Asian Journal of Chemistry Vol. 12, No. 2 (2000), 541–547

Determination of Melatonin by Differential Pulse Voltammetry at Modified Carbon Paste Electrode

G.E. BEKHEIT*
Chemistry Department, Faculty of Science,
New Damietta, Mansoura University, Damietta, Egypt

A direct sensitive and reproducible electroanalytical procedure for the determination of melatonin is developed at a modified and unmodified carbon paste electrode. A linear relationship between the current and concentration was found at the two electrode surfaces. The method was employed for the analysis of melatonin in urine samples. The open circuit preconcentration/medium exchange/voltammetric scheme was used to eliminate interference from sample solutions.

INTRODUCTION

The non-electrochemical accumulation of electroactive compounds on the working electrode by adsorption and other mechanisms has been exploited for the development of adsorptive stripping voltammetry (ADSV) procedures. The theoretical and practical aspects of ADSV have been reported[1-3]. Carbon paste electrode (CPE) was used widely as a working electrode in ADSV for the determination of electrochemically oxidisable analyte at positive potentials.

Melatonin, N-acetyl-5-methoxytryptamine, is a hormone synthesized by the pineal gland from serotonin by N-acetylation and O-methylation. The bulk of melatonin is produced and secreted at night[4]. This circadian rhythm is controlled by the pacemaker in the suprachiasmatic nucleus that regulates the amount of noradrenaline release from the sympathetic nerve terminals synapsing with the pineal gland[5].

Melatonin is a well known electroactive compound that is readily oxidised at a CPE and glassy carbon electrode[6-8]. Therefore, the present paper was aimed to develop a simple, sensitive and precise electroanalytical carbon paste electrode with oleic acid

EXPERIMENTAL

An Oxford potentiostate equipped with a Philips PM 8043 X-Y recorder was used for recording the cyclic voltammograms. The electrochemical cell was a 20 mL glass vessel with Ag/AgCl/3.5 M KCl, a separate salt bridge, a glassy carbon auxiliary electrode and a carbon paste working electrode (diameter 3 mm). The differential pulse voltammograms have been recorded using Sargent-Welch (4001) voltammetric analyzer. A Schott Gerate pH with glass combination electrode was used for pH determination.

All reagents were of analytical-reagent grade. Melatonin was dissolved in 10 mL methanol to give 10^{-2} M stock solution. All solutions were prepared with double distilled water in a quartz apparatus. Britton-Robinson buffers (B.R.)

**Present address*: Teacher College of Education, Madinah, P.O. Box 1343, Saudi Arabia.

(acetic, phosphoric and boric acids, all at 0.04 M; pH adjusted with NaOH) were used as supporting electrolytes.

Procedures: The electrodes were prepared by mixing the graphite with the modifier or nujol (5 : 3 w/w) in a mortar and pestle. The CPE was thoroughly cleaned before packing with new paste by sonication in chloroform to remove all the paste from previous experiments. After being dried with tissue paper, the electrode was then packed tightly with carbon paste and the surface was smoothed by a clean weighing paper until there were no cracks apparent. For direct measurements the electrode was placed into the cell and preconcentration of the analyte was started by stirring the solution at *ca.* 400 rpm, by a magnetic stirrer and stirring bar 1 cm long for given period of time. At the end of the preconcentration period, the stirring was stopped and a rest period of 15 seconds was given before the voltammogram was recorded. For experiments involving the medium exchange procedure, the electrode was washed with water after the preconcentration step, and then transferred to a blank solution; the voltammogram was then recorded in the anodic direction.

Regeneration of the electrode surface was achieved after each measurement by two successive voltage sweeps over the same range of potentials in a blank solution. Occasional scans in blank solution were done to check the quality of the base line. In the case of presistent current peaks indicating an irreversible electrode poisoning, the electrode surface was renewed.

RESULTS AND DISCUSSION

Melatonin showed three well defined oxidation peaks in aqueous buffered solution in the pH range 1–12 at carbon paste or glassy carbon electrodes. In the reverse scan, a well defined reduction peak is observed coupled with an anodic peak on the second forward scan. The mechanism of oxidation of melatonin has already been discussed[6]. Figure 1 shows the cyclic voltammogram of 6×10^{-5} M melatonin at pH 5 at carbon paste electrode modified with oleic acid at 50 mV s^{-1} scan rate. A peak appeared at a potential of 0.8 V *vs.* Ag-AgCl electrode.

The influence of scan rate was critically investigated by linear scan voltammetry to assess whether the process on the modified and unmodified carbon paste electrodes proceeds under diffusion or adsorption control. When the peak current was plotted versus the square root of the scan rate, a linear response was recorded for the two electrodes, confirming the diffusion-controlled process. The results are summarized in Table-1. The main conclusion to be drawn form these results is that the rate controlling process is the diffusion of the melatonin from one part of the modified layer to another[9].

The variation of the peak current height as a function of pH using linear sweep voltammetry at 6×10^{-5} M melatonin, showed maximum responses between pH 4 and 6, while at other pHs, a 50% depression of the peak current intensities was observed. Therefore, the measurements were recorded at pH 5 in the subsequent work.

The dependence of the stripping peak current on the preconcentration potential was examined for CPEMO over the range +0.4 V to –0.4 V *vs.* Ag/AgCl electrode

at pH 5. Melatonin showed the highest extraction capacity at open circuit potential.

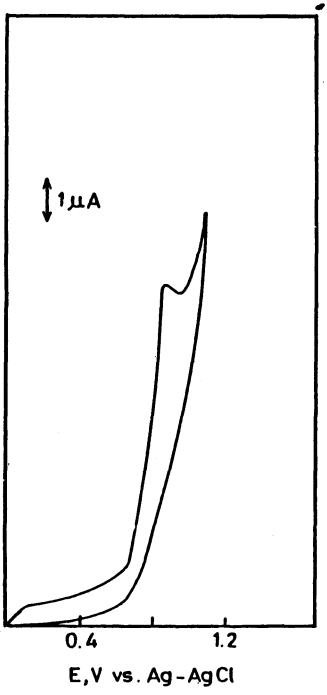

Fig. 1. Cyclic voltammogram of 6×10^{-5} M melatonin, in pH 5 at (CPEMO), scan rate 50 mV s^{-1}

TABLE-1
CHARACTERIZATION OF THE RATE-CONTROLLING STEP FOR 6×10^{-5} M MELATONIN USING VOLTAMMETRY FOR THE TESTED ELECTRODES AT pH = 5 AT OPEN CIRCUIT (scan rate range 10–500 mV/s)*

Electrode	Equation
CPE	ip (μA) = 17.795 $V^{1/2}$ (μA $volt^{-1/2}$ $s^{1/2}$) + 0.05477 r = 0.9561, n = 6
CPEMO	ip (μA) = 15.6507 $V^{1/2}$ (μA $volt^{-1/2}$ $s^{1/2}$) + 1.8896 r = 0.9740, n = 6

*CPE = carbon paste electrode
CPEMO = carbon paste electrode modified with oleic acid

Figure 2 shows plots of anodic peak current (ip_a) of linear sweep voltammetry versus accumulation time (t) for the two different concentrations of melatonin at pH 5 using carbon paste electrode modified with oleic acid. At first, the anodic peak current increased approximately linearly with time and at longer preconcentration time, more melatonin was retained. However, at 120 sec accumulation time the peak current tended to level off, illustrating that the adsorptive

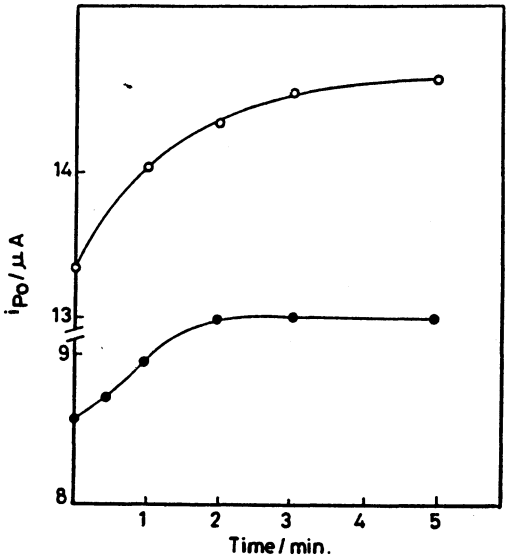

Fig. 2. Effect of preconcentration time on stripping peak current for two different concentrations of melatonin on CPEMO at pH = 5, scan rate 50 mV s^{-1}: (a) 3×10^{-6} M and (b) 6×10^{-6} M

equilibrium of melatonin on the electrode surface was achieved[10]. On the other hand, carbon paste electrode showed no accumulation of melatonin on it as previously reported[11].

Analytical application

The differential pulse determination of melatonin at carbon paste CPE and modified CPEMO electrode is expressed in terms of the slope of the corresponding working curve (peak of differential currents *vs.* concentrations). For the two electrodes a linear response was obtained in B.R. buffer at pH = 5 in the concentration range 6×10^{-6} to 3×10^{-5} M. Employing the least square method the analytical data are obtained as given in Table-2.

TABLE-2
SENSITIVITY OF THE DETERMINATION OF MELATONIN EXAMINED AT CARBON PASTE ELECTRODE (CPE) AND MODIFIED CARBON PASTE ELECTRODE (CPEMO) USING DIFFERENTIAL PULSE VOLTAMMETRY AT pH = 5
(scan rate 33.33 mV/s and excitation volt = 20 mV.)

Electrode	The regression equation (μA/mM)	Sr*	S_B†
CPE	ip (μA) = 25 C (mM) + 0.25 R = 0.94, n = 5	0.122	6.45×10^{-3}
CPEMO	ip 37.3 C(mM) –0.015 R = 0.9665, n = 5	0.066	3.49×10^{-3}

Sr* The standard deviation about the regression[12]
S_B† The standard deviation of the slope.

Figure 3 shows the differential pulse voltammograms of melatonin at various concentrations on carbon paste electrode modified with oleic acid at pH 5.

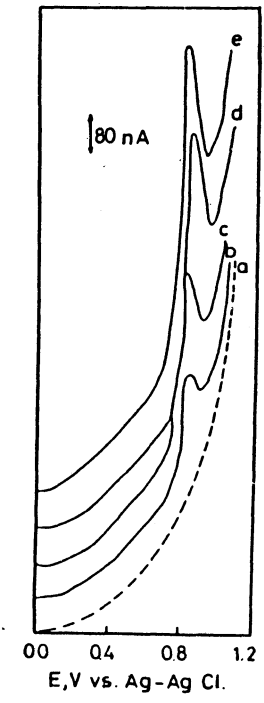

Fig. 3. Differential pulse voltammograms of melatonin of various concentrations on CPEMO electrode at pH 5, scan rate 33.33 mV s^{-1}, excitation potential 20 mV: (a) blank; (b) 6×10^{-6} M; (c) 12×10^{-6} M; (d) 18×10^{-6} M and (e) 24×10^{-6} M

Because of its high adsorption and selectivity oleic acid modified carbon paste electrode (CPEMO) was assessed for the determination of melatonin in complex media, *e.g.*, urine samples. For this purpose the working electrode (with the accumulated analyte) is transferred from the human urine sample to an electrolytic blank solution between the preconcentration and measurement steps[13, 14]. The selectivity improvement obtained by such medium exchange procedure is illustrated in Figure 4. The voltammogram recorded in a sample containing diluted (1 : 20) urine solution and 6×10^{-6} M melatonin using CPEMO does not permit quantitation of the latter due to large overlapping urine oxidation peak (Figure 4A). Using the medium exchange procedure, in contrast, the urine interference is eliminated; this allows convenient quantitation of melatonin (Figure 4B). The effective correction of oxidation currents, associated with solution-phase sample components, is indicated from the different current scales used in the "direct" vs. "exchange" voltammograms shown in Figure 4.

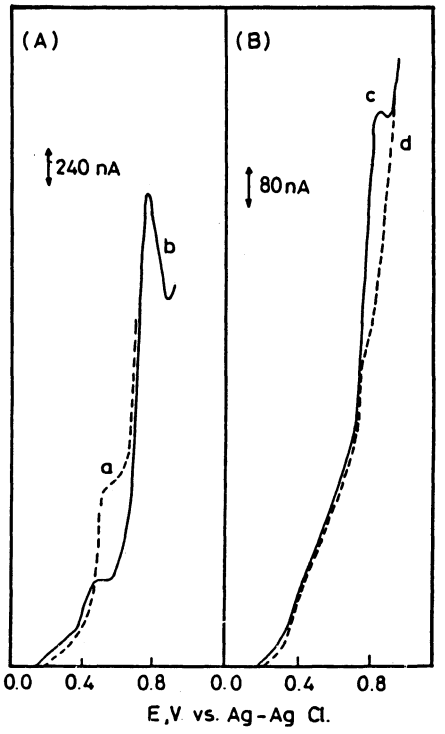

Fig. 4. Differential pulse measurements of 6×10^{-5} M melatonin using carbon paste electrode modified with oleic acid at scan rate 33.33 mV s^{-1}: (a) precocentration for 3 min. in urine solution only; (b) preconcentration for three min. in urine $+6 \times 10^{-5}$ M melatonin; (c) measurement after medium exchange to a blank solution for (b), and (d) measurement after medium exchange to a blank solution for (a), preconcentration at 0.0 V, excitation 20 mV.

Conclusion

Adsorptive stripping voltammetry at a modified carbon paste electrode with oleic acid has been shown to be suitable for the determination of trace amounts of melatonin. The sensitivity is significantly enhanced by adsorption of melatonin on the electrode surface. The open circuit preconcentration/medium exchange/ voltammetric scheme eliminates interference of urine solution-phase species.

REFERENCES

1. R. Kalvoda, *Anal. Chim. Acta*, **138**, 11 (1982).
2. J. Wang, *Int. Lab.*, **15**, 68 (1985).
3. ————, Stripping Analysis VCH, Deerfield Beach, FL, p. 61 (1985).
4. R.J. Reiter, *News Physiol. Sci.*, **1**, 20 (1986).
5. K.F. Tipton and M.B.H. Youdim, in: M.B.H. Youdim, W. Lovenberg and K.F. Tipton (Eds.), Biomedical and Pharmacological Aspects of Depression: Topics in Neurochemistry and Neuropharmacology, Taylor and Francis, Basingstoke, p. 91 (1989).

6. A. Radi and G. Bekheit, *Bioelectrochem. Bioenerg.*, **45**, 275 (1998).
7. F. Cresphi, E. Ratti and D.G. Trist, *Analyst*, **119**, 2193 (1994).
8. A. Radi, *Anal. Commun.*, **36**, 43 (1999).
9. D. Boyd, J.R. Rodriguez, A.J. Ordieres and P.T. Blanco, *Analyst*, **119**, (1994).
10. L. Qilong and C. Shouai, *Anal. Chim. Acta*, **282**, 145 (1993).
11. N.E. Zoulis, D.P. Nikolelis and C.E. Efstathiou, *Analyst*, **115**, 291 (1990).
12. Douglas, Principles of Instrumental Analysis, Saunders College Publishing, London, U.K. (1984).
13. J. Wang and B.A. Freiha, *Anal. Chem.*, **55**, 1285 (1983).
14. ————, *Anal. Chim. Acta*, **148**, 79 (1983).

(*Received*: 19 November 1999; *Accepted*: 14 December 1999) AJC-1944