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Applications in drug analysis of carbon paste electrodes modified by fatty acids*

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Abstract: The oxidation of promethazine as a model compound has been studied by adsorptive stripping voltammetry at carbon paste electrodes (CPE). A modification of the carbon paste matrix with fatty acids allows greater preconcentration of the molecule at the electrode surface. Several fatty acids of different chain length have been tested. The modification of the CPE with lauric acid has been successfully applied in the quantiative analysis of promethazine in standard serum samples. The influence of several parameters affecting the accumulation step has been investigated such as: pH, ionic strength, interfering ions, paste composition. The detection limit in phosphate buffer at pH 9.0 (t_{acc} = 5 min) has been found to be 1×10^{-10} M.

Keywords: *Electrodes modified with lipids; promethazine analysis; carbon paste electrode; electrochemistry.*

Introduction

Since the early eighties there has been a great deal of interest in electroanalytical techniques operating in the adsorptive preconcentration mode [1, 2]. The procedures consist on accumulating the analyte onto or into the electrode surface by convection forces prior to voltammetric analysis. Originally developed and successfully applied to the determination of traces of heavy metals at mercury and mercury-coated electrodes, the technique can also be advantageously used for the determination of traces of inorganic and organic compounds at solid electrodes [1–5]. When applied to analysis in complex biological media, solid electrodes offer serious advantages due to their inherent mechanical stability which allows the application of a medium exchange technique. This procedure consists of performing a selective preconcentration of the analyte onto the electrode surface in the biological matrix then, after transferring the electrode, voltammetric measurements are performed in an appropriate electrolyte. Recently, in order to improve the selectivity and efficiency of the preconcentration step, various

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surface modification procedures have been proposed and the resulting electrodes applied to the analysis of several inorganic species and some organic compounds [6, 7]. Generally the modification procedures consisted of changing the chemical nature of the electrode surface by attaching chemical reagents to it, on the basis that the electrode surface would take on the properties of the attached reagents [6]. In previous work, it has been shown that the modification of carbon-based electrodes with lipids (phospholipids or fatty acids) improved the preconcentration step of several pharmacologically interesting compounds [8–11]. Because a stearic acid-modified carbon paste electrode gave the highest responses in the determination of promethazine (detection limit: 1×10^{-9} M) [10], the present study was undertaken in order to optimize the electrode response in the determination of promethazine by varying the nature of the incorporated fatty acids.

Experimental

Apparatus

Two 50-ml cells were used, one containing the sample solution to be measured and the other containing the blank solution. The working electrode was a carbon paste prepared by pressing the paste into the well of the Teflon body of home-made electrodes (2 mm depth and 3 mm diameter). The carbon paste was from Metrohm (AG) Switzerland and prepared from spectroscopic grade carbon powder and UVASOL liquid paraffin (Metrohm EA 207C). A saturated calomel electrode (S.C.E.) served as reference and a platinum wire as auxilliary electrode. Monitoring of cell output was accomplished with a BAS CV 27 for cyclic voltammetry and a Brüker E100 voltammograph for linear scan and differential pulse measurements. The output of the data was plotted with the aid of a HP 7090 A recorder.

Reagents and solutions

All reagents used were of analytical grade. Deionized water was used to prepare the solutions. Lauric acid (C_{12}), stearic acid (C_{18}) and tetracosanoic acid (C_{24}) were obtained from Sigma. Promethazine hydrochloride (*N*-2-dimethylamino-*n*-propylphenothiazine hydrochloride) was of pharmacopoeial quality and used without further purification. Standard samples of serum were from Sigma. Buffer solutions (0.1 M) were prepared from disodium monohydrogen phosphate (Merck P.A.) and the pH was adjusted with NaOH or HCl solutions. Stock solutions of promethazine (0.01 M) at pH 7.0 were prepared daily and kept in the dark. All measurements were carried out at 20 ± 1°C.

Electrode preparation

Mixing of the paste with fatty acids (5% w/w) was carried out in a mortar in the presence of the minimum amount of chloroform (lauric and stearic acid) or chloroform–ethanol 50:50 v/v (tetracosanoic acid) and allowing the solvent to evaporate overnight at room temperature. The surface of the carbon paste electrode was smoothed against clean paper, and a new surface prepared for each experiment; no electrochemical pretreatment was required. For direct measurements, the electrode was placed into the cell and the preconcentration of the analyte was started in open circuit by stirring the solution (≈500 r.p.m.) for a selected time. At the end of the preconcentration period, the stirring was stopped and the initial potential was applied for 30 s before recording the voltammogram. The optimum operating parameters in the differential pulse mode were found to be; 50 mV pulse amplitude, 0.5 s pulse duration and 5 mV s^{-1} scan rate. For

experiments involving medium exchange, the electrode was washed by water after the preconcentration stage, and then transferred to a blank solution (phosphate buffer pH 9.0); the voltammogram was recorded between +0.3 V and +1.1 V vs S.C.E. Cleaning of the electrode was carried out by immersing the probe in 1.0 M HCl solution and after 5 min of stirring it was transferred to the blank solution and the voltammogram was recorded between +0.3 and +1.1 V vs S.C.E. three times (to obtain reproducible residual currents). Quantitative analysis was performed by the standard addition method.

Results and Discussion

The preconcentration and subsequent oxidation of promethazine was studied on carbon paste (CPMet) and on lipid-modified carbon paste electrodes (LMCPE). The positive potential limits and the oxidation peak characteristics of the molecule at different electrodes at pH 9.0 are summarized in Table 1. The presence of lipids had little effect on the electrochemical properties of the carbon paste in acidic media. By increasing the pH values above 7.0 slight increases in background currents concomitant with restricted available positive potential limits were observed. After preconcentration, the oxidation of promethazine occurred at less positive potentials at the LMCPEs except

Table 1
Electrochemical characteristics of carbon paste and lipid-modified carbon paste electrodes in phosphate buffers, (cut-off potential at a current in excess of 1 μA) and oxidation peak characteristics of promethazine (2.5×10^{-5} M, pH 9.0, linear scan: 10 mV s^{-1})

Electrode	Anodic pH 3	Limit pH 9	Peak potential Ep(V)	Peak width Ep − Ep/2 (mV)
CP Met(graphite + Nujol)	+1.30	+1.30	+0.61	110
CP Tet(graphite + tetracosanoic acid)	+1.30	+1.30	+0.71	130
CP Ste(graphite + stearic acid)	+1.40	+1.20	+0.57	60
CP Lau(graphite + lauric acid)	+1.30	+1.10	+0.57	60

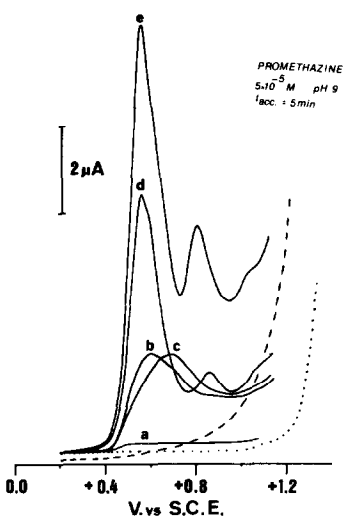

Figure 1
Linear scan voltammetric oxidation curves of promethazine (5×10^{-5} M) at different carbon paste electrodes. Phosphate buffer pH 9.0, accumulation time (t.acc.) = 5 min, scan rate: 10 mV s^{-1}. a; CPMet t.acc = 0, b; CPMet + acc., c; CPTet + acc., d; CPSt + acc., e; CPLau + acc., . . . blank at CPMet, ---- blank at CPLau.

on the carbon paste electrode with C_{24} (CPTet). Moreover, at the former, the electrode process can be regarded as a rapid phenomenon as suggested by the sharp oxidation peak (Ep-Ep/2). In contrast the oxidation peaks exhibited by the CPMet and the CPTet were less sharp (Table 1 and Fig. 1). Referring to the response at the CPMet without accumulation (Fig. 1 curve a), by preconcentration it was possible to observe enhanced signals as illustrated in Fig. 1 for a 5×10^{-5} M solution of promethazine at pH 9.0 and for 5 min accumulation time. The responses were markedly enhanced at the carbon paste electrodes with C_{12} and C_{18} (CPLau and CPSte) with gain factors of 37 and 22 respectively (Fig. 1 curves d and e). Furthermore, the gain factors were considerably improved by applying the differential pulse mode giving respectively 67- and 40-fold enhancements. Due to improved detection at the LMCPEs, new oxidation peaks situated at more positive potentials were also observed. Considering these results, further experiments were performed at the CPLau.

Influence of pH

The pH of the solution had a marked effect on the electrode response, i.e. on the preconcentration step (Fig. 2). Little accumulation was observed at pH values lower than 7.0. A sharp increase in response was observed in basic media with a maximum at pH 9.0. Further increase in pH resulted in a progressive decrease of the response which may be attributed to the destruction of the mechanical integrity of the paste. A similar pH influence has already been described in previous work on stearic acid-modified carbon paste electrodes [10]. The phenomenon can be explained by a higher tendency of the promethazine (neutral form) to leave the bulk solution and go to the electrode interface: as the pH values of the solutions approach the pK_a value of promethazine (9.08) more free base will be found in solution [11–13]. By observing the shape of the voltammo-

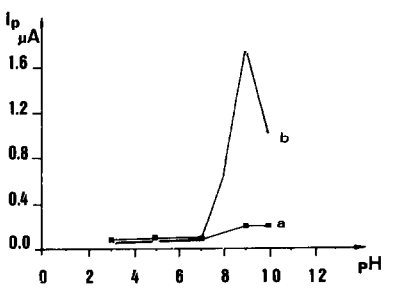

Figure 2
Differential pulse peak current (I) as a function of pH. Promethazine 1×10^{-6} M, t.acc. = 5 min, scan rate: 5 mV s^{-1}, a = CPMet, b = CPLau.

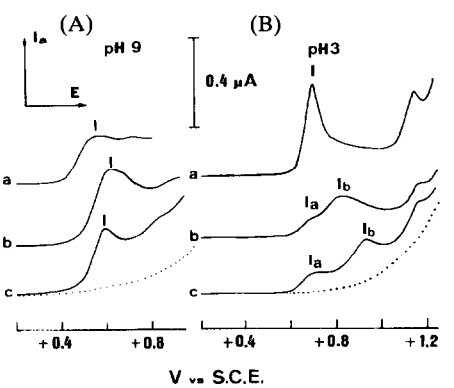

Figure 3
Linear scan voltammograms as a function of pH. Promethazine 5×10^{-5} M, scan rate: 10 mV s^{-1}. A = pH 9.0, B = pH 3.0. a = CPMet, b = CPSt, c = CPLau.

grams as a function of pH at the CPMet and at the LMCPEs, interesting differences in the first oxidation step of promethazine were noted (Fig. 3). Indeed, by decreasing the pH value at the LMCPEs to 3, peak I splits into two steps that are well separated at the CPLau (Fig. 3 curve C). At the non-modified electrode, the splitting of the first peak was observed only at pH values lower than 1.0. By carrying out cyclic voltammetric experiments in acidic media, it was possible to show the reversible character of peak Ia and to observe a better separation between peaks Ia and b. On the basis of the well-known oxidation mechanisms of phenothiazine, peak Ia was assigned to the formation of promethazine cation radical and peak Ib to the further oxidation to the corresponding sulphoxide (Fig. 4). These results suggest an improved stability of the cation radical at the LMCPE which might be attributed either to interactions with the negatively charged lipids or to a higher apparent acidity at the LMCPE interface due to possible hydrogen ion competition for the anionic sites [15].

Influence of preconcentration time and lipid content

The study of the different pulse peak current intensity as a function of accumulation time at pH 9.0 at the CPLau showed a marked current increase within the first 5 min (Fig. 5). A progressive levelling off of the curves occurred with longer preconcentration times. The strength of the accumulation was examined using the medium exchange

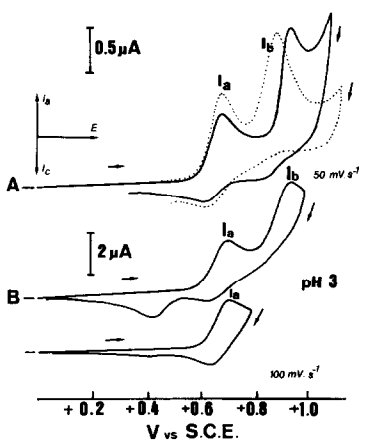

Figure 4
Cyclic voltammograms of promethazine (5×10^{-5} M) A:H_2SO_4 1 M, scan rate = 50 mV s^{-1}, —— CPLau, . . . CPMet. B: Phosphate buffer pH 3.0, scan rate = 100 mV s^{-1}, CPLau.

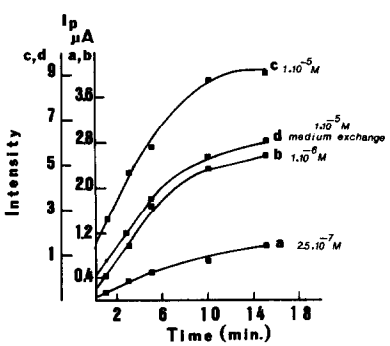

Figure 5
Differential pulse peak current (I) at CPLau as a function of accumulation time at pH 9.0. Promethazine: a, 2.5×10^{-7} M; b, 1×10^{-6} M; c, 1.0×10^{-5} M; d, 1.0×10^{-5} M (medium exchange).

technique. The results showed that a loss in sensitivity by a factor of three approximately occurs at short accumulation times and by a factor of two at longer periods.

The electrode response was examined with respect to paste composition by selecting three pastes containing, respectively, 2.5, 5 and 10% lauric acid. By increasing the lipid content and after accumulation the current corresponding to promethazine increased. In contrast, background currents were only a little higher: the 5% composition was found to give the best signal to background current ratio. By varying the phosphate buffer capacity from 0.001 to 0.5 M at pH 9.0, it was observed that the response improved by increasing the ionic strength up to 0.10 M but above this value it decreased.

The precision of the technique was calculated for a series of seven measurements of 1×10^{-7} M solutions of promethazine at pH 9.0: the relative standard deviation was found to be 5%. Under optimum conditions (pH 9.0, 0.1 M phosphate buffer, 5 min accumulation) the differential pulse response showed a linear relationship as a function of promethazine concentration in the range 1×10^{-6}–1×10^{-8} M with a correlation coefficient of 0.999 and a detection limit (S/N = 3) of 1×10^{-10} M.

Interferences

The influence of both calcium(II) and copper(II) ions which are present in biological fluids at relatively high concentrations was investigated. The presence of copper ions in the ratio 1:10 [Cu(II):promethazine] depressed the response by about 30% and the presence of 1×10^{-4} M calcium ions, i.e. in the ratio 4:1 (Ca(II):promethazine) depressed the response by the same percentage. Fortunately, the addition of 2×10^{-4} M disodium ethylene diamine tetraacetic acid (sodium edetate; $EDTANa_2$) restored the electrode response completely without affecting the peak shape. Ascorbic acid and uric acid which were oxidized at similar potentials to those of promethazine were completely eliminated by the medium exchange technique. This method was successfully applied in quantitative measurements: the electrode response showed a linear dependence between peak current and promethazine concentration (conditions as in Fig. 5) in the range 5×10^{-8}–5×10^{-6} M with a correlation coefficient of 0.9998.

Application to biological samples

The medium exchange technique was applied to the analysis of the drug in standard serum samples. The latter were diluted with phosphate buffer pH 9.0 containing $EDTANa_2$ (2×10^{-4} M). After a preconcentration period of 5 min, the electrode was transferred to the blank electrolyte prior to voltammetric oxidation. The electrode response was linearly related to the concentration of promethazine within the range 4×10^{-6}–9×10^{-5} M and obeyed the equation: i_p (μA) = 0.55 + 0.38 C (10^{-5} M) (r = 0.998).

Conclusions

The modification of the carbon paste electrode by appropriate fatty acids is proposed. The modified electrodes improve significantly the preconcentration step of promethazine. By varying the nature of the fatty acids it has been possible to demonstrate that the smaller the acyl chain length of the lipid the higher is the efficiency of the accumulation. This might be related to a higher amount of polar head groups at the electrode interface for a given quantity of incorporated lipid. This approach is very promising for new developments in electrode modification and for the study of interactions of drugs at

the charged interface. Finally, the ease and selectivity of analysis combined with the high sensitivity render the preconcentration–medium exchange technique at LMCPEs a suitable method of analysis in complex biological media. The analysis of other pharmaceutically interesting compounds at LMCPEs is at present under investigation [14].

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