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Application of Stripping Voltammetry to the Determination of Guanethidine

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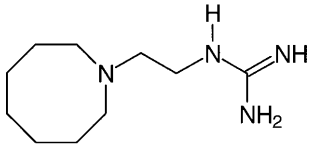
Summary. Adsorptive stripping voltammetry for the determination of guanethidine is employed. The drug is adsorbed onto the hanging mercury drop electrode, and the reduction current of the accumulated drug is measured by scanning the potential in the cathodic direction. The adsorption and redox behaviour are explored by cyclic voltammetry. Optimum experimental conditions include preconcentration potential, solution *pH*, potential scan rate, and preconcentration time. A detection limit of guanethidine of $0.992\,ng/cm^3$ was obtained.

Keywords. Stripping voltammetry; Hanging mercury drop electrode; Cyclic voltammetry; Guanethidine.

Introduction

Guanethidine (**1**) is an antihypertensive agent (adrenergic neurone blocking agent) [1]. Methods for the determination of **1** in the bulk or in dosage forms can hardly to be found in the literature. Spectrophotometry [2], chromatography [3–6], and fluorescence measurements [7] together with other drugs in tablets and in dosage form have been reported for this purpose. A quantitative determination of guanethidine in biological fluids and in non-aqueous medium using thin layer chromatography has also been described [8].



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The aim of the present work was to study the determination of guanethidine in pure drug and biological samples (urine and serum) using the cathodic stripping voltammetric technique. This is an important method for the determination of many biological compounds which show adsorption onto mercury surfaces [9–11]. It is highly sensitive, rapid, precise, reproducible, simple, and cheap.

Results and Discussion

The adsorptive peak current of **1** is strongly affected by the type of supporting electrolytes. The effect of sodium nitrate, sodium perchlorate, sodium borate, sodium citrate, sodium acetate–acetic acid buffer, perchloric acid, nitric acid, phosphoric acid, and phosphate buffer were studied. The observed peak merges into the supporting electrolyte decay peaks for all these electrolytes except for phosphate buffer, where it gave the highest signal. The effect of the concentration of phosphate buffer as supporting electrolyte (0.001–0.1 *M*) and the influence of *pH* from 6.0 to 11.0 was studied. **1** showed a very small peak current at low *pH* values, the peak current increased with increasing *pH* values and reached a maximum at *pH* 7.1–7.4 as shown in Fig. 1. This phosphate buffer concentration gave the highest signal at a concentration of 0.01 *M*.

Figure 2 shows cyclic voltammograms for $1 \cdot 10^{-7}\,M$ guanethidine in 0.01 *M* phosphate buffer ($pH = 7.3$) and a preconcentration time of 120 s. It is obvious that the voltammogram consists of one cathodic peak at about -1.99 V which is related to the reduction of the –C=NH group; no anodic peak appeared in the reverse direction. Repetitive cyclic voltammograms indicated that the peak current decreases sharply in the second and third cycle. The current signal disappears because all of the analyte accumulated at the electrode is reduced. The influence of this deposition potential on the peak height was studied in the range of -0.6 to

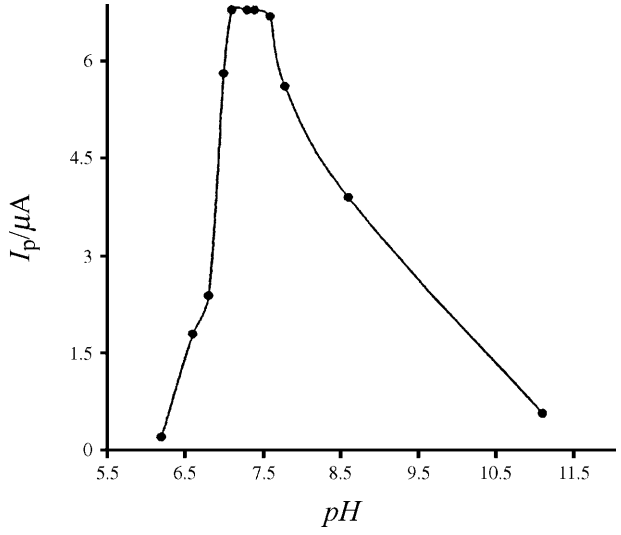

Fig. 1. Dependence of the peak current of $1 \cdot 10^{-6}\,M$ **1** on the *pH* values of phosphate buffer at -1.4 V preconcentration potential, 60 s preconcentration time, and a scan rate of 100 mV/s

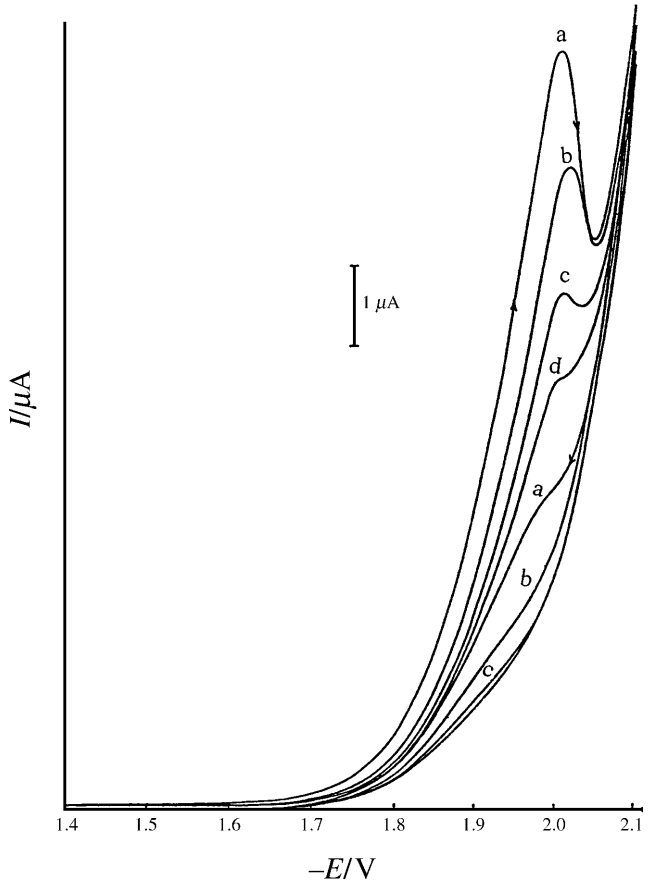

Fig. 2. Repetitive cyclic voltammograms for $1 \cdot 10^{-7} M$ **1** in 0.01 *M* phosphate buffer ($pH = 7.3$) at an accumulation potential of -1.4 V, an accumulation time 120 s, and a scan rate of 100 mV/s; (a) first cycle, (b) second cycle, (c) third cycle, (d) fourth cycle

-1.9 V. **1** exhibits a strong adsorption at -1.4 V; accordingly, this value was selected for further studies.

The effect of the potential scan rate ν on the peak current or the peak potential was also examined. The peak current increases with increasing scan rate (10 to 200 mV/s), and the peak potential is shifted to more negative values. At higher scan rates, the peak current shape was destroyed. Upon plotting $\log I_p$ *vs.* $\log \nu$, a straight line was obtained with a slope of 0.8 and a correlation coefficient of 0.997 (Fig. 3). This slope is in close agreement with a slope of 1.0 that was to be expected for the ideal reaction of the surface species [12–13].

The amount of **1** accumulated on the electrode surface increased with increasing deposition time and concentration of **1** ($1–9 \cdot 10^{-8} M$ and $1–5 \cdot 10^{-7} M$) as shown in Fig. 4. A linear relation was found between accumulation time and peak height of **1** up to 360 and 300 s for $1 \cdot 10^{-8}$ and $3 \cdot 10^{-8} M$ solutions, respectively. A deviation from the linearity was observed at accumulation times above 180 s for $5 \cdot 10^{-8}$, $7 \cdot 10^{-8}$, and $1 \cdot 10^{-7} M$ solutions and above 45 or 30 s for $3 \cdot 10^{-7}$ and $5 \cdot 10^{-7} M$ **1** (Fig. 5).

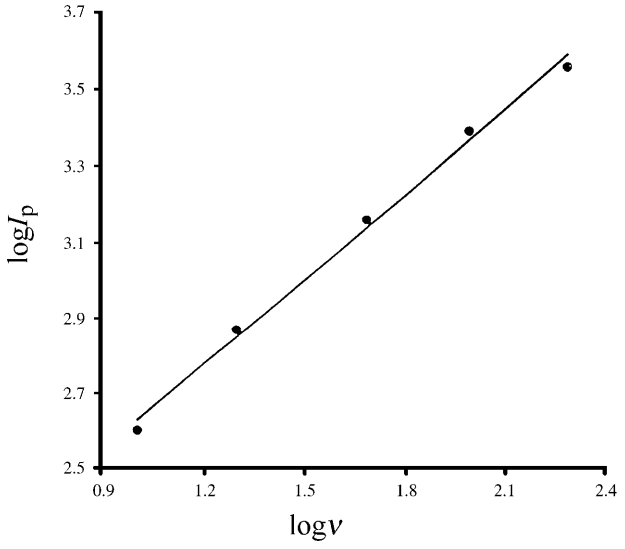

Fig. 3. Dependence of the logarithm of the peak current on the logarithm of the scan rate for $1 \cdot 10^{-6}$ *M* **1** in 0.01 *M* phosphate buffer ($pH = 7.3$); accumulation potential -1.4 V, preconcentration time 30 s

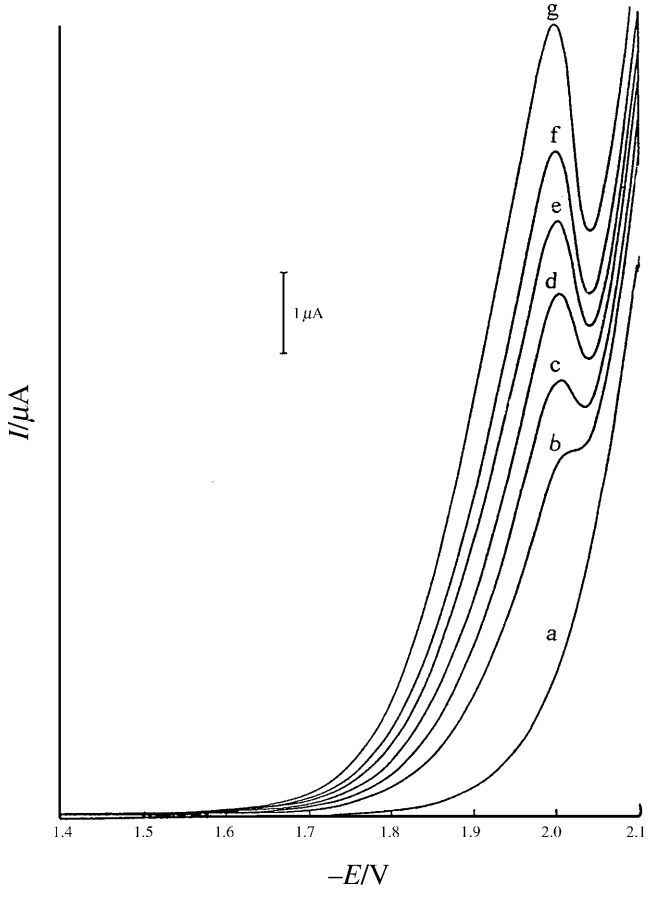

Fig. 4. Typical linear sweep cathodic stripping voltammograms for $5 \cdot 10^{-8}$ *M* **1** in 0.01 *M* phosphate buffer ($pH = 7.3$) at 100 mV/s and different accumulation times: (a) 0, (b) 60, (c) 90, (d) 120, (e) 150, (f) 180, (g) 300 s

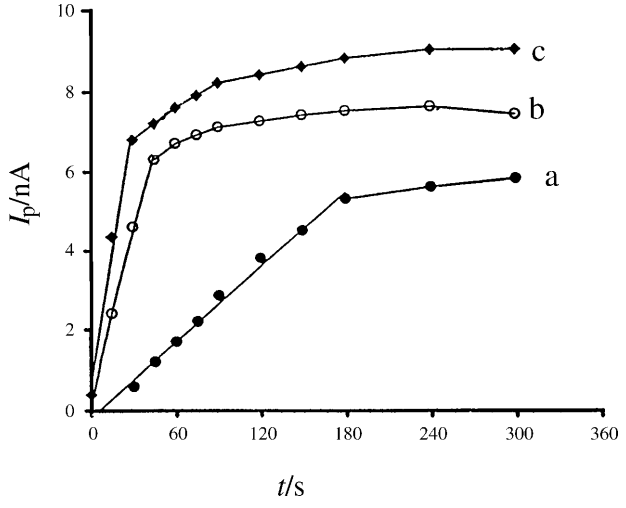

Fig. 5. Current *vs*. time plots from linear sweep cathodic stripping voltammograms of different concentration of **1** in 0.01 *M* phosphate buffer ($pH = 7.3$); accumulation potential -1.4 V, scan rate 100 mV/s; (a) $1 \cdot 10^{-7}$, (b) $3 \cdot 10^{-7}$, (c) $5 \cdot 10^{-7}$ *M*

To select the suitable mode for the determination of **1**, the results were treated statistically using the equation $Y = A + B_x$. From the results it can be observed that the linear regression mode gives satisfactory results using an accumulation time of 120 s.

Upon plotting the peak current *vs*. the square root of the accumulation time for $5 \cdot 10^{-8}$ *M* **1**, a straight line was obtained with a correlation coefficient of 0.9974. This behaviour was expected for mass-transport controlled adsorption where the quantity of adsorbed material is proportional to the square root of the adsorption time [14].

Reproducibility of the adsorption process was tested using a $5 \cdot 10^{-8}$ *M* solution of **1**. The voltammogram was measured five times obtaining a mean value of the peak current of 0.62 μA and a relative standard deviation of 1.52%. A detection limit of $5 \cdot 10^{-9}$ *M* (0.992 ng/cm^3) was achieved after 120 s preconcentration time; the lower limit of detection was 0.198 ng/cm^3, the limit of quantitation [15] 27.39 ng/cm^3.

Generally, the major source of interferences in adsorptive stripping measurements are likely to be organic compounds and surfactants that compete with the drug for space on the mercury surface as well as other metal ions that may form adsorbable electroactive species. Interference of several metal ions and some amino acids were tested for $1 \cdot 10^{-7}$ *M* **1** in the presence of 0.01 *M* phosphate buffer. Upon addition of $1 \cdot 10^{-7}$ to $1 \cdot 10^{-5}$ *M* of Cu(II), Ni(II), Co(II), Zn(II), Cd(II), Pb(II), and some amino acids such as glycine, *L*-ascorbic, and *L*-aspartic acid individually or in admixture to $1 \cdot 10^{-7}$ *M* **1** solution, no change of the current signal was observed. In addition, the reduction of the metal ions which precede the reduction of **1** has no effect on the current signal, although a change in the electrode surface occurs, *i.e*. amalgamated ions.

The determination of **1** in urine samples was demonstrated as follows: the sample was diluted (1:1000) with 0.01 *M* phosphate buffer ($pH = 7.3$) and increasing concentrations of **1**. The peak current increased with increasing drug

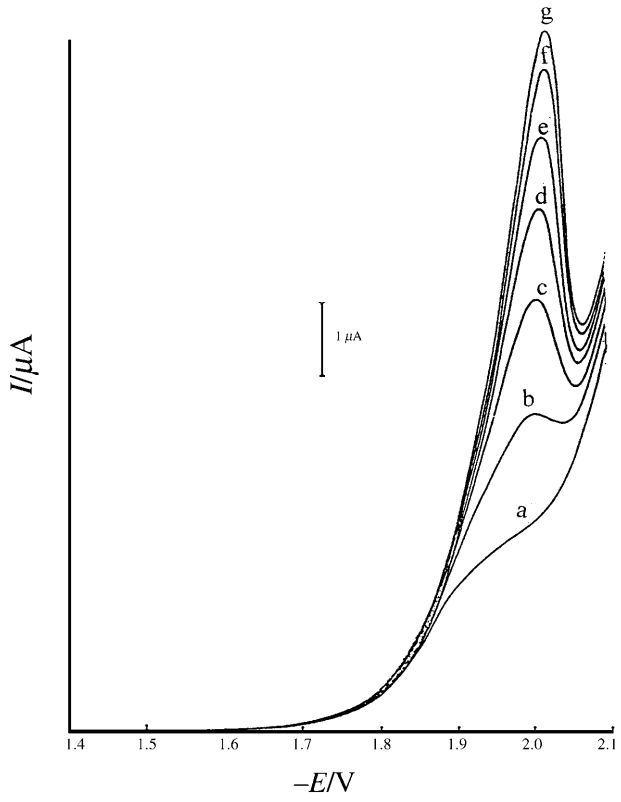

Fig. 6. Typical linear sweep cathodic stripping voltammograms of an urine sample (1:1000) in 0.01 *M* phosphate buffer ($pH = 7.3$); accumulation potential -1.4 V, preconcentration time 30 s, scan rate 100 mV/s; (a) urine sample, (b) a $+ 1{\cdot}10^{-7}$ *M* **1**, (c) a $+ 2{\cdot}10^{-7}$ *M* **1**, (d) a $+ 3{\cdot}10^{-7}$ *M* **1**, (e) a $+ 4{\cdot}10^{-7}$ *M* **1**, (f) a $+ 5{\cdot}10^{-7}$ *M* **1**, (g) a $+ 6{\cdot}10^{-7}$ *M* **1**

concentration as shown in Fig. 6. As can be seen from these voltammograms, the original sample of urine (diluted 1:1000) does not show any electroactive species. Upon adding **1** alone, the peak current increased with increasing concentration of **1**. The resulting peak current showed a linear behaviour with respect to analyte concentration from $1{\cdot}10^{-7}$ *M* to $6{\cdot}10^{-7}$ *M* at 30 s accumulation time with a correlation coefficient of 0.9991; the recovery was 96.3%. Therefore, a concentration as low as $1{\cdot}10^{-4}$ *M* of **1** can be determined successfully in the original urine sample, *i.e.* 19.8 μg/cm^3.

The daily dose of **1** required for a satisfactory antihypertensive response varies greatly among individual patients [16]. For this reason, therapy is usually initiated at low dose, *e.g.* 10 mg/d. The bioavailability of **1** is low and variable, and only 3 to 50% of an oral dose reaches the systemic circulation. The drug is rapidly transported to its intraneuronal site of action from which it is eliminated with a half-life of days. About 50% of the drug is metabolized, and the remainder is excreted unchanged in the urine. From the above it is obvious that the new method can be used to determine **1** in urine. The analysis of **1** was also studied in a more complicated medium, *i.e.* serum. However, it could not be determined successfully because the peak of **1** disappeared in this case.

Conclusions

The present study describes an effective time-saving and cheap method for the determination of **1**. It is more rapid and highly sensitive, with a lower limit of detection of 0.992 ng/cm^3, and also gives good results for the determination of **1** in pure material and in spiked urine.

Experimental

Apparatus and reagents

The voltammograms were obtained on an EG&G PAR 264A voltammetric analyzer with a PAR 303A static mercury drop working electrode. A medium drop size (0.014 cm^2 surface area) was used. The polarographic cell (PAR Model K0060) was fitted with a Ag/AgCl (saturated KCl) reference electrode and a Pt wire counter electrode. A magnetic stirrer (PAR 305) and a stirring bar (1 cm long, 2 mm thick) provided the connective transport during the accumulation step. A PAR model RE 0089 X-Y recorder was used for the collection of experimental data. All solutions were prepared using deionized H_2O. The stock solution of **1** (0.01 *M*, Sigma) was prepared daily by dissolving the appropriate amount of **1** in bidistilled H_2O and stored in the dark at 8°C; the other concentrations (1 m*M*, 0.1 m*M*, and 0.01 m*M*) were prepared daily by dilution from the stock solution.

Procedure

For voltammetric measurements, 10 cm^3 of the supporting electrolyte solution was added to the cell and degassed with N_2 for 16 min. The deposition potential (-1.4 V) was then applied at the electrode for 120 s. The stirring was stopped, and after a rest period of 15 s the voltammograms were recorded by applying a liner scan (100 mV/s) in the cathodic direction. The appropriate concentration of the drug solution was spiked using an automatic pipettor (10–100 mm^3). The solution was stirred while purging with N_2 and then proceeded through the accumulation and stripping step as before. All results were obtained at room temperature (25$\pm$1°C) with an N_2 atmosphere maintained over the solution surface.

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