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Amperometric and differential pulse voltammetric determination of 5-Hydroxy-L-tryptophan in pharmaceutical samples using gold modified pencil graphite electrode

H. C. B. Kalachar[a], Y. Arthoba Naik[a]*, S. Basavanna[b], R. Viswanatha[a], T. G. Venkatesha[a] and T. Sheela[a]

[a]*Department of Chemistry, School of Chemical Science, Kuvempu University, Shankaraghatta, India*
[b]*Solid State and Structural Chemistry Unit, Indian Institute of Science, Bangalore, India*

ABSTRACT

Quantitative evaluation of 5-Hydroxy-L-tryptophan (5-HTP) in seed powder of Griffonia simplicifolia (pharmaceutical tablet source) using gold modified pencil graphite electrode (GPGE) was carried out by differential pulse voltammetric technique (DPV) and amperometry. The percentage of 5-HTP detected by DPV and amperometry is concurrent with that of Uv-Vis spectroscopic results. GPGE shows high electro-catalytic activity and excellent analytical performance towards the oxidation of 5-HTP.

Key words: Amperometric determination; Differential pulse voltammetry; Gold modified pencil graphite electrode; Griffonia simplicifolia; 5-Hydroxytryptophan.

INTRODUCTION

The synthesis of major and essential neurotransmitter serotonin from tryptophan proceeds via the intermediary 5-HTP. 5-HTP is an aromatic amino acid naturally produced in human body from the essential amino acid tryptophan. The seed powder of *Griffonia simplicifolia* is administered as a natural source of 5-HTP when plasma tryptophan is significantly lower in patients with major depression [1, 2]. *Griffonia simplicifolia* is a medium sized tropical non-climbing shrub native to West Africa and belongs to the family Caesalpinaceae [3]. The seeds contain non protein amino acid 5-HTP in appreciable concentration (6-14%), because of evolutionary importance. The seeds are proved to be an extremely toxic to the larvae of *prodenia eridania* and the larvae of *callosobruchus maculates* [4, 5]. 5-HTP can decrease the food intake and reduce the body weight in case of deprived and stressed rats [6] and also negatively affects the sexual behavior in female rats [7]. 5-HTP is used for human welfare in modern therapecitic applications

including the treatment for anxiety, depression, insomnia, fibromyalgia, binge eating associated with obesity and chronic headache [8-10].

5-HTP can easily absorb through intestine and has the capacity to cross the blood-brain barrier. By the action of enzyme, aromatic L-amino acid decarboxylase, it converts into serotonin in the brain and perform its neurological functions. Because of this reason *Griffonia simplicifolia* seeds have huge commercial demand in the herbal market.

Quantitative analysis of 5-HTP in the seeds of *Griffonia simplicifolia* was studied using different methods like UV-Vis [11], LC-MS [12], HPLC [13], electrochemical and enzymatic oxidation [14] and pulse radiolysis technique [15]. Electro analytical methods of detection have gained considerable attention for their high sensitivity and selectivity. Some of them are electrochemical oxidation of 5-HTP in the sol gel matrix [16], oxidation and flow-injection amperometric determination of 5-HTP at an electrode modified by electrochemically assisted deposition of a sol-gel film with templated nanoscale pores [17], simultaneous determination of femtomole quantities of 5-HTP, serotonin and 5-hydroxyacetic acid in brain using HPLC with electrochemical detection [18].

Varieties of nanoparticals have been utilized for the modification of electrode surface to improve sensitivity and selectivity. Among these, gold nanoparticals have attracted increasing attention for the application in catalysis and biosensors [19-23]. The importance of gold modified electrode is because of its high biocompatibility and bio recognition [24]. Pencil graphite electrode (PGE) and modified PGE were used for the detection of electro active compounds is attracted for its easy preparation, economical and easily available [25-28]. In the present work, gold nanostructures obtained by electrodeposition method were used for sensing of 5-HTP present in pharmaceutical sample, i, e, seed powder of *Griffonia simplicifolia*.

EXPERIMENTAL SECTION

2.1 Reagents and Apparatus

5-HTP was procured from Sigma–Aldrich (USA). Potassium dihydrogen phosphate and di-potassium hydrogen phosphate were procured from Merck, (Mumbai, India). All the chemicals used were of analytical grade and used for the experiments without purification. Double distilled water was used for the preparation of all the solutions. 5-HTPη-50 tablet (which contains seed powder of *Griffonia simplicifolia*) was procured from Molekule Pvt. Ltd. (Mumbai, India).

All the electrochemical measurements were performed using electrochemical workstation (CHI 660D, USA). All the experiments were conducted in a standard three-electrode assembly incorporating GPGE, platinum wire and Ag/AgCl (KCl_{Sat}) as working, auxiliary and reference electrodes respectively. UV-Vis spectrophotometric measurements were performed using Ocean optics Inc Ltd. (Model USB 4000, USA). The surface morphology of the modified electrode was characterized by scanning electron microscope (FEI-Quanta 200).

2.2 Preparation of PGE and GPGE.

The working electrode was constructed with 2B pencil graphite lead (0.5 mm diameter) procured from Camlin Ltd, (Mumbai, India). Electrical contact to the lead was achieved by wrapping a metallic wire around the pencil. Then the pencil lead was inserted in the plastic tube and filled with epoxy resin. After 24 hours (time required for the setting of epoxy resin), the anterior end of the electrode was scarped using a sharp knife and polished using emery papers followed by butter sheet.

The prepared PGE was subjected to electrodeposition using chronoamperometry in 1 mM chloroauric acid solution at the potential of -1.0 V against Ag/AgCl (KCl_{Sat}) with pulse width of 300 seconds. After electrodeposition the electrode was rinsed with double distilled water and used for the analysis [29].

2.3. Preparation of real samples.

A known quantity (80 mg) of 5-HTPη-50 tablet powder was dissolved in 100 mL of phosphate buffer solution (PBS) of pH 7.0. The insoluble residue was filtered off to get a clear stock solution. The prepared real samples was used for electrochemical and UV-Vis spectroscopic studies.

RESULTS AND DISCUSSION

3.1 Electrochemical behavior of $[Fe(CN)_6]^{3-/4-}$ at PGE/GPGE

$[Fe(CN)_6]^{3-/4-}$ are valuable and convenient probes to characterize the electrochemical performance of the modified electrode. Fig. 1, shows the cyclic voltammograms of 5 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl at PGE/GPGE. There was a pair of well defined anodic (E_{pa}) and cathodic (E_{pc}) peaks observed at potential 275 and 215 mV respectively at GPGE. The cyclic voltammograms of $[Fe(CN)_6]^{3-/4-}$ couple show a peak to peak separation of 121 mV at PGE, and 60 mV at GPGE. The gold nanostructures are responsible for the decrease in peak to peak separation and increase in the peak current at GPGE, than that of PGE.

3.2 Electro-oxidation of 5- HTP on GPGE

Fig. 2A shows the electrochemical response of PBS of pH 7.0 on PGE /GPGE. The baseline is only due to the charging and discharging of the electrode double layer. In the applied potential range no redox peaks were observed. Electrochemical oxidation of 5-HTP at PGE/GPGE is shown in Fig. 2B. At PGE electrode, a poor electrochemical response and hence, a weak oxidation peak is observed at the potential 387 mV (curve a). By comparison, the oxidation of 5-HTP at GPGE occurred at a lower potential of 368 mV. The reduction in oxidation peak potential and enhancement of peak current (6.3 times) is due to the good electro catalytic activity and enhanced active surface area of GPGE.

3.3 DPV response of 5-HTP at GPGE

The Fig. 3 shows DPVs for the quantitative analysis of 5-HTP in PBS of pH 7.0 at GPGE. According to the electrochemical response, the oxidation peak current increases with increase in concentration of the analyte. In CV, 5-HTP was oxidized at 368 mV but in case of DPV oxidation of 5-HTP was observed at 328 mV. This difference in oxidation potential is attributed to pulse amplitude and pulse width applied in DPV. The calibration curve of current response versus 5-HTP concentration is linear in the range 10 to 60 µM. The linear regression equation is depicted as $y = 0.1176 x + 0.8839$ and the correlation coefficient is 0.9879

3.4UV-Vis spectrophotometric behavior of 5-HTP.

UV-Vis spectroscopy is one of the powerful tools for the quantitative estimation of organic molecules. Fig. 4A shows the characteristic absorption patterns for 5-HTP and solution of 5-HTPη-50 tablet in 0.1 M PBS of pH 7.0. 5-HTP exhibits two absorption peaks in the UV region, one sharp peak at 229.6 nm and another broad peak at 275 nm. A prominent shoulder peak was observed at 296 nm. The observed absorption peaks are due to conjugation present in 5-Hydroxyindole skeleton of 5-HTP. An identical absorption peak is observed for 5-HTP present in tablet.

The calibration graph of concentration of 5-HTP versus absorption was plotted and is linear in the range 80 - 560 µM (Fig.4B). The linear regression equation is depicted as y = 0.0024 x - 0.0113 and the corresponding correlation coefficient is 0.9994. The observed absorption for the successive addition of 5-HTP is proportional to the concentration of the analyte.

3.5 Amperometric response of 5-HTP at GPGE

The typical amperometric experiments were carried out for the oxidation of 5-HTP at GPGE in 0.1 M PBS of pH 7.0. Fig. 5A shows the amperometric i - t curve obtained for the oxidation of 5-HTP in a homogeneously stirred solution at the applied potential of 368 mV. Initial current response was due to the presence of 0.5 µM of 5-HTP and on the successive addition of 0.5 µM of 5-HTP with a sample interval of 50 s, the current response was linearly increased with linear regression equation is depicted as y = 0.0221 x – 0.655 and the correlation coefficient is found to be 0.9992.

The calibration graph of concentration of 5-HTP versus current was plotted and is linear in the range 0.5 - 3.5 µM (Fig. 5B). The linear regression equation is depicted as y = 2.2509 x – 0.7617 and the correlation coefficient is found to be 0.9998. The observed absorption for the successive addition of synthetic 5-HTP is proportional to the concentration of the analyte.

3.6 Determination of 5-HTP in pharmaceutical samples.

In order to evaluate the validity of GPGE for the determination of 5-HTP in 5-HTPη-50 tablet, the DPV studies were carried out. A Known amount (80 mg) of 5-HTPη-50 tablet was dissolved in 100 mL PBS of pH 7.0 and about 300 µL of this solution were diluted to 10 mL using PBS. Fig. 6 (curve a) shows the DPV curves for varying volume of 5-HTPη-50 stock solution. From the calibration curve, the concentration of 5-HTP in the tablet powder was found to be 21.19 µM which corresponds to 20.16 weight percent of 5-HTP. The oxidation of 5-HTP was observed at 0.332 V which is in good agreement with the experimental results obtained for synthetic 5-HTP.

To confirm the observed anodic peak was due to 5-HTP, the sample was spiked with 10 µM solution of synthetic 5-HTP. An increase in the peak current confirmed that the peak observed at 332 mV was due to the oxidation of 5-HTP as shown in Fig. 6 (curve b). The recovery rate of the spiked sample was 99.40 %.

Fig. 7 (curve a) shows the UV absorption peak for 5-HTP (from 5-HTPη-50 tablet). From the calibration curve the percentage of 5-HTP in 5-HTPη-50 (pharmaceutical sample) is found to be 21.12%. To confirm the observed absorption peak was due to 5-HTP, the sample was spiked with 40 µM synthetic 5-HTP and the corresponding UV-Vis absorption spectrum was recorded Fig. 7 (curve b). An increase in the absorption peak confirmed the presence of 5-HTP in the pharmaceutical sample the same. The recovery rate of the spiked sample was 99.89 %.

The determination of 5-HTP in commercial 5-HTPη-50 tablet powder by amperometry using GPGE has been carried out. The amperometric experiments were conducted with the addition of known amount (1 µM) (steps 1-2) of 5-HTP followed by 20 µL 5-HTPη-50 (pharmaceutical sample) with successive intervals in 0.1 M PBS of pH 7.0 (steps 3-4) and 0.5 µM and 10 µL synthetic 5-HTP and 5-HTPη-50 (pharmaceutical sample) (steps 5-6) as shown in Fig. 8. From the calibration curve, the concentration of 5-HTP present in tablet powder was found to 0.763 µM which corresponds to 21.08 weight percent of 5-HTP.

To confirm the observed current was due to 5-HTP, the sample was spiked with 0.5 µM 5-HTP. An increase in the current confirmed that the peak observed at 368 V was due to the oxidation of 5-HTP. The recovery rate of the spiked sample was 100 %.

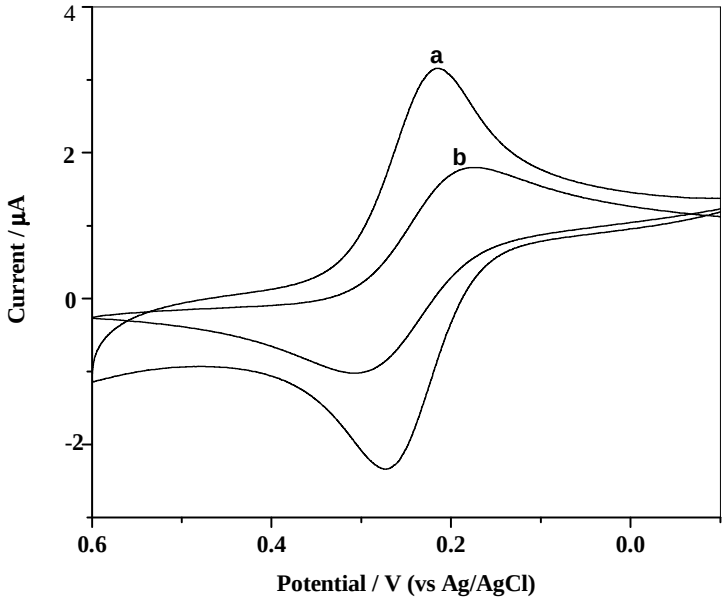

Fig.1.Cyclic voltammograms of 0.1 M KCl solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$ at the GPGE (a) and PGE (b) at the scan rate of 100 mV/s.

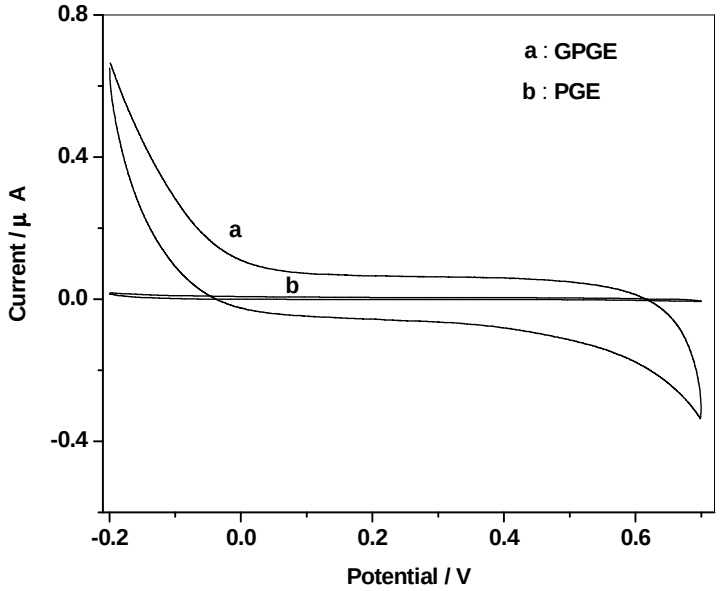

Fig. 2A. Cyclic voltammogram of 0.1M phosphate buffer at GPGE (a) and PGE (b) at the scan rate of 100 mV/s.

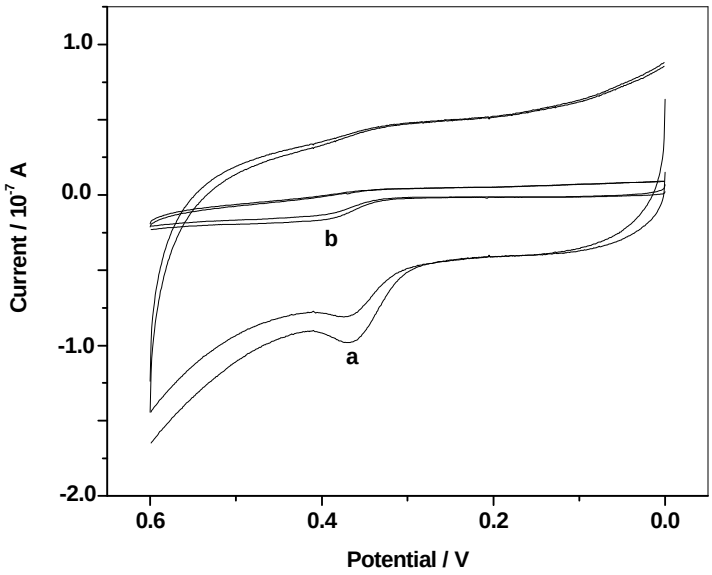

Fig. 2B. Cyclic voltammograms of 20 µM 5-HTP at GPGE (a) and PGE (b) in 0.1 M phosphate buffer of pH 7.0.

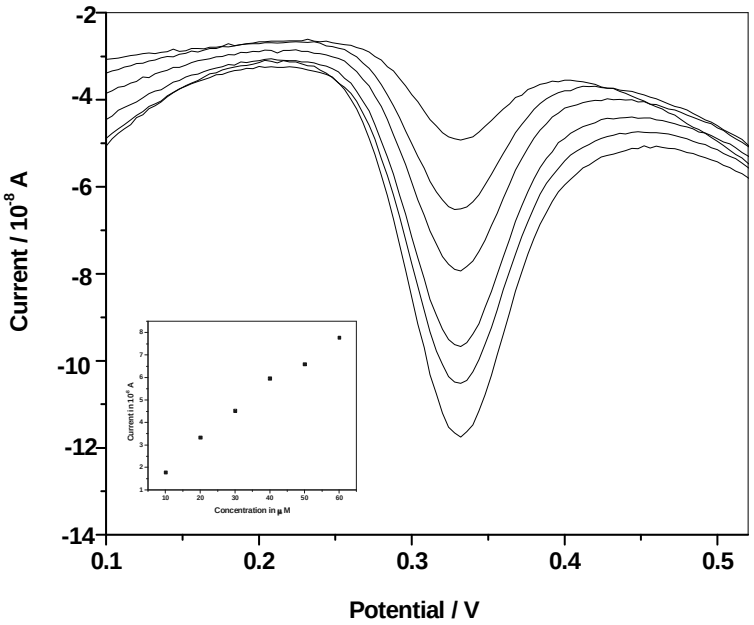

Fig. 3. DPV graphs of 5-HTP (a) 10; (b)20; (c) 30; (d) 40;(e)50; (f) 60 µM in 0.1 M phosphate buffer of pH 7.0 on GPGE. Inset: shows the calibration plot of current response versus 5-HTP concentration at the GPGE.

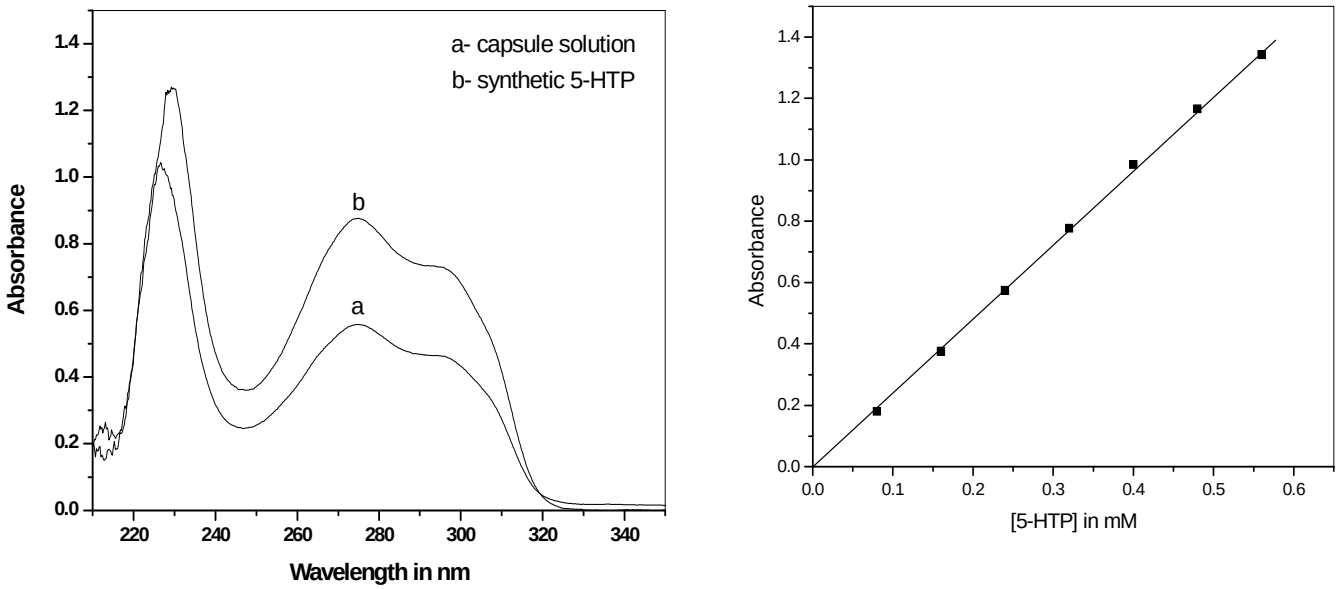

Fig. 4A. UV absorption patterns of 5-HTP in5-HTPη-50 stock solution (a) and (b) 5-HTP (left image). Fig. 4B.The calibration plot of absorbance versus 5-HTP concentration of 5-HTP (right image).

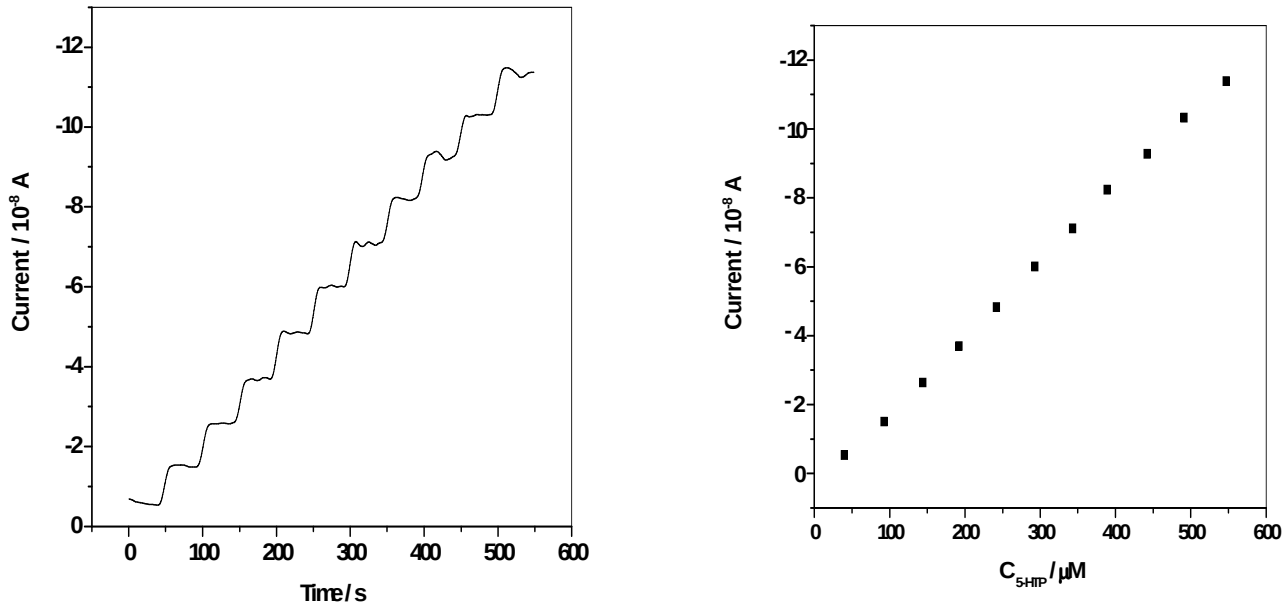

Fig.5A. Amperometric detection of 5-HTP by GPGE in 0.1 M PBS of pH 7.0, each addition of 0.5 µM (current was measured at constant potential of 368 mV in the time interval of 50 s in a stirred system). Fig. 5B. Calibration curve for the determination of 5-HTP at the GPGE by amperometric technique.

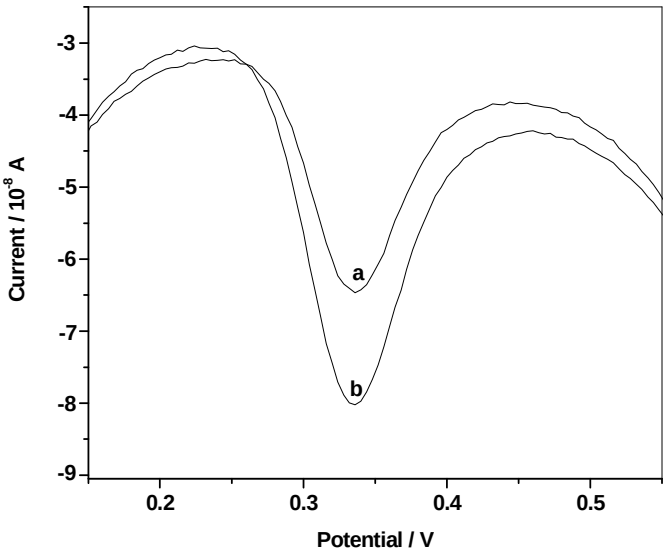

Fig. 6. DPV curves of 5-HTPη-50 stock solution (a) and addition of 10 µM 5-HTP spiked to 5-HTPη-50 stock solution (b) in 0.1 M phosphate buffer of pH 7.0 on GPGE.

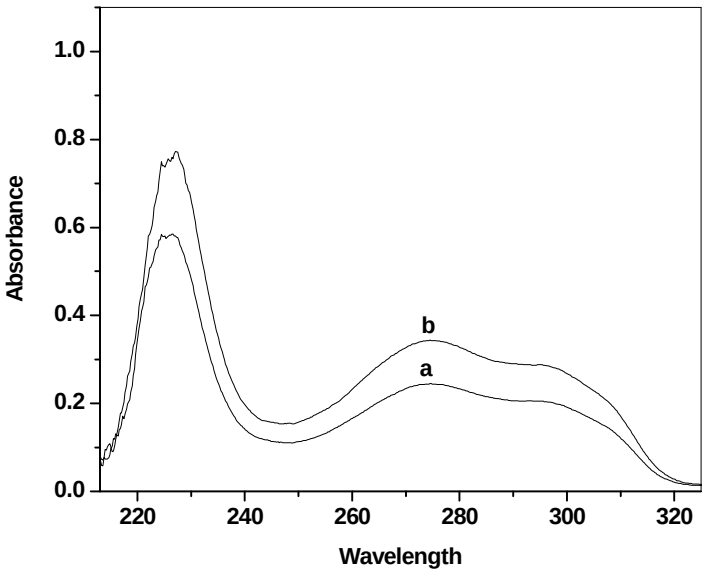

Fig. 7. UV absorption spectra of 5-HTPη-50 stock solution (a) and spiked with 40 µM synthetic 5-HTP into 5-HTPη-50 stock solution (b).

CONCLUSION

In the present study the amperometric and differential pulse voltammetric determination of 5-HTP present in the real sample (5-HTPη-50 tablet) using GPGE has been demonstrated. The modified electrode showed a good reproducibility and fast response for the determination of 5-HTP. The larger surface area and good catalytic activity of gold nanoparticles are responsible for the improved performance of GPGE. The observed DPV and amperometric result is consistent with UV-Vis spectrophotometric results. The results of the present work indicated that the modified electrode is suitable for quantative determination of 5-HTP. The method described is rapid, simple and sensitive and it can be used for the electrochemical estimation of 5-HTP from pharmaceutical samples.

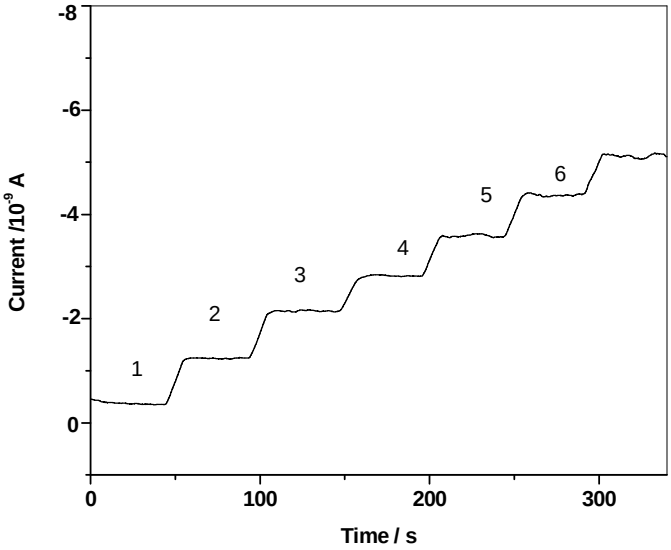

Fig. 8. Typical amperometric response of GPGE to successive addition of 5-HTP (0.5 µM) (1-2 steps), 20µL 5-HTPη-50 stock solution (3-4 steps) and spike which is the mixture of 5-HTP and 5-HTPη-50 stock solution (5-6 steps).

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