The Apparent Lipophilicity of Quaternary Ammonium Ions Is Influenced by Galvani Potential Difference, Not Ion-Pairing: A Cyclic Voltammetry Study

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Purpose. This work examines whether ion-pairing contributes to the apparent lipophilicity of cations, which is seen by a shake-flask or titrimetic method to be influenced by the nature and concentration of counter-ions.

Methods. To solve this problem, the lipophilicity of several quaternary ammonium drugs was measured by cyclic voltammetry in the 1,2-dichloroethane/water system. The standard ionic partition coefficient values so obtained (log $P^{\circ,C}_{\text{dce}}$) were correlated with log P_{oct} values calculated by the CLOGP algorithm for the respective neutral molecules.

Results. The standard (i.e., intrinsic) lipophilicity values are shown to depend on a, the structure of the ion (nature, volume, charge), and b, on the Galvani potential difference at the ITIES (interface between two immiscible electrolyte solutions).

Conclusions. The standard lipophilicity values were not influenced by counter-ions. In contrast, simulations showed that the increased apparent lipophilicity of cations, as measured by the shake-flask method in the presence of lipophilic anions, is fully accounted for by the resulting increase in the Galvani potential difference.

KEY WORDS: quaternary ammonium ions; lipophilicity; ion-pairing; cyclic voltammetry; ITIES.

INTRODUCTION

A large majority of pharmaceutical compounds are ionized at physiological pH and may show decreased therapeutic usefulness due to poor oral absorption caused by their charge. In attempts to improve the absorption of ions, several studies have shown that the partition of ionic drugs can be increased by the addition of lipophilic counter-ions (1–7). The physicochemical mechanisms governing this particular type of adjuvant-mediated transfer remain elusive, but several explanations have been given such as a specific ion-membrane interactions which could modify the structure of the membrane and even damage it (1), or the formation of lipophilic ion-pairs. This second hypothesis is the most popular and the most extensively studied (8–10). But due to a variety of experimental artifacts, we still lack conclusive evidence that highly ionized drugs may be absorbed in the form of ion-pairs. Furthermore, the literature on ion-pair permeation remains controversial (11).

To shed light on this problem, we examined the lipophilicity of 18 quaternary ammonium drugs and model compounds (Fig. 1) using cyclic voltammetry at the 1,2-dichloroethane/water interface, by far the best method to measure the standard partition coefficient of ions (12). Evidence is given that the partition of such ions is not influenced by the formation of ion-pairs. The increase in lipophilicity reported in some studies when a lipophilic anion is added in excess is re-interpreted by the theory of equilibrium at an interface between two immiscible electrolyte solutions (ITIES), and a discrimination is made between apparent partition coefficients of ions measured by the shake-flask method, and standard (i.e., intrinsic) partition coefficients determined by cyclic voltammetry.

THEORY

The partition of salts can be described by a system consisting in two immiscible liquid phases α and β (for example water and 1,2-dichloroethane) and containing n ions X_i:

$$\begin{array}{c|c} \alpha(V_\alpha) & \beta(V_\beta) \\ \displaystyle\sum_{i=1}^{n} X_i^\alpha \rightleftarrows & \displaystyle\sum_{i=1}^{n} X_i^\beta \end{array}$$

where V_α and V_β are the volumes of phases α and β.

At equilibrium, the electrochemical potential of each ion X_i is the same in both phases α and β:

$$\tilde{\mu}_i^\alpha = \tilde{\mu}_i^\beta \tag{1}$$

The electrochemical potential of the system is the sum of a chemical and an electrical term:

$$\tilde{\mu}_i^\alpha = \mu_i^\alpha + z_i F_\phi^\alpha \tag{2}$$

where μ_i^α is the chemical potential of ion X_i in phase α, z_i is the charge of ion X_i, F is the Faraday constant and ϕ^α the Galvani potential of phase α (i.e., the bulk potential of phase α).

The chemical potential is defined by:

$$\mu_i^\alpha = \mu_i^{\circ,\alpha} + RT \ln a_i^\alpha \tag{3}$$

where $\mu_i^{\circ,\alpha}$ and a_i^α are respectively the standard chemical potential and the activity of X_i in phase α.

From Eq. 1, we obtain:

$$\Delta_\beta^\alpha \phi = \phi^\alpha - \phi^\beta = \frac{\mu_i^{\circ,\beta} - \mu_i^{\circ,\alpha}}{z_i F} + \left(\frac{RT}{z_i F}\right) \ln \left(\frac{a_i^\beta}{a_i^\alpha}\right) \tag{4}$$

where $\Delta_\beta^\alpha \phi$ is the Galvani potential difference between phases a and β and represents the potential difference across the Interface between two immiscible electrolyte solutions (noted ITIES) α/β.

Eq. 4 can be written as:

$$\Delta_\beta^\alpha \phi = \Delta_\beta^\alpha \phi_i^\circ + \left(\frac{RT}{z_i F}\right) \ln \left(\frac{a_i^\beta}{a_i^\alpha}\right) \tag{5}$$

where $\Delta_\beta^\alpha \phi_i^\circ$ is the standard transfer potential of ion X_i between phases α and β. Supposing that the ion transfers with-

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out formation of any ion-pair and without any chemical or redox reaction, $\Delta_\beta^\alpha\phi_i^\circ$ can be expressed by:

$$\Delta_\beta^\alpha\phi_i^\circ = \frac{\mu_i^{\circ,\beta} - \mu_i^{\circ,\alpha}}{z_iF} = \frac{\Delta G_{\mathrm{tr},i}^{\circ,\alpha\to\beta}}{z_iF} \tag{6}$$

where $\Delta G_{\mathrm{tr},i}^{\circ,\alpha\to\beta}$ is the standard Gibbs energy of transfer of ion X_i from α to β.

The 1,2-dichloroethane/water partition coefficient of ion X_i (noted $\log P_{\mathrm{dce}}^{i}$) between phases α and β is potential-dependent and is given by Eq. 7:

$$\log P_{\mathrm{dce}}^{i} = \log\left(\frac{a_i^\beta}{a_i^\alpha}\right) = -\frac{z_iF}{RT\ln 10}\Delta_\beta^\alpha\phi_i^\circ + \frac{z_iF}{RT\ln 10}\Delta_\beta^\alpha\phi \tag{7}$$

which can be written as:

$$\log P_{\mathrm{dce}}^{i} = \log P_{\mathrm{dce}}^{\circ,i} + \frac{z_iF}{RT\ln 10}\Delta_\beta^\alpha\phi \tag{8}$$

where $\log P_{\mathrm{dce}}^{\circ,i}$ is the standard partition coefficient of the ionic species X_i. $\log P_{\mathrm{dce}}^{\circ,i}$ represents the partition coefficient of i when the interface is not polarized and depends only on the chemical structure of X_i. The standard partition coefficient is given by:

$$\log P_{\mathrm{dce}}^{\circ,i} = -\frac{z_iF}{RT\ln 10}\Delta_\beta^\alpha\phi_i^\circ \tag{9}$$

Since cyclic voltammetry at the ITIES is a potential-controlled electrochemical method, the standard partition coefficient obtained by this method is independent on the Galvani potential difference between the two phases ($\Delta_\beta^\alpha\phi$) and corresponds to $\log P_{\mathrm{dce}}^{\circ,i}$. At the opposite, the shake-flask method is not a potential-controlled method. Each experiment corresponds to a particular value of $\Delta_\beta^\alpha\phi$, according to particular experimental conditions (nature and concentration of all ions, phase volumes). The partition coefficient measured by the shake-flask method is thus an apparent partition coefficient (noted $\log P_{\mathrm{dce}}^{i}$).

By noting n_i^{T} the total number of moles of ion X_i in the two phases α and β, the law of conservation of mass gives:

$$V_\alpha c_i^\alpha + V_\beta c_i^\beta = n_i^{\mathrm{T}} \tag{10}$$

The electroneutrality in both phases α and β gives:

$$\sum_{i=1}^{n} z_i c_i^\alpha = 0 \tag{11}$$

$$\sum_{i=1}^{n} z_i c_i^\beta = 0 \tag{12}$$

$$\sum_{i=1}^{n} z_i n_i^{\mathrm{T}} = 0 \tag{13}$$

where c_i^α and c_i^β are the concentrations of X_i in phases α and β, respectively.

Eq. 5 can be written as:

$$c_i^\beta = c_i^\alpha \frac{\gamma_i^\alpha}{\gamma_i^\beta}\exp\left[\frac{z_iF}{RT}(\Delta_\beta^\alpha\phi - \Delta_\beta^\alpha\phi_i^\circ)\right] \tag{14}$$

where γ_i^α and γ_i^β are the activity coefficients of X_i in phases α and β, respectively.

When this expression is used in Eq. 10 to substitute c_i^β, we obtain:

$$c_i^\alpha = \frac{n_i^{\mathrm{T}}}{V_\alpha + V_\beta \frac{\gamma_i^\alpha}{\gamma_i^\beta}\exp\left[\frac{z_iF}{RT}(\Delta_\beta^\alpha\phi - \Delta_\beta^\alpha\phi_i^\circ)\right]} \tag{15}$$

Since it is not c_i^α but n_i^{T} which is known, the substitution of c_i^α in Eq. 11 by its expression in Eq. 15 gives:

$$\sum_{i=1}^{n} \frac{z_i n_i^{\mathrm{T}}}{V_\alpha + V_\beta \frac{\gamma_i^\alpha}{\gamma_i^\beta}\exp\left[\frac{z_iF}{RT}(\Delta_\beta^\alpha\phi - \Delta_\beta^\alpha\phi_i^\circ)\right]} = 0 \tag{16}$$

Eq. 16 allows the Galvani potential difference at the ITIES α/β to be determined, when concentrations, activity coefficients, and standard Gibbs transfer energies of all the ions in the system, volumes of each phase and temperature are known. When $n \geq 3$, Eq. 16 has no general analytical solution but can be solved in special cases with simplifying assumptions presented in References (13,14). In this paper, Eq. 16 is used below to understand the influence of the Galvani potential difference on the partitioning behavior of quaternary ammonium ions in a biphasic system.

MATERIAL AND METHODS

Compounds and Reagents

Methylhomatropine bromide, pyridostigmine bromide, neostigmine bromide, propantheline bromide, trantheline bromide, homidium bromide, N-methylderamciclane iodide, N-methylquinidine iodide, and 14-methylrutecarpine chloride were graciously supplied by K. Takács-Novák (Budapest, Hungary). Acetylcholine chloride and 1-ethylquinoline iodide were purchased respectively from Fluka (Buchs, Switzerland) and Kodak (Rochester, NY). Carbamoylcholine chloride, homatropine hydrobromide and S-butyrylthiocholine chloride were purchased from Sigma (St Louis, MO). N-Methylderamciclane iodide, N-methylquinidine iodide, and 1-ethylquinoline iodide were transformed in the chloride salt on an anion-exchanger column Amberlite IRA-400 (Fluka) to avoid possible oxidation of iodide at the electrodes. The organic solvent was 1,2-dichloroethane (Romi Ltd, Cambridge, UK). It was used without further purification and handled with all necessary precautions (15). Bis(triphenylphosphoranylidene)ammonium tetrakis-(4-chlorophenyl)borate (BTPPATPBCl) and bis(triphenylphosphoranylidene)-ammonium tetraphenylborate (BTPPATPB) were prepared by metathesis of potassium tetraphenylborate or potassium tetrakis(4-chlorophenyl)borate (Fluka) and of bis(triphenylphosphoranylidene)ammonium chloride (Aldrich, Milwaukee, WI).

Cyclic Voltammetry

The experimental set-up was a home-made four-electrode potentiostat, as previously described (16), with full ohmic drop compensation. The scanning of the applied potential was preformed by a waveform generator (VA-scanner E 612, Metrohm, Herisau, Switzerland), coupled to an X–Y recorder (Bausch & Lomb, Rochester, NY). Both the cell and the four-electrode potentiostat were housed in a Faraday

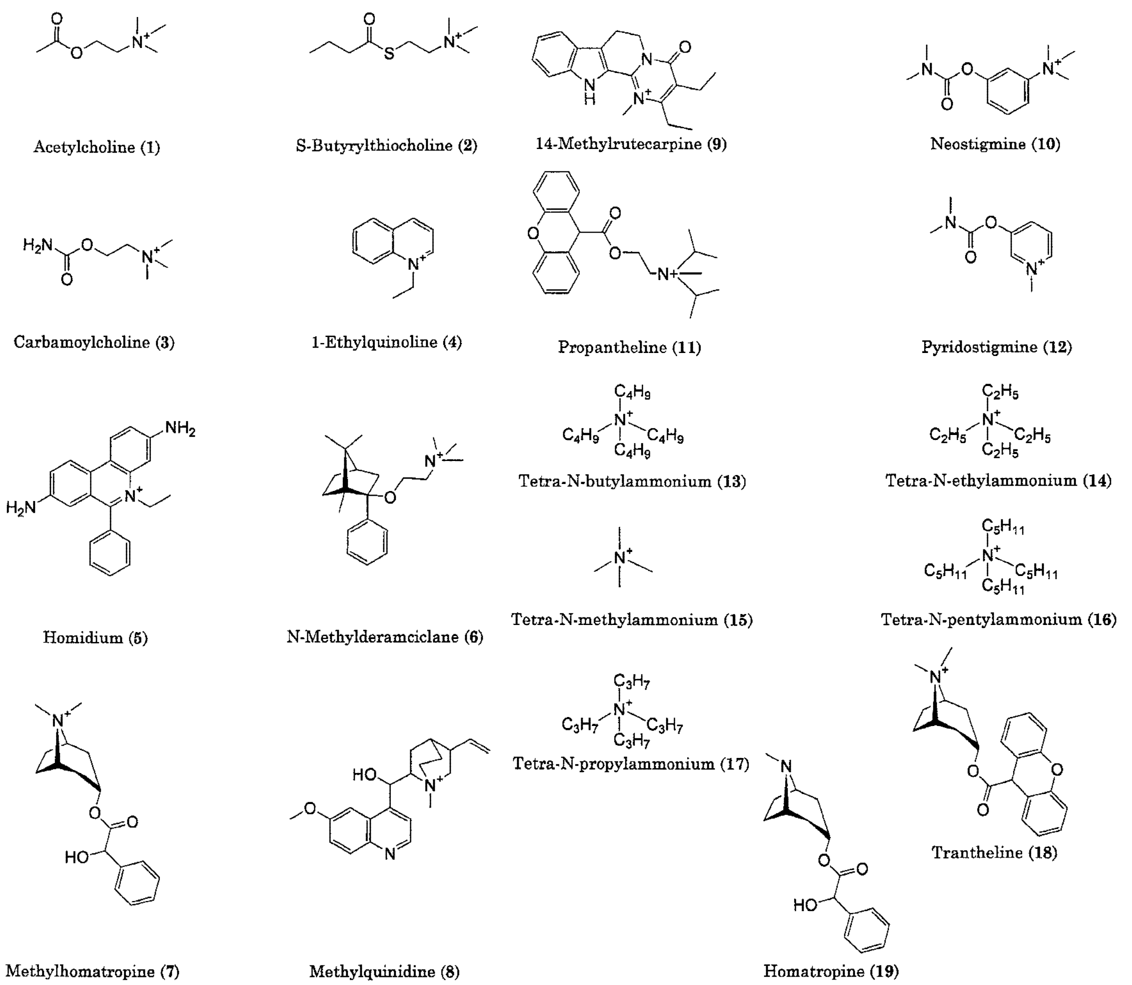

Fig. 1. Chemical structure and numbering of the 19 investigated compounds.

cage. All experiments were carried out at room temperature (25 ± 1°C).

In the organic phase, two different electrolyte salts were used: BTPPATPBCl (cell 1) and BTPPATPB (cell 2).

The following electrochemical chain represents the set-up used:

Ag	Ag_2SO_4	Li_2SO_4, 10 mM + QAS, 0.5 mM in H_2O + ME_4N^+	BTPPATPBCl (cell 1) or BTPPATPB (cell 2), 10 mM in DCE	LiCl, 10 mM in H_2O + BTPPACl, 1 mM	AgCl	Ag
		Aqueous phase	*Organic phase*	*Aqueous reference*		

The quaternary ammonium salts (noted QAS) were dissolved in the aqueous phase, The pH of these solutions was adjusted to the desired value with H_2SO_4 or LiOH. All half-wave measured potentials (noted $\Delta_o^w\phi_i^{1/2}$) were referred to the half-wave potential of tetra-N-methylammonium cation (noted $\Delta_o^w\phi_{Me_4N^+}^{1/2}$). Thus, the standard transfer potential of an ion X_i (noted $\Delta_o^w\phi_i^\circ$) can be calculated using Eq. 17 (12).

$$\Delta_o^w\phi_i^{1/2} - \Delta_o^w\phi_i^\circ = \Delta_o^w\phi_{Me_4N^+}^{1/2} - \Delta_o^w\phi_{Me_4N^+}^\circ \qquad (17)$$

Since the value of $\Delta_o^w\phi_{Me_4N^+}^\circ$ is known (160 mV on the tetraphenylarsonium tetraphenylborate scale (17)), the standard Gibbs transfer Gibbs transfer energy of ion X_i ($\Delta G_{tr,i}^{\circ,w\rightarrow o}$) and its standard partition coefficient ($\log P_{dce}^{o,i}$) can be calculated by using Eq. 6 and 9.

RESULTS AND DISCUSSION

Lipophilicity of Quaternary Ammonium Ions

Cell 1 (BTPPATPBCl as electrolyte in the organic phase and Li_2SO_4 in the aqueous phase) was used to measure the standard transfer potential of quaternary ammonium ions at a 1,2-dicloroethane/water interface ($\Delta_o^w\phi_C^\circ$). These potentials are reported in Table I and were used to calculate the standard partition coefficients in the 1,2-dichloroethane/water system ($\log P_{dce}^{o,C}$) by using Eq. 6 and 9 (see Table I). The standard transfer potential of tetramethylammonium, tetraethylammonium, tetrapropylammonium, tetrabutylammonium, and tetraphentylammonium were taken from the literature (17,18).

The apparent partition coefficients of seven of the studied compounds, measured in the octanol/water system by the shake-flask method (in Sörensen buffer pH 7.4, with constant ionic strength 0.27 and phase volumes (V_o/V_w) varying between 1:10 to 50:5) were found in the literature (19). The range of standard partition coefficients is much broader in the 1,2-dichloroethane/water system ($\log P_{dce}^{o,C}$ between −3.5 and 6.1) than in the octanol/water system ($\log P_{oct}^{C}$ between −1.68

Table I. Physicochemical Parameters of Quaternary Ammonium Ions

Compound[a]	$\Delta_o^w\phi_C^\circ$[mV][b]	log $P_{dce}^{o,C}$[c]	CLOGP[d]	log $P_{est,dce}^{N}$[e]	log P_{oct}^{C}[f]
1	210	−2.0	1.196	1.88	
2	25	−0.4	2.439	3.31	
3	210	−3.5	0.344	0.90	
4	5	−0.1	3.554	4.58	
5	−192	3.2	5.772	7.11	−1.10
6	−225	3.8	6.311	7.76	−1.12
7	42	−0.7	2.323	3.18	−1.68
8	−115	1.9	3.807	4.89	−1.31
9	−12	0.2	3.441	4.47	−1.30
10	36	−0.6	2.602	3.50	<−3
11	−218	3.7	6.076	7.50	−1.07
12	55	−0.9	1.307	2.01	<−3
13	−225[g]	3.8	7.392	9.01	
14	19[g]	−0.3	2.920	3.87	
15	160[g]	−2.7	0.924	1.57	
16	−361[h]	6.1	9.628	11.58	
17	−91[h]	1.5	5.156	6.44	
18	−163	2.8	4.752	5.97	−1.45

[a] For structures, see Figure 1.

[b] Standard transfer potential of cation C at the 1,2-dichloroethane/water interface measured by cyclic voltammetry in cell 1.

[c] Standard partition coefficient of cation C at the 1,2-dichloroethane/water interface.

[d] CLOGP of the corresponding neutral form of the ammonium cation C, calculated by the PCmodel software (23) as explained in the text.

[e] Calculated from CLOGP by Eq. 21.

[f] Apparent partition coefficient in the octanol/water system taken from (19) (measured by the shake-flask method at pH 7.4).

[g] Taken from (17).

[h] Taken from (18).

and −1.10). Moreover, the partition coefficients of neostigmine and pyridostigmine were measurable by cyclic voltammetry (whose range is −6 and 4) but not by the shake-flask method, due to their low value.

Eq. 8 allows to break down the partition coefficient of an ion at an ITIES into a sum of two terms. The first term is the standard partition coefficient of the ion (log $P_{dce}^{o,C}$), which depends exclusively on the temperature, as well as on the chemical structure of the ion (i.e., its nature, volume, and charge). In other words, the standard partition coefficient is an intrinsic property of each ion for two immiscible solvents. The second term is proportional to the Galvani potential difference at the ITIES ($\Delta_o^w\phi$) and thus depends on the temperature, the volume of each phase, and notably on all species in the system (i.e., their concentration and standard transfer potential, as per to Eq. 16). In order to confirm the ability of log $P_{dce}^{o,C}$ to give a reliable description of the "true" lipophilicity of an ion, the CLOGP values of the neutral molecular structures closest to the cations were calculated by a, removing one alkyl group $-(CH_2)_nCH_3$ from the corresponding quaternary nitrogen, b, calculating the CLOGP value of this neutral molecule, and c, adding to it the value of the $-(CH_2)_nCH_3$ fragment. Figure 2 shows the good correlation between this hypothetical CLOGP and log $P_{dce}^{o,C}$ values (see Table I and Eq. 18):

$$\log P_{dce}^{o,C} = 1.02\ (\pm 0.06)\ \mathrm{CLOGP} - 3.11\ (\pm 0.29)$$
$$n = 18;\ r^2 = 0.94;\ s = 0.64;\ F = 267 \tag{18}$$

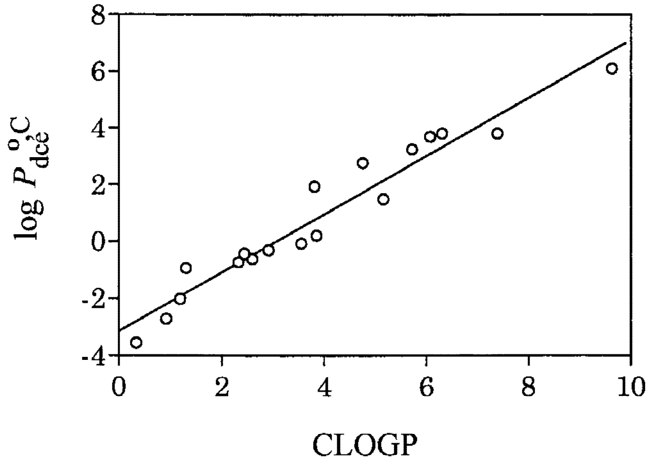

Fig. 2. Relation (Eq. 18) between the standard partition coefficient log $P_{dce}^{o,C}$ of quaternary ammonium ions and CLOGP value of hypothetical tertiary amino isomers (for explanations: see text).

In this and the following equations, 95% confidence limits are given in parenthesis; n is the number of compounds, r^2 the squared correlation coefficient, s the standard deviation, and F the Fischer's test.

These results demonstrate that cyclic voltammetry is an accurate method to measure the intrinsic lipophilicity of quaternary ammonium ions, mainly because log $P_{dce}^{o,C}$ (contrary to log P_{oct}^{C}) as determined by this method does not depend on the Galvani potential difference between the organic and aqueous phases. Indeed, log P_{oct}^{C} values (and log P^C values in any other solvent system) measured by a shake-flask or titrimetic method, depend on the nature and concentrations of counter-ions; they are thus apparent and not standard values.

Solvation of Quaternary Ammonium Ions

Equations for the solvation energy of ions are derived from physical models, which are intended to represent certain selected features of an ion-solvent interaction in a simplified manner. The solvation energy of ions is often divided into electrostatic and non-electrostatic terms (20). In the simple model for the electrostatic component introduced by Born, an ion of crystallographic radius r and the charge z is identified with a rigid sphere of the same radius and charge. The solvent, which in truth is not only structured, but also polarized in the vicinity of an ion, is represented by a structureless continuum of uniform dielectric constant ε_r, corresponding to its bulk value. The Gibbs interaction energy of a ion C in a solvent S (noted ΔG_{CS}^{α}) is computed as the net electrostatic work of discharging the sphere of vacuum and then recharging it to the same charge as the ion in a medium α of dielectric constant. Δg_{CS}^{α} can be expressed as:

$$\Delta G_{CG}^{\alpha} = -\frac{z^2 e^2 N_A}{8\,\pi\varepsilon_0 r}\left(1-\frac{1}{\varepsilon_r^{\alpha}}\right) \tag{19}$$

where N_A is Avogadro's number. ε_0 and ε_r^{α} are the dielectric constants of the vacuum and of medium α respectively. Born's theory neglects the dielectric saturation and assumes that the dielectric constant around the ion is equal to that in the bulk, resulting in a overestimation of ion-solvent interactions values. Nevertheless, Born's equation provides good estimates of ionic solvation energies. A recent paper (21) has shown that since partition coefficients represent the difference in solvation energy between two solvents, the log P difference between a neutral and a charged species in the 1,2-dichloroethane/water system can be used to approximate $\Delta G_{CS}^{dce} - \Delta G_{CS}^{w}$ according to Eq. 20.

$$\begin{aligned}\Delta G_{CS}^{dce} - \Delta G_{CS}^{w} &= \frac{z^2e^2N_A\,(\varepsilon_r^{dce} - \varepsilon_r^{w})}{8\pi\varepsilon_0\varepsilon_r^{dce}\varepsilon_r^{w}}\cdot\frac{1}{r_0}\\ &= RT\ln 10\cdot(\log P_{dce}^{N} - \log P_{dce}^{o,C})\\ &= RT\ln 10\cdot diff\,(\log P_{dce}^{N\text{-}C}) \qquad (20)\end{aligned}$$

where ΔG_{CS}^{dce} (resp. ΔG_{CS}^{w}) is the ion-solvent interaction energy in 1,2-dichloroethane (resp. water), z and r_0 are respectively the charge and the molecular radius of the ionized drug, N_A is the Avogadro constant and ε_r^{dce} (resp. ε_r^{w}) is the dielectric constant of 1,2-dichloroethane (resp. water). When *diff* ($\log P_{dce}^{N\text{-}C}$) values are concerned, the correction for the dielectric saturation becomes negligible so that Born's model and more complex models (20,22) yielded the same results (21).

In order to estimate the parameter $\Delta G_{CS}^{dce} - \Delta G_{CS}^{w}$ for quaternary ammonium ions, the CLOGP values calculated in Table I, which corresponds to log P values in the n-octanol/water system (23), were used to assess the partition coefficients of the corresponding molecular structures in the 1,2-dichloroethane/water system (noted $\log P_{est,dce}^{N}$) using the solvatochromic analysis of neutral compounds in the 1,2-dichloroethane/water system (24). This study has shown that for non-H-bond donor compounds such as aromatic and aliphatic amines, $\log P_{oct}^{N}$ and $\log P_{dce}^{N}$ are strongly related (Eq. 21):

$$\log P_{dce}^{N} = 1.15\ (\pm 0.10)\log P_{oct}^{N} + 0.51\ (\pm 0.16) \qquad (21)$$
$$n = 19;\ r^2 = 0.89;\ s = 0.50;\ F = 128$$

The $\log P_{est,dce}^{N}$ values given in Table I were calculated from the n-octanol/water CLOGP values, using Eq. 21. A linear relationship (Eq. 22) was found between the measured standard partition coefficient of the ionic forms and the partition coefficient of the corresponding neutral forms, as shown in Figure 3.

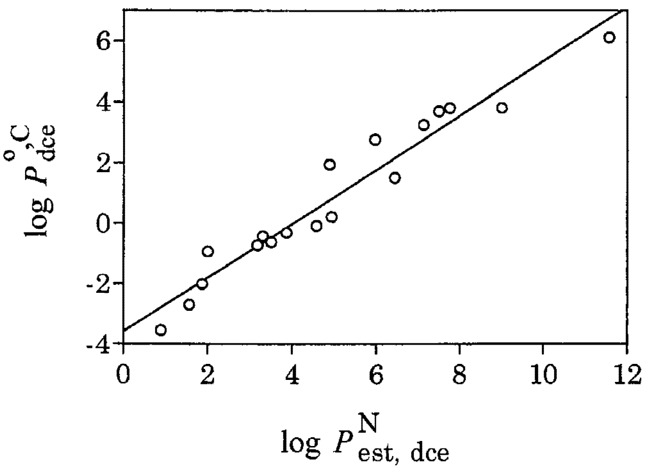

Fig. 3. Relation (Eq. 22) between the standard partition coefficient of the 18 quaternary ammonium ions in 1,2-dichloroethane/water system ($\log P_{dce}^{o,C}$) and the partition coefficient of their corresponding neutral form ($\log P_{est,dce}^{N}$) estimated from Eq. 21.

$$\log P_{dce}^{o,C} = 0.89\ (\pm 0.05)\log P_{est,dce}^{N} - 3.58\ (\pm 0.32) \qquad (22)$$
$$n = 18;\ r^2 = 0.94;\ s = 0.65;\ F = 255$$

The equivalent relation to Eq. 21 for H-bond donors (24) were also used to calculate the $\log P_{est,dce}^{N}$ of compounds **7, 8,** and **9** but the obtained values were too low to be correlated with $\log P_{dce}^{o,C}$, proving that these three compounds do not express their H-bond capacity in the 1,2-dichloroethane/water system.

Eq. 22 makes it possible to calculate the parameter $\log P_{dce}^{N} - \log P_{dce}^{o,C} = diff\,(\log P_{dce}^{N\text{-}C}) \approx 3.6$ for quaternary ammonium ions and their corresponding neutral form. Thus for quaternary amines, one can estimate that $\Delta G_{CS}^{dce} - \Delta G_{CS}^{w}$ is equal to 21 kJ · mol^{-1}.

In order to prove the reliability of this value, the physicochemical parameters of homatropine, a protonable tertiary amine (compound **19**), were measured by potentiometry and cyclic voltammetry (see Table II). We found *diff* ($\log P_{dce}^{N\text{-}C}$) = 4 and $\Delta G_{CS}^{dce} - \Delta G_{CS}^{w} = 23$ kJ · mol^{-1}, which confirm that Born's solvation model is a good first approximation to estimate the intrinsic lipophilicity of quaternary ammonium cations.

Increased Apparent Lipophilicity of Quaternary Ammonium Ions

A number of papers report an increase in the apparent lipophilicity of quaternary ammonium ions in the presence of an anion added in excess. It has often been suggested that such an increase is due to the formation of neutral ion-pairs between the cation and the lipophilic anion (3,11). But according to the theories of Bjerrum and Fuoss (10), the dielectric constant of water is too high ($\varepsilon_r = 78.5$) to allow the formation of ion-pairs. To confirm the lack of ion-pair formation in 1,2-dichloroethane ($\varepsilon_r = 10$) between the quaternary ammonium cations and TPBCl$^-$ (in excess in the organic phase), cell 2 (BTPPATPB as electrolyte in the organic phase) was used to measure the standard transfer potential ($\Delta_\beta^\alpha\phi_C^o$) of the model compound homidium (compound **5**). No difference in the standard transfer potential was found between cell 1 ($\Delta_o^w\phi_5^o = -192 \pm 3$ mV) and cell 2 ($\Delta_o^w = -195 \pm 5$ mV), indicating that the nature of the counter-anion in the organic phase does not influence the standard transfer potential of homidium. Moreover, to check the influence of the counter-ion concentration on the half-wave potential of homidium ($\Delta_o^w\phi_5^{1/2}$), the concentration of BTPPATPBCl was varied from 1 mM to 30 mM in cell 1 (25,26). The results in Table III show that the half-wave potential and thus the standard transfer potential of homidium were not influenced by

Table II. Physicochemical Parameters of Homatropine (Compound 19)

Parameter	pK_a^{a}	CLOGP^b	log P_{oct}^{N} ^{a}	log P_{dce}^{N} ^{a}	log $P_{dce}^{o,C}$ ^{c}	*diff* ($\log P_{dce}^{N\text{-}C}$)^d
Value	9.81	1.45	1.63	1.61	–2.4	4.0

[a] Measured by potentiometry (30).
[b] Calculated by the PCmodel Software (23).
[c] Measured by cyclic voltammetry.
[d] *diff* ($\log P_{dce}^{N\text{-}C}$) = $\log P_{dce}^{N} - \log P_{dce}^{o,C}$.

Table III. Variations of $\Delta_o^w\phi_5^{1/2}$ with BTPPA-Concentration in the Organic Phase

c_{BTPPA}-[mM]^a	1	2	5	10	30
$\Delta_o^w\phi_5^{1/2}$ [mV]^b	−192 ± 3	−193 ± 3	−194 ± 4	−192 ± 3	−192 ± 3

^{a} Concentration of BTPPA$^-$ in the organic phase.
^{b} Half-wave potential of homidium measured by cyclic voltammetry (average on 4 values).

the anion concentration. These results are not compatible with the hypothesis of ion-pair formation.

The equations presented in the theoretical part and all calculations reported here are based on the hypothesis that no formation of ion-pair between the organic cation and an anion had occurred. With this assumption, the experimental observations that the lipophilicity of quaternary ammonium ions increases when lipophilic anions are added can be explained by the theory of ITIES presented below and by the evaluation of the Galvani potential difference ($\Delta_o^w\phi$) between the organic and the aqueous phase.

In the particular case of the salt of a quaternary ammonium compound partitioning between an aqueous and an organic phase:

$$\begin{array}{c|c} \text{water } (V_w) & \text{1,2-dichloroethane } (V_o) \\ A^-C^+ \quad \rightleftarrows & \quad A^-C^+ \end{array}$$

and assuming that $V_w = V_o$ and that n_{AC}^T is sufficiently small to justify the approximation of infinitely diluted solutions, then $\gamma_C^o = \gamma_C^w = 1$ and Eq. 16 becomes:

$$\Delta_o^w\phi = \frac{\Delta_o^w\phi_A^o + \Delta_o^w\phi_C^o}{2} \tag{23}$$

As an example, in the case of the partition of propantheline bromide: $\Delta_o^w\phi_A^o = -446$ mV and $\Delta_o^w\phi_C^o = -218$ mV (27) and thus $\Delta_o^w\phi = -332$ mV. The potential of the aqueous phase is lower than the potential of the organic phase and the apparent partition coefficient of propantheline in the 1,2-dichloroethane/water system (calculated from Eq. 9) is log $P_{\text{dce}}^{\text{app,C}} = -1.91$.

When another salt is added to the propantheline bromide solution (for example a sodium salt, NaY), Eq. 16 cannot be simplified and must be solved numerically, as a function of the ratio of the total Y$^-$ concentration to the total C$^+$ concentration (noted r). Figure 4 illustrates the evolution of the apparent partition coefficient of propantheline (log $P_{\text{dce}}^{\text{app,C}}$) in the 1,2-dichloroetane/water system calculated by Eq. 16, when different concentrations of NaY are added to the system. The apparent partition coefficient of propantheline increases with r until a plateau is reached, which corresponds to an excess of anion Y$^-$. In this case, Eq. 16 can be rewritten as:

$$\Delta_o^w\phi\,[\text{mV}] = \frac{\Delta_o^w\phi_Y^o + \Delta_o^w\phi_{\text{Na}}^o}{2} = \frac{\Delta_o^w\phi_Y^o + 591}{2} \tag{24}$$

with $\Delta_o^w\phi_{\text{Na}}^o = 591$ mV (28). The Galvani potential difference becomes constant and identical to that of a biphasic system containing exclusively NaY (see Eq. 23). In other words, the increase in log $P_{\text{dce}}^{\text{app,C}}$ is entirely accounted for by the increase in the Galvani potential difference.

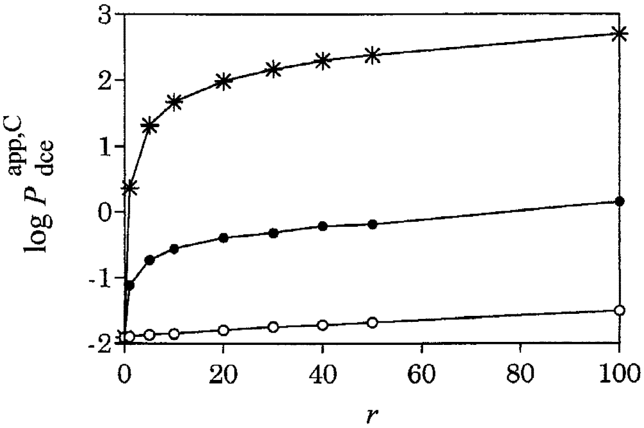

Fig. 4. Evolution of the apparent partition coefficient of propantheline (log $P_{\text{dce}}^{\text{app,C}}$) calculated from Equations 16 and 8 in function of the ratio of the total Y$^-$ concentration to the total C$^+$ concentration (noted *r*). Different lipophilicities were chosen for Y$^-$: log $P_{\text{dce}}^{\text{o,Y}} = -3$ (stars), −6 (black circles) and −9 (white circles).

In order to study the influence of the lipophilicity of Y$^-$ on the log $P_{\text{dce}}^{\text{app,C}}$ of propantheline, different values of log $P_{\text{dce}}^{\text{o,Y}}$ were chosen corresponding to those usually obtained for the conjugated anions of lipophilic acids (see (29)). The higher the lipophilicity of Y$^-$, the more positive $\Delta_o^w\phi_Y^o$ and $\Delta_o^w\phi$ become, and the higher the apparent log $P_{\text{dce}}^{\text{app,C}}$ too. This simulation explains easily why an increase in the apparent lipophilicity of quaternary ammonium cations is often observed only when large and lipophilic anions are added in excess in the system (1,2,19).

CONCLUSION

The theory of liquid/liquid interfaces presented here shows that the partitioning of ions between an aqueous and an organic phase is governed by the Galvani potential difference between the two phases. As this potential difference depends on the volume of phases and on all ions in solution, particularly the support electrolytes, the apparent partition coefficients measured by the shake-flask method will strongly depend on experimental conditions. The increase in lipophilicity of quaternary ammonium ions observed when a lipophilic anion is added in excess is only governed by the Galvani potential difference and does not depend on any ion-pair formation. In contrast to the shake-flask method, cyclic voltammetry yields a standard value of the partition coefficient which does not depend on experimental conditions and must be considered as an intrinsic value which can be used as reference.

The evidence reported here also has biochemical and pharmacological implications, given the ubiquity of ion permeation across biological membranes. Whereas the effect of counter-ions on the apparent lipophilicity of ions is a well-recognized phenomenon, our re-interpretation in terms of the Galvani potential difference renders doubtful any mechanistic hypothesis based on ion-pair permeation. There is now a clear need for extensive electrochemical studies of and at biological membranes.

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REFERENCES

1. J. Van Gelder, M. Witvrouw, C. Pannecouque, G. Henson, G. Bridger, L. Naesens, E. De Clercq, P. Annaert, M. Shafiee, G. van den Mooter, R. Kinget, and P. Augustijns. Evaluation of the potential of ion pair formation to improve the oral absorption of two potent antiviral compounds. *Int. J. Pharm.* **186**:127–136 (1999).
2. A. Fini, G. Fazio, M. Gonzalez-Rodriguez, C. Cavallari, N. Passerini, and L. Rodriguez. Formation of ion-pairs in aqueous solutions of diclofenac salts. *Int. J. Pharm.* **187**:163–173 (1999).
3. M.Kadono, K. Kubo, H. Miyazaki, N. Tojyo, S. Nakagawa, K. Myashita, T. Imanishi, J. H. Rytting, and T. Mayumi. Enhanced *in vitro* percutaneous penetration of salicylate by ion pair formation with alkylamines. *Biol. Pharm. Bull.* **21**:599–603 (1998).
4. C. Valenta, U. Siman, M. Kratzel, and J. Hadgraft. The dermal delivery of lignocaine: influence of ion pairing. *Int. J. Pharm.* **197**:77–85 (2000).
5. T. Hatanaka, T. Kamon, S. Morigaki, K. Katayama, and T. Koizumi. Ion pair skin transport of a zwitterionic drug, cephalexin. *J. Control Release* **66**:63–71 (2000).
6. J. D. Meyer and M. C. Manning. Hydrophobic ion pairing: Altering the solubility properties of biomolecules. *Pharm. Res.* **15**:188–193 (1998).
7. P. Liu, T. Kurihara-Bergstrom, F. H. Clarke, N. Gonella, and W. R. Good. Quantitative evaluation of aqueous isopropanol enhancement of skin flux of terbutaline (sulfate). I. Ion associations and species equilibria in the formulation. *Pharm. Res.* **9**:1035–1042 (1992).
8. R. M. Diamond. The aqueous solution behavior of large univalent ions. New type of ion-pairing. *J. Phys. Chem.* **67**:2513–2517 (1963).
9. R. Neubert. Ion pair transport across membranes. *Pharm. Res.* **6**:743–747 (1989).
10. C. A. Kraus. The ion-pair concept: Its evolution and some applications. *J. Phys. Chem.* **60**:129–141 (1956).
11. J. H. G. Jonkman and C. A. Hunt. Ion pair absorption of ionized drugs–fact or fiction? *Pharmac. Weekblad Sci. Ed.* **5**:41–48 (1983).
12. F. Reymond, V. Gobry, G. Bouchard, and H. H. Girault. Cyclic voltammetry as a new tool to study the partitioning and proton transport behavior of ionized drugs. In B. Testa, H. van de Waterbeemd, G. Folkers, and R. H. Guy (eds.), *Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical and Computational Strategies,* Wiley-VHCA, Zurich, 2001 pp. 327–349.
13. L. Q. Hung. Electrochemical properties of the interface between two immiscible electrolyte solutions. Part I. Equilibrium situation and Galvani potential difference. *J. Electroanal. Chem.* **115**:159–174 (1980).
14. T. Kakiuchi. Limiting behavior in equilibrium partitioning of ionic components in liquid-liquid two-phase systems. *Anal. Chem.* **68**:3658–3664 (1996).
15. *1,2-Dichloroethane (Environmental Health Criteria N° 176).* World Health Organization, Geneva, 1995.
16. F. Reymond, G. Steyaert, P.-A. Carrupt, B. Testa, and H. H. Girault. Mechanism of transfer of a basic drug across the water/1,2-dichloroethane interface: The case of quinidine. *Helv. Chim. Acta* **79**:101–117 (1996).
17. T. Wandlowski, V. Marecek, and Z. Samec. Galvani potential scales for water-nitrobenzene and water-1,2-dichloroethane interfaces. *Electrochim. Acta* **7**:1173–1175 (1990).
18. J. Czapkiewicz and B. Czapkiewicz-Tutaj. Relative scale of free energy of transfer of anions from water to 1,2-dichloroethane. *J. Chem. Soc. Faraday Trans. 1* **76**:1663–1668 (1980).
19. K. Takács-Novák and G. Szasz. Ion-pair of quaternary ammonium drugs: The influence of counter ions of different lipophilicity, size, and flexibility. *Pharm. Res.* **16**:1633–1638 (1999).
20. M. H. Abraham and J. Liszi. Calculation of ionic solvation. V. The calculation of partition coefficients of ions. *J. Inorg. Nucl. Chem.* **43**:143–151 (1981).
21. F. Reymond, P.-A. Carrupt, B. Testa, and H. H. Girault. Charge and delocalisation effects on the lipophilicity of protonable drugs. *Chem. Eur. J.* **5**:39–47 (1999).
22. T. Osakai and K. Ebina. Non-bornian theory of the Gibbs energy of ion transfer between two immiscible liquids. *J. Phys. Chem.* **102**:5691–5698 (1998).
23. DAYLIGHT Software 4.41. Daylight Chemical Information System, Inc., Irvine, California, 1995.
24. G. Steyaert, G. Lisa, P. Gaillard, G. Boss, F. Reymond, H. H. Girault, P.-A. Carrupt, and B. Testa. Intermolecular forces expressed in 1,2-dichloroethane/water partition coefficient: A solvatochromic analysis. *J. Chem. Soc. Faraday Trans.* **93**:401–406 (1997).
25. A. K. Kontturi, K. Kontturi, L. Murtomäki, and D. J. Schiffrin. An electrochemical study of long-chain aliphatic amines. *Acta Chem. Scand.* **46**:47–53 (1992).
26. A. K. Kontturi, K. Kontturi, J. A. Manzanares, S. Mafé, and L. Murtomäki. Ion pairing in the analysis of voltammetric data at the ITIES: RbTPB and RbTPBCl in 1,2-dichloroethane. *Ber. Bunsenges, Phys. Chem.* **99**:1131–1136 (1995).
27. A. Sabela, V. Marecek, Z. Samec, and R. Fuoco. Standard Gibbs energies of transfer of univalent ions from water to 1,2-dichloroethane. *Electrochim. Acta* **37**:231–235 (1992).
28. Y. Shao, A. A. Stewart, and H. H. Girault. Determination of the half-wave potential of the species limiting the potential window: Measurement of Gibbs transfer energies at the water/1,2-dichloroethane interface. *J. Chem. Soc. Faraday Trans.* **87**:2593–2597 (1991).
29. F. Reymond, V. Chopineaux-Courtois, G. Steyaert, G. Bouchard, P.-A. Carrupt, B. Testa, and H. H. Girault. Ionic partition of ionisable drugs: pH-lipophilicity profiles, transfer mechanisms and charge effects on solvation. *J. Electroanal. Chem.* **462**:235–250 (1999).
30. A. Avdeef. Assessment of distribution-pH profiles. In V. Pliska, B. Testa, and H. van de Waterbeemd (eds.), *Lipophilicity in Drug Action and Toxicology,* VCH Publishers, Weinheim, 1996 pp. 109–139.