



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

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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 1.0×10^{-4} M to 1.0×10^{-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-(4-morpholin) propoxy] and It has the molecular formula $C_{22}H_{24}ClFN_4O_3$, a relative molecular mass of 446.9 and is a white-colored 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. 6,7 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 study⁸ failed to demonstrate a survival advantage for gefitinib in the treatment of non-small cell lung cancer (NSCLC).^{9,10} Literature survey reveals that few assay methods, for estimation of gefitinib, like liquid chromatography and mass spectrometry, enzyme-linked immunosorbent assay were reported.^{11,12}

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 (1.0×10^{-4} 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 electronics 200X-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 $25 \pm 1^\circ C$, pH measurements were carried out with a Hanna instruments (Italy) pH meter.

Recommended procedure

A recommended standard stock solution (1.0×10^{-4} 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

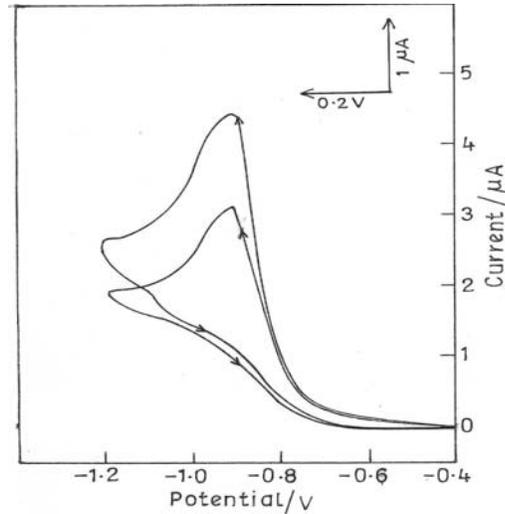


Fig. 1: Typical cyclic voltammogram at gefitinib at HMDE accumulation time: 60sec; pH: 4.0; accumulation potential: -0.84V; rest time: 10sec; stirring rate: 400rpm; pulse amplitude: 25 mV, concentration: 1.0×10^{-4} M; scan rate: 40 mVs^{-1}

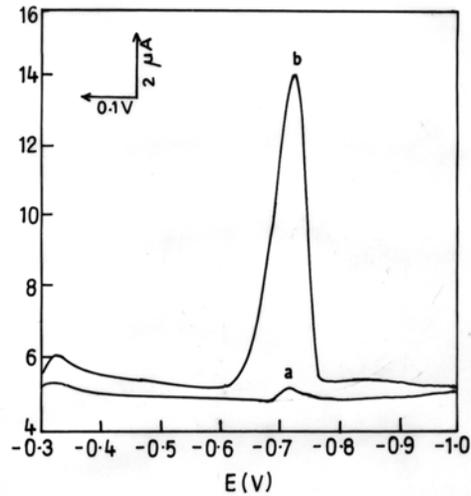


Fig. 2: Adsorptive stripping voltammogram at gefitinib at HMDE, accumulation time: 60sec; pH: 4.0; accumulation potential: -0.84V; rest time: 10sec; stirring rate: 400rpm; pulse amplitude: 25 mV, scan rate: 40 mVs^{-1} ; concentration : (a) blank (b) 1.0×10^{-4} M.

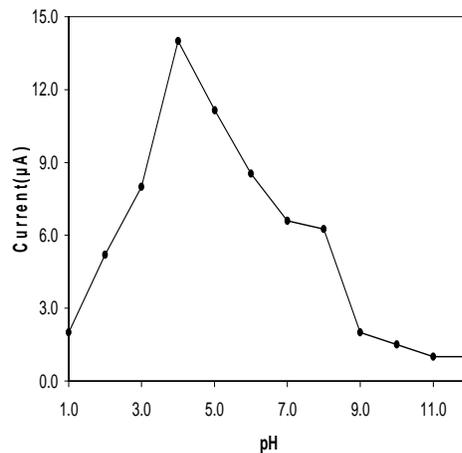


Fig. 3: Effect of pH on the AdSV of Gefitinib at HMDE accumulation time: 60 sec; rest time: 10sec; scan rate: 40 mVs^{-1} ; accumulation potential: -0.84V; Stirring rate: 400 rpm; Pulse amplitude: 25 mV.

Effect of accumulation time

The dependence of the peak on the accumulation time t_{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 indicates further that when t_{acc} 60sec and peak current (i_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 of 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 1.0×10^{-4} to 1.0×10^{-8} M with lower detection limit of 1.2×10^{-8} M. The correlation coefficient and standard deviation were 0.996 and 1.4% respectively.

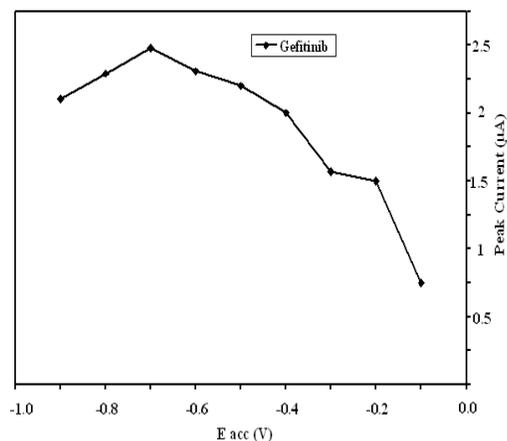


Fig. 4: Effect of accumulation potential on the AdSV of gefitinib accumulation time: 60 sec; pH: 4.0; rest time: 10 se; stirring rate: 400 rpm; scan rate: 40mVs⁻¹; pulse amplitude: 25 mV.

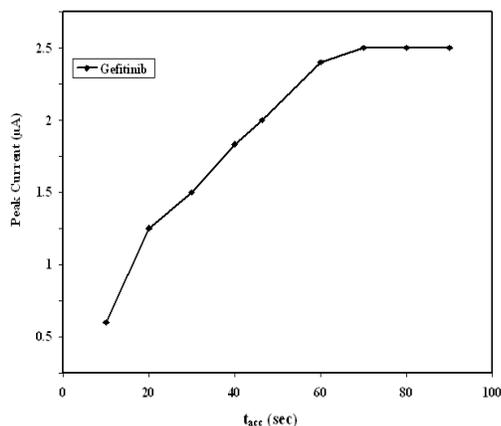


Fig. 5: Effect of accumulation time on the AdSV of gefitinib at pH: 4.0; accumulation potential:-0.84 V; rest time: 10 sec; stirring rate: 400 rpm; scan rate: 40mVs⁻¹; pulse amplitude: 25 mV.

Table 1: Typical cyclic voltammetric data of gefitinib

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

Table 2: Determination of gefitinib in pharmaceutical formulations

Name of the formulation	Labeled amount (mg)	Amount found (mg)	Average recovery (%)	Standard deviation
Iressa	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 in terms of selectivity and simplicity.

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