Electroanalysis, 1 (1989) 501–505

Preconcentration and Determination of Promethazine at Lipid-Modified Carbon Paste Electrodes

Mahmoud Khodari
Faculty of Sciences, Chemistry Department, Assiut University, Kena Branch, Kena, Egypt

Jean-Michel Kauffmann and Gaston J. Patriarche[1]
Université Libre de Bruxelles, Institut de Pharmacie, Campus Plaine, CP 205/6, Boulevard du Triomphe, B-1050 Bruxelles, Belgium

Mahmoud A. Ghandour
Faculty of Sciences, Chemistry Department, Assiut University, Assiut, Egypt
Received March 8, 1989.

ABSTRACT

Lipid-modified carbon paste electrodes were electrochemically characterized and their potential for drug analysis was compared with conventional carbon paste electrodes. The presence of lipids (phospholipids, fatty acids) in the paste matrix provided enhanced current responses with improved reproducibility. With 5 minutes of accumulation, phenothiazines exhibit a 40-fold enhancement of the response compared to that obtained without accumulation. As a result, a detection limit of 1×10^{-9} M was attained. The response was characterized with respect to pH, preconcentration potential, accumulation time, paste composition, possible interferences, and other variables. The determination of the tranquilizer in serum and urine required no preliminary treatment other than dilution with phosphate buffer and medium exchange. Detection limits are 5×10^{-8} M in urine and 2×10^{-7} M in serum.

INTRODUCTION

Previous studies of the surface modification of a glassy carbon electrode with a multilayer coating of phospholipids have shown the potential of this new, modified electrode for the analysis of pharmacologically active compounds [1, 2]. The technique consists of accumulating the analytes into the lipid layer prior its voltammetric oxidation. By varying the nature of the phospholipid, the processes governing the accumulation step have been shown to be electrostatic and hydrophobic. Indeed, positively charged hydrophobic organic compounds accumulate to a greater extent into the lipid layer; the more the lipids were negatively charged, the higher were the electrode responses [1, 2]. The sensitivity of this type of electrode is based on the preconcentration into the lipid layer, and the stability of the latter determines the performance of the probe. Because the electrode response is depressed with long accumulation periods (more than 3 minutes), we propose an alternative approach that improves the lipid-modified electrode's stability.

One possible way of increasing the lipid layer stability while maintaining its inherent property is to solubilize the lipid into paraffin oil. The proposed modified electrode construction consists of mixing a Nujol paste (liquid paraffin plus graphite) with an appropriate amount of lipids. The use of a carbon paste matrix is dictated by its interesting mechanical and electrochemical properties as well as by its ability to preconcentrate organic molecules [3–13]. In order to increase the sensitivity and selectivity of the preconcentration step, a variety of modifiers were incorporated into the carbon paste matrix. These modified carbon paste electrodes (MCPEs) have been applied to the determination of several inorganic [14–24] and organic [25–28] compounds. Generally, the modifier is an appropriate ligand that possesses a high affinity for the analyte or an ion exchanger [29].

In this study, several modified carbon paste electrodes were prepared by mixing the carbon paste matrix with lipids such as phospholipids and fatty acids. The lipids, added in appropriate ratios, remain stably incorporated while maintaining the mechanical integrity of the paste. The aim of the dissolution of the lipid into a paraffin paste liquid was to produce a functionalized electrode surface with polar groups at the electrode–solution interface. Moreover, due to the amphipathic structure of the modifiers, a decrease of the interfacial tension (which should aid in the extractive accumulation process) was expected.

[1] To whom correspondence should be addressed.

1040-0397/89/$1.00+.25

Several pharmacologically important compounds are known to interact and accumulate strongly with phospholipid layers and fatty acids present in membrane structures [30]. Recently, a stearic acid-modified carbon paste electrode (CPSt) was proposed [31] and applied [32] to the selective voltammetric measurements of dopamine and ascorbic acid; this electrode takes advantage of the electrostatic repulsion between the anionic carboxyl groups on the surface and the ascorbate ion. Phenothiazines are known to accumulate at biological membranes, and this surface activity may be at least partly a factor in determining their pharmacological activity [35]. The proposed lipid-modified electrodes have been applied to the preconcentration and oxidation of the phenothiazine derivative, promethazine hydrochloride [*N*-(2-dimethylamino-*n*-propyl)phenothiazine hydrochloride], which is known for its electroactivity [10, 33, 34].

EXPERIMENTAL

Apparatus and Instrumentation

A conventional, three-electrode cell was used with a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as the auxilliary electrode. Monitoring of cell output was accomplished with a Bioanalytical System Model CV27 for cyclic voltammetric analysis and a Bruker E100 for linear scan and differential pulse measurements. Data were plotted with the aid of an HP 7090 A X-Y recorder. All potentials are referred to the SCE.

Reagents and Solution

All solutions were prepared from deionized and distilled water and analytical grade materials. Stearic acid and asolectin (L-α-phosphatidylcholine, type II-S, extracted from soybean) were obtained from Sigma. Promethazine (pharmaceutical quality) was used without further purification. Standard serum samples were obtained from Sigma. Buffer solutions (0.1 M) were prepared from sodium monohydrogenophosphate (Merck P.A.) with pH adjustments obtained with hydrochloric acid or sodium hydroxide solutions. Lipid solutions in chloroform and the 0.01 M promethazine stock solution (pH 7.0) were prepared daily and kept in the dark. All measurements were carried out at 20 ± 1°C.

Electrode Materials and Procedures

Three commercially available pastes were purchased from Bioanalytical Systems (West Lafayette, Indiana). These pastes were graphite with paraffin oil (CPO), graphite with silicone grease (CPS) and graphite with ceresin wax (CPW). One commercially available paste, which was obtained from Metrohm AG (Switzerland), was prepared from spectroscopic grade carbon powder and UVASOL liquid paraffin (CPMet) (Metrohm EA 207C). The ratio of graphite to binder was not specified by the manufacturers. Mixing the paste (CPMet) with the lipid (5% w/w) was accomplished in a mortar in the presence of the minimum amount of chloroform; the solvent was allowed to evaporate overnight at room temperature. The working electrodes were prepared by pressing the paste into the well of the Teflon body of homemade electrodes (2 mm deep, 3 mm in diameter). The surfaces were smoothed against clean paper. Unless stated otherwise, a fresh surface was utilized for each experiment.

Following this, the electrode was placed in the cell and the preconcentration of the analyte was initiated. The accumulation proceeded at open circuit for a selected time. At the end of the preconcentration period, the solution stirring was stopped and the initial potential (+0.3 V) was applied for 30 seconds before recording the voltammogram. The best operating conditions (in the differential pulse mode) were as follows: 50-mV pulse amplitude, 0.5-second pulse duration, and 5 mV/s scan rate.

For experiments involving medium exchange, the electrode was rinsed with water (after preconcentration) and then transferred to the electrolytic blank solution (phosphate buffer pH 9.0); the voltammogram was recorded between +0.3 and +1.1 V (versus SCE). The cleaning procedure consisted of dipping the electrode into a stirred solution of 1.0 M hydrochloric acid for 5 minutes and transferring it to a blank solution where the response was recorded between +0.3 and +1.1 V versus SCE three times to reach reproducible residual currents. Quantitative analysis were performed by the standard-addition method.

RESULTS AND DISCUSSION

Background currents and available potential ranges at the different electrodes were investigated in the linear scan mode considering potential limits at currents in excess of 1 μA. As reported in Table 1, the lipid-modified electrodes exhibit similar positive potential limits in acidic media but less positive values in basic media. Residual currents are low and of comparable magnitude; it should be pointed out that, with our experimental conditions, the CPO, CPW, and CPS electrodes developed low-intensity interfering peaks (less than 2.0 μA), which precluded measurements of traces of promethazine. In terms of peak shape ($E_p - E_{p/2}$), the oxidation of promethazine produced a well-defined, sharp oxidation peak, which suggested a rapid electrode process. In contrast, the unmodified electrodes exhibited broader, peaks (Table 1). After 5 minutes of accumulation at pH 9.0, the signal enhancement was compared with the response at the electrode without accumulation. Table 1 lists the gain factors observed using the linear scan and differential pulse methods. With the former, the lipid-based electrodes—CP–asolectin (CPAso) and CPSt—exhibited significantly greater current enhancement. The differential pulse mode produced even higher currents at the lipid MCPE and emphasized the gain factors as compared to the unmodified electrodes. This phenomenon agrees with the differences in the reaction rates ($E_p - E_{p/2}$).

TABLE 1 Positive Potential Limits in Phosphate Buffers, Oxidation Peak Shape ($E_p - E_{p/2}$) of Promethazine (2.5×10^{-5} M) at pH 9.0 and Gain Factors after 5 Minutes of Accumulation[a]

	Anodic Limit			*Gain Factor*	
Electrode	*pH 3*	*pH 9*	*Peak Width (mV), $E_p - E_{p/2}$*	*LSV*	*DPV*
CPAso	+1.35	+1.00	80	30	45
CPSt	+1.40	+1.20	60	22	40
CPMet	+1.30	+1.30	110	5.5	9
CPO	+1.30	+1.20	120	7	8
CPW	+1.40	+1.30	150	5.5	6
CPS	+1.30	+1.30	170	4	4

[a] See Figure 1 and the text for abbreviations.

The pH of the solution influenced the accumulation process at the MCPE. Using the differential pulse mode, the investigation of the extent of preconcentration over a pH range of 3.0–10.5 showed little current enhancement with acidic media; a marked increase in response, however, was observed above pH 7, with a maximum response at pH 9.0 (Figure 1). At higher pH values, the signal decreased and peaks became distorted.

These observations are in accordance with literature reporting surface pressure experiments with chlorpromazine [35]. The results can be explained in terms of the surface tension and the pK_a of the tertiary amine group of promethazine. At low pH values, the predominant positive charge of the electrode surface repels the positively charged drug. When the pH is increased, (1) more carboxylic groups of the lipid are ionized, which decreases the interfacial tension [36], and (2) above pH 7 the promethazine-protonation equilibrium is shifted in favor of the neutral form of the molecule. The result of these two effects is a higher affinity of the compound toward the surface. Destruction of the mechanical integrity of the paste due to swelling [36] can explain a progressive decrease in response at pH values higher than 9. The study of differential pulse peak current intensity as a function of stirring time at pH 9.0 at the MCPE showed a rapid increase in the electrode response at short accumulation periods with a progressive leveling off (but with continuous increases) with stirring longer than 6 minutes (Figure 2). This indicates an electrode process governed first by surface adsorption and subsequently by diffusion into the pasting liquid [10].

The strength of adsorption and the extent of extraction at pH 9.0 have been estimated by measuring the signal after preconcentration and medium exchange. As illustrated in Figure 2 (curves c′ and b′), the electrode

FIGURE 1. Differential pulse peak currents (DPV) as a function of pH. Promethazine, 2.5×10^{-5} M; $t_{acc} = 5$ minutes. (a) CP–Metrohm (CPMet); (b) CP–stearic acid (CPSt); (c) CP–asolectin (CPAso).

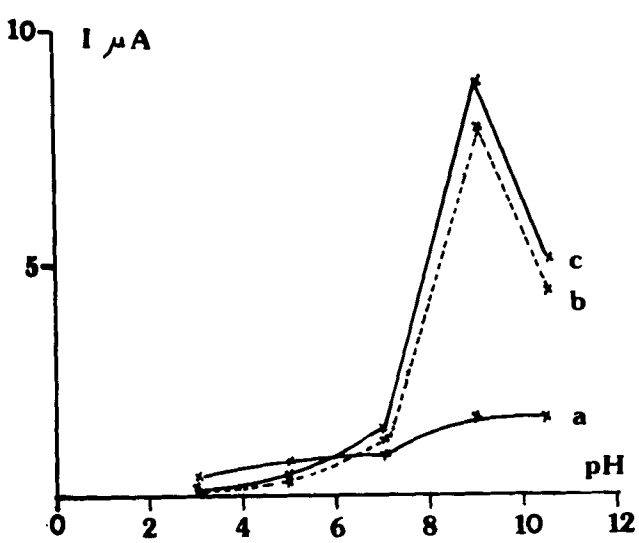
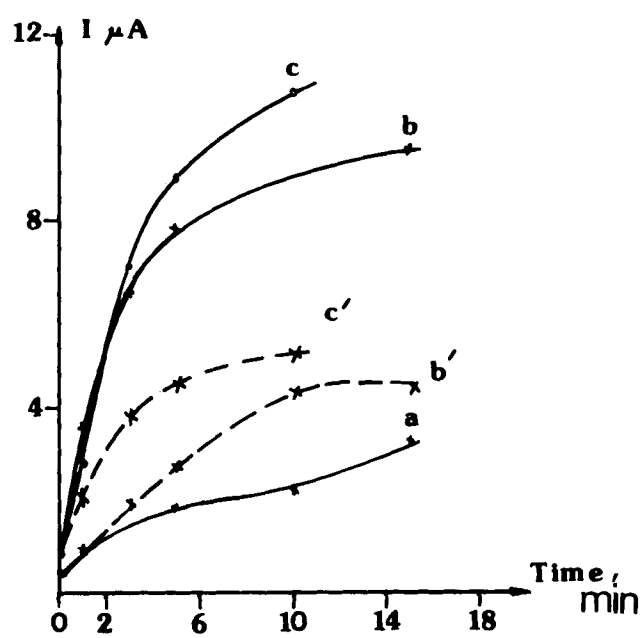


FIGURE 2. Peak currents (DPV) as a function of accumulation time. Promethazine, 2.5×10^{-5} M, pH 9.0 (a) CPMet; (b) CPSe; (c) CPAso; (c′) CPAso medium exchange; (b′) CPSte medium exchange.



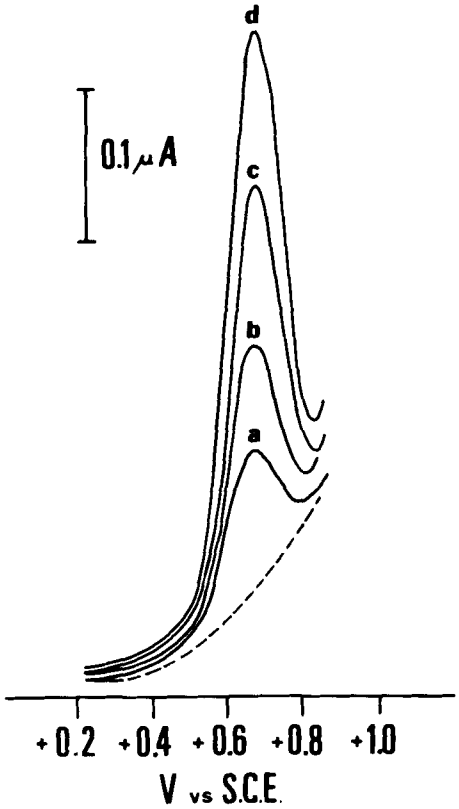


FIGURE 3. Typical responses (DPV) at the CPSte electrode as a function of promethazine concentration in diluted serum (1 : 2), pH 9.0, t_{acc} = 5 minutes, medium exchange (a) 1×10^{-5} M; (b) 2×10^{-5} M; (c) 3×10^{-5} M; (d) 4×10^{-5} M.

response was depressed (approximately by a factor of four at short stirring times and by a factor of two at longer periods). Such a relatively small loss of accumulated species (i.e., in sensitivity after medium exchange) is of considerable interest for trace analysis in complex biological samples (see the following discussion). Taking into account the facts that phospholipids have a tendency to swell in aqueous solutions [37] and that the CPAso electrode exhibited lower available potential ranges, subsequent experiments were conducted using the fatty acid-modified electrode (CPSt). Optimization of the latter was realized at pH 9.0 and stirring for 5 minutes with a 2.5×10^{-5} M solution of promethazine. The efficiency of the preconcentration step was studied at several applied potentials (−0.2, 0.0, and +0.2 V versus SCE) and at open circuit. Peak current intensitities decreased by 30, 18, and 35%, respectively, compared to the value obtained using the open-circuit operation.

Various paste compositions containing up to 10% stearic acid have been studied, and that with 5% stearic acid produced a better response. The reproducibility of the measurements depends on the surface smoothing and controlling the stirring rates. The coefficient of variation calculated for six measurements at two different concentrations of promethazine (2.5×10^{-5} and 5.0×10^{-9} M) were 3.2 and 4.1%, respectively. Differential pulse peak currents recorded after 5 minutes of stirring showed a linear dependence on the promethazine concentration between 1×10^{-6} and 1×10^{-8} M; the correlation coefficient was 0.999. Based on the signal-and-background characteristics (and considering a signal-to-noise ratio of 3 [38]), the calculated detection limit was 1×10^{-9} M (5 minutes of accumulation).

Interferences

The influence of copper and calcium ions, which may be present in biological fluids, was investigated. We observed that the presence of copper in a ratio of 1 : 100 [Cu(II) : promethazine] gave a peak current decrease of 20%, while with ratio of 1 : 10, a 50% depression of the signal was observed. This is probably due to complex formation between copper ions and promethazine [39] as well as to electrostatic association between surface carboxylic groups and the dications which hinder the accumulation process. The presence of 1×10^{-4} M calcium ions (with a Ca(II) : promethazine ratio of 4 : 1) caused depression of the promethazine current: 50% at the MCPE and 10% at the CPMet electrode. Adding 2×10^{-4} M of sodium ethylenediaminetetracetic acid (EDTA) restored the electrode signal completely without affecting the peak shape. Urea, which is known to interact with fatty acids [40], has no effect on the electrode response. Ascorbic acid and uric acid, which are potent interfering compounds present in biological samples, where successfully eliminated by the medium-exchange technique. Using the latter, quantitative measurements were successfully applied. With 5 minutes of stirring at pH 9.0, a linear dependence between peak current and promethazine concentration between 5×10^{-5} and 1×10^{-7} M (correlation coefficient, 0.999).

Applications to Biological Samples

The method was applied to the determination of promethazine in urine. The sample was diluted with the supporting electrolyte (1 : 10) and the pH was adjusted to 9.0 in the presence of 2×10^{-4} M EDTA to mask any cations which may interfere. A linear dependence on the promethazine concentration was observed between 1×10^{-6} and 3×10^{-7} M (r = 0.999) with a detection limit of 5×10^{-8} M (5 minutes accumulation). Determination of the drug was also successfully accomplished in serum samples using the stearic acid MCPE and the differential pulse mode (Figure 3). The procedure consisted of diluting the serum sample with the supporting electrolyte (1 : 2), adjusting to pH 9.0 in the presence of 2×10^{-4} M EDTA, and then transferring to the blank electrolyte after 5 minutes of stirring. The electrode signal was linearly related to the concentration of promethazine between 4×10^{-5} and 4×10^{-6} M (r = 0.994) with a detection limit of 2×10^{-7} M.

CONCLUSIONS

Modifying carbon paste electrodes with lipids offers new electrode models for the analysis of drugs that exhibit a high affinity for living cell membranes. We observed enhanced sensitivity (compared to unmodified electrodes) as a result of improved adsorption/extraction steps. Lipid-modified electrodes are thus particularly suitable for trace analysis of organic compounds. Due to the ease of electrode preparation and sample analysis, the electrochemical approach constitutes an alternative method, compared to more sophisticated techniques, for studies dealing with drug–membrane interactions.

ACKNOWLEDGMENTS

Thanks are expressed to le Fonds National de la Recherche Scientifique (FNRS Belgium) for assistance to one of us (G.J.P), to the SPPS (Belgium Politic Research, ARC, contract 86/96-89), and to the Egyptian government for the grant provided to M. Khodari.

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