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Interaction of triflupromazine with distearoylphosphatidylglycerol films studied by surface pressure isotherms and cyclic voltammetry at a 1,2-dichloroethane/water interface

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ABSTRACT

The effect of triflupromazine (TFP) on molecular packing of distearoylphosphatidylglycerol (DSPG), adsorbed at the water/1,2-dichloroethane interface, was investigated using cyclic voltammetry (CV) and surface pressure–molecular area isotherm. TFP partition in the DSPG monolayer changes the structure of the film. The results indicate that a fluidizing effect, dependent on the time and the drug concentration, takes place leading to an increase in the permeability of the film. This effect is produced by TFP either from the organic or from the aqueous phase due to the amphiphilic nature of this drug. Nevertheless, the expansion of the film is enhanced when TFP acts from the aqueous phase.

1. Introduction

Triflupromazine (TFP) is a phenothiazine which acts at the central nervous system. It is used in the treatment of disorganized and psychotic thinking and also to treat false perceptions (e.g. hallucinations or delusions) as well as to control violent behavior during acute episodes of psychotic disorders. The antipsychotic effects produced by TFP are due to the blocking of dopamine receptors.

TFP, as well as phenothizine drugs in general, are amphiphilic molecules with hydrophobic phenothiazine ring system and hydrophilic ionizable amine tail group (see Scheme 1). This structural property explains the ability of these drugs to partition into biological membranes inducing several lateral effects. For this reason several authors have studied the interaction of phenothiazines with zwitterionic and anionic phospholipids employing different techniques. Chlorpromazine penetration into the lipid core of membranes has been demonstrated trough measurements on lipid monolayers and the intrinsic binding constant has been calculated by surface pressure and electrophoretic mobilities measurements on Langmuir monolayers and liposomes, respectively [1]. Broniec et al. [2] demonstrated that trifluperazine had little effect on monolayers of dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanol-amine but increased the mean molecular area of dipalmitoylphosphatidylserine monolayers and this increase was proportional to trifluperazine concentration. Those authors concluded that this drug interacts more strongly with phospholipids located in the inner layer of the plasma membrane, while it does not interact with phospholipids located in the outer region. Hendrich et al. studied the interaction between a trifluperazine analog and bilayers composed by zwitterionic lipids employing fluorescence spectroscopy, differential scanning calorimetry and electron spin resonance [3] showing that this drug produces changes in the lipid bilayers properties increasing their permeability. These changes explain the enhancement produced by phenothizines in the rate of passive anticancer drugs' influx contributing to their accumulation in cancer cells. The interaction of phenothiazines with phosphatidylcholine was also analyzed from the partition coefficient of these drugs between bilayers of unilamellar vesicles and water, determined by second-derivative spectrophotometry [4]. Hidalgo et al. studied the cooperative interaction of trifluperazine and chlorpromazine with phospholipid monolayers employing surface pressure and surface potential isotherms [5]. They observed that monolayers formed by charged phospholipids (dipalmitoylphosphatidylglycerol) became more expanded with the drug incorporation. Alves Pinto et al. investigated the kinetic and thermodynamic of the promethazine and thioridazine interaction with dipalmitoylphosphatidylcholine and dipalmitoylphosphatidic acid Langmuir films [6]. They concluded that drug molecular size, interface mechanical properties and thermodynamic stability are the most important parameters

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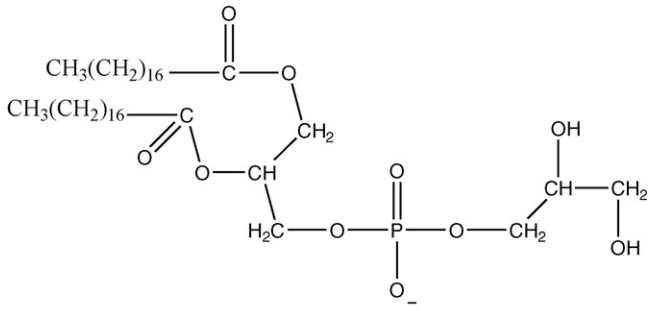
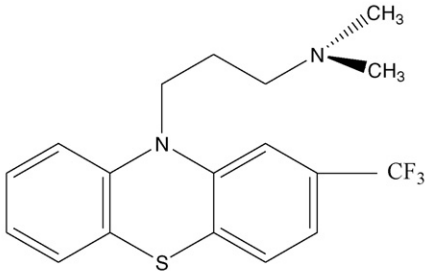


DSPG $pK_{a1} = 1$



Trifluprom azine $pK_a = 9.4$

Scheme 1. Chemical structure of TFP and DSPG.

controlling the adsorption–desorption rate and the affinity binding constant of these drugs into monolayers. Liu et al. studied the concentration and time dependant behavior of chlorpromazine interaction with supported bilayer lipid membrane on glassy carbon electrode using cyclic voltammetry and ac impedance spectroscopy [7]. They postulated three stages for the interaction global process: drug penetration into the membrane, defects formation and solubilization of the membrane.

Electrochemical measurements applied to liquid–liquid interfaces modified by films of different nature, have been carried out with the aim of developing new biomimetic membrane models [8–32]. In this sense, the adsorption of lipid monolayers [25,27–29,32], proteins [11], surfactants [24] or polyelectrolytes [15] has been studied and the properties of these films have been characterized by cyclic voltammetry, electrochemical impedance spectroscopy and surface tension measurements. These electrochemical techniques were also employed to determine the porosity and thickness of alumina membranes [20]. One aspect of special interest has been the study of the interaction or the complex formation between phospholipid monolayers with alkaline and alkaline earth cations [18], trivalent cations [30], peptides [9] and different organic anions [31] with the aim to analyze the blocking effect produced by these species on the monolayer structure. The study of the incorporation of pharmaceutical drugs as antibiotics [16,17] or anxiolytic [21–23] into phospholipid monolayers adsorbed at liquid/liquid interfaces are very important for their contribution to the knowledge of the interaction between drugs and biological membranes components. On the another hand, the rates of electron transfer between two redox species trough a monolayer adsorbed at liquid interfaces were measured by scanning electrochemical microscopy [8,10]. The electrochemical study of monolayers generated by the Langmuir [12] or Langmuir–Blodgett methods [13,14,19,26] represent an important progress in the knowledge and control of the state of these films.

In previous papers, we studied the transfer of phenothiazine derivatives (promazine, chlorpromazine, trifluprom azine, methotrimeprazine, perphenazine and fluphenazine) across the water/1,2-dichloroethane interface, using cyclic voltammetry. The partition coefficients of ionic species of phenothiazines, were calculated from the transfer potentials measured at $pH < pK_a$. These values were correlated with the Hammet parameter of substituents in order to explain the differences in their biological activity based on the electronic effect of electron acceptor groups present in their structure [33]. We have also investigated the formation of promazine cation radical in 1,2-dichloroethane followed by π-mers complexation, using cyclic voltammetry at the ITIES and UV–visible spectrophotometry [34].

Although several authors have reported the fluidizing effect of phenothiazines, evidenced by the formation of a more expanded monolayer, with increased mean molecular area and increased permeability, to our knowledge, no studies were conducted to compare this fluidizing effect caused by supramolecular interactions taking place on both sides of lipids layers with the phenothiazine, i.e. at the polar head groups and near the hydrophobic chains. In this sense, electrochemical techniques applied at the interface between two immiscible electrolyte solutions (ITIES) are ideal to follow dynamic changes in the lipid layer compactness and interfacial interactions at a hydrophobic/hydrophilic boundary. For this purpose, cyclic voltammetry experiments are carried out, in the present paper, to analyze the effect of TFP on a distearoylphosphatidylglycerol (DSPG, Scheme 1) layer adsorbed at the water/1,2-dichloroethane interface, when TFP is added to the organic or the aqueous side of the interface. The results are correlated with surface pressure – molecular area measurements for DSPG monolayers adsorbed at the air/water interface.

2. Experimental

2.1. Materials and electrochemical cell

Cyclic voltammetry (CV), performed in a four-electrode system using a conventional glass cell of 0.18 cm^2 interfacial area, were conducted to characterize the film. Two platinum wires were used as counter electrodes and the reference electrodes were Ag/AgCl. The reference electrode in contact with the organic solution was immersed in an aqueous solution of 1.0×10^{-2} M tetraphenylarsonium chloride (TPAsCl, Sigma). Potential values (ΔE) reported in this work are those which include $\Delta\phi^{\circ}_{tr,TPAs+} = 0.364$ V for the transfer of the reference ion $TPAs^+$.

The base electrolyte solutions were 1.0×10^{-2} M $CaCl_2$ (p.a. grade) in ultrapure water and 1.0×10^{-2} M tetraphenyl arsonium dicarbollyl cobaltate (TPAsDCC) in 1,2-dichloroethane (DCE, Dorwil p.a.). TPAsDCC was prepared by metathesis of TPAsCl and cesium dicarbollyl cobaltate (Lachema p.a.). The pH of the aqueous phase was 6.00. In all the experiments, 2.00 mL of organic and aqueous phases were used to fill the cell.

The electrochemical cell used was as follows:

		TPAsCl	TPAsDCC	$CaCl_2$		
Ag | AgCl		$1x10^{-2}$ M	$1x10^{-2}$ M	$1x10^{-2}$M	AgCl |	Ag
		(w′)	(o)	(w)		
			Working interface			

Trifluprom azine (TFP, Hoffman – La Roche) at 5.0×10^{-4} M concentration was added to the aqueous phase in some experiments. At pH = 6.00 this drug is mainly present in its protonated form, $HTFP^+$, given that its pK_a value is 9.4. Chemical structure of TFP is shown in Scheme 1.

In other experiments TFP was added to the organic phase by injection of different volumes of a concentrated solution to

obtain a final concentration in the range between 2.5×10^{-5} M and 5.0×10^{-4} M.

Distearoylphosphatidylglycerol (DSPG) was of analytical grade (Sigma). A solution of 0.8 mg/mL DSPG was prepared in 1:2 methanol:chloroform. In order to form the lipid film, different volumes (50 or 100 μL) of DSPG solution were injected at the liquid/liquid interface, using a Hamilton microsyringe. Two methods were followed: (a) injection of phospholipid solution at the interface on the organic side after both phases were put in contact in the electrolytic cell and (b) addition of phospholipid solution in the organic phase before filling the cell. Methods (a) and (b) were found to be equivalent to the spread and adsorption methods, respectively, described by Kakiuchi et al. [35]. The experiments shown in this paper were done following the method (a). After the injection of lipid solution, a time equal to 60 min was required to obtain invariant and reproducible voltammetric response, indicating that a stable lipidic film had been formed. As a consequence, all experiments were performed after this equilibration time at room temperature equal to 25 ± 1 °C. Temperature was controlled with a temperature/humidity monitor.

TFP was added to 2.00 mL of the organic or the aqueous phase by injection of different volumes, ranged from 10 to 200 μL, of a 5.0×10^{-3} M solution of this drug, after t = 60 min from DSPG injection, time required to form a stable film. The injection was carried out far enough from the interface to ensure that it is not disrupted. The TFP concentrated solution was prepared employing water or DCE as solvents, depending on the phase were it was injected. It is important to point out that at pH = 6.00 DSPG polar head groups are negatively charged (pK_a = 1.0, Scheme 1).

CV was performed using a four-electrode potentiostat with periodic current interruption for automatic elimination of solution resistance. The voltage ranged from 0.200 to 0.700 V with a potential sweep generator (L y P Electrónica Argentina). Voltammograms were recorded employing a 10 bit computer board acquisition card connected to a personal computer.

2.2. Langmuir monolayers

2.2.1. Surface pressure – molecular area isotherms

Surface pressure – area isotherms were recorded with a Minitrough II from KSV Instruments Ltd. (Helsinki, Finland). The surface tension was measured according to the Wilhelmy plate method, using a platinum plate.

The aqueous subphase, contained in a Teflon trough (364 mm × 75 mm effective film area), was a 1.0×10^{-2} M $CaCl_2$ solution of pH = 6.00 with or without TFP at different concentrations.

To prepare DSPG monolayers at the air–water interface a 50 μL volume of DSPG in 1:2 methanol:chloroform (0.8 mg/mL) was carefully spread at the surface with a Hamilton micro-syringe. Before spreading DSPG solution, the subphase surface was cleaned by sweeping it with a Teflon barrier and then, any surface contaminant was removed by suction from the interface. The cleaning of the surface was checked by recording an isotherm in absence of DSPG and verifying a surface pressure value lower than 0.2 mN/m. After 10 min of spreading, to allow the evaporation of the solvent [6,13,14], the film was compressed with two barriers on each side of the trough at a compression speed of 5 cm/min while the automatic measurement of the lateral surface pressure (Π) was carried out.

All experiments were performed at a temperature of 25.0 ± 0.1 °C using a thermostat.

2.2.2. Drug penetration experiments

Penetration experiments were carried out at constant surface areas for two different initial lateral surface pressures ($\Pi_{i(1)}$ = 40 mN/m and $\Pi_{i(2)}$ = 15 mN/m) at 25.0 °C. After forming the monolayer as described above, different volumes of a TFP solution were injected into the subphase containing $CaCl_2$ 1.0×10^{-2} M, to obtain concentration values equal to 1.0×10^{-3} M, 5.0×10^{-4} M and 3.0×10^{-4} M. Then, the temporal dependence of surface pressure was recorded.

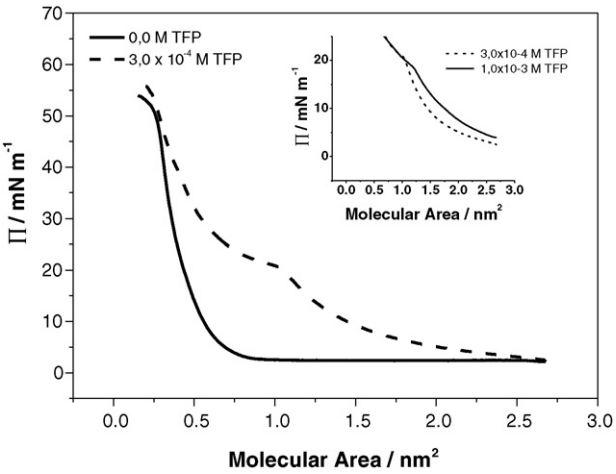


Fig. 1. Surface pressure (Π) as a function of the mean molecular area for DSPG monolayer at the air–subphase interface. Subphase composition: 1.0×10^{-2} M $CaCl_2$, pH = 6.00 + (—) 0.0 M or (- - - -) 3.0×10^{-4} M TFP. *Inset*: Effect of TFP concentration on surface pressure (Π) – mean molecular area isotherm for DSPG monolayer at the air–subphase interface. Subphase composition: 1.0×10^{-2} M $CaCl_2$ + (—) 1.0×10^{-3} M or (- - -) 3.0×10^{-4} M TFP, pH = 6.00.

3. Results and discussion

3.1. Surface pressure isotherms

The effect of TFP on the anionic DSPG monolayer can be noted in Fig. 1. This figure shows the surface pressure–area isotherm obtained at 25 °C in absence or in presence of TFP in the subphase. In the first case the change in slope observed in the isotherm corresponds to the liftoff due to the gaseous–liquid condensed phase transition. The monolayer collapse is evident at surface pressures around of 50 mN/m, with a mean molecular area of 0.26 nm^2, in agreement with the results obtained by other authors [36]. Important changes in the surface pressure–area isotherm are visible when DSPG monolayer was spread on a subphase containing TFP. In this figure, the area is given in terms of area per DSPG molecule and, therefore, any changes can be attributed to the interaction between DSPG and TFP molecules in the monolayer. As it can be noted in the figure, the surface pressure–area isotherm was shifted toward larger areas per molecule, indicating that TFP has been incorporated into the film. Thus, the liftoff of the surface pressure started at a larger area per molecule. Moreover, a change in the shape of the isotherm is evident, appearing a plateau between the first liftoff corresponding to the gaseous–liquid expanded phase transition and the second one due to liquid expanded–liquid condensed transition. As the film was further compressed, the isotherm of the mixed monolayer asymptotically approached that of the phospholipid monolayer. At pressures close to that corresponding to the collapse, no difference are observed in both curves, indicating that TFP molecules are probably squeezed out from the monolayer at these high pressures.

The changes above described are evidencing a considerable expansion of the monolayer caused by the presence of TFP and, as can be noted in the inset, they are enhanced when TFP concentration increases. Similar expanding effects were found by

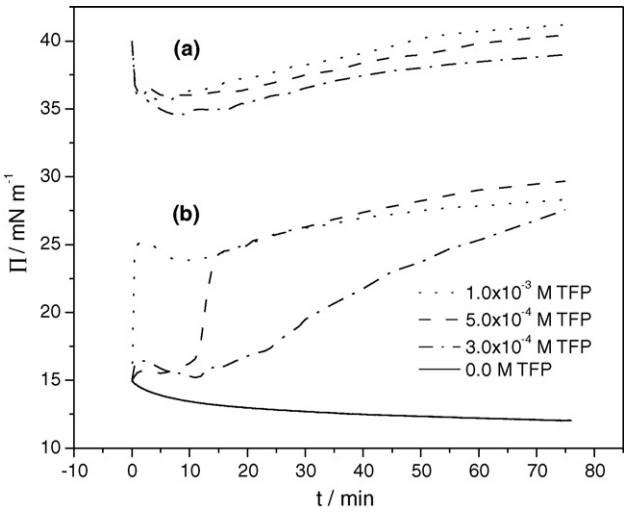


Fig. 2. Effect of TFP concentration and time on TFP penetration into DSPG monolayer at the air–subphase interface for two initial pressure (Π_i): (a) $\Pi_{i(1)}$ = 40 mN/m; (b) $\Pi_{i(2)}$ = 15 mN/m. Subphase composition: 1.0×10^{-2} M $CaCl_2$ + (—) 0.0 M, (-·-·-·-) 3.0×10^{-4} M, (- - - -) 5.0×10^{-4} M or (. . . . . .) 1.0×10^{-3} M TFP, pH = 6.00.

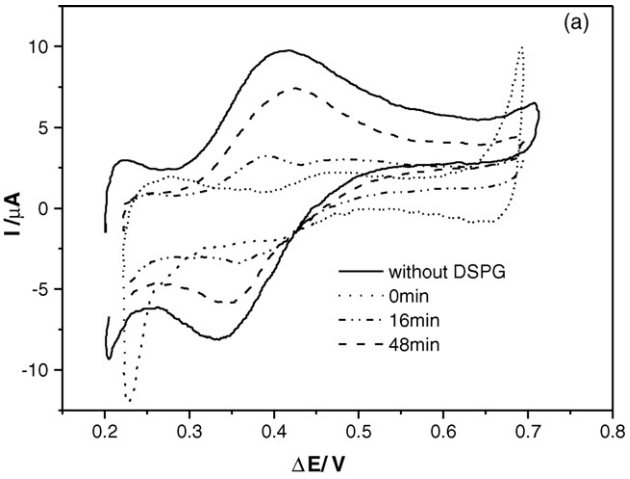


Fig. 3. Cyclic voltammograms for HTFP$^+$ transfer across the unmodified (—) and modified liquid–liquid interface at different times elapsed from the injection of TFP solution to the aqueous phase: (. . . . . .) t = 0 min; (-·-·-·-·-) t = 16 min; (- - -) t = 48 min. Aqueous phase composition: 1.0×10^{-2} M $CaCl_2$, 5.0×10^{-4} M TFP (added after DSPG film formation), pH = 6.00. Organic phase composition: 1.0×10^{-2} M TPAsDCC ν = 0.050 V s^{-1}. DSPG monolayer was formed by injecting 50 μL of 0.8 mg/mL DSPG solution near the interface.

other authors for other phenotizine derivatives, as well other neurotrophic drugs and proteins [5,6,36].

The adsorption of TFP from the subphase into the monolayer as a function of the time elapsed from spreading phospholipids solution was investigated. The results obtained at two surface pressure values and different TFP concentrations are shown in Fig. 2. These penetration experiments were performed as described in Section 2. The results are significantly different for both initial pressure values analyzed. At high pressures values no effect of drug concentration or time elapsed since drug injection is observed, indicating that no TFP penetration can occur when the monolayer is tightly compressed. On the contrary, when the initial pressure is lower than that corresponding to the first inflection point in the isotherm (Fig. 1), a general pressure increase from 15 to a steady state value close to 28 mN/m is observed, indicating drug penetration into the monolayer. At this initial pressure, the monolayer is in the liquid expanded state, making TFP penetration easier. As expected, the slope of pressure increase, that is the rate of TFP penetration, decreases with time and decreasing drug concentration. It is interesting to note that, the final pressure reached is almost independent of the TFP concentration in the aqueous phase.

From the results shown up to here we can partially conclude that TFP can penetrate or adsorb from an aqueous phase into a DSPG monolayer. This adsorption is enhanced with drug concentration when the monolayer is not tightly compressed (liquid expanded state). Considering that this state is reached when a DSPG lipid layer is adsorbed at liquid–liquid interfaces [35,37], cyclic voltammetry experiments at these interfaces were performed with the aim to correlate with surface pressure measurements.

3.2. Cyclic voltammetry

The effect of TFP on DSPG monolayer was analyzed by CV adding the drug to the aqueous or to the organic phase. The results obtained are compared below in Sections 3.2.1 and 3.2.2. This comparison allows evaluating the ion permeability of the lipid layer, and therefore, its degree of compactness.

3.2.1. Adsorption of TFP from aqueous phase

Fig. 3 shows the voltammetric response corresponding to HTFP$^+$ transfer across the bare interface (solid line) and in the presence of DSPG monolayer after several times elapsed since the injection of TFP solution to the aqueous phase (time elapsed ranged from 0 to 48 min, after which a constant response was observed). Solid line corresponds to the reversible diffusion controlled behavior of HTFP$^+$ transfer process across the bare liquid–liquid interface previously reported [33]. A forward current peak at E_p = 0.400 V and the corresponding backward process with a peak to peak separation ΔE_p = 0.060 V can be observed. The peak current, I_p, is linear with $\nu^{1/2}$ in the whole range of sweep rates analyzed (not shown). If this response is compared with that obtained when the DSPG molecules are present at the interface, an important decrease in current and a shift of 0.075 V in E_p towards more positive values can be noticed at t = 0 min. These changes are evidencing a blocking effect of the layer to the drug transfer since it can be assumed that the transfer potential shift is due to the increase in Gibbs energy on transfer caused by the work of permeation of species across the film. However this effect decreases as time elapses and at t = 48 min it almost disappears, recovering a voltammetric response close to the original one. This is a demonstration that HTFP$^+$ produces a fluidizing effect on the film minimizing its blocking effect on drug transfer.

It is worthwhile to discuss the voltammetric behavior observed at t = 16 min. Two processes are present under these conditions: one of them at 0.475 V is coincident with the response at t = 0, and the other at 0.400 V corresponds to the HTFP$^+$ transfer across the bare interface. From these results it is possible to postulate that the incorporation of HTFP$^+$ into the monolayer produces, at short times, a not uniform distribution of DSPG covering molecules, with blocked and bare domains. In this case the ion transfer can occur through the clean surface spots (E_p = 0.400 V) or through the covered zones (E_p = 0.475 V). At longer times bare domains predominates, and the process at E_p = 0.400 V prevails.

Fig. 4 summarizes the effect of HTFP$^+$ on the DSPG monolayer structure as a function of time. The variation of current values at E_p = 0.400 V is plotted vs the time elapsed from TFP injection in absence (□) and in presence (●) of the monolayer. As expected, the current values in absence of the film are independent of time. When the monolayer is present, a sharply current increase, from almost zero to values close to that observed in absence of the film, is evident at short times. After t = 20 min, the increase is slight. The inset of this figure shows the difference between I_p for HTFP$^+$ transfer obtained in the absence and in the presence of the film normalized by I_p in absence of DSPG vs time. At t = 0 no fluidizing effect of HTFP$^+$

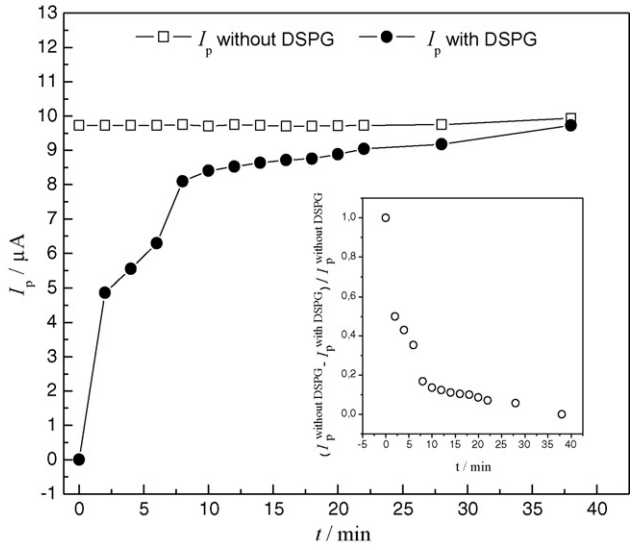


Fig. 4. Plot of I_p vs time elapsed from TFP injection to the aqueous phase. The experimental conditions are the same than in Fig. 3. *Inset:* Variation of $(I_p^{\text{without DSPG}} - I_p^{\text{with DSPG}})/I_p^{\text{without DSPG}}$ with time.

is still observed, the blocking effect of the monolayer remains, giving the biggest value for the difference in I_p. As time elapses the fluidizing effect takes places and the I_p differences fall reaching very small values (close to zero) at time near to 40 min, after which the difference in I_p values remains constant.

3.2.2. Adsorption of TFP from organic phase

In this series of experiments, TFP was added to the organic phase, by injection of different volumes of a concentrated solution, after the film was formed, as it was described in Section 2.

With the aim to evaluate the transfer mechanism of TFP (transfer of positively charged species, $HTFP^+$ or proton assisted transfer), when it is present in the organic phase, cyclic voltammetry experiments at different pH values were carried out, similar to that previously reported for $HTFP^+$ transfer from the aqueous phase [33]. Fig. 5a shows the voltammetric response obtained when an organic solution containing TFP at a concentration 5.0×10^{-4} M were put in contact with the same volume of an aqueous phase containing the base electrolyte at pH = 6.00. Also under these conditions, a reversible transfer process with a forward current peak at $E_p = 0.412$ V and the corresponding backward process with a peak to peak separation $\Delta E_p = 0.060$ V is observed. The peak current, I_p, was linear with $v^{1/2}$ in the whole range of sweep rates analyzed. The similarity observed in peak potentials and peak current values between Figs. 3 and 5a, taking into account that the same volume was used for the organic and the aqueous phase, suggest that the transfer process correspond to $HTFP^+$ species generated, in the case of the experiment in Fig. 5a, by partition of TFP to the aqueous phase and consequent protonation. To corroborate this hypothesis, the voltammetric analyses were carried out at different pH values. Fig. 5b shows the ionic partition diagram for this drug. This kind of diagrams, described by Reymond et al. [38,39], defines the domains of predominance of all the species present in both phases. The presence of ionic form of triflupromazine ($HTFP^+$) in aqueous phase is favored at $pH < pK_a - \log P_X$ ($pK_a = 9.40$ and $\log P_{TFP} = 2.21$ [33]) within the lower part of the diagram (below boundary line 1). These $HTFP^+$ species transfer into organic phase (above line 1) upon increasing the interfacial Galvani potential difference. For pH values higher than $pK_a - \log P_X$ the neutral form of chlorpromazine increases in organic phase and the assisted proton transfer reaction occurs, so that E_p shifts by 2.303 RT/zF per pH unit (line 2). The

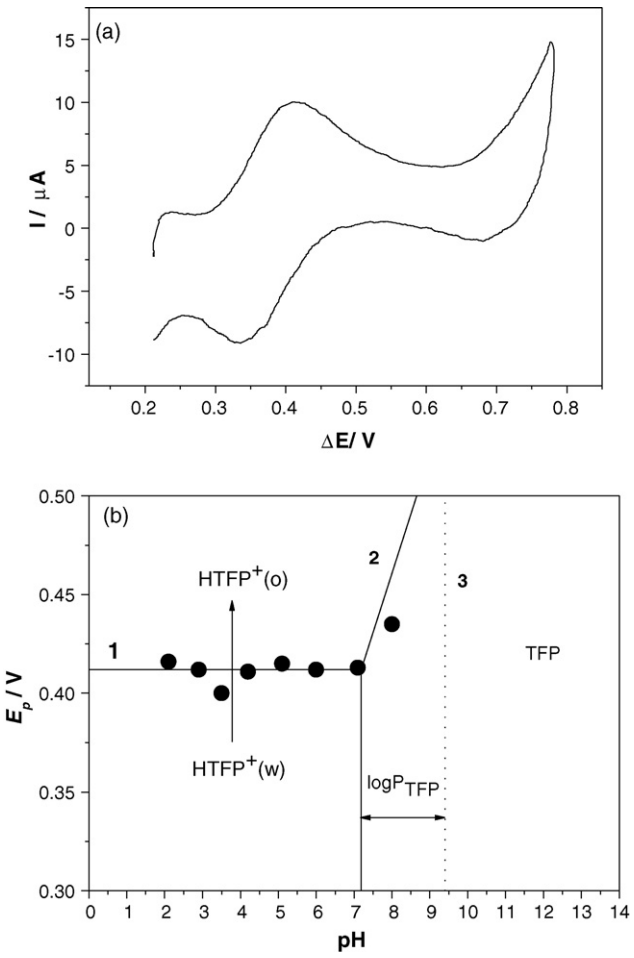


Fig. 5. (a) Cyclic voltammetry obtained when TFP was added to 2.00 mL of organic phase in contact with 2.00 mL of aqueous base solution $v = 0.050\ V\ s^{-1}$. Aqueous phase composition: 1×10^{-2} M $CaCl_2$, pH = 6.00. Organic phase composition: 1×10^{-2} M TPAsDCC + 5×10^{-4} M TFP. (b) Ionic partition diagram in water/DCE interface for triflupromazine. Solid line: theoretical boundary line according to [38], (●): experimental values obtained from the voltammograms recorded at different pH values of the aqueous phase. Organic phase composition is the same than in (a).

experimental E_p values obtained for triflupromazine are shown in the diagram: at $pH < pK_a - \log P_X$, the positive peak potential, E_p^+, and the peak potential difference, $\Delta E_p = E_p^+ - E_p^- = 60$ mV, are constant and independent of pH and, according to a direct transfer mechanism. Considering this result, we could confirm that, even in the case that TFP was initially present in organic phase, the mechanism occurring at pH = 6.00 is the direct transfer of $HTFP^+$ species from the aqueous to the organic phase.

In the presence of DSPG, the electrochemical behavior is similar to that observed when TFP is added to the aqueous phase and shown in Fig. 3, i.e. an initial blocking effect of the monolayer is evidenced by a decrease in current peak and a shift in peak potential, which is gradually vanished by the fluidizing effect of TFP. Fig. 6 summarizes the variation of the relationship $(I_p^{\text{without DSPG}} - I_p^{\text{with DSPG}})/I_p^{\text{without DSPG}}$ with TFP concentration in organic phase for two different times elapsed since the injection of TFP solution. As expected, the fluidizing effect of TFP increases with time and TFP concentration.

Finally, Fig. 7 compares the fluidizing effect of TFP when it is added to the aqueous or to the organic phase. As it can be noted, the relationship $(I_p^{\text{without DSPG}} - I_p^{\text{with DSPG}})/I_p^{\text{without DSPG}}$ decreases more sharply when it is present in the aqueous phase. Due to their amphiphilic nature, phenothiazines are able to interact with DSPG monolayer by hydrophilic forces, mainly electrostatic attraction

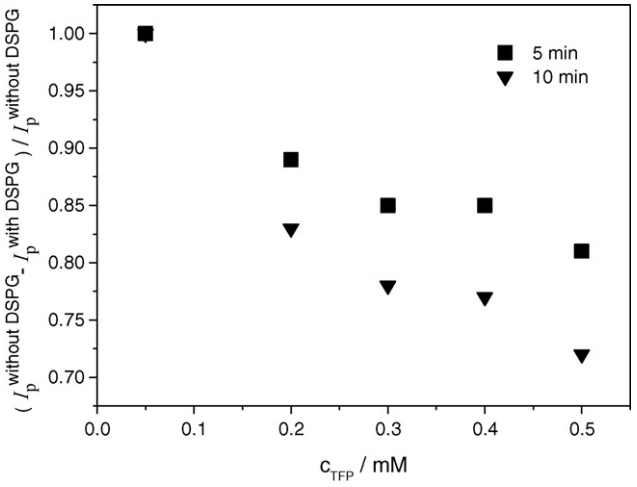


Fig. 6. Variation of $(I_p^{without\ DSPG} - I_p^{with\ DSPG})/I_p^{without\ DSPG}$ with TFP concentration added to the organic phase for two different times elapsed from TFP injection to the organic phase: (■) 5 min and (▼) 10 min. Aqueous phase composition: 1.0×10^{-2} M $CaCl_2$, pH = 6.00. Organic phase composition: 1.0×10^{-2} M TPAsDCC + *x*M TFP (added after film formation) $\nu = 0.050\ V\ s^{-1}$. DSPG monolayer was formed by injecting 50 μL of 0.8 mg/mL DSPG solution near the interface.

between the negatively charged polar groups of DSPG and the positive amine group of the drug, or by hydrophobic interactions between the phenothiazine tricyclic ring system and the hydrocarbon chains. The results obtained in this work allow us to state that interactions with the polar head groups lead to a fluidizing effect more efficient than hydrophobic forces. Nevertheless, it is also worthwhile to consider that the fact that TFP requires more time to produce an effect on the monolayer from the organic side of the interface, may have other origins besides differences in the hydrophilic or hydrophobic interaction forces. In this sense, it could be assumed that the presence of TFP in the organic phase produces a delay in the global process, due to diffusion, partition and protonation steps previous to the $HTFP^+$ electrochemical transfer. However, no evidence of this delay was observed in the experiments shown in Fig. 5 in absence of the monolayer. On the contrary, the presence of the monolayer slows down the global process, leading to the

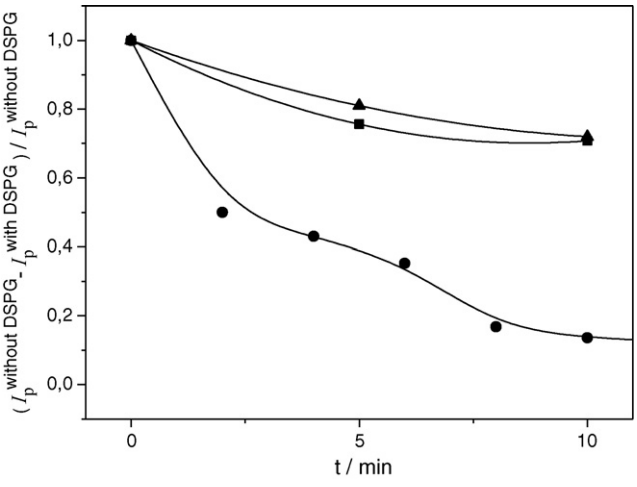


Fig. 7. Comparison of the variation of $(I_p^{without\ DSPG} - I_p^{with\ DSPG})/I_p^{without\ DSPG}$ with the time elapsed since TFP injection to the organic (■, ▲) or to the aqueous (●) phase to obtain a final concentration of 5.0×10^{-4} M. Aqueous phase composition: 1.0×10^{-2} M $CaCl_2$, pH = 6.00 with or without 5.0×10^{-4} M TFP. Organic phase composition: 1.0×10^{-2} M TPAsDCC with or without 5.0×10^{-4} M TFP $\nu = 0.050\ V\ s^{-1}$. DSPG monolayer was formed by injecting (●, ▲) 50 μL or (■) 100 μL of 0.8 mg/mL DSPG solution near the interface.

conclusion that additional forces must be exerted by the drug onto the film to be able to cross it and partition into the aqueous phase for the later electrochemical transfer. In other words, a disorganization of the monolayer is needed for reaching the TFP equilibrium distribution and the presence of the drug in the organic side of the interface is not able to produce the fluidizing effect required for the global process occurrence. When TFP is present at the aqueous phase, it is possible to observe the disappearance of the blocking effect of the monolayer almost completely after 20 min this is not longer observed when TFP is added to the organic phase.

So that, it can be concluded that the differences found in both sides of the interface, for TFP effect on the monolayer compactness, can be attributed to specific interactions with different zones of the monolayer.

4. Conclusions

The combination of surface pressure–molecular area and cyclic voltammetry experiments demonstrates that TFP partitions in a DSPG monolayer adsorbed at air/water and water/1,2-dichloroethane interfaces changing the structure of the film. The results indicate that a fluidizing effect, dependent on the time and the drug concentration, takes place leading to an increment in the permeability of the film. This effect can be produced by TFP either from the organic or from the aqueous phase due to the amphiphilic nature of this drug. Nevertheless, the expansion of the lipid layer is enhanced when TFP acts from the aqueous phase.

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