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An Electrochemical Sensor based on conductive polymers/Graphite Paste Electrode for Simultaneous Determination of Dopamine, Uric acid and Tryptophan in Biological Samples

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A graphite paste electrode (GPE) modified with chemically synthesized Polythiophene nanostructures (PTh) was proposed for simultaneous voltammetric detection of Dopamine (DA), Uric acid (UA) and Tryptophan (Trp). The prepared electrode (PTh/GPE) showed a good improvment in catalytic activity of electrochemical oxidation DA, UA and Trp, leading to significant improves in the supposed peak currents and decreasing the peak potentials. In the calibration curve obtained from Linear sweep voltammetry it was illustrated the peak currents of DA, UA and Trp enhanced linearly with their concentration. The concentration range was found for DA, UA and Trp, 10–180 μmol L^{-1} , 6-180 μmolL^{-1} and 6–180 μmol L^{-1} in 0.1 M phosphate buffer solution (pH 4.0) respectively. The anodic peak height of analytes was investigated in different pH and scan rates and the optimum pH and scan rate was obtained. The LODs (S/N= 3) were 1, 0.57 and 0.61 μM for DA, UA and Trp, respectively. Consequently, the applicability of the voltammetric sensor was evaluated by simultaneous determination of DA, UA and Trp in biological samples. This present sensor showed valuable characteristics such as stability and repeatability during analytical experiments.

Keywords: Graphite paste electrode, Polythiophene nanostructures, Simultaneous determination, Electrochemical oxidation

1. INTRODUCTION

Determination and evaluation of electro-active compounds such as ascorbic acid (AA), dopamine (DA), xanthine (XA), uric acid (UA), Tryptophane (Trp), acetaminophen (APAP) and nicotinamide adenine dinucleotide (NADH), in real samples, such as serum and urine, are necessary to

detect and diagnose many health problems, besides providing information about the interactions involving these species in the physiological processes.

DA is a significant mediator component in Tyrosine (Tyr) conversion of adrenalin which shows neuro-degenerative processing, as it's a factor of Parkinson's disease [1,2], schizophrenia [3-5] and human immunodeficiency virus (HIV) infection [4,6]. Its normal concentration is very low (0.01–1μmol L^{-1}) in blood. Uric Acid (UA) is one of the main catabolites of guanine and adenine dissociation, while UA and other purines are the main physiological components that is related to signs of some disorders, mostly gout, hyperuricaemia and Lesch–Nyhan syndrome. The level of UA in human serum is 120–450μmol L^{-1} and in urine is 2mmol L^{-1} approximately [7-9]. Tryptophan (Trp) is classified as a necessary amino acid in the body and also is a precursor of serotonin, melatonin, and niacin [10]. To remove some possible dietary deficiencies, Trp is used in food fortifiers and pharmaceutical formulations. [11].

Theoretically, DA, UA, and Trp co-exist in biological samples such as human serum and urine. For instance, DA is coexisting in mammalian brain along with several neurotransmitters including Trp and also UA while they have more or less same electrochemical properties. Normally, it is difficult to measure them at the same time by routine electroanalytical tools, as the oxidation peaks of mentioned compounds are at a closed potential and overlapped. Thus, it is highly desirable to promote a method for the sensitive and selective determination of DA, UA and Trp for analytical purposes and diagnostic research [12]. The aim of this work is to present a nonenzymatic electrochemical sensor for the simultaneous analysis of DA, UA and Trp. Though, there are many reports for the simultaneous quantification of these biocompounds, GPE modified with chemically synthesized PTh nanostructures has not been reported yet.

GPE is a specific type of heterogeneous graphite electrode consisting of mixture prepared from graphite and an appropriate water-immiscible or non-conducting binder [13]. Adams in 1958 reported application of carbon past as an electrode for the first time [14]. Chemically modified graphite paste electrodes (CMGPEs) have received notable consideration in recent years as they have benefits of low cost, simplicity in preparation, extended potential window, and easy surface renewal process, feasible miniaturization, good stability and sensitivity [15]. The application of electroactive materials as modifier into a graphite paste electrode is beneficial and has been greatly applied in the electroanalytical chemistry. One of the significant advantages of modifier is reducing the redox potential of the electrochemical reaction and rising the sensitivity and selectivity of that proposed method [16]. Conducting polymers (CPs) as a new generation of synthetic materials, they come with especial properties which make them different form the rest of materials ever synthesized [17-19]. Among all, a significant and positive property of CPs is the ability to increase the rate of redox process belongs to the media species [20]. This case is said to be potentially beneficial in electrosynthesis and electroanalysis. Furthermore, these materials by being inexpensive and easy prepared become more desirable comparing the traditional electrocatalysts like, e.g., completely dispersed platinum group metals [21]. Among conducting polymers, PThs are polymerized thiophenes, a sulfur heterocycle. A conducting polymer become conductive when oxidized it. In this study PTh nanofibers were synthesized chemically in and utilized as modifier in GPE.

To the best of our knowledge, no study has been reported on the electroanalysis and simultaneous determination of DA, UA and Trp using a modified GPE with synthesized PTh nanofibers as a conducting polymer. This work has sought to investigate the capability of GPEs modified by chemically synthesized PTh for the detection of DA, UA and Trp in biological samples. The proposed electrode showed a low LOD and high sensitivity for these three species. The evaluation of this electrode was investigated for the simultaneous determination of DA, UA and Trp using cyclic voltammetry. Eventually, this sensor were used for the determination of these analytes in biological samples successfully.

2. EXPERIMENTAL

2.1. Chemicals and solutions

Dopamine, Uric acid and Tryptophan were reagent grade from Merck. Nafion (5%) (NF) was provided from Aldrich. All the solutions were provided with doubly distilled water (DDW). Appropriate amounts of Dopamine hydrochloride and Tryptophan were dissolved in DDW to prepare fresh solutions of DA (0.01 M) and Trp (0.001 M) daily. For the UA stock solution (0.01 M) the solid were dissolved in a small volume of 0.1 mol L^{-1}NaOH solution, then it was diluted to reach its proposed level. To prevent the solution changes, it was kept in low temperature in no light condition. Serial dilution was utilized for the preparation of more dilute solutions using phosphate buffer solutions (PBS). A series of PBS solutions were prepared using H_3PO_4 and *p*Hs were adjusted using NaOH (0.1 M) covering limit of 2.0 to 8.0. The electrolyte solutions of experiments were bubbled by N_2 for deoxygenation, before each electoanalytical analysis. All the analysis were carried out under N_2 at 25°C.

2.2. Apparatus

Electrochemical experiments were carried out with an SAMA500 Electroanalyser (SAMA Research Center, Iran) controlled by a PC. The three-electrode cell system consisted of GPE, a saturated calomel electrode (SCE) as the reference electrode and a Pt as the counter electrode were used. All the experiments were performed under N_2 gas at 25 ºC. TEM images of both the chemically synthesized polymer and prepared electrode were taken using a TEM-Philips CM120 with 2.5˚A resolution. FTIR spectrophotometer, manufactured by Bruker Tensor 27 Germany was utilized for the FTIR study of synthesized polymer.

2.3. Synthesis of PTh nanostructures

For the synthesis of PTh nanostructures, two separate solutions in organic phase and aqueous phase were prepared; in the organic phase, 2.5 mL of Thiophene monomer was as monomer was transferred to 12 mL isobutyl methyl ketone, and for the aqueous phase, 5.0 g of potassium persulfate

in 50 mL doubly distilled water was dissolved. Afterward, prepared organic phase was transferred to the aqueous phase and was fixed in ultrasonic bath for 2 hours. The mixed solutions were stirred by magnetic stirring apparatus for 3 hours at room temperature. Next, the aqueous phase was removed from organic phase by a decanter and the organic phase was dried in vacuum oven for 2 hours at 60 °C. Finally, the synthesized polymer was washed by DDW and dried at room temperature.

2.3. Modification of the working electrode

PTh/GPE was constructed by mixing 0.10 g of PTh with 0.9 g graphite powder using a mortar and pestle. Next, 0.9 mL of paraffin was injected to the prepared composite and carefully mixed for 30 min until a uniform paste was acquired. The prepared homogeneous paste was fixed into of a glass tube and concentrated. Then, by inserting a Cu wire into the graphite paste the electrical contact was issued. When need, a cleaned surface was provided by pushing the paste out of the tube a few, then polishing it on a weighing paper. For comparison, bare GPE was also made in the same way.

2.4. Preparation of real sample

Real samples (urine and blood) without any pretreatment were provided from Omid Laboratory (Zahedan, Iran). Both samples (urine and blood) were stored in -4 ºC directly after collection. A specific amount of real samples (10 mL) was centrifuged during a limited time (20 min) at rotation speed of 2000 rpm. The supernatant liquid was filtered using a 0.45 μm filter and then diluted (ten times) with PBS in optimum pH (4.0). The solution was added into the voltammetric cell for the analysis without any pretreatment. To calculate recovery of the proposed method (for DA, UA and Trp in urine and human serum samples), spiking method was applied.

3. RESULTS AND DISCUSSION

3.1. FT-IR study

The chemical structures of synthesized PTh nanostructures were determined by FT-IR spectroscopy (Figure 1), which has furnished desirable information about the formation of nanostructured PTh. The FT-IR transmission spectrum of the products exhibited characteristic vibration at 2923.49 cm^{-1} for C-H stretching vibration band. The bands at 1669.19 and 1399.19 cm^{-1} are corresponding to C=C asymmetric and symmetric stretching vibrations of thiophene ring, respectively. The vibration band observed at 1044.10 cm^{-1} is due to C– H in-plane bending band. Furthermore, the band at 3443.62 cm^{-1} originated from O–H stretching of water in KBr. Results of the FT-IR studies clearly indicates polymerization of Thiophene.

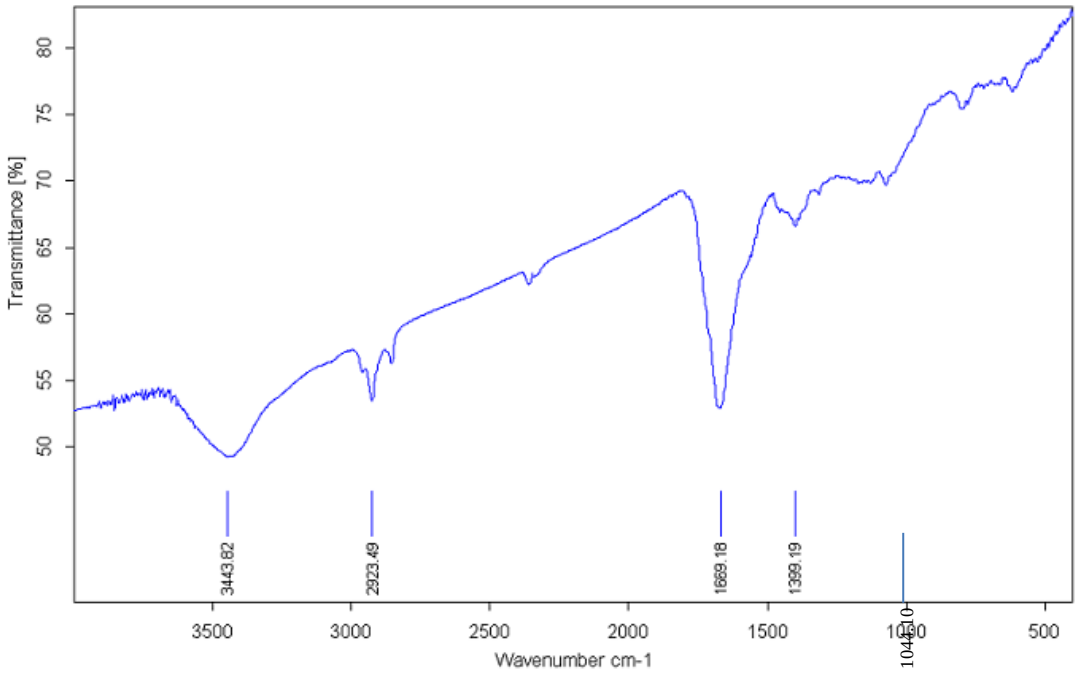
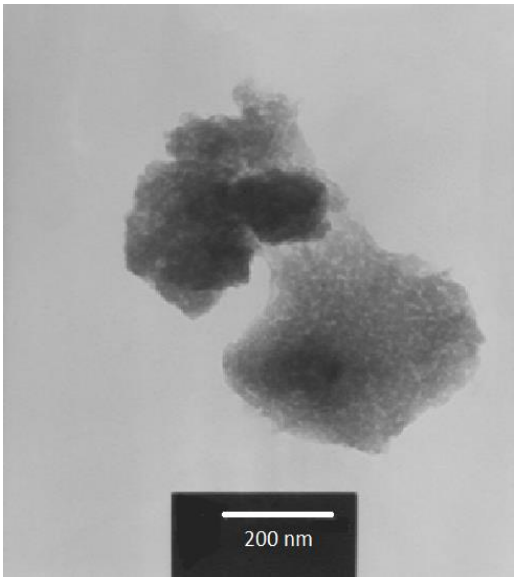


Figure 1. FT-IR spectrum of synthesized PTh

3.2. TEM characterization

Fig.2a. shows TEM of synthesized nanostructured polythiophene. As it can be seen, the scales of these polymeric fibers are almost between 40 – 80 nm which proves its nanostructures by the proposed synthesis. Fig. 2b shows the TEM images of PTh/GPE. Based on this image, the PTh was attached on the graphite.



A

B

Figure 2.a) TEM of chemically synthesized PTh nanostructures, b) TEM image of PTh/GPE

3.3. Study of modified electrode on the oxidation of DA, UA and Trp

In Figure 3. the comparison of LSV voltammograms obtained from a) GPE and b) PTh/GPE modified electrode in a solution of DA, UA and Trp (pH 4.0) has been reported. Figs. 3a and b displays the cyclic voltammograms of a ternary solution of DA, UA and Trp in 0.10 M PBS (pH 4.0) at GPE and PTh/GPE. As it is shown in Fig. 3b, the PTh/GPE showed a desirable growth in the Ip_a of DA, UA and Trp. The separations of Ep of AA–DA, DA–UA and UA–Trp were of 177 and 332 mV, respectively. These results indicate that the simultaneous detection of DA, UA and Trp is feasible.

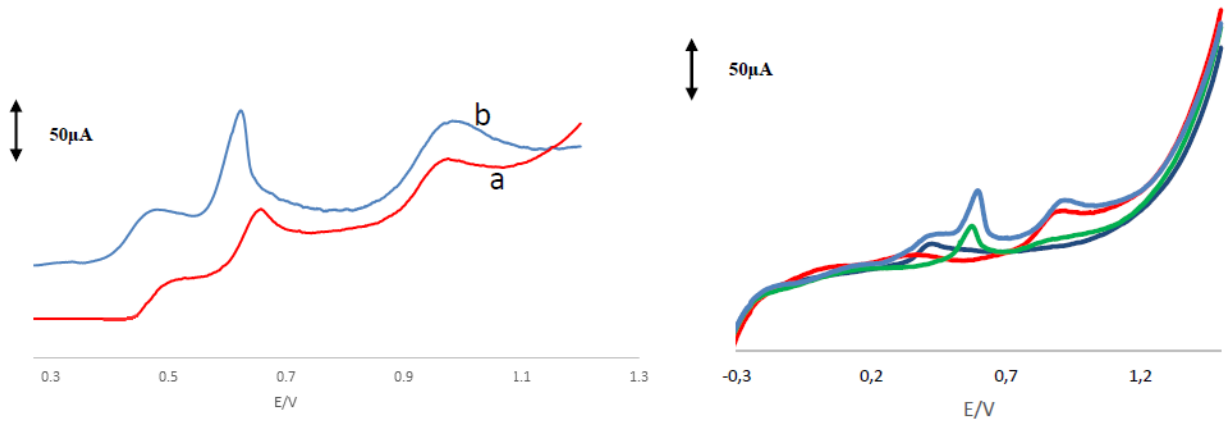


Figure 3. LSVs at (a) GPE unmodified (b) PTh/GPE in DA, UA and Trp mixture in PBS (0.1 M) at pH (4.0). Scan rate: 50 mV s^{-1}.

3.4. Effect of scan rate

The kinetics of the overall process were studied by running cyclic voltammetry on PTh/GPE in PBS at pH 4.0 in the mixture of DA, UA and Trp at different scan rates (Fig.4a). As there is a linear correlation between Ip and $v^{1/2}$ (Fig.4b), it can be predicted that DA, UA and Trp is controlled by mass transfer from bulk to the electrode. It is considerable that E_p, for each of these three analytes changes moderately to higher potentials when the scan rate enhances. Therefore, according these results it can be concluded that at higher scan rates a kinetic limitation occurs because of the reaction of the PTh/GPE blend and DA, UA and Trp. The scan rates more than 50mV.s^{-1} couldn't be reported as GPE were not able to work properly there. Consequently, due to our efforts to achieve highest performance for peak separation and peak currents, 50 mV s^{-1} was used for the all the experiments.

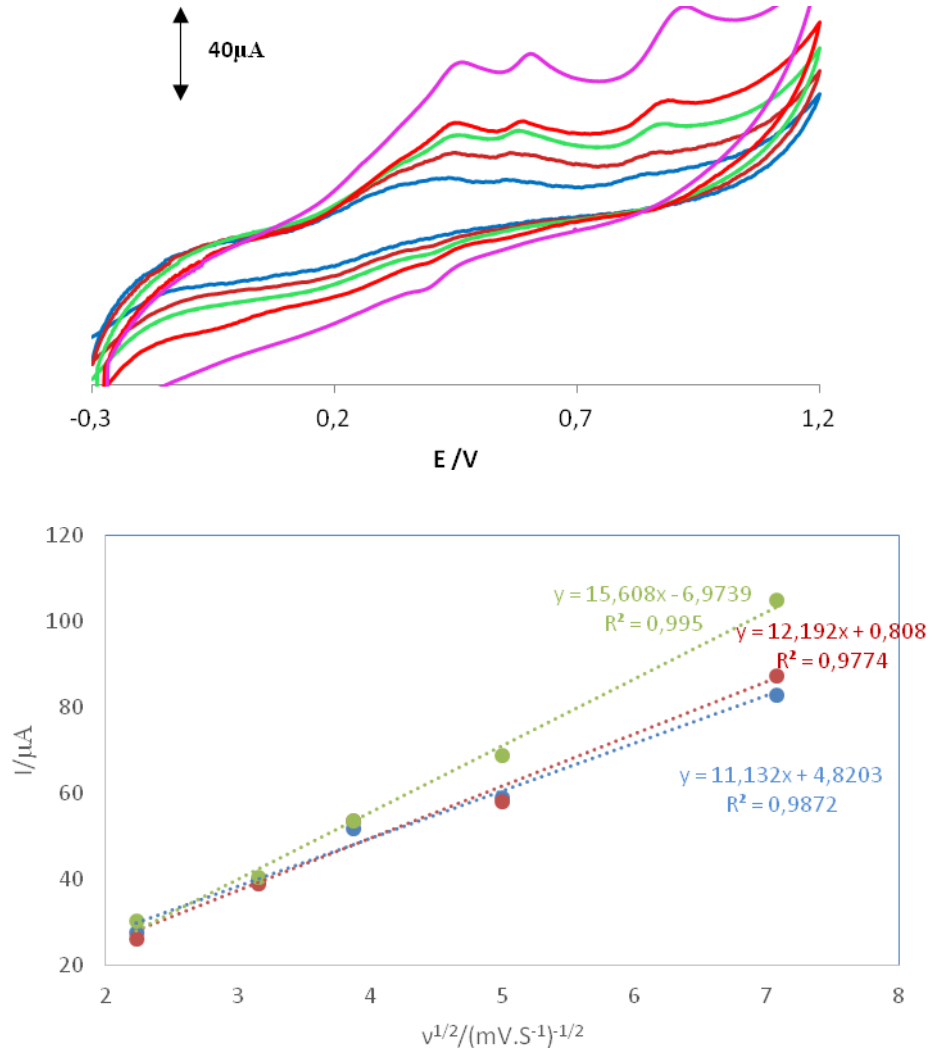


Figure 4. Cyclic voltammograms of PTh/GPE in phosphate buffer (pH 4.0) in the mixture of DA, UA and Trp at different scan rates 5, 10, 15, 25 and 50 mVs^{-1}, B) the plot of Ip vs. $v^{1/2}$

3.5. Effect of pH on the oxidation of DA, UA and Trp

To study the effect of pH in the solution on electrochemical response of the PTh/GPE for the simultaneous determination of DA, UA and Trp, phosphate buffer (PBS) (0.1 mol L^{-1}) and acetate buffer (0.1 mol L^{-1}) solutions with different *p*Hs were prepared and used as supporting electrolyte. The differences of peaks separation and peaks current related to the alteration in the *p*H of the solution in the *p*H range from 2.0 to 8.0 are reported in Fig. 5a-g.

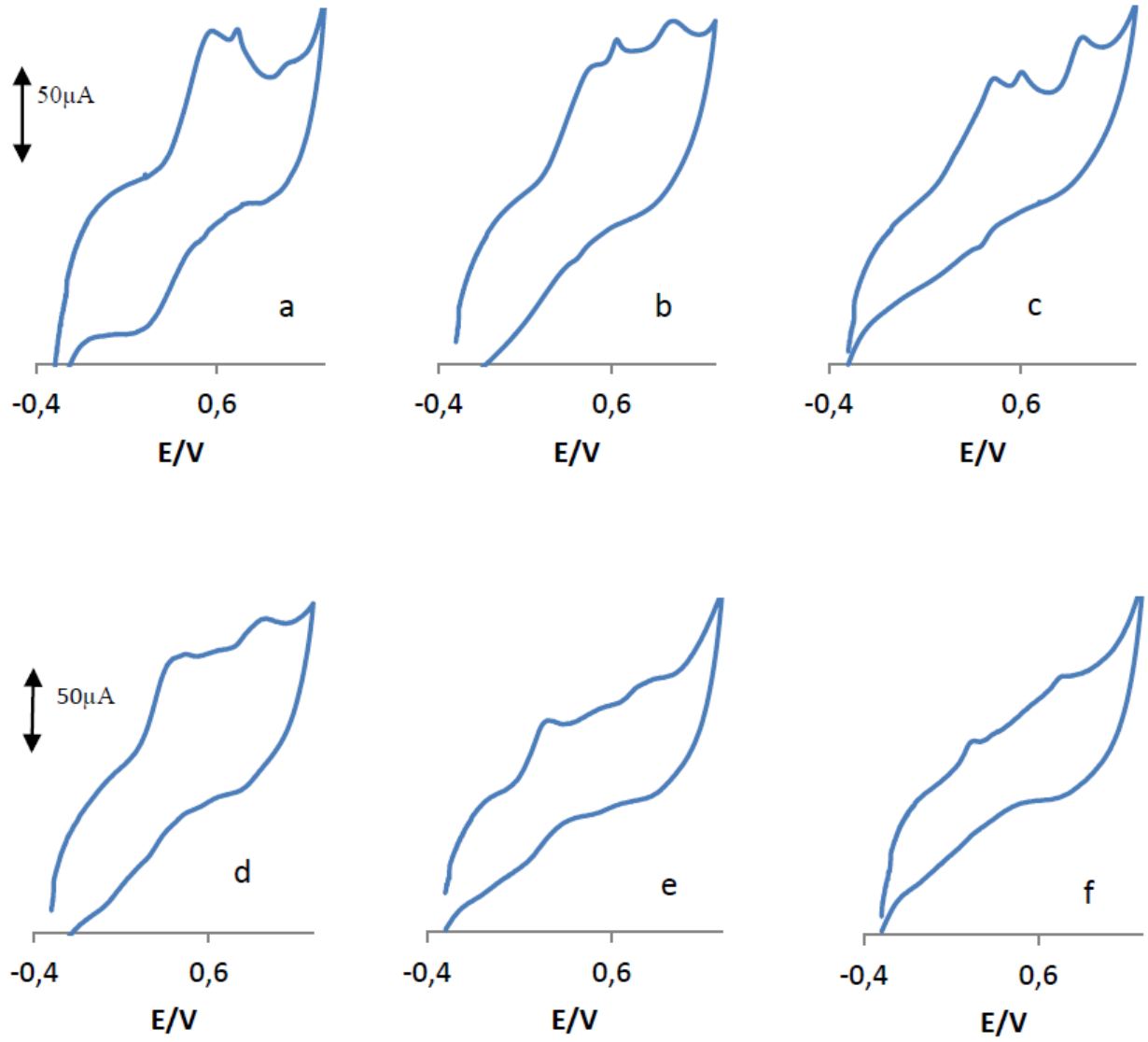


Figure 5. CVs of PTh/GPE in the mixture of DA, UA and Trp at different *p*Hs: (a) 2, (b) 3, (c) 4, (d) 5, (e) 6, (f) 8.

The effect of *p*H on the anodic peak potentials of DA UA and Trp has been indicated in Fig. 7. As shown, the anodic peak potentials of each three analytes (DA, UA and Trp) were declined linearly with rising *p*H from 2.0 to 8.0, indicating the proton transfer in the electrode processes. Clearly, it was detected that the anodic peak potentials of DA, UA and Trp changed to negative potentials by rising

*p*H to more positive values. This was expected because of the presence of proton(s) in the oxidation reactions of DA, UA and Trp. The redox reaction can be discussed as follow:

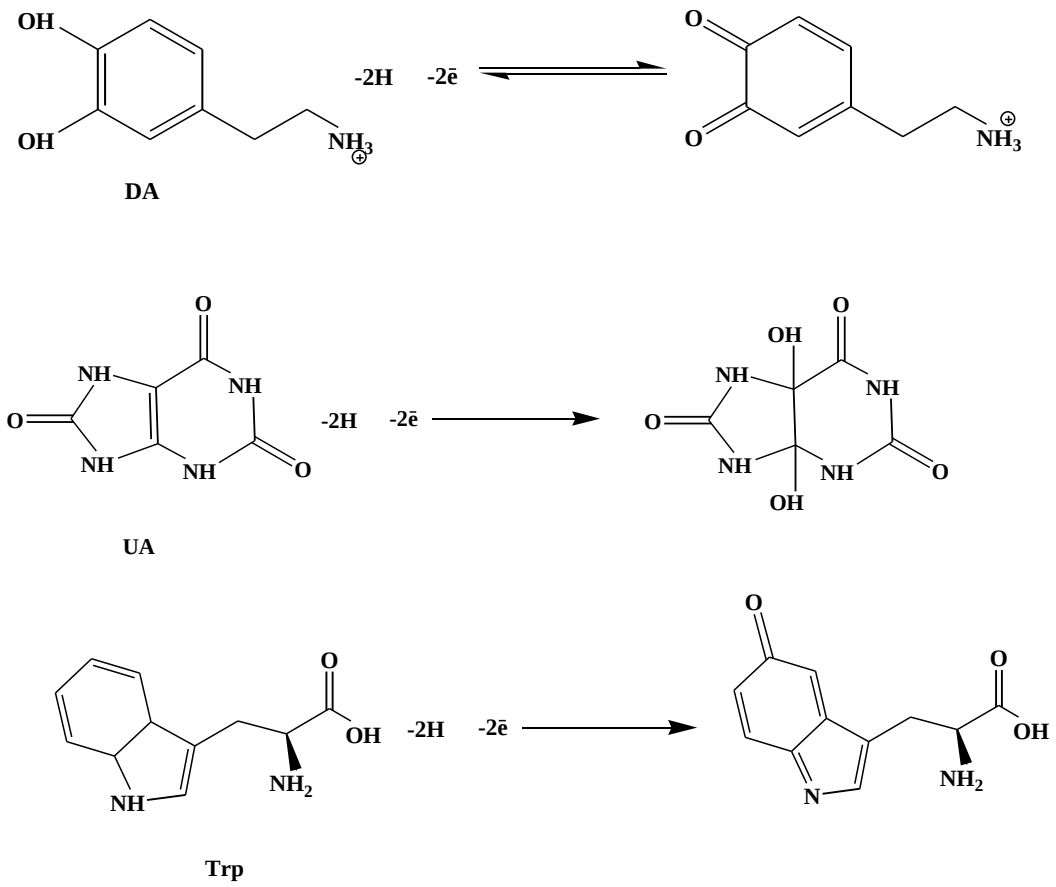


Figure 6. proposed mechanism for the electrooxidation of DA, UA and Trp at PTh/CPE

$$Red - mH^{+} - ne \rightleftharpoons Ox \qquad (1)$$

Where Red is DA, UA and Trp; Ox present the responding products; m and n are the number of protons and electrons transferred in the redox process. The anodic peak potentials for peak Red, is given by [22]:

$$E'_{p(Red)} = E_{p(Red,\ pH=0)} - \frac{2.303mRT}{nF}pH \qquad (2)$$

where $E_{p(Red,\ pH=0)}$ is the anodic peak potential for the reduced form at pH= 0.0, and R, T, and F have their common meanings. The values of $E_{p(Red)}$ for DA, UA and Trp are plotted in Fig. 7. As shown, in Fig. 7, $E'_{p(Red)}$ were moved to negative potentials (slopes 0.062, 0.065 and 0.044 V/pH for DA UA and Trp, respectively), which are in agreement with the theoretical slope ($-\frac{2.303mRT}{nF}$) of 0.059($\frac{m}{n}$) V/pH. According these results, it can be concluded that the oxidation process of DA, UA and Trp are containing an equal number of protons (m) and electrons (n) (m=n) [23, 24] (Fig6).

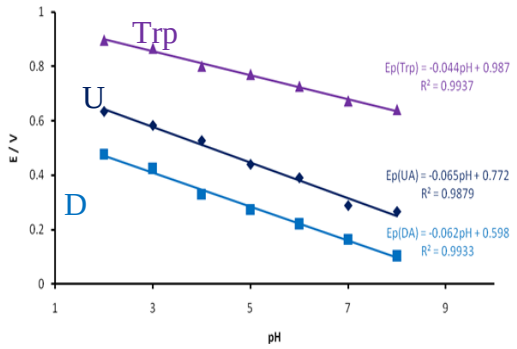
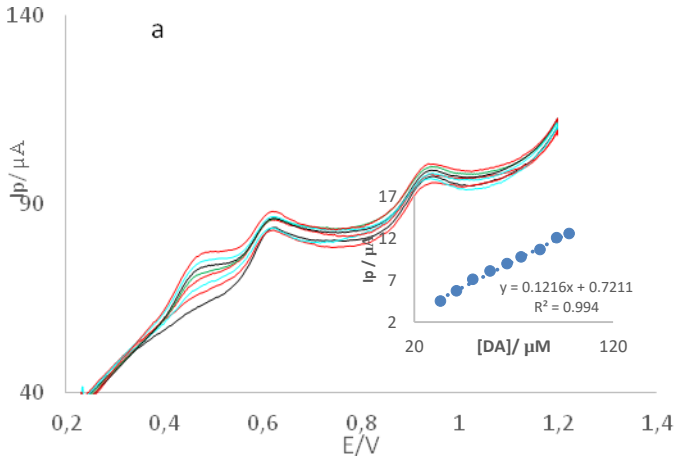


Figure 7. plot of peak potential *vs. p*H for the oxidation of three proposed analytes for CVs of Figure 5.

Finally, due to satisfactory peak separation and highest anodic peak current for the simultaneous determination of proposed analytes (DA, UA and Trp), a phosphate buffer with pH 4.0 was utilized as the optimal *p*H.

3.6. Interference studies

One of the important problems in electroanalytical methods especially in simultaneous determinations are the interference caused by some electrochemically active compounds which can be oxidized under the same conditions, in biological samples. Thus, study on interferences needs to be taken in account carefully. Therefore, most usual interference being derived from any are the tyrosine (Try), cysteine (Cys) and glucose (Glu). Among all other compounds, the interference caused by DA, UA and Trp on each other is much important than others as their oxidation peak potential is near to each other and they mainly coexist in real biological samples [25,26]. Therefore, to study these interfering agents and their effects, the mixed solution technique [25, 26] was used on the simultaneous quantification of DA, UA and Trp.



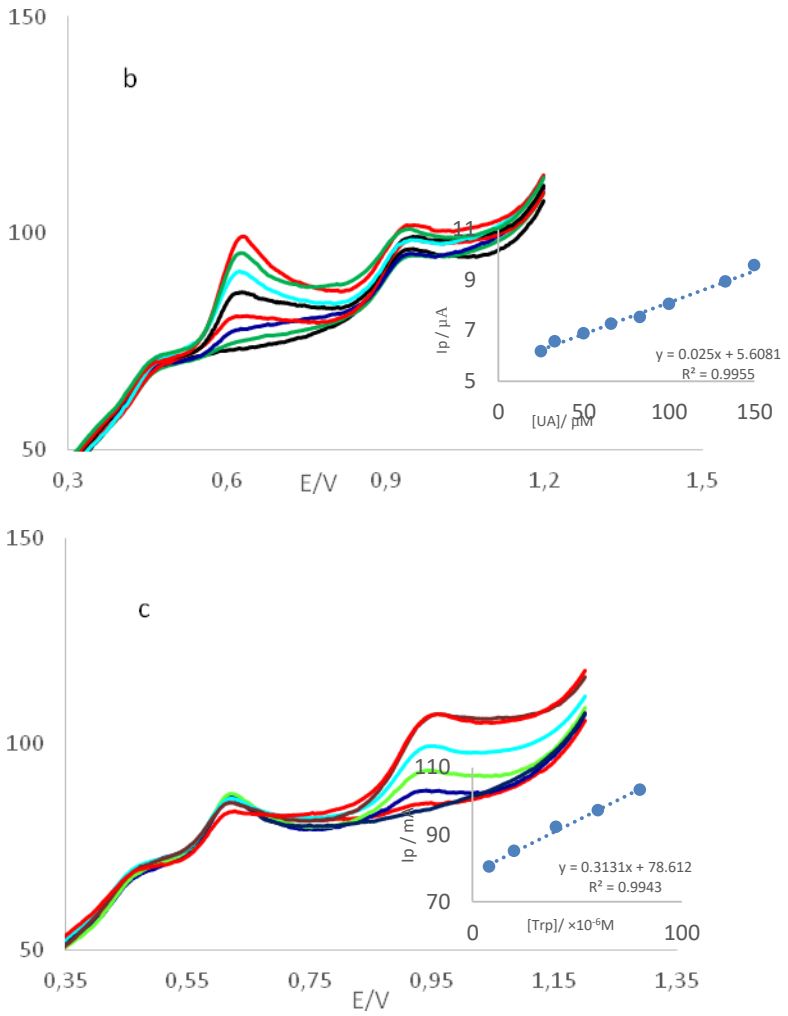


Figure 8. CVs at the PTh/GPE in 0.1 M PBS pH=4.0 (a) containing UA (103.3 µM), (Trp (80.5 µM) and different concentrations of DA (from inner to outer):33.3, 43.1, 49.2, 58.5, 66.1, 683.1, 73.2, 78.5, 88.4 µM (b) Containing DA (105.5 µM) ,(Trp (80.5 µM) and different concentrations of UA (from inner to outer): 33.3, 50.0, 83.3, 100.0, 133.3, 150.0, 160.7 µM, (c) Containing DA (100.0 µM), UA (103.3 µM) and different concentrations of Trp (from inner to outer): 13.3, 33.3, 50.0, 66.6, 116.6 µM,

The electro-oxidation processes of DA, UA and Trp at PTh/GPE in the solution have also been studied in the way that in the mixture of these three analyts the level of one species changed, while two of other is kept constant. The results are shown in Fig. 8. As can be seen, the (Fig. 8a) shows the effect of different concentrations of DA interferences on a constant amount of UA and Trp. Similarly, as shown in Fig. 8b and c the effect of interference of UA and Trp were investigated by keeping the constant concentration of two of other species. The other biological species, such as Try, Glu up to 1200 µM and Cys up to 900 µM did not significantly interfered with the determination of DA and UA (100 µM). In addition, determination of DA, UA and Trp (100 µM each) was performed with no significant interference from the following compounds: NaCl and KCl (3500 µM), $CaCl_2$ and $ZnCl_2$ (4000 µM) and $MgSO_4$ (3500 µM). Following the above results it was concluded that the proposed

sensor is desirable for the selective quantification of DA, UA and Trp in presence of other possible existing compounds in real biological sample.

3.7. Calibration curve

According our experimental results mentioned above, when the proposed electrode (PTh/GPE) was used for the detection of proposed analytes in a mixture, the oxidation peaks of them (DA, UA and Trp) were monitored as three separated peaks. It can be seen that, if the concentrations of DA, UA and Trp increased synchronously, the peak currents at the PTh/GPE enhance according as shown in Fig. 9. The calibrations were linear in the range of 10–180 µM, 6–180 µM and 6–180 µM, for DA, UA and Trp, respectively. LOD were assessed using the equation $Y_{LOD} = X_B + 3S_B$, where Y_{LOD} is the signal [27] for the detection limit, X_B is the symbol of blank signal and S_B the standard deviation of the blank signal. The theoretical LODs of the proposed sensor for DA, UA and Trp were 1, 0.57 and 0.61 µM, respectively. In the Table 1. the comparison of proposed work (figures of merit, such as linear range and LOD) for the detection of DA, UA and Trp with those from other published works on modified electrodes has been reported.

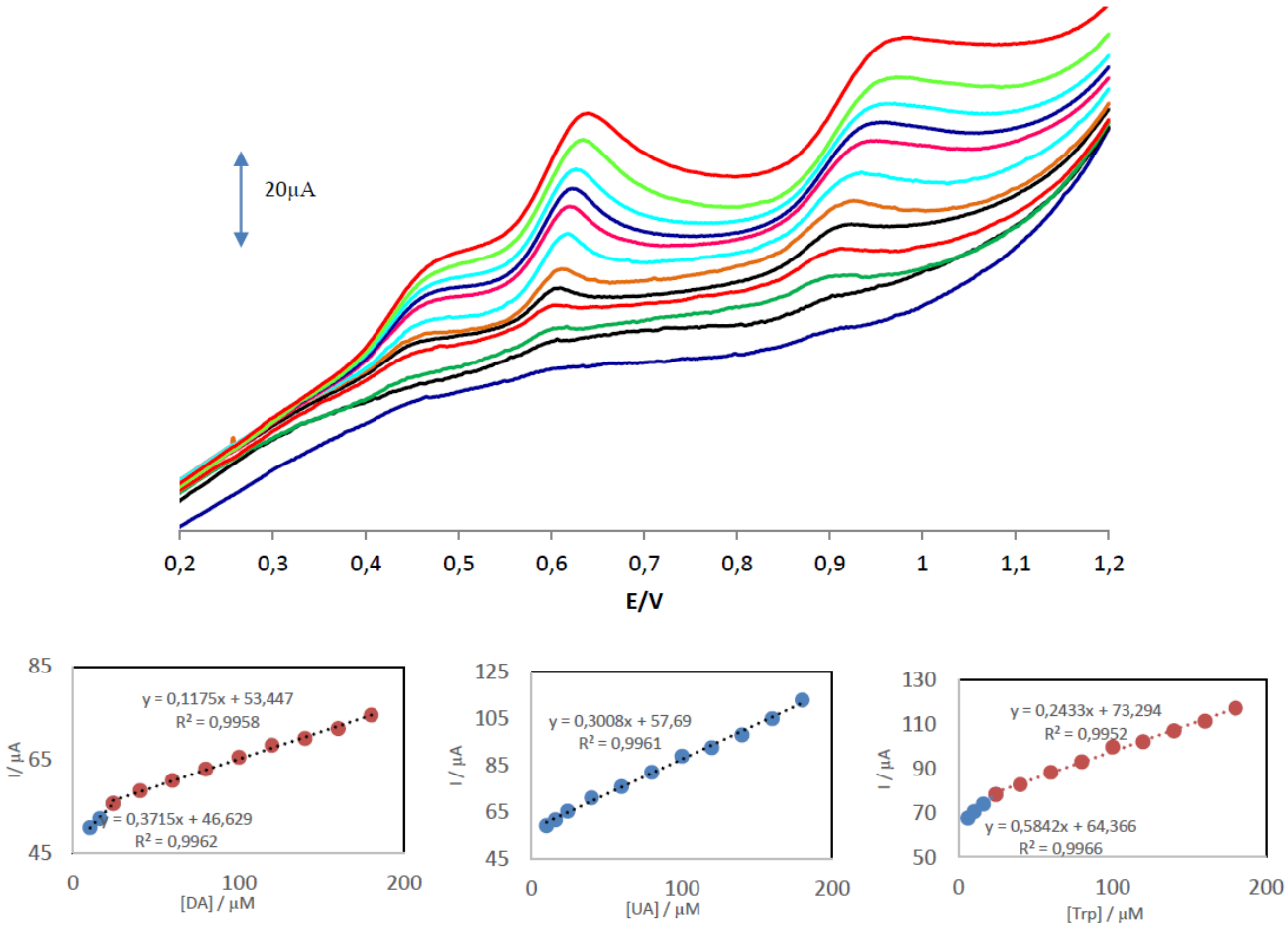


Figure 9. LSVs of the solution containing AA, DA and UA at the PTh/CPE in PBS (pH 4.0) at the scan rate of 50 mV s^{-1}, (Inset, Calibration curves for analytes).

Table 1. Comparison of the proposed sensor with other electroanalytical methods for the simultaneous determination of DA, UA and/or Trp

Electrode	Modifier	Method	pH	Analyte	Linear range (μM)	Detection limit (μM)	Ref.
GCE	TiO_2-graphene/poly(4 amino benzenesulfonic acid)	DPV	7	DA	-	-	[28]
				UA	1-400	0.1	
				Trp	1-400	0.3	
GCE	Poly (Evans Blue)	DPV	4.5	DA	1-10	0.25	[29]
				UA	30-110	2	
				Trp	-	-	
Pyrolytic graphite	Dopamine	DPV	6.5	DA	-	0.11	[30]
				UA	2.5-20	1.4	
				Trp	-	-	
Carbon nanofibers	Palladium nanoparticle	DPV	7	DA	0.5-160	0.7	[31]
				UA	2-200	4.5	
				Trp	-	-	
Carbon paste	Multi-walled carbon nanotubes	DPV	3	DA	2-170	0.36	[15]
				UA	0.4-100	0.27	
				Trp	0.6-100	0.065	
GCE	Poly (vinyl alcohol)	LSV/CV	7	DA	2-70	1.4	[32]
				UA	2-50	0.6	
				Trp	-	-	
FTO	fMWCNT/RuRe-NF	LSV/CV	3	DA	1.3-283.3	0.15	[33]
				UA	1.3-433.3	0.14	
				Trp	1.3-433.3	0.14	
Carbon paste	Polythiophene	Lsv/CV		DA	10-180	1	This work
				UA	6-180	0.57	
				Trp	6-180	0.61	
Carbon fiber microdisk electrode	Graphen oxide	LSV/CV		GSH	0.8-600	0.6	[34]
				UA	1-600	0.6	
				Trp	0.6-600	0.1	
GCE	cetyltrimethylammonium bromide	CV	7	DA	0.5-1000	0.11	[35]
				UA	1-1000	0.33	
				Trp	1-1000	0.44	
				TP	0.5-1000	0.11	

3.8. Analytical application

Table 2. The analytical application of PTh/CPE for simultaneous determination of DA. UA and Trp in human serum and urine samples (n=3)

sample	Analyte	Detected(μM)	Spiked(μM)	Found(μM)	RSD*%
Serum	DA	-	30.0	29.43±1.0	98.0
	UA	11.7±1.3	50.0	63.5±2.7	102.9
	Trp	-	50.0	47.8±2.1	95.6
Urine	DA	-	30.0	28.8±1.2	96.0
	UA	18.2±2.3	50.0	69.3±2.6	101.6
	Trp	-	50.0	48.0±2.3	96.0

*RSD relative standard deviation

To demonstrate the applicability of the PTh/GPE, the proposed electrode was applied for the simultaneous determination of DA, UA and Trp in real biological samples such as blood and urine. The real sample dilution was done with 0.1 M PBS (pH 4.0) and then proper amount of prepared samples were added to the electrochemical cell for the determination of these three analytes using the proposed sensor. The simultaneous measurement of DA, UA and Trp was also done in a mixture sample, 0.1 M PBS (pH 4.0) spiked with appropriate amounts of real samples. Table 2 summarized the analytical results. It can be seen that the proposed electrode demonstrated recovery of the spiked samples in a range between 95.6% and 102.9% and (RSD%) of all three species (n=3), were less than 3.0 % (Table 2).

4. CONCLUSION

A novel polythiophene modified graphite paste electrode was designed. The proposed electrode exhibited good electrocatalytic activity of DA, UA and Trp. It was found that pH of solution can strongly affect electrochemical behavior of the proposed electrode. As DA, UA and Trp coexist in (real) samples, then they can be analyzed by the proposed modified electrode in this work. It was also found that the separation peak potential for the detection of mentioned analytes are quite desirable. Therefore, simultaneous detection of these three analytes are feasible without any interference. Finally, feasibility of the proposed sensor was investigated by determination of DA, UA and Trp in blood and urine samples with satisfactory results.

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