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Anodic adsorptive stripping voltammetric determination of the anesthetic drug: methohexital sodium

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Abstract

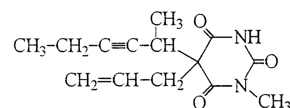
Methohexital (MS) determination is based on the formation of insoluble mercury salt on a hanging mercury drop electrode after preaccumulation by adsorption. This property was exploited in developing a highly sensitive stripping voltammetric procedure for the determination of the drug. The anodic current of adsorbed compound is measured by linear sweep anodic stripping voltammetry (LSASV), preceded by a period of preconcentration. The effect of various parameters such as supporting electrolyte composition, pH, initial potential, scan rate, accumulation time and ionic strength are discussed to characterize the interfacial and redox behavior. The detection limit was found to be 2×10^{-7} M (56.8 ppb) with 180-s accumulation time. The interference of some amino acids, ascorbic acid and some metal ions was investigated. The application of this method was tested in the determination of methohexital in spiked urine samples. The precision of the method is satisfactory with a relative standard deviation of 2.5%. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Methohexital sodium; Anodic stripping voltammetry; Urine

1. Introduction

As methohexital is one of the barbiturate group used in clinical practice as depressants of the central nervous system, it is important to develop a rapid, sensitive and reproducible method for its determination.

The structural formula of methohexital is



The compound has been determined by gas chromatography with either flame-ionization detector [1] or electron-capture detector [2]. It was also determined by HPLC/UV in plasma [3] or whole blood [4]. Other barbiturates have been determined using titrimetric [5] and spectrophotometric methods [6,7]. A derivative spectrophotometric technique was used for determination of

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phenobarbitone and phenytone sodium in combined tablet preparation [8,9]. The fluoroimmunoassay technique was used for determination of phenytone and phenobarbitone [10,11]. Chromatographic methods including HPLC with UV detector [12–15] and gas chromatography [16] have been used. Capillary electrophoresis has also been used for determination of some barbiturates [17]. Potentiometric determination of some barbiturates using solid-state iodide ion selective electrode has been reported [18]. The polarographic behavior of some barbiturates like amobarbital, barbital, barbituric acid, diallylbarbituric acid, pentobarbital and phenobarbital has been studied using AC polarography [19]. DC and AC polarography were chosen as methods of determination of phenobarbital in non aqueous medium [20]. Differential pulse polarography was used for estimation of phenobarbitone after nitration [21] and in a mixture with *N*-alkyl phenobarbitone [22]. The same technique was used for determination of other barbiturates [23,24]. In the present work an effort has been made to utilize the linear sweep anodic stripping voltammetric method (LSASV) for simultaneous determination of methohexital sodium (MS) as pure and in spiked urine samples.

2. Experimental

2.1. Apparatus and reagents

Stripping and cyclic voltammograms were obtained using an EG and G Princeton Applied Research Corporation (PAR) model 264A stripping analyzer. The working electrode was PAR model 303A static mercury drop electrode (SMDE) with a drop area of 0.014 cm^2. The polarographic cell (PAR model K 0060) was fitted with an Ag/AgCl/sat. KCl reference electrode and a platinum wire counter-electrode. A PAR 305 stirrer was connected to the 303A SMDE. A PAR model RE 0089 X–Y recorder was used to collect the experimental data. The pH of the solution was adjusted using an Orion 601A Precision Research Ion analyzer digital pH meter. Stock solutions (1×10^{-3} M) of methohexital sodium (Lilly Research Centre, UK) were prepared daily by dissolving appropriate amounts of the drug in double-distilled water. All other solutions were prepared using double-distilled water and analytical grade reagents. Urine samples from healthy volunteers were used in the analysis.

2.2. Procedure

In the ideal experimental conditions, 10 ml of 0.01 M sodium acetate–acetic acid buffer (pH 4.2) was deoxygenated by passing nitrogen for 15 min and the voltammogram was recorded after equilibration for 15 s. The preconcentration potential was -0.4 V. The scan rate was 100 mV/s for both cyclic voltammetry (CV) and linear sweep anodic stripping voltammetry (LSASV). The pulse amplitude was 25 mV and pulse repetition time was 1 s. The same procedure was repeated after addition of analyte to the solution. All data were obtained at room temperature (25 ± 2°C).

For application of the method to urine, we use the standard addition method where urine samples from volunteers, taking no drugs, were diluted (1:100) and (1:1000) times with 0.01 M acetate buffer (pH 4.2) and the drug was spiked in the range of 1×10^{-6}–1.2×10^{-5} M.

3. Results and discussion

The optimum conditions for the determination of methohexital sodium were investigated. The influence of different supporting electrolytes including sodium nitrate, sodium perchlorate, disodium hydrogen phosphate, borate, citrate and sodium acetate–acetic acid buffer were studied in order to obtain a reproducible current for the peak of the drug. The observed peak merges into the supporting electrolyte decay peaks for all buffers investigated, except for sodium acetate–acetic acid buffer. It gave the highest signal owing to its low background current. The effect of different pH (2.8, 4.2, 5.1 and 8.3) on peak height were tested in sodium acetate–acetic acid medium. The optimum pH for the determination of methohexital sodium was about 4.2 as shown in Fig. 1. Also the effect of sodium acetate–acetic

acid concentration at constant pH 4.2 was examined. The sodium acetate buffer concentration which gave the highest signal was 0.01 M. However, 0.01 M sodium acetate–acetic acid buffer at pH 4.2 was selected as the best supporting electrolyte for the determination of methohexital sodium. At this pH, the separation of the drug peak and electrolyte decay peak were also at a maximum.

Methohexital determination was based on the formation of insoluble mercury salt on a hanging mercury drop electrode after preaccumulation by adsorption [22]. This property was exploited in developing a highly sensitive stripping voltammetric procedure for the determination of the drug. Looking at the literature [25] it is evident that in 0.1 N NaOH barbituric acid, derivatives were decomposed due to hydrolysis even during the time of deoxygenation. The influence of deposition potential on peak height was tested using linear sweep anodic stripping voltammetric technique where the drug exhibits strong adsorption at -0.4 and -0.5 V. Below and above these values the height of the peak current decreased, indicating lower adsorption as shown in Fig. 2. Hence, the deposition potential was fixed at -0.4 V for all further experimental measurements.

The interfacial and redox behavior of the drug can be evaluated from the cyclic voltammetric measurements. Fig. 3 shows repetitive cyclic

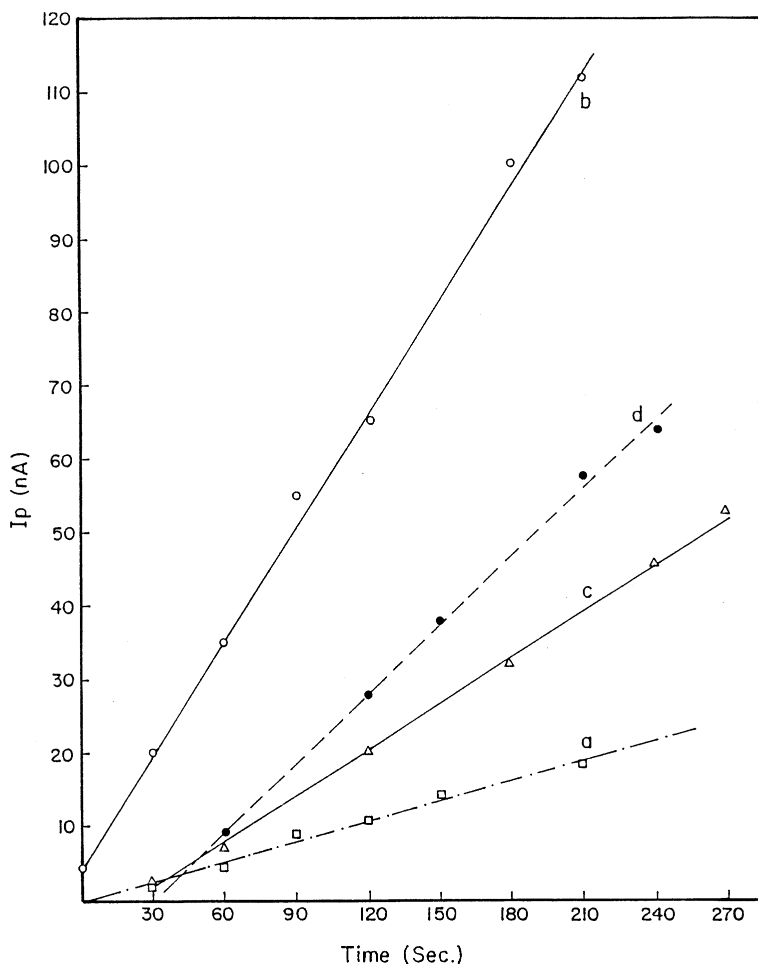

Fig. 1. Current–time plots of 1×10^{-6} M methohexital sodium in 0.01 M acetate–acetic acid buffer with pH (a) 2.8, (b) 4.2, (c) 5.1 and (d) 8.3.

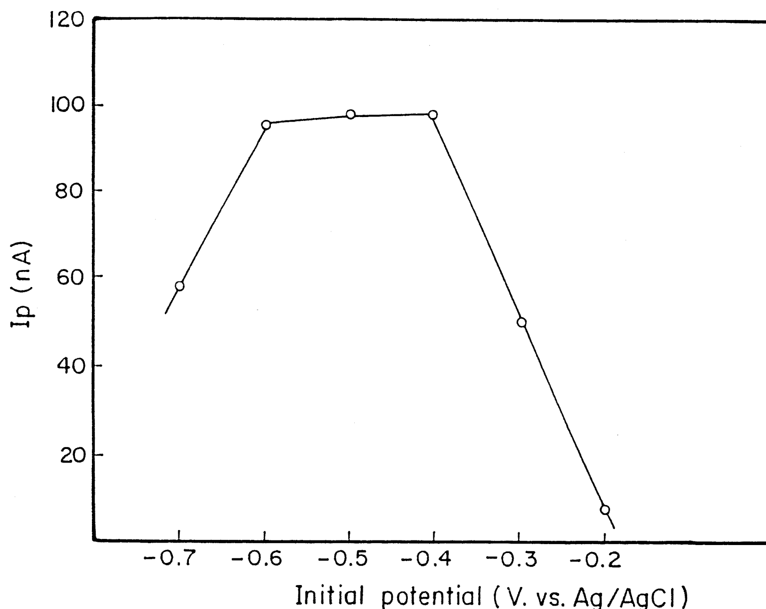

Fig. 2. Effect of accumulation potential on the peak current of 2×10^{-6} M MS in the presence of 0.01 M sodium acetate–acetic acid buffer (pH 4.2) and 20 s accumulation time.

voltammograms for 2×10^{-6} M methohexital in the presence of 0.01 M acetate–acetic acid buffer (pH 4.2) after a deposition time of 10 s at an accumulation potential of -0.4 V. One anodic peak was obtained at -0.04 V and no peak was observed on scanning in the negative direction. Subsequent scans yielded smaller anodic peaks which represent a rapidly desorbed product which inhibited the appearance of the reduction product. However, it can be assumed that the drug is concentrated onto the electrode surface at the deposition potential.

On scanning in the anodic direction, the analyte reacts with mercuric ion, liberated from the electrode, to form MS salt i.e. the anodic signal is due to the formation of mercuric salt of MS accumulated onto the electrode surface. The same electrode reaction mechanism was operative for the measurement of anodic wave corresponding to the formation of insoluble almost barbiturate derivatives. It is also concluded that the mercury atoms are bound to the barbiturates via the nitrogen atoms [24]. The effects of potential scan rate V on the peak current and potential were evaluated as shown in Fig. 4. A log I_p vs. log V plot was linear over the range 10–200 mV/s (Fig. 4a) with a slope of 0.75, which is in agreement with that expected for irreversible reaction of surface species [26]. A 40-mV positive shift in the peak potential was observed upon increasing the scan rate in the given range. The plot of E_p vs. log V (Fig. 4b) was also linear (correlation coefficient 0.995).

Fig. 5 shows the effect of preconcentration time in the presence of different concentrations of the drug. The peak current increased linearly with preconcentration time up to 270, 240 and 210 s for 2×10^{-7}, 5×10^{-7} and 1×10^{-6} M (MS), respectively. A deviation from linearity was observed at accumulation times longer than 45 and 30 s for 5×10^{-6} and 1×10^{-5} M respectively. Table 1 illustrates the data collected. From the investigation of peak current characteristics, changes of peak current and peak potential with different drug concentrations were noticed. The peak current and peak potential depended on drug concentration very markedly (Fig. 6). The peak potentials shifted toward more positive values as the concentration of methohexital increased, but this shift is lower at higher concentration (Fig. 6).

The relationship between peak potential and the logarithm of concentration was linear (correlation coefficient 0.990). This behavior is a very striking aspect of electrode processes based on mercury salt formation [24].

A well defined stripping peak was observed over the concentration range 1×10^{-6}–1×10^{-5} M after 10, 15 and 20 s with stirring at -0.4 V. The resulting calibration plots for these concentrations are shown in Fig. 7. The graphs show positive deviation from linearity at concentrations higher than 8×10^{-6}, 6×10^{-6} and 4×10^{-6} M methohexital respectively. This phenomenon and the change in the slope of the response might be attributed to surface effects of the investigated molecule [24]. The data obtained were collected in Table 2.

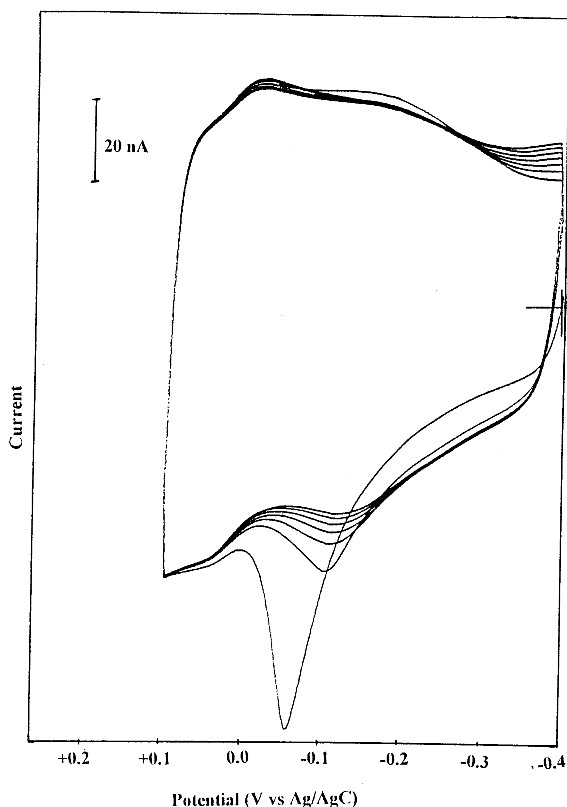

Fig. 3. Cyclic voltammogram of 2×10^{-6} M MS in 0.01 M sodium acetate–acetic acid buffer (pH 4.2), accumulation potential -0.4 V, scan rate 100 mV s^{-1} and $t_{acc}=10$ s.

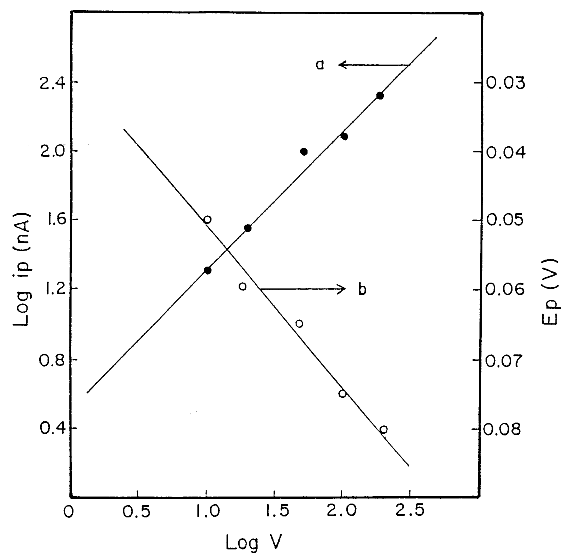

Fig. 4. Log I_p vs. log V (scan rate) (a) and E_p vs. log V (b) for 1×10^{-6} M methohexital sodium in 0.01 M sodium acetate–acetic acid buffer (pH 4.2), $t_{acc}=120$ s.

The reproducibility of the adsorption process was tested by repeating 10 experiments on 1×10^{-6} M of the drug at a preconcentration time of 20 s. The relative standard deviation was calculated to be 2.5%.

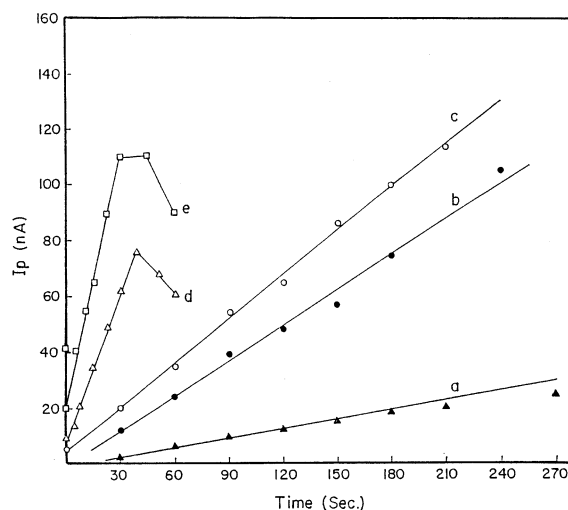

Fig. 5. Effect of accumulation time on linear sweep voltammetric response of (a) 2×10^{-7}, (b) 5×10^{-7}, (c) 1×10^{-6}, (d) 5×10^{-6} and (e) 1×10^{-5} M MS in 0.01 M sodium acetate–acetic acid buffer (pH 4.2).

Table 1
Characteristics of current–time curves established using different methohexital (MS) concentrations with 0.01 M sodium acetate–acetic acid (pH 4.2)

[MS] (M)	Linearity range (s)	Correlation coefficient	Slope (nA s^{-1})	Intercept (nA)
2×10^{-7}	30–270	0.9878	0.1404	0.0
5×10^{-7}	15–240	0.9798	0.4000	0.0
1×10^{-6}	0–210	0.9887	0.5185	5.0
5×10^{-6}	0–45	0.9974	1.6666	8.0
1×10^{-5}	0–30	0.9989	3.6666	20.0

Temizer and Solak [24] reported that some barbiturates were in the region of the first reduction peak of oxygen. The peak of the drugs was strongly affected by the dissolved oxygen in solution. Deoxygenation was carried out by passing a nitrogen stream through the solution. Reduction of the oxygen was prevented after a definite concentration of the drug (4 μM). However, in the present work, with a proper selection of potential (−0.4 V) and by passing a nitrogen stream through the solution for a long time (16 min) and for 2 min during each run, such oxygen contribution was completely eliminated and the drug peak was readily detected. Linear sweep anodic stripping voltammetry (LSASV) is the best method for such determination of MS at lower concentration where as low as 2×10^{-7} M (56.8 ppb) was estimated with 180 s preconcentration time using standard additions.

The influence of ascorbic acid and some amino acids including glutamic acid, L-valine, β-alanine, L-serine and aspartic acid, which are potent interfering compounds present in biological samples, were investigated. It was found that an equimolar concentration of each of them had no effect on the peak response of methohexital. However, at a higher molar excess (10:1) of ascorbic acid, a complete depression of the drug peak was observed. Also, the addition of amino acids in the ratio of 100:1 causes reduction of the peak height of the drug by 25%. The effect of some metal ions (Cu and Pb) that form chelates with the drug were also investigated, for 1×10^{-6} M methohexital with 30 s accumulation time in the presence of 0.01 M acetate buffer (pH 4.2). Addition of Cu and Pb ions, individually in the range of $(1-5)\times10^{-7}$ M to 1×10^{-6} M drug have no effect on the peak height of the drug. But on further addition of each metal ion individually in the range of 6×10^{-7}–2×10^{-6} M to 1×10^{-6} M drug, the signal of MS peak decreased gradually, and a new

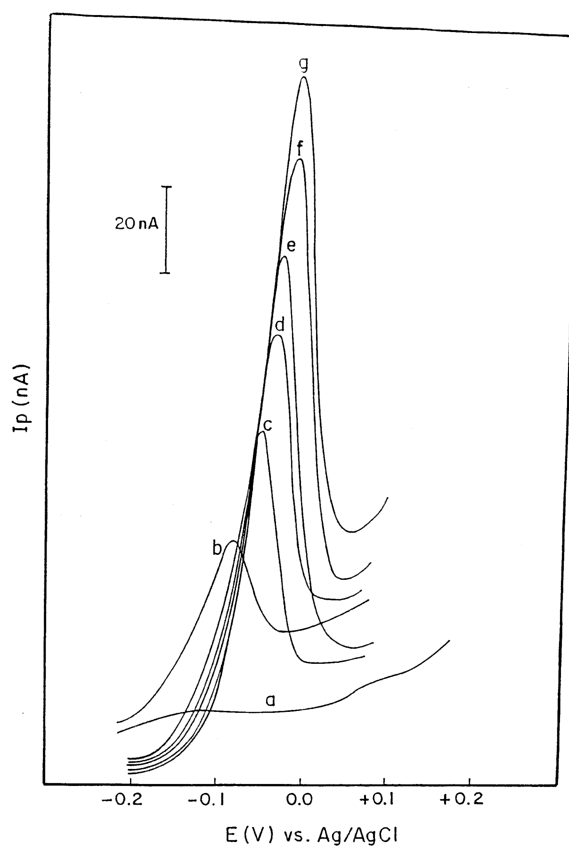

Fig. 6. Effect of concentration on the peak potentials and peak currents of MS in the presence of 0.01 M sodium acetate–acetic acid (pH 4.2) and accumulation time, 10 s; (a) blank, (b) 1×10^{-6}, (c) 2×10^{-6}, (d) 4×10^{-6}, (e) 6×10^{-6}, (f) 8×10^{-6} and (g) 1×10^{-5} M MS.

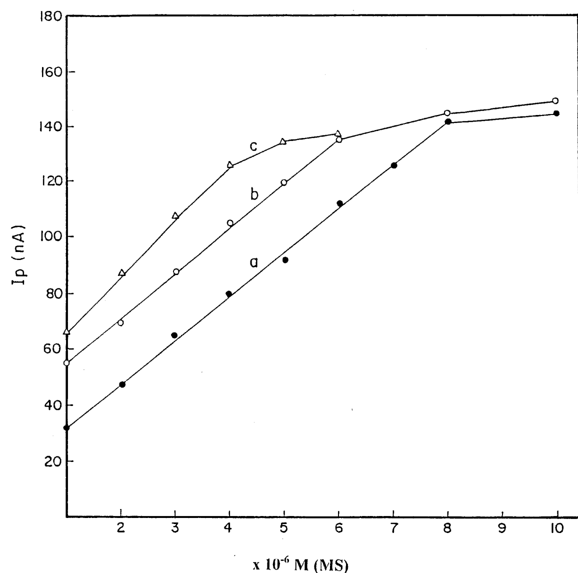

Fig. 7. Current–concentration graphs after pre-concentration times (a) 10, (b) 15 and (c) 20 s for methohexital sodium in the presence of acetate buffer (pH 4.2).

peak current of metal–drug complexes appeared with a positive shift in peak potential. On the other hand, when 6×10^{-7} M Cu or Pb ions were added individually to 1×10^{-6} M MS, this lead to the formation of a metal–drug complex and the drug peak was completely depressed at 2×10^{-6} M of each metal ion addition.

4. Application of the LSASV method for assay in urine

Methohexital is excreted through the kidney by glomerular filtration [27]. Measurement of the drug in a spiked urine sample was demonstrated and voltammograms were recorded (Fig. 8A). A linear dependence on the MS concentration was observed, with urine samples diluted (1:10) with supporting electrolyte, between 1×10^{-6}–1.2×10^{-5} M and 1×10^{-6}–6×10^{-6} M, after 5 and 15 s respectively ($r=0.997$) using the standard additions method. Also, when the spiked urine was diluted (1:100) and at 5 s accumulation time, a linear relation was observed over the range 4×10^{-6}–2×10^{-5} M of methohexital ($r=0.998$) as shown in Fig. 8B.

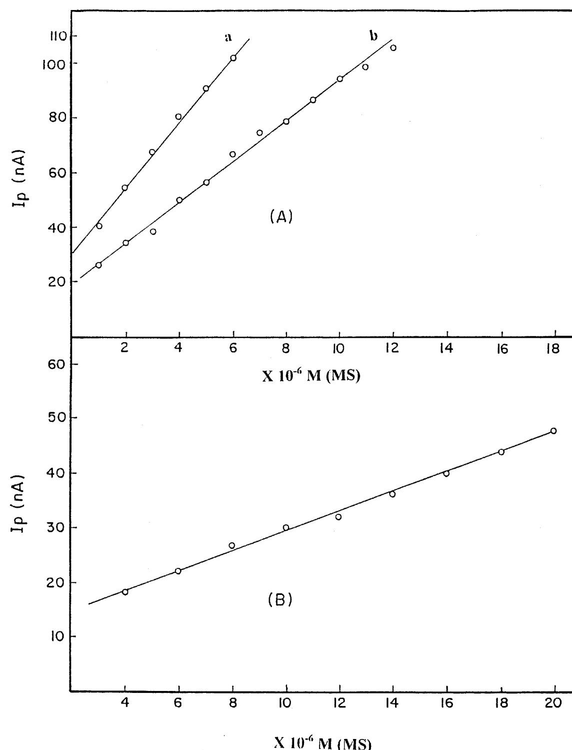

Fig. 8. (A) Plot of I_p vs conc. of MS added to the urine sample solution (0.01 ml/10 ml H_2O) in 0.01 M acetate buffer (pH 4.2), at (a) 5 and (b) 15 s accumulation times. (B) Plot of I_p vs conc. of MS added to urine sample solution (0.1 ml/10 ml H_2O), in the presence of acetate buffer (pH 4.2) and 5 s accumulation time.

Table 2
Characteristics of calibration curves established using different deposition times with sodium acetate–acetic acid (pH 4.2)

Deposition time (s)	Linearity range (M)	Correlation coefficient	Slope (nA/10^{-6} M)	Intercept (nA)
10	1×10^{-6}–8×10^{-6}	0.9784	0.7143	32
15	1×10^{-6}–6×10^{-6}	0.9717	0.8333	55
20	1×10^{-6}–4×10^{-6}	0.9975	0.8571	66

5. Conclusion

A major objective in this investigation was to evaluate methohexital at lower concentrations than that of oxygen levels (4 μM), using anodic adsorptive stripping voltammetry. A detection limit of 2×10^{-7} M MS (56.8 ppb) was obtained. The method was applied to biological samples (urine). A linear dependence of the peak current on methohexital concentration was observed over the range 1×10^{-6}–1.2×10^{-5} M of the drug with a correlation coefficient of 0.9985. The reproducibility of the method was tested with R.S.D. of 2.5% ($n = 10$).

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