Eur Biophys J (1995) 23: 447–452

European Biophysics Journal

Interaction of chlorpromazine with phospholipid membranes

An EPR study of membrane surface potential effects

C. Anteneodo[1], **P. M. Bisch**[1], **J. Ferreira Marques**[1, 2]

[1] Centro Brasileiro de Pesquisas Físicas/CNPq, Rua Dr. Xavier Sigaud 150, 22290 RJ, Rio de Janeiro, Brazil (Fax: (0055) (021) 541-2047)
[2] Université Libre de Bruxelles, Bruxelles, Belgium

Received: 31 May 1994 / Accepted in revised form: 24 November 1994

Abstract. The interaction of chlorpromazine (CPZ) with artificial membranes (egg-yolk phosphatidylcholine liposomes) has been studied. Measurements of the surface electric potential, which is modified in the presence of the ionized form of the drug, were obtained by electron paramagnetic resonance spectroscopy (EPR) using a positively charged amphiphilic spin-probe. This probe partitions between the aqueous and lipidic phases depending on the surface potential and on the structural state of the membrane. The surface potential was measured as a function of drug concentration in the range where the spectral line-shapes are not affected by the incorporation of the drug. From these experimental results and through an appropriate formalism we obtain information on the binding of the drug to the lipid bilayer and on the ionization of the drug in the lipidic phase.

Key words: Phospholipid vesicles – Surface potential – Chlorpromazine – Drug binding – Electron paramagnetic resonance

Introduction

Chlorpromazine (CPZ) is clinically used as a tranquilizer; however, CPZ and related compounds have been shown to be very potent local anesthetics (Lee 1977). These substances are amphiphiles which affect the conductance of excitable cells by interacting with hydrophobic sites of biological membranes (Seeman 1972). However, the precise mechanism and site of action are still unknown.

Even if the targets of anesthetic action are probably membrane proteins, such as ion channels or pumps, it is also plausible that these drugs affect proteins by first interacting with lipids. Binding of anesthetics to the lipidic matrix may produce changes in membrane fluidity (Papahadjopoulos et al. 1975; Boggs et al. 1975), induce phase transitions in lipid bilayers (Hornby and Cullis 1981; McIntosh et al. 1983), modify membrane permeabilities (Escudero and Gutierrez-Merino 1987) or micellize the bilayer (Fernandez 1981; Frezzati et al. 1986). Since the activity of membrane proteins is highly sensitive to the chemical composition and to the structural phase of the surrounding lipids (Lee et al. 1983; Simmonds et al. 1982), it could be affected secondarily. In spite of the importance of drug-lipid interactions, attempts to correlate these effects with the anesthetic potency at clinically relevant concentrations have not been successfull or at least sufficient to describe the phenomenon of anesthesia.

Moreover, anesthetics, such as chlorpromazine (CPZ), with a tertiary amine group are protonated or not depending upon the pH value, and, therefore, the adsorption of the charged form of the drug modifies the surface potential of the membrane through change of the surface charge density. Alterations in the surface potential modify the concentration of charged substances close to the membrane surface and may consequently disturb the function of membrance proteins. Modifications in the surface potential by charged drugs have been studied (Ohki 1984; Lee 1978; Eriksson and Westman 1981; Barthel et al. 1988) and a wide discussion was opened about which form (protonated or not) is the biologically active one.

In studying drug interactions with membranes it is essential to know the fraction of drug which is bound to the membrance, and what is the partition of the charged and uncharged species between the bulk and the membrane. By using an appropriate formalism, the concentration of both species in the lipidic phase may be determined from measurements of the membrane surface potential (Goormaghtigh et al. 1981). This is evaluated in the present work through EPR experiments, by employing an amphiphilic spin-probe with net positive charge (Cafiso and Hubbell 1981).

Owing to the complex composition of biological membranes, artifitial liposomes are convenient model systems which allow the incorporation of drugs and the observation of their behaviour in the presence of a membraneous environment, making it possible to study the lipid contri-

Correspondence to: C. Anteneodo

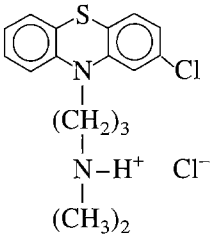
bution in the interaction of drugs with the biological membrane.

Materials and methods

Preparation of multilamellar phospholipid vesicles

From a stock solution of egg-PC in chloroform/methanol (purchased from Lipid Products, grade I) 100 mg/ml, solvent was removed by a flow of nitrogen gas to form a lipid film and the sample was dried in a vacuum dessicator for at least two hours. Potassium phosphate buffer (8 mM, pH 7) with or without NaCl (100 mM) was added to give a final lipid concentration of 50 mM and the solution stirred with a vortex mixer for 5 min at room temperature (above the lipid phase transition temperature).

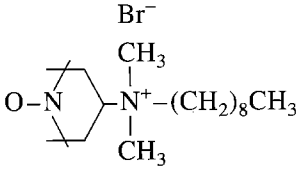
Chlorpromazine hydrochloride, whose structure is shown below, was obtained from Sigma Chemical Co.



In the case of CPZ containing vesicles, appropriate aliquots of a stock solution of CPZ (20 mM in chloroform) was added to the stock solution of egg-PC, following thereafter the same procedure above.

Sample preparation for EPR measurements

The amphiphilic and positively charged spin-probe S^+ (N,N-dimethyl-N-nonyl-N-tempoylammonium bromide), whose structure is shown below, was employed as a probe for EPR measurements.



After incubating the liposomes for 20 min, an aliquot of stock solution of spin-label (250 μM in the buffer solution) is incorporated and the sample incubated for an hour before introduction into a quartz capillary for EPR measurements.

EPR experiments were carried out on an X-band Varian E-9 spectrometer at 10 mW and a modulation field of 1 G. Sample preparation and experiments were carried out at 25 °C.

Evaluation of the surface electric potential

In the presence of vesicles, the amphiphilic spin-probe S^+ partitions between the aqueous and the lipidic phases as a function of surface potential, membrane structural state and lipid concentration. In the lipidic phase, the spin-probe mobility is limited and the EPR spectral lines are broader than in the aqueous phase. The resulting spectrum is the superposition of the spectra corresponding to each population (Fig. 1). Spectral analysis yields the spin-probe distribution in both phases (Cafiso and Hubbell 1981; Castle and Hubbell 1976).

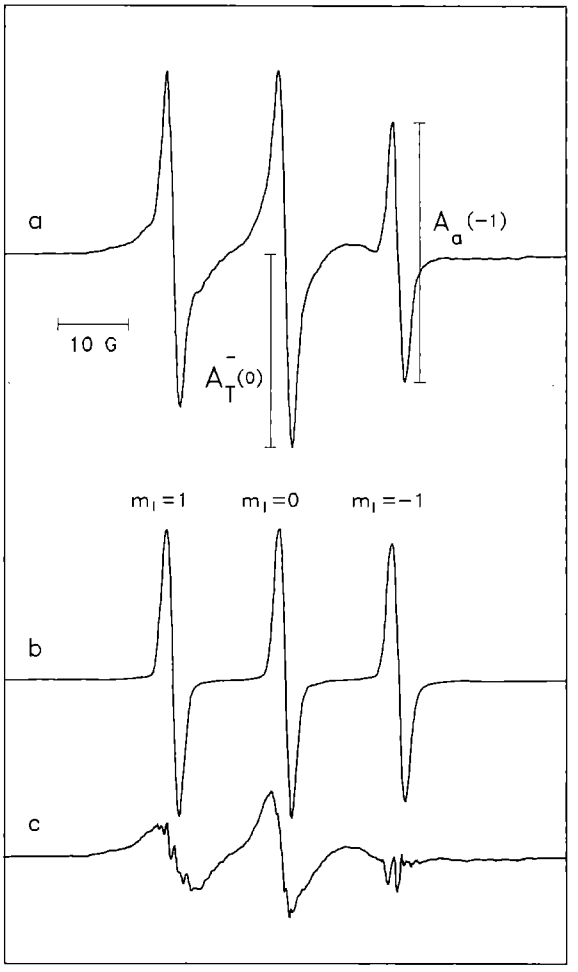


Fig. 1. EPR spectra of samples containing 20 μM S^+ in phosphate buffer 8 mM, pH 7. *a* in the presence of egg-PC vesicles, *b* in aqueous solution (×0.32) and *c* resulting from the substraction of spectrum *b* from *a*

The peak-to-peak intensity of the spectral line corresponding to $m_I=-1$ ($A_a(-1)$) (Fig. 1) is proportional to the quantity of spin-probe in the aqueous phase (n_a), while the base-to-peak intensity of the line $m_I=0$ ($A_T^-(0)$) is proportional to the quantity of total spin-label (n_T).

Consequently, it is possible to obtain the parameter $P = \frac{A_T^-(0)}{A_a(-1)} = \beta \frac{n_v}{n_a}$, where n_v is the quantity of spin-probe in the lipidic phase and β a constant which is related to spectral line-widths, being, therefore, characteristic of the lipidic phase structure.

Since the spin-probe does not cross the lipid bilayer (Castle and Hubbell 1976; Cafiso and Hubbell 1978), equilibrium partition is established between the aqueous phase

and the external surface of the liposomes depending on the surface potential, such that, the surface potential of the vesicle (ψ_s) may be calculated through the following expression:

$$\psi_s = \frac{k_B T}{e} \ln \frac{P}{P_0}, \quad (1)$$

where k_B is the Boltzmann constant, e the electron charge, T the temperature in degrees Kelvin and P_0 the value of P in the absence of charges in the vesicles (Eriksson and Westman 1981; Cafiso and Hubbell 1981).

The sensitivity of this technique allows one to work with spin-probe concentrations that do not affect the intrinsic charge of the membrane within the measurements accuracy.

Results and discussion

In the absence of a lipid environment, the number of probes in the aqueous phase (n_a) is proportional to the intensity of each spectral line, so that: $n_a = k_a^0 A_a^-(0) = k_a^{-1} A_a(-1)$. We have measured the values of $A_a^-(0)$ and $A_a(-1)$ for different spin-label concentration within the range 20 μM – 100 μM obtaining from the slopes of these linear plots $\frac{A_a^-(0)}{A_a(-1)} = 0.54 \pm 0.01$.

The value of β was determined from the spectra of vesicles (lipid concentration 50 mM) containing different spin-probe concentrations in the range 20–100 μM. We obtained $\beta = 0.15 \pm 0.02$ which is within the range of values reported in the literature (Eriksson and Westman 1981; Castle and Hubbell 1976). The same value was obtained for samples containing lipid concentrations in the range 20–100 mM and 20 μM of spin-probe.

Spectra of vesicles (lipid concentration 50 mM) containing the spin-probe (20 μM) and different drug concentrations were collected. Values of the parameter P, defined above, were obtained from spectral analysis and are displayed in Fig. 2 as a function of the ratio $[A]_T/[L]_T$ (total drug concentration divided by total lipid concentration). It can be observed that P decreases as drug concentration increases. If the line-shape of the immobilized spectral component is not altered by the presence of the drug, then the decrease of P indicates the decrease of the fraction of spin-probe incorporated in the vesicles, as a consequence of the increase of the adsorbed positively charged drug. The parameter β is used to evaluate line shape changes.

For samples containing CPZ, the value of β was calculated and compared to the value obtained in the absence of the drug (Fig. 3). It can be seen that for low values of $[A]_T/[L]_T$ (up to 0.09 and 0.05, in the absence and presence of NaCl, respectively) the value of β remains nearly constant (0.15±0.02) at the value observed in the absence of the drug, while, for higher CPZ concentrations, the value of β increases with drug concentration. A variation in the value of β may be due to the alteration of the spectral component corresponding to the spin-probe in the vesicles or to the presence of a new spectral component. In the first case, the modification of β indicates a modification in the structural state of the membrane. For instance, if the bilayer structure is modified such that the spin-probe is less immobilized, the line-widths of the spectral component corresponding to the spin-probe in the membrane decreases and, therefore, β increases. In the second case, a new component may be a consequence, for example, of the drug micellization at concentrations of the drug above its critical micellar concentration. However, spectral analysis of samples of CPZ 10 mM in the absence of vesicles and in phosphate buffer 8 mM, NaCl 100 mM, pH 7, conditions under which the drug is micellized, with 20 μM spin-probe, does not show any line broadening. This indicates that no incorporation of the spin-probe in micelles of the drug occurs. We conclude that the fact of β increasing with drug concentration is presumely due to the in-

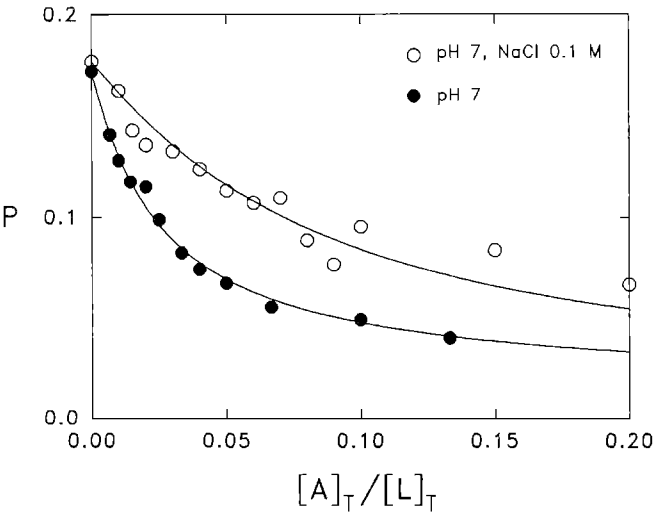


Fig. 2. P as a function of $[A]_T/[L]_T$, for samples in phosphate buffer 8 mM, pH 7, without NaCl (•) and with NaCl 100 mM (○). Lipid concentration was 50 mM. The maximum error in the determination of P is 10% as estimated from the reproducibility of EPR spectra recordings. Symbols correspond to the mean value of at least two measurements

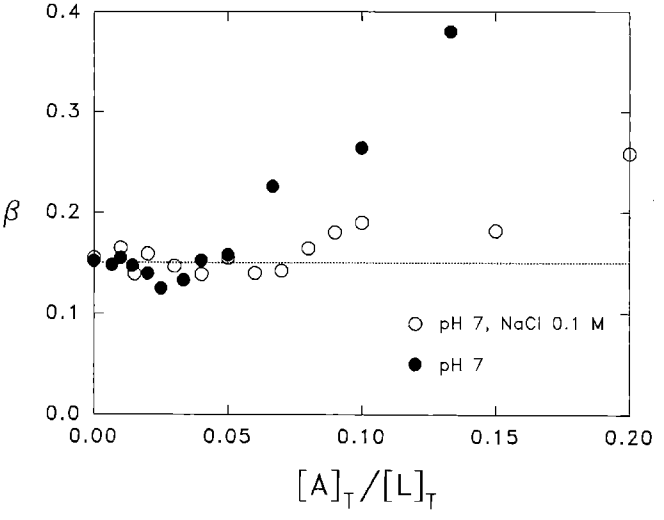


Fig. 3. β as a function of $[A]_T/[L]_T$, for samples in phosphate buffer 8 mM, pH 7, without NaCl (•) and with NaCl 100 mM (○). Lipid concentration was 50 mM. The error in β is less than 10%

crease of either membrane fluidity or disorder, taking place at the level of head-groups, which is the probed region. This effect could also be a consequence of the formation of mixed micellar aggregates producing membrane rupture. Whatever the case, if the structural state of the membrane is altered, the incorporation of the spin-probe in the vesicles will not only be a function of the electric surface potential. In this case, it is also expected that the adsorption constants of the drug would be altered as more drug molecules are incorporated. Then, in order to fit the model, we consider only the region where β is constant, so that the spin-probe incorporation is governed by electrostatic effects. In this case, the spin-probe incorporation is only a function of the surface electric potential of the membrane. Values of β in the range 0.15 ± 0.02 are within the experimental error, consistent with those observed by other authors (Eriksson and Westman 1981; Castle and Hubbell 1976), even in the absence of drugs.

The value of the surface electric potential ψ_s was obtained, from Eq. (1), for different CPZ concentrations ($[A]_T$) corresponding to the range in which β is contant. For all samples, the concentration of lipids was 50 mM. In Fig. 4, we display the plots ψ_s vs. $[A]_T/[L]_T$ obtained for samples at pH 7, with and without NaCl, as well as the best fits of the model described in the Appendix. It can be observed that these curves show saturation for high CPZ concentrations. The presence of a positive net charge produces a surface potential which will repel other charged drug molecules; thus, the concentration of the drug at the membrane/solution interface will diminish as more positively charged drug molecules are incorporated. On the other hand, the saturation of the lipidic sites available for drug incorporation also contributes to the shape of the curve. The electrostatic effect is also shown by the addition of salt to the solution, as shown in Fig. 4, higher salt concentration produces a lower surface potential due to electrostatic shielding.

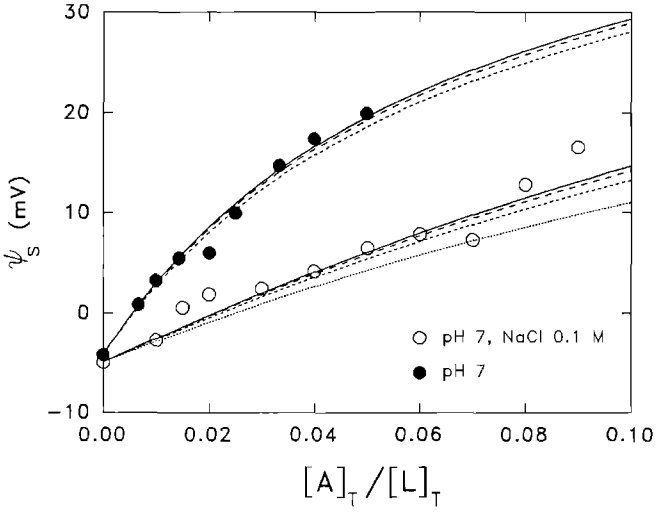


Fig. 4. ψ_s as a function of $[A]_T/[L]_T$, for samples in phosphate buffer 8 mM, pH 7, without NaCl (•) and with NaCl 100 mM (○). Lipid concentration was 50 mM. ψ_s was calculated from expression (1). The error in ψ_s is less than 5 mV. Fitted curves correspond to: $pK_i^v=7.9$ e K_{AH^+} (l/mol)=10^2 (···), $10^{2.5}$ (---), 10^3 (–––), ∞ (——)

For vesicles containing S^+, 20 μM in phosphate buffer pH 7, we have obtained P values as a function of NaCl concentration (results not shown), observing that P decreases as salt concentration increases. If the membrane were electrically neutral, then, the charge introduced by the spin-probe (positively charged) would produce a positive surface potential which would decrease as ionic strength increases. In that case P would also increase. However, the effect observed is opposite. This fact may be explained if we assume the presence of impurities with negative charge in the membrane. Quantitatively, we found that the impurity corresponds to 1% of the lipids present, which is consistent with the specifications of the product which was not further purified. The presence of this impurity was taken into account in our calculations. The value of P in the absence of charges in the membrane (P_0) would correspond to $P_0=0.146\pm0.008$. Owing to the presence of negatively charged impurities the value of ψ_s in the absence of the drug is negative (see Fig. 4). When NaCl is incorporated into the samples the electric potential is still lower ($\simeq -1$ mV) owing to the adsorption of Cl^-. The value $K_{Cl}=0.17$ l/mol was determined from the value of the electric potential in the absence of drug when NaCl is present and it confirms values in the literature (Eriksson and Westman 1981).

The plots ψ_s vs. $[A]_T/[L]_T$ were fitted with an expression resulting from the formalism described in the Appendix. The ionization constant of the drug incorporated in the vesicles (K_i^v) was determined from the best fits. We obtained $pK_i^v=7.9$ which corresponds to $\Delta pK_i=pK_i^w-pK_i^v=1.4$, where K_i^w is the constant of ionization of the drug in the aqueous phase. That value is in good agreement with those obtained through other techniques (Rooney and Lee 1983). Since the value of pK_i in the vesicles is lower than that corresponding to the aqueous phase, it indicates that the ionization process in the vesicles is displaced such that the neutral form is favoured. Moreover, this result indicates that the adsorption constant for the neutral form of the drug (K_A) is higher than that for the cationic (K_{AH^+}), since they are in the ratio

$$\frac{K_{AH^+}}{K_A}=\frac{K_i^w}{K_i^v},$$ as shown in the Appendix.

For the adsorption constant K_{AH^+} (expressed in l/mol), we find no significative difference between curves obtained for $K_{AH^+}\geq 10^3$, within the range of concentrations studied. Other techniques (Barthel et al. 1988; Rooney and Lee 1983; Welti et al. 1984) estimate the association constant for the charged form of the drug to be of the order of 10^4 which is consistent with our results. However, at least for drugs such as CPZ with a strong adsorption to phospholipid vesicles, the present method seems to be not sufficiently precise to give more accurate values. Nevertheless, with more sensitive equipment, it would be possible to work using lower spin-label concentrations and, consequently, lower lipid and drug concentrations, such that saturation would be reached without substantial structural modifications. In that case, a more complete curve would be fitted, eliminating the inaccuracy which arises from considering almost only the linear region of the curve ψ_s vs. $[A]_T/[L]_T$.

In order to estimate the surface potential we assumed that the multilamelar vesicles formed in the absence of the drug as well as in its presence are always characterized by the same mean ratio of exposed lipids in the external layer (6%). This assumption may be valid at least in the range where β is constant. The drug can reach both sides of the membrane, since it can cross the membrane in the neutral form and, thereafter, the ionization equilibrium may be reached inside the vesicles. However, the spin-label incorporated after the vesicles are formed does not cross the membrane, so that only a fraction of the total lipidic surface is accessible. In order to estimate this fraction, we performed measurements of P in the absence of the drug (50 mM lipids with spin-probe 20–100 μM and 20–100 mM lipids with spin-probe 20 μM) and we calculated ψ_s, through Eq. (1). These data were fitted with our model, for the case in which the drug is absent, and the best fits were obtained by assuming 6% of exposed lipids. Moreover this value is consistent with those found in the literature for similar preparations (Szoka and Papahadjopoulos 1980; Schwartz and McConnell 1978). Besides, the value of the constant of association of the spin-label for the vesicles calculated with that value of exposed lipids is of the same order as that found in the literature (Castle and Hubbell 1976; Eriksson and Westman 1981). However, if the concentration of exposed lipids changes with drug concentration, the value of P_0 would change and, therefore, the determination of ψ_0 would be affected. The present systematic study shows clearly the scope of the technique, which allows one to distinguish structural from electrostatic effects, and demonstrates the applicability of the method, which, even if not sufficiently precise for CPZ, may be applied and improved for the study of other drugs.

Acknowledgements. We thank CNPq-Brazil for financial support.

Appendix

Determination of the association constants

Since, in the aqueous phase, we have $AH^+ \overset{K_i^w}{\rightleftharpoons} A + H^+$, the bulk concentrations of the uncharged and charged species ($[A]_b$ and $[AH^+]_b$, respectively) are in proportions given by:

$$K_i^w = \frac{[A]_b\,[H^+]_b}{[AH^+]_b}, \tag{A.1}$$

where $[H^+]_b$ ($=10^{-\mathrm{pH}}$) is the bulk concentration of protons expressed in mol/l and K_i^w is the ionization constant of the drug. For chlorpromazine, $\mathrm{p}K_i^w = -\log K_i^w \simeq 9.3$ (Rooney and Lee 1983; Welti et al. 1984), thus, at physiological pH, the drug is charged. Similarly, in the membrane surface where the drug is adsorbed, we have:

$$K_i^v = \frac{[LA]\,[H^+]_s}{[LAH^+]}, \tag{A.2}$$

where $[LA]$ and $[LAH^+]$ are, respectively, the volumetric concentrations (in moles/liter of solution) of each species adsorbed to the membrane, K_i^v is the ionization constant of the drug in the membrane and the subscript s corresponds to concentrations at the membrane/buffer interface. In the presence of a surface electric potential ψ_s the concentration of an ionic species X with charge ze, at the interface, is given by the Boltzmann equation:

$$[X]_s = [X]_b\, \mathrm{e}^{-\frac{ze\psi_s}{k_B T}}. \tag{A.3}$$

If we consider that only one molecule of drug per lipid can be adsorbed and $[L]$ is the concentration of free lipidic sites, then the total concentration of lipids is $[L]_T = [L] + [LA] + [LAH^+]$. Thus, we may write

$$[LAH^+] = \gamma^+ [L]_T$$
$$[LA] = \gamma^\circ [L]_T,$$

where γ^+ and γ° are the fraction of lipidic sites occupied by the charged and neutral adsorbed drug, respectively.

In the case of zwitterionic lipids, such as phosphatidylcholine, the surface charge density σ is given by the charged drug adsorbed to the membrane, so that if $\mathcal{A}$ is the average area per lipid (we considered $\mathcal{A} \sim 70\ \text{Å}^2$), we have:

$$\sigma = \gamma^+ e/\mathcal{A}. \tag{A.4}$$

On the other hand, from the Poisson-Boltzmann equation, the dependence of the surface charge density on the surface electric potential of the membrane is given by:

$$\sigma = \sqrt{\frac{\varepsilon N_A 10^{-3} k_B T}{2\pi}} \left(\sum_i c_i \left\{ \mathrm{e}^{-\frac{z_i e \psi_s}{k_B T}} - 1 \right\} \right)^{1/2}, \tag{A.5}$$

where c_i is the molar concentration of the ion i with charge z_i.

Consistently with the hypothesis that only one molecule of drug per lipid can be adsorbed, the binding of both forms of the drug may be described by the following processes:

$$AH_s^+ + L \overset{K_{AH^+}}{\rightleftharpoons} LAH^+ \qquad K_{AH^+} = \frac{[LAH^+]}{[L][AH^+]_s}$$

$$A_s + L \overset{K_A}{\rightleftharpoons} LA \qquad K_A = \frac{[LA]}{[L][A]_s},$$

where AH_s^+ and A_s represent the charged and uncharged forms of the drug at the interface, whose concentrations are related to the respective bulk concentrations through Eq. (A.3), and K_{AH^+} and K_A are, respectively, the association constants of the charged and uncharged forms of the drug with the lipid membrane, which may be rewritten as:

$$K_{AH^+} = \frac{\gamma^+}{1-\gamma^\circ-\gamma^+} \frac{1}{[AH^+]_s} \tag{A.6}$$

$$K_A = \frac{\gamma^\circ}{1-\gamma^\circ-\gamma^+} \frac{1}{[A]_s}. \tag{A.7}$$

These expressions correspond to the classical Langmuir adsorption isotherms.

From Eqs. (A.1), (A.2), (A.6) and (A.7), it may be shown that the association and ionization constants are related according to:

$$\frac{K_{AH^+}}{K_A} = \frac{K_i^w}{K_i^v}. \tag{A.8}$$

The fact that the ratio between the two species in the membrane may be different from the ratio of the two species in the aqueous environment may also be interpreted as being due to the fact that the partition constant between the membrane and the bulk is different for each form of the drug.

Considering (A.1)–(A.6) and the conservation of the total number of molecules of drug and lipids, one may build a system of equations which allows one to find ψ_s as a function of $[A]_T/[L]_T$. K_{AH^+} and K_i^v are the parameters which are obtained by fitting that relation to the experimental data. Once these parameters are known, K_A may be obtained through the relation (A.8).

Since it is also necessary to take into account the adsorption of Cl^- to the lipidic membrane, we considered the additional reaction $Cl_s^- + L \overset{K_{Cl^-}}{\rightleftharpoons} LCl^-$, where K_{Cl^-} is the adsorption constant of Cl^- to the lipidic membrane and it is given by:

$$K_{Cl^-} = \frac{\gamma^-}{1-\gamma^\circ-\gamma^+-\gamma^-}\frac{1}{[Cl^-]_s}, \tag{A.9}$$

with γ^- equal to $[LCl^-]/[L]_T$.

In this case, we have $\sigma \mathcal{A}/e = \gamma^+ - \gamma^-$ and

$$K_{AH^+} = \frac{\gamma^+}{1-\gamma^\circ-\gamma^+-\gamma^-}/[AH^+]_s.$$

The charge introduced by the presence of impurities and the spin-label itself was also included in an additional corrective term to the surface charge.

References

Barthel D, Zschoernig O, Lange K, Lenk R, Arnold K (1988) Interaction of electrically charged drug molecules with phospholipid membranes. Biochim Biophys Acta 945:361–366

Boggs JM, Yoong T, Hsia JC (1975) Site and mechanism of action: I. Effect of anesthetics and pressure on fluidity of spin-labeled lipid vesicles. Mol Pharmacol 12:127–135

Cafiso DS, Hubbell WL (1978) Estimation of transmembrane potentials from phase equilibria of hydrophobic paramagnetic ions. Biochemistry 17:187–195

Cafiso DS, Hubbell WL (1981) EPR determination of membrane potentials. Annu Rev Biophys Bioeng 10:217–244

Castle JD, Hubbell WL (1976) Estimation of membrane surface potential and charge density from the phase equilibrium of paramagnetic amphiphile. Biochemistry 15:4818–4831

Eriksson LEG, Westman J (1981) Interaction of some charged amphiphilic drugs with phosphatidylcholine vesicles. A spin label study of surface potential effects. Biophys Chem 13:253–264

Escudero B, Gutiérrez-Merino C (1987) Effects of local anesthetics on the passive permeability of sarcoplasmic reticulum vesicles to Ca^{++} and Mg^{++}. Biochim Biophys Acta 902:374–384

Fernandez MS (1981) Determination of surface potential in liposomes. Biochim Biophys Acta 646:27–30

Frezzati WA, Toselli WR, Schreier S (1986) Spin label study of local anesthetic-lipid membrane interactions. Phase separation of the uncharged form and bilayer micellization by the charged form of tetracaine. Biochim Biophys Acta 860:531–538

Goormaghtigh E, Caspers J, Ruysschaert JM (1981) Lipid-drug electrostatic interactions in model membranes. J Colloid Interface Sci 80:163–170

Hornby AP, Cullis PR (1981) Influence of local and neutral anaesthetics on the polymorphic phase preferences of eff yolk phosphatidylethanolamine. Biochim Biophys Acta 647:285–292

Lee AG (1977) Local anesthesia: the interaction between phospholipids and chlorpromazine, propranolol and practolol. Mol Pharmacol 13:474–487

Lee AG (1978) Effects of charged drugs on the phase transition temperatures of phospholipid bilayers. Biochim Biophys Acta 514:95–104

Lee AG, East JM, Jones OT, McWhirter J, Rooney EK, Simmonds AC (1983) Binding of dansyl propranolol to the $(Ca^{++}+Mg^{++})$-ATPase. Biochim Biophys Acta 732:441–454

McIntosh TJ, McDaniel RV, Simon SA (1983) Induction of an interdigitated gel phase in fully hydrated phosphatidylcholine bilayers. Biochim Biophys Acta 731:109–114

Ohki S (1984) Adsorption of local anesthetics on phospholipid membranes. Biochim Biophys Acta 777:56–66

Papahadjopoulos D, Jacobson K, Poste G, Shepherd G (1975) Effects of local anesthetics on membrance properties: I. Changes in the fluidity of phospholipid bilayers. Biochim Biophys Acta 394:504–519

Rooney EK, Lee AG (1983) Binding of hydrophobic drugs to lipid bilayers and to the $(Ca^{++}+Mg^{++})$-ATPase. Biochim Biophys Acta 732:428–440

Seeman P (1972) The membrane actions of anesthetics and tranquilizers. Pharmacol Rev 24:583–655

Schwartz MA, McConnell HM (1978) Surface areas of lipid membranes. Biochemistry 17:837–840

Simmonds AC, East JM, Jones EK, Rooney EK, McWhirter J, Lee AG (1982) Annular and non-annular binding sites on the $(Ca^{++}+Mg^{++})$-ATPase. Biochim Biophys Acta 693:398–406

Szoka F Jr, Papahadjopoulos D (1980) Comparative properties and preparation of lipid vesicles (liposomes). Annu Rev Biophys Bioeng 9:467–508

Welti R, Mullikin LJ, Yoshimura T, Helmkamp GM (1984) Partition of amphiphilic molecules into phospholipid vesicles and human erythrocyte ghosts: measurements by ultraviolet difference spectroscopy. Biochemistry 23:6086–6091