Lipophilicity Profiles of Ampholytes

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I. Introduction

A. Significance of Amphoteric Drugs and Biomolecules

The relation between drug distribution and physicochemical properties such as lipophilicity has received considerable attention and is discussed in numerous reviews.[1−4] In contrast, no specific and comprehensive treatment has been devoted to amphoteric compounds (i.e., ampholytes) despite their frequent occurrence in medicinal chemistry and biochemistry.

Ampholytes are encountered in drug research either as drugs or as their metabolites. In the latter case, a metabolic reaction creates an additional ionizable group in the molecule, resulting in a different ionization behavior. When a second ionic center is introduced whose charge is opposite to that of the preexisting group, an ampholyte is formed. The consequences of metabolic transformations on lipophilicity have been discussed in an informative review[1] and classified according to their influence on the ionization behavior, namely, (a) reactions that leave the ionization pattern unchanged and (b) reactions that alter it by either introducing or removing an ionizable group. Thus, reactions of functionalization can oxidize a primary alcoholic group to a carboxylic group, whereas glucuroconjugation in many cases (the exception being formation of acylglucuronides) and sulfation always introduce an acidic group in the substrate molecule. But be they drugs or metabolites, ampholytes are expected to display physicochemical properties and a pharmacokinetic behavior distinct from those of compounds with only acidic or basic group(s).

In biochemistry also, ampholytes are the rule more than the exception when one considers amino acids, peptides, proteins, a number of neurotransmitters such as dopamine, many metabolic intermediates (e.g., creatine and porphobilinogen), fatty acid derivatives such as various leukotrienes, complex lipids (e.g., phosphatidylcholine and sphingomyelin), numerous nucleotides, coenzymes such as pyridoxal phosphate, and countless other compounds. It cannot be fortuitous that nature created and so extensively relies on ampholytic compounds as metabolic intermediates, messengers, and building blocks for a practically infinite variety of complex molecular assemblies. The deep biological significance of the specific properties of ampholytes is perhaps linked to their broad property space, which allows polymorphic behavior and hence adaptability.[5−7]

Any rationalization in the specific physicochemical properties of ampholytes should thus be beneficial in medicinal chemistry and biochemistry, to name but two branches of chemistry. Lipophilicity is precisely one such property of biochemical and pharmacological significance. It is our objective in this writing to review recent advances in this field and to clarify a number of concepts.

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Alessandra Pagliara was born in Foggia, Italy. She studied Pharmaceutical Sciences at the University of Turin (Italy) where she was awarded degrees in Pharmaceutical Chemistry and Technology (1993) and Pharmacy (1995). In 1994, she joined the Institute of Medicinal Chemistry at the University of Lausanne to undertake doctoral research on lipophilicity and other physicochemical properties of relevance in molecular pharmacology and drug design. Her current research interests center on QSAR and on molecular modeling of drugs and receptors, and her Ph.D. thesis is in the writing.

Pierre-Alain Carrupt graduated as a chemist and hold a Ph.D. from the University of Lausanne. Following early work in synthetic chemistry, he soon became interested in computational chemistry with applications in reaction mechanisms and organic physical chemistry. A tenured senior lecturer in Medicinal Chemistry, he now specializes in molecular modeling techniques as applied to drug design and molecular pharmacology. He is the author or co-author of about 150 research papers and 30 chapters in scientific books.

Giulia Caron is a native of Turin, Italy. She obtained her degrees in Pharmaceutical Chemistry and Technology (1992) and Pharmacy (1994) from the University of Turin. In 1997, she was awarded a Ph.D. from the University of Lausanne for a thesis on the physicochemical determinants of drug binding and distribution. In this work, she used experimental and computational techniques to explore new research fronts in lipophilicity. She is currently postdoctoral fellow and senior teaching assistant at the Institute of Medicinal Chemistry of the University of Lausanne.

B. Partitioning of Amphoteric Drugs: Zwitterions Compared to Ordinary Ampholytes

Amphoteric compounds (i.e., ampholytes) can be classified conveniently into two main categories, namely, the ordinary and the zwitterionic ampholytes. All ampholytes have an acidic and a basic group but what distinguishes them is the relative acidity of the two functional groups. Indeed, zwitterionic ampholytes can form an internal salt since the acidic and the basic group can be simultaneously ionized. For zwitterionic ampholytes, the relation $pK_a^{acidic} < pK_a^{basic}$ is true. In contrast, the two groups cannot ionize simultaneously in ordinary ampholytes, since here the relation $pK_a^{acidic} > pK_a^{basic}$ holds (see section II.A).[8]

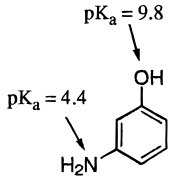
As far as their lipophilicity is concerned, amphoteric drugs have not been widely discussed in the literature. Contradictory results have even been published.[9] In ordinary ampholytes, which in solution and for all practical purposes exist only in neutral or singly charged form, it is trivial knowledge that the intrinsic lipophilicity of the neutral species is greater than that of the associated cation or anion. In contrast, much controversy surrounds the lipophilicity of zwitterions, which is very low according to some authors but marked according to others.[9] In principle, any ionized species has some affinity, however small, for an organic phase. The issue is to know when zwitterion partitioning can be neglected and when not.

II. Theoretical Background

A. Acid–Base Equilibria

1. Ordinary Ampholytes

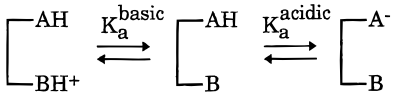
The protolysis equilibria of ordinary ampholytes ($pK_a^{acidic} > pK_a^{basic}$) are straightforward. The pK_a values can be readily assigned by ordinary titration, and the pattern of ionization can be described as an *unbranched* two-step protolysis system[10] as shown in Scheme 1, where A and B are the acidic and basic groups, respectively. When the pH is increased, the first group to lose its proton is the basic one.

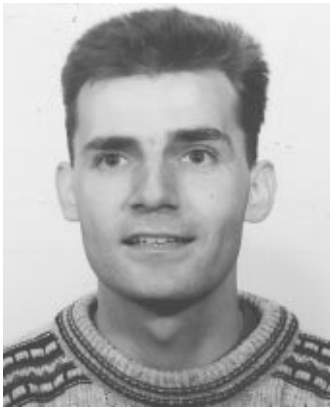


m-aminophenol (**1**)

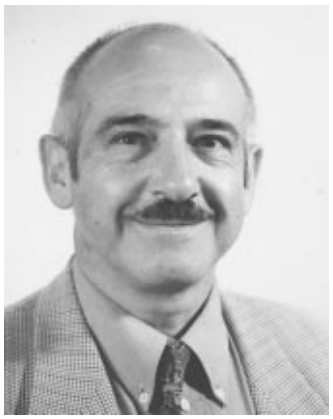
Figure 1 shows the ionic populations of *m*-aminophenol (**1**), an ordinary ampholyte having pK_a values of 4.4 and 9.8 for the basic amino group and the acidic phenolic group, respectively.[8] This ordi-

Scheme 1





Patrick Gaillard studied chemistry at the University of Lausanne and obtained his chemist diploma in 1989. From 1990 to 1994, he was a graduate student the Institute of Medicinal Chemistry, where he pursued computational investigations in the field of drug design and molecular modeling. His major achievement was the successful development of the molecular lipophilicity potential. He received a Ph.D. in 1994 and remained in the same institution until recently as a postdoctoral fellow and senior teaching assistant. He is currently Head of the Quality Control Department of a chemical company in Bex, Switzerland. He has (co)-authored 20 publications.



Bernard Testa studied pharmacy because he was unable to choose between medicine and chemistry. Because he was incapable of working in a community pharmacy, he undertook a Ph.D. thesis on the physicochemistry of drug–macromolecule interactions. Because he felt himself ungifted for the pharmaceutical industry, he went for 2 years to Chelsea College, University of London, for postdoctoral research under the supervision of Prof. Arnold H. Beckett. And because these were easy times, he was called as assistant professor to the University of Lausanne (Switzerland), to become full professor and Head of Medicinal Chemistry in 1978. Since then, he has tried to repay his debts by fulfilling a number of local and international commitments, e.g., Dean of the Faculty of Sciences (1984–86), Director of the School of Pharmacy since 8 years, and vice-president of the Senate of the University. He has written three books and edited 25 others and (co)-authored over 300 research and review articles in the fields of drug design and drug metabolism. A member of the Editorial Board of several leading journals, he is the Editor-Europe of Pharmaceutical Research, the flagship journal of the American Association of Pharmaceutical Scientists (AAPS). He holds a Honorary Doctorate from the University of Montpellier (France) and is a Fellow of the Royal Society of Chemistry and of the AAPS and a member of numerous scientific societies such as the American Chemical Society and the New Swiss Chemical Society. His hobbies, interests, and passions include jogging, science-fiction, epistemology, teaching, and scientific exploration.

nary ampholyte can thus exist in at least three different electrical states, namely, as a cation, a neutral compound, and an anion. This is trivial knowledge repeated here merely for comparison purposes with what follows.

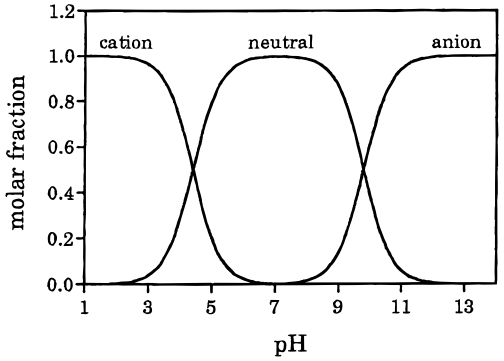


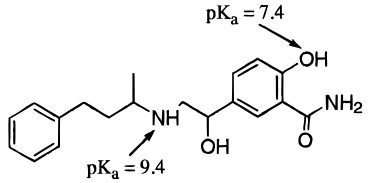
Figure 1. Distribution of ionic species for the ordinary ampholyte *m*-aminophenol (**1**).

The above discussion neglects the possibility of simultaneous ionization of the two groups. When the difference between pK_a^{acidic} and pK_a^{basic} (noted ΔpK_a) is greater than 3 as with *m*-aminophenol, only one kind of group can be ionized at a time to any noteworthy proportion. When, however, ΔpK_a is smaller than 3, an overlap of the two equilibria begins to be felt. At some pH values the ionization of the other group will no longer be negligible, allowing the existence of a small proportion of the zwitterionic species.

2. Zwitterionic Ampholytes: Microdissociation Equilibria and Micro-pK_a

As explained below, the dissociation equilibria of the zwitterionic ampholytes ($pK_a^{acidic} < pK_a^{basic}$) are more complex than those of the ordinary ampholytes. And when the two pK_a are relatively close (small value of ΔpK_a), it is not possible to decide *a priori* which of the two protonated sites dissociates first. Further investigation is necessary to assign pK_a values (see sections III.A.1 and III.A.2).

A zwitterionic ampholyte can exist in solution in four different electrical states, namely, as a cation (**C**), as a charged but globally neutral form called a zwitterion (**Z**), as a neutral and uncharged form simply called the neutral form (**N**), and as an anion (**A**), as shown in Figure 2 taking the drug labetalol (**2**) as an example. In such cases, the pattern of



labetalol (**2**)

ionization is described as a *branched* system[10] (Scheme 2).

In such a scheme, the acid−base equilibria are defined in terms of macroscopic constants (or *macroconstants*, K_a^{acidic} and K_a^{basic}) which refer to the stoichiometric ionization, and of microscopic constants (or *microconstants*) which refer to the ionization of individual forms. In this work, the following notation will be used to characterize the microconstants (see also the Glossary):

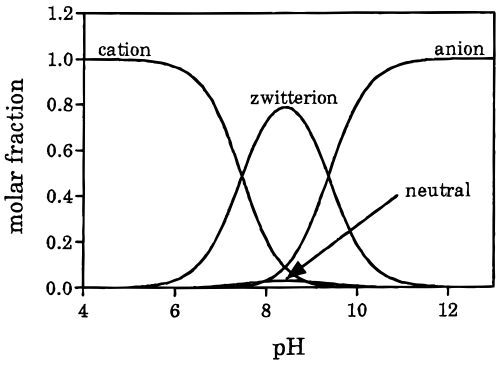
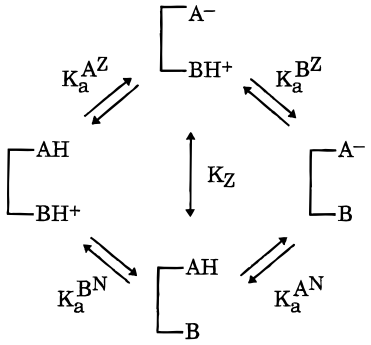


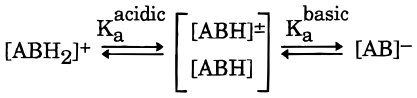
Figure 2. Distribution of ionic species for the zwitterionic ampholyte labetalol (**2**), where $pK_a^{acidic} = 7.4$; $pK_a^{basic} = 9.4$; $K_Z = 29$.

Scheme 2

Microdissociation equilibria



Stoichiometric equilibria



The superscripts A and B refer to the dissociation of the acidic and basic group, respectively.

The superscript Z in the microconstants K_a^{AZ} and K_a^{BZ} means that they describe a protolysis equilibrium leading to the formation or dissociation of the zwitterion (upper part of Scheme 2).

The superscript N of the microconstants K_a^{BN} and K_a^{AN} denotes that they are involved in the protolysis equilibria leading to the formation or dissociation of the neutral uncharged species (lower part of Scheme 2).

It must be pointed out that the above notation is one among several which can be used to specify the various macroscopic and microscopic pK_a as well as the species involved in the equilibria. In most cases, and mainly with more than two sites of protonation, the macroscopic pK_as are called pK_{a1}, pK_{a2}, pK_{a3}, ..., in order of increasing value, but no information is thus given about the acidic or basic nature of the sites. The latter notation is helpful at an early stage of investigation when pK_a assignment has not yet been made.[8] Regarding the notation of microscopic pK_a used above, they can be gainfully compared with the vast variety of superscripts and subscripts found in the literature.[8,9,11−14]

In stoichiometric equilibria, the two globally neutral microspecies (i.e., the charged and the uncharged one) are treated collectively as being two tautomeric

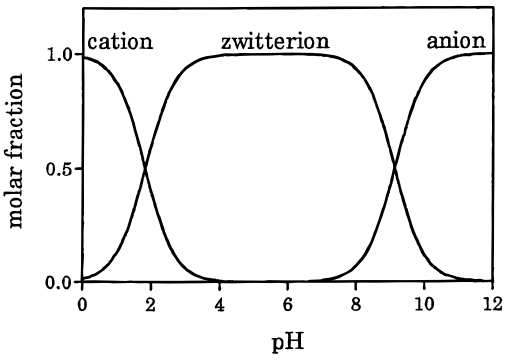


Figure 3. Distribution of ionic species for the α-amino acid phenylalanine (**3**) ($\Delta pK_a > 5$).

representations of a single neutral form, since a titration will yield only the two macro-pK_a values. Due to the simultaneous ionization of the two groups, the macro-pK_as are a composite of the underlying microconstants as made explicit in Adams' equations[8] (eqs 1−3):

$$K_a^{acidic} = K_a^{AZ} + K_a^{BN} \quad (1)$$

$$\frac{1}{K_a^{basic}} = \frac{1}{K_a^{BZ}} + \frac{1}{K_a^{AN}} \quad (2)$$

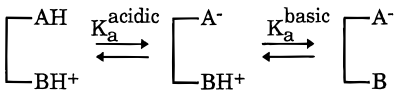
$$K_Z = \frac{K_a^{AZ}}{K_a^{BN}} = \frac{K_a^{AN}}{K_a^{BZ}} \quad (3)$$

As can be seen, each macroconstant is defined by two microconstants, but there is no direct relationship between the macroconstants and the *constant of tautomeric equilibrium* K_Z, i.e., the ratio of concentrations of the two neutral microspecies (zwitterion/neutral).[15] As a consequence, it is not sufficient to know the values of the two macro-pK_a to understand the microscopic behavior of a zwitterionic ampholyte. Knowledge of at least one micro-pK_a or of the tautomeric constant K_Z is also indispensable. Only then can one apply Adams' equations to calculate all other microconstants.

3. Zwitterionic Ampholytes Having a Large Value of K_Z

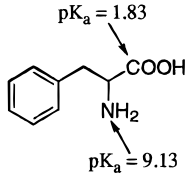
When the two ionizable groups (A and B) have very different pK_a values ($\Delta pK_a >$ ca. 5), as is the case with many amino acids, the ionization scheme may be simplified to an *unbranched* one, and it will be sufficient to know the two macroscopic pK_a values[8] (Scheme 3):

Scheme 3



Here as with the ordinary ampholytes, a simple titration will yield enough information to describe the system, but in this case the electrical species existing

in solution are the cation, the zwitterion, and the anion, as shown in Figure 3 for phenylalanine (**3**).



phenylalanine (**3**)

B. Partitioning of Zwitterions

1. General Assumptions for Ionizable Solutes

Lipophilicity is conventionally expressed as the logarithm of the *partition coefficient* (written as log *P*), a measure of the concentration ratio of a solute present in a single electrical state and in equilibrium between two immiscible solvents (water and an organic solvent). When more than one electrical species are present in solution, as can be the case with ionizable solutes, the observed ratio of concentrations is the *distribution coefficient* (log *D*), which takes into account the intrinsic lipophilicity of the different electrical species present and their relative concentrations. At a given pH, the log *D* of an ionizable solute is thus defined by eq 4:

$$\log D = \log[f^{N}P^{N} + \sum(f^{I}P^{I})] \quad (4)$$

where f^N and f^I are the respective fractions of the neutral and ionized forms, themselves a function of pH and pK_a values; P^N and P^I are the partition coefficients of the neutral and ionized forms, respectively (see also the Glossary).

At this point, it must be stressed that when we refer to partition coefficients of cationic or anionic species (written here as log P^C and log P^A, respectively), we refer to the monocharged forms in the presence of specific counterions. For theoretical and practical reasons, partition coefficients cannot be measured in the absence of counterions (see section III.A.3), implying that the influence of counterions on the partitioning of monocharged species cannot be neglected. The notation log P^C and log P^A used here must therefore be taken as a convention and a simplification. However, the influence of counterions on log P^I should not be overestimated. As demonstrated for chloropromazine,[16] a sixfold increase in chloride counterion concentration caused an increase in log *D* of only 0.5 unit.

For ionizable solutes, their log *D* value at a given pH (e.g., at physiological or gastric pH) is often a critical parameter in quantitative structure−activity relationships and quantitative structure−permeation relationships. Any error in the determination of log *D* may therefore lead to misleading conclusions. Hence the distribution profile (better called the *lipophilicity profile* and defined as the variation of log *D* as a function of pH, see Figure 4) becomes essential to interpret pharmacokinetic, toxicokinetic, and even pharmacodynamic properties and should contribute to our understanding of biochemical regulations.

For ionizable solutes, the log *P* of the neutral species (log P^N) is usually 2−5 units larger than that of the ionized species, depending on solute, counterion concentration, and solvents.[16−18] This difference is usually considered large enough to neglect the partitioning of charged species, allowing eq 4 to be simplified to eq 5.

$$\log D = \log(f^{N}P^{N}) \quad (5)$$

In most cases reported in the literature, the log P_{oct} value (i.e., measured in the octanol/water system) of ionizable solutes was derived from eq 5 by using a log *D* value measured at a single pH. However, such log *P* values can be overestimates due to the assumption in eq 5. Similarly, when log *D* at a given pH is calculated from the log *D* at another pH, neglect of ionic partitioning can yield a misleading result.

This problem is explained graphically in Figure 4, using simulations for *diff*(log P^{N-I}) = 3, namely, for a difference of 3 units between log P_{oct} of the neutral form and log *P* of the ionized form. Figure 4 demonstrates the error introduced by eq 5 in log P^N when the latter is calculated from log *D* data obtained in pH regions increasingly distant from the pK_a value (i.e., with increasing contribution of the charged form to partitioning). As can be seen, the artifactual error becomes larger than the experimental error (taken to be 0.2 unit) at pK_a − pH > 3 for a base (Figure 4a) or pH − pK_a > 3 for an acid (Figure 4b).

2. General Assumptions for Zwitterionic Ampholytes

For a zwitterionic ampholyte that can exist in solution as four different electrical species, log *D* is defined by eq 6:

$$\log D = \log (f^{N}P^{N} + f^{Z}P^{Z} + f^{C}P^{C} + f^{A}P^{A}) \quad (6)$$

where *f* and *P* have the meaning given above (see also the Glossary).

Around the isoelectric pH, the two neutral microspecies (the +/− ionized and the uncharged species) always coexist. Neglecting the partitioning of the cationic and anionic species (a reasonable assumption given their very low fractions), log *D* is described by eq 7:

$$\log D^{pH_i} = \log (f^{N}P^{N} + f^{Z}P^{Z}) \quad (7)$$

The terms f^N and f^Z are related to the tautomeric constant K_Z (Scheme 2) as shown in eq 8.

$$\log D^{pH_i} = \log \left[P^{N}\left(\frac{1}{1+K_Z}\right) + P^{Z}\left(\frac{K_Z}{1+K_Z}\right)\right] \quad (8)$$

Although it is common-sensical to expect that the intrinsic lipophilicity of the neutral uncharged form (N) will be larger than that of the zwitterion (Z), the contribution of the latter cannot be neglected *a priori*. Indeed, zwitterionic ampholytes often exhibit incompletely understood intramolecular effects which increase their lipophilicity, e.g., charge delocalization, internal electrostatic bonds, and folding, as discussed and exemplified in section IV.A.2.

A correct derivation of log P^N and/or log P^Z around the isoelectric pH calls for the knowledge of K_Z.

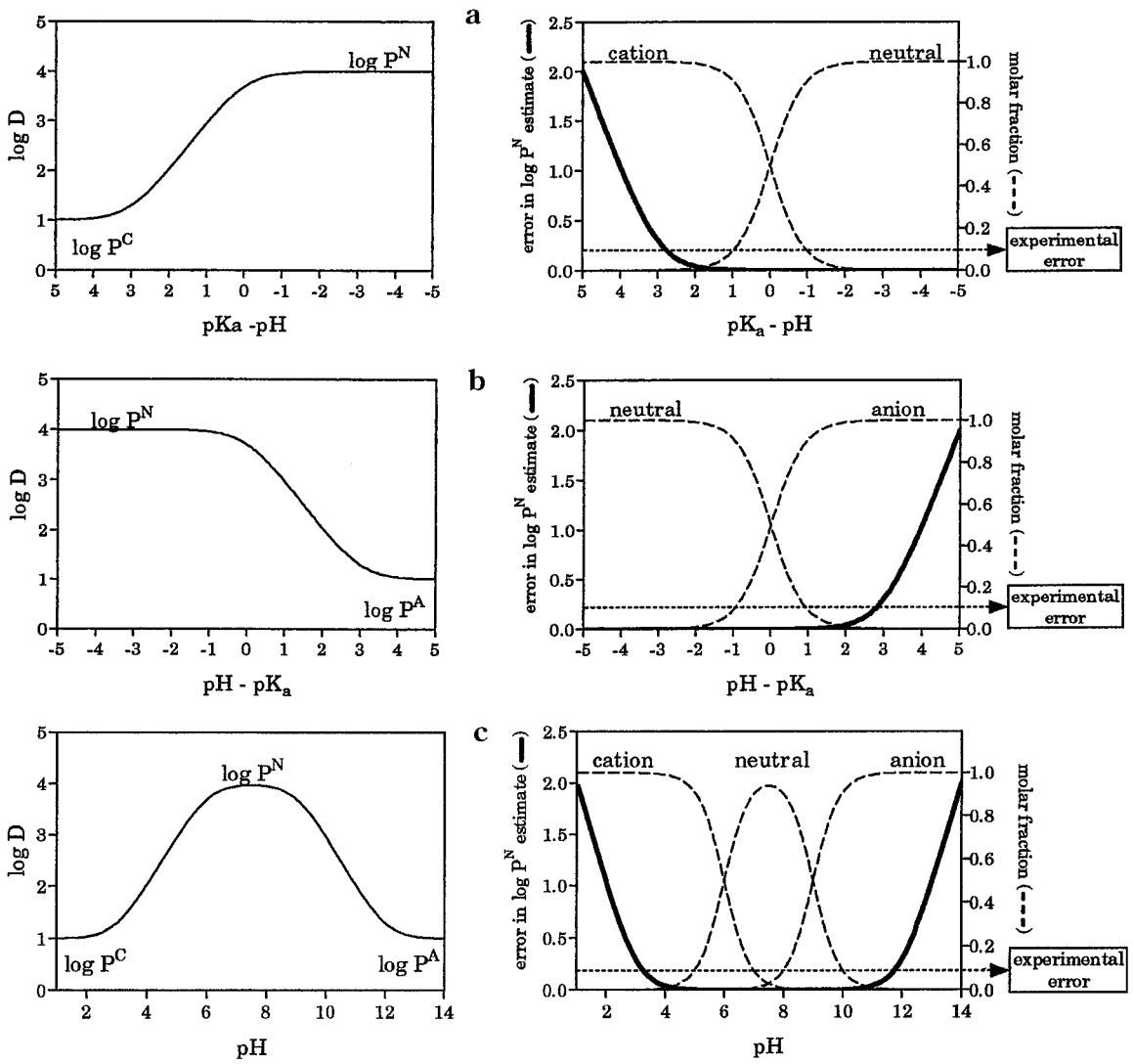


Figure 4. Lipophilicity profiles of ionizable solutes and errors produced in the calculation of log P^N from log D at a given pH when neglecting the partition of charged species: (a) the case of a monobase with *diff*(log P^{N-C}) = 3; (b) the case of a monoacid with *diff*(log P^{N-A}) = 3; (c) the case of an ordinary ampholyte with *diff*(log P^{N-C}) = *diff*(log P^{N-A}) = 3.

When both species contribute to log D, eq 8 is applicable. Unfortunately, because the zwitterion and the neutral uncharged species coexist in solution, no direct experimental method is available to determine separately log P^N and log P^Z. In contrast, the actual values of log P^N and log P^Z can be estimated indirectly by measuring K_Z in the organic phase using a suitable method such as NMR or UV spectroscopy. However, the K_Z values so obtained are estimates since they differ from those prevailing in the partitioning experiments where the solvent conditions are different.[15]

As for the *diff*(log P^{N-Z}) parameter (i.e., the difference between the partition coefficients of the neutral and zwitterionic species), its value may differ among solutes due to different intramolecular interactions. By neglecting the partition of the uncharged or zwitterionic form, errors can be made whose magnitude depends on K_Z. The examples in Figure 5 demonstrate that K_Z has a critical influence on the accuracy of log P^N estimates from log D^{max} when log P^Z is neglected. When the value of K_Z increases, the contribution to partitioning of the zwitterionic species also increases, and its neglect leads to ever larger overestimates of log P^N.

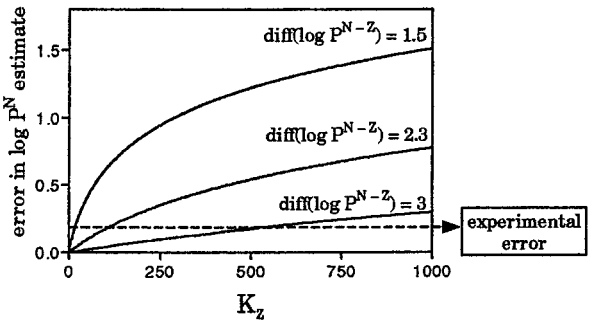


Figure 5. Error in log P^N estimates from log D^{max} as a function of K_Z for three values of *diff*(log P^{N-Z}).

In conclusion, an increase in K_Z means that the zwitterionic fraction increases, whereas the fraction of the uncharged species decreases. This implies that, due to the contribution of the zwitterionic form, it is not always possible to obtain experimentally a reliable value of log P^N. However, independent estimates of log P^N can be obtained by computational approaches, especially for the octanol/water system. These points are exemplified in sections III.B.1 and III.B.2.

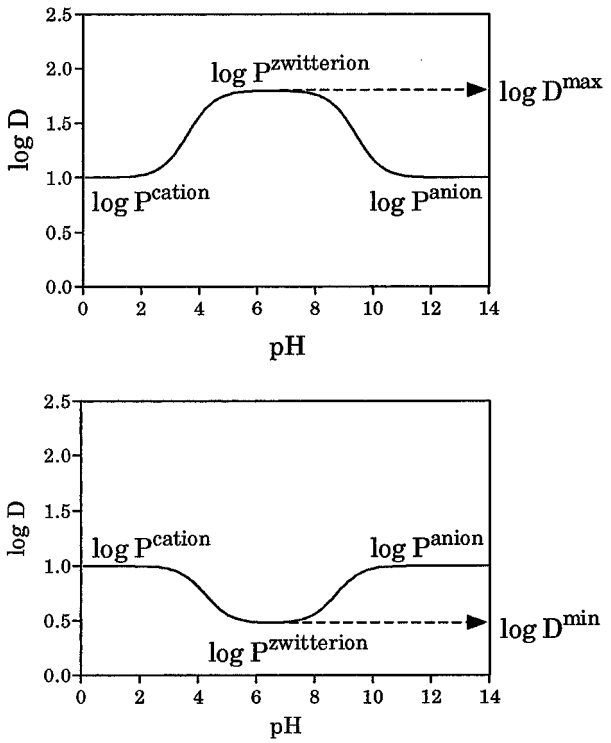


Figure 6. Two extreme lipophilicity profiles for zwitterionic compounds with a very large value of K_Z.

3. Zwitterions Having a Large Value of K_Z

The behavior of zwitterionic ampholytes becomes simpler when the value of ΔpK_a is large (5 or greater) and both ionizable centers are completely ionized (>99.7%) at isoelectric pH. For such ampholytes, K_Z is very large (>ca. 10^4), and the neutral species is present in such low proportions that it does not contribute detectably to the observed distribution coefficients. Thus log D around the isoelectric pH becomes the log P of the zwitterionic species and is experimentally measurable (eq 9):

$$\log D^{pH_i} = \log(f^Z P^Z) \qquad (9)$$

As shown in Figure 6, log P^Z corresponds to log D^{max} (i.e., the maximum value of log D) when the lipophilicity profile is *bell-shaped*, and it corresponds to log D^{min} when the lipophilicity profile curve is *U-shaped*. The literature contains examples of both cases, log P^Z = log D^{max} and log P^Z = log D^{min}. Why and when this occurs is discussed and exemplified in sections IV.A.2 and IV.B.

III. Relevant Techniques[19]

A. Experimental Techniques

1. Measurements of pK_a Values

The experimental determination of macro-pK_a values can be performed by potentiometric or spectrometric methods (UV−vis, NMR). Potentiometric titrations at different concentrations of cosolvent (generally methanol or ethanol) can frequently help to define whether the compound under study is a zwitterionic or an ordinary ampholyte. Organic cosolvents having a dielectric constant lower than that of water weaken both acidic and basic groups. As a consequence, in a mixture of water and cosolvent the pK_a of an acidic group is raised whereas that of a basic group is lowered. However, not all ionization equilibria are affected to the same extent by a change in the solvent's dielectric constant, and the pK_a values of some zwitterions are not affected by a cosolvent.[8]

2. Investigation of Microdissociation Equilibria: Assignment of Micro-pK_a and K_Z

Different approaches exist to investigate microdissociation equilibria. These approaches fall into two main categories, namely, the *deductive* and the *direct* methods.[20] Unfortunately, none of these methods is simultaneously precise and assumption-free, meaning that the results must usually be regarded as mere estimates.[10]

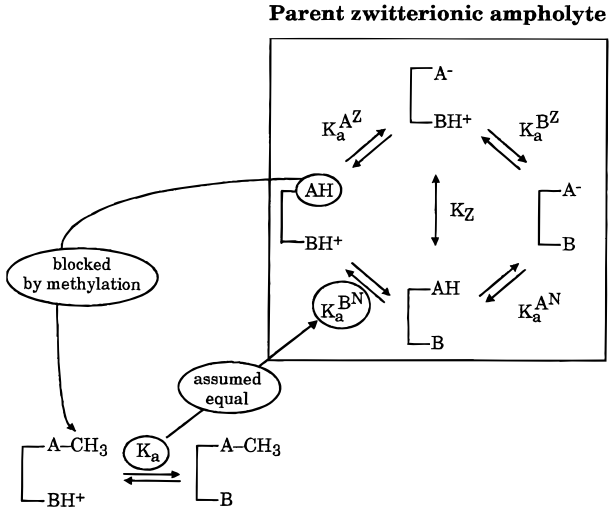
The deductive method, which is the oldest in use, is based on the assumption that blocking by chemical transformation an ionizable group in a zwitterionic compound unveils the micro-pK_a value of the other group without affecting it.[12,20] Scheme 4 offers a generalized example of this method.[21] However, the transformation of an ionizable group into a nonionizable one changes the electrostatic interactions within the molecule, and this in turn may affect the estimate of the pK_a value by the deductive method.

The direct methods measure the micro-pK_a or the tautomeric constant K_Z. These approaches, however, are not assumption-free, again leading to estimates. A very simple method consists of a direct measure of K_Z by UV spectrometry.[12,20,22,23] At isoelectric pH the predominant species of a zwitterionic ampholyte are the zwitterion and the neutral uncharged form. The basic assumption in this case is that only the neutral nonpolar form is stable enough to exist in an organic solvent of low dielectric constant such as dioxane. By examining differences in spectra recorded in dioxane−water mixtures covering a dioxane range of 0−100%, it is considered possible to determine the tautomeric constant K_Z according to eq 10:

$$K_{Z(\%)} = (A_\% - A_d)/(A_w - A_\%) \qquad (10)$$

where $K_{Z(\%)}$ is the equilibrium constant of tautomerism in a given solvent mixture, $A_\%$ the absorbance of the compound in a solvent mixture, A_d the absorbance

Scheme 4



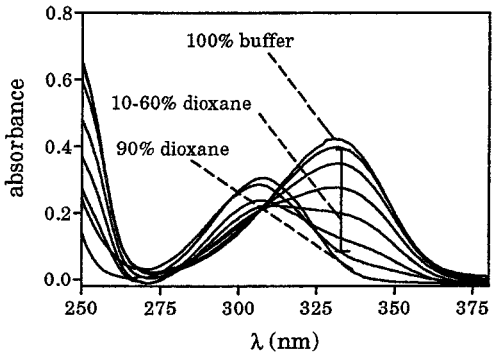


Figure 7. UV spectra of the zwitterionic ampholyte labetalol (**2**) in various mixtures of Tris/HCl buffer (pH^i = 8.4) and dioxane.

of the compound in dioxane, and A_w its absorbance in an aqueous buffer at isoelectric pH.[24]

Figure 7 illustrates the case of the drug labetalol (**2**) (see structure in Figure 2). As can be observed, the isosbestic points are not sharp enough. This is probably due to the small difference between the two pK_a ($\Delta pK_a = 2$, see below section IV.A.1), which implies the existence of other chemical equilibria in addition to the zwitterion/uncharged tautomerism. The K_Z value so obtained ($K_Z \approx 29$) must be considered as an approximation.

Other direct approaches can sometimes be used to determine either the micro-pK_a or the K_Z value, namely IR, Raman, or NMR spectrometry.[10] However, some of these methods lack precision, while others require high solute concentrations and sophisticated instrumentation.

3. Measurement of Lipophilicity Profiles

The lipophilicity profiles of zwitterionic ampholytes can be determined by standard methods such as the *shake-flask* (SF) technique[25] or *centrifugal partition chromatography*[26] (CPC). These methods allow only single point measurements each at a given pH value, whereas the *pH-metric method* usually yields a complete lipophilicity profile from each measurement.[27]

B. Computational Techniques

1. Two-Dimensional Fragmental Systems

A number of approaches have been developed to allow the direct calculation of partition coefficients from the 2D molecular structure.[28] The most common fragmental methods to predict the log P_{oct} of neutral compounds have been developed by Rekker[29] and Hansch and Leo (CLOGP),[30,31] respectively. In these approaches, the calculation of lipophilicity for zwitterionic compounds starts with the fragmental values of the neutral fragments, to which a "*zwitterionic correction factor*" is added, which takes the charged groups and their proximity into account (eq 11):

$$\log P^{Z} = \log P^{N} - \text{zwitterionic correction factor} \quad (11)$$

This correction factor, which is equal to 2.3 in the CLOGP software, is in fact the *diff*(log P^{N-Z}) value mentioned in section II.B.2. However, there is evidence that this correction factor is not universally applicable and should be decreased or raised depending on intramolecular effects.

2. Calculation of Virtual log P Values

The MLP (molecular lipophilicity potential) calculated with the CLIP 1.0 software[32] allows one to calculate log P_{oct} values as a function of the 3D molecular structure, giving access to the theoretical log *P* of all conformers identified in an exploration of the conformational space of a molecule.[33] In our laboratory, quenched molecular dynamics (QMD) is used to explore the conformational space; the MLP integrated on the solvent-accessible surface area (SASA)[34] then serves to *back-calculate* log P_{oct} values by using eq 12 obtained with rigid solutes:[33]

$$\log P_{oct} = (2.86 \times 10^{-3})(\pm 0.24 \times 10^{-3})\sum \mathrm{MLP}^{+} + (1.52 \times 10^{-3})(\pm 0.22 \times 10^{-3})\sum \mathrm{MLP}^{-} - 0.10\ (\pm 0.23)$$
$$n = 114;\quad r^2 = 0.94;\quad s = 0.37;\quad F = 926 \quad (12)$$

In this equation, ΣMLP^+ and ΣMLP^- represent the hydrophobic and polar parts of the molecule, respectively, i.e., the regions of the SASA where positive and negative atomic increments of lipophilicity are expressed.

We have called *virtual log P* the theoretical log *P* calculated for a given conformer.[34] By comparing the virtual log *P* values of all conformers identified by QMD, the *lipophilicity range* accessible to a given solute in a given electrical state can be assessed. In other words, the lipophilicity range encompasses the ensemble of all virtual log *P* values of a solute, whereas its experimental log *P* is the weighted average of an unknown number of virtual log *P* values.[34]

IV. Lipophilicity Profiles of Zwitterionic Ampholytes

The pH-partitioning profiles of ampholytes can be grouped in two main classes, namely, bell-shaped and U-shaped curves.

Solutes displaying *bell-shaped* curves include (a) ordinary ampholytes (since the neutral form predominates in the median pH range between the two pK_as), (b) zwitterionic ampholytes with a small K_Z, and (c) some zwitterionic ampholytes with a large K_Z, when the lipophilicity of the zwitterionic form is increased by intramolecular effects.

Solutes displaying *U-shaped* curves are all zwitterionic ampholytes displaying a large K_Z and modest intramolecular interactions between the two ionic groups.

A. Bell-Shaped Distribution Profiles

1. Zwitterions with a Small K_Z

Takács-Novác and her co-workers have actively investigated the acid−base equilibria and partitioning behavior of zwitterionic drugs such as oxicams, niflumic acid, and quinolone antibacterials.[9,20,23,35−37]

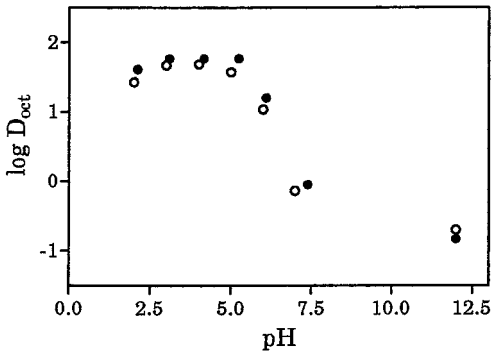
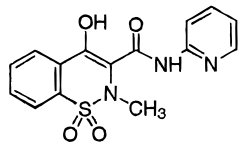


Figure 8. Lipophilictity profile of piroxicam (**4**) in octanol/phosphate buffer systems (● taken from ref 13; ○ taken from ref 39).

Most compounds were zwitterions with a small K_Z where the neutral uncharged form was able to contribute detectably to the observed lipophilicity. There is, however, a problem here regarding what can be taken as the intrinsic lipophilicity of ampholytes. Whereas log P^N describes the intrinsic lipophilicity of neutral compounds, we fail to see how it can represent the intrinsic lipophilicity of all amphoteric compounds, given that the distribution coefficient measured at the top of the bell-shaped curve (designated log D^{max}) is a mixture of log P^N and log P^Z in unknown proportions and that these values are not experimentally measurable (see below). We thus believe that when it comes to biological correlations, the intrinsic lipophilicity of zwitterionic ampholytes is best characterized by their experimental log D^{max} values.

In a recent study on the brain extraction of four oxicams,[38] log D^{max} was the only lipophilicity parameter useful for deriving the polarity factor Λ_{oct}, which proved inversely correlated to brain efflux.

An important point here is the difficulty of characterizing the microprotonation equilibria of zwitterions with a small K_Z. Consequently, it becomes practically impossible to derive accurate value of log P^N (see section II.B.2). This is illustrated with piroxicam (**4**), a long-acting nonsteroidal antiinflam-



piroxicam (**4**)

matory drug whose microscopic equilibria and partitioning behavior were exhaustively investigated.[13,20,39] The lipophilicity profile of piroxicam (**4**) in octanol/water is reported in Figure 8, showing that its log D^{max} is indeed found in the region between the two pK_as (pK_{a1} = 1.86; pK_{a2} = 5.46). Depending on the method used to investigate the microscopic equilibria, K_Z was found to vary between 6 (a value obtained by the deductive method) and 37 (a value obtained by the direct UV spectrometric method).[20] Using either K_Z value and assuming that the neutral uncharged species is much more lipophilic than the zwitterionic form, one could venture to calculate a log P^N from log D^{max} (eq 5).

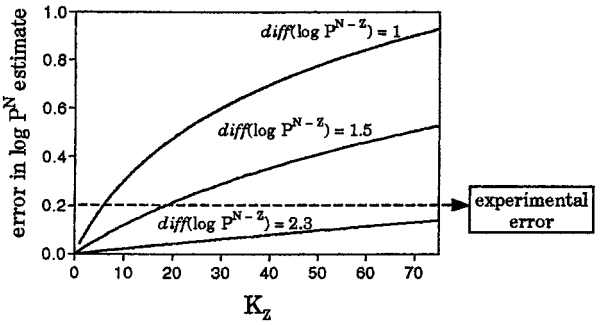


Figure 9. Error in log P^N estimates for piroxicam (**4**) from log D^{max} as a function of K_Z for three values of *diff*(log P^{N-Z}).

Before so doing, however, two important questions must be raised. First, how realistic is the assumption that the partitioning of the zwitterionic species can be neglected? And second, can the K_Z value be considered sufficiently accurate to allow a fair derivation of log P^N? The answers to these two questions are intimately related, since they both depend on the value of K_Z. Unfortunately, and perhaps excepting the approximate determination of K_Z in organic media (see above), no direct experimental method allows the separate measurement of log P^N and log P^Z, but a reasonable solution can be based on the *diff*(log P^{N-Z}) parameter (see section II.B.2). The relation between *diff*(log P^{N-Z}) and K_Z is shown in Figure 9 for three values of the former parameter. As can be seen, the smaller K_Z and the larger the difference between log P^N and log P^Z, the smaller the error in determining log P^N from log D^{max} with neglect of log P^Z.

But even when assuming that log P^Z is negligible, the uncertainty on K_Z can affect the calculation of log P^N, which for piroxicam (**4**) is calculated to be 2.63 (for $K_Z = 6$) or 3.34 (for $K_Z = 37$). To choose one result over the other is far from obvious. The log P^N so derived can be compared with that obtained by computational methods, but the more complex the molecular structure, the more difficult it is to have highly reliable computational results. For piroxicam (**4**), the only tool available to estimate log P^N is the CLOGP software, which yields a value of 2.7, whose agreement with the value of 2.63 cited above suggests that K_Z should be closer to 6 than to 37.

Another good example of a zwitterionic compound having a small value of K_Z is labetalol (**2**). Our reinvestigation of its protonation equilibria by potentiometric titration has led to an acidic and a basic pK_a of 7.48 and 9.39, respectively, whereas UV spectrometry yielded a K_Z of 29 at isoelectric pH (Figure 7). The lipophilicity profile of labetalol (**2**) in octanol/buffer[40] is shown in Figure 10.

A log P^N of 2.6 can be calculated from the log D at isoelectric pH by neglecting the partitioning of the zwitterionic species (eq 5). This value is in good agreement with the log P^N value of 2.5 calculated with the CLOGP software. It seems hence reasonable to classify labetalol (**2**) as a zwitterionic compound with a small K_Z and having a zwitterionic form that does not contribute detectably to the distribution coefficient. This does not mean that the zwitterion fails to partition into octanol but simply that in this case its log P^Z can be neglected.

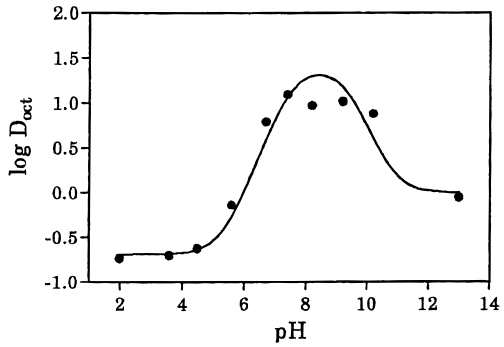


Figure 10. Lipophilicity profile of labetalol (**2**) in octanol/buffer systems (● taken from ref 40). The solid line is the curve obtained by nonlinear fitting of experimental data using eq 4 (see section II.B).

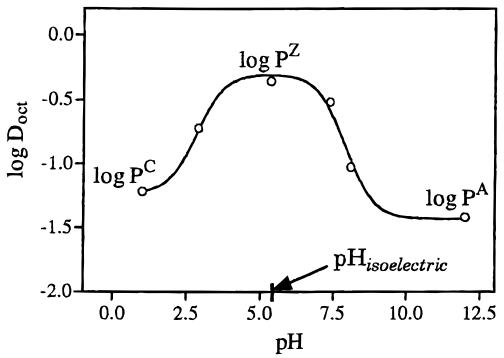


Figure 11. Lipophilicity profile of Trp-Phe (**5**) in octanol/phosphate buffer (0.067 M) systems. The solid line is the curve obtained by nonlinear fitting of experimental data using eq 4 with $f^N = 0$ (see section II.B).

In summary, and because K_Z is pH-independent, the neutral uncharged form can never exist alone and is always in equilibrium with its zwitterionic tautomer. The log D^{max} hence represents the maximum expression of the global neutral form (uncharged plus charged) in solution. For this reason a log D value measured at the isoelectric pH appears as a much more reliable QSAR parameter than log P^N.

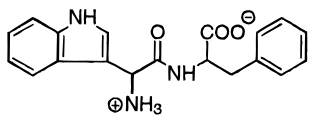
2. Zwitterions with a Large K_Z

As mentioned in section II.B.3, for large values of K_Z (>ca. 10^4) the log P of the zwitterionic species becomes experimentally accessible and corresponds to the log D measured at the isoelectric pH. Common sense suggests additivity of the polarity of the two charges, but as shown below this is the exception rather than the rule. In most cases, a bell-shaped lipophilicity profile is seen (log P^Z = log D^{max}). The greater lipophilicity of the doubly charged form compared to that of a singly charged species is explained by a partial intramolecular neutralization of the two opposite charges of the zwitterion. The intramolecular effects which play a role in enhancing the lipophilicity of the zwitterionic species can be classified in three main categories: charge proximity effects operating through σ-bonds, conformational effects favoring internal ionic bonds, and charge delocalization operating through π-bonds.

Very often two such interactions operate together, and their relative influence is difficult to assess.

a. Charge Proximity Effects Operating through σ-Bonds. In recent years analogues of amino acids and small peptides have attracted attention as potential drugs. Such compounds raise an interesting pharmacokinetic problem, since their transport through biological membranes is usually not restricted to a passive diffusion of the neutral uncharged form but also involves an active, carrier-mediated transport of an ionized form.[41]

The main intramolecular effect governing the lipophilicity of α-amino acids and dipeptides is believed to be charge neutralization through σ-bonds.[42] Such an effect would render the zwitterionic species markedly more lipophilic than the singly charged cation and anion. However, this phenomenon may be hidden, since the partitioning of monocharged species is somewhat influenced by the background salt concentration[16,43,44] (see section II.B.1). This was seen for example with the dipeptide Trp-Phe (**5**) (results not shown).[27,43] To clarify this point we



Trp-Phe (**5**)

measured the lipophilicity profile of Trp-Phe (**5**) in octanol/phosphate buffer systems of pH 1 to 12 (Figure 11).

A bell-shaped curve was indeed obtained, with a log P^Z about one unit above either log P^A and log P^C. Thus, although counterions (0.067 M phosphate buffer in this case) do have some influence on the partitioning of the cation and anion, the zwitterion is indeed more lipophilic than the singly charged species. A decreased ion-pair partitioning would simply have amplified the bell shape of the curve by affecting log P^A and log P^C.

The log P^N value of Trp-Phe (**5**) calculated with the CLOGP software was 1.99, yielding a *diff*(log P^{N-Z}) of 2.35, which is similar to the correction factor of 2.3 applied to α-amino acids in the CLOGP software. This suggests that charge proximity effects operating through σ-bonds may indeed occur in dipeptides and influence their lipophilicity profile.

b. Conformational Effects Favoring Internal Ionic Bonds. In two excellent reviews, Manners and coworkers[1,45] have discussed the lipophilicity of zwitterionic metabolites of some basic drugs, namely, *O*-sulfate conjugates of propranolol and the carboxylic metabolite of tiaramide. Here, the neutral uncharged species was not present in a significant amount at any pH, and the introduction of an additional charge did not produce the expected decrease in lipophilicity relative to the parent compound. All investigated conjugates thus displayed a constant and maximal lipophilicity around their isoelectric pH, and extensive internal charge neutralization was inferred.

One of the examples investigated by Manners *et al.*[45] is reported here, namely, the β-blocker propranolol (**6**) and its two zwitterionic metabolites, pro-

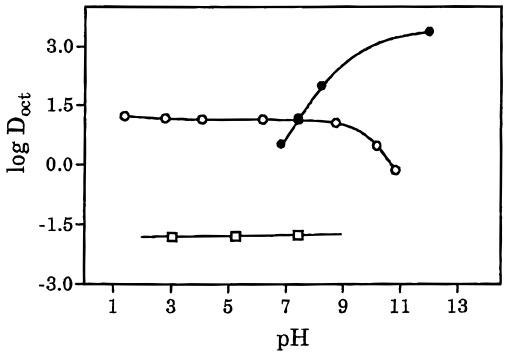
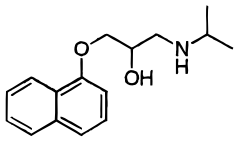
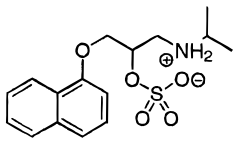


Figure 12. Lipophilicity profiles of propranolol (**6**) (●), propranolol *O*-sulfate (**7**) (○), and 4′-hydroxypropranolol 4′-*O*-sulfate (**8**) (□) in octanol/buffer systems.[45]

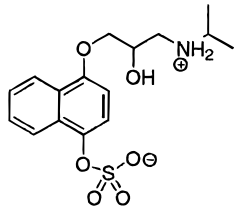
pranolol *O*-sulfate (**7**) and 4′-hydroxypropranolol 4′-*O*-sulfate (**8**). Their lipophilicity profiles (Figure 12)



propranolol (**6**)



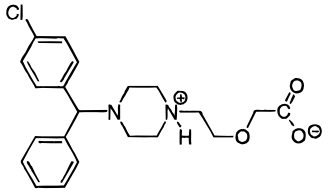
propranolol O-sulfate (**7**)



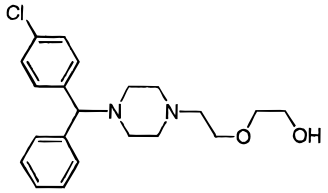
4'-hydroxypropranolol 4'O-sulfate (**8**)

show that the latter metabolite (log $D^{max} = -1.8$) is less lipophilic than the former (log $D^{max} = 1.1$) by as much as 2.91 log *P* units, an unexpected difference considering their structural similarity. The difference in lipophilicity between the two metabolites appears to be due to their different 3D geometries. In propranolol *O*-sulfate (**7**), intramolecular effects operate through space and to some extent also through σ-bonds to partly neutralize the two opposite charges. Indeed its log $D^{7.4}$ is similar to that of the parent compound (**6**) (Figure 12), showing that in this case sulfation does not increase polarity as expected. Such intramolecular effects cannot operate in 4′-hydroxypropranolol 4′-*O*-sulfate (**8**), where the two opposite charges cannot come close together. As a result, this metabolite is far more hydrophilic than the parent compound (3 log *D* units at physiological pH) as shown in Figure 12.

The H_1-receptor antagonist cetirizine (**9**) belongs also to the class of zwitterionic compounds where partial charge neutralization may occur by formation of an internal ionic bond. The acid-base properties and lipophilicity profile of cetirizine (**9**) have recently been reinvestigated,[46] showing that this drug is in zwitterionic form over a wide pH range (from pH 3.5 to 7.5) and has a large K_Z (>36 000). The large difference between the two higher pK_a values (pK_a^{acid} = 2.91, pK_a^{basic} = 8.0, ΔpK_a = 5.07)[46] and the



cetirizine (**9**)



hydroxyzine (**10**)

large K_Z allows the protolysis equilibria to be simplified to an unbranched system (see section II.A.3).

The lipophilicity profile of cetirizine (**9**) in octanol/phosphate buffer (0.06 M) shows a broad plateau in the pH range between the two higher pK_a values (Figure 13) and drops outside this pH range due to an increased proportion of the cations and anion. A low value above pH 11 characterizes the log *P* of the anionic form in the presence of the phosphate buffer. In contrast, the log *P* of the cation could not be deduced from the lipophilicity profile because this species was never alone in solution, being in equilibrium with the dication (the lesser basic center in cetirizine has a pK_a = 2.19[46]). The log P^C was thus calculated by a nonlinear fitting procedure of the log *D*/pH curve and measured by the pH-metric method, the values so obtained being 1.01 and 1.12, respectively.[46]

The difference between the log *P* of the neutral and zwitterionic forms of cetirizine, i.e., *diff*(log P^{N-Z}), proved again to be a structural parameter yielding useful insight on intramolecular effects. A convincing estimate of the log P_{oct} of neutral uncharged cetirizine (**9**) (log P^N = 3.7) was obtained with Rekker's fragmental method starting from the experimental log *P* value of the analogue hydroxyzine (**10**) (3.5)[46] and using eq 13:

$$\log P^N(\text{cetir}) = \log P^N(\text{hydrox}) - f(\text{OH}) - f(\text{CH}_2) - 2C_M + f(\text{COOH}) + 3C_M \quad (13)$$

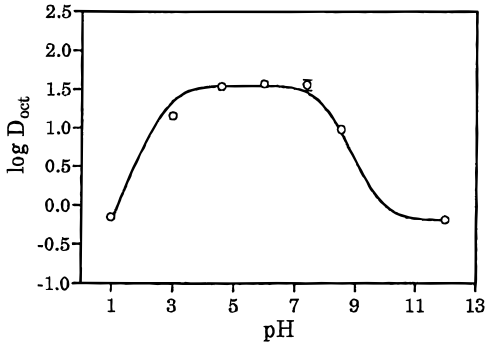
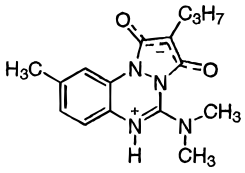


Figure 13. Lipophilicity profile of cetirizine (**9**) in octanol/buffer systems as measured by centrifugal partitioning chromatography. The solid line is the curve obtained by fitting experimental data (○; n = 3) with eq 4 with f^N = 0.[46]

In the above equation the magic constant (C_M) contributions code for proximity effects; the fragmental system used was the original one with a C_M value of 0.289. The advantage of this method was that the backbone of hydroxyzine (**10**) is very similar to that of cetirizine (**9**), implying that the calculated log P^N value was largely based on an experimental value. A *diff*(log P^{N-Z}) of 2.2 was found, suggesting that the two opposite charges in the zwitterion undergo partial neutralization as a result of an internal ionic bond made possible by a folded conformation. Indeed a *diff*(log P^{N-Z}) of approximately 6 units is expected in the absence of any internal neutralization, starting from the mere estimate that the monocharged species are each at least 3 log *P* units less lipophilic than the uncharged one.[47] When partial charge neutralization occurs, some polarity is lost, and *diff*(log P^{N-Z}) is expected to decrease with increasing charge neutralization.

To confirm this hypothesis, an exploration of the conformational space of zwitterionic cetirizine (**9**) was performed by quenched molecular dynamics,[48,49] and the virtual log *P* of each conformer was calculated by the MLP.[46] Two main classes of conformers were identified, namely, extended and folded (with internal ionic bond) ones. The virtual log *P* values ranged from 0.3 for extended conformers to 1.3 for folded conformers. This value of 1.3 is close to the experimental log P^Z of 1.5,[46] suggesting that folded conformers of zwitterionic cetirizine dominate its partitioning behavior in the octanol/water system.

c. Charge Delocalization Operating through One or More π-Bonds. A variety of experimental and computational methods were used to examine the multiple protonation/deprotonation equilibria, tautomerism, and pH-lipophilicity profiles of the nonsteroidal antiinflammatory drug azapropazone (**11**).[50]



azapropazone (**11**)

This zwitterionic compound demonstrated a large value of K_Z ($\Delta pK_a > 5$), and here again a broad plateau between the two pK_a was obtained in octanol/water and dodecane/water. Unfortunately, the region below the acidic pK_a is not experimentally accessible, thus hiding the full bell shape of these curves. Evidence for the partitioning of the zwitterionic species was deduced from the large difference between the two plateaux of Figure 14 (Δ log $D_{oct-alk}$ = 3.3), which should be much smaller if the compound were to partition as the neutral species as does its acidic analogue phenylbutazone (Δ log $D_{oct-alk}$ = 0.68). The log P_{oct} of the zwitterionic form of azapropazone was relatively high (log D^{max} = 1.5), suggesting a partial cancellation of polarity of the two opposite charges due to charge delocalization across the aromatic rings.

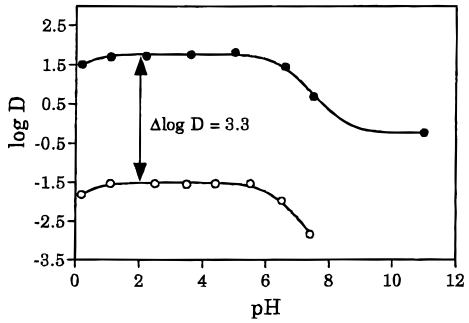


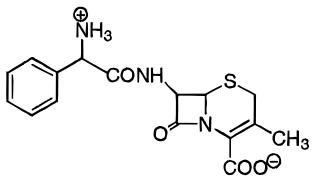
Figure 14. Lipophilicity profiles of azapropazone (**11**) in octanol/buffer (●) and dodecane/buffer systems (○). The solid lines are the curves obtained by nonlinear fitting of experimental data using eq 4 with $f^N = 0$.

To estimate the log P_{oct} of the neutral uncharged form was not straightforward considering the structural complexity of azapropazone (**11**). Due to some unavailable fragmental values, the CLOGP value could not be computed. However, a log P^N of 2.4 could be estimated from the virtual log P calculated in the current MLP parametrization,[33] leading to a *diff*(log P^{N-Z}) of 0.9. This small value is indeed indicative of partial charge neutralization (which here should be mainly via π-electron delocalization).

B. U-Shaped Distribution Profiles

In the absence of strong intramolecular interactions, and as mentioned above, the zwitterion is expected to be more hydrophilic than the corresponding anion and cation. As a result, a U-shaped curve will be obtained, with log P^Z = log D^{min}. Such U-shaped curves have indeed been observed, but less commonly than bell-shaped lipophilicity profiles. As mentioned in section IV, the only compounds that may display this behavior are zwitterions with a very large value of K_Z, because the lipophilicity profile is always bell-shaped when the neutral form is present to a marked extent (small K_Z).

For a series of β-lactam antibiotics, Purich *et al.*[51] found that minimal partitioning occurred in the isoelectric region, suggesting that the zwitterion partitioned less than the cation or anion. Similarly, U-shaped curves were seen with cephalexin (**12**) both



cephalexine (**12**)

for its membrane permeability coefficient versus pH and in its lipophilicity profile.[52] Indeed, in the case of cephalexin (**12**) and other β-lactams, the β-lactam nucleus does not permit detectable charge neutralization and favors a highly polar zwitterion.

To add another relevant example where the two opposite charges cannot interact, the acid−base equilibrium and the lipophilicity profile of the zwitterionic

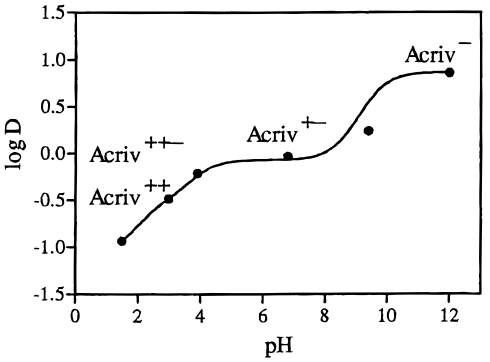
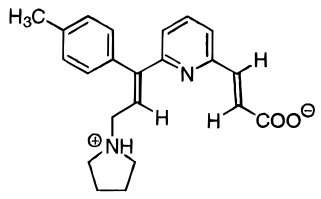
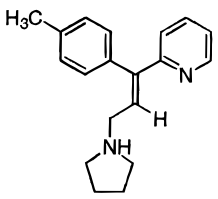


Figure 15. Lipophilicity profile of acrivastine (**13**) in octanol/phsphate buffer (0.067 M) systems. The solid line is the curve obtained by nonlinear fitting of experimental data using eq 4 with $f^N = 0$.

H_1-receptor antagonist acrivastine (**13**) were investigated. This drug possesses a rigid backbone that



acrivastine (**13**)



triprolidine (**14**)

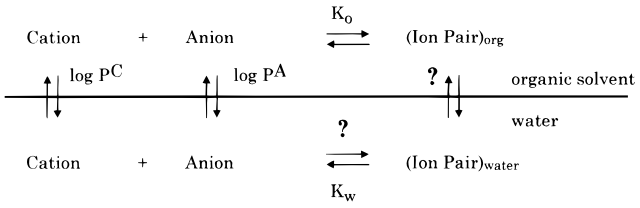
prevents the two opposite charges from creating an internal ionic bond. As can be seen in Figure 15, the shape of the lipophilicity profile determined in octanol/phosphate buffer systems was not exactly U-shaped but rather S-shaped because below pH 5 a mixture of multicharged species exists. Indeed acrivastine (**13**) has one acidic and two basic groups ($pK_{a1} = 2.2$, $pK_{a2} = 3.89$, and $pK_{a3} = 9.55$). Hence the zwitterionic species predominates between pH 5 and 8. The point of relevance here is that the anion is about one log P unit more lipophilic than the zwitterion, and the difference remains significant even if the counterion can affect its partitioning.

The log P_{oct} of the neutral uncharged form of acrivastine (**13**) can be calculated with the CLOGP software (3.4) or Rekker's method (4.09), starting in the latter case from the experimental log P^N of the dibasic analogue triprolidine (**14**). Substracting the experimental value of log P^Z (−0.01) yielded a *diff*(log P^{N-Z}) of 3.41 or 4.1, respectively. Such values, which are markedly larger than the *diff*(log P^{N-Z}) found in our earlier examples, are consistent with limited internal charge neutralization.

C. Counterion Effects on the Partitioning of Cations and Anions

As stressed in section IV.A.2.a, the nature of the counterion can effectively increase the partitioning of a singly charged species, be it an anion or a cation. However, it remains difficult to clearly define the effect of counterions on partition coefficients. Indeed, as illustrated in Scheme 5, only two equilibrium constants can be unambiguously defined, namely, the two log *P*s of the charged species. They differ from the definition given in this review (section II.B.1) since they are the true partition coefficients of singly

Scheme 5



charged species which can be obtained by extrapolation to zero concentration of counterions. The other equilibrium constants cannot be determined correctly since ion pair formation is doubtful. Moreover, the molecular structure of ion pairs is expected to be different in water and in organic phases, implying that one cannot define a partition coefficient for a complex which differs in the two phases.

The contribution of ion pair formation to the partitioning of singly charged species may result in U-shaped lipophilicity profiles of extrinsic rather than intrinsic origin. Such effects were observed in octanol/water systems and could be an experimental artifact. However, they may also be important in biological environments where numerous couterions are present even though the literature on ion pair absorption remains controversial.[53]

V. Conclusions

A. Physicochemical Outlook

A number of physicochemical parameters have been considered in this review, with a range of behavior patterns arising from combinations of their values. To help the reader acquire a global view, we offer Figure 16 as a summary of these combinations and their outcomes. However, the price to pay in drawing a decision tree of this nature is its schematic character in neglecting intermediate cases and assigning a somewhat arbitrary borderline value to K_Z. Yet despite its limitations, this figure should give a clarifying framework of heuristic and utilitarian value: heuristic because it may provide researchers with a platform for future progresses and utilitarian because it may allow sounder predictions of partitioning behavior.

Lipophilicity is a molecular property of double interest in medicinal chemistry and biochemistry. First, it has proven its value in innumerable structure−activity, structure−affinity and structure−permeation relations. This is the empirical—and best recognized—importance of lipophilicity. Its second contribution is of a more fundamental nature, since lipophilicity has proven to be a probe of irreplaceable potential to unravel biologically relevant intramolecular interactions and intermolecular forces of recognition.[54] Rationalizing the pH-dependent lipophilicity of ampholytes should thus improve our understanding of the interplay between their intramolecular and intermolecular interactions. The biological and particularly pharmacokinetic relevance of these phenomena is discussed below.

B. Pharmacokinetic Relevance

The ability of drugs to diffuse passively through biological membranes is influenced to a major extent

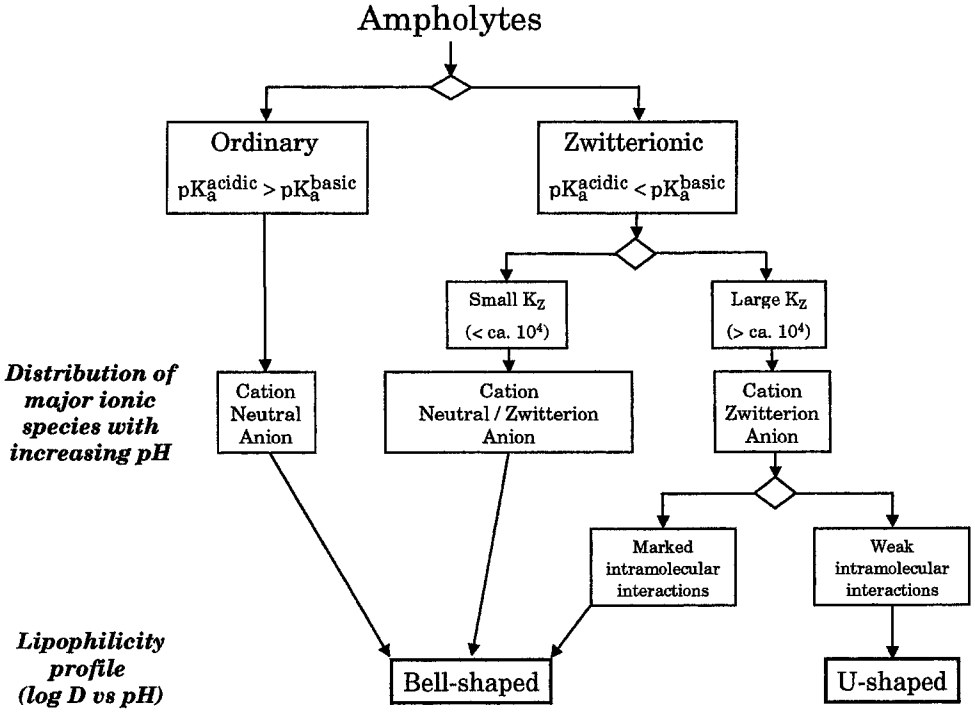


Figure 16. Schematic classification of ampholytes, their ionic distributions, and lipophilicity profiles.

by their lipophilicity, a key factor in determining drug disposition.[55−60] Zwitterionic compounds, whether they have a large or small value of K_Z, exhibit an intriguing common feature, namely, a practically *constant value of lipophilicity* in the region around the isoelectric pH. Since the pH values of various biological compartments varies between approximately pH 1 (the stomach) and 8, it might indeed prove valuable to have zwitterionic drugs or biomolecules that display a constant value of lipophilicity in the weakly acidic to neutral pH range. In contrast, organic acids and bases display constant values of lipophilicity at pH values below or above their pK_a, but this is usually outside the physiologically interesting pH range.

To put it differently, zwitterionic compounds behave as "*lipophilicity buffers*" in a pH range of stability defined by the two pK_a values. Not fortuitously, this range overlaps with the physiologically important pH region. It is also interesting to note that the notion of *micro-pH* is one that is gaining recognition in biochemistry, molecular pharmacology, and pathological states.[61−69] Thus, a bioactive compound that interacts with functional macromolecules such as receptors or enzymes may encounter different micro-pH environments, and one can speculate whether a lipophilicity buffer capacity could not be advantageous in terms of ligand−receptor recognition. Specifically, the model we have in mind is that of an approaching ligand whose ionic charges and hence molecular fields remain constant during long-distance recognition and then docking.

Zwitterionic compounds with a small K_Z present an additional interest since the neutral uncharged species and the zwitterion coexist in significant proportions at and around the isoelectric pH. Both tautomers have their own intrinsic lipophilicity (log P^N and log P^Z, respectively), and they may both contribute to membrane permeation. In such a view, the uncharged species should permeate preferably across bilayers and the zwitterion along aqueous pores which exist in many membranes. In other words, zwitterions with small K_Z have a dual intrinsic lipophilicity that might allow them to adapt better to biological barriers when crossing them. As a result of the dynamic equilibrium between the two species, a molecular "pump" would be established in the pH range between the two pK_as.

Even if somewhat speculative, such considerations may convince our readers that the molecular polymorphism of ampholytes in general and zwitterions in particular makes them fascinating objects of study and versatile carriers of pharmacological and biological information.

VI. Acknowledgments

We are indebted to the Swiss National Science Foundation for support. Insightful discussions with Prof. Kristina Takács-Novák (Budapest) are acknowledged.

VII. Glossary

K_a	Dissociation constant of an equilibrium of protolysis.
pH^i	pH measured at the isoelectric point, where the zwitterion (and the neutral form) are present at maximum concentration.
K_a^{acidic}	Macrodissociation constant associated with the stoichiometric equilibria of a zwitterionic ampholyte. It denotes the loss of the first proton from the molecule.
K_a^{basic}	Macrodissociation constant associated with the stoichiometric equilibria of a zwitterionic ampholyte. It denotes the loss of the second proton from the molecule.

$K_a^{A^Z}$	Microscopic dissociation constant of the cation/zwitterion equilibrium. The notation A^Z indicates that the acidic group (A) is involved in the formation of the zwitterionic microspecies (Z) by losing its proton.
$K_a^{B^Z}$	Microscopic dissociation constant of the zwitterion/anion equilibrium. The notation B^Z indicates that the basic group (B) is involved in the dissociation of the zwitterionic microspecies (Z) by losing its proton.
$K_a^{B^N}$	Microscopic dissociation constant of the cation/neutral equilibrium. The notation B^N indicates that the basic group (B) is involved in the formation of the neutral microspecies (N) by losing its proton.
$K_a^{A^N}$	Microscopic dissociation constant of the neutral/anion equilibrium. The notation A^N indicates that the acidic group (A) is involved in the dissociation of the neutral microspecies (N) by losing its proton.
K_Z	Tautomeric equilibrium constant defined as the ratio of concentrations of the zwitterionic and neutral forms ([Z]/[N]). It does not depend on pH.
ΔpK_a	Difference between two pK_a values. In the specific case of zwitterions, it represents pK_a^{basic} minus pK_a^{acidic}.
P	Partition coefficient expressed as the concentration ratio of a solute present in a single electrical state and in equilibrium between two immiscible solvents (water and an organic solvent).
D	Distribution coefficient expressed as the concentration ratio of a solute present in more than one electrical states and in equilibrium between two immiscible solvents (water and an organic solvent).
lipophilicity profile	Synonymous with distribution profile. Variation of log D as a function of pH.
log D^{max}	Maximum value of log D on a bell-shaped lipophilicity profile.
log D^{min}	Minimum value of log D on a U-shaped lipophilicity profile.
log D^{pHi}	log D measured at the isoelectric pH.
log $D^{7.4}$	log D measured at the physiological pH 7.4.
P^N, P^Z, P^C, P^A	Partition coefficients of the neutral (N), zwitterionic (Z), cationic (C), and anionic (A) forms, respectively.
f^N, f^Z, f^C, f^A	Fractions of the neutral (N), zwitterionic (Z), cationic (C), and anionic (A) forms, respectively.
diff(log P^{N-I})	Difference between the log P values of the neutral and an ionized form (I) of a solute, measured in same solvent system. If not specified, the octanol/water system is implied.
$\Delta\log P_{oct-alk}$	Difference between the log P_{oct} and log P_{alk} (alk = *n*-alkane) of a solute.
$\Delta\log D^{7.4}_{oct-alk}$	Difference between the log D_{oct} and log D_{alk} (alk = *n*-alkane) of a solute measured at pH 7.4.
zwitterionic correction factor	Synonymous with *diff*(log P^{N-Z}). Difference between the log P values of the neutral and zwitterionic forms of a solute. Refers usually to the octanol/water system.
virtual log P	Theoretical log P value calculated for a given conformer by a 3D method (e.g., the MLP).
MLP	Molecular lipophilicity potential.
QMD	Quenched molecular dynamics.

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