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Properties of amiodarone monolayer spread at the air–water interface

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Surface pressure measurements demonstrate that, over a wide range of pH, amiodarone forms a stable monolayer at the air–water interface. The area found to be occupied by an amiodarone molecule was compared with a theoretical prediction based on a conformational analysis, making it possible to assemble amphiphilic molecules. The intrinsic ionization constant of amiodarone in an aqueous environment $pK_i = 8{\cdot}7 \pm 0{\cdot}5$ was evaluated from surface potential measurements.

Amiodarone (Fig. 1) is a potent antianginal and antiarrhythmic drug (Marcus et al 1981; Singh 1983), highly soluble in chloroform and poorly soluble in water. It forms micelles in ultra pure water with a critical micelle concentration of 0·05%; however, its solubility decreases sharply with increasing anion concentration which limits its study in an aqueous medium relevant to the biological environment (Ravin et al 1969, 1975). A possible way to overcome these solubility difficulties is to spread amiodarone as a monolayer at the air–water interface. This paper reports some surface properties of amiodarone, namely the area occupied by each amiodarone molecule at the air–water interface, the drug's pK_i values, and its conformation at the air–water interface.

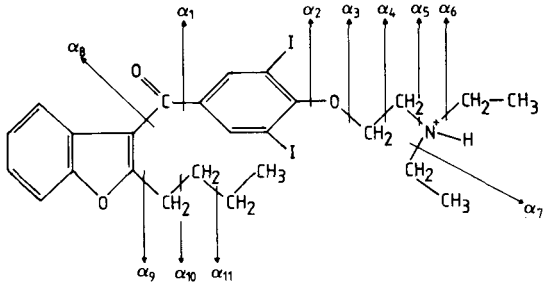

FIG. 1. Chemical structure of amiodarone. Definition of torsional angles.

EXPERIMENTAL SECTION

Materials and methods

Amiodarone (2-butyl-3[4-(2-diethylaminoethoxy)-3,5-diiodobenzoyl]benzofuran), obtained from Labaz-Sanofi, was dissolved in chloroform (0·33 mg litre^{-1}) and spread at the air–water interface with an Agla microsyringe (Burroughs Welcome and Co., UK). The ionic strength of the buffered subphase (Tris–HCl, acetate–acetic acid, $NaHCO_3$–NaOH) was maintained at a constant value (0·1 or 0·15 M NaCl) by addition of NaCl. Water was triple-distilled and the pH of the solutions was measured with a pH meter to ± 0·05 pH units. The temperature was maintained at 20 °C. Surface potential was measured using the vibrating plate technique (Kinloch & MacMullen 1959; Caspers et al 1981; Noblet et al 1984). Surface pressure measurements were made with a Wilhelmy plate using a Cahn Electro-Balance (Cahn Instrument Company USA) as previously described (Jaffé et al 1967). Accuracy was ±0·01 mN m^{-1} for surface pressure measurements and ±2 mV for surface potential measurements.

Conformational analysis

The conformational analysis procedure was based on a strategy discussed by Brasseur et al (1981). The total conformational energy of the molecule at the air–water interface was empirically calculated as the sum of all contributions resulting from local interactions—i.e. the Van der Waals' energy, the torsional potential, the electrostatic interaction and the transfer energy. The electrostatic energy was calculated as a function of the dielectric constant. To simulate the interface, the dielectric constant of the hydrophobic and hydrophilic media were taken as 3 and 30, respectively. Between these two media, the dielectric constant was assumed to increase linearly. The transfer energy for distinct moeities of the molecule has been determined experimentally by numerous authors, as summarized by Tanford (1972). The values used for the valence angles, bond lengths and atomic charges are those currently used in conformational analysis (Hopfinger 1973).

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In the calculation procedure, changes of 60° each were first imposed on each of n torsional angles, yielding 6^n conformers. The internal energy was calculated for each of these conformers. The most probable configurations were taken as those yielding the lowest internal energy; such a selection was based on one statistical weight associated with the individual configurations. A simplex minimization procedure (Nelder & Mead 1965) was eventually used to reduce further the total internal energy of selected conformations and the molecule was orientated at the air–water interface taking into account the positions of the hydrophobic and hydrophilic centres (Brasseur et al 1983b). Finally, molecules were assembled in monolayers as described by Brasseur et al (1983a). Calculations were performed on a CDC-Cyber 170 Computer coupled to a Calcomp drawing table.

RESULTS AND DISCUSSION

Surface pressure measurements

Fig. 2 illustrates the influence of the surface charge density on the organization of the spread monolayer. Decreasing the pH of the subphase, for a given surface concentration (C_s), caused an increase in the surface pressure due to electrostatic repulsion between positive amine residues. From the isotherms $\pi = f(C_s)$, where π represents the difference of surface tension measured in the absence and presence of the spread monolayer, the area (A) occupied per molecule in a close-packed surface monolayer was evaluated at 0·44 nm^2, using the classical extrapolation procedure shown in Fig. 2. The fact that this area does not depend on the degree of ionization (Fig. 2) suggests that the spread molecules form a stable monolayer and that no desorption occurs, even in the ionized form (i.e. at low pH). The collapse surface pressure was constant as a function of time, up to 40 min (data not shown).

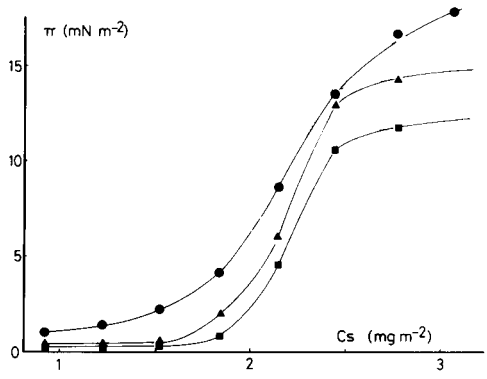

FIG. 2. Surface pressure isotherms of amiodarone spread on an aqueous phase at different pH (●, pH 4·8; ▲, 7·4; ■, 8·8). NaCl concentration, 0·15 M; temperature, 20 ± 0·5 °C. The surface concentration (C_s) of amiodarone in the close-packed state is 2·47 mg m^{-2}.

Conformational analysis

An initial systematic study was performed on the torsional angles, α_1, α_2, α_3, α_4, α_8, α_9, α_{10}, which were given successive increments of 60° (Fig. 1). This approach yielded 6^7 or 279 936 different conformations. Six structures with maximal probability i.e. with the lowest energies were selected (Table 1).

Table 1. Torsional angles after systematic analysis.

Conformer	Torsional angles α_1	α_2	α_3	α_4	α_8	α_9	α_{10}	Probability[a] %	ΔE^b (KJ mol^{-1})
A	180	240	180	180	120	180	180	15·51	0·0
A	0	60	180	180	120	180	180	15·51	0·0
B	180	300	180	180	120	180	180	6·5	2·13
B	0	120	180	180	120	180	180	6·5	2·13
C	0	300	180	180	120	180	180	4·2	3·22
C	180	120	180	180	120	180	180	4·2	3·22

Torsional angles (α_5, α_6 and α_7) were fixed at 180°.
[a] The probability of existence is given by a Boltzman distribution.
[b] ΔE is the energy above minimal value.

Only two structures remained after application of the simplex minimization procedure (Nelder & Mead 1965) and orientation of the molecule at the air–water interface (Brasseur et al 1983a). The values of the torsional angles are listed in Table 2, along with the distance (Δ) between the hydrophobic and the hydrophilic centres and the hydrophobic and hydrophilic transfer energy (E_{pho}, E_{phi}). The distance between the hydrophobic and hydrophilic centres and the hydrophobic–hydrophilic balance of amiodarone are similar to those obtained for several amphiphilic molecules forming insoluble monolayers at the air–water interface (Brasseur et al 1982, 1983b). Fig. 3a, b shows the stereoviews of the two most probable structures obtained. These are identical to each other with respect to the conformation of the 2-butyl-3-[3,5-diiodobenzoyl]benzofuran moiety. They differ only by the conformation of the 2-diethylaminoethoxy moiety attached in position 4 on the benzoyl. These two structures were assembled in monolayers. The mean molecular area occupied by each structure is given in Table 2. A systematic analysis was performed for conformers A and B on the angles α_2, α_3, α_4, α_5, α_6, α_7 which were given successive increments of 60°. This analysis showed that the amino group and its two ethyl substituents moved freely (angles α_5, α_6 and α_7) and that

Table 2. Most probable conformers after minimization and orientation at the air–water interface. The mean molecular area was calculated for each molecule from both the area occupied by the drug molecule and the intermolecular area in a cluster of 7 molecules. This area is estimated after projection on the X–Y interface plane, using a grid of squares, each with a 0·1 nm side.

Conformers	Torsional angles α_1	α_2	α_3	α_4	α_5	α_6	α_7	α_8	α_9	α_{10}	α_{11}	Δ nm	E^{tr}_{pho}	E^{tr}_{phi}	Mean area $nm^2\,mol^{-1}$
A	180	−87	182	179	62	141	259	120	157	173	181	0·239	206·2	44·6	0·63
B	0	−83	166	184	61	141	259	120	159	176	183	0·240			0·56

Δ is the distance between the hydrophobic and hydrophilic centres.
E^{tr}_{pho} and E^{tr}_{phi} are respectively the sum of the hydrophobic transfer energy and the sum of the hydrophilic transfer energy ($KJ\,mol^{-1}$).

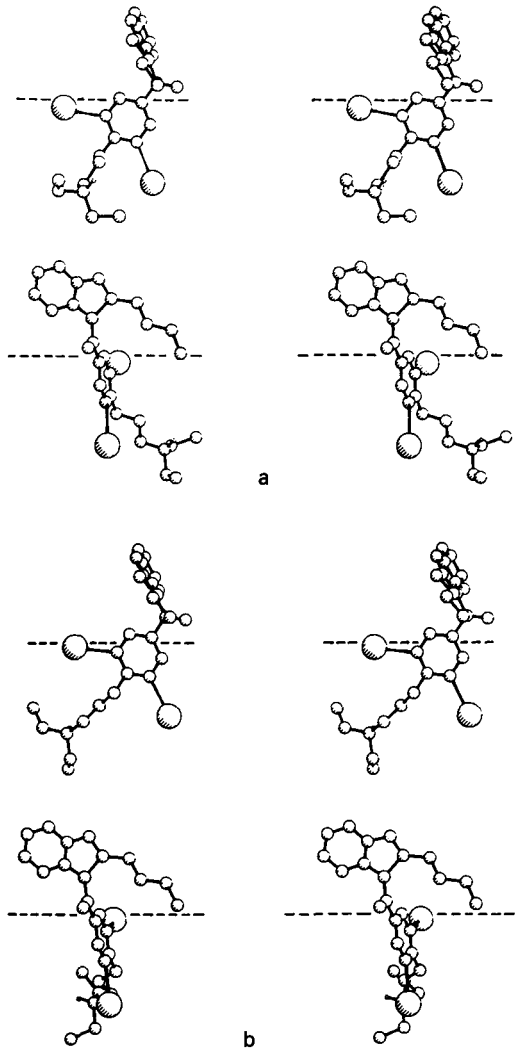

FIG. 3. Stereoviews of the most probable conformers of amiodarone after minimization and orientation at the air–water interface. The two views are taken at 90° of one another. Dotted line delineates the interface plane. Figures a and b represent the conformers A and B, respectively.

consequently the values given for these angles in Table 2 correspond only to one possible structure and that other possible structures could exist even though they are not represented in Table 2. The fact remains, however, that the free rotation around angles α_5, α_6, α_7 will not modify the orientation of the isolated amiodarone molecule at the interface and of the benzofuran and benzene rings. The high value of the calculated area (A = 0·56 − 0·63 nm^2) compared with the experimental area (A = 0·44 nm^2) must be explained in terms of the procedure used in the conformational analysis. At this stage of the development of the technique, the assembling process does not take into account a possible structural reorganization resulting from interactions between adjacent molecules. The calculated area (0·56–0·63 nm^2) corresponds to very low surface pressures (1–2·5 $mN\,m^{-1}$) associated with a state of monolayer organization in which the distance between adjacent spread molecules renders improbable such a structural reorganization.

Surface potential measurements

Fig. 4 shows the surface potential modification as a function of the subphase pH, for an amiodarone monolayer spread in a close-packed state. In agreement with the classical behaviour of ionized monolayers (Caspers et al 1974), ΔV and the electrostatic potential calculation Ψ decrease as a function of the subphase pH. Indeed, the measured surface potential ΔV (in mV) is given by the equation (Schulman & Hughes 1932):

$$\Delta V = \frac{1}{\varepsilon_0 A}[\mu_0(1-\alpha) + \mu_1\alpha] + \Psi \qquad (1)$$

in which μ_0 and μ_1 are the vertical contributions to the dipolar moment of the basic and acid forms, respectively, of the spread molecule, A is the area occupied per molecule in a close-packed monolayer, α the ionization degree of the spread molecule, Ψ the

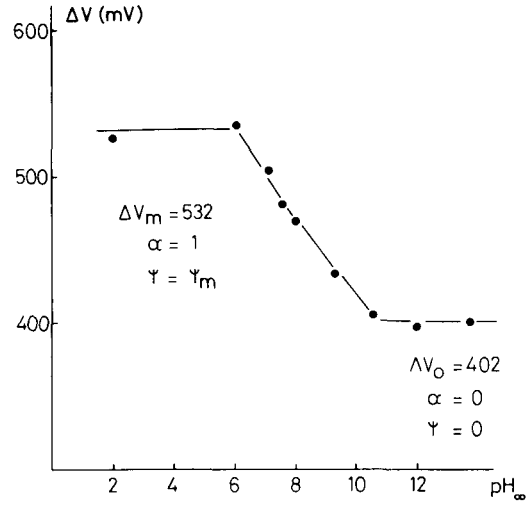

FIG. 4. Evolution of the surface potential (ΔV) of an amiodarone monolayer spread in the close-packed state ($A = 0{\cdot}44\ nm^2\ mol^{-1}$) as a function of the pH of the subphase. NaCl concentration, 0·10 M; temperature, 20·0 ± 0·5 °C.

value of the double-layer potential in the plane of charges, and ε_0 the permittivity of the vacuum.

For the non-ionized film ($\alpha = 0$):

$$\Delta V_0 = \frac{1}{\varepsilon_0 A}\mu_0 \tag{2}$$

For the fully ionized film ($\alpha = 1$):

$$\Delta V_m = \frac{1}{\varepsilon_0 A}\mu_1 + \Psi_m \tag{3}$$

From the combination of equations 1 to 3,

$$\Delta V = \alpha(\Delta V_m - \Delta V_0 - \Psi_m) + \Delta V_0 + \Psi \tag{4}$$

From equation 4 and the $\Delta V = f$ (pH) curve, the intrinsic ionization constant (K_i) can be calculated (Ferreira 1983). Since amiodarone is spread at the air–water interface on subphases of different pH, the following interfacial reaction does occur:

$$RNH^+ \rightleftarrows RN + H^+ \tag{5}$$

where RNH^+ and RN are the acidic and basic forms, respectively, of amiodarone spread at the interface. H^+ can be exchanged with the aqueous subphase. The equilibrium constant of this interfacial reaction is given by the equation:

$$K_i = \frac{(1-\alpha)[H^+]_s}{\alpha} \tag{6}$$

where $[H^+]_s$ is the H^+ concentration at the interface. $[H^+]_s$ is related to the bulk concentration $[H^+]_\infty$ through a Boltzmann distribution:

$$[H^+]_s = [H^+]_\infty\, e^{-e\Psi/kT} \tag{7}$$

where e is the electronic charge, k the Boltzmann constant, and Ψ the electrostatic potential at the interface.

The electrostatic potential ($\Psi > 0$) associated with the monolayer is given by the Gouy-Chapman theory of the electrical double-layer (Gaines 1966). At 20 °C and in the presence of a univalent electrolyte,

$$\Psi = \frac{2\,kT}{Ze}\,\mathrm{Sh}^{-1}\frac{\sigma}{(8\,kT\varepsilon\,\varepsilon_0 C)^{\frac{1}{2}}} \tag{8}$$

in which σ is the surface charge density, ε the relative permittivity of the solution, ε_0 the permittivity of the vacuum, Z the charge carried by the ion, and C the molar salt concentration of the subphase; the other parameters have the usual meaning. σ is directly related to the ionization degree through the relation:

$$\frac{\sigma}{e} = \frac{\alpha}{A} \tag{9}$$

where A is the area occupied per molecule in the close-packed state. From equation 6,

$$pK_i = pH_s + \log\frac{\alpha}{1-\alpha} \tag{10}$$

where pK_i is the interfacial pK, and pH_s is given by equation 7 which can be expressed as

$$pH_s = pH_\infty + \frac{e\Psi}{2{\cdot}3\,kT} \tag{11}$$

From 10 and 11 the following is obtained:

$$pK_i = pH_\infty + \log\frac{\alpha}{1-\alpha} + \frac{e\,\Psi}{2{\cdot}3\,kT} \tag{12}$$

Equation 12 defines an 'intrinsic pK_i' which does not depend on α or the ionic strength.

When the electrostatic contribution ($e\,\Psi/2{\cdot}3\,kT$) is not taken into account, an apparent pK_a is defined by

$$pK_a = pH_\infty + \log\frac{\alpha}{1-\alpha} \tag{13}$$

pK_i can be calculated from α and pH_s. These two parameters are related to Ψ (equations 8, 9, 11) which can be evaluated from surface potential measurements (equation 4). In the present work, the procedure of calculation is the following (Table 3):

1. Several values between 0 and 1 are imposed for α. For each α value, Ψ is calculated from equations 8 and 9. Ψ_m is calculated for $\alpha = 1$.

Table 3. pK_i and pK_a values calculated from the experimental curve $\Delta V = f(pH)_\infty$ using the procedure described in the text.

α	σ	Ψ (mV) eqn 8, eqn 9	ΔV eqn 4	pH Fig. 2	pH_s eqn 11	pK_i eqn 12	pK_a eqn 13
0·05	$1{\cdot}14\ 10^{-3}$	23	424	9·45	9·84	8·56	8·17
0·10	$2{\cdot}27\ 10^{-3}$	42	442	8·90	9·62	8·66	7·94
0·20	$4{\cdot}54\ 10^{-3}$	70	468	8·10	9·30	8·70	7·49
0·30	$6{\cdot}80\ 10^{-3}$	88	484	7·60	9·11	8·74	7·20
0·40	$9{\cdot}10\ 10^{-3}$	102	496	7·10	8·85	8·67	6·90
0·50	$1{\cdot}136\ 10^{-2}$	114	508	6·80	8·76	8·76	6·80

2. ΔV_0 and ΔV_m are obtained from the experimental $\Delta V = f(pH)_\infty$ (Fig. 4). For $pH_\infty < 6$, $\alpha = 1$ and $\Delta V = \Delta V_m = 532$ mV. For $pH_\infty > 10$, $\alpha = 0$ and $\Delta V = \Delta V_0 = 402$ mV.

3. From the α, Ψ, Ψ_m, ΔV_0 and ΔV_m values, ΔV can be estimated for each imposed α value (equation 4).

4. The experimental curve $\Delta V = f(pH)_\infty$ gives the pH_∞ value corresponding to each calculated ΔV value.

5. pH_s is calculated from pH_∞ and Ψ, and from equation 11 for each α value.

6. The pK_i and the pK_a values are obtained from equations 12 and 13.

Such a procedure applied to the amiodarone monolayer, suggests that the pK_i values do not depend on α, demonstrating the validity of the method, and that the extrapolated pK_i and pK_a values at $\alpha = 0$ are identical (8·7 ± 0·5) (Fig. 5, Table 3).

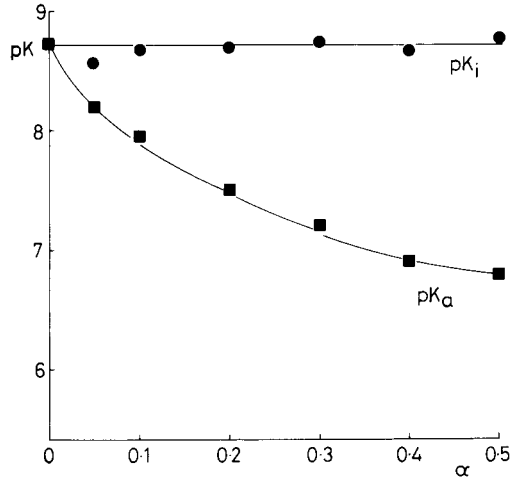

FIG. 5. Relationship between the calculated pK_i and pK_a values and the degree of ionization α. pK_i and pK_a were determined from Table 3.

There are several reported pK_a values for amiodarone: 5·6 (Andreasen et al 1981), 7·4 (Canada et al 1981) and 6·56 (Bonati et al 1984). The dispersion of the results and the low pK_a value obtained for a ternary amine might reflect the difficulties encountered in the determination of the aqueous dissociation constants of amiodarone, because of its low water solubility using classical potentiometric or UV spectrophotometric methods. This is also illustrated by the recent determination of a pK_a value of 9·12 obtained in a mixture of water and organic solvent (Gachon 1981). The determination of the pK_i of amiodarone performed in the present work is not subject to the difficulties encountered when working in a solution, since this operation is not affected by the low water solubility of the compound or by the use of a mixture of water and organic solvent. It should thus be more reliable than previous determinations. It is also closer to the expected value for any ternary amine.

In summary, several characteristics of amiodarone were determined at the air–water interface using the unique property of the compound to form a stable monolayer. These characteristics are the area occupied per molecule in the close-packed state (0·44 nm^2/molecule) and the pK_i (8·7). This approach combined with a semi-empirical conformational analysis gives a molecular picture of the amiodarone conformation and orientation at the air–water interface.

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