

ISSN- 0975-1491

Vol 3, Suppl 3, 2011

**Research Article** 

# VOLTAMMETRIC BEHAVIOR OF GEFITINIB AND ITS ADSORPTIVE STRIPPING VOLTAMMETRIC DETERMINATION IN PHARMACEUTICAL FORMULATIONS AND URINE SAMPLES

## C.NAGESWARA REDDY, P.REDDY PRASAD AND N.Y.SREEDHAR\*

\*Electroanalytical Lab, Department of Chemistry, S.V.University, Tirupati, A.P,India Email: sreedhar\_ny@rediffmail.com, chennupallireddy@gmail.com

## Received: 25 Feb 2011, Revised and Accepted: 23 March 2011

## ABSTRACT

Gefitinib is a drug that is used to treat several types of lung cancer and in particular, it is used alone for the treatment of patients with a specific type of lung cancer termed non-small cell lung cancer (NSCLC). An adsorptive stripping voltammetric method was developed for the quantitative analysis of gefitinib gives a peak at -0.78 V at HMDE. From the structural point of view gefitinib contains a >C=N- moiety which can be electrochemically reduced at universal buffer (pH 4.0). Millicoulometric experiment is performed successfully in estimating the number of electrons and proton to understand reduction mechanism. The adsorptive stripping voltammetric peak was adequately well-resolved, reproducible and linear dependent with the gefitinib concentration. For quantification the calibration plot method for gefitinib concentrations ranging between  $1.0 \times 10^{-4}$  M to  $1.0 \times 10^{-8}$  M at pH 4.0 was selected. The proposed AdSV method was successfully applied to the determination of gefitinib in pharmaceutical formulations and urine samples.

Keywords: Gefitinib, AdSV, Pharmaceutical formulations and urine samples.

## INTRODUCTION

Gefitinib(iressa) is an anilinoquinazoline with the chemical name 4-Quinazolinamine, N-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-4morpholin) propoxy] and It has the molecular formula C22H24ClFN4O3, a relative molecular mass of 446.9 and is a whitecolored powder. Gefitinib is a free base. Gefitinib can be defined as sparingly soluble at pH 1.0, but is practically insoluble above pH 7.0, with the solubility dropping sharply between pH 4.0 and pH 6.0. In non-aqueous solvents, gefitinib is freely soluble in glacial acetic acid and dimethyl sulphoxide, soluble in pyridine, sparingly soluble in tetrahydrofuran, and slightly soluble in methanol, ethanol (99.5%), ethyl acetate, propan-2-ol and acetonitrile. The recommended daily dose of iressa is one 250 mg tablet with or without food. Higher doses do not give a better response and cause increased toxicity. The mechanism of the clinical anti tumor action of gefitinib is not fully characterized. Gefitinib inhibits the intracellular phosphorylation of numerous tyrosine kinases associated with trans membrane cell surface receptors, including the tyrosine kinases associated with the epidermal growth factor receptor (EGFR-TK). EGFR is expressed on the cell surface of many normal cells and cancer cells. No clinical studies have been performed that demonstrate a correlation between EGFR receptor expression and response to gefitinib. Gefitinib is approved for patients with advanced non-small cell lung cancer. The compound is designed to target and block the activity of the tyrosine kinase that signals the epidermal growth factor receptor (EGFR).1-3 EGFR promotes the growth, division, and spread of cancer cells.4 Specific method for determination of gefitinib in human plasma, mouse plasma and tissues using high performance liquid chromatography coupled to tandem mass spectrometry.5

Gefitinib is the first selective inhibitor of epidermal growth factor receptor's (EGFR) tyrosine kinase domain. The target protein (EGFR) is also sometimes referred to as Her1 or ErbB-1 depending on the literature source. EGFR is over expressed in the cells of certain types of human carcinomas - for example in lung and breast cancers. This leads to inappropriate activation of the anti-apoptotic signal transduction cascade, eventually leading to uncontrolled cell proliferation. Research on gefitinib-sensitive non-small cell lung cancers has shown that a mutation in the EGFR tyrosine kinase domain is responsible for activating anti-apoptotic pathways.<sup>6,7</sup> While gefitinib has yet to be proven to be effective in other cancers, there is certainly potential for its use in the treatment of other cancers where EGFR over expression is involved. In 2004, Astra

Zeneca informed the United States Food and Drug Administration (FDA) that a large randomized study<sup>8</sup> failed to demonstrate a survival advantage for gefitinib in the treatment of non-small cell lung cancer (NSCLC).<sup>9,10</sup> Literature survey reveals that few assay methods, for estimation of gefitinib, like liquid chromatography and mass spectrometry, enzyme-linked immunosorbent assay were repored.<sup>11,12</sup>

In the present study, a simple and rapid method was developed for trace analysis of gefitinib in pharmaceutical formulations and urine samples by AdSV. Therefore, electrochemical behavior of gefitinib in aqueous buffered media to arrive at the information on the mechanistic aspects and the electrode kinetics concerned and an attempt has also been made to determine the title compound in pharmaceutical formulations and urine samples by using adsorptive stripping voltammetry without any prior separations.

## EXPERIMENTAL

## Materials

All chemicals were reagent grade chemicals (Merk, India), doubly distilled water was used in preparation of all solutions. Stock solution  $(1.0 \times 10^{-4} \text{ mol dm}^3)$  was prepared by dissolving Gefitinib in dimethylformamide. All dilute solutions were freshly prepared daily from the stock solution. Universal buffer solution ranging from pH 2.0 to 12.0 were prepared using 0.2M boric acid, 0.01M citric acid and 0.1M trisodium orthophosphate.

#### Instrumentation

Adsorptive stripping Voltammetric measurement were carried on Metrohm 757 VA computrace Model AFRDE 4 potentiostat and MSRX speed control unit supplied from Pine Instrument Company (USA) coupled with digital electronics200X-y/t recorder. All the solutions were degassed prior to analysis by bubbling purified nitrogen gas through the cell for 10 min. All the experiments were performed at  $25\pm1^{\circ}$ C, pH measurements were carried out with a Hanna instruments (Italy) pH meter.

## **Recommended procedure**

A recommended standard stock solution (1.0x10<sup>-4</sup> M) was prepared by dissolving the required amount of the gefitinib in dimethylformamide. Standard solutions are prepared by dilution of the stock solution with suitable amount of dimethyl formamide. In voltammetric cell 1 ml of standard solution is transferred and made up to 10 ml with 9 ml of the universal buffer of pH 4.0 and deoxygenated with pure nitrogen gas for 15 min. After obtaining the voltammogram small additions of (0.2 ml) of standard solutions are added and voltammogram recorded after each addition under

similar experimental parameters. The optimum conditions for the determination of gefitinib in pH 4.0 is found to be a drop time of 2 sec, a pulse amplitude of 25 mV and applied potential of -0.84 V.



#### Scheme 1: Reduction mechanism of gefitinib

## **RESULTS AND DISCUSSIONS**

## Cyclic voltammetric studies

In the present investigation, gefitinib found to exhibit two well separated peaks of almost equal heights over the pH range 2.0 to 6.0. The first and second peaks are attributed to the reduction of gefitinib with two-electron signals and which are found to change with pH. In acidic media (pH 2.0 to 6.0) a four electron reduction is noticed and first step might be due to hydrolysis of -C=N- bond. The imine thus formed is then protonated and further reduction with an addition of four electrons could result in the second signal. This is primarily due to the saturation of azomethine group. Typical cyclic voltammogram is shown in Fig. 1.

The linear plot of  $i_p$  vs v<sup>1/2</sup> passing through the origin, indicate the electrode process to be mainly diffusion controlled and adsorption controlled. The irreversibility of the electrode process is verified by logarithmic analysis of the peak. Absence of anodic peak in the reverse scan, indicates the over all electrode processes to be irreversible. The shift in  $E_p$  values towards more negative potentials with an increase in concentration of electroactive species also suggests the irreversible nature of the peaks. The peak potentials of gefitinib are found to be pH dependent and shift towards more negative values with increase in pH indicating the involvement of protons in the reduction process. The  $\alpha$  n<sub>a</sub> values for the reduction of azomethine group in gefitinib is found to be in the order of 0.96. The number of protons participated in the rate determining step is found to be two.

The total number of electrons involved in the electrode process has been established experimentally by millicoulometry. From the comparison of the peak heights observed, the number of electron involved in the reduction process of the gefitinib is four. Controlled potential electrolysis experiments are carried out at -0.84 V vs SCE and pH 4.0 also agree with the above results. The results of our investigations as well as on data from the literature, the electrode mechanism is proposed for electrochemical reduction of gefitinib (Scheme 1)

#### Kinetic data

Typical kinetic parameters evaluated from cyclic voltammetric technique are reported in the Table 1. The diffusion and adsorption

controlled nature of the electrode process is clearly evidenced from the nearly equal diffusion efficient values obtained from the cyclic voltammetric technique. The 'D' values are seen to gradually decrease which account for decrease in diffusion current with increase in pH due to less availability of protons. The forward rate constant values are observed to be low in basic media (pH 8.0) when compared to that obtained in acidic media, since the unprotonated azomethine being the electroactive species in the acidic region. The k<sup>0</sup><sub>fh</sub> values are found to decrease with increase in pH indicating that the electrode reaction becomes more and more irreversible with increase in pH of the solution.

## Adsorptive stripping voltammetric studies

The present investigation, Fig.2.shows adsorptive stripping voltammetric response for  $1.2 \times 10^{-4}$  M gefitinib in universal buffer of pH 2.0 to 12.0 after preconcentration of the drug onto the HMDE for 60 sec. Two cathodic irreversible peaks are obtained over the pH range 2.0 to 6.0.

The first peak recorded within the pH range 2.0 to 6.0 is due reduction and second peak is due to >C=N- bond. A Single much more developed and sharp compared to both the first and second peaks was generated in solutions of pH 4.0. Therefore the latter peak obtained in universal buffer of pH 8.0 was considered in the present analytical study.

#### Effect of pH

The effect of pH on the adsorptive stripping voltammetric response for  $1.0 \times 10^{-4}$  M gefitinib was examined in universal buffer of pH 2.0 to 12.0 (Fig.3) after pre-concentration of the chemotherapeutic drug onto the HMDE for 60 sec. At pH 4.0, one cathodic irreversible peak was obtained.

Therefore, the first peak obtained in universal buffer at pH 4.0 was considered in the present analytical study.

## Effect of accumulation potential

The influence of the accumulation potential on the peak height was studied from -0.0 to-1.0V and a strong adsorption at -0.84V (Fig.4) was observed, so this potential was used as the accumulation potential for all the measurements.



Fig. 1: Typical cyclic voltammogram at gefitinib at HMDE accumulation time: 60sec; pH: 4.0; accumulation potential: -0.84V; rest time: 10sec; stirring rate: 400rpm; pulse amplitude: 25 mV, concentration: 1.0×10<sup>-4</sup>M; scan rate: 40mVs<sup>-1</sup>



Fig. 2: Adsorptive stripping voltammogram at gefitinib at HMDE, accumulation time: 60sec; pH: 4.0; accumulation potential: -0.84V;rest time: 10sec; stirring rate: 400rpm; pulse amplitude: 25 mV, scan rate: 40mVs<sup>-1</sup>; concentration : (a) blank (b) 1.0×10<sup>-4</sup>M.



Fig. 3: Effect of pH on the AdSV of Gefitinib at HMDE accumulation time: 60 sec; rest time: 10sec; scan rate: 40 mVs<sup>-1</sup>; accumulation potential: -0.84V: Stirring rate: 400 rpm; Pulse amplitude: 25 mV.

## Effect of accumulation time

The dependence pf the peak on the accumulation time  $t_{\rm acc}$  (sec.), on the adsorptive stripping peak current of the first peak of gefitinib in universal buffer of pH 4.0 is investigated. As shown in Fig. 5. accumulation time of 60 sec. at -0.84 V generated a much more peak current.

The peak current increased with an increasing scan rate. The experiment indicate further that when  $t_{acc}$  60sec and peak current( $i_{\rm p})$  show a linear relationship with illustrating that the reduction of gefitinib is diffusion controlled adsorption process.

## Applications

The developed analytical procedure has been successfully employed for the determination of gefitinib in pharmaceutical formulations namely iressa. The equivalent to 2 and 10 mg of gefitinib were accurately weighed dissolved in pure dimethylformamide and transferred into 10 ml calibrated flasks. A 0.5 ml aliquot of the clear supernatant liquid was diluted to 10 ml with the supporting electrolyte (pH 4.0) and voltammograms were recorded. In order to establish the reliability and suitability of the proposed method, recovery experiments were performed. The amount of the compound in portion of the sample taken was calculated by reference to the calibration plot. The assay results for gefitinib in pharmaceutical formulation at pH 4.0 are given in Table.2. The recoveries are in the range 93.50 % to 99.40 % for gefitinib for sample respectively. The reliability of the method for that determination of gefitinib in urine is checked by using different urine samples. Direct determinations in undigested urine may yield for certain probands unreliable data due to trace metal trapping by natural chelating substances. An aliquot of 1.5 ml of urine without treatment were diluted with water to 25 ml in a volumetric flask; a 0.1 ml aliquot of this solution was transferred into a 25 ml calibrated flask; 5 ml of universal buffer pH 4.0 were added and diluted to the mark with water. The samples were measured according to the above described procedure. The voltammograms of samples without gefitinib do not show any signal that can interfere with direct determination.

The intention of our experiments with urine samples is to study various possibilities to overcome a biomatrix effect and to find the best solution but to explore the way for simple, reliable, fast determination of gefitinib in urine by its dilution on employing AdSV. The potential interference of some urine ingredients both organic and inorganic salts is checked without adding depolarizer. None of these ingredients did affect the determination gefitinib. The recoveries are in the range of urine samples 93.73% to 99.60%. The obtained results were presented in Table 3.

Adsorptive stripping voltammetry has been used for the quantitative determination of gefitinib using both standard addition and calibration methods in pharmaceutical formulation and urine samples. Quantitative measurements were successful in the concentration range of  $1.0 \times 10^{-4}$  to  $1.0 \times 10^{-8}$  M with lower detection limit of  $1.2 \times 10^{-8}$  M. The correlation coefficient and standard deviation were 0.996 and 1.4% respectively.



Fig. 4: Effect of accumulation potential on the AdSV of gefitinib accumulation time: 60 sec; pH: 4.0; rest time: 10 se; stirring rate: 400 rpm; scan rate: 40mVs<sup>-1</sup>; pulse amplitude: 25 mV.



Fig. 5: Effect of accumulation time on the AdSV of gefitinib at pH: 4.0; accumulation potential:-0.84 V; rest time: 10 sec; stirring rate: 400 rpm; scan rate: 40mVs<sup>-1</sup>; pulse amplitude: 25 mV.

PH of the Supporting Electrolyte	$\frac{-Ep}{V}$	$rac{i_p}{\mu A}$	$\alpha_{na}$	$\frac{Dx10^6}{cm^2S^{-1}}$	$\frac{K^0f,h}{CmS^{-1}}$
2.0	0.61	4.07	0.85	5.91	9.57x10-9
4.0	0.70	4.02	0.82	5.86	7.39x10 <sup>-10</sup>
6.0	0.76	3.68	0.72	5.58	7.22x10 <sup>-12</sup>
8.0	0.84	3.52	0.55	5.32	6.18x10 <sup>-14</sup>
10.0	0.98	3.33	0.52	5.14	3.86x10 <sup>-15</sup>
12.0	1.22	3.39	0.49	5.07	9.98x10 <sup>-18</sup>

## Table 1: Typical cyclic voltammetric data of gefitinib

Table 2: Determination of gefitinib in pharmaceutical formulations

Labeled amount (mg)	Amount found (mg)	Average recovery (%)	Standard deviation	
5.0	4.87	97.40	0.011	
10.0	9.94	99.40	0.015	
20.0	18.70	93.50	0.013	
	Labeled amount (mg) 5.0 10.0 20.0	Labeled amount Amount found   (mg) (mg)   5.0 4.87   10.0 9.94   20.0 18.70	Labeled amount (mg) Amount found (mg) Average recovery (%)   5.0 4.87 97.40   10.0 9.94 99.40   20.0 18.70 93.50	Labeled amount (mg) Amount found (mg) Average recovery (%) Standard deviation   5.0 4.87 97.40 0.011   10.0 9.94 99.40 0.015   20.0 18.70 93.50 0.013

## Table 3: Determination of gefitinib in urine Samples

Sample	Labeled amount (mg)	Amount found (mg)	Recovery (%)	Standard deviation
1	5.0	4.92	98.40	0.012
2	10.0	9.96	99.60	0.016
3	15.0	14.06	93.73	0.015

## CONCLUSION

In conclusion in this work a simple low cost and low sample consumption adsorptive stripping voltammetric method can be used satisfactorily for determination of gefitinib in urine and pharmaceutical formulations. The result obtained revealed that the proposed procedure as good accuracy. The proposed method using AdSV not only is one of the most sensitive method for the determination of gefitinib but also excellent interms of selectivity and simplicity.

## REFERENCES

- 1. F. Ciardello, G.A. Tortora, Clin Cancer Res (2001)7,2958-70
- 2. F. Ciardiello, R.Caputo, R.Bianco, et al. Clin Cancer Res (2000) 6,
- 3. F.M.Sirotnak, Semin Oncol, 2003(1) 12-20.
- F.Cappuzzo, E.Magrini, G.L.Ceresoli, S. Bartolini, E.Rossi, V. Ludovini, V.Gregorc, C.Ligorio, A.Cancellieri, S.Damiani, A.Spreafico, C.T. Paties, L.Lombardo, C. Calandri, G.Bellezza. M.Tonato, and L.Crino, J. Natl.Can. Inst., (2004), 96, 1133.

- Z. Ming, H. Carol; J. Antonio; L. Jing, H. Ping, Z. Yelena, M. Hidalgo, S.D.Baker, Journal of chromatography. 819 (2005) 73.
- W. Pao, V. Miller, M. Zakowski, J. Doherty, K. Politi, I. Sarkaria, Proc Natl Acad Sci. 101 (2004) 13306.
- R. Sordella, D.W. Bell, D.A. Haber, J. Settleman, Science. 305 (2004) 1163.
- 8. S. Rossi, editor. Australian Medicines Handbook (2004) Adelaide, Australian Medicines Handbook (2004).
- 9. G. Giaccone, RS, Herbst, C.Magegold, G.Scagliotti, R.Rosell, V.Miller, RB. Natle, J. Clin Oncol (2004), 22, 777-84.
- 10. RS.Herbst, G.Giaccone, JH.Schiller, RB.Natale, J Clin Oncol (2004), 22,785-94.
- M. Zho, C. Hartke, A.Jimeno, J. Li, P.He, Y.Hidalgo, S.D.Baker, J Chromatogr B Analyt Technol Biomed Life Sci, (2005) 819(1),73-80.
- 12. S.Tetsuya, H.Fujito and M.Masato, Biological and Pharmaceutical Bulletin, (2005), 28,1833-137.