

# Electrochemical studies of tamsulosin hydrochloride using multiwalled carbon nanotube-modified glassy carbon sensor

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A differential pulse voltammetric sensor for the determination of tamsulosin hydrochloride (TAM) using multiwalled carbon nanotubes (MWNTs)–Nafion-modified glassy carbon electrode (GCE) has been developed. MWNTs were dispersed in water with the help of Nafion and were used to modify the surface of GCE via solvent evaporation. At MWNT-modified electrode, TAM gave a well-defined oxidation peak at a potential of 1084 mV in 0.1 M acetate buffer solution of pH 5. Compared to the bare electrode, the peak current of TAM showed a marked increase and the peak potential showed a negative deviation. The determination conditions, such as the amount of MWNT–Nafion suspension, pH of the supporting electrolyte and scan rate, were optimised. Under optimum conditions, the oxidation peak current was proportional to the concentration of TAM in the range  $1 \times 10^{-3}$  M– $3 \times 10^{-7}$  M with a detection limit of  $9.8 \times 10^{-8}$  M. The developed sensor showed good stability, selectivity and was successfully used for the determination of TAM in pharmaceutical formulations and urine samples.

**1. Introduction:** Recent years have witnessed significant interest in nanomaterials, such as nanocrystals, nanowires and nanotubes for their wide applications. Carbon nanotubes (CNTs) have been at the forefront of novel nano-scale investigations owing to their unique structure-dependent electronic and mechanical properties. Their small dimensions, closed topology and lattice helicity have enabled nanotubes to influence broad areas of science and technology, ranging from super strong nanocomposites to nanoelectronics [1]. Certainly, in the studies of electrochemical properties of biologically relevant molecules such as hydrogen peroxide [2], nicotinamide adenine dinucleotide [3], dopamine [4] and so on, multiwalled carbon nanotube (MWNT)-modified electrodes have shown superior performances compared to other carbon electrodes. Also, their subtle electronic properties suggest that MWNTs have the stability to promote the direct electron transfer reaction of some important biomolecules, such as microperoxidase [5], horseradish peroxidase [6], haemoglobin [7], glucose oxidase [8] and so on.

Tamsulosin hydrochloride (TAM) is a potent and selective  $\alpha_1$ -adrenoceptor antagonist. This drug is used as an oral medication to ameliorate the bladder-outlet obstruction associated with prostatic hypertrophy. Prostatic and urethral smooth muscle tone is maintained by stimulation of postjunctional  $\alpha_1$ -adrenoceptors through the release of noradrenaline from the adrenergic nerves in both animals and humans [9]. Benign prostatic hyperplasia is associated with a bladder-outlet obstruction that has been postulated to occur via both mechanical compression exerted by the increased bulk of the prostate and alterations in the neural control of the prostatic smooth muscle. In recent years,  $\alpha_1$ -adrenoceptor antagonists have been increasingly used for the symptomatic treatment of lower urinary tract symptoms, suggestive of benign prostatic obstruction [10].

Several analytical methods have been reported for the analysis of TAM. These include liquid chromatography-mass spectrometry [11], liquid chromatography with fluorescence detection [12] and radio receptor assay [13]. The major drawbacks of these reported methods include potential loss of drug in the re-extraction procedure, lengthy, tedious and time-consuming plasma sample preparation and extraction processes and interference of endogenous substances. Above all, these methods also require sophisticated and expensive instrumentation. In this context electrochemical detection methods are often advantageous as they offer high sensitivity, ease of use, portability and relatively inexpensive equipment.

The main electrochemical methods involve reaction at different solid electrodes such as glassy carbon electrode (GCE), platinum, diamond, gold and transition metal-oxide electrode. However, the application of these electrodes is limited, because several species can poison the electrode surface and decrease the sensitivity and accuracy. The performance characteristics of these electrodes can be enhanced by using chemically modified electrodes.

In continuation to our work on electroanalysis [14–17], an attempt has been made to study the electrochemical oxidation of TAM at an MWNT-modified GCE and to develop a convenient and sensitive method for the determination of TAM by differential pulse voltammetry (DPV) using the developed sensor.

## 2. Experimental

**2.1. Apparatus:** Voltammetric measurements were performed with a PC-controlled BAS Epsilon electrochemical analyser (Bio-analytical System, USA). A three-electrode cell system consisting of MWNT–Nafion-modified GCE as working electrode, an Ag/AgCl reference electrode and a platinum wire auxiliary electrode were used. Fourier transform infrared (FTIR) spectra of the MWNT were recorded on JASCO-4100 FTIR spectrometer using KBr discs. Scanning electron microscopic (SEM) images were obtained on a JOEL 6390 LV.

Pure TAM was obtained as a gift sample and was used as received. Tablets of TAM, Veltam capsules (Intas Pharmaceuticals, India) were purchased from local drug stores. MWNTs (6–20 nm diameter and 1–5  $\mu$ m length) and Nafion were purchased from Aldrich. MWNT was refluxed in concentrated  $\text{HNO}_3$  for 48 h to cause segmentation and carboxylation [18]. All the chemicals used were of analytical reagent grade quality (Merck or Sigma) and they were employed without further purification and all solutions were prepared with Millipore water.

**2.2. Preparation of MWNT-modified GCE:** Prior to modification, the GCE was mechanically polished with alumina slurry down to 0.05  $\mu$ m on a polishing cloth. Then the electrode was sonicated in methanol, water,  $\text{HNO}_3$  solution and acetone, respectively. Acid treatment of MWNT was carried out as described in the literature [18]. As a result of this treatment, length of MWNT got shortened and they gained functional groups which made them hydrophilic. The peaks at 1703 and 1564  $\text{cm}^{-1}$  of the FTIR spectrum demonstrated that carboxy groups and carboxylate groups are present on the surface of treated MWNTs.

MWNTs are generally insoluble in common solvents; therefore the key step is to disperse insoluble MWNTs in suitable solvents to perform the electrochemical measurements by using MWNT-modified electrodes. However, Nafion, a sulfonated tetrafluoroethylene copolymer was found to effectively disperse MWNTs. Nafion possesses several advantages such as excellent ion-exchange characteristics, thermal stability, chemical inertness, mechanical strength and has been widely used for the MWNT modification of electrodes [19]. Hence Nafion was selected as the suitable solvent for this study.

5 mg of acid-treated MWNT was dispersed in 2 mL of 0.5% Nafion–water solution with the aid of ultrasonic agitation to form a black homogenous suspension. 4  $\mu$ L of dark suspension was dropped onto the surface of cleaned GCE. The electrode was then allowed to dry in air to obtain the MWNT-modified GCE. The SEM images of the bare and MWNT-modified electrodes clearly showed that the electrode surface was effectively modified with MWNT.

**2.3. Differential pulse voltammetric determination of TAM:** A 10 mL acetate buffer solution (0.1 M, pH 5) containing specific amount of sample solution was transferred to a voltammetric cell and then purged with purified nitrogen for 3 min to remove oxygen. DPV was recorded from 800 to 1300 mV, at a scan rate of 100 mV/s. All the voltammetric experiments were carried out at room temperature. After each measurement the modified electrode was subjected to successive sweeping at pH 5 to give reproducible electrode surface.

**3. Results and discussion:** To study the electrochemical behaviour of TAM, we tried different modification techniques for various electrodes. It was first studied on bare gold electrode but no response was obtained. MWNT-modified gold electrode was then tried and that also gave no response for TAM. However TAM gave an oxidation peak on bare GCE at 1232 mV. To reduce the overpotential, we tried various chemical modifications on GCE. At Prussian blue-MWNT colloid-modified GCE, an oxidation peak was obtained at 1164 mV with a peak current of 10  $\mu$ A. However, at MWNT–Nafion-modified GCE, TAM gave a well-defined oxidation peak at 1084 mV with a peak current of 36.7  $\mu$ A. For all these studies, a  $1 \times 10^{-3}$  M solution of TAM was used. Since a better sensitivity for TAM was obtained with MWNT–Nafion-modified GCE, the choice is justified.

The cyclic voltammograms of TAM at the bare GCE and MWNT–Nafion-modified GCE are shown in Fig. 1. It can be seen that the oxidation peak at the bare GCE was weak while the response was considerably improved at the MWNT-modified GCE. The peak potentials of TAM at the bare electrode and the

MWNT-modified GCE were almost the same. However, the peak was sharper and the peak current increased significantly, for the latter.

DPV was also applied to investigate the electrochemical behaviour of TAM at the bare and modified GCEs. Fig. 1 shows the differential pulse voltammograms obtained for  $1 \times 10^{-3}$  M TAM at bare and MWNT-modified GCEs. As can be seen, the oxidation peak of TAM at bare GCE was broad due to slow electron transfer [20], whereas at MWNT-modified electrode, an improved response was observed. In the case of bare GCE, TAM gave an oxidation peak at 1232 mV and the peak current was about 1.87  $\mu$ A, whereas at the MWNT-modified GCE, the oxidation peak was at 1084 mV with a peak current of 36.7  $\mu$ A. Compared with that of bare GCE, the oxidation potential of TAM showed a negative shift of about 148 mV and also a 36-fold increase in peak current. These results undoubtedly proved the electrocatalytic activity of MWNT–Nafion GCE towards the oxidation of TAM. Since DPV showed a better response, this was selected for further studies.

The surface area of both bare and MWNT–Nafion GCE were determined by recording the cyclic voltammograms at different scan rates. 2 mM  $K_3Fe(CN)_6$  was taken as a probe to measure the microscopic areas. For a reversible system the relationship between the current and scan rate is given by the Randles Sevcik equation [21]

$$I_p = 2.69 \times 10^5 An^{3/2} D_R^{1/2} C v^{1/2}$$

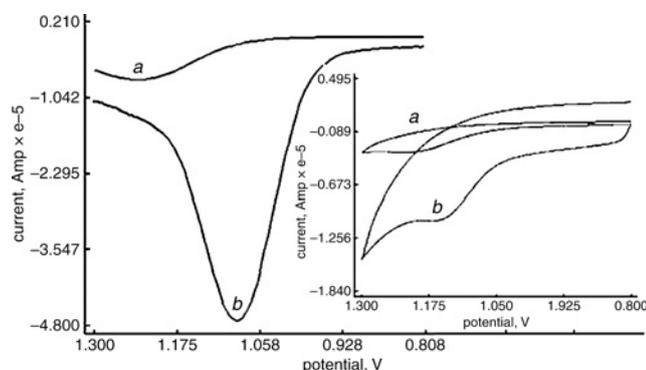
where  $I_p$  refers to the peak current,  $n$  the number of electron transferred,  $A$  the surface area of the electrode,  $D_R$  the diffusion coefficient,  $C$  the concentration of  $K_3Fe(CN)_6$  and  $v$  the scan rate. For  $K_3Fe(CN)_6$ ,  $n = 1$  and  $D_R = 7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ . The surface area can be calculated from the slope of  $I_p$  against  $v^{1/2}$ . Surface area for bare electrode was found to be 0.129  $\text{cm}^2$ . On modification with MWNT, the effective surface area was increased to 0.287  $\text{cm}^2$ . This enhancement in surface area resulted in the remarkable catalytic activity of the modified electrode.

The electrochemical behaviour of TAM in various media such as 0.1 M solutions of phosphate buffer, acetate buffer,  $H_2SO_4$ , NaOH and tetra-*n*-butyl ammonium chloride were studied by DPV. The best oxidation response was obtained in acetate buffer solution as the peak current is the highest. So 0.1 M acetate buffer was taken as the experimental medium for the determination of TAM.

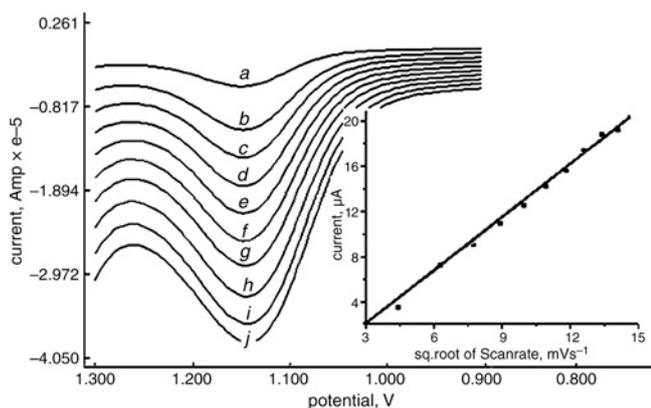
The electrochemical studies of  $1 \times 10^{-3}$  M TAM in acetate buffer were carried out in the pH range of 3–9 using DPV. Peak current showed a gradual increase with maxima at pH 5. An increase of pH beyond this value caused a marked decrease in the peak current. Hence the optimum pH for buffer medium was selected to be 5.

The relationship between the amount of MWNT–Nafion and the oxidation peak current of TAM was studied. When 4  $\mu$ L of suspension was used to cast on the electrode surface, the anodic peak current reached its maximum value. This enhancement of current indicates that the specific surface area and the number of catalytic sites increase with an increasing amount of MWNT. Further increase in the amount of MWNT–Nafion suspension caused a decrease in the anodic peak current. This is because Nafion is an insulator and this along with excess of MWNT blocks electron transfer. As a result, the peak current decreases when the MWNT–Nafion film is too thick. So the amount of MWNT–Nafion dispersion was fixed to be 4  $\mu$ L.

Scan rate studies were carried out using square wave voltammetry to assess whether the process at the MWNT–Nafion-modified GCE was under diffusion or adsorption control. When the scan rate was varied from 20 to 200 mV/s in  $1 \times 10^{-3}$  M solutions of TAM, (Fig. 2) a linear dependence of the peak current upon the square root of the scan rate was found, demonstrating a diffusional behaviour.



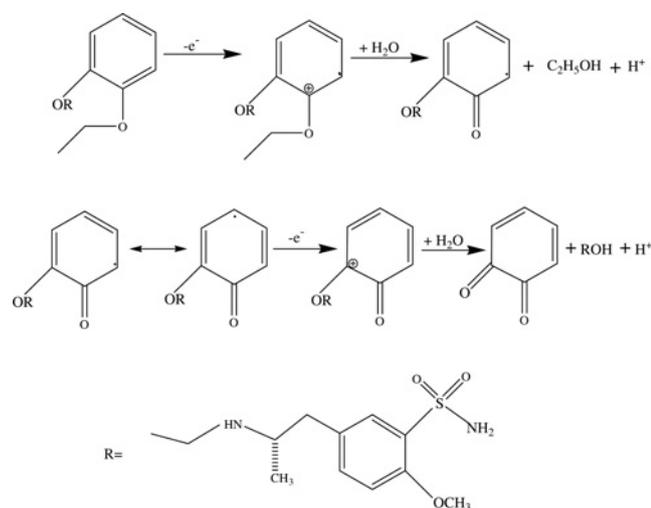
**Figure 1** Differential pulse voltammograms of  $1 \times 10^{-3}$  M TAM  
 a At bare GCE  
 b MWNT-modified GCE  
 Inset is the cyclic voltammograms of  $1 \times 10^{-3}$  M TAM at a bare GCE  
 b MWNT modified



**Figure 2** Overlay of square wave voltammograms of  $1 \times 10^{-3}$  M TAM at MWNT-modified GCE in 0.1 M acetate buffer (pH 5) at different scan rates  
 a  $20 \text{ mV s}^{-1}$   
 b  $40 \text{ mV s}^{-1}$   
 c  $60 \text{ mV s}^{-1}$   
 d  $80 \text{ mV s}^{-1}$   
 e  $100 \text{ mV s}^{-1}$   
 f  $120 \text{ mV s}^{-1}$   
 g  $140 \text{ mV s}^{-1}$   
 h  $160 \text{ mV s}^{-1}$   
 i  $180 \text{ mV s}^{-1}$   
 j  $200 \text{ mV s}^{-1}$   
 Inset is the plot of peak currents as a function of the square root of scan rates

The effect of scan rate on peak current was also examined under the above conditions with a plot of  $\log I$  against  $\log v$ , giving a straight line within the same scan rate range. The slope (0.61) of the curve was close to the theoretically expected value (0.5) for an ideal reaction of solution species (diffusion controlled electroc process) [22]. The number of electrons involved in the reaction ( $n_a$ ) can be calculated from the scan rate study using the Laviron's equation [23]. It was found that  $E$  varies linearly with the  $\ln v$ . The slope of this plot (b) is equal to  $RT/\alpha n_a F$ , where  $\alpha$  of the totally irreversible electrode process is assumed to be 0.5. For the irreversible oxidation of TAM,  $n_a$  value was calculated to be around 2. It indicates that two electrons are involved in the oxidation process of TAM.

The anodic oxidative behaviour of TAM may be due to oxidation of alkoxybenzene [24, 25]. The alkoxybenzene is converted into quinone by undergoing a  $2e^-$ ,  $2H^+$  oxidation as explained in Fig. 3. After optimising the conditions for effective electro-oxidation, an electrochemical sensor was developed based on the



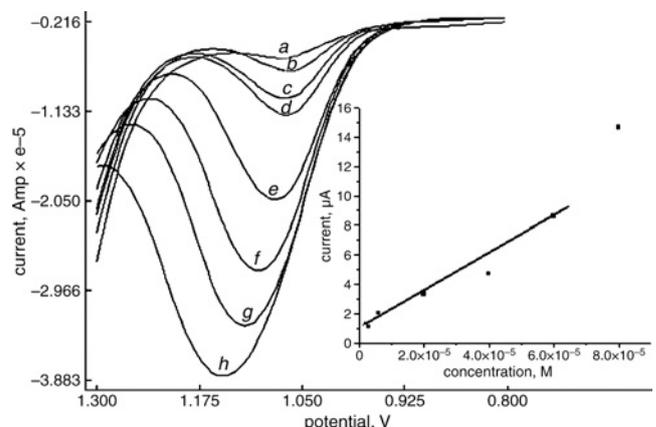
**Figure 3** Reaction mechanism

electrode reaction of TAM. The anodic peak current of TAM increased linearly with its concentration. The experimental results showed that the peak current was linear with the concentration of TAM in the range  $2 \times 10^{-6}$ – $3 \times 10^{-7}$  M (Fig. 4). The detection limit was found to be  $2 \times 10^{-8}$  M. The determination of TAM at a concentration level of  $1 \times 10^{-3}$  M was analysed repeatedly at a MWNT–Nafion film coated GCE for five times. The relative standard deviation of peak potential was obtained as 2%. This result demonstrated the good reproducibility of the developed sensor.

To evaluate the interference of foreign materials on the determination of TAM, a systematic study was carried out. It was found that glucose, urea,  $K^+$ ,  $SO_4^{2-}$ ,  $Na^+$  and  $Cl^-$  almost did not interfere with the performance of the sensor (signal change below 5%); however, ascorbic acid interfered severely.

The developed sensor was successfully applied for the determination of TAM in a commercially available pharmaceutical formulation (Veltam (Intas Pharmaceuticals, India)). The determination was performed as follows: Ten tablets were weighed and finely powdered. Then, required amount of the sample to prepare a  $1 \times 10^{-4}$  M solution of TAM was weighed and transferred to a beaker. Powder was dissolved in methanol and solutions of different concentrations were prepared by serial dilution of the stock with acetate buffer. DPV was recorded for each of these concentrations. The obtained oxidation current in each case was plotted against the previously obtained calibration graph shown in Fig. 3. The results are shown in Table 1. The results obtained are in good agreement with the declared TAM content and showed a high degree of precision.

The developed sensor was also applied for the determination of TAM in urine samples. An adequate amount of TAM corresponding



**Figure 4** Differential pulse voltammograms of TAM at different concentrations at MWNT-modified GCE  
 a  $3 \times 10^{-7}$  M  
 b  $8 \times 10^{-7}$  M  
 c  $2 \times 10^{-6}$  M  
 d  $6 \times 10^{-6}$  M  
 e  $3 \times 10^{-5}$  M  
 f  $9 \times 10^{-5}$  M  
 g  $4 \times 10^{-4}$  M  
 h  $1 \times 10^{-3}$  M  
 Inset is the plot of peak currents as a function of concentration of TAM

**Table 1** Determination of TAM in tablets

Sample	Declared amount (mg/tablet)	Found <sup>a</sup> (mg/tablet)	S.D. <sup>a</sup>	C.V. <sup>a</sup>
Veltam (Intas Pharmaceuticals, India)	0.20	0.19	1.4	0.71

<sup>a</sup>Average of six replicates

**Table 2** Determination of TAM in urine sample

TAM added, M	TAM found, M	Recovery, %	S.D. <sup>a</sup>
$2.00 \times 10^{-3}$	$1.95 \times 10^{-3}$	98	0.43
$4.00 \times 10^{-3}$	$4.08 \times 10^{-3}$	102	0.98
$6.00 \times 10^{-3}$	$5.99 \times 10^{-3}$	100	0.67

<sup>a</sup>Average of three replicates

to  $1 \times 10^{-2}$  M was added to the urine sample. This solution was quantitatively diluted using acetate buffer solution to obtain various concentrations. DPV was recorded and the unknown concentrations were determined from the calibration graph. The results are shown in Table 2, which showed that the developed sensor can determine the drug in urine samples with high percentage recovery.

**4. Conclusions:** Electrochemical oxidation of TAM was studied on a MWNT-modified GCE. TAM gave a well-defined oxidation peak due to a  $2e^-$  oxidation of the alkoxybenzene. The oxidation reaction was found to be diffusion controlled. Based on the electrochemical behaviour, a sensor is developed for the determination of TAM using DPV. The developed sensor has been successfully applied for the determination of TAM in dosage forms and also that present in urine samples. The developed method is a good alternative for the determination of TAM because it is simple, fast, cost effective and also has sufficient precision, accuracy and sensitivity.

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## 6 References

- [1] Balasubramanian K., Burghard M.: 'Electrochemically functionalized carbon nanotubes for device applications', *J. Mater. Chem.*, 2008, **18**, pp. 3071–3083
- [2] Wang J., Musameh M., Lin Y.H.: 'Solubilization of carbon nanotubes by nafion toward the preparation of amperometric biosensors', *J. Am. Chem. Soc.*, 2003, **125**, pp. 2408–2409
- [3] Chen J., Bao J., Cai C., Lu T.: 'Electrocatalytic oxidation of NADH at an ordered carbon nanotubes modified glassy carbon electrode', *Anal. Chim. Acta*, 2004, **516**, pp. 29–34
- [4] Britto P.J., Santhanam K.S.V., Ajayan P.M.: 'Carbon nanotube electrode for oxidation of dopamine', *Bioelectrochem. Bioenergy*, 1996, **41**, pp. 121–125
- [5] Wang M., Shen Y., Liu Y., ET AL.: 'Direct electrochemistry of microperoxidase 11 using carbon nanotube modified electrodes', *J. Electroanal. Chem.*, 2005, **578**, pp. 121–127
- [6] Yan Y., Zheng W., Zhang M., Wang L., Su L., Mao L.: 'Bioelectrochemically functional nano hybrids through co-assembly of proteins and surfactants onto carbon nanotubes: facilitated electron transfer of assembled proteins with enhanced faradic response', *Langmuir*, 2005, **21**, pp. 6560–6566
- [7] Cai C.X., Chen J.: 'Direct electron transfer and bioelectrocatalysis of hemoglobin at a carbon nanotube electrode', *Anal. Biochem.*, 2004, **325**, pp. 285–292
- [8] Guiseppi-Elie A., Lei C., Baughman R.H.: 'Direct electron transfer of glucose oxidase on carbon nanotubes', *Nanotechnology*, 2002, **13**, pp. 559–564
- [9] Andersson K.E., Sjogren C.: 'Aspects on the physiology and pharmacology of the bladder and urethra', *Progr. Neurobiol.*, 1982, **19**, pp. 71–89
- [10] Chapple C.R.: 'Pharmacotherapy for benign prostatic hyperplasia – the potential for  $\alpha_1$ -adrenoceptor subtype specific blockade', *Br. J. Urol.*, 1998, **81**, pp. 34–47
- [11] Matsushima H., Takanuki K., Kamimura H., Watanabe T., Higuchi S.: 'Highly sensitive method for the determination of tamsulosin hydrochloride in human plasma dialysate, plasma and urine by high performance liquid chromatography-electrospray tandem mass spectrometry', *J. Chromatogr. B*, 1997, **695**, pp. 317–327
- [12] Soeishi Y., Kobori M., Kobayashi S.I., Higuchi S.: 'Sensitive method for the determination of tamsulosin in human plasma using high performance liquid chromatography with fluorescence detection', *J. Chromatogr. B*, 1990, **533**, pp. 291–296
- [13] Taguchi K., Schafers R.F., Michel M.C.: 'Radioreceptor assay analysis of tamsulosin and terazosin pharmacokinetics', *Br. J. Clin. Pharmacol.*, 2002, **45**, pp. 49–55
- [14] Joseph R., Girish Kumar K.: 'Electrochemical reduction and voltammetric determination of metronidazole benzoate at modified carbon paste electrode', *Anal. Lett.*, 2009, **42**, pp. 2309–2321
- [15] Isaac S., Girish Kumar K.: 'Voltammetric determination of sulfamethoxazole at a multiwalled carbon nanotube modified glassy carbon sensor and its application studies', *Drug Test Anal.*, 2009, **1**, pp. 350–354
- [16] Joseph R., Girish Kumar K.: 'Differential pulse voltammetric determination and catalytic oxidation of sulfamethoxazole using [5,10,15,20-tetrakis (3-methoxy-4-hydroxy phenyl) porphyrinato] Cu (II) modified carbon paste sensor', *Drug Test Anal.*, 2010, **2**, pp. 278–283
- [17] Laina L., Girish Kumar K.: 'Carbon nanotube based sensor for the differential pulse voltammetric determination of pyridine-2-aldoxime methochloride', *Sens. Lett.*, 2011, **9**, pp. 541–545
- [18] Tsang S.C., Chen Y.K., Harris P.J.E., Green M.L.H.: 'A simple chemical method of opening and filling carbon nanotubes', *Nature*, 1994, **372**, pp. 159–162
- [19] Tsai Y., Chen J., Li S., Marken F.: 'Electroanalytical thin film electrodes based on a nafion(TM) – multi-walled carbon nanotube composite', *Electrochem. Commun.*, 2004, **6**, pp. 917–922
- [20] Nan C.G., Feng Z.Z., Li W.X., Ping D.J., Qin C.H.: 'Electrochemical behavior of tryptophan and its derivatives at a glassy carbon electrode modified with hemin', *Anal. Chim. Acta*, 2002, **452**, pp. 245–254
- [21] Randles J.E.B.: 'Cathode-ray polarograph', *Trans Faraday Soc.*, 1948, **44**, pp. 322–327
- [22] Laviron E., Roullier E., Degrand C.: 'A multilayer model for the study of space distributed redox modified electrodes. Part: II. Theory and application of linear potential sweep voltammetry for a simple reaction', *J. Electroanal. Chem.*, 1980, **112**, pp. 11–23
- [23] Laviron E.: 'Surface linear potential sweep voltammetry. Equation of the peaks for a reversible reaction when interactions between the adsorbed molecules are taken into account', *J. Electroanal. Chem.*, 1974, **52**, pp. 395–402
- [24] Demircigil B.T., Ozkan S.A., Coruh O., Yilmaz S.: 'Electrochemical behaviour of formoterol fumarate and its determination in capsules for inhalation and human serum using differential pulse and square wave voltammetry', *Electroanalysis*, 2002, **14**, pp. 122–127
- [25] Bermejo E., Zapardiel A., Perez-Lopez J.A., Chicharro M., Sanchez A., Hernandez L.: 'Electrochemical study of mefexamide at glassy-carbon electrodes and its determination in urine by differential pulse voltammetry', *J. Electroanal. Chem.*, 2000, **481**, pp. 52–61