Current State of the Art in HPLC Methodology for Lipophilicity Assessment of Basic Drugs. A Review

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Abstract: HPLC provides a user’s friendly, rapid, and compound sparing methodology, which is successfully applied to determine drug lipophilicity. Under suitable chromatographic conditions isocratic and extrapolated retention factors correlate well with octanol-water partition or distribution coefficients. The present review provides an overview of the stationary and mobile phases, which are preferably used for lipophilicity assessment mainly in the case of basic compounds. Difficulties raised by the interference of silanophilic interactions in the partition mechanism, and the ways proposed to face this problem are discussed. Attention has been given to the extrapolation procedure and the standardization of conditions to obtain 1:1 correlation between extrapolated retention factors and logP or logD. Other chromatographic indices encoding information on the lipophilic behavior are briefly presented. A separate section refers to recent advances in IAM Chromatography, its similarities/dissimilarities with reversed phase HPLC and the octanol-water system, as well as its potential to mimic specific interactions with phospholipids.

Keywords: Lipophilicity indices, Basic drugs, n-Octanol-water system, Reversed-phase HPLC, IAM chromatography, Extrapolated retention factors

INTRODUCTION

Lipophilicity, expressed by the logarithm of octanol-water partition coefficient logP or distribution coefficient logD, if ionized molecular species are present,
constitutes a physicochemical property of paramount importance for the medicinal chemist. It plays an important role in ADME (Absorption, Distribution, Metabolism, and Elimination) characteristics of drugs, while affecting their pharmacodynamic and toxicological profile, as well.\textsuperscript{1–3} Although lipophilicity is essential for penetration across biological membranes and hydrophobic interactions with receptors, high logP/logD values are associated with undesired drug features, like extensive and unpredictable metabolism, high plasma protein binding, or accumulation to tissues.\textsuperscript{4}

Basic compounds represent the major fraction in drug related databases rendering their lipophilicity assessment as an urgent requirement in drug design. Computed lipophilicity values are often inaccurate, especially if they refer to ionized or partially ionized molecules and, although valuable for screening virtual libraries, they should be replaced by measured data early in the drug discovery process.\textsuperscript{5,6} Different experimental protocols for logP or logD determination have been reported in literature. The classical shaking flask method for direct partitioning experiments is tedious and time consuming, not suitable for degradable compounds, less amenable to automation, while it presents limitations concerning the logP/logD range which can be reliably measured.\textsuperscript{3,7,8} The dual phase potentiometric titration suitable for ionisable drugs, on the other hand, requires special equipment, not always available in an analytical laboratory.\textsuperscript{9,10}

Reversed-phase high performance liquid chromatography (RP-HPLC) has proven to simulate octanol-water partitioning and is considered as a popular alternative for lipophilicity assessment. It offers several practical advantages, including speed, reproducibility, insensitivity to impurities or degradation products, broader dynamic range, on line detection, and reduced sample handling and sample sizes.\textsuperscript{11–13} These advantages have attracted considerable interest and the literature is rich in research articles, which investigate the relationship of chromatographic retention with octanol-water partitioning and the common factors underlying the two processes.\textsuperscript{11–16}

The criticism towards octanol as an isotropic medium with only a superficial similarity to biomembranes and the difficulties associated with the use of liposomes as more representative models,\textsuperscript{17} have triggered the development of immobilized artificial membrane (IAM) stationary phases for use in HPLC. IAM chromatography has unfolded new perspectives in the application of HPLC as a tool to mimic specific interactions with phospholipids.\textsuperscript{18,19}

The major part of the present review considers the chromatographic conditions that are more suitable for lipophilicity assessment focusing on basic drugs, evaluates associated difficulties, and provides an overview on the relation between RP-HPLC and the reference octanol-water partitioning system. In a separate section, analogous aspects in respect to IAM chromatography and its potential as a tool for rapid evaluation of drug permeation and/or interactions with biological membranes are discussed.
LIPOPHILICITY INDICES IN REVERSED-PHASE HPLC

The lipophilicity index measured by HPLC is derived by the retention time \( t_r \) that is converted to the logarithm of the retention factor \( \log k \) according to Equation (1):

\[
\log k = \log \left( \frac{t_r - t_0}{t_0} \right)
\]

where \( t_0 \) being the retention time of an unretained solute.

Isocratic retention factors represent a relative scale of lipophilicity. They are preferred by some authors since they require fewer experiments. However, extrapolated retention factors \( \log k_w \), corresponding to pure water as mobile phase, are considered as more representative lipophilicity indices, their values being of the same order of magnitude as octanol-water \( \log P/\log D \).[11–16] Extrapolated \( \log k_w \) values are derived using the linear part of the \( \log k/\phi \) relationships, where \( \phi \) is the concentration of the organic modifier in the mobile phase. This issue is discussed extensively in the Extrapolation Procedure section.

Both isocratic \( \log k \) and extrapolated \( \log k_w \) values are directly correlated to octanol-water \( \log P/\log D \) via Collander-type equations (Equation (2):

\[
\log P/\log D = a \log k(w) + b
\]

where \( a, b \), constants derived by linear regression analysis.

Equations of type 2 are constructed using compounds with known \( \log P/\log D \) values and can serve as calibration equations for further \( \log P/\log D \) calculations.[21] In many cases, when \( \log k_w \) values are used, \( a \) and \( b \) in Equation (2) tend to approach 1 and 0, respectively. In such cases, retention and octanol water partitioning are considered as homoenergetic processes. The quality of type 2 equations, however, depends both on the chromatographic conditions and the nature of the solutes.[20,21] Solvatochromic analysis has revealed differences in the balance of factors involved in octanol-water partitioning and reversed phase retention,[22,23] while conformational effects have also been manifested.[24] In this aspect, care should be taken in the selection of the training set of solutes for \( \log P/\log D \) estimation by HPLC. Nevertheless, considerable research efforts are directed towards the standardization of chromatographic conditions, which attenuate dissimilarities between retention and octanol-water partitioning and guarantee 1:1 correlation between \( \log k_w \) and \( \log P/\log D \) values for structurally diverse compounds.[25–27]

CHROMATOGRAPHIC CONDITIONS FOR LIPOPHILICITY ASSESSMENT

Stationary Phases

C18 silanized silica gel is the most preferred packing material for reversed phase columns in the chromatographic analysis of basic drugs. The same
material is appropriate for drug lipophilicity assessment, as well. However, the interference of silanophilic interactions in the partitioning mechanism of RP-HPLC has been recognized as a serious drawback, especially in the case of basic drugs.\[28,29\] Silanophilic interactions are attributed to the remaining free silanol sites and include hydrogen bonding as well as electrostatic forces, especially in the case of positively charged basic compounds, producing considerable increases in retention.\[28–32\] They also depend on the degree of ionization of the silanol groups, being less pronounced at low pH.\[33,34\]

The problem of silanophilic interactions is partially faced by the development of columns with reduced free or accessible silanol sites. End-capping of the silanol residues by trimethylchlorosilane (TMCS) or hexamethyldisilazane (HMDS) is usually performed during the manufacturing process, leading to a higher degree of silanization.\[31,32\] Hence, base deactivated silica represents a packing material more suitable (e.g., BDS C18) for basic solutes. In addition, recent technology has led to the development of polar embedded and polar endcapped stationary phases, which are considered to be further deprived from silanophilic effects.\[26,35–37\] With respect to embedded columns, a polar functional group, such as amide, carbamate, ether, or sulfonamide, is incorporated at the bottom of the alkyl bonded chains. This functional group provides electrostatic shielding to the surface silanol sites. The LC-ABZ+ and the Discovery-RP-Amide-C16 stationary phases belong to these types of columns. However, these packing materials, which have been used for lipophilicity determination, may exhibit other polar interactions with analytes, such as the strong interaction between the polar embedded groups and the phenolic analytes.\[38\] On the other hand, the need of a masking agent in the case of basic compounds (see below) with these types of columns suggests that silanophilic interactions still persist.\[26\] With respect to polar endcapped columns, a second reaction is used to bond a short carbon chain (usually C3-C4) with a polar end to the surface silanol sites. A favourable advantage for both types is the fact that a higher degree of orientation for the alkyl chains is achieved and, thus, they can be used with mobile phases containing high amounts of water or even pure water without the problem of hydrophobic collapse.\[39,40\]

The pH limitation of the above mentioned columns lies in the range 2.5 to 7.5. Thus, in the case of basic compounds, they do not allow determination of retention factors corresponding to the neutral form. In this case, log\(k_w\) of the neutral form can be estimated from the apparent log\(k_{w}^{app}\) by using Equation (3) adapted from the analogous logP/logD relationship.

\[
\log k_w = \log k_{w}^{app} + \log(1 + 10^{pK_a-pH}) \quad (3)
\]

Equation (3) is assumed to be valid for isocratic logk values, as well. Nevertheless, whether the effect of ionization in octanol-water and HPLC system is similar remains to be clarified, in as much as secondary interactions
and the influence of organic modifier may lead to deteriorations in the logk/pH profile (see further details in sections below).

Recently bidentate stationary phases (e.g., Zorbax-extend C18) that include a propylene bridge, as well as surface modified silica columns (e.g., XTerra C18), where organic functional groups have become a constituent of the silica backbone, allow the use of mobile phases with pH up to 12.[41] However, the applicability of such columns in the lipophilicity assessment of basic pharmaceuticals has not been systematically investigated yet. In a recent publication, 1:1 correlation has been reported between logkw and logP for 40 basic compounds measured at pH 10.5 on a Zorbax-extend C18 column without addition of any masking agent.[42]

As an alternative choice, the polymer based octadecyl-poly(vinyl alcohol) (ODP) stationary phase, which is completely devoid of reactive silanol groups, has also been used for lipophilicity measurements.[15,43] The ODP column presents stability to acidic and strongly basic conditions (at pH between 2 and 13).[44] However, it has been reported that the retention mechanism on ODP stationary phase compared to octanol-water partitioning is controlled by a different balance of forces. Thus, the derived data may not be so suitable to reproduce the classical log P or logD values.[45]

Mobile Phases

The most extensively used mobile phases in RP-HPLC are mixtures of water or buffer with an organic modifier, usually methanol, acetonitrile, or THF. However, acetonitrile was found to produce the most asymmetrical peaks in the analysis of organic bases.[46] This fact was attributed to the inability of acetonitrile to form hydrogen bonds with residuals silanols, in contrast to methanol and THF. In terms of lipophilicity assessment, methanol seems to be the most suitable organic modifier for RP-HPLC, since it does not disturb the hydrogen bonding network of water. Moreover, during equilibration, methanol molecules associate with the stationary phase forming a monolayer, which provides a hydrogen bonding capability in better agreement with n-octanol.[47]

It should be taken into account that organic modifiers are capable of affecting the pKa of ionized solutes, as well as the acidity of the surface silanol groups and the pH of the mobile phase. In general, the pKa of bases decreases as the organic modifier concentration increases. However, substantial structure-dependent differences in pKa shifts for bases at a given organic solvent composition as well as pH variations at different organic solvent compositions have been reported.[48–50] These effects are minimized in the extrapolation procedure, providing a further argument for the use of logkw values instead of isocratic logk in the case of basic compounds.

The buffer composition of the aqueous component in the mobile phase also plays an active role in the retention of protonated basic compounds,
which may form ion pairs with the counter ions. Morpholinepropanesulfonic acid (MOPS), is considered as the buffer of choice for lipophilicity assessment by HPLC.\(^{[26,51,52]}\) It exhibits a large buffering capacity coupled to poor ion pair formation ability due to its zwitterionic nature and, thus, it does not interfere either with solutes or with stationary phase. On the other hand, the partitioning experiments for logD determination are usually performed in phosphate buffer or in phosphate buffered saline (PBS), containing NaCl and KCl at a total concentration of approximately 0.16 M, to mimic the isotonic physiological conditions.\(^{[53]}\) Hence, this choice is often used in HPLC as well. However, phosphate and, especially, the chloride anions are capable of forming ion pairs with protonated molecules with extraction constants that may differ from those in octanol-water.

In the case of basic drugs, the addition of small amounts (0.15–0.20% v/v) of amines to the mobile phase is a critical prerequisite in order to suppress silanophilic interactions, even if polar embedded or polar end capped stationary phases are used. Hydrophobic amines, such as n-decylamine and N,N-dimethyloctylamine, are considered to be the most suitable masking agents combined with methanol as organic modifier.\(^{[15,26]}\) The effect of hydrophobic amines on retention is less evident with acetonitrile as organic modifier. Acetonitrile, as a weak hydrogen bonding solvent, is not capable of solvating the stationary phase with sufficient water, thus presumably preventing the positively charged amine to be dragged on the column and to exert its role as a masking agent.\(^{[15]}\)

Recently, room temperature ionic liquids of the imidazolium tetrafluoroborate family, such as 1-butyl-3-methylimidazolium (BMIM BF\(_4\)), have been reported to be suitable for suppressing silanophilic interactions for a set of \(\beta\)-blockers.\(^{[54–56]}\) In particular, this type of mobile phase additive combines the silanol masking effect of the imidazolium cation with the chaotropic character of the BF\(_4\) anion, providing a promising masking agent which may release new perspectives for the optimization of lipophilicity determinations.\(^{[54–56]}\)

**EXTRAPOLATION PROCEDURE**

If the entire organic modifier range is considered, the relationship between retention factors and the fraction of the organic modifier \(\phi\) follows the Schoenmaker’s solubility parameter model according to Equation (4).\(^{[57]}\)

\[
\log k = A\phi + B\phi^2 + E\sqrt{\phi} + \log k_w
\]

(4)

A, B, and E are fitting coefficients and \(\log k_w\) is the intercept corresponding to 100% aqueous phase. The \(B\phi^2\) term accounts for the curvature (concave) at higher organic modifier concentrations partly attributed to silanophilic interactions, while \(E\sqrt{\phi}\) accounts for a curvature (concave or convex)
observed at water rich mobile phases ($\phi < 0.2$) due to stationary phase solvation problems. The error in the $\log k_w$ values produced as a result of the curvature at lower fractions of organic modifier has recently been investigated by Tate et al.\cite{58} The correct estimation of extrapolated chromatographic indices also depends on the stationary phase. Indeed, a hybrid based polar embedded column produced a slightly smaller error in extrapolation procedure than a polar endcapped and a conventional non-polar endcapped column, as a result of both lower surface area and less surface silanols.\cite{58} Nevertheless, N-N lone pair interactions between amide embedded groups and the solutes containing nitrogen atoms, like basic compounds, seem to affect the retention characteristics and thus the extrapolation accuracy.\cite{58}

Quadratic extrapolation using the higher organic modifier concentration range may also lead to erroneous values in respect to lipophilicity.\cite{15,27} Nevertheless, when methanol is used as organic modifier at fractions $>0.2$ coupled to a masking agent, the linear part of Equation (4) is sufficiently wide. Hence, it can be used to derive extrapolated $\log k_w$ values according to Snyde’s linear solvent strength model\cite{59} via Equation (5):

$$\log k = -S\phi + \log k_w$$

Linear extrapolation is generally preferred to obtain $\log k_w$ values representative of lipophilicity. It is assumed that linearity holds better for modifier concentrations that produce $0 < \log k < 1$.\cite{60,61}

STANDARDIZATION OF THE CHROMATOGRAPHIC CONDITIONS FOR THE LIPOPHILICITY ASSESSMENT OF BASIC DRUGS

Attempts to optimize the chromatographic conditions in the aim to simulate better octanol-water partitioning have already been reported 30 years ago by Unger et al.\cite{62,63} These authors suggested a reversed-phase C$_{18}$ packing material as stationary phase previously coated with n-octanol and the use of pure n-octanol saturated buffer as mobile phase. A good correlation between $\log P$ values and $\log k_w$ was obtained; however, the basic compounds were strongly retained in the column interacting with the surface silanol sites and disrupting the correlation.\cite{62} A hydrophobic amine, N,N-dimethylectylamine, was further added to suppress the silanophilic interactions, leading to a very good correlation.\cite{63} However, a rather limited set of basic drugs, including phenothiazines and tricyclic antidepressants, was used. Moreover, retention factors were less reproducible due to column instability and bleeding. Recently, the use of n-octanol was revisited not as a principal solvent component of the stationary phase, but as a mobile phase additive. In fact, the addition of 0.25% n-octanol in the methanol fraction of mobile phase coupled to n-octanol saturated MOPS buffer produced a very good
correlation for a C₈ column. Triethylamine or n-decylamine was used as
masking agent, with the latter to be advantageous concerning logP/logKw
relationships. However, the data set was limited, including nonfunctional
solutes only, while the dynamic range of logD values did not exceed the
three log units.

Based on this evidence, Lombardo et al. proposed a LC-ABZ⁺ column as
stationary phase and mobile phase conditions similar to Minick (methanol as
organic modifier +0.25% n-octanol, MOPS as n-octanol saturated buffer and
0.15% n-decylamine in respect to the total volume). For a set of 163 struc-
turally diverse basic and neutral drugs, a calibration curve has been obtained
reflected in Equation (6), which covers a dynamic range broader than seven
log units. To achieve the potential for automation, three isocratic logk
values were used for the extrapolation to logKw according to three lipophilicity
ranges.

\[
\begin{align*}
\log D_7.4 &= 1.08 (\pm 0.02) \log K_w + 0.20 (\pm 0.04) \\
n &= 163, \quad r^2 = 0.949, \quad s = 0.369, \quad F = 3000
\end{align*}
\] (6)

Equation (6) represents a general practically 1:1 correlation with a slope
close to unity and an intercept close to zero and is used to calculate logD
values at pH 7.4. The method, introduced as ElogD₇.₄, has been validated
for a large number of neutral and basic drugs.

To this point, a conventional base deactivated silica column (BDS C₁₈)
has successfully been applied for the lipophilicity assessment of 64 structu-
rally diverse basic and neutral drugs using analogous mobile phase con-
ditions. Correlation of 1:1 with high statistics was obtained according to
Equation (7), which covers a dynamic range of six log units.

\[
\begin{align*}
\log D_7.4 &= 1.03 (\pm 0.03) \log K_w + 0.14 (\pm 0.07) \\
n &= 64, \quad r^2 = 0.937, \quad s = 0.288, \quad F = 908
\end{align*}
\] (7)

When basic drugs were analyzed separately from neutral ones, an
analogous equation was obtained (Equation 8).

\[
\begin{align*}
\log D_7.4 &= 1.07 (\pm 0.04) \log K_w + 0.00 (\pm 0.09) \\
n &= 40, \quad r^2 = 0.943, \quad s = 0.278, \quad F = 632
\end{align*}
\] (8)

If only n-decylamine is added in the mobile phase Equation (9) even
better statistics was obtained accompanied, however, by a high intercept,
which could be attributed to the presence of silanophilic interactions despite
the use of the masking agent.

\[
\begin{align*}
\log D_7.4 &= 1.08 (\pm 0.03) \log K_w - 0.64 (\pm 0.08) \\
n &= 40, \quad r^2 = 0.970, \quad s = 0.201, \quad F = 1250
\end{align*}
\] (9)
The difference in logD_{7.4} versus logk_w relationships in the absence and presence of n-octanol in the mobile phase is illustrated in Figure 1.

The comparison between Equations (8) and (9) further supports the addition of n-octanol in the mobile phase as a crucial factor, favorable for the establishment of similar energetics between retention and bulk octanol-water partitioning. In fact, as the stationary phase becomes solvated by the mobile phase during equilibration, n-octanol, as a lipophilic component, associates with the stationary phase providing additional masking of the free silanols and octanol-like character in respect to hydrogen bonding capability. Nevertheless, in the presence of n-octanol, careful consideration should be taken concerning the range of organic modifier concentration for the extrapolation procedure, since, in water rich mobile phases, it seems to affect more markedly the linearity of the logk/\phi relationship leading to downward curves.\cite{27} Analogous findings were described earlier by Minick et al.\cite{52}

The downward curvature has been observed for both strongly ionized basic drugs at pH 7.4 and neutral compounds at volume percentages greater than 60% water, depending, however, on the solute as well. Therefore, to avoid an underestimation of logk_w indices, careful selection of the methanol fraction range was proposed and the use of at least five isocratic logk values for the extrapolation procedure.\cite{27}

OTHER CHROMATOGRAPHIC DATA AS LIPOPHILICITY RELEVANT EXPRESSIONS

The slope S of the linear Equation (5) is considered to encode significant information on the lipophilic behaviour of the solute. By some authors, the slope S

![Figure 1](Image)

*Figure 1.* Relationships of logD_{7.4} values versus logk_w values, in presence of decylamine (■) and in presence of decylamine + n-octanol in the mobile phase (△) (Data taken from reference[27]).
is considered to reflect the solute/solvent interactions during the retention process and is related to the specific hydrophobic surface area.\textsuperscript{[65]} The strong influence of volume in the slope $S$ was demonstrated for a series of substituted coumarins using PLS analysis.\textsuperscript{[24]} If, within a series of compounds there are no considerable differences in the forces involved in solute/stationary phase interactions (mainly concerning hydrogen bonding or the extent of silanophilic interactions), a good relationship between the slope $S$ and the intercept $\log k_w$ is anticipated (Equation (10):

$$S = a \log k_w + b$$  \hspace{1cm} (10)

The organic modifier concentration $\phi_o$, which produces an equal molar distribution between the stationary and mobile phase leading to $\log k = 0$, has been proposed as a measure to rank lipophilicity.\textsuperscript{[66]} The $\phi_o$ indices correspond to the quotient:

$$\phi_o = \log k_w/S$$  \hspace{1cm} (11)

Based on the $\phi_o$ concept, a fast gradient method has been proposed by Valko et al. to determine the chromatographic hydrophobicity index (CHI) as a high throughput alternative to the other lipophilicity measures.\textsuperscript{[67,68]} For this purpose, gradient retention times ($t_g$) are measured and converted to CHI values by means of a calibration equation, derived by a set of standards with well determined CHI ($\phi_o$) values:

$$\text{CHI} = \text{slope} \times t_g + \text{intercept}$$  \hspace{1cm} (12)

The absolute magnitude of the CHI parameter depends on the values assigned to the set of standards. The method has the advantage that, once the calibration equation has been established, the retention parameter is obtained from a single fast gradient run, thus saving time and solvents. The CHI parameter has been reported to correlate satisfactorily with $\log P$. The reported chromatographic conditions involve acetonitrile as organic modifier and the use of ammonium acetate as buffer, without addition of any masking agent. It should be noted that only few basic compounds were included in the data set and they were measured at elevated pH mainly as uncharged species.\textsuperscript{[69]} The effect of organic solvent composition on mobile phase starting pH and on solutes pKa in gradient chromatography and its consequences in CHI indices of ionisable compounds, has been further investigated.\textsuperscript{[70]} The CHI/pH profile for a number of basic drugs was established using 2,2,2 trifluoro-ethanol as organic modifier and either ammonium acetate or butylamine buffer as the aqueous component of the mobile phase.\textsuperscript{[71]} The focus of that study was to solve the problem of a substantial drop in pH during gradient elution, especially at high starting pH, which implies that the neutral form in the case of strong bases cannot be achieved. The authors suggest the use of 50 mM butylamine as the aqueous component of the mobile phase to overcome this drawback. In the presence
of butylamine, minimization of pH variation during gradient elution is achieved, permitting the determination of the CHI index of the neutral species.

IMMOBILIZED ARTIFICIAL MEMBRANE CHROMATOGRAPHY

IAM chromatography has been introduced as a promising alternative to simulate liposome/water partitioning and cell membrane permeation.\textsuperscript{[17,18,72]} It is prepared by phospholipids covalently bonded to a propylamino silica support at monolayer densities. Remaining propylamine residues are treated in a second step to suppress an undesired basic function on the silica backbone. Moreover, free silanol groups, although not easily accessible, may interfere in secondary interactions. The most frequently used IAM column is IAMPC, which contains phosphatidylcholine. In fact, three different types of IAMPC have been introduced in the market, the single chain IAMPC-DD, the double chain IAMPC-MG, and IAMPC-DD-2, which differ on the way the remaining propylamine residues are treated. It is reported that double chain IAM surfaces better simulate natural phospholipids and the resulting chromatographic indices correlate better with permeability data.\textsuperscript{[73,74]}

IAM columns permit the use of aqueous mobile phases without addition of organic modifier, leading to directly measured log$k_w$ values and reducing considerably the time of analysis. The buffer of choice is phosphate buffered saline in order to mimic physiological conditions. The pH limitations of the column restrict measurement in the pH range 2.5 to 7.4. Many authors prefer the use of pH 7.0, which is close to physiological pH and safer for the column.\textsuperscript{[71,73]} In the case of compounds with strong affinity for the IAM surface, acetonitrile up to 30% is preferably added and log$k_w$ values are obtained by linear extrapolation. The use of methanol as organic modifier is avoided, since it affects the stability of the column, causing methanolysis of the phospholipids. Nevertheless, the ageing of the column should be checked from time to time, using standard compounds.\textsuperscript{[75 – 77]}

According to Ong and Pidgeon,\textsuperscript{[78]} partitioning seems to be the principal retention mechanism in IAM retention, implying that besides hydrophobic interactions, polar interactions with the solvated layer(s) of the stationary phases and the head groups of the immobilized phospholipids should be considered. The latter constitute specific electrostatic interactions with ionized species.\textsuperscript{[79]} Such interactions are very important in the case of protonated basic compounds, which are more strongly retained as a result of their interaction with the phosphate anions of the stationary phase. It is reported that due to the involvement of electrostatic forces, the IAM retention of protonated $\beta$-blockers was stronger compared to isolipophilic neutral compounds.\textsuperscript{[80]} In another study concerning structurally diverse basic and neutral compounds, the degree of protonation had to be considered in order to obtain a good
correlation between log\(k_{wIAM}\) and logD values at pH 7.4.\(^{[81]}\) Otherwise, a better correlation was obtained with logP values, implying that the decrease in the retention due to ionization was compensated by the electrostatic interactions.\(^{[80]}\) In the same study, IAM retention was compared to reversed phase chromatographic retention. Very characteristically, the strong base metformin, fully protonated at pH 7.4, eluted with the dead time in reversed phase HPLC, while it was retained in IAM chromatography due to the electrostatic interactions of its positively charged center with the phosphate anions.\(^{[81]}\)

IAM chromatographic indices have been successfully correlated with liposomes partitioning data; however, the balance between electrostatic and hydrophobic interactions is considered to differ between the two systems. Nevertheless, such studies include a rather limited number of compounds.\(^{[82]}\) In the case of basic drugs, silanophilic interactions have been reported to affect the log\(k_{wIAM}/pH\) profile as compared to the corresponding pH/partition diagram in liposomes. Thus, log\(k_{wIAM}\) values of propranolol, measured on a double chain IAM.PC.DD2 column, was increased between pH 6–7, while in liposome partitioning a plateau was reached at pH below 8.\(^{[76]}\)

The potential of IAM chromatographic indices to predict passive transport through various biological barriers, as well as to estimate pharmacokinetic properties and certain pharmacological activities, has recently been reviewed by Barbato.\(^{[83]}\) Nevertheless, in a parallel study on the similarity between IAM columns, conventional HPLC columns, octanol-water partitioning, and biopartitioning systems by means of solvatochromic analysis, published by Lazaro et al., the belief that IAM chromatography should be considered to be always the best choice for modelling biological processes, is disputed.\(^{[84]}\)

**CONCLUSIONS**

HPLC provides a user’s friendly, rapid, and compound sparing methodology, which is successfully applied to determine drug lipophilicity. Although, in the case of basic drugs, silanophilic interactions may interfere in the partition mechanism, leading to overestimated or erroneous lipophilicity, there are ways to reduce such secondary interactions and to obtain extrapolated retention factors, log\(k_w\), which reproduce octanol-water logD values in a satisfactory manner. Nevertheless, protonated bases are considered to develop specific interactions with biological membranes, which are not encoded in octanol-water partitioning or reversed-phase chromatographic retention. The development of IAM stationary phase has opened new perspectives in the use of HPLC to investigate such interactions in a fast and reproducible way. The greatest potential of IAM Chromatography is the estimation of passive
transport and in this aspect it may offer a high throughput screening method for drug candidates in drug discovery and the development process.

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