

Lipophilicity of AMPHOteric and zwitterionic compounds: A comparative study of determination methods

Clara Ràfols, Xavier Subirats, Javier Rubio, Martí Rosés, Elisabeth Bosch



PII: S0039-9140(16)30790-1
DOI: <http://dx.doi.org/10.1016/j.talanta.2016.10.038>
Reference: TAL16958

To appear in: *Talanta*

Received date: 24 June 2016
Revised date: 4 October 2016
Accepted date: 8 October 2016

Cite this article as: Clara Ràfols, Xavier Subirats, Javier Rubio, Martí Rosés and Elisabeth Bosch, Lipophilicity of AMPHOteric and zwitterionic compounds: A comparative study of determination methods, *Talanta*, <http://dx.doi.org/10.1016/j.talanta.2016.10.038>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting galley proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

LIPOPHILICITY OF AMPHOTERIC AND ZWITTERIONIC COMPOUNDS: A COMPARATIVE STUDY OF DETERMINATION METHODSClara Ràfols*, Xavier Subirats¹, Javier Rubio², Martí Rosés³, Elisabeth Bosch⁴

Departament de Química Analítica and Institut de Biomedicina (IBUB), Universitat de Barcelona, Martí i Franquès 1-11, E-08028 Barcelona, Spain

crafols@ub.edu

xavier.subirats@ub.edu

jr@masso.com

marti.roses@ub.edu

e.bosch@ub.edu

*Corresponding author. Phone: (+34) 934 034 874, Fax: (+34) 934 021 233.

Abstract

Common drugs intended for action in plasma (antibacterials, antiallergics, diuretics...) often display both acidic and basic behavior, and some of these amphoteric compounds can appear as zwitterions. In such cases, accurate profiling of lipophilicity vs. pH, which plays a fundamental role in drug pharmacokinetics, might be complex. In the present work two common lipophilicity estimation methods based on the drug distribution between 1-octanol and aqueous buffer i.e. phase equilibration (shake-flask) and two-phase titration (potentiometry), were compared with a high-throughput lipophilicity index, the Chromatographic Hydrophobicity Index (CHI). The results were also compared with log $D_{o/w}$ pH-profiles calculated by different algorithms from ACD/Labs. Accurate and similar results were obtained for both octanol-water approaches but, due to the lower determination times and the absence of different ion-pairing buffers, potentiometry was shown to be the most convenient method. CHI vs. pH profiles provide rapid and efficient information, which is very convenient for lipophilicity screening purposes, but may differ slightly from shake-flask and potentiometric results.

Abbreviations

CHI: chromatographic hydrophobicity index

¹ Phone: (+34) 934 039 119, Fax: (+34) 934 021 233.² Present address: Massó Analítica, S.A., Montsià 4, 08820 El Prat de Llobregat, Spain, Phone: (+34) 932 859 797, Fax: (+34) 932 198 165.³ Phone: (+34) 934 039 275, Fax: (+34) 934 021 233.⁴ Phone: (+34) 934 021 284, Fax: (+34) 934 021 233.

Keywords: Lipophilicity, ampholyte, zwitterion, shake-flask, potentiometry, chromatographic hydrophobicity index (CHI).

1. Introduction

Most compounds of pharmaceutical interest are weak acids or bases. In such cases, changes in the pH of the medium (for instance along the gastrointestinal tract) influence the ionization state of the molecule and consequently its solubility in aqueous solution and its partition in lipidic media. Water-soluble drugs are needed in the case of oral administration and when they have to be transported through body fluids, and it is well known that ionization favors solubility. Once in solution, the drug's lipophilicity needs to be increased in order to cross lipidic membranes to reach the drug target and thus the presence of the neutral form, which is more lipophilic, is preferable. Thus, many drug molecules contain acid-base groups that help to change their degree of ionization during the uptake process. Molecules containing carboxylic or phenolic groups can be easily identified as weak acids, or in the case of aliphatic or aromatic amines, as bases. However, nitrogen atoms can lack acid-base properties (e.g., amides), or even exhibit weakly acidic behavior due to delocalization onto adjacent carbonyl groups and resonance stabilization (e.g. barbiturates) or due to the electron-withdrawing effect of sulfo groups (e.g. sulfonamides) [1]. Very often a drug presents amphiprotic characteristics, having both acidic and basic functional groups. The difference between the two pK_a values has a remarkable impact on the achievable maximum mole fraction of the neutral species. For instance, as shown in Fig. 1, an amphiprotic molecule in which the acidic and basic groups are only separated by 1 pK_a unit can lead at a maximum mole fraction of neutral species of about 60%. This fraction is increased to 83% with a pK_a distance of 2 units, but will only reach a value close to unity beyond a separation of 5 pK_a units. This might be an issue when measuring the lipophilicity of neutral species of an amphoteric molecule. In the particular case of an acidic group that is stronger than a basic one (i.e., acidic $pK_a <$ basic pK_a) zwitterionic species appear, these being electrically neutral but bearing both a negative and a positive charge.

Prediction software can be very useful and convenient for a preliminary and rapid estimate of the lipophilicity of potential drug candidates. In general terms and for the most commonly used programs, the lipophilicity of the whole molecule is calculated from the individual contributions of separate atoms and/or structural fragments, and in some cases from intramolecular interactions [2, 3]. In combination with calculated pK_a values, such

software provides a rough estimate of lipophilicity profiles ($\log D_{o/w}$ vs. pH), which is very useful as a starting point in experimental designs of accurate $\log P_{o/w}$ determination.

In the present study the lipophilicity profiles of several amphoteric drugs were determined using the classical methods based on potentiometry [4, 5] and shake-flask [6, 7] over a wide range of pH values, and the results were compared with those derived from determination methods based on the fast gradient chromatographic hydrophobicity index (CHI_{MeCN}) [8, 9], as well as the prediction made by two different approaches using the common ACD/Labs software.

2. Material and methods

2.1 Reagents

Buffers were prepared from glacial acetic acid (Baker, >99%), phosphoric acid (Merck, >85%), citric acid (Fluka, >99.5%), sodium (Baker >99.5%) and potassium dihydrogen phosphate (Merck, >99.5%), sodium hydrogen phosphate (Merck, >99%), sodium or ammonium hydrogen carbonate (Fluka, >99%), sodium carbonate (Panreac, >99.5%), ammonia (Merck, 25%), and anhydrous ammonium acetate (Baker, >99.5%). When necessary, small volumes of hydrochloric acid (Merck, 25%) or concentrated sodium hydroxide solutions (prepared from pellets, Merck, >99%) were used for pH adjustment. Water was obtained from a Milli-Q plus system (Millipore, Billerica, USA) with a resistivity of 18.2 M Ω cm. The organic modifiers used in HPLC mobile phases were acetonitrile and methanol (Panreac, HPLC grade). Potassium bromide (Merck, >99%) was used as a dead time marker. Dimethyl sulfoxide (DMSO) (Merck, >99.9%) and *n*-octanol (Merck, >97%) were also employed. Titrisol potassium hydroxide and hydrochloric acid from Merck were used as titrants for potentiometric measurements, and potassium chloride (Sigma, >99%) as an electrolyte for ionic strength adjustment. The drugs (Table 1) were purchased from Sigma–Aldrich, all of high purity grade ($\geq 98\%$).

2.2 Instrumentation

pH was measured using a Crison 5014 combined electrode connected to a GLP 22 potentiometer from Crison (Alella, Spain), and standard aqueous solutions (pH 4.01, 7.00, and 9.21) were used for calibration. Partitions were equilibrated using a movil-ROD rotation shaker from Selecta (Abrera, Spain) in closed test tubes at 25°C.

pK_a and $\log P_{o/w}$ potentiometric measurements were carried out in a GLpKa/D-PAS automatic titrator (Sirius Analytical Instruments Ltd., Forest Row, UK).

HPLC measurements were performed on a Shimadzu (Kyoto, Japan) HPLC system consisting of two LC-10ADvp pumps, a SIL-20ACHT auto-injector, an SPD-M10AVvp diode array detector and a CTO-10ASvp oven at 25°C and a SCL-10Avp controller. A 5 μ m, 50 x 4.6 mm XTerra RP18 (Waters, Milford, EUA) column was employed. Injection volumes were between 10 and 50 μ L, and flow rates in the range 1.0–1.5 mL min⁻¹.

UHPLC data were recorded using a Shimadzu Nexera system with two LC-30AD pumps, a DGU-20A₅ online degasser, a SIL-30AC autosampler, a SPD-M20A diode array detector, a CTO-10ASvp oven at 25°C, and a CBM-20Alite controller. A 1.7 μ m, 50 x 2.1 mm Waters (Milford, MA, USA) Acquity BEH C18, 50 mm x 2.1 mm column was employed, at a flow rate of 0.5 mL min⁻¹ and injection volume of 0.2 μ L.

2.3 Determination of pK_a and $\log P_{o/w}$ by potentiometry

For potentiometric pK_a determination about 5 mg of sample were dissolved in 15 mL of water or methanol/water mixtures containing 0.15 M of KCl, followed by pre-acidification or pre-basification, and finally titrated with KOH 0.5 M or HCl 0.5 M. When mixed solvents were used, extrapolated aqueous pK_a values were calculated according to the Yasuda-Shedlovsky procedure. For spectrophotometric pK_a determinations, a 5 mM solution of the compound was prepared in DMSO, and 50 μ L of this stock solution were diluted with 250 μ L of KH₂PO₄ 0.015 M/KCl 0.15 M and 10 mL of KCl 0.15 M. A blank reference titration is required [10-12].

For $\log P_{o/w}$ determination about 5 mg of sample were dissolved in the appropriate volume ratio of *n*-octanol (saturated with 0.15 M KCl aqueous solution) and 0.15 M KCl aqueous solution (saturated with *n*-octanol), followed by titration with KOH 0.5 M or HCl 0.5 M [4].

All measurements were performed at 25°C, under an inert gas atmosphere, and at least three titrations were performed for each compound.

2.4 Determination of $\log P_{o/w}$ by shake-flask method

Volumes of octanol-saturated aqueous buffer and water-saturated octanol were selected according to the procedure proposed in a former study [13]. Briefly, a 10 mM sample stock solution in DMSO was diluted in the corresponding buffer, 1:100 for regular compounds (-1.0

$< \log D_{o/w} < 3.5$) and 1:40 for hydrophilic substances ($\log D_{o/w} < -1.0$), followed by the addition of octanol in a volume ratio depending on the expected lipophilicity of the compound, and then the mixture was stirred for 1 hour at 25°C. Finally, the analyte concentration was measured by HPLC in the aqueous phase before and after equilibration with octanol in the case of regular compounds, or in the octanolic phase for hydrophilic compounds. Thus, for a compound of regular lipophilicity $\log D_{o/w}$ values were calculated according to the following expression:

$$\text{Regular compounds: } \log D_{o/w} = \log \left(\left(\frac{A_{st} v_{inj(w)}}{A_w v_{inj(st)}} - 1 \right) \frac{V_w}{V_o} \right) \quad (1)$$

where V_w/V_o is the volume ratio between the aqueous and the octanolic phases, $v_{inj(w)}$ and $v_{inj(st)}$ are the injection volumes, and A_w and A_{st} are the chromatographic peak areas; the subscript *st* refers to the standard solution, i.e. the aqueous solution of the analyte of known concentration before the addition of octanol, and *w* stands for the aqueous phase after the equilibration step with octanol. In the case of poorly lipophilic compounds:

$$\text{Hydrophilic compounds: } \log D_{o/w} = -\log \left(\frac{A_{st} v_{inj(o)}}{A_o v_{inj(st)}} - \frac{V_o}{V_w} \right) \quad (2)$$

where $v_{inj(o)}$ is the injection volume of the octanolic phase after equilibration.

Several aqueous buffers at ionic strength of 0.10 M were prepared in order to cover a pH range between 1.90 and 11.00 (1.90 – 3.10, H_3PO_4/NaH_2PO_4 ; 3.75 – 5.75, $CH_3COOH/NaCH_3COO$; 6.21 – 8.21, NaH_2PO_4/Na_2HPO_4 ; 7.00 – 9.20, $TrisHCl/Tris$; 9.30 – 11.00, $NaHCO_3/Na_2CO_3$). These aqueous buffer solutions were saturated in octanol before use for at least 24 hours. Selected volumes of aqueous buffers and *n*-octanol are presented in Table 2 according to the expected $\log D_{o/w}$ value. The chromatographic elution was performed isocratically in the range between 20 and 80% of methanol, depending on the analyte, in mobile phases buffered with phosphate at pH 7.4.

2.5 Determination of Chromatographic Hydrophobicity Index

CHI is a high-throughput methodology lipophilicity descriptor [8] originally intended for screening purposes, based on the retention of compounds in a reversed-phase column under a fast gradient elution. In the present work CHI measurements were carried out via the UHPLC method as described elsewhere, using acetonitrile as the organic modifier and aqueous solutions of 50 mM ammonium acetate at the desired pH value (3.0, 7.4, and 11.0) [14]. In the

case of neutral species, CHI values were converted into a $\log P_{o/w}$ lipophilicity scale by means of the following relations [15]:

$$\log P_{o/w,CHI} = 0.059 CHI_{MeCN} + 103[HDCA - 1/TMSA] - 0.15[HOMO - LUMO] - 0.80 \quad (3)$$

where HDCA-1/TMSA and HOMO-LUMO are CODESSA [16] molecular descriptors accounting for hydrogen-bond acidity and polarizability, respectively (Table 3) [17].

3. Results and discussion

As mentioned in the introduction, the acidic or basic properties of a molecule, and therefore its degree of ionization, play a fundamental role in its lipophilic behavior. In fact, ionization can easily reduce the lipophilicity of a substance by about 3 or 4 $\log P_{o/w}$ units [12]. This is just a rule of thumb, since ionic species partition into the oil phase as ion-pairs and the lipophilicity also depends on the nature and concentration of the counter-ion. Consequently, determining the lipophilicity of polyprotic compounds is very often a challenging enterprise in the drug discovery process, especially in the case of ordinary and zwitterionic ampholytes. Thus, for drugs with some structural complexity, software packages may calculate a variety of lipophilicity values depending on the differences in their algorithms [2]. Even similar computational programs offered by the same provider may show significantly different lipophilicity values for the same compound [3].

Two different predictive approaches from the ACD/Labs software, Classic and GALAS, were used in this work to calculate the $\log D_{o/w}$ drug profile over a wide range of pH [18]. Since distribution ratios depend on the partition ratios of individual species, $\log P_{o/w}$ values of neutral and ionized forms are first calculated by the software, and then estimated pK_a values of the molecule are used to define the distribution of the species within the pH range. Classic $\log P_{o/w}$ are calculated from the contributions of atoms, structural fragments and intramolecular interactions, whereas GALAS $\log P_{o/w}$ are based on a training set compiled from experimental data. For pK_a estimation, the Classic approach involves Hammett-type equations and electronic substituent constants, whereas the GALAS procedure is based on a database of ionization centers and interaction constants of ionized groups to neighboring ionization centers.

As shown in Fig. 2, pK_a values obtained by both approaches were very similar (inflection points in calculated $\log D_{o/w}$ vs. pH curves), but this was not the case for $\log P_{o/w}$ values. For instance, there was an almost constant $\log D_{o/w}$ shift of more than one unit along the whole pH range for benazepril and ciprofloxacin, and this distance increased to over 2 \log

$D_{o/w}$ units for telmisartan. On the other hand, the $\log P_{o/w}$ of neutral molecules was comparable, whereas this was not the case for ionized species such as esomeprazole, mebendazole and sulfamethoxazole. In all cases, the extent of the plateau of maximum lipophilicity, corresponding to the $\log P_{o/w}$ of the neutral species, depended on the difference in pK_a between the acidic and the basic groups. For esomeprazole, haloperidol, mebendazole, pantoprazole, and sulfamethoxazole, all of them with $\Delta pK_a \geq 4$, the plateau was clearly visible, whereas for substances with $\Delta pK_a \leq 2$ it was simply reduced to an apex.

Thus, computer programs provide an estimation of the lipophilicity profile of acid-base compounds, but the proper establishment of this profile requires experimental determination. There are several measurement methods and the most common (shake-flask, potentiometry, chromatography through CHI) were used in this work, with the results obtained for a selected group of amphoteric and zwitterionic compounds being compared between methods and with computer estimations.

As shown earlier, the $\log D_{o/w}$ profile of a polyprotic compound depends on the pH of the medium, the pK_a of the successive dissociation steps, and the partition ratios ($\log P_{o/w}$ values) of individual species between the aqueous and the octanolic phases, usually expressed by:

$$\log D_{o/w} = \log \frac{P_{H_n X^z} + \sum_{i=1}^n P_{H_{n-i} X^{z-i}} \cdot 10^{i \text{pH} - \sum_{j=1}^i pK'_{aj}}}{1 + \sum_{i=1}^n 10^{i \text{pH} - \sum_{j=1}^i pK'_{aj}}} \quad (4)$$

where $H_n X^z$ refers to the fully protonated species (neutral form of a weak acid, or cationic species of a weak base) since n is the total number of ionizable groups and z is the maximum number of positive charges ($z = 0$ for acids, $z \geq +1$ for bases), and pK'_{aj} stands for the mixed pK_a of the subscript species (i.e. the activity of the proton measured by the glass electrode, but the concentration of the acid/base species). In Eq. (4) ionic species are considered to partition into the octanolic phase as ion-pairs, normally taking any of the buffer ions as a counter-ion. At this point two issues should be taken into consideration: 1) the $\log P_{o/w}$ values of ion-pairs depend on the nature and concentration of the counter-ion [12]; and 2) pK'_{aj} values obtained from fittings to Eq. (4) depend on the thermodynamic pK_a and the ionic activity coefficients, which in turn are influenced by the ionic strength of the medium.

The acidity constants from shake-flask measurements fitted to Eq. (4) and those determined by potentiometry are presented in Table 4. It must be pointed out that the latter are

reported on a concentration scale (pK_{aj}'' , i.e. the acidity constant expressed in terms of concentrations, including that of hydrogen ions, instead of activities, and at different ionic strengths: 0.10 M for shake-flask and 0.15 M for potentiometry). Both sets of values showed generally good consistency (most differences were less than 0.5 pK_a units), indicating that, in most cases, side interactions with the buffer components in the pH range considered for the shake-flask fittings are not significant.

As shown in Table 5, shake-flask and potentiometric methods led to very similar $\log P_{o/w}$ values in most instances. Consequently, as the acidity constants were similar, the $\log D_{o/w}$ profiles shown in Fig. 2 matched quite nicely. Slight differences were observed for the zwitterionic telmisartan and the positively charged ciprofloxacin species, but they are quite large for labetalol and haloperidol below pH 3.5. In fact, $\log D_{o/w}$ values obtained by the shake-flask in these acidic conditions were not included in the fittings of Eq. (4). Differences for ionized species might be attributed to diverse ion pairing effects between the buffering agent in the case of the shake-flask and the potassium chloride used for the potentiometric method. The lowest pK_a of haloperidol corresponded to the weak tertiary amine, and had a value of 8.48. There was no apparent reason for the decrease in the shake-flask lipophilicity below pH 3.5, except for the presence of phosphoric acid and dihydrogen phosphate as buffering species (Table 2); above pH 3.75, acetic acid and acetate were used instead.. However, the particular reasons for this behavior remain unclear and require further investigation. For the ciprofloxacin there was a slight difference between the shake-flask and the potentiometric profiles in the acidic region corresponding to the cationic acid species. In fact, the pK_{a1}' value fitted from shake-flask data (Table 4), which exhibited a relatively high fitting error, was about 2 units lower in relation to the pK_a determined in the present work by the reference potentiometric method and the values found in the literature [19]. Thus, high pK_{a1}' difference was likely due to the very low $\log P_{o/w}$ values of both neutral and cationic species of ciprofloxacin and the small difference between them, which led to high fitting errors. Measured $\log D_{o/w}$ values for this fluoroquinolone in the region buffered by acetic acid/acetate were slightly higher than expected, suggesting some kind of analyte–buffer interaction.

In summary, assuming that consistent lipophilicity values are obtained by both the shake-flask and the potentiometric methods, the latter seems to be more convenient because of the shorter determination times. In addition, potentiometry removes the need for the

preparation of several pH buffers and avoids the effect of different buffer counter-ions on the lipophilicity of ionized acid-base compounds.

Similarly to Eq. (4), a CHI profile can be defined as a function of the CHI of individual species, acid-base constants, and pH of the medium [20]:

$$CHI = \frac{CHI_{H_nX^z} + \sum_{i=1}^n CHI_{H_{n-i}X^{z-i}} \cdot 10^{\sum_{j=1}^i s_j (pH - pK'_{aj})}}{1 + \sum_{i=1}^n 10^{\sum_{j=1}^i s_j (pH - pK'_{aj})}} \quad (5)$$

where s is an empirical parameter accounting for the dynamics of the gradient elution [21]. The solvent change during the run implies continuous variations in the mobile phase pH and the analyte pK_a . In most cases CHI values can be rapidly obtained for neutral and ionized species, the pH range of applicability only being limited by the stability of the chromatographic column. Since CHI_{MeCN} measurements are based on a fast gradient using acetonitrile as the organic modifier, pK_a values of Eq. (5) are an average of the pK_a values in these hydro-organic solvent mixtures and do not match the potentiometric (or shake-flask) aqueous pK_a (Table 4). This is why the CHI profiles did not match the $\log D_{o/w}$ profiles, as shown in Fig. 2, although they showed a similar trend. In the case of mebendazole, which exhibited a difference between consecutive pK_a values of 6.35 units, no significant changes were observed in CHI value (from 60 to 62) in the studied pH range. The CHI profile obtained for labetalol was consistent with that obtained in a previous study [20], showing that CHI values are indeed independent of the particular C18 column employed.

Although general conversion between chromatographic and $\log P_{o/w}$ lipophilicity scales is in principle possible [22, 23], it can only be applied to neutral compounds and requires the calculation of molecular descriptors (Eq. (3), Table 3). Even so, we applied Eq. (3) to convert the CHI to the $\log D_{o/w}$ scale for all obtained CHI data for a particular compound. This conversion is illustrated in Fig. 2, i.e. the obtained CHI value of the drug can be read on the righthand y-axis and the corresponding calculated $\log D_{o/w}$ (from Eq. 3 and the particular drug descriptor (Table 3)) on the lefthand y-axis. However as shown in Table 5, $\log P_{o/w}$ values obtained from CHI measurements differed slightly from those measured by shake-flask and potentiometry. The main reasons may lie in the nature of the CHI approach, since it is an indirect method for $\log P_{o/w}$ determination, and in addition, for most of the zwitterionic compounds, there is the issue of the reduction of the molar fraction of neutral species when acetonitrile is added to the aqueous buffer to prepare the mobile phase. Thus, acidic groups

increase their pK_a values with the content of organic modifier, whereas basic groups show the opposite trend. For instance, benazepril has two functional groups with acid-base properties, a carboxylic aliphatic acid and secondary amine of aqueous pK_a values of 3.35 and 5.43, respectively. Thus, in pure water, the neutral form of this compound is a zwitterion. When 30% of acetonitrile is added, the first pK_a is expected to shift up to 3.95 and the second one to shift down to 4.96 [24], reducing the distance between pK_a values from 2.1 to 1.0 units. When the percentage of acetonitrile in the hydro-organic mixture exceeds 60%, the pK_a variation continues until the amine becomes stronger (4.33) than the carboxylic acid (4.75), and thus the main neutral compound is no longer a zwitterion. The shorter the pK_a distance, the lower the mole fraction of neutral species found in solution. Moreover, both the pK_a and the pH of the mobile phase change with the organic modifier, which requires an accurate pH measurement of the mobile phase or alternatively some previous calculations in order to find the most appropriate pH for the aqueous buffer [24]. Therefore, even under the most favorable mobile phase pH, it is not possible to measure the retention of the isolated neutral species, but only a mixture of neutral and ionized forms.

A second reason for the lower accuracy of chromatographic methods in relation to shake-flask and potentiometry arises from the molecular descriptors. They are always calculated from the uncharged form, even in the case of zwitterionic species, and this must affect the charge distribution and the geometry of the molecule, and consequently the accuracy of the molecular descriptors.

4. Conclusions

For the studied amphoteric compounds, the shake-flask and potentiometric methods led to very similar $\log D_{o/w}$ pH-profiles, and consequently to comparable $\log D_{o/w}$ profiles. Differences for ionized species between the two methods (e.g. haloperidol or labetalol at acidic pH) might be attributed to differential ion pairing effects between the buffering agent in the case of the shake-flask and the potassium chloride required for the potentiometric method. Since the phase equilibration method is extremely time consuming and requires buffers of different nature according to their buffer capacity and working pH, the potentiometric methods seems to be more advantageous.

Prediction software can be very useful for defining the start-up conditions for the experimental determination, since they provide a preliminary estimate of $\log D_{o/w}$ profiles over the whole pH range. However, their accuracy compared with experimental values may

not be very good, and different prediction algorithms may lead to diverse results for the same compound.

The CHI methodology is very convenient for lipophilicity screening purposes, due to its rapidity and wide pH range of applicability, but the $\log P_{o/w}$ values from CHI measurements are expected to be less accurate than those obtained using the shake-flask and potentiometric methods.

Conflict Of Interest Statement

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the Ministry of Economy and Competitiveness of Spain (project CTQ2014-56253-P).

References

- [1] D. Cairns, Essentials of Pharmaceutical Chemistry, 4th ed., Pharmaceutical Press, London, UK, 2012.
- [2] I.V. Tetko, G.I. Poda, C. Ostermann, R. Mannhold, Large-Scale Evaluation of $\log P$ Predictors: Local Corrections May Compensate Insufficient Accuracy and Need of Experimentally Testing Every Other Compound, Chem. Biodivers., 6 (2009) 1837-1844.
- [3] J.M. Pallicer, M. Rosés, C. Ràfols, E. Bosch, R. Pascual, A. Port, Evaluation of $\log P_{o/w}$ values of drugs from some molecular structure calculation software, ADMET & DMPK, 2 (2014) 107-114.
- [4] A. Avdeef, pH-Metric $\log P$. Part 1. Difference Plots for Determining Ion-Pair Octanol-Water Partition Coefficients of Multiprotic Substances, Quant. Struct-Act. Rel., 11 (1992) 510-517.
- [5] A. Avdeef, pH-metric $\log P$. II: Refinement of partition coefficients and ionization constants of multiprotic substances, J. Pharm. Sci., 82 (1993) 183-190.
- [6] OECD, Guideline for the testing of chemicals. Method 107. Partition coefficient (n -octanol/water): shake flask method, 1995.
- [7] EPA, Product Properties Test Guidelines. OPPTS 830.7550 Partition coefficient (n -octanol/water), shake flask method, United States, 1996.

- [8] K. Valkó, C. Bevan, D. Reynolds, Chromatographic Hydrophobicity Index by Fast-Gradient RP-HPLC: A High-Throughput Alternative to $\log P/\log D$, *Anal. Chem.*, 69 (1997) 2022-2029.
- [9] M. Rosés, E. Bosch, C. Ràfols, E. Fuguet, Chromatographic hydrophobicity index (CHI), *Advances in Chromatography*, CRC Press, Boca Raton, FL, U.S., 2012, pp. 377-414.
- [10] A. Avdeef, J.E.A. Comer, S.J. Thomson, pH-Metric $\log P$. 3. Glass electrode calibration in methanol-water, applied to pK_a determination of water-insoluble substances, *Anal. Chem.*, 65 (1993) 42-49.
- [11] K.Y. Tam, K. Takács-Novák, Multiwavelength spectrophotometric determination of acid dissociation constants: a validation study, *Anal. Chim. Acta*, 434 (2001) 157-167.
- [12] A. Avdeef, *Absorption and Drug Development: Solubility, Permeability, and Charge State*, 2nd edition, Wiley, Hoboken, NJ, US, 2012.
- [13] A. Andrés, M. Rosés, C. Ràfols, E. Bosch, S. Espinosa, V. Segarra, J.M. Huerta, Setup and validation of shake-flask procedures for the determination of partition coefficients ($\log D$) from low drug amounts, *Eur. J. Pharm. Sci.*, 76 (2015) 181-191.
- [14] J.M. Cabot, X. Subirats, E. Fuguet, M. Rosés, High throughput determination $\log P_{o/w}/pK_a/\log D_{o/w}$ of drugs by combination of UHPLC and CE methods, *ADMET & DMPK*, 2 (2014) 98-106.
- [15] X. Subirats, M. Rosés, E. Bosch, High-throughput $\log P_{o/w}$ determination from UHPLC measurements: revisiting the Chromatographic Hydrophobicity Index *J. Pharm. Biomed. Anal.*, 127 (2016) 26-31.
- [16] CODESSA, CompuDrug, Bal Harbor, Florida, USA. www.compudrug.com.
- [17] A.R. Katritzky, V.S. Lohanov, M. Karelson, CODESSA, Reference Manual V 2.13, Semichem and the University of Florida, 1997.
- [18] ACD/Labs, Advanced Chemistry Development, Inc., Toronto, Canada. <http://www.acdlabs.com/products/percepta/predictors/logd/>.
- [19] G.-M. Cárdenas-Youngs, J.-L. Beltrán, Dissociation Constants and Octanol–Water Partition Equilibria for Several Fluoroquinolones, *J. Chem. Eng. Data*, 60 (2015) 3327-3332.
- [20] E. Fuguet, C. Ràfols, E. Bosch, M. Rosés, Chromatographic hydrophobicity index: pH profile for polyprotic compounds, *J. Chromatogr. A*, 1216 (2009) 7798-7805.
- [21] I. Canals, K. Valkó, E. Bosch, A.P. Hill, M. Rosés, Retention of Ionizable Compounds on HPLC. 8. Influence of Mobile-Phase pH Change on the Chromatographic Retention of Acids and Bases during Gradient Elution, *Analytical Chemistry*, 73 (2001) 4937-4945.

- [22] K. Valko, C.M. Du, C. Bevan, D.P. Reynolds, M.H. Abraham, Rapid method for the estimation of octanol/water partition coefficient ($\log P_{\text{oct}}$) from gradient RP-HPLC retention and a hydrogen bond acidity term ($\Sigma\alpha_2^{\text{H}}$), *Curr. Med. Chem.*, 8 (2001) 1137-1146.
- [23] J.M. Pallicer, R. Pascual, A. Port, M. Rosés, C. Ràfols, E. Bosch, The contribution of the hydrogen bond acidity on the lipophilicity of drugs estimated from chromatographic measurements, *Eur. J. Pharm. Sci.*, 48 (2013) 484-493.
- [24] X. Subirats, M. Rosés, E. Bosch, On the Effect of Organic Solvent Composition on the pH of Buffered HPLC Mobile Phases and the pK_a of Analytes - A Review, *Sep. Purif. Rev.*, 36 (2007) 231-255.

FIGURE CAPTIONS

Figure 1. Maximum advisable mole fraction of neutral species for an amphoteric compound depending on the pK_a distance between acidic and basic groups.

Figure 2. Lipophilicity profiles of some ordinary and zwitterionic ampholytes of pharmaceutical interest measured by different methods: a) (●) shake-flask with chromatographic UV detection; b) (solid lines) potentiometry; c) (■) CHI; d) (dotted lines) estimated from ACD/Labs, Classic approach; e) (dashed lines) estimated from ACD/Labs, GALAS approach. For shake-flask and CHI, symbols represent experimental measurements and lines are the fittings to corresponding models (Eqs. (4) and (5)). The scale of the right CHI axis has been set to match that of the left $\log P_{\text{o/w}}$ axis by means of Eq. (3) and the molecular descriptors presented in Table 3.

TABLES

Table 1. Studied compounds.

Compound	Compound
Benazepril	Mebendazole
Ciprofloxacin	Nalidixic acid
Esomeprazole	Pantoprazole
Haloperidol	Sulfamethoxazole
Labetalol	Telmisartan

Table 2. Selected partition volumes of the shake-flask procedure.

log $D_{o/w}$ range	V_w/V_o (V in μL)
log $D_{o/w} < -1$	200/5000, 500/5000, 500/500
$-1 < \log D_{o/w} < 0$	500/5000, 500/500
$0 < \log D_{o/w} < 1.5$	500/5000, 500/500, 1000/100
$1.5 < \log D_{o/w} < 3.0$	1000/10, 3000/10, 5000/10
$3.0 < \log D_{o/w} < 3.5$	3000/10, 5000/10

Table 3. CODESSA molecular descriptors used in the log $P_{o/w}$ determination from chromatographic measurements.

Compounds	HDCA-1/TMSA	HOMO-LUMO
Benazepril	$3.64 \cdot 10^{-3}$	9.03
Ciprofloxacin	$8.69 \cdot 10^{-3}$	8.26
Esomeprazole	$3.46 \cdot 10^{-3}$	8.11
Haloperidol	$3.47 \cdot 10^{-3}$	8.42
Labetalol	$9.33 \cdot 10^{-3}$	9.17
Mebendazole	$8.64 \cdot 10^{-3}$	8.35
Nalidixic acid	$8.70 \cdot 10^{-3}$	8.47

1-Naphthoic acid	$7.24 \cdot 10^{-3}$	8.16
Pantoprazole	$5.75 \cdot 10^{-3}$	8.38
Sulfamethoxazole	$4.99 \cdot 10^{-3}$	8.67
Telmisartan	$3.25 \cdot 10^{-3}$	7.80

Table 4. Comparison between the potentiometric reference acidity constants and the fitted ones from shake-flask (Eq. 4) and CHI (Eq. 5) vs. pH profiles.

Compound	Class ^a	Potentiometric ^b	Shake-flask ^c	CHI	
		pK_{aj}''	pK_{aj}'	pK_{aj}'	<i>s</i>
Benazepril	H ₂ X ⁺	3.35±0.25	2.73±0.18	-	-
	HX [±]	5.43±0.02	5.87±0.11	4.86±0.08	1.12±0.16
Ciprofloxacin	H ₂ X ⁺	6.20±0.05	4.08±0.50	-	-
	HX [±]	8.56±0.04	8.85±0.17	7.34±0.27	0.49±0.18
Esomeprazole	H ₂ X ⁺	4.60±0.08	5.37±0.20	3.46±0.96	0.84±0.71
	HX [±]	8.86±0.04	8.69±0.08	10.13±0.23	0.89±0.19
Haloperidol	H ₂ X ⁺	8.54±0.09	8.12±0.13	8.15±0.09	0.70±0.09
	HX	10.98±0.15	-	-	-
Labetalol	H ₂ X ⁺	7.41±0.04	7.59±0.32	8.00±0.59	0.72±0.46
	HX [±]	9.37±0.08	9.32±0.23	9.99±0.08	1.82±0.94
Mebendazole	H ₂ X ⁺	3.53±0.04	3.59±0.16	-	-
	HX	9.88±0.06	9.43±0.17	-	-
Nalidixic acid	HX	6.00±0.03	6.41±0.11	7.58±0.04	1.04±0.11
Pantoprazole	HX	8.22±0.09	8.38±0.06	9.23±0.05	1.03±0.13
Sulfamethoxazole	HX	5.65±0.03	5.83±0.04	6.47±0.09	0.64±0.06
Telmisartan	H ₃ X ⁺	3.01±0.09	3.28±0.28	-	-
	H ₂ X [±]	4.39±0.02	4.60±0.44	4.61±0.04	0.92±0.05
	HX ⁻	6.02±0.03	5.61±0.35	-	-

^aThe acidic form of the compound is shown; ^b*I*=0.15M (KCl); ^c*I*=0.10M (several buffers).

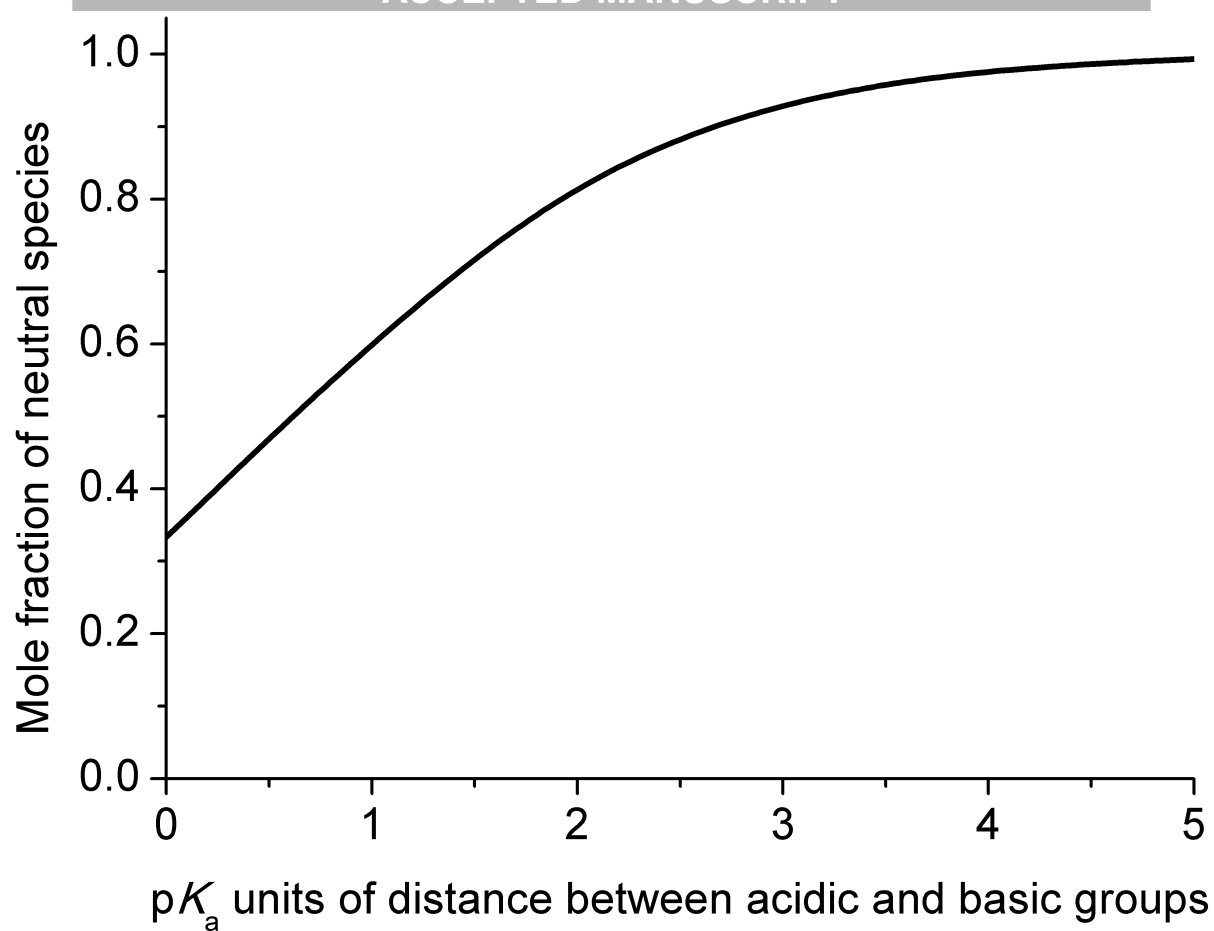
Table 5. Measured lipophilicity for the studied amphoteric and zwitterionic compounds.

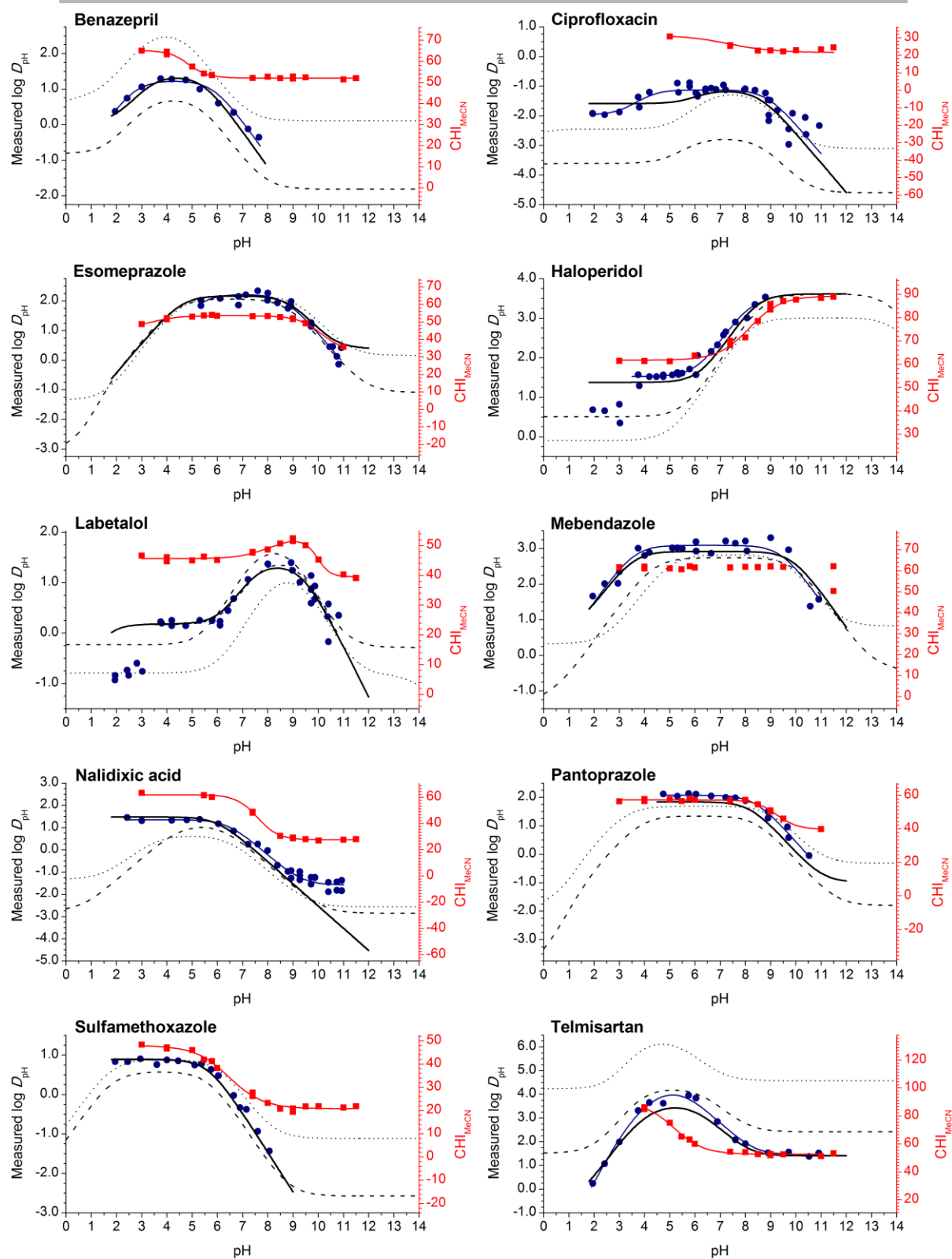
Compound	Class	Shake-flask	Potenciometric	CHI method	
		$\log P_{o/w}$ (<i>I</i> =0.10M)	$\log P_{o/w}$ (<i>I</i> =0.15M)	CHI _{MeCN}	$\log P_{o/w}$
Benazepril	H ₂ X ⁺	-	0.07±0.10	-	-
	HX [±]	1.24±0.08	1.38±0.01	65.3±0.5	2.07
	X ⁻	-	-	52.2±0.2	-
Ciprofloxacin	H ₂ X ⁺	-1.98±0.21	-1.59±0.12	-	-
	HX [±]	-1.13±0.09	-1.15±0.03	31.5±0.7	0.71
	X ⁻	-	-	21.6±0.8	-
Esomeprazole	H ₂ X ⁺	-	-	46.8±5.3	-
	HX [±]	2.23±0.05	2.17±0.01	53.6±0.3	1.50
	X ⁻	-	0.39±0.04	32.8±2.6	-
Haloperidol	H ₂ X ⁺	1.53±0.04	1.38±0.03	61.8±0.7	-

	HX	3.52±0.09	3.61±0.01	89.3±0.9	3.56
Labetalol	H ₂ X ⁺	0.17±0.08	0.18±0.03	45.6±0.4	-
	HX [±]	1.45±0.17	1.37±0.02	52.7±2.8	1.89
	X ⁻	-	-	39.6±0.7	-
Mebendazole	H ₂ X ⁺	-	0.71±0.14	61.6±0.4	-
	HX	3.09±0.07	2.92±0.10	-	2.47
Nalidixic acid	HX	1.36±0.08	1.48±0.02	61.9±0.5	2.48
	X ⁻	-1.58±0.07	-	27.5±0.4	-
Pantoprazole	HX	2.07±0.04	1.84±0.01	57.2±0.2	1.91
	X ⁻	-	-0.98±0.28	39.6±0.7	-
Sulfamethoxazole	HX	0.86±0.02	0.90±0.01	47.9±0.7	1.24
	X ⁻	-	-	20.8±0.5	-
Telmisartan	H ₃ X ⁺	-	1.45±0.29	-	-
	H ₂ X [±]	4.18±0.29	3.54±0.11	90.3±2.4	2.44
	HX ⁻	1.46±0.09	1.41±0.05	52.7±0.4	-

Highlights

- Lipophilicity-pH profiling of amphoteric and zwitterionic drugs might be complex.
- Octanol/water shake-flask and potentiometry approaches lead to similar results.
- Drug-buffer interactions might be relevant in octanol/water shake-flask approach.
- CHI vs. pH profiles provide rapid and efficient information for screening purposes.





Graphical abstract

Haloperidol

