Anal. Bioanal. Electrochem., Vol. 9, No. 7, 2017, 841-861

Analytical & Bioanalytical Electrochemistry

2017 by CEE
www.abechem.com

Full Paper

Development of Gold Modified Disposable Pencil Graphite Electrode for the Electrochemical Investigation of Acetaminophen Present in Pharmaceutical formulations and Biological samples

Manjunatha Puttaiah and Arthoba Nayaka Yanjerappa*

Department of Chemistry, School of Chemical Science, Kuvempu University, Shankaraghatta - 577 451, Karnataka, India

*Corresponding Author, Tel.: +91 9448855078; Fax: +91 08282 256255
E-Mail: drarthoba@yahoo.co.in

Received: 4 July 2017 / Received in revised form: 31 August 2017 / Accepted: 5 September 2017 / Published online: 15 November 2017

Abstract- A novel electrochemical sensor based on the electrodeposition of gold nanoparticles on disposable pencil graphite electrode (GMPGE) has been developed and employed for the electrochemical investigation of acetaminophen. The developed GMPGE has been characterized by scanning electron microscopy (SEM), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The modified electrode showed excellent electrocatalytic activities with respect to acetaminophen redox behavior, with good reproducibility. The electrochemical parameters such as, surface concentration (Γ), electron transfer coefficient (α) and the standard rate constant (k^0) of acetaminophen at the GMPGE were calculated. Under the optimized experimental conditions, the proposed sensor exhibited a rapid response to acetaminophen in a linear range from 1.0×10^{-8} M to 0.9×10^{-6} M with a limit of detection (LOD) and limit of quantification (LOQ) was found to be 1.95×10^{-9} M and 6.47×10^{-9} M respectively. The analytical applicability of GMPGE was illustrated for the determination of acetaminophen present in pharmaceutical formulations, human serum and urine samples as real samples with good accuracy and precision. The UV-Vis spectroscopy has been adopted to confirm the presence of acetaminophen in pharmaceutical formulations, human serum and urine samples.

Keywords - Gold Modified Pencil Graphite Electrode, Acetaminophen, Scanning Electron Microscope, Electrochemical Impedance Spectroscopy, Cyclic Voltammetry, Differential Pulse Voltammetry

1. INTRODUCTION

Acetaminophen is an acylated aromatic amide, commonly known as paracetamol (N-acetyl-p-aminophenol, 4-acetamidophenol or Tylenol). It is a non-narcotic, non-carcinogenic drug with analgesic and antipyretic effect as that of aspirin [1]. It is an effective substitute especially for the patients those who are sensitive to aspirin [2]. It is also being used for therapeutic purposes for the treatment of fever, cough, cold, headache, migraine headache, muscular aches, chronic pain, neuralgia, backache, joint pain, tooth ache [3], arthritis and post-operative pains [4]. Sometimes it is also being used for the management of cancer pain, dysmenorrhoea, neuropathic pain, osteoarthritis therapy and sciatica [5]. The recent research suggests that the acetaminophen may help to protect from changes leading to hardening of arteries that cause cardiovascular disease [6]. It also remains the analgesic of choice for people with asthma [7]. There is also some evidence to suggest that acetaminophen may offer some protection against ovarian cancer [8]. Generally, acetaminophen does not exhibit any harmful side effects but overdoses of acetaminophen produce toxic metabolite accumulation that causes acute hepatic necrosis, inducing morbidity and mortality in humans [9]. However, hypersensitivity leads to the formation of some liver and nephrotoxic metabolites. Moreover, the hydrolytic degradation product is 4-aminophenol, which can cause teratogenic effect and nephrotoxicity [10-12]. Hence, the qualitative and quantitative investigations of acetaminophen present in pharmaceuticals and biological samples have great importance.

Various analytical methods for therapeutic monitoring have been reported in the literature for the determination of acetaminophen such as, chromatography [13,14], spectrophotometry [15,16], Voltammetry [17-19], chemiluminescence [20,21], capillary electrophoresis [22,23], high performance liquid chromatography [24-26], colorimetry [27], titrimetry [28], FTIR and Raman spectroscopy [29], UV-Vis spectrophotometry [30,31], spectrofluorimetry [32,33], Flow-injection [34,35], thin layer chromatography [36], capillary electrophoresis [37,38] and liquid chromatography mass spectrometry [39]. However, these methods suffer from some of the disadvantages such as high cost, long analysis time, lengthy procedure for sample pretreatment etc. Also in some cases, because of their low sensitivity and selectivity towards the detection of acetaminophen samples at low concentrations makes them unsuitable for routine analysis. Since, acetaminophen is an electroactive molecule, from the past few decades, the development of electrochemical sensors and their analytical applications for the determination of acetaminophen have received considerable importance [40-42]. Most electrochemical methods rely on the use of modified electrodes, some of those are, boron-doped diamond thin film electrodes [43], single-wall carbon nanotubes-dicetyl phosphate film modified glassy carbon electrode [44], polyaniline-multiwalled carbon nanotubes composites modified electrode [45], cobalt hexacyanoferrate modified graphite wax composite electrodes [46], carbon film resistor electrode [47], C_{60} modified glassy carbon electrodes [48], amino acids modified glassy carbon electrodes [49], carbon nanotubes based

nanoelectrode arrays: Fabrication, Evaluation and Application in voltammetric analysis [50], multiwalled carbon nanotubes modified basal plane pyrolytic graphite electrode [51], nanogold modified indium tin oxide electrode [16], Nafion/TiO_2-graphene modified glassy carbon electrode [28], etc. However, carbon nanotubes modified electrodes have been used for the determination of numerous biologically important molecules [51-54]. To the best of our knowledge there is no report on the usage of gold modified pencil graphite electrode for the determination of acetaminophen.

Till date, the electrochemical techniques have been widely explored for the determination of acetaminophen detection in regular clinical and industrial applications, which have the advantages of high sensitivity, less time-consuming and low costs over other analytical methods. However, at traditional electrode surface, acetaminophen exhibits sluggish voltammetric response. Thus, a considerable efforts have been devoted to develop chemically modified electrodes (CMEs) to enhance the voltammetric response and analytical performance for acetaminophen detection [55-58] because the CMEs has been their ability to catalyze the electrode process via significant decreasing of overpotential with respect to unmodified electrodes and with respect to relatively selective interaction of electron mediator with the target analyte [59,60]. Thus, the development of novel, simple, sensitive and cost-effective modified electrode for the investigation of acetaminophen present in pharmaceutical formulations and biological samples is of great importance.

Hence, in this article, we developed a gold modified pencil graphite electrode (GMPGE) for the sensitive determination of acetaminophen present in pharmaceutical formulations and biological samples using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques.

2. EXPERIMENTAL

2.1. Chemicals and reagents

Acetaminophen was purchased from Sigma-Aldrich, Bangalore, India and used as received without purification. A stock solution of 1 mM acetaminophen was prepared in phosphate buffer solution (PBS) of pH 7.0. The tablets calpol, dolo and colgin containing acetaminophen content were purchased from local Pharmaceutical shops, Shivamogga, India. The chloroauric acid, potassium dihydrogen phosphate (KH_2PO_4) and di-potassium hydrogen phosphate (K_2HPO_4) were procured from Merck, Mumbai, India. Potassium ferricyanide ($K_3[Fe(CN)_6]$) was procured from Merck, Darmstadt, Germany. All the chemicals used were of analytical grade. The 0.1 M phosphate buffer solution was prepared and pH of the solutions was adjusted either by using H_3PO_4 or NaOH. Double distilled water was used for the preparation of all the reagents.

2.2. Instrumentations

The electrochemical measurements were performed using electrochemical workstation (CHI660D, CH Instruments, USA) and all the experiments were conducted in a standard three-electrode assembly, incorporating pencil graphite electrode (PGE) or gold modified pencil graphite electrode (GMPGE) as working electrode, platinum wire as auxiliary electrode and Ag/AgCl as reference electrode. The UV-Vis spectroscopic studies were carried out using UV-Vis spectrophotometer (Model: USB 4000, Ocean Optics, USA). The surface morphology of the modified electrode has been analyzed by field emission scanning electron microscope (Model: VEGA3 TESCAN, Bangalore, India).

2.3. Analytical procedure

The stock solution of acetaminophen (1 mM) was prepared by dissolving the required amount of acetaminophen in 0.1 M phosphate buffer solution of pH 7.0. The cyclic voltammograms were recorded in the sweep rates ranging from 25−300 mV s^{-1} with an initial sweep towards positive potentials. For differential pulse voltammetry, the optimized parameters used were as follows: initial potential +100 mV: final potential +500 mV, amplitude 0.05 V, pulse width 0.2 Sec. sampling width 0.02 Sec. pulse period 0.5 Sec. quit time 2 Sec.

2.4. Preparation of unmodified and modified electrode (PGE/GMPGE)

The gold modified pencil graphite electrode (GMPGE) was prepared by using 2B pencil graphite lead (0.5 mm diameter, Kokuyo Camlin Ltd.). One end of the pencil graphite lead was connected to a copper wire to establish the electrical contact and it was inserted in a small Teflon tube of 1 mm inner diameter and a height of 6 cm. Then the Teflon tube was filled with epoxy resin without allowing any air gap and kept at room temperature for 24 h for setting. After setting of epoxy resin, the anterior end of the electrode was cut using a sharp knife and polished using emery paper followed by butter sheet. The mirror polished pencil graphite electrode (PGE) was washed with double distilled water and subjected to electrodeposition by chronoamperometry technique by using an electrolyte solution containing 1 mM chloroauric acid with the applied potential of −1.0 V *vs*. saturated Ag/AgCl electrode and a pulse width of 300 seconds. Finally, the prepared electrode was washed with double distilled water and used for electrochemical studies.

2.5. Real samples preparations

A suitable quantity of calpol (80 mg), dolo (95 mg) and colgin (80 mg) tablets were powdered separately. The powders were separately placed in a conical flasks containing 50ml phosphate buffer solution of pH 7.0 and sonicated for about 30 min with room temperature.

The resultant supernatant solutions was filtered off through Whatman filter paper and diluted to 100 mL using same phosphate buffer solution of pH.7.0 in a calibration flask.

2.6. Urine and serum pretreatment

The human serum and urine samples were taken from the patients undergoing a treatment with calpol (acetaminophen) at Kuvempu University Health Centre, Shivamogga, India. The collected samples were used shortly after collection. The urine sample was centrifuged at ~5000 rpm for ~15 minutes (to reduce matrix complexity) and filtered before use. The serum sample was treated with 3 mL methanol as protein precipitating agent. The precipitated proteins were separated out by centrifugation for ~10 min at 5000 rpm by using REMI cooling centrifuge (Model No. C-24PLUS Sr. No. -5330). The supernatant layer was filtered through 0.45 µm Millipore filter to obtain a protein free serum sample.

3. RESULTS AND DISCUSSION

3.1. Surface Morphology of PGE/GMPGE

The surface morphologies of PGE and GMPGE were characterized by scanning electron microscopy (SEM).

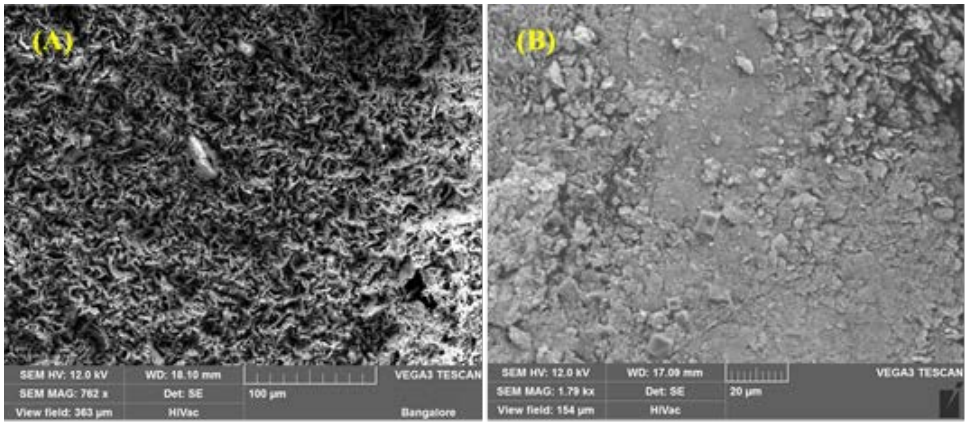


Fig. 1. Scanning electron microscopy images of (A) PGE and (B) GMPGE

Scanning electron microscope (SEM) is one of the powerful techniques to characterize the surface features of different composites. The SEM features of PGE and GMPGE were compared with each other. Fig. 1A illustrates the surface morphology of pencil graphite electrode (PGE), it shows that the surface of PGE is heterogeneous in nature and it contains many micro cavities and stacked flakes. Fig. 1B illustrate the surface morphology of gold modified pencil graphite electrode (GMPGE), it shows that the gold nanoparticles are uniformly assembled on the surface of PGE. The uniform deposition of gold nanoparticles on

the surface of PGE were evidently increases the surface area of the electrode and hence better electron transfer rates between the electrode surface and bulk analytical solution. Furthermore, the increase in surface area and the electron transfer capacity has been verified by the electrochemical studies of potassium ferricyanide ($K_3[Fe(CN)_6]$).

3.2. Electrochemical behavior of potassium ferricyanide at PGE/GMPGE

The redox behavior of $K_3[Fe(CN)_6]$ depends on the nature of electrode and it is one of the important probe to verify the modification of electrodes. Fig. 2A illustrate the cyclic voltammograms of 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl at PGE (a) and at GMPGE (b). The PGE showed a reversible redox peak with a peak to peak separation of 179.2 mV *vs.* Ag/AgCl.

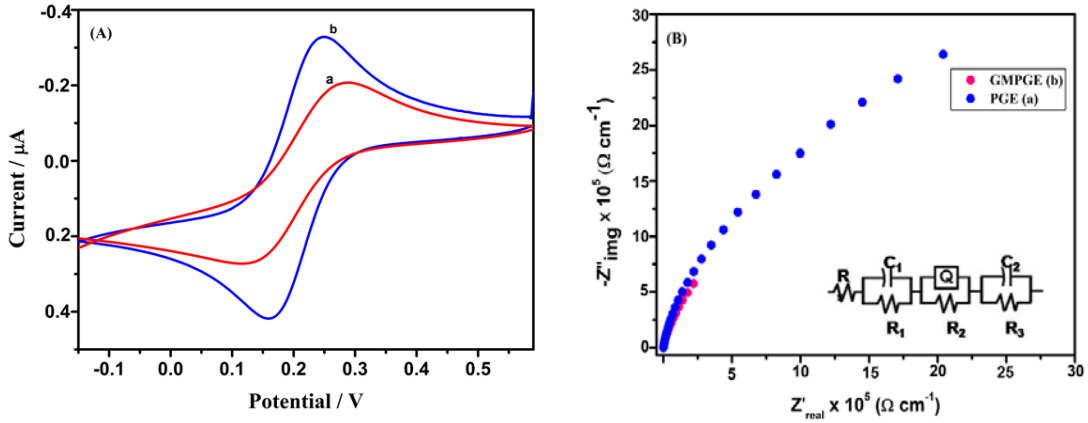


Fig. 2. (A) illustrates the cyclic voltammograms of 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl solution at PGE (a) and GMPGE (b) at a scan rate of 100 mV s^{-1}; **(B)** EIS at PGE (a) at GMPGE (b) in 0.1 M KCl solution containing 1 mM $K_3[Fe(CN)_6]$ solution with a frequency range: 10 kHz–0.1 Hz. Inset: Randle's equivalent circuit

As compare to PGE, the GMPGE showed a well-defined reversible redox peak with an increased magnitude of ~1.2 fold of peak current and reduction in the peak to peak potential (ΔE_p) of 83.6 mV *vs.* Ag/AgCl. The significant enhancement in redox peaks current with a subsequent reduction in ΔE_p is attributed to the good electrocatalytic activity of electrochemically assembled gold nanoparticles on GMPGE [61].

The electrochemical impedance spectroscopy (EIS) is an effective technique to study the characteristic features of surface modification of electrode and it plays a crucial role in studying the electrical conductivity, capacitance of the electrodes. The EIS is also being used to determine the electrochemical behavior of electrode surface in the modification processes with individual or mixed components [62]. Thus, the EIS was employed to study the

interfacial properties of PGE and GMPGE using 0.1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl. Fig. 2B shows the EIS of 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl at PGE (a) and GMPGE (b). The EIS of PGE showed a big curve as compare to that of GMPGE (b) and the value of charge transfer resistance (R_{ct}) was determined from the Randles equivalent circuit (Inset of Fig. 2B). The R_{ct} value of PGE (a) was calculated and found to be 2151 kΩ and for GMPGE (b) it was calculated and found to be of 265 kΩ. The reduction in the R_{ct} value clearly indicate the surface modification of GMPGE and it signifying that the significant acceleration of $K_3[Fe(CN)_6]$ redox reaction fissile due to the presence of gold nanoparticles at the surface of GMPGE. The resistance was very low at GMPGE is due to the better conductivity and diffusion-limited electrochemical behavior of redox couple at the surface of GMPGE and it confirmed the surface modification of GMPGE with gold nanoparticles. The impedance plots obtained were in good agreement with the voltammetric behavior of Fig. 2A. The surface inhomogeneity (*n*) of the GMPGE was found to be 0.8656 and 0.3901 for PGE. The higher value of surface inhomogeneity of GMPGE is due to the formation of a layer of gold nanoparticles on GMPGE as a result of electrochemical fine deposition of gold nanoparticles on the surface of GMPGE during surface modification and it further confirmed the surface modification of GMPGE (also observed in Fig. 1A & 1B).

3.3. Determination of surface area of PGE/GMPGE

The active surface area of the modified and unmodified electrodes has been determined by cyclic voltammetry using 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl at GMPGE and PGE at different scan rates. For a reversible process, the following Randles-Sevcik equation can applies:

$$I_p=(2.69\times10^5)\, n^{3/2} A D_o^{1/2} C_o^* \upsilon^{1/2}$$

where, i_p is the anodic peak current in A, n is the number of electrons transferred; A is the surface area of the electrode in cm^2, D_o is diffusion coefficient in $cm^2\ s^{-1}$, υ is the scan rate in V s^{-1} and C_o^* is the concentration of potassium ferricyanide in mol L^{-1}. For 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl solution, n=1 and $D_o=7.6\times10^{-6}$ $cm^2\ s^{-1}$, then from the slope of the plot of i_{pa} *vs.* $\upsilon^{1/2}$, the active surface area of the electrode has been calculated. The area of PGE and GMPGE was calculated and found to be 0.01835 and 0.02670 cm^2, respectively.

3.4. Determination of surface concentrations (Γ)

The surface concentration (Γ) of electroactive species, was determined using the slope of the plot of i_{pa} *vs.* υ [63]. The Γ can be calculated using the formula $I_p=n^2F^2\upsilon A\Gamma/4RT$, where, n is the number of electrons transferred, F is the Faraday's constant (C mol^{-1}), A is the area of the electrode(cm^2), Γ is the surface concentration of the analyte and υ is the scan rate (V s^{-1}).

The surface concentration (Γ) of acetaminophen was found to be 1.77×10^{-10} mol cm^{-2} and 1.59×10^{-11} mol cm^{-2} for GMPGE and PGE respectively. The higher value at GMPGE clearly proved that the presence of gold nanoparticles on the modified electrode surface has incresed the surface concentration of acetaminophen.

3.5. Electrochemical behavior of acetaminophen at PGE/GMPGE

The baseline peak current of GMPGE was higher than that of the PGE indicate the more sensitivity of GMPGE. The electrochemical response of acetaminophen in 0.1 M phosphate buffer solution of pH 7.0 at PGE (curve c) showed poor electrochemical response and hence the acetaminophen oxidized at higher potential with lesser peak current as compare to GMPGE. The oxidation and reduction potentials at PGE were found at +0.401 V and −0.156 respectively with a peak to peak separation of ~246 mV. The electrochemical response of acetaminophen at GMPGE (curve d) in the same experimental conditions, noticed better electrochemical performance as comes pare to that of PGE. At GMPGE the acetaminophen oxidized at lower potential with higher peak current. The oxidation and reduction peak potentials were found at +0.336 V and −1.062 V respectively with a peak to peak separation of ~56 mV, and with an increased peak current of 2-fold higher than that of PGE. The remarkable enhancement in peaks current with a subsequent reduction in peak-to-peak separation (ΔE_p) is attributed to the excellent electrocatalytic activity of electrochemically assembled gold nanoparticles on the surface of GMPGE.

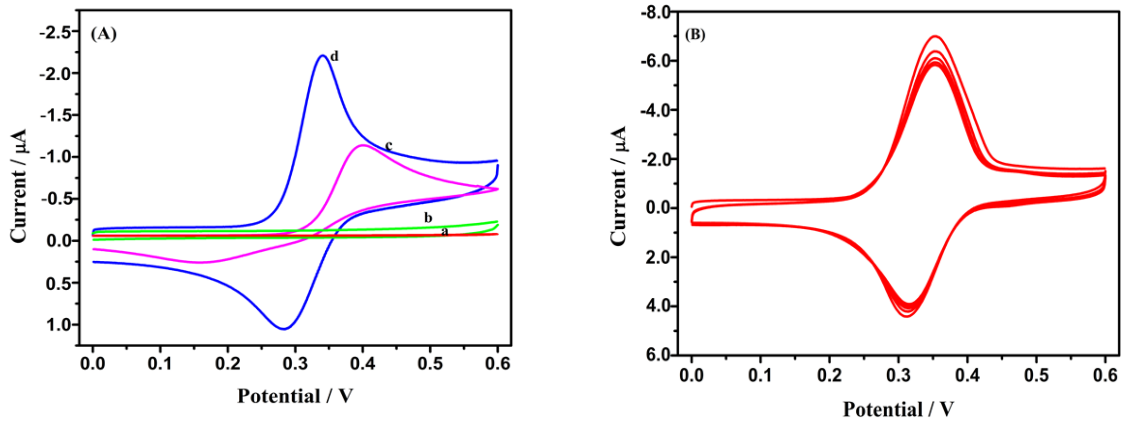


Fig. 3. (A) Depicts the cyclic voltammograms of phosphate buffer solution at PGE (a) and GMPGE (b), cyclic voltammograms obtained for 1mM acetaminophen at PGE (c) and GMPGE (d) in 0.1 M phosphate buffer solution of pH 7.0 at a scan rate of 100 mV s^{-1}; **(B)** Depicts the successive votammograms (10 cycles) of 1 mM acetaminophen at GMPGE in 0.1 M PBS of pH 7.0 at a scan rate of 100 mV s^{-1}

However, for the successive cyclic voltammograms of acetaminophen there is a tiny decrease in the redox peak current without any change in the redox peak potential is attributed to the good selectivity, sensitivity, stability and reproducibility of GMPGE towards the determination of acetaminophen. Fig. 3B showed the successive voltammograms of 1 mM acetaminophen in 0.1 M PBS of pH 7.0. The tiny decrease in redox peak current occurs with an increased number of successive sweeps could be ascribed to the adsorption of oxidation product of acetaminophen on the electrode surface, which leads to the saturation of active surface area of the electrode [65] and hence to achieve the more accuracy and precession the voltammogram corresponding to the first cycle was recorded.

3.6. Effect of pH

The pH of the solution has a significant influence on the peak current (i_p) and peak potential (E_p) in electrochemical studies. Hence, the effect of solution pH on the electrochemical behavior of acetaminophen at GMPGE has been studied in the pH range of 4.0-10.0 using differential pulse voltammetry. Fig. 4A illustrates the relationship between oxidation peak current of acetaminophen (20 µM) in 0.1 M phosphate buffer solutions of different pH, range from 4.0 to 10.0 at GMPGE.

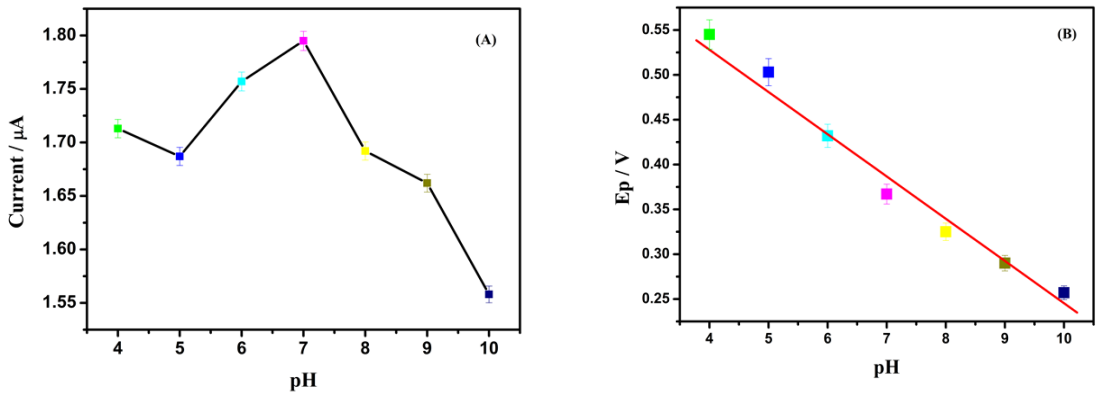
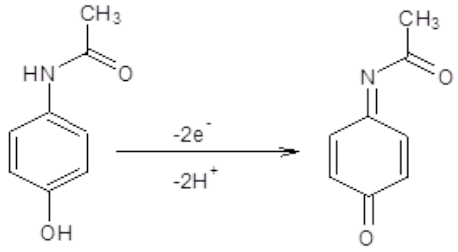


Fig. 4. (A) Illustrates the effect of pH (4.0, 5.0. 6.0, 7.0, 8.0, 9.0 and 10.0) on peak current of acetaminophen (20 µM) at GMPGE in 0.1 M phosphate buffer solutions; **(B)** Illustrates the effect of pH on the peak potential of acetaminophen (20 µM) at GMPGE in 0.1 M different phosphate buffer solutions

The maximum oxidation peak current was observed at pH 7.0 and hence the pH 7.0 was selected as a suitable analytical medium for further electrochemical studies. Fig. 4B illustrates the relationship between pH of the solution on the oxidation peak potential (E_p) of acetaminophen in 0.1 M phosphate buffer solutions of different pH, ranging from 4.0 to 10.0 at GMPGE. The peak potential shifted negatively with increasing the solution pH from 4.0 to 10.0, indicating that the protons are involved in the redox process of acetaminophen [66,67].

There is a good linear relationship established between the formal potential (E^0) and pH of the solution with corresponding linear regression equation as represented by: E_p (pH 4.0 to 10.0)=(0-.73768)+0.04989 pH; with correlation coefficient (R^2) = 0.99092 *vs.* Ag/AgCl. In order to substantiate the percentage of deviation of E_p values from linearity, error bars have been included and the deviation is well within 2%. Therefore, from the equation $dE_p/dpH = 0.059\chi/\alpha n$, the proton number ($\chi$) was calculated and found to be 2 and hence, the electrochemical reaction of acetaminophen at GMPGE is a two-electron and two-proton process ($2e^-$, $2H^+$), which is in good agreement with the earlier reports [68-70].



Scheme 1. Proposed mechanism for the redox reaction of acetaminophen

3.7. Effect of scan rate

In order to validate the nature of the electrochemical process occurring at the electrode surface, the cyclic voltammograms of 1 mM acetaminophen in 0.1 M phosphate buffer solution of pH 7.0 on GMPGE at different scan rate of 25, 50, 75, 100, 125, 150 and 175 mV s^{-1} have been recorded. Fig. 5A shows the voltammograms of 1 mM acetaminophen in 0.1 M phosphate buffer solution at various scan rates at GMPGE. As the scan rate increased from 25 to 175 mV s^{-1}, the anodic and cathodic peak potentials (E_{pa} and E_{pc}) were shifted to positive and negative values respectively, with a linear increase in the anodic and cathodic peak current (i_{pa} and i_{pc}). The plot of square root of scan rates ($\upsilon^{1/2}$) *vs.* anodic and cathodic peak current (i_{pa} and i_{pc}) showed a linear relationship, which is the typical electrochemical behavior of a diffusion-controlled electrode process of reversible nature [71] and the corresponding equations can be expressed as, i_{pa} (µA)=0.17461 ($\upsilon^{1/2}$)+(−0.06019) and i_{pc} (µA)=0.07596 ($\upsilon^{1/2}$)+(−0.1031) with a correlation coefficients (r^2)=0.99867 and 0.99945 respectively. In order to substantiate the percentage of deviation of i_{pa} and i_{pc} values from linearity, the error bars have been included and the deviation is well within 1%. Fig. 5B illustrates the relationship between anodic and cathodic peak current with square root of scan rate. The results clearly indicated that the electrochemical behavior of acetaminophen at GMPGE is a diffusion-controlled process [72]. The plot of log i_{pa} *vs.* log υ showed relationship and the corresponding linear equation can be expressed as log i_{pa} (µA)=0.492 log υ+(−0.737) with a correlation coefficient (r^2)=0.99746. The slope value of 0.492 is very close to the theoretical value of 0.5, which clearly confirms that the redox reaction of

acetaminophen at GMPGE is a diffusion-controlled process [73]. The error bar was inserted to notice the deviation from the linearity and the deviation is within 1%. As the scan rate increases, the oxidation peak potential shifts to more positive and reduction peak shifts to negative values respectively.

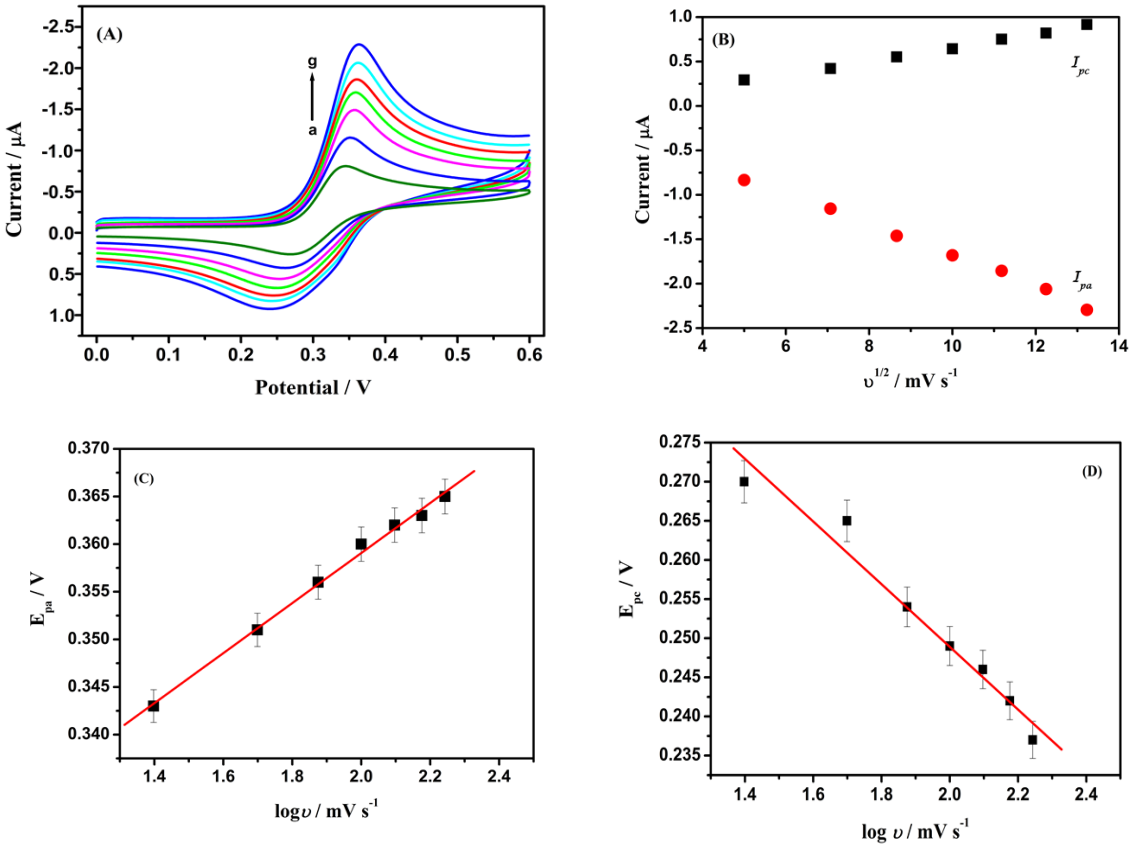


Fig. 5. (A) Illustrates the cyclic voltammograms of 1 mM acetaminophen at GMPGE in phosphate buffer solution of pH 7.0 with different scan rate (a) 25, (b) 50, (c) 75, (d) 100, (e) 125, (f) 150, (g) 175 mV s^{-1}; **(B)** Illustrates the relationship between anodic and cathodic peaks currents with square root of scan rates; **(C)** Illustrates the relationship between the anodic peak potential with logarithmic scan rate; **(D)** Illustrates the relationship between the cathodic peak potential with logarithmic scan rate

Fig. 5C and 5D shows the linear relationship between oxidation peak potential (E_{pa}) and reduction peak potential (E_{pc}) *vs.* logarithm of scan rate (log υ) of 25, 50, 75, 100, 125, 150 and 175 mV s^{-1}. The corresponding linear regression equations can be expressed as: E_{pa} (V)=0.0307 log υ + (−0.2663) and E_{pc} (V)=0.03968 log υ + (−0.32832) with a correlation coefficients (r^2)=0.9976 and 0.98193 respectively. The inserted error bar showed a deviation is well within 1%.

For reversible electrode process, according to the Laviron's equation [75], the slope of E_{pa} and E_{pc} *vs*. log υ is equal to $2.303RT/(1-\alpha)nF$ and $-2.3RT/\alpha nF$ for anodic and cathodic peak respectively [76]. Based on the slope of the lines $2.303RT/(1-\alpha)nF$ and $-2.3RT/\alpha nF$ the value of charge-transfer coefficient (α) was calculated [71] and found to be 0.56 and the electron transfer number (*n*) was estimated to be ~1.9. Furthermore, the standard rate constant (k^0) was find out according to the given equation.

$$\log k^0 = \alpha \log(1-\alpha) \log \alpha - \log(\frac{RT}{nF\upsilon}) - \alpha(1-\alpha)\frac{nF\Delta E_p}{2.3RT}$$

where, α is the charge transfer coefficient, k^0 is the standard rate constant of the reaction (s^{-1}), *n* is the number of electrons transferred, υ is the scan rate (mV s^{-1}), *R* is the universal gas constant (J K^{-1} mol^{-1}), *F* is the Faraday's constant (C mol^{-1}). Taking T=298 K, *R*=8.314 J K^{-1} mol^{-1}, and F=96,480 C mol^{-1}, generally α is assumed to be 0.5 in a total reversible electrode process, the number of electrons (*n*) transferred in the electro-oxidation of acetaminophen was found to be 2. Therefore, the standard rate constant of the reaction (k^0) was found to be 1825.9 s^{-1}.

3.8. Differential pulse voltammetry (DPV)

The differential pulse voltammetry (DPV) is one of the simple, rapid and sensitive techniques and provides well-defined analytical peaks even at lower concentrations for the given analyte with a lower background current and improved resolution [77,78].

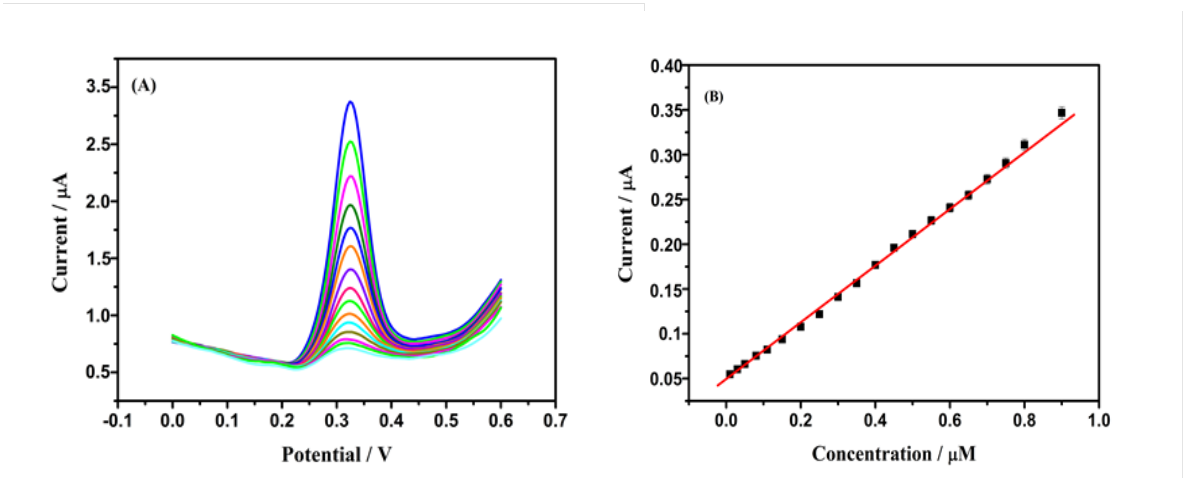


Fig. 6. (A) Illustrates the differential pulse voltammograms of acetaminophen at GMPGE in the range of 1.0×10^{-8} M to 0.9×10^{-6} M in phosphate buffer solution of pH 7.0; **(B)** Illustrates the relation between anodic peak current (i_{pa}) *vs*. various concentration (*C*) of acetaminophen in 0.1 M phosphate buffer solution of pH 7.0

Therefore, the DPV technique has been applied for the quantification of acetaminophen in the optimized potential range of 0.0 V to +0.6 V at GMPGE with respect to dependence of oxidation peak current (I_{pa}) with concentration (C) of acetaminophen.

Fig. 6A illustrates the differential pulse voltammograms (DPVs) of various concentrations of acetaminophen in 0.1 M phosphate buffer solution of pH 7.0 at GMPGE. The electrochemical responses of peak current (I_{pa}) of acetaminophen were increased linearly with its concentration (C). Therefore, the developed sensor is highly selective and sensitive towards the acetaminophen detection owing to the large peak current. The response of peak currents (I_{pa}) of acetaminophen at GMPGE was linear with the concentration (C) in the range of 1.0×10^{-8} M to 0.9×10^{-6} M.

3.9. Validation of the developed method

The validation of the proposed method has been demonstrated with respect to electrochemical parameters such as linearity, limit of detection (LOD), limit of quantification (LOQ), specificity/selectivity, accuracy and precision, stability, reproducibility and analytical applicability.

3.9.1. Linearity

The dependence of oxidation peak current (i_{pa}) with respect to concentration (C) of acetaminophen has been studied using GMPGE. Fig. 6B illustrates the relationship between anodic peak current (i_{pa}) *vs*. various concentrations of acetaminophen in 0.1 M phosphate buffer solution of pH 7.0. Under the optimized conditions, a very good linear correlation was observed between the peak current (i_{pa}) and acetaminophen concentration (C). The linear calibration was obtained in the concentration range of 1.0×10^{-8} M to 0.9×10^{-6} M and the corresponding linear regression equation was expressed as follows:

i_{pa} (μA)=3.26298 C+0.466428 with a correlation coefficient (r^2)=0.99926.

In order to substantiate the percentage of deviation of i_{pa} values from linearity, the error bar have been inserted and the deviation is well within 1.5%.

3.9.2. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were determined from the linear calibration plot of peak currents (i_{pa}) *vs*. various concentration (C) of acetaminophen (Fig. 6B). The LOD and LOQ were calculated using the equations, LOD=3σ/m; and LOQ=10 σ/m. where, σ is the standard deviation of the intercept and m is the slope of the calibration curve. The calculated LOD and LOQ were found to be 1.95×10^{-9} M and 6.47×10^{-9} M respectively. Therefore, the obtained LOD and LOQ values of present work clearly signified the sensitivity of the proposed method.

3.9.3. Specificity/selectivity

Generally the pharmaceutical and physiological samples contain many excipients, which may affect the specificity/selectivity of the sensors towards the given analysis. The specificity is the ability of the sensor to measure the analyte response (I_{pa} and E_{pa}) in the presence of common excipients. Hence, in order to verify the specificity/selectivity of GMPGE towards the determination of acetaminophen was examined in the presence of some probable interfering substances such as ascorbic acid, uric acid, glucose, sodium and potassium. The results showed that the presence of interfering substances did not affect the electrochemical behavior of acetaminophen even the concentrations of interfering substances were ten-fold higher than that of the acetaminophen. This clearly indicates that the GMPGE has got good efficacy towards the electrochemical determination of acetaminophen in presence of probable interfering species. Therefore, the developed electrode can be successfully used for the specific/selective and sensitive determination of acetaminophen present in various real samples.

3.9.4. Stability of GMPGE

The stability of the GMPGE was evaluated by measuring the peak current (I_{pa}) and oxidation peak potential (E_{pa}) response of fixed concentration of acetaminophen (10 µM) in 0.1 M phosphate buffer solution of pH 7.0. The GMPGE was stored for a period of 90 days and the voltammograms were recorded for a regular intervals of 5 days using the same GMPGE, it was noticed that, only a minimal variation of peak current without change in the oxidation peak potential. The relative standard deviation (RSD) was found to be 2.65%, which is attributed to the excellent stability of electrochemically assembled gold nanoparticles on the surface of GMPGE. Thus the results indicated the GMPGE have excellent log storage stability during the electrochemical investigation of acetaminophen in aqueous solutions.

3.9.5. Accuracy and precision

The accuracy and precision of the proposed method at GMPGE has been verified. The intra-day precision of the method was evaluated by repeating six experimental measurements for fixed concentration of acetaminophen of 10 µM and the RSD was found to be 1.5% and during the real sample analysis, the good recovery percent were observed in the range 96.30 to 102.3%, indicating that the proposed method at GMPGE have excellent accuracy. Further, the inter-day precision was also investigated by measuring the peak current response of same concentration (10 µM) of acetaminophen at GMPGE after a period of five days and the RSD was found to be 1.95%. Thus, the results were clearly demonstrated that there is a good reproducibility of the proposed method using GMPGE for the determination of acetaminophen. Hence, the proposed method can be successfully applied for the electrochemical determination of acetaminophen present in real samples.

3.10. Comparison of analytical performance of present method with literature

The comparison between the analytical performances of present method with previously reported methods for the determination of acetaminophen is shown in Table 1. The statistics reveals that, the GMPGE showed superior analytical performance with respect to LOD, wide linear dynamic range, high selectivity, sensitivity, stability and reproducibility over the methods reported in the literature. In addition, the present method is simple, does not require any pretreatments, easy procedure for the development of GMPGE and low-cost.

3.11. Analytical applications

3.11.1. Analysis of Pharmaceutical formulations

The analytical applicability of the proposed method has been illustrated by the determination of acetaminophen present in pharmaceutical formulations such as calpol, dolo and colgin tablets. The stock solutions from the experimental sections (*2.5*) are used for further studies. The differential pulse voltammograms were recorded under the optimized experimental conditions for all the samples and correlated with the calibration curve (Fig. 6B) in order to found out the concentration of acetaminophen. The calculations were made using standard addition method and the results are tabulated in Table 2. The RSD of each sample for five parallel measurements were less than 3.0%. The percentage recovery of calpol, dolo and colgin tablets formulations has been investigated and the recovery values were found to be 99.95%, 102.30%, 99.05 respectively. Thus the recovery percent clearly indicated that the determination of acetaminophen at GMPGE is an effective method and can successfully apply for the quantitative estimation in pharmaceutical formulations.

3.11.2. Determination of acetaminophen in human serum and urine samples

In order to verify the significance of the proposed method using GMPGE, the estimation of acetaminophen present in human serum and urine samples has been carried out using differential pulse voltammetric method. The serum and urine samples were obtained from two volunteers of different age groups. Aliquots of the samples were centrifuged at ~ 10,000 rpm for about 10 min at room temperature. The resultant serum sample was diluted to 5 times and urine sample was diluted to 10 times using 0.1 M phosphate buffer solution of pH 7.0 and subjected to differential pulse voltammetric analyses. The DPV studies were carried out by spiking serum samples with a known amount of standard acetaminophen and the results so obtained are given in Table 3. The recoveries of acetaminophen for serum and urine samples were found to be 96.30% and 99.95% respectively. Therefore, the recovery rate suggests that, the proposed method for the estimation of acetaminophen using GMPGE has free from

interference of sample matrix and the proposed method is a reliable method that can be applied for the determination of acetaminophen present in real samples.

Table 1. Comparison of analytical parameters of several modified electrodes for the determination of acetaminophen with reported literatures

Modified Electrode Type	**Linear rage (mol L^{-1})**	**Limit of detection (µ mol L^{-1})**	**Ref.**
SWNT/EPPGE	5.0×10^{-9}–1.0×10^{-6}	2.9×10^{-9}	[5]
MWCNT-BPPGE	1.0×10^{-8}–2.0×10^{-6}	1.0×10^{-8}	[51]
Nafion/TiO_2-graphene/GCE	1.0×10^{-6}–1.0×10^{-9}	2.1×10^{-7}	[70]
MWCNTs-NHNPs/GCE	6.0×10^{-8}–2.6×10^{-5}	1.7×10^{-8}	[79]
AuNP/MWCNT/GCE	0.9×10^{-7}–3.5×10^{-5}	0.3×10^{-7}	[80]
Pd/Graphene oxide/GCE	0.5×10^{-7}–80×10^{-6}	2.2×10^{-9}	[81]
CS-CPE	8.0×10^{-7}–2.0×10^{-4}	5.08×10^{-7}	[82]
CoNPs/MWCNT/GCE	5.2×10^{-9}–4.5×10^{-7}	1.0×10^{-9}	[83]
PAY/nano-TiO_2/GCE	1.2×10^{-5}–1.20×10^{-4}	2.0×10^{-6}	[84]
MCPE-PtMWCNTs-TX100	9.0×10^{-8}–1.0×10^{-5}	1.77×10^{-10}	[85]
BDDE electrode	5.0×10^{-7}–8.3×10^{-5}	4.9×10^{-7}	[86]
GMPGE	1.0×10^{-8}- 0.9×10^{-6}	1.95×10^{-9}	**This work**

Table 2. Determination of concentrations of acetaminophen present in pharmaceutical samples (n=3)

Analyte	**Detected (nM)**	**Spiked (nM)**	**Found (nM)**	**Recovery (%)**
Tablet 1 (Calpol)	11.99	40	51.97	99.95
Tablet 2 (Dolo)	116.97	40	157.89	102.3
Tablet 3 (Colgin)	241.53	40	241.53	99.05

Table 3. Determination of concentration of acetaminophen present in human serum and urine samples (n=2)

Analyte (%)	**Detected (nM)**	**Spiked (nM)**	**Found (nM)**	**Recovery**
Human serum sample	55.87	20	75.13	96.30
Human urine sample	41.12	20	61.11	99.95

3.12. UV-vis spectrophotometry

The UV-Vis spectrophotometry is one of the significant techniques for the qualitative and quantitative determination of biologically important molecules, as they give characteristic

absorption spectra (λ_{max}) [87]. Hence, the UV-Vis spectrophotometric studies were carried out to confirm the presence of acetaminophen in pharmaceutical formulations and in biological samples. The UV-Vis spectra were recorded for standard acetaminophen sample, pharmaceutical formulations, human serum and urine samples in 0.1 M phosphate buffer solution of pH 7.0. The UV-Vis spectrum showed the characteristic absorption (λ_{max}) for standard acetaminophen at 246.79 nm. The pharmaceutical formulations and biological samples such as calpol, dolo, colgin, human serum and urine samples give λ_{max} at 246.51, 246.66, 246.72, 247.37 and 247.59 nm respectively. For all the samples, the characteristic absorption spectra's are appeared at almost same wavelength confirmed the presence of acetaminophen in pharmaceutical and biological samples.

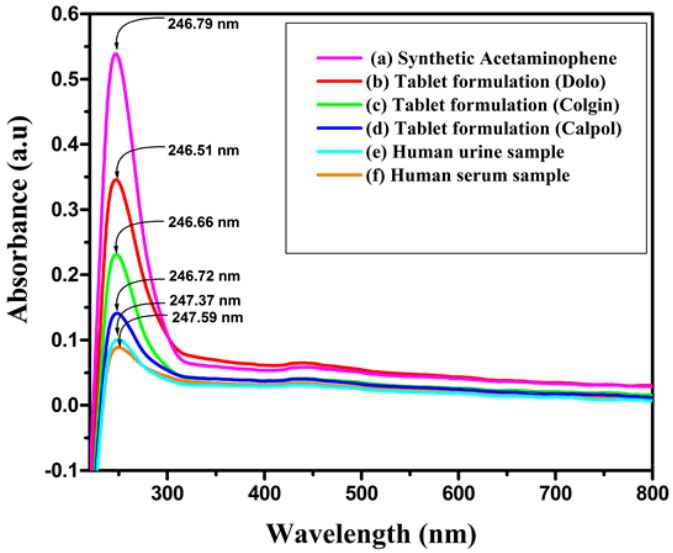


Fig. 7. Illustrate the UV-Vis absorption spectra's of (a) standard acetaminophen, (b) Tablet formulation of dolo, (c) Tablet formulation of colgin, (d) Tablet formulation of calpol, (e) Human urine sample, (f) Human serum sample in 0.1 M phosphate buffer solution of pH 7.0.

4. CONCLUSION

A novel gold modified disposable pencil graphite electrode (GMPGE) has been developed to be a convenient and reliable electrochemical sensor for the determination of acetaminophen. The GMPGE has been used satisfactorily for the determination of acetaminophen present in different pharmaceutical products and biological samples by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The fabrication of GMPGE is easy, requires low-cost and it possess good reproducibility, high stability, high sensitivity and rapid response. The GMPGE displayed reproducible performances with highly stable and not susceptible to interference of some common biological excipients. Acetaminophen exhibits better electrocatalytic activity and showed quasi-reversible redox peak at GMPGE. The

oxidation peak was found at 0.339 V and reduction at 0.285 V (*vs*. Ag/AgCl) in 0.1 M phosphate buffer solution of pH 7.0. This indicates that, the modified electrode has significantly lowered the over-potential (ΔE_p) and enhanced the diffusion current (I_d). At GMPGE the electron transfer kinetics of the acetaminophen was found to be diffusion-controlled possess. In differential pulse voltammetry, the linear calibration curves were obtained in the range of 1.0×10^{-8} M to 0.9×10^{-6} M with a limit of detection (LOD) 1.95×10^{-9} M and limit of quantification (LOQ) 6.47×10^{-9} M. Furthermore, the applicability of GMPGE has been successfully illustrated for the determination of acetaminophen present in pharmaceutical formulations and biological samples. Thus, the proposed modified electrode can be used in the quantitative estimation of acetaminophen present in various real samples with good accuracy.

REFERENCES

[1] M. Espinosa Bosch, A. J. Ruiz Sanchez, F. Sanchez Rojas, and C. Bosch Ojeda, J. Pharm. Biomed. Anal. 42 (2006) 291.

[2] Q. Chu, L. Jiang, X. Tian, and J. Ye, Anal. Chim. Acta 606 (2008) 246.

[3] C. J. Nikles, M. Yelland, C. D. Marc, and D. Wilkinson, Am. J. Therap. 12 (2005) 80.

[4] R. M. D. Carvalho, R. S. Freire, S. Rath, and L. T. Kubota, J. Pharm. Biomed. Anal. 34 (2004) 871.

[5] R. N. Goyal, V. K. Gupta, and S. Chatterjee, Sens. Actuators B 149 (2010) 252.

[6] A. A. Taylor, and O. Shoheiber, Wiley Online Library 9 (2003) 324.

[7] T. El Ouafy, A. Chtaini, and H. Oulfajrite, Acta Tehnic. Corviniensis–Bulletin Eng. 8 (2015) 100.

[8] D. W. Cramer, B. L. Harlow, L. T. Ernstoff, K. Bohlke, W. R. Welch, and E. R. Greenberg, Lancet 351 (1998) 104.

[9] N. R. Goyal, K. V. Gupta, M. Oyama, and N. Bachheti, Electrochemistry Communications 7 (2005) 803.

[10] A. German Messina, E. Irma De Vito, and J. Raba, Anal. Chim. Acta 559 (2006) 152.

[11] F. Andre Lavorante, K. Cherrine Pires, F. Boaventura, and Reis, J. Pharm. Biomed. Anal. 42 (2006) 423.

[12] N. Rajendra Goyal, and P. Sudhanshu Singh, Electrochim. Acta 51 (2006) 3008.

[13] M. J. Gómez, M. Petrovic, A. R. Fernández-Alba, and D. Barceló, J. Chromatogr. A 1114 (2006) 224.

[14] E. McEvoy, S. Donegan, J. Power, and K. Altria, J. Pharm. Biomed. Anal. 44 (2007) 137.

[15] J. F. Chiou, S. L. Chen, S. M. Chen, S. S. Tsou, C. Y. Wu, J. S. Chu, and T. Z. Liu, J. Food Drug Anal. 16 (2008) 36.

[16] H. Filik, I. Sener, S. D. Cekic, E. Kilic, and R. Apak, Chem. Pharm. Bull. 54 (2006) 891.
[17] B. C. Lourenc, R. A. Medeiros, C. Romeu, R. Filho, L. H. Mazo, and O. Fatibello-Filho, Talanta 78 (2009) 748.
[18] A. Ozcan, and Yucel, Anal. Chim. Acta 685 (2011) 9.
[19] Y. Fan, J. H. Liu, H. T. Lu, and Q. Zhang, Coll. Surfaces B 85 (2011) 289.
[20] W. Ruengsitagoona, A. S. Liawruangrath, and Townshend, Talanta 69 (2006) 976.
[21] S. Zhao, W. Bai, H. Yuan, and D. Xiao, Analytica Chimica Acta 559 (2006) 195.
[22] Maria-Elisa Capella-Peir, Devasish Bose, M. Font Rubert, and J. Esteve-Romero, J. Chromatogr. B 839 (2006) 95.
[23] S. Zhao, W. Bai, H. Yuan, and D. Xiao, Anal. Chim. Acta 559 (2006) 195.
[24] J. T. Franeta, D. Agbaba, S. Eric, S. Pavkov, M. Aleksic, and S. Vladimirov, Farmaco 57 (2002) 709.
[25] S. S. Narwade, J. Appl. Chem. 7 (2014) 46.
[26] O. Calinescu, I. A. Badea, L. Vladescu, V. Meltzer, and E. Pincu, J. Chromatogr. Sci. 50 (2012) 335.
[27] M. Knochen, J. Giglio, and B. F. Reis, J. Pharm. Biomed. Anal. 33 (2003) 191.
[28] G. Burgot, and J. L. Burgot, Anal. Chim. Acta 343 (1997) 125.
[29] N. Al-Zoubi, J. E. Koundourellis, and S. Malamataris, J. Pharm. Biomed. Anal. 29 (2002) 459.
[30] N. Erk, Y. Ozkan, E. Banoglu, S. A. Ozkan, and Z. Senturk, J. Pharm. Biomed. Anal. 24 (2001) 469.
[31] C. Andres, C. Soledad, G. Mercedes, and V. Miguel, Talanta 53 (2000) 417.
[32] E. J. Llorent-Martínez, D. Satínsky, P. Solich, P. Ortega-Barrales, and A. Molina-Díaz, J. Pharm. Biomed. Anal. 45 (2007) 318.
[33] B. Dejaegher, M. S. Bloomfield, J. Smeyers-Verbeke, and Y. V. Heyden, Talanta, 75 (2008) 258.
[34] P. Fanjul-Bolado, P. J. Lamas-Ardisana, D. Hernández-Santos, and A. C. Arcía, Anal. Chim. Acta 638 (2009) 133.
[35] M. L. S. Silva, M. B. Q. Garcia, J. L. F. C. Lima, and E. Barrado, Electrochim. Acta 24 (2006) 261.
[36] A. A. Shirkhedkar, A. M. Shaikh, and S. J. Surana, J. Anal. Chem. 3 (2008) 244.
[37] S. Zhao, W. Bai, H. Yuan, and D. Xiao, Anal. Chim. Acta 559 (2006) 195.
[38] M. E. Capella-Peiro, D. Bose, M.F. Rubert, and J. Esteve-Romero, J. Chromatogr. B 839 (2006) 95.
[39] C. H. Latha Saranya, J. C. Thejaswini, B. M. Gurupadayya, and B. Y. K. Sruthi, IOSR J. Pharm. 4 (2014) 12.
[40] A. Gutes, F. Cespedes, S. Alegret, and M. D. Valle, Talanta 66 (2005) 1187.

[41] M. L. S. Silva, M. B. Q. Garcia, J. L. F. C. Lima, and E. Barrado, Anal. Chim. Acta 573 (2006) 383.
[42] S. F. Wang, F. Xie, and R. F. Hu, Sens. Actuator B 123 (2007) 495.
[43] N. Wangfuengkanagul, and O. Chailapakul, J. Pharm. Biomed. Anal. 28 (2002) 841.
[44] D. Sun, and H. Zhang, Microchim. Acta 158 (2007) 131.
[45] M. Li, and L. Jing, Electrochim. Acta 52 (2007) 3250.
[46] S. J. R. Prabakar, and S. S. Narayanan, Talanta 72 (2007) 1818.
[47] S. F. Fabiana, M. A. B. Christopher, and L. Angnes, J. Pharm. Biomed. Anal. 43 (2007) 1622.
[48] R. N. Goyal, and S. P. Singh, Electrochim. Acta 51 (2006) 3008.
[49] C. Wang, C. Li, F. Wang, and C. Wang, Microchim. Acta 155 (2006) 365.
[50] Y. Tu, Y. Lin, W. Yantasee, and Z. Ren, Electroanalysis 17 (2005) 79.
[51] R. T. Kachoosangi, G. G. Wildgoose, and R. G. Compton, Anal. Chim. Acta 618 (2008) 54.
[52] Z. Z. Yin, G. C. Zhao, and X. W. Wei, Chem. Lett. 34 (2005) 992.
[53] M. P. Siswana, K. I. Ozoemena1, and T. Nyokong, Electrochim. Acta 52 (2006) 114.
[54] X. Yu, B. Munge, V. Patel, G. Jensen, A. Bhirde, J. D. Gong, S. N. Kim, J. Gillespie, J. S. Gutkind, F. Papadimitrakopoulos, and J. F. Rusling, J. Am. Chem. Soc. 128 (2006) 11199.
[55] X. Kang, J. Wang, H. Wu, J. Liu, I. A. Aksay, and Y. Lin, Talanta 81 (2010) 754.
[56] N. F. Atta, M. F. El-Kady, and A. Galal, Sens. Actuators B 141 (2009) 566.
[57] D. Nematollahia, H. Shayani-Jam, M. Alimoradi, and S. Niroomand, Electrochim. Acta 54 (2009) 7407.
[58] J. I. Gowda, D. G. Gunjiganvi, N. B. Sunagar, M. N. Bhat, and S. T. Nandibewoor, RSC Adv. 5 (2015) 49045.
[59] T. H. Tsai, S. H. Wang, and S. M. Chen, J. Electroanal. Chem. 659 (2011) 69.
[60] R. Sivasubramanian, and M. V. Sangaranarayanan, Talanta 85 (2011) 2142.
[61] A. Cernat, M. Tertis, R. Sandulescu, F. Bedioui, A. Cristea, and C. Cristea, Anal. Chim. Acta 886 (2015) 16.
[62] C. Zhou, M. Yang, S. S. Li, T. J. Jiang, J. H. Liu, X. J. Huanga, and X. Chen, Electrochim. Acta 231 (2017) 238.
[63] H. Ibrahim, and Y. Temerk, Sens. Actuators B 206 (2015) 744.
[64] A. J. Bard, and L. R. Faulkner, second addition, John Wiley & Sons, INC, New York, (2001).
[65] S. Zhang, K. Wu, and S. Hu, Talanta 58 (2002) 747.
[66] M. Boopathia, M. S. Won, and Y. B. Shim, Anal. Chim Acta 512 (2004) 191.
[67] H. Yang, B. Liu, Y. Ding, L. Li, and X. Ouyang, J. Electroanal. Chem. 757 (2015) 88.

[68] Y. He, Y. Dong, W. Huang, X. Tang, H. Liu, H. Lin, and H. Li, J. Electroanal. Chem. 759 (2015) 167.
[69] A. Özcan, and Y. Sahin, Biosens. Bioelectron. 25 (2010) 2497.
[70] Y. Fan, J. H. Liu, H. T. Lu, and Q. Zhang, Coll. Surfaces B 85 (2011) 289.
[71] M. L. Zhanga, D. K. Huang, Z. Cao, Y. Q. Liu, J. L. He, J. F. Xiong, Z. M. Feng, and Y. L. Yin, LWT–Food Sci. Technol. 64 (2015) 663.
[72] H. Heli, A. Jabbari, S. Majdi, M. Mahjoub, A. A. Moosavi Movahedi, and Sheibani, J. Solid State Electrochem. Acta 13 (2009) 1951.
[73] D. K. Gosser, Wiley−VCH Publishers: New York (1993) 27.
[74] E. Laviron, J. Electroanal. Chem. 101 (1979) 18.
[75] B. C. Lourenc, R. A. Medeiros, R. C. Rocha-Filho, L. H. Mazo, and O. Fatibello-Filho, Talanta 78 (2009) 748.
[76] W. Yunhua, J. Xiaobo, and H. Shengshui, Bioelectrochemistry 64 (2004), 91.
[77] B. Uslu, S. A Ozkan, and Z. Senturk, Anal. Chim. Acta 555 (2006) 341.
[78] A. Babaei, M. Sohrabi, and A. R. Taheri, J. Electroanal. Chem. 698 (2013) 45.
[79] T. Madrakian, E. Haghshenas, and A. Afkhami, Sens. Actuators B 193 (2014) 451.
[80] J. Li, J. Liu, G. Tan, J. Jiang, S. Peng, M. Deng, D. Qian, Y. Feng, and Y. Liu, Biosens. Bioelectron. 54 (2014) 468.
[81] Y. EL Bouabi, A. Farahi, N. Labjar, S. El Hajjaji, M. Bakasse, and M. A. El Mhammedi, Mater. Sci. Eng. C 58 (2016) 70.
[82] A. Kutluay, and M. Aslanoglu, Anal. Chim. Acta 839 (2014) 59.
[83] S. Ashok Kumar, C. F. Tang, and S. M. Chen, Talanta 76 (2008) 997.
[84] O. J. D'Souza, R. J. Mascarenhas, T. Thomas, B. M. Basavaraja, A. Kumar Saxena, K. Mukhopadhyay, and D. Roy, J. Electroanal. Chem. 739 (2015) 49.
[85] T. A. Enache, and A. M. Oliveira-Brett, Bioelectrochemistry 81 (2011) 46.
[86] V. Rodenas, M. S. Garcia, C. Sanchez-Pedren, and M. I. Albero, Talanta 52 (2000) 517.