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Electrochemical sensor for the detection of mefenamic acid in pharmaceutical sample and human urine at glassy carbon electrode

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ABSTRACT

The electrochemical oxidation of mefenamic acid has been investigated by cyclic, linear sweep and differential pulse voltammetry in phosphate buffer of different pH range 3-11, at glassy carbon electrode. Cyclic voltammetric studies were performed in a wide range of sweep rates and various concentration of mefenamic acid. The effect of surfactant was studied. The anodic peak was characterized and the process was adsorption controlled. The linear relationship between peak current and the mefenamic acid concentration were studied using differential pulse voltammetric technique, for the quantitative determination of mefenamic acid. The linear response was obtained in the range of 8 x 10^{-5} to 2 x 10^{-3} M with detection limit of MFA 1.49 x 10^{-7} with good selectivity and sensitivity. Furthermore, the proposed method was applied to in-vitro determination of mefenamic acid in pharmaceutical sample, spiked human urine by adopting the differential pulse voltammetric technique.

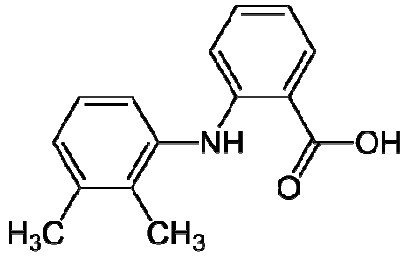
Keywords: Mefenamic acid, Voltammetry, Glassy carbon electrode, Electro-oxidation, Electro-analysis.

INTRODUCTION

Mefenamic acid, (MFA) 2-(2, 3-dimethylphenyl) amino benzoic acid (Scheme1) is a non- steroidal anti-inflammatory drug used to treat pain, including menstrual pain. It is a member of fenamate group of non-steroidal anti-inflammatory drug. The compound is in the form of white or light gray powder odorless and bitter to taste. It is practically insoluble in water and soluble in acetone chloroform and alcohols [1]. Mefenamic acid is used in treatment of menstrual related migraine (MRM) headache which is common in women and associated with substantial disability. Compared to non-menstrual migraine, MRM attacks are more severe, longer in duration, and have a poorer response to analgesics [2]. Mefenamic acid is metabolized by cytochrome P450 enzyme CYP2C9 to 3-hydroxymethyl mefenamic acid (Metabolite I). Further oxidation to a 3-carboxymefenamic acid (Metabolite II) occurs [3]. A peak plasma level approximating 20mcg/ml was observed at 3 hours for the hydroxy metabolite and its glucuronide after a single 1-gram dose. Similarly, a peak plasma level of 8mcg/ml was observed at 6-8 hours for the carboxy metabolite and its glucuronide. Approximately fifty-two percent of a mefenamic acid dose is excreted into urine primarily as glucuronide of mefenamic acid (6%), 3-hydroxymefenamic acid (25%) and 3-carboxymefenamic acid (21%). The fecal route of elimination, account for up to 20% of the dose, mainly in the form of unconjugated 3-carboxymefenamic acid. The elimination half-life of mefenamic acid is approximately two hours [4]. Several methods have already been reported for the determination of mefenamic acid in pharmaceutical formulation and

clinical sample including potentiometric [5], Flow injection analysis [6], and spectophotometric [7], [8], [9], [10] HPLC method [11], RP-HPLC [12], Spectofluorimetry method [13].

In earlier reports we have studied the determination of antiviral drug, acyclovir on fullerene modified glassy carbon electrode [14] and trazodone at multi-walled carbon nanotube-modified glassy carbon electrode [15]. No literature was found on the voltammetric method of determination of mefenamic acid at glassy carbon electrode. The aim of this study is to establish the suitable experimental conditions, to investigate the oxidation mechanism of mefenamic acid by cyclic, linear sweep voltammetry and determination of MFA in pharmaceutical dosage forms and urine by differential-pulse voltammetric technique. The most striking feature of the method is that as applied to the urine, no prior extraction step is needed.



Scheme 1: Chemical structure of MFA

MATERIALS AND METHODS

Reagents and chemicals

The MFA was gifted and was used without further purification. A 1 mM stock solution was made in ethanol and stored in dark at low temperature. The phosphate buffer between pH 3.0-11.0 was used as the supporting electrolytes (I= 0.2M). All the chemicals and reagents were of analytical grade and used without further purification and double distilled water was used throughout the experiment.

Apparatus

Electrochemical experiments were performed with CHI Company, USA (Model D630) electrochemical analyzer. The voltammetric measurement were carried out in a 10 ml single compartment three-electrode glass cell with a glassy carbon electrode as working electrode , an Ag/AgCl (3M KCl) as a reference electrode and a Platinum wire as a counter electrode. The pH of the buffer solution was measured using Elico pH meter (Elico Ltd., India). All experiment were carried out at an ambient temperature of 25 ± 0.1 ^{0}C. The area of the electrode was obtained by cyclic voltammetric method using 1.0 mM $K_3Fe(CN)_6$ as a probe at different scan rate. For a reversible process at T = 298 K, the Randles – Sevcik formula has been used:

$$I_{pa} = 0.4463 (F^3/RT)^{1/2} n^{3/2} A_0 D_0^{1/2} \upsilon^{1/2} C_0 \qquad (1)$$

In equation (1), the value of 0.4463 $(F^3/RT)^{1/2}$ is equal to 2.687 X 10^5 at T = 298 K, Hence equation (1) is written as,

$$I_{pa} = (2.687 \times 10^5) n^{3/2} A_0 D_0^{1/2} \upsilon^{1/2} C_0$$

Where I_{pa} refers to the anodic peak current, n is the number of electron transferred. A_0 is the surface area of the electrode, D_0 is the diffusion coefficient, υ is the scan rate and C_0 is the concentration of $K_3Fe(CN)_6$. For 1.0 x 10^{-3} M $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, n =1, D_0 = 7.6 x 10^{-6} cm^2 s^{-1} . Then from the slope of the plot of I_{pa} vs. $\upsilon^{1/2}$, the area of the electrode surface can be calculated. In our experiment area of the electrode was found to be 0.0423 cm^2.

Analytical procedure

The polishing was done on micro cloths glued to flat mirror. The particle size of alumina used was 3.0 µm. Before transferring the electrode to the solution, it was washed with high purity water.

The experimental conditions for differential pulse voltammetry were initial potential: 0.2 V, final potential: 0.8 V, sensitivity: 1.0 X 10^{-4} A/V, pulse width: 0.06 s, pulse increment of 4 mV, pulse period: 0.2 s, pulse amplitude of 50 mV.

Procedures for pharmaceutical preparations

Ten pieces of MFA tablets were powdered in a mortar. A portion equivalent to a stock solution of a concentration of about 1.0 X 10^{-3} M was accurately weighed and transferred in to a 100 ml calibrated flask and completed to the volume with ethanol. The content of the flask were sonicated for 10 minutes to affect complete dissolution. Appropriate solution was prepared by taking suitable aliquots of the clear supernatant liquid and diluting with buffer solution of pH 5. The differential-pulse voltammogram was subsequently recorded following the optimum condition. The content of the drug in the tablet was determined referring to the calibration graph or regression analysis.

To study the accuracy of the proposed method, and check the interference from exepients used in the dosage forms, recovery experiment were carried out by the standard addition method. This study was performed by addition of known amounts of MFA to known concentration of the tablets. The resulting mixture was analyzed as in pure MFA.

Analysis of urine

Human urine was obtained from healthy volunteers of similar sex and age. Aliquots were centrifuged at 7000 rpm for 5 min at room temperature (25 ± 0.1^0C). These urine samples were analyzed immediately or they were stored at low temperature until analysis.

RESULTS AND DISCUSSION

Voltammetric behavior of MFA

In order to understand the electrochemical process occurring at glassy carbon electrode, cyclic and linear sweep voltammetry were carried out. MFA was oxidized on glassy electrode between the pH 3.0-11.0, producing one well defined irreversible oxidation peak. Figure 1 shows cyclic voltammogram of MFA at pH 5 phosphate buffer. The blank solution was shown by curve (a) and anodic peak corresponding to MFA oxidation appeared at 0.670 V as shown in curve (b), which corresponds to the oxidation of MFA.

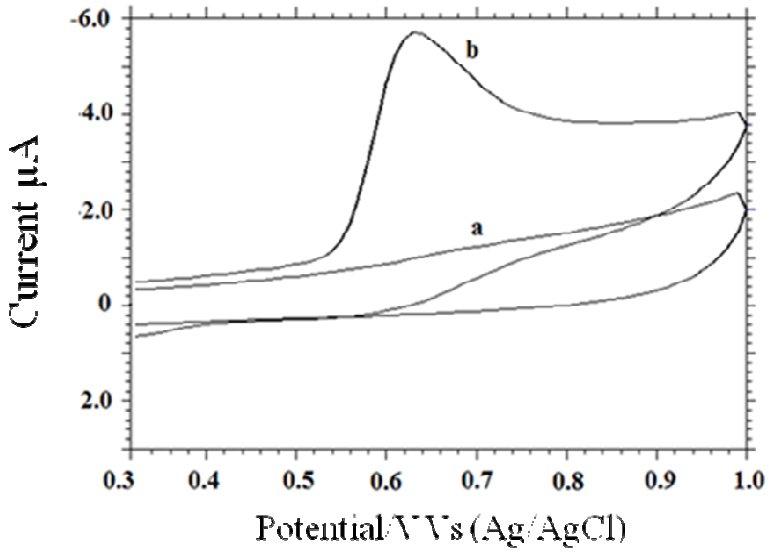


Figure 1: Cyclic voltammogram of 1.0 x 10^{-4} M MFA on glassy carbon electrode in pH 5, phosphate buffer (I = 0.2 M) (a) blank and (b) MFA run at 0.05 Vs^{-1}

Effect of pH

Cyclic voltammograms of MFA recorded from pH 3.0-11.0 at a scan rate of 0.05 Vs^{-1} was presented in Figure 2 with the increase of pH of the solution, the peak potentials shifted to less positive value and the linear relationship between pH and E_p was observed as shown in Figure 2a. The pH of solution influenced peak current considerably and is shown in Figure 2b. Above pH 9 there was no oxidation peak. The result showed that the high peak obtained in buffer solution of pH = 5, hence we selected pH 5 for remaining studies.

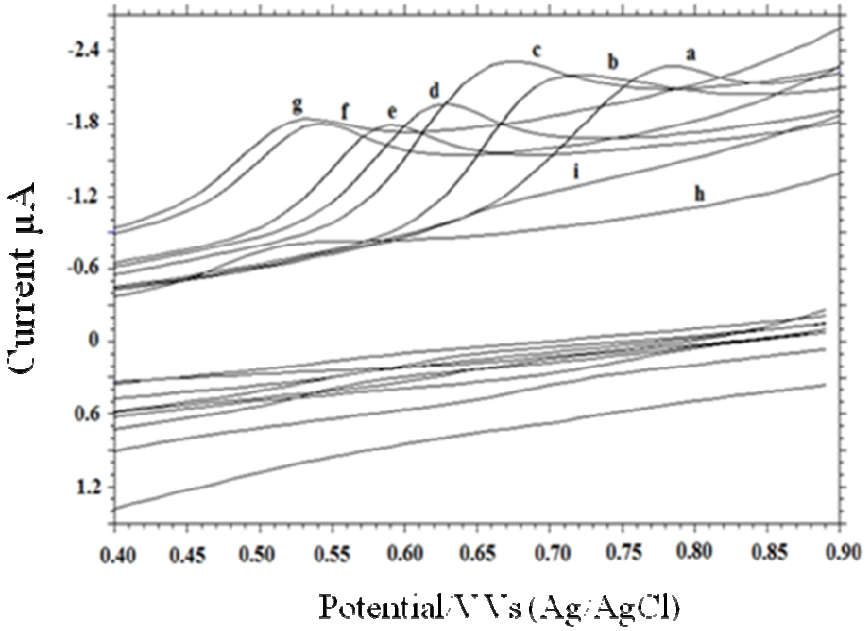


Figure 2: Cyclic voltammogram obtained for 1.0 x 10^{-4} M MFA in buffer solution at (a) pH 3; (b) pH4; (c) pH 5.0; (d) pH 6.0; (e) pH 7.0; (f) pH 8.0; (g) pH 9.0; (h) pH 10.0; (i) pH 11

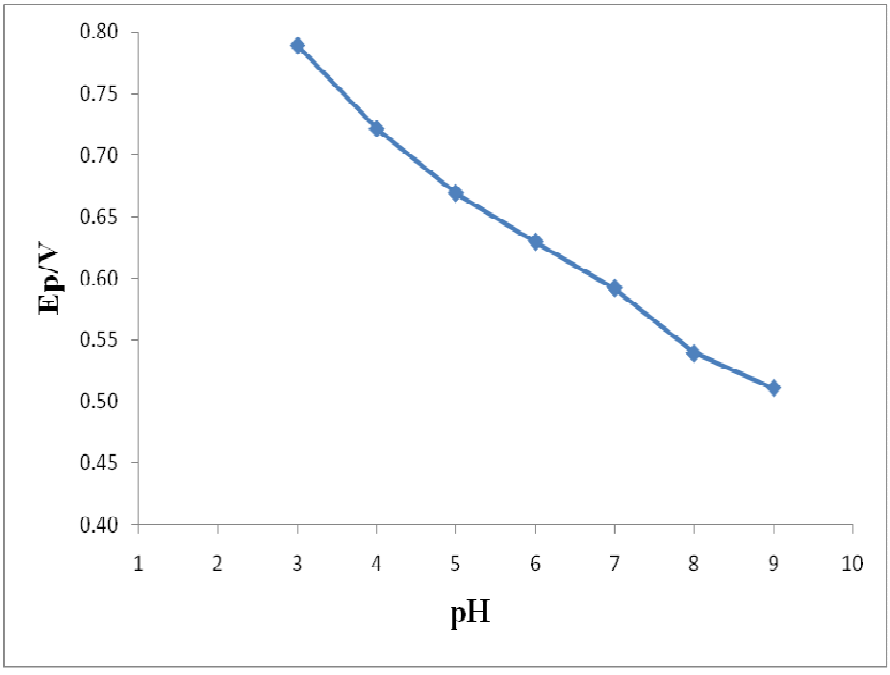


Figure 2a: Influence of pH on the peak potential E_p/V of MFA

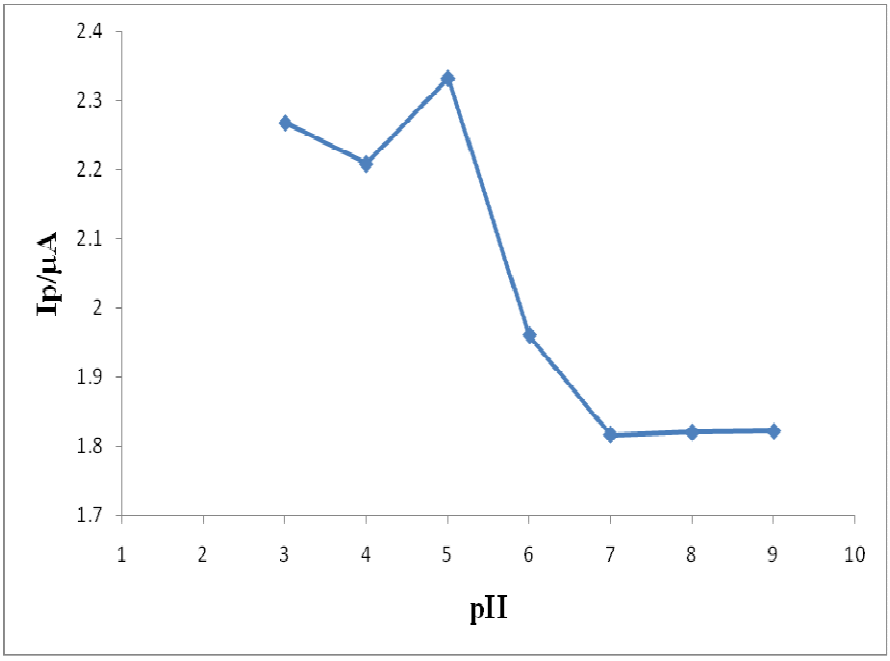


Figure 2b: Variation of peak currents I_p/µA of MFA with pH

Effect of Scan rate

Useful information involving electrochemical mechanism usually can be acquired from relationship between peak current and scan rate. Therefore the electrochemical behavior of MFA at different scan rates from 20 to350 mVs^{-1} was also studied at pH 5 by cyclic voltammetry Figure 3 and linear sweep voltammetry Figure 4.

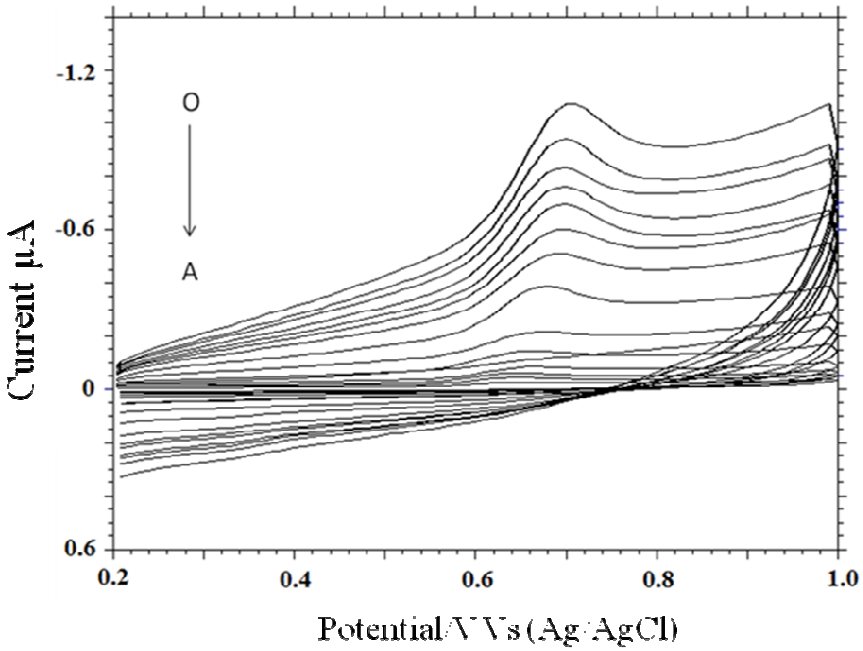


Figure 3: Cyclic voltammogram of 1.0 x 10^{-4} M MFA in buffer solution of pH 5 (I = 0.2 M) at scan rate of : (a) blank; (b) 4; (c) 6; (d) 10 ; (e) 20; (f) 40 ; (g) 80; (h) 100; (i) 120; (j) 150; (k)180; (l) 200; (m) 240 (n) 280 (o) 300 mV s^{-1}

There is a good linear relationship between peak current and scan rate. The equation representing this was I_p = 32.03 υ + 1.061; R^2 = 0.995 as shown in Figure 3a. This shows that electrode process was controlled by adsorption rather than diffusion at pH buffer 5. In addition to this there was a linear relationship between log I_p and log υ, corresponding to the following equation: log I_p = 0.825 log I_p + 1.474; R^2 = 0.995 Figure 3b. The slope of 0.825 was close to theoretical value of 1.0 for adsorption controlled.

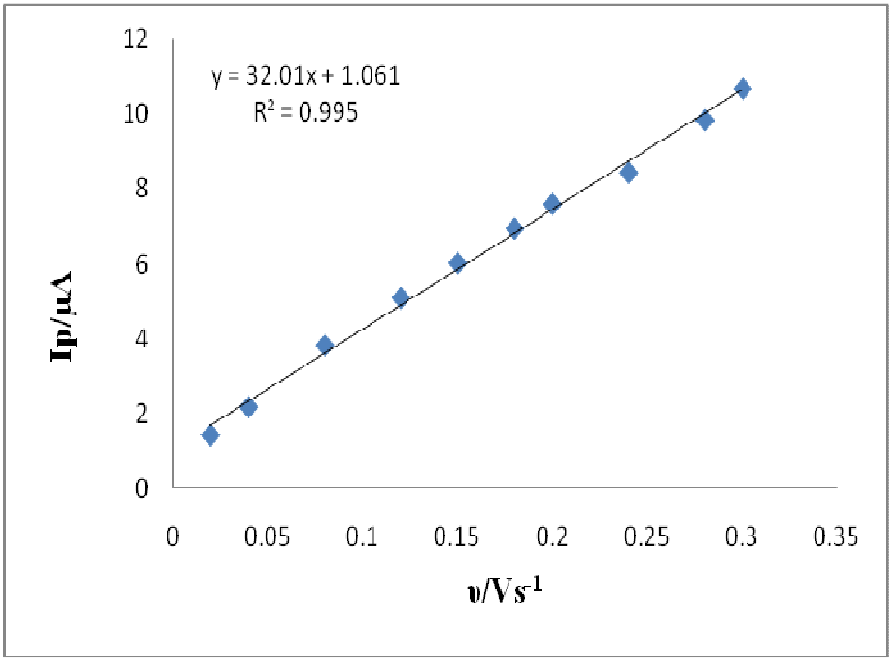


Figure 3a: Dependence of peak current I_p/µA on the scan rate υ/Vs^{-1}

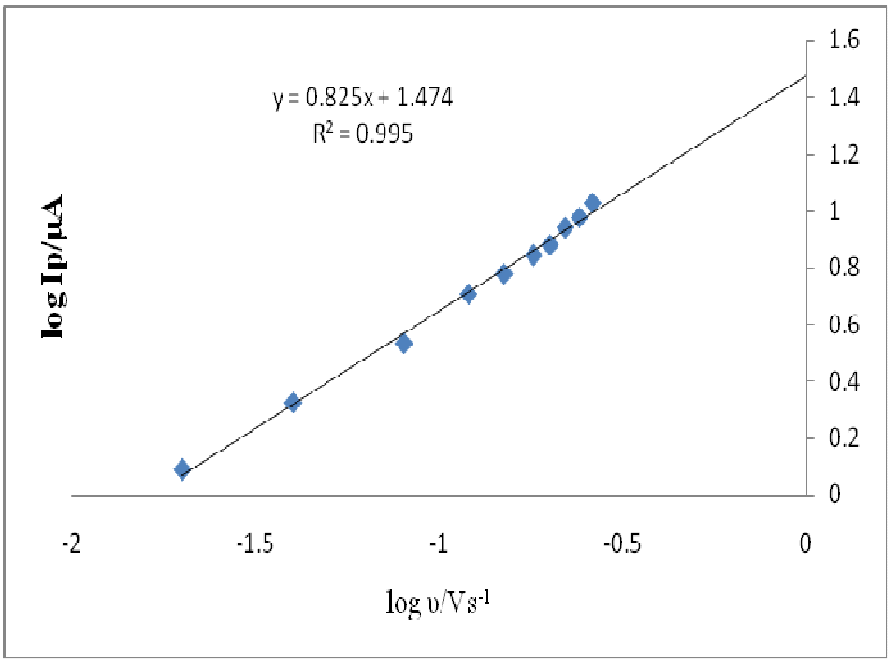


Figure 3b: Plot of logarithm of peak current log I_p/µA versus logarithm of scan rate log υ/Vs^{-1}.

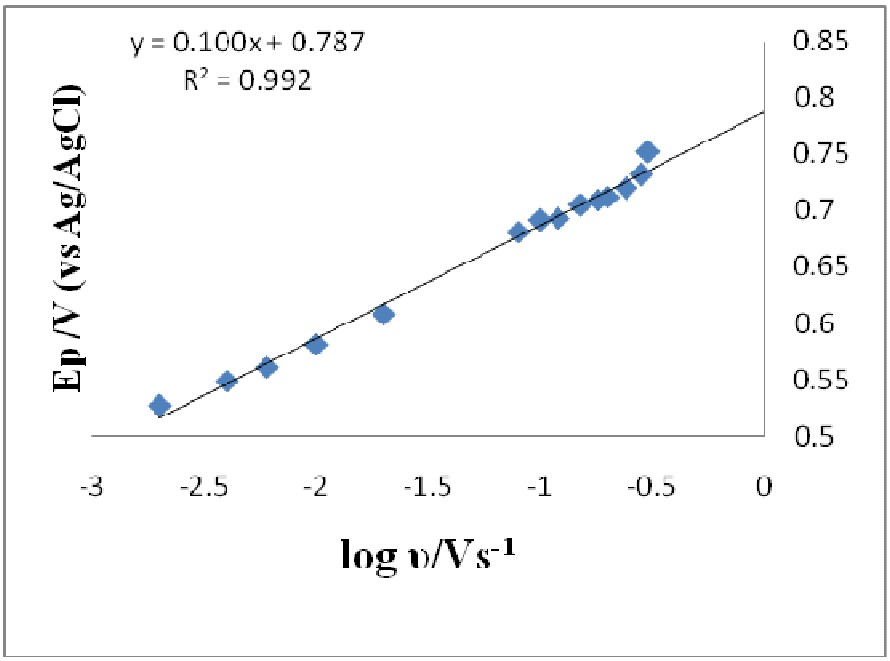


Figure 3c: Plot of variation of peak potential E_p/V with logarithm of scan rate log υ/Vs^{-1}.

The peak potential shifted to more positive value with increasing the scan rates. The linear relationship between peak potential and logarithm of scan rate can be expressed as $E_p = 0.1 \log \upsilon + 0.787$; $R^2 = 0.992$ Figure 3c.

For an adsorption controlled and irreversible electrode process according to Laviron, E_p is defined by following equation

$$E_p = E_0 + \left(\frac{2.303RT}{\alpha nF}\log\right)\left(\frac{RTk_t^0}{\alpha nF}\right)\left(\frac{2.303RT}{\alpha nF}\log\upsilon\right) \quad (2)$$

Where E_p is the transfer coefficient, k^0 the standard heterogeneous rate constant of the reaction, n is the number of electrons transferred, υ the scan rate and E_0 is the formal redox potential. Other symbols have their usual meaning. Thus the value of k^0 can be easily from the slope of E_p vs. log υ. In this system, the slope was 0.1 taking T = 298 K, R = 8.314 J mol^{-1} K^{-1} and F = 96485, αn was calculated to be 0.59 According to Bard and Faulker [16], α can be given as,

$$\alpha = \frac{47.7\text{mV}}{E_p - E_{p/2}} \quad (3)$$

Where $E_{p/2}$ is the potential where the current is half the peak value. Generally, α is assumed to be 0.55 in totally irreversible electrode process [17]. Further the number of electron (n) transferred in the electro oxidation of MFA was calculated to be 1.07 ≈ 1. The value of k^0 can be determined from the intercept of above plot if the value of E_0 is known. The value of E_0 in equation (2) can be obtained from the intercept of E_p versus υ curve by extrapolating to the vertical axis at υ = 0 [18]. The intercept for E_p versus log υ plot was 0.587 and E_0 was obtained to be 0.587 the k^0 was calculated to be 361.8 s^{-1}.

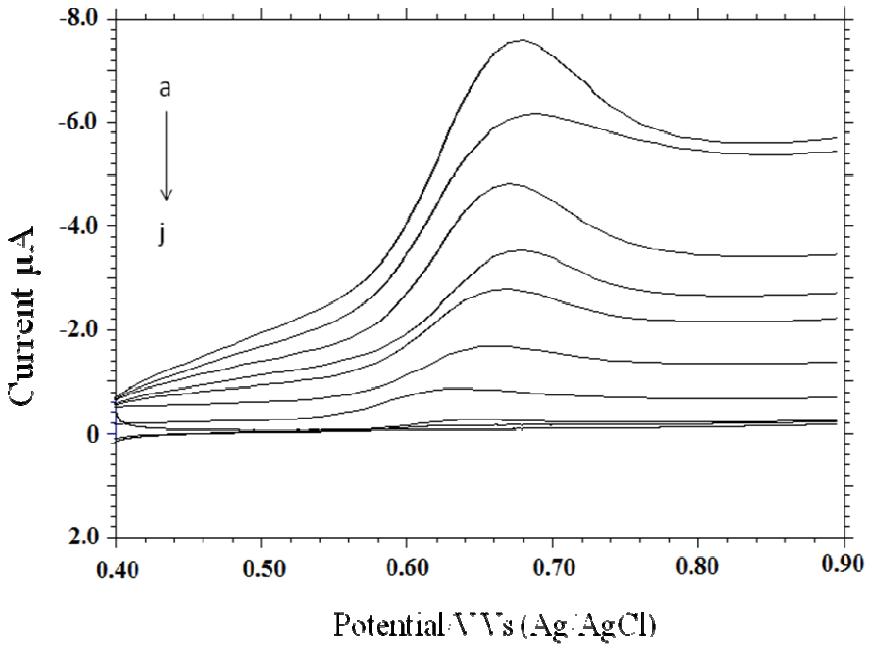


Figure 4: Linear sweep voltammogram of 1.0 x 10^{-4} M MFA in phosphate buffer solution of pH 5 at scan rate of: (a) blank; (b) 3; (c) 4; (e) 8; (f) 20; (g) 40; (h) 60; (i) 80; (j) 120; (k) 150 mVs^{-1}

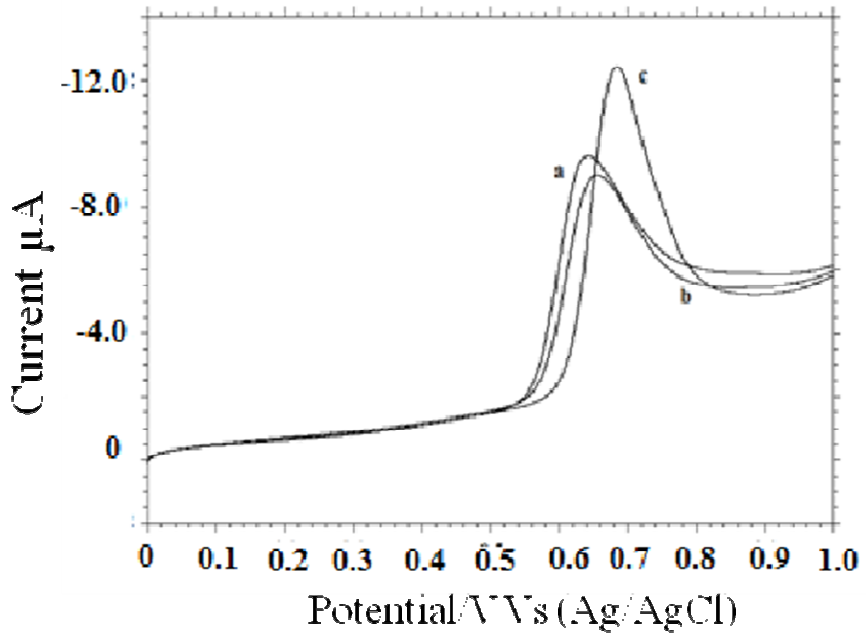


Figure 5: Linear sweep voltammogram of 1.0 x 10^{-4} M MFA solution at pH 5 phosphate buffer and effect of surfactant. (a) without surfactant (b) anionic surfactant (sodium dodecyl sulfate (SDS)) 10 x 10^{-4} M; (c) cationic surfactant (cetrimide) 10 x 10^{-4} M

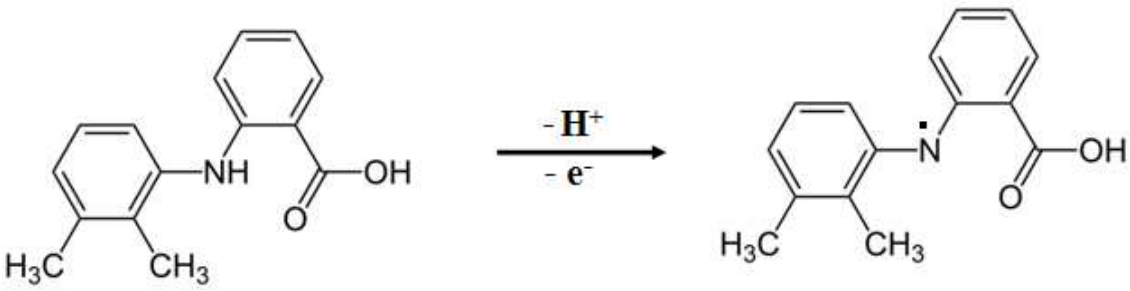
Effect of surfactant

Surfactants even in trace quantities can exert a strong effect on the electrode process. Adsorption of such substance at the electrode may inhibit the electrolytic process; bring about the irregularity in the voltammograms, and causes shift in the wave to more negative potentials [19]. Surface active substances have the common tendency of accumulation at interfaces. The lack of affinity between hydrophobic portions of the surfactant and water leads to a

repulsion of these substances from the water phase as a consequence of the oxidation of the microscopic MFA-water interface. It was found that addition of the cationic surfactant cetrimide shifted peak potential of MFA to more positive value and also with increase in current. Whereas the anionic surfactant sodium dodecycle sulphate shifted peak potential of MFA to positive value with decrease in current. The non ionic surfactant triton X-100 had no effect on voltamogramme as shown in Figure 5.

Oxidation mechanism

The anodic peak present on reverse scan, corresponding to MFA oxidation. In this irreversible system, the results suggests a one electron transfer process in the rate determining step in the mechanism of oxidation of MFA. From the results mentioned above, the following mechanism can be presented for the oxidation of MFA at the surface of electrode. The reaction mechanism is given in Scheme 2.



Scheme 2: Reaction mechanism for oxidation of MFA

Calibration Curve

In order to develop a voltammetric method for determining the MFA, the differential-pulse voltammetric method was adopted, since the peaks are sharper and better defined at lower concentration of MFA than those obtained by cyclic voltammetry, with a lower background current, resulting in improved resolution. According to the obtained results, it was possible to apply this technique to the quantitative analysis of MFA. The phosphate buffer solution of pH 5 was selected as the supporting electrolyte, for the quantification as MFA which gave maximum peak current at pH 5. Differential pulse voltammogram obtained with increasing amounts of MFA showed that peak current increased linearly with increasing concentrations, as shown in Figure 6.

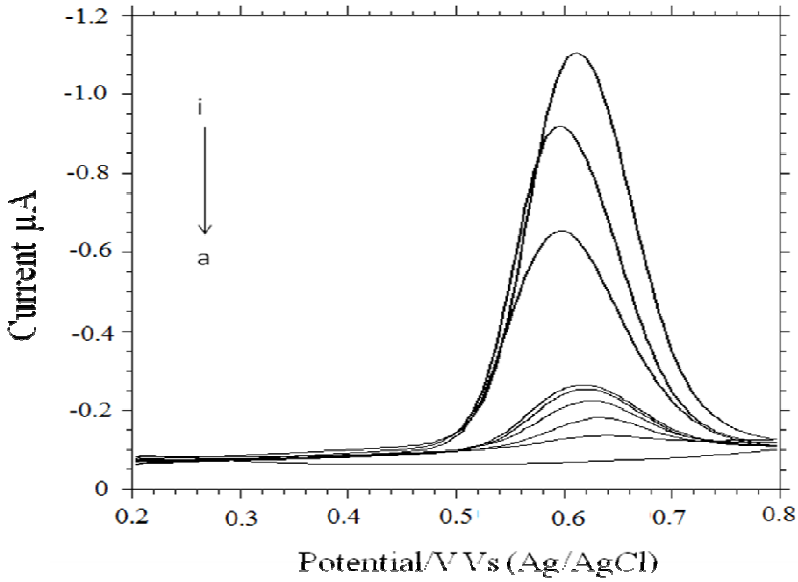


Figure 6: Differential-pulse voltammogram with increasing concentrations of MFA in pH 5, phosphate buffer solution on glassy carbon electrode (a) blank; (b) 1 x 10^{-6}; (c) 3 x 10^{-6}; (d) 5 x 10^{-6} (e) 7 x 10^{-6}; (f) 9 x 10^{-6}; (g) 1 x 10^{-4} ; (h) 2 x 10^{-4} ; (i) 3 x 10^{-4} M

Using the optimum condition described above, linear calibration curves were obtained for MFA in the range of 8 X 10^{-5} M to 2 X 10^{-3} M.

The linear equation was I_p (µ A) = 1.849 C + 0.121; R^2 = 0.996. Deviation from linearity was observed for more concentrated solutions, due to the adsorption of MFA its oxidation product on the electrode surface. Related statistical data of the calibration curve were obtained from five different calibration curves. The sensitivity of the analytical method is determined by LOD and LOQ values. LOD is defined as the lowest concentration of the analyte that can be detected but cannot be accurately quantified. LOQ is the lowest concentration that can be precisely and accurately quantified by the proposed analytical method. The LOD and LOQ of MFA were 1.49 X 10^{-7} and 4.8 X 10^{-7} respectively (Table 1). The LOD and LOQ of MFA were calculated using the following equations:

LOD = 3s/m ; LOQ =10s/m (5)

Where s is the standard deviation of the peak current of the blank (five runs), and m is the slope of the calibration curve. This method was better as compared with other reported electrochemical sensors in Table 2.

Table 1: Characteristics of MFA calibration plot using differential pulse voltammetry at glassy carbon electrode

Linearity range (M)	1.0 X 10^{-6} to 1.0 X 10^{-3}
Slope of the calibration plot	1.849
Intercept (µA)	0.121
Number of data points	05
LOD (M)	1.49 X 10^{-7}
LOQ (M)	4.8 X 10^{-7}

Table 2: Comparison of analytical characteristics for determination of MFA at several reported electrochemical methods

Method	Linear range	LOD	Reference
Potentiometric	9 x 10^{-5} - 1 X10^{-2}	4.5 X 10^{-5} mol/l	[5]
Flow injection analysis	0.05-6.0 µg/ml	2.1 X 10^{-7} M	[6]
Spectrophotometric	10-60 µg/ml	2.16 µg/ml	[7]
Spectrofluorimetric	0.05-5.0 mg/l	0.006 mg/l	[8]
Chemiluminescence	0.05-6.0 mg/l	0.051 mg/l	[11]
HPLC (with UV detection)	0.025-40 mg/l	-	[20]
Differential pulse voltammetry	1.49 x 10^{-7}	4.8 x 10^{-7} (LOQ)	This work

Tablet Analysis

In order to evaluate the applicability of the proposed method in the pharmaceutical sample analysis, two commercial medicinal samples containing MFA were studied. The results are in good agreement with the content marked in the label in Table 3.

Table 3: Determination of MFA in tablets

Sample	Declared (mol/L)	Detected by this method (mol/L)	Recovery (%) Meftal – p Sample 1
	0.03 x 10^{-3}	0.0287 x 10^{-3}	95.6
Meftal – p Sample 2	0.1 x 10^{-3}	0.0985 x 10^{-3}	98.5
Meftal – p Sample 3	0.3 x 10^{-3}	0.299 x 10^{-3}	99.3
Meftal – p Sample 4	1.0 x 10^{-3}	0.997 x 10^{-3}	99.7

The recovery test of MFA ranging from 1.0 x 10^{-4} to 3.0 x 10^{-5} was performed using differential-pulse voltammetry. Recovery studies were carried out after the addition of known amount of drug to the various pre-analyzed formulations of MFA. The recoveries in different samples were found to lie in the range from 95.6 to 99.8.

Detection of MFA in Spiked Urine Samples

The developed differential pulse voltammetric method for the MFA determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known amounts of MFA. The urine samples were diluted 100 times with phosphate buffer solution before analysis without further pretreatments. A quantitative analysis can be carried out, by adding standard solution of MFA into the direct system of urine sample. The calibration graph was used for determination of spiked MFA in urine samples. The detection results of three urine samples obtained are listed in Table 4. The analysis was carried out ranging from 1.0 x 10^{-4} to 5.0 x 10^{-5} using differential pulse voltammetry. The recovery determination was in the range from 96.3 to 101.3.

Table 4: Determination of MFA in urine

Sample	Declared (mol/L)	Detected by this method (mol/L)	Recovery (%)
Urine Sample 1	1.0×10^{-4}	0.968×10^{-3}	96.86
Urine Sample 2	3.0×10^{-4}	3.039×10^{-3}	101.3
Urine Sample 3	9×10^{-4}	0.0983×10^{-3}	98.3

CONCLUSION

The oxidation of MFA at glassy carbon electrode surface was investigated by cyclic voltammetry. MFA undergoes one electron and one proton change with adsorption-controlled process. The effects of surfactants were studied. A suitable mechanism was proposed. The proposed differential pulse voltammetric procedure can be used successfully to determine MFA in pharmaceutical samples. It compares reasonably well with the reported methods and can be a good alternative for the analytical determination of MFA. The proposed method offered the advantages of accuracy and time saving as well as simplicity of reagents and apparatus. In addition, the results obtained in the analysis of MFA in spiked urine demonstrated the applicability of the method for real sample analysis.

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