Effects of Charge and Intramolecular Structure on the Lipophilicity of Nitrophenols

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Abstract: The lipophilicity of a series of phenolic compounds was studied in the 1,2-dichloroethane (1,2-DCE)/water system. Cyclic voltammetry at the ITIES was used to study the transfer characteristics of the charged species, and their partition coefficient was deduced from their formal transfer potential. For the neutral species, log P_{DCE} values were measured by a two-phase pH-metric method. The results are compared to those previously obtained in octanol/water and by linear solvation energy relationships (LSER) in the two solvent systems. It is shown that nitrophenols with intramolecular H-bonding deviate from the solvatochromic equation for the 1,2-DCE/water system, and discrepancies between both approaches are discussed on the basis of conformational and steric effects. When charged however, all the species have approximately the same partition coefficients because the effect of the intramolecular H-bond disappears and the differences in measured lipophilicity arise from the variation of the intramolecular charge delocalization due to resonance equilibria. Some biological implications of these properties are discussed.

I. Introduction

Nitro-substituted phenols are weak hydrophobic acids for which the toxicity is due primarily to their ability to uncouple the cellular energy production. 2,4-Dinitrophenol (2,4-DNP), 3-nitrophenol (3-NP), and, to a lesser degree, 2-nitrophenol (2-NP) and 4-nitrophenol (4-NP) increase the ATPase activity of mammalian skeletal muscle in the presence of different cations.[1] 2,4-DNP also acts as uncoupler for oxidative phosphorylation in mitochondria,[2] and in the presence of Mg^{2+}, it mimics the effect of actin.

The study of their lipophilicity is of particular interest to understand their mechanisms of skin permeability and hence their biological activity.[3] Phenolic compounds may be transported across the skin barrier by diffusion through the appendages, hair follicles, sweat glands, and sebaceous glands or by diffusion through the stratum corneum itself.[4] Until now, the intrinsic partition coefficient of phenols has been determined in the reference *n*-octanol/water system,[5] and their permeability has been classified.[4] However, the target sites of many toxic compounds are biological membranes constituted of distinct hydrophilic and hydrophobic regions, where both neutral and charged species may interact with the membrane.[6] Thus, studies taking only neutral species into consideration to investigate the partitioning behaviors can be too restrictive.

The partition of ionic species has been taken into account only recently in the evaluation of the lipophilicity of drugs and compounds of pharmaceutical interest. Then it has been possible to measure the partitioning of charged species by studying the variation of the distribution coefficient of ionizable compounds as a function of pH.

Electrochemistry at the interface between two immiscible electrolyte solutions (ITIES) appears as a new efficient technique in the field of lipophilicity to study the transfer and partition mechanisms of ionic compounds.[7,8] At thermodynamic equilibrium, the distribution of an ion is determined by the equality of its electrochemical potentials in the two phases, and it is given by the Nernst equation at the ITIES,[9] which can be expressed in terms of the partition coefficient log P_I (log P of ion) as

$$\log P_I = \log\left(\frac{c_I^o}{c_I^w}\right) = \frac{zF}{RT \ln 10}(\Delta_o^w\phi - \Delta_o^w\phi_I^{0'}) \qquad (1)$$

where $\Delta_o^w\phi$ is the applied Galvani potential difference between the organic (o) and the aqueous (w) phase, c_I is the concentration of the ion I, and $\Delta_o^w\phi_I^{0'}$ is the formal transfer potential of I.

In dilute solutions, the formal ion-transfer potential is related to the standard Gibbs energy of transfer by

$$\Delta_o^w\phi_I^{0'} = \frac{-\Delta G_{tr,I}^{0,w\rightarrow o}}{z_I F} = \frac{-RT \ln 10}{z_I F} \log P_I^{0'} \qquad (2)$$

$\Delta G_{tr,I}^{0,w\rightarrow o}$ represents the difference of standard Gibbs energy of solvation between water and the organic phase and $P_I^{0'}$ repre-

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sents the formal ionic partition coefficient when the interface is not polarized, so that it is a direct measure of lipophilicity.

For neutral species, log P_N is a constant independent of the potential, defined as the activity ratio between the two phases:

$$\log P_N = \log \frac{a_N^o}{a_N^w} = -\frac{\Delta G_{tr,N}^{0,w\rightarrow o}}{RT \ln 10} \quad (3)$$

Although *n*-octanol is the most commonly used solvent in lipophilicity measurements, 1,2-DCE is chosen in our electrochemical experiments because the range of Gibbs energy of transfer from water to octanol for ionic species is restricted. Furthermore, 1,2-DCE/water appears as an interesting system to predict the biodistribution because the partition coefficients obtained in this solvent system encode information on the solute−solvent interactions via both H-bond donor and acceptor capacity. Solvatochromic parameters allow assessing the intermolecular forces governing the partition mechanisms of neutral organic solutes: nonspecific dipolarity−polarizability π^*, hydrogen-bond donor acidity α, hydrogen-bond acceptor basicity β, and the van der Waals volume (V_w). The effect of each of these parameters is introduced by a linear solvation energy relationships (LSER). The partition coefficient for a solvent pair can be expressed as[10]

$$\log P = vV_w + p\pi^* + a\alpha + b\beta + c \quad (4)$$

A recent solvatochromic analysis of 1,2-DCE/water yielded a LSER[11] demonstrating the difference between intermolecular interactions encoded in octanol/water and 1,2-DCE/water partition coefficients.[12,13] For 1,2-DCE, LSER gives the following equation:

$$\log P_{DCE} = (3.4 \pm 0.4) \times 10^{-2} V_w - (2.75 \pm 0.38)\alpha - (5.66 \pm 0.93)\beta + (0.72 \pm 0.40)$$
$$n = 37;\ q^2 = 0.96;\ r^2 = 0.97;\ s = 0.27;\ F = 330 \quad (5)$$

(n is the number of compounds, q^2 the cross-validated correlation coefficient, r^2 the square correlation coefficient, s the standard deviation, and F the Fischer's test) where only three terms are important, namely, the volume term and the H-bonding donor and acceptor capacities. Correlations between log P_{OCT} and log P_{DCE} could be established following Collander's type equation considering H-bond donors and non-H-bond donors solutes separately (eqs 6 and 7, respectively):

$$\log P_{OCT} = (0.92 \pm 0.14)\log P_{DCE} + (0.95 \pm 0.18)$$
$$n = 19;\ q^2 = 0.93;\ r^2 = 0.95;\ s = 0.28;\ F = 310 \quad (6)$$
$$\log P_{OCT} = (0.79 \pm 0.13)\log P_{DCE} - (0.26 \pm 0.23)$$
$$n = 25;\ q^2 = 0.88;\ r^2 = 0.90;\ s = 0.35;\ F = 200 \quad (7)$$

These two regression lines differ in intercepts mainly because the contribution of the H-bond donor α term differs in the two biphasic solvent systems. Their slopes are not identical due to the slightly different contributions of the polarizability π^* and the H-bond acceptor β term. Equations 6 and 7 can be used in a first approximation to estimate log P_{DCE} from the partition coefficients in octanol/water. The comparison between the log P_{DCE} predicted by eqs 6 and 7 and the experimental values and/or the position of compounds in the graph log P_{OCT} vs log P_{DCE} yields an important indicator of intramolecular effects.

In the present paper, partition coefficients of mono- and disubstituted nitrophenols are compared to these solvatochromic relations. The results are discussed from a structural point of view showing how the position of the nitro groups affects lipophilicity. For the deprotonated species, log $P_I^{0'}$ is much more negative than for neutral compounds, and it will be shown that the change of lipophilicity between charged and neutral species can be qualitatively explained by the delocalization of the charge and by the loss of intramolecular H-bond stabilization. This work relies on ionic partition diagram representations[14] and agrees with a previous study by Reymond et al. dealing with the influence of charge and delocalization effects on the lipophilicity of protonable compounds.[15]

II. Experimental Section

All reagents were purchased from *Fluka* and were analytical grade or purer. The aqueous phase was deionized water (Milli-QSP reagent water system, Millipore), whereas 1,2-dichloroethane (1,2-DCE) of the highest available purity (Merck, Germany) was used as the organic phase and was handled with all necessary precautions to avoid inhalation and skin contact.[16] The organic supporting electrolyte was prepared by metathesis of bis(triphenylphosphoranylidene) ammonium chloride (BTPPACl) and potassium tetrakis(4-chlorophenyl)borate (KTPBCl) (Lancaster, U.K.) in a 1:1 methanol/water mixture, providing BTPPATPBCl precipitate, which was filtered and recrystallized twice from methanol before use. LiCl was the aqueous electrolyte, and HNO_3 or LiOH was added to the aqueous phase of electrochemical cell 1 to fix the pH at the desired value. The experiments were carried out at room temperature.

Ag	AgCl	10 mM LiCl 1mM BTPPACl	10mM BTPPATPBCl	1mM X 10mM LiCl +HNO_3 or LiOH to desired pH	AgCl	Ag

Cell 1

where X stands for each compound studied.

For 2,4-dinitrophenol (DNP), experiments were repeated using cell 2, where DNP^- was dissolved in the organic phase after formation of a 1,2-DCE-soluble salt with $BTPPA^+$.

Ag	AgCl	10 mM LiCl 1mM BTPPACl	10mM BTPPATPBCl 1mM BTPPADNP	10 mM LiCl +HNO_3 or LiOH to desired pH	AgCl	Ag

Cell 2

The electrochemical apparatus used was a homemade four-electrode potentiostat in a Faraday cage to minimize background electrical noise. The scanning of the applied potential was performed by a waveform generator PPR1 (HiTek Instruments, U.K.), and the current−potential curves were monitored on an X-Y recorder (Bryans Instruments, U.K.). Each measurement was referenced by adding a few drops of 10 mM $(TMA)_2SO_4$ to the aqueous phase, and the formal ion-transfer potentials were deduced from the following relation between the measured half-wave potentials and the absolue formal transfer potentials of the ion I

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Table 1. Partition Coefficients in Octanol/Water and 1,2-DCE/Water Systems of Various Nitrophenols in Their Neutral Form

	phenol	2-NP	3-NP	4-NP	2,4-DNP	2,5-DNP
log P_{OCT}[a]	1.46	1.77	2.00	1.96	1.52	1.75
log P_{DCE}[b]	0.61 ± 0.02[c]	2.81 ± 0.02	0.92 ± 0.02	0.72 ± 0.03	2.46 ± 0.05	2.49 ± 0.03

[a] Intrinsic partition coefficients from ref 3. [b] Measured by the Sirius pH-metric method. [c] From ref 11.

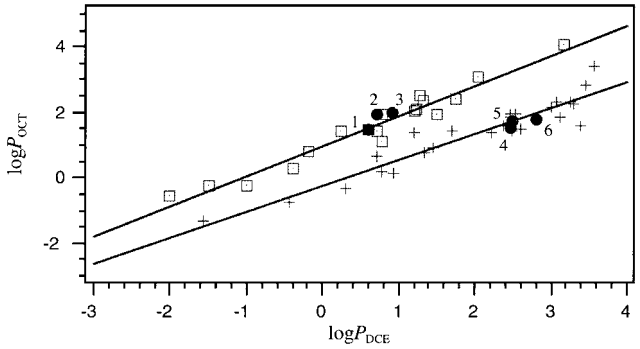

Figure 1. Experimental results obtained for nitrophenols (1 = phenol, 2 = 4-nitrophenol, 3 = 3-nitrophenol, 4 = 2,4-dinitrophenol, 5 = 2,5-dinitrophenol, 6 = 2-nitrophenol) represented on the graph log *P* in 1,2-DCE vs log *P* in octanol. The two regression lines stand for H-bond donor solutes (□) and non-H-bond donor solutes (+) (eq 6 and 7, respectively).

and TMA^+:

$$\Delta_o^w\phi_I^{1/2} - \Delta_o^w\phi_I^{0'} = \Delta_o^w\phi_{TMA^+}^{1/2} - \Delta_o^w\phi_{TMA^+}^{0'} \quad (8)$$

where the formal potential of transfer of TMA^+ is expressed on a tetraphenylarsonium tetraphenylborate (TATB) scale[17] ($\Delta_o^w\phi_{TMA^+}^{0'}$ = 160 mV[18]). The partition coefficients of the neutral species and the acid/base dissociation constants were determined by a pH-metric two-phase titrator (Sirius PCA101, Sirius Analytical Instruments Ltd., Riverside, U.K.) . The organic phase presaturated with the aqueous phase was added manually to the titration vial. The distribution coefficient (log *D*) was measured as a function of aqueous pH, and the partition coefficients of the neutral species were deduced by a fitting procedure.

III. Results and Discussion

III.1. Partition Coefficients of Neutral Species. The log P_{DCE} values obtained for the neutral form of the nitrophenols studied are given in Table 1, where those found in the literature for the octanol/water system have been added.

Two classes emerge from the position of nitrophenols in the graph log P_{OCT} vs log P_{DCE} shown in Figure 1. Only phenol, 3-nitrophenol, and 4-nitrophenol approach the H-bond donors LSER regression line, while 2-nitrophenol, 2,4-dinitrophenol, and 2,5-dinitrophenol approach the H-bond acceptors LSER regression line. For the first class, the lower log P_{DCE} relative to log P_{OCT} is consistent with the fact that 1,2-DCE neither has a permanent dipole moment nor forms H-bonds with solutes. This effect manifests itself in eq 5 by the high negative values of the coefficients related to the hydrogen-bond capacities α and β.

However, log P_{DCE} values for the second class are significantly larger than expected for compounds having a H-bond-donating group, indicating that other important factors are not encoded into the LSER parameters. The position of these compounds near the regression line of solutes having only H-bond acceptor substituents indicates that the H-bond donor capacity of these solutes is not expressed in the 1,2-DCE/water biphasic system. The compounds of the second class are all phenols substituted by a nitro group in the *ortho* position able to form a strong intramolecular hydrogen bond between the oxygen of the nitro group and the hydrogen of the phenolic function.

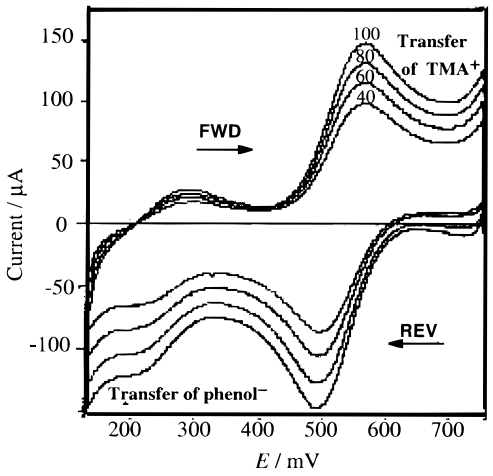

Figure 2. Typical cyclic voltammogram of the transfer from water to oil of $phenol^-$ (on the left) and of the reference ion TMA^+ (on the right) at four scan rates (40, 60, 80, and 100 mV/s) for pH 11.70.

The strong stabilization of this intramolecular hydrogen bond in nonpolar environments, already described for the partitioning of ortho-nitrophenols in alkane/water systems,[19] renders these compounds much more lipophilic than other nitrophenols in the 1,2-DCE/water system, confirming previous experiments[20] and ab initio MO calculations.[21] In the octanol/water system, the large amount of water existing in the organic phase offers additional possibility of intermolecular hydrogen-bond interactions to the phenolic group. Thus the influence of the intramolecular hydrogen bond on partitioning is largely diminished, leading to a different ranking of theses compounds (Table 1).

III.2. Partition Coefficients of Charged Species. The transfer behavior of the deprotonated forms of all compounds was studied by cyclic voltammetry employing cell 1. Figure 2 shows a typical voltammogram obtained at pH = 11.70 and various scan rates. For the transfer of phenolate and of the reference ion TMA^+ across the water/1,2-DCE interface, the forward scan goes from left to right, and the reverse scan from right to left.

The formal transfer potentials were deduced from the voltammograms and were further used to determine the partition coefficients. The results obtained are only given in the form of ionic partition diagrams that show the domains of predominance of each species either in the aqueous or in the organic phase.[14] The transfer of the phenolic species was studied over the whole pH range, and the pH dependence of the formal transfer potential is displayed in Figure 3 for two of these compounds.

This potential−pH representation illustrates quite well the lipophilicity of the different species. The boundary lines are

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Table 2. Physicochemical Parameters Obtained by Cyclic Voltammetry for the Transfer of Phenols and Phenolates across the 1,2DCE/Water Interface

	phenol	2-NP	3-NP	4-NP	2,4-DNP	2,5-DNP
pK_a^{w} [a]	9.99	6.92	8.10	6.90	3.96 ± 0.04	4.97 ± 0.01
$\Delta_o^w\phi_I^{0'}$/mV	-138 ± 8	-121 ± 9	-144 ± 10	-154 ± 8	-101 ± 9	-137 ± 10
$\log P_I^{0'}$	-2.3 ± 0.1	-2.0 ± 0.1	-2.4 ± 0.2	-2.5 ± 0.1	-1.7 ± 0.1	-2.3 ± 0.2
diff($\log P_{I-N}^{0'}$)	-2.9 ± 0.1	-4.8 ± 0.1	-3.3 ± 0.2	-3.3 ± 0.1	-4.2 ± 0.1	-4.8 ± 0.2
partial charge	-0.53	-0.42	-0.49	-0.45	-0.34	-0.39

[a] Measured by the Sirius pH-metric method.

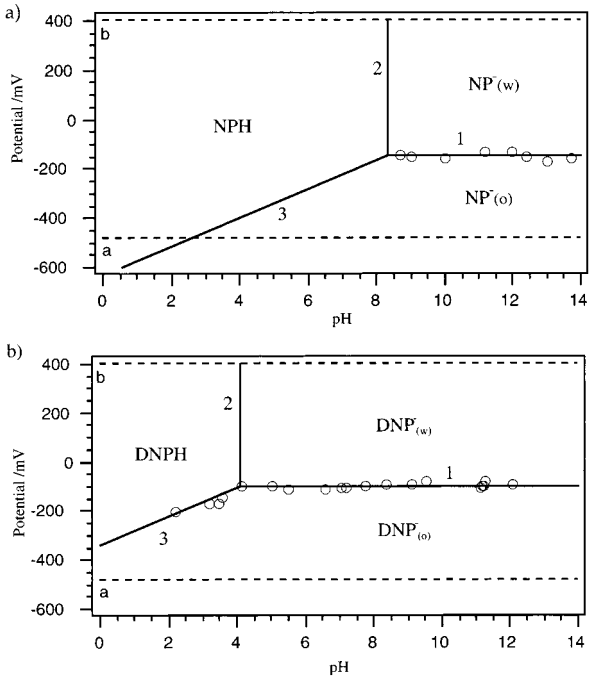

Figure 3. Ionic partition diagrams of (a) 4-nitrophenol and (b) 2,4-dinitrophenol at 25 °C in 1,2-DCE/water. Lines a and b are respectively the lower and the upper detection limits for electrochemical cells 1 and 2.

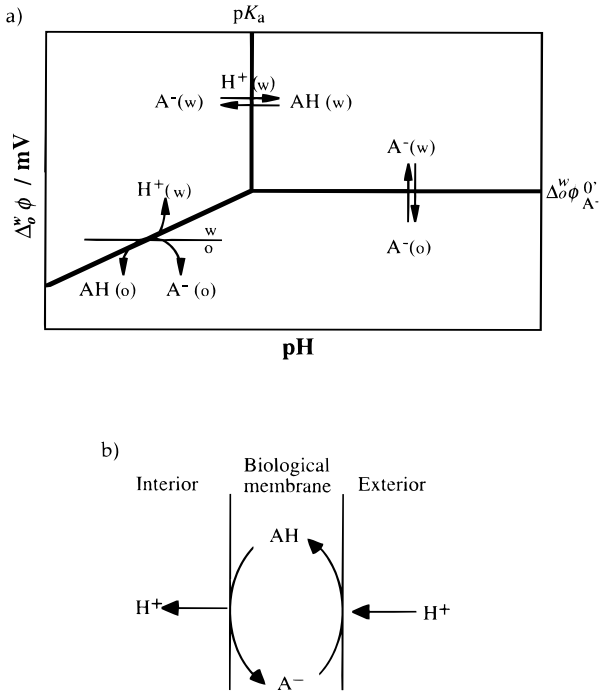

Figure 4. Schematic representation of an ionic partition diagram with the corresponding transfer mechanisms (a) and transposition of the assisted proton transfer to the biological facilitated transport through biological membranes by an uncoupler (AH and A^-) (b).

directly correlated to the ion-transfer reactions and to the associated energy needed to cross the 1,2-DCE/water interface. For pH values above pK_a the anionic species are predominant. The recorded current results from a displacement of the thermodynamic equilibria at the interface upon increase of potential generating a flow of ions across the 1,2-DCE/water interface. In this range of pH, the horizontal boundary line in Figure 3 corresponds to the formal transfer potential. For pH values lower than the pK_a, the boundary line results from a facilitated proton transfer in which the deprotonated species, present in the organic phase, acts as a proton acceptor, as shown in Figure 4a.

The potential resulting from the proton transfer is pH dependent, as illustrated by the line equation[2]

$$\Delta_o^w\phi = \Delta_o^w\phi_{A^-}^{0'} - \frac{RT}{F}\ln\left(\frac{c_{H^+}^w c_{A^-}^o}{c_{AH}^o}\right) \qquad (9)$$

This particular mechanism is of great importance for the biological activity of nitrophenol, as discussed in section III.3. The various physicochemical parameters deduced from our experiments are summarized in Table 2.

The comparison of the various $\log P_I$ values obtained shows that the two classes observed for the neutral species do not remain, the position of the nitro group affecting the lipophilicity of phenolates in smaller proportion than for the neutral species. Since an intramolecular H-bond does not exist in deprotonated species, the lipophilicity variations of the phenolates should be due to variations in the charge distribution. As indicated by the partial Mulliken charges (Table 2), the negative charge on the phenolate oxygen is lowered by *ortho* and *para* nitro groups due to well-known inductive and resonance effects. This charge delocalization leads to ions (2,4-DNP, 2-NP) that are more lipophilic than the unsubstituted phenolate.

For the other compounds, the charges are too similar to be fully discussed, resulting from a balance of effects.

For phenol itself, the difference in log *P* between neutral and anionic species, diff($\log P_{I-N}^{0'}$) of -2.9, is smaller than the values of -5 usually found for the 1,2-DCE/water system. This difference is due to the delocalization of the phenolate charge in the aromatic ring, which is in agreement with Born's solvation model.[15] Indeed, the charge delocalization induces weaker attractive forces between the ions and solvating water molecules, so that the replacement of water by the organic solvent molecules upon transfer is easier than with well-localized charges.

For the 3-NP and 4-NP, diff($\log P_{I-N}^{0'}$) are both equal to -3.3, reflecting both the increase in ion polarity due to topological effects and the small increase of the lipophilicity of the neutral form.

For the *ortho*-derivatives, the diff($\log P_{I-N}^{0'}$) is larger; it includes principally the enhanced lipophilicity of neutral species

due to the intramolecular bonding. The smaller value (−4.2) for 2,4-DNP reflects the larger stabilization of its phenolate form.

In conclusion, much care must be taken when using diff(log $P^{0'}_{I-N}$) to interpret solvation differences since it may encode intramolecular effects operating differently on the neutral and ionized forms.

III.3. Biological Implications. The results reported here may help in understanding some biological properties of nitrophenols, in particular their cutaneous permeability and their uncoupling effects.

The skin and stratum corneum permeability of phenols shows that phenol and 4-NP have a low and almost identical permeability, in contrast to 2-NP and 2,4-DNP, for which the permeability is high and comparable. This trend is not reflected in the log P_{OCT} scale, but is indeed found in the log P_{DCE} results (Table 1). Such a result once more confirms the key role played in membrane permeation by the H-bonding capacity, as encoded in log P_{DCE} and not in log P_{OCT} values.

Nitro-substituted phenolic compounds, like numerous organic substances, are known to act as uncouplers of oxidative phosphorylation in mitochondria.[22] In the presence of an uncoupling agent, electron transport and H^+ pumping continue at a fast rate, but no H^+ gradient is generated. The explanation for this effect is that these uncouplers are soluble in energy-transducing membranes such as mitochondria and chloroplasts, where they act as protonophores. Thus, they provide an alternative pathway to the ATP synthesis for the flow of protons across the inner mitochondrial membrane. As a result of this "short-circuiting",[23] the proton-motive force is dissipated completely, and ATP can no longer be produced.[22,24]

Both the neutral and the charged species can diffuse across the lipid bilayer by passive diffusion. Because of the electrochemical proton gradient, nitrophenols such as 2,4-DNP carry more protons in than out, until the proton-motive force has completely disappeared. The effect of the interfacial potential difference at the two membrane/solution interfaces has been explicitly taken into account to derive an equation for the facilitated transport of protons across biological membranes when an uncoupler is added to the medium on the inside or on the outside of the membrane.[8] Results obtained at the nitrobenzene/water interface[2] indicate that the physicochemical properties of uncouplers, such as the dissociation constant in the membrane, the standard ion-transfer potential of the anionic form at the membrane/aqueous solution interface, and the proton dissociation constant in the aqueous medium, are the main factors determining their uncoupling activity (see Figure 4b). These results agree well with those obtained here at the 1,2-DCE/water interface since ionic partition diagrams in Figure 3 show that a relatively large potential difference is needed to let charged species cross the membrane. Indeed, compounds containing highly lipophilic groups (such as *tert*-butyl, −Cl, $-CF_3$) are generally more potent uncouplers[25] because they can bind better to mitochondria and remain within the membrane during their uncoupling action. Moreover, from Figure 3, it is deduced that compounds of higher pK_a values should be more effective, since the pH domain where they act as protonophore is located at values closer to 7. This confirms previous quantitative structure−activity relationship studies[26−29] showing the importance of lipophilicity and acidity for uncoupling activity.

IV. Conclusion

This study illustrates the influence of intramolecular interactions on the partition of ionizable compounds. The 1,2-DCE/water system appears as a promising means to identify intramolecular H-bonds acting on the lipophilicity of neutral species and the charge delocalization diminishing the polarity of ions. It also underlines that the comparison between log P_{OCT} and log P_{DCE} is suitable to identify intermolecular interactions not included in solvatochromic equations.

Moreover, the electrochemical method allows the comparison between neutral and charged species in order to better understand transfer phenomena between two immiscible phases, which is a key factor for the pharmacokinetic behavior of drugs. This study points out the complexity of the distribution of ionizable compounds and offers a new approach to relate the structure of such compounds to their passive transport across biological membranes.

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