Theoretical and Experimental Exploration of the Lipophilicity of Zwitterionic Drugs in the 1,2-Dichloroethane/Water System

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Purpose. This work examines the lipophilic behavior of various zwitterions and shows how distribution profiles in biphasic systems and ionic partition diagrams may improve our understanding of pH-absorption profiles of drugs.

Methods. The lipophilicity of various zwitterionic drugs was examined by potentiometry and cyclic voltammetry in the 1,2-dichloroethane/water system to study the intramolecular interactions and conformational effects affecting absorption and activity of zwitterions, as well as to draw their theoretical and experimental ionic partition diagrams.

Results. Different theoretical partition diagrams are reported according to the tautomeric constant of the zwitterion. Shifts of apparent pK_a are obtained in the ionic partition diagrams of raclopride and eticlopride and compared to the deviations from pH-absorption profile described in the literature for lipophilic drugs. The physicochemical origin of these shifts is discussed.

Conclusions. The comparison between pH-absorption profiles and ionic partition diagrams of zwitterions is shown here to be of value for a better mechanistic understanding of absorption processes, thus opening new perspectives in studying pH-absorption profiles of ionizable drugs.

KEY WORDS: cyclic voltammetry; 1,2-dichloroethane; lipophilicity; pH-absorption profiles; zwitterions.

INTRODUCTION

In drug research, ampholytes occur in various therapeutic classes, such as non-steroidal anti-inflammatory drugs, antihistaminic drugs, and antibacterial agents, and they may also be produced as metabolites (1). From a chemical viewpoint, it is critical to distinguish 1) ordinary ampholytes, which are both weak bases and weak acids and hence are essentially uncharged at neutral pH and 2) zwitterions, which have a positive and a negative charge at neutral pH by virtue of the strength of their ionizable groups. Be they drugs or metabolites, ampholytes possess physicochemical properties and a pharmacokinetic behavior distinct from those of compounds with only acidic or basic groups. Ordinary ampholytes display a lipophilicity behavior resembling that of acids and bases. In contrast, zwitterions display an original partitioning because they behave as ″lipophilicity buffers″ (constant lipophilicity) in a pH range of stability defined by their two pK_a values (1). This behavior results from intramolecular and intermolecular interactions specific to zwitterions and of particular importance for their bioavailability.

The present study was undertaken to explore the pH-partitioning behavior of zwitterions in the 1,2-dichloroethane/water system, which is particularly well suited to reveal the intramolecular interactions and conformational behaviors that affect drug absorption and activity (2,3). This work also examines how distribution profiles in biphasic systems and ionic partition diagrams may improve our understanding of pH-absorption profiles.

THEORY

Acid-Base Equilibria

In contrast with ordinary ampholytes ($pK_a^{acidic} > pK_a^{basic}$) whose pK_a values can be readily assigned by ordinary titra-

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ABBREVIATIONS: $A_{\%}$, A_d, A_w, the absorbance of a compound in a solvent mixture, in dioxane and in an aqueous buffer, respectively; K_a, dissociation constant of an equilibrium of protolysis; K_a^{acidic}, K_a^{basic}, macrodissociation constant associated with the stoichiometric equilibria of a zwitterionic ampholyte; $K_a^{A^Z}$, 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; f^A, f^C, f^N, f^Z, aqueous molar fraction of the neutral (N), zwitterionic (Z), cationic (C) and anionic (A) forms, respectively; P, partition coefficient (P) 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 (D) 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); $\Delta_o^w\phi$, Galvani potential difference between the aqueous (w) and the organic (o) phases; $\Delta_o^w\phi_i^0$, standard potential of transfer of ion i between the phases w and o; $\Delta_o^w\phi_i^{1/2}$, half-wave potential of ion i between the phases w and o; log P_{oct}^N, partition coefficient of the neutral form of an ionizable solute in the *n*-octanol/water system; log P_{dce}^N, partition coefficient of the neutral form of an ionizable solute in the 1,2-dichloroethane/water system; log P_{dce}^I, apparent partition coefficient of the ionic form of an ionizable solute in the 1,2-dichloroethane/water system; log $P_{dce}^{O,I}$, standard partition coefficient of the ionic form of an ionizable solute in the 1,2-dichloroethane/water system; Δlog $P_{oct\text{-}dce}^N$, difference between log P_{oct}^N and log P_{dce}^N; *diff*(log P_{dce}^{N-I} $P_{dce}^{O,I}$, difference between log P_{dce}^N and log $P_{dce}^{O,I}$; pH^i, pH measured at the isoelectric point, where the zwitterion (and the neutral form) are present at maximum concentration; K_Z, tautomeric equilibrium constant defined as the ratio of concentrations of the zwitterionic and unionized forms ([Z]/[N]). It does not depend on pH; QMD, quenched molecular dynamics; MLP, molecular lipophilicity potential.

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tion, the ionization equilibria of zwitterionic ampholytes ($pK_a^{acidic} > pK_a^{basic}$) are more complex. A zwitterionic ampholyte in solution can exist in four different electrical states, namely as a cation (C) at low pH, as an ionized but globally neutral form called a zwitterion (Z), as a neutral and unionized form simply called the neutral form (N), and as an anion (A) at high pH. The pattern of ionization of such a zwitterion in an aqueous phase is described in Fig. 1 (1,4). In such a scheme, acid-base equilibria are defined in terms of macroscopic constants (or macroconstants, K_a^{acidic} and K_a^{basic}), which refer to stoichiometric ionization, and of microscopic constants (or microconstants), which refer to the ionization of individual forms. In stoichiometric equilibria, the two globally neutral species are treated collectively as being two tautomers of a single form because a titration will yield only the two macroscopic pK_a values. As a result of the simultaneous ionization of the two groups, macroscopic pK_a are a composite of the underlying microconstants as made explicit in Eqs. (1), (2), and (3), which are called Adam's equations (1,4).

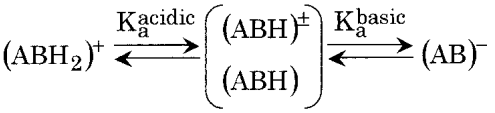
$$K_a^{acidic} = K_a^{A^Z} + K_a^{B^N} \tag{1}$$

$$\frac{1}{K_a^{basic}} = \frac{1}{K_a^{B^Z}} + \frac{1}{K_a^{A^N}} \tag{2}$$

$$K_Z = \frac{K_a^{A^Z}}{K_a^{B^N}} = \frac{K_a^{A^N}}{K_a^{B^Z}} \tag{3}$$

Each macroconstant is defined by two microconstants, but there is no direct relationship between macroconstants and the constant of tautomeric equilibrium K_Z, i.e., the ratio of concentrations of the two neutral microspecies (zwitterion and unionized).

Stoichiometric equilibria



Microdissociation equilibria

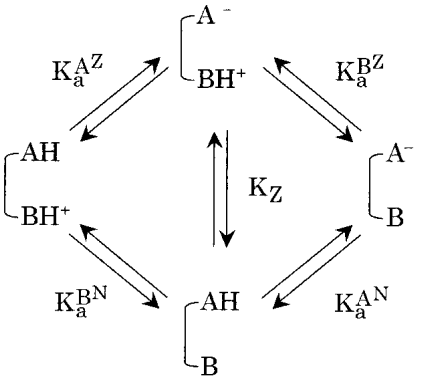


Fig. 1. Ionization scheme of a zwitterion (1).

The Tautomeric Equilibrium Constant

The solvent dependence of the tautomeric equilibrium constant K_Z has been the subject of many experimental studies by NMR and UV spectrometry (5,6). It has been well established that solvents with large dielectric constants favor the more polar tautomers (7). Recently, efforts have been made to simulate tautomeric processes in solvents using the self-consistent reaction field theory, coupled with *ab initio* molecular orbital calculations. Of the theoretical studies that take the medium into account, most are restricted to solvation in aqueous solutions (8,9). Much less attention has been paid to tautomeric equilibria in nonaqueous solutions, but all available results confirm the influence of solvent effects on conformational and tautomeric equilibria (10).

Partitioning of Zwitterions

For a zwitterionic ampholyte that can exist in solution in four different electrical states, the distribution coefficient log D^{pH} is defined by Eq. (4):

$$\log D^{pH} = \log (f^N \cdot P^N + f^Z \cdot P^Z + f^C \cdot P^C + f^A \cdot P^A) \tag{4}$$

where f represents the aqueous molar fraction of each form and P its partition coefficient.

The isoelectric pH (noted pH_i) is defined as the pH at which the effective net charge on a molecule is zero and can be calculated by Eq. (5):

$$pH_i = \frac{pK_a^{acidic} + pK_a^{basic}}{2} \tag{5}$$

At pH_i, the two neutral microspecies always coexist. When $pK_a^{basic} - pK_a^{acidic} \geq 2$, f^C and f^A can be neglected and log D^{pH_i} is described by Eq. (6):

$$\log D^{pHi} = \log (f^N \cdot P^N + f^Z \cdot P^Z) \tag{6}$$

The terms f^N and f^Z are related to the tautomeric constant K_Z as shown in Eq. (7):

$$\log D^{pH_i} = \log \left[\left(\frac{1}{1+K_Z} \right) \bullet P^N + \left(\frac{K_Z}{1+K_Z} \right) \bullet P^Z \right] \tag{7}$$

Equation (7) shows the importance of K_Z to determine log P^N and log P^Z. Indeed, 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. Equation (7) is the only way to estimate the values of log P^N and log P^Z.

For zwitterions with a large K_Z (> ca. 10^4), the neutral species is present in so low proportions that it does not contribute to the observed distribution coefficient. In such cases, the log P of the zwitterionic species becomes experimentally measurable using Eq. (8):

$$\log D^{pHi} = \log P^z \tag{8}$$

For zwitterions with a small K_Z (< ca. 10^4), neither log P^Z nor log P^N can be measured directly. Only estimates of these two parameters can be given, as shown below.

Ionic Partition Diagram of Zwitterions

The ionic partition diagram of an ionizable compound in a 1,2-dichloroethane/water system represents the domains of

predominance of its various electrical species as a function of aqueous pH and of the Galvani potential difference between the two phases ($\Delta_o^w\phi$) (11). Because for a zwitterionic drug two different electrical species (Z and N) can coexist, two cases must be considered according to the value of K_Z.

By considering the transfer of an ion i at a 1,2-dichloroethane/water interface, the Galvani potential difference between the two phases ($\Delta_o^w\phi$) is related to the standard transfer potential of i ($\Delta_o^w\phi_i^0$) by Eq. (9):

$$\Delta_o^w\phi = \Delta_o^w\phi_i^0 + \left(\frac{RT}{z_iF}\right) \bullet \ln\left(\frac{a_i^o}{a_i^w}\right) \qquad (9)$$

where z_i is the charge of i, and a_i^o (resp. a_i^w) its activity in the organic (resp. aqueous) phase.

The ionic partition diagram of an ionizable compound is drawn by rewriting Eq. (9) according to the electrical species that predominates in the biphasic system. By using Debye-Hückel approximation for infinitely diluted solutions, Eq. (9) gives:

$$\Delta_o^w\phi = \Delta_o^w\phi_i^0 + \left(\frac{RT}{z_iF}\right) \bullet \ln\left(\frac{c_i^o}{c_i^w}\right) \qquad (10)$$

where c_i^o (resp. c_i^w) is the concentration of ion i in the organic (resp. aqueous) phase. Given that the predominant species between the two pK_a varies according to the K_Z value, two different cases must now be considered.

Case 1: Zwitterions with a Large K_Z

The ionization scheme of such compounds can be simplified as follows:

$$C \overset{K_a^{acidic}}{\rightleftharpoons} Z \overset{K_a^{basic}}{\rightleftharpoons} A$$

For $pH < pK_a^{acidic}$, the drug exists as a cation C and Eq. (10) becomes:

$$\Delta_o^w\phi = \Delta_o^w\phi_C^0 + \left(\frac{RT}{F}\right) \bullet \ln\left(\frac{c_C^o}{c_C^w}\right) \qquad (11)$$

When $\Delta_o^w\phi > \Delta_o^w\phi_C^0$, the major species in the system is the cation C in 1,2-dichloroethane [noted C(o)]. When $\Delta_o^w\phi < \Delta_o^w\phi_C^0$, the major species in the system is the cation C in water [noted C(w)]. In the ionic partition diagram, the first boundary line (line **a**, in Fig. 2A) is obtained when $c_C^w = c_C^o$. This equiconcentration line corresponds to:

$$\Delta_o^w\phi = \Delta_o^w\phi_C^0 \qquad \text{[line a, Fig. 2A]}$$

When the pH increases, C is deprotonated into Z. Because $\log P_{dce}^Z$ is low, the major electrical species is Z in water [Z(w)], hence:

$$\frac{c_C^o}{c_C^w} = \frac{c_C^o\, K_a^{acidic}}{c_Z^w\, c_H^w} \qquad (12)$$

Using Eq. (11), $\Delta_o^w\phi$ can be rewritten as follows:

$$\Delta_o^w\phi = \Delta_o^w\phi_C^0 + \left(\frac{2.3RT}{F}\right) \bullet \left[\log\left(\frac{c_C^o}{c_Z^w}\right) - pK_a^{acidic}\right] + \left(\frac{2.3RT}{F}\right) \bullet pH \qquad (13)$$

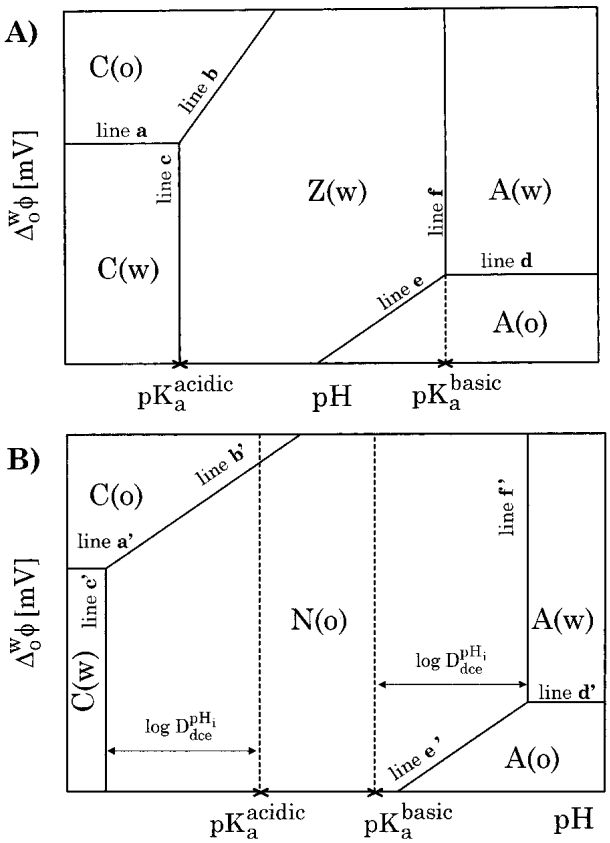


Fig. 2. Theoretical ionic partition diagram of (A) a zwitterion with a large K_z and (B) a zwitterion with a small K_z.

When $pH = pK_a^{acidic}$, the boundary line corresponding to $c_C^o = c_Z^w$ is given by line **b** (Fig. 2A) and corresponds to the interfacial acidic/basic equilibrium between C(o) and Z(w).

$$\Delta_o^w\phi = \Delta_o^w\phi_C^0 - \left(\frac{2.3RT}{F}\right) \bullet pK_a^{acidic} + \left(\frac{2.3RT}{F}\right) \bullet pH \qquad \text{[line b, Fig. 2A]}$$

Line **c** (Fig. 2A) corresponds to the equiconcentration $c_C^w = c_Z^w$ obtained when $pH = pK_a^{acidic}$.

In the alkaline range, the major species in solution is the anionic form A of the zwitterionic compound. According to Eq. (10), $\Delta_o^w\phi$ is then expressed by:

$$\Delta_o^w\phi = \Delta_o^w\phi_A^0 - \left(\frac{RT}{F}\right) \bullet \ln\left(\frac{c_A^o}{c_A^w}\right) \qquad (14)$$

and the equiconcentration line $c_A^w = c_A^o$ (line **d**) corresponds to:

$$\Delta_o^w\phi = \Delta_o^w\phi_A^0 \qquad \text{[line d, Fig. 2A]}$$

When pH decreases below pK_a^{basic}, A is protonated into Z and Eq. (14) becomes:

$$\Delta_o^w\phi = \Delta_o^w\phi_A^0 - \left(\frac{2.3RT}{F}\right) \bullet \left[\log\left(\frac{c_A^o}{c_Z^w}\right) + pK_a^{acidic}\right] + \left(\frac{2.3RT}{F}\right) \bullet pH \qquad (15)$$

The boundary line **e** corresponding to $c_A^o = c_Z^w$ is given by:

$$\Delta_o^w\phi = \Delta_o^w\phi_A^0 - \left(\frac{2.3RT}{F}\right) \bullet pK_a^{basic} + \left(\frac{2.3RT}{F}\right) \bullet pH$$

[line **e**, Fig. 2A]

Line **e** corresponds to the interfacial acidic/basic equilibrium between A(o) and Z(w). In addition, line **f** (Fig. 2A) corresponds to the equiconcentration $c_A^w = c_Z^w$ obtained when $pH = pK_a^{basic}$. The theoretical ionic partition diagram of zwitterionic compounds with a large K_Z is thus schematized in Fig. 2A.

Case 2: Zwitterions with a Small K_Z

The ionization scheme of such compounds (Fig. 1) can no longer be simplified and both neutral forms (zwitterionic and non-charged) must be considered.

For low $pH << pK_a^{acidic}$, the zwitterionic drug exists as a cation C and the first boundary line of the diagram remains:

$$\Delta_o^w\phi = \Delta_o^w\phi_C^0 \qquad \text{[line } \mathbf{a}', \text{ Fig. 2A]}$$

When pH increases, C is deprotonated into N or Z. The concentration of C in the aqueous phase can be deduced from microscopic constants $K_a^{A^Z}$ or $K_a^{B^N}$ by Eq. (16):

$$\frac{c_C^o}{c_C^w} = \frac{c_C^o\, K_a^{B^N}}{c_N^o\, c_H^w} = \frac{c_C^o\, K_a^{A^Z}}{c_N^w\, c_H^w} \tag{16}$$

By using Eqs. (2) and (3), we obtain Eq. (17):

$$\frac{c_C^o}{c_C^w} = \frac{c_C^o\, K_a^{acidic}}{c_N^o\, c_H^w} \bullet \frac{P_{dce}^N}{1+K_Z} = \frac{c_C^o\, K_a^{acidic}}{c_Z^w\, c_H^w} \bullet \frac{K_Z}{1+K_Z} \tag{17}$$

Case 2A

In this case, K_Z is small enough to simplify Eq. (7) as follows:

$$\log D^{pH_i} = \log\left[\frac{P_{dce}^N}{1+K_Z}\right] \tag{18}$$

and $\Delta_o^w\phi$ is given by Eq. (19):

$$\Delta_o^w\phi = \Delta_o^w\phi_C^0 + \left(\frac{2.3RT}{F}\right) \bullet \left[\log\left(\frac{c_C^o}{c_N^o}\right) - pK_a^{acidic} + \log D_{dce}^{pH_i}\right] + \left(\frac{2.3RT}{F}\right) \bullet pH \tag{19}$$

The boundary line **b′** (Fig. 2B) corresponding to $c_C^o = c_N^o$ is:

$$\Delta_o^w\phi = \Delta_o^w\phi_C^0 + \left(\frac{2.3RT}{F}\right) \bullet [-pK_a^{acidic} + \log D_{dce}^{pH_i}] + \left(\frac{2.3RT}{F}\right) \bullet pH$$

[line **b′**, Fig. 2B]

and the equiconcentration line $c_C^w = c_N^o$ (line **c′**, Fig. 2B) is obtained when $pH = pK_a^{acidic} - \log D_{dce}^{pH_i}$.

At very basic pH values, the major species in solution is the anion A and the equiconcentration $c_A^w = c_A^o$ (line **d′**) is given by:

$$\Delta_o^w\phi = \Delta_o^w\phi_A^0 \qquad \text{[line } \mathbf{d}', \text{ Fig. 2B]}$$

When the pH decreases, A is protonated into N and Eq. (14) becomes:

$$\Delta_o^w\phi = \Delta_o^w\phi_A^0 - \left(\frac{2.3RT}{F}\right) \bullet \left[\log\left(\frac{c_A^o}{c_N^o}\right) + pK_a^{basic} + \log D_{dce}^{pH_i}\right] + \left(\frac{2.3RT}{F}\right) \bullet pH \tag{20}$$

The boundary line **e′** corresponding to $c_A^o = c_N^o$ is:

$$\Delta_o^w\phi = \Delta_o^w\phi_A^0 - \left(\frac{2.3RT}{F}\right) \bullet [pK_a^{basic} + \log D_{dce}^{pH_i}] + \left(\frac{2.3RT}{F}\right) \bullet pH$$

[line **e′**, Fig. 2B]

Line **f′** (Fig. 2B) corresponds to the equiconcentration $c_A^w = c_N^o$ obtained when $pH = pK_a^{basic} - \log D_{dce}^{pH_i}$. The theoretical ionic partition diagram of zwitterionic compounds with a very small K_Z is thus schematized in Fig. 2B.

Case 2B. When K_Z is not low enough to neglect the zwitterionic species, the ionic partition diagram will depend on the ratio between P_{dce}^N and $K_Z \bullet P_{dce}^Z$.

- When $P_{dce}^N << K_Z \bullet P_{dce}^Z$, the ionic partition diagram will be the same as for zwitterions with large K_Z.
- When $P_{dce}^N >> K_Z \bullet P_{dce}^Z$, the ionic partition diagram will be the same as for zwitterions with very small K_Z.
- For intermediate cases, no calculation is possible since neither P_{dce}^N nor P_{dce}^Z can be measured with enough precision.

MATERIAL AND METHODS

Compounds and Reagents

Azapropazone was supplied by Siegfried (Zofingen, Switzerland). Cetirizine was donated by UCB (Braine-l' Alleud, Belgium). Tenoxicam was supplied by Hoffman-La Roche Ltd. (Basel, Switzerland). Raclopride and eticlopride were kindly given by Astra Arcus AB (Södertälje, Sweden). *n*-Octanol, methanol, KCl, KOH, and HCl were purchased from Fluka (Buchs, Switzerland). 1,2-Dichloroethane (Romil, Cambridge, UK) was used without further purification and handled with all necessary precautions (12). BTPPATPBCl (bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)borate) was prepared by metathesis of potassium tetrakis(4-chlorophenyl)borate (Fluka) and of bis(triphenylphosphoranylidene)ammonium chloride (Aldrich, Milwaukee, WI, USA).

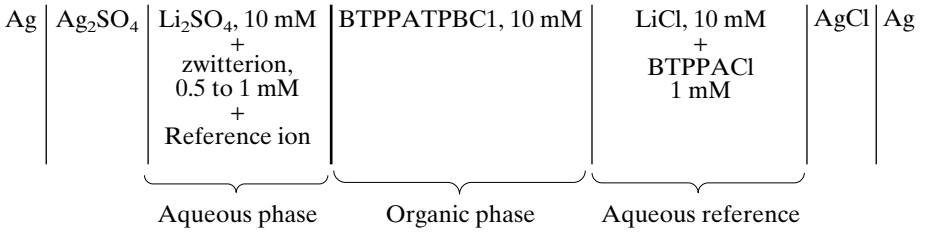
Potentiometric Determination of Ionization Constants and Partition Coefficients

The ionization constants and the partition coefficients in *n*-octanol/water and 1,2-dichloroethane/water were determined by potentiometric titrations using the GLpK$_a$ apparatus of Sirius Analytical Instruments Ltd (Forest Row, East Sussex, UK). The detailed experimental procedures can be found elsewhere (13).

Cyclic Voltammetry Measurements

The experimental setup used was a home-made four-electrode potentiostat, as described in reference (11), with ohmic drop compensation. The scanning of the applied po-

tential was performed by a waveform generator (VA-scanner E 612, Metrohm, Herisau, Switzerland), coupled to an X-Y recorder (Bausch & Lomb, Rochester, NY, USA). All experiments were carried out at room temperature (25°C). The following electrochemical chain was used:



1,2-Dichloroethane and water were mutually saturated. The drugs were dissolved in the aqueous phase (azapropazone, cetirizine, labetalol) or in the organic phase (tenoxicam, isoxicam, raclopride, eticlopride) according to their solubility. The pH of the aqueous solution was adjusted to the desired value with H_2SO_4 or LiOH. All measured half-wave potentials (noted $\Delta_o^w\phi_i^{1/2}$) were referred to the half-wave potential of the tetramethylammonium cation. The detailed procedure to calculate $\Delta_o^w\phi_i^0$ and $P_{dce}^{0,i}$ can be found elsewhere (3).

Determination of Tautomeric Constants

The tautomeric constant K_Z of raclopride and eticlopride were measured by UV spectrometry (1,14) using a Hewlett Packard 8452A spectrophotometer (Waldbronn, Germany). Spectra were recorded in dioxane-buffer mixtures containing 0.3 mM of drug and covering a dioxane range of 0–100%. A phosphate buffer ($pH_i = 7.8$) was used for raclopride and a glycine/KOH buffer ($pH_i = 9.2$) for eticlopride.

log P Calculations by MLP

The conformational space of the molecules was explored by QMD (3). For each conformer, the MLP was integrated on the solvent-accessible surface area by using the CLIP 1.0 software (15) and served to calculate the corresponding log P_{oct} (16).

LIPOPHILICITY AND IONIC PARTITION DIAGRAMS OF ZWITTERIONS

Zwitterions with Large K_z

Azapropazone

Azapropazone (Fig. 3) is a nonsteroidal anti-inflammatory drug with a low toxicity and a good gastrointestinal tolerance. Its ionization equilibria, tautomerism and lipophilicity profiles in *n*-octanol/water were examined in a recent work to shed light on its molecular and distribution equilibria (17).

Azapropazone demonstrated a large value of K_Z because the difference between its two pK_a is larger than 5. As a result, azapropazone exists as a zwitterion and an anion at physiological pH. Its physicochemical parameters in 1,2-dichloroethane/water are given in Table I. The low value of log P measured by potentiometry between the two pK_a confirms that azapropazone partitions as a zwitterion and not as a non-ionized molecule. The low pK_a^{acidic} did not allow to measure log $P_{dce}^{0,C}$ by cyclic voltammetry. Owing to the low value of log P_{dce}^{Z}, the difference in lipophilicity between the zwitterionic and the anionic forms (*diff*(log P_{dce}^{Z-A}) = 3.2) is lower than usually observed for classical monoacids.

The ionic partition diagram obtained for azapropazone is given in Fig. 4. This diagram is not complete since the value of log P_{dce}^{C} is not known, but it shows a good consistency between experimental points and theoretical lines calculated as explained above for zwitterions with large K_Z. Between the two pK_a, the predominant species is the zwitterionic form in the aqueous phase, except if the Galvani potential difference between the two phases is high (in this case, the major species is the cation in the organic phase due to the protonation of Z(w) according to line **b** in Fig. 2A. At higher pH (> pK_a^{basic}), azapropazone exists as an anion either in the aqueous or in the organic phase according to the Galvani potential difference between the two phases.

Cetirizine

The lipophilicity behavior of the zwitterionic antihistaminic cetirizine in 1,2-dichloroethane/water (Fig. 3) as well as its ionic partition diagram were recently investigated (3). Its lipophilicity parameters are recalled in Table I. The low difference Δ log $P_{oct-dce}^{Z}$ confirms that, like in the *n*-octanol/water system (18), cetirizine partitions between 1,2-dichloroethane and water as a zwitterion and not as an unionized molecule.

Moreover, the relatively high value of log P_{dce}^{Z} suggests that conformational effects lower the polarity of zwitterionic cetirizine. The low value of *diff*(log P_{dce}^{Z-C}) shows that the positive charge in cationic cetirizine is masked by intramolecular interactions between the protonated nitrogen and the oxygen atom of the carboxylic group, whereas the negative charge in the anionic form is more localized. These results coupled with quenched molecular dynamic simulations confirm that strong conformational effects act on the partitioning of the different electrical species of cetirizine and may influence its pharmacokinetic behavior.

The ionic partition diagram of cetirizine is in agreement with the theoretical diagram presented in Fig. 2A, but addi-

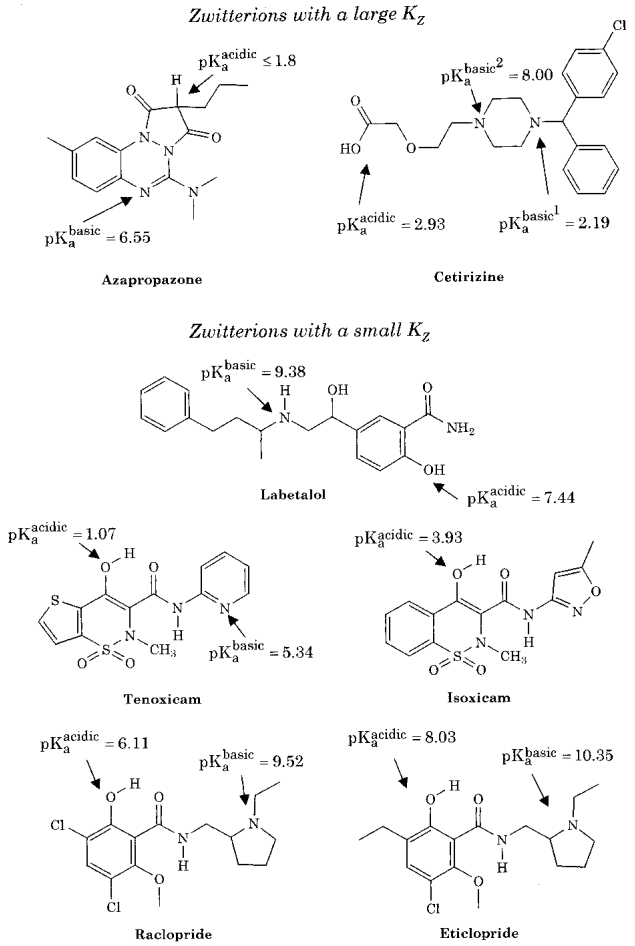


Fig. 3. Chemical structure and ionization constants of the investigated zwitterions.

tional boundary lines should be considered because cetirizine has not one but two basic centers.

Zwitterions with Small K_z

Labetalol

The antihypertensive agent labetalol consists of an approximate equimolar mixture of four stereoisomers (19). The protonation equilibria and the *n*-octanol/water partitioning behavior of labetalol (see Fig. 3) were published (1). A UV spectrophotometric study in various mixtures of Tris/HCl buffer (at isolectric pH) and dioxane yielded a K_Z of 29, showing that between the two pK_a both two neutral forms of labetalol (zwitterionic and unionized) coexist. Consequently neither log P^N_{dce} nor log P^Z_{dce} can be obtained directly, and only the distribution coefficient of labetalol could be measured here (see Table II).

Knowing K_Z, log P^N_{dce} can be evaluated by using the expression of the distribution coefficient at isoelectric pH (log $D^{pH_i}_{dce}$) and by neglecting the partitioning of the zwitterionic species [see Eq. (7)]. This evaluation yielded a log P^N_{dce} of 1.7. The solvatochromic analysis of neutral compounds in the 1,2-dichloroethane/water system produced in good correlations between log P^N_{dce} and log P^N_{oct} (20). The correlation for H-bond donors applied to labetalol yielded a log P^N_{oct} of 2.5 which is

Table I. Physicochemical Parameters of Zwitterions with a Large K_Z

Compound	Azapropazone	Cetirizine (3,18)
log $P^{Z\ a}_{oct}$	1.8 (17)	1.5
log $P^{Z\ b}_{dce}$	0.2	0.7
Δlog $P^{Z\ c}_{oct\ -\ dce}$	1.6	0.8
log $P^{O,\ Cd}_{dce}$	n.m.[h]	0.7
log $P^{O,\ Ae}_{dce}$	−3.0	~−3.1
diff(log $P^{Z\ -\ C}_{dce}$)[f]	n.m.[h]	0.7
diff(log $P^{Z\ -\ A}_{dce}$)[g]	3.2	~3.8

[a] Log P of Z in *n*-octanol/water, measured by potentiometry.
[b] Log P of Z in 1,2-dichloroethane/water, measured by potentiometry.
[c] $\Delta\log P^Z_{oct\ -\ dce} = \log P^Z_{oct} - \log P^Z_{dce}$.
[d] Standard log P of C, measured by cyclic voltammetry in 1,2-dichloroethane/water.
[e] Standard log P of A, measured by cyclic votammetry in 1,2-dichloroethane/water.
[f] $diff(\log P^{Z\ -\ C}_{dce}) = \log P^Z_{dce} - \log P^C_{dce}$.
[g] $diff(\log P^{Z\ -\ A}_{dce}) = \log P^Z_{dce} - \log P^A_{dce}$.
[h] Not measurable.

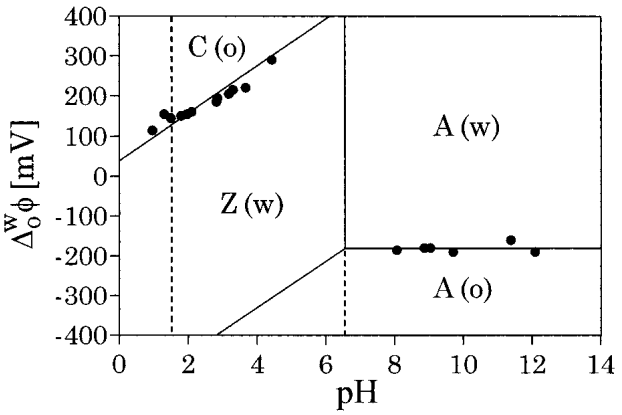


Fig. 4. Partial ionic partition diagram of azapropazone. Black circles are experimental points measured by cyclic voltammetry. Solid lines are theoretical lines.

Table II. Physicochemical Parameters of Labetalol

Compound	Labetalol
log $D^{pH_i}_{oct}$	2.6[a]
log $D^{pH_i}_{dce}$	0.2[b]
K_Z	29[a]
log P^N_{dce}	1.7[c]
log $P^{O,\ C}_{dce}$	−2.6[d]
log $P^{O,\ A}_{dce}$	−4.8[d]
diff(log $P^{N\ -\ C}_{dce}$)[e]	4.3
diff(log $P^{N\ -\ A}_{dce}$)[f]	6.5
log P^N_{oct}	2.5[g]
CLOGP[h]	2.5

[a] Taken from (1).
[b] Measured by potentiometry.
[c] Calculated by Equation 7 by neglecting log P^Z_{dce}.
[d] Measured by cyclic votammetry.
[e] $diff(\log P^{N\ -\ C}_{dce}) = \log P^N_{dce} - \log P^C_{dce}$.
[f] $diff(\log P^{N\ -\ A}_{dce}) = \log P^N_{dce} - \log P^A_{dce}$.
[g] Calculated from log P^N_{dce} (20).
[h] Calculated by the PCModels software (21).

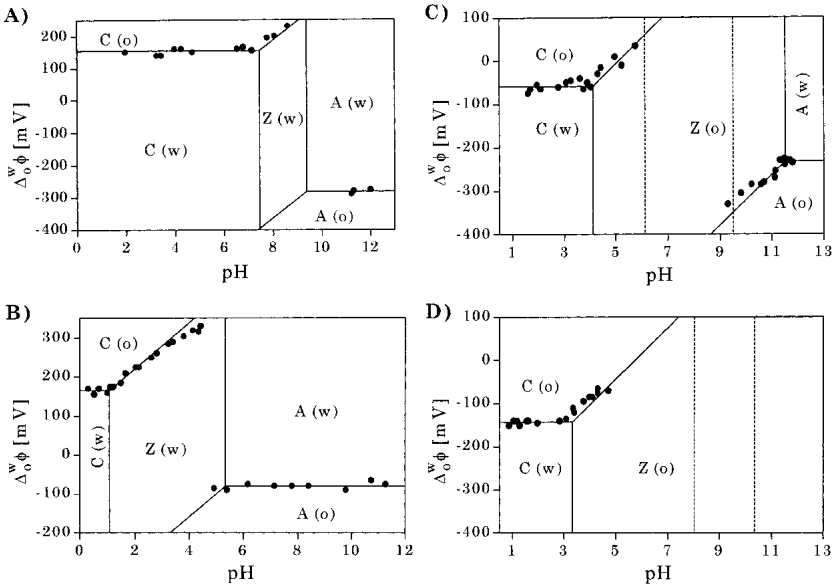


Fig. 5. Ionic partition diagram of zwitterions with small K_Z. (A) labetalol; (B) tenoxicam; (C) raclopride; and (D) eticlopride. Black circles are experimental points measured by cyclic voltammetry. Solid lines are theoretical lines. Dotted lines are aqueous pK_a.

exactly equal to the value obtained by the CLOGP software (21) and confirms the relevance of our estimated value of log P^N_{dce}. Thus labetalol behaves as an H-bond donor compound despite the H-bond between the O atom of the phenolic group and one of the proton of the NH_2 group, which exists in all electrical species (N, Z, C and A).

The parameters *diff*(log P^{N-C}_{dce}) and *diff*(log P^{N-A}_{dce}) are respectively equal to 4.3 and 6.5. These values are consistent with those usually obtained respectively for basic tertiary amines (22) and for phenolic acids deprived of intramolecular interactions stabilizing the ionized species. This does not mean that the zwitterion fails to partition into 1,2-dichloroethane but simply that, in this case, its log P^Z_{dce} can be neglected.

Because K_Z is large enough to deduce that the zwitterionic species is predominant between the two pK_a, the ionic partition diagram of labetalol (Fig. 5A) was drawn as in Fig. 2A. Experimental points are consistent with theoretical lines, confirming once again that log P^Z_{dce} is negligible.

Tenoxicam and Isoxicam

Tenoxicam (Fig. 3) belongs to the nonsteroidal anti-inflammatory drug oxicams. Its ionization and partitioning behavior in *n*-octanol/water and heptane/water were published (23). In this paper, the microscopic ionization constants of tenoxicam were determined by analogy with piroxicam, yielding a K_Z of 150. This K_z value is large enough to deduce that zwitterionic tenoxicam is the species predominating between the two pK_a, but the relatively high value of log $D^{pH_i}_{dce}$ (see Table III) indicates clearly that the unionized species contributes markedly to the distribution of tenoxicam. Its log $D^{pH_i}_{dce}$ is higher than log $D^{pH_i}_{oct}$ suggesting that tenoxicam behaves as a non-H-bond donor. This observation is consistent with the existence of an internal H-bond between the enolic OH and the amide C=O group described in (23) for both electrical species of tenoxicam. The same H-bond also exists in neutral isoxicam, a monoacidic analogue whose structure (Fig. 3) is very close to that of tenoxicam. As for labetalol, log P^N_{dce} can be deduced from log $D^{pH_i}_{dce}$ and is equal to 4.0. Thus *diff*(log P^{N-A}_{dce}) is equal to 5.3. The study of isoxicam yielded a *diff*(log P^{N-A}_{dce}) of 5.0, confirming the relevance of the log P^N_{dce} value estimated for tenoxicam. The corresponding correlation between log P^N_{dce} and log P^N_{oct} was then used (20) to estimate log P^N_{oct} . The value obtained (2.9) is close to the log P^N_{oct} of isoxicam (2.8) and is consistent with the log P^N_{dce} value estimated for tenoxicam because the CLOGP algorithm gave the same value of CLOGP for isoxicam and tenoxicam (CLOGP = 2.4).

The ionic partition diagram of tenoxicam is given in Fig. 5B. Its pK^{acidic}_a is too low to allow log $P^{0,C}_{dce}$ to be measured. According to the K_Z value, theoretical lines were drawn, as for labetalol, by considering the zwitterionic tenoxicam as the predominant species between the two pK_a. Experimental

Table III. Physicochemical Parameters of Isoxicam and Tenoxicam

Compound	Isoxicam	Tenoxicam
log $D^{pH_i}_{oct}$		0.8[a]
log $D^{pH_i}_{dce}$		1.8[a]
K_Z		150[b]
log P^N_{dce}	4.0[a]	4.0[c]
log $P^{O,C}_{dce}$		n.m.[d]
log $P^{O,A}_{dce}$[e]	–1.0	–1.3
diff(log P^{N-A}_{dce})[f]	5.0	5.3
log P^N_{oct}	2.8[a]	2.9[g]
CLOGP[h]	2.4	2.4

[a] Measured by potentiometry.
[b] Taken from (23).
[c] Calculated by Equation 7 by neglecting log P^Z_{dce}.
[d] Not measurable.
[e] Measured by cyclic votammetry.
[f] *diff*(log P^{N-A}_{dce}) = log P^N_{dce} – log P^A_{dce}.
[g] Calculated from log P^N_{dce} (20).
[h] Calculated by the PCModels software (21).

points are consistent with theoretical lines, supporting this assumption.

Raclopride and Eticlopride

Raclopride and eticlopride (see Fig. 3) belong to the family of neuroleptic orthopramides. The effects of solvation on their ionization and conformation were extensively studied (7). Raclopride was shown to exist in zwitterionic form at physiological pH while its unionized form predomines in organic phases such as *n*-octanol, heptane and possibly also in the receptor phase.

The tautomeric constant of raclopride and eticlopride was investigated by UV-spectrometry at isoelectric pH in various dioxane/water mixtures (see Material and Methods). In the case of raclopride, a sharp isobestic point allowed to determine the K_Z value by extrapolation to 0% dioxane ($K_Z = 14$) and indicated that at isoelectric pH, raclopride exists as a mixture of zwitterionic and unionized forms. In the case of eticlopride, no spectral change was observed. The absorbance increased with dioxane percentage to reach its maximum at 100% dioxane, showing that the unionized form of eticlopride always predominates whatever the dioxane percentage, probably due to the low pK_a difference ($\Delta pK_a = 2.32$).

Results of the potentiometric and voltammetric studies are given in Table IV. An exploration of the conformational space of each neutral unionized drug was performed by QMD and the log P^{MLP} of each conformer was calculated by the MLP. These log P^{MLP} values ranged from 3.2 to 3.7 for raclopride and from 3.7 to 4.2 for eticlopride. The log P^{MLP} of the lower-energy conformer is given in Table IV. Thus the log $D_{oct}^{pH_i}$ of eticlopride corresponds to the partition coefficient of its unionized form whereas the log $D_{oct}^{pH_i}$ of raclopride corresponds to its two neutral species, zwitterionic and unionized. This observation explains the large difference (2.6) measured between the log $D_{oct}^{pH_i}$ of raclopride and eticlopride since the difference between the log P_{oct}^{N} values of these two drugs calculated by the CLOGP algorithm is only of 0.6. Thus the log $D_{oct}^{pH_i}$ value of eticlopride can be attributed exclusively to its unionized species allowing its log P_{dce}^{N} to be calculated as 4.7.

The distribution coefficients at pH_i of raclopride and eticlopride are higher in 1,2-dichloroethane/water than in *n*-octanol/water, indicating that the two compounds behave as non-H-bond donors due to the two internal H-bonds existing in their zwitterionic and unionized species (7) (Fig. 6).

For eticlopride, *diff*(log P_{dce}^{N-C}) = 2.4. This difference, which is lower than expected for a tertiary amine (22), is accounted for by two intramolecular H-bonds in the cation, as demonstrated by QMD. Likewise in zwitterionic raclopride, one internal H-bond is between the phenolic oxygen and the NH of the amide group, and the other between the protonated quaternary nitrogen and the carbonyl oxygen.

The ionic partition diagrams of raclopride and eticlopride are given in Fig. 5C and D, respectively. The pK_a^{basic} of eticlopride is too high to allow the transfer of the anion to be observed. The experimental lines are shifted by a value equal to log $D_{oct}^{pH_i}$, in accordance with the theory given above for zwitterions with small K_Z. These two diagrams show that the domain of predominance of the neutral form of a zwitterionic drug with small K_Z is much larger than expected in this biphasic system. This shift of the ionic partition diagrams could explain some deviations from the expected pH/gastrointestinal absorption curves mentioned in the literature for various drugs such as β-lactam antibiotics (24).

Table IV. Physicochemical Parameters of Raclopride and Eticlopride

Compound	Raclopride	Eticlopride
log $D_{oct}^{pH_i}$[a]	1.3	3.9
log $D_{dce}^{pH_i}$[a]	2.0	4.7
K_Z[b]	11	n.m.[c]
log P_{oct}^{MLP}[d]	3.4	3.9
CLOGP[e]	4.11	4.73
log $P_{dce}^{O,C}$[f]	1.0	2.4
log $P_{dce}^{O,A}$[f]	–3.9	n.m.[c]
diff(log P_{dce}^{N-C})[g]	n.m.[c]	2.3
diff(log P_{dce}^{N-A})[h]	n.m.[c]	n.m.[c]

[a] Measured by potentiometry.
[b] Measured by UV spectrophotometry in dioxane/water mixtures.
[c] Not measurable.
[d] Log P of the lower-energy conformer calculated by the CLIP1.0 software (15).
[e] Calculated by the PCModels software (21).
[f] Measured by cyclic voltammetry.
[g] *diff*(log P_{dce}^{N-C}) = log P_{dce}^{N} – log P_{dce}^{C}.
[h] *diff*(log P_{dce}^{N-A}) = log P_{dce}^{N} – log P_{dce}^{A}.

APPLICATION TO PH-ABSORPTION PROFILES

According to the simple pH-partition theory (25), the rate of absorption of an ionizable substance is proportional to its degree of dissociation. When the pH varies, the absorption rate changes in parallel with the fraction of the neutral form

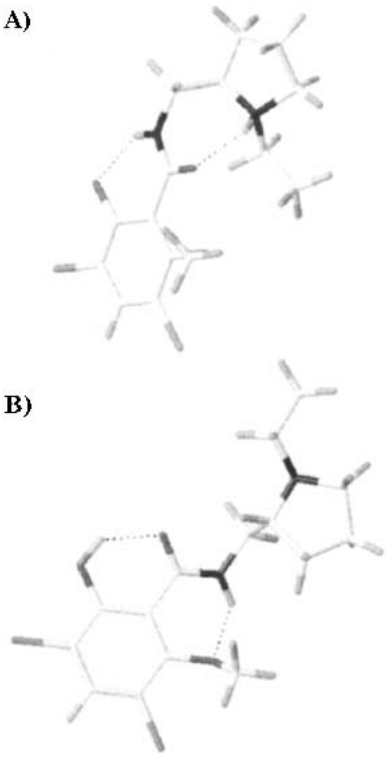


Fig. 6. Intramolecular H-bonds in (A) zwitterionic and (B) unionized raclopride.

in solution. In the simplest case, absorption curves so obtained (pH-absorption curves) have the well-known sigmoidal shape of dissociation curves. In other words, they decline from a maximal value, cross the half-maximal absorption rate at pH = pK_a and asymptotically approach zero (26). Deviations from this simple pH-absorption have been observed for the gastrointestinal absorption of some highly lipophilic compounds (24,27). In such cases, the pH-absorption profiles do not overlap with pH partition profiles but are shifted significantly relative to the dissociation curves, to the right for acids and to the left for bases. The higher the lipophilicity of the neutral species, the higher the observed shift (28).

Similar shifts of apparent pKa are reported here in the ionic partition diagrams of raclopride and eticlopride in the 1,2-dichloroethane/water biphasic system. These diagrams are shifted to the right for pK_a^{basic} and to the left for pK_a^{acidic}, with shifts corresponding to the value of log $D_{dce}^{pH_i}$. The similarity between these biologic (pH-absorption) and physicochemical (dissociation) observations suggests that the two shifts may have the same physicochemical origin and could contribute to a better mechanistic understanding of the absorption and bioavailability of ionizable drugs.

Various explanations have been proposed for the deviations in pH-absorption curves (assuming passive transport), including a microclimate-pH on mucosal surfaces, binding to components of the intestinal mucosa, or a permeation-retarding unstirred layer (24,27). Without questioning the relevance of such proposals, Kubinyi favored distribution coefficients to explain these deviations and to describe the dependence of drug absorption on the degree of ionization (and hence on pH and pK^a) and on the lipophilicity of each electrical species (28). The pH-shift can easily be explained by assuming an aqueous diffusion layer at the aqueous/organic interface. Neutral species rapidly enter the organic phase from the aqueous/organic interface and are steadily regenerated within the aqueous diffusion layer from the ionized species, much faster than the neutral molecules can diffuse from the bulk solution into this layer.

The same assumption of an aqueous diffusion layer at the aqueous/organic interface accounts for pH-shifts in ionic partition diagrams (29). Neutral species rapidly enter the organic phase from the aqueous/organic interface and are steadily regenerated from the ionized species within the aqueous diffusion layer, much faster than the neutral molecules can diffuse from the bulk solution into this layer. Thus, ionic partition diagrams and pH-absorption profiles alike depend on pH, pK_a, and log D (and hence on the lipophilicity of both electrical species).

This view, although not excluding alternative mechanisms such as microclimate pH, affords a simple explanation to the biologic and physicochemical deviations from the hydrodynamic diffusion theory discussed above.

CONCLUSION

Our study shows that cyclic voltammetry is an informative method to study the lipophilicity of cationic and anionic forms of ampholytes and to understand the intramolecular interactions responsible for the lipophilic behavior of these drugs. Moreover, the comparison between pH-absorption profiles and ionic partition diagrams of zwitterions is shown here to be of value for a better mechanistic understanding of absorption processes, thus opening new perspectives in studying pH-absorption profiles of ionizable drugs.

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