Physicochemical Characterization of Sildenafil: Ionization, Lipophilicity Behavior, and Ionic-Partition Diagram Studied by Two-Phase Titration and Electrochemistry

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Sildenafil (*Viagra*™) was examined for its ionization and lipophilicity by two-phase titration and electrochemistry at the interface between two immiscible electrolyte solutions (ITIES) in the 1,2-dichloroethane/H_2O system. The dissociation constants (basic $pK_a = 6.78$, acidic $pK_a = 9.12$) and partition coefficients of the various species, together with the effects of electrical potential, were used to construct an ionic partition diagram (pH-potential representation). This allowed to interpret the transfer mechanisms of sildenafil at liquid/liquid interfaces, suggesting in particular that an intramolecular H-bond influences the lipophilicity of the neutral and cationic species. Conformational calculations confirmed this hypothesis.

1. Introduction. – Selective inhibitors of phosphodiesterase (PDE) type 5 can efficiently be used as therapy for a range of cardiovascular disorders. PDE Type 5 being also the predominant cGMP-hydrolyzing activity in the cytosolic fraction from human *corpus cavernosum*, these inhibitors improve erection by enhancing relaxation of the *corpus cavernosum* smooth muscle [1][2]. Sildenafil (*Viagra*™; 1-{[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl}-4-methylpiperazine) appears to be such a potent and highly selective inhibitor of PDE type 5 [3]. Following oral administration, sildenafil has to cross biological barriers and compartments before reaching its target sites. Given that the pharmacokinetic behavior of sildenafil must be influenced by its physicochemical properties, it was of interest to study the influence of pH and electrical potential on the partitioning of the various electrical species. A recent study indicates that sildenafil has a single basic group ($pK_a = 6.5$) and is relatively lipophilic with a distribution coefficient in the octan-1-ol/water system at pH 7.4 ($\log D_{OCT}^{7.4}$) equal to 2.7 [4]. As our study shows, these results are incomplete.

The interfacial properties of sildenafil are investigated here by electrochemistry at the interface between two immiscible electrolyte solutions (ITIES) in a 1,2-dichloroethane/H_2O system. It has already been shown in previous studies that partition data obtained with this system are in good agreement with the pharmacokinetic behavior of ionizable compounds [5][6]. With this aim, preliminary studies are needed to determine the dissociation constants of the drug, as well as the partition coefficients of the various species in octan-1-ol/H_2O and 1,2-dichloroethane/H_2O. Conformational calculations confirm the presence of an intramolecular H-bond able to influence the distribution of the neutral and cationic species.

2. Theory. – Ionic-partition diagrams based on *Pourbaix* diagrams [7] consist in a representation of the predominance area of the various species of a given ionizable compound as a function of the *Galvani*-potential difference and the pH [8]. The resulting diagram represents the equiconcentrations or boundary lines defining predominance area. By convention, in the case of a monobasic solute B that can be protonated in acidic solution to BH^+, the concentration of neutral species in the aqueous phase is chosen as variable [8], so that only the domain of predominance of B^w appears on the diagram. For lipophilic compounds, however, the concentration of neutral species in the aqueous phase is negligible compared to that in the organic phase. In such a case, another choice must be made, taking into account B^o to derive the equations defining the boundary lines.

As an example, let us consider a lipophilic monobasic compound B ($\log P^N_{DCE} > 0$; DCE = 1,2-dichloroethane) partitioning between two immiscible phases. The first boundary line corresponding to the equiconcentration of the two contiguous charged species BH^{+w} and BH^{+o} is defined by the *Nernst* equation for ion-transfer reactions (see *Eqn. 1*). The acid/base equilibrium between the charged species in the aqueous phase and the neutral species in the organic phase is given by *Eqn. 2*. Hence the dissociation constant can be expressed according to the standard partition coefficient of B, $\log P^o_B = a^o_B/a^w_B$, the pH, and the ratio $a^o_B/a^w_{BH^+}$, *i.e.*, by *Eqn. 3*.

$$\Delta^w_o \phi = \Delta^w_o \phi^{o'}_{BH} \tag{1}$$

$$B^o + H^{+w} \rightleftarrows BH^{+w} \tag{2}$$

$$K^w_a = \frac{a^w_B a^w_{H^+}}{a^w_{BH^+}} = \frac{a^o_B}{a^w_{BH^+}} \cdot \frac{a^w_{H^+}}{P^o_B} \tag{3}$$

The standard partition coefficient can be evaluated as the ratio of the concentrations instead of the activities for dilute solutions, and equal volumes of the two phases are used. In the present case, the volumes of the two phases are equal, and so the ratio between the organic and the aqueous phases, $r = V^o/V^w$, is equal to one. Thus the dissociation constant can be rewritten as *Eqn. 4*. Hence, the boundary line between B^o and BH^{+w} is defined by *Eqn. 5*.

$$pK^w_a = -\log\left(\frac{a^o_B}{a^w_{BH^+}}\right) + pH + \log P^o_B \tag{4}$$

$$pH = pK^w_a - \log P^o_B \tag{5}$$

The boundary between BH^{+o} and B^o is similarly defined by developing the ratio $a^o_{BH^+}/a^o_B$ from the partition coefficient of the neutral and the charged species. This latter partition coefficient is defined by *Eqn. 6*, where $\Delta^w_o \phi$ is the *Galvani*-potential difference and $\Delta^w_o \phi^{o'}_{BH^+}$ is the apparent standard transfer potential (also called formal transfer potential) of the cation BH^+. Including the expression of K_a in P_{BH^+} yields *Eqn. 7*. The partition coefficient is a function of the *Galvani*-potential difference, itself established by the partition coefficient of all the other ionic species present. Thus *Eqn. 6* can be rewritten as *Eqn. 8*. At the equilibrium condition $a^o_{BH^+} = a^o_B$ and equation for the boundary line is given by *Eqn. 9*. The corresponding potential *vs.* pH representation is shown in *Fig. 1*.

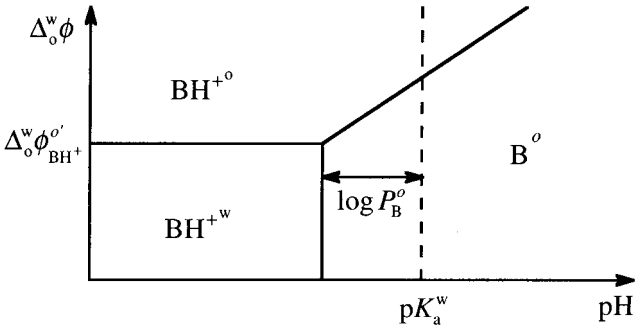

Fig. 1. *Theoretical ion-partition diagram for a lipophilic monobasic compound*

$$\Delta_o^w \phi = \Delta_o^w \phi^{o'}_{BH^+} + \frac{RT}{zF}\log\left(\frac{a^o_{BH^+}}{a^w_{BH^+}}\right) \quad (6)$$

$$P_{BH^+} = \frac{a^o_{BH^+}}{a^o_B} \cdot \frac{P^o_B K^w_a}{a^w_{H^+}} \quad (7)$$

$$\Delta_o^w \phi = \Delta_o^w \phi^{o'}_{BH^+} + \frac{RT}{zF}\log\left(\frac{a^o_{BH^+}}{a^o_B}\right) + \frac{RT}{zF}\log\left(\frac{P^o_B K^w_a}{a^w_{H^+}}\right) \quad (8)$$

$$\Delta_o^w \phi = \Delta_o^w \phi^{o'}_{BH^+} + \frac{RT}{zF}\mathrm{pH} + \frac{RT}{zF}(\log P^o_B - \mathrm{p}K^w_a) \quad (9)$$

3. Materials and Methods. – 3.1. *Compounds.* Sildenafil citrate was graciously supplied by *Pfizer* (Zurich, Switzerland). For potentiometric experiments, deionized H_2O was employed, and KCl (*Fluka*, Buchs, Switzerland) was used as supporting electrolyte. The pH was adjusted by adding HCl (*Fluka*), and titrations were carried out in anal. grade 1,2-dichloroethane (*Merck*, Darmstadt, Germany) [9] and octan-1-ol (*Fluka*). Electrochemical experiments were carried out in 1,2-dichloroethane (DCE), deionized H_2O being employed throughout. Lithium sulfate monohydrate ($Li_2SO_4 \cdot H_2O$) (*Fluka*) was used as aq. supporting electrolyte and bis(triphenylphosphoranylidene)ammonium tetrakis(4-chlorophenyl)borate (BTPPATPBCl) as org. supporting electrolyte. It was prepared by metathesis of bis(triphenylphosphoranylidene)ammonium chloride (BTPPACl) (*Fluka*) and potassium tetrakis(4-chlorophenyl)borate (KTPBCl) (*Lancaster*, Strasbourg, France) and recrystallized from MeOH.

A four-electrode cell was used for electrochemistry at the ITIES where the interface was polarized by two reference electrodes, and the current was measured with two counter-electrodes. The org.-phase reference electrode was an ion-selective electrode consisting of a silver electrode covered with silver chloride (Ag/AgCl). It was immersed in an aq. soln. of BTPPACl. In the aqueous phase, an Ag/Ag_2SO_4 electrode was used due to the supporting electrolyte Li_2SO_4.

The surface area of the interface was *ca.* 1 cm^2. The interface was positioned between two lugging capillaries to facilitate minimization of the IR drop.

The potential was applied with a *PPR1-Waveform* generator (*HI-TEK Instrument*, UK) connected to a home-made four-electrode potentiostat, and the potential and current output signals were monitored with an *XY* recorder (*Advance Instruments*, UK).

For all experiments, the liquid/liquid cell was placed in a *Faraday* cage to minimize the background noise. The cell was kept at room temperature.

The cell configuration for the study of ion transfer was:

$$\mathrm{Ag}\left|\mathrm{AgCl}\right|\mathrm{10mM\ BTPPACl}\left|\begin{matrix}\mathrm{10\,mM\ BTPPATPB}\\ \mathrm{in\ DCE}\end{matrix}\right|\left|\begin{matrix}\mathrm{5\,mM\ Li_2SO_4 + 0.5\,mM\ analyte}\\ \mathrm{+LiOH\ and/or\ HNO_3}\\ \mathrm{to\ desired\ pH}\end{matrix}\right|\left|\mathrm{Ag_2SO_4}\right|\mathrm{Ag}$$

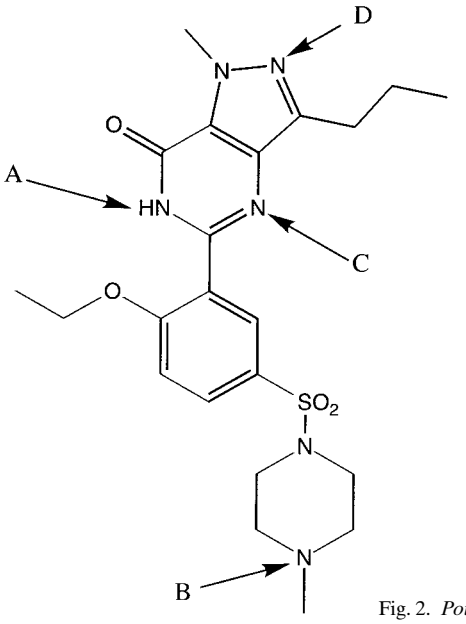

Fig. 2. *Potential ionization sites of sildenafil*

3.2. *Determination of Ionization Constants.* The ionization constants of sildenafil were determined by potentiometric titration in H_2O/MeOH with the GlpK_a apparatus of *Sirius Analytical Instruments* (Forrest Row, UK) [10]. All titrations were conducted under Ar to exclude atmospheric CO_2 and at $25 \pm 0.1°$. Six separate semi-aq. solns. in 18.87–36.49% (*w*/*w*) MeOH of 0.43–0.75 mM sildenafil citrate and 0.15M KCl (to adjust ionic strength) were acidified with 0.1M HCl to pH 2. The solns. were then titrated with standardized KOH to pH 12. *Bjerrum* difference plots were deduced from each titration curve and used to calculate approximate values of the apparent ionization constants in the mixed-solvent p_sK_a [11]. These approximate values were used as 'seed' values in a weighted nonlinear least-squares procedure to refine the pK_a values by including previously determined pK_a values of citric acid as unrefined contributions ($pK_{a1} = 5.60$, $pK_{a2} = 4.32$, and $pK_{a3} = 2.96$). Then the refined values were extrapolated to zero co-solvent by the *Yasuda-Shedlovsky* procedure.

3.3. *Determination of the Standard Partition Coefficients.* The standard partition coefficients in octan-1-ol/H_2O and 1,2-dichloroethane/H_2O were measured by the same potentiometric method. Sildenafil solns. (0.5–0.8M) with KCl (0.15M), initially acidified with HCl to pH 2, were titrated from pH 2 to 11 in the presence of different volumes of org. phase (volume ratio oil/H_2O between 0.02 and 1). Titrations were conducted under Ar at $25 \pm 0.1°$.

The values were estimated from difference *Bjerrum* plots [12] and refined by a nonlinear least-squares procedure [13] by including previously determined pK_a values as unrefined contributions. The detailed experimental procedures can be found elsewhere [14][15].

3.4. *Conformational Calculations.* An adaptation of quenched molecular-dynamics (QMD) simulations [16–18] was used to explore the main valleys of a conformational space. Starting geometries of neutral and charged sildenafil were built according to the CONCORD algorithm [19] and their geometry optimized with the TRIPOS force field [20] with *Gasteiger-Marsili* partial atomic charges [21] to remove initial high-energy interactions. High-temperature molecular-dynamics (MD) calculations were carried out at 2000 K. The 200 randomly selected conformers were energy-optimized, and their conformational similarity was investigated by comparing all pairs of conformers. When two conformers were considered similar according to the force-field energy and the RMS distance difference calculated by the option MATCH of SYBYL over all heavy atoms and polar H-atoms, the conformer with the higher energy was eliminated. All calculations were run on *Silicon Graphics Indy R4400*, *O2 R500*, or *Origin2000* workstations with the SYBYL 6.5 molecular modelling package (*Tripos Associates*, St. Louis, MO, USA).

4. Results and Discussion. – 4.1. *Potentiometric Determination of Dissociation Constants and Standard Partition Coefficients. Acid-Base Equilibria of Sildenafil.* By analogy with its constituting moieties pyrimidinone, pyrazole, and piperazine, sildenafil was expected to have a maximum of four ionization sites [22–24]: an acidic site (pyrimidinone A), a strongly basic site (B), and two sites of uncertain basicity (C and D) as shown in *Fig. 2*. Potentiometry showed only two ionization constants.

In *Fig. 3*, the first constant (pK_{a1}) had a positive slope characteristic of an acidic group A. The second constant (pK_{a2}) had a negative slope and was attributed to the basic group B [15]. The two pK_a values of sildenafil are 9.12 and 6.78 for the ionization sites A and B, respectively; the acid-base equilibria are depicted in *Fig. 4*.

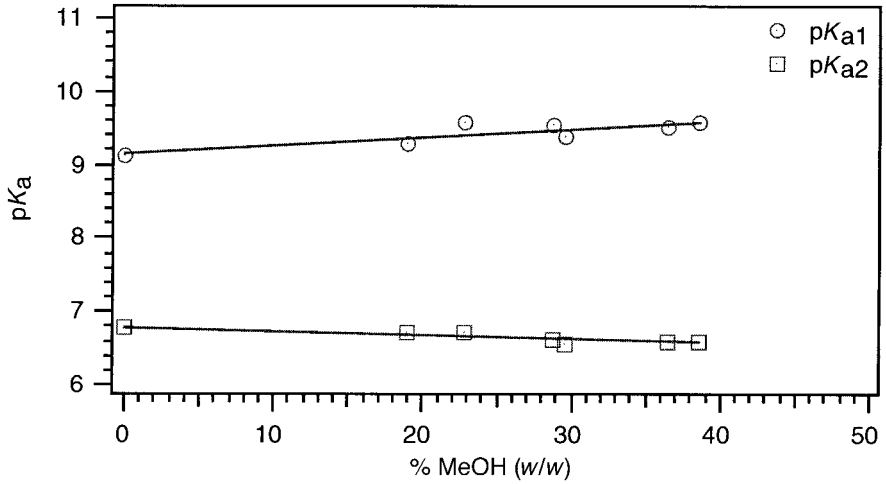

Fig. 3. *pK_a Variations of sildenafil as a function of MeOH concentration*

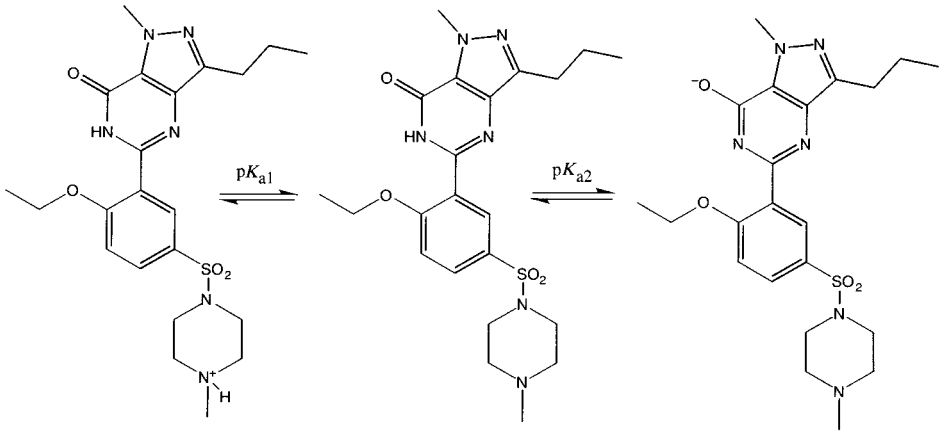

Fig. 4. *Ionization equilibria of sildenafil*

From these data, a distribution of ionic species in H_2O was calculated, showing that between pK_{a1} and pK_{a2}, the predominant species is neutral sildenafil. Around pH 8, the two equilibria described in *Fig. 4* overlap, but the proportion of the zwitterion is too small to be taken into account. Hence sildenafil behaves as an ordinary ampholyte [25] as shown in *Fig. 5*.

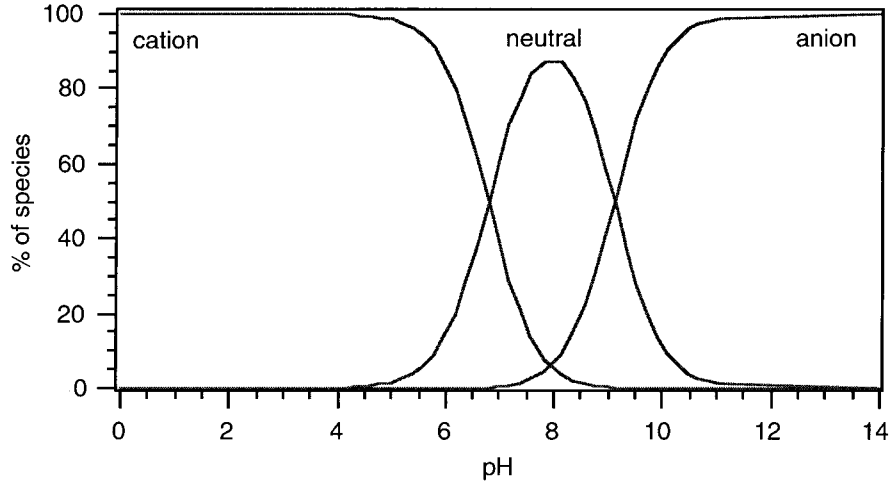

Fig. 5. *Distribution of the ionic species of sildenafil*

Lipophilicity of Sildenafil. The pH-metric method was used to study the lipophilicity of sildenafil in octan-1-ol/H_2O and DCE/H_2O. Only the partition coefficient of the cationic form (C) and the standard partition coefficient of the neutral form (N) of sildenafil were measured, since the log *P* of the anionic form was too low to be detected by potentiometry. The results are given in *Table 1*.

Table 1. *Standard Partition Coefficients of the Neutral* (N) *and Partition Coefficient of the Cationic Species* (C) *of Sildenafil Measured by Potentiometry in Octan-1-ol/0.15*M *KCl and in 1,2-Dichloroethane* (DCE)*/0.15*M *KCl*

	Octanol/H_2O	DCE/H_2O
$\log P^{N^\circ}$	3.18 ± 0.01	3.75 ± 0.05
$\log P^{C}$	0.32 ± 0.10	-0.11 ± 0.17

The difference between $\log P^{N^\circ}_{OCT}$ and $\log P^{N^\circ}_{DCE}$ for the neutral species ($\Delta\log P^{N^\circ}_{OCT-DCE} = -0.57$) is negative, meaning that the H-bond donating capacity of the neutral sildenafil is not expressed in the DCE | H_2O system [5][26]. This result suggests the existence of an intramolecular H-bond between the H-atom of the acidic N-atom at the pyrimidinone moiety and the O-atom of the ethoxy group at the phenyl ring. This hypothesis was confirmed by a conformational study of sildenafil by quenched molecular dynamics (QMD). The conformers of neutral sildenafil could be separated into two groups according to the distance between the ethoxy O-atom and the acidic H-atom at the pyrimidinone moiety. A distance < 2 Å, corresponding to the formation of an internal H-bond, is typical for conformers having the lowest energy (*Fig. 6*). The conformers with no internal H-bond have higher energies.

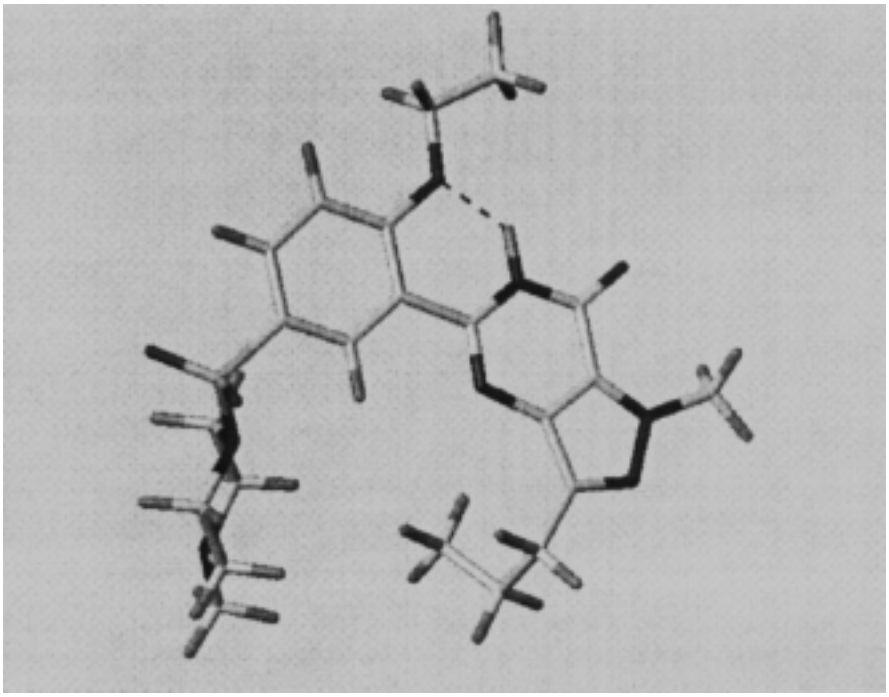

Fig. 6. *Visualization of the internal H-bond of neutral sildenafil*

In the DCE | H_2O system, the difference between the neutral and cationic forms of sildenafil ($diff(\log P^{N-I}) = 3.65$) is smaller than the difference observed for simpler protonable compounds. This result suggests that the positive charge in the cationic sildenafil is less localized than for regular cations [27] [28]. This hypothesis is supported by molecular-dynamics simulations. Indeed, for protonated sildenafil, the minimal-energy conformers are characterized by two internal H-bonds as shown in *Fig. 7*. The H-bond between the ethoxy O-atom and the acidic H-atom at the pyrimidinone moiety is present as in the neutral sildenafil, but an additional H-bond exists between one O-atom of the sulfonamide function and the proton of the basic N-atom.

Measurements and molecular-dynamics simulations confirm the capacity of neutral and cationic sildenafil to form internal H-bonds and may explain their higher-than-expected lipophilicity for the two electrical species of sildenafil. The expected polarity of the anionic sildenafil in which no internal H-bond is possible, should also be noted.

4.2. *Ionic-Partition Diagram of Sildenafil and Its Interpretation*. As the drug is a citrate salt, sodium citrate was first examined over the full pH range. Due to its high hydrophilicity, citrate did not appear in our potential window and, therefore, could not interfere with the signals of sildenafil. The partition diagram of sildenafil was calculated from the results above and is shown in *Fig. 8*. Sildenafil being an ampholyte, its diagram is divided into three regions with the cation predominating in the first region (I), the neutral species in the second region (II), and the anion in the third region (III).

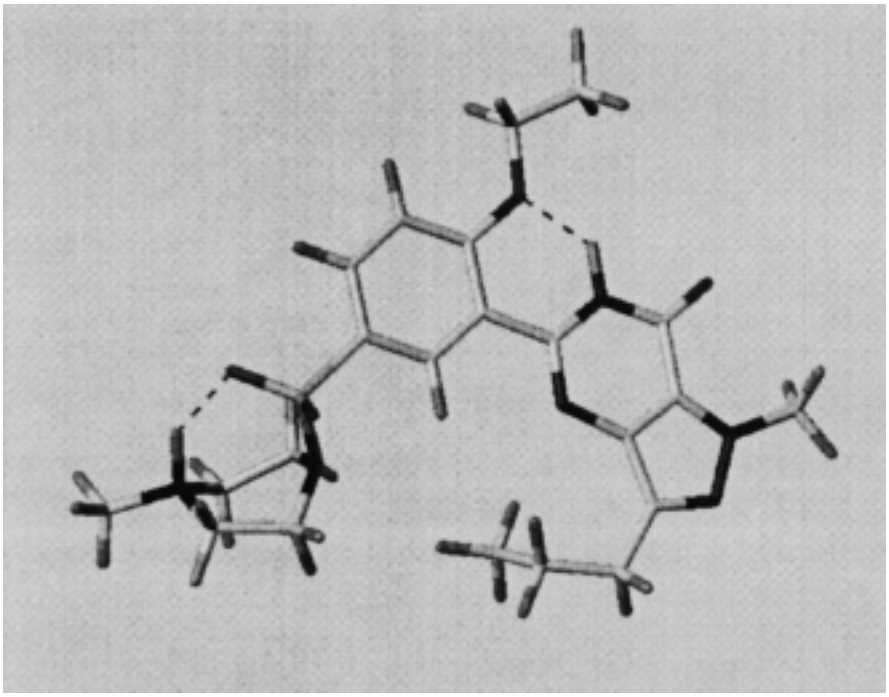

Fig. 7. *Visualization of the two internal H-bonds of protonated sildenafil*

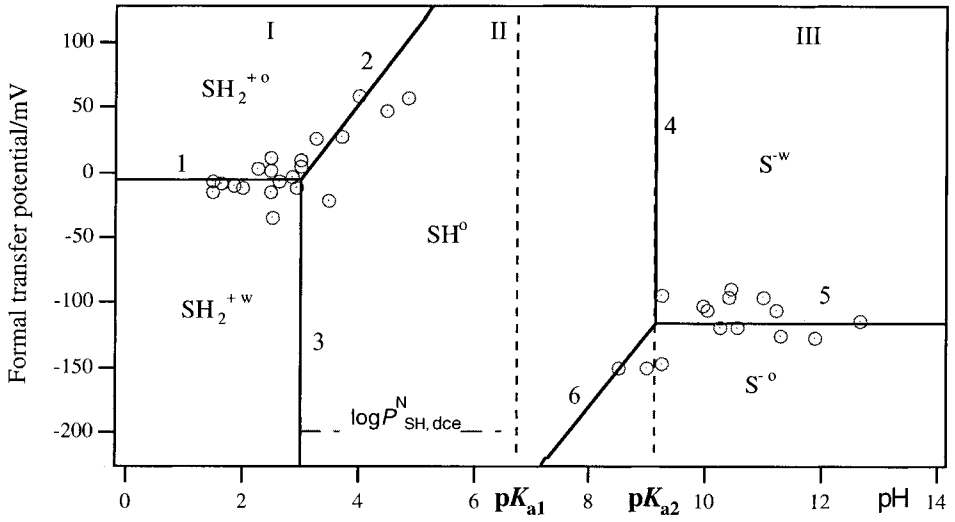

Fig. 8. *Ion-partition diagram of sildenafil showing the variations of the formal transfer potential with aqueous pH.* The solid lines represent the experimental boundaries while the dotted lines are the calculated boundaries for the cation.

Region III is a typical diagram of a weak acid [6] with three boundary lines (4, 5, and 6). Line 4 is the equiconcentration boundary of both the neutral and the anionic species. Line 5 corresponds to the partition of the anion from the aqueous to the organic phase. At pH lower than the pK_a, the concentration of the neutral species is no longer negligible, and the observed decrease in the formal transfer potential (line 6) corresponds to a facilitated proton-transfer reaction in which neutral sildenafil acts as a proton acceptor, facilitating the transfer of a proton into the organic phase.

On the other hand, neutral sildenafil being lipophilic, the boundary lines 2 and 3 of the first region (I) are shifted according to the theoretical model discussed above. It must be stressed that the lipophilic character of sildenafil is strongly expressed under our experimental conditions, since the volume of the two phases is equal. The observed shift is, therefore, caused by the simultaneous occurrence of sildenafil lipophilicity and of experimental conditions.

The formal transfer potentials are directly linked to the standard partition coefficient of the various species. In fact, the standard partition coefficient of the neutral species is related to the *Gibbs* energy $\Delta G_{tr,i}^{o,w\rightarrow o}$ by *Eqn. 10*.

$$\log P_i^{N^o} = \log\left(\frac{a_i^o}{a_i^w}\right) = -\frac{\Delta G_{tr,i}^{o,w\rightarrow o}}{RT\ln 10} \tag{10}$$

Table 2 summarizes the physicochemical parameters obtained in this study.

Table 2. *Physicochemical Parameters of the Various Species of Sildenafil Obtained by Cyclic Voltammetry in DCE*

	$\Delta_o^w \phi_i^{o'}$/mV[a])	$\log P_{DCE}^o$[b])	*diff*($\log P^{N-I}$)[c])
Cation	– 5.5	0.1	3.65
Anion	– 115.7	– 2.7	6.45

[a]) Formal transfer potential of the charged species. [b]) Standard partition coefficient with DCE. [c]) *diff*($\log P^{N-I}$) = $\log P^N - \log P^I$ = difference between the partition coefficient of the neutral species $\log P^N$ and that of the cation or anion $\log P^I$.

5. Pharmacokinetic Implications. – In contrast to a recent study [4], sildenafil is shown here to be an ordinary ampholyte with modest basicity ($pK_{a1} = 6.78$) and weak acidity ($pK_{a2} = 9.12$). The compound is thus mostly neutral at physiological pH, with little of the cation and zwitterion present. Both the neutral and cationic forms have a relatively high lipophilicity due, in part, to internal H-bonds.

In humans and laboratory animals, sildenafil undergoes complete intestinal absorption and extensive biotransformation, and shows good tissue affinity and significant plasma-protein binding [4]. This pharmacokinetic behavior is consistent with the extensive physicochemical characterization of the drug reported here.

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