“Happy are those who dream dreams and are ready to pay the price to make them come true.”

~Leon J. Suenes

PART B

VOLTAMMETRIC STUDIES
“All knowledge that the world has ever received comes from the mind; the infinite library of the universe is in our own mind.”

~Swami Vivekananda

Voltammetric oxidation and determination of loop diuretic furosemide at a multi-walled carbon nanotubes paste electrode

Electrochimica Acta

60 (2012) 95-101
5.1. INTRODUCTION

Furosemide (FUR) is a diuretic which is an anthranilic acid derivative. Chemically, it is 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid.\(^1\) It is used in the treatment of edema associated with renal impairment,\(^2\) nephrotic syndrome, hypertension, heart failure,\(^3\) hepatic cirrhosis,\(^4\) adjunct in cerebral/pulmonary edema where rapid diuresis is required and also used in the management of severe hypercalcemia in combination with adequate rehydration. The high degree of efficacy is largely due to this unique site of action. Furosemide with a prompt action is fairly and rapidly absorbed after oral administration and shows a strong diuretic effect of short duration.\(^5\) Its bioavailability ranges from 60 to 70 % and its plasma half-life is about 1-2 h,\(^6\) with structural formula shown in Scheme 1.

![Structural formula of furosemide]

**Scheme 1**

A number of methods for the individual determination of these diuretics in both pharmaceutical preparations and biological fluids have been reported. Thus, furosemide is normally determined by liquid chromatography with spectrophotometric,\(^7\) spectrofluorimetric detection,\(^8\) chemiluminescent\(^9\) and micellar electrokinetic chromatographic methods.\(^10\) Most often, the procedures
involves some extraction, solvent-usage intensive, expensive devices and are thus time consuming.

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 include 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. This drug was also be quantified with electrochemical methods using glassy carbon electrode, gold electrode, hanging mercury drop electrode and graphite electrode.

A limit of detection close to $1.5 \times 10^{-7}$ M is found at glassy carbon electrodes, but with the necessity of renewal of the electrode surface after each measurement, in order to achieve reproducible responses; limits of detection of $1.7 \times 10^{-7}$ M (using flow injection analysis) and $5.5 \times 10^{-7}$ M using HPLC with electrochemical detection were found in at glassy carbon electrodes. In the latter case, the oxidation peak potential had a relatively high value, close to $+1.25$ V vs. Ag/AgCl (1 M KCl), electrode surface pre-treatment being needed before each measurement. For electrochemical determinations, the main problem which causes most difficulties is adsorption of the analyte or its
reaction products on the electrode surface. Apart from solid carbon electrodes, such as glassy carbon, paste electrodes have also been investigated for use in oxidative electroanalytical procedures.

Carbon nanotubes (CNTs) continue to receive remarkable attention in electrochemistry. Since their synthesis by Iijima in 1991 using transmission electron microscopy, CNTs have been the subject of numerous investigations in chemical, physical and material areas due to their novel structural, mechanical, electronic and chemical properties. The subtle electronic properties suggest that CNTs have the ability to promote charge transfer reactions when used as an electrode. The modification of electrode substrates with multi-walled carbon nanotubes (MWCNTs) for use in analytical sensing has been documented to result in low detection limits, high sensitivities, reduction of over potentials and resistance to surface fouling. MWCNTs have been introduced as electrocatalysts and CNTs-modified electrodes have been reported to give super performance in the study of a number of biological species. MWNTs consist of multiple rolled layers (concentric tubes) of graphite. Generally there are two ways to fabricate CNTs based electrodes. One way is to cast CNTs suspension on the surface of solid electrodes such as Pt, Au and glassy carbon electrodes to make CNTs film modified electrodes. Another method is to mix CNTs with bonds such as nujol, bromoform or mineral oil, and then pack the mixture into a pipe to prepare paste electrodes. Naturally, the characteristics of CNTs paste electrodes depend
on the type of CNTs and the bonds used. Especially, the bonds show greater influence on the accumulation efficiency of electroactive species and blank current due to their different hydrophobicity. Every bond has its characteristics and suits for some purposes. Paraffin oil is commercially available and paraffin oil based paste electrode exhibits some characteristics; hence it is frequently used as bond.\textsuperscript{29} Although CNTs modified electrodes are commonly used, carbon nanotubes paste electrodes are rarely used in electro oxidation.

To our knowledge, voltammetric determination of FUR using multi-walled carbon nanotubes-paraffin oil paste electrode (CNTPE) has not been reported yet. The objective of the present work is to develop a convenient and sensitive method for the determination of FUR by multi-walled carbon nanotubes-paraffin oil paste electrode. Here we report the electrochemical oxidation of FUR on CNTPE in this chapter. The ability of the modified electrode for voltammetric response of selected compound was evaluated. Finally, this modified electrode was used for the analysis of FUR in pharmaceutical and urine samples. The resulted biosensor exhibits high sensitivity, rapid response, good reproducibility and freedom of other potentially interfering species.

\textbf{5.2. EXPERIMENTAL}

\textbf{5.2.1. Reagents and chemicals}

A stock standard solution of furosemide (Sigma Aldrich), 1 mM was
prepared in HPLC grade methanol (S.D. Fine-Chem.) and stored in the dark under refrigeration (4 °C) to avoid possible decomposition. More dilute solutions (10^{-6} to 10^{-4} M) were prepared by diluting the stock solution. Multi-walled carbon nanotubes were from Sigma–Aldrich (>90 %, O.D.: 10–15 nm, I.D.: 2–6 nm, length: 0.1–10 μm). Britton-Robinson (BR) buffer (0.04 M) was prepared and used as supporting electrolyte. Buffer solutions were adjusted by adding the necessary amounts of KOH or HCl in order to obtain the appropriate pH value. Furosemide tablets i.e., Lasix (40 mg per tablet) were purchased from Aventis Pharma. Limited, India. Rest of the reagents was of analytical-reagent grade, and millipore water was used throughout the experiment.

5.2.2. Instrumentation

Electrochemical measurements were carried on a CHI-1110A electrochemical analyzer (CH Instruments Ltd. Co., USA, version 4.01) coupled with a conventional three-electrode cell. A three-electrode system consisting of a Ag/AgCl as reference electrode, a Pt wire as counter electrode and a self-made carbon nanotubes paste electrode as a working electrode were used. All the potentials in this paper are given against the Ag/AgCl (3 M KCl). The pH measurements were made by Elico LI120 pH meter (Elico Ltd., India) and Nicolet Impact - 410 FTIR, Varian CARY 50 Bio UV-vis spectrophotometer were used to identify the product.
5.2.3. Preparation of electrode

The carbon nanotubes paste was prepared by mixing multi-walled carbon nanotube powder and paraffin oil in an agate mortar in a ratio of 60.0 % nanotubes powder to 40.0 % paraffin oil (w/w) and this mixture was then homogenized. The ratio was used due to its success in previous applications.\textsuperscript{30} A portion of the resulting paste was packed firmly into a cavity of a polytetrafluoro ethylene tube (PTFE). The surface of the electrode was smoothed against weighing paper and rinsed with water. Unless otherwise stated, the paste was carefully removed prior to pressing a new portion into the electrode after every measurement. The resulting electrode was noted as CNTPE.

The area of the electrode was obtained by the cyclic voltammetric method using 1.0 mM $\text{K}_3\text{Fe(CN)}_6$ in 0.1 M KCl by recording the current voltage curve at different scan rates. For a reversible process, the following Randles-Sevcik formula can be used:\textsuperscript{31}

$$I_{pa} = (2.69 \times 10^5) n^{3/2} A D_R^{1/2} \nu^{1/2} C_0$$

where $I_{pa}$ refers to the anodic peak current, $n$ is the number of electrons transferred, $A$ is the surface area of the electrode, $D_R$ is diffusion coefficient, $\nu$ is the scan rate and $C_0$ is the concentration of $\text{K}_3\text{Fe(CN)}_6$. For 1.0 mM $\text{K}_3\text{Fe(CN)}_6$ in 0.1 M KCl electrolyte, $n = 1$, $D_R = 7.6 \times 10^{-6}$ cm$^2$ s$^{-1}$, then from the slope of the plot of $I_{pa}$ versus $\nu^{1/2}$, relation, the electro active area was
calculated. In our experiment the slope was $7.58 \times 10^{-6} \mu A (Vs^{-1})^{-1/2}$ and the area of electrode was calculated to be 0.102 cm$^2$.

5.2.4. Analytical procedure

The CNTPE was first activated in BR buffer (pH 5.0) by cyclic voltammetric sweeps between 0.0 to 2.0 V until a stable cyclic voltammogram was obtained. Then electrodes were transferred into another cell of BR buffer (pH 5.0) containing proper amount of FUR. The potential scan was initiated and cyclic voltammograms were recorded between +0.60 and +1.60, with a scan rate of 50 mVs$^{-1}$. All measurements were carried out at room temperature of 25.0 ± 0.1 °C.

5.2.5. Sample preparation

A quantity of 10 tablets were weighed and ground to a homogeneous fine powder in a mortar. A portion equivalent to a stock solution of a concentration of about 0.01 M was accurately weighed and transferred into a 100 ml calibrated flask and completed to the volume with methanol. The contents of the flask were sonicated for 10 min to affect complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquid and diluting them with the BR buffer solutions. Each solution was transferred to the voltammetric cell and analyzed by standard addition method. The differential pulse voltammograms were recorded
between 0.60 and 1.60 V. The oxidation peak current of FUR was measured. The parameters for differential pulse voltammetry (DPV) were pulse width of 0.06 s, pulse increment of 4 mV, pulse period of 0.2 s, pulse amplitude of 50 mV and scan rate of 20 mVs$^{-1}$. To study the accuracy of the proposed method and to check the interferences from excipients used in the dosage form, recovery experiments were carried out. The concentration of FUR was calculated using standard addition method.

5.3. RESULTS AND DISCUSSION

5.3.1. Cyclic voltammetric behavior of FUR

The voltammetric behavior of furosemide at the CNTPE was examined. Figure V (i) (p. 175) shows the cyclic voltammograms of 1.0 $\times$ 10$^{-3}$ M furosemide in BR buffer of pH 5.0 in the potential range of 0.60 to 1.60 V, with a scan rate of 50 mVs$^{-1}$. Furosemide exhibited two anodic peaks, one at 1.09 V with anodic current of 6.28 $\mu$A and another at 1.26 V with anodic current of 10.2 $\mu$A.

No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction was a totally irreversible process. Nevertheless, it was found that the oxidation peak current of FUR showed a remarkable decrease during the successive cyclic voltammetric sweeps (Figure V (ii) (p. 176)). After the second sweep, the peak current decreased greatly and finally remained unchanged. This phenomenon may be due to the fact that the adsorption of
Figure V (i)

(a) Cyclic voltammogram of CNTPE taken out from $1.0 \times 10^{-3}$ M FUR, (b) Cyclic voltammogram of CNTPE. Scan rate: 50 mV s$^{-1}$; supporting electrolyte: 0.04 M BR buffer with pH 5.0
Successive cyclic voltammograms of $1.0 \times 10^{-3}$ M FUR on CNTPE. Scan rate: 50 mVs$^{-1}$; supporting electrolyte: 0.04 M BR buffer with pH 5.0
FUR or its oxidative product occurs at the electrode surface. Therefore, the voltammograms corresponding to the second cycle and peak B were generally recorded, since peak B was intense than A.

5.3.2. Influence of pH

Within the range of pH 3.0 - 11.2, the peak potential shifted to less positive values for both the peaks, together with a decrease in peak currents with increasing the pH of the buffer solution (Figure V (iii) (p. 178)). However, by increasing the pH, the potential of the peak B is shifted to less positive values till pH 7.0, then becomes almost pH independent (Figure V (iv) (p. 179)). Basically, two linear regions are obtained, one between pH 3.0 and 7.0 with a slope of 50 mV/pH and another between pH 7.0 and 11.2 with a slope of 27 mV/pH. The maximum peak current value was obtained at acidic pH value i.e., the intensity of peak B was increased to a high value at pH 5.0, and then the peak intensity decreases continuously. The highest peak intensity for the peak A was obtained around pH 7.0. Above pH 9.0, the peak A was no longer present. This effect of pH on the electrochemical properties of soluble ions in solution can be attributed to the acid-base equilibrium constants of this diuretic due to the iminium, carboxylic and sulphonamidic groups. As compared to peak B, peak A was less intense and moreover it can be observed within pH 9.0.
Figure V (iii)

Influence of pH on the shape of anodic peak. pH: 3.0 (a), 4.0 (b), 5.0 (c), 6.0 (d), 7.0 (e), 8.0 (f) and 9.0 (g). Scan rate: 50 mVs\(^{-1}\); supporting electrolyte: 0.04 M BR buffer with pH 5.0
Figure V (iv)

Influence of pH on the peak potential of FUR for peaks A and B. Scan rate: 50 mVs\(^{-1}\); supporting electrolyte: 0.04 M BR buffer with pH 5.0
5.3.3. Influence of scan rate

Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behavior of FUR at different scan rates from 10 to 200 mVs$^{-1}$ (Figure V (v) (p. 182)) was also studied. It was noticed that the first oxidation peak became broader and almost disappeared at higher scan rates. There is a good linear relationship between peak current and scan rate. The equations are $I_{pa1} = 98.51 \nu + 1.4702; r = 0.9870$ and $I_{pa2} = 129.59 \nu + 2.5953; r = 0.9865$, for peaks A and B, respectively. In addition, there was a linear relation between log $I_p$ and log $\nu$, corresponding to the following equation: log $I_{pa1} = 0.7846 \log \nu + 1.8533; r = 0.9955$ and log $I_{pa2} = 0.8028 \log \nu + 2.0144; r = 0.9953$, for peaks A and B, respectively (Figure V (vi A) (p. 183)). The slopes of 0.7846 and 0.8028 are close to the theoretically expected value of 1.0 for an adsorption controlled process.\footnote{32} This indicates that the electrode process was controlled by adsorption rather than diffusion.

The peak potential shifted to more positive values with increasing the scan rates. The linear relation between peak potential and logarithm of scan rate can be expressed as $E_{pa1} = 1.2108 + 0.0898 \log \nu; r = 0.9902$ and $E_{pa2} = 1.372 + 0.0985 \log \nu; r = 0.9951$, for the peaks A and B, respectively (Figure V (vi B) (p. 183)). 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.\footnote{33}
As for an irreversible electrode process, according to Laviron,\textsuperscript{34} $E_p$ is defined by the following equation

$$E_p = E^{0'} + \frac{2.303RT}{\alpha nF} \log \left( \frac{Rk^0}{\alpha nF} \right) + \frac{2.303RT}{\alpha nF} \log \nu$$  \hspace{1cm} (2)

where $\alpha$ (alpha) is the transfer coefficient, $k^0$ the standard heterogeneous rate constant of the reaction, $n$ the number of electrons transferred, $\nu$ (nu) the scan rate and $E^{0'}$ is the formal redox potential. Other symbols have their usual meanings. Thus the value of $\alpha n$ can be easily calculated from the slope of $E_{pa1}$ versus $\log \nu$ and $E_{pa2}$ versus $\log \nu$. In this system, for first plot slope is 0.0898, therefore, the $\alpha n$ calculated to be 0.66 and for the second plot slope is 0.0985, therefore $\alpha n$ calculated to be 0.60, taking $T = 298$, $R = 8.314$ and $F = 96480$.

According to Bard and Faulkner,\textsuperscript{35} $\alpha$ can be given as

$$\alpha = \frac{47.7}{E_p - E_{p/2}} \text{ mV}$$ \hspace{1cm} (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 $\alpha$ to be 0.56. Further, the number of electron ($n$) transferred in the electro oxidation of FUR, was calculated to be 1.1~1 for first peak and 1.07~1 for second peak. Totally the number of electron ($n$) transferred in the electro oxidation of FUR was found to be two. The value of $k^0$ can be determined from the intercept of the 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 $E_{pa1}$.
Figure V (v)

Cyclic voltammograms of $1.0 \times 10^{-3}$ M FUR on CNTPE with different scan rates. (a) – (g) were 10, 30, 50, 80, 100, 180 and 200 mVs$^{-1}$. Scan rate: 50 mVs$^{-1}$; supporting electrolyte: 0.04 M BR buffer with pH 5.0
Figure V (vi A)

(A) Dependence of the logarithm of peak current on logarithm of scan rate

(B) Relationship between peak potential and logarithm of scan rate
versus \( \nu \) curve by extrapolating to the vertical axis at \( \nu = 0 \). For peak A, the intercept for \( E_{pa1} \) versus log \( \nu \) plot was 1.2108 and \( E^0 \) was obtained to be 1.057, the \( k^0 \) was calculated to be \( 0.941 \times 10^3 \) s\(^{-1}\). Similarly for the peak B, \( k^0 \) was \( 1.321 \times 10^3 \) s\(^{-1}\).

**5.3.4. Mechanism and identification of product of electrolysis**

The chemical oxidation of FUR by dimethyldioxirane in acetone, which involves a Mannich-like reaction for the formation of different products, is proposed by earlier workers. But based on the voltammetric experiment, the number of electrons transferred (n) was calculated and found to be 2. The IR spectrum of the product shows a 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 1.0 mM FUR in BR buffer solution at pH 5.0, before and after electrolysis are shown in Figure V (vii) (p.186). 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 \( \pi-\pi^* \) 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, confirming the product.
Based on the spectral characterization, the electro-chemical oxidation product of FUR in BR buffer was identified as 2 chloro-4-[furan-2ylmethylene]-amino]-benzenesulfonamide. Hence the proposed mechanism is shown in Scheme 2. The voltammetric studies show that oxidative pathways of electrochemical and chemical process are different.

![Scheme 2](image_url)

**Scheme 2**

### 5.3.5. Calibration curve

In order to develop a voltammetric method for determining the drug, we selected the differential pulse voltammetric mode, because the peaks are sharper and better defined at lower concentration of FUR 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 FUR. The BR buffer solution of pH
Figure V (vii)

UV spectra of 1 mM FUR in 0.04 M BR buffer solution at pH 5.0, (a) Before electrolysis; (b) After electrolysis; (c) Buffer
5.0 was selected as the supporting electrolyte for the quantification as FUR gave maximum peak current at pH 5.0. Differential pulse voltammograms obtained with increasing amounts of FUR showed that the peak current increased linearly with increasing concentration, as shown in Figure V (viii) (p. 188). It was found that the plot of $I_{pa1}$ and $I_{pa2}$ versus concentrations showed linearity over the drug concentration range of $8.0 \times 10^{-6}$ to $2.0 \times 10^{-4}$ M (from DPV) suggesting further that the electron de process was adsorption controlled. By selecting the anodic peak ($I_{pa2}$) of FUR, the DPV procedure was developed. The linear equation was $I_{pa} (\mu A) = 41.21 + 0.236 C$ ($r = 0.9970$, C is in mM). Deviation from linearity was observed for more concentrated solutions, due to the adsorption of FUR or its oxidation product on the electrode surface. Related statistical data of the calibration curves were obtained from the five different calibration curves. Limit of detection (LOD) and quantification (LOQ) were calculated based on the peak current ($I_{pa2}$) using the following equations shown below.

$$LOD = 3 \ s/m; \quad LOQ = 10 \ s/m.$$ 

where $s$ is the standard deviation of the peak currents of the blank (five replicates), and $m$ is the slope of the calibration curve. The LOD and LOQ values were calculated to be $2.92 \times 10^{-7}$ and $9.73 \times 10^{-7}$ M, respectively. Low values of the both LOD and LOQ values confirmed the sensitivity of the proposed method as compared to reported electrochemical method performed by graphite-polyurethane composite electrode. A comparison between the
Differential pulse voltammograms of CNTPE in FUR solution at different concentrations: blank (1), 0.08 (2), 0.1 (3), 0.3 (4), 0.5 (5), 0.8 (6), 1.0 (7), 1.5 (8) and 2.0 (9) \times 10^{-4} \text{ M}. Inset: plot of the peak current against the concentration of FUR.
analytical parameters obtained by various electrochemical procedures is presented in Table V (i) (p. 190). The LOD and LOQ values calculated by the present method are better compared to the reported work. Analyzing five replicates, for the process of the validation within–day variations and for intraday assay were studied.

5.3.6. Reproducibility of the multi-walled carbon nanotubes paste electrode

The regeneration and reproducibility of the electrode was investigated. It was found that after determination the surface of the CNTPE could be regenerated by successively cycling between 0.6 and 1.6 V in 5.0 pH with 0.04 M Britton-Robinson buffer for 5 cycles, respectively. As an example, a 1.0 mM FUR solution was measured successively for 10 times with the same electrode regenerated through such procedure after every determination, the relative standard deviation (RSD) of the peak current was 3.54 %. As to the reproducibility between days, it was similar to that of within a day if the temperature was kept almost unchanged. Owing to the adsorption of FUR or its oxidative products on to the electrode surface, the current response of the modified electrode would decrease after successive use. In this case, the electrode should be prepared again.
**Table V (i)**

Electroanalytical procedure for determination of furosemide in the literature

<table>
<thead>
<tr>
<th>Detection</th>
<th>Media</th>
<th>LOD (M)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amperometric detection at carbon fiber microelectrodes (+1.25 V vs. Ag/AgCl) coupled to HPLC and FIA</td>
<td>Acetonitrile - water (25:75) with 5 mmol L(^{-1}) NaH(_2)PO(_4) (HPLC), and 5 mmol L(^{-1}) NaH(_2)PO(_4) pH 6.5 (FIA)</td>
<td>5.5×10(^{-7}) (HPLC), 1.7×10(^{-7}) (FIA)</td>
<td>16</td>
</tr>
<tr>
<td>Graphite-polyurethane composite electrode (+ 1.0 V vs. SCE)</td>
<td>1.0 mmol L(^{-1}) NaOH</td>
<td>2.8×10(^{-6})</td>
<td>41</td>
</tr>
<tr>
<td>Multi-walled carbon nanotubes-paraffin oil paste electrode</td>
<td>Methanol - water (10:90)</td>
<td>2.9×10(^{-7})</td>
<td>Present work</td>
</tr>
</tbody>
</table>
5.3.7. Interference

FUR was formulated in single as well as multi-component tablets. The oxidation peaks of interferents should not appear where the peak corresponds to FUR appears. So in order to investigate the effect of co-formulated substances such as glucose, starch, dextrose, sucrose etc. on the voltammetric response of FUR, this study was carried out. Differential pulse voltammetric experiments were carried out for $1.0 \times 10^{-4}$ M FUR in the presence of 0.1 mM of each of the interferents. The results are listed in Table V (ii) (p. 193). It was observed that 10 folds of dextrose, gum acacia, lactic acid and sucrose did not interfere; however, citric acid, glucose, oxalic acid and starch interfered with the voltammetric signal of FUR by $-11.08\%$, $-10.34\%$, $-10.94\%$ and $-10.49\%$, respectively.

5.3.8. Tablet analysis

In order to evaluate the applicability of the proposed method in the pharmaceutical sample analysis, a commercial medicinal sample containing FUR i.e., Lasix (40 mg per tablet) was used. The tablets were grounded to powder, dissolved in methanol and then further diluted so that FUR concentration falls in the range of calibration plot. The contents of the flask were sonicated for 10 min to affect complete dissolution. Differential pulse voltammograms were then recorded under exactly identical conditions that were employed while recording differential pulse voltammograms for plotting.
calibration plot. The results are in good agreement with the content marked in the label. The detected content was 39.3 mg per tablet with 98.2 % recovery.

The recovery test of FUR ranging from $3.0 \times 10^{-5}$ to $2.0 \times 10^{-4}$ M was performed using differential pulse voltammetry. Recovery studies were carried out after the addition of known amounts of the drug to various pre-analyzed formulations of FUR. The recoveries in different samples were found to lie in the range from 98.5 % to 100.8 %, with RSD of 1.0 %.

5.3.9. Detection of FUR in urine samples

The applicability of the proposed method for the determination of FUR in biological fluid of human urine was attempted. Furosemide is a potent diuretic which, if given in excessive amounts, can lead to a profound diuresis with water and electrolyte depletion. It is detectible in urine 36-72 hrs following injection. Therefore, it analyzes directly in biological fluids. Drug-free human and urine samples, obtained from healthy volunteers, filtrated through a filter paper and stored frozen until the assay. The developed differential pulse voltammetric method for the FUR determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known amounts of FUR. The urine samples were diluted 100 times with the BR buffer solution before analysis without further pretreatments. A quantitative analysis can be carried out by adding the standard solution of FUR into the detect system of urine sample. The calibration graph was used for
Table V (ii)

Influence of potential interferents on the voltammetric response of $1.0 \times 10^{-3}$ M FUR

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Concentration (mM)</th>
<th>Signal change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>1.0</td>
<td>-11.1</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.0</td>
<td>-1.47</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0</td>
<td>-10.3</td>
</tr>
<tr>
<td>Gum acacia</td>
<td>1.0</td>
<td>-6.43</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>1.0</td>
<td>+1.76</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>1.0</td>
<td>-10.9</td>
</tr>
<tr>
<td>Starch</td>
<td>1.0</td>
<td>-10.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.0</td>
<td>-3.21</td>
</tr>
</tbody>
</table>

Table V (iii)

Determination of FUR in urine samples

<table>
<thead>
<tr>
<th>Urine</th>
<th>Spiked $(10^{-4}$ M)</th>
<th>Detected$^{[a]}$ $(10^{-4}$ M)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.3</td>
<td>0.30</td>
<td>99.4</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.5</td>
<td>0.49</td>
<td>98.8</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.6</td>
<td>0.59</td>
<td>98.6</td>
</tr>
<tr>
<td>Sample 4</td>
<td>1.0</td>
<td>0.99</td>
<td>98.7</td>
</tr>
</tbody>
</table>

$^{[a]}$ Average of five determinations
the determination of spiked FUR in urine samples. The detection results of four urine samples obtained are listed in Table V (iii) (p. 193). The recovery determined was in the range from 98.72 % to 99.43 % and the RSD was 0.38 %. Good recoveries of FUR were achieved from these matrices, denoting that application of the proposed method to the analysis of FUR in biological fluid could be easily assessed.

5.4. IMPORTANCE OF CHAPTER V

In this work, multi-walled carbon nanotubes paste electrode has been successfully used for the oxidation of FUR in BR buffer solution (pH = 5.0). Based on the study, influence of several physico-chemical parameters like potential scan rate, pH and concentration were investigated. A probable reaction mechanism was proposed. The oxidation of FUR was found to be an irreversible, two electrons and two protons process with adsorption controlled character. This method has been successfully used to determine FUR in the pharmaceutical sample. 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 FUR in spiked urine samples demonstrated the applicability of the method for real sample analysis. Further this method may be considered as a suitable alternative to the existing chromatographic methods.
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