PREPARATION AND APPLICATION OF PLATINUM COMPOSITE MICROELECTRODE (PCM) FOR THE ROUTINE ANALYSIS OF ACETAMINOPHEN IN PHARMACEUTICAL PRODUCTS

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ABSTRACT

Preparation and application of platinum composite microelectrode (PCM) for the routine analysis of acetaminophen in pharmaceutical products has been carried out. This electrode was prepare by Pt powder and PVC in 4 mL tetrahydrofuran (THF) solvent and swirled flatly to homogeneous followed by drying in an oven at 100 °C for 3 h. The mixture was placed in 0.5 cm diameter stainless steel mould and pressed at 10 ton/cm^2. The cyclic voltammetry were performed in a three electrodes system using PCM as a working electrode, an Ag/AgCl (saturated KCl) as reference electrode and platinum wire as the counter electrode. Electroanalysis of acetaminophen was performed in 0.1 M H$_2$SO$_4$ as an electrolyte. The result of the study showed that the correlation of determination using PCM electrode for electroanalysis acetaminophen was R^2 = 0.999. Precision, recovery, LOD and LOQ of the PCM towards acetaminophen were found to be 1.04%, 100.54%, 19.52 mg/L and 65.08 mg/L, respectively. As a conclusion, the methods can be used for routine analysis of acetaminophen in pharmaceutical product. Simplicity of sample preparation and use of low cost reagents are the additional benefit of this method.

Keywords: electroanalysis; platinum composite microelectrode; acetaminophen; cyclic voltammetry

ABSTRAK

Telah dilakukan penelitian tentang pembuatan dan aplikasi elektroda platinum komposit microelectrode (PCM) untuk analisis rutin acetaminophen dalam berbagai produk farmasi. Elektroda PCM dibuat dengan mencampurkan serbuk logam Pt dan PVC dengan pelarut 4 mL tetrahidrofuran (THF) dan diaduk sampai homogen, kemudian dikeringkan pada 100 °C selama 3 jam. Campuran ditempatkan dalam cetakan stainless steel dengan diameter 0,5 cm dan ditekan pada tekanan 10 ton/cm^2. Metode voltametri siklik dilakukan dalam sel elektrolisis dengan sistem tiga elektroda yaitu PCM sebagai elektroda kerja, Ag/AgCl (KCl jenuh) sebagai elektroda referensi dan kawat platina sebagai elektroda counter/pembanding. Elektroanalisis acetaminophen dilakukan dalam larutan 0,1 M H$_2$SO$_4$ sebagai elektrolit. Hasil penelitian menunjukkan bahwa nilai R^2 dari persamaan regresi linear untuk analisis asetaminofen mengunakan elektroda PCM adalah sebesar 0,999. Hasil validasi metode uji meliputi parameter presisi, akurasi, LOD dan LOQ diperoleh hasil masing-masing sebesar 1,04%, 100,54%, 19,52 mg/L dan 65,08 mg/L. Dari hasil penelitian dapat disimpulkan bahwa metode ini dapat digunakan untuk analisis rutin asetaminofen dalam berbagai produk farmasi. Kesederhanaan persiapan sampel dan penggunaan reagen yang sedikit merupakan keuntungan lain dari metode ini.

Kata Kunci: elektroanalisis; platinum composite microelectrode; asetaminofen; voltametri siklis

INTRODUCTION

Acetaminophen is widely used as an antipyretic and analgesic drug, proven effective to relieve pain associated with arthralgia, neuralgia, headaches and even for patients who suffer from gastric symptoms [1]. Because acetaminophen easily processed in metabolism, usually do not show any harmful side effects. However, the use of high doses causes accumulation of toxic metabolites that cause damage to the kidneys, liver disorders, skin rashes and inflammation of the pancreas [2]. From some of the research reported many techniques have been developed for the determination of acetaminophen such as spectrophotometry [3-5], spectrofluorometry [6-7], reverse phase high performance liquid chromatography (RP-HPLC) [8], raman spectrometry [9], titrimetry [10], a flow injection with multicommutation and chemiluminescence [11]. However, many of these techniques require a process that requires the destruction of a complicated, long analysis time, high cost, sophisticated instruments and

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skilled operators. Electroanalytical technique has the advantage of a simple, rapid, time-saving, inexpensive, sensitive, and in-situ detection of traces of various analytes and is widely used in the detection of drugs such as acetaminophen.

Electrochemical techniques have been used for the determination of a wide range of drug compounds. Electrochemical techniques also include determination of the drug's electrode mechanism. Redox properties of drugs can provide insight into their metabolic fate, there in vivo redox processes, and their pharmacological activity [12]. Electrochemical techniques are extensively applied due to their sensitive properties and usually they do not require sample pretreatment that is time consuming and difficult [13]. Furthermore, in process control and routine analysis of acetaminophen there is a need for methods that allow determination to be performed with some requirements such as speed, accuracy, high degree of automation and cost-effectiveness.

Composite materials typically consist of two or more components that modify the surface of electrodes. There is clear that the potential for several differences in the preparation (the type of carbon, binder and presence of modifiers) can affect the selectivity and sensitivity of electrochemical response of the modified electrode. The binder can be taken a simple mineral oil, polymers, wax or epoxy, or ionic liquid [14]. Modification of electrode surfaces has played an important role in the study of electron transfer kinetics and electrocatalytic reactions. It has involved the formation of an electrocatalytic system in which redox species are capable of undergoing a rapid and reversible electrode reaction, reducing the over-potential required for either the oxidation or reduction of compounds [15].

In the present work, a new technique, simple and precise method is proposed for the determination of acetaminophenin at pharmaceutical product. The method is based on electrochemical oxidation of acetaminophenin in 0.1 M H_2SO_4 solution at room temperature using platinum composite microelectrode (PCM). One of the techniques of making the porous electrode is by incorporating polymer material like polyvinyl chloride (PVC) with the powder of respected metals. The addition of certain percentage of polymer powder into platinum powder will enhance the electrode reactivity. PCM electrode is the simple and low cost the electrode fabrication, high speed, reproducibility, high stability, wide linear dynamic range and high sensitivity.

EXPERIMENTAL SECTION

Materials

All solutions were prepared by dissolving their analytical grade reagent (Merck) in deionised distilled water. Pt powder (Aldrich) and polyvinyl chloride (PVC) and tetrahydrofuran (THF) from Merck was used for preparation of a PCM electrode. Buffer phosphate solution was prepared using 0.1 M KH_2PO_4 (Merck) and 0.1 M KOH (Merck). Acetaminophen solutions were prepared by dilution of absolute acetaminophen (Merck) with deionised distilled water. The calibration curve was made by using acetaminophen concentration of 100-1000 mg/L.

Instrumentation

The surface and cross section characterization of the PCM electrode using SEM was performed on the JSM 5400 microscope equipped with a microprobe Voyager Noran system. PGSTAT 100 N 100 V/250 mA (Metrohm Autolab) was used for electroanalysis measurements.

Procedure

#### *Preparation of a platinum composite microelectrode (PCM)*

Pt powder (< 2 micron in size and 99.9% purity) and PVC in 4 mL THF solvent and swirled flatly to homogeneous followed by drying in an oven at 100 °C for 3 h. The mixture was placed in 0.5 cm diameter stainless steel mould and pressed at 10 ton/cm^2. A typical pellet contained approximately amount of Pt (95%) powder, and approximately 5% of PVC polymer.

Electroanalysis of acetaminophen

The electrochemical process of acetaminophen was performed in 0.1 M H_2SO_4 solution at room temperature. The electrochemical studies by cyclic voltammetry (CV) were performed in 50 mL capacity glass electrochemical cell. The cyclic voltammetry experiments were performed in a three electrodes system using PCM as a working electrode, an Ag/AgCl (saturated KCl) as reference electrode and platinum wire as the counter electrode. All potentials given are with respect to the Ag/AgCl reference electrode.

Calibration and validation method

Calibration curves were obtained by plotting anodic peak height (current) versus acetaminophen concentration. Validation parameters including linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy were assessed. Cyclic voltammograms (CVs) of acetaminophen solutions were recorded in a wide range of concentrations (100-1000 mg/L) in 0.1 M H_2SO_4 solution at room temperature.
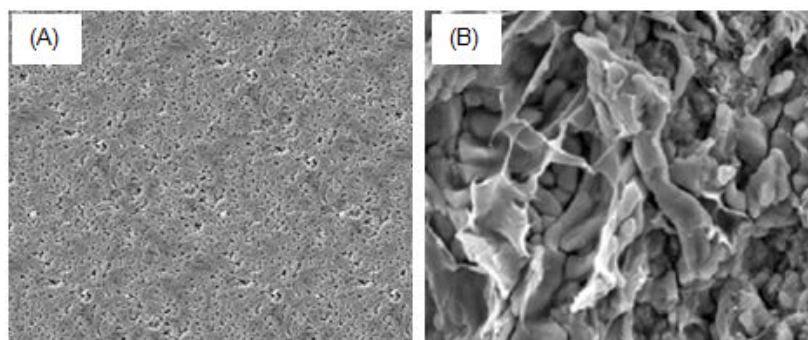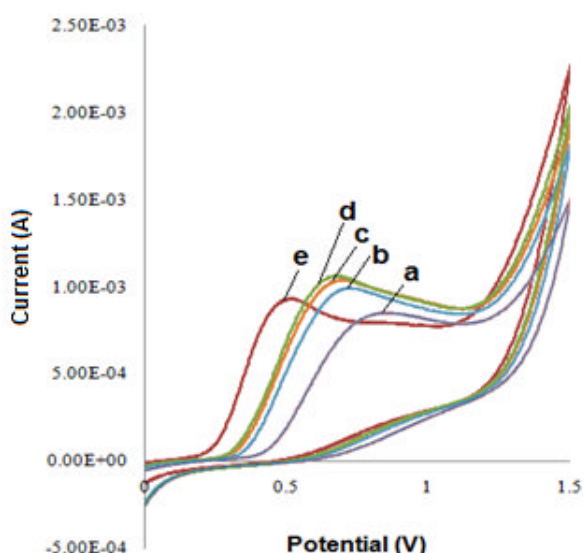
Fig 1. SEM micrographs at electrode surface (A) and cross section (B) of PCM with magnification x 2000
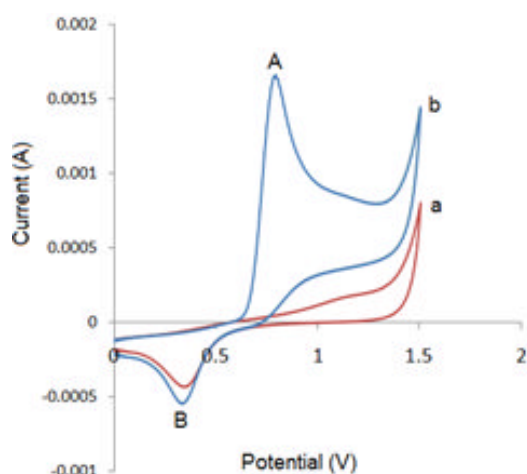
Fig 2. Cyclic voltammetry of (a) 0.1 M H_2SO_4 (without acetaminophen) and (b) 0.1 M H_2SO_4 + 1.0 g/L acetaminophen. Scan rate 100 mV/sec
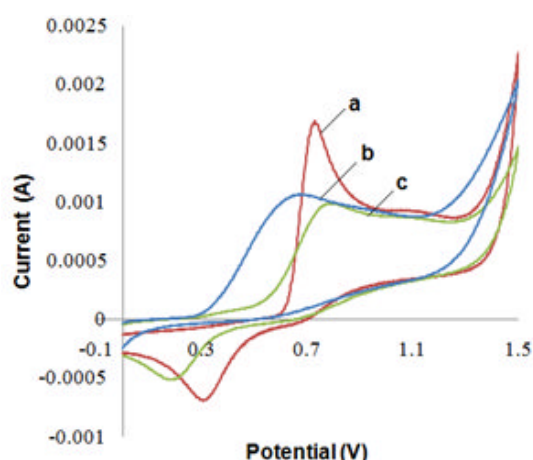
Fig 3. Cyclic voltammetry of (a) 0.1 M H_2SO_4 (b) buffer phosphate pH 9 (c) 0.1 M KNO_3, in 1.0 gL^{-1} acetaminophen. Scan rate 100 mV/sec

Fig 4. Cyclic voltammetry 1.0 gL^{-1} acetaminophen in buffer phosphate solution with various pH solutions: (a) 6 (b) 7 (c) 8 (d) 9 and (e) 10. Scan rate 100 mV/sec

RESULT AND DISCUSSION

### Characterization of Platinum Composite Microelectrode (PCM)

The scanning electron microscopic image of PCM was recorded and shown in Fig. 1. In accordance with this Fig. 1, PCM have porous structure. Therefore, prepared platinum-PVC mixture can present porous structure. PVC has been used as a binder, so that the electrode has a high stability and porosity.

Electrolyte Effect on Cyclic Voltammetry Responses

Fig. 2(a) shows the cyclic voltammogram of 0.1 M H_2SO_4 (without acetaminophen) with the sweep potential from potential 0 mV to +1500 mV, and then return from +1500 mV to 0 mV. Fig. 2(b) shows the cyclic voltammogram of 1.0 g/L acetaminophen in 0.1 M H_2SO_4. The A peak represent the anodic peak. These peaks related to the oxidation of acetaminophen.
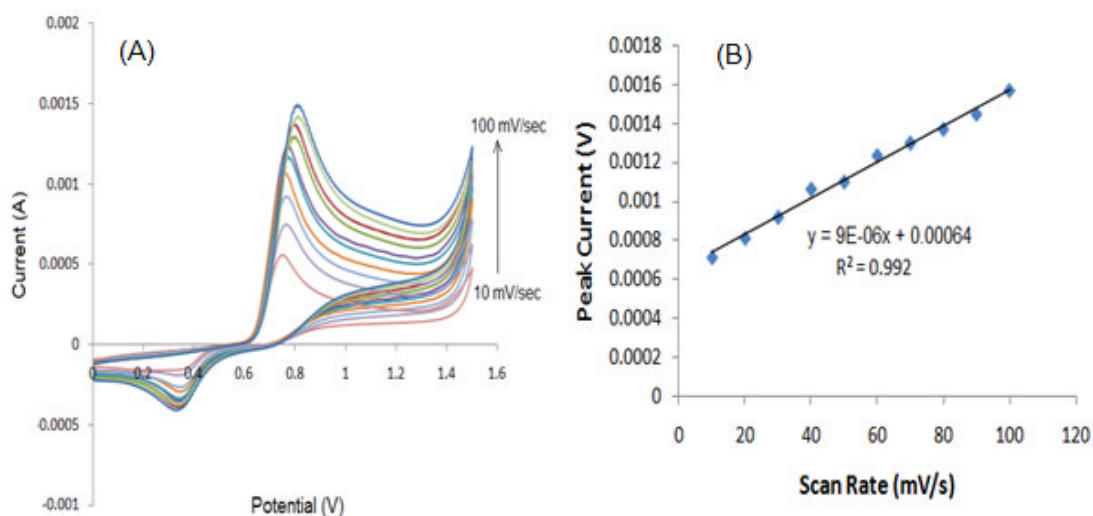
Fig 5. Cyclic voltammetry (A) and plot of peak currents as a function of scan rate of potential (B) in 0.1 M H_2SO_4 solution in 1.0 gL^{-1} acetaminophen with variation scan rate 10-100 mV/sec
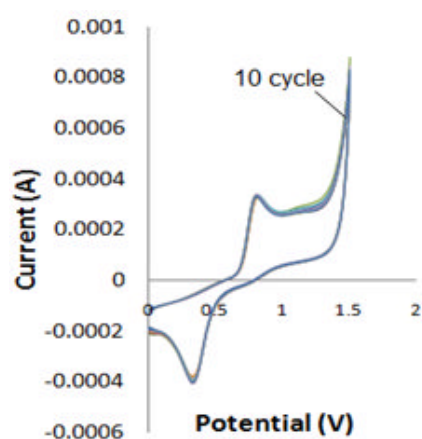
Fig 6. Cyclic voltammetry in 1.0 g/L acetaminophen in 0.1 M H_2SO_4 solution with 10 cycles and scan rate 100 mV/sec

The oxidation reaction that occurred at A peak (Fig. 2(a)) were represent at Fig. 2. The B peak in Fig. 2(b) represents the cathodic peak. These peaks related to the reduction of acetaminophen. Cyclic voltammetry response was found after the addition of acetaminophen, is characterized by detection of anodic and cathodic peak. The anodic and cathodic peaks showed character of the cyclic voltammetry of acetaminophen are $E_{pa} = \pm 0.77$ V and $E_{pc} = \pm 0.38$ V, respectively. The cyclic voltammogram of the electrochemical characteristic of acetaminophen was found to be a quasi reversible process. As shown in Fig. 2 the acetaminophen can be oxidized *via* two electrons and two protons processes.

Fig. 3 showed the cyclic voltammetry of 1.0 gL^{-1} acetaminophen with different electrolyte. Fig. 3 (a, b and c) showed the cyclic voltammetry of 1.0 gL^{-1} acetaminophen at in 0.1 M H_2SO_4, buffer phosphate

pH 9, 0.1 M KNO_3, respectively. Sulfuric acid, buffer phosphate pH 9 and KNO_3 were selected based on the study of Li and Chen [16]. Li and Chen have reported the electrochemical oxidation of acetaminophen in different pH using the bare glassy carbon electrode [16]. Fig. 3 shows the effect of the addition of electrolyte to the anodic and cathodic peaks. Based on the cyclic voltammetry result of the anodic peak from 0.1 M H_2SO_4, buffer phosphate pH 9 and 0.1 M KNO_3 are 0.0017 A, 0.0011 A and 0.00099 A, respectively. H_2SO_4 electrolyte was chosen as the electrolyte at the application of the analysis acetaminophen in this study. The solution of 0.1 M H_2SO_4 is a good electrolyte, because it produces a high oxidation peak. High oxidation peak will be increase the sensitivity of the method. Fig. 3(a) shows oxidation of acetaminophen in acid pH (0.1 M H_2SO_4). According to Li and Chen [16], this is expected because of the participation of proton(s) in the oxidation reaction of acetaminophen to N-acetyl-p-benzoquinone-imine, and vice-versa within a quasi-reversible two-electron process.

pH Effect on Cyclic Voltammetric Responses

Fig. 4(d) shows the highest peak of 1.0 gL^{-1} acetaminophen in buffer phosphate at pH 9 with current peak $I_{pa} = 1.06 \times 10^{-3}$ A. While in Fig. 4 anodic peak potential (Epa) showed a decrease as the pH increases. Li and Chen [16] reported a decrease Epa as pH increased due to the inclusion of protons in the oxidation reaction to N-acetyl ACOP-p-benzoquinone-imine (NAPQI). In this study the potential relationship with pH linier as successfully demonstrated the correlation of determination $R^2 = 0.902$ (not shows in this paper). Fig. 4(d) showed in base solution, there is
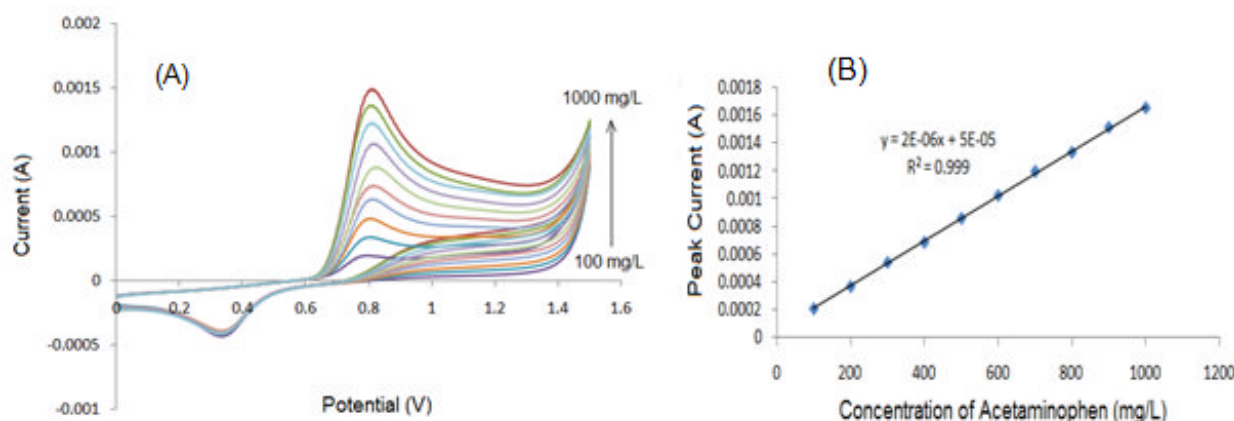
Fig 7. Cyclic voltammetry (A) and calibration curve (B) 0.1 M H_2SO_4 solution in various concentration of acetaminophen 100-1000 mg/L. Scan rate 100 mV/sec

a strong relation between basicity and instability of N-acetyl-p-benzoquinone-imine. This is expected because of the participation of hydroxide ions in reaction mechanism (EC mechanism or ECE mechanism).

Effect of Scan Rate on the Peak Currents of Acetaminophen

The effect of potential scan rate on peak current of 1.0 g/L acetaminophen in 0.1 M H_2SO_4 solution was investigated. Fig. 5(A) shows the cyclic voltammograms of the 1.0 g/L acetaminophen using the PCM electrode at different scan rate in the potential range of 10-100 mVs^{-1}. Scan rate is directly proportional to anodic peak (I_{pa}) of the acetaminophen. Scan rate is directly proportional to the speed of electrolysis that occurs on the surface of the working electrode. The higher the scan rate the higher the reaction rate. Anodic peak current is directly proportional to the scan rate. In this study also obtained a linear correlation is shown in Fig. 6(B) with the correlation of determination R^2 = 0.992. Slope value shows sensitivity of the electroanalysis of acetaminophen using PCM electrode. R^2 value shows good effect of scan rate on the peak current. If the value of R^2 closes to one, then it can choose the most high scan rate. The higher of scan rate, the higher peak currents and faster analysis time. Besides the R^2 value indicates the type of electron migration and stability of the electrode. From Fig. 5 it is easy to conclude that, as the scan rate increases the peak-to-peak separation also changes. The ratio of the anodic peak current to the cathodic peak current is also different from unity. Hence the electrochemical reaction of acetaminophen is irreversible.

Fig. 6 showed cyclic voltammogram 1.0 g/L acetaminophen in 0.1 M H_2SO_4 solution with 10 cycles. The cyclic voltammogram obtained in Fig. 7 shows that PCM electrode is a good stability in 0.1 M H_2SO_4 solution.

Calibration Curves

The calibration curves for acetaminophen were found by the results of cyclic voltammetric measurements (Fig. 7(A)). Fig. 7(B) shows the calibration curve for the concentration of acetaminophen with current for PCM electrodes. The cyclic voltammetric obtained for acetaminophen with various concentrations that were linear over the concentration range of 100-1000 mg/L. In this Fig. 7, anodic current of acetaminophen were plotted against the concentration of acetaminophen and linear regression analysis completed on the resulting curve.

From the calibration curve obtained using PCM electrodes, the correlation of determination (R^2) recorded is 0.999. Linear regression equation (Fig. 7(B)) is $y = 2.10^{-6}x + 5.10^{-5}$. The linear regression equation can be used to determine the concentration of acetaminophen in pharmaceutical product.

Validation Parameters

Precision is a measure of the closeness of the analytical results obtained from a series of replicate measurements of the same measure under the conditions of the method. It reflects the random errors which occur in a method. Precision is usually measured as the coefficient of variation or relative standard deviation of analytical results obtained from independently prepared quality control standards [17]. The precision of a measurement is a measure of the reproducibility of a set of measurements. Precision has been obtained by the analysis of samples by the same method as much as 10 replications. Results of the analysis of the precision values are 1.04%. This method has a good precision as below the limit of 2%.

Recovery experiments should be performed by comparing the analytical results for extracted samples at three concentrations. Recovery of the analyte need

Table 1. Analytical application of proposed method. Acetaminophen present = 500 mg/tablet

No	Pharmaceutical product	Acetaminophen measured (mg/tablet)	Recovery* (%)
1	Pyrexin	503.33	100.67
2	Indofarma	504.62	100.92
3	Sanbefarma	500.17	100.03

*Average of three determinations

Table 2. Comparison of electroanalytical technique and LOD for determination of acetaminophen using different electrodes

No	Modified electrode	Electroanalytical technique	LOD (mg/L)	Reference
1	MCPE/PR	Differential pulse voltammetry	80.12	[18]
2	Bi_2O_3/GCE	Cyclic voltammetry	30.23	[19]
3	PEDOT/GCE	Differential pulse voltammetry	170.81	[20]
4	Pt/PVC	Cyclic voltammetry	19.52	This work

not be 100%, but the extent of recovery of an analyte and of the internal standard should be consistent, precise, and reproducible. Table 1 showed electro-analysis acetaminophen in 0.1 M H_2SO_4 solution using PCM electrode have a good recovery is close to 100%.

The LOD is defined as the lowest concentration that can be distinguished from the background noise with a certain degree of confidence. Limit of detection (LOD) and limit of quantification (LOQ) are two important performance characteristics in method validation. LOD and LOQ are terms used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure [17]. LOD and LOQ of the electrode towards acetaminophen were found to be 19.52 mg/L and 65.08 mg/L, respectively. A comparison of the response characteristics of different modified electrode towards the detection of acetaminophen is tabulated in Table 2.

CONCLUSION

From this research we can conclude that the developed electroanalysis methods using PCM electrode are accurate, precise, reproducible and inexpensive with acceptable correlation of determination (R^2), RSD (%), LOD, LOQ and recovery (%). The methods can be used for routine analysis of acetaminophen in pharmaceutical product. Simplicity of sample preparation and use of low cost reagents are the additional benefit of this method. The correlation of determination using PCM electrode for electroanalysis of acetaminophen was R^2 = 0.999. Precision, LOQ, LOD and recovery of the PCM towards acetaminophen were found to be 1.04%, 19.52 mg/L, 65.08 mg/L and 100.54%, respectively.

ACKNOWLEDGEMENT

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