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GLYCINE AND GOLD NANOPARTICLES FOR THE ELECTROCHEMICAL DETERMINATION OF AN ANTI-PARKINSON'S DRUG IN A TERTIARY MIXTURE

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ABSTRACT: A sensitive sensor for the electrochemical determination of anti-Parkinson's disease drug; entacapone (EN) was constructed using carbon paste electrode modified *via* electro-polymerization of glycine (GL) incorporate gold nanoparticles (GNP) and sodium dodecyl sulphate (SDS). Different voltammetric techniques, electrochemical impedance spectroscopy (EIS), and scanning electron microscopy (SEM) techniques were used to characterize the properties of the modified electrode. The linear response was obtained for EN in the ranges of 4.1×10^{-7} to 0.5×10^{-3} mol L⁻¹ with correlation coefficient 0.9990. The lower limit of detection was found to be 2.82×10^{-9} mol L⁻¹. The effectiveness of this sensor was established for the determination of EN in real samples. Also the simultaneous determination of EN with Levodopa LD and Carbidopa CD that are present in Stalevo tablets gives a good separated peak potential. Very low detection limit, simplicity of preparation and surface restoration, make the proposed modified electrode very useful for precise determination of EN either alone or in the tertiary mixture.

INTRODUCTION: Entacapone (EN) is an adjunct to standard preparations of levodopa / benserazide or levodopa / carbidopa to be used in patients with Parkinson's disease who have end-of-dose motor fluctuations which cannot be stabilized on those combinations¹. Entacapon drops the GLabolic loss of levodopa to 3-O-GLhyldopa. Therefore EN increases the bioavailability of levodopa to the brain by 5-10%².

Recently, electrochemical sensors and biosensors have found broad applications in many fields. Due to the high request for having a device to check the concentration of species in different samples, the construction of new and simple sensors and biosensors has occurred among the researchers to obtain low detection limits, a wide linear response range, and good stability and reproducibility. Transduction of a biological or chemical signal to an electrical signal can be performed by amperometry, voltammetry, potentiometry, or conductometry^{3, 4}. Nowadays, application of different nanoparticles and polymers in manufacture of sensors⁵ and biosensors as a modifier causes to improve their thermal, electrical, and mechanical properties.

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Several analytical procedures have been used for EN determination such as spectrophotometric methods^{6,7}, HPLC⁸⁻¹¹ and electrochemical method¹²⁻¹⁵. These procedures have some negatives such as sample handling, long analysis time, and high prices. So in this work a voltammetric sensor that is used for the analysis of several organic and inorganic analytes in different matrices will be performed, due to its non-destructive properties because only a very minor amount of the analyte is consumed at the surface of the working and auxiliary electrodes. Glycine (GL) is the smallest amino acid and neurotransmitter found in the central nervous system. It is amphoteric with a pKa of about 2.4 and 9.6¹⁶. Glycine is contributed in the electro-deposition of metals due to its biocompatibility, high buffering properties and its ability to stabilize the pH on the electrode surface during the electro-deposition process^{17, 18}, these properties makes glycine importance in sensor and biosensor applications.

Gold nanoparticles have been impressively used to modify electrodes in order to detect several neurotransmitters and drugs such as carbamazepine, paracetamol, dopamine and noradrenaline¹⁹⁻²¹. It was also used in the detection of heavy metals due to their noble conductivity, biocompatibility and high surface-to-volume ratio²². No work has been reported using glycine with gold nanoparticles; so the aim of this work is to develop a new and simple voltammetric method based on carbon paste electrode modified with glycine, gold nanoparticles and surfactant to constitute an accurate and validated method for the simultaneous determination of entacapone with levodopa and carbidopa in Stalevo tablets.

MATERIALS AND METHODS:

Apparatus and Chemicals: The voltammetric measurements were performed using computer-driven, AEW2 Analytical Electrochemical Workstation with ECprog3 electrochemistry software, manufactured by SYCOPEL SCIENTIFIC LIMITED (Tyne & Wear, UK). A conventional three electrode cell was used, A platinum wire, Ag/AgCl (3M NaCl) and GNP-GL/CPE were employed as the auxiliary, reference and working electrode, respectively. A Cyber scan 500 digital (EUTECH Instruments, USA) pH-meter with a glass combination electrode served to carry

out the pH measurement. Scanning electron microscopy (SEM) measurements were carried out using a JSM-6700F scanning electron microscope (Japan Electro Company). For impedance spectroscopy measurements; an electrochemical workstation IM6e Zahner - elektrik, GmbH, (Kronach, Germany) was used. All solutions were prepared with doubly distilled water. Entacapone was supplied from Novartis Pharmaceutical Co., Egypt, glycine and sodium dodecyl sulphate (SDS) were obtained from Sigma. Britton-Robinson buffer solution (B-R buffer) was prepared by mixing H₃PO₄, C₂H₄O₂ and H₃BO₃ with 0.2N NaOH to obtain the desired pH value.

Fabrication of Modified CPE: Modified CPE was prepared by electro-deposition of 4.0×10^{-2} mol L⁻¹ GL by cyclic sweeping from -0.5 to 1.8 V at 50 mV s⁻¹ for 10 cycles²³ to form glycine modified CPE (GL/CPE). Then the modified electrode was immersed into 6×10^{-3} mol L⁻¹ hydrogen-tetrachloroaurate HAuCl₄ solution containing 0.1M KNO₃, and a constant potential of -0.4 V versus Ag/AgCl was applied for 400 s²⁴⁻²⁶ to form gold nanoparticles - GL/CPE (GNP-GL/CPE). Then 10 μ L of 1×10^{-2} mol L⁻¹ SDS was added to the solution to improve the peak current.

Application to Human Urine: Calibration samples were prepared by diluting urine samples 400 times to reduce urine matrices²⁷ with B-R buffer and adequate amounts of 1.0×10^{-2} mol L⁻¹ EN standard solution were added to prepare 5mL aliquots of the sample, using the standard additions technique. Then the current against the concentration were plotted to achieve the calibration graph.

Validation in Pharmaceutical Samples: To evaluate the validity of the proposed method, five tablets of Stalevo (200mg / Tablet) were weighed and crushed to provide a uniform powder and the average mass per tablet was determined, and then dissolved in deionized water.

RESULTS AND DISCUSSION:

Structural Characterization: SEM micrographs with a characteristic three-dimensional appearance of A) CPE, B) GL/CPE and C) GNP-GL/CPE were made. The surface of the CPE was characterized by uniform and smooth shaped separated layers. On

the other hand, the SEM image of GL/CPE shows a porous granular film. The morphological features of the deposited gold nanoparticles gives random distribution aggregations of spherical nanoparticles in the SEM image of GNP-GL/CPE exhibiting a large surface area. The effect of electro-deposition

time of gold nanoparticles was studied in 1.0×10^{-3} mol L⁻¹ EN in B-R buffer pH 2, at scan rate 100 mVs⁻¹, (Fig. 1D) the results show that 400 s was the optimum time needed to deposit gold nanoparticles.

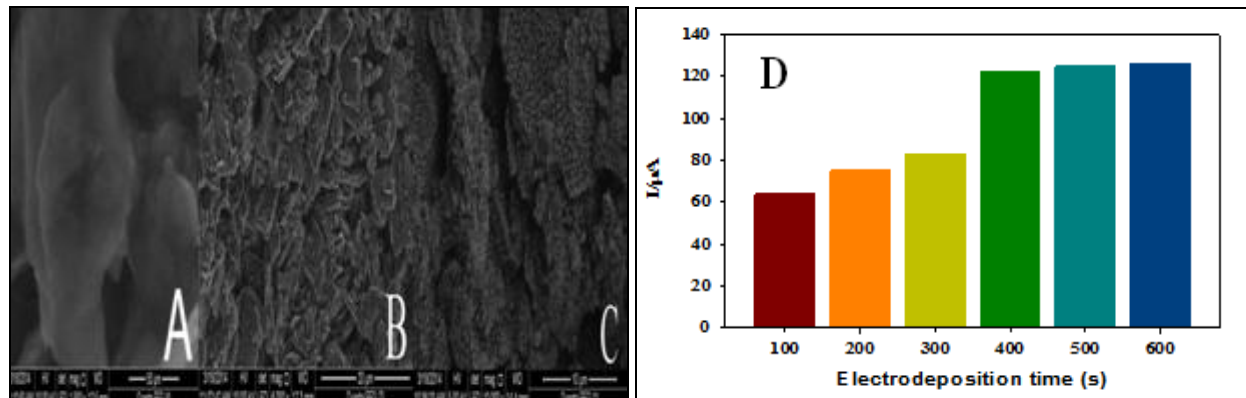


FIG. 1: SCANNING ELECTRON MICROSCOPE IMAGES OF A) BARE CPE, B) GL/CPE AND C) GNP-GL/CPE, D) THE EFFECT OF CHANGING THE ELECTRODEPOSITION TIME OF GOLD NANOPARTICLES ON GL/CPE SURFACE

Electrocatalytic Oxidation of EN: The cyclic voltammograms of 1.0×10^{-3} mol L⁻¹ EN in B-R buffer pH 2, at scan rate 100 mVs⁻¹ (Fig. 2) were recorded at three different working electrodes (*i.e.* bare CPE, and GNP-GL/CPE in absence and presence of SDS). At bare CPE the anodic peak current (ca. 64.8 μA) of EN appears at 0.627 V. The porosity of GL makes gold nanoparticles attaches easily to the electrode surfaces and creates self-assembled monolayer²⁸.

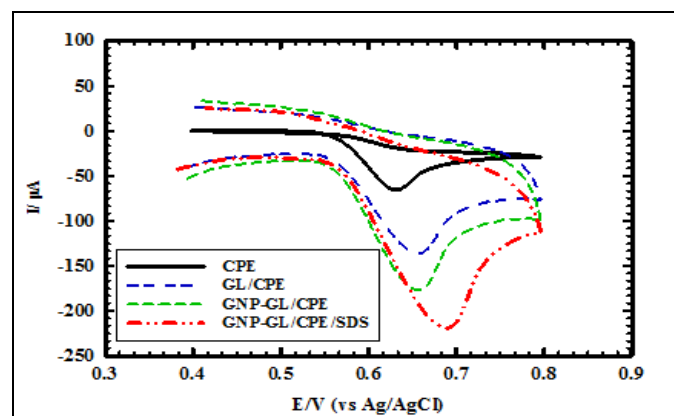
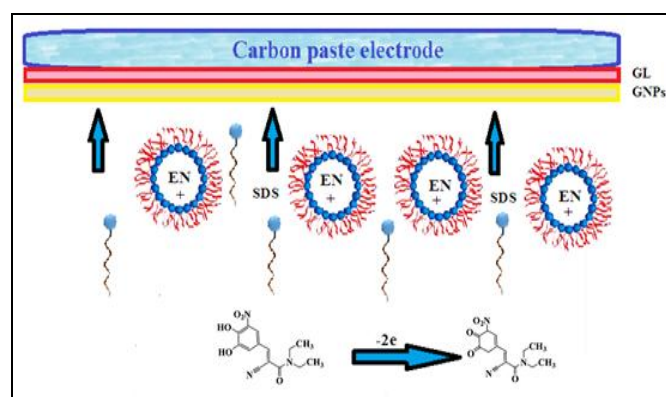


FIG. 2: CYCLIC VOLTAMMOGRAMS OF 1.0×10^{-3} MOL L⁻¹ EN IN B-R BUFFER pH 2 AT A SCAN RATE OF 100 MV S⁻¹ RECORDED AT THREE DIFFERENT WORKING ELECTRODES

Up on the electro-deposition of GNP the current increases to a value of 117.5 μA, which is twice the current observed at bare CPE, due to the low lattice coordination number of gold atom.

It is assumed that, on oxidation, it acquires a relatively large ligand coordination sphere; also the protrusion of GNP from the surface gives the larger surface area of the modified electrode which increases the electrode kinetics which makes it reactive from a chemical, redox and catalytic point of view²⁴.

The addition of SDS causes a higher current response of 198.16 μA to appear, where the sensitivity of GNP film is influenced by the aggregates of SDS that accumulates onto the surface and helps the attraction of EN. So the catalytic effect of GL, GNP and SDS combined together to increase the current response of EN. The suggested oxidation mechanism and the electrochemical diagram of EN are presented in Schematic 1.



SCHEMATIC: 1

Methods of Analysis:

Influence of pH: The effect of solution pH on the electrochemical response of EN at the GNP-GL/CPE in the presence of SDS was recorded in B-R buffers and was found to be discerning over the pH range of 2–9 (Fig. 3). The peak potentials decrease gradually in solutions as pH is raised, manifesting that protons have taken part in the electrode reaction processes. The relationships between oxidation potentials (E_p) and pH gives linear regression equation of $E_{pa}(V) = 0.788 - 0.060$ pH, with a correlation coefficient of $r = 0.9944$. A slope of 59 mV/pH was obtained in the investigated pH range indicating the $2e^-/2H^+$ process. The (inset) shows that at pH 2 the anodic peak current gave the maximum response which is detected for all electrodes.

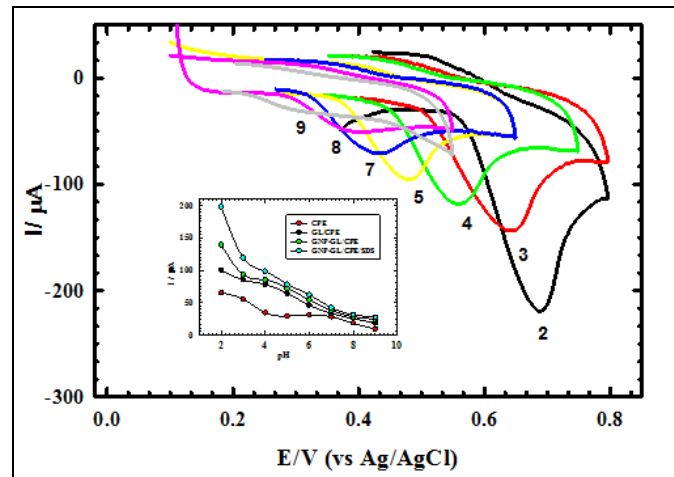


FIG. 3: CYCLIC VOLTAMMETRIC RESPONSE OF 1.0×10^{-3} MOL L $^{-1}$ EN AT DIFFERENT pH VALUES USING 0.04M B-R BUFFERS USING GNP-GL/CPE/SDS

The Inset: plot of the anodic peak current values of EN versus pH values using different electrodes.

Influence of Scan Rate: With an increasing scan rate ranging from 0.1 to 1.5Vs $^{-1}$, the peak currents of 1.0×10^{-3} mol L $^{-1}$ EN using GNP-GL/CPE in the presence of SDS in B-R buffer (pH 2) vary linearly up to scan rate 100 mVs $^{-1}$ then deviation occurs (Fig. 4) showing a surface-controlled reaction. The inset shows the linearly increasing relation between the anodic peak currents and the scan rate (inset A), and the linear regression equation was $i_{pa} (10^{-6}A) = 24.75 v^{1/2} (V s^{-1})^{1/2} - 56.21$ ($n = 5$, $r = 0.9977$). A plot of log peak current versus log scan rate was performed giving a linear relationship with a slope of 0.906 (inset B), suggesting that the oxidation of EN is primarily controlled by adsorption with some

diffusion support. The surface areas of the modified sensors were evaluated by Randles-Sevcik equation^{29, 30}, from which also the diffusion coefficient values were calculated and were found to be 1.26×10^{-7} cm 2 s $^{-1}$, 1.46×10^{-7} cm 2 s $^{-1}$, 6.89×10^{-7} cm 2 s $^{-1}$ and 2.64×10^{-6} cm 2 s $^{-1}$ in case of bare CPE, GL/CPE, GNP-GL/CPE and GNP-GL/CPE/SDS, respectively.

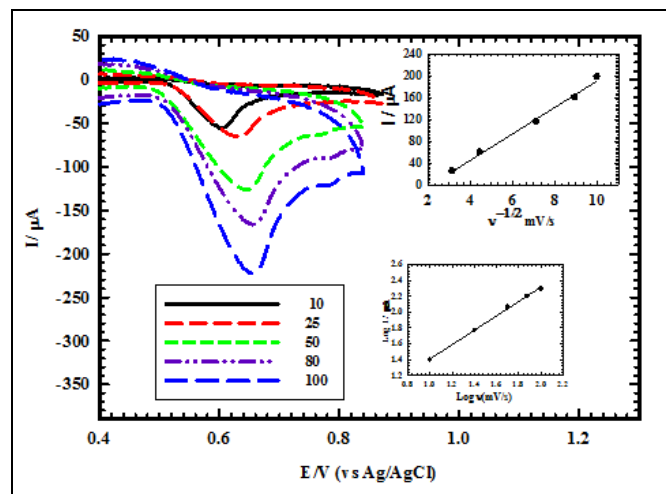


FIG. 4: THE EFFECT OF DIFFERENT SCAN RATES (FROM 10 TO 100 MVS $^{-1}$) ON THE CURRENT RESPONSE OF EN (1.0×10^{-3} MOL L $^{-1}$) USING GNP-GL/CPE/SDS IN B-R BUFFER (pH 2)

The Insets: A) Relation between the anodic peak currents of EN and the square root of the scan rate. B) Relation between log the anodic peak currents of EN and log the scan rate.

Chronoamperometric Measurements: Fig. 5 shows the chronoamperometric measurements of EN, carried out by making the working electrode at a constant DC potential of 690 mV using GNP-GL/CPE/SDS in B-R buffer (pH 2). Once the applied potential reaches the threshold value, all the current densities initially experience a sharp decrease and then keep constant. The inset shows the relation between currents at permanent time and EN concentration. The current of an electrochemical reaction can be described by Cottrell's equation³¹:

$$I(t) = nFAC^0(\sqrt{\frac{D}{\pi t}}) \quad (1)$$

Where I is the current (A), n is the number of electrons, F is a Faraday constant, 96,485 C/mol, A is the area of the electrode in cm 2 , c^0 is the concentration of the analyte in mol/cm 3 ; D is

the diffusion coefficient of EN in cm^2/s and t is time in seconds. The plot of I versus $t^{-1/2}$, showed a straight line (inset) and from its slope, the diffusion coefficient of EN was calculated to be $1.15 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$.

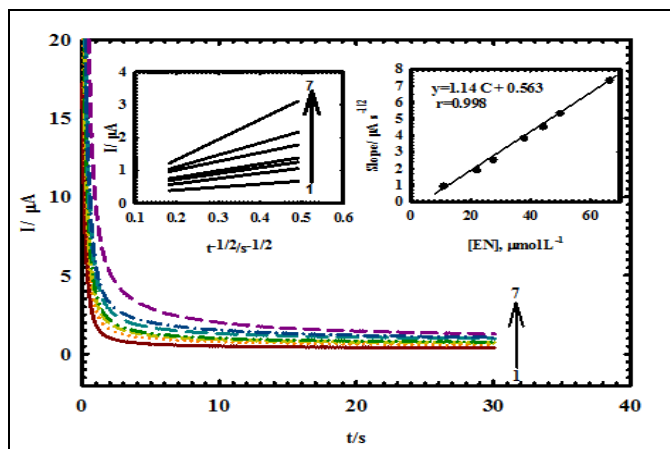


FIG. 5: CHRONOAMPEROGRAMS FOR THE OXIDATION OF DIFFERENT CONCENTRATIONS EN AT GNP-GL/CPE/SDS IN BR BUFFER, pH 2, FOR A POTENTIAL STEP OF +0.690 V VS. Ag/AgCl

The numbers 1 to 7 in Cottrell's plot (inset) correspond to 11.1, 22.2, 27.7, 38.0, 44.4, 50.0 and $66.6 \times 10^{-6} \mu\text{mol L}^{-1}$ of EN, respectively. Inset shows the variation of chronoamperometric currents at $t=30 \text{ s}$ vs. EN concentration.

Electrochemical Impedance Spectroscopy (EIS)

Studies: Electrochemical impedance spectroscopy (EIS) was applied to monitor the whole process of the electrode modification. The EIS scans of the four electrodes; A) bare CPE, B) GL/CPE, C) GNP-GL/CPE and D) GNP-GL/CPE/SDS, are shown in **Fig. 6a, b** as bode and Nyquist plots,

respectively, at potential of 650 mV. The $|Z|$ and Θ_{max} value were found to be in the following order $A > B > C > D$ as shown in **Fig. 6a**, indicating that bare CPE electrode with $\Theta_{\text{max}} \approx 81^\circ$, is almost inert with lowest current as obtained from CV data, however, GNP-GL/CPE in the presence of SDS is the most conductive electrode with $\Theta_{\text{max}} \approx 45^\circ$ that is corresponding to a diffusion control process³². Nyquist plots (**Fig. 6b**) also show that the highest diffusion process is obtained for GNP-GL/CPE in the presence of SDS that confirms well scan rate calculations and Bode plots.

The impedance data were thus simulated to the appropriate equivalent circuit for the cases with two time constants for all tested electrodes (inset in **Fig. 6a**) with 3% error. The model consists of two circuits in series from R_1C_1 and Z_wC_2 parallel combination and both are in series with the solution resistance (R_s). C_1 is related to inner layer capacitance and C_2 is related to outer layer capacitance, while R_1 is related to the inner layer resistance^{33, 34} and Z_w is related to Warburg impedance which corresponding to the linear region at the lower frequencies in the Nyquist plot which is related to diffusion phenomena^{32, 33}. Thus an equivalent circuit with Warburg component Z_w is more applicable. This specifies that the mechanism is organized not only by a charge-transfer process but also by a diffusion process. Analysis of the experimental spectra were made by best fitting to the corresponding equivalent circuit using Thales software provided with Zahner electric workstation³⁵.

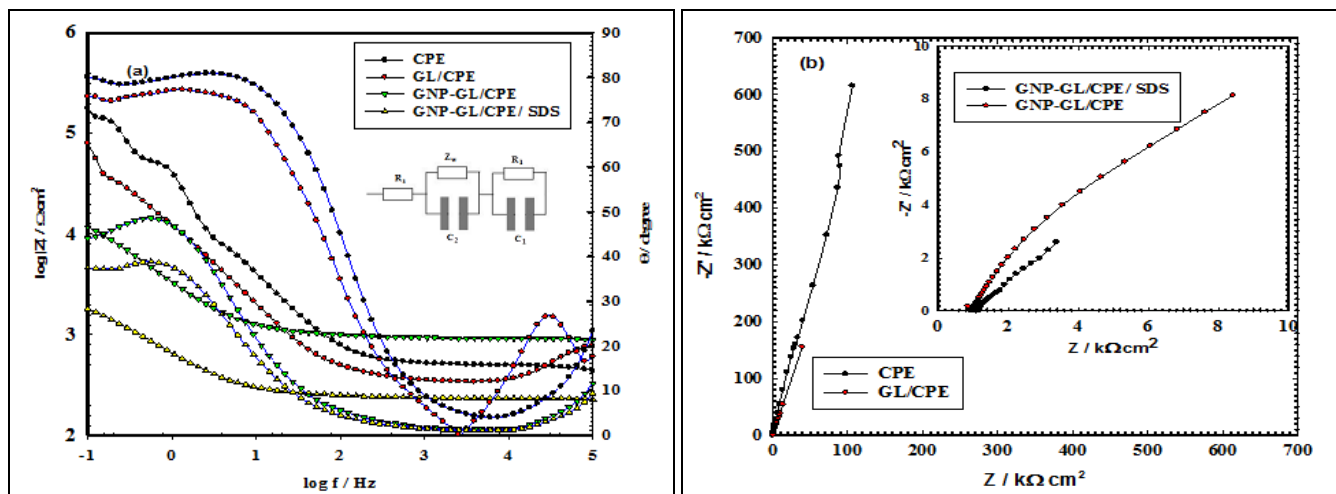


FIG. 6: A) BODE AND B) NYQUIST PLOTS FOR THE FOUR TESTED ELECTRODES THE INSET: THE EQUIVALENT CIRCUIT FOR TWO TIMES CONSTANT THAT FITS THE DATA

In this difficult formula a realistic exponent (α), in the range between 0 and 1, is introduced to account for the deviation from the ideal capacitive performance due to surface uniformities and roughness. An ideal capacitor corresponds to $\alpha = 1$ while $\alpha = 0.5$ becomes the constant phase element in a Warburg component. Meanwhile, the results show that the phase angle has values less than 90° ($\theta = 50 - 81^\circ$) which gives more confirmation for the non-ideality of the capacitive behaviour.

Calibration Curve: Differential pulse voltammetry (DPV) method was used to determine the concentration of EN using GNP-GL/CPE/SDS in B-R buffer pH 2 (Fig. 7) at scan rate = 10 mV s^{-1} . The corresponding calibration plot (inset) was linearly related to EN concentration over the ranges of 4.1×10^{-8} to $0.5 \times 10^{-4} \text{ mol L}^{-1}$ with a regression equation of $I_p(\mu\text{A}) = 0.667c(\mu\text{M}) + 0.0445$ and a correlation coefficient equals 0.9990. The LOD and LOQ³⁶⁻³⁸ were calculated and they were found to be $2.82 \times 10^{-9} \text{ mol L}^{-1}$ and $9.40 \times 10^{-9} \text{ mol L}^{-1}$, respectively. Intra-day and inter-day ($n = 5$) relative standard deviations of samples concentrations (10, 60, and $100 \mu\text{mol L}^{-1}$) of EN were calculated to be 0.02 and 0.03, respectively. Statistical study of the results achieved by applying the suggested and the reported methods for the analysis of EN is presented in Table 1.

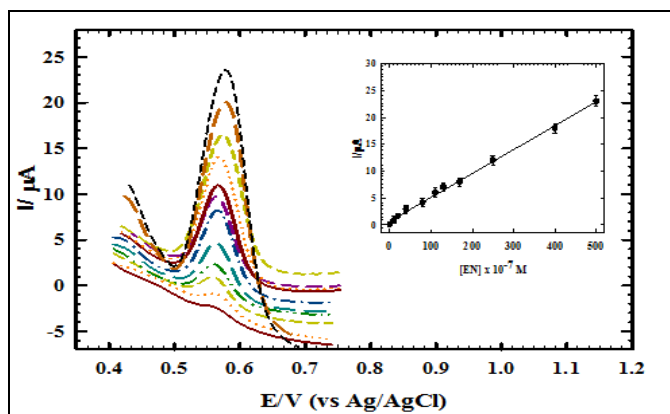


FIG. 7: THE EFFECT OF CHANGING THE CONCENTRATION OF EN, USING DPV MODE AT GNP-GL/CPE/SDS IN B-R BUFFER pH 2 AND SCAN RATE 10 mV/s

The Inset: The calibration plot of EN.

The relative standard deviation of different batches of GNP-GL/CPE/SDS for the determination of EN was less than 1.1%. It was concluded that the presence of the nanoparticles especially gold

nanoparticles on the surface of sensors plays an important role in long-term stability of the catalyst film. GNP-GL/CPE/SDS sensor was stable for about 45 days.

TABLE 1: STATISTICAL ANALYSIS OF THE RESULTS OBTAINED BY APPLYING THE PROPOSED AND THE REPORTED METHODS FOR THE ANALYSIS OF EN

Values	Proposed method	Reported method
Mean \pm S.D.	100.5 ± 1.02	99.10 ± 0.99
N	5	5
Variance	2.56	0.98
t (2.776)	1.61	-
F (6.390)	2.61	-

*Values between parentheses are the theoretical values of t and F at confidence 95%.

Simultaneous Determination of Entacapone, Carbidopa and Levodopa: The main object of this study was to detect EN, CD and LD simultaneously using GNP-GL/CPE in the presence of SDS, using DPV technique. The voltammetric results showed well-defined anodic peaks at potentials of 650, 488 and 320 mV, corresponding to the oxidation of successive addition of the three constituents with a ratio of 8:4:1, respectively, which is the ratio of the three analytes in the Stalevo tablet, giving three calibration curves with a correlation coefficient of 0.9981, 0.9986 and 0.9988 for EN, LD and CD, respectively. These result indicated that the simultaneous determination of these compounds is feasible at GNP-GL/CPE/SDS as shown in Fig. 8.

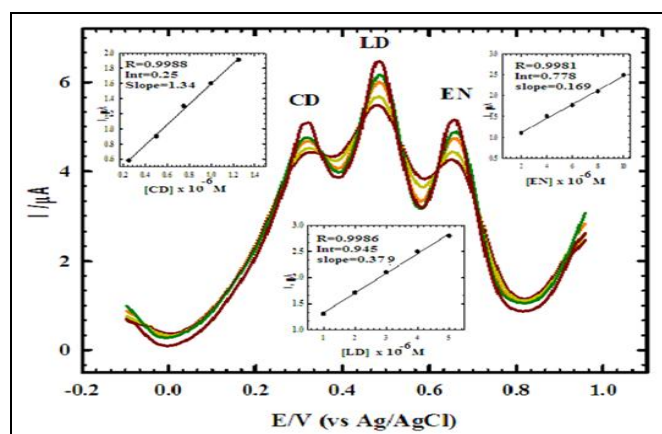


FIG. 8: CYCLIC VOLTAMMETRIC RESPONSE OF SUCCESSIVE ADDITION OF EN, LD AND CD, IN B-R BUFFER (pH 2) USING GNP-GL/CPE/SDS AND SCAN RATE 10 mV/s .

The insets represent three calibration curves for EN, LD and CD.

Analytical Application:

Analysis of Stalevo Tablets: Five Stalevo tablets were weighed and powdered to obtain 1.0×10^{-3} mol L⁻¹ solution. Aliquots of the drug solution were introduced and the general method was carried out built on the average of three replicate measurements. The recovery and standard

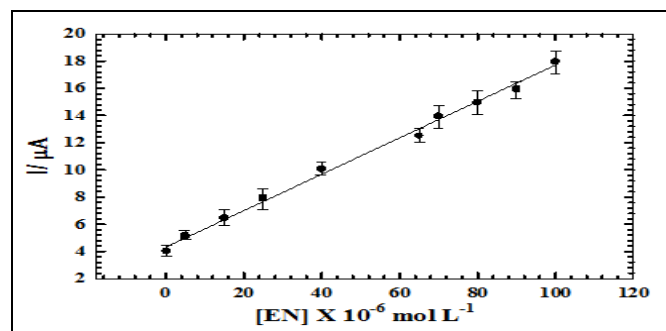
deviation were calculated in **Table 2**. The results suggested that GNP-GL/CPE in the presence of SDS has high reproducibility and that there were no important matrix interferences for the samples analyzed and it would be useful electrode for quantitative analysis of EN in pharmaceutical formulations.

TABLE 2: APPLICATION OF THE PROPOSED METHOD TO DETERMINATION OF EN IN ITS PHARMACEUTICAL PREPARATION STALEVO®

Parameter	Taken 10 ⁻⁶ mol L ⁻¹	Found 10 ⁻⁶ mol L ⁻¹	Recovery* %
EN	20.00	19.20	99.00
	60.00	61.00	101.16
	100.00	99.50	99.50
	Mean ± S.D.		99.88±1.13
CD	2.50	2.50	100.00
	7.50	7.40	98.67
	12.50	12.61	100.88
	Mean ± S.D.		99.85±1.11
LD	10.00	10.01	100.10
	30.00	30.00	100.00
	50.00	49.80	99.60
	Mean ± S.D.		99.90±0.26

* Average of three determinations

Validation Method in Urine: The suggested method was also studied in human urine samples in B-R buffer pH 2, at scan rate 10 mV/s using DPV. The calibration curve (**Fig. 9**) gave a straight line in the linear range 2×10^{-7} mol L⁻¹ – 1×10^{-4} mol L⁻¹, $r = 0.998$, LOD = 3.18×10^{-8} mol L⁻¹ and LOQ = 1.06×10^{-7} mol L⁻¹. The recovery of the spiked samples ranged between 99.88% and 100.6%. To evaluate the accuracy and precision of the proposed method; four different concentrations on the calibration curve were chosen to be repeated for five times (**Table 3**).

**FIG. 9: CALIBRATION PLOT OF EN IN URINE, USING DIFFERENTIAL PULSE MODE AT GNP-GL/CPE/SDS IN B-R BUFFER pH 2 AND SCAN RATE 10 mV/s****TABLE 3: ACCURACY AND PRECISION OF THE PROPOSED METHOD IN URINE**

[EN] added (M) x 10 ⁻⁶	[EN] Found ^a (M) x 10 ⁻⁶	Recovery (%)	SD x 10 ⁻⁷	S. E ^b x 10 ⁻⁷	C. L. ^c x 10 ⁻⁷
5.00	5.01	100.2	0.14	0.07	0.22
25.00	24.97	99.88	0.55	0.27	0.88
40.00	40.27	100.6	5.25	2.62	8.35
80.00	80.10	100.10	2.94	1.47	4.68

^amean for five determinations

^bStandard error = SD/√n

^cC. L. confidence at 95% confidence level and 4 degrees of freedom (t = 2.776)

CONCLUSION: In the present study, the suitability of carbon paste electrode modified with glycine, gold nanoparticles and sodium dodecyl sulphate was investigated for the simultaneous determination of EN in the presence of LD and CD. The CV and DPV investigations showed effective

electrocatalytic activity, high sensitivity, selectivity and reproducibility of the voltammetric responses. Very low detection limit, simplicity of preparation and surface restoration, make the proposed modified electrode very useful for precise determination of EN in real samples.

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CONFLICTS OF INTEREST: Nil.

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