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Cyclic voltammetric studies of the antipsychotic chlorpromazine using an alkylthiol/phospholipid-modified gold electrode

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Abstract Self-assembled monolayers of alkanethiols on gold have been reported to be highly stable for voltammetry experiments in aqueous electrolyte. In this work a gold electrode has been modified by first depositing one layer of an alkylthiol (S-C_{18}) and then coating by phospholipid multilayers. Voltammetric oxidation of the antipsychotic chlorpromazine at this two-step modified electrode was followed by means of cyclic voltammetry measurements. The results give important information concerning the behaviour of the pharmacological agent at the lipid-water interface. Measurements made using the pre-concentration method allow good sensitivity improvement after 5 min accumulation time. The ability of chlorpromazine to penetrate inside the phospholipid multilayers has also been investigated under different conditions such as the nature of the phospholipid and the pH of the medium. The accumulation process seems to be closely related to the charge carried by the phospholipid and by the molecule, while the incorporation process seems to be independent of the charge carried by the phospholipid and dependent of the degree of fluidity of their hydrocarbon chains. We found through this work that the acid-base equilibrium of chlorpromazine together with its amphiphilic properties (as compared with the results of similar studies on phenothiazine) could be responsible for governing the principal aspects of the drug's behaviour toward biological membranes.

Key words Chlorpromazine · Cyclic voltammetry · Modified gold electrode · Phospholipid · Penetration

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Introduction

Some organic compounds of biological and pharmacological interest exert their activity through interactions with the lipid components of living cell membranes. Thus, modification of surface electrodes by coating with lipid films becomes an attractive analytical method, since it offers the possibility of using these electrodes for following electron transfer reactions of electroactive drugs in an environment that resembles biological membranes.

We have demonstrated in previous reports that drug-lipid interaction studies can help in better understanding the behaviour of a wide range of pharmaceuticals within real biomembranes [1, 2]. Since important progress has been achieved in the field of lipid-modified electrodes as affinity biosensors [3–5], our approach here is illustrated within the framework of voltammetric measurements using the electrical properties of the antipsychotic chlorpromazine (CPZ) at a novel lipid-modified gold electrode.

Chlorpromazine (2-chloro-10-[3-dimethylamino-propyl] phenothiazine) ($C_{17}H_{19}ClN_2S \cdot HCl$) is still considered the pharmaceutical of first choice in psychosis and various treatments of mental illnesses [6–8]. However, one of the most important side effects of this drug is "lipidosis", meaning a high accumulation of phospholipids into lysosomes [9, 10]. Some studies have demonstrated the existence of a close relationship between lipidosis and the presence of a high level of chlorpromazine inside the lysosomes [11]. It has also been shown that the amphiphilic properties, together with the weak base character of the drug, make it undergo a high and specific interaction with phospholipids inside the lysosomes [9, 12].

The antipsychotic agent can be electrochemically oxidized via two one-electron steps [13]. The first oxidation step produces the ($CPZ^{+\cdot}$) cation radical, while the second involves the oxidation of $CPZ^{+\cdot}$ to produce the sulfoxide. The electrochemical sensing of CPZ is

followed here by cyclic voltammetry measurements using a previously reported two-step gold electrode modification [14] in which the electrodes are made by first covering the gold with a covalently attached monolayer of an alkylthiol and then casting phospholipid multilayers on the initial thiol layer. We have also demonstrated that this system gives better mechanical stability and improved sensitivity [14]. Furthermore, self-assembled monolayers of alkanethiols on gold have been reported to be highly stable systems for voltammetry experiments in aqueous electrolytes [15, 16].

The interaction of chlorpromazine with phospholipids, the main subject of this paper, is explored here by following the drug's incorporation and anodic oxidation peak at the "S-C_{18}-lipid"-modified gold electrode. The influence of parameters such as the nature of the lipid, pH and CPZ accumulation time have been investigated. We have also explored and compared the electrochemical behaviour of the hydrophobic phenothiazine derivative (PTH) and CPZ at pH 9 in phosphatidylcholine (PC) multilayers.

Experimental

Apparatus

Cyclic voltammograms were obtained with a Princeton Applied Research Model 173 universal programmer, a Model 175 potentiostat, and a Model 179 digital coulometer equipped with positive feedback circuitry for IR compensation and recorded with a Soltec VP-6423S X-Y recorder. The working electrode was a disc of gold (0.6 cm^2 geometric area), and the auxiliary electrode was a platinum wire. A sodium chloride-saturated calomel electrode (SSCE) built in our laboratory was used as the reference electrode. The gold electrode was polished mechanically with alumina on a smooth cloth before each experiment. The scan rate was 50 mV/s for all experiments.

Reagents

Chlorpromazine (CPZ), egg yolk phosphatidylcholine (PC), phosphatidylglycerol (PG), DL-α-dipalmitoylphosphatidylcholine (DPPC) and L-α-dipalmitoylphosphatidyl-DL-glycerol (DPPG) were purchased from Sigma. PC, DPPC and DPPG were solids, while PG was in the form of a 10 mg/ml 95:5 chloroform:methanol solution. Phenothiazine (PTH) and octadecylmercaptan were purchased from Aldrich. The supporting electrolyte solution was a 0.05 M phosphate buffer adjusted to the desired pH. Distilled water was further purified by passage through a Barnstead Nanopure four-cartridge system. All the solvents, buffers and salts used were "pro-analysi".

Preparation of the sensor

The gold electrode was immersed overnight in a 1 mM ethanolic solution of octadecylmercaptan. The next day the electrode was washed gently with a few milliliters of ethanol and allowed to dry in air. 10 μl of a 10 mg/ml phospholipid chloroform solution was placed on the thiol-covered gold electrode (to cover the active area and its surroundings). The chloroform was then allowed to evaporate at room temperature by slowly rotating the electrode (100 rpm) in order to obtain maximum homogeneity of the lipid multilayers. The resulting lipid coating represents about 23 bilayers.

Procedure

The electrochemical sensing of CPZ and PTH was based on the pre-concentration procedure. The modified electrode was placed in 15 ml of a solution of the drug after previous removal of oxygen from this solution by passing purified nitrogen for 20 min through the solution and then passing it over the solution during all experiments. Accumulation of the phenothiazine derivatives on the gold-modified electrode was accomplished by stirring the drug solution at a constant rate (200 rpm) for 5 min with an open circuit. After the stirring and a 10-s rest period, cyclic voltammograms were recorded with the electrode in the same solution.

Results and discussion

The accumulation of CPZ at the novel gold/alkylthiol/phospholipid-modified electrode followed by the drug incorporation into the lipid multilayers can be easily studied by using the electroactive properties of the molecule and by measuring the oxidation peak intensity corresponding to the resulting formation of cation radical $CPZ^{+\cdot}$. We have studied the effect of different parameters such as the phospholipid charge and the solution pH on the electrode reaction, mainly governed by the process of incorporation of CPZ.

Accumulation time study

The stirring time for accumulation of a 10^{-4} M solution of chlorpromazine at pH 9, using a PC coating of 3.5 μg/mm^2, was varied over the range 1–7 min. The electrode was cleaned in the same solution by applying a potential of 0.9 V for 1 min, while the solution was stirred. The constant potential facilitates the removal of CPZ from the lipid layers, as was indicated by control cyclic voltammogram measurements performed in the same solution. Figure 1 show a significant increase in the CPZ oxidation peak during the first minutes with a maximum accumulation time of 5 min. It is also important to notice from Fig. 1 the improvement of sensitivity after 5 min as compared to the response obtained under the same experimental conditions on bare gold electrode (data not shown). When the stirring time exceeds 5 min there is a decrease in the peak current intensity. We think that this is probably due to the surfactant properties of CPZ, which above 10^{-5} M, the drug CMC [17], can lead to a drug-phospholipid comicellisation [18]. As a result of this a partial solubilisation and then a loss of some lipid layers can be expected.

Effect of lipid nature and pH

Optimum accumulation conditions and amount of lipid (5 min, 3.5 μg/mm^2) were used in the following experi-

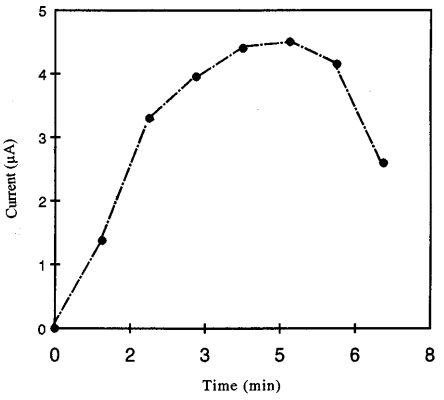

Fig. 1 Dependence of the peak current on the accumulation time for 10^{-4} M chlorpromazine at the Au/S-C_{18}/PC-modified gold electrode (3.5 μg lipid/mm^2). Conditions: scan rate 50 mV/s, supporting electrolyte 0.05 M phosphate buffer (pH 9)

ments. Since most of CPZ-induced lipidosis models have implicated a close interaction between this drug and acidic phospholipids inside the lysosome and since the charge of both drug and lipids seem to play an important role in the side effect process [9], we have studied here the electrochemical behaviour of CPZ toward two different modified gold electrodes. The first was an Au/s-C_{18}/PG and the second an Au/s-C_{18}/PC. CPZ accumulation as a function of the drug concentration was measured on the PG modified electrode at neutral pH and at pH 9. Accumulation on PC-modified electrode was studied only at the drug neutral conditions.

Figure 2 displays cyclic voltammograms at the Au/s-C18/PC electrode (pH 9) obtained after successive standard additions of CPZ, each effecting a 6×10^{-6} M increase in concentration. The reproducibility of the measurements depends on the phospholipid deposit, quality of buffer solutions and control of stirring rates. The coefficient of variation calculated for three measurements at 3×10^{-5} M and 1.2×10^{-5} M of CPZ were 1.57 and 1.62% respectively. According to the *p*Ka of CPZ, most of the drug at pH 9 is in its neutral form. The sharp oxidation peaks (Fig. 2) clearly indicate a rapid electrode process due to the high level of incorporation of the antidepressant drug into the lipid layers, which can be also confirmed by the shift of the peak current intensity (130 mV) to more positive values. Calibration curves obtained from the data of Fig. 2 show a good linear relationship between peak height and concentration within the range 6×10^{-6} and 5×10^{-5} M (Fig. 3), with a correlation coefficient of 0.997 and a slope of 0.217 μA/μM. Our observations here are in accordance with literature reports of the passive permeation of biological membranes to CPZ in neutral form [8].

Electrochemical oxidation of the antidepressant drug has also been explored under different experimental conditions. Figure 4A shows the reaction of an Au/S-C_{18}/PG electrode exposed to increasing concentrations of CPZ in a supporting electrolyte solution at pH 7. The

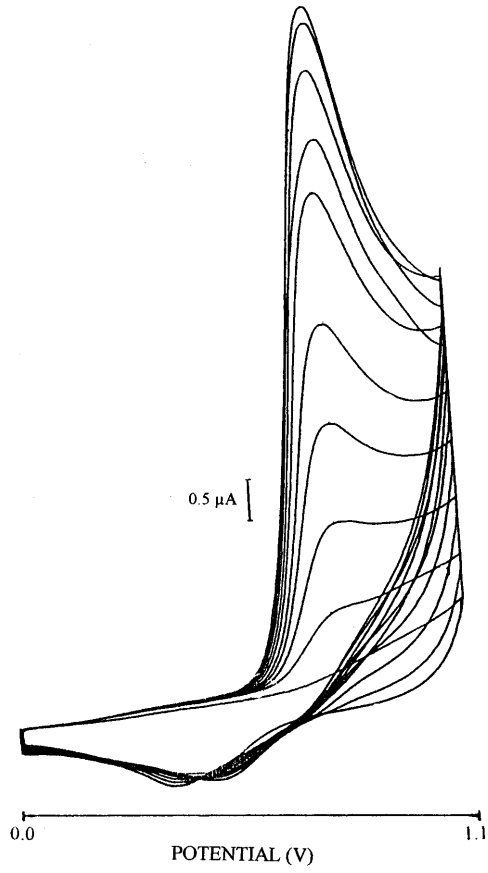

Fig. 2 Voltammetric response of an Au/S-C_{18}/PC electrode at pH 9. Cyclic voltammograms were recorded for successive additions of 6×10^{-6} M of chlorpromazine; 5 min accumulation time; other conditions as in Fig. 1

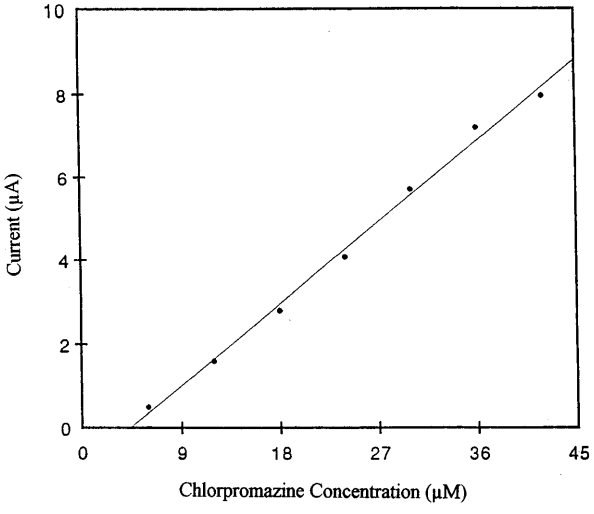

Fig. 3 Dependence of the peak current on the concentration of chlorpromazine. Conditions are those of Fig. 2

cyclic voltammograms recorded under these conditions show very poor anodic peaks, and no significant improvement in response were observed by increasing the drug concentration. Since most of the drug is in the

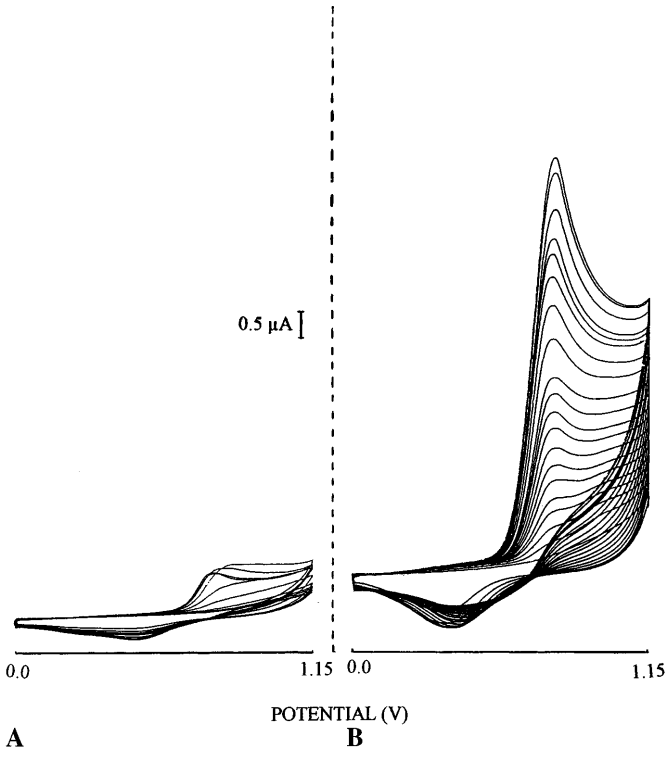

Fig. 4A, B Voltammetric response of an Au/S-C_{18}/PG electrode (3.5 μg lipid/mm^2). Cyclic voltammograms were recorded after each standard addition of 2×10^{-6} M chlorpromazine in (**A**) supporting electrolyte, 0.05 M phosphate buffer (pH 7) and (**B**) supporting electrolyte, 0.05 M phosphate buffer (pH 9). Conditions: scan rate 50 mV/s, accumulation time 5 min

protonated form at pH 7, this result can be explained in terms of a high electrostatic interaction occurring between CPZ cations and the negatively charged phosphate groups of phosphatidylglycerol, leading to stable complexes. As a result of this, CPZ molecules will be trapped at the lipid solution interface, and only a small number will be able to reach the electrode surface.

When the same electrode is immersed in a CPZ solution at pH 9, and experiments are performed using the same procedure (Fig. 4B), improved signals corresponding to high CPZ incorporation into the PG multilayers are obtained. Taking these observations and the results above into consideration, it seems that when the acid-base equilibrium of CPZ is shifted to its neutral form the charge carried by the phospholipid does not play an important role in the drug penetration inside lipid membranes. However, a different accumulation behaviour can be observed through the concentration dependence of the peak currents obtained after successive standard additions (2×10^{-6} M increase) of the drug into the solution. The calibration curve (Fig. 5), exhibits a change in the slope at concentrations higher than 2.4×10^{-5} M. Below this concentration the slope is 0.1612 μA/μM and the correlation coefficient is 0.998. The second portion of the curve has a slope of 0.2488 μA/μM with a correlation coefficient of 0.996. Such profiles reflect a two-step incorporation process, probably due to the remaining CPZ cations. For three trials, the coefficient of variations calculated at two different concentrations of CPZ (1.8×10^{-5} M and 2.6×10^{-5} M) were respectively 2.89 and 5.17%. Conditions for reproducibility were the same as those cited for Fig. 2.

Effect of the fluidity of the phospholipids on CPZ incorporation

Figure 6 shows the cyclic voltammograms for the electrochemical oxidation of CPZ with a gold working electrode modified as follows: (A) Au/s-C_{18}/DPPC and (B) Au/s-C_{18}/DPPG. The oxidation waves obtained in the two cases represent very low and broad intensities, and almost the same response is obtained for either the neutral DPPC or the negatively charged DPPG. Knowing that the two phospholipids are in their rigid physical state under the experimental conditions and considering their respective transition temperatures (41.8 °C and 41 °C), this result suggests a slow and poor incorporation process of CPZ under these rigid conditions. Therefore, we think that the fluidity of the lipid hydrocarbon chains may help the drug to reach the electrode surface more easily and rapidly.

Effect of the amphiphilic property on the drug incorporation

Figure 7 shows the electrochemical oxidation of phenothiazine (PTH), a hydrophobic derivative of CPZ at the Au/s-C_{18}/PC electrode (pH 9). The small and broad voltammogram peaks obtained suggest a slow electrode process due to the poor incorporation of this compound inside PC multilayers as compared to that found in the

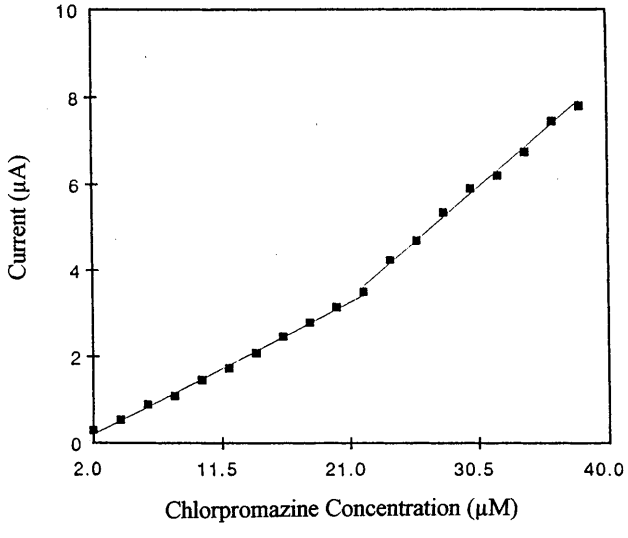

Fig. 5 Dependence of the peak current on the concentration of chlorpromazine. Conditions are those of Fig. 4B

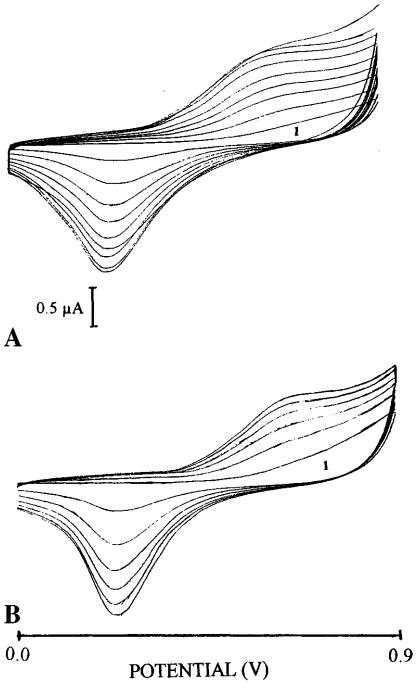

Fig. 6 Cyclic voltammograms of CPZ (10^{-5} M) at (**A**) Au/S-C_{18}/DPPC and (**B**) Au/S-C_{18}/DPPG modified gold electrode. Scans recorded every 5 min. First scan is 1. Other conditions are as in Fig. 1

similar experiments performed with CPZ (Fig. 2). The high hydrophobicity of PTH probably blocks its diffusion through the multilayers by stabilizing the drug inside the first hydrocarbon regions of the lipids. This result clearly supports the role played by the hydrophilic-hydrophobic balance of the antipsychotic agent in its mode of interaction with biological membranes.

Conclusions

The improved analytical performance of the double modified gold electrode has enabled us to show that the interaction of the antipsychotic chlorpromazine (CPZ) with lipid layers is mainly governed by the pH of the medium. According to our results, CPZ penetrates more easily inside lipid layers in the non-protonated form independently of the charge of the phospholipid. At alkaline pH, the negatively charged phospholipid PG seems to play only an attractive role, increasing the drug concentration at the membrane-water interface. The state of fluidity of the lipid hydrocarbon chains also appears to be a determining factor in the drug incorporation process.

When CPZ is positively charged at pH 7, our results are consistent with findings by other groups in the fact that a strong electrostatic interaction between PG and CPZ seems to form a stable complex at the lipid-water interface. This result is in complete agreement with earlier CPZ-phospholipid interaction studies [19].

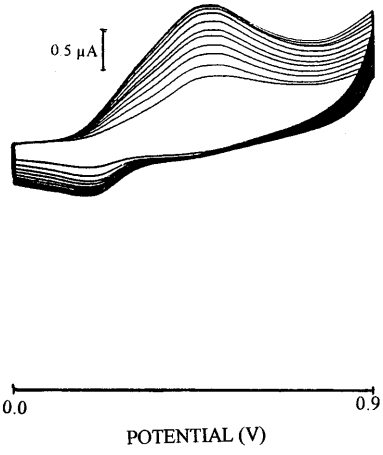

Fig. 7 Cyclic voltammetric oxidation of phenothiazine (PTH) at an Au/S-C18/PC electrode. Conditions are as in Fig. 2

Because of the facility and stability of this new electrode preparation, the electrochemical approach here can provide, as an alternative method, important information regarding drug-lipid interactions. The use of these electrodes for voltammetric drug analysis in biological fluids is under investigation.

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