Effect of gold nanoparticle attached multi-walled carbon nanotube-layered indium tin oxide in monitoring the effect of paracetamol on the release of epinephrine

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ABSTRACT

A gold nanoparticle attached multi-walled carbon nanotube-layered indium tin oxide (AuNP/MWNT/ITO) electrode has been used for monitoring the effect of paracetamol (PAR) on the release of epinephrine (EPI) in human urine. The modified electrode shows an excellent electrocatalytic activity for the oxidation of EPI and PAR with acceleration of electron transfer rate as compared to MWNT/ITO and AuNP/ITO. An apparent shift of the oxidative potential towards less positive potential with a marked increase in peak currents is observed in square wave voltammetry at AuNP/MWNT/ITO electrode. The calibration curves for the simultaneous determination of PAR and EPI showed an excellent linear response, ranging from 5.0×10^{-9} mol L^{-1} to 80.0×10^{-9} mol L^{-1} for both the compounds. The detection limits for the simultaneous determination of PAR and EPI were found to be 46×10^{-10} mol L^{-1} and 42×10^{-10} mol L^{-1} respectively. The proposed method has been successfully applied for the simultaneous determination of PAR and EPI in human urine. It is observed that gold nanoparticles attached with multi-wall carbon nanotube catalyze the oxidation of EPI and PAR.

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1. Introduction

Epinephrine (EPI) (adrenaline, I) is one of the most crucial neurotransmitter which controls nervous chemical processes [1]. EP is considered as one of the emergency health care medicine to treat cardiac arrest, dysrhythmias and as a bronchodialator for asthma patients [2]. In perceived emergency situations, it prepares the body for, boosting the supply of oxygen and energy-giving glucose to the brain and muscles [3]. EP has also been found to elevate the blood sugar level by increasing catalysis of glycogen to glucose in the liver [3]. Due to these important actions of EP, it is considered as a potent doping agent and hence, it is also banned in competitive games by World Anti Doping Agency [4,5]. It is also used to treat anaphylaxis and sepsis because of its suppressive effect on the immune system [6]. In recent years, the change of EP concentration in nervous tissues and body fluids has been considered as diagnostic symptoms of several diseases [7]. The amount of EP present in blood, plasma or serum is considered to monitor therapeutic administration or to identify the causative agent in potential poisoning victims [8].

Paracetamol (PAR) (acetaminophen, II) is an analgesic and antipyretic drug. It is a safe and effective pain killer employed for the far-flung relief of moderate pain associated with backache, headache, arthritis and postoperative pain [9]. PAR intoxication results in the induction of a stress syndrome in the humans and hence increases EPI release from the adrenal medulla, thereby activating hepatic glycogenolysis [10]. Many methods have been reported for the determination of EPI, such as spectrophotometry [11], thermal-lens microscopy [12] and voltammetry [13], etc.

Multi-walled carbon nanotube (MWNT) exhibits fascinating electronic, chemical and mechanical properties with a wide range of possible applications [14]. In nanostructured network with unusual charge/mass transport mechanisms, nanoparticles could improve charge and mass transfer [15,16]. Thus, it was considered interesting to modify ITO surface with MWNT as well as by nanogold particles to get the benefit of both in electrocatalytic activity. In this study, we have described the use of gold nanoparticle-attached MWNT-layered indium tin oxide (AuNP/MWNT/ITO) electrode for the simultaneous electrochemical determination of PAR and EPI in biological fluids. As a feature of the present preparation method, carboxylated MWNTs were casted on ITO surfaces, in place of untreated MWNTs which caused a formation of homogeneous layer of MWNT. The use of carboxylated MWNTs derivatization is also effective for attaching AuNPs on the MWCT layer. It is also expected that AuNP/MWNT/ITO electrode has higher effective surface area and nanogold as well as MWNT layer will be useful for the effective electrocatalysis. The literature

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survey indicates that no attempt has been made to determine the effect of PAR on the release of EPI. Hence, in this paper, the effect of PAR on the release of EPI has been studied at this newly developed electrode.

(I)   **(II)**
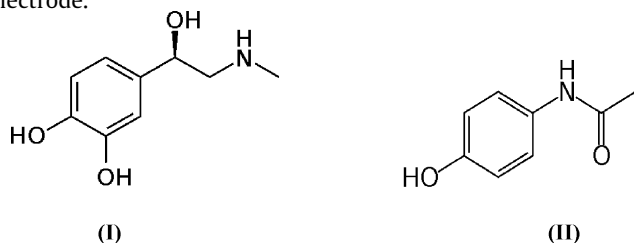
2. Experimental

2.1. Reagents

Indium tin oxide (ITO) electrode spurted glass sheets of size $10\,mm \times 20\,mm \times 1.1\,mm$ and resistivity $30\,\Omega\,cm^{-2}$ were obtained from Geomatec, Japan. MWNT (purity > 98%, outer diameter 10–15 nm and inner diameter 2–6 nm) and $HAuCl_4$ was purchased from Aldrich (USA). PAR was received as a gift from Sri Krishna Pharmaceuticals Ltd., Hyderabad and EPI was incured from Sigma Aldrich, Germany. Ascorbic acid was purchased from Wako pure chemicals industries Ltd., Japan. All solutions were prepared in double distilled water.

2.2. Instrumentation

The square wave voltammetric experiments were carried out at room temperature ($27 \pm 2\,°C$) using three-electrode single compartment cell equipped with a AuNP/MWNT/ITO electrode as working, platinum wire as counter and Ag/AgCl (3 M NaCl) as reference electrode. Phosphate buffers in the pH range 2.4–11.0 ($\mu = 1.0\,M$) were prepared according to the reported method [17]. BAS (Bioanalytical systems, West Lafayette, USA) CV-50W Voltammetric analyzer was used for measurements. The pH measurements were performed using a Century India Ltd. Digital pH-meter (Model CP-901) after due standardization with 0.05 M potassium hydrogen phthalate (pH 4.0 at 25 °C) and 0.01 M borax (pH 9.2 at 25 °C). Optimized square wave voltammetry (SWV) parameters used were: initial E: −200 mV, final E: 600 mV, square wave amplitude (E_{sw}): 25 mV, potential step (E): 4 mV, square wave frequency (f): 15 Hz. Cyclic voltammograms were recorded in the sweep range 10–1000 mV s^{-1} with initial sweep to positive potentials. The solutions were deoxygenated by bubbling high-purity nitrogen for 12–15 min before recording the cyclic voltammograms.

2.3. Procedures

Carboxylation of MWNT was carried out according to previous report for making them water soluble [18]. For preparing the gold nanoparticle (AuNP) solution, simply Au^{3+} ions were reduced to Au^0 by ascorbic acid (AA) [19,20]. In brief 50 mL of 2.2 mM AA (aq.) was added to 50 mL of 1.34 mM $HAuCl_4$ (aq.) under stirring. The change of colour solution from yellow to deep red indicated the formation of AuNP which was confirmed by recording FE-SEM images.

For modification of ITO, initially 100 μL of 1 mg/mL carboxylated MWNT (aq.) was dropped on clean ITO electrode (10 mm × 10 mm × 1.1 mm) and dried at 60 °C. Afterward, 100 μL solution of the synthesized AuNP was dropped on the MWNT-layered ITO, followed by drying at 60 °C. A AuNP/MWNT/ITO electrode is fabricated by connecting a piece of thus-prepared AuNP/MWNT/ITO with a strip of copper adhesive tape, and then covering with a scotch tape that is made to have a 2 mm-
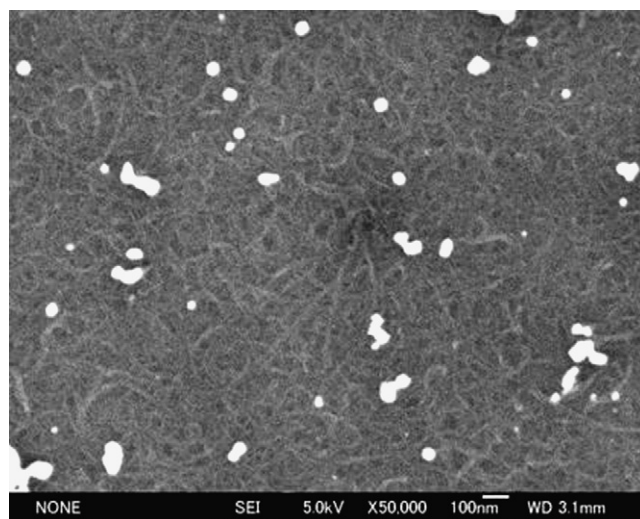

Fig. 1. A typical FE-SEM image of AuNP-attached multi-wall carbon nanotube-layered ITO surface used as working electrode.

diameter hole. Thus, the geometrical electrode surface area of the AuNP/MWNT/ITO was 0.0314 cm^2. The other modified ITO electrodes were fabricated in the same manner.

A typical FE-SEM image of the modified ITO electrode is presented in Fig. 1. The grain parts observed in white are AuNPs, and, on the background, it is recognized that the MWNT layer is formed homogeneously on the surface of ITO. In comparison with the SEM image observed just after the formation of only the MWNT layer (the data is not shown), we could confirm the formation of AuNPs after the treatment mentioned above.

The urine samples of smokers undergoing treatment with PAR in the hospital of Indian Institute of Technology Roorkee were collected after 4 h of oral administration of 500 mg tablet of PAR.

3. Results and discussions

3.1. Determination of surface area

To determine the efficacy of surface modification procedure, the surface area of bare AuNP/ITO, MWNT/ITO and AuNP/MWNT/ITO electrode was calculated. For this purpose, cyclic voltammograms were recorded for 1 mM $K_3Fe(CN)_6$ at different scan rates using 0.1 M KCl as the supporting electrolyte. A well defined redox couple due to the presence of Fe^{3+}/Fe^{2+}, was observed at all the three electrodes. The peak potentials of the redox couples were 296/190, 270/196, 242/188 mV at sweep rate of 50 mV s^{-1} at AuNP/ITO, MWNT/ITO and AuNP/MWNT/ITO electrodes respectively. Thus, the peak separation of anodic and cathodic peaks was minimum at AuNP/MWNT/ITO electrode indicating reversible system. There was an enhancement in the peak current values at AuNP/MWNT/ITO electrode in comparison to the bare AuNP/ITO and MWNT/ITO electrodes. For a reversible process, assuming semi-infinite planar diffusion, the peak current is defined as:

$$i_p = 0.4463 \left(\frac{F^3}{RT} \right)^{1/2} An^{3/2} D_R^{1/2} C_0 v^{1/2} \qquad (1)$$

where i_p refers to the peak current in Ampere, A is the electrode surface area in cm^2, F is Faraday's constant (96,485 C/mol) and R is the universal gas constant (8.314 J/mol K). For 1 mM $K_3Fe(CN)_6$, $n = 1$, $D_R = 7.6 \times 10^{-6}$ cm^2 s^{-1}, C_0 is the concentration of $K_3Fe(CN)_6$ in M and v is the scan rate in V s^{-1}. The slope of i_p versus $v^{1/2}$ plot was then used to calculate the surface area of bare AuNP/ITO, MWNT/ITO and AuNP/MWNT/ITO electrode which were found as
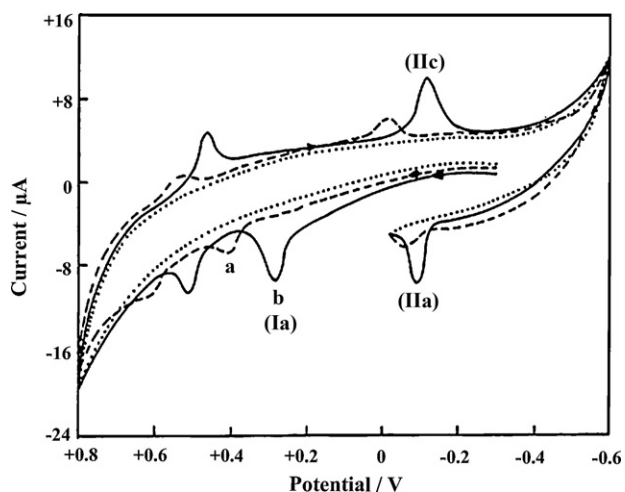
Fig. 2. Cyclic voltammograms of binary mixture of PAR (60 nM) and EPI (60 nM) using (a) MWNT/ITO (- - - -) and (b) AuNP/MWNT/ITO (—) electrode at pH 7.20, and dotted line is the response of AuNP/MWNT/ITO electrode in blank PBS of pH 7.20.

Fig. 3. Square wave voltammograms of binary mixture of PAR (40 nM) and EPI (40 nM) using bare MWNT/ITO electrode (- - - -), AuNP/ITO (-··-··-) and AuNP/MWNT/ITO electrode (—) at pH 7.20. The dotted line is the response of AuNP/MWNT/ITO electrode in blank PBS of pH 7.20.

0.0315 cm^2, 0.0614 cm^2 and 0.1247 cm^2 respectively. The effective working area of the AuNP/MWNT/ITO modified electrode is ∼4 and 2 times larger than that of the bare AuNP/ITO, MWNT/ITO electrode respectively, thereby, indicating that the bare MWNT/ITO electrode has been modified efficiently by nanogold particles. In recent studies, the increased dc current signals in cyclic voltammetry have been observed for the ferri/ferro cyanide couple at nanotubes modified glassy carbon electrode. Such an increase in current has been assigned to the effect of diffusion within a porous thin layer. Thus, at AuNP/MWNT/ITO electrode it seems reasonable to conclude that apart from semi-infinite planar diffusion of ferri/ferro cyanide at the nanotubes modified electrode, thin layer diffusion effects are also likely to operate.

3.2. Electrooxidation of PAR and EPI

3.2.1. Cyclic voltammetry

The cyclic voltammograms for the binary mixture of 60 nM PAR and EPI were recorded at the MWNT modified ITO (MWNT/ITO) and AuNP/MWNT/ITO electrode in 1 M phosphate buffer solution at pH 7.2. A typical voltammogram observed is illustrated in Fig. 2. On going to positive potentials, well defined oxidation peaks at ∼508 mV and ∼300 mV were obtained for PAR and EPI respectively at AuNP/MWNT/ITO electrode, which shifted to more positive potentials with a marked decrease in peak current at the MWNT/ITO electrode. These results clearly reveal that not only the kinetic factor but also the variation of adsorption structure can shift the oxidation potential of both EPI and PAR. In the reverse sweep, EPI exhibits a reversible couple with peak potentials of −118(II$_c$)/−74(II$_a$) mV. At AuNP/MWNT/ITO electrode, a well defined oxidation peak of PAR is obtained at 508 mV which formed a quasi-reversible couple with peak at 469 mV. To ascertain the nature of electrode reaction, sweep rate studies were performed in the range 10–1000 mV s^{-1}. The peak current of PAR and EPI was found to increase with increasing sweep rates and the plot of $i_p/v^{1/2}$ versus log v clearly indicated that the electrode process is adsorption controlled [21,22]. As SWV is considered to be a more sensitive technique in comparison to cyclic voltammetry, hence it is used for the further determination of PAR and EPI.

3.2.2. Comparison at bare gold nanoparticle modified indium tin oxide (AuNP/ITO) and AuNP/MWNT/ITO electrodes

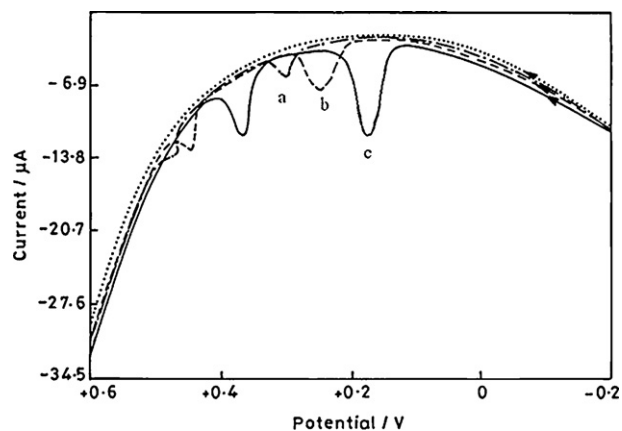Initially square wave voltammograms were recorded for a binary mixture of EPI and PAR at AuNP/ITO, MWNT/ITO and AuNP/MWNT/ITO working electrodes at pH 7.2 as shown in Fig. 3. At all the three electrodes, two well defined peaks were observed corresponding to the oxidation of EPI and PAR. The shape of peaks at AuNP/ITO electrode was rather broad and peak potentials were slightly more positive as compared to MWNT/ITO electrode. However, the two well-separated peaks with shift of peak potential towards less positive potential with a significant enhancement in peak current at AuNP/MWNT/ITO electrode clearly revealed that the proposed voltammetric sensor acts as a very efficient promoter to enhance the kinetics of the electrochemical process as compared to MWNT/ITO and AuNP/ITO electrodes. Hence, AuNP/MWNT/ITO electrode has been utilized for further detailed studies.

3.3. Individual determination of PAR and EPI

3.3.1. Effect of pH

The effect of pH on the oxidation of EPI and PAR was studied in the pH range 2.4–11.0 using SWV. It was found that the peak potential shifted towards less positive potentials with increase in pH. The dependence of E_p on pH obeys the relation:

$$E_p = [549.89 - 52.987 \text{ pH}] \text{ mV versus Ag/AgCl for EPI} \quad (2)$$

$$E_p = [766.34 - 53.618 \text{ pH}] \text{ mV versus Ag/AgCl for PAR} \quad (3)$$

having correlation coefficient 0.994 for both the analytes. The slope of E_p versus pH plots for EPI and PAR is close to 60 mV pH^{-1}, hence, suggests that equal number of protons and electrons are involved in the electrode reactions.

3.3.2. Effect of square wave frequency

The dependence of oxidation peak current (i_p) on the square wave frequency (f) for EPI or PAR was studied in the range of 5–100 Hz. The peak current was found to increase linearly with square wave frequency for both the analytes. The linear relation between i_p and f for EPI and PAR can be expressed by the equation:

$$i_p(10^{-5} \text{ A}) = 0.0248f + 0.1455 \quad \text{for EPI} \quad (4)$$

$$i_p(10^{-5} \text{ A}) = 0.0183f + 0.1044 \quad \text{for PAR} \quad (5)$$

having correlation coefficient 0.997 and 0.996 for EPI and PAR respectively. This behavior indicates that the oxidation occurs at the surface of AuNP/MWNT/ITO electrode and the electrode reactions are governed by the adsorption process for both the compounds [23–25].
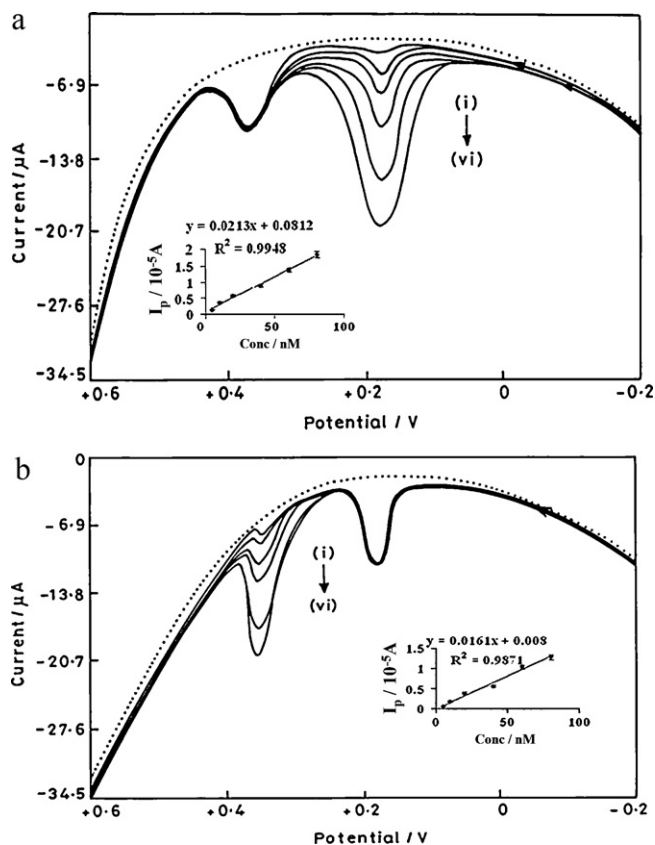
Fig. 5. Square wave voltammograms of (i) phosphate buffer solution (·····) and (ii) urine sample, as control, of patient not consuming PAR (—–), (iii) urine sample of patient treated with PAR (-·-··-·-), (iii) urine sample of patient spiked with known concentration of PAR and EPI (—) at pH 7.2 using AuNP/MWNT/ITO electrode.

by the addition of EPI and the peak current of EPI increased. Similarly, the concentration of PAR was varied in the concentration range 5.0–80.0 nM keeping the concentration of EPI fixed at 40 nM as shown in Fig. 4b. The oxidation peak current of PAR increased whereas, the peak current of EPI remained unaltered. The current observed in both the cases for varied components was same as observed during the individual compound study and obeyed the calibration plot. These interesting and new results prompted us to use the proposed voltammetric sensor for the simultaneous determination of EPI and PAR in human body fluids.

3.5. Stability and reproducibility of the modified electrode

The long-term stability of the AuNP/MWNT/ITO electrode was evaluated by measuring the voltammetric current response of fixed concentration of EPI (60 nM) and PAR (60 nM) after the modified electrode was stored for approximately 1 week. Only a minimal decrease of current sensitivity with a relative standard deviation (R.S.D.) of ~3.82% for PAR and 4.45% for EPI was observed. This indicated excellent stability of the modified electrode. The reproducibility of the modified electrode has also been investigated by evaluating intra-day and inter-day precision. The R.S.D. for both PAR and EPI were found to be in the range of 0.74–4.48%. Thus, it demonstrated the good reproducibility of the method.

3.6. Effect of interferents

Ascorbic acid, uric acid, xanthine and hypoxanthine are common biological metabolites present in living systems which can intervene in the electrochemical studies of PAR and EPI by influencing their peak potential and peak current response. The effect of these metabolites on the voltammetric peak response of 60 nM PAR and 60 nM EPI was studied. The tolerance limit was defined as the concentrations of foreign substances, which gave an error less than ±5.0% in the detection of the drug. It was observed that up to 10-fold excess of each of the interferents there was no remarkable change in the peak current response. This indicates that the method can be safely applied to the determination of PAR and EPI in biological fluids.

3.7. Real sample analysis

3.7.1. Determination of PAR and EPI in human urine
The effect of PAR intake on release of EPI was examined in human urine samples obtained from patients undergoing treat-

Fig. 4. (a) Voltammograms of a binary mixture of PAR and EPI, keeping the concentration of PAR constant (40 nM) and concentration of EPI was (i) 5, (ii) 10, (iii) 20, (iv) 40, (v) 60 and (vi) 80 nM at pH 7.2 (—) using AuNP/MWNT/ITO electrode and dotted line is the response of AuNP/MWNT/ITO electrode in blank PBS of pH 7.20. The observed calibration curve is presented as inset. (b) Voltammograms of a binary mixture of PAR and EPI, keeping the concentration of EPI constant (40 nM) and concentration of PAR is (i) 5, (ii) 10, (iii) 20, (iv) 40, (v) 60 and (vi) 80 nM at pH 7.2 (—) using AuNP/MWNT/ITO electrode. The dotted line is the response of AuNP/MWNT/ITO electrode in blank PBS of pH 7.20. The observed calibration curve is presented as inset.

3.3.3. Concentration study

The peak current (i_p) was found to be linearly dependent on concentration in the range of 5–80 nM for EPI and PAR. The linear regression equations having correlation coefficients 0.995 and 0.987 for EPI and PAR respectively are presented as:

$$i_p(10^{-5} \text{ A}) = 0.0213C(\text{nM}) + 0.081 \quad \text{for EPI} \tag{6}$$

$$i_p(10^{-5} \text{ A}) = 0.0161C(\text{nM}) + 0.008 \quad \text{for PAR} \tag{7}$$

where C is the concentration of EPI and PAR. The detection limits were calculated by using the relation $3\sigma/b$, where σ is the standard deviation of the blank and b is the slope of the calibration curve. The detection limits of EPI and PAR determination were calculated as 42×10^{-10} M and 46×10^{-10} M and limit of quantification were found as 13.9×10^{-9} M and 15.3×10^{-9} M respectively. The sensitivities of EPI and PAR determination were calculated as 213 and 161 nA nM^{-1} respectively.

3.4. Simultaneous determination of EPI and PAR

The main objective of the present investigation is to monitor the effect of PAR on the release of EPI. Hence it was necessary to simultaneously determine the concentration of EPI and PAR using AuNP/MWNT/ITO electrode. Firstly, the concentration of PAR was kept constant at 40 nM and EPI was varied in the range 5.0–80.0 nM as shown in Fig. 4a. The oxidation peak of PAR remained unaltered
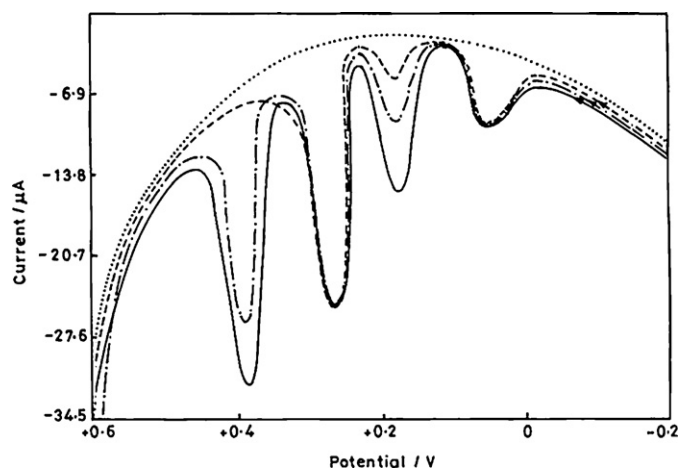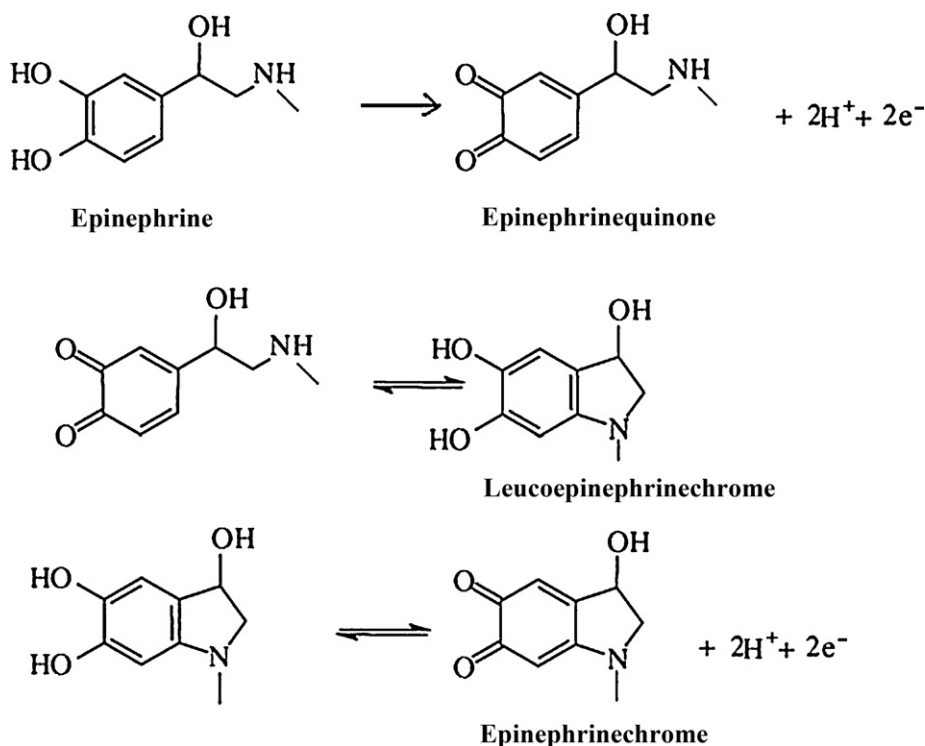
Scheme 1.

ment with PAR. The samples were used for analysis after 10 times dilution with phosphate buffer solution (pH 7.2) to minimize matrix complexity. PAR intoxication has been reported in the induction of a stress syndrome [10] and also increases brain serotonin (5-HT) levels in the humans, which plays a crucial role in the release of catecholamines in the body [26]. Fig. 5 presents voltammograms observed for the control urine sample. A peak at ∼183 mV was observed corresponding to oxidation of EPI. The other peaks in urine samples are due to the presence of major endogenous metabolites i.e. ascorbic acid and uric acid. While the urine sample collected from the patients undergoing treatment with PAR exhibited a well-defined peak ∼380 mV corresponding to oxidation of PAR along with an enhanced additional peak at ∼183 mV, which confirmed that PAR intoxication leads to the ooze out of EPI in human urine. The average increase in EPI for three samples was found to be ∼155%. To confirm that the peak at ∼183 and 380 mV are due to EPI and PAR respectively, voltammograms were also recorded after

spiking urine sample with known concentrations of PAR and EPI. To reconfirm the results urine samples of three patients were spiked with known concentrations of standard PAR and EPI and in all the cases the peak at ∼183 mV was increased. The results obtained for urine samples of three different patients are tabulated in Table 1.

4. Conclusions

This work describes an extremely sensitive electroanalytical procedure for simultaneous determination of PAR and EPI based on SWV utilizing AuNP/MWNT/ITO electrode. The modified electrode not only shifted the peak potentials of the oxidation of PAR and EPI towards lower positive values but also increased the peak currents significantly in comparison to bare AuNP/ITO electrode. The oxidation of EPI occurs in a 2e, 2H+ reaction at hydroxyl groups to give corresponding o-quinone. The reversible couple observed is attributed to the formation of epinephrinechrome/leucoepinephrinechrome couple as shown in Scheme 1 [27,28]. The electrocatalytic activity of MWNT has been assigned to the embedded metallic impurities [29,30]. The increased dc current observed for the ferri/ferro cyanide couple at nanotubes modified glassy carbon electrode has been assigned to the effect of diffusion within a porous thin layer [31,32]. Thus, at AuNP/MWNT/ITO electrode it seems reasonable to conclude that apart from semi-infinite planar diffusion at the nanotubes modified electrode, thin layer diffusion effects are also likely to operate. However, the nanogold particles appear to further catalyze the oxidation due to their high specific surface area, superhydrophobicity and surface enhanced Raman scattering [33].

The modified electrode showed a stable and reproducible response towards the simultaneous determination of both the compounds. The concentration of EPI in normal human urine (control) has been reported as 60 nM [34], where as in smokers it is ∼105 nM [28]. The studies reveal that oral administration of a single tablet of 500 mg of PAR causes an increase in EPI release by ∼5 times in urine as compared to control.

Table 1
Simultaneous determination of EPI and PAR in urine samples of patients treated with PAR using MWNT/AuNP/ITO electrode.

Added	Paracetamol (μM)			Epinephrine (μM)		
	Found[a]	Actual	Recovery	Found[a]	Actual	Recovery
Sample 1						
0.00	1.20	1.20	–	0.31	0.31	–
0.30	1.55	1.25	103.33	0.63	0.33	103.28
0.50	1.68	1.18	98.82	0.85	0.35	104.94
Sample 2						
0.00	1.23	1.23	–	0.36	0.36	–
0.30	1.55	1.25	101.30	0.67	0.37	101.51
0.50	1.70	1.20	98.27	0.84	0.34	97.67
Sample 3						
0.00	1.18	1.18	–	0.33	0.33	–
0.30	1.51	1.21	102.03	0.65	0.35	103.17
0.50	1.70	1.20	101.19	0.81	0.31	97.59

[a] The R.S.D. value for the determination of EPI and PAR was less than ±3.2% for n = 3.

Acknowledgements

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