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SHORT COMMUNICATION

Voltammetric quantitation of nitazoxanide by glassy carbon electrode

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Abstract The present study reports voltammetric reduction of nitazoxanide in Britton–Robinson (B–R) buffer by cyclic and square-wave voltammetry at glassy carbon electrode. A versatile fully validated voltammetric method for quantitative determination of nitazoxanide in pharmaceutical formulation has been proposed. A squrewave peak current was linear over the nitazoxanide concentration in the range of 20–140 μg/mL. The limit of detection (LOD) and limit of quantification (LOQ) was calculated to be 5.23 μg/mL and 17.45 μg/mL, respectively.

1. Introduction

Intestinal parasitic infections rank among the most significant causes of morbidity and mortality in the world today. Nitazoxanide (Scheme 1) is a new nitrothiazole benzamide compound notable for its activity in treating both intestinal protozoal and helminthic infections [1]. Nitazoxanide in humans has been reported to be effective against a broad range of parasites, including *Giardia lamblia*, *Entamoeba histolytica and Cryptosporidium parvum* [2].

Literature survey reveals few analytical methods including high-performance liquid chromatography (HPLC) [3], spectrophotometry [4], stability indicating high performance thin layer chromatography (HPTLC) [5] and colorimetric method [6] for the quantification of nitazoxanide. These methods are time consuming and require large number of complicated steps to follow on for analysis. For this purpose the desirable technique for the analysis of drugs should be rapid, simple, low cost and of high sensitivity in analysis. Therefore, in order to have better technique for analysis of drugs electrochemical methods have been applied [7,8]. There are already reported electrochemical methods for the analysis of the nitazoxanide [9–12], these methods show low sensitivity compared to proposed voltammetric method for the detection of nitazoxanide. The purpose of the present work is to study the voltammetric behavior of nitazoxanide by employing cyclic and squarewave voltammetry and to establish the methodology for the determination of nitazoxanide in pharmaceutical formulation.

2. Experimental

2.1. Instrumentation

Electrochemical measurements were performed using a μ-Autolab type III (Eco-Chemie B.V., Utrecht, The Netherlands) potentiostat–galvanostat with 757 VA computrace software. The

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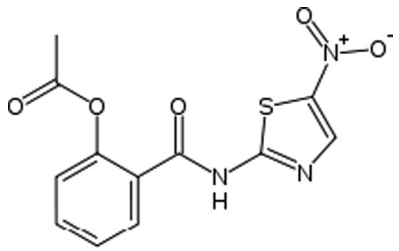


Scheme 1 Structure of nitazoxanide.

utilized electrodes were glassy carbon as working electrode, Ag/AgCl (3 M KCl) as reference electrode and platinum wire as auxiliary electrode. The electrochemical cell was a Metrohm 663 VA stand (Metrohm AG, The Netherlands). Controlled potential coulometric experiments were carried out using an Autolab Potentiostat/Galvanostat PGSTAT Metrohm 663 VA stand as electrochemical cell, fitted with a PC provided with the appropriate GPES 4.2 software. All the solutions examined by electrochemical technique were purged for 10 min with purified nitrogen gas. All pH-metric measurements were made on a Decible DB-1011 digital pH meter fitted with a glass electrode and a saturated calomel electrode as reference, which was previously standardized with buffers of known pH.

2.2. *Reagents and materials*

Nitazoxanide (99% purity) was obtained from Alembic limited India and used as received. Capsules containing nitazoxanide (*Nitacure*) labeled 500 mg were obtained from commercial sources. The solvents (1,4 dioxane, dimethyl formamide (DMF), acetonitrile (ACN) and ethanol) and surfactants (cetrimide and tween-20) were purchased from Sigma-Aldrich, India. Britton–Robinson (B–R) supporting buffers of different pH values were prepared by dissolving appropriate volumes of boric acid and glacial acetic acid in distilled water.

2.3. *Procedure for pharmaceutical preparation*

The contents of 10 capsules were weighed and powdered in a mortar. A weighed portion of the powder was transferred to a 250 mL volumetric flask containing dioxane to form a stock solution of 1 mg/mL and then flask was put in ultrasonic bath for 5 min. A portion of this solution was centrifuged and the supernatant was filtered through a filter paper and filtered solution was then transferred to the voltammetric cell and voltammograms were recorded. Potassium chloride (1.0 M) solution was prepared in distilled water and used as supporting electrolyte. The content of the drug in pharmaceutical formulation was determined using calibration curve.

2.4. *Pretreatment of glassy carbon electrode*

Before each measurement, the glassy carbon electrode (GCE) was polished on a polishing micro-cloth with 0.5 μM alumina powder and rinsed thoroughly with redistilled water, followed by sonication for 5 min in an ultrasonic bath. The electrode was then transferred to the supporting electrolyte and potential in the range from −0.1 to −1.6 V was applied in a regime of cyclic voltammetry for 20 cycles until a stable voltammograms was achieved.

3. Results and discussion

3.1. *Optimization of the experimental conditions*

Square-wave voltammetry was used to optimize a rapid and sensitive electroanalytical procedure for the determination of nitazoxanide in a pharmaceutical dosage form. The square-wave voltammetric response depends markedly on instrumental parameters. To obtain a high sensitivity (peak current), the optimum instrumental parameters, viz., pulse amplitude and frequency, were studied for 20 μg/mL nitazoxanide solution. The frequency is varied from 50 to 140 Hz. Although the response to nitazoxanide increased with frequency up to 70 Hz after that the peak current was masked by a large residual current. When the pulse amplitude was varied in the range of 10–100 mV, the peak current increased with increasing pulse amplitude. Analysis of the data showed a linear increase in the peak height for amplitudes ESW $\leq$ 50 mV. When the pulse amplitude was greater than 50 mV, the peak width increased at the same time. Hence, the best peak was obtained when 70 Hz square-wave frequency, 50 mV pulse amplitude and 10 mV scan rate were employed.

3.2. *Effect of pH*

The pH effect on the peak current of 100 μg/mL nitazoxanide was studied in B–R buffer in the pH range from 2.0 to 11.0. A well-defined cathodic peak was obtained at pH 3.0 and hence this pH was taken as optimum for complete investigation. The cathodic peak shifted towards more negative potential with increase in pH indicates participation of proton in the electrode process.

3.3. *Effect of different solvents*

The effect of different solvent systems viz., dimethyl formamide, 1,4 dioxane, acetonitrile, ethanol and surfactants (cetrimide and tween-20) on the reduction of nitazoxanide was investigated by the proposed voltammetric procedure. On comparing the voltammetric behavior of nitazoxanide in different solvent systems, it was observed that nitazoxanide showed substantial increase in peak current and the limit of detection (LOD) was found to be lower in 1,4 dioxane. While in other organic solvents nitazoxanide adsorbed at the electrode surface to varying degrees and hence hampered the kinetics of redox process at the electrode surface. Thus 1,4 dioxane was selected for the analysis of nitazoxanide by the proposed voltammetric procedure.

3.4. *Cyclic voltammetric behavior*

Cyclic voltammograms (Fig. 1) of nitazoxanide yield single well-defined reduction peak at −0.75 V in B–R buffer at pH 3.0 and no oxidation peak was observed in reverse scan, indicating the irreversibility of the electrode process. The effect of the scan rate (ν) on the peak current (I_p) was studied in the range of 50–250 mV/s. A linear plot was obtained between the log I_p and log ν expressed by the equation: $\log I_p = 0.4937\nu$ (mV/s)+0.8536 ($r^2 = 0.984$), a slope of 0.49 obtained is close to the 0.5 which reflects that reduction process is diffusion controlled [13].

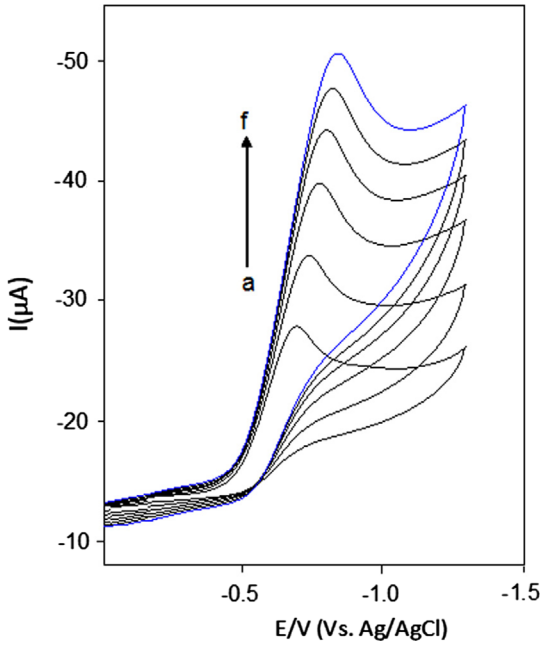


Fig. 1 Cyclic voltammograms of 100 μg/mL nitazoxanide in dioxane at different scan rates; 50, 100,150, 200, and 250 mV/s.

3.5. *Validation of the proposed method*

The analytical method was validated with respect to parameters such as LOD, LOQ, specificity, precision and accuracy [14].

3.5.1. *LOD*

The LOD is calculated by the equation LOD = 3(*SD*/*b*), where *SD* is the standard deviation of the intercept and *b* is the average slope of the regression line. The calculated LOD for the standard solution was 5.23 μg/mL.

3.5.2. *LOQ*

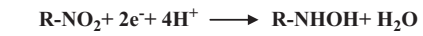
The LOQ is examined by the equation LOQ = 10(*SD*/*b*). The LOQ for the standard solution was found to be 17.45 μg/mL.

3.5.3. *Specificity*

Specificity is the ability of method to measure analytical response in the presence of all potential ingredients present in pharmaceutical formulation. For specificity test, voltammograms of standard solution containing excipients (starch, gelatin, lactose, and magnesium stearate) were recorded under selected conditions. Response of analyte in this mixture was compared with the response of pure nitazoxanide. It was found that assay results were not affected by the presence of excipients in pharmaceutical formulation.

3.5.4. *Accuracy and precision*

The accuracy of a method is the degree of the nearness of the real value to observed analysis results. The accuracy of the analysis was determined by calculating the percentage relative error between the measured mean concentration and the nominal concentration. Precision is expressed in terms of the relative standard deviation. The values of both accuracy and precision were found to be less than 5%, indicating the high precision and accuracy of the method and the confidence in its repeatability [15].



Scheme 2 Reduction of nitazoxanide.

3.6. *Analytical application*

To check the applicability of the proposed method, a commercial tablet formulation containing nitazoxanide was analyzed. Because of high concentration of nitazoxanide in its dosage form no accumulation time was needed and tablet assay was conducted using the square-wave voltammetry. The variation of the peak current with the nitazoxanide concentration was investigated by recording square-wave voltammograms at different concentrations of nitazoxanide. The relationship between peak current and concentration was found to be linear over the range of 20–140 μg/mL. Linear equation is expressed as I_p (μA) = (0.0871) $C_{\text{nitazoxanide}}$ (μg/mL)+0.4371, $r^2 = 0.9938$, where I_p is the peak current in μA, C is the concentration in μg/mL and r is the correlation coefficient.

3.7. *Controlled potential electrolysis and reaction mechanism*

By using controlled potential coulometry, the number of electrons n transferred was calculated from the charge consumed by the desired concentration of nitazoxanide. The charge consumed was determined in acidic medium. For this purpose 2 mL of 5 μg/mL solution of the electroactive species was placed in the cell and electrolysis was carried out at a potential of −0.75 V against Ag/AgCl reference electrode. During the electrolysis, solutions were continuously stirred and purged with nitrogen. Number of electrons n was calculated using the equation $Q = nFN$, where Q is the charge in coulombs, F is the Faradays constant and N is the number of moles of the substrate. The calculated number of electrons is found to be two for the cathodic peak of nitazoxanide.

On the basis of cyclic voltammetry, squarewave voltammetry and coulometric studies, the following mechanism has been postulated for the reduction of nitazoxanide in acidic conditions (Scheme 2).

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