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A novel surfactant silica gel modified carbon paste electrode in micellar media for selective determination of diminazene aceturate in presence of its stabilizer

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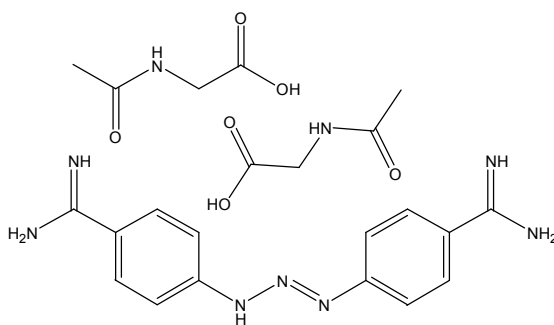
Abstract

A novel irreversible anodic voltammetric method was introduced for selective determination of diminazene aceturate in presence of phenazone as stabilizer. A sensor based on carbon paste electrode modified with silica gel in micellar media was introduced in universal buffer solution (pH 2.0). The effect of various experimental parameters was investigated. Under optimized conditions, the differential pulse voltammetry exhibited acceptable analytical performances in terms of linearity (2.0×10^{-6} to 3.0×10^{-4} mol L⁻¹) with correlation coefficients of; 0.9998 and 0.9996, detection limits; 5.27×10^{-9} and 1.2×10^{-8} mol L⁻¹ and reproducibility (RSD < 2.0%) for the solution containing diminazene aceturate and phenazone respectively. The method is selective, sensitive and could be applied for simultaneous determination of diminazene aceturate and phenazone in veterinary preparation, spiked cattle's milk, and urine with satisfactory results.

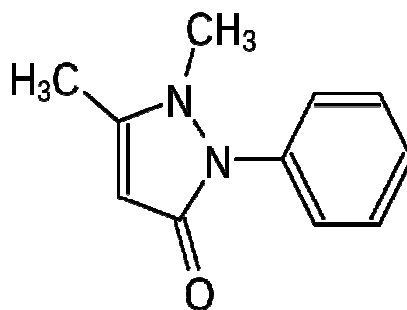
Keyword: Diminazene aceturate; Sensor; Silica gel; Sodium dodecyl sulphate; Carbon paste electrode; Phenazone.

INTRODUCTION

Diminazene aceturate; 4,4'-(diamino)dibenzamide diacetate is used extensively as a veterinary trypanocide and babesiacide^[1,2]. It is successfully employed in the treatment of early stage of human African sleeping sickness^[3]. It has the following molecular structure.



Phenazone; 1,2-Dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one is added to diminazene aceturate as a stabilizer in ratio of; 1.1 to 0.9 in most formulations^[4]. It has the following molecular structure.



The literature comprises few methods for analysis of diminazene aceturate in dosage forms, plasma and animal tissues includes; spectrophotometry^[5,6], ¹⁴C radioactive^[7], high performance liquid chromatography (UV and/or MS detector)^[1,3,8,9], GC-MS^[10] and Enzyme Linked Immuno Sorbent Assay (ELISA)^[11].

Electrochemical methods such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) has proved to be fast, simple, cheap, and sensitive for the determination of organic molecules that undergo oxidation or reduction reactions, including drugs and related molecules in pharmaceutical preparations and biological fluids^[12-15]. Chemically modified electrodes (CME) have recently attracted much attention due to their significant advantages such as increasing peak current and decreasing over potential for redox systems. Modification of electrodes with various modifiers such as carbon nanotubes^[16,17], pyrolytic graphite electrode^[18], fullerene-C₆₀ coated gold electrode^[19], fullerene-C₆₀ coated electrode^[20]. The poly (vinyl chloride) matrix membranes^[21-23] and ion-selective electrodes^[24,25] has also been reported in recent years as modified electrode.

Silica gel (CME) is an inexpensive material with high adsorption capacity, insolubility in most solvent and thermal stability. It can be grafted with a variety of function groups to enrichment the surface properties^[26-29]. Adsorption of surfactants (sodium dodecyl sulfate) on electrodes and solubilization of electrochemical active compounds in micellar aggregates might significantly change the redox potential, charge transfer and diffusion coefficients of electrode processes^[30-33]. A literature survey revealed that no attempt has

been made to study the CV and DPV behavior of diminazine acetate. Therefore, the aim of this study was directed to investigate and optimize the experimental conditions for simultaneous determination of diminazine acetate and phenazone in drug substances, veterinary preparation, spiked cattle's milk and urine.

EXPERIMENTAL

Materials and method

Diminazine acetate was kindly supplied from Pharma Sweden Co., Egypt with 99.89 % purity^[34]. Silica gel, phenazone, sodium dodecyl sulphate, chitosan and tetracyanoethylene were purchased from Aldrich (Germany). Britton-Robinson buffer was prepared by mixing different volumes of 0.04 mol L⁻¹ in H₃PO₄ (Adwic Co., Egypt), 0.04 mol L⁻¹ acetic acid (Loba-Chemic Co., India) and 0.04 mol L⁻¹ boric acid (Polski EODZNN Chemiczne S.A. Co., Poland) with the appropriate amount of 0.2 mol L⁻¹ NaOH (Adwia Co., Egypt) to obtain the desired pH (2.0 – 9.0). Buffer solutions were kept in a refrigerator^[35]. All solutions were prepared from analytical grade chemicals. Milli-Q deionized water was used.

Preparation of electrodes

Carbon paste electrode

Carbon paste electrode was prepared by mixing graphite powder (0.5 g) with nujol oil (0.3 mL) in a glassy mortar. The carbon paste was packed into the hole of the electrode body and smoothed on a filter paper until its shiny appearance.

Modified silica gel/ carbon paste electrode

Modified carbon paste electrode was prepared by mixing graphite powder with 5% of its weight silica gel. For better homogeneity the resulting composite was dispersed in ethanol and stirred on a magnetic stirrer till the solvent completely evaporated then nujol oil was added. The prepared silica gel modified carbon paste electrode composite was used in the same way as the unmodified electrode, and the addition of sodium dodecyl sulphate surfactant in the solution gives surfactant silica gel modified carbon paste electrode in micellar media.

INSTRUMENTAL AND EXPERIMENTAL SET UP

All voltammetric measurements were performed using computer-driven, AEW2 Analytical Electrochemical Workstation with ECprog3 electrochemistry software, manufactured by SYCOPEL SCIENTIFIC LIMITED (Tyne & Wear, UK). One compartment cell and the three electrodes were connected to the electrochemical workstation through a C3-stand from BAS (USA). A platinum wire counter electrode (MW -1032, BAS model) was employed as the auxiliary electrode. The electrode potentials were measured with respect to the reference electrode Ag/AgCl/ 3 mol L⁻³ NaCl (MW-2063, BAS model). Solutions were degassed using pure nitrogen prior and throughout the electrochemical measurements. Sigma plot 11 software was used for the transformation of the initial signal. A JENWAY 3510 pH meter (England) with glass combination electrode was used for pH measurements. The electrochemical experiments were performed at an ambient temperature of 25 °C.

Recommended differential pulse voltammetric procedure

Aliquots equivalent to, 20 µL – 3000 µL, Diminazene and phenazone solutions were transferred separately into a series of 10-mL volumetric flasks using micro pipette. Six µL of 10⁻² mol L⁻¹ sodium dodecyl sulphate solution were added and the volume was completed to the mark with B-R buffer pH 2. Quantitatively 5 mL was transferred to the electrolytic cell, and differential pulse voltammograms were recorded. The peak current at working silica modified electrode was measured at scan rate of 10 m Vs⁻¹ using DPV method. A blank of the same buffer containing sodium dodecyl sulphate was used.

Application of the proposed differential pulse voltammetric method

Veterinary preparation

Commercial veterinary samples containing diminazene and phenazone were analyzed to evaluate the validity of the proposed method. It was labeled to contain 445 mg diminazene acetate and 555 mg phenazone. The content of vial was weighed, finely pulverized and diluted to obtain the concentration of diminazene and phenazone in the working range and then DPV were recorded using surfactant silica gel modified carbon paste electrode.

Spiked urine and milk

Urine and milk samples were obtained from healthy cattle without any pretreatment, diluted 400 times with Britton-Robinson buffer (pH 2.0). This dilution can reduce the matrix effect of real samples. Aliquots equivalent to, 40 – 1500 μL and 35 – 1500 μL of diminazene and phenazone respectively were spiked in urine, and equivalent to 45 – 1500 μL of each drug were spiked in milk. Then the calibration graphs were constructed by plotting the peak current against drug concentrations.

RESULTS AND DISCUSSION

Electrochemical behavior of the investigated drugs

Preliminary investigation using CV shows an irreversible oxidation of, $1.0 \times 10^{-3} \text{ mol L}^{-1}$ of diminazene in Britton-Robinson buffer pH 2 at scan rate 100 mVs^{-1} and no cathodic peak in the reverse scan. The CV was carried out in positive direction with applied potential range +0.8 to +1.25 V at room temperature at bare carbon paste electrode, silica gel modified carbon paste electrode and surfactant silica gel modified carbon paste electrode (Figure 1). It is clear that diminazene gives an anodic peak current at +1.084 V in B-R buffer pH 2 at bare CPE. The oxidation peak currents are in the order, $48.2 > 43.2 > 23.59 \mu\text{A}$ for bare carbon paste electrode > silica gel modified carbon paste electrode > silica gel modified carbon paste electrode in micellar media, respectively. The potential is shifted negatively to less positive potentials, due to the enhancement of the electron transfer process and a larger intrinsic surface area of the modified electrode. The anodic process has been attributed in literature to the oxidation of primary amine group.

Optimization of cyclic voltammetric conditions

Effect of pH

Effect of pH on cyclic voltammograms of the oxidation peak currents of diminazene in Britton–Robinson buffer with different pH values (2 \rightarrow 9) and scan rate 100 mV s^{-1} was investigated. The peak current values are obtained by subtracting the background current density obtained in the pure supporting electrolyte solution from the anodic peak current density obtained from oxidation of diminazene. A comparison between the anodic peak

potential at different pH values of, bare carbon paste electrode, silica gel modified carbon paste electrode and silica gel modified carbon paste electrode in micellar media shows that the pH of the solution has a significant influence on the peak potential of the oxidation of diminazene aceturate, i.e. the anodic peak potentials shifted negatively with the increase of the solution pH, indicating that the oxidation at the modified electrode is a pH-dependent. The relationship between the anodic peak potential and the solution pH (2 – 9) could be fit to the linear regression equation of $E_{pa} \text{ (V)} = 1.1973 - 0.0562 \text{ pH}$, with a correlation coefficient of $r = 0.998$. The slope was found to be -56.2 mV/pH units over the pH range from 2 to 9, which is close to the theoretical value of -59 mV . This indicates that the number of protons and electrons transferred involved in the oxidation mechanism is equal^[36,37]. The highest oxidation peak current^[36] is obtained at pH 2 (Figure 2). Also the anodic peak current at different pH values of the three electrodes (the inset) shows that upon using silica gel and sodium dodecyl sulphate, the oxidation of diminazene displays higher anodic current than that of bare carbon past electrode. The pKa values of diminazene are 18.96 and 12.07^[38] proves that diminazene carries positive charge at pH values lower than these pKa, and the attraction forces between these positive charges and negative charges (sodium dodecyl sulphate and silica) responsible for catalytic oxidation processes.

Effect of different modifiers

The cyclic voltammogram of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ diminazene in the electrolytic cell, in Britton-Robinson buffer (pH 2) was recorded at a scan rate of; 100 mVs^{-1} . Comparative studies of different modifiers; teter acyano ethylene, chitosan and silica gel were investigated. The study shows that the anodic peak current is in order, silica gel/ sodium dodecyl sulphate > silica gel > chitosan > teteracyanoethylene (Figure 3).

Effect of silica concentration

Cyclic voltammogram of $1 \times 10^{-3} \text{ mol L}^{-1}$ diminazene, in Britton-Robinson buffer (pH 2) is recorded at scan rate 100 mVs^{-1} using, different amounts of silica gel. The anodic peak current is increased by the amount of silica gel added, in the range of 5.0 – 10.0 % and above these concentrations the anodic peak current is decreased (Figure 4).

Effect of sodium dodecyl sulphate concentration on silica gel modified carbon paste electrode

Different amounts of sodium dodecyl sulphate (2 – 6 μL) are added and the voltammetric response of $1 \times 10^{-3} \text{ mol L}^{-1}$ diminazene is examined in Britton-Robinson buffer (pH 2) at scan rate 100 m Vs^{-1} using silica gel modified carbon paste electrode. Upon addition of sodium dodecyl sulphate, the anodic peak current is increased and gave its highest response when sodium dodecyl sulphate concentration reached to $1.2 \times 10^{-5} \text{ mol L}^{-1}$ (Figure 5). These could be explained by the electrostatic interaction between the positively charged drug and the negatively adsorbed surfactant (sodium dodecyl sulphate); which will promote the pre-concentration of the drug on the electrode surface. As a result, the surface concentration of the drug was increased and the oxidation process was improved.

Effect of accumulation time

The effect of accumulation time ranging from 0 to 90 seconds on the anodic peak current of $1 \times 10^{-3} \text{ mol L}^{-1}$ diminazene in Britton-Robinson buffer (pH 2) using surfactant silica gel modified carbon paste electrode under open circuit condition was investigated. The peak currents were increased with time up to 30 seconds, and become stable to 60 seconds. After that the anodic peak current decreases as shown in Figure 6.

Effect of scan rate

The effect of different scan rates ($10 - 250 \text{ mV s}^{-1}$) on the current response of diminazene ($1 \times 10^{-3} \text{ mol L}^{-1}$) in Britton-Robinson buffer (pH 2) was studied. The interfacial reaction at each electrode was identified by recording the cyclic voltammograms of $1 \times 10^{-3} \text{ mol L}^{-1}$ DA at bare carbon paste electrode, silica gel modified carbon paste electrode and surfactant silica gel modified carbon paste electrode (Figure 7). A plot of scan rate against potential gave a straight line which was realized up to 100 mVs^{-1} followed by a deviation with the increase of scan rate. A plot of i_{pa} versus $v^{1/2}$ gave a straight line up to a scan rate of 100 mVs^{-1} . The relation between anodic peak current, i_{pa} (μA), diffusion coefficient of the electro active species $D_0(\text{cm}^2\text{s}^{-1})$, and scan rate $v(\text{Vs}^{-1})$, is given by Randles- Sevcik equation^[39].

$$i_{pa} = (2.69 \times 10^5) n^{3/2} A C_o * D_o^{1/2} v^{1/2}, \text{ where the constant has units } (2.69 \times 10^5 \text{ C mol}^{-1} \text{ v}^{-1/2})$$

In this equation: i_{pa} is the peak current density ($\mu\text{A cm}^{-2}$), n is the number of electrons appearing in half-reaction for the redox couple, v is the scan rate at which the potential is swept (V s^{-1}), C_o is the analyte concentration ($1 \times 10^{-6} \text{ mol cm}^{-3}$), A is the electrode area (0.0706 cm^2), and D is the electroactive species diffusion coefficient ($\text{cm}^2 \text{ s}^{-1}$). Apparent surface area used in the calculations, do not take into account the surface roughness.

The apparent diffusion coefficients, D_{app} , of diminazene using bare carbon paste electrode, silica gel modified carbon paste electrode and silica gel modified carbon paste electrode in micellar media in Britton-Robinson buffer (pH 2) were calculated from cyclic voltammetry(CV) experiments and was found to be 1.95×10^{-6} , 4.65×10^{-6} and $8.13 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ respectively. The results indicate that the silica in presence of SDS improves the electron transfer kinetics at the electrode/solution interface^[40,41].

Direct relationship between log current, and log scan rate in range from 10 - 100 mVs^{-1} , was obtained and gave the following equations:

$$\log I_p = 0.363 + 0.498 \log v \quad r = 0.9998 \text{ for bare carbon paste electrode,}$$

$$\log I_p = 0.692 + 0.409 \log v \quad r = 0.9994 \text{ for silica gel modified carbon paste electrode}$$

$$\log I_p = 0.725 + 0.463 \log v \quad r = 0.9996 \text{ for surfactant silica gel modified carbon paste electrode}$$

The value of the slope of the obtained linear relations is less than 0.5, which implies that the electro active species are transported by a diffusion controlled process^[42].

Method validation

Linearity

Under the above optimum conditions, the linearity was carried out with good correlation between the peak current (I_p) and concentration, in ranges of $2.0 \times 10^{-6} - 3 \times 10^{-4} \text{ M}$ for both diminazene and phenazone at surfactant silica gel modified carbon paste electrode,

in drug substances(Figure 8), spiked urine and spiked milk. The corresponding regression equations were calculated and the results were presented in Table (1).

Limits of detection (LOD) and quantitation (LOQ)

The limits of detection(LOD) and quantitation(LOQ) were calculated by using the following equations: $LOD = 3.3 \sigma /S$, and $LOQ = 10 \sigma /S$ where " σ " is the mean of standard deviation of intercept and "S" is the mean of the slope of calibration curve. The LOD and LOQ were presented in Table (1).

Accuracy

The procedure under linearity was repeated three times for five different concentrations of both drugs and the concentrations were calculated from the corresponding regression equations. The mean percentage recoveries were evaluated and satisfactory results were obtained in Table (1).

Precision

The intra-day precision was assessed by analyzing five concentration levels in triplicate in a single assay run for the drug and calculated as RSD%. The inter-day precision was assessed by analyzing the same concentration, in triplicate, in three separate assay runs. RSDs% were less than 2% (Table 1). The level of precision o was adequate for the quality control analysis of the studied drugs.

Specificity and selectivity

The specificity of the proposed voltammetric method was proven by its ability to determine diminazene in veterinary preparation without interference from commonly present excipient; phenazone.

Robustness

The robustness of the proposed method was demonstrated by constancy of the peak current with deliberated minor changes in the experimental parameter. The studied variables included; the change in pH (2.0 ± 0.5), the time considered before each

measurement ($30s \pm 5s$). These minor changes did not affect the peak current intensity of the studied drugs, indicating the reliability of the proposed method during normal usage.

Statistical analysis of the results obtained by the proposed DPV method and official or comparison methods for the determination diminazene aceturate and phenazone in their drug substances are shown in Table (2).

Stability of the modified electrode

In order to investigate the stability of surfactant silica gel modified carbon paste electrode the CV for $1.0 \times 10^{-3} \text{ mol L}^{-1}$ diminazene in Britton-Robinson buffer (pH 2.0) solution were recorded every five minutes for a total of fifty runs. It was found that the anodic peak currents remain the same. Moreover, repetitive measurements indicate that this electrode has a good reproducibility and does not suffer from surface fouling during the voltammetric measurements.

Analytical applications

Simultaneous determination of diminazene and phenazone

Aliquots equivalent to; $1.0 \times 10^{-3} \text{ mol L}^{-1}$ and $1.0 \times 10^{-3} \text{ mol L}^{-1}$ of diminazene and phenazone, respectively were individually and simultaneously investigated in Britton-Robinson buffer (pH 2.0) with differential pulse voltammetry, at both bare carbon paste electrode and surfactant silica gel modified carbon paste electrode. The anodic peak potentials were found to be, 1.029 V and 1.162 V for diminzene and phenazone at bare caebon paste electrode. While at surfactant silica gel modified carbon paste electrode the anodic peak potentials of diminazene is shifted to less negative potential at i.e. 1.006 V but for phenazone it was remain the same indicating that the surface of surfactant silica gel modified carbon paste electrode improved the separation of two peaks. The results showed a relatively large separation between anodic peak potentials of diminazene and phenazone. The mediated oxidation peak current of diminazene depends on its concentration.

Veterinary preparation

The proposed voltammetric method was applied for simultaneous determination of diminazene and phenazone in veterinary preparation. Under the optimum experimental conditions mentioned above, the relationship between the anodic peak current and the concentration of diminazene and phenazone were investigated in Britton-Robinson buffer pH 2.0 at scan rate 10 mV/s, using silica gel modified carbon paste electrode in micellar media. Table (3), represents the recovery data obtained by the standard addition technique for diminazene and phenazone. We can also see that surfactant silica gel modified carbon paste electrode has higher reproducibility and that there is no important matrix interferences for the samples analyzed by the DPV mode and it could be a useful electrode for quantitative analysis of diminazene and phenazone in veterinary preparation.

Real samples of cattle urine and milk

Validation of the procedure for the quantitative assay of the diminazene in cattle urine and milk are examined in Britton-Robinson buffer pH 2, at scan rate 10 mV/s using silica gel modified carbon paste electrode in micellar media (Table 4). The calibration curve in urine give a straight line in the linear dynamic range, $8 \times 10^{-6} \text{ mol L}^{-1}$ to $3 \times 10^{-4} \text{ mol L}^{-1}$ with correlation coefficient, $r = 0.9997$, and LOD is $6.36 \times 10^{-7} \text{ mol L}^{-1}$. While the calibration curve in milk give a straight line in the linear dynamic range, $6 \times 10^{-5} \text{ mol L}^{-1}$ to $30 \times 10^{-4} \text{ mol L}^{-1}$ with correlation coefficient, $r = 0.9997$, and LOD is $7.13 \times 10^{-7} \text{ mol L}^{-1}$. It is clear from the results that a wide concentration range of diminazene could be determined by the investigated DPV method and give stable results and good recoveries as shown in Table (4).

CONCLUSION

In the present work, a biosensor based on carbon past electrode modified with silica gel and sodium dodecyl sulphate is used for electrochemical determination of diminazene aceturate. The advantages of silica gel is the enhancement of sensitivity at carbon past electrode significantly. The present study employed accurate, simple and sensitive, cyclic and differential pulse voltammetric method for determination of diminazene aceturate in drug substance, drug product and clinical preparations (cattle urine and milk), using the most popular and simpler working electrode, bare carbon paste electrode and silica gel

modified carbon paste electrode in micellar media . Different parameters are tested to optimize the determination conditions. The analytical procedure has been fully validated regarding linearity, precision, accuracy, reproducibility, sensitivity and selectivity. The developed method are compared with the reported one and it has been found that it is a satisfactory alternative for the fast, clean quantitative determination of diminazene in different matrices. A complete resolution between differential pulse voltammetry peak potentials of diminazene and phenazone (with more than 130 mV peak potentials) provides a very suitable and effective method for simultaneous determination of these compounds.

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TABLE 1. Regression and some validation data of the calibration curves for the quantitative determination of diminazene aceturate and phenazone in pure, spiked urine and milk by DPV technique

Parameters	surfactant silica gel modified carbon paste electrode					
	Diminazene	Diminazene in spiked urine	Diminazene in spiked milk	Phenazone	Phenazone in spiked urine	Phenazone in spiked milk
Linearity (mol L ⁻¹)	2 x 10 ⁻⁶ – 3 x 10 ⁻⁴	8 x 10 ⁻⁶ – 3x 10 ⁻⁴	9 x 10 ⁻⁶ – 3 x 10 ⁻⁴	2 x 10 ⁻⁶ – 3 x 10 ⁻⁴	7 x 10 ⁻⁶ – 3 x 10 ⁻⁴	9 x 10 ⁻⁶ – 3 x 10 ⁻⁴
LOD (mol L ⁻¹)	5.72 x 10 ⁻⁹	6.36 x 10 ⁻⁷	7.13 x 10 ⁻⁷	1.2 x 10 ⁻⁸	2.1 x 10 ⁻⁷	3.4 x 10 ⁻⁷
LOQ (mol L ⁻¹)	1.91 x 10 ⁻⁸	2.12 x 10 ⁻⁶	2.38 x 10 ⁻⁶	4 x 10 ⁻⁸	7 x 10 ⁻⁷	1.13 x 10 ⁻⁶
Slope	0.508	0.476	0.525	0.673	0.675	0.603
SE of slope	0.0030	0.0051	0.0071	0.0057	0.0058	0.0067
Intercept	1.399	1.574	1.44	7.297	7.399	6.891
SE of Intercept	0.0056	0.0647	0.0894	0.0943	0.1125	0.1245
Correlation coefficient (r)	0.9998	0.9997	0.9997	0.9996	0.9996	0.9994
Accuracy ^(a) Mean± RSD%	99.98± 1.557			99.86± 0.587		
Precision ^(b) (RSD %)						
Repeatability	0.865					0.679
Intermediate precision	0.929					0.885

^an=6.

^bn=9.

TABLE 2. Statistical analysis of the results obtained by the propose DPV and reported or official methods for the determination of Diminazene and phenazone in their drug substances

Parameters	Drug substances			
	Diminazene		Phenazone	
	SSGMCPE ^a	Reported Method ^b	SSGMCPE	official Method ^c
Mean%	100.21	101.23	99.86	99.97
SD	1.497	3.365	0.587	0.724
Variance	2.241	11.323	0.345	0.524
n	5	5	5	5
t-test (2.306) ^d	0.619		0.264	
F-test (6.400) ^d	5.053		1.519	

^a SSMCPE is surfactant silica gel modified carbon paste electrode

^b HPLC reported method using a symmetry RP 18 column & detected by UV absorbance at 250 nm^[43].

^c HPLC official method (BP 2013)^[44].

^d Values between parenthesis are the tabulated values of t and F at p=0.05.

TABLE 3. Application of the proposed DPV method for determination of Diminazene and phenazone in their veterinary preparation

Parameter	Diminazene			phenazone		
	Conc. taken 10^{-5} M	Conc. Found 10^{-5} M	Recovery ^a %	Conc taken 10^{-5} M	Conc. Found 10^{-5} M	Recovery ^a %
	Veterinary preparation (445 mg Diminazene and 555 mg Phenazone)	0.6	0.59	98.33	2.04	2.02
	5	4.97	99.40	17.0	17.1	100.59
	9	8.88	98.67	30.6	30	98.04
Mean recovery \pm RSD %	98.80 \pm 0.55			99.22 \pm 1.29		

^aMean of three determinations.

TABLE 4. Determination of diminazene in spiked urine and milk cattle samples by the proposed DPV method

Parameters	Conc. Added X 10 ⁻⁵ M	Conc. Found X 10 ⁻⁵ M	Recovery
Spiked urine	10	10.1	101.00
	16	15.9	99.38
	24	24.02	100.08
Mean% ±RSD			100.15±0.81
Spiked milk	10	9.8	98.00
	16	15.72	98.25
	24	23.6	98.33
Mean% ±RSD			98.19±0.17

Figures Captions:

Figure 1. Cyclic voltammograms of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ diminazene in B-R buffer pH 2 at scan rate 100 mVs^{-1} recorded at three electrode (1) bare carbon paste electrode (CPE), (2) silica gel modified carbon paste electrode (SGMCPE ----) and (3) surfactant silica gel modified carbon paste electrode (SSGMCPE —).

Figure 2. Cyclic voltammograms of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ of diminazene in B-R buffer at scan rate 100 mVs^{-1} recorded at different pH values using silica gel modified carbon paste electrode/micellar media.

The inset: the relation between pH and anodic peak current recorded at three different electrodes.

Figure 3. Effect of different modified electrodes on the oxidation peak of $1 \times 10^{-3} \text{ mol L}^{-1}$ diminazene in B-R buffer pH 2 at scan rate 100 mV s^{-1} .

Figure 4. Effect of silica concentration on the oxidation peak of $1 \times 10^{-3} \text{ mol L}^{-1}$ diminazene in B-R buffer pH 2 at scan rate 100 mV s^{-1} .

Figure 5. Effect of different volumes of sodium dodecyl sulphate solution on the voltammetric response of diminazene in B-R buffer, pH 2 at silica gel modified carbon paste electrode. The inset: the relation between sodium dodecyl sulphate concentration and the anodic peak current.

Figure 6. Accumulation time effect of diminazene in B-R buffer pH 2 using silica gel modified carbon paste electrode/micellar media at scan rate 100 mV s^{-1} . The inset: the relation between time (Sec) and the anodic peak current.

Figure 7. Effect of scan rate on anodic peak current response of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ of diminazene solution in B-R buffer pH 2.

Figure 8. Differential pulse voltammograms for different concentrations of diminazene and phenazone using silica gel modified carbon paste electrode/micellar media in B-R buffer pH 2 at scan rate 10 mV s^{-1} .

The inset: Calibration curve of diminazene and phenazone using silica gel modified carbon paste electrode/micellar media in B-R buffer pH 2 at scan rate 10 mV s^{-1} .

Figure 1

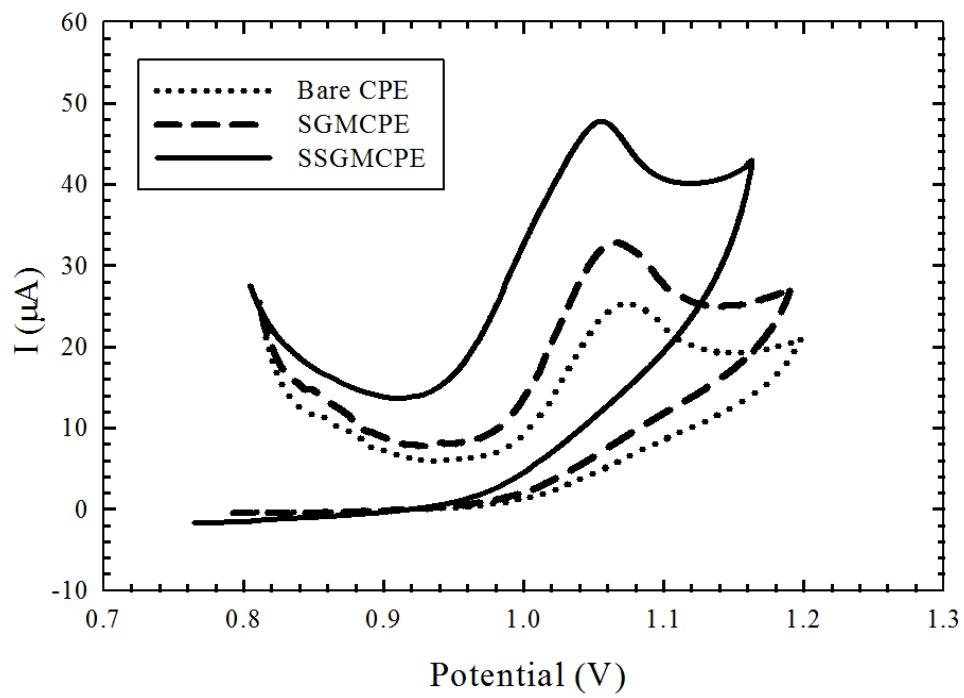


Figure 2

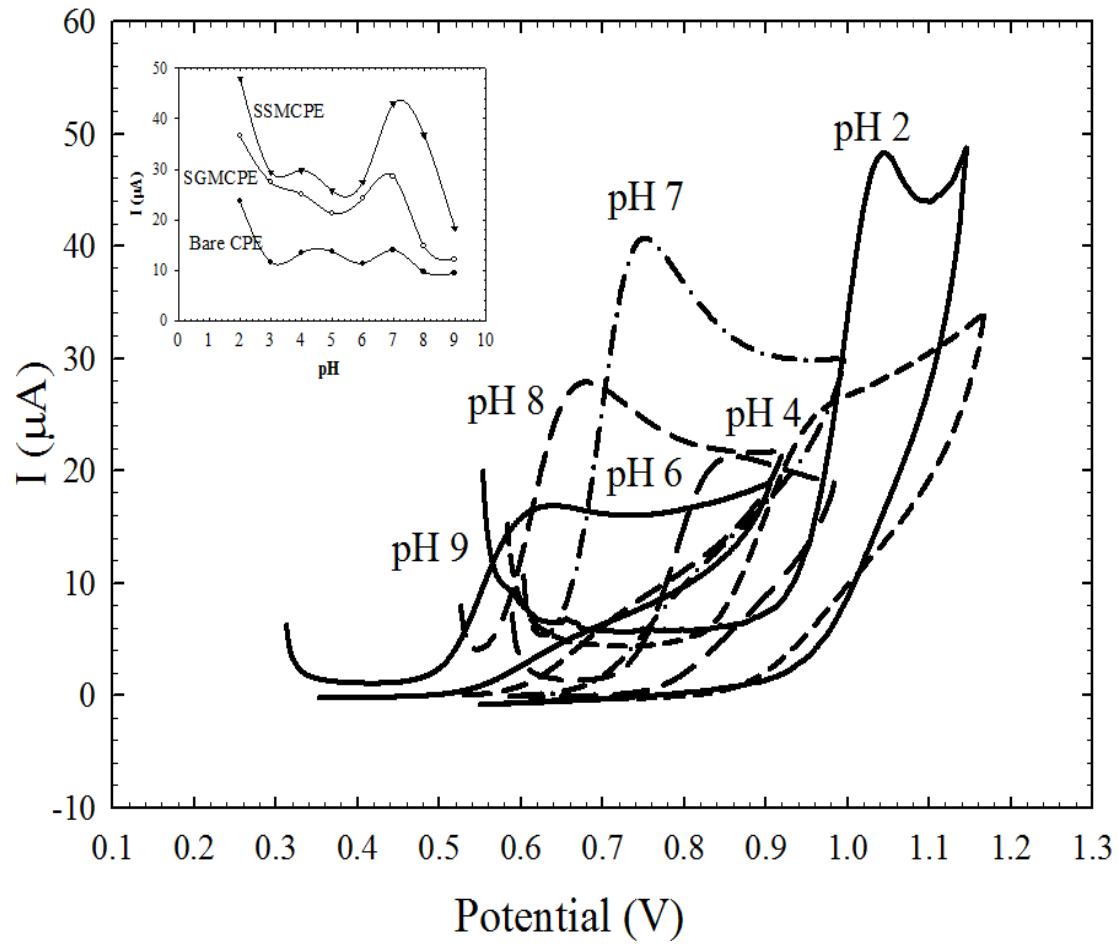


Figure 3

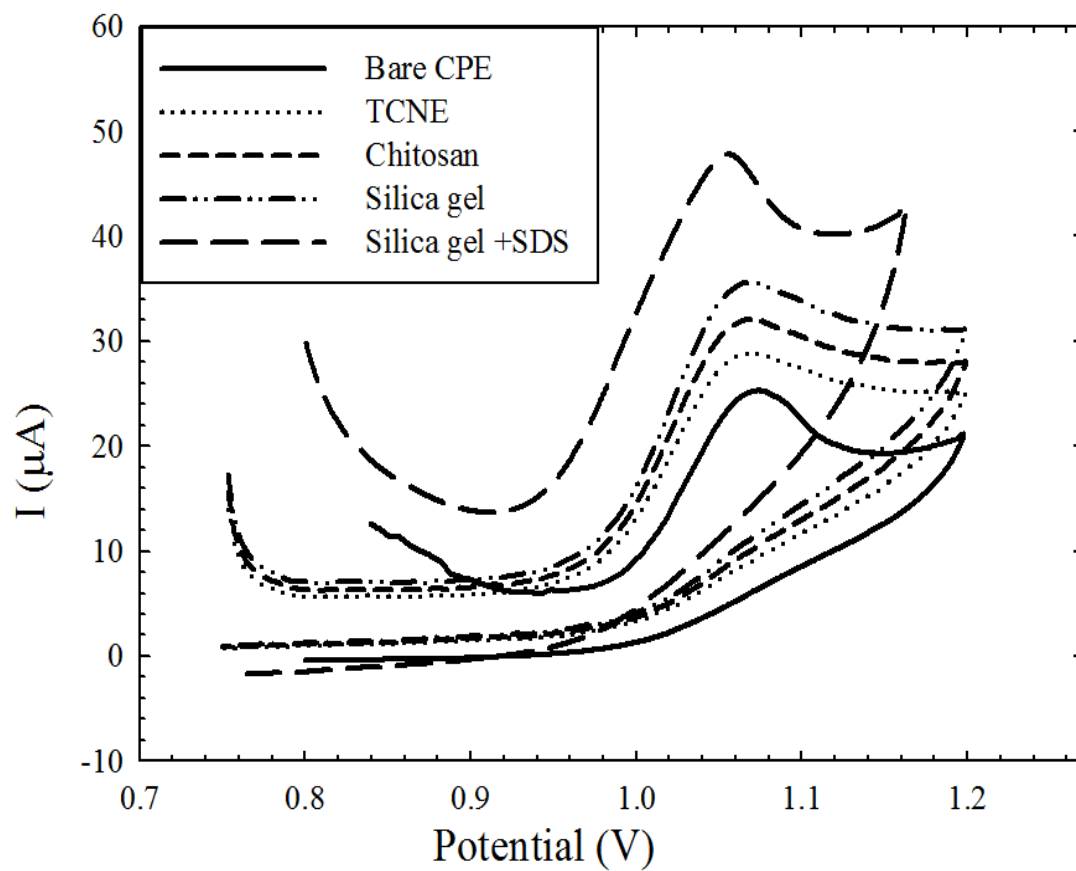


Figure 4

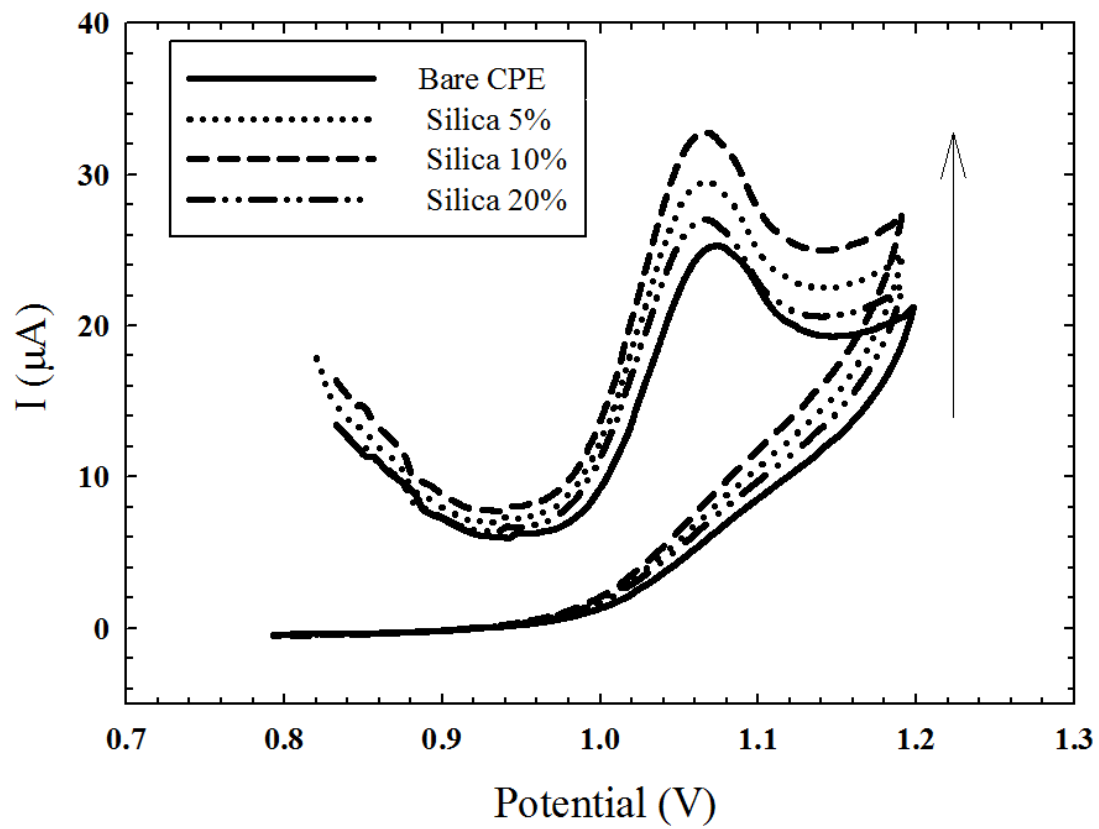


Figure 5

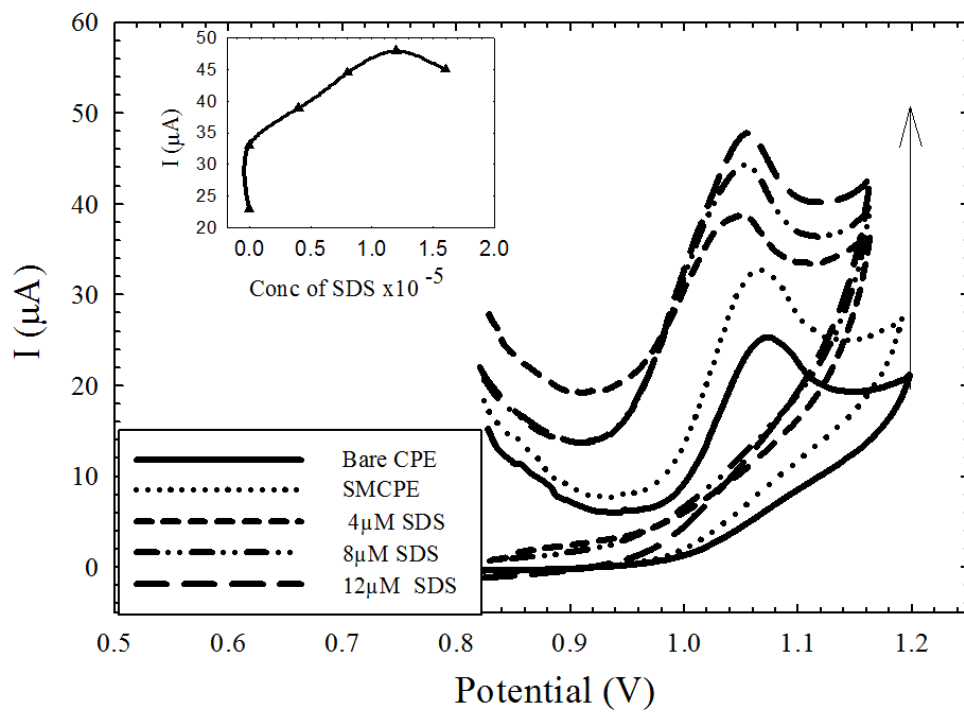


Figure 6

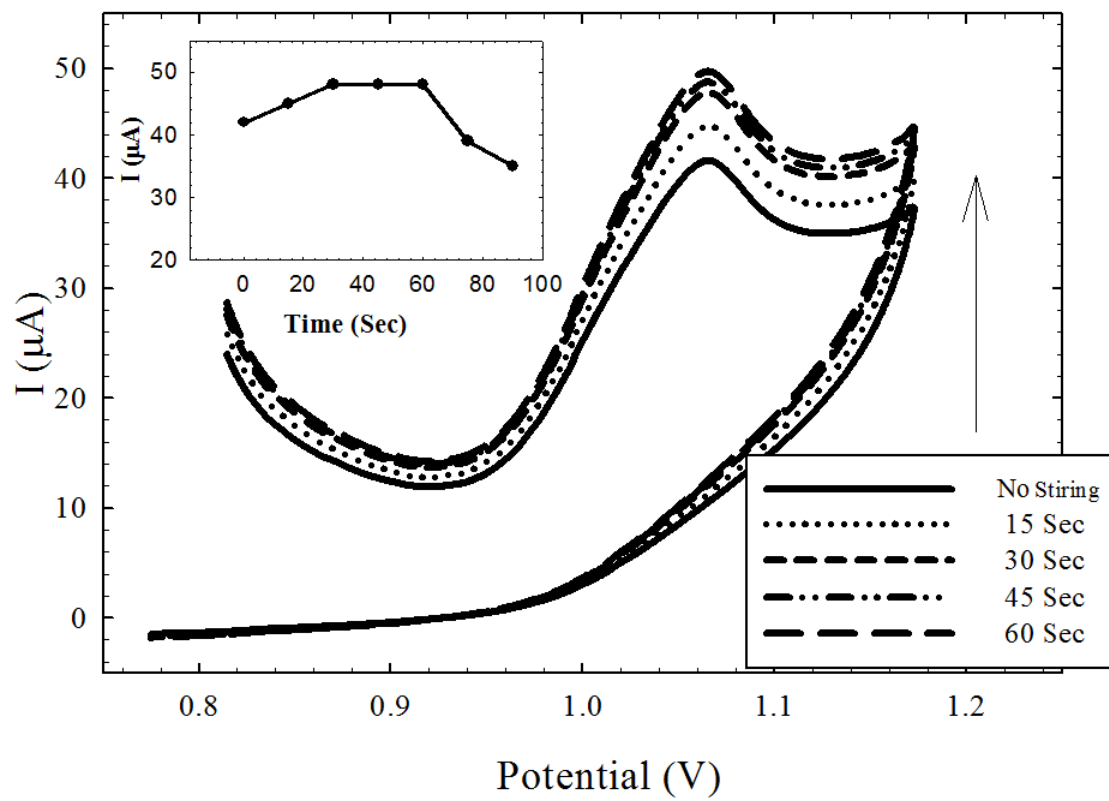


Figure 7

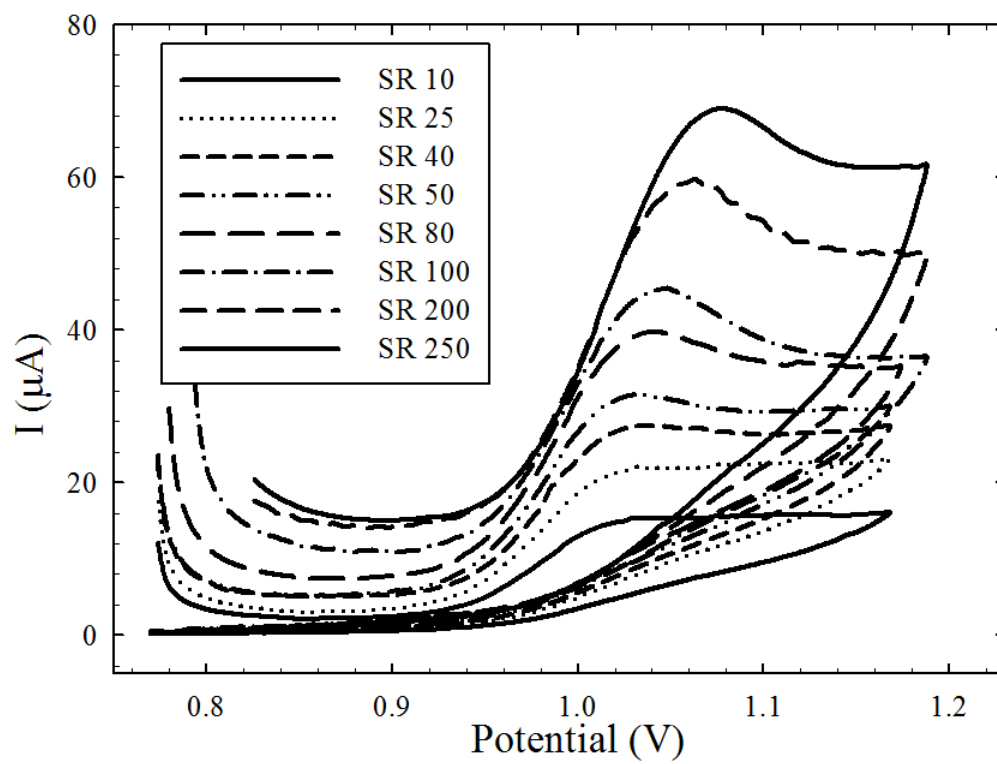


Figure 8

