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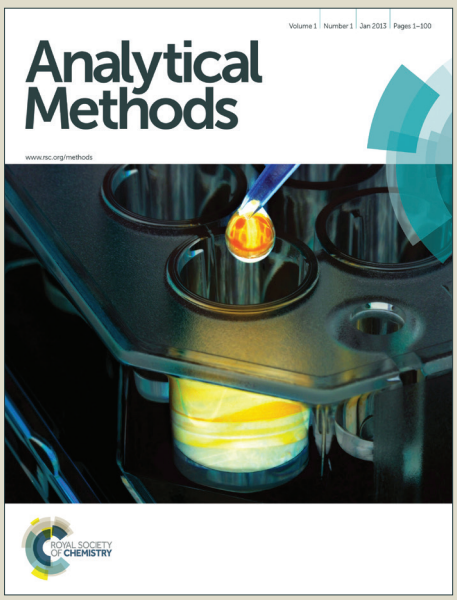

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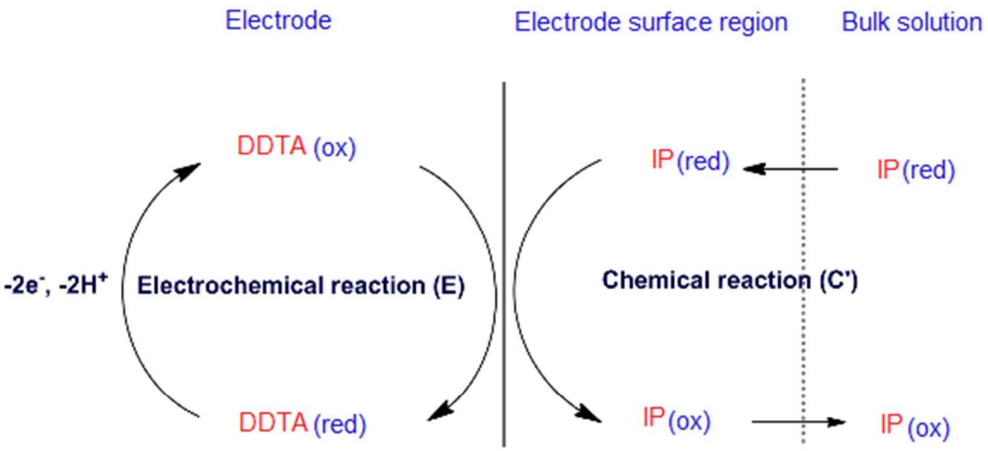

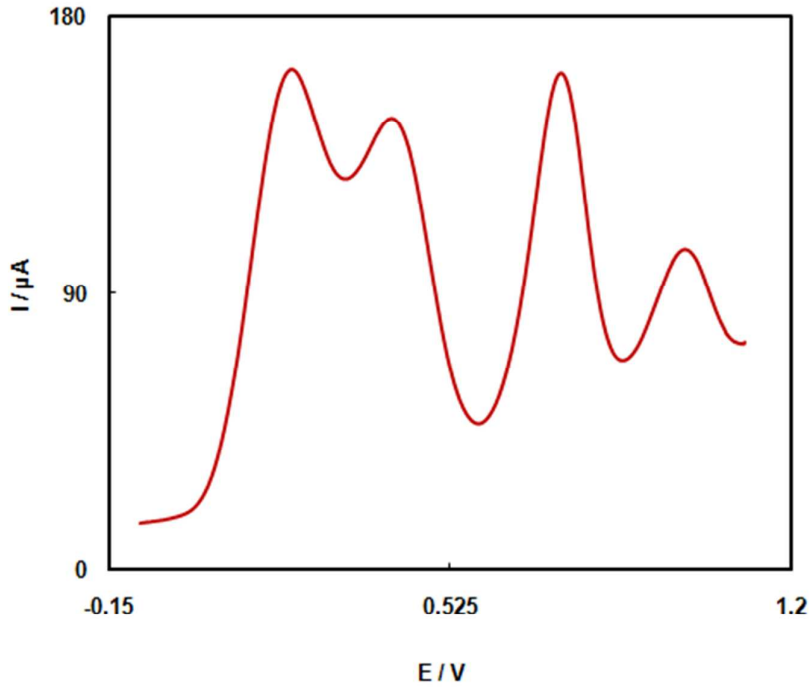

A chemically modified electrode with hydroquinone derivative based on carbon nanoparticles for simultaneous determination of isoproterenol, uric acid, folic acid and tryptophan

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Abstract

A nanostructure electrochemical sensor with carbon paste electrode has been used to study the electrocatalytic oxidation of isoproterenol (IP) in the presence of uric acid, folic acid and tryptophan. In this work carbon nanoparticles (CNPs) and 7-(3, 4-dihydroxyphenyl)-10,10-dimethyl-9,10,11,12-tetrahydrobenzo[c]acridin-9 (7H)-one (DDTA) were used for investigation of electrochemical behaviour of IP. The apparent charge transfer rate constant of k_s=1.99 s^{-1}, and transfer coefficient of $\alpha = 0.51$, for electron transfer between the modifier and the carbon paste electrode were calculated. In the optimum pH (pH = 9.0) the oxidation of IP at modified electrode occurs at a potential about 298 mV less positive than unmodified carbon paste electrode. The catalytic rate constant (k = 134.28 M^{-1} s^{-1}) and diffusion coefficient (D= 3.7×10^{-5} cm^2 s^{-1}) were calculated for IP by chronoamperometry. Differential pulse voltammetry (DPV) exhibited two linear dynamic ranges of 0.25 to 20.0 μM and 20.0 to 2000.0 μM for IP. There was a decrease in sensitivity of the second linear segment due to kinetic limitation. Also the modified electrode was used to determine IP in the presence of uric acid (UA), folic acid (FA) and tryptophan (TRY) by differential pulse voltammetry. Simultaneous determination of these four compounds was performed on the proposed sensor for the first time. This sensor was used for the determination of IP in the ampoule sample. The detection limit of the IP was calculated as 0.075 μM.

Keywords: Carbon paste. Carbon nanoparticles. Isoproterenol. Uric acid. Folic acid, Tryptophan

1. Introduction

Modified electrodes have been developed in electrochemistry since 1970s. In these electrodes conducting support material is used in order to produce an electrode suited to a particular function, whose properties are different from those of the unmodified electrodes. Unmodified electrodes have disadvantages such as high overvoltage, slow kinetics, etc. Due to these

problems, chemically modified electrodes have received great attention in recent years.[1-3] Because of unique physical and chemical properties such as high sensitivity, stability and large catalytic surface nanomaterials are used in the chemically modified electrodes.[4,5]

Isoproterenol (IP) is a catecholamine drug that is widely used for bronchitis, cardiac shock and heart attack. Nevertheless, the excess of the drug may cause heart failure and arrhythmias.[6] Some methods have been reported for the determination of IP such as liquid and gas chromatographic methods based on fluorimetry[7], spectrometry[9], and electrochemical detection methods. [8, 10, 11]

Uric acid (2,6,8-trihydroxypurin, UA) is major product of the catabolism of the purine nucleosides, adenosine and guanosine. Purines from catabolism of dietary nucleic acids are converted to uric acid directly. Abnormal levels of uric acid in body fluids will lead to some diseases. Therefore some electrochemical methods for the determination of uric acid were established.[12, 13]

Folic acid (FA) or B_9 vitamin that helps build healthy cells and its biological importance is due to tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid in the liver. Because of FA is an electro active compound, some electrochemical methods have been reported for its determination.[14, 15]

Tryptophan (TRY) is a precursor of the neurotransmitter serotonin.[16] Also, tryptophan is an essential amino acid for humans and herbivores scarcely present in vegetable products. It is a vital constituent of proteins and indispensable to human nutrition for establishing and maintaining a positive nitrogen balance. Some electrochemical methods have been reported for the determination of TRY.[17,18]

Simultaneous determination of IP, UA, FA and TRY is important because the mentioned compounds are vital biomedical compounds and have an important role in human metabolism. During the present study (with attention to the our previous studies)[12-15] we prepared a novel nanostructure electrochemical sensor for simultaneous determination of IP, UA, FA and TRY. According to our knowledge no study has been reported on the simultaneous determination of these four compounds with carbon paste electrodes (CPEs) modified by CNPs and DDTA. Therefore, in the present work, we introduced initially the preparation of a CPE modified with both DDTA and CNPs (DDTA/CNPs/CPE) as an electrode in the electrocatalysis and determination of IP and then we evaluated the analytical performance of the modified electrode for simultaneous determination of IP, UA, FA and TRY. The DDTA/CNPs/CPE was used to resolve the overlapping of the anodic peaks of these compounds in DPV. This modified electrode was quite effective not only to detect IP,

UA, FA and TRY but also in simultaneous determination of these compounds in a mixture. The detected peak potential difference between these four compounds at this electrode is large enough to allow simultaneous determination of isoproterenol, uric acid, folic acid and tryptophan in mixtures without significant interferences. In addition high sensitivity, selectivity and low detection limit, with the ease of preparation and the reproducibility of electrode, make the proposed modified electrode very suitable for voltammetric detection of IP. The detection limit and linear range for IP in this paper are comprisable with previously reported works.

2. Experimental

2.1. Apparatus and reagents

The electrochemical experiments were carried out using a potentiostat/galvanostat μAutolab Type III (Eco Chemie B. V.) with GPES 4.9 software coupled with a personal computer. A modified electrode (DDTA/CNPs/CPE) was used as working electrode. An Ag/AgCl (KCl, sat.) electrode and a platinum wire were used as reference and auxiliary electrode, respectively. pH measurements were carried out with a Metrohm model 691 pH/mV meter. All solutions were freshly prepared with double distilled water. IP, UA, FA, TRY and other reagents were analytical grade (Merck, Darmstadt, Germany). Phosphate buffer solutions (0.1 M) were prepared from 0.1 M H_3PO_4–NaH_2PO_4, and the pH was regulated with 0.1 M H_3PO_4 or NaOH. Graphite powder (Merck, Darmstadt, Germany) and paraffin oil (DC 350, Merck, and Darmstadt, Germany) were used as binding agents for graphite pastes.

2.2. General procedure for the synthesis of 7-(3,4-dihydroxyphenyl)-10,10-dimethyl-9,10,11,12-tetrahydrobenzo[c]acridin-8(7H)-one

A mixture of dimedone (1 mmol), 3, 4- di hydroxybezaldehyde (1 mmol) 1-naphthylamine (1mmol) and BM-400 (50 mg) was refluxed in EtOH (4 mL). After completion of the reaction (monitored by TLC, eluent; n-hexane:EtOAc, 7:3), the catalyst was separated and washed with EtOH (3×2 mL). Further purification was achieved by recrystallization in EtOH. MP: 242-244 °C,

Spectroscopic data: FT-IR (KBr) υmax: 3492, 3169, 3046, 2957, 1643, 1605, 1480, 1282, 758 cm^{-1}. ^{1}HNMR (400 MHz, CDCl3): δ=1.02(s, 3H), 1.08 (s, 3H), 2.06 (d, 2H, J=16 Hz), 2.23 (d, 2H, J=16 Hz), 2.63 (d, 2H, J=16 Hz), 2.72 (d, 2H, J=16 Hz), 5.01 (s, 1H), 6.47 (d, 1H, J=8 Hz), 6.50 (t, 1H, J=8 Hz), 6.59 (d, 1H, J=8 Hz), 7.57 (d, 1H, J=8 Hz), 7.57 (d, H, J=8

Hz), 7.83 (d, 1H, J=8 Hz), 8.40 (d, 1H, J=8 Hz), 8.50 (1H, OH), 8.58 (1H, OH), 917 (s, 1H, NH).

2.3. Preparation of modified working electrode

In order to prepare the carbon paste a mixture of 0.48 g of graphite powder, 0.005 g of DDTA, 0.015 g of CNPs and ~ 0.7 mL of paraffin oil was blended by hand mixing in a mortar and then paste was packed in to the end of a glass tube (internal radius: 2 mm and 10 cm long). A copper wire inserted into the carbon paste provided an electrical contact. A fresh electrode surface was generated rapidly by extruding the paste with a small copper plug and smoothing the resulting surface on white paper until a smooth shiny surface was observed.

2.4. Procedure of real samples preparation

One mL of an IP ampoule (0.2 mg mL^{-1}, Galena Siena Srl, Italy) was diluted to volume 10.0 mL by phosphate buffer solution (pH=9.0). This solution was used for the analysis.

3. Results and discussion

3.1. Electrochemical characteristics of DDTA/CNPs/CPE

The DDTA that is used in this research is insoluble in aqueous solution and it can be incorporated into carbon paste without leaching from the surface electrode. Therefore DDTA/CNPs/CPE was prepared and its electrochemical properties were investigated in an aqueous solution using cyclic voltammetry (CV). We anticipated that the electrochemical response of mediator would depend on pH, since DDTA has a hydroquinone structure. The effect of pH on the electrochemical behavior of a modified electrode in 0.1 M phosphate buffer solutions with different pH values was studied by CV. As can be seen in the inset of Figure 1 the formal potential ($E^{o'}$) of DDTA/CNPs/CPE was pH dependent. Since one straight line was obtained with a slope value of -53.5 mV per pH in the pH ranges of 4.0–11.0, there is a transfer of two electrons and two protons in the redox reaction of DDTA in the pH range of 4.0–11.0.[19]

Here Figure1

The effect of the potential scan rate on electrochemical behavior of the DDTA/CNPs/CPE was studied in 0.1 M phosphate buffer solution (Figure 2). The anodic and cathodic peaks potentials (E_{pa} and $E_{pc)}$ were 0.11 and 0.01 V respectively. The peak separation potential, $\Delta E_p = (E_{pa} - E_{pc}) = 0.10$ V is more than excepted value for a reversible system. The apparent charge transfer rate constant, k_s, and the transfer coefficient, α, of a surface-confined redox

modifier were evaluated from CV experiments and by using the variation of anodic and cathodic peak potentials with the logarithm of the scan rate, according to the procedure of Laviron.[20] We found out that the E_p values are proportional to the logarithm of the potential scan rate, for scan rates higher than 100 mV s^{-1} (Figure 2C). The slopes of the plots can be used to extract the kinetic parameter α. The slope of the linear segment is equal to $2.303RT/(1-\alpha_a)\, n_\alpha F$ for the anodic peaks and $2.303RT/\alpha_c n_\alpha F$ for the cathotic peaks. The calculated value for the average transfer coefficient (α) is 0.51. The following equation can be used to determine the electron transfer rate constant between the modifier (DDTA) and CPE:

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log(RT/n_\alpha F v) - \alpha(1-\alpha)n_\alpha F\Delta E_p/2.3RT \quad (1)$$

The value of k_s was evaluated as 1.99 s^{-1} using eq. (1). Since the electron transfer rate constant between DDTA and CPE was about 1.99 s^{-1}, it can be used as electron transfer mediator for electrocatalytic processes. As can be seen in Figure 2D, the plots of the anodic and cathodic peak currents (I_p) were linearly dependent on ν from 30 mV s^{-1} to 800 mV s^{-1}, indicating a surface-confined redox process. An approximate estimate of the surface coverage of the electrode was made by adopting the method used by Sharp.[21] According to this method, the peak current is related to the surface concentration of the electroactive species, Γ, by the following equation:

$$I_p = n^2F^2A\Gamma v/4RT \quad (2)$$

where n represents the number of electrons involved in the reaction, A is the surface area (0.096 cm^2) of the electrode, Γ (mol cm^{-2}) is the surface coverage, and the other symbols have their usual meanings. From the slope of the anodic peak currents versus the scan rate (Figure 2D), the calculated surface concentration of is 3.27×10^{-10} mol cm^{-2} for n = 2.

Here Figure 2

3.2. Electrocatalytic oxidation of isoproterenol at the DDTA/CNPs/CPE

Electrocatalytic oxidation of IP at DDTA/CNPs/CPE can be seen in Figure 3. For comparison between different electrodes the CVs of DDTA/CNPs/CPE, DDTA/CPE, CNPs/CPE and bare CPE were recorded in a buffered aqueous solution (pH=9.0) in the presence and absence of IP. Figure 3 shows a decrease in the over potential of IP oxidation from 430 mV obtained at a bare CPE to 132 mV at a DDTA/CNPs/CPE. In addition, an approximately three-fold increase in oxidation peak current of IP was obtained when a DDTA/CNPs/CPE was used relative to a bare CPE. Figure 3a demonstrates that the electro-oxidation of IP can be

catalyzed by DDTA/CNPs/CPE since the anodic peak current of the modifier was very increased in the presence of IP and the cathodic peak of the modifier disappeared on the reverse scan of the potential. This behavior is typical of that expected for electrocatalysis at chemically modified electrodes. Figure 3b shows oxidation of IP at a bare electrode that exhibits a peak current lower than that observed with DDTA/CNPs/CPE and CNPs/CPE. So, a decrease in the overpotential of ca. 298 mV and an enhancement of peak current are obtained with the modified electrode. Figure 3c indicates that the oxidation peak current of IP at the CPE modified with only carbon nanoparticles (CNPs/CPE) is smaller than that at DDTA/CNPs/CPE. On the other hand oxidation of IP at the CNPs/CPE is more difficult than at DDTA/CNPs/CPE.

Here Figure 3

The effect of the potential scan rate on the electrocatalytic properties of DDTA/CNPs/CPE in 0.1 M phosphate buffer solution containing 50.0 μM IP was investigated. Based on inset A of Figure 4, the anodic oxidation current of IP is proportional to the square root of the scan rate. Therefore the reaction is controlled by diffusion.[19] Inset B of Figure 4 shows the variation of the scan rate normalized current (I_p/v) with the scan rate expected for an electrocatalytic (EC') reaction as shown in scheme 1.[22] The inset C of Figure 4 shows a Tafel plot that was drawn from the CVs. A Tafel slope of 0.0942 V was obtained in this case, calculating a charge transfer coefficient of $\alpha = 0.37$.

Here Figure 4

3.3. Chronoamperometric measurements

The chronoamperometric behavior of DDTA/CNPs/CPE was examined in the absence and in the presence of IP. Chronoamperometric measurements of solutions with different concentrations of IP, were done by setting the working electrode potential at 250 mV. Chronoamperograms obtained are depicted in Figure 5. For IP with a diffusion coefficient of D, the current for the electrochemical reaction is described by the Cottrell equation;[19]

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \quad (3)$$

where D is the diffusion coefficient ($cm^2\ s^{-1}$) and C_b is the bulk concentration (mol cm^{-3}) of IP. For different concentrations of IP, the experimental plots of I versus $t^{-1/2}$ are depicted in Figure 5A. Then slopes of the obtained straight lines were plotted versus the IP concentration (Figure 5B). Based on the Cottrell equation and using the slope of the linear segment in Figure 5B, we can estimate the diffusion coefficient of 3.7×10^{-5} $cm^2\ s^{-1}$ for IP. We have also

used the chronoamperometric method of Galus to evaluate the catalytic rate constant, k ($M^{-1}S^{-1}$), for the reaction between IP and the DDTA/CNPs/CPE;[23]

$$I_{Cat}/I_L = \gamma^{1/2}[\pi^{1/2}\mathrm{erf}(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}] \quad (4)$$

Where I_C is the catalytic current of IP at the DDTA/CNPs/CPE, I_L is the limited current in the absence of IP and $\gamma = kC_bt$ (C_b is the bulk concentration of IP) is the argument of the error function. In the cases where γ exceeds 2, the error function is almost equal to 1 and the above equation can be reduced to:

$$I_{Cat}/I_L = \pi^{1/2}\gamma^{1/2} = \pi^{1/2}(kC_bt)^{1/2} \quad (5)$$

Where t is the time elapsed. We can obtain k based on the slope of the I_C/I_L vs. $t^{1/2}$ plot, for given IP concentrations. Such plots obtained from the chronoamperograms in Figure 5 are shown in inset C of this figure. From the values of the slopes, the average value of k was found to be k =134.28 M^{-1} s^{-1}.

Here Figure 5

3.4. Interference studies

In the optimized conditions the influence of various interfering species for the determination of IP was studied. The tolerance limit was taken as the maximum concentration of the foreign substances that caused an error of less than ±5% in the determination of IP. According to the results, Na^+, Cl^-, Mg^{2+}, K^+, l-lysine, glucose, captopril, N-acetyl cysteine and uric acid did not show interference in the determination of IP.

3.5. Calibration plot and detection limit

Since differential pulse voltammetry (DPV) has a much higher current sensitivity than CV, it was used to estimate the lower limit of detection and the linear range of IP. Voltammetry response increased linearly with increasing the concentration of IP. The responses were linear with IP concentrations in two linear segments. From the lower liner segment data, the detection limit of IP was calculated to be 0.075 μM.

3.6 Simultaneous determination of IP, UA, FA and TRY

DDTA/CNPs/CPE was used for the simultaneous determination of IP, UA, FA and TRY by simultaneously changing their concentrations in the solution. From four well-distinguished

anodic peaks, it was found that simultaneous determination of these compounds is feasible at the DDTA/CNPs/CPE as shown in Figure 6. The sensitivity of the modified electrode towards the oxidation of IP in the mixture of these compounds was found to be 0.0823 μA μM^{-1}. This is very close to the value obtained in the absence of UA, FA and TRY (0.0827). These results indicate that the oxidation processes of these compounds at the DDTA/CNPs/CPE are independent and therefore, simultaneous determination of their mixtures is possible without significant interferences. IP, UA, FA and TRY are compounds of great biomedical interest, playing a potential role in human metabolism. For the first time simultaneous determination of these compounds was performed by the proposed sensor in this work.

Here Figure 6

3.7. Determination of IP in a real sample

This method was found to work well under the laboratory conditions. To assess the applicability of this method in real samples, an attempt was made to determine IP in an ampoule purchased from local sources. In order to prevent any matrix effect the concentration of IP was measured by standard addition method. The average amount of IP in the injection was found to be 0.192 ± 0.022 mg which is in good agreement with the accepted value (0.2 mg). In order to evaluate the analytical capability of the proposed method, also it was applied to the determination of IP, UA, FA and TRY in human blood serum. The results are given in Table 1. Satisfactory recovery percent was found for IP, UA, FA and TRY.

Here Table 1

3.8. Repeatability, reproducibility and stability of DDTA/CNPs/CPE

The electrode capability for the generation of a repeatable response was examined using DPV. Data were obtained for five measurements and RSD for two concentration of IP (100 and 400 M) was calculated 2.1 and 1.9 %, respectively. DPV experiments of five separately prepared DDTA/CNPs/CPE were examined for testing the reproducibility. The calculated RSD for DPV peak current is about 1.9% for the same concentration of IP, indicating that reproducibility of preparation is good. The long term stability of the DDTA/CNPs/CPE was tested over a four-week period. In the DPV measurements the peak potential for IP oxidation was unchanged and the current signals showed less than 1.8% decrease relative to the initial response after four weeks.

Table 2 shows some analytical parameters such as detection limit and linear range for IP by the proposed electrode in comparison with some other electrochemical procedures.

According to the Table 2 detection limit and linear rang at this work are comparable with other works.

Here Table 2

4. Conclusions

In this work, we initially prepared the DDTA/CNPs/CPE and then its electrochemical behavior was investigated by DPV. Also its application for the simultaneous determination of IP, UA, FA and TRY was investigated. Simultaneous determination of four compounds, high sensitivity, low detection limit, fast response, the ease of preparation, high repeatability and stability of the modified electrode are the advantages of the proposed electrode in comparison with our previous researches.[27,28] The peak potential of IP is shifted by 298 mV to a less positive potential at the surface of the DDTA/CNPs/CPE. The modified electrode displays higher selectivity in voltammetric measurements of IP, UA, FA and TRY in their mixture solution. Simultaneous determination of the four compounds has been carried out in Ampule sample.

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Figure Captions:

Figure 1. CVs at DDTA/CNPs/CPE in various buffer solutions from pHs 4.0-11.0 at scan rate 50 mV s^{-1}). Inset: plot of E_p vs. pH.

Figure 2. Cyclic voltammograms obtained at DDTA/CNPs/CPE in 0.1 M phosphate buffer (pH 9.0) at various scan rates from 200 to 800 mV s^{-1}. Insets: (A) CVs in the same condition at various scan rates from 30 to 150 mV s^{-1}. (B) Variation of *E* versus the logarithm of scan rate, (C) variation of *E* versus the logarithm of scan rate for scan rates higher than 100 mV s^{-1}. (D) Variations of I_{pa} versus scan rate

Figure 3. CVs obtained at: (a) DDTA/CNPs/CPE (b) unmodified CPE (c) CNP/CPE, (d) DDTA/CPE, in 0.1 M phosphate buffer solution (pH 9.0), at a scan rate of 50 mV s^{-1} in a solution of 50 μM IP. (e) DDTA/CNPs/CPE in 0.1 M phosphate buffer solution (pH 9.0)

Figure 4. CVs obtained at DDTA/CNPs/CPE in 0.1 M phosphate buffer (pH 9.0) containing 50 μM IP at scan rates 5, 10, 25, 35, 45 and 55 mV s^{-1}, (A) variation of the electrocatalytic currents with the square root of the scan rate (B) variation of the scan rate normalized current (I_p/v) with scan rate (C) the Tafel plot derived from the CV at scan rate 10 mV s^{-1}.

Figure 5. Chronoamperograms obtained at DDTA/CNPs/CPE in 0.1 M phosphate buffer solution (pH 9.0) for IP concentrations of 0.0, 0.8, 1.0, 1.5 and 2.0 mM. Insets: (A) plots of *I*

vs. $t^{-1/2}$ obtained from the chronoamperogram data, (B) plot of the slope of the straight lines against the IP concentration and (C) dependence of I_C/I_L derived from the data of chronoamperograms.

Figure 6. DPVs obtained DDTA/CNPs/CPE in 0.1 M phosphate buffer solution (pH 9.0) containing different concentrations of IP, UA, FA and TRY (from inner to outer) mixed solutions of 1200.0+1000+2000+24000, 1000.0+800.0+1600.0+2000, 800.0+600.0+1300.0+1500.0, 600.0+550.0+1000.0+1000.0, 500.0+300.0+600.0+800.0, 400.0+150.0+550.0+400.0 μM, respectively. Insets: A) plot of the peak currents as a function of IP concentration B) Plot of the peak currents as a function of UA concentrations, C) Plot of the peak currents as a function of FA concentrations. D) Plot of the peak currents as a function of TRY concentrations. Scan rate: 20 mV s^{-1}, modulation time: 0.05 s, pulse amplitude: 0.02 V.

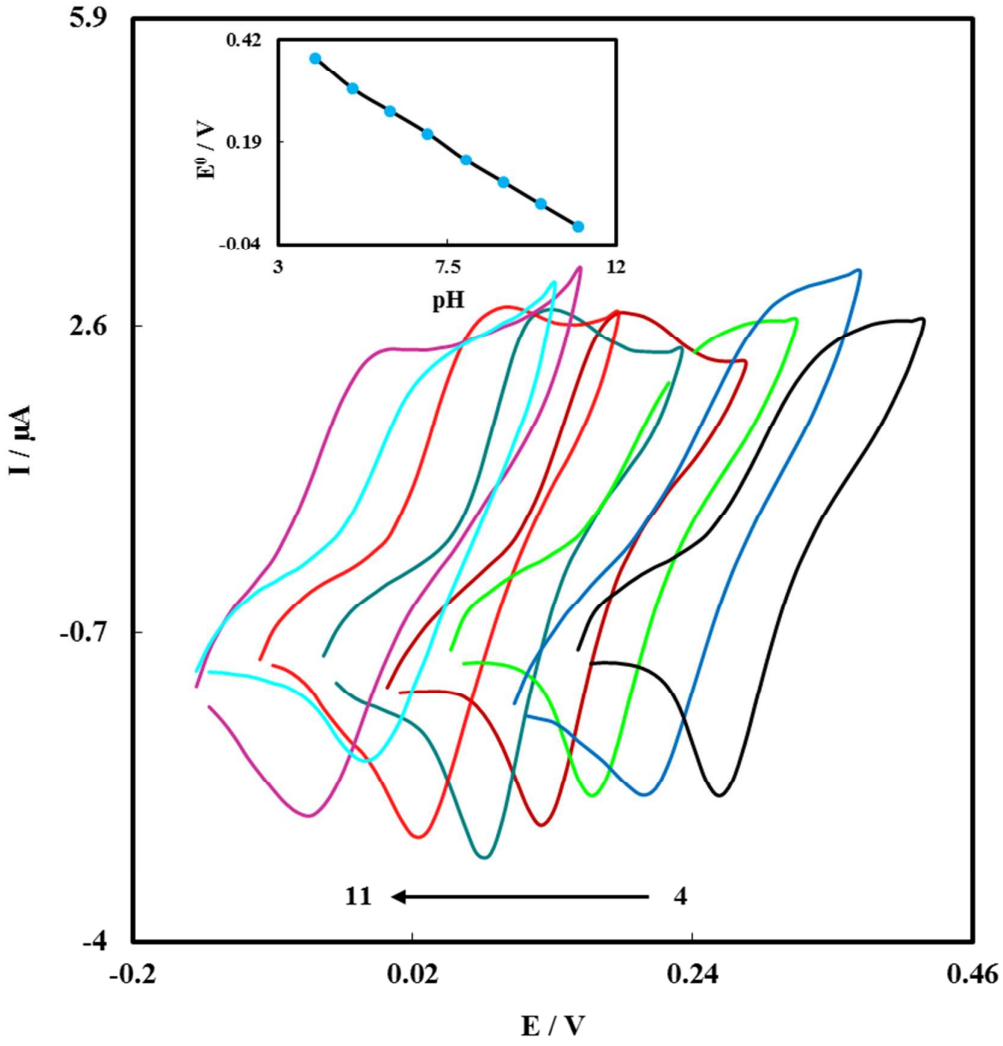


Fig. 1

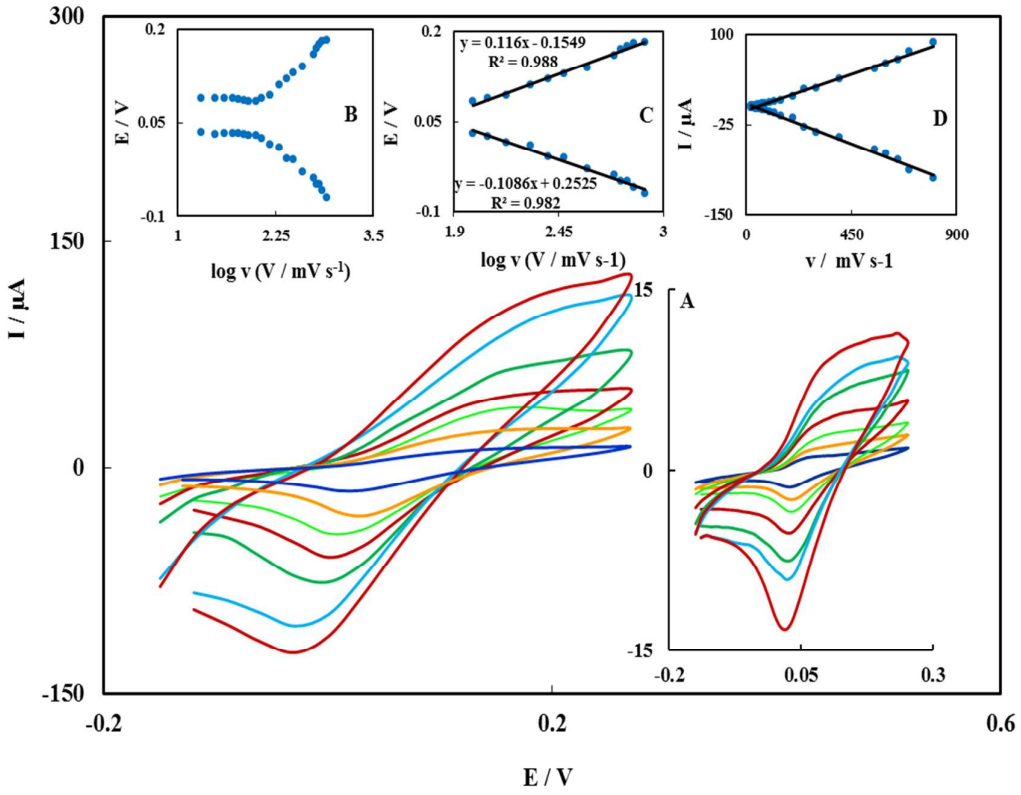


Fig. 2

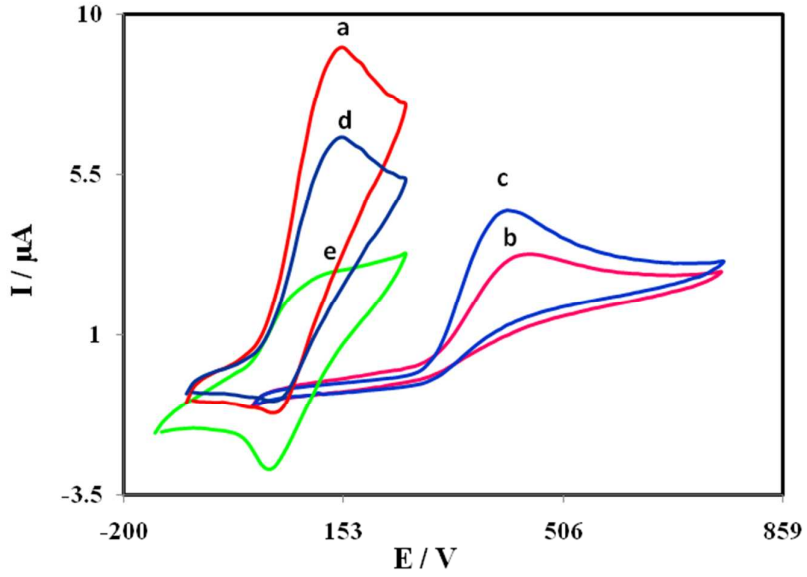


Fig. 3

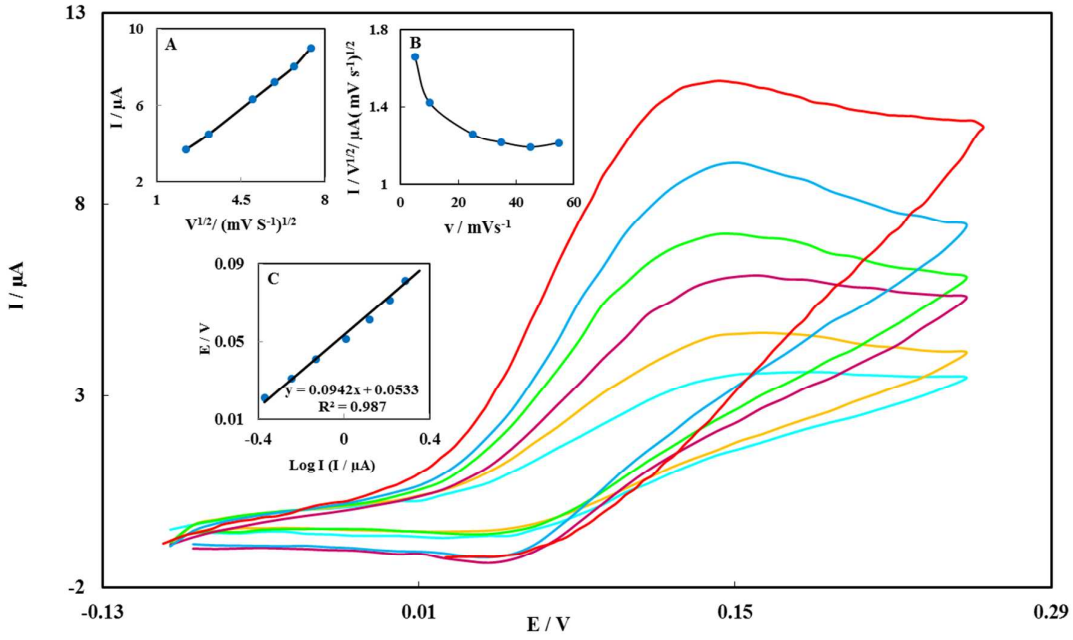


Fig. 4

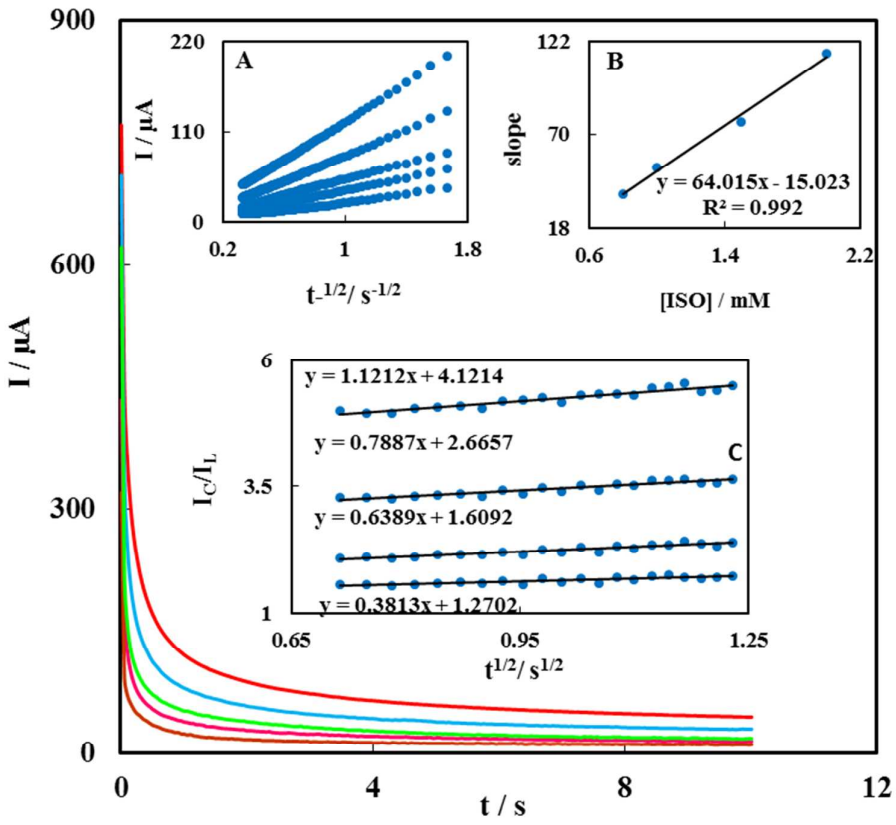


Fig. 5

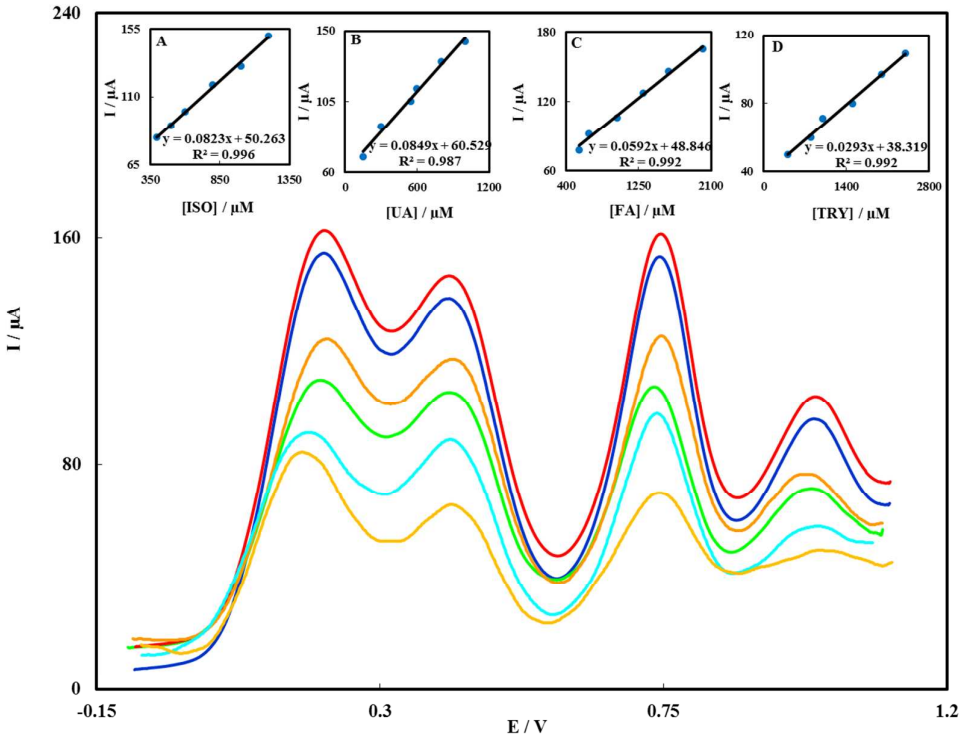


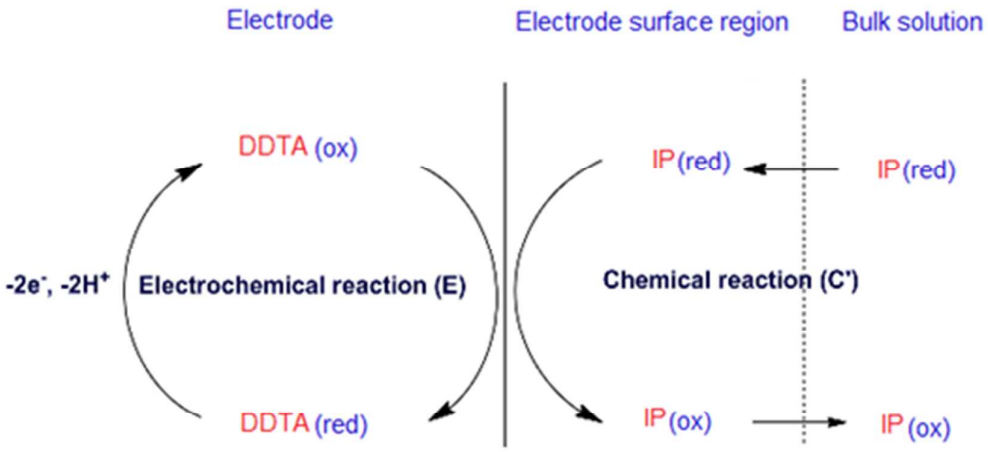
Fig. 6

Table 1 Determination of IP, UA, FA and TRY using DDTA/CNP/CPE by standard addition method in ampoule sample and human blood serum.

Sample	Spiked (μM)				Found (μM)				Recovery%			
	IP	UA	FA	TRY	IP	UA	FA	TRY	IP	UA	FA	TRY
Ampoule	0	0	0	0	5.2	-	-	-	-	-	-	-
	5.0	10.0	10.0	10.0	10.4	10.2	9.9	10.2	101.9	102.0	99.0	102.0
	10.0	15.0	15.0	15.0	15.1	15.2	14.9	14.8	99.3	101.3	99.3	98.6
	20.0	20.0	20.0	20.0	25.5	19.9	20.3	20.4	101.1	99.5	101.5	102.0
Human blood serum	0	0	0	0	ND	14	ND	ND	-	-	-	-
	5	15	20	25	5.2	29.3	19.7	25.2	104.0	101.0	98.5	100.8
	15	25	30	40	14.8	40.0	29.8	40.1	98.6	102.5	99.3	100.2
	20	30	40	65	21.0	44.2	40.8	64.3	105.0	100.4	102.0	98.9

Table 2 Comparison of some electrochemical procedures used in the determination of IP.

Electrode	Modifier	pH	Linear range (μM)	Detection limit (μM)	Peak potential shift (mV)	Ref.
CPE	P-chloranil	10.5	0.015-100	0.009	250	24
CPE	FerrocenMultiwall carbon nanotubes	5.0	0.1-5.0	0.07	140	25
GCE	Poly(1-methylpyrrole)-DNA	4.0	2.0-50	0.16	334	26
CPE	DDTA/CNP/CPE	9.0	0.25-2000.0	0.075	298	This work



Scheme 1: Mechanism of electrocatalytic reaction