Glassy carbon electrode modified with polyaniline based nanosensors for electrochemical determination of aurone flavonoid

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Abstract

The redox characteristics of the flavonoid aurone was determined at glassycarbon electrode (GCE) modified with polyaniline based nanosensors in human plasma. Here the determination of aurone by differential pulse voltammetry (DPV) and cyclic voltammetry (CV). In Phosphate buffer of pH 2.0–10.0, an irreversible and diffusion-controlled reduction wave was developed. The dependence of the CV response of the developed anodic peak on the sweep rate (ν) and on depolizer concentration was typical of an electrode-coupled chemical reaction mechanism (EC) in which an irreversible first-order reaction is interposed between the charges. The values of the electron-transfer coefficient (α) involved in the rate determining step calculated from the linear plots of in the pH range investigated. In Phosphate buffer of pH 5.0, a well defined reduction wave was developed and the plot of peak current height of the DPV against aurone concentration at this peak potential was linear in the range 1.5×10^{-6} to 2.25×10^{-3} $molL^{-1}$ with lower limits of detection (LOD) and quantification (LOQ) of 3.9×10^{-9} and 4.5×10^{-7} mol L^{-1}, respectively. A relative standard deviation of 1.8% (n=5) was obtained for the flavonoid. These DPV procedures were successfully used for analysis of aurone in human plasma. The method was successfully applied for the determination aurone in human plasma by differential pulse voltammetry using polyaniline doped glassy carbon electrode.

Key words: aurone, human plasma, polyaniline, differential pulse voltammetry and glassy carbon electrode.

INTRODUCTION

Flavonoids correspond to a large class of natural products in the plant kingdom, exhibiting numerous biological deeds [1]. aurones take part in an significant role in the pigmentation of some flowers and fruits and supply particularly to the bright yellow color of flowers [2]. Leishmaniasis, a contagious disease caused by protozoan parasites belonging to the genus Leishmania (L), is transmitted to humans from side to side the bite of female phlebotomine sand flies infected with the parasite. This disease is manifested in three forms: cutaneous leishmaniasis (CL), which is the most common form, mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL) [3]. VL is the most serious form of the disease which inevitably leads to death if left untreated [4]. Moreover aurones shows a strong and broad variety of biological activities. For instance they have been described as antifungal agents [5], as insect antifeedant agents [6], as inhibitors of tyrosinase [7], and as antioxidants [8]. The antileishmanial activity of a series of aurones were active against the parasites while they showed moderate cytotoxicity [9].

Recently, fabrication of electrodes for detection of trace heavy metals by means of conductive polymers have external substantial consideration due to their superior electrical conductivities, good adhesion properties and suitable structural characteristics [10,11]. Due to its facile preparation, high conductivity and good environmental stability [12], conductive polyaniline (PAN) can be electrochemically layered on the surfaces of glassy carbon electrodes (GCE) and forms an absorbent coating [13,14]. PAN doping is stable and remain intact for a long time as long as they are not mechanically broken [15]. The microstructure of PAN coatings can be controlled by fabrication methods and conditions such as temperature, monomer concentration, deposition potential and time, which then greatly influences their electrical conductivities [16].

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

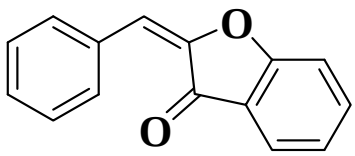


Fig.1 Molecular structure of aurone

Experimental

Reagents

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

Preparation of samples

An accurately weighed amount (5 mg) of aurone was quantitatively transferred into a 25mL calibrated flask, dissolved in 20 mL methanol, completed to volume with the same solvent to obtain a stock solution. This stock solution was further diluted till obtain a working standard solution 1.5×10^{-6}M.

Materials

Electrochemical studies were carried out by Autolab PG STAT 101 supplied by Metrohm, Autolab, Netherlands. electrochemical workstation, having a conventional three-electrode cell configuration with GCE or PAN/GCE of a diameter of 3 mm as the working electrode, saturated calomel electrode (SCE) as a reference electrode and platinum wire as a counter electrode obtained from Metrohm, was employed. An Elico LI-120 pH meter supplied by Elico Ltd, Hyderabad, India was used to determine the pH of the buffer solution.

Electrode preparation

Thoroughly polished GCE surfaces using alumina slurry on a soft cloth were sonicated in first ethanol and then doubly distilled water for 3 min each to remove possible contaminants. The PANI coatings were formed on the GCE surfaces by dipping the polished GCEs and electrochemically deposited at a constant potential of 0.80 V for 120 s in an aqueous solution of 0.1 M LiClO4 and 0.1 M carbonate containing 0.15 M polyaniline as well as in a 0.25M H2SO4 electrolyte containing 7.3 mM aniline monomers via a CV process from −0.2 to 0.9 V at a scan rate of 50 mV/s for cycles under a nitrogen environment. After the polymerization of PAN [17], the fabricated PAN/GCEs were dipped into doubly distilled water for 3 min to remove unpolymerized aniline monomers remaining in the PAN coatings if any. After each polishing, the electrode was sonicated in ethanol and doubly distilled water for 5 min, successively, in order to remove any adsorbed substances on the electrode surface. Finally, it was dried under nitrogen atmosphere ready for use. The electrode was then transferred into 0.1 M $HClO_4$ solution for 12 h aging. The polyaniline modified electrode was denoted as PAN/GCE.

Plasma sample Preparation

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

RESULTS AND DISCUSSION

Cyclic voltammetric studies

In order to understand the electrochemical process occurring at the polyaniline modified glassy carbon electrode, cyclic voltammetry was carried out. Aurone was reduced on PAN/GCE. The effect of pH on the cyclic voltammetry was investigated by recording the current v/s voltage curves for aurone in phosphate buffer systems over the pH range 2.0 to 12.0. Aurone exhibits a single well defined wave/peak in the pH range 2.0 to 6.0 throughout the study which corresponding to the reduction of carbonyl group. Typical cyclic voltammogram have been shown for aurone in Fig.2. Aurone was readily adsorbed onto the modified glassy carbon electrode. Fig.2 (c) displays a cyclic voltammogram of 1.5×10^{-6} M aurone in phosphate buffer pH 4.0 on a modified glassy carbon electrode. A well-defined peak observed at PAN/GCE than at bare GCE. A large definite cathodic peak, corresponding the reduction of the adsorbed aurone is observed at -0.96 V. No peaks are observed in the anodic scan, indicating that the aurone reduction is an irreversible process. The cathodic peak may be attributed

to the irreversible reduction of the carbonyl moiety of aurone, in accordance with the redox mechanism postulated in Scheme.

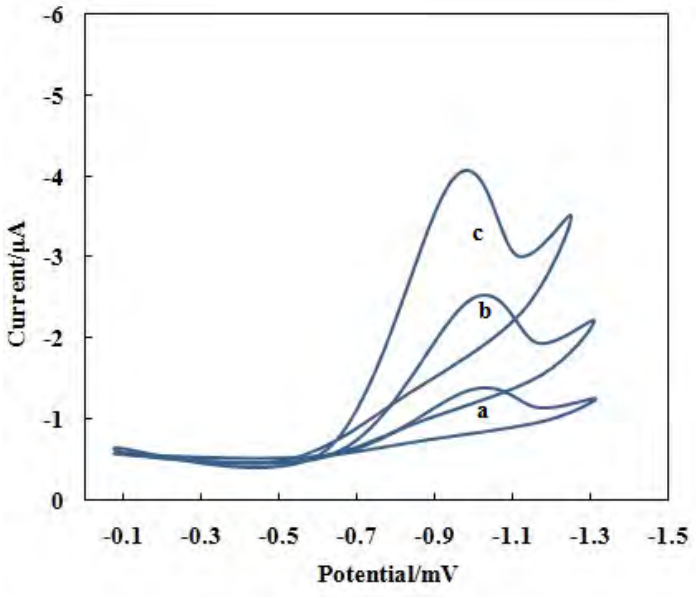


Fig.2 Typical cyclic voltammograms of aurone, concentration 1.5×10^{-6} M at (a) blank (b) bare GCE and (c) PAN/GCE.

Effect of scan rate

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

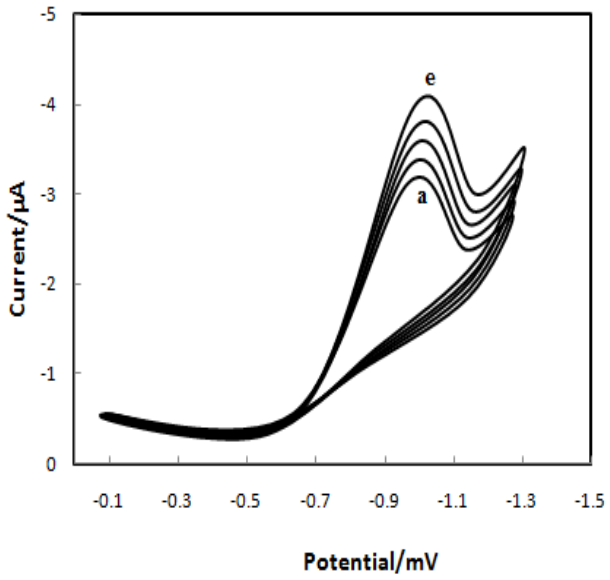


Fig.3. Effect of scan rate on peak current of aurone, concentration 1.5×10^{-6} M, scan rate (a) 20 mVs^{-1} to (e) 100 mVs^{-1} under optimum conditions.

Effect of pH

The influence of pH on the reduction behaviour of aurone was performed at different electrodes and different pH values using differential pulse voltammetry. The relation between pH verses current curves were shown in figure 4. It can be seen that the peak current reaches a maximum at pH 4.0. Therefore pH = 4.0 was selected as the optimum pH, at this pH, the sensitivity was highest and the peak was well defined. According to the structure of aurone, 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 aurone on the surface of the modified electrode in the accumulation step.

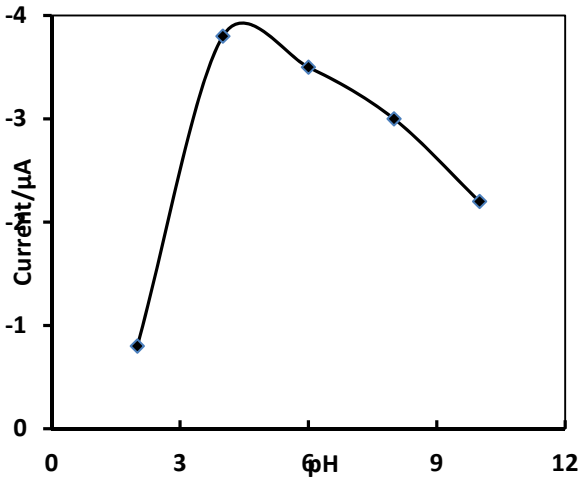


Fig.4. Effect of pH of aurone, concentration 1.5×10^{-6}M on peak signal.

Nature of the electrode process

The diffusion controlled nature of the electrode process is evidenced from the linear plots of i_p *vs.* $V^{1/2}$ that passes through the origin indicating the electrode process to be mainly diffusion controlled and adsorbed on the electrode surface in all the pH measurements. The experimental constancy of i_p / $v^{1/2}$ with scan rate (v) has shown the electrode process to be free from any kinetic complications. Conventional log-plot analysis and the variations of E_p values towards more negative potentials upon increasing the concentration of aurone indicate the irreversibility of electrode process.

Differential pulse voltammetric studies

The application of the differential pulse voltammetry for the determination of aurone in human plasma was investigated. Because the peaks are sharper and better defined at lower concentration of aurone, than those obtained by cyclic voltammetry, with low background current, resulting in improved resolution. According to the obtained results, it was possible to apply these techniques to the quantitative analysis of aurone. The direct determination of aurone in plasma was found to be possible by employing a high dilution of the sample with the supporting electrolyte. It is well known that DPV is suitable for the analysis of electrochemically active substances because relatively small difference in peak potentials of the analytes is needed for their determination. The peak current depends on pH of the medium, concentration and chemical composition of the buffer solution, and instrumental parameters. Differential pulse voltammograms of aurone at bare GCE and at PAN/GCE, different concentrations of aurone was also studied by DPV at PAN/GCE was shown in Fig.5.

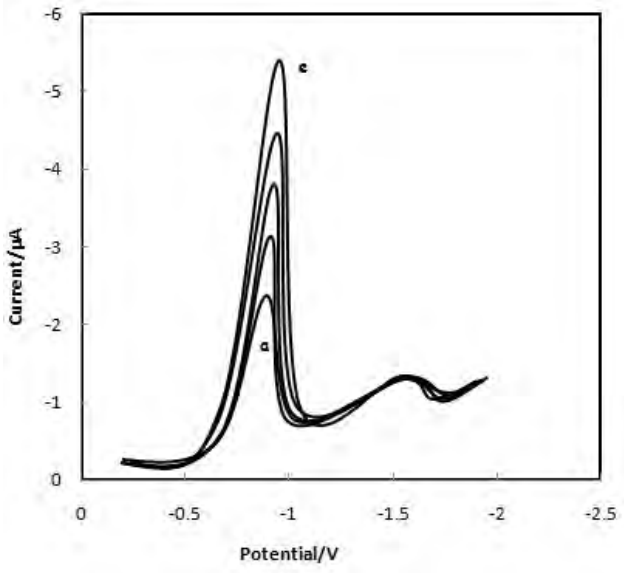


Fig.5. Differential pulse voltammogram of aurone at different concentrations at PAN/GCE from 20 µM to 100 µM (a to e)

Fig.5. displayed the differential pulse voltammetric response of aurone at GCE/PAN. Well resolved peaks proportional to the concentration of corresponding aurone was observed in the range of 20-100 µM. Limit of detection (LOD) was found to be 3.9×10^{-9} M. The peak current linearly increases with increase in concentration. In a similar manner, DPV studies of aurone at bare GCE and at modified systems were carried out. Recovery and relative standard deviations were calculated and results were presented in Table.1.

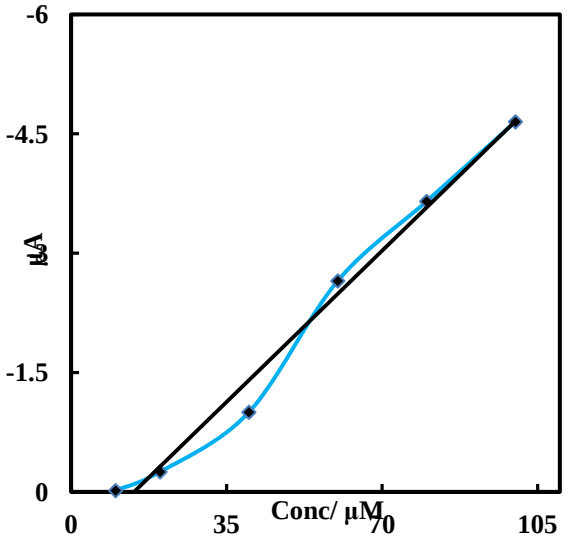


Figure. 6. DPV responses of aurone at PAN/GCE in PBS buffer solution with increasing concentrations of 10-90 µM.

An accurate volume (10 mL) of the phosphate buffer at the required pH 2.0–12.0 was transferred to the electrochemical cell and the electrodes were immersed in test solutions through which a stream of pure nitrogen was passed for 15 min before recording the voltammograms. The scans were initiated in the negitive direction of the applied potential from 0 V to -2 V. After recording the voltammogram of the blank solution, an accurate volume (0.5–2.0 mL) of the aurone solution was added. The anodic potential sweep was then recorded under different operating conditions of pH, sweep rate, and pulse amplitude. Before each measurement the GCE was polished manually with a paste of 0.5 mm alumina in distilled water on a smooth polishing cloth and gently dried with a tissue paper (31, 32). The effect of scan rate (ν=10–200 mV s^{-1}) on the voltammograms were determined using the same solution.

Recovery study

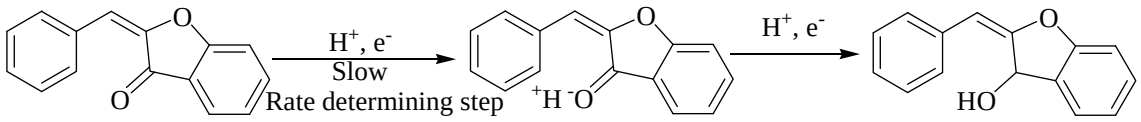
The recovery tests of aurone ranging from 1.5 x 10^{-6} to 1.0 x 10^{-4} M were performed using DPV. The results are listed in Table 1. The recoveries lie in the range from 97.3% to 99.5%. The relative standard deviation was 1.45%. The recovery test of aurone in spiked plasma samples at concentration 1.5 x 10^{-6} were performed by DPV, the results shown in table 1. The recoveries lie in the range from 98 % to 99 %.

Table.V. 2 Recoveries of aurone in spiked human plasma samples

Sample	Amount added in µg	Found µg	*Recovery (%)	Relative Standard deviation (%)
plasma sample1	5	4.96	99.20	1.764
	10	9.87	98.70	1.631
	15	14.80	98.66	1.629
plasma sample2	5	4.98	99.60	1.818
	10	9.91	99.10	1.625
	15	14.78	98.53	1.456

*Average of three determinations

Mechanism



Scheme.1 Electrochemical reduction mechanism of aurone

Conclusions

The voltammetric behavior of aurone was investigated at a PAN/GCE by CV in phosphate buffer solution (pH = 4.0). Based on the study, influence of several physicochemical parameters like potential scan rate, pH and concentration were investigated. The reduction was found to be an irreversible two-electron and two-proton process with diffusion character. The PAN/GCE shows excellent electrocatalytic activity towards the reduction of aurone at concentration 1.5×10^{-6} M under the optimum conditions. It exhibits irreversible cathodic peak at potentials over the pH 2.0–10.0 in phosphate buffer solutions. This method has been successfully used to determine aurone in the plasma sample. The proposed method offered the advantages of accuracy and time saving as well as simplicity of reagents and apparatus.

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