Anal. Bioanal. Electrochem., Vol. 6, No. 2, 2014, 159 - 169

Analytical & Bioanalytical Electrochemistry

Full Paper

Electroanalysis of Mefenamic Acid Using Platinum Powder Composite Microelectrode (PPCM)

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Received: 18 February 2014/ Accepted after minor revision: 10 April 2014/ Published online: 30 April 2014

Abstract- Electroanalysis of mefenamic acid using platinum powder composite microelectrode (PPCM) by cyclic voltammetry (CV) method in buffer phosphate has been carried out. PPCM 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 hours. The mixture was placed in 0.5 cm diameter stainless steel mould and pressed at 10 ton/cm^2. Cyclic voltammetry method was performed in a three electrodes system using PPCM as a working electrode, an Ag/AgCl (saturated KCl) as reference electrode and platinum wire as the counter electrode. Electroanalysis of mefenamic acid was performed in a buffer phosphate solution (pH 4). The results of the study showed that the correlation of determination using PPCM electrode for electroanalysis of mefenamic acid was R^2=0.995. Precision, LOQ, LOD and recovery of the PPCM towards mefenamic acid were found to be 1.01%, 27.94 ppm, 93.13 ppm and 100.88%, respectively. As a conclusion, PPCM electrode is very good for electroanalysis of mefenamic acid in pharmaceutical product.

Keywords- Electroanalysis, Platinum powder composite microelectrode (PPCM), Mefenamic acid

1. INTRODUCTION

Mefenamic acid [2-(2,3-dimethyl phenyl)amino] benzoic acid is a non-steroidal drug which has analgesic, anti-inflammatory and antipyretic actions and it is used specially in the treatment of rheumatoid arthritis and osteoarthritis and other muscular-skeletal diseases [1,2]. Various methods have been reported for the determination of mefenamic acid as pure and in dosages forms. These methods include titrimetric, chromatographic (HPLC) [3], spectrofluorimetric [4] and spectrophotometric methods [1,5]. Also spectrophotometric methods have been described for the simultaneous determination of mefenamic acid in the mixture with other active drugs in the same pharmaceutical preparation [1,6]. Drug analysis has an important role on public health and due to extensive use of non-steroidal anti-inflammatory drugs for their devastating effects on pains like: headache, post-operative, dental pain and some diseases including arthritis, sport injuries and other rheumatic diseases, many researches were done for developing of sensitive and rapid technique for the analysis of these drugs [7]. Overdoses of mefenamic acid generate toxic metabolite accretion that causes severe hepatic necrosis, including morbidity and death in patients [8]. Due to the vital importance of mefenamic acid, it is essential to develop a simple, rapid and reliable technique for the determination of mefenamic acid. However, some of these procedures suffer from one or another disadvantage such as extraction into organic solvent, requiring non-aqueous medium and others need control of temperature.

Mefenamic acid (MFA) is used to relieve the symptoms of many diseases such as rheumatoid arthritis, non-articular rheumatism, and sport injuries [7]. It is used to treat mild to moderate pain, including headache, dental pain, post-operative and post-partum pain, dysmenorrhoea, as well as musculoskeletal disorders and joint disorders such as osteoarthritis. Overdoses of MFA produce toxic metabolite accumulation that causes acute hepatic necrosis, inducing morbidity and mortality in humans. Due to the vital importance of the assay of MFA for pharmaceutical formulations and biological fluids, several analytical methods have been developed for the quantitative determination of this drug in both pharmaceutical and biological samples. Therapeutic mefenamic acid level is 10 μg/mL [7].

Furthermore, in process control and routine analysis of mefenamic acid 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**.** The cyclic voltammetry method was shown to be a good alternative compared to the most popular method, HPLC, because it is simpler, faster, less expensive, does not involve sample preparation techniques, and differences between the results obtained by the two methods are not statistically significant. The alternative method produces smaller amounts of residual solutions compared with HPLC. The total volume per analysis by the voltammetric method is 10 mL given that all standard solutions are prepared in the electrochemical cell, whereas large volumes of mobile phases

are needed for HPLC analysis. When comparing both methods for routine analysis, the voltammetric method also has an advantage considering the waste management issue [9].

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 [7]. Electrochemical techniques are extensively applied due to their sensitive properties and usually they do not require sample pretreatment that is time consuming and difficult. 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 [8]. 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 [10].

In the present work a new, simple and precise method is proposed for the determination of mefenamic acid in pharmaceutical product. The method is based on electrochemical oxidation mefenamic acid in buffer phosphate solution at room temperature using platinum powder composite microelectrode (PPCM). PPCM electrode is the simple and low cost the electrode fabrication, high speed, reproducibility, high stability, wide linear dynamic range and high sensitivity.

2. EXPERIMENTAL

2.1. Solution

All solutions were prepared by dissolving their analytical grade reagent (Merck) in deionised distilled water. Nitrogen was used to deaerate the solutions and to keep an inert atmosphere over the reaction solution during the analysis process. Buffer phosphate solution (pH 2-5) was prepared using 0.l M KH_2PO_4 (Merck) and 0.1 M HCl (Merck), while pH 7 was prepared using 0.l M KH_2PO_4 (Merck) and 0.1 M NaOH (Merck). Mefenamic acid solutions were prepared by dilution of absolute mefenamic acid (Merck) with deionised distilled water. The calibration curve was made by using mefenamic acid concentration of 100-1000 mg/L.

2.2. Preparation of a platinum powder composite microelectrode (PPCM)

Pt powder (< 2 micron in size and 99.9% purity, Aldrich Chemical Company) and PVC in 4 mL tetrahydrofuran (THF) solvent and swirled flatly to homogeneous followed by drying in

an oven at 100°C for 3 hours. 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.

2.3. Characterization of PPCM

The surface characterization of the electrode using SEM was performed on the JSM 5400 microscope equipped with a microprobe Voyager Noran system.

2.4. Electroanalysis of mefenamic acid procedure

The electrochemical process of mefenamic acid was performed in solution a buffer phosphate solution pH 4 at room temperature. The electrochemical studies by cyclic voltammetry (CV) were performed in 50 mL capacity glass electrochemical cell. PGSTAT 100 N 100 V/250 mA (Metrohm Autolab) was used for electrochemical behavior measurements; data acquisition was accomplished using the software from Metrohm. Cyclic voltammetry experiments were performed in a three electrodes system using PPCM 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.

2.5. Calibration and validation method

Calibration curves were obtained by plotting anodic peak height (current) versus mefenamic acid concentration. Validation parameters including linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy were assessed. Cyclic voltammograms (CVs) of mefenamic acid solutions were recorded in a wide range of concentrations (100-1000 mg/L) in buffer phosphate solution at room temperature.

3. RESULT AND DISCUSSION

3.1. Characterization of platinum powder composite microelectrode (PPCM)

The scanning electron microscopic image of PPCM was recorded and shown in Fig. 1. In accordance with this Fig. 1, PPCM 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.

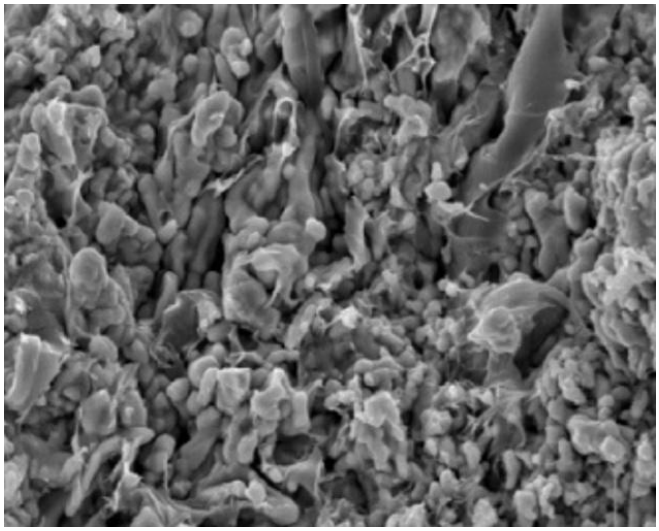

Fig. 1. SEM micrographs at cross section of platinum powder composite microelectrode (PPCM) with magnification x 2000

3.2. Electrolyte effect on cyclic voltammetric responses

Fig. 2 showed mechanism of electrooxidation of mefenamic acid at electrode surface. In electrochemistry, oxidation is loss of electrons or hydrogen. Based on mechanism of electrooxidation (Fig. 2), mefenamic acid can be oxidized to radical mefenamic acid with loss of one electron and hydrogen [7]. Radical mefenamic acid can also be reduced back to mefenamic acid again by adding hydrogen to it. A possible reducing agent is buffer phosphate solution (pH 4).

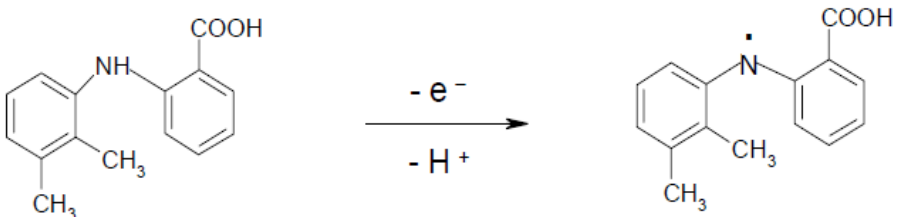

Fig. 2. Mechanism of electrooxidation of mefenamic acid at electrode surface [7]

Fig. 3C shows the cyclic voltammogram using the PPMC electrode in 1.0 g/L mefenamic acid in buffer phosphate pH 4 solution. Fig. 3A, 3B and 3C showed no peak in the cyclic voltammetry without the addition of 1.0 g/L mefenamic acid. When 1.0 g/L mefenamic acid was added (Fig. 3D), a new peak was observed (peak A), which represents the electrochemical oxidation of mefenamic acid peak (Ep anodic). Peak B at Fig. 3D related to the reduction of mefenamic acid. Cyclic voltammogram of the electrochemical characteristic of mefenamic acid was found to be a quasi reversible process. Our results revealed that the numbers of protons in the processes are equal to the number of the transferred electrons. As shown in Fig. 2, this conclusion is in accordance with the known electrochemical reactions of

mefenamic acid. As shown in Fig. 2 and Fig. 3 (peak A & B) mefenamic acid can be oxidized via one electron and one protons processes [8].

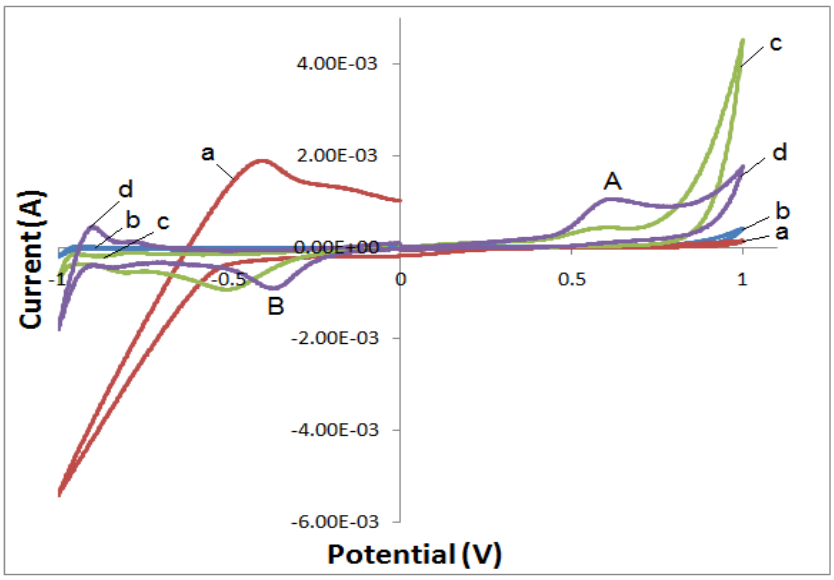

Fig. 3. Cyclic voltammetry of (A) buffer phosphate solution (pH 4); (B) distilled water; (C) distilled water+buffer phosphate solution (pH 4) without mefenamic acid; (D) distilled water+buffer phosphate solution (pH 4)+1.0 g/L mefenamic acid and at scan rate 100 mV/sec

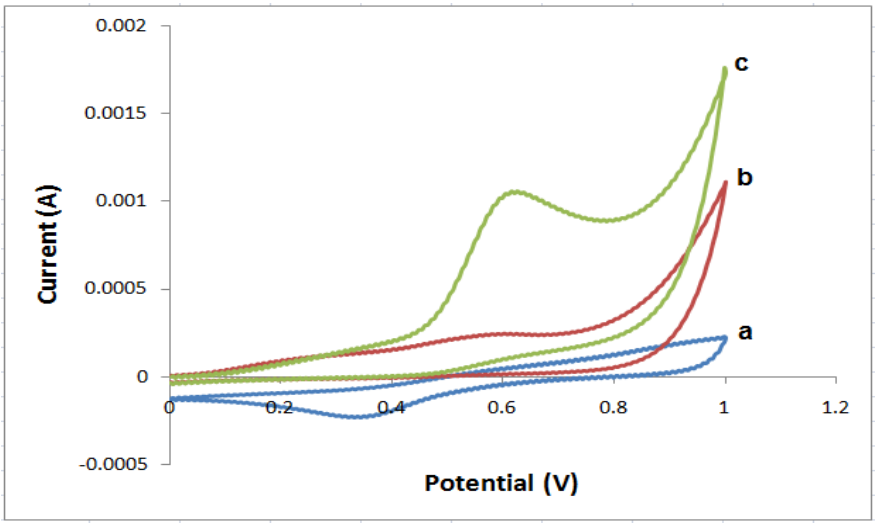

Fig. 4. Cyclic voltammetry of (a) 0.2 M acid sulfuric (b) 0.1 M KNO_3 (c) buffer phosphate pH 4 solution, in 1.0 g/L mefenamic acid at scan rate 100 mV/sec

Fig. 4 showed cyclic voltammetry of 1.0 g/L mefenamic acid at scan rate 0.1 V/sec with different electrolyte. Buffer phosphate solution (pH 4) is a very good electrolyte,

because it produces a high oxidation peak. High oxidation peak will be increase the sensitivity of the method.

3.3. pH effect on cyclic voltammetric responses

Fig. 5. showed cyclic voltammetry 1.0 g/L mefenamic acid in buffer phosphate solution with variation pH. The influences of pH on the peak current and peak potential of mefenamic acid are shown in Fig. 5. The oxidation of mefenamic acid is a pH dependent, 1-electron, 1-proton, which is illustrated in Fig. 2. Cyclic voltammetry of mefenamic acid at pH 2 and pH 3, indicated very low anodic peak, because the concentration of H^+ ions are abundant. Buffer phosphate solution with pH 4 is a good electrolyte for electroanalysis of mefenamic acid using PPMC electrode. Hence buffer phosphate solution with pH 4.0 is chosen as the supporting electrolyte. In addition, the effect of pH is related to the electrochemical reaction, since proton transfer is involved in it [11].

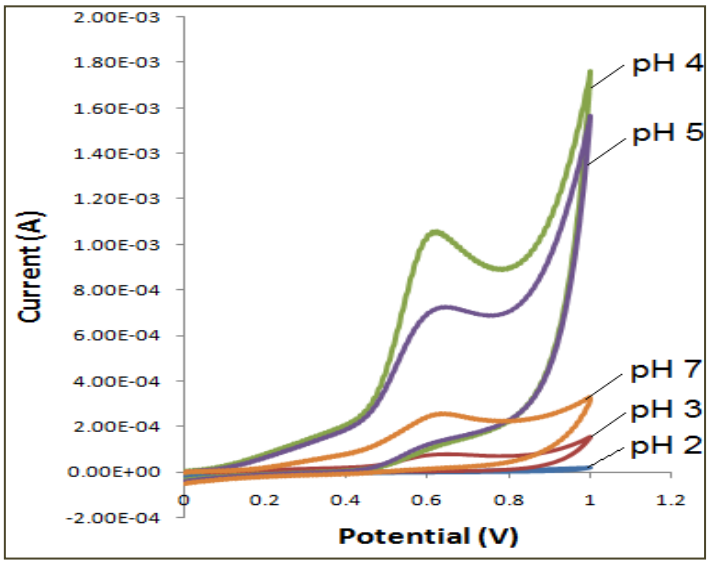

Fig. 5. Cyclic Voltammetry in buffer phosphate solution with pH 2, 3, 4, 5 and 7, in 1.0 g/L mefenamic acid at scan rate 100 mV/sec

3.4. Effect of scan rate on the peak currents of mefenamic acid

The effect of potential scan rate on peak current of 1.0 g/L mefenamic acid in buffer phosphate solution (pH 4.0) was investigated. Fig. 6A shows the cyclic voltammograms of the 1.0 g/L mefenamic acid using the PPCM electrode at different scan rate in the potential range of 10 to 300 mV/s. The results showed that the anodic peaks current of 1.0 g/L mefenamic acid were proportional to the scan rate over the range 10–300 mV/s (Fig. 6B) indicating adsorptive properties of the electrochemical process [5].

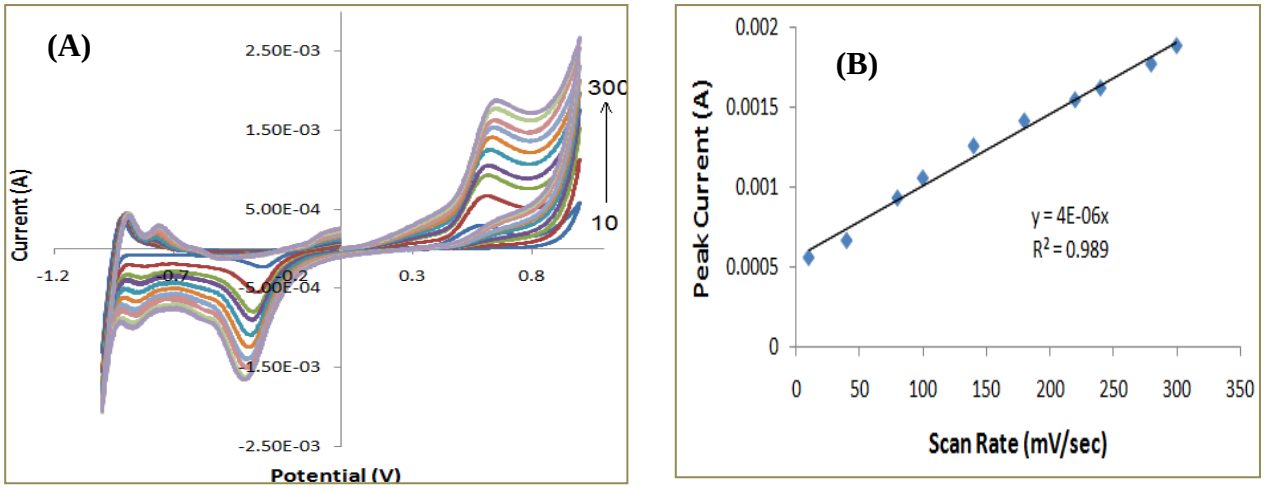

Fig. 6. Cyclic Voltammetry **(A)** and plot of peak currents as a function of scan rate of potential **(B)** in buffer phosphate solution with pH 4 in 1.0 g/L mefenamic acid with variation scan rate 10, 40, 80, 100, 140, 180, 220, 240, 280 and 300 mV/s

Fig. 7. showed cyclic voltammetry 1.0 g/L mefenamic acid in buffer phosphate solution (pH 4) with 10 cycle. The cyclic voltammogram obtained in Fig. 7 shows that PPMC electrode is very good stability in buffer phosphate solution (pH 4).

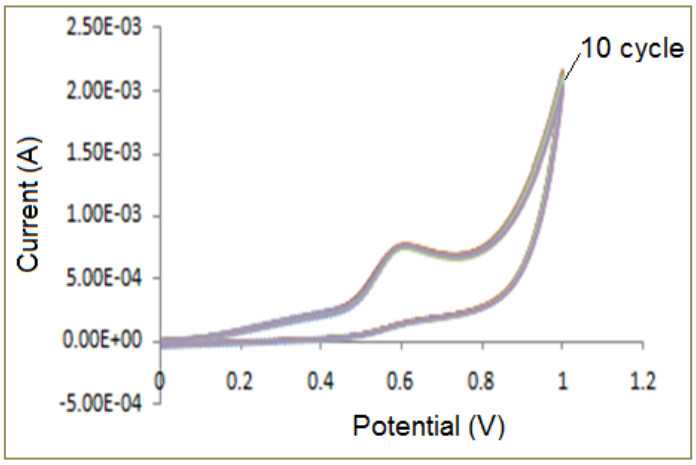

Fig. 7. Cyclic voltammetry in 1.0 g/L mefenamic acid in buffer phosphate solution (pH 4) with 10 cycle and scan rate 100 mV/s

3.5. Calibration Curves

The calibration curves for mefenamic acid were found by the results of cyclic voltammetric measurements. Fig. 8A shows the calibration curve for the concentration of mefenamic acid with current for PPCM electrodes. The cyclic voltammetric obtained for mefenamic acid with various concentrations that were linear over the concentration range of

100-1000 mg/L. In this Fig. 8, anodic current of mefenamic acid were plotted against the concentration of mefenamic acid and linear regression analysis completed on the resulting curve.

From the calibration curve obtained using PPCM electrodes, the correlation of determination (R^2) recorded is 0.995. Linear regression equation (Fig. 8A) is $y=8.10^{-7}x$ with slope 8×10^{-7}. The linear regression equation can be used to determine the concentration of mefenamic acid in pharmaceutical product.

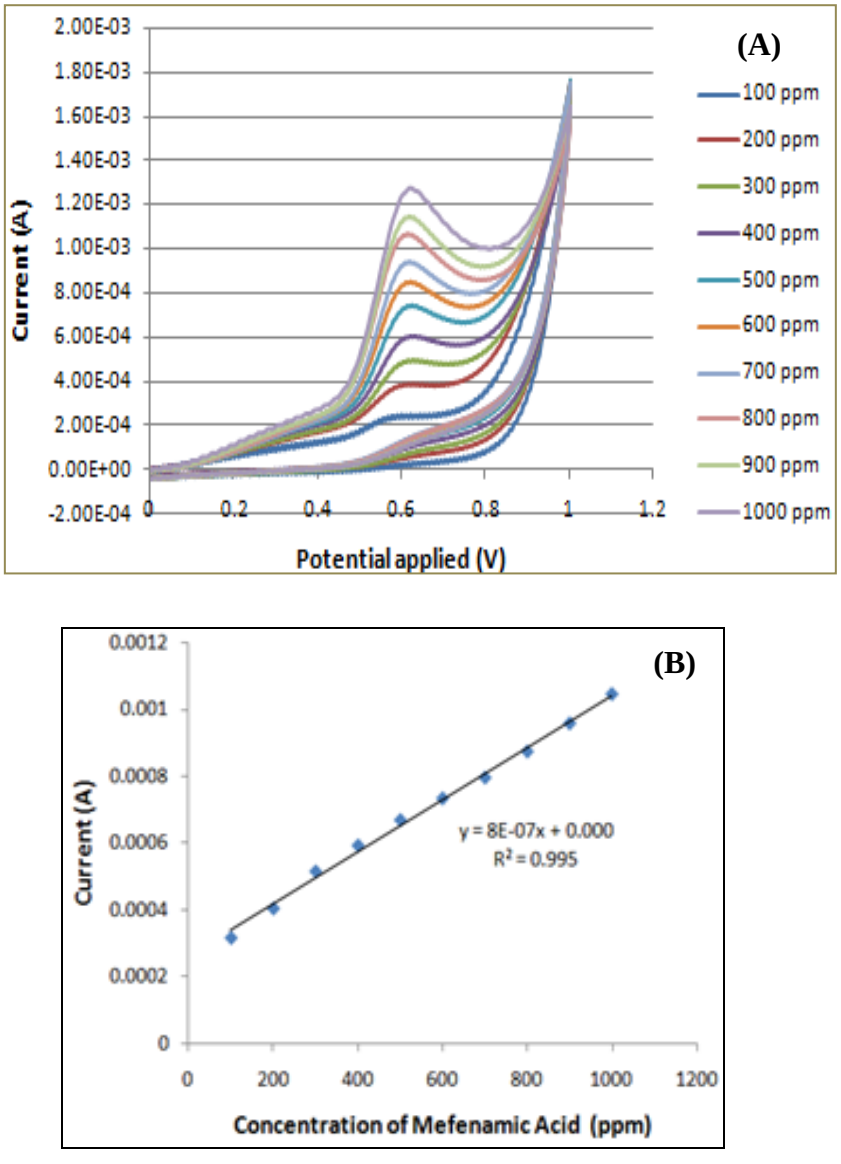

Fig. 8. Cyclic Voltammetry **(A)** and calibration curve **(B)** in buffer phosphate solution with pH 4 in mefenamic acid with variation concentration of 100-1000 ppm, scan rate 100 mV/s

3.6. 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 [12]. 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 1.01%. This method has a good precision as below the limit of 2%.

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 [12]. LOD and LOQ of the electrode towards mefenamic acid were found to be 27.94 mg/L and 93.13 mg/L, respectively.

Recovery experiments should be performed by comparing the analytical results for extracted samples at three concentrations. Recovery of the analyte need 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 mefenamic acid in buffer phosphate solution (pH 4) using PPCM electrode have a good recovery is close to 100%.

Table 1. Analytical application of proposed method

No.	Pharmaceutical sample	Mefenamic acid present (% w/w)	Mefenamic acid measured (% w/w)	Recovery*(%)
1	Sample A	87.23	87.57	100.39
2	Sample B	76.19	76.43	100.31
3	Sample C	70.38	71.74	101.94

*Average of three determinations

4. CONCLUSIONS

Platinum Powder Composite Microelectrode (PPCM) 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 hours. PPCM electrode has porous structure. This electrode is very good for electroanalysis of mefenamic acid buffer phosphate (pH 4) in pharmaceutical product. The correlation of determination using PPCM electrode for electroanalysis of

mefenamic acid was R^2=0.995. Precision, LOQ, LOD and recovery of the PPCM towards mefenamic acid were found to be 1.01%, 27.94 ppm, 93.13 ppm and 100.88%, respectively.

Acknowledgment

This research was supported by DP2M DIKTI (Directorate of Higher Education) Ministry of Education, Indonesia through "Hibah Fundamental" Research Grant 2013 for the financial support.

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