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Modified Graphite Paste Electrode with Strontium Phen-Dione Complex for Simultaneous Determination of a Ternary Mixture of 4-aminophenol, Uric Acid and Tryptophan (Part I)

S.Narouie, M. Shahbakhsh, Z. Hashemzaei, A. Nouri, H. Saravani and M. Noroozifar*

Department of Chemistry, University of Sistan and Baluchestan, Zahedan, P.O. Box 98135-674, Iran *E-mail: <u>mnoroozifar@chem.usb.ac.ir</u>

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For the first time, the $[Sr(OH_2)_4(phen-dione)_2](Cl)_2$ complex (Sr-Phen) was synthesized and characterized by different spectroscopy techniques such as ¹H-NMR, UV-Vis, FT-IR and conductometry as well as elemental analysis. The prepared complex was used for modification of graphite paste electrode for simultaneous determination of 4-aminophenol (AP), uric acid (UA), and tryptophan (Trp). Detailed investigations by electrochemical impedance spectroscopy and electrochemistry methods were used in order to elucidate the properties of the modified graphite paste electrode with Sr-Phen. The proposed modified electrode displays intense and indelible electrooxidation responses for simultaneous determination of AP, UA, and Trp to three well-separated peaks in potential range from 0.4 to 1.1V using cyclic and differential pulse voltammetry methods in pH 2.0. Based on the experimental results the detection limits of 3.40, 1.06 and 1.32 μ M were obtained for AP, UA and Trp, respectively. Finally, the proposed modified electrode was used for simultaneous determination of AP, UA and Trp in real samples.

Keywords: Strontium Phen-dione, Modified graphite paste electrode, Simultaneous electrochemical determination, 4-Aminophenol, Uric acid, Tryptophan.

1. INTRODUCTION

Paracetamol is an analgesic and anti-pyretic drug prescribed for various mild pain and fever conditions [1]. In the human body, the material is initially degraded to its de acetylated metabolite, 4-Aminophenol (AP) [2]. 4-AP is the last synthetic intermediate of Paracetamol [3]. Given the nephrotoxicity of 4-AP, both the European and the United States Pharmacopeia specify with a

maximum level of 0.005 % w/w or 50 ppm of 4-AP in pharmaceutical drugs [4]. Uric acid (UA), is an important anti-oxidant produced naturally by purine metabolism in the human body [5]. High concentration of UA in the human urine and blood are showing of a disorder in purine metabolism [6]. Tryptophan (Trp) is an important amino acid in the human body for the synthesis of many biomolecules (hormones and neurotransmitters) [7]. Due to disorder in the metabolism, abnormal levels of Trp can cause hallucinations, delusions and hepatic disease [8]. Therefore, it is critical to establish simple, selective, sensitive, low cost, and rapid techniques for simultaneous determination of these analytes in order to inspect their physiological functions and diagnose disease. Among various analytical methods, electroanalytical methods are characterized by their selectivity, simplicity, sensitivity and rapidity, making them good candidates for this purpose [9] Bare electrodes, on the other hand, have poor sensitivity and selectivity; thus, they need to be chemically modified to enhance their selectivity and sensitivity. Fine-tuning of redox potentials creates a good match between redox levels of intermediates and the electrochemical reactions of analytes which can be accessible by many variables such as pH changes, coordination environment modification, structural variables and design of systems with multiple electron transfers. Electro active species with multiple electron transfers produce high-energy intermediates which can catalyze the electrochemical reaction of desired analytes [10].

1, 10-phenanthroline-5, 6-dione (Phendione) was synthesized from oxidation of phenanthroline [11]. It shows ortho-quinone-based, two-electron, pH-dependent redox peaks in aqueous media [10]. Phendione mostly forms stable $N \cap N$ complexes with metal ions [12]. Its redox properties can be controlled through metal complex formation as well as pH [10]. Complex formation shifts the oxidation-reduction peak potentials of the ortho quinone moiety of phedion to more positive potentials because of the stronger π acidity of the complex [12]. Given the high affinity of phendione ligand to form complexes with metal ions and the inevitable presence of metal ions in the real samples, a sensible approach is modify the electrode using phendione stable complexes instead of phendione ligand so that the electrochemical determination of analytes is performed in a stable, correct and exact manner. Transition metal complexes of phendione exhibit good electrocatalytic activity toward Nicotinamide adenine dinucleotide (NADH) oxidation both in solution and as immobilized film on electrode surface[13]. Hasanpour et al. used tris (1, 10-phendione-5, 6-Dione) Zinc (II) hexafluro phosphate modified graphite paste electrode for electro catalytic determination of penicillamine [14]. By complex formation the oxidation /reduction potentials of ortho quinone groups in Phendione structure shift to more positive values and the electron affinity of these functional groups increases.

For investigation the effect of the complex formation on the electrocatalytic activity of phendione, in this work, for the first time we synthesized a Strontium-phendione complex (Sr-Phen) for modification of the graphite paste electrode (GPE/Sr-Phen). The GPE/Sr-Phen exhibits electrocatalytic activity toward oxidation of AP, UA and Trp. Finally, the GPE/Sr-Phen was used for simultaneous electrochemical determination of AP, UA and Trp in human real samples.

2. EXPERIMENTAL

2.1. Reagents

Aminophenol (AP), Uric acid (UA), and Tryptophan (Trp) were purchased from Sigma-Aldrich Company. Phosphoric acid, 1,10-phenanthroline 5,6-dione, Ethanol (99%), SrCl₂ .6H₂O were purchased from Merck Company. Fresh urine and serum samples were obtained from the Omid Clinical Laboratory (Zahedan, Iran) without any pretreatments.

2.2. Instrumentation

All electrochemical measurements were carried out with a SAMA 500 Electroanalyzer (SAMA Research Center, Iran). A conventional three-electrode cell including a platinum wire electrode, a silver/silver chloride electrode (Ag/AgCl) and a modified graphite paste electrode were used as the counter, the reference and the working electrodes in this study. Electrochemical impedance spectroscopy (ESI) was performed with an Autolab PGSTAT 128N (EcoChemie, Netherlands) potentiostat/galvanostat controlled by NOVA 1.11 software. All EIS study were performed in 5 mM [Fe (CN) $_{6}$]^{3-/4-} prepared in 0.1 M KCl. EIS was performed over a frequency range of 0.1 Hz to 10 kHz with 0.02 V amplitude (rms). Elemental analyses were obtained by Heraeus CHN-O Rapid elemental analyzer. Conductivity measurements of the complexes were carried out on a Systronics conductivity bridge 305, using a conductivity cell of cell constant 1.0. IR spectra were recorded as KBr pellets on an FTIR Perkin Elemer spectrophotometer and electronic spectra on a JASCO V-570 spectrophotometer while ¹H NMR spectra were recorded on a Bruker DRX-300 MHZ spectrometer at ambient temperature in DMSO-d6. A Metrohm pH meter, model 744 was also used for pH measurements.

2.3. Preparation of modifiers

2.3.1 Synthesize of [Sr(OH₂)₄(phen-dione)₂](Cl)₂ complex

The $[Sr(OH_2)_4(phen-dione)_2](Cl)_2$ was synthesized by adding a 266 mg (or 1mmol) of $SrCl_2$ in water to a (420 mg (or 2mmol) 1,10-phenanthroline 5,6-dione in ethanol. The contents of the reaction were then heated to a 40–45 °C under constant stirring for 10 h. After several days, a yellow compound $[Sr(OH_2)_4(phen-dione)_2](Cl)_2$, was precipitated at room temperature. Yield: 92%. Anal. Calc. for $C_{24}H_{20}C_{12}N_4O_8$: C , 44.28; H, 3.10; N, 8.61. Found: C, 44.32; H, 3.15; N, 8.57%.

2.3.2 Preparation of modified graphite paste electrode

6 mg of [Sr $(OH_2)_4$ (phen-dione) 2] (Cl) 2 and 194 mg graphite were mixed together and then 5 mg paraffin oil was added. This mixture was ground by mortar and pestle for 10 min. Next, the resulting paste was packed into a glass tube and a copper wire as electrical contact was inserted into

the paste of glass tube. Polishing by weighing paper was used for preparation of the new surface of the graphite paste.

3. RESULT AND DISCUSSION

3.1. Fourier transforms infrared (FTIR), Conductometry, ¹H-NMR spectroscopy and UV-Vis spectroscopy characterization of synthetic Strontium Phen-dione complex

Figure 1S shows the FT-IR spectrum of free phen-dione clearly shown a band at 1675 cm⁻¹ which may be assigned to a stretching frequency of the C=O bonds on the phen-dione [10]. The mentioned band did a slightly shift in the FTIR of complex, because the C=O groups are not a coordination site of this ligand with the Sr. The same results have been reported in the literature [10, 11]. Although, in some metal complexes with chemical formula M(O, O'-phen-dione)₃ (where M is Ti or V), the phen-dione ligand is coordinated via oxygen atoms to the metal ion. In these complexes the carbon –oxygen stretching vibration was shifted about 200–300 cm⁻¹ to the lower wavenumbers but in the $Zn(N,N'-phen-dione)_3$ and $[Ni(bpy)_2(phen-dione)](PF_6)_2$ complexes, the phen-dione ligand is coordinated to the metal ion via nitrogen atoms [12, 15]. Based on the literature and results in Fig. 1S, the *phen*-dione ligand is coordinated to Sr *via* nitrogen atoms. In other hand, the band at 1639 cm⁻¹ and 1606 cm⁻¹ are ascribed to the C=N vibrations of the imidazole group of the free ligand but this band was changed to 1544 cm^{-1} for C=N stretching vibration in the ligand in the complex, which is at a lower wavenumber than that in the free ligand due to the bond formation with the metal ion. Based on these results, the C=N group in the ligand shifted to the lower wavenumber due to the bond formation with the metal ion and the fact that the charge delocalization or conjugation increases due to the coordination of the ligand to the metal ion. The similar results reported in literature [16-20].

The electronic spectrum of the phendione ligand and the strontium phendione complex were measured in the ethanol solution (Figure 2S). Phendione ligand showed absorption bands in the UV region 206, 236 nm ($\pi \rightarrow \pi^*$) and 258 nm ($n \rightarrow \pi^*$), the strontium complex showed absorption bands in the UV region 222, 254 nm ($\pi \rightarrow \pi^*$) and 273 nm (($n \rightarrow \pi^*$) [21-25]. Moreover, it exhibited lowerenergy absorptions band (313 nm) indicating that solvent dependency is not present in the spectra of the ligand but there is in the complex spectra. This effect was called metal-to-ligand charge transfer transitions [10, 26]. The electrolytic conductivity of an aqueous solution of strontium Phendione complex (10⁻⁴ M) was measured and its molar conductivity (λ) was calculated. The molar conductivity of one molecule dissolved in aqueous solution and dissociated to forms ions, increases with the number of ions. The ranges of molar conductivity values for various electrolytes that form two, three, four and five ions are given in Table (1) [27]. The molar conductivity of strontium phendione complex was calculated 274 µs. By comparing the obtained molar conductivity and the molar conductivity ranges in Table (1), it was concluded that the strontium phendione complex forms three ions (one complex ion and two chloride ions). The presence of Cl⁻ ions outside the coordination sphere was confirmed by the addition of AgNO₃ solution. The ¹H NMR of the free phen-dione and [Sr(phendione)₂(OH₂)₄](Cl)₂ was recorded in DMSO-d6. The ¹H NMR spectrum of the free phen-dione showed three signals in 7.6-8.9 ppm range and $[Sr(phen-dione)_2(OH_2)_4](Cl)_2$ complex showed three signals in 7.7-9.1 ppm range, this shift is due to the metal connection. The relative intensities of these signals were congruent with the proposed structure. This is consistent with its structure. Also, this complex displays diamagnetic behavior at room temperature [28]. According to the results, the proposed formula for this complex is given in Figure (1).

Molar conductivity	Number of ions		
100 - 150	2		
200 - 300	3		
400 - 450	4		
> 500	5		



Figure 1. The proposed structure of [Sr(phen-dione)₂(OH2)₄](Cl)₂ complex.

3.2. Electrochemial charaterization of modified GPE

As shown in Figure 2, the electrocatalytic behavior of the electrodes in the stepwise modification of GPE was investigated. $[Sr(OH_2)_4(phen-dione)_2](Cl)_2$ modified GPE exhibited three well-defined peak for three analytes and showed maximum peak separation among various electrodes. Figure 2(A) displays the DPVs of a ternary mixture of AP, UA and Trp in 0.1 M phosphate buffer solution (PBS, pH = 2.0) at BGCE, phendione modified GPE and GPE/Sr-Phen. BGPE showed a broad overlapped oxidation peak for AP, UA and Trp at 0.56, 0.75 and 1.14 V, respectively. Also the

phendione modified GPE show a broad overlapped oxidation peak for AP, UA and Trp at 0.55, 0.71 and 1.02 V, respectively. But the $[Sr(OH_2)_4(phen-dione)_2](Cl)_2$ modified GPE showed strong well separated oxidation peak for AP, UA and Trp at 0.55, 0.69 and 1.09V, respectively. The first peak in the CVs of modified electrode with Phen at 0.35 V and Sr-Phen at 0.33 V was assigned to redox peak of free phen and Phen in Sr-Phen [10]. Therefore the $[Sr(OH_2)_4(phen-dione)_2](Cl)_2$ modified GPE has highest sensitivity and selectivity for simultaneous electrochemical determination of AP, UA and Trp. Based on these results GPE/Sr-Phen exhibits higher electrocatalytic activity toward electrooxidation of these analytes, because the coordinated ortho quinone groups in GPE/Sr-Phen structure have higher electron affinity compare to free ortho quinone groups in GPE/Phen structure [12]. Figure 2. (B) display the DPVs of individual solutions of AP, UA and Trp and a mixture solution of AP, UA and Trp in the same potential range and condition. Based on this Figure, the peaks potential and current of AP, UA and Trp are constant in simultaneous and individual conditions.



Figure 2. (A) CVs at (a) BGPE (b GPE/ Phen (c) GPE/Sr-Phen in 0.1M PBS (pH = 2) containing AP (200 μ M), UA (150 μ M) and Trp (120 μ M) and (B) DPVs of GPE /Sr-Phen in 0.1 M PBS(pH = 2) containing (a) AP (220 μ M), (b) UA (150 μ M), (c) Trp (130 μ M) (d) AP (220 μ M), UA (150 μ M) and Trp (130 μ M).

3.3. EIS measurements

The CVs of at BGPE and different modified electrodes with Phen-dione and Strontium Phendione complex in 5 mM Fe (CN) $_{6}^{3-/4-}$ in 0.1 M KCl were shown in Fig. 3(A). The results indicated a remarkable decrease in the ΔE_P accompanied by increases in the peak currents of different electrodes as follows: GPE / Sr-Phen > GPE / Phen > BGPE. Supporting evidence for these modified electrodes was found by EIS, a powerful technique to study electrode-electrolyte interfacial features. Based on the Fig. 3, the Nyquist plot of BGPE comprises two parts: one semicircle, whose diameter equals charge transfer resistance (R_{ct}), at higher frequencies indicates charge transfer limitations while a straight line appears in low frequencies signaling mass transfer limitations [29]. The NOVA software was used for fitting and simulation of EIS data and also the Randles equivalent circuit illustrated in the inset of Fig. 3(B) (inset) was selected as the equivalent circuit for fitting and simulation of EIS data. The R_{ct} for GPE / Sr-Phen, GPE / Phen and BGPE were obtained as 380, 520, 2500 Ω respectively. The R_{ct} values of the electrodes decrease as follow: GPE / Sr-Phen < GPE / Phen < BGPE.



Figure 3. (A) CVs of 0.1M KCl containing 5 mM Fe $(CN)_6^{3-/4-}$ at (a) BGPE (b) Phen / GPE and (c) Sr-Phen / GPE. Scan rate: 100 mVs⁻¹; (B) Nyquist plots of various electrodes (a-c) in 0.1M KCl containing 5 mM Fe $(CN)_6^{3-/4-}$; Fig (B) (inset) equivalent circuit. R_s : solution resistance , R_{ct} : charge transfer resistance, W: Warburg element and CPE: Constant Phase Element.

3.4. The effective electroactive surface area study

The microscopic surface areas of the BGPE and GPE / Sr-Phen were determined by using the randles sevcik equation[30];

 $I_{\rm p} = 2.69 \times 10^5 \, {\rm n}^{3/2} \, {\rm AC_0} \, {\rm D_R}^{1/2} \upsilon^{1/2} \tag{1}$

where I_{pa} (µA) is the anodic peak current; n is the number of transferring electrons; A is the microscopic area (cm²); C₀ (mM) is the bulk concentration of probe ion; D_R is the diffusion coefficient (cm².s⁻¹); and υ (Vs⁻¹) is the scan rate. For 1mM K₃Fe(CN)₆ in 0.1 M KCl the number of transferring electron equals one (n=1) and D_R= 7.6 × 10⁻⁶ cm².s⁻¹ [31, 32]. Figure 3S(A) and 3S(B) shows the plots of I_{pa} vs. $\upsilon^{1/2}$ for BGPE and GPE / Sr-Phen. From the slopes of the trend lines, microscopic surface areas were obtained as 0.154 and 0.236 cm² for BGPE and GPE / Sr-Phen, respectively.

3.5. The effect of scan rates

The CVs of modified electrode, GPE / Sr-Phen, at different scan rates was studied and the results were shown in Fig. 4S(A). The presence of redox peaks (anodic and cathodic peaks) with nearly equal peak currents indicates the presence of diffusionless, reversible surface confined process [33]. The presence of these peaks is attributed to the ortho quinone groups of Strontium-Phen dione

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complex. The Surface concentration of electro active species at GPE / Sr-Phen was determined via the Sharp equation explained with following equation [34]:

$$I_{\rm P} = \frac{n^2 F^2 A \Gamma \upsilon}{4 R T} \tag{2}$$

where I_p (μ A) is the peak current; n = 2 is the number of transferring electrons; A is the geometric surface area (here 0.0314 cm²); υ (mV/s) is the scan rate; Γ (mol cm⁻²) is the surface concentration of electro active species; R (8.314 jmol⁻¹k⁻¹), F (96485 C/mol) and T (here 298 K) have their normal meanings. As shown in Fig. 4S(B), from the slope of the trend line in the plot of I_p vs. υ , Γ was estimated as (3.1×10^{-9} mol.cm⁻²). Figure 5S(C) was shown the effect of scan rate on the on the electrochemical oxidation of AP, UA and Trp at the GPE/ Sr-phen by LSVs. (A), Fig. 5S(B) and Fig 5S(C), respectively. Based on this Figures, with increasing the potential scan rate the anodic peak currents for AP, UA and Trp increase and also the oxidation peak potentials were shifted to more positive values due to the kinetic limitations of the electrochemical reaction. As shown in plot in Fig. 6S(E), the peak currents for Ap, UA and Trp, the $I_p vs. v^{1/2}$ were linearly on the scan rate in the range of 5 to 500 mV s⁻¹ as expected for diffusion control processes.

Moreover, charge transfer coefficients (α) of analytes were determined by using the Tofel equation [35];

$$E_{pa} = E^{0'} + \frac{2.303RT}{(1-\alpha)nF} - \frac{2.303RT}{(1-\alpha)nF} \log v^{1/2}$$
(3)

In this equation, E_{pa} , v, α and n are the anodic peak potential (V), the scan rate (V/s), the charge transfer coefficient and the number of electrons transferred in the rate determining step (here, we assume n= 1 (25), respectively. They others parameters such as R (8.314 jmol⁻¹k⁻¹), T (here 298 K) and F (96485 C/mol) have their normal meanings. Based on Fig. 5S(D), the plot of E_p *vs.* logarithm *v* and the slope of trend lines for AP, UA and Trp, α value were obtained 0.89, 0.89 and 0.94 for AP, UA and Trp, respectively.

3.6. Chronoamperometric studies

As shown in Figures (6S-8S), diffusion coefficients of analytes were determined by chronoamperometry and using Cottrell equation which explained the variation of current with time for diffusion controlled process [36].

$$I = nFACD^{1/2}\pi^{-1/2}t^{-1/2}$$
(9)

where D and C are the diffusion coefficient $(\text{cm}^2 \text{ s}^{-1})$ and the concentration (mol.cm^{-3}) , respectively. On the other hand, A and F are the surface area of the working electrode (0.13 cm^2) and Faraday constant (96,485 C mol⁻¹). Under diffusion control (mass transport), a plot of I *vs.* t^{-1/2} is linear, and the value of D can be obtained from its slope. The values of D were found 4.52×10^{-5} , 1.50×10^{-4} and $4.0 \times 10^{-4} \text{ cm}^2.\text{s}^{-1}$ for AP, UA and Trp, respectively.

3.7. pH effect

As shown in Figure 4, pH effect on the electrochemical response of the modified GPE in 0.1 M PBS containing AP (120 μ M), UA (85 μ M), and Trp (60 μ M) was investigated. Peak potential for the three analytes and surface confined species [Sr(OH₂)₄(phen-dione)₂](Cl)₂ shifted to negative potentials with increasing solution pH pointing to the contribution of protons in their electrochemical reaction. For species in whose oxidation reaction protons are transferred, the following formulas explained the relationship between peak potential and pH [36].

$$E'_{p(Red)} = E_{p(Red,pH=0)} - 2.303 \frac{mRT}{nF} pH$$
(6)

$$\frac{dE_p}{dpH} = -2.303 \frac{mRT}{nF}$$
(7)

$$\frac{dE_p}{dpH} = -0.059 \frac{m}{n}$$
at $25c^0$ (8)

where $E_{p(Red, pH=0)}$ is the peak potential for AP, UA and Trp at pH=0; m and n are the numbers of protons and electrons, respectively; R (8.314jmol⁻¹k⁻¹), T (K) and F (96485c.mol⁻¹) have their normal meanings.



Figure 4. (A) DPVs of a solution containing AP(120µM),UA(85µM) and Trp (60µM) at Sr-Phen / GPE in 0.1M PBS at various pH values (2-7) (B) plots of peak potential versus pH for three analytes; (C) plots of peak current versus pH for three analytes.

Figure 4(B) was shown linear relationships between peak potentials and pH values for all species. Based on this Figure, the plot E *vs.* pH showed of one linear lines with slopes of 0.0589, 0.0623 and 0.0631 (based on Eqs. 9-11) that is close to theoretical slope 0.059 (Eq. 8). Therefore, it was suggested that the oxidation reaction of these analytes in the pH range of 2-7 involves two protons and two electrons (see reactions of 12-14). Based on Fig 4(A), DPV related to pH=2 exhibits maximum peak separation as well as the strongest and sharpest oxidation peaks for AP, UA and Trp, therefore pH=2 was selected as the optimum pH.

$E_{(pa, AP)}(V) = 1.0849 - 0.0589 \text{ pH}$	$(r^2 = 0.9964)$	(9)
$E_{(pa, UA)}(V) = 0.587 - 0.0623 \text{ pH}$	$(r^2 = 0.9998)$	(10)
$E_{(pa, Trp)}(V) = 0.7691 - 0.0631 \text{ pH}$	$(r^2 = 0.9973)$	(11)



3.8. Simultaneous determination of AP, UA and Trp

Based on our experimental results above, the simultaneous determination of AP, UA and Trp can be done using DPV in potential range of 0.4 to 1.1 V. Under optimum conditions, the electrocatalytic peak current of AP, UA and Trp increase linearly with increasing the concentration of AP, UA and Trp. Figs. (5 A-B) show the DPVs and calibration curves of AP, UA and Trp at GPE / Sr-Phen. The calibration curve was linear for AP in the range from 56 to 360 μ M. The dynamic ranges

were linear with UA and Trp concentrations in the range from 26 to 267.5 μ M and 10.5 to 262 μ M, respectively. The detection limit was calculated based on relationship LOD = $3S_{blank} / m$, where S_{blank} is the relative standard deviation of blank signals (n=10) and m is the slope of the calibration plot. The theoretical detection limits for AP, UA and Trp were 3.40, 1.06 and 1.32 μ M, respectively. The interference studies for the electrooxidation of AP, UA, and Trp in a mixture were also investigated with intermolecular effect method. In this method, the concentration of one species was changed and the others were kept constant. The results for interference studies of the AP, UA and Trp were shown in Figs.10-12S. In a certain range of concentrations, one analyte has no interference if it causes relative error lower or equal to \pm 5 (%). Examination of Fig. 9S(A) shows that the peak current of AP increased (56 to 360 μ M) with increasing AP concentration (see Fig.9SB), whereas the concentrations of UA (65 μ M) and Trp (35 μ M) remained constant. Similar studies for UA and Trp were shown in Fig. 10S and 11S.Based on Fig. 11S for UA and Fig. 12S for Trp, the I_p for UA or Trp increased with increasing concentration of UA or Trp while the concentration of the other two compounds are constant. It was found that AP, UA and Trp had no interference in simultaneous determination of one another in the linear ranges of AP, UA and Trp.



Figure 5. (A) DPVs of Sr-Phen / GPE in 0.1 M PBS (pH =2) containing mixed concentration of AP, UA and Trp; [AP]:56 – 360 μ M, [UA]: 26 –267.5 μ M, [Trp]:10.5 – 262 μ M; (B) plots of anodic peak current vs. concentration of AP, UA and Trp.

3.9. Real sample analysis

To evaluation of the proposed modified electrode for simultaneous determination of Ap, UA and Trp in real sample, the GPE / Sr-Phen was examined for the simultaneous determination of AP, UA and Trp in the human urine and serum samples. The samples were diluted with PBS and DPVs were used for the simultaneous determination of AP, UA and Trp using standard addition method. As

depicted in Table 2, acceptable recovery values were obtained that indicates possibility of the proposed modified electrode for trace amounts of AP, UA and Trp in the real sample analysis.

Sample	Analyte	Detected (µM)	Spiked (µM)	Found (µM)	Recovery (%)	RSD (%)
Urine	AP	_	180	172.8	96	1.3
	UA	_	120	112.8	94	1.5
	Trp	-	135	137.7	102	1.2
Serum	AP	_	210	199.5	95	1.6
	UA	_	140	144.2	103	2.0
	Trp	_	150	145.5	97	1.3

Table 2. Simultaneous determination of AP, UA and Trp in human urine and serum samples (n = 3)

4. CONCLUSIONS

In this work, a new modifier, Sr-Phen, was used for modification of graphite paste electrode. The proposed modified electrode GPE/Sr-Phen was used for the simultaneous determination of a ternary mixture of AP, UA and Trp. Based on our results; the Sr-Phen not only improves the selectivity but also increased the electrochemical catalytic activities towards the oxidation of AP, UA and Trp in the simultaneous determination. The calibration curve were liner up to 360, 276.5 and 262 μ M with a detection limits 3.40, 1.06 and 1.32 μ M for AP, UA and Trp, respectively. Also, based on the Table 3, the GPE/Sr-Phen seems to provide a favorable alternative for the simultaneous determination of AP, UA and Trp with better or satisfactory results than those described in the literature. The preparation method for modified electrode is very simple, cheap and the proposed modified electrode is an attractive candidate for practical applications for individual and simultaneous determination of AP, UA and Trp. Finally, the proposed modified electrode can be applied to determination of trace amounts of AP, UA and Trp in human urine and serum samples with satisfactory results.

Table 3. Comparison of the proposed modified electrode with others modified electrode in literature for the simultaneous determination of AP, UA and/or Trp

Electrode	Modifier	Method	рН	Analyte	Linear range(µM)	Detection Limit(µM)	Ref	
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GCE	Delphindin immdoilized on silver nanoparticle	DPV	7	UA Trp	51.9-132 129-330	-	[37]
Au	Au/Nafion/Au- MWCNTs–Nafion:Cu cells	CV	-	AP	0.2-1.6	0.09	[38]
Carbon Paste	Polythiophene	LSV/CV	4	UA Trp	6-180 6-180	0.57 0.61	[39]
GCE	Poly(Evans Blue)	DPV	4.5	UA	3-110	2	[40]
Pyrolytic graphite	Dopamine	DPV	6.5	UA	2.5-20	1.4	[41]
Carbon nanofibers Carbon Paste	Palladium nanoparticle	DPV	7	UA	2-200	4.5	[42]
	Natural phosphate	CV	7	AP	450-2290	-	[43]
GCE	Poly(3,4- etyiendioxythiophene)	CV/DPV	7	AP	4-320	1.6	[44]
Graphite Paste	Strontium Phen-Dione Complex	CV/DPV	2	AP UA Trp	56-360 26-267.5 10.5-262	3.4 1.06 1.32	This work

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SUPPLEMENTARY DATA:



Figure 1S. FT-IR spectra of phen-dion ligand (a) and complex (b).



Figure 2S. Absorption spectrum of phen–dion ligand (A) and complex (B) in ethanol.



Figure 3S. CVs of (A) BGPE and (B) the modified GPE with Sr- phen in the presence of $[Fe(CN)_6]^{3-1/4-}(1.0 \text{ mM})$ in 0.5 M KCl, at various scan rates (from inner to outer curve): 5,10,25,50,75,100,150,200 and 250 mV s⁻¹; (C) Plots of anodic and cathodic peak currents for each electrode vs. $v^{1/2}$.



Figure 4S. CVs of (A) modified GPE with Sr-Phen in 0.1 M PBS (pH=2) at various scan rates (from 50 to 500 mV.s⁻¹) (B) plot of anodic and cathodic peak currents vs. scan rate.



Figure 5S. LSVs of modified GPE in 0.1 PBS (pH =2) containing AP (100 μ M) (A), UA (120 μ M) (B) and Trp (80 μ M) (C) at various scan rates (5 to 500 mVs⁻¹) ;Figure (D) plots of anodic peak



Figure 6S. (A) Chronoamperograms obtained at the modified GPE containing different concentrations of AP: (1) 70, (2) 150, (3) 250 μ M in buffer solution (pH=2.0). (B) Cottrell's plots of I vs. t^{-1/2} obtained from chronoamperograms and (C) plots of the slope of the straight lines against analyte concentration.





Figure 7S. (A) Chronoamperograms obtained at the modified GPE containing different concentrations of UA: (1) 50, (2) 130, (3) 220 μ M in buffer solution (pH=2.0). (B) Cottrell's plots of I vs. t^{-1/2} obtained from chronoamperograms and (C) plots of the slope of the straight lines against analyte concentration.



Figure 8S. (A) Chronoamperograms obtained at the modified GPE containing different concentrations of Trp: (1) 50, (2) 130, (3) 250 μ M in buffer solution (pH=2.0). (B) Cottrell's plots of I vs. t^{-1/2} obtained from chronoamperograms and (C) plots of the slope of the straight lines against analyte concentration.





Figure 9S. (A) DPVs at the modified GPE in 0.1 M (pH =2.0) containing UA (65 μ M), and Trp (35 μ M) and different concentrations of AP in the range of 56-360 μ M (from inner to outer). (B) Plots of anodic peak current vs. concentration of AP.



Figure 10S (A) DPVs at the modified GPE in 0.1 M (pH=2.0) containing AP (65μ M) and Trp (36.5μ M) and different concentrations of UA in the range of 26-267.5 (from inner to outer). (B) Plots of anodic peak current vs. concentration of UA.



Figure 11S. (A) DPVs at the modified GPE in 0.1 M (pH =2.0) containing AP (140 μ M), UA (85 μ M),) and different concentrations of Trp in the range of 10-262 (from inner to outer). (B) Plots of anodic peak current vs. concentration of Trp.

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