

# Determination Of Sorafenib In Spiked Human Urine By Differential Pulse Polarography At Dropping Mercury Electrode

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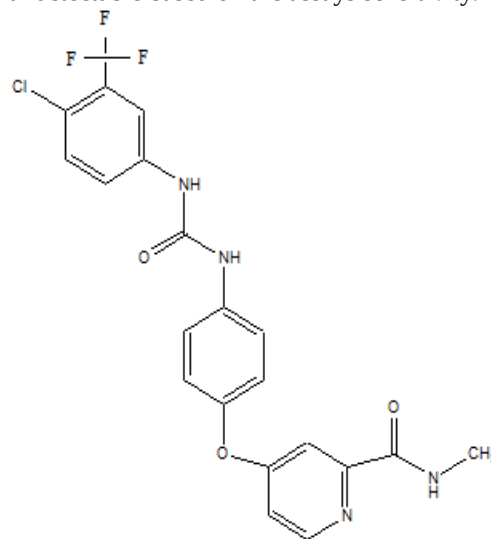
**Abstract-** The electrochemical reduction behavior and determination of sorafenib was studied by differential pulse polarography at dropping mercury electrode. A linear response was obtained over the concentration range  $5.0 \times 10^{-8}$  to  $1.0 \times 10^{-5}$  M with lower detection limits  $4.2 \times 10^{-8}$  M for sorafenib. Sorafenib exhibits well defined cathodic waves in universal buffers over the pH range 2.0 to 6.0. The carbonyl group getting reduced to the saturated compound in a four electron processes and reduction mechanism has been proposed. The kinetic parameters such as diffusion coefficients (D), transfer coefficients ( $\alpha_{na}$ ) and heterogeneous forward rate constants ( $K^0_{fh}$ ) are evaluated and reported. The relative standard deviation and correlation coefficient value was found to be 0.326% and 0.65 respectively. Differential pulse polarography was employed for determination of the sorafenib in trace levels using both standard addition and calibration methods.

**Keywords-** Sorafenib, polarography, dropping mercury electrode, formulations and urine.

## I. INTRODUCTION

Sorafenib (4[4[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-N-methylpyridine-2-carboxamide) is a multikinase inhibitor currently approved by the FDA for the treatment of advanced renal-cell carcinoma (RCC) and unresectable hepatocellular carcinoma (HCC), and by the EMEA for the treatment of HCC and advanced RCC. Sorafenib is available as a tablet formulation. Phase I trial of sorafenib in combination with gefitinib<sup>1</sup> and combination with carboplatin and paclitaxel in patients in lung cancer.<sup>2</sup> Phase II trial of first-line treatment with sorafenib versus interferon alfa-2a in patients with metastatic renal cell carcinoma<sup>3</sup> and patients with metastatic or recurrent sarcomas.<sup>4</sup> The determination of sorafenib and sorafenib-glucuronide in mouse plasma and liver homogenate was developed.<sup>5</sup> The HPLC-UV method for sorafenib determination in human plasma and application to cancer patients.<sup>6</sup> Sorafenib triggers antiproliferative and pro-apoptotic signals in human esophageal adenocarcinoma cells.<sup>7</sup> Angiogenesis and signaling through the RAF/mitogen-activated protein/extra cellular signal-regulated kinase (ERK) cascade was reported to play important roles in the development of hepatocellular

carcinomas (HCC)<sup>8</sup> LC-MS/MS assay for the determination of sorafenib in human plasma.<sup>9,10</sup> Sorafenib concentrations in the samples from the patients with hand foot skin reaction were undetectable based on the assays sensitivity.<sup>11</sup>



Scheme1: Structure of sorafenib

## II. INSTRUMENTATION

Direct current polarography and differential pulse polarography were performed with a model 362 polarographic analyzer supplied by Elico Ltd, Hyderabad. Polarographic analyzer connected with Epson LX – 300<sup>+</sup> printer. The electrode assembly consisted of a dropping mercury electrode of surface area  $0.026 \text{ cm}^2$  as the working electrode, a saturated Ag/AgCl(S),  $\text{Cl}^-$  as the reference electrode and a platinum wire as the auxiliary electrode were used. Metrohm unit E 506 polarecord coupled with E612 VA-scanner, E 648 VA – controller and digital electronics x-y/t recorder are used for cyclic voltammetry. Dissolved air from the solutions was removed by degassing with oxygen free nitrogen for 10 – 15 minutes before polarograms taken. pH measurements were carried out with Elico digital pH meter. Potentiostat was supplied by Tec.Ino.Electronics, Lucknow, India used to perform controlled potential electrolysis.

## III. REAGENTS

Sorafenib was kindly provided by Manusaktteva, India. Drugs containing sorafenib labeled to 200 mg per drug were obtained from commercial sources were used without prior

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purification. Sorafenib stock standard solutions ( $1 \times 10^{-3}$  M) were prepared daily by direct dissolution in dimethyl sulfoxide. Human urine samples were obtained from healthy volunteers. The universal buffers of pH 2.0 to 12.0 were prepared by using 0.2M boric acid, 0.05M citric acid and 0.1M trisodium orthophosphate. Triple distilled water was used throughout the experiments. All the experiments were carried out at  $27^\circ\text{C}$  temperature.

#### IV. RECOMMENDED PROCEDURE

From the stock solution, 1.0 ml of electrolyte solution were transferred into a polarographic cell, 9.0 ml of the supporting electrolyte of pH 4.0 were added and deoxygenated with nitrogen gas for 15 minutes. After recording the polarograms small increments (0.2 milliliter) of standard solutions were added and polarograms were recorded after each addition under the similar conditions. The optimum conditions for the determination of sorafenib at pH 2.0 were found to be a drop time 2 sec. pulse amplitude 50 mV and applied potential of -1.31V respectively. The above described analytical procedure has been employed for the determination of sorafenib in pharmaceutical formulations and urine samples.

#### V. ANALYSIS OF DRUG

Ten tablets were weighed and powdered in an Agate Mortar. Portion equivalent to a stock solution of a concentration about  $1 \times 10^{-3}$  M was accurately weighed and transferred into a 100 ml standard flask containing buffer. The content was stirring magnetically for 15 minutes to affect complete dissolution and then diluted to the mark with the selected supporting electrolyte appropriate solutions were prepared by taking suitable aliquots of the clear supernatant and diluted with a buffer solution. Each solution was transferred into a polarographic cell and polarograms were subsequently recorded following the optimized conditions. The content of the drug in tablet was determined referring to standard addition and calibration methods.

#### VI. ANALYSIS OF DRUG IN URINE SAMPLE

Transfer 1.0 ml of human urine sample into a centrifugation tube adds aliquots of sorafenib stock solution and mix well using vortex mixer. Transfer the contents of the centrifugation tube quantitatively into a 25 ml beaker add 9.0 ml of universal buffer solution then transfer the whole contents into a polarographic cell and pass the nitrogen gas for 15 min. and the content of drug in urine sample was determined referring to the calibration graphs.

#### VII. RESULTS AND DISCUSSION

##### a) Influence of pH effect on the polarographic peaks

The polarographic behavior of sorafenib was studied in universal buffer solution. The electrochemical reduction of sorafenib was giving a single well defined cathodic peak at a dropping mercury electrode in pH 2.0 to 6.0. Fig.1 shows a typical d.c.polarogram of  $2.0 \times 10^{-5}$  M solution of sorafenib at pH 4.0 and drop time 2 seconds. When the concentration of the sorafenib increases, the half wave/peak potential

values are found to change to more negative values. Fig.2 shows the cyclic voltammograms of sorafenib at pH 2.0 and scan rate  $40 \text{ mVs}^{-1}$  and concentration  $2.0 \times 10^{-5}$  M. A peculiar behavior was observed i.e., absence of anodic peak in reverse scan and cathodic peak was obtained which may be due to the reduction of carbonyl compound to hydroxyl derivative. The peak height decreased with increase in pH and gave a characteristic  $E_p$  in all the buffer systems because of the decreased available of protons. Fig.3 shows the typical differential pulse polarogram of sorafenib at pH 4.0, concentration  $2.0 \times 10^{-5}$  M and drop time two seconds. There was no peaks obtained in the basic medium (pH 8.0 to 12.0) due to the precipitation of the electroactive species. The simultaneous reduction of the two carbonyl groups into the corresponding hydroxyl derivative in a four electrons process. The peaks were developed in acidic medium (pH 2.0 to 6.0).

##### b) Nature of the electrode process

The electrode process was found to be diffusion controlled in all the buffer systems studied, as shown by the linear dependence of limiting current on  $h^{1/2}$ ,  $v^{1/2}$  and  $t^{2/3}$ . All the plots are observed to be passing through origin indicating the absence of adsorption complications. When the concentrations of the sorafenib increases, the  $E_{1/2}$ ,  $E_p$  and  $E_m$  values are found to change to more negative values indicated the irreversibility of the electrode process. The marginal variation of peak potential ( $E_m$ ) with concentration, nonlinearity in the plot of  $i_m$  vs  $(1-\sigma/1+\sigma)$  in differential pulse polarography and disobedience of Tomes' criterion also confirm the irreversible nature of the electrode process. Millicoulometry employed at pH 2.0 to find out the number of electrons involved in the electrode process. The results showed the number of electrons to be four for sorafenib. From the slope of  $E_{1/2}$  vs pH plot, the number of protons involved in the rate determining step of the electrode process in found to be two. Controlled potential electrolysis experiments are carried out at -0.27V vs saturated calomel electrode at pH 4.0. The isolated product was identified as hydroxyl product and confirmed by I.R. spectral method (absence of C=O stretch  $1690 - 1650 \text{ cm}^{-1}$ , O-H stretch  $3420 \text{ cm}^{-1}$ , O-H bend  $1360 \text{ cm}^{-1}$  and C-O stretch  $1240 \text{ cm}^{-1}$ ). For the irreversible process, the value of  $\alpha_{na}$  was calculated from the equation.

$$E = E_{1/2} - \frac{0.0542}{\alpha_{na}} \log \left( \frac{i}{i_d - i} \right),$$

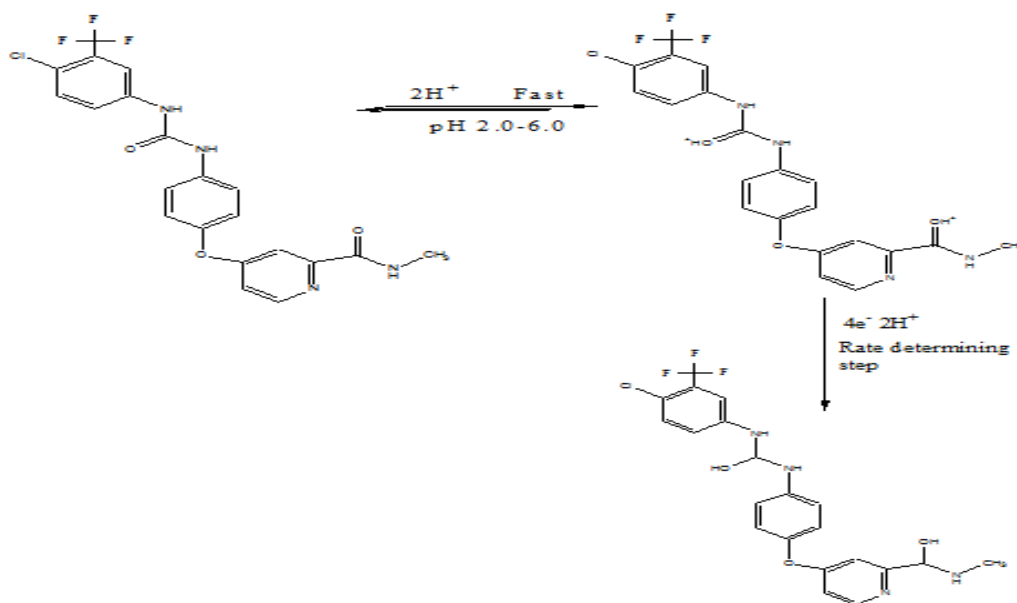
where,  $i$  was the cathodic current in  $\mu\text{A}$ ,  $i_d$  was the cathodic diffusion current in  $\mu\text{A}$  and the  $\alpha_{na}$  are illustrated in Table 1. In polarography, the theoretical equation for the maximum diffusion current obtained with a dropping mercury electrode, which was first derived by Ilkovic<sup>12-16</sup> was given by  $i_d = 708 \text{ nCD}^{1/2} m^{2/3} t^{1/6}$ . The diffusion coefficients values were obtained in a good agreement indicating the diffusion controlled and adsorption free nature of the electrode process. The variation of diffusion current with the pH of the supporting electrolyte influences the diffusion

coefficient values also to vary in the same manner. The reason for slight variation in diffusion coefficient values with increase in pH may be attributed to the decrease in the availability of protons with increase in pH of the supporting electrolyte. The number of protons ( $Z$ ) involved in the rate determining step of the electrode reaction is given by  $\Delta E_{1/2} / \Delta \text{pH} = -0.059P/\alpha_{\text{na}}$ . The number of proton was determined to be 1.45, i.e. two protons were probably consumed in the rate determining step of the electrode reaction. The heterogeneous forward rate constant values ( $k_{\text{th}}^0$ ) are found

to decrease with increasing pH indicating that the electrode reaction in more and more irreversible with increasing pH of the solution.

### c) Electrode mechanism

Based on the experimental results obtained from all the techniques employed, a possible electrochemical reduction mechanism has been suggested on the basis of protons and electrons involved in the reduction as follows.



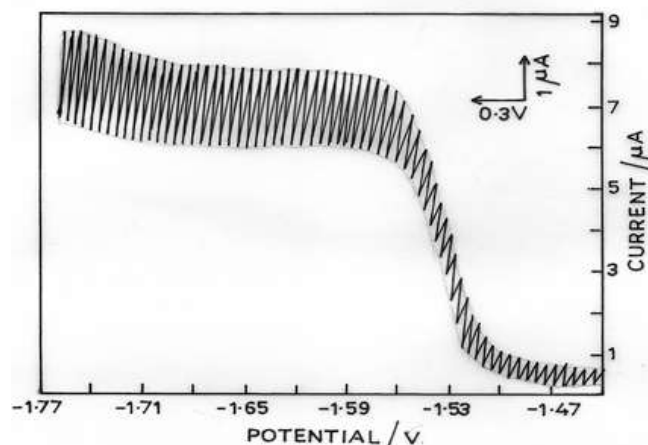
**Scheme 2: Electrode mechanism of sorafenib**

The differential pulse polarography was used for the determination of the sorafenib. Both calibration and standard addition methods are used. The polarographic peaks obtained in the pH range 2.0 to 6.0 are well resolved and reproducible. Calibration plots are linear for sorafenib in the concentration over the range from  $5.0 \times 10^{-8}$  to  $1.0 \times 10^{-5}$  M, the height of the peak was a linear function of the concentration at any pH value. The lower detection limit was calculated as  $4.2 \times 10^{-8}$  M using the expression  $dl = 3 \times Sd/m$ , where  $Sd$  was the standard deviation and  $m$  was the slope of the calibration plot.

### VIII. RECOMMENDED ANALYTICAL PROCEDURE

A stock solution ( $1 \times 10^{-3}$  M) was prepared by dissolution of the appropriate amount of the electroactive species in dimethyl sulfoxide due to the low solubility of the drug in water. 1.0 ml of the standard solution was transferred into polarographic cell and made up with 9 ml of the supporting electrolyte and then deoxygenated with nitrogen gas for 10 min. After recording the polarogram small increments (0.3 ml) of standard solution were added, and the polarograms are recorded after each addition under similar conditions. In the present study, the best precision was obtained at pH 4.0 with a drop time of 2 sec, pulse

amplitude of 50 mV, and peak potential of -1.54 V for sorafenib respectively. The relative standard deviation and correlation coefficients values are found to 0.326 and 0.65 for the respective compound for 10 replicates.



**Fig.1 Typical d.c. polarogram of sorafenib at pH 4.0  
Concentration:  $2.0 \times 10^{-5}$  M, Drop time: 2 Sec**

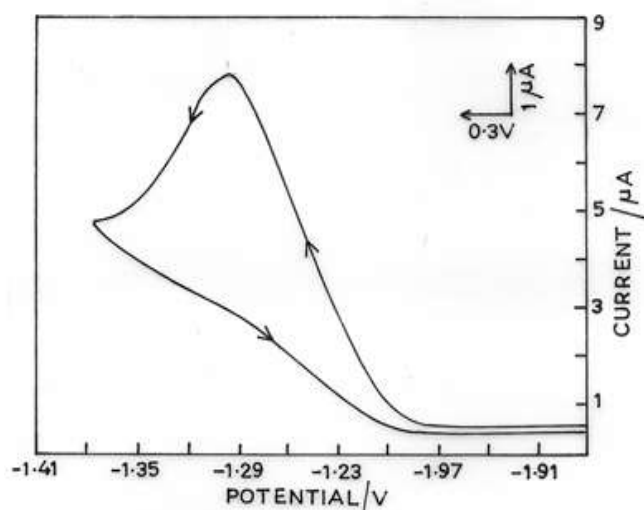


Fig. 2. Typical cyclic voltammogram of sorafenib at pH 2.0, Concentration:  $2.0 \times 10^{-5}$  M, Scan rate:  $40 \text{ mVs}^{-1}$

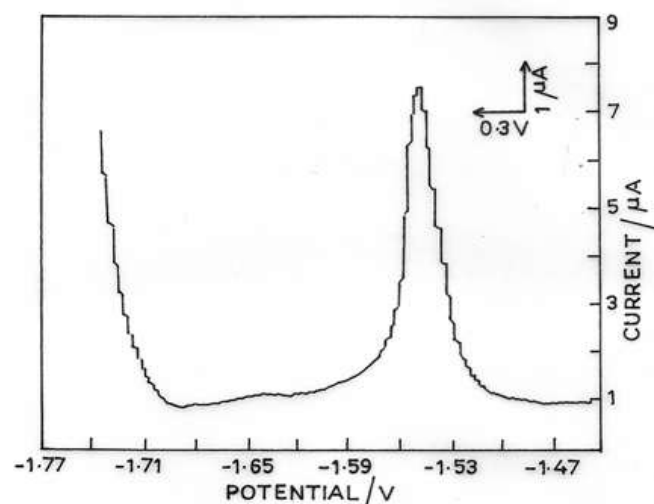


Fig. 3. Typical differential pulse polarogram of sorafenib at pH 4.0, Concentration:  $2.0 \times 10^{-5}$  M, Drop time: 2 sec, Pulse amplitude: 50 mV.

| pH of the supporting electrolyte | $-E_{1/2}/\text{V}$ | $I_d/\mu\text{A}$ | $\Delta\text{pH}$ | $\Delta E_{1/2}$ | $\frac{\Delta E_{1/2}}{\Delta\text{pH}}$ | $\alpha_{na}$ |
|----------------------------------|---------------------|-------------------|-------------------|------------------|------------------------------------------|---------------|
| 2.0                              | 1.32                | 8.40              | 1                 | 0.15             | 0.15                                     | 0.82          |
| 3.0                              | 1.47                | 7.95              |                   |                  |                                          | 0.77          |
| 4.0                              | 1.55                | 7.30              | 1                 | 0.08             | 0.08                                     | 0.72          |
| 5.0                              | 1.62                | 6.70              | 1                 | 0.07             | 0.07                                     | 0.67          |
| 6.0                              | 1.67                | 6.10              | 1                 | 0.05             | 0.05                                     | 0.58          |

Table 1: – Effect of pH on the polarographic behavior of sorafenib

| pH of the supporting electrolyte | D.C. Polarography   |                                                     |                                    | Cyclic voltammetry |                                                     |                                    | Differential pulse polarography |                                                     |                                    |
|----------------------------------|---------------------|-----------------------------------------------------|------------------------------------|--------------------|-----------------------------------------------------|------------------------------------|---------------------------------|-----------------------------------------------------|------------------------------------|
|                                  | $-E_{1/2}/\text{V}$ | $\frac{D \times 10^{-5}}{\text{Cm}^2\text{s}^{-1}}$ | $\frac{K_{cb}^0}{\text{Cms}^{-1}}$ | $-E_p/\text{V}$    | $\frac{D \times 10^{-5}}{\text{Cm}^2\text{s}^{-1}}$ | $\frac{K_{cb}^0}{\text{Cms}^{-1}}$ | $-E_m/\text{V}$                 | $\frac{D \times 10^{-5}}{\text{Cm}^2\text{s}^{-1}}$ | $\frac{K_{cb}^0}{\text{Cms}^{-1}}$ |
| 2.0                              | 1.32                | 2.82                                                | $8.54 \times 10^{-4}$              | 1.30               | 2.79                                                | $7.26 \times 10^{-4}$              | 1.31                            | 2.86                                                | $7.86 \times 10^{-4}$              |
| 3.0                              | 1.47                | 2.64                                                | $6.26 \times 10^{-4}$              | 1.45               | 2.68                                                | $4.82 \times 10^{-4}$              | 1.46                            | 2.62                                                | $2.48 \times 10^{-4}$              |
| 4.0                              | 1.55                | 2.47                                                | $4.25 \times 10^{-5}$              | 1.56               | 2.51                                                | $5.52 \times 10^{-5}$              | 1.54                            | 2.38                                                | $6.84 \times 10^{-5}$              |
| 5.0                              | 1.62                | 2.24                                                | $1.62 \times 10^{-5}$              | 1.64               | 2.10                                                | $3.10 \times 10^{-5}$              | 1.63                            | 2.18                                                | $2.26 \times 10^{-5}$              |
| 6.0                              | 1.67                | 1.97                                                | $5.47 \times 10^{-6}$              | 1.65               | 2.06                                                | $6.26 \times 10^{-6}$              | 1.66                            | 2.02                                                | $4.12 \times 10^{-6}$              |

TABLE 2: - Typical kinetic data of sorafenib

| S.No. | Sample    | Type                       | Labeled amount mg/L | Average amount found mg/L | Recovery % | Standard deviation | %Relative Standard deviation |
|-------|-----------|----------------------------|---------------------|---------------------------|------------|--------------------|------------------------------|
| 1     | sorafenib | Pharmaceutical formulation | 200                 | 199.20                    | 99.60      | 0.40               | 0.200                        |
| 1     | sorafenib | Urine                      | 200                 | 198.70                    | 99.35      | 0.65               | 0.326                        |

**TABLE 3: - Polarographic assay of sorafenib by DPP in pharmaceutical formulations and in spiked human urine samples.**

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