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Electrochemical Oxidation of Loop Diuretic Furosemide at Gold Electrode and its Analytical Applications

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Electrochemical oxidation of loop diuretic furosemide at gold electrode was investigated by cyclic, linear sweep and differential pulse voltammetric (DPV) techniques. The electrochemical studies were carried out in methanol: water (10:90) in 4.8 pH with 0.04 mol/l Britton-Robinson buffer as supporting electrolyte at 25±0.2^0 C. The effects of scan rate, concentration, surfactant and temperature were evaluated. The electrochemical process was observed to be adsorption controlled, irreversible and involving two-electron oxidation. Effects of anodic peak potential (E_{pa}), anodic peak current (i_{pa}) and heterogeneous rate constant (k^0) have been discussed. A DPV method with good precision and accuracy was developed for the determination of furosemide in pharmaceutical formulations. The linear response was obtained in the range of 6.0 x 10^{-6} to 8.0 x 10^{-4} M with detection limit of 4.12 x 10^{-8} M. The proposed method was successfully applied to the individual tablet dosage form.

Keywords: Furosemide; Electrochemical studies; Gold electrode; Pharmaceutical formulation; Surfactant

1. INTRODUCTION

Drugs that facilitate diuresis are widely used for the treatment of edematous conditions and in the management of hypertension and other conditions for which the increase in urinary flow can relieve symptoms [1]. 5-Aminosulphonyl-4-chloro-2-furanylmethyl acid (furosemide)is a potent and widely used diuretic in the treatment of edematous states associated with cardiac, chronic renal failure [2,3], hypertension, congestive heart failure [4,5] and cirrhosis of the liver [6]. Their principal site of action is the thick ascending limp of the loop of Henle. Furosemide with a prompt action is fairly rapidly absorbed after oral administration and shows a strong diuretic effect of short duration [7]. Its

bioavailability ranges from 60 to 70% and its plasma half-life is about 1-2 h. [8], with structural formula shown in Scheme 1.

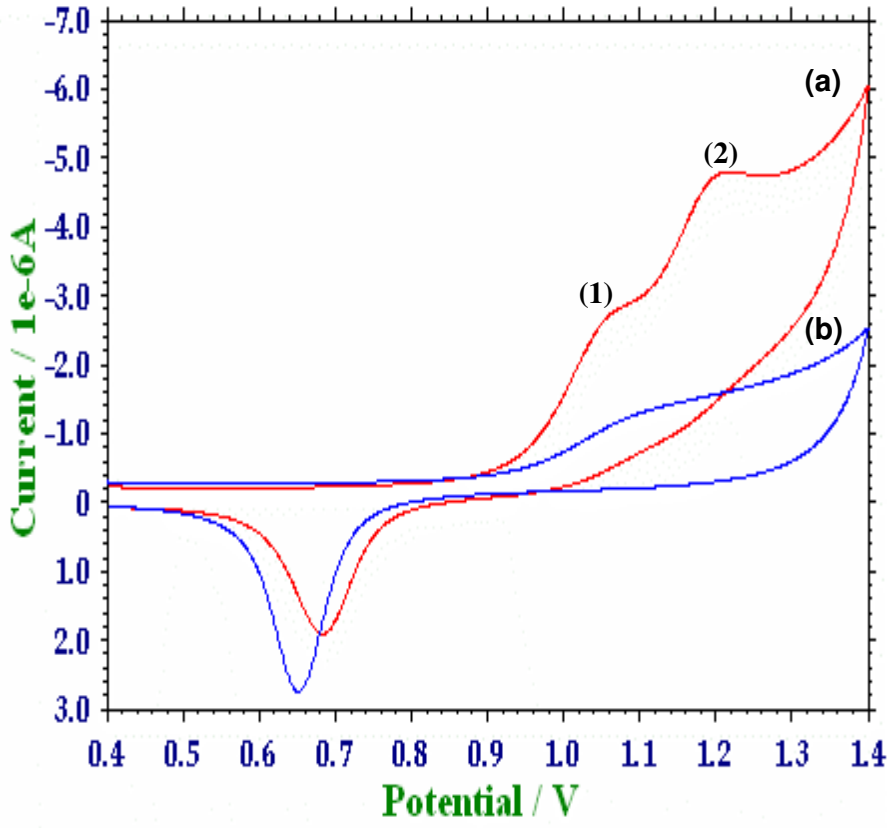


Figure 1. (a) Cyclic voltammograms of 0.4 mM FUR solutions in methanol-water (10:90) at pH 4.8 and a scan rate 6mMs^{-1} at gold electrode; (1) first oxidation peak; (2) second oxidation peak; (b) blank.

A number of methods for the individual determination of these diuretics in both pharmaceutical preparations and biological fluids have been reported. Thus, furosemide (FUR) is normally determined by liquid chromatography with spectrophotometric [9-11], spectrofluorimetric detection [12-14], chemiluminescent [15] and micellar electrokinetic chromatographic methods [16]. Most often, the procedures involve some extraction and are thus time consuming. This drug was also be quantified with electrochemical methods using glassy carbon electrode [17], hanging mercury drop electrode and graphite electrode [18].

In recent years, the electrochemical techniques have led to the advancement in the field of analysis because of their sensitivity, low cost and relatively short analysis time, as compared with other techniques. Electrochemical have proven to be useful for development of very sensitive and selective methods for the determination of organic molecules including drugs. In addition application of electro analytical techniques includes the determination of electrode mechanisms. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmaceutical activity [19-21].

Surfactants even in trace quantities can exert a strong effect on the electrode process. Adsorption of such substances at the electrode may inhibit the electrolytic process, bring about the irregularity in the voltammograms and cause shift in the wave to more positive or negative potential [22]. The applications of surfactants in electrochemistry and electro analytical chemistry have been widely reported [23]. Many of the studies of modified electrodes were undertaken simply because electrochemists were curious about new species attached to electrode surface behavior compared to these species in solution [24].

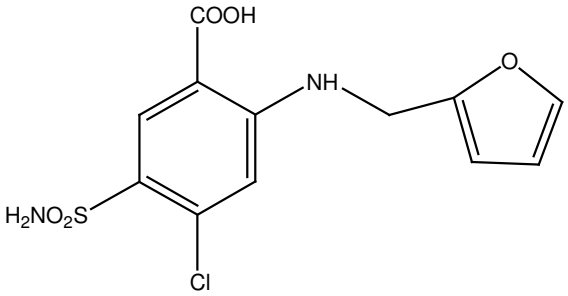
The gold electrode has been widely used in electrochemical studies and electro analysis for various substrates for a long time because of its stability, wide potential window and fast electron transfer rate. [25,26].

Since, there is no report on the electrochemical oxidation process of furosemide on gold electrode, here we have investigated an electrochemical oxidation process of furosemide on gold electrode. Further, differential pulse voltammetric (DPV) method with good precision and accuracy was developed for the determination of furosemide in pharmaceutical formulations.

2. EXPERIMENTAL PART

2.1. Reagents and Chemicals

A stock standard solution of furosemide (Sigma Aldrich), 5mM was prepared in HPLC grade methanol (S.D. Fine) and stored in the dark under refrigeration (4^0C) to avoid possible decomposition. Britton-Robinson (BR) buffer (0.04 mol/l) was prepared and used as supporting electrolytes. Buffer solutions were adjusted by adding the necessary amounts of KOH or HCl in order to obtain the appropriate pH value. In this study, Sodium lauryl sulphate (S.D. Fine), Hexadecyl trimethyl ammonium bromide (Hi-media) and Triton X-100 were used as anionic, cathodic and nonionic surfactants respectively. Rest of the reagents were of analytical-reagent grade, and millipore water was used through out the experiment.



Scheme 1. Structure of furosemide

Furosemide, Scheme 1 (Indian Pharmacopoeia), furosemide tablets (40 mg furosemide per tablet) were purchased from Aventis Pharma Limited in India.

A standard stock solution (5mM) of furosemide was prepared by dissolving an accurate mass of FUR in an appropriate volume of water, and then stored in refrigerator in dark place. More dilute solutions (10^{-6} to 10^{-4}M) were prepared by diluting the stock solution.

2.2. Apparatus

Electrochemical experiments were performed with CHI-1110a electrochemical Analyzer (CH Instruments Ltd. Co., USA, version 4.01). A three-electrode system consisting of a gold electrode (2mm diameter) as the working electrode, an Ag/AgCl (3 M KCl) as reference electrode and a platinum wire as the auxiliary electrode was used. The pH measurements were made by Elico pH meter model LI120 and Nicolet Impact -410 FTIR, Varian CARY 50 Bio UV-visible Spectrophotometer and Helvet Packard 1100 reverse phase high performance liquid chromatography (HPLC) system with a phenomenes C18 column, hp 1100 series diode array UV/Visible detector and hp 1100 MSD series mass analyzer was used in the experiment to identify the product.

The experimental conditions for differential pulse voltammetry (DPV) were: Initial E: 0.7 V, Final E: 1.4 V, sensitivity: 1.0 x 10^{-5}µA/V, pulse amplitude: 50mV, sample width: 20 ms, pulse width: 50 ms, pulse period: 200ms.

2.3. Pretreatment of working electrode

To provide a reproducible active surface and to improve the sensitivity and resolution of the voltammetric peaks, the gold electrode was polished to a mirror finish with 0.3 micron alumina on a smooth polishing cloth and then rinsed with methanol and millipore water prior to each electrochemical measurements. All the measurements were carried out at room temperature (25 ± 0.2^0 C).

2.4. Procedures for pharmaceutical preparations

Ten tablets were weighed and the average mass per tablet was calculated. A total of 400mg of furosemide was accurately weighed and transferred to a 100ml beaker containing 10 ml of methanol and 50 ml of water. The sonication process performed for 5min to dissolve the FUR and then the solution was next filtered with filter paper. The desired concentrations of the drug were obtained by accurate dilutions with highly purified water. The prepared sample solution was directly measured according to the proposed procedure without any pretreatment or extraction steps.

2.5. Area of the electrode

The area of the electrode was calibrated using 1mM $K_4Fe(CN)_6$ in 0.1 M KNO_3 by recording the current voltage curve. From the cyclic voltammetric peak current (7.2µA) and the diffusion coefficient of $Fe(CN)_6^{4-}$, the area of the electrode was calculated [27,28] by using the equation (1)

$$i_{pa} = (2.69 \times 10^5)\, n^{3/2}\, A\, Do^{1/2}\, \nu^{1/2}\, C_o^* \quad (1)$$

where n = number of electrons transferred i.e. 1, A = surface area of the electrode, D = diffusion coefficient (9.382×10^{-6} cm^2 s^{-1}), ν = sweep rate ($0.1 Vs^{-1}$) and C_o^* = concentration of electro active species (1 mM). The surface area of the electrode was found to be 0.026 cm^2.

3. RESULTS AND DISCUSSION

3.1. Electro-oxidation of furosemide (FUR)

Scheme 1, shows the molecular structure of FUR. Fig. 1, represents a cyclic voltammogram of 4.0×10^{-4} M FUR in BR buffer of pH 4.8 using gold electrode in the potential range of +0.4 to 1.4 V, with a sweep rate of 6 mV s^{-1}. In cyclic voltammograms, FUR exhibited two anodic peaks, one at 1.08mV with anodic current of 2.82μA and another at 1.18mV with anodic current of 4.85 μA. The fact that no peak was observed in the reverse scan suggests that the oxidation process is an irreversible one. In potential range of 0.6 to 0.8 V, gold itself undergoes reduction (Fig.1) [25]. The multisweep cyclic voltammograms of FUR (0.4mM) in RB buffer of 4.8 pH at scan rate 6mV/s are shown in Fig.2. The decrease in peak current with succeeding potential scans suggested the formation of adsorbed species on the electrode surface [29].

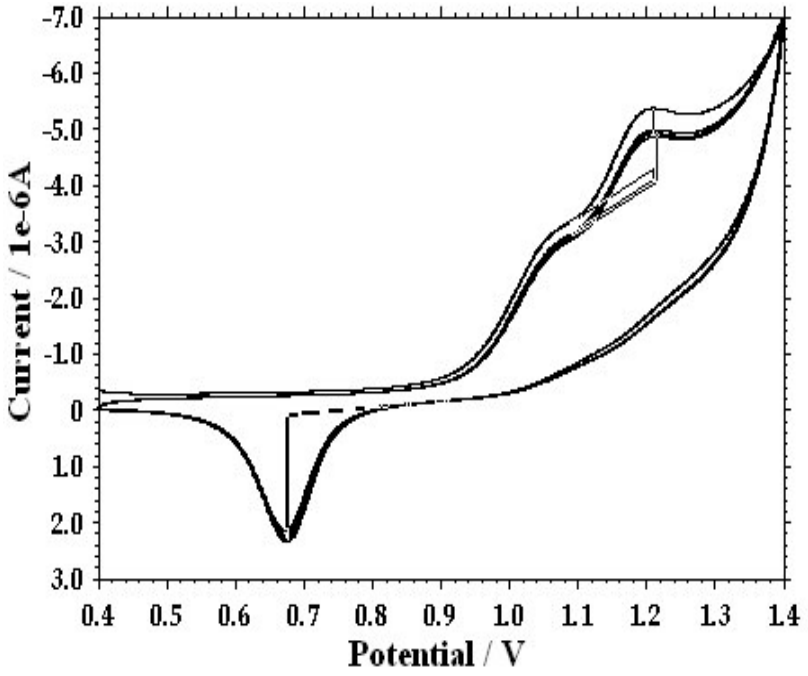


Figure 2. The multisweep cyclic voltammograms of FUR (0.4mM) in RB buffer of 4.8 pH at scan rate 6mV/s

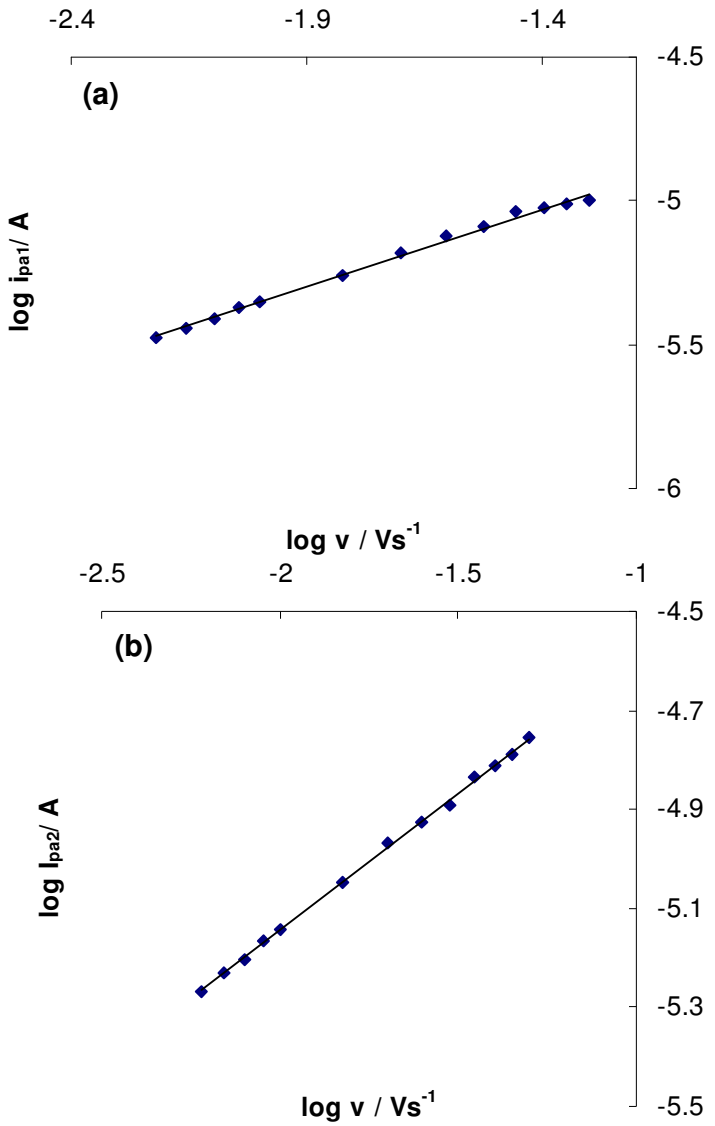


Figure 3. (a) Variation of the logarithm of peak current (i_{pa1}) with the logarithm of the sweep rate for 0.4mM FUR. (b) Variation of the logarithm of peak current (i_{pa2}) with the logarithm of the sweep rate for 0.4mM FUR.

3.2. Effect of scan rate

The effect of scan rate (υ) on the peak currents (i_{pa1} and i_{pa2}) and the peak potential (E_{pa1} and E_{pa2}) of FUR was studied. A linear relationship was observed between the oxidation peak current (i_{pa1}) and the square root of the scan rate with a significant correlation coefficient of 0.9895 for first peak. Further, it was noticed that the first oxidation peak became broader and almost disappeared at higher scan rates. The plot of log of peak current (log i_{pa1}) versus log of scan rate (log υ) gives a slope value of 0.7389 in the scan rate range of 1 to 100 mVs^{-1} (Fig. 3a). This was close to the theoretical value of

1.0 reported for an ideal reaction for the adsorption-controlled electrode process [30]. The corresponding equation is,

$$i_{pa1}\ (\mu\ A) = 0.7389\ \upsilon^{1/2}\ (mVs^{-1})^{1/2} - 4.28; \qquad R^2 = 0.996$$

A linear relationship was observed between the oxidation peak current (log i_{pa2}) and the square root of the scan rate (log υ) with a significant correlation coefficient of 0.9973 for second peak. The plot of log of peak current versus log of scan rate gives a slope value of 0.7821 in the scan rate range of 1 to 100 mVs^{-1} (Fig. 3b). This was close to the theoretical value of 1.0 reported for an ideal reaction for the adsorption-controlled electrode process [30]. The corresponding equation is,

$$i_{pa2}\ (\mu\ A) = 0.7821\ \upsilon^{1/2}\ (mVs^{-1})^{1/2} - 4.08\ ; \qquad R^2 = 0.9973$$

The E_{pa1} and E_{pa2} of the oxidation peak were also dependent on the scan rate. The plot of E_{pa} versus log υ was linear having a correlation coefficient of 0.9977 for first peak and 0.9973 for second peak (Fig. 4a and 4b) and this behavior was consistent with the electrochemical nature of the reaction in which the electrode reaction is coupled with an irreversible follow-up chemical step [31]. The relation between E_{pa} and υ can be expressed by the equation,

$$E_{pa1}\ (V) = 0.0582 \log \upsilon + 1.1818$$
$$E_{pa2}\ (V) = 0.0759 \log \upsilon + 1.3707$$

As for an irreversible electrode process, according to Laviron [32], E_p is defined by the following equation

$$E_p = E_0 + \left(\frac{2.303RT}{\alpha nF}\right) \log\left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) \log \nu \qquad (2)$$

where α is the transfer coefficient, k^o is the standard heterogeneous rate constant of the reaction, n is the number of electron transferred, υ is the scan rate and E_o is the formal redox potential. Other symbols have their usual meanings. Thus the values of αn can be easily calculated from the slope of E_{p1} versus log υ and E_{p2} versus log υ. In this system, for first plot slope is 0.0582, therefore, the αn calculated to be 0.7725 and for the second plot slope is 0.0759, therefore αn calculated to be 0.91, taking T = 298, R = 8.314 and F = 96480. According to Bard and Faulkner [27], α can be given as

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

where $E_{p/2}$ is the potential where the current is at half the peak value. So, from this we got the value of α to be 0.75. Further, the number of electron (n) transferred in the electro oxidation of FUR, was calculated to be 1.04 ~ 1 for first peak and 1.21 ~ 1 for second peak. Totally the number of electron (n) transferred in the electro oxidation of FUR was found to be two.

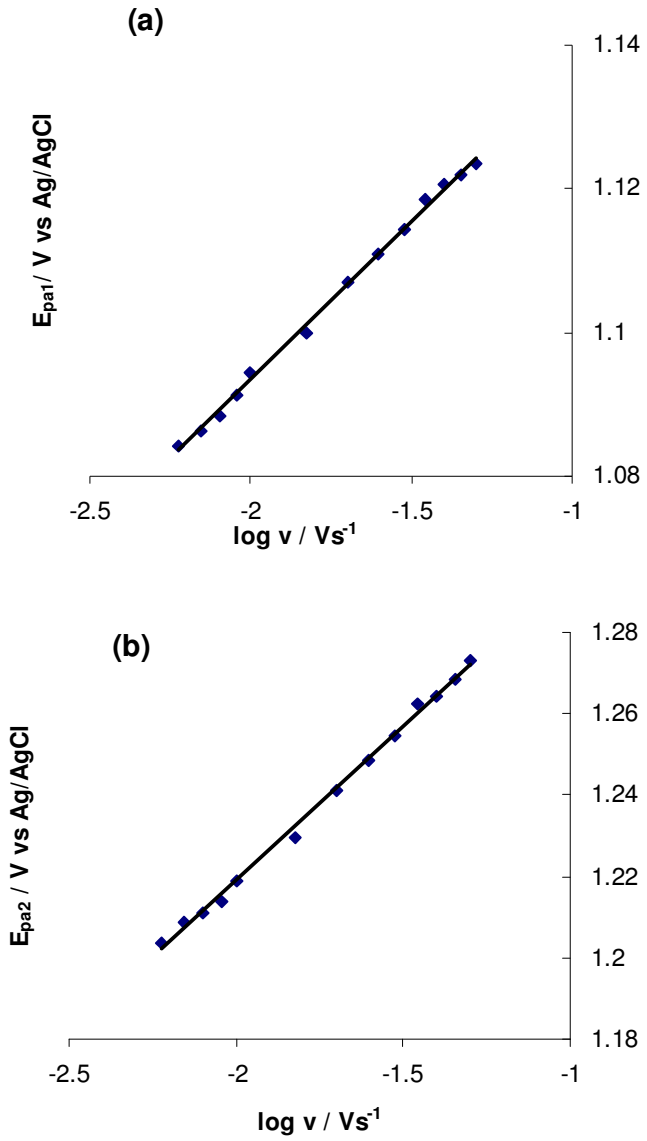


Figure 4. (a) Dependence of Ep_1 on log ν for 0.4mM FUR; (b) Dependence of Ep_2 on log ν for 0.4mM FUR.

3.3. Effect of pH

The FUR oxidized in an aqueous alcoholic medium (methanol : water, 10:90) in the pH range 2.0 -11.0 at gold electrode in 0.04 ml/l BR buffer as supporting electrolyte gives rise to voltammetric peaks whose potentials and currents are pH dependent. The peak at less positive

potentials occurs in the whole pH range studied, but the second voltammetric peak exits in a limited range (pH 3.0- 7.5). The pH study is almost same as in earlier literature [17]. In acidic pH, i.e. in 4.8 pH the maximum peak current value was obtained.

3.4. Effect of surfactant

Electrochemical oxidation of FUR was also studied over anionic, cationic and neutral surfactants in the range 2.5 x 10^{-6} to 1.0 x 10^{-5} with 4.0 x 10^{-4} FUR and BR buffer of pH 4.8. Except sodium lauryl sulphate (anionic surfactant), cationic and neutral surfactants did not affect the oxidation peak, which remain same. Anionic surfactant particles accumulate at the surface of the electrode and thus increase the current (Fig. 5a and Fig. 5b). Addition of anodic surfactant results in the shift of E_{pa} towards more positive potential. This may be attributed to the direct adsorption of the surfactants at the gold electrode surface, which was replaced by the surfactant molecules. Surface-active substances have the common tendency of accumulation at interfaces. The lack of affinity between the hydrophobic portion of the surfactant and water leads to a repulsion of these substances from the water phase as a consequence of oxidation of the microscopic FUR water interface [33,34].

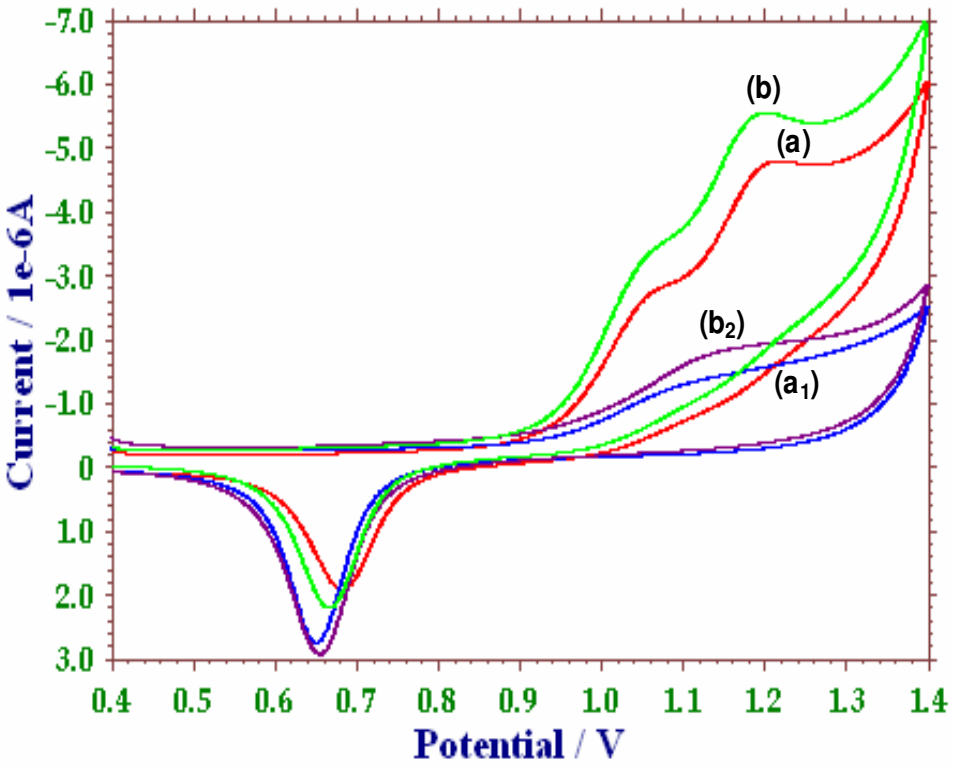


Figure 5a. (a) Cyclic voltammograms of 0.4mM FUR solutions in methanol-water (10:90) at pH 4.8; (a_1) Respective blank; (b) Cyclic voltammograms of 0.4mM FUR solutions in methanol-water (10:90) at pH 4.8 with 0.01mM anodic surfactant (sodium laurly sulphate); (b_1) Respective blank.

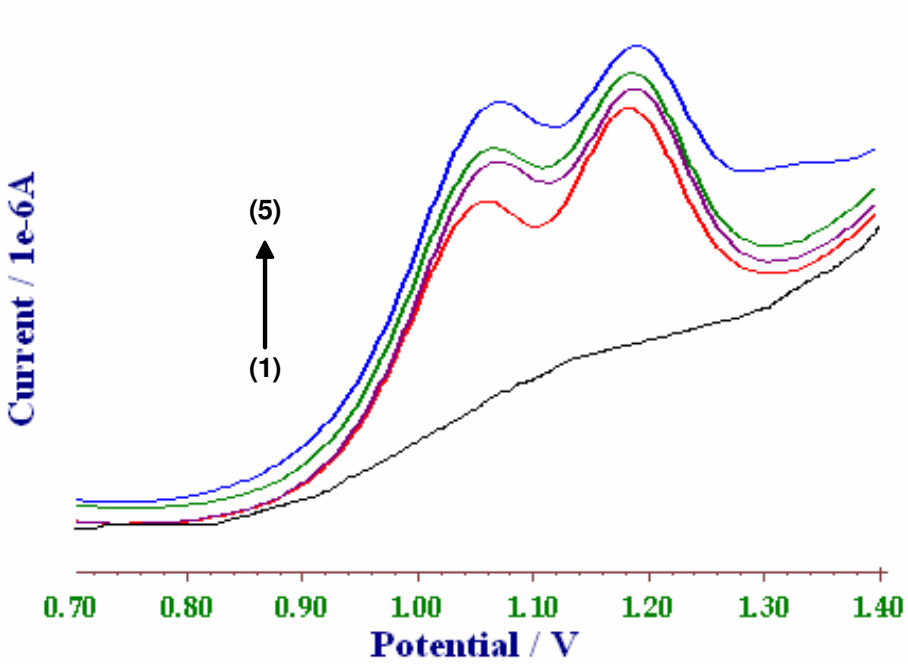


Figure 5b. Differential pulse voltammograms of 0.4mM FUR solutions at pH 4.8 in methanol-water (10:90) and effect of different concentrations (mol/l) of anodic surfactant (sodium laurly sulphate) at gold electrode; (1) blank; (2) without anodic surfactant; (3) 2.5 x 10^{-6}; (4) 5x10^{-6}; (5) 1 x 10^{-5}.

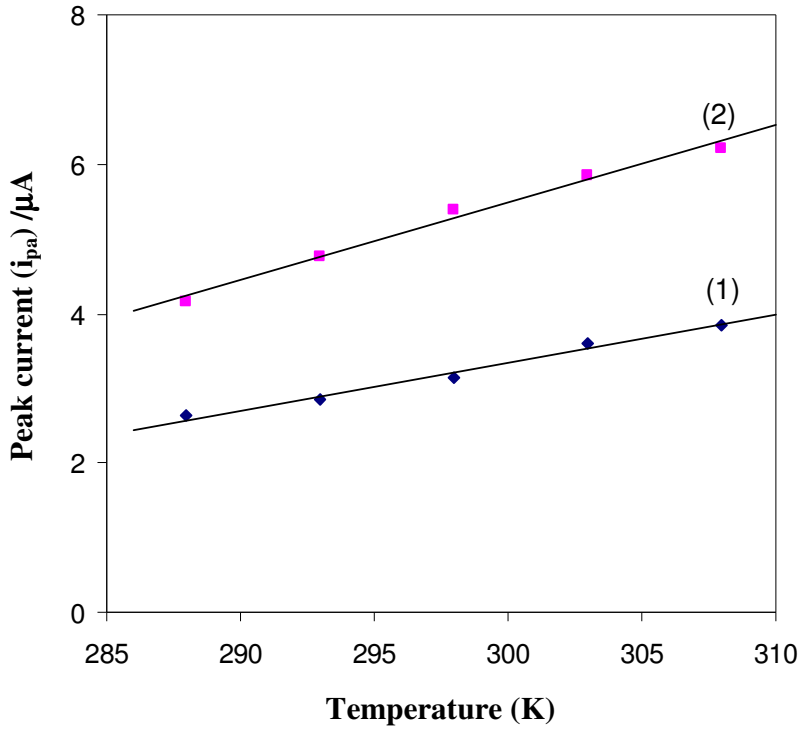


Figure 6. Observed dependence of peak current (i_{pa}) on different temperature for 0.4mM FUR at gold electrode; (1) i_{pa1} versus Temperature (K); (2) i_{pa2} versus Temperature (K).

3.5. Effect of temperature

Linear sweep voltammograms of furosemide (4.0×10^{-4} M) and 0.04 mol/l of pH 4.8 BR buffer were recorded at five different temperatures (288, 293, 298, 303 and 308 K). The both anodic peak current (i_{pa1} and i_{pa2}) increased linearly with correlation coefficient 0.9997 and 0.9968 (Fig.6). The standard heterogeneous rate constants (k^0) were calculated at different temperatures by using the equation (2).

Table 1. Standard heterogeneous rate constants at different temperatures for 0.4mM furosemide with scan rate $6mVs^{-1}$ at gold electrode

Temperature (*K*)	i_{pa1} /μA	i_{pa2} /μA	$k^0_1 \times 10^{-2}$	$k^0_2 \times 10^{-2}$
288	2.45	4.26	1.95	0.88
293	2.86	4.76	2.67	1.36
298	3.14	5.39	3.85	1.73
303	3.61	5.89	5.06	2.65
308	3.89	6.22	6.25	3.34

*i_{pa1}- peak current for the first oxidation peak
*i_{pa2}- peak current for the second oxidation peak
*k^0_1- heterogeneous rate constant for the first oxidation peak
*k^0_2- heterogeneous rate constant for the second oxidation peak

Table 2. Thermodynamic activation parameters for the electro oxidation of 0.4mM furosemide at gold electrode in 298K

Thermodynamic activation parameters	Values for 1^{st} peak	Values for 2^{nd} peak
Ea ($kJmol^{-1}$)	43.78	47.28
$\Delta H^{\#}$ ($kJmol^{-1}$)	41.30	44.80
$\Delta S^{\#}$ ($JK^{-1}mol^{-1}$)	-56.63	-52.10
$\Delta G^{\#}$ ($kJmol^{-1}$)	58.18	60.16
logA	10.26	10.52

The rate constants (k^0) for first and second peak were tabulated in Table 1. The energy of activation (Ea) for both peaks were evaluated from the Arrhenius plots for log k^0_1 versus 1/T and log k^0_2 versus 1/T, which were linear with the slope -2288.8 and -2471.9 respectively (Fig. 7). The other activation parameters were obtained from Ea values and were tabulated in Table 2. The less value of $\Delta H^{\#}$ indicates the electro oxidation of furosemide might be taking place through physical adsorption. The more negative value of $\Delta S^{\#}$ indicates the electro oxidation of furosemide might be taking place via the formation of an activated adsorbed complex [35,36] before the products were formed. Such adsorbed intermediate complex is more ordered than the reactant molecules themselves.

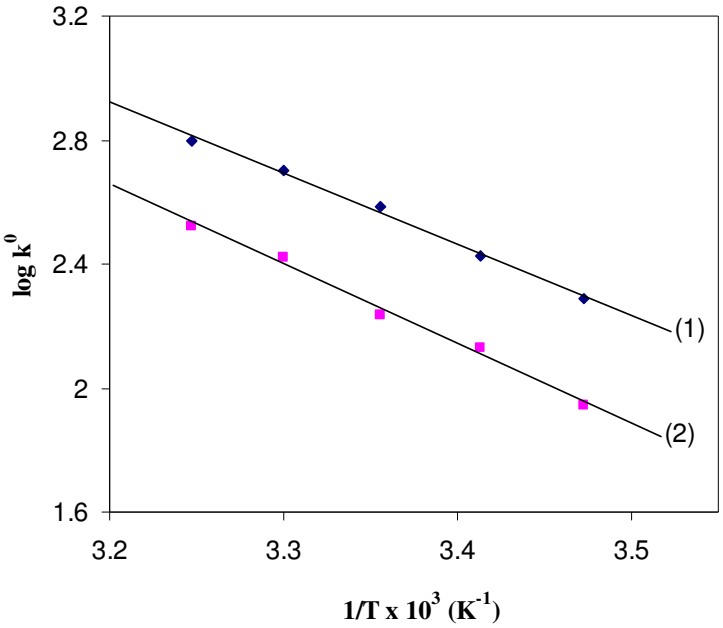
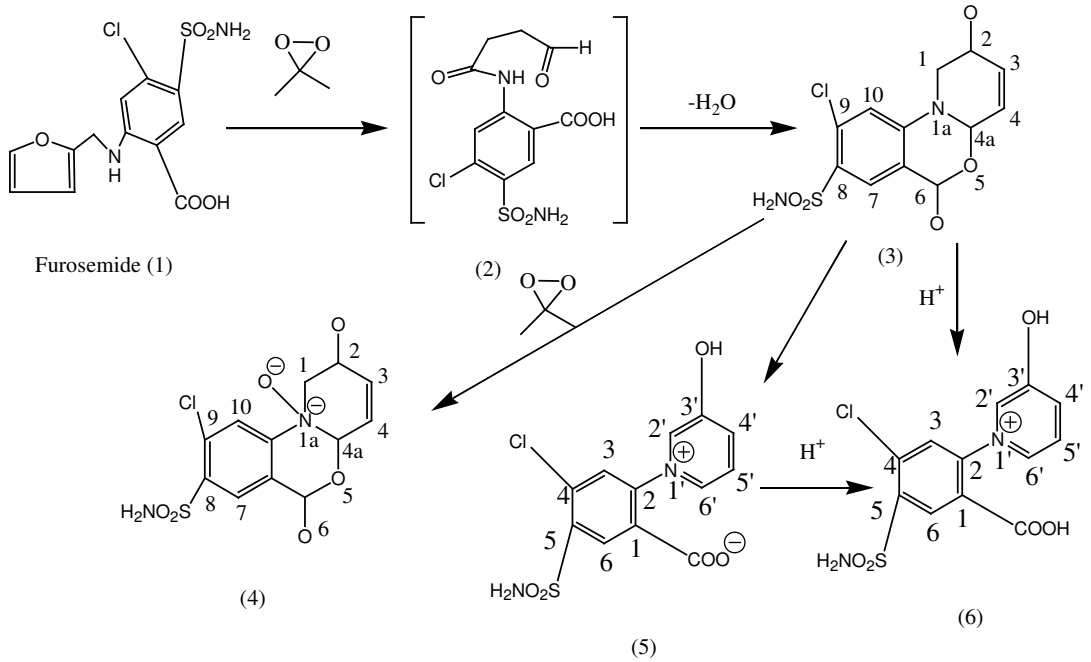


Figure 7. Effect of temperature on the electro-oxidation of 0.4mM FUR with scan rate 6mVs^{-1} on gold electrode (1) Plot of log k^0_1 versus 1/T; (2) Plot of log k^0_2 versus 1/T.



Scheme 2. Chemical oxidation of furosemide (1) by dimethyldioxirane in acetone, which involves a Mannich-like reaction for the formation different products. (3) 9-Chloro-2,6-dioxo-8-sulfamoyl-1-2,4a,6tetrahydro-1H-benzo[d]pyrido[2,1-b][1,3]oxazine; (4) 9-Chloro -2,6-dioxo-8-sulfamoyl-1-2,4a,6tetrahydro-1H-benzo[d]pyrido[2,1-b][1,3]oxazine 1a-oxide; (5) 4-Chloro-2-(3'-hydroxypyridinium-1'-yl)-5-sulfamolybenzoate; (6) 4-Chloro-2-(3'-hydroxypyridinium-1'-yl)-5-sulfamoylbenzoic acid.

3.6. Mechanism and identification of product of electrolysis

As given in literature [37], the chemical oxidation of FUR by dimethyldioxirane in acetone, which involves a Mannich-like reaction for the formation of different products, was shown in Scheme 2.

But based on the voltammetric experiment, the number of electrons transferred (n) was calculated and found to be two. The IR spectrum of the product shows an sharp intense band at 1705 cm^{-1} due to C=O stretching frequency of carboxylic group, a broad band at 3401 cm^{-1} due to acidic OH; two sharp band at 3055 and 3021 cm^{-1} due to NH_2 stretching, a band at 1625 cm^{-1} due the presence of C=N which was absent in the IR spectrum of furosemide.

The UV spectra of 5mM FUR in 0.04 mol/l BR buffer solution at pH 4.8, before and after electrolysis are shown in Fig. 8. Three absorption peaks are found at 226,271 and 316 nm (curve a), but after depleting electrolysis the relative absorption peak, a slight blue shift to 229, 277 and 329 nm occurs (curve b). The electro oxidation might have led to excitation of π- π^* transitions due to the formation of C=N bond. Further, LC-ESI-MS analysis of product showed a molecular ion peak m/z at 329 (Fig. 9).

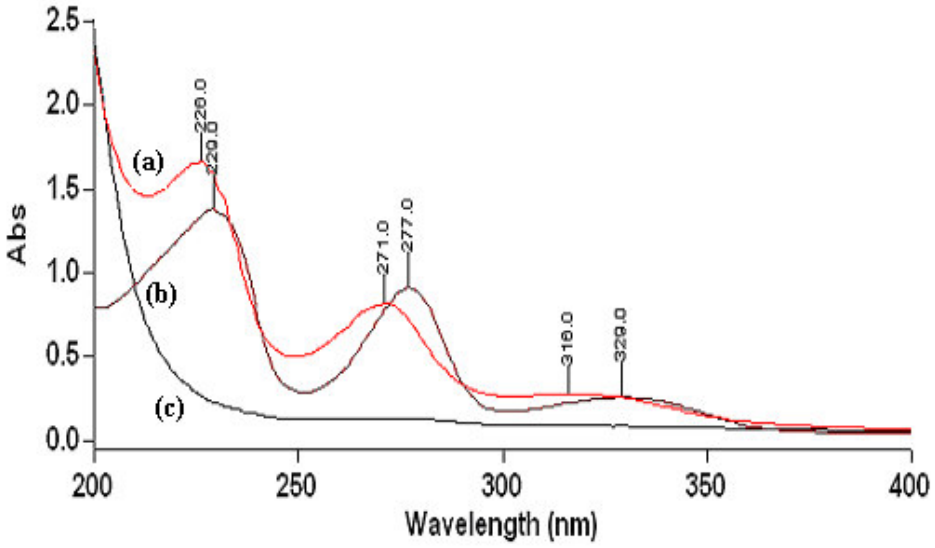


Figure 8. UV spectra of 5mM FUR in 0.04ml/l BR buffer solution at pH 4.8. (a) before electrolysis; (b) after electrolysis; (c) buffer.

Based on the spectral characterization of electro-chemical oxidation product of FUR in BR buffer was identified as 2 chloro-4-[furan-2lymethylene)-amino]-benzenesulfonamide. Hence the proposed mechanism is shown in Scheme 3. These voltammetric studies show that oxidative pathways of electrochemical and chemical process are different.

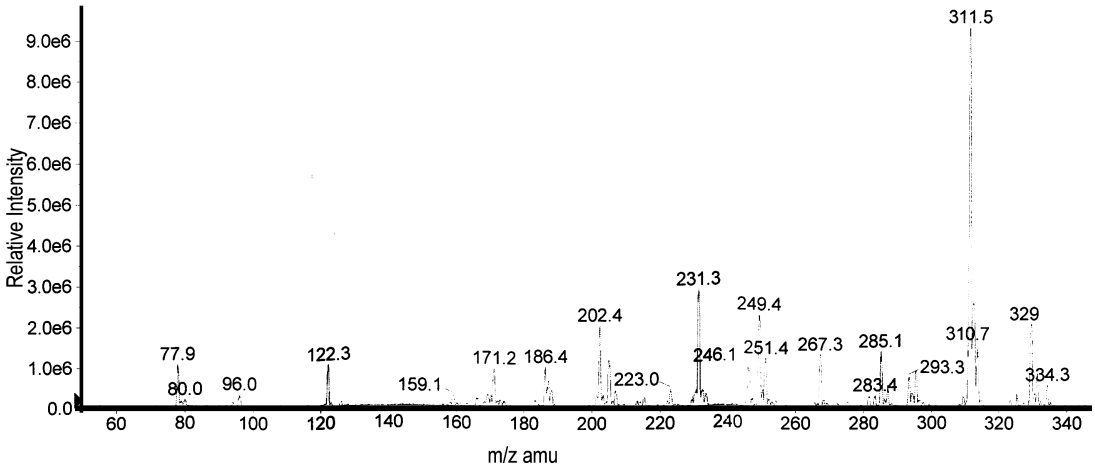
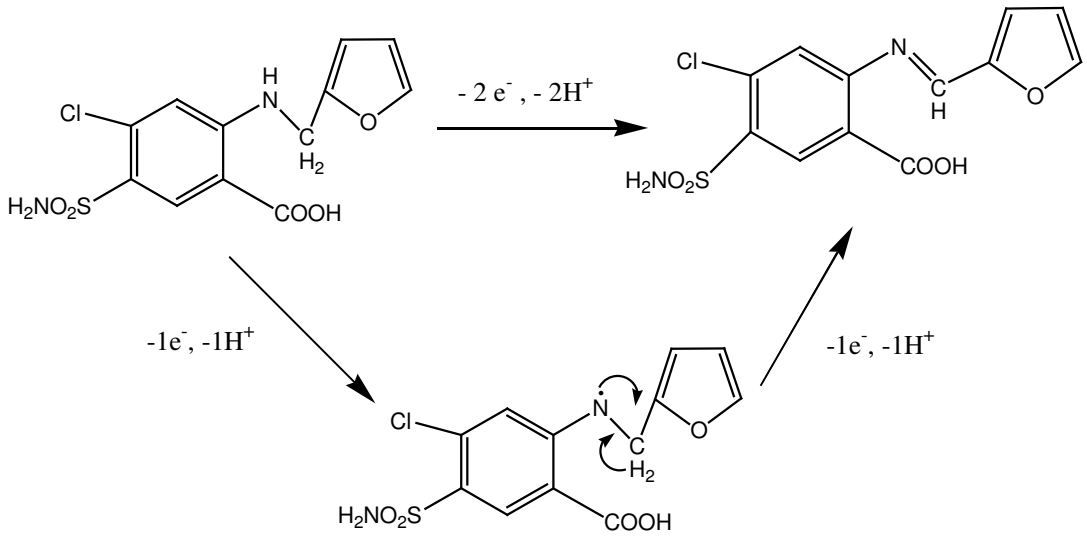


Figure 9. LC-ESI-MS analysis of product shows a molecular ion peak m/z at 329.



Scheme 3. Electro-oxidation of 0.4mM furosemide solutions at pH 4.8 in methanol-water (10:90) with scan rate 6mVs^{-1} at gold electrode.

Controlled potential electrolysis was carried out using H type cell separating the anode and cathodic compartments by a fine glass sinter. The rate of electrolysis was enhanced by using gold electrode with a larger surface area, as the working electrode in the cathodic compartment and applying a potential 1.3V using Ag/AgCl electrode as reference electrode. The potential is usually fixed slightly higher than that obtained in CV experiments. Platinum gauze acted as anode in the other compartment. Ag/AgCl electrode used as a reference electrode was placed in the same compartment along with gold electrode. The electrolysis was carried out for 10 hrs. for complete oxidation using

5mM furosemide and 4.8 pH BR buffer as supporting electrolyte under hydrodynamic conditions in order to speed up the mass transport. All measurements were carried out at laboratory ambient temperature controlled at 25 ± 0.2^0 C.

3.7. Effect of concentration

The influence of concentration of furosemide on peak currents at gold electrode was also studied with 0.04 mol/l of pH 4.8 BR buffer as supporting electrolyte [Fig. 10]. It was found that the plot of i_{pa1} and i_{pa2} versus concentrations showed linearity over the drug concentration range of 5.98 x 10^{-6} to 8.01 x 10^{-4} M (from DPV) suggesting further that the electron de process was adsorption controlled [36,38]. Above this concentration, loss of linearity was noticed and this was due to adsorption of FUR on the electrode surface [39].

i_{pa1} (μ A) = 9.96 x C*, where C* is in mM/l.
i_{pa2} (μ A) = 15.29 x C*, where C* is in mM/l.

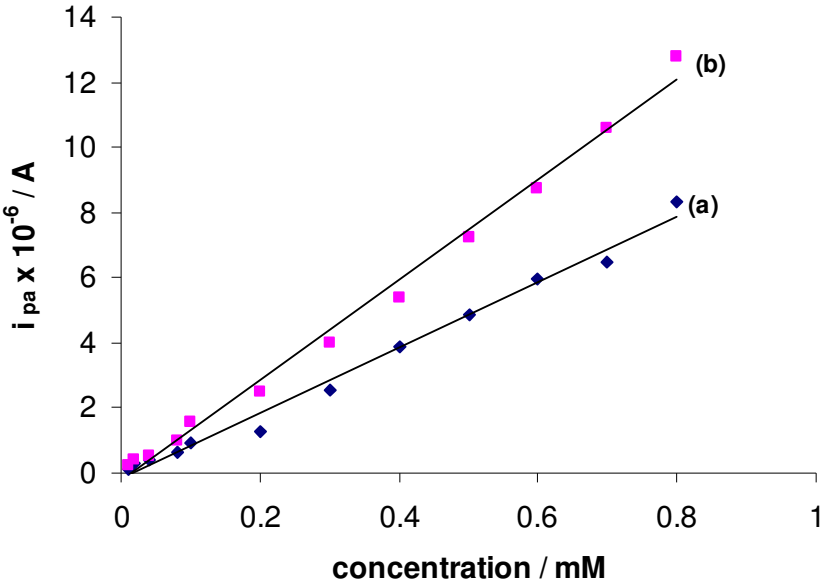


Figure 10. The linear relationship between the peak current (i_{pa}) and concentration of FUR in the range 1.0 x 10^{-5} to 8.0 x 10^{-4}; (a) and (b) corresponds to i_{pa1} and i_{pa2} respectively.

4. ANALYTICAL APPLICATIONS

4.1. Validation of the analytical procedure

Based on the electrochemical oxidation of furosemide at gold electrode, an analytical method, differential pulse voltammetry (DPV) was developed for the determination of this drug. The analytical characteristics of the calibration plot are summarized in Table 3. Validation of the optimized procedure

for the quantitative assay of furosemide was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and recovery. LOD and LOQ were calculated based on the peak current (i_{pa2}) using the following equations shown below [40,41].

$$LOD = 3\ S/m;\ LOQ = 10\ S/m$$

Where *S* is the standard deviation of the peak current (i_{pa2}) (five replicates) and *m* is the slope of the calibration curve. The LOD and LOQ values were calculated to be 4.12×10^{-8} M and 1.37×10^{-7} respectively. Low values of the both LOD and LOQ values confirmed the sensitivity of the proposed method. Analyzing five replicates, for the process of the validation within–day variations and for intra-day assay were studied. The corresponding percentage of RSD values are tabulated in Table 3. The percentage of RSD values indicating good repeatability and reproducibility as compared to earlier method [17].

Table 3. Characteristics of furosemide calibration plot using differential pulse voltammetry at gold electrode.

Linearity range (M)	5.98×10^{-6} to 8.01×10^{-4}
Slope of the calibration Plot (μ A M-1)	1.561×10^{-5}
Intercept (μ A)	1.033
Correlation coefficient (r)	0.9969
RSD of slope (%)	1.358
RSD of intercept (%)	1.033
Number of data points	6
LOD (M)	4.12×10^{-8}
LOQ (M)	1.37×10^{-7}
Repeatability (RSD %)	1.154
Reproducibility (RSD %)	1.217

4.2. Determination of FUR in pharmaceutical dosages

The developed DPV method was applied successfully for the assay of FUR in tablets. The results of analysis of FUR are recorded in Table 3. In order to validate and to obtained the precision and accuracy of the developed method, recovery studies have been carried out at different concentration levels of the drug. These studies were carried out by standard addition method. For this, known quantities of pure FUR were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The average percent recoveries obtained were found to be quantitative 98.3 % indicating good recovery of the drug.

The effects of excipients *viz*., talc, glucose, starch, lactose, dextrose, gum acacia and magnesium stearate on voltammetric assay of FUR were investigated. It was noticed that none of these interfered in the determination at the levels normally found in dosage forms. The results shown in

Table 4 revealed that the proposed method is selective and accurate and hence, could be adopted for the quality assurance.

Table 4. Analysis of furosemide in tablet by DPV and recovery studies

	Lasix Tablet
Labeled claim (mg)	40.0
Amount found (mg)*	39.4
RSD (%)	1.07
Added (mg)	5.0
Found (mg)*	4.93
Recovered (%)	98.3
RSD (%)	1.17

* Average of 5 determination

5. CONCLUSIONS

An electrochemical study step by step has been presented on the oxidation of FUR, a loop diuretic drug at a gold electrode. Based on the study of the influence of several physico-chemical parameters (potential scan rate, pH, temperature, concentration…) were investigated. A Probable reaction mechanism was proposed. A two electrons, two proton mechanism, irreversible and adsorption controlled were observed for the oxidation reaction of FUR. By selecting the anodic peak (i_{pa2}) of FUR, the DPV procedure was developed for its assay in tablets. The recoveries obtained for pharmaceutical formulations show the applicability of this technique to control analysis of FUR drug. Further this method may be considered as a suitable alternative to the existing chromatographic methods. Finally the methods were not time-consuming and less expensive than the HPLC one.

References

1. G.O. Rankin, D.A. Williams, T.L. Lemke (Eds.) Foye's Principles of Medicinal Chemistry , 5th ed., Lippicott Williams, Wilkins, Baltimore, MD, 2002, p. 518, 529.
2. L.L.B. Laura, D.S. Ronald, *Clin. Pharmacokinet.*, 18 (1990) 81.
3. F. Cantarovich, C. Galli, L. Benedeti, *Br. Med. J.*, 4 (1973) 449.
4. H.S. Frazier, H. Yager, *New Engl. J. Med.*, 288 (1973) 449.
5. K. Dikshit, J.K. Vyden, J.S. Forrester, K. Chatterjee, R. Prakash, H.J.C. Sawn, *New Engl. J. Med.*, 288 (1973) 1087.
6. F. Sadikal, *Br. J. Clin. Pract.*, 27 (1973) 222.
7. B. Beermann, *Clin. Pharmacol. Ther.*, 32 (1982) 584.
8. E.S. Waller, S.W. Hamilton, J. E. Masserella, M.A. Sharanevyeh, R.V. Smith, G.J. Yakatan, and J.T. Doluisio, *J. Pharm. Sci.*, 71 (1982) 1105.
9. A.A. Nava-Ocampo, E.Y. Velazquez-Armenta, H. Reys-Perez. E. Ramirez-Lopez, H. ponce-Monter, *J. Chromatogr., B*, 730 (1999) 49.
10. S. Carda-Brosch, J. Esteve-Romero, M.C. Garcia-Alvarez-Coque, *J. Pharm. Biomed. Anal.*, 23 (2000) 803.

11. M.C.F. Ferraro, P.M. Castellano, T.S. Kaufman, *J. Pharm. Biomed. Anal.*, 26 (2001) 443.
12. T.B. Vree., M. van den Biggelaar-Martea, C.P.W.G.M. Verwey-van Wissen, *J. Chromatogr., B*, 655 (1994) 53.
13. P.C.Ioannon, N.V. Rusakova, D.A. Andrikopoulou. K. M. Glynon. G.M. Tzompanaki, *Analyst*, 123 (1998) 2839.
14. M.L. Luis, J.M.G. Fraga, A.I. Jimenez, F. Jimenez, O. Hernandez, J.J. Arias, *Talanta*, 62 (2004) 307.
15. Y. Rao, X.R. Zhang, G. Luo, W.R.G. Baeyens. *Anal. Chim. Acta.*,396 (1999) 273.
16. M. Luz. S. Corujedo, D. Blanco, J.M.G. Fraga, A.I. Jimenez, F. Jimenez, J.J. Arias, *Talanta*, 57 (2002) 23.
17. M.B.Barroso, R.M. Alonso, R.M. Jimenez, *Anal. Chim. Acta.*,305 (1995) 332.
18. I. Baranowska, P. Markowski, A. Gerie, j. Baranowski, *Bioelechem.*, 73 (2008) 5.
19. J.M. Kauffmann, J.C. Vire, *Anal. Chim. Acta.*, 273 (1993) 329.
20. J. Wang (Ed.), Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine, VCH, New York, 1996.
21. P.T. Kissinger, W.R. Heinemann, Laboratory Techniques in Electroanalytical Chemistry, 2nd Ed., Marcel Dekker, New York, 1996.
22. J. Herovsky, J. Rute, Principles of Polarography, (Academic Press, New York) 1965.
23. J.F. Rusling, *Acc.Chem. Res.*, 24 (1991) 75.
24. A. J. Bard, *J.Chem. Educ.,* 60 (1983) 302.
25. E.E. Ferapontova, *Electrochim. acta*, 49 (2004) 1751.
26. H.Y. Xia, X. Y. Hu, *Anal. Letters*, 38 (2005) 1405.
27. A.J. Bard, L.R. Faulkner, Elecrochemical Methods Fundamentals and Applications, Wiley, New York, 2nd edn, 2004.
28. D.K. Gosser (Ed), Cyclic Voltametry, VCH, New York, 1994.
29. S.S. Kalanur, J. Seetharamappa, G.P. Mamatha, M.D. Hadagali, P.B. Kandagal, *Int. J. Electrochem. Sci.*, 3 (2008) 756.
30. Greef, R. Peat, L.M. Pletcher, J. Robison, Instrumental Methods in Electrochemistry, Ellis Horwood, Chicherster, 1985, p.185
31. E.R. Brown, R. F. Large, in: A. Weissberger, B.W. Rossiter (Eds.), Physical Methods of Chemistry, Wiley Interscience. Rochester New York, 1964, p.423.
32. E. Laviron, *J. Electroanal. Chem.* 101 (1979) 19.
33. R. Jain, S. Gupta, *Bull. Electrochem.*, 14 (19980) 57.
34. M.Panduranga Char, E. Niranjana, B.E. Kumara Swamy, B.S. Sherigara, K. Vasantkumar Pai, *Int. J. Electrochem. Sci.*, 3 (2008) 588.
35. R.N. Hegde, B.E. Kumara Swamy, B.S. Sherigara, S.T. Nandibewoor, *Int. J. Electrochem. Sci.*, 3 (2008) 302.
36. W.J. Moore, Physical Chemistry, 5th ed, Orient Longman Pvt Ltd, New Delhi, 2004, p.502.
37. L.J. Chen, L. T. Burka, *Chem. Res. Toxicol.*, 20 (2007) 1741.
38. R. N. Nicholson, I. Shain, *Anal. Chem.*, 36 (1964) 722.
39. S.S. Kalanur, J. Seetharamappa, U. Katrahalli, P.B. Kandagal, *Int. J. Electrochem Sci*., 3 (2008) 711.
40. C.M. Riley, T.W. Rosanske, Development and Validation of analytical Methods, Elsevier Science Ltd., New York, 1996.
41. M.E. Swatz, I.S. Krull, Analytical Method Development and Validation, Marcel Dekker, New York, 1997.