



Electrochemical Behaviour of Lenalidomide and its Determination Using Glassy Carbon Electrode Modified with Polyaniline and Multi Walled Carbon Nanotubes in Pharmaceutical, Human Urine and Serum Samples

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ABSTRACT

The electrochemical determination of lenalidomide (LND) in pharmaceutical, human urine and serum samples using polyaniline (PAN) and multiwall carbon nanotubes (MWCNT) modified glassy carbon electrode (PAN/MWCNT/GCE), as a working electrode. Electrochemical behaviour and surface characteristics of the PAN/MWCNT/GCE was studied using scanning electron microscopy (SEM) and cyclic voltammetry (CV). The PAN/MWCNT/GCE shows excellent electrocatalytic activity towards the reduction of LND under the optimum conditions. It exhibits irreversible cathodic peak at potential -0.71V over the pH 2.0–10.0 in Britton–Robinson buffer. The effects of Initial scan potential, pulse amplitude accumulation potential, accumulation time and pulse width were examined for the optimization of voltammetric conditions. The differential pulse voltammetric method was successfully applied to commercial tablets of 5.5×10^{-7} M LND in pharmaceutical human urine and serum samples with average percentage recovery and relative standard deviations (RSD) are 98.85% and 1.45% respectively.

Keywords: Lenalidomide (LND), polyaniline, MWCNT/GCE, Cyclic voltammetry, DPV

INTRODUCTION

Lenalidomide (3-(4-amino-1-oxo-1,3-dihydro-2H-isoindol-2-yl) piperidine-2,6-dione) shown in fig.1 is an orally available thalidomide analogue, which is showing both anti-angiogenic and immune modulatory/anti-inflammatory properties. It is used for the treatment of myelodysplastic syndrome (MDS) patients if may cause thrombocytopenia or neutropenia.¹⁻⁷ Lenalidomide (LND) demonstrated remarkable clinical activity in treatment of multiple myeloma disease via a multiple-pathways mechanism.^{4, 8-15} Few methods have been reported for the determination LND in bulk material and capsules by Spectrophotometric method as well as two HPLC methods.^{1, 16-19}

Improvement of nanostructures afforded by conducting polymers, viz. Polyaniline (PANI) and polypyrrole (PPy) proceeds independently and includes the preparation of nanotubes.^{20,21} Carbon nanotubes coated with conducting polymers have been proposed for widely differing applications. Both single-wall CNT and multi-wall CNT have been coated with PANI or polypyrrole (PPy) in situ during the polymerization of the respective monomers.²²⁻²⁶ Among all these polymers, polyaniline (PAN) is a promising conducting polymer due to high environmental stability, high conductivity in the oxidized state.²⁷

The present work focused to the voltammetric performance of LND at GCE modified with MWCNT and PAN. The resulted electrochemical sensors exhibits high sensitivity, rapid response, good reproducibility, low detection limit, renewal of the surface and freedom from other potentially interfering species. With best of my knowledge there is no any electroanalytical method for determination of LND.

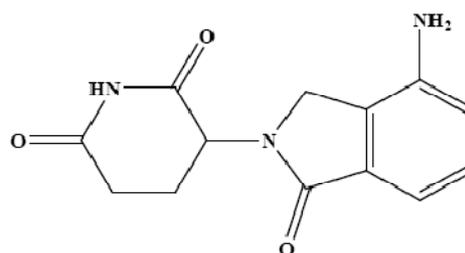


Figure 1: Lenalidomide

MATERIALS AND METHODS

Apparatus

Electrochemical studies were carried out by Autolab PG STAT101 supplied by Metrohm Autolab B.V. The Netherlands. A three electrode system comprising of a glassy carbon electrode modified with polyaniline and multi walled carbon nanotubes (PAN/MWCNT/GCE) as a working electrode. Glassy carbon electrode (GCE-3mm) obtained from Metrohm India Ltd Chennai. Saturated Ag/AgCl/KCl as a reference electrode and Pt wire as a counter electrode. Electrode surface morphology study was carried out by SEM instrument model OXFORD instrument INCA PENTA FETX3 CARL ZEISS from Japan. An Elico LI-120 pH meter supplied by Elico Ltd, Hyderabad, India was used to determine the pH of the buffer solution.

Reagents and solutions

Lenalidomide (LND), free base (3-(4'-aminoisoindoline-1'-one)-1-piperidine-2,6-dione) was obtained from Micro Labs, Bangalore, India. Revlimid® labelled to contain 5 mg LND per capsule was obtained from the local pharmacy. Multi walled carbon nanotubes (MWCNT) powder was obtained from Sigma and Sodium dodecyl sulphate (SDS)

from Merck. Polyaniline (AR-Merck). Double distilled water was used throughout the work. All other solvents and materials used throughout this study were of analytical grade.

Preparation of samples

Lenalidomide (LND) standard solution

An accurately weighed amount (5 mg) of LND was quantitatively transferred into a 25mL calibrated flask, dissolved in 20 mL dimethyl sulfoxide (DMSO), completed to volume with the same solvent to obtain a stock solution of 1 mg/mL. This stock solution was further diluted with deionised water to obtain a working solution containing 5.5×10^{-7} M.

Preparation of PANI/MWCNT/GCE

To get reproducible results, great care was taken in the electrode pre-treatment. The GCE was pre-treated in two ways: (i) mechanical polishing over a velvet micro-cloth with 0.3 and 0.05m alumina slurry (Metrohm Autolab.) and (ii) electrochemical treatment by applying a potential of 1.25V for 2 s. The electrochemical pre-treatment was done in the same supporting electrolyte solution in which the measurement was carried out. The current was recorded for each electrolyte. The obtained results were clearly verified by voltammogram under optimum identical conditions at various time intervals. After that 1mg of MWCNT powder was dispersed in 1mL of 0.1M sodium dodecyl sulphate using an ultra sonication to give black suspensions. The films were prepared by placing 5 μ L of the MWCNT surfactant suspensions on GCE and then evaporating it in an oven at 50°C. Polyaniline was deposited by electro oxidation of 0.1M aniline, in presence of 1M sulphuric acid as a supporting electrolyte by applying a potential between -0.5 and 0.5 V and finally the resulting electrode was prominent as GCE/MWCNT/PAN. After GC electrode surface pre-treatment, covalent attachment binder of MWCNT and MWCNT-PAN was accomplished in the modification with PAN was in the same way, the electrode schematic diagram shown in figure 2. The area of the electrode was calculated by the cyclic voltammetric method using 1.0 M $K_3Fe(CN)_6$ in 0.1 M KCl by recording the current voltage curve at different scan rates. For a reversible process, the following Randles-Sevcik formula can be used.²⁸

$$I_p = (2.69 \times 10^5) n^{3/2} A D_R^{1/2} V^{1/2} C_0$$

Where 'I_p' refers to the cathodic peak current, 'n' is the number of electrons transferred, 'A' is the surface area of the electrode, 'D_R' is diffusion coefficient, 'V' is the scan rate and 'C₀' is the concentration of $K_3Fe(CN)_6$. For 1.0 M $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, n = 1, D_R = 7.6 $\times 10^{-6}$ cm² s⁻¹, then from the slope of the plot of I_p versus V^{1/2}, relation, the electro active area was calculated. In our experiment the slope was 0.651 and the area of electrode was calculated to be 0.110 cm².

RESULTS AND DISCUSSION

Morphology study of modified electrode

Morphology and surface investigation experiments were performed for PANI/MWCNT/GCE. Polyaniline and MWCNT powder was dispersed on the GCE surface by electro polymerisation process. Figure 3 shows the photography of SEM images of PANI/GCE (A), MWCNT/GCE (B), PANI/MWCNT/GCE (C). The tube size of the nanomaterial is 20 nm. The cyclic voltammetric studies examine that LND undergo adsorption diffusion controlled process. Hence it was through to study the morphological change of adsorbed LND drug on the suitable modified system which resulted in better signal. Maximum peak current was observed at PANI/MWCNT/GCE system at potential -0.71. Here we compared the results of the drug LND at different modified electrode systems by CV and DPV studies at optimum conditions.

Electrochemical behaviour of LND

In the beginning studies the electrochemical behaviour of LND was investigated on glassy carbon electrode modified with polyaniline and multi walled carbon nanotubes (PANI/MWCNT/GCE) and Ag/AgCl/KCl as reference electrolyte by cyclic voltammetry. The drug exhibits well defined reduction peak in the potential range -0.2 V to -1.4V. This clearly shows that LND undergo reduction at C=O on the glassy carbon modified electrode surface. CV was carried out with the potential sweep rate the range of -50 to -300 mV s⁻¹. As the potential sweep rate is increased, the peak current also increased and peak potential shifted to more negative value and no peak appeared in the reverse sweep, indicating irreversible nature of the reaction process. In addition, the peak current depends linearly on the corresponding square root of potential sweep rate. From the linear dependency of the peak current on the square root of potential sweep rate, ip can be calculated using the Randles-Sevcik equation for totally irreversible electron transfer process.

$$i_p = [2.99 \times 10^5] n \alpha^{0.5} A C^* D^{0.5} V^{0.5}$$

Where 'α' is the Electron transfer coefficient, 'n' is the number of electron exchanged, 'A' surface area of the working electrode, 'C*' and 'D' are the bulk concentration and diffusion coefficient of the electro reactant species respectively, and 'V' is the potential sweep rate.

Cyclic voltammetric study of Lenalidomide (LND)

The voltammetric response of 5.5×10^{-7} M LND at GCE and MWCNT/GCE at pH 6.0 in Britton- Robinson buffer the potential range of -0.20 to -1.40V, with a scan rate of 50 mV s⁻¹ were compared with each other. At GCE a poorly defined reduction peak was observed and the peak current was smaller the voltammogram shown in figure 4(A). The LND exhibits well defined cathodic peak at -0.71V at PAN/GCE, the voltammogram shown in figure 4(B). The reduction peak current increases greatly at PAN/MWCNT/GCE the voltammogram shown in Figure

4(C). It indicates that PANI/MWCNT/GCE can make the electron transfer of LND easily and no oxidation peak was

observed in the reverse scan, suggesting that the electrochemical reaction was totally irreversible process.

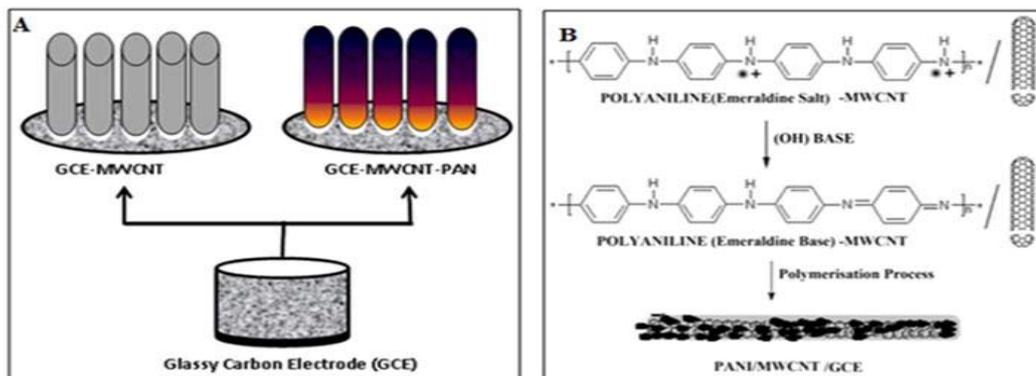


Figure 2: Schematic diagram of preparation of PANI/MWCNT/GCE (A) and Mechanism of polymerisation process of electrode modification (B).

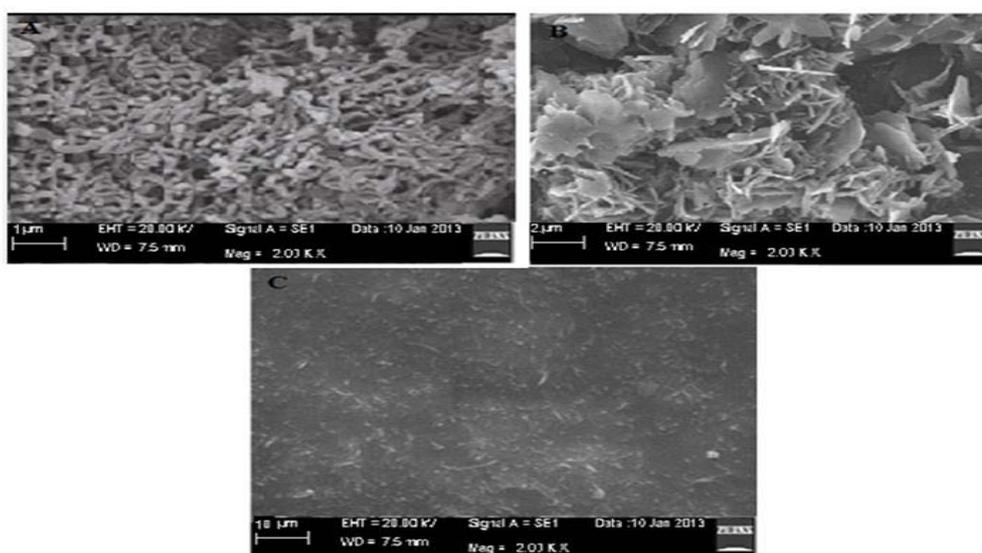


Figure 3: SEM images of (A) PANI/GCE, (B) MWCNT/GCE, (C) PANI/MWCNT/GCE

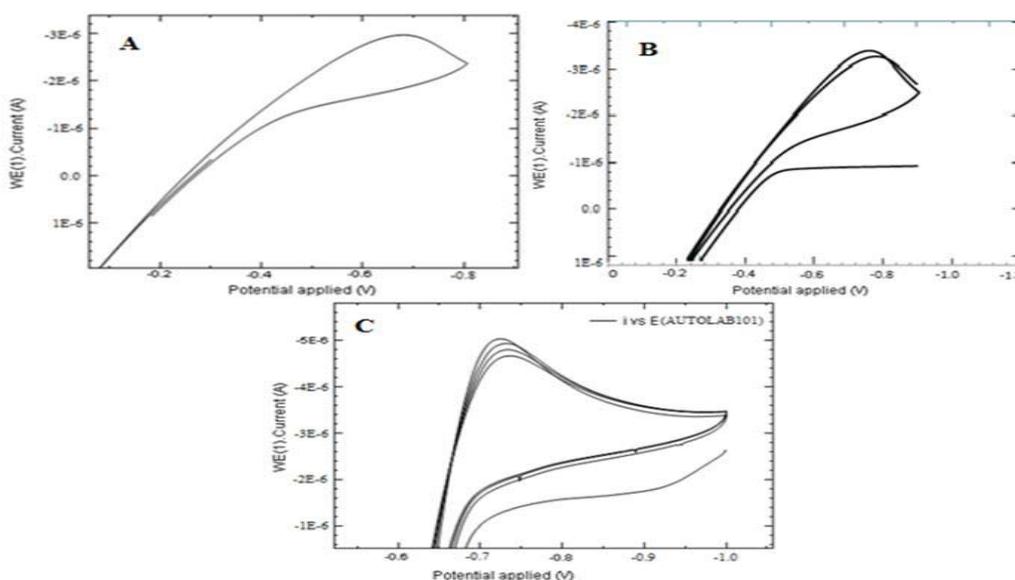


Figure 4: Typical Cyclic voltammograms of Lenalidomide (LND) at GCE(A), PAN/GCE(B), PANI/MWCNT/GCE(C) concentration $5.5 \times 10^{-7} \text{M}$ pH-6.0, Scan rate-50 mV/s.

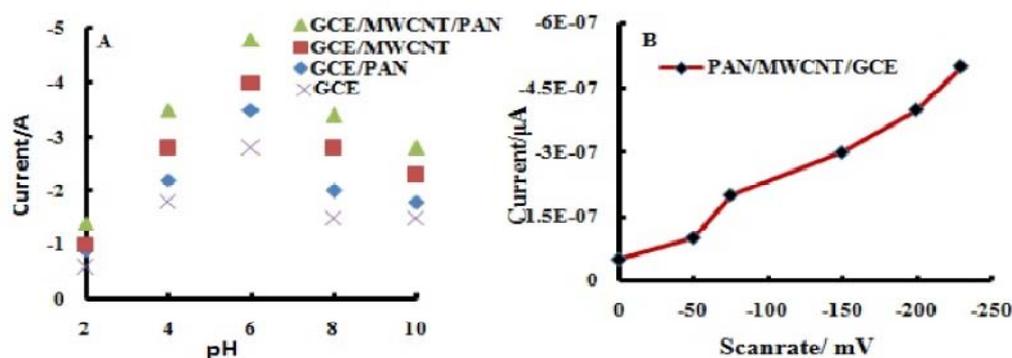


Figure 5: Effect of pH on GCE, GCE/PAN, GCE/MWCNT, GCE/MWCNT/PAN(A), Linear plot of current Vs scan rate for 5.5×10^{-7} M LND at PAN/MWCNT/GCE in BR buffer of pH 6.0(B)

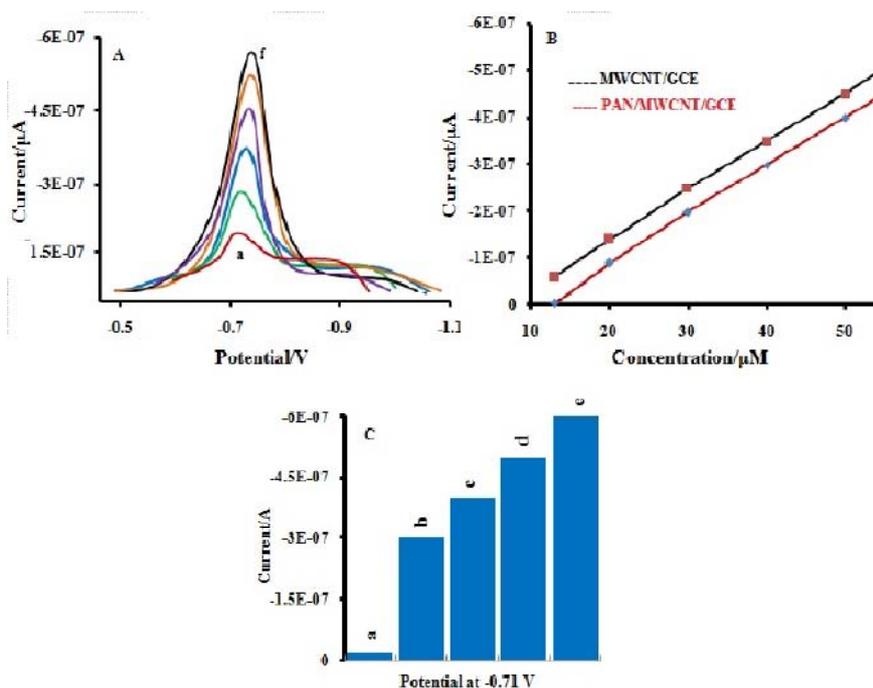


Figure 6: Differential pulse voltammogram of LND at different concentrations at PAN/MWCNT/GCE (a) blank, (b) 10 μM (c) 20 μM, (d) 30 μM, (e) 40 μM, (f) 50 μM(A), Plot of the Concentration Vs peak current of LND at MWCNT/GCE and PAN/MWCNT/GCE (B), Comparison of peak current at glassy carbon modified electrodes (C.)

Effect of pH

The influence of pH on the reduction behaviour of LND was performed at different electrodes and different pH values using differential pulse voltammetry. The relation between pH versus current curves were shown in figure 5(A). It can be seen that the peak current reaches a maximum at pH 6.0. Therefore pH = 6.0 was selected as the optimum pH, at this pH, the sensitivity was highest and the peak was well defined. According to the structure of LND, 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 LND on the surface of the modified electrode in the accumulation step.

Effect of Scan rate

The effect of scan rate (V) on the peak current and peak potential of LND at PAN/MWCNT/GCE shown in Figure 5(B). The influence of the square root of the scan rate on

the peak current showed a linear relationship between -25 to -300 mV s^{-1} , which is of typical diffusion controlled process.²⁹ The slope of the theoretical value and expected value is close to each other. This indicates that the electrode process was controlled by diffusion controlled process. The peaks are shifted to negative values with increasing the scan rates.

Differential pulse voltammetric studies

An accurate volume of the clear supernatant liquor was transferred into the electrochemical cell containing 10 mL of BR buffer of pH 6.0 to yield a final concentration of approximately 5.5×10^{-7} M of LND. The DPV was recorded under the optimum experimental conditions at PAN/MWCNT/GCE. A typical differential pulse voltammogram recorded in various concentration ranges at GCE modified with Polyaniline and multi walled carbon nanotubes. The differential pulse voltammograms are shown in figure 6(A).

Standard Addition Method

Alternatively, the standard (spiking) method was used. Known volumes (0.5 – 1.0 mL) of the test drug solution at pH 6.0 were transferred to the electrochemical cell, with the current height being measured before and after addition of the standard. The change in the current height was recorded and used to determine the drug, the voltammogram shown in figure 6(A).

Calibration Curve

In order to grow the voltammetric method for influential of the drug, we choose the differential pulse voltammetric method, for the reason that the peaks are sharper and better distinct at lower concentration of LND than those obtained by CV, with a lower background current, resulting in enhanced resolution. In keeping with obtained results, it was feasible to apply this technique to the quantitative analysis of LND. The Britton-Rabinson buffer solution of pH 6.0 was selected as the supporting electrolyte for the quantification as LND gave maximum peak current. Differential pulse voltammograms obtained with increasing amounts of lenalidomide showed that the peak current increased linearly with increasing concentration the calibration plot was shown in Fig.(6B). Interrelated statistical data of the calibration curves were procure, from the five different calibration curves. Detection limit (LOD) and quantification (LOQ) were calculated based on the peak current using the following equations shown below.

$$\text{LOD} = 3\alpha/a; \text{LOQ} = 10\alpha/a$$

Where 'α' is the standard deviation of the peak currents of the blank (five replicates), and 'a' is the slope of the calibration curve. The LOD and LOQ values are 4.55×10^{-9} M, and 2.55×10^{-7} M correspondingly.

Specificity & Selectivity

Commercial LND tablets in dosage form are usually coated with excipients, like lactose, anhydrous, cellulose, microcrystalline, croscarmellose sodium, magnesium stearate. It was therefore essential to analyze the uncoated tablets by the proposed DPV method at a GCE/MWCNT/PAN at pH 6.0 to determine whether the coating materials would interfere with the analysis. Each excipient (0.25– 0.75 g) was added according to the manufacturer's batch formula to known amounts of LND ($0.052 \mu\text{g ml}^{-1}$). The tolerable limit was defined as the concentration of the excipient causing a deviation of less than $\pm 3\%$ in the cathodic peak current of the solution containing the pure active ingredient. The recovery data indicated that the magnitude of the peak current of LND did not deviate by more than $\pm 3\%$ from the peak current of the solution containing no interfering additives. Confirming that the developed DPV method is free from serious interferences from the tested excipients. The developed DPV procedure can therefore be regarded as a selective method for determination of the analyte.

Robustness

The robustness of the method was checked by evaluating the influence of small variations of some of the most important procedure variables including pH and potential range. The results indicated that none of these variables significantly affect the recovery of LND. This provided an indication of the dependability of the proposed process for the assay of LND, and the projected method could be measured robust.

Ruggedness

The effect of different analysts (two) on the results for 0.55 and 4.75 mg ml^{-1} standard samples was evaluated. The results showed good agreement, with RSD 1.45%.

Recovery test of LND

The recovery tests of LND ranging from 5.5×10^{-7} to 1.0×10^{-4} M were performed using DPV. The results are listed in Table 1. The recoveries lie in the range from 97.3% to 99.5%. The relative standard deviation was 1.45%. The recovery test of LND in spiked urine and serum samples at concentration 5.5×10^{-7} were performed by DPV, the results shown in table 1. The recoveries lie in the range from 98 % to 99 %.

Table 1: Determination of Lenalidomide (LND)

Pharmaceutical samples			
Added (M)	Found (M)	*Recovery (%)	RSD
5.5×10^{-7}	5.45×10^{-7}	99.5	1.45
4.5×10^{-6}	4.35×10^{-6}	98.3	1.52
3.5×10^{-5}	3.33×10^{-5}	98.2	1.85
2×10^{-4}	1.97×10^{-4}	97.5	2.0
1×10^{-4}	0.97×10^{-4}	97.3	2.10
Urine and Serum Samples			
Sample	Spiked (10^{-6} M)	Detected (10^{-6} M)	*Recovery (%)
Urine Sample-1	0.5	0.48	98.0
Urine Sample-2	1.0	0.98	98.4
Serum Sample-1	0.5	0.49	98.3
Serum Sample-2	1.0	0.99	99.0

*Average of five determinations

Reproducibility of the modified electrode

The renewal and reproducibility of the electrode was investigated. It was found that after determination the surface of the PANI/MWCNT/GCE could be regenerated by successively cycling between 0.8 and 1.2 V in 2.5 pH with 0.2 M Britten-Robinson buffer. As an example, a 1.0 mM LND solution was measured successively for 5 times with the same electrode regenerated through such procedure after every determination, the relative standard deviation (RSD) of the peak current was 1.45%-2.10%. As to the reproducibility between some days, it was similar to that of within a day, if the temperature was

kept almost unchanged. Owing to the adsorption of LND or its reductive products on to the electrode surface, the current response of the modified electrode would decrease after successive use. In this case, the electrode should be prepared again.

Determination of LND in Biological samples

The applicability of the proposed method for the determination of LND in biological fluid of human urine and serum samples were attempted. Drug free human urine and serum samples, obtained from healthy volunteers, filtrated through a filter paper and stored frozen until the assay. The developed differential pulse voltammetric method for the LND determination was applied to urine and serum samples. The recoveries from urine and serum were measured by spiking drug free urine and serum with known amounts of LND. The urine and serum samples were diluted with the Britton-Robinson buffer solution before analysis without further pre-treatment. A quantitative analysis can be carried out by adding the standard solution of LND into the detect system of urine and serum samples. The calibration graph was used for the determination of spiked LND in urine samples. The detection results of two urine and samples are listed in Table 1. The recovery determined was in the range from 97.3% to 99.5% and the RSD was 1.45%. Good recoveries of LND were achieved from these matrices, denoting that application of the proposed method to the analysis of LND in biological fluid could be easily determined.

Analytical applications

The high sensitivity of the developed reduction peak ($E_{p,c} = -0.71V$) at the GCE modified with MWCNT and PAN at pH 6.0, suggests possible application of the developed DPV method for analysis of LND in dosage forms. Under the optimum experimental conditions of pH 6.0, pulse amplitude 50 mV, pulse width 30 ms, and scan rate -50 mV s^{-1} LND shows that the peak current increased linearly on increasing the drug concentration in the range 5.5×10^{-7} to 1.5×10^{-4} M by DPV method. A lower limit of detection (LOD) was found to be $4.55 \times 10^{-9} \text{ M}$, was obtained by use of the formula $LOD = 3\alpha/a$ (where ' α ' is the standard deviation of residuals and ' a ' is the slope of the calibration plot). The LOQ was found to be $2.5 \times 10^{-7} \text{ M}$ and the relative standard deviation (RSD) of LND at a concentration of 5.5×10^{-7} was 1.45%. The peak potentials and peak heights of the given compound at concentration $5.5 \times 10^{-7} \text{ M}$ was compared at GCE modified electrode shown in fig.(6C). The detection limits are compared at different modified electrodes each other.

CONCLUSION

The voltammetric behaviour and reduction mechanism of LND was investigated at a PAN/MWCN/GCE by CV in britton-rabinson buffer solution of pH.6.0. Based on this study, influence of several physicochemical parameters like potential scan rate, pH and concentration were investigated. The reduction of LND was found to be an

irreversible 1:1 electron and proton process with diffusion character. MWCNTs showed electrocatalytic action for the reduction of LND, characterizing by the enhancement of the peak current and the reduction of the peak potential, which was probably due to the larger effective surface area of MWCNTs and PAN. This method was successfully used to determine LND in the pharmaceutical, human urine and serum samples. The proposed method offered the advantages of accuracy and time saving as well as simplicity of reagents and apparatus. In addition, the results obtained in the analysis of LND in spiked urine and serum samples demonstrated the applicability of the method for real sample analysis.

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