Differential pulse voltammetric determination of diminazene in urine and serum samples using polyaniline modified GCE

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Abstract** - sensitive and reliable differential pulse voltammetric method was developed to determine diminazene drug. The developed method is based on the accumulation of the drug at a glassy carbon electrode and then a negative sweep was initiated, which yield a well-defined cathodic peak at –850.0 mV. To achieve high sensitivity, various experimental and instrumental variables were investigated such as pH, accumulation potential and potential time, scan rate, pulse amplitude and working electrode area. The monitored adsorptive current was directly proportional to the concentration of diminazene and it shows a linear response in the range from 10-100 µM of diminazene (correlation coefficient = 0.998) and the detection limit (S/N = 3) is 3.9×10^{-9} mol·l^{-1} at an accumulation time of 30 s. The developed adsorptive stripping voltammetry (AdSV) procedure shows a good reproducibility, the relative standard deviation RSD% (n = 8) at a concentration level of 1.25×10^{-6} mol·l^{-1} of diminazene was 0.13%, whereas the method accuracy was indicated via the mean recovery of 99.9% ± 1.414. The developed procedure of this approach was proved by the determination of the drug in biological fluids such as serum and urine.

Index Terms— Drug, differential pulse voltammetry, diminazene and biological fluids

I. INTRODUCTION

Diminazene (4,4-diamidinodiazoaminobenzene diaceturate tetrahydrate) (Fig. 1.) is an aromatic diamidine as used extensively as a veterinary trypanocide and babesiacide [1,2] in the affected areas of the World. Although not licensed for human use, it has been successfully employed in the treatment of early stage cases of human African sleeping sickness [3]. However, its efficacy has been curtailed by widespread drug resistance [4]. Despite it's used for over four decades, there are no pharmacopoieal specifications for the quality control of the product. The expiry of patent protection of the innovator product (Berenil) and the introduction of generic formulations necessitates the development of quality control techniques to safeguard the quality and subsequently the efficacy of the product. The assay of diminazene in biological fluids by liquid chromatography has been associated with poor peak shape (tailing peaks), the use of complex mobile phases and poor recoveries [5,6].

The presence of two highly basic amidino groups (pKa 11) in diminazene makes it very susceptible to residual interactions with silanol groups of standard silica-based reversed phase stationary phases. Adsorption of basic compounds such as amidines, to laboratory glassware and equipment is well known [7] and contributes to poor recoveries. Diminazene residues are known to persist in bovine plasma [8], therefore collecting and analyzing the plasma of animals for residues at the time of slaughter is an effective method to monitor for possible use of this drug. LC–UV methods are available to quantify diminazene in bovine plasma [9, 10] and milk with detection limits in the range of 10–25 ng/mL [11, 12]. Immunochemical methods applied in pharmacological tests, e.g., enzyme linked immunosorbent assay (ELISA) [13], require no expensive recording instruments, are highly sensitive and specific (by contrast to microbiological methods), and avoid lengthy sample preparation (by contrast to, e.g., high-performance liquid chromatography (HPLC) [14-18], gas chromatography [19], or capillary zone electrophoresis [20]). Recently, there has been interest in colloidal gold (CG) as a vector for direct delivery of drugs to the inflammation foci and affected organs of a macroorganism [21-24]. Pow and Crook [25] suggested attaching haptens to a carrier protein before conjugation with CG. The antibodies thus obtained were reported to have greater specificities for the haptens under study and higher (or, as the authors of [24, 25] put it, "extremely high") titers in comparison with the antibodies produced routinely (conjugation with protein).

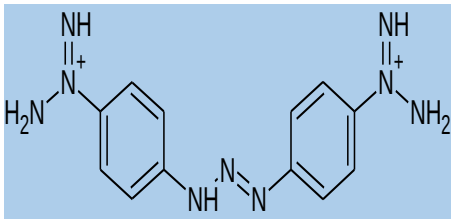


Fig. 1. Molecular structure of diminazene

. In the present work focused on an electrochemical analysis of diminazene in human urine and serum samples with polyaniline modified glassy carbon electrode. It was chosen to get the reduction mechanism of azomethine group by employing electrochemical techniques such as cyclic voltammetry, differential pulse voltammetry. Therefore, a rapid and sensitive voltammetric method has been applied for the determination of diminazene in human urine samples.

II. EXPERIMENTAL

Apparatus and Reagents

Diminazene was purchased from Triveni Aromatics and Perfumery Private Limited and used without further purification and dissolved in methanol. The stock solution of diminazene was prepared by dissolving in methanol. All the chemicals and reagents used in this study were of AR grade. Phosphate buffer solutions prepared using double distilled water used throughout the experiment.

Preparation of samples

An accurately weighed amount (5.0 mg) of diminazene was quantitatively transferred into a 25mL calibrated flask, dissolved in 20 mL methanol, completed to volume with the same solvent to obtain a stock solution of 1.0×10^{-3} mol·l^{-1}. This stock solution was further diluted till obtain a working standard solution containing 2.5×10^{-7} mol·l^{-1}.

Electrode preparation

Thoroughly polished GCE surfaces using alumina slurry on a soft cloth were sonicated in first ethanol and then doubly distilled water for 3 min each to remove possible contaminants. The PAN coatings were formed on the GCE surfaces by dipping the polished GCEs and electrochemically deposited at a constant potential of 0.80 V for 120 s in an aqueous solution of 0.1 M $LiClO_4$ and 0.1 mol·l^{-1} carbonate containing 0.15 mol·l^{-1} polyaniline as well as in a 0.25 mol·l^{-1} H_2SO_4 electrolyte containing 7.3 mM aniline monomers via a CV process from −0.2 to 0.9 V at a scan rate of 50 mV/s for cycles under a nitrogen environment. After the polymerization of PAN, the fabricated PAN/GCEs were dipped into doubly distilled water for 3 min to remove unpolymerized aniline monomers remaining in the PAN coatings if any. After each polishing, the electrode was sonicated in ethanol and doubly distilled water for 5 min, successively, in order to remove any adsorbed substances on the electrode surface. Finally, it was dried under nitrogen atmosphere ready for use. The electrode was then transferred into 0.1 M $HClO_4$ solution for 12 h aging. The polyaniline modified electrode was denoted as PAN/GCE.

Sample preparation

Human blood samples were collected in dry and evacuated tubes (which contained saline and sodium citrate solution) from same healthy volunteer. The samples were handled at room temperature and were centrifuged for 10 min at 1500 rpm for the separation of serum within 1 hour of collection. The samples were then transferred to polypropylene tubes and stored at 20 °C until analysis. The serum samples, 0.2 mL, were deproteinized with 2 mL of methanol, vortexed for 15 minutes centrifuged at 6000 rpm for 15 min, and supernatants were collected. The supernatants were spiked with an appropriate volume of drug.

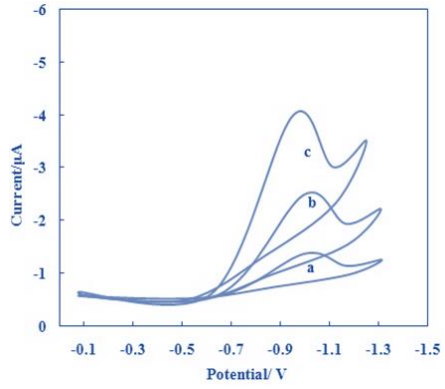
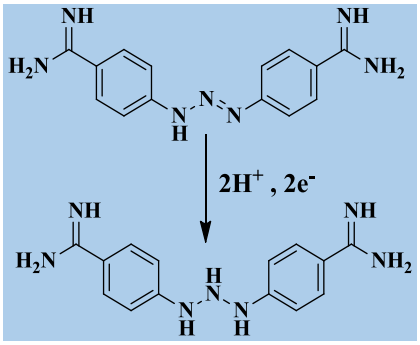


Fig. 2. Typical cyclic voltammograms of diminazene, concentration 2.5×10^{-7} M at (a) blank (b) bare GCE and (c) PAN/GCE.

III. RESULTS AND DISCUSSION

Cyclic voltammetric studies

In order to understand the electrochemical process occurring at the polyaniline modified glassy carbon electrode, cyclic voltammetry was carried out. Diminazene was reduced on PAN/GCE. The effect of pH on the cyclic voltammetry was investigated by recording the current v/s voltage curves for diminazene in phosphate buffer systems over the pH range 2.0 to 10.0. Diminazene exhibits a single well defined wave/peak at pH 4.0 throughout the study which corresponding to the reduction of azomethine group.



Scheme.1 Electrochemical reduction mechanism of diminazene

Typical cyclic voltammograms have been shown for diminazene in Fig. 2. Diminazene was readily adsorbed onto the PAN/GCE. Cyclic voltammogram of 2.5×10^{-7} mol·l^{-1} diminazene in phosphate buffer pH 4.0 on a modified glassy carbon electrode. A well-defined peak observed at PAN/GCE than at bare GCE. A large definite cathodic peak, corresponding the reduction of the adsorbed diminazene is observed at -0.96 V. No peaks are observed in the anodic scan, indicating that the diminazene reduction is an irreversible process.

Effect of scan rate

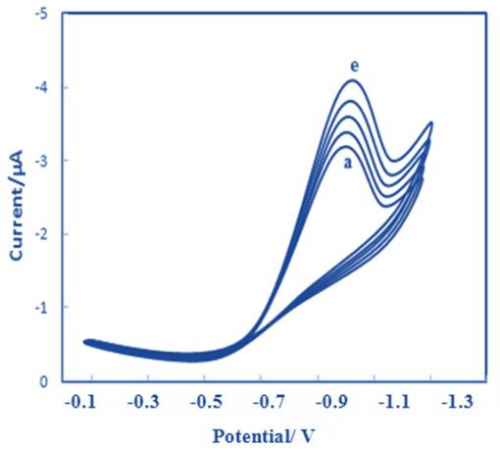


Fig. 3. Effect of scan rate on peak current of diminazene, conc: 2.5×10^{-7} M, scan rate (a) 20 mVs^{-1} to (e) 100 mVs^{-1} under optimum conditions.

The effect of varying scan rate on the reduction peak current of diminazene was examined. The reduction peak current increased linearly with the scan rate at the range from 20 mVs^{-1}-100 mVs^{-1} (Fig. 3.). Better sensitivity is observed at 50 mVs^{-1} and the same was applied for analytical calculations.

Effect of pH

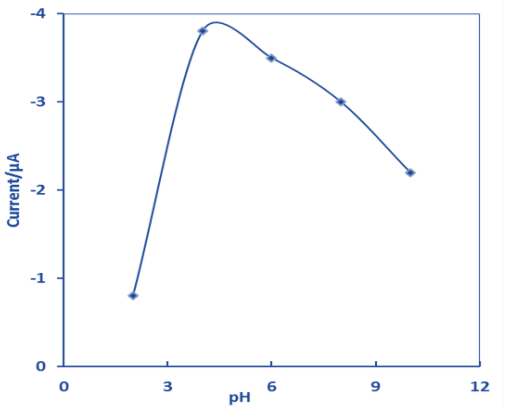


Fig. 4. Effect of pH of diminazene, concentration 2.5×10^{-7} M, on peak signal at phosphate buffer solution at different pH values.

The influence of pH on the reduction behaviour of diminazene was performed at different electrodes and different pH values using differential pulse voltammetry. The relation between pH verses current curves were shown in Fig. 4. It can be seen that the peak current reaches a maximum at pH 4.0. Therefore, pH = 4.0 was selected as the optimum pH, at this pH, the sensitivity was highest and the peak was well defined. According to the structure of diminazene which does not have any strong acidic or basic groups, pH changes do not cause a change on the structure. It shows that, pH change causes adsorption of diminazene on the surface of the modified electrode in the accumulation step.

Differential pulse voltammetric studies

The application of the differential pulse voltammetry for the determination of diminazene in human urine and serum samples were investigated. The peaks are sharper and better defined at lower concentration of DMZ, than those obtained by cyclic voltammetry, with low background current, resulting in improved resolution. According to the obtained results, it was possible to apply these techniques to the quantitative analysis of DMZ. The direct determination of diminazene in given samples were found to be possible by employing a high dilution of the sample with the supporting electrolyte. It is well known that DPV is suitable for the analysis of electrochemically active substances because relatively small difference in peak potentials of the analytes is needed for their determination of given compound. The peak current depends on pH of the medium, concentration and chemical composition of the buffer solution, and instrumental parameters. Differential pulse voltammograms of diminazene at bare GCE and at PAN/GCE, different concentrations of diminazene was also studied by DPV at PAN/GCE.

Fig. 5 displayed the differential pulse voltammetric response of diminazene at GCE/PAN. Well resolved peaks proportional to the concentration of corresponding diminazene was observed in the range of 10-100 µM. Limit of detection was found to be 3.9×10^{-9} $mol\cdot l^{-1}$. The peak current linearly increases with increase in concentration (Fig. 6). In a similar manner, DPV studies of diminazene at bare GCE and at modified systems were carried out.

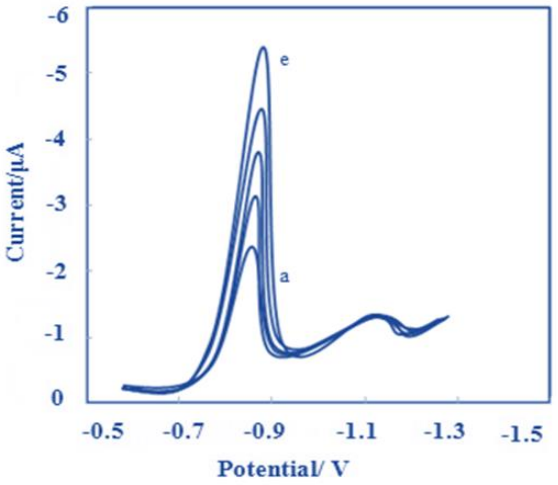


Fig. 5. Differential pulse voltammogram of diminazene at different concentrations at PAN/GCE from 20 µM to 100 µM (a to e).

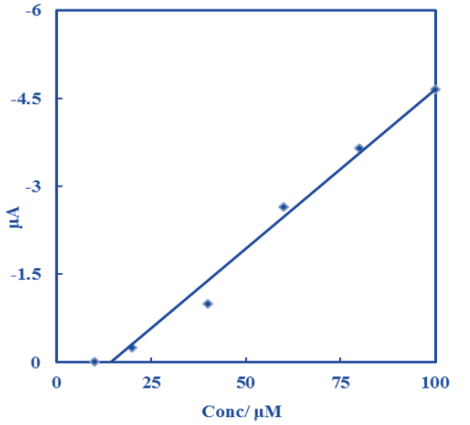


Fig. 6. Calibration plot of diminazene at PAN/GCE in PBS buffer solution with increasing concentrations of 10-100 µM.

An accurate volume (10 mL) of the phosphate buffer at the required pH (2.0–10.0) was transferred to the electrochemical cell and the electrodes were immersed in test solutions through which a stream of pure nitrogen was passed for 15 min before recording the voltammograms. The scans were initiated in the negative direction of the applied potential from 0.0 to -2.0 V. After recording the voltammogram of the blank solution, an accurate volume (0.5–2.0 mL) of the drug solution was added. The anodic potential sweep was then recorded under different operating conditions of pH, sweep rate, and pulse amplitude. Before each measurement the GCE was polished manually with a paste of 0.5 mm alumina in distilled water on a smooth polishing cloth and gently dried with a tissue paper. The effect of scan rate (ν=10–

200 mV s^{-1}) on the voltammograms were determined using the same solution.

Recovery study

Table. 1. Recoveries of diminazene in spiked human urine and serum samples

Sample	Amount added (µg)	Found (µg)	Recovery (%)	RSD (%)
Urine sample	5	4.95	99.00	1.720
	10	9.86	98.60	1.759
	15	14.82	98.80	1.640
Serum sample	5	4.97	99.40	1.821
	10	9.92	99.20	1.466
	15	14.79	98.60	1.628

*Average of three determinations

The recovery tests of diminazene ranging from 2.5×10^{-7} mol·l^{-1} to 1.0×10^{-3} mol·l^{-1} were performed using DPV. The recoveries of urine samples lie in the range from 98.60 to 99.00%. The relative standard deviation was 1.46%. The recovery test of diminazene in spiked urine and serum samples at concentration 2.5×10^{-7} mol·l^{-1} were performed by DPV, the results are shown in Table 1. The recoveries of serum sample are in the range from 98.60% to 99.40 % and the results are presented in Table 1.

CONCLUSIONS

The voltammetric behavior of diminazene was investigated at a PAN/GCE by cyclic voltammetry in phosphate buffer solution (pH = 4.0). Based on the study, influence of several physicochemical parameters like potential scan rate, pH and concentration were investigated. The reduction was found to be an irreversible two-electron and two-proton process with diffusion character. The PAN/GCE shows excellent electrocatalytic activity towards the reduction of diminazene at concentration 2.5×10^{-7} mol·l^{-1} under the optimum conditions. It exhibits irreversible cathodic peak at potentials over the pH 2.0–10.0 in phosphate buffer solutions. This method has been successfully used to determine diminazene in the urine and serum sample. The proposed method offered the advantages of accuracy and time saving as well as simplicity of reagents and apparatus.

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