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Cathodic adsorptive stripping voltammetric determination of muscle relaxant: gallamine triethiodide (flaxedil)

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Abstract

A senstive and simple voltammetric method of analysis is developed for the determination of trace amounts of gallamine triethiode in phosphate media. This method is based on controlled adsorptive preconcentration of the relaxant onto a Hanging Mercury Drop Electrode (HMDE) whereby mercurous iodide salt(s) are formed. The technique used is Cathodic Linear Sweep Stripping Voltammetry (CLSSV). The adsorptive response was evaluated with respect to preconcentration time and potential. As little as 3×10^{-9} mol dm^{-3} i.e. 2.7 ppb flaxedil (proconcentration time 300 seconds) can be determined successfully. The application of this method was tested in the determination of flaxedil in pharmaceutical preparation (ampoules).

Keywords: Flaxedil (gallamine triethiodide); Cathodic linear sweep; Adsorptive stripping voltammetry; Flaxedil ampoule

1. Introduction

Several techniques have been developed for the determination of flaxedil in human plasma and rat serum including high performance liquid chromatography [1–3], potentiometry with an ion-selective membrane electrode [4] and thin layer chromatography [5]. In an earlier study, a polarographic method was used to investigate the interfacial activity of certain healing substances used in medicine including flaxedil. No results were obtained for flaxedil either due to its chemical structure or because of its different mode of action [6]. Almost 100% of a dose is exerted unchanged in the urine in 24–30 h. Negligible amounts are excreted in the bile [7]. To the best of our knowledge, no polarographic or voltammetric investigation on flaxedil has appeared in the literature. The adsorptive stripping voltammetric technique has been used for trace determination of many inorganic and organic substances [8]. This technique eliminates both time-consuming solvent extraction steps and calculations of recovery common to ion selective electrode and chromatographic methods while the resulting accuracy and precision are at

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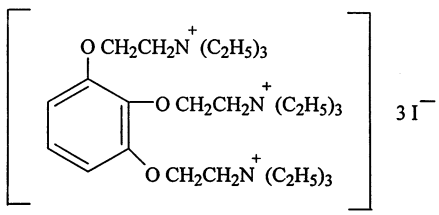
least comparable if not better than the above mentioned methods [9].

The aim of this work was to investigate the behaviour of flaxedil (I) [7] at the hanging mercury drop electrode (HMDE) using a cathodic linear sweep stripping voltammetric (CLSSV) technique. The method was applied to the analysis of the drug in a pharmaceutical formulation (ampoule).



Flaxedil (Gallamine triethiodide)

(I)

2. Experimental

2.1. Apparatus

Stripping and cyclic voltammograms were carried out by a Polarographic Analyzer, Model 264A (EG&G, Princeton Applied Research; Princeton, NJ, USA), coupled with a PAR 303A Static Mercury Drop Electrode (SMDE), (drop size: medium, area of the drop: 0.014 cm^2). The polarographic cell bottom (PAR Model K0060) was fitted with an Ag/AgCl (saturated KCl) reference electrode and a platinum wire used as a counter electrode. A PAR 305 stirrer was connected to the 303A SMDE. A PAR Model RE 0089 X-Y recorder was used for recording the voltammograms.

pH measurements were made with an Orion 601A Precision Research ionalyzer digital pH meter.

2.2. Materials and reagents

A 10 mM aqueous stock solution of flaxedil was prepared daily by dissolving appropriate amounts of gallamine triethiodide (from Alexandria Pharmaceutical Company, Egypt) in bidistilled water. Solutions of 10 mM copper(II), lead(II), zinc nitrate (Merck), sodium chloride and ascorbic acid were prepared and used in the interference studies. A total of 10 mM solutions from *N*-cetyl-*N*,*N*,*N*-trimethyl ammonium bromide (TAB), sodium dodecylsulphate (SDS), cationic and anionic surface active substances (SAS), respectively, were prepared by dissolving the appropriate weight in bidistilled water and used in interference studies.

2.3. Supporting electrolytes

A 0.2 M phosphate buffer mixture from sodium dihydrogen phosphate (H_2A^-) and sodium monohydrogen phosphate ($HA^=$) was prepared by dissolving the appropriate weight from H_2A^- and $HA^=$ in redistilled water. The desired pH value is attained by adding 10 mM carbonate-free sodium hydroxide. All other solutions were prepared from doubly distilled water and analytical grade reagents were used.

2.4. Procedure

A total of 0.25 ml of 0.2 M mono- and disodium hydrogen phosphate buffer (H_2A^-, $HA^=$, 5 mM each, pH = 7.0) were transferred to the voltammetric cell and diluted to 10 ml. The solution was deaerated by passing pure nitrogen for 16 min and the preconcentration potential (usually + 0.1 V) was applied to a fresh mercury drop while the solution was stirred. Scans were carried out using scan rate of 100 mV s^{-1} in the cyclic voltammetry (CV) and (CLSSV). After the accumulation step, and further 15 s equilibrium time, the voltammogram was recorded. A known concentration of the analyte (flaxedil) was added using a micropipette (Volac, UK) to the same cell. The solution was stirred while purging with nitrogen for 2 min and the procedure was completed as above. All measurements were carried out at (25 ± 1)°C with a nitrogen atmosphere maintained over the solution surface.

2.5. Analysis of gallamine triethiodide 'flaxedil' in pharmaceutical preparation

A total of 50 μl of Gallamine aqueous solution in its pharmaceutical preparation (each 2-ml ampoule contains 40 mg Gallamine in pyrogenic water), (Alexandria Pharmaceutical Company, Egypt) was diluted to 100 ml with bidistilled water, then 100 μl of the diluted drug solution was added to the voltammetric cell (10 ml) containing (H_2A^- and $HA^=$, 5 mM each, pH = 7.0). The analysis was done using the cathodic linear sweep voltammetric technique.

3. Results and discussion

The optimal conditions for studying the behaviour of 1×10^{-6} M flaxedil were investigated. The influence of different supporting electrolytes, i.e. nitric, phosphoric, perchloric acids, sodium acetate-acetic acid mixture and sodium dihydrogen phosphate and sodium mono-hydrogen phosphate buffers were studied in order to obtain a reproducible current peak for the drug. The highest signal was obtained in the presence of a mixture from [H_2A^-, $HA^=$] buffer. The effect of pH values 3, 5, 7, 8 and 10 on the peak height and peak potential were tested by the addition of phosphoric acid and/or sodium hydroxide to obtain the required pH. It was noticed that there is no effect of pH on the peak height. The pH of 7.0 was selected to carry out this study. Also, the effect of H_2A^- and $HA^=$ concentrations was examined at constant pH $\simeq$ 7.0. The mixture of H_2A^- and $HA^=$ (5 mM each) gives the highest peak signal. In the same manner, no shift in the peak potential ($E_p = -0.25$ V) was observed, indicating that the electrochemical process does not involve hydrogen ions.

The influence of the accumulation potential on the peak height was tested over a range (+ 0.2– − 0.15 V) using CLSSV technique. Flaxedil exhibits a sharp adsorption peak at accumulation potentials (+ 0.1– − 0.0V). The peak height decreased as the initial potential become more negative (− 0.05– − 0.15 V). Therefore, a potential of + 0.1 V was used as the accumulation potential for all the experimental measurements.

The cyclic voltammograms of 1×10^{-6} M flaxedil in the presence of of H_2A^- and $HA^=$, (5 mM each), pH = 7.0, initial potential + 0.1 V, 30 s of accumulation time (1st cycle) and scan rate 100 mV s^{-1} have been recorded. A single reduction peak was observed at − 0.25 V as shown in Fig. 1; subsequent scans have the same peak potential and decreased peak height, leading to a complete disappearance of the peak. This may be attributed to the adsorption of insoluble mercurous iodide salt(s). It is well known that many compounds such as cyanides, halides, thiols and selenide depolarize the Hg electrode [10–12]. No peak was observed in the positive scanning direction, indicating that the electrochemical process is irreversible. Fig. 2. shows cyclic voltammograms using 90 s of accumulation time for the 1st cycle. In the first cycle, two cathodic peaks appeared. In the second cycle, one of the two peaks, i.e. that is located at the more positive potential has completely disappeared, this may be attribute either to the existence of another Hg salt which is less adsorbable or which exists in a very minute amount.

3.1. Effect of scan rate

The effect of scan rate ν (from 10 to 200 mV s^{-1}) on the peak current and peak potential of 1×10^{-6} M flaxedil was studied. The log i_p versus log ν gives straight line with slope 0.856, this value is expected for an ideal reaction of surface species [13]. A 20 mV negative shift in the peak potential was observed upon increasing the scan rate in the range given. This is further evidence for the adsorption of the drug onto the electrode surface [14]. The plot of E_p versus log ν is also linear. The value of the linear correlation coefficient is 0.998.

3.2. Effect of accumulation time

Fig. 3 shows the plots of peak current versus preconcentration time for 1×10^{-7}, 3×10^{-7}, 5×10^{-7} and 1×10^{-6} M flaxedil in the presence of H_2A^-, and $HA^=$ (5 mM each), pH $\simeq$ 7.0) and an initial potential + 0.1 V; a straight line with slope 1.66 nA s^{-1}. was obtained for 1×10^{-7} M flaxedil. However at higher concentrations, the straight lines curve at 150, 90 and 30 s for 3 ×

10^{-7}, 5×10^{-7} and 1×10^{-6} M flaxedil, respectively. The breaks at certain accumulation times indicate that surface coverage attained. The slopes of the straight lines are 3, 5 and 13 nA s^{-1} for 3, 5 and 10×10^{-7} M flaxedil, respectively, and the increase in the slope is due to an increase in the concentration. All the lines intersect with the current axis at zero preconcentration time indicating that the adsorption takes place during the rest period 15 s [15]. Further evidence is that with increasing concentration, the amount of current produced at zero accumulation time is increased.

3.3. Effect of flaxedil concentration

Flaxedil yields a well defined peak at -0.25 V; the peak current increases with increasing flaxedil concentration indicating the formation of the more insoluble mercuric iodide salt on the mercury surface. Plotting the peak current against the flaxedil concentration in the range of (1–10) 10^{-7} M at an accumulation time 30 s gives a straight line with two section's. The first section is within the range of (1–4) 10^{-7} M flaxedil and the second section range is (4–10) 10^{-7} M flaxedil. This phenomenon may be due to the kinetic electrochemical process which is controlled by the chemical reaction rate, i.e. the formation of mercury iodide salt(s) or complexes; (1–10) 10^{-8} and (5–10) 10^{-9} M flaxedil concentrations gave straight lines. (The statistical parameters for different flaxedil concentrations are given in Table 1). These results indicate that CLSSV can be used for the determination of flaxedil and at concentration

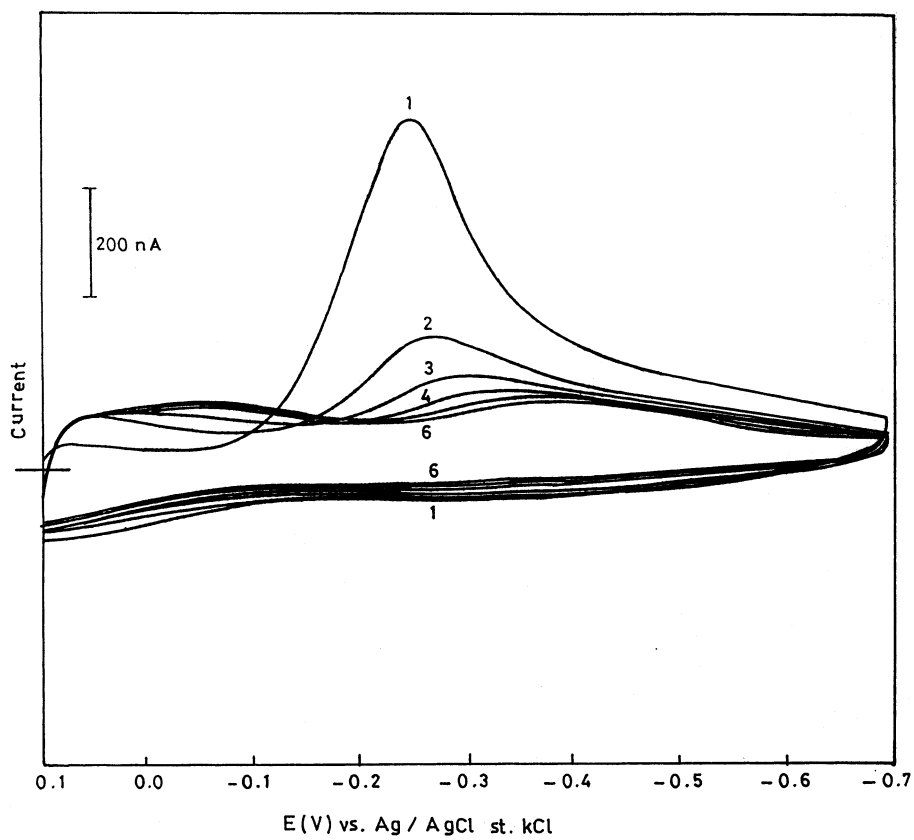


Fig. 1. Repetitive cyclic volammograms for 1×10^{-6} M flaxedil in the presence of (H_2A^- and $HA^=$, 5 mM each pH = 7.0) accumulation potential +0.1 V. scan rate 100 mV s^{-1} and deposition time 30 s (1) first cycle; (2) second cycle; (3) third cycle; and (4) fourth cycle.

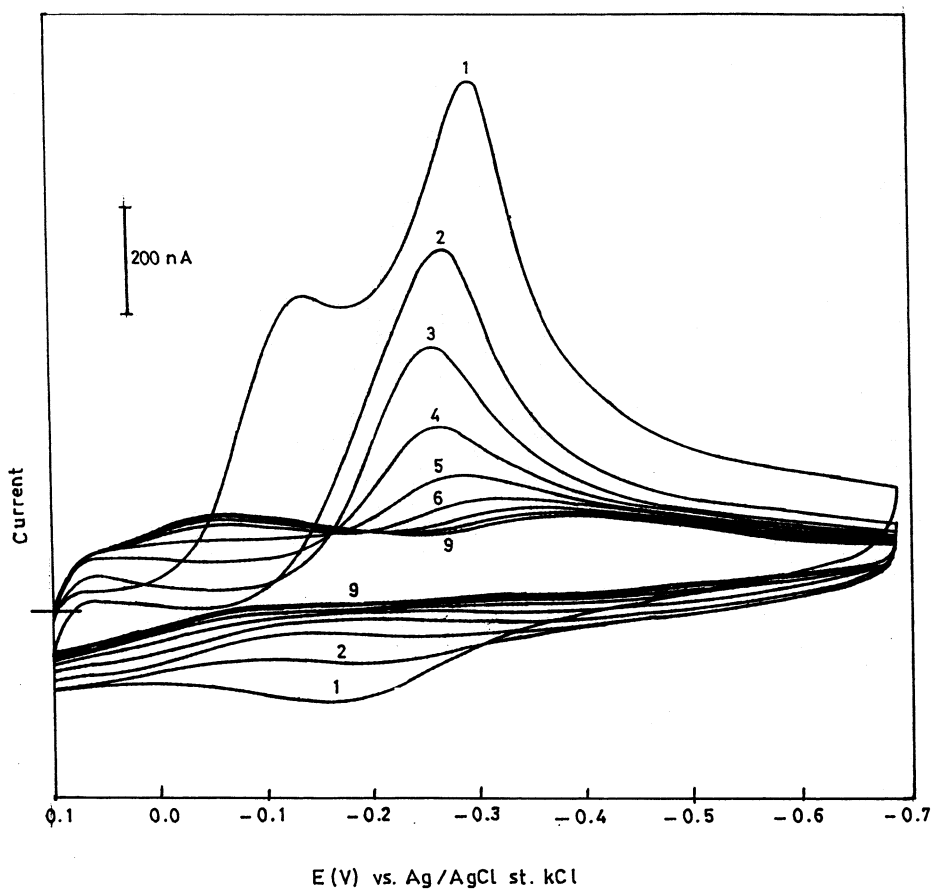


Fig. 2. Repetitive cyclic voltammograms for 1×10^{-6} M flaxedil in the presence of (H_2A^- and $HA^=$, 5 mM each pH = 7.0) accumulation potential +0.1 V scan rate 100 mV s^{-1} and deposition time 90 s (1) first cycle; (2) second cycle; (3) third cycle; (4) fourth cycle; and (5) fifth cycle.

low as 3×10^{-9} M (2.7 ppb) using a preconcentration time 300 s). The general definition given in the literature ($y_B + 3\sigma_B$) was used for the estimation of the limit of detection (L.O.D) [16–18]. The calculated L.O.D of flaxedil in aqueous solutions is 5×10^{-10} M, which is equal to 0.45 μg l^{-1} of flaxedil. According to our result, the detection limit obtained is less than that obtained by other method [1–5]. Also LOQ (limit of qauntitation) was estimated using the following equation $LOQ = 10\sigma/S$ where σ was the standard deviation and S was the slope [19], the results are give in Table 1.

Voltammograms were recorded for 3×10^{-7} M flaxedil at different accumulation times. It is found that the peak potentials shift towards more positive values as the accumulation time increases. On the other hand, the same phenomenon is observed when the concentration of flaxedil increased at constant preconcentration time. This behaviour is a striking aspect of the electrode process based on mercury salt formation [20,21]. To confirm that the adsorption peak of the Hg-drug is related to the adsorption and reduction of the mercury iodide compound, the voltammetric behaviour of the potassium iodide was studied

using 1.1×10^{-6} M KI under the same conditions as above; the voltammograms are identical. The reproducibility of the adsorption process was tested by repeating five experiments on 1×10^{-7} M flaxedil at a deposition time of 30 s in the presence of (H_2A^-, and $HA^=$ (5 mM each), pH $\simeq$ 7.0). The relative standard deviation is 2.7% and the coefficient of variation is 0.9984

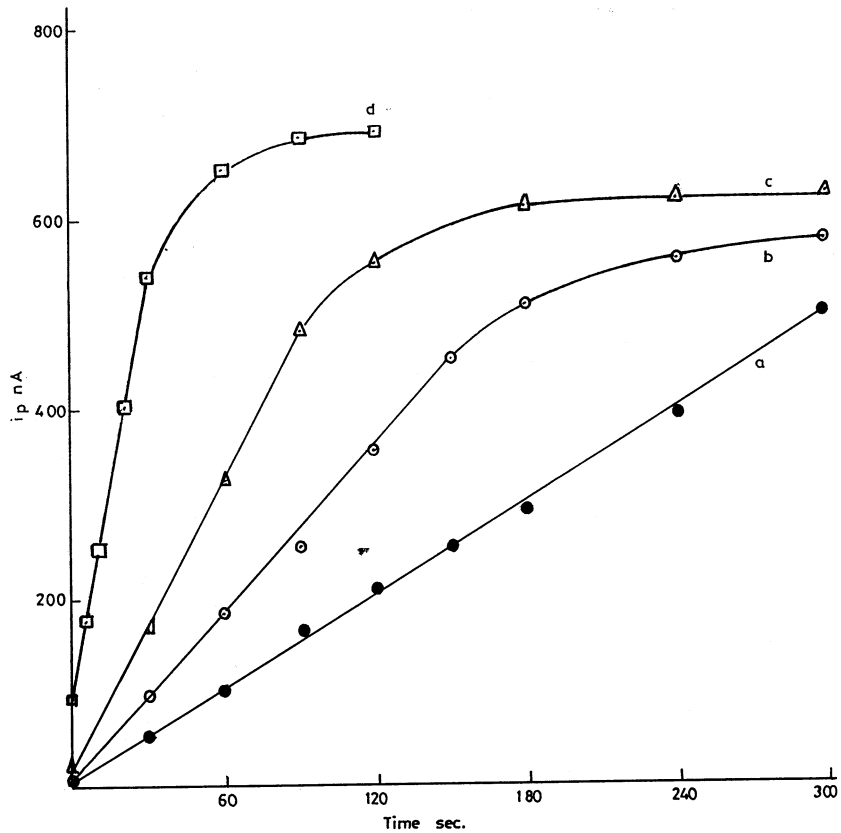


Fig. 3. Peak current versus accumulation time in the presence of (H_2A^- and $HA^=$, 5 mM each pH = 7.0) and scan rate 100 mV s^{-1} for (a) 1; (b) 3; (c) 5; and (d) 10×10^{-7} M flaxedil.

Table 1
Statistical parameters for different flaxedil concentrations

Concentration	Slope	RSD for slope	St. dev.	Corr. coef.	Confidence	LOQ
(1–4) 10^{-7} M	30.500 nA/10^{-7} M	0.125	1.2910	0.9998	1.2651	3.8 ppb
(4–10) 10^{-7} M	50.410 nA/10^{-7} M	0.031	2.1602	0.9993	1.6003	3.9 ppb
(1–10) 10^{-8} M	3.5815 nA/10^{-8} M	0.011	3.0277	0.9990	1.8765	7.6 ppb
(5–10) 10^{-9} M	2.9143 nA/10^{-9} M	0.027	1.8708	0.9954	1.4969	0.6 ppb

3.4. Interferences

Interferences of several metal ions, chloride ion and ascorbic acid were tested in a solution containing 1×10^{-6} M flaxedil, (H_2A^-, $HA^=$, 5 mM each, pH = 7.0) and the accumulation period 60 s. It was noticed that the addition of 1×10^{-6} M Cu(II), Zn(II), Pb(II), Cl^- and ascorbic acid individually or in admixtures, (each 1×10^{-6} M) caused no change in the current signal was observed in the determination of flaxedil. If you only used 1×10^{-6} M Cu(II), Zn(II), Pb(II), Cl^-, ascorbic acid, you cannot make this statement were these substances all are 5×10^{-6} M. This means that no interference occur with 5-fold molar excesses of the mentioned compounds.

3.5. Effect of surface active substance (SAS)

The adsorptive cathodic peak of 1×10^{-6} M flaxedil is increased to one third of its original value by the addition of 1×10^{-3}% cationic (TAB), whereas its height is decreased and splitted into two peaks in the presence of 1×10^{-3}% anionic (SDS). This indicates that the peak height of the drug depends on the type of the (SAS) (and its concentration). In the case of cationic (SAS) the adsorbed species of the Hg-drug compound probably carries a negative charge, and the anionic (SAS) repels the negatively charge compounds.

4. Application

4.1. Determination of flaxedil in an ampoule

In the experiment described previously, flaxedil in an ampoule was determined using the CLSSV technique by means of standard-addition method. The determination of flaxedil in its pharmaceutical preparation was performed with a mean recovery of (103 ± 1.2)% ($n = 5$) and relative standard deviation of 2.16%. Our described method for the determination of flaxedil in an ampoule provided results in good agreement with those obtained by USP methods [22].

5. Conclusion

The proposed method described in this paper, resulting from an almost detailed investigation of the electrochemical properties of gallamine triethiodide (flaxedil), was found to be rapid, sensitive, selective and accurate for the determination of the drug in pharmaceutical preparation (ampoule). The experimental limit of detection obtained for this method, especially in ampoule is sufficiently low that the proposed method can be used in drug monitoring.

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