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Complete List of Authors:	Rajabi, Hossein; aAnalytical Research Laboratory, Department of Chemistry, University of Sistan and Baluchestan; Noroozifar, Meissam; University of Sistan and Baluchestan, Khorasani-Motlagh, Mozhgan; University of Sistan and Baluchestan,

Modified graphite paste electrode by Lewatit® FO36 nano-resin for simultaneous determination of ascorbic acid, acetaminophen and tryptophan

Hossein Rajabi*[1], Meissam Noroozifar *[a], Mozhgan Khorasani-Motlagh[b]

[a]*Analytical Research Laboratory, Department of Chemistry, University of Sistan and Baluchestan, Zahedan, P.O. Box 98135-674, Iran*

[b]*Inorganic Research Laboratory, Department of Chemistry, University of Sistan and Baluchestan, Zahedan, Iran*

* To whom correspondence should be addressed.

E-mail: h_rajabi8086@yahoo.com

E-mail: mnoroozifar@chem.usb.ac.ir, Tel: +98-54-3344-6565 , Fax: +98-54-3344-688

Abstract

In this study, a novel modified graphite paste electrode (GPE) with Lewatit® FO36 resin (LFOR) for the simultaneous determination of ascorbic acid (AA), acetaminophen (AC) and tryptophan (Trp) is described. A detailed investigation by transmission electron microscopy and electrochemistry is performed in order to elucidate the preparation process and properties of the GPE/LFOR. The electrochemical response characteristics of the modified GPE/LFOR toward AA, AC and Trp were investigated by cyclic voltammetry, linear sweep voltammetry (LSV) and Chronoamperometry. Under the optimum conditions, the detection limits were obtained 2.08 μM, 0.842 μM and 0.385 μM for AA, AC and Trp, respectively. Some kinetic and thermodynamic parameters for electrochemical oxidation of AA, AC and Trp including electron transfer coefficients and diffusion coefficients, are also determined. The LSV is used for the simultaneous determination of AA, AC and Trp at the modified electrode. Also, the GPE/LFOR is successfully used to determine the concentrations of AA, AC and Trp in real samples.

Keywords: Lewatit FO36 resin; Graphite carbon paste; Ascorbic acid; Acetaminophen; Tryptophan

1. Introduction

L-Ascorbic acid (AA) is naturally organic compound with anti-oxidant properties. It is widely present in many foods, biological systems and in multivitamin preparations. Its shortage leads to the development of scurvy syndrome. It is administered in the treatment of many disorders, including Alzheimer's disease, cancer, atherosclerosis, infertility and HIV infections. Due to its importance, many modified electrodes have been developed and reported for the electrochemical determination of this compound.[1-3] N-acetyl-p-aminophenol or acetaminophen,[4,5] (AC) is a mild, safe, widely used analgesic and antipyretic substitute of aspirin. However, it causes liver necrosis in humans and experimental animals when high doses are administered.[6] L-Tryptophan (Trp) is an essential amino acid with diverse physiological roles, functioning both independently or via incorporation into the high molecular weight such as proteins. It is a precursor for biologically important molecules, such as the neurotransmitter serotonin (NS) and the neurohormone melatonin (NM).[7] Abnormal levels of NS and NM have been shown to be associated with depression and Alzheimer's and Parkinson's diseases, respectively. It has been shown that the control of dietary intake of Trp through food or supplements has a positive effect on the regulation of the serotonin synthesis.[8] The regulation of the synthesis of serotonin leads to the controlled synthesis of melatonin,[9] which promotes sleep. Following the lifting of the USA Food and Drug Association ban on Trp, this amino acid has been increasingly available in food supplement forms, and in some formulations co-administered with melatonin. Given the far reaching role of this amino acid, fast, simple and cheap determination methods for Trp's detection in food processing, pharmaceutical formulations and biological fluids are of great importance.[10]

Carbon paste electrodes (CPEs) are a special kind of heterogeneous carbon electrode consisting of mixture prepared from carbon powder (as graphite, glassy carbon and carbon

nanotubes) and a suitable water-immiscible or non-conducting binder.[11,12] Application of CPEs was initially reported by Adams in 1958.[13] In afterward researches have been used different modifiers including enzymes,[14–16] a wide variety polymer such as Nafion® and Chitosan,[17-20] and nanomaterials,[21-23] in CPEs composite. Because of some advantages such as very low background current, facility to prepare, low cost, large potential window, simple surface renewal process and easiness of miniaturization, CPEs are widely applicable in electrochemical studies and electroanalysis.[24–26]

Application of modified electrodes using ion exchanger such as; zeolite and resin for electroanalytical purposes have attracted much attention.[27–28] Lewatit FO36 resin (LFOR) is a weak basic macroporous anion exchanger based on the polystyrene-divinylbenzene copolymer of 0.35 mm beads containing an iron nanooxide. The rigid macro pores of the LFOR are filled with iron oxide particles and the iron oxide is distributed in the pores of the ion exchange resin in a layer with a few nanometers thick. The combination of the ion exchange resin and iron nanooxide is termed a hybrid system. The "hybrid" nature of Lewatit FO36 is unique. It is the result of a special process developed by LANXESS company, that provides a nanoscale, finely distributed and highly reactive iron nanooxide layer plated on the inside of the resin pores. This resin has a content of approximately 15 % iron measured on dry weight base. Sorbents based on hydrated ferric hydroxide such as LFOR and natrolite zeolite–iron oxyhydroxide system,[29] are primarily used just for anions and cation removal such as arsenic,[30–36] chromate,[37]phosphate,[38] Pb,[39] and Cu, [40] from waters and wastewaters. There are a few reports on the using ion exchangers "resin" for preparation of modified electrode. Teixeira et al. examined using copper (II) phosphate immobilized in a polyester resin for determination of AA.[41] Lupetti et al. has been used anion-exchange resin with triiodide carbon paste electrode for the determination of adrenaline.[42]

To the best of our knowledge, no study has been reported on the electroanalysis and simultaneous determination of AA, AC and Trp using a modified graphite paste electrode with LFOR. This paper includes for the first time a simple method for preparation of a new modified graphite paste electrode based on LFOR dispersed in paraffin. Low detection limits and high sensitivity for these three species were obtained due to the high electrocatalytic properties of LFOR. The analytical performance of this sensor was evaluated for simultaneous determination of AA, AC and Trp by cyclic voltammetry. Finally, this sensor has been used for the successful determination of these compounds in AC and AA tablets, human serum and urine samples.

2. Experimental

2.1. Reagents

LFOR was purchased from LANXESS Deutschland GmbH, Germany. Ascorbic acid (AA), acetaminophen (AC) and tryptophan (Trp) was purchased from Sigma-Aldrich Company. Graphite powder and paraffin oil (DC 350, density = 0.88 g/cm^3) as binding agents, sodium hydroxide and phosphoric acid were purchased from Merck Company.

2.2. Apparatus

A potentiostat/galvanostat (SAMA 500, electroanalyzer system) was used for carrying out the electrochemical experiments. A conventional three electrode cell was used at 25 ± 1°C. A saturated calomel electrode (SCE), platinum wire, and a modified graphite paste electrode (GPE) with LFOR were used as reference, auxiliary and working electrodes, respectively. Electrochemical impedance spectroscopy (ESI) was performed with a potentiostat (Metrohm, The Netherlands) controlled by a personal computer. ESI measurements were controlled using the Frequency Response Analysis (FRA) system software. Electrochemical measurements were

performed in 10 mM $[Fe(CN)6]^{3-/4-}$ prepared in 0.1 M KCl. EIS was performed over a frequency range of 0.1 Hz−10 kHz with 0.005 V amplitude (rms). Transmission electron microscopy (TEM) images were taken using a Philips CM120 transmission electron microscopy with 2.5 ˚A resolution. A Metrohm pH meter, model 744 was also used for pH measurements.

2.3. Preparation of modified graphite paste electrode with LFOR

The GPE/LFOR was prepared by mixing LFOR (0.01 g) and hand mixing with 95 times its weight of graphite powder using a mortar and pestle. Paraffin oil (~ 0.4 ml) was added and the mixture was ground for 20 min until a uniform paste was obtained. The paste was packed into the end of a glass tube with an internal diameter of 3.0 mm and length of 10 cm. A copper wire was inserted into the carbon paste as an electrical contact. When necessary, a new surface was obtained by pushing some of the paste out of the tube and polishing the end with weighing paper. Also, unmodified GPE was prepared in the same way without adding LFOR to the mixture. Unmodified GPEs were used for the purpose of comparison.

2.4. Preparation of practical solutions

A series of buffer solutions including H_3PO_4 were prepared and pHs were adjusted using HCl or NaOH titrasol solution (0.1 M) in the range from 1.0 to 5.0. All experiments were performed under nitrogen atmosphere at room temperature. The stock solutions of AA (0.01 M), AC (0.01 M) and Trp (0.01 M) were freshly prepared by dissolving ascorbic acid, acetaminophen and tryptophan hydrochloride in doubly distillated water (DDW). All electrolyte solutions were

prepared with DDW and deoxygenated with nitrogen bubbling before each voltammetric experiments at room temperature.

All experiments were performed in compliance with the relevant laws and institutional guidelines. Also, we include a statement that informed consent was obtained for any experimentation with human subjects. For the analysis of AA and AC in tablets, five tablets were accurately weighed and ground to a fine powder. An amount of powder equivalent to the weight of one tablet was dissolved in DDW and then diluted with PBS (0.1 M, pH 3.0) to produce a solution of AA and AC with a concentration of 100 μM. LSV, in conjunction with standard addition technique was used for the determination of the AA, AC and Trp content of the sample. Measurements of the samples were carried out immediately after preparation steps. Human urine and blood serum samples were obtained from the Sina Clinical laboratory, Zahedan, Iran. The samples were prepared from the body by the medical examiner. The samples were frozen at –20 °C immediately after collection and were shipped after retrieval at the earliest by the medical examiner's office to prevent loss of analytes by degradation processes. The blood serum and urine samples were 10 and 5 times diluted with PBS prior to measurement, respectively. Recovery of the AA, AC and Trp in human urine and blood serum were obtained just like tablet samples.

3. Results and discussion

3.1. TEM and EIS characterization of GPE/LFOR and GPE

Fig. 1A shows TEM images for GPE/LFOR. The TEM images of the GPE/LFOR shows the elliptic geometry of the LFOR granules with particles size bigger than 100 nm, because of resin swelling in aqueous environment. For comparison, TEM of GPE was shown in Fig. 1B.

3.2. Electrochemical behavior of AA, AC and Trp at GPE/LFOR

In order to prove catalytic effect of LFOR on biologic compounds AA, AC and Trp was used of cyclic voltammetry. As for redox pair Fe^{III} /Fe^{II} in structure LFOR could predicted electrochemical reaction with mechanism on surface of electrode:

$$R\text{-}[CH_2\text{-}N(CH_3)_2 / Fe^{II}O](OH)_{(s)} \rightleftharpoons R\text{-}[CH_2\text{-}N(CH_3)_2 / Fe^{III}O](OH)_{(s)} + e \quad (1)$$

Electrochemical behaviors of AA, AC and Trp on the surface of the modified electrode with LFOR is shown in the following equations:

$$2R\text{-}[CH_2\text{-}N(CH_3)_2/Fe^{III}O](OH)_{(s)} + AA_{(Red)} \longrightarrow R\text{-}[CH_2\text{-}N(CH_3)_2/Fe^{II}O](OH)_{(s)} + AA_{(Ox)} + 2H^+ \quad (2)$$

$$2R\text{-}[CH_2\text{-}N(CH_3)_2 / Fe^{III}O](OH)_{(s)} + AC_{(Red)} \longrightarrow 2R\text{-}[CH_2\text{-}N(CH_3)_2 / Fe^{II}O](OH)_{(s)} + AC_{(Ox)} + 2H^+ \quad (3)$$

$$2R\text{-}[CH_2\text{-}N(CH_3)_2 / Fe^{III}O](OH)_{(s)} + Trp_{(Red)} \longrightarrow 2R\text{-}[CH_2\text{-}N(CH_3)_2 / Fe^{II}O](OH)_{(s)} + Trp_{(Ox)} + 2H^+ \quad (4)$$

Fig. 2A.a depicts CVs for the electrochemical oxidation of a mixture of AA, AC and Trp (0.2 mM each) on the surfaces of different prepared electrodes in a 0.1M PBS of pH 3.0 at GPE and GPE/LFOR. Based on Fig. 2A.a, GPE electrode showed three oxidation peaks at 330, 575 and 905 mV for AA, AC and Trp, respectively. Based on Fig. 2A.b, the GPE/LFOR showed an enhancement of the oxidation peak currents of AA, AC and Trp, so that three well-defined peaks are observed at potentials of 275, 550 and 855 mV, respectively and the respective peak separations between AA–AC and AC–Trp were 275 and 305 mV. The presence of LFOR in the matrix of the modified electrode (Fig. 2B) has been shown an effective catalytic role in the electro-oxidation of AA, AC and Trp. This catalytic effect caused to a considerable enhancement in the kinetic of anodic peak current of AA, AC and Trp. The resulted sensitivity in three anodic peak current is sufficient enough to achieve the accurate simultaneous determination of AA, AC and Trp in mixture samples. The GPE/LFOR showed an enhancement in the oxidation peak

currents of AA, AC and Trp by about 9, 15 and 20 μA, respectively, compared with that at a GPE electrode.

The microscopic areas of the GPE and GPE/LFOR were obtained by CV using 1 mM $K_3Fe(CN)_6$ as a probe at different scan rates. For a reversible process, the Randles-Sevcik equation has being used:

$$i_{pa} = 2.69 \times 10^5 n^{3/2} A C_0 D_R^{1/2} v^{1/2} \quad (5)$$

where i_{pa} refers to the anodic peak current, n the electron transfer number, A the surface area of the electrode, D_R the diffusion coefficient, C_0 the concentration of $K_3Fe(CN)_6$ and v is the scan rate. For 1 mM $K_3Fe(CN)_6$ in the 0.1 M KCl electrolyte: n = 1 and $D_R = 7.6 \times 10^{-6}$ cm s^{-1} , then from the slope of the i_{pa}–$v^{1/2}$ relation, the microscopic areas were calculated. In bare GPE, the electrode surface was found 0.071 cm^2 and for GPE/LFOR the surface was 0.334 cm^2 (nearly 4.7 times greater than GPE).

Fig. 2C was shown the Nyquist plots for different electrodes. If the Nyquist plot of impedance spectra includes a semicircle portion and a linear portion, the semicircle portion at higher frequencies corresponded to the electron transfer limited process and the linear portion at lower frequencies corresponded to the diffusion process. Based on the Fig. 3C, a gradual decrease in R_{CT} (the diameter of semicircle) was observed from GPE and GPE/LFOR. These results indicate that the presence of GPE/LFOR is effective in enhancing the rate of electron transfer and firmly modified the electrode surface. The improved performance of the GPE/LFOR which employs that the presence of LFOR exhibited good conductivity and the electro n transfer rate at the electrode/solution interface was greatly increased.

3.3. Influence of pH on the simultaneous oxidation of AA, AC and Trp

The effect of solution pH on the electrochemical response of the GPE/LFOR toward the simultaneous oxidation of AA in the presence of AC and Trp was also studied . Figure 3A shows the CVs of AA, AC and Trp in various pHs. Based on the results, the anodic peak currents of AA, AC and Trp increase, with an increase in the solution pH until it reaches to 3.0 and then the anodic peak current of AA, AC and Trp decreases with decreasing pH until pH reaches to 5.0 (see Fig. 3B). In addition, the anodic peak potentials for the oxidation of AA, AC and Trp were decreased linearly with increasing pH from 1.0 to 5.0, showing the proton involvement in the electrode processes. It was found that the anodic peak potentials for AA, AC and Trp shifted to negative potentials by increasing pH. This was expected because of the participation of proton(s) in the oxidation reactions of AA, AC and Trp. Based on Fig. 3C, the anodic peak potentials for the oxidation of AA, AC and Trp shifted towards more negative potential with an increase in pH from 1.0 to 5.0, showing that protons have taken part in their electrode reaction processes. The oxidation reaction can be explained as follows;

$$\text{Red} - mH^{+} - ne \rightleftharpoons \text{Ox} \qquad (6)$$

where Red stands for AA, AC and Trp; Ox stands for the responding products; m and n are the number of protons and electrons involved in the reaction. The anodic peak potentials for peak Red, is given by:[43]

$$E'_{p(Red)} = E_{p(Red,\, pH=0)} - \frac{2.303mRT}{nF} pH \qquad (7)$$

where $E_{p(Red,\ pH=0)}$ is the anodic peak potential for Red at *p*H= 0.0, and R, T, and F have their usual meanings. The plots of $E_{p(Red)}$ values *vs.* *p*H for AA, AC and Trp in the working pH range

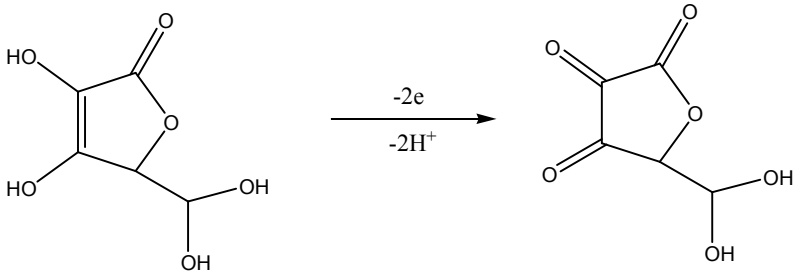
were drawn and the E_p of three compounds (see Fig. 3C) shows a linear relationship with pH of the buffer solution regarding following equations:

$$E_p\,(\mathrm{AA}) = -0.064pH + 0.542 \qquad R^2 = 0.990 \qquad (8)$$

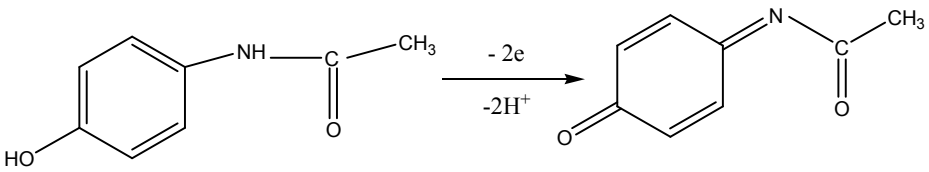
$$E_p\,(\mathrm{AC}) = -0.050pH + 0.718 \qquad R^2 = 0.993 \qquad (9)$$

$$E_p\,(\mathrm{Trp}) = -0.050pH + 1.025 \qquad R^2 = 0.994 \qquad (10)$$

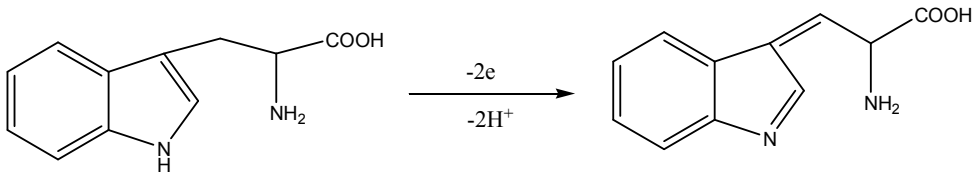
As can be seen in Fig. 3C and equations 3-5, $E'_{p(Red)}$ were shifted to negative potentials with the slopes of 0.064, 0.05, and 0.05 V/pH for AA, AC and Trp, respectively, which are in agreement with the theoretical slope ($-\frac{2.303mRT}{nF}$) of 0.059($\frac{m}{n}$) V/pH. These results suggest that oxidation of AA, AC and Trp involves an equal number of protons and electrons (m=n).[44, 45] This conclusion is in accordance with the known electrochemical reactions of AA, AC and Trp at the surface of GPE/LONR were illustrated in Eqs. 11-13.



(11)



(12)



(13)

Out of these, the PBS with pH 3.0 gave the best response in terms of peak current and peak shape and negatively shifts, hence was chosen as the optimal pH for further studies.

3.4. The effect of scan rate and stability of modified electrode

The effect of scan rate on the electrochemical oxidation of AA, AC and Trp at GPE/LFOR was investigated by CV (Fig. 4A). The oxidation peak potential for AA, AC and Trp shifted to a more positive value with increasing scan rate, confirming the kinetic limitation of the electrochemical reaction. Also, a plot of peak height (I_p) *vs.* the square root of scan rate ($\nu^{1/2}$) was linear in the range of 10–250 mV/s, suggesting that at sufficient overpotential, the process is diffusion rather than surface controlled (Fig. 4B). Also, the stability of GPE/LFOR for the electrochemical oxidation of AA, AC and Trp at GPE/LFOR was investigated by linear sweeping voltammetry (LSV) after 1, 7, 30 and 90 days was shown in Fig. 4C. Based on the LSVs, the peak currents of AA, AC and Trp were decreased 1.5, 4.5 and 5 % after 7, 30 and 90 days, respectively. These results was indicated the excellent stability of GPE/LFOR for simultaneous determination of AA, AC and Trp. Figs. 5B, 5C and 5D shows the Tafel plot AA, AC and Trp for the sharp rising part of the voltammogram at the scan rate of 20 mVs^{-1} (Fig. 5A). The slopes of Fig. 5B, 5C and 5D plots can be used to extract the kinetic parameters α_c (cathodic transfer coefficient) and $\alpha_a = 1-\alpha_c$ (anodic transfer coefficient). The slope of the linear segment is equal to $2.3RT/\alpha n_\alpha F$ and $2.3RT/ ((1–\alpha)n_\alpha F)$ for the cathodic and anodic peaks,[46] while the evaluated values for the transfer coefficients are 0.41, 0.75 and 0.80 for AA, AC and Trp, respectively.

3.5. Chronoamperometric measurements

Chronoamperometric measurements of AA, AC and Trp at GPE/LFOR were carried out by setting the working electrode potential at 0.385, 0.650 and 0.930 V respectively, at the first potential step *vs.* SCE for various concentrations of AA, AC and Trp in PBS (pH 3.0) singly, as presented in Fig. 6A, 6B and 6C for electroactive materials (AA, AC and Trp) with a diffusion coefficients D, the current observed for the electrochemical reaction under mass transport-limited conditions can be described by the Cottrell equation.[46] Experimental plots of I *vs.* $t^{-1/2}$ were drawn, and the best fits for different concentrations of AA, AC and Trp were determined (Insets Figs. of 6A, 6B and 6C). The slopes of the resulting straight lines were then plotted against AA, AC and Trp concentration (Insets Figs. of 6A, 6B and 6C). From the resulting slopes and Cottrell equation, the values of D were found to be 1.99×10^{-5}, 5.22×10^{-5} and 6.02×10^{-5} cm^2/s, respectively.

The rate constants for the electrochemical reactions AA, AC and Trp at surface of GPE/LFOR, K_h, can be evaluated by chronoamperometry according to the method of Galus[47];

$$\frac{I_C}{I_L} = \pi^{1/2}\gamma^{1/2} = \pi^{1/2}\,(K_h C_b t)^{1/2} \qquad (14)$$

where I_C is the catalytic currents of analytes, AA, AC and Trp, at GPE/LFOR, I_L is the limited current in the absence of analytes, and t is the time elapsed (s). The above equation can be used to calculate the rate constants of the catalytic process K_h. Based on the slope of the $\frac{I_C}{I_L}$ *vs.* $t^{1/2}$ plots. The results were shown if Fig. 7. The K_h can be obtained for a given AA, AC, Trp concentrations. Based on the values of the slopes, the average values of K_h for AA, AC and Trp were found to be equal to 2.137×10^3, 3.527×10^3 and 4.157×10^3 M^{-1} s^{-1}, respectively.

3.6. Linear range, detection limit and simultaneous determination

From LSV was used for simultaneous determination of AA, AC and Trp (Fig. 8A). In order to get the best sensitivity under the specific conditions, an amplitude scan rate of 20 mV and pH 3.0 were selected, respectively. the responses were linear with AA concentration in the range from 10.0 to 290.0 μM and the current sensitivity was 0.051μA/μM (Fig. 8B), while the dynamic range was linear with AC concentration in the range 7.0 to 300 μM and the current sensitivity was 0.126 μA/μM (Fig. 9B). The plot of peak current *vs.* Trp concentration consisted of two linear segments with slopes of 0.276 and 0.164 μA/μM in the concentration ranges of 5.0–100.0 μM and 100.0–310.0 μM, respectively. The decrease in sensitivity (slope) of the second linear segment is likely due to kinetic limitation (Fig. 8B). Detection limits were determined as 2.08 μM AA, 0.842 μM AC and 0.385 μM Trp based on $Y_{LOD}=Y_B+3\sigma$.[48] On the other hand, the electro oxidation processes of AA, AC and Trp in mixtures were also investigated when the concentration of one species changed and the other was kept constant, and the results are shown in Fig. 9. Examination of Fig. 9A shows that the peak current of AA increased (20.0-300.0 μM) with increasing AA concentrations, whereas the concentrations of AC and Trp remained constant (200.0 μM). Similarly, as shown in Fig. 9B and 9C, the oxidation peak currents of AC or Trp increased linearly with the increase of the concentration in the presence of a constant concentration (250.0, 300.0 μM) of other two compounds.

3.7. Analytical application

Applicability of the GPE/LFOR was examined for the simultaneous determination of AA, AC and Trp in vitamin C tablets, acetaminophen tablets, human serum and urine samples. The linear sweep voltammetries were obtained by spiking appropriate samples in diluted solution using GPE/LFOR at optimum conditions as described earlier. The results are shown in Tables 1,

2 and 3. The recoveries were acceptable, showing that the proposed methods could be efficiently used for the determination of trace amounts of these compounds in biological systems and pharmaceutical preparations.

4 Conclusions

In this work, for the first time a simple modified electrode was prepared by the Lewatit FO36 resin and applied to the electrochemical determination of AA, AC and Trp. The GPE/LFOR enhanced the oxidation peak current of AA, AC and Trp obviously. The results showed wide liner concentration range, low detection limit and good selectivity. In addition, this proposed method can be applied to the determination of AA, AC and Trp in real samples with satisfactory results.

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Figures captions

Fig. 1. (A) TEM image of GPE/LFOR and (B) GPE

Fig. 2. (A) Cyclic voltammograms of 0.1 mM AA + 0.1 mM AC + 0.1 mM (Trp) at the surface of (a) GPE and (b) GPE/LFOR with scan rate 25 mV s^{-1} at the same conditions (a). (B) Cyclic voltammograms of (a) GPE and (b) GPE/LFOR in 0.1M PBS (pH 3.0) and the presence of 0.1 mM AA (c) 0.1 mM AC (d) 0.1 mM (Trp) and (e) 0.1 mM AA + 0.1 mM AC + 0.1 mM (Trp) and (C) Nyquist plots showing the step-wise modification of GPE (a) and GPE/LFOR (b). Electrochemical measurements were performed in 10 mM $[Fe(CN)6]^{3-/4-}$ prepared in 0.1 M KCl. EIS was analyzed over a frequency range of 0.1 Hz−10 kHz.

Fig. 3. (A) CVs of GPE/LFOR in presence 0.1 mM AA + 0.1 mM AC + 0.1 mM (Trp) at a scan rate of 100 mV/s^{-1} with pHs 1 to 5 (B) Influence of pH values on the current response of three species at the GPE/LFOR (C) Variation of E_p versus the various buffered pHs: 1, 2, 3, 4, and 5.

Fig. 4. (A) CVs of the GPE/LFOR in the presence of 0.1 mM AA, AC and Trp and pH 3.0 at various scan rates; 10, 20, 30, 40, 60, 80, 100, 125, 150, 175, 200 and 250 mVs^{-1} (1 to 11). (B) Variation of the anodic peak currents versus $v^{1/2}$ and (C) the LSV of the GPE/LFOR in the presence of AA (0.22mM), AC (0.18 mM) and Trp (0.15 mM) after (a) first, (b) 7, (c) 30 and (d) 90 days.

Fig. 5. (A) Cyclic voltammogram of GPE/LFOR AA, AC and Trp (0.1 mM) at pH 3.0 and scan rate 20 mVs^{-1}. Tafel plot derived from the CV at scan rate 20 mVs^{-1} from AA (B), AC (C) and Trp (D).

Fig. 6. Chronoamperograms obtained at GPE/LFOR in 0.1M PBS (pH 3.0) (A) for different concentrations of AA; (1) 0.0, (2) 0.1, (3) 0.2, (4) 0.3, (5) 0.4 and (6) 0.5 mM (B) for different concentrations of AC; (1) 0.0, (2) 0.1, (3) 0.2, (4) 0.3, (5) 0.4 and (6) 0.5 mM AC and (C) for different concentrations of Trp; (1) 0.0, (2) 0.32, (3) 0.44, (4) 0.56 and (5) 0.66 mM in the buffer solution (pH 3.0). (Insets) Cottrell's plots of *I vs.* $t^{-1/2}$ obtained from chronoamperograms and plot of the slope of the straight lines against analytes concentration.

Fig. 7. Plot of $\frac{I_c}{I_l}$ *vs.* $t^{1/2}$ for (A) AA, (B) AC and (C) Trp.

Fig. 8. (A) LSVs of GPE/LFOR 0.1 mol/L PBS (pH 3.0) containing different concentrations of AA, AC, and Trp. Concentrations of AA+AC+Trp (μmol/L): (1) 0; (2) 5.0 + 7.0 + 10.0; (3) 20.0 + 20.0 + 20.0; (4) 30.0 + 30.0 + 30.0; (5) 40.0 + 40.0 + 40.0; (6) 50.0 + 50.0 + 50.0; (7) to (29)

add 10 μM (AA+AC+Trp), (30) 290.0 + 290.0 + 290.0 (31) 300.0 AC + 300.0 Trp; (32) 310.0 Trp, (B) plots of Ip vs. concentration of AA, AC, and Trp, respectively.

Fig. 9. (A) LSVs at the GPE/LFOR in 0.1 M pH 3.0 (A) containing AC and Trp (200.0 μM each) and different concentrations of AA (from inner to outer): 0, 25.0, 40.0, 60.0, 80.0, 100.0, 120.0, 140.0, 160.0, 180.0, 200.0, 220.0, 240.0, 260.0, 280.0 and 300.0 μM (B) containing AA and Trp (250.0 μM each) and different concentrations of AC (from inner to outer) 0, 25.0, 40.0, 60.0, 80.0, 100.0, 120.0, 140.0, 160.0, 180.0, 200.0, 220.0, 240.0, 260.0, 280.0 and 300.0 μM(C) containing AA and AC (300.0 μM each) and different concentrations of Trp (from inner to outer) 0, 25.0, 40.0, 60.0, 80.0, 100.0, 120.0, 140.0, 160.0, 180.0, 200.0, 220.0, 240.0, 260.0, 280.0 and 300.0 μM

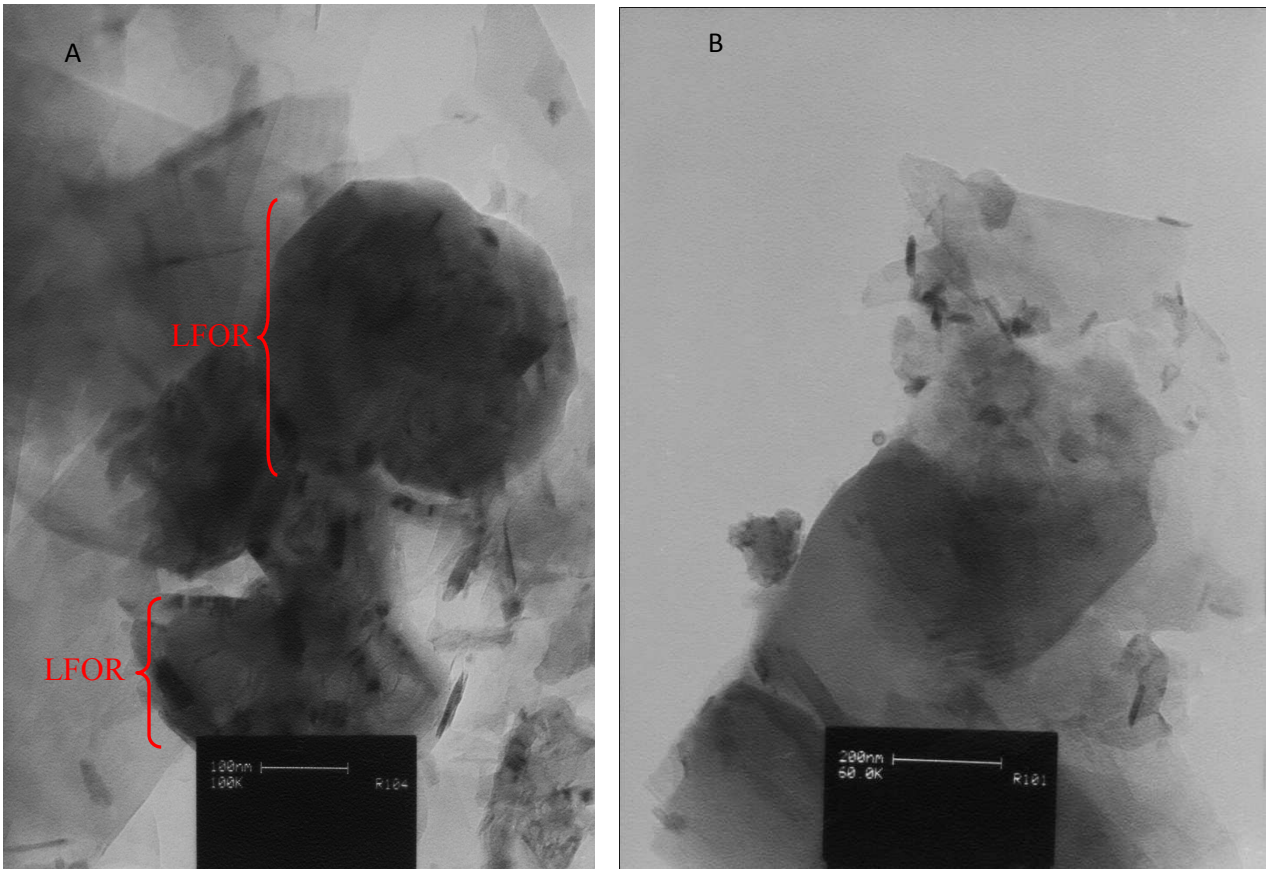


Fig. 1.

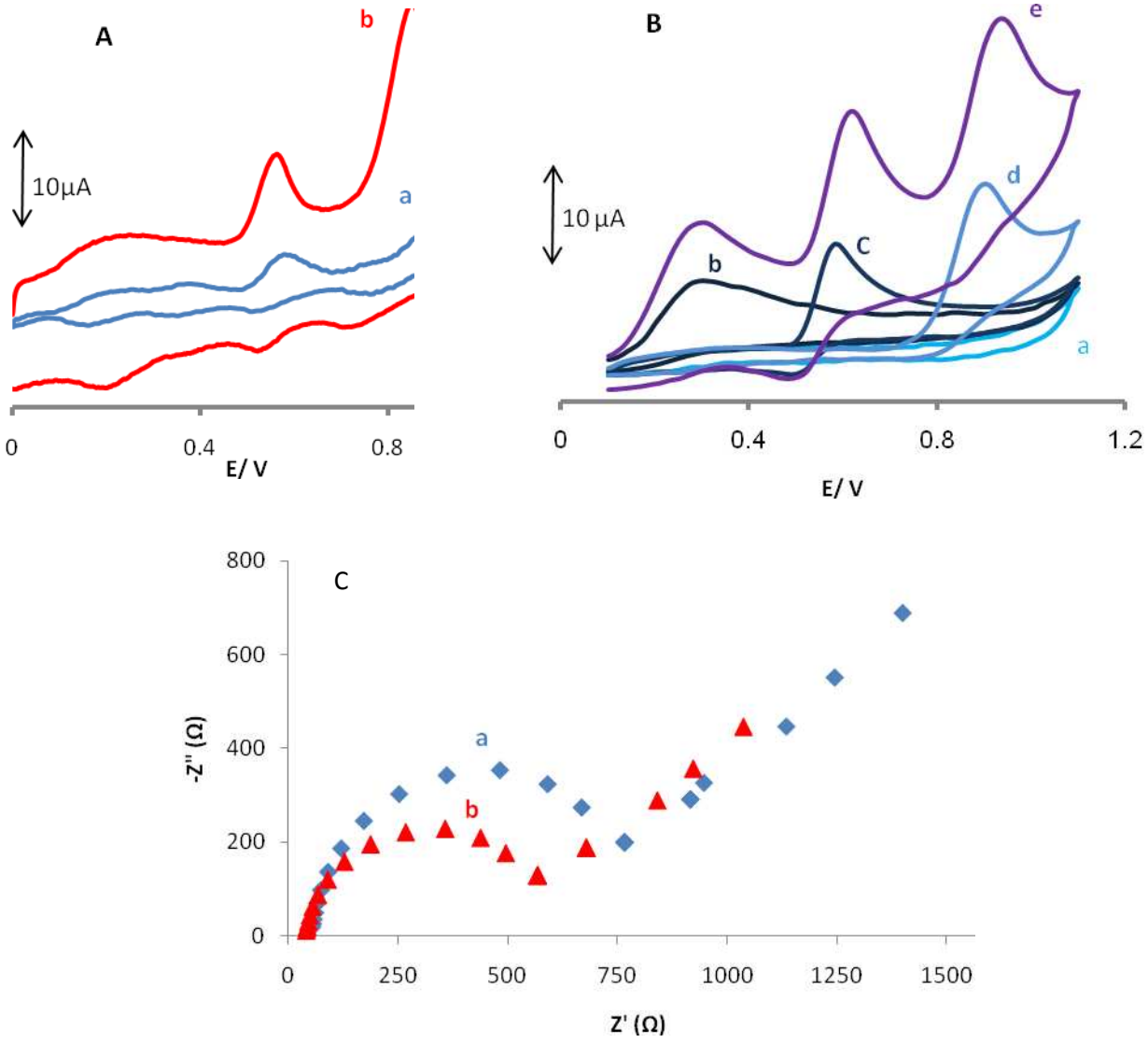

A
b
a
10μA
0
0.4
0.8
E/ V
B
e
d
C
b
a
10 μA
0
0.4
0.8
1.2
E/ V
C
800
600
400
200
0
-Z'' (Ω)
a
b
0
250
500
750
1000
1250
1500
Z' (Ω)

Fig. 2

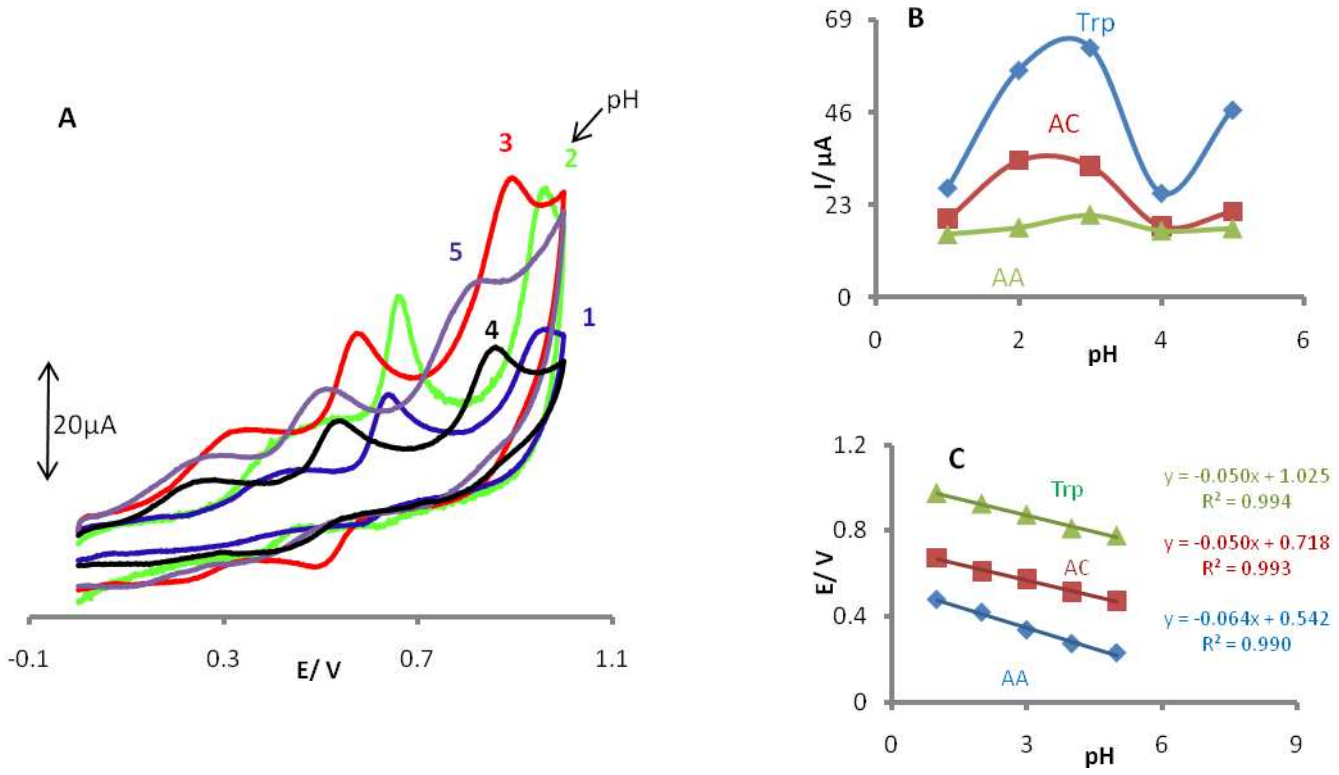

A
pH
3
2
5
1
4
20µA
-0.1
0.3
E/ V
0.7
1.1
B
69
46
23
0
I/ µA
Trp
AC
AA
0
2
4
6
pH
C
1.2
0.8
0.4
0
E/ V
Trp
AC
AA
y = -0.050x + 1.025
R² = 0.994
y = -0.050x + 0.718
R² = 0.993
y = -0.064x + 0.542
R² = 0.990
0
3
6
9
pH

Fig. 3.

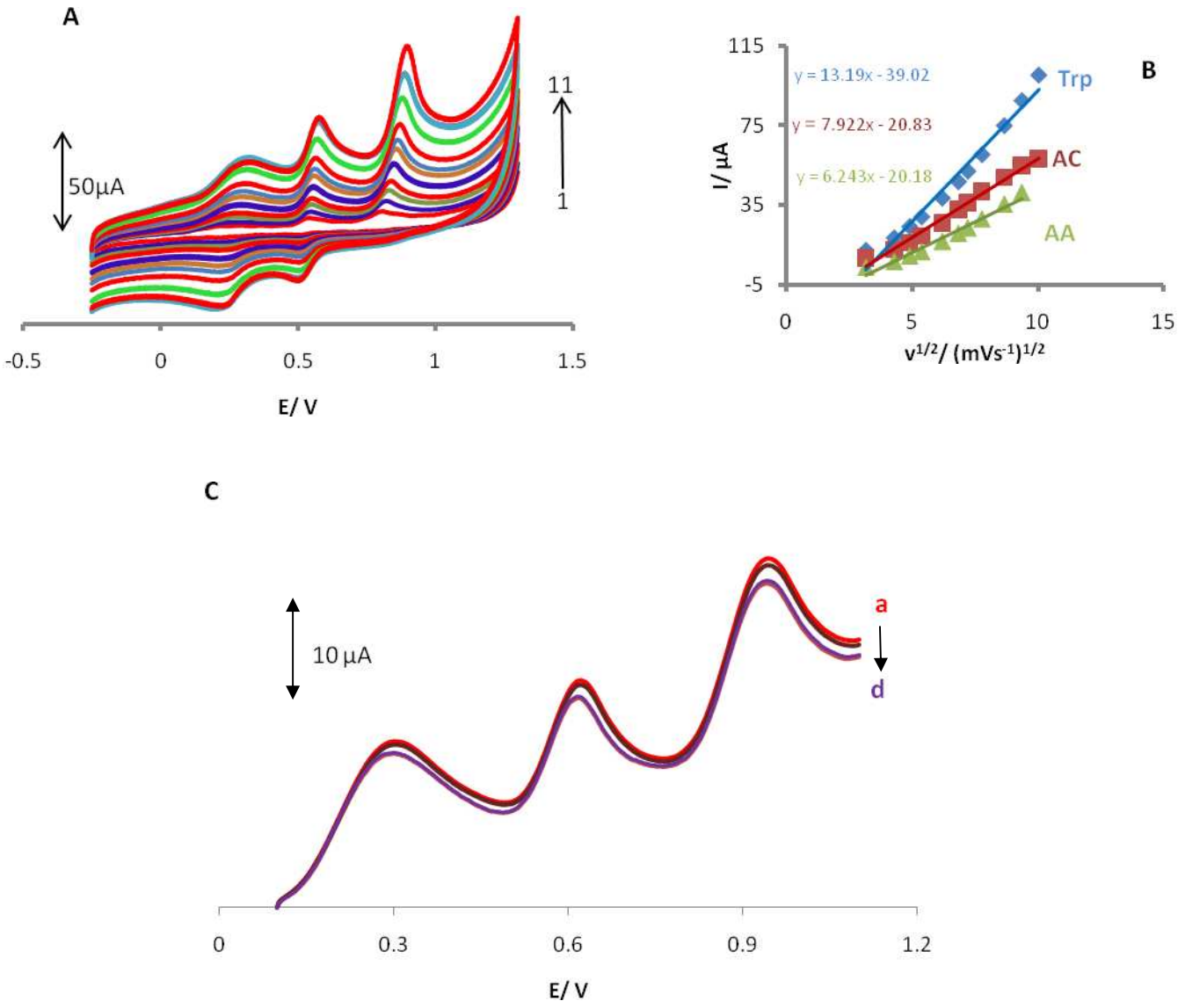
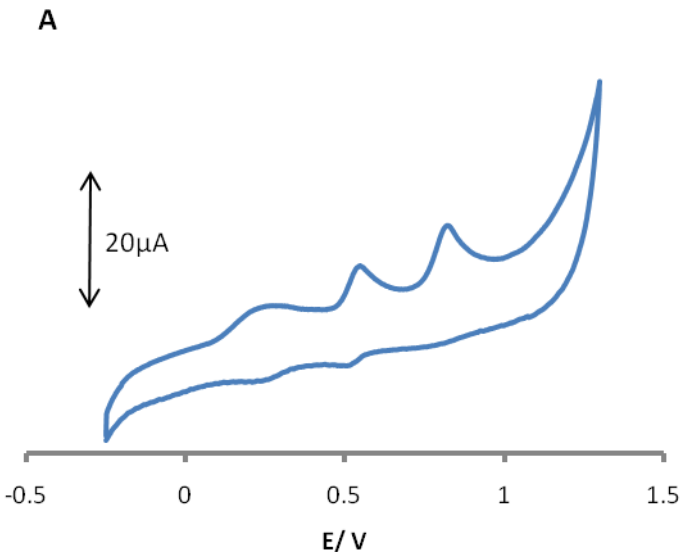
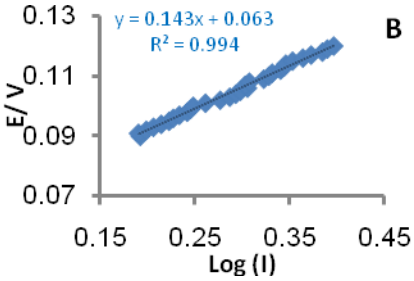
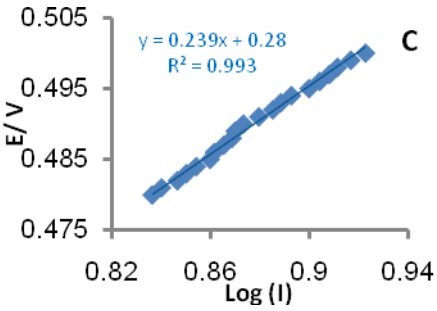

A
50µA
11
1
-0.5
0
0.5
1
1.5
E/ V
B
115
75
35
-5
I/ µA
y = 13.19x - 39.02
y = 7.922x - 20.83
y = 6.243x - 20.18
Trp
AC
AA
0
5
10
15
v1/2/ (mVs-1)1/2
C
10 µA
a
d
0
0.3
0.6
0.9
1.2
E/ V

Fig. 4.


A
20µA
-0.5
0
0.5
1
1.5
E/ V


B
y = 0.143x + 0.063
R² = 0.994
0.13
0.11
0.09
0.07
E/ V
0.15
0.25
0.35
0.45
Log (I)


C
y = 0.239x + 0.28
R² = 0.993
0.505
0.495
0.485
0.475
E/ V
0.82
0.86
0.9
0.94
Log (I)

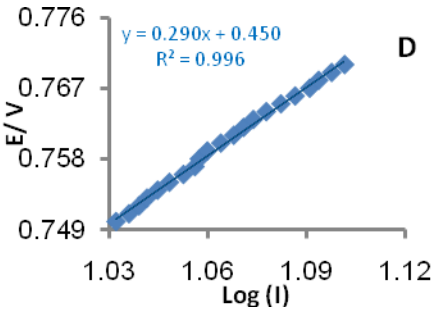

D
y = 0.290x + 0.450
R² = 0.996
0.776
0.767
0.758
0.749
E/ V
1.03
1.06
1.09
1.12
Log (I)

Fig. 5.

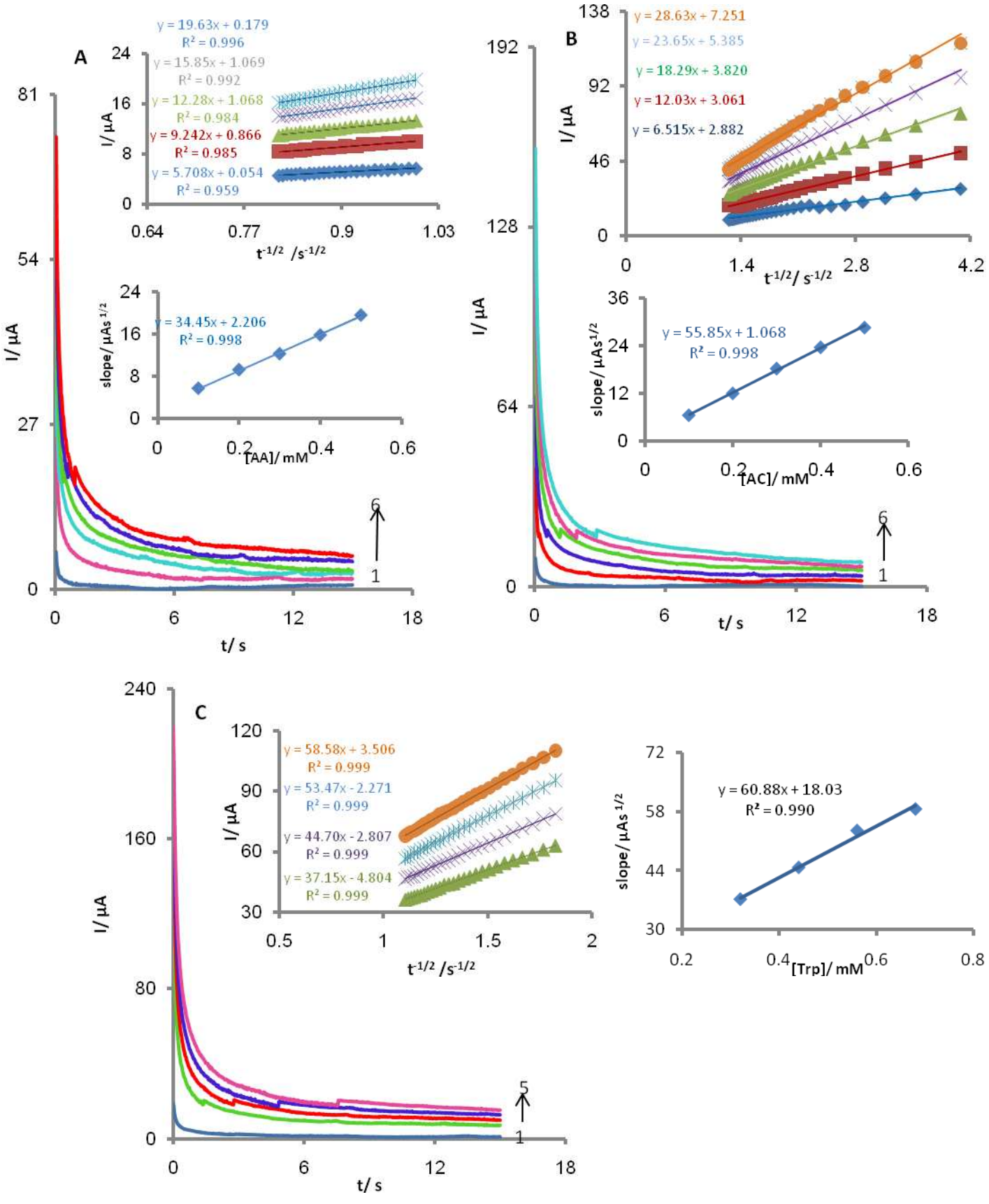

A
y = 19.63x + 0.179
R² = 0.996
y = 15.85x + 1.069
R² = 0.992
y = 12.28x + 1.068
R² = 0.984
y = 9.242x + 0.866
R² = 0.985
y = 5.708x + 0.054
R² = 0.959
I/ µA
t^-1/2 /s^-1/2
y = 34.45x + 2.206
R² = 0.998
slope/ µAs^1/2
[AA]/ mM
I/ µA
t/ s
B
y = 28.63x + 7.251
y = 23.65x + 5.385
y = 18.29x + 3.820
y = 12.03x + 3.061
y = 6.515x + 2.882
I/ µA
t^-1/2/ s^-1/2
y = 55.85x + 1.068
R² = 0.998
slope/ µAs^1/2
[AC]/ mM
I/ µA
t/ s
C
y = 58.58x + 3.506
R² = 0.999
y = 53.47x - 2.271
R² = 0.999
y = 44.70x - 2.807
R² = 0.999
y = 37.15x - 4.804
R² = 0.999
I/ µA
t^-1/2 /s^-1/2
y = 60.88x + 18.03
R² = 0.990
slope/ µAs^1/2
[Trp]/ mM
I/ µA
t/ s

Fig. 6.

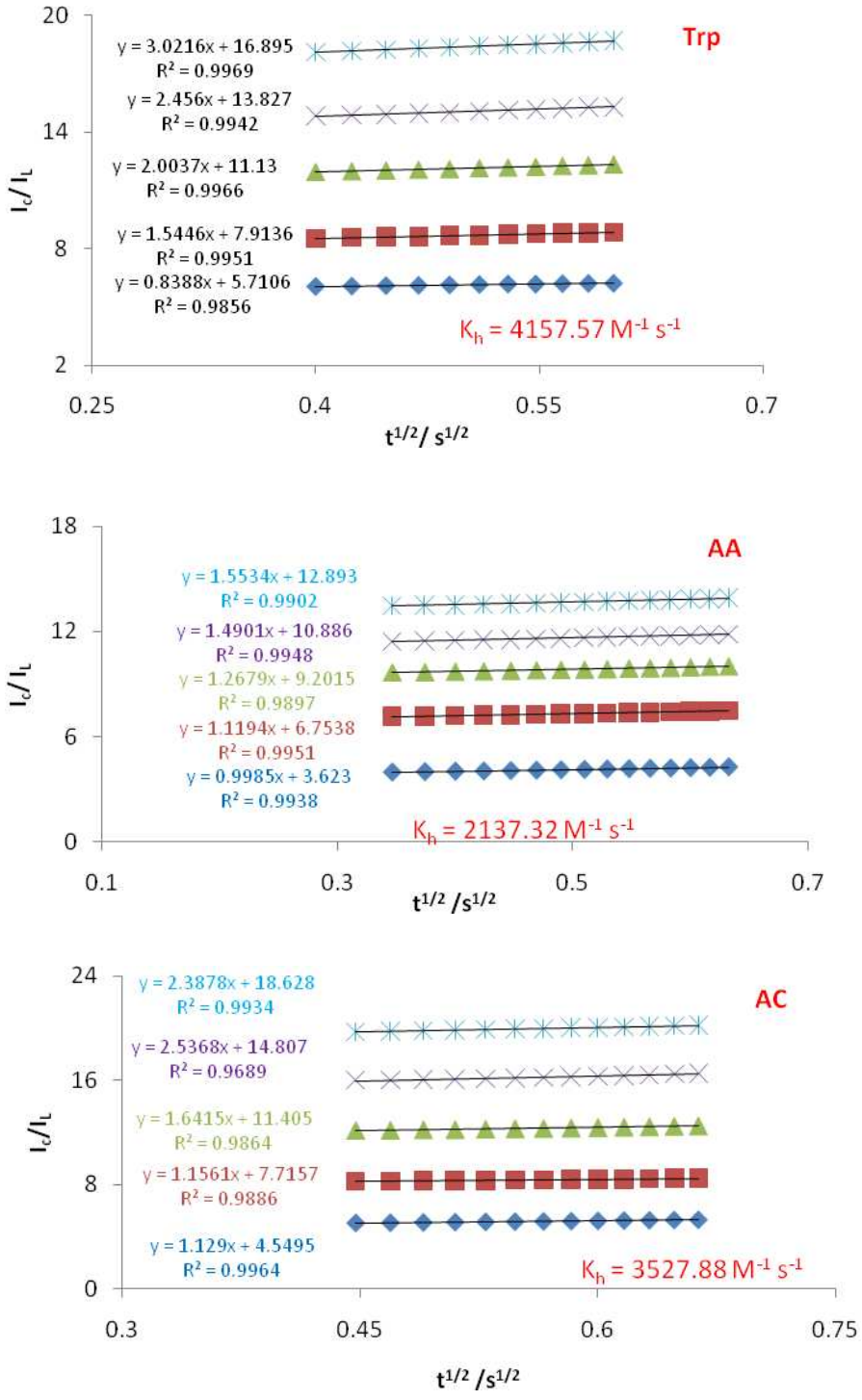

Trp
y = 3.0216x + 16.895
R² = 0.9969
y = 2.456x + 13.827
R² = 0.9942
y = 2.0037x + 11.13
R² = 0.9966
y = 1.5446x + 7.9136
R² = 0.9951
y = 0.8388x + 5.7106
R² = 0.9856
K_h = 4157.57 M^-1 s^-1
I_c/I_L
t^1/2/ s^1/2
AA
y = 1.5534x + 12.893
R² = 0.9902
y = 1.4901x + 10.886
R² = 0.9948
y = 1.2679x + 9.2015
R² = 0.9897
y = 1.1194x + 6.7538
R² = 0.9951
y = 0.9985x + 3.623
R² = 0.9938
K_h = 2137.32 M^-1 s^-1
AC
y = 2.3878x + 18.628
R² = 0.9934
y = 2.5368x + 14.807
R² = 0.9689
y = 1.6415x + 11.405
R² = 0.9864
y = 1.1561x + 7.7157
R² = 0.9886
y = 1.129x + 4.5495
R² = 0.9964
K_h = 3527.88 M^-1 s^-1

Fig. 7.

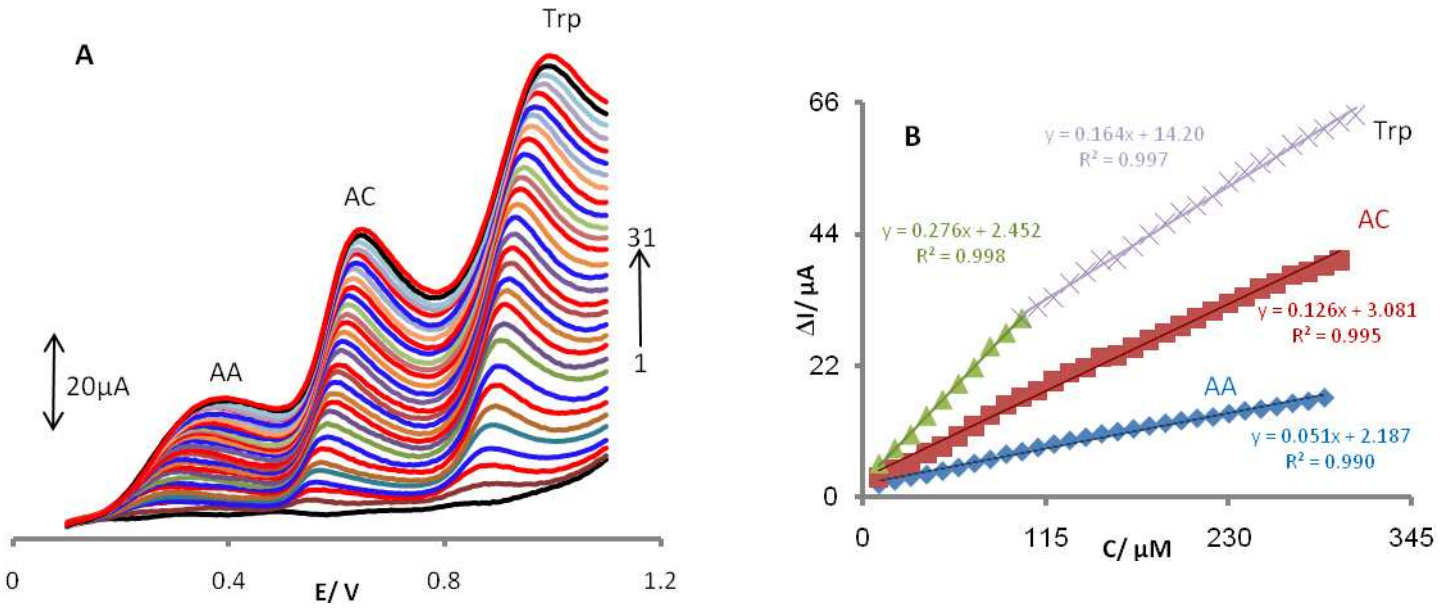

A
Trp
AC
AA
20μA
31
1
0
0.4
E/ V
0.8
1.2
B
66
44
22
0
ΔI/ μA
y = 0.164x + 14.20
R² = 0.997
Trp
y = 0.276x + 2.452
R² = 0.998
AC
y = 0.126x + 3.081
R² = 0.995
AA
y = 0.051x + 2.187
R² = 0.990
0
115
230
345
C/ μM

Fig. 8.

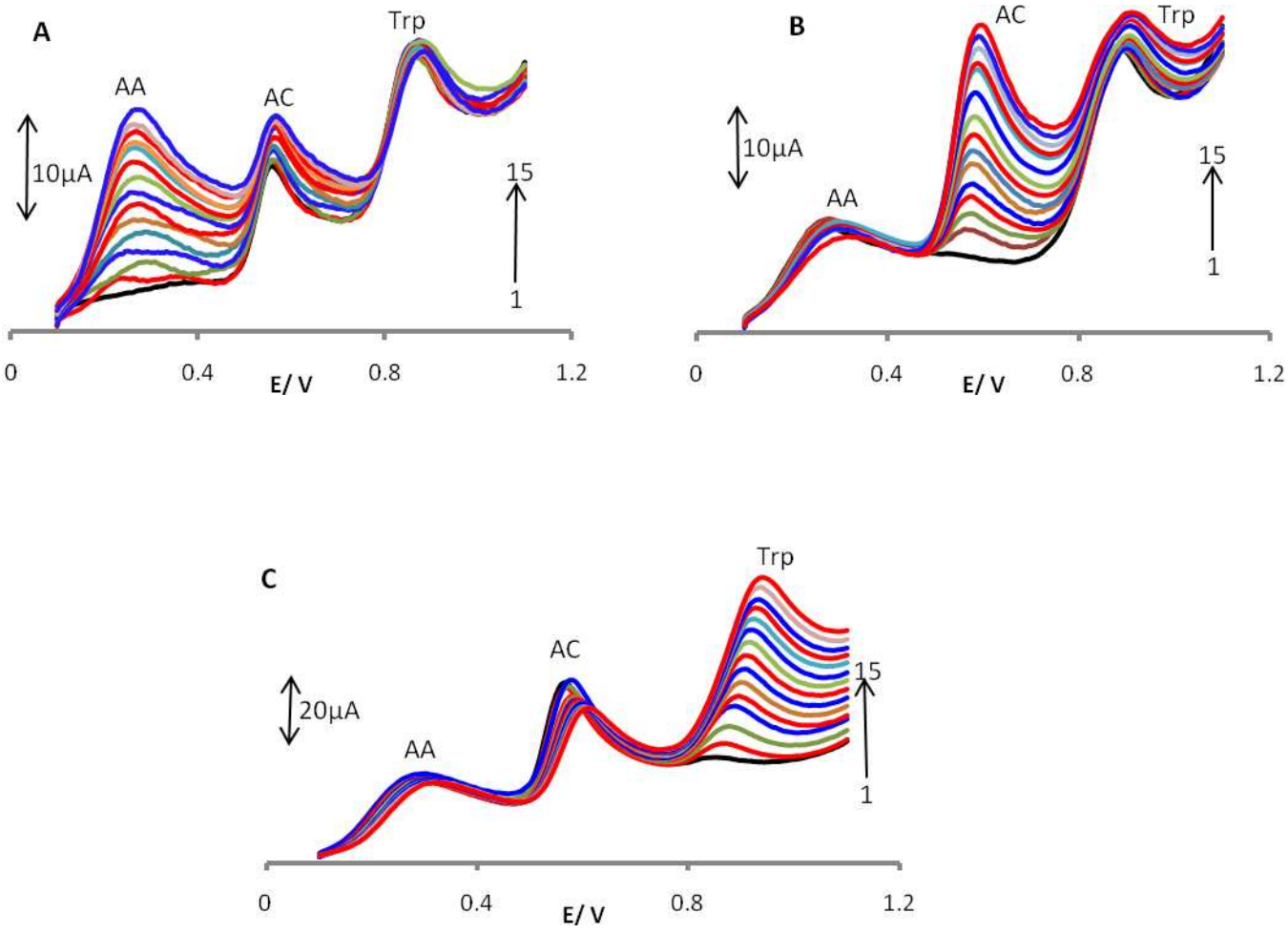

A
AA
AC
Trp
10μA
15
1
0
0.4
0.8
1.2
E/ V
B
AC
Trp
10μA
AA
15
1
0
0.4
E/ V
0.8
1.2
C
Trp
AC
20μA
AA
15
1
0
0.4
E/ V
0.8
1.2

Fig. 9.

Table 1: Determination of AA, AC and Trp in vitamin C tablets at pH 3.0 (n=3)

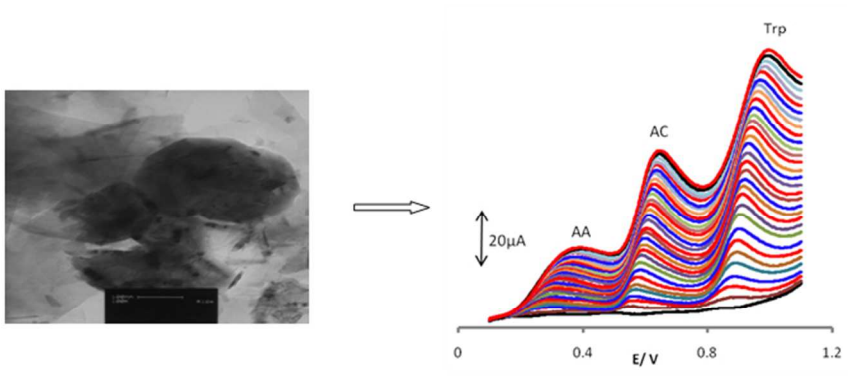
AA	AC	Trp	AA			AC			Trp		
tablet (μM)	Added (μM)	Added (μM)	Found (μM)	Recovery (%)	RSD (%)	Found (μM)	Recovery (%)	RSD (%)	Found (μM)	Recovery (%)	RSD (%)
20	–	–	19.6	98	2.8	–	–	–	–	–	–
70	35	25	70.5	100.7	3.6	35.7	102	1.6	24.8	99.2	3.6
90	55	45	91.3	101.4	1.3	55.3	100.5	2.7	44.7	99.3	1.4
130	70	55	130.7	100.5	2.4	69.4	99.1	3.8	55.8	101.5	2.3
170	85	65	168.9	99.4	2.1	84.6	99.5	4.1	64.2	98.8	0.9
190	95	75	189.4	99.7	1.7	94.3	98.8	1.3	76.3	101.7	1.4
210	105	85	208.7	99.4	3.3	106.7	101.6	2.9	86.6	101.9	1.8
240	115	95	243.1	101.3	4.1	112.8	98.1	2.3	94.2	99.2	2.8
270	125	115	272.3	100.9	1.8	126.4	101.1	1.7	116.2	101	2.6
290	160	135	289.1	99.7	1.2	162.2	101.4	3.6	134.7	99.8	1.1

Table 2: Determination of AA, AC and Trp in acetaminophen tablets at pH 3.0 (n=3)

AC	AA	Trp	AC			AA			Trp		
Tablet (μM)	Added (μM)	Added (μM)	Found (μM)	Recovery (%)	RSD (%)	Found (μM)	Recovery (%)	RSD (%)	Found (μM)	Recovery (%)	RSD (%)
20	–	–	20.7	103.5	3.6	–	–	–	–	–	–
40	20	15	41.2	103	3.2	19.7	98.5	2.6	15.3	102	1.8
60	40	35	58.7	97.8	1.3	40.6	101.5	2.3	35.6	101.7	4.1
75	65	55	75.6	100.8	2.4	65.6	100.9	3.8	54.2	98.5	3.7
85	85	70	86.4	101.6	1.6	84.3	99.2	1.1	70.6	100.9	3.6
105	105	85	103.9	99	2.1	105.7	100.7	1.6	84.3	99.2	2.8
125	120	95	126.4	101.1	1.7	119.2	99.3	0.9	96.7	101.8	1.3
135	150	110	133.7	99	2.4	150.8	100.5	2.8	111.2	101.1	1.8
145	175	125	143.2	98.8	0.7	173.9	99.4	3.2	124.6	99.7	2.6
160	195	135	162.4	101.5	3.1	196.2	100.6	3.1	136.7	101.3	2.2

Table3: The application of GPE/LFOR for simultaneous determination of AA, AC and Trp in human blood serum and urine (n=3).

Sample	Spiked (μM)			Found (μM)			Recovery (μM) %			RSD %		
	AA	AC	Trp	AA	AC	Trp	AA	AC	Trp	AA	AC	Trp
Serum	0	0	0	ND	ND	ND	–	–	–	–	–	–
	10	15	20	10.2	14.6	19.5	102	97.3	97.5	2.60	1.76	2.93
	30	45	60	30.9	45.6	62.3	103	101.3	103.8	2.40	1.54	2.44
Urine	0	0	0	ND	ND	ND	–	–	–	–	–	–
	10	15	20	9.7	15.7	20.4	97	104.7	102	1.56	2.13	3.12
	30	45	60	29.4	46.2	59.1	98	102.7	98.5	1.68	1.34	2.78



TEM of surface of graphite paste electrode, modified with LFOR for simultaneous determination of ascorbic acid, acetaminophen and tryptophan