrm{p}K_{\mathrm{a}} - \mathrm{pH}) > 1$, $\log D_{\text{bases}} \approx \log P - \mathrm{p}K_{\mathrm{a}} + \mathrm{pH}$

Approximation when the compound is largely un-ionized:

$$\log D \approx \log P$$

The most common procedures for the measurement of lipophilicity are the “shake-flask” and “stir-flask” techniques. In these methods, the solute concentration in each phase of water–organic mixture is determined by spectrophotometric or chromatographic methods. Among them, separation (chromatographic systems, membranes), optical and electrochemical techniques are using. Apart from the experimental methods, the lipophilicity of novel drugs can be estimated using various chemical software products, based on the different mathematic methods.

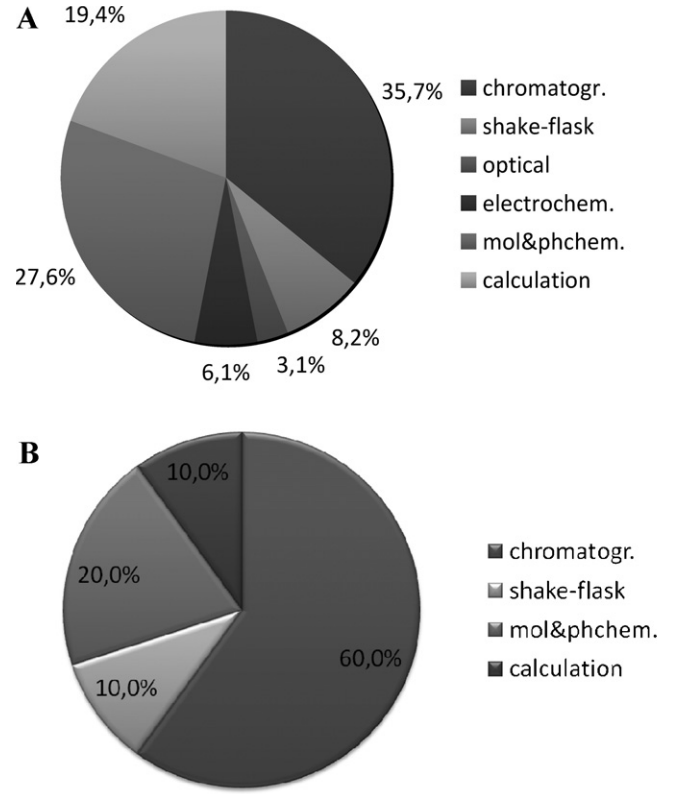

Fig. 1. Different techniques employed for determination of (A) NSAIDs, (B) cephalosporins.

This manuscript presents different techniques applied for the determination lipophilicity of non-steroidal anti-inflammatory drugs (NSAIDs) and cephalosporin antibiotics. Presented drugs differed in respect of the chemical structures and the pharmaceutical actions, but they are often used in the therapy together. This review covers over hundred methods, like chromatographic, shake-flask, optical, electrochemical, calculation procedures, molecular and physicochemical properties, used for researches of lipophilicity specified compounds. The percentage of their utility is shown in Fig. 1, from which it can be seen that chromatographic methods and molecular and physicochemical properties have been used most extensively sustainable for determination lipophilicity of NSAIDs and cephalosporin antibiotics.

The objective of this article was to describe the concepts and theories that form the foundation of the traditional and contemporary approaches to measurement of lipophilicity, and miscellaneous ways offered by different methods.

2. Chromatographic methods

High performance liquid chromatography (HPLC) and reversed phase thin layer chromatography (RP-TLC) are considered very popular techniques for the assessment of lipophilicity, the physicochemical property of primary interest for the evaluation of ADME characteristics in drug design. During the last decades numerous publications report correlations between chromatographic retention factors and octanol–water (o/w) log *P*, the reference lipophilicity parameter. Chromatographic methods, in particular HPLC and RP-TLC can be used as alternative, indirect, methods. A faster method of log *P* determination makes of HPLC (5–10 min per sample). Disadvantages of the method are: the solute's chemical structure must be known beforehand; since the value of log *P* is determined by linear regression several compounds with similar structures must have known log *P* values; different chemical classes will have different correlation coefficients and between-class comparisons are not significant.

2.1. High performance liquid chromatography (HPLC)

The log *P* of a solute was determined by correlating its retention time with similar compounds with known log *P* values [6]. The physicochemical properties investigated during the study include the molecular mass, ionization of the drug at physiological pH, the lipid/water partition coefficient and the solubility constraint (and melting point) in the stratum corneum.

Beetge et al. determined a correlation between the absorption of selected NSAIDs and their physicochemical and pharmacokinetic properties by HPLC method. The log *P* values obtained from literature for piroxicam, ketoprofen, naproxen, ibuprofen and indomethacin, (1.8, 0.97, 3.22, 3.6 and 3.8, respectively) correlated with the area under the plasma-time curve (AUC) values. The AUC values determined were 527.00 (piroxicam), 269.45 (ketoprofen), 258.65 (naproxen), 243.22 (indomethacin), and 88.09 (ibuprofen) $\mu g\,mL^{-1}\,h^{-1}$. It was concluded that the most reliable parameter for transdermal absorption was the lipophilic character of a drug (log *P* value) [7]. The glucose and mannose esters of flurbiprofen, ibuprofen, ketoprofen and naproxen were prepared from glucose and mannose in enzyme catalysed retention. The NSAIDs and NSAID glycosides were assayed by HPLC method. Solutions of the NSAIDs and their glycosides ($30\,\mu g\,mL^{-1}$) were prepared in the pre-saturated n-octanol phase. Partition coefficients were calculated as the ratio of drug concentration in the n-octanol phase to that in the buffer phase. ACDLabs and KowWin prediction softwares were used to predict the partition coefficients for the NSAIDs and glycosides. Obtained results confirmed that transdermal flux was greatly determined by partition coefficient and aqueous solubility as the parent NSAIDs with log *P* values closer to 2–3 and higher aqueous solubility at pH 7.0 than that of the glycoside derivatives presented with higher transdermal flux [8].

The unbound fraction in serum (f_u) is a critical parameter in describing and understanding the pharmacokinetics of NSAIDs. Authors compared f_u values for six different NSAIDs using ultrafiltration serum at pH 7.4 and 24 °C. HPLC was used to measure drug concentrations in serum and ultrafiltrate. Direct injection of ultrafiltrate and serum (diluted 250×) permitted quantitation down to approximately 70 nM for most of the NSAIDs. Assuming binding only to albumin, the data were fitted to a model of two classes of binding sites with dissociation constants *K*1 and *K*2. The lowest *K*1 (highest affinity) was found with ibuprofen, 0.0658 μM, an 80-fold difference. At low drug concentrations, f_u becomes virtually constant and approaches a lower limit, f_u^{min}. Large differences exist between NSAIDs with regard to parameters describing the serum protein binding. This translates into large differences in f_u values and with regard to potential for saturation of binding sites. Diclofenac and ketoprofen were used at low, nonsaturating plasma levels. Their f_u values were constant within an individual. Also, they were not potential displayers of others drugs. The f_u values of naproxen varies eightfold over the range of concentrations observed in therapy, making its half-life a function of dose. Naproxen is predicted to be a potent displacer of drugs that are bound to the same binding site. The maximum variation in f_u values at clinical doses of fenoprofen, flurbiprofen, and tolmentin is modest, approximately twofold. The NSAIDs also different widely with regard to the extent of variation in f_u values within the range of therapeutic concentrations, and hence with regard to their potential as displacers of other drugs [9].

Siraki et al. investigated the physicochemical requirements of twenty one NSAIDs (e.g., aspirin, salicylic acid, flufenamic acid, tolfenamic acid, mefenamic acid, niflumic acid, diclofenac, fenoprofen, flurbiprofen, naproxen, ketoprofen, ibuprofen, fenbufen, indomethacin, piroxicam, nimesulide) for glucuronidation and cytoxicity by quantitative structure toxicity relationships (QSTR) in isolated rat hepatocytes. Lipophilicity is a frequently used parameter for QSTR modeling. The uptake of small organic chemicals (such as NSAIDs) to their site of action occurs by passive diffusion, which depends on their lipophilicity and is quantified by the logarithm of the octanol–water partition coefficient, $\log P$. Authors have investigated the contrast in physicochemical variables that correlated with NSAID-induced hepatocyte cytotoxicity when glucuronidation was inhibited with borneol. The competitive inhibition of hepatocyte p-nitrophenol glucuronidation by NSAIDs was determined by HPLC technique [10].

The method reported by Donovan et al. of estimating the $\log P_{\mathrm{ow}}$ from HPLC method, differs from usual published methods in a number of ways: they used a very fast methanol gradient instead of a series of isocratic runs, a very short octadecyl-poly(vinyl alcohol) column instead of a regular length C_{18} silica column, and the use of two internal standards in each injection. The method has the capacity to measure the $\log P_{\mathrm{ow}}$ at any given pH between 2 and 13, can be carried out on impure material and does not require a radiolabeled compound. The majority of interests for this study were compounds with a $\log P_{\mathrm{ow}}$ between 2 and 6 (e.g., naproxen, ibuprofen, ketoprofen) [11].

Research of a correlation between the absorption of selected NSAIDs and their physicochemical and pharmacokinetic properties, such as the $\log P$ values, molecular mass of the respective drugs, percentage unionized moiety, solubility constraint of the drug in the stratum corneum, were conducted. Plasma concentrations of indomethacin, ketoprofen and piroxicam were determined using a HPLC method [12].

A rapid HPLC method with ultraviolet (UV) and mass spectroscopy (MS) detection, that provides estimates of log octanol–water distribution coefficient ($\log D$) at pH 7.4 and integrity vs. purity at the rate of 5.5 min per compound for application in pharmaceutical profiling was described. The lipophilicity of components (e.g, acetylsalicylic acid, salicylic acid, ketoprofen, flurbiprofen, ibuprofen, naproxen, ceftriaxone and cephalexin) was estimated from a calibration curve [13].

A gradient HPLC method to measure human serum albumin (HSA) binding for discovery research compounds (e.g., aspirin, diclofenac, flurbiprofen, ibuprofen, indomethacin, ketoprofen, naproxen, piroxicam, cefazoline, ceftazidime, cephalexin) was described. The obtained results were suitable for deriving quantitative structure property relationships (QSPR). The effect of positive and negative charge on the albumin binding and membrane affinity has been revealed. It was found that negatively charged compounds bind more strongly to HSA than it would be expected from the lipophilicity of the ionized species at pH 7.4. Several compounds showed stronger HSA binding than can be expected from their lipophilicity alone, and comparison between predicted and experimental binding affinity allows the identification of compounds that have good complementarities with any of the known binding sites [14].

The linear solvent strength (LSS) model of gradient HPLC elution has been applied to estimate parameters of lipophilicity and acidity of a series of drugs (e.g., acetylsalicylic acid, ibuprofen, ketoprofen, salicylic acid) and model chemicals. Apparent dissociation constant ($\mathrm{p}K_{\mathrm{a}}$) values and lipophilicity index ($\log k_{\mathrm{w}}$) values for individual analytes were determined in 2–3 gradient runs. The first experiment used a wide-range organic modifier gradient with pH chosen for suppressed ionization of the analyte. Authors used mathematical equations to present correlations between $\mathrm{p}K_{\mathrm{a}}$ and pH and retention times. The following experiment was carried out with pH-gradient of the aqueous component of the eluent that was sufficient to overlap the possible $\mathrm{p}K_{\mathrm{a}}$ value of the analyte. Obtained results indicated that gradient HPLC can be used for rapid screening of hydrophobicity of analytes including drugs and drugs candidates; two gradient runs suffice to obtain a reliable measure of analyte lipophilicity; estimates of $\mathrm{p}K_{\mathrm{a}}$ of analytes can be obtained from two gradient runs, one with modifier gradient and the other with pH gradient, however the conditions must be adjusted to individual analytes; further experiments were required to apply gradient as a routine method of determination of acidity constants [15].

X-ray crystallography showed that meloxicam crystallized in four different prototropic forms: the anion, the acidic enol, the zwitterions and the cation forms, dependent on pH and used solvents. As determined by proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR), meloxicam in neutral or weakly basic solution exists in the anion form. An equilibrium between the enol and zwitterions forms, dependent upon solvent polarity, was indicated. The $\log P$ of meloxicam and the other NSAIDs (such as piroxicam, tenoxicam, diclofenac sodium, ibuprofen, naproxen, indomethacin, ketoprofen and acetylsalicylic acid) between n-octanol and 0.1 M phosphate buffer at different pH values were determined by HPLC method using UV detection. The studies on $\mathrm{p}K_{\mathrm{a}}$ values, solubility and $\log P$ values demonstrated remarkable differences between meloxicam in comparison with other analyzed drugs. At low pH meloxicam was more lipophilic than piroxicam or tenoxicam and similar to ketoprofen and naproxen [16].

Two different, reversed phase high performance liquid chromatography (RP-HPLC) methods for the determination of analyte (e.g., diclofenac, fenbufen, indomethacin, ketoprofen, naproxen, nimesulide, piroxicam, salicylic acid, tramadol) dissociation constant and lipophilicity parameters, were presented. They differ in the number of required experiments and the accuracy of the obtained results. The validity of the presented methods was experimentally verified on a set of basic and acidic analytes of different physicochemical properties. Both methods are theoretically founded and give insight into the mechanism of partition of an analyte between used the stationary and the mobile phases and its changes with changing pH of the eluent [17].

The influence of n-octanol in the mobile phase on estimation of lipophilic parameters using linear solvation/free energy relationship (LSER) were examined. Used a Phenomenex Gemini C18 column is a new generation silica-based hybrid column with an extended pH range capability (pH 2–12) due to the introduction of saturated hydrocarbons on the surface of the particles. The solutes set can be classified into two groups: neutral test solutes and basic (local anesthetics, β-blockers), acidic (NSAIDs: naproxen, flurbiprofen, indoprofen, fenbufen, fenprofen, ibuprofen) and neutral (steroid hormones) drugs. The inclusion of n-octanol in the chromatographic system should better mimic o/w partition system [18].

Lipophilicity was evaluated using a RP-HPLC stationary phase with and without n-octanol added to the mobile phase. A set of 46 drugs (e.g., aspirin, diclofenac, fenbufen, flufenamic acid, flurbiprofen, ibuprofen, ketoprofen, mefenamic acid, naproxen, sulindac) and flavonoids characterized by a broad structural diversity and a wide $\log P_{\mathrm{ow}}$ range (from -0.69 to 5.70) was selected. Using the Discovery-RP-Amide-C16 stationary phase, linear relationships between isocratic $\log k$ values and the volume fraction of methanol in the eluent in the presence and absence of n-octanol were found. The correlation between the derived $\log k_{\mathrm{w}}$ and the slope (S) was highly significant when a n-octanol-enriched eluent was used, implying that under such conditions the two parameters encode the same intermolecular forces. In contrast, no significant correlation between these two parameters was seen in the absence of n-octanol. The addition of n-octanol to the mobile phase was a key factor to obtain a lipophilicity index, $\log k_{\mathrm{w}}$ highly correlated with $\log P_{\mathrm{ow}}$ values ($\log P_{\mathrm{ow}}$ ranging from -0.69 to 5.70). This implies that the RP-HPLC method with proper stationary and mobile phases was of value to derive $\log P_{\mathrm{ow}}$ values for neutral drugs, and for drugs with acidic and ampholytic functionalities, which were maintained neutral at the experimental conditions [19].

The effect of different amounts of n-octanol, as mobile phase additive, on the retention of structurally diverse acidic and ampholytic compounds (e.g., acetylsalicylic acid, diclofenac, fenbufen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, mefenamic acid, meloxicam, naproxen, nimesulide, paracetamol, piroxicam, salicylic acid, tenoxicam), when being present in their neutral (or zwitterionic) form and the best n-octanol concentration for lipophilicity assessment were analyzed. For this purpose, a conventional BDS C18 column was used. Furthermore, the retention in presence and absence of n-octanol was studied at physiological pH, and correlated with $\log D_{7.4}$ values, in an attempt to establish relevant model equations for ionized acidic compounds. In this case, the maximum concentration of 0.25% n-octanol in the methanol fraction was used in order to compensate for the high affinity of anions to the stationary phase. The obtained results indicated that n-octanol may act as a weak masking agent, attenuating silanophilic (mainly hydrogen bonding) interactions even if its concentration was restricted to the saturated buffer. This behaviour was more evident in the case of strong acidic compounds. In addition, the effect of n-octanol in retention decreases with increase of lipophilicity for compounds with $\log P$ less than 2. At low pH, in which acidic compounds exist in their neutral species, merely the use of n-octanol saturated buffer as aqueous component of the mobile phase, led to 1:1 correlation with $\log P$. At physiological pH, 1:1 correlation was obtained between $\log D_{7.4}$ and $\log k_{\mathrm{w}}$ indices upon addition of 0.25% n-octanol, in the case of weak acids. For strongly ionized compounds, a good correlation was also established under the same conditions [20].

A possible relationship between lipophilicity and binding to human serum albumin was investigated for eleven arylpropionate NSAIDs (flurbiprofen, ibuprofen, pirprofen, tiaprofenic acid, alminoprofen, carprofen, fenbufen, fenoprofen, indoprofen, ketoprofen, suprofen). The hydrophobic parameter was measured as the logarithmic chromatographic retention factor values ($\log k_{\mathrm{w}}$) by RP-HPLC method. The binding of arylpropionic acids to HSA was studied in vitro by equilibrium dialysis. For each compound, a Scatchard analysis was performed, allowing the determination of the number of binding sites and the association constants (K). Binding of arylpropionic acids to HSA increased linearly with lipophilicity. This feature was restricted to the presented experimental conditions, and to the NSAIDs with lipophilic parameter $\log k_{\mathrm{w}}$ between 2.57 (suprofen) and 3.85 (carprofen). Under other experimental conditions, some factors including electrostatic and conformational forces may play an increasing role, so that the linear relationship between lipophilicity and total affinity to HSA may become inappropriate [21].

Lipophilicity values were measured with the silica-based Discovery-RP-Amide-C16 and the polymer-based ODP-50-4B stationary phases. A set of solutes with well-defined structural parameters were selected. This set included simple monofunctional compounds and complex drugs (e.g., flurbiprofen, ibuprofen, indomethacin, ketoprofen, naproxen) covering a broad range of structural parameters and $\log P_{\mathrm{ow}}$. The relationship between $\log k_{\mathrm{w}}$ and S were investigated. A LSER approach was applied to unravel the retention mechanisms of the solutes on the two stationary phases and to compare them with the partitioning mechanism in o/w. This analysis showed that retention on the Discovery-RP-Amide-C16 phase and partitioning in o/w were controlled by the same balance of structural properties, namely van der Waals volume, H-bond acceptor basicity, and dipolarizability. In contrast, the retention mechanism on the ODP-50-4B phase was governed by a different balance of structural properties [22].

Valko et al. described the application of RP-HPLC methods for the characterization of lipophilicity of potential drug molecules. A correlation study was carried out between the fast gradient retention time values and the isocratic index of hydrophobicity values obtained from isocratic runs for a large set of structurally unrelated compounds (e.g., acetylsalicylic acid, paracetamol). The defined chromatographic hydrophobicity index (CHI) can be obtained from the gradient retention time after calibrating the system with a test mixture. The CHI values showed good correlation to the hydrophobicity values obtained from several isocratic runs. From the gradient retention time obtained by incorporating acidic and basic buffers in solvent A, the acid or base character of the compounds can be revealed. The CHI (un-ionized) values showed good correlation to the $\mathrm{Clog}\,P$ values for a structurally unrelated set of compounds [23].

The CHI obtained from high-throughput gradient elution RP-HPLC with ODS column has been shown to be well correlated with $\log k$ values obtained by isocratic elution in the same system. The correlation coefficient between CHI and $\log k_{50}$ was 0.99 for a very diverse set of 55 compounds (e.g., paracetamol, salicylic acid). Results showed that the CHI value obtained by the rapid gradient elution method encodes the same information as the isocratic $\log k_{30-70}$ values [24].

A RP-HPLC has been viewed in terms of partition of a solute between a polar, aqueous mobile phase and a nonpolar stationary phase appeared especially suitable for lipophilicity (hydrophobicity) determination. Relationships between chromatographic parameters were reviewed from the point of view of convenient and reliable lipophilicity measurements. The advantages and disadvantages of the stationary phase materials, which were presently employed for the determination of lipophilicity as well as those of specific HPLC systems and procedures, were critically reported. Role of lipophilicity in drug–biomolecule interactions was discussed in terms of quantitative structure retention relationship (QSRR). Reports were analyzed on systemic information which can be extracted by multivariate methods of data processing, like principal component analysis, from sets of lipophilicity parameters determined in diverse HPLC systems [25].

Pehoureq et al. described a study of the lipophilicity of eleven cephalosporin derivatives (cefazoline, cefonicid, cefoperazone, ceforanide, cefotetan, cefotiam, cefoxitin, cefpiramide, cephalotin, cephapirin, latamoxef) by RP-HPLC method. Authors measured the binding affinity of these compounds to HSA by equilibrium dialysis in order to establish a possible structure-binding relationship. Obtained results suggest that binding affinity depends on lipophilic character of the drugs. A significant parabolic relationship between the affinity constant $\log K_{\mathrm{a}}$ and $\log k_{\mathrm{w}}$ was found [26].

Investigation of the influence of lipophilicity on the diffusion of cephalosporins into the cerebrospinal fluid (CSF) by quantitative structure activity relationship (QSAR) study, was described. The lipophilicity was expressed by $\log k_{\mathrm{w}}$ and determined by RP-HPLC method. The penetration of eight cephalosporins into CSF was studied in male Wistar rats receiving the drugs intramuscularly. A significant parabolic relationship was sought between lipophilicity ($\log k_{\mathrm{w}}$) and the capacity of diffusion across the BBB, expressed as $\log(C_{\mathrm{CSF}}/C_{\mathrm{P}})$. The cephalosporins exhibiting a moderate lipophilicity diffused well into CSF. The $\mathrm{AUC_{CSF}}/\mathrm{AUC_P}$ ratio presented a maximum value for a strongly albumin bound cephalosporin, ceftriaxone. In the experimental conditions, the ideal lipophilicity ($\log k_{\mathrm{w}}$) range for diffusion of cephalosporins from plasma into CSF was between 1.6 and 1.8 [27].

Koufopoulou et al. applied the ion pair concept in the o/w partitioning of cefepime and cefpirome to monitor the action of counter anions as lipophilicity modulators at different pH and compare it to their effect on the retention profile in reversed phase ion pair HPLC, and to establish relationships between the apparent lipophilicity and the counter anion concentration and use them in order to determine the “intrinsic” $\log D_0$. $\log D$ were determined in presence of different concentrations of sodium octanesulphonate. The $\log D_x$

values within the linear part of the $\log D$ vs. $[X^-]$ relationships were extrapolated to $\log D_0$ values corresponding to the partitioning in absence of the counter ion. Measurements were feasible at pH values close to the isoelectric points of the acidic and basic functions. A minor effect of the counter ion in the extrapolated $\log D_0$ values was reflected in a small positive difference compared to direct measurements in the case of cefpirome and the reference compound CGX-0057364 [28].

2.2. Immobilized artificial membrane (IAM) chromatography

Standardization of the chromatographic conditions in HPLC have been suggested in order to obtain calibration curves for $\log P$ prediction, the ultimate goal being the estimation of solute transport across the biological barriers. The development of IAM chromatography unfolded new perspectives in the application of HPLC for the rapid evaluation of drug partitioning into cell membranes. IAMs are monolayers of phospholipid molecules covalently bonded to a solid matrix, the surface of silica particles. IAM retention factors have been successfully used to correlate drug permeability data. The functional groups of the bonded phospholipids are considered to play an important role in retention especially if charged molecules are analyzed. For small neutral compounds the intermolecular forces resemble those underlying partitioning in octanol–water and retention in RP-HPLC. Thus besides the hydrophobic/solvophobic interactions, polar interactions, mainly expressed as H-bond acceptor basicity, are the predominant factors in IAM retention. According to the results of LSER analysis the hydrophobic term seems to have a smaller positive contribution in IAM retention compared to its contribution in o/w partitioning.

Salminen et al. measured IAM chromatographic retention factors for a set of structurally diverse drugs (acetylsalicylic acid, salicylic acid, ibuprofen, ketoprofen, indomethacin). The relationship between IAM retention and lipophilicity, molecular size and acid/base character of the drugs, and the relationship between brain distribution and IAM retention, were analyzed. IAM retention was increased with increases in lipophilicity and solute size, and decreased by the ionization of acidic groups. Ionization of basic groups had no significant effect. The concentration ratio between brain and blood was only weakly correlated with the IAM chromatographic retention or o/w partitioning [29].

The retention behaviour of 43 structurally diverse neutral and basic drugs (e.g., paracetamol) in IAM chromatography was investigated and compared to the reversed phase retention and o/w partitioning. IAM chromatography was performed using morpholinepropanesulfonic acid (MOPS) or phosphate buffer saline at pH 7.4 as the aqueous component of the mobile phase. The differences in the retention factors were attributed to increased electrostatic interactions in the MOPS environment, dependent on the fraction of charged species. The similarities/dissimilarities between the different IAM and reversed-phase retention factors and their relationships with lipophilicity data were discussed [30].

The in vivo tissue distribution of seventeen drugs (e.g., indomethacin) has been modeled by using estimated o/w and membrane/water distribution coefficients. The $\log P$ and $\mathrm{p}K_a$ data for used compounds were obtained from the Pomona 95 database. The membrane affinities were estimated using IAM column chromatography. Parameter $\Delta(\log D_{(\mathrm{o/w-membrane/water})})$, which measures a hypothetical equilibrium of the drug between of n-octanol and membrane phase was a better model of in vivo tissue distribution, as measured by adipose tissue storage index, than either o/w or membrane/water distribution coefficients alone. This demonstrates the importance of membrane distribution coefficients as a complementary descriptor of lipophilicity to $\log D$, in modeling in vivo distribution of drugs [31].

Quantitative retention activity relationship (QRAR) models of the biological activities and pharmacokinetic properties of NSAIDs (e.g., acemetazin, flurbiprofen, nabumetone, ibuproxam, piketoprofen, fenbufen, indomethacin, sulindac, fentiazac, ketoprofen, tolmetin) with predictive and interpretative ability were reported. These models were compared with the IAM column data, taken from the literature [32].

Berbato et al. considered a set of seventeen NSAIDs, including structurally unrelated compounds supporting a carboxylic function and piroxicam, with the aim to investigate their interaction with phospholipids. Drugs were examined by HPLC method on an IAM column. The chromatographic retention factors extrapolated to 100% aqueous phase ($\log K_w^{\mathrm{IAM}}$) were compared with n-octanol/buffer lipophilicity parameters. The study of the influence of different experimental conditions on the IAM chromatographic behaviour of drugs could provide insight not only into chromatographic retention mechanisms, but also into the in vivo interactions with biomembranes. The affinity indexes for phospholipids by IAM have been compared with $\log P$ values to verify their capability in describing biological activity data. The interactions with phospholipids were much better predicted from the intrinsic partition coefficient ($\log P$) than from the apparent partition value ($\log D_{7.4}$), indicating that phospholipids can counteract the influence of electrically charged functions of analytes on lipophilic interactions. The $\log K_w^{\mathrm{IAM}}$ and $\log P$ values for both NSAIDs and structurally unrelated neutral compounds result in unique scale if uniquely partition-based mechanisms take place. However, an electrostatic repulsion component was observed for the NSAIDs bearing the carboxylic function directly linked to the aromatic ring. The presented relationship indicated that a uniquely lipophilic mechanism occurs for the ten NSAIDs. It also confirmed that phospholipids could counteract the negative influence of the electric charges on the solutes. This phenomenon was already observed for basic molecules, which also showed attractive extra-interactions with the charged moiety of phosphatidylcholine, and which was different from the acidic molecules considered. Furthermore, in contrast with the HPLC parameters obtained on hydrocarbon stationary phases, the IAM measures of lipophilicity and $\log P$ values are on a unique scale, when considering both neutral and structurally unrelated ionizable molecules. To further investigate the influence of the electric charge on the NSAIDs/phospholipids interaction, for some tests compounds, with a different ionization degree was conducted. The obtained results confirmed the higher the ionization degree of the molecule, and the higher the disturbance of the electric charge on the lipophilic interaction. They suggest that membrane affinity may constitute an important prerequisite only for the specific binding NSAID/COX-2, and support the hypothesis that the two isoforms of COX have different subcellular localization [33].

Pehoureq et al. compared different methods of lipophilicity determination in a homologous series of compounds supporting a carboxylic function (carprofen, fenoprofen, indoprofen, ketoprofen, naproxen, pirprofen, soprofen, alminoprofen, flurbiprofen, ibuprofen, tiaprofenic acid). The $\log k$ of those NSAIDs was determined at pH 7.4 of the eluent, using two stationary phases e.g., octadecylsilane and an IAM packing. The chromatographic retention factors extrapolated to 100% aqueous phase ($\log k_{\mathrm{wODS}}$ and $\log k_{\mathrm{wIAM}}$) were correlated with $\log P$ and with o/w partition coefficients corrected for ionization at pH 7.4 ($\log D_{7.4}$). In this series of compounds the lipophilicity values ranked as follow: $\log k_{\mathrm{wODS}} > \log k_{\mathrm{wIAM}} > \log D_{7.4}$. The $\log k_{\mathrm{wIAM}}$ parameter determined with a buffer of physiological pH correlated well with the ionization-corrected reference lipophilicity parameter from the o/w system. Moreover, correlations between pharmacokinetic data reported for analyzed NSAIDs demonstrated the performance of $\log k_{\mathrm{wIAM}}$ to be as good as that of the reference partition coefficient in predicting bioactivity [34].

The quantitative relationship between the diffusion of arylpropionic acid NSAIDs (carprofen, fenoprofen, ketoprofen, naproxen, suprofen, flurbiprofen, ibuprofen, tiaprofenic acid) into CSF and their lipophilicity expressed as chromatographic retention factor ($\log k_{\mathrm{IAM}}$) was reported. The influence of two additional physicochemical parameters, molecular weight (MW) and $\mathrm{p}K_{\mathrm{a}}$ were also studied. The lipophilicity was expressed as $\log k_{\mathrm{IAM}}$ determined by HPLC method on an IAM column. A significant parabolic relationship was sought between lipophilicity (k_{IAM}) and the capacity of diffusion across the BBB ($r = 0.928$). The arylpropionic acid NSAIDs exhibiting a lipophilicity value between 1.1 and 1.7 entered the CSF easily. The MW was included in this parabolic relationship by means of a multiple regression analysis. Based on the results, diffusion of arylpropionic acid NSAIDs into CSF appears to depend primarily on their lipophilicity and MW [35].

The interactions of anionic species under the same chromatographic conditions for a set of drugs including acidic drugs and ampholytes (e.g., paracetamol, ketoprofen, naproxen, nimesulide, piroxicam, tenoxicam, cephadroxil, cefepime, cefpirome, ceftazidime), were discussed. Measurements for the entire set were also performed at pH 5.0 in order to further investigate the effect of ionization in the retention of basic and acidic drugs. The relationship of IAM chromatographic indices with the corresponding $\log D$ values was investigated. Authors attempted to apply LSER analysis incorporating the degree of ionization as an additional parameter, in the aim to gain insight in the balance of forces between IAM retention and o/w partitioning [36].

2.3. *Biopartitioning micellar chromatography (BMC)*

The main oral drug absorption barriers are fluid cell membranes and generally drugs are absorbed by a passive diffusion mechanism. BMC is a mode of micellar liquid chromatography that uses micellar mobile phases under adequate experimental conditions and can be useful to mimic the drug partitioning process in biological systems.

Escuder-Gilabert et al. made attempts to identify the main molecular properties responsible for the retention of a compound in BMC, and therefore to predict the retention based on its structural properties. The retention data of a set of 151 structurally unrelated compounds (e.g., diclofenac sodium, fenbufen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, nabumetone, naproxen, piketoprofen, salicylic acid, sulindac) with different hydrophobicity, molecular size, hydrogen bonding and acid–base properties were obtained, and several multivariate QSRR models were tested. The chromatographic retention of any molecule in BMC, independently of its family, in terms of the logarithm of the retention factor, can be adequately described by its hydrophobicity. The structural parameters molar refractivity, molar volume, parachor and polarizability have a negligible influence on retention. Among QSRR models, the functional nonlinear model was adequate for describing retention for both ionic and neutral compounds. The multiple linear regression models based on $\log P$ and hydrophobicity factors provide similar results to the functional model and they are simpler and easier to fit [37].

2.4. *Immobilized liposome chromatography (ILC)*

Liposomes are composed of lipid bilayers surrounding aqueous compartments. For attempts to predict drug absorption through cell membranes by use of a chromatographic model system, liposomes or biological membrane vesicles can be sterically immobilized by entrapment in the pores of gel beads upon freeze–thaw fusion of small liposomes or vesicles.

Drugs were applied to ILC columns to study the partitioning of drugs into liposomes, which were immobilized by freeze–thawing in small agarose-dextran gel beads. Drugs of various structures (e.g., acetylsalicylic acid, salicylic acid) and with a wide range of $\log P_{\mathrm{ow}}$ values were applied to the columns and showed widely different retention volumes. The ILC results were compared with $\log P$ determined with free liposomes, and with data on drug absorption through monolayers of cultured epithelial cells of the cell line Caco-2, and on absorption of orally administered doses [38].

2.5. *Reversed-phase thin-layer chromatography (RP-TLC)*

The determination of the lipophilicity by reversed phase techniques can have very high practical importance, especially when high biological activity is also a consideration. RP-TLC is a rapid, easy to perform technique which requires small quantities of the sample and enables simultaneous analysis of several compounds. The lipophilicity determined by RP-TLC can be expressed by R_{M} parameter value (obtained from retention factor values). It may be calculated using the formula: $R_{\mathrm{M}} = \mathrm{R}_{\mathrm{MW}} - S\varphi$, where R_{MW} is the theoretical value of R_{M} of analyte extrapolated to zero concentration of organic modifier in mobile phase, S is the slope of regression curve, φ is the volume fraction of organic modifier in the mobile phase. TLC method frequently has been used to investigate QSAR and QSPR.

A RP-TLC retention behaviour of structurally diverse drugs (e.g., nimesulide) has been investigated. Phosphate buffer, phosphate buffered saline, and MOPS, with or without addition of n-decylamine, at pH 7.4, and phosphate buffer at pH 11.0 were used with different proportions of methanol as the mobile phase. The buffer constituents of the mobile phase play an active role in retention, affecting, at constant pH 7.4, extrapolated R_{MW} values, the corresponding S, and their relationship. No further effect was observed on addition of a masking agent. Although ionization did not seem to facilitate migration significantly, R_{MW} values correlated better with $\log D_{7.4}$ than with $\log P$. Use of $\log D$ combined with the ionization-correction term, led to improved correlation and revealed a reduced net effect of ionization in retention. Finally, MOPS was found to be more suitable than phosphate buffer for lipophilicity assessment [39].

Giaginis et al. discussed the role of the aqueous component of the mobile phase in retention behaviour, extending the data set to include sixteen basic and ten neutral drugs (e.g., paracetamol). Phosphate buffer at pH 11.0, phosphate buffered saline, MOPS at pH 7.4, and pure water were used with different proportions of methanol as the mobile phase. Use of n-octanol as mobile phase additive was also investigated. R_{MW} values obtained under different mobile phase conditions were evaluated for their performance as lipophilicity indices by comparison with $\log P_{\mathrm{ow}}$ or $\log D$. LSER were established for all data sets. Apart from the reduced effect of ionization, hydrogen-bond basicity and volume were found to affect to an almost equal extent o/w partitioning and RP-TLC retention under all conditions at pH 7.4, indicating close similarity between the different processes. At pH 11.0 a lower negative contribution of hydrogen-bond basicity to retention compared with o/w partitioning, was noticed [40].

The lipophilicity and specific hydrophobic surface area of eighteen NSAIDs drugs have been determined by RP-TLC for subsequent studies of QSAR. The mobile phases were mixtures of water and methanol containing 50 mM acetic acid, sodium acetate, or sodium chloride. The lipophilicity and specific hydrophobic surface area were calculated from the linear relationship between the actual R_{M} value of the solutes and the concentration of methanol in the mobile phase. The spectral mapping technique indicated that eluent additives increase the apparent lipophilicity and specific hydrophobic surface area of drugs [41].

Ionization constants and lipophilicity, expressed as $\log P$ from the o/w system, or as R_{M} values from RP-TLC, were determined and discussed in relation to structural characteristics of some aryl-(amino or hydroxyl)ethylamino ketones. It was found that both experimental expressions for the tested basic compounds were in agreement. The influence of functional groups, and R_M values can be used as a fast, convenient and reliable method for the evaluation of the lipophilicity of similar structures. Acute toxicity and anti-inflammatory activity tests, using the adjuvant induced disease (AID) model, were also conducted. The tested compounds possess protective, as well as curative properties in AID rats [42].

Sarbu et al. investigated the feasibility of the scores, obtained by principal component analysis using RP-TLC retention data, as a measure of lipophilicity in correlation with biological activity of NSAIDs (aspirin, indomethacin, ibuprofen, ketoprofen, naproxen, diclofenac, piroxicam, tenoxicam, phenylbutazone, niflumic acid), eigenvector analysis, also affords a useful graphical tool, since scatter plots of the scores onto a plane described by the first two components will have the effect of separating the compounds one from each other most effectively, obtaining in this way a “congeneric lipophilicity chart” [43].

The chromatographic behaviour of the phenolic drugs (e.g., ibuprofen), has been investigated on $RP8F_{254}$ and $RP18F_{254}$ TLC plates with methanol-water mixtures in different volume proportions as mobile phases. Linear relationships were obtained between the R_M values of the drugs and the volume fraction of methanol in the mobile phase. R_M values were extrapolated to zero methanol content and the obtained lipophilicity values $R_{MW(RP8)}$ and $R_{MW(RP18)}$ obtained were compared both with measured $\log P_{exp}$ and with P calculated using different software products (AlogPs, IAlogP, ABlogP, COSMOFag, miLogP, KowWin, and xlogP). The results indicate that chromatographic lipophilicity R_{MW} can be used as a measure of the lipophilicity of investigated phenolic drug [44].

3. Shake-flask method

The classical and most reliable method of $\log P$ determination is the shake-flask method, which consists of dissolving some of the solute in a volume of octanol and water, then measuring the concentration of the solute in each solvent. The most common method of measuring the distribution of the solute is UV/vis spectroscopy. There are a number of benefits (most accurate method, accurate for broadest range of solutes, neutral and charged compounds applicable, chemical structure does not have to be known beforehand), and defects to this method (time consuming, above 30 min per sample), octanol and water must be premixed and equilibrated (takes at least 24 h to equilibrate), complete solubility must be attained, and it can be difficult to detect small amounts of undissolved material and the concentration vs. UV/vis response must be linear over the solute's concentration range. If the compound is extremely lipophilic or hydrophilic, the concentration in one of the phases will be exceedingly small, and thus difficult to quantify, relative to chromatographic methods, large amounts of material are required.

The drug niflumic acid is an amphoteric substance with overlapping pK_a values. The acid–base chemistry of the molecule has been characterized in terms of protonation macroconstants (with reference to stoichiometric ionizations) and microconstants (with reference to ionizations of individual species). The proton-binding sites were assigned using ^1H and ^{13}C NMR spectroscopy. The apparent partition coefficients (P_{app}) of niflumic acid were measured in o/w solution by the shake-flask method over a wide pH range. The lipophilicity profile shows a parabolic shape near its maximum at the isoelectric point. A relationship derived between P_{app}, micropartition coefficient of the uncharged microspecies and P of the anion is valid for amphoteric drugs, in cases where the partition of the unionized form and the ion–pair partition of anion can be confirmed. The $\log P$ values of microspecies indicate the high lipophilicity of niflumic acid, which is consistent with its good skin penetration and absorption [45].

The lipophilicity of potential prodrugs was evaluated both via traditional experimental parameters, such as P (by the shake-flask method) and R_M value (by RP-TLC method) and by predictive computational methods KowWin. Synthesize and predict the permeation profiles of a few NSAID (diclofenac, ibuprofen, ketoprofen, tiaprofenic acid, tolmetin) derivates designed to increase their access to the brain, were reported. From experimental parameters, all prodrugs were more lipophilic when compared to their corresponding parent compounds and consequently a better BBB penetration was hypothesized. The correlation between experimental R_M and $\log P$ values (calculated by KowWin's method) was good for all compounds with the exception of one compound, a prodrug of tiaprofenic acid [46].

Medic-Saric et al. described a correlation the experimentally determined by shake-flask method and calculated $\log P$ values, for salicylamide using nine different computer programs (HyperChem, xlogP, KowWin; MlogP; ClogP; milogP, AlogPs, IAlogP; CSlogP). The results of analysis demonstrated good agreement between the experimentally observed $\log P$ value, and the value calculated by the CSlogP program, based on topoplogical structure descriptors and electrotopological indices [47].

The set of compounds (e.g., carprofen, flurbiprofen, ibuprofen, naproxen, pirprofen, suprofen, ketoprofen, indomethacin) was used to develop a solvatochromic equation. The compounds were rigid ones covering a broader range of van der Waals volumes, dipolarity/polarizability, H-bond-acceptor basicity and H-bond-donor acidity values, than the compounds in the initial set. Moreover, the comparison between solvatochromic equations in the o-nitrophenyl octyl ether (o-NPOE)/water, 1,2-dichloroethane (DCE)/water, and o/w system was discussed. Partition coefficients in o-NPOE/water ($\log P_{NPOE}$) were measured by the shake-flask or the potentiometric method. The partitioning mechanism of the investigated compounds in o-NPOE/water was controlled by the same structural properties as it is in DCE/water. Difference between $\log P_{oct}$ and $\log P_{NPOE}$ express mainly dipolarity/polarizability and H-bond-donor acidity [48].

The apparent intrinsic clearance of thirteen drugs (e.g., indomethacin, ketoprofen, oxaprozin, piroxicam, sulindac, diclofenac, ibuprofen) were determined using rat liver microsomes at three different concentrations of microsomal protein. The kinetics was studied using the in vitro half-life method. Presented studies used a variety of microsomal protein concentrations, and the resulting data were consequently unsuitable for finding predictive quantitative relationships between the physicochemical properties of the compounds and the extent of microsomal binding. To find such relationships, data were required on a set of compounds with a variety of physicochemical properties, and where the binding was measured under identical conditions of microsomal protein concentration. Partitioning of compounds between octanol and 0.02 M phosphate buffer, pH 7.4, at 20 ◯C was determined using a standard shake-flask method. Samples were analyzed by HPLC method with MS detection of both layers of the partition mixture [49].

Dellis et al. investigated the physicochemical profile of nimesulide over a broad pH range by means of experimental and calculative procedures. Research plan included: investigation of the lipophilicity and solubility profile of nimesulide as a function of pH, and comparison with theoretical profiles and calculated values, comparison between direct partitioning/pH and retention/pH profiles, in presence and absence of octanol as mobile phase additive, exploration of the appropriateness of the lipophilicity and solubility profiles to generate the pK_a value of nimesulide. Lipophilicity was assessed by direct partitioning experiments in the o/w system using the shake-flask method, as well as by RP-HPLC, using methanol as organic modifier, with or without addition of octanol. Both lipophilicity and solubility/pH profiles of nimesulide showed deviations from the theoretically expected behaviour, as dictated by the Henderson–Hasselbach equation, concerning the difference between the corresponding values of the neutral and ionized species in the case of a weak acid. Since deviations affected mostly the values at increased ionization, the $\mathrm{p}K_\mathrm{a}$ value of nimesulide could be accurately calculated using part of both lipophilicity and solubility profiles [50].

The possibility of using the combination of two descriptors representing the lipophilicity (as measured by octanol–buffer partitioning and RP-HPLC techniques) and relative hydrophobicity (as measured by aqueous two-phase partitioning) of organic compounds for analysis of drugs known to cross or not to cross the BBB, was discussed. Relative hydrophobicity and lipophilicity of different compounds (e.g., ibuprofen, indomethacin) with known permeability through the BBB was examined by partitioning in aqueous dextran-poly(ethylene glycol) two-phase system and octanol–buffer system, and by gradient RP-HPLC at pH 7.4. Combination of the relative hydrophobicity was estimated. $\mathrm{N(CH_2)}$ obtained by aqueous two-phase partitioning and the lipophilicity ($\log D_\mathrm{exp}$ or $\log D_\mathrm{HPLC}$) values, obtained by the shake-flask technique or HPLC technique allows one to differentiate between compounds capable of crossing the BBB and those cannot. Moderate levels of both relative hydrophobicity and lipophilicity values were required for compounds to be able to cross the BBB; more lipophilic compounds must be more sensitive toward their aqueous environment to cross the BBB. Authors suggest that the combination of the relative affinity of a compound for a cellular membrane (represented by $\log D/\log P$ value) and that for the different aqueous media in blood and in interstitial fluid in brain (represented by $\mathrm{N(CH_2)}$-value) may be predictive of the ability of a compound to permeate the BBB by passive diffusion [51].

Molecular lipophilicity can be expected by $\log P$ or, more conveniently, by $\log k_\mathrm{w}$, determined by the traditional shake-flask technique, or by RP-HPLC. The measured $\mathrm{p}K_\mathrm{a}$ values of the twelve NSAIDs were compared. The lipophilicities of the unionized forms must be measured at pH below 2.0. The values of $\log k_\mathrm{w}$ were obtained by a series of isocratic measurements, at various compositions of binary acetonitrile–water eluents. Extrapolation of the relationship between $\log k_\mathrm{w}$ and volume fraction of organic solvent, to 100% water was made. The $\log P_\mathrm{ow}$ of analyzed NSAIDs were determined by shake-flask technique using a conventional methodology. A significant linear relationship was obtained between $\log P_\mathrm{ow}$ and $\log k_\mathrm{w}$ with a slope close to unity, indicating similar intrinsic thermodynamic behaviour of these drugs for the two partitioning processes. This excellent correlation prompted authors to validate this polymer-based column, to be useful for the determination of other acidic drug lipophilicity [52].

Hatanaka et al. analyzed a skin permeation behaviour of zwitterionic drugs. Cephalexin, a β-lactam antibiotic with an amino and a carboxyl group, was selected as a model of drug. Distribution coefficients of cephalexin between octanol and buffer solutions were determined by a shake-flask method. The apparent diffusion coefficients of cephalexin in 0.1 mM phosphate buffer solution at various pH (3.0–7.0) were determined by the capillary-cell method. The permeability coefficient of cephalexin decreased with increase in zwitterions fraction and decrease in fractions of cation and anion, suggesting that each ionic species has different skin permeability. The octanol/buffer distribution coefficient and diffusion coefficient were also lower for zwitterion than for cation and anion. The results demonstrated that pH-dependent skin permeation of cephalexin may reflect the perm selective property of skin due to the different lipophilicity and diffusivity of each ionic species [53].

4. Optical methods

In the recent past some experiments using polarized liquid interfaces have been used to examine the thermodynamics and kinetics of the transfer of charged species from one phase to another. Two main methods exist; ITIES, interfaces between two immiscible electrolyte solutions which for example has been used at Ecole Polytechnique Federale de Lausanne; and droplet experiments which have been used by Alan Bond and Frank Marken and also by the team at the Ecole Polytechnique Federale de Lausanne [54–56]. A reaction at a triple interface between a conductive solid, droplets of a redox active liquid phase and an electrolyte solution have been used to determine the energy required to transfer a charged species across the interface [57].

Optical tramping Raman microscopy is a powerful analytical method that allows the investigation of membrane interactions with drugs or other compounds. This technique allows membrane fluidity, permeability, and drug localization to be assessed while simplifying sample preparation and eliminating effects associated with labeling. Fox et al. presented study about these capabilities with small-molecule drugs and model phosphatidylcholine membrane vesicles. Although both, salicylate and ibuprofen led to membrane disorder according to drug concentration, only ibuprofen preferentially accumulated in the membrane itself. The extent of ibuprofen accumulation determined from its scattering intensity compared to the solution correlated well with its P_ow. The results indicate that ibuprofen accumulates in the hydrophobic portion of the membrane, whereas salicylate interacts with the lipid head groups. Although high drug concentrations are often needed to detect a Raman signal, it is important to note that the effects of the drug on the membrane lipids can be detected in Raman scattering at much lower concentrations [58].

4.1. Transmission electron microscopy (TEM)

Transmission electron microscopy is a powerful and unique technique for structure characterization. The most important application of TEM is the atomic-resolution real-space imaging of nanoparticles.

Charkaboty et al. showed that even in absence of net surface charges different prototropic forms of the drugs, viz. global neutral and anionic forms of piroxicam and meloxicam are capable of partitioning in the dimyristoylphosphatidylcholine (DMPC) vesicles. They have also tried to find the reason behind the differential partitioning of piroxicam and meloxicam in the DMPC vesicles. TEM was used to characterize small vesicles formed under different experimental pH. The changes in the intrinsic fluorescence properties of the different structural forms of drugs have been used to study the interaction of piroxicam and meloxicam with DMPC vesicles. Both the prototropic forms of meloxicam, viz. global neutral and anionic, show higher partitioning into the vesicles was attributed to the difference in chemical nature of the third ring of the two drug molecules. For bio-membranes having no net surface charges, hydrophobic effects would play a principal role in guiding these drugs to their targets [59]. The phototautomerism of piroxicam was studied in free aqueous solution, and in the confined aqueous interior of the reversed micelles of Aerosol-OT in iso-octane, using both steady-state and picosecond time-resolved fluorescence microscopy, at $\lambda = 384\,\mathrm{nm}$ [60].

5. Electrochemical methods

The effect of drug lipophilicity on in vivo iontophoretic transdermal absorption was evaluated. NSAIDs (salicylic acid, ketoprofen, naproxen, indomethacin) were selected as model drugs with a wide range of lipophilicity. The log *P* values at pH 7.4 are in the range of 2.18–1.32. Cathodal ionthophoresis was conducted in rats, and drug concentrations in skin, cutaneous vein and systemic vein were determined. Presented study demonstrated the effect of drug lipophilicity on in vivo iontophoretic transdermal absorption of NSAIDs. Absorption into the skin was higher in the case of lipophilic drugs, whereas drug transfer from skin to cutaneous vein decreased with an increase in lipophilicity. Skin concentrations of analyzed NSAIDs were higher in the case of lipophilic drugs, whereas cutaneous plasma concentrations decreased with an increase in lipophilicity. The dependence of lipophilicity on cutaneous plasma concentration was similar to systemic plasma concentration [61].

Sodium salicylate, naproxen sodium, diclofenac sodium, diclofenac potassium and ibuprofen sodium are completely dissociated in aqueous solutions. Their anions are weakly hydrated due to its hydrophobicity, whereas the hydratation of cations depends on their charge densities. The electric conductivities of aqueous solutions of those NSAIDs were measured in the temperature range from 278.15 to 315.15 K (in steps of 5 K) and in the concentration range from 3×10^{-4} to 0.007 M. Data analysis based on the low concentration chemical model of electrolyte solutions yielded the limiting molar conductivity, and the *K* value. Using the known data of the limiting conductivities of sodium and potassium ions the limiting conductivities anions was evaluated, and the radii of anions in water were estimated. Total dissociation of the investigated salts in water was evident and the considerable differences in the anion mobility were observed [62].

An approach for the determination of liposomal membarane–water *P* and lipophilicity profiles of ionizable drugs (e.g., ibuprofen, diclofenac) were presented. The results shown that the liposomal membrane–water *P* as derived from the pH-metric technique are consistent with those obtained from alternative methods, such as ultrafiltration and dialysis. In liposome system partitioning of the ionized species was significant and was influenced by electrostatic interaction with the membranes [63].

A simple procedure for deriving log *P* values readily and reproducibly, by carrying out several linked acid–base titrations with an ordinary laboratory titrator, was provided. It described requires comparatively little financial investment or training, and gives access to the log *P* of an ionizable compound within a few hours. The versatility of potentiometric log *P* determination was illustrated for 12 ionizable organic compounds (comprising carboxylic acids, phenols, sulfonamides, amines and quinoline derivative; e.g., ibuprofen, paracetamol). The research outlined how o/w (and liposome–water) partition coefficients can be evaluated for ionizable compounds, without the need to resort to expensive, specialized equipment or software. There were a few practical, albeit not fundamental limitations: the method works well for monoprotic and diprotic ionizable compounds with pK_a values in the range of 3–10, which are more easier to analyze than compounds with more extreme pK_a values; compounds have to be soluble in aqueous KCl or octanol at millimolar concentrations throughout the whole pH range [64].

The lipophilicity of 28 acidic compounds with various functional groups (e.g., isoxicam, suprofen, naproxen, pirprofen, flurbiprofen, ibuprofen, carprofen, indomethacin, sulindac, phenylbutazone) was studied by potentiometry and cyclic voltammetry, in the o/w and DCE/water systems in order to complement understanding of the lipophilicity of neutral and ionized acids, and to clarify the solvation mechanisms responsible for their partition. The parameter $\Delta(\log P_{\mathrm{DCE}}^{\mathrm{N-A}})$ (e.g., log *P* of the neutral acid minus standard log *P* of the conjugated anion in DCE/water) was calculated. The potentiometric method was based on the observation, when an ionizable compound in aqueous solution was titrated in the presence of an organic phase, the titration curve was shifted to the right for acids and to the left for bases. This shift is related to the pK_a and the lipophilicity of both, the neutral and ionized forms of the solute, and to the volume ratio of the two phases. Cyclic voltammetry has recently introduced to determine the lipophilicity of ions and study their mechanisms of transfer at the interface. This method requires the use of a polarizable interface, excluding the o/w system. Given interesting properties, the system usually used DCE/water. The parameter $\Delta(\log P_{\mathrm{DCE}}^{\mathrm{N-A}})$ depends not only on intramolecular interactions and conformational effects in the neutral and anionic forms, but also on the delocalization of the negative charge on the anion. The value of this parameter decreases when the delocalization of the negative charge increases, due to the increased stabilization of the anion in the organic phase [65].

Hosseinzadeh et al. discussed an interactions of diclofenac with bovine serum albumin (BSA), that play an important role in understanding drug transport, distribution and interaction, using diclofenac selective membrane electrode. The process showed positive cooperation in both binding sets for all of the studied conditions. The positive cooperation in first binding set represented that both electrostatic and hydrophobic interactions contribute in this set. The positive cooperation in the second binding set, referred to essentially hydrophobic nature of interaction in this set. The binding strength of the first and second binding sets decreased by increasing of BSA concentration from 0.5 to 2 mg mL^{-1}. Also comparison between binding strength in second binding set showed that binding strength, strongly affected by variation of protein concentration, and indicated that at high concentration of BSA aggregation of protein have been occurred [66].

6. Calculation procedures

Lipophilicity, widely expressed by log *P* or log *D*, plays an important role of several ADME, as well as in the pharmacodynamic and toxicological profile of drugs. A rich arsenal of calculation procedures has been developed for rapid estimation of log *P*.

QSPR algorithms calculate a log *P* in several different ways. The simplest method for prediction of log *P* is parameterizing the contributions of various atoms to the over all molecular *P* values using constrained least squares fitting to a training set of compounds with experimentally measured *P* values [67–69]. In order to get reasonable correlations, the common elements contained in drugs (hydrogen, carbon, oxygen, sulfur, nitrogen, and halogens) are divided into several different atom types depending on the environment of the atom within the molecule. While this method is generally the least accurate, the advantage is that it be able to provide at least a rough estimate for a wide variety of molecules. Fragment based prediction (group contribution) has been shown, that the log *P* of a compound can be determined by the sum of its non-overlapping molecular fragments (defined as one or more atoms covalently bound to each other within the molecule). Fragmentary log *P* values were determined in a statistical method analogous to the atomic methods (least squares fitting to a training set). In addition, Hammett type correlations were included to account of electronic and steric effects. This method in general gives better results than atomic based methods, but cannot be used to predict *P* values for molecules containing unusual functional groups, for which the method has not yet been parameterized [70,71]. A typical data mining based prediction uses support vector machines [72], decision trees, or neutral networks [73]. This method is usually very successful for calculating log *P* values when used with compounds that have similar chemical structures and known log *P* values. Molecule mining approaches apply a similarity matrix based prediction or an automatic fragmentation scheme into molecular substructures. Furthermore, there exist also approaches using maximum common subgraph searches or molecule kernels. Estimation of log *D* (at a given pH) from log *P* and pK_a, and prediction of pK_a were studied [5]. For prediction of pK_a which in turn can be used to estimate log *D*, Hammett type equations have frequently been applied [74].

There are many log *P* calculators or predictors available both commercially and for free, e.g., ACDLogP DB (a commercial application that calculates log *P* values and includes the largest commercially available database of experimental log *P* values with calculation of rule-of-five parameters), ACDLogP Freeware Download (free log *P* calculator), AlogPs (free online calculations and comparison of ten log *P* methods), ClogP and others.

The relationship between physicochemical property and toxic effectiveness using rat red blood cells (RBCs) was explored. The toxic effectiveness of NSAIDs (e.g., aspirin, diclofenac, flurbiprofen, ibuprofen, indomethacin, ketoprofen, mefenamic acid, salicylic acid) was systematically examined by the depletion of intracorpuscular adenosine triphosphate, glutathione, and hemoglobin at various doses. The RBCs were incubated with those drugs. Authors created the interaction between the toxic effects and physicochemical properties of the desired drugs. The interaction would contribute to a potential therapeutic utility. Physicochemical properties of drugs were obtained using a QMPRPlus^{TM}, which is a state-of-the-art computer program: designed to estimate certain ADME properties of a drug from its structure, log *P*, effective permeability, apparent permeability, molar volume, diffusivity, and percent unbound to blood plasma proteins. Experimental log *P* and pK_a were obtained from a CS ChemDraw Ultra, CS ChemOffice, Drawing, Modeling and Informations, Cambridge Soft [75].

Hadgraft et al. presented the penetration pathways of a selection of NSAIDs (e.g., diclofenac, flufenamic acid, flurbiprofen, ibuprofen, indomethacin, ketoprofen, mefenamic acid, naproxen, piroxicam, tiaprofenic acid) through the skin. The calculations were based on physicochemical parameters (MW, melting points, log *P*, P_{app}), that can be predicted using commercially available software. The bio-effectiveness of the drug will be a function of both, its penetration through the skin and its potency. pH partition behaviour into the skin has been considered together with the relative impact of decreased permeation, but increased solubility with degree of ionization [76].

The study of the solubility of ibuprofen and naproxen in aqueous 1,2-propanediol (PG) at several temperatures had as its purpose the modeling of the solubilities in terms of the solvent composition. The data required for the application of the IKBI (the Inverse Kirkwood–Buff Integral) and QLQC (the quasi-lattice quasi-chemical approaches) methods in the present instance were first of all the solubilities of the ibuprofen and naproxen in the solvent mixtures, water and PG. These were provided at five temperatures, between 20 and 40 °C at six solvent compositions. Ibuprofen has a hydrophilic head, the carboxylic group, and a hydrophobic tail. Naproxen has in addition the hydrophilic head also a methoxy group in the, albeit more bulky, hydrophobic tail. Neat PG solvated both drugs many times better than water. The neat solvents have a network of hydrogen bonds, more pronounced and stiff for water than for PG, so that the formation of cavity in the solvents to accommodate the bulky solutes in an endoergic process, more in water than in PG. In the mixtures, the interactions between PG and water moderate the difference in the solvation abilities of the neat solvents. This interaction loosens the tight hydrogen bond network of the water and facilitates the inclusion of the drug molecules, in addition to the direct solvation of the polar groups. The result was a preferential solvation of both drug molecules by PG at all compositions, but not as much as would have been suggested by the differences in the solvation abilities of the neat solvent components [77].

D values were calculated from the microscopic dissociation constants (microconstants), *P* values of the microspecies, and the counterion concentration. Different compounds (e.g., ketoprofen, mefenamic acid) were used. In order to enable predictions of *D* value at any pH, a program module called PrologD has been developed. The method estimated log *D* from the two-dimensional structure of the microspecies compounds [78].

Fini et al. studied the surfactant behaviour of 10 NSAIDs solutions (alclofenac, diclofenac, fenclofenac, indomethacin, sulindac, fenbufen, ibuprofen, indoprofen, ketoprofen, naproxen). The drugs were chosen within the acetic and propionic acid classes and were used in the form of sodium salts or, in the case of low solubility of this salt form, as salts with a hydrophilic organic base. Calculation of the log *P* values for each drug was performed by ClogP software. The log *P* of the anions was calculated from that of the acids substracting contribute of the carboxyl group and adding that of the carboxylate. In order to calculate the hydrophilic/hydrophobic ratio of the anions, authors attributed the main hydrophilic contribution to the carboxylate group and the hydrophobic weight to the remaining part of the molecule [79].

Evaluation the enhancement effect of the ethanol/water (60/40) binary vehicle with lauric acid on the skin permeation of various drugs (salicylic acid, alclofenac, ketoprofen, ibuprofen) having a wide range of lipophilicity as indicated by the log *P* (from −0.95 to 4.40) was discussed. log *P* values were obtained from literature or by the calculation, which was made by using Hansch values. The permeation rate increased with an increase in the hydrophilicity of the drugs. The relationship between lipophilicity and skin permeability of the drugs from the ethanol/water (60/40) binary vehicle with lauric acid showed a parabolic shape, with its peak at a more hydrophilic range (log *P* = 0.19) compared with other past references (log *P* = 2–3). The ethanol/water (60/40) binary vehicle with lauric acid appears to be a good candidate as a vehicle for transdermal therapeutic systems for hydrophilic drugs [80].

Huuskonen et al. determined the efficacy of atom-type electrotopological state indices for estimation of log *P* values in a set of drug compounds or related complex chemical structures (e.g., ketoprofen, flufenamic acid, salicylic acid). Multilinear regression analysis and artificial neutral networks were used to construct models, based on MW and atom-type electrotopological state indices. Atom-type electrotopological state indices offered valuable parameters for fast evaluation of log *P* that can be applied to screen large databases of chemical compounds, such as combinatorial libraries [81].

Characteristic of the barrier function of the stratum corneum and the viable epidermis and prediction of their influences on the skin permeability and the maximum fluxes of the NSAIDs (diclofenac, flufenamic acid, ibuprofen, ketoprofen, naproxen, nabumetone, piroxicam, tenoxicam, aspirin, diflunisal), by model equations were described. The permeability of the human skin for those drugs applied in a lipophilic vehicle is a function of their hydrophilicity e.g., of their *P* between phosphate buffer (pH 7.4) and the lipophilic vehicle, while the maximum flux is primarily dependent on their vehicle solubilities. The experimentally determined permeabilities P_{skin} generally decrease with increasing lipophilicity of the drugs expressed as P_{ow}. For the development of cutaneous preparations, drugs with high vehicle solubilities and skin permeabilities, as high as possible, should be preferred [82].

Different chemical structures have been found to possess different anti-inflammatory activities. In view of the complexity and multitude of biochemical factors involved in inflammatory events, few general correlations of chemical structures and physicochemical properties with biological activities would be expected. In the lead optimization phase of the synthetic project various QSAR procedures with the aid of computer-technology have been proposed. Among them, the classical Hansch approach has been widely used. The QSAR procedures are based on physical organic concepts and involve calculational operations. Since inflammation is a complex phenomenon involving interrelationships between humoral and cellular reactions through a number of inflammatory mediators, there is not much evidence on QSAR studies. Several QSAR studies have been reported obtaining only partial results. It was found that substituents which contribute to the high lipophilicity, were favorable to the activity. Substituents of short length (H, CH_3) have also a favorable effect. Satisfactory relationships between the in vivo activities and deprotonation energies, the HOMO energies and lipophilicities were found [83].

Selective COX-2 inhibitors (e.g., rofecoxib) have attracted much attention in recent times in the design of NSAIDs, which were devoid of the common side effects of classical NSAIDs. QSAR studies have been performed on a series of diaryl furanoes that acts as selective COX-2 inhibitor using Molecular Operating Environment. The researches were carried out on forty three analogues. The COX-2 inhibitory activity was found to show a positive correlation with lipophilicity. System determined $\log P$ including implicit hydrogens and was calculated from a linear atom type model. COX is a membrane-based enzyme and the entry of the inhibitor in the enzyme requires that molecule should be lipophilic in nature. Thus increase in lipophilicity increases inhibitory activity. In addition, quantitative structure activity analysis was reported for diaryl furanoes. Thus such studies may help for the design and synthesis of better selective COX-2 inhibitors [84].

Charkaboty et al. explored QSAR for COX-2 bringing of 1,2-diarylimidazoles using E-state index as the electronic parameter, and hydrophobic and steric substituent constants of the aryl ring substituents as the lipophilic and steric parameters, respectively along with appropriate indicator variables. The results were compared to the equations obtained from Hammett substituent constants as electronic parameters. Lipophilicity of ortho, meta and para substituents of the aryl ring increases the binding affinity, while molar refractivity of ortho and meta substituents of the aryl ring decreases the binding affinity. Again, electron-withdrawing substituents at meta and para positions of the aryl ring increase the binding affinity. Additionally, a 4-fluoro substituent on the aryl ring, a trifluoromethyl substituent at R_3 position and simultaneous presence of 3-chloro and 4-methyl groups on the aryl ring are conductive to the binding affinity. Also, an amino substituent is preferred over a methyl group at R_2 position suggesting preference of the sulfonamide moiety over the methyl sulfone moiety for the COX-2 binding affinity. Furthermore, importance of E-state values of different atoms in the generated relations suggests the influence of electron density distribution over the 1,2-diarylimidazole nucleus for the binding affinity. For this data set of 1,2-diarylimidazoles, E-state parameters perform better as electronic parameter, in comparison to Hammett sigma parameters. E-state parameters could explain the variance of COX-2 binding affinity to satisfactory extent, when used in combination with lipophilic and steric substituent constants and suitable indicator parameters. When hydrophobic whole molecular descriptor ClogP was used, instead of hydrophobic substituent constant, quality of the models increased substantially [85].

The QSAR of two hundred thirty two structurally diverse drugs (e.g., nabumetone, acetylsalicylic acid, diclofenac, flurbiprofen, ibuprofen, indomethacin, ketoprofen, naproxen, oxaprozin, salicylic acid, tenoxicam) was studied to evaluate the feasibility of constructing a predictive model for the human oral bioavailability of prospective new medicinal agents. The oral bioavailability determined in human adults was assigned one of four ratings, and analyzed in relation to physicochemical and structural factors by the ORMUCS (ordered multicategorical classification method using the simplex technique) method. Lipophilicity, expressed as D values at pH of blood (7.4) and the small intestine (6.5), was found to be a significant factor influencing bioavailability [86].

Khoshneviszadeh et al. conducted a QSAR study on some of NSAIDs (e.g., celecoxib, rofecoxib) to find quanitative relationship between their molecular structure and COX-2 inhibitor activity, which help to find structural requirement for more selective inhibition. A combination of different chemometrics methods including multiple linear regression with factor analysis as the data preprocessing (FA-MLR), principal component regression analysis (PCRA), and genetic algorithm partial least squares (GAPLS) analysis were employed. The study suggests the importance of lipophilicity and shape of molecules for COX-2 activity, which is in agreement with previous docking studies of some COX-2 inhibitors on the active site of COX-2 enzyme. Presence of methyl or methoxy substitutes on para position of the phenyl ring enhances hydrophobic interaction with the enzyme and therefore increases compound potency. Authors found a quadratic effect for lipophilicity, which indicates that it should have an optimum value. By taking partial derivative from the resulted QSAR models having quadratic term of lipophilicity the optimum values of $\log P$ were obtained between 1.5 and 2.0. In addition, the results indicated that increasing the molecular surface could be resulted in enhanced ligand–receptor interaction [87].

A QSAR model for corneal permeability applicable to all compound classes was used. A parabolic relationship with good correlation between lipophilicity, $\log P_{\mathrm{ow}}$ and the permeability in individual analyses of compound (e.g., ibuprofen) was obtained. Even with the addition of miscellaneous compounds, such as methanol and ibuprofen, the $\Delta\log P$ and lipophilicity terms were still significant. However, small molecules were likely to be underestimated, which was consistent with penetration via another pathway besides that governed by $\Delta\log P$ and lipophilicity [88].

The synthesis of a series of 2-anilinophenylacetic acids, close analogues of diclofenac, was described. These compounds were tested in two models used for evaluating the activity of NSAIDs, inhibition of COX enzyme activity in vitro, and adjuvant-induced arthritis in rats. Statistically significant correlations were found between the inhibitory activities of the compounds, indicating that COX inhibition seems to be the underlying mechanism for the anti-inflammatory activity of these compounds. QSAR analysis revealed that the crucial parameters for activity in both models were the lipophilicity and the angle of twist between the two phenyl rings. Optimal activities were associated with halogen or alkyl substituents in both ortho positions of the aniline ring. Compounds with OH groups in addition to two ortho substituents or compounds with only one or no ortho substituents were less active [89].

In a series of 2-acetoxyphenyl alkyl sulfides, most potent compound selectively inactivates COX-2 by acetylating the same serine residue that aspirin acetylates and has ability to attenuate growth of COX-2 expressing colon cancer cells. Therefore, QSAR studies have been done, in order to explore the substitutional requirement of S-alkyl chain carbonyl moiety of 2-acetoxyphenyl alkyl sulfides. Lipophilicity affects both COX-1 and COX-2 inhibition in different manner and indicator variables like aromatic ring and triple bond, play an important role in COX-2 selectivity. Branching in the molecule, higher path length six rich in polarizability, and lesser number of carbonyl groups would be favorable for COX-2 inhibition. Fourth highest eigenvalue of burden matrix corresponding to atomic mass would be favorable for COX-2 inhibition and sixth lowest eigenvalue of burden matrix corresponding to Sanderson electronegativities is conductive for COX-1 inhibition. Lower path length three rich in atomic mass and lesser degree of unsaturation in the molecule would be favorable for COX-1 inhibition [90].

Ghose et al. showed how atomic parameters can be developed for successfully evaluating the molecular $P_{\mathrm{o/w}}$. They report more refined atomic values of compounds. Carbon, hydrogen, oxygen, nitrogen, sulfur and halogens were divided into 110 atom types of which 94 atomic values are evaluated from 830 molecules by least squares. A standard deviation and correlation coefficient were used simultaneously to model intermolecular interactions. Authors suggested that the charge density on the heteroatoms in conjugated systems in strongly affected by the presence of similar atoms in the conjugation which suggests it should be incorporated as a separate parameter in evaluating the partition coefficient [91].

Thirteen NSAIDs (aspirin, diclofenac, diclofenac sodium, diflunisal, flufenamic acid, ibuprofen, ibuprofen sodium, ketoprofen, nabumetone, naproxen, piroxicam, tenoxicam and indomethacin) were selected as model drugs to construct a QSPR model to predict the skin permeability for transdermal delivery by considering the solubility parameter of those model drugs, by assuming the penetration of drugs with $\mathrm{Clog}\,P$ or $\log P_{\mathrm{ow}} > 2$ and <2 via hydrophobic (nonpolar) and hydrophilic (polar) pathways, respectively, which would encounter different characteristics determined by the biological parameters of individual skin samples. An empirical model of QSPR to predict P_{app} based on the hydrophilicity of the model drugs, was statistically improved with standard deviation, and by taking the biological parameters of the skin into consideration [92].

Oprea et al. described a quantitative extension of the “rule of five” scheme, which can be achieved with minimal computational efforts. The descriptors used to correlate oral absorption were chosen on the basis of three criteria: they are relatively fast and easy to calculate; they have a direct physicochemical interpretation that is intuitive to the practicing chemist; and their use is intended for the combinatorial chemistry and/or HTS framework (i.e., efficient handling of hundreds of thousands of compounds). Furthermore, these descriptors are consistent with the current QSAR paradigm for oral absorption. Using data available from the literature, authors proposed an easy-to-interpret model that was stable for two independent measures of oral absorption, namely the percent human intestinal absorption, and the apparent Caco-2 cell P_{app}. Different compounds were used in the model e.g., cefatrizine, cefuroxime, cefuroxime axetil, cephalexin, ibuprofen, ketoprofen, naproxen, acetylsalicylic acid. In presented model several properties were found to be relevant: variables related to H-bonding capacity, that reveal inverse correlations to permeability, and variables related to hydrophobic transferability, that reveal direct correlations to permeability. The parsimony principle was applied in several aspects, as follows: single conformations were used to compute molecular surface areas; the definitions “polar” and “non-polar” surfaces were made in a simplistic fashion; simple and fast 2D descriptors were used to estimate other properties; and the 1-PC model was selected from the projection analysis. All these aspects indicate an approach toward what we call “rule of five” test, for example, in a combinatorial library synthesis planning mode, by helping the chemist to focus on compounds with increased probability of oral absorption [93].

7. Supplementary methods

Charkaboty et al., showed that NSAIDs e.g., piroxicam, tenoxicam and meloxicam can include fusion in membrane mimetics. As a first line of study, they have chosen small unilamellar vesicles (SUV) formed by the phospholipid, DMPC that serve as a convenient membrane mimetic system that is easy to prepare, and whose size can be more readily controlled. Authors were aware that large unilameller vesicles (LUV) could be a better model of membranes and the high curvature of SUVs could facilitate spontaneous destabilization of vesicles. Carefully designed controls, both positive and negative, have therefore been included to show that within their studies time frame and experimental conditions used, spontaneous membrane fusion does not contribute significantly. DMPC has been used as the phospholipid, since phosphatidylcholine is a zwitterionic head group and at pH 7.4 it does not have any net charge, thereby providing no electrostatic advantage to the fusion process. It is also the principal lipid component of mitochondria. In addition, all NSAIDs used in this work were known to interact with DMPC vesicles. Three oxicams viz. piroxicam, tenoxicam and meloxicam can induce fusion of membrane mimetic systems. Even though oxicam NSAIDs are structurally related, they show differential fusogenic behaviour as shown by the kinetics of content mixing and leakage [94].

An experimental method, in which the chamber was modified with an addition of 5% dimethyl sulfonide (DMSO) to both sides of chamber (DMSO-MS), was proposed. Firstly, the effects of the DMSO on the membrane integrity were evaluated by the measurement of released enzymes and membrane conductance. Secondly, the effects of DMSO on the membrane permeability were investigated using apparent P_{app} of fluorescein isothiocyanatelabeled dextra 4000, sulfasalazine, antipyrine, and rhodamine-123. Finally, the relationship between P_{app} values of seven poorly water-soluble drugs (e.g., indomethacin), obtained through DMSO-MS and their fraction absorbed values in humans was investigated. Obtained results showed that DMSO-MS was a useful method for predicting the absorbability of poorly water-soluble drugs [95].

Loidl-Stahlhofen et al. presented the use of solid-supported lipid membranes to assess lipophilicity. An single phospholipids bilayer is no covalently attached to a solid support; the lipids retain their physiological fluidity, but because of the higher density of the support material, phase separation is easily achieved. Therefore, the material for lipophilicity determination combines both physiological membrane conditions and the option for high throughput screening of pharmaceuticals. The reliability and reproducibility of the model system for quantification of membrane affinity, when applied to neutral and charged molecules, was studied. Eighteen pharmaceuticals (e.g., diclofenac, ibuprofen, indomethacin, ketoprofen, piroxicam) have been analyzed for their lipophilicity at physiological pH in an automated setup. Phase separation of lipid and aqueous phase can be achieved simply by a short low-speed configuration or filtration [96].

Diadermatic dosage form, using various polymeric gel and ointment bases containing 1% piroxicam were prepared to study the in vitro release of the drug. The general rank order for the in vitro drug release from all the bases evaluated was: gel base > hydrophilic base > emulsion base. The in vitro release data were treated with various kinetic principles to assess the relevant parameters, such as, diffusion coefficient, P_{app}, P, zero order and first order rate constants [97].

Idowu et al. developed a simple and elegant artificial biological interface, a specially for modeling the biomembrane in lipophilicity determination of drugs and xenobiotics. The biodevice was potentially useful as a predictive analytic tool in early-stage drug discovery. Authors reported the refinement and optimization of the crude leucaena oil to obtain technical grade oil for routine use in engineering a planar thin film for biomembrane modeling. The crude and refined oil were also characterized by standard physicochemical properties, in order to delineate the impact of purification process on oil composition. Retention behaviour of four structurally diverse model compounds (e.g., naproxen, nabumetone) representing four broad classes of varying molecular polarities and aqueous solubility behaviour, was investigated on the lipid film, liquid paraffin, and octadecylsilane layers. Optimal lipid film thickness was designed by using liquid paraffin film on silica support as benchmark. The surface chemistry of the lipid film based on refined leucaena oil was evaluated by computational analysis, thermodynamic analysis and digital image analysis of the lipid layer under UV light (254 and 365 nm). Effect of solute-type, layer-type and variables interactions on retention behaviour was delineated by two-way analysis of variance and QSPR. Validation of the lipid film was implemented by statistical correlation of a unique chromatographic metric with log P_{ow} and several calculated molecular descriptors of bulk and solubility properties [98].

Liposomes were immobilized in capillary continuous beds with covalently linked C_4 or C_8 alkyl ligands for chromatographic analysis of drug interaction with phospholipid bilayers, as reflected by drug retention volumes and calculated differences in interaction free energies. The procedure was a high-resolution micro-scale version of ILC for prediction of diffusion of drugs across biological membranes. The assumption, that liposomes immobilized in a continuous bed can be used as a model to evaluate or predict the partitioning or absorption properties of drugs, by the linear correlation between log k and log P values for structurally unrelated drugs (e.g., aspirin, salicylic acid), was validated [99].

The correlation of artificial membrane permeability to human fraction absorption was closely compared with dose of Caco-2 permeability, lipophilicity (log P and log D), prostate specific antigen (PSA), as well as QSPR predicted P_{app} using a diverse set of commercial drugs (e.g., acetylsalicylic acid, diclofenac, ibuprofen, indomethacin, ketoprofen, naproxen, piroxicam, salicylic acid, cefadroxil, ceftriaxone, cephalexin). Researches explored the use of a hydrophilic filter membrane (lecithin-dodecane) as the supporting material for phospholipids membrane. The use of hydrophilic filter membrane increased the rate of permeation significantly, and reduced the transport time to 2 h or less as compared with over 10 h [100].

Znovar et al. described using microemulsion buffers to separate solutes based on both their hydrophobicities and electrophoretic mobilities. A microemulsion system comprising Mygliol 812, Labrasol, Plurol Oleque and double distilled water was selected to improve the solubility of a specific COX-2 inhibitor, celecoxib. Celecoxib is a weakly acidic ($pK_a = 11.1$) and hydrophobic ($\log P = 3.5$) drug, almost insoluble in water (3–7 $\mu g\,mL^{-1}$, at pH 7.0 and 40 °C). The gradual changes in the microstructure of the unloaded and drug-loaded systems on dilution have been investigated by conductivity, viscosity, density and surface tension measurements, and the influence of the microstructure on release behaviour of dissolved celecoxib has been evaluated. Additionally the influence of celecoxib on the stability of drug-loaded systems during 3 months storage at 40 °C has been examined. Authors concluded that a microemulsion, containing less than 27% of water, as well as oil continuous, e.g., bicontinuous, and finally, at more than 45% of water was of o/w type. On incorporation of celecoxib at 1% concentration, the microemulsions remain stable and optically clear, with no phase separation or considerable microstructure changes [101].

A multitasking fluorescent device were obtained by forming micelles of Triton X-100, containing a lipophilic macrocyclic Cu^{2+} complex and the coordinating fluorophore Coumarin 343 (C343), which features a COOH moiety. Authors demonstrated that micellar multicomponent molecular system was able to carry out multiple tasks, that is, to signal with a fluorescence reviving if the pH is drifting out of a sharp window (that includes the physiological pH interval) and, inside this window, to measure with a fluorescence “off-on” modality the lipophilicity of carboxylate-containing molecules, including the widespread drug family of NSAIDs (ibuprofen, tolmetin, naproxen, mefenamic acid, flufenamic acid, diclofenac, flurbiprofen). The device successfully measures their lipophilicity, expressing it with an “off-on” type fluorescent signal, as demonstrated by the correlation of the fluorescence increase with the logarithmic water/octanol partition coefficient (log P) and with the difference between the pK_a observed in micelles and that measured in water [102].

Montenegro et al. investigated the effects of oil phase lipophilicity on in vivo drug release from topical o/w microemulsions (MEs) containing low percentages of emulsifiers. Three different lipids, isopropyl myristate, isopropyl palmitate, and isopropyl stearate, were used as oil phase to prepare o/w MEs. Three active compounds: naproxen, idebenone, and butylmethoxydibenzoylmethane were selected as model drugs, and their release rates from MEs were evaluated using Franz-type diffusion cells. The results suggest that the choice of proper combinations of oil phase lipids and emulsifiers may allow achieving drug controlled delivery from topical o/w MEs with low emulsifier content [103].

A migration index (MI) was applied to anionic solutes. The obtained MI values of anionic solutes correlated very well with the log P_{ow}, whereas the log k_w, which is also used as a hydrophobicity scale, correlated very little with log P for the tested anionic solutes. To investigate the biological meaning of the MI values in comparison with log P, a quantitative migration-activity relationship (QMAR) study on human skin permeability was performed for NSAIDs (aspirin, bufexamac, diclofenac, flufenamic acid, flurbiprofen, ibuprofen, ketoprofen, indomethacin, salicylic acid, salicylamide), whose MI and log P values deviated from the linear relationships between the MI and log P of other compounds that have no carboxylic group [104].

Seedher and Bhatia made an attempt to investigate the solubility of four COX-2 inhibitors (celecoxib, rofecoxib, meloxicam, and nimesulide) using a series of solvents and solvent–cosolvent mixtures. Water, alcohols, glycols, glycerol, and polyethylene glycol 400 were used as solvents, and water–ethanol, glycerol–ethanol, and polyethylene glycol–methanol were used as mixed-solvent systems. Physicochemical properties of the solvent such as polarity, intermolecular interactions, and the ability of the solvent to form a hydrogen bond with the drug molecules were found, to be the major factors involved in the dissolution of drugs by pure solvents [105].

Four hydrazide compounds: ibuprofen, aspirin, lauric acid and itaconic acid, were presented to discern molecular properties that effectuate bacterial inhibition (*Mycobacterium* tuberculosis). Partitioning into the central nervous system via BBB penetration, indicated by values of log BBB was observed. Results of this study elucidated the influence of various molecular properties (i.e., formula weight, log P, polar surface area, number of atoms) [106].

The values of log P for twenty two drugs (e.g., flufenamic acid) were calculated by the simple method using seven parameters such as: summation of weighted numbers of carbon and halogen atoms, total number of nitrogen and oxygen atoms, proximity effect of nitrogen/oxygen, number of unsaturated bonds including semi-polar bonds, number of aromatic polar substituents, indicator variable for the presence of ring structures except benzene and its condensed rings, and number of nitro groups. The results were compared with literature data [107].

Cordero et al. compared the intrinsic transdermal permeation properties of a series of NSAIDs (indomethacin, ketoprofen, diclofenac, piroxicam, tenoxicam, ketorolac, and aceclofenac) across human skin. The potential of the individual NSAIDs studied for transdermal delivery was assessed. Authors attempted to establish correlations between the physicochemical parameters of the studied drugs (MW, P, melting point, n-octanol/buffer pH 6.6, and intrinsic P) as well as their permeation constants to determine the predictive potential of these physicochemical parameters. The results showed that these drugs have low intrinsic permeation capacities. Therefore, formulation with enhances would have to be considered in an attempt to increase the fluxes of this NSAIDs. The higher transdermal permeability through human skin has been suggested to correlate with the greater intrinsic lipophilicity of the studied drugs [108].

The relationship between the physicochemical properties and uncoupling activity of flufenamic acid and its structural analogues, which vary in position 3′ of the phenyl ring, has been investigated. The partition coefficients, determined in n-heptane/water, n-hexane/water, and chloroform/water at pH 7.0, increased with introduction of hydrophobic groups, such as $-CF_3$, $-CH_3$, and $-C_6H_5$ into N-phenylanthranilic acid, and decreased with introduction of hydrophilic groups such as $-OH$, $-NH_2$, and $-SO_3H$. Compounds with large P values had high uncoupling activity, and this correlation was highest in the chloroform/water system. The formation of a dimer in the organic solvent phase was strongly suggested in chloroform, but not in n-hexane or n-heptane, with compounds possessing $-H$, $-CF_3$ $-CH_3$, or $-NO_2$ at position 3′. Higher uncoupling activity was correlated with larger pK_a values. Also, an important role of the hydrophobic nature of the acids with respect to uncoupling was confirmed for compounds that had similar ionization constants, but different uncoupling activities [109].

Luger et al. monitored the physicochemical properties of meloxicam. The drug was soluble at neutral pH, but became rapidly insoluble with decreasing pH. At very low pH its solubility increased, indicating a second pK_a value and the existent of a cation species. The very low solubility of meloxicam in acidic environments suggests it may cause few local gastrointestinal adverse events. The $\log P_{ow}$ values of meloxicam and other NSAIDs were estimated. At low pH meloxicam was more lipophilic than piroxicam or tenoxicam and similar to ketoprofen and naproxen [16].

Considering the lipophilicity of aspirin ($\log P = -1.15$), a significant contributor to its action mechanism, interaction of the drug with the whole lipids of goat blood have been investigated, using phospholipids binding and lipid peroxidation phenomena, as the parameters under investigation. The lipid content change along with the peroxidation induced by aspirin, and its suppression with ascorbic acid had been quantitatively measured. Significant loss in phospholipid was observed after incubation of whole blood with aspirin in varying periods of time. This may be ascribed to binding affinity of aspirin with lipid constituents in blood, which may have potential role in this therapeutic effect [110].

Obata et al. tried to use other parameters than $\log P$ and solubility, to represent lipophilicity to assess the structure of compounds and promoting activity (structure-activity relationship). The effect of 35 synthesized O-ethylmenthol derivatives on percutaneous absorption of ketoprofen in rats was investigated. Calculated $\log P$, MW, steric energy, van der Waals area, van der Waals volume, dipole moment, highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) were used as factors to determine the structural nature of cyclohexanol derivatives. Among these parameters, $\log P$, steric energy and LUMO significantly affected the prediction of enhancement factor (E_f) and total irritation score. The contribution index of $\log P$ was about 50% in the prediction of E_f, suggesting that lipophilicity among physicochemical properties contributes most of the promoting activity of these compounds [111].

Using the five oxicams Tsai et al. showed that isosteric replacements result in remarkable changes in the physicochemical and structural properties of congeners. Thus, the acidity of the phenolic OH groups is relatively higher in the oxicams containing a pyridinyl moiety, e.g., piroxicam, tenoxicam, and lornoxicam, due to their zwitterionic nature. This consequently influences their lipophilicity profile at different ionization states. Furthermore, partitioning behaviour in o/w and heptane/water systems suggests an internal H-bond between the enolic OH and the amide $C{=}O$ group. Only the P values of oxicams measured in the heptane/water system, correlate with their transfer across the BBB [112].

Solubility and solvation of some NSAIDs (ibuprofen, diflunisal, ketoprofen, flurbiprofen, naproxen) were studied in their non-ionic (aqueous buffers of pH 2.0) and ionic molecular form (pH 7.4) over a wide temperature interval. Absolute scale values for the thermodynamic terms (Gibbs energy, enthalpy and entropy) were obtained. The thermodynamic characteristics of solvation (hydration) of (+) and (±)-ibuprofen in the buffers show a difference, which was larger than the experimental error. This may be explained by differences in the association states of the molecules in solution. For the other studied NSAIDs, a correlation between the Gibbs energy of transfer and the pK_a value, and a compensation effect between the enthalpic and entropic terms have been revealed. The following points were discussed: significance of using o/w systems ($\log P$ as a measure of drug lipophilicity) to describe biological membranes (lipid systems); differences in thermodynamic aspects of the partitioning/distribution processes of these systems; advantages of the present transfer method approach in comparison with temperature dependencies of $\log P$ to analyze the driving forces of partitioning/distribution [113].

Q_H and $Q_{O,N}$ parameters were used to develop a noncongeneric model of the corneal permeability from a training set of miscellaneous compounds (e.g., ibuprofen) by Yoshida and Topliss. Q_H and $Q_{O,N}$ were defined as the sum of the absolute values of the net atomic charges of oxygen and nitrogen atoms and the sum of net atomic charges of hydrogen atoms attached to these heteroatoms, respectively. The model was suitable for the rapid prediction of the corneal permeability of drugs [114].

The effects of an ethanol/panasate800 system as a lipophilic vehicle, and an ethanol/water system as a hydrophilic vehicle, with or without permeation enhancers for in vitro skin permeation and in vivo skin absorption of tagafur, alclofenac and ibuprofen with different lipophilicity, were evaluated. The two vehicles showed contrastive properties in relation to the extent of permeation enhancement of these drugs. When lauric acid, as a permeation enhancer, was added to both of the binary vehicles, the in vitro and in vivo skin permeability of drugs further increased, and the in vivo absorption rate from the ethanol/water system was larger than that from the ethanol/panastate800 system. The results suggested that the skin absorption of lipophilic drugs can be improved by the use of the ethanol/water hydrophilic binary vehicle with or without lauric acid as permeation enhancer [115].

The human epidermal permeabilities of different NSAIDs (salicylic acid, indomethacin, naproxen, diclofenac, piroxicam) from aqueous solutions is dependent on the drug's lipophilicity. A parabolic relationship was observed when the logarithms of those drugs permeability coefficients were plotted against $\log P_{ow}$; the optimum $\log P$ being around 3. The local tissue concentrations of these drugs after dermal application in aqueous solutions were determined in a rat model. A comparison of the predicted tissue concentrations of each drug after its application to human epidermis was then made by a convolution of the epidermal and underlying tissue concentration–time profiles [116].

Some enhancing effects promoted by O-acylmenthol enhancers synthesized from 1-menthol and excipient acids (lactic acid, cinnamic acid, salicylic acid, oleic acid) were observed. Their promoting activity on the percutaneous absorption of five model drugs (e.g., ketoprofen, indomethacin) which were selected based on their lipophilicity, represented by $\log P_{ow}$, were tested in vitro across full thickness rat skin with each of the evaluated drugs in saturated donor solution. The lipophilicity of O-acylmenthol enhancers had a significant effect on the percutaneous permeation of the model drugs notwithstanding lipophilicity was not the only determinant factor. Model drug lipophilicity had a significant impact on the derivative enhancer promoting activity, and a parabolic relationship was found between the enhancement ratio for the P (ER_P) and $\log P_{ow}$ of the model drugs tested, except lidokaine [117].

The influence of Carbopol polymers and the penetration enhancer oleic acid on transdermal penetration of ketoprofen, through a fullthickness human skin was investigated. Ketoprofen patches were fabricated, and permeation parameters such as the permeability coefficient, ER_p, lag time, and P values were determined [118].

Quantitative relationships between lipophilicity (P_{ow}) and binding to both human plasma proteins and blood cells have been studied, in a group of model anionic drugs (e.g., salicylic acid, tolfenamic acid). Protein binding in plasma and accumulation in blood cells in suspension increases with increasing lipophilicity. Lipophilicity was determined as the log D of each compound between n-octanol and 0.5 M HCl. In both plasma and blood cell suspension the free fraction decreases with increasing lipophilicity. In the binding to plasma proteins the binding ratio approximately correlates with the P_{ow}. In contrast the binding ratio to blood cells was correlated with the P_{ow} to the power 0.685. This was the reason for the increase in the relative proportion of the compounds bound to plasma proteins and for the decrease both in blood cell uptake, and proportion of unbound drug in whole blood with increasing lipophilicity [119].

Acosta et al. evaluated the analgesic efficacy of nepafenac measuring the reduction in the nerve impulse activity evoked by repeated chemical noxious stimulation of corneal polymodal nociceptive fibers of anesthetized cats and compared the effects of nepafenac with those of diclofenac and ketorolac, two NSAIDs extensively used for the treatment of postsurgical ocular pain and inflammation. The possibility that analgesic effects of nepafenac were mediated by a reduction of sodium ion channel activity at corneal sensory nerve terminals was also explored by measuring sodium currents in patch-clamped cultured trigeminal ganglion neurons. The effects of nepafenac and its active metabolite, amfenac, were compared with equivalent doses of diclofenac. One of the unique properties of nefapenac is its ability to penetrate ocular tissue rapidly. This property is largely attributable to the carboxamide function of nepafenac. This structural change not only eliminates the compound's anionic character, but also simultaneously enhances its P value. Although no assessments were made to identify the rate-determining barrier of cornea for nepafenac, previous data indicate that the epithelium is the rate-determining barrier for compounds exhibiting P between 0 and 1.6, a range that includes NSAIDs with arylacetic acid structure. Compounds with log P values in the range of 1.6–2.5 increasingly find the stroma as the major barrier for penetration. Nepafenac, is on the lower side of this range and has characteristics that circumvent the barrier function of both the epithelium and the stroma. Thus, the greater lipophilicity of nepafenac compared with diclofenac and ketorolac provides a more rapid saturation of the epithelium and a minimal lag time before the drug crosses the cornea (7.5×10^{-5} cm s^{-1}). Diclofenac and ketorolac, as anionic drug species, exhibit significantly lower penetration coefficients (1.4×10^{-5} and 1.0×10^{-6} cm s^{-1}, respectively), due to an apparently slower saturation of the epithelial barrier, which results in a significant lag before drug crosses the cornea. Thus, the rapid onset of nepafenac's analgesic action is probably due to a rapid saturation of the corneal epithelial layer, highly innervated by nociceptors. Although nepafenac is a significantly weaker inhibitor of COX than its active metabolite, amfenac, corneal epithelial concentrations may initially be sufficient to attenuate corneal nociceptor prostaglandin formation without invoking an immediate conversion to amfenac [120].

Seven piperazinylalkyl ester prodrugs of ketorolac were synthesized to enhance its skin permeation. The prodrugs' aqueous solubility was measured in buffer solution (pH 5.0 and 7.4), and log P was measured by partitioning between buffer solutions and n-octanol. The prodrugs were more lipophilic than ketorolac at pH 7.4. The results attributed to higher lipophilicity at pH 7.4 and higher aqueous solubility at pH 5.0 compared to ketorolac [121].

Partitioning of a variety of organic compounds (e.g., ceftriaxone, cephalexin, cephalotin Na–salt, cefamandole Na–salt, cefuroxime Na–salt, cefazoline Na-salt, cefaclor, cephradine), was examined in an aqueous dextran-polyethylene glycol (Dex-PEG) two-phase system containing 0.15 M NaCl in 0.01 M sodium phosphate buffer at pH 7.3 and in octanol–buffer (0.15 M NaCl in 0.01 M sodium phosphate buffer at pH 7.3) system. The possibility of introducing compounds to be partitioned in an aqueous two-phase system with dimethyl sulfonide, and the effect of this solvent on the solute partitioning was explored. Comparison of the results obtained for several subsets of compounds in the octanol–buffer and in aqueous Dex-PEG two-phase systems clearly demonstrates the advantage of aqueous two-phase partitioning for the hydrophobicity measurements over partitioning in octanol–buffer system. The results indicated that the aqueous PEG-salt systems, such as PEG-sodium sulfate and PEG-sodium/potassium phosphate, in particular, may not be viewed as suitable for the relative hydrophobicity measurements, likely due large differences between the electrostatic properties of the two phases in these systems. It is possible, however, the information provided by partitioning in PEG-salt systems may be importance for better understanding of the solute-solvent interactions in aqueous environment under conditions simulating certain situations occurring in vivo [122].

8. Conclusions

Lipophilicity is very important from both physicochemical and biological viewpoints and has been widely recognized as important parameter in today's drug discovery research. This property provides discovery scientists with insights on the tendency of the compound to partition into lipid vs. aqueous environments. Such insights are useful for correlation important pharmaceutical processes such as gastrointestinal absorption, membrane permeation, solubility, volume of distribution, and protein binding. In drug discovery research, various measured or computationally calculated properties have been used to assess the oral absorption potential for drug candidates with various degrees of success. Presented review highlighted some recent developments and different techniques that have been used for determination of lipophilicity of two various drugs groups, NSAIDs and cephalosporins. Consideration of the concepts outlined in this review may facilitate future advancement of different methodologies and encourage the participation of scientists not presently engaged in this line of research, which is by necessity multi-disciplinary in nature.

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