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Stripping voltammetric behaviour of toxic drug nitrofurantoin

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ABSTRACT

A simple, sensitive and reproducible squarewave cathodic adsorptive stripping voltammetric method has been developed for the determination of nitrofurantoin in solubilized system. The objective of the present paper is to investigate the redox behaviour of nitrofurantoin by using different voltammetric techniques and to establish the methodology for its determination in the presence of surfactants. Voltammograms of the drug with cetrimide in phosphate buffers of pH 2–11 exhibited a single well-defined reduction peak which may be attributed to the reduction of $-NO_2$ group. The reduction process is irreversible over the entire pH range studied. The mechanism of reduction has been postulated on the basis of controlled potential electrolysis, coulometry and spectral analysis. The proposed SWCAdSV voltammetric method allows the determination of nitrofurantoin in linear concentration range 2×10^{-5} to 1×10^{-7} mol L^{-1}. The lower limit of detection (LOD) and lower limit of quantification (LOQ) are 0.06 and 0.27 ng/mL, respectively.

1. Introduction

Nitrofurantoin [N-(5-nitro-2-furyldine)-1-aminohydantoin] (Scheme 1) is a widely utilized urinary antimicrobial drug which has been associated with pulmonary fibrosis, neuropathy, hepatitis and hemolytic anemia in patients with glucose-6-phosphate dehydrogenase deficiency [1]. Although the molecular mechanism leading to nitrofurantoin included cell toxicity is still uncertain, the antimicrobial activities as well as other clinical toxicities of nitrofurantoin may be due to the reductive metabolic activation of 5-nitro function to the anion radical, nitroso and hydroxylamine derivatives [2].

Several analytical methods have been described for the estimation of nitrofurantoin including colorimetry [3], reductive flow injection amperometry [4,5], spectrophotometry [6–8], high performance liquid chromatography [9–11], polarography [12], and cathodic adsorptive stripping voltammetry [13]. The published, colorimetric, flow injection amperometric, spectrophotometric and CAdSV methods did not offer a sufficient quantification limit for the determination of drug. In addition the chromatographic methods for the determination of the drug required sample pre-treatment and time-consuming extraction steps that lead to prolonged exposure of the drug to light.

Literature survey revealed that no electroanalytical method has been published in solubilized system for the determination of nitrofurantoin in pharmaceutical and bulk form. Electrochemical methods such as differential pulse polarography (DPP) [14,15], adsorptive stripping voltammetry (AdSV) [16–20], and differential pulse voltammetry (DPV) [21,22] have been widely applied for the determination of pharmaceuticals in dosage forms and wastewater. Thousands of pollutants including organic, inorganic compounds and biological identities are present in water. Among various contaminants, the presence of drugs and pharmaceuticals in the water and wastewater is alarming and dangerous as some of them can disturb enzymatic, hormonal and genetic systems of human beings. Many drug residues have been found in water [23,24]. In the present study a voltammetric method has been developed for the analysis of nitrofurantoin.

Furthermore, additions of surface active agents have proven an effective role in the electroanalysis of biological compounds and drugs. It has been shown that surfactants are highly effective in stabilizing the voltammetric response of serotonin by protecting the electrode surface from fouling. In another study, it was shown that anionic surfactants could also be used to improve the accumulation of some electroactive organic molecules such as ethopropazine at gold electrodes. Recently, the influence of micelles in the simultaneous determination of two components was also demonstrated, as in the case of ascorbic acid and dopamine and catechol and hydroquinone [25]. It is well established that interaction between aggregates and solutes in the solution phase is controlled by diffusion and takes place in the microsecond time place [26]. Electrode surfaces with hydrophobic characters interact with surfactants, namely through surface adsorption. Thus, electrode modified with surfactants proved to be useful for the determination of both inorganic species and biological compounds [27].

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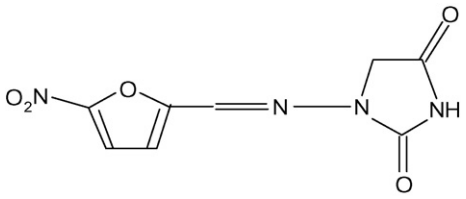


Scheme 1. Structural formula of nitrofurantoin.

2. Experimental

2.1. Materials and methods

Nitrofurantoin (99% pure) was a gift from Glaxo Smithkline Limited Pharmaceuticals, Mumbai, India. Tablets containing nitrofurantoin (Furadantin) 100 mg were obtained from commercial sources. KCl (1 mol L^{-1}) solution was prepared in distilled water and used as a supporting electrolyte. The mass of 10 tablets was determined and finely powdered, and then the required amount of sample to prepare a solution of 1×10^{-4} M was transferred into a 50 mL of standard flasks. After that 40 mL of Tween 20, cetrimide, sodium lauryl sulphate and water were added separately to each flask to dissolve the active material. The contents of the flasks were stirred magnetically for 30 min. and then diluted to volume with same solvents. After dilution the solutions were centrifuged. An aliquot of the supernatant liquid was then transferred into a calibrated flask and a series of dilutions were prepared with phosphate buffers in pH range 2.5–12.0 and mixed 1.0 mL potassium chloride as supporting electrolyte. The contents of the drug in pharmaceutical formulation were determined using calibration graph.

2.2. 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 utilized electrodes were hanging mercury drop electrode (HMDE) as working electrode, Ag/AgCl (3 M KCl) as reference electrode and a graphite rod as an auxiliary electrode. The electrochemical cell was a Metrohm 663 VA stand. 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, after which a continuous stream of nitrogen was passed over the solutions during the measurements. 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.

Table 1
Electrochemical parameters of nitrofurantoin in solubilized systems.

Electrolyte (phosphate buffer pH 6)	E_{pc}/mV (vs. Ag/AgCl)	i_{pc}/μA
1.0×10^{-4} mol L^{-1} nitrofurantoin + 1.48×10^{-3} mol L^{-1} cetrimide	299.0	4.33
2.0×10^{-3} mol L^{-1} nitrofurantoin + Tween 20 2.43×10^{-3} mol L^{-1}	196.0	1.26
2.0×10^{-3} mol L^{-1} nitrofurantoin + 1.73×10^{-3} mol L^{-1} sodium lauryl sulphate	240.0	2.08

3. Results and discussion

The electrochemical behaviour of nitrofurantoin was studied by cyclic voltammetry, differential pulse voltammetry and cathodic adsorptive stripping voltammetric techniques on HMDE. In all electrochemical methods nitrofurantoin gave one well-defined reduction peak in solubilized system, which is attributed to the reduction of $-NO_2$ group at HMDE.

3.1. Voltammetric behaviour of nitrofurantoin in the presence and absence of surfactant

There has been substantial interest in the electrochemistry in micellar systems in the past decade. Adsorption of surfactants on electrodes and the solubilization of electrochemically active compounds in micellar aggregates might significantly change the redox potential, charge transfer coefficients and diffusion coefficients of the electrode processes, as well as the stability of the electrogenerated intermediates. For example Rusling [28] has successfully used micelles and other surfactant microstructures to catalyze the electrochemical dehalogenation of organic halides. Kaifer and Bard [29] reported significant changes in the redox potential and peak current of methylviologen in the presence of the anionic micelle sodium dodecylsulphate (SDS). It has been seen that surfactant play a very important role in electrode reactions, not only in solubilizing organic compounds but also by providing specific orientation of the molecules at the electrode interface [30]. In addition, micellar systems are considered to be primitive model systems for biological membranes [31,32]. Electrochemical parameters of nitrofurantoin determined at HMDE in solubilized systems are shown in Table 1.

The voltammetric response of nitrofurantoin with cetrimide leads to the increase in peak current. The suggested mechanism for the aggregation of surfactants on the electrode surface in the forms of bilayers, cylinders or surface micelles (in the case of relatively higher concentrations added of cetrimide) could explain the increase in current in the presence of surfactants [33]. The electron transfer process will take place when the electroactive species approaches the vicinity of the electrode surface. Two main possibilities allow the transfer of charge, first is the displacement of the adsorbed surfactant by the analyte, and second is the approach of the analyte to the surface of the electrode with in the moieties. Furthermore, a possible mechanism suggests the formulation of ion-pair that anchor onto the surface of the electrode that should posses some hydrophobic character. Thus the resulting ion-pair of the charged surfactant and drug tend to adhere to the surface through the lipophilic parts in both moieties. On comparing the voltammograms of nitrofurantoin in the presence and absence of surfactant, it was observed that nitrofurantoin show an increase in peak current and the limit of detection is also found to be lower in cetrimide in comparison with water, used as a solvent under similar experimental conditions (Fig. 1). The effect of cetrimide concentration was also examined with nitrofurantoin and it was found that nitrofurantoin with 0.05% cetrimide solution gave well-defined reduction peak with maximum peak height. CV, DPV and CAdSV curves show one reduction wave due to the reduction of the nitro group to the hydroxylamine stage.

3.2. Effect of pH

The shape and characteristics of voltammograms were dependent on various electrolyte and pH of the medium. Britton-Robinson, acetate, borate and phosphate, buffers were used in the study and the best results with respect to peak height and shape were obtained with phosphate buffers.

The voltammetric behaviour of 1×10^{-4} mol L^{-1} nitrofurantoin in 1.48×10^{-3} mol L^{-1} cetrimide was examined in the pH range

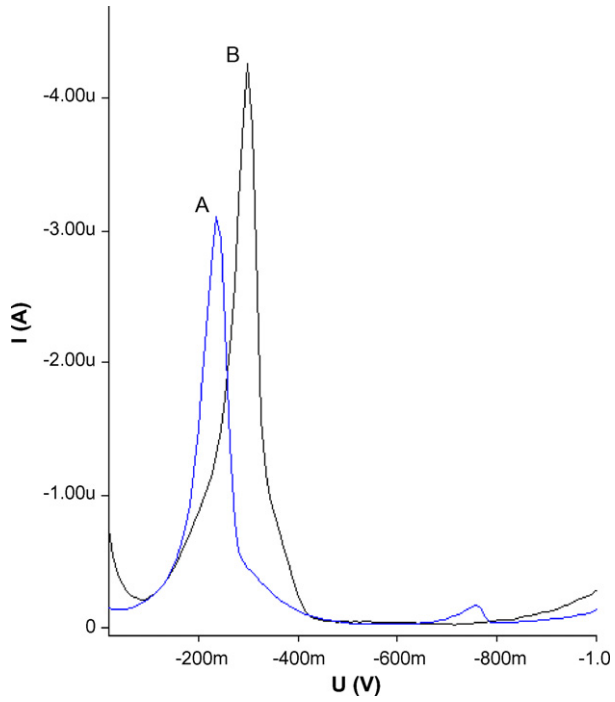


Fig. 1. Square-wave polarogram of 1×10^{-4} mol L^{-1} nitrofurantoin solution in phosphate buffer, pH 6 (A) without addition of 1.48×10^{-3} mol L^{-1} cetrimide (B) after addition of 1.48×10^{-3} mol L^{-1} cetrimide showing enhancement in peak current.

2.5–12 employing CV, DPCAdSV and SWCAdSV techniques. Nitrofurantoin exhibit well-defined cathodic peak over the entire pH range 2.5–12. Stripping peak potential shifted towards more negative potential with increase in pH, indicating involvement of proton in the electrode process. Fig. 2 shows the influence of the pH on the peak height. The absolute value of i_p where peak shape is well defined passes through a maximum at pH 6.

3.3. Optimization of operational parameters

Cathodic adsorptive and differential pulse voltammetric techniques were applied for the quantification and estimation of nitrofurantoin in cetrimide at the HMDE. Surprisingly both the techniques gave comparable results; stripping square-wave voltammetry has been chosen as it is much more sensitive than the other technique, but both techniques require some parameter adjustment. The optimum instrumental conditions for frequency (f), scan increment (Δs), pulse amplitude (E_{sw}), accumulation time (t_{acc}), etc., were examined.

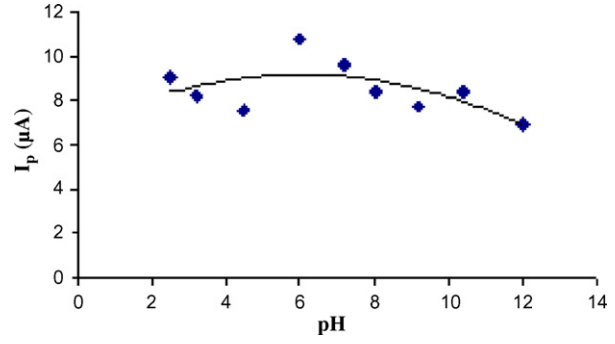


Fig. 2. Influence of pH on the cathodic adsorptive peak current response for 1×10^{-4} mol L^{-1} nitrofurantoin in 1.48×10^{-3} mol L^{-1} cetrimide in phosphate buffer (pH 2.5–12) after 150 s preconcentration time; frequency (f) = 100 Hz, Δs = 10 mV and pulse amplitude 50 mV at E_{acc} = −1.0 V.

Table 2
The optimized experimental conditions of the proposed procedure for the determination of nitrofurantoin with cetrimide.

Variable	Optimized value
pH	6.0
Buffer type	Phosphate buffer
Strength of the buffer (M)	0.2
Temperature (0 °C)	25–30
Purge time (s)	300
Accumulation potential (V)	−1.0
Preconcentration time (s)	150
Rest period (s)	10
Mercury drop size (cm^2)	4
Stirring rate (rpm)	2000
Frequency (Hz)	100
Scan increment (mV)	100
Pulse amplitude (mV)	50

A well-defined stripping peak was obtained with peak potential −0.299 V vs. Ag/AgCl. The square-wave cathodic adsorptive stripping peak height for 1×10^{-4} mol L^{-1} nitrofurantoin depends strongly on the accumulation time, suggesting an effective adsorption of nitrofurantoin on the HDME. The peak current increases with the increase in accumulation time and after reaching a certain accumulation time, the peak current leveled off illustrating that the adsorptive equilibrium of nitrofurantoin. Thus a considerable increase in sensitivity can be achieved by the application of adsorptive stripping voltammetric determination of nitrofurantoin.

The influence of accumulation potential (E_{acc}) on the cathodic peak current (i_p) of nitrofurantoin was also examined over the potential range −0.2 to −1.3 V. Maximum development of the peak current was achieved over the potential range of −0.6 to −1.0 V vs. Ag/AgCl. Hence an accumulation potential of −1.0 V was used throughout the present study. When the potential was made more negative, the peak height decreased due to reduction in the amount of adsorbed nitrofurantoin.

Frequency was varied from 5 to 100 Hz using a scan increment of 100 mV pulse amplitude of 50 mV, and 150 s accumulation time. A linear relationship was observed between peak current and frequency of the signal up to 100 Hz and hence it was chosen to improve the sensitivity without any distortion of the peak or the baseline.

Study of the effect of scan increment on adsorptive cathodic peak current of the drug in phosphate buffer pH 6 revealed that the peak current enhanced on the increase of scan increment (2–10 mV). A scan increment of 10 mV was preferable in the present study. At pulse amplitude 50 mV, the peak current was found to be much more sharp and defined.

Several instrumental parameters, which directly affect the voltammetric response were also optimized, e.g., mercury drop size, stirring rate and rest period. The working conditions decided upon were drop size 4 cm^2, and 2000 rpm. The stripping current was not significantly affected when the rest period was varied, since it was found that 10 s was sufficient for the formation of a uniform concentration of the reactant on the mercury drop. All the experimental conditions are summarized in Table 2.

3.4. Validation of the proposed method

A calibration graph for nitrofurantoin was recorded to estimate the analytical characteristics of the developed method when the most ideal and suitable chemical conditions and instrumental parameters for the voltammetric determination were established. The calibration plot of the peak current vs. the concentration was found to be linear over the range of 2×10^{-5} to 1×10^{-7} mol L^{-1} in the square-wave voltammetric method. The linearity was checked by preparing standard solution at 10 different

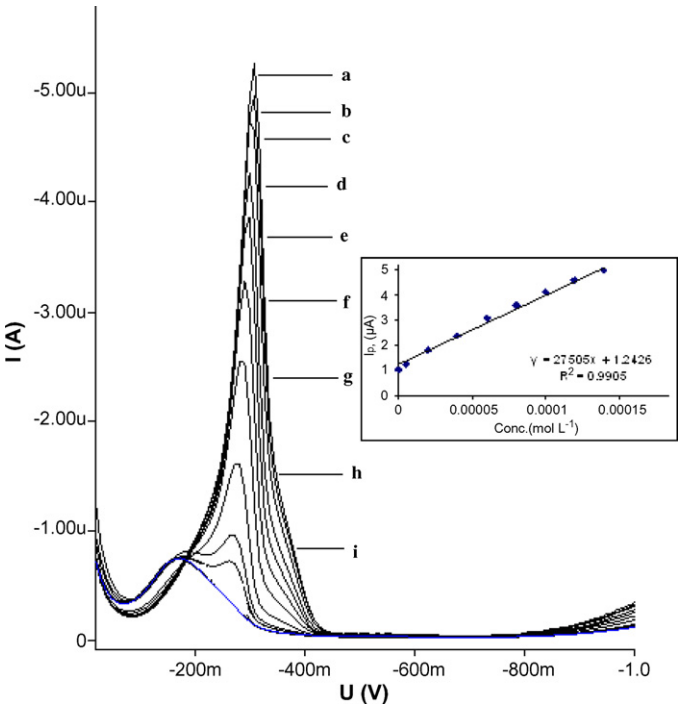


Fig. 3. The dependence of SWCAdS voltammetric current response for nitrofurantoin in 1.48×10^{-3} mol L^{-1} cetrimide at different concentrations; phosphate buffer, pH 6, equilibrium time = 10 s; frequency f = 100 Hz; Δs = 10; pulse amplitude ΔE_{sw} = 50 mV. E_{acc} = −1.0 V; and t_{acc} = 150 s. Insert: plot of i_p, μA vs. Conc. (mol L^{-1}); phosphate buffer, pH 6.0 (0.2 M), equilibrium time = 10 s; frequency f = 100 Hz; Δs = 10 mV; pulse amplitude ΔE_{sw} = 50 mV.

concentrations. The peak current deviated from linearity and then remains constant when nitrofurantoin concentrations were higher than 5×10^{-8} mol L^{-1}. This result showed that nitrofurantoin was strongly adsorbed on the electrode surface and limited due to surface saturation.

The following linear equation was obtained [i_p (μA) = (2.7×10^6) C (mol^{-1}) + 1.2426], r^2 = 0.9905 where $y(i)$ is the peak current in μA, C is the concentration and r is the correlation coefficient. Good linearity is evident from the value of the correlation coefficient (r^2 = 0.9905). Fig. 3 illustrates the square-wave voltammetric response to different concentrations of nitrofurantoin with cetrimide.

The linearity of the differential pulse voltammetric method was found over the range 2×10^{-4} to 4×10^{-5} mol L^{-1} for nitrofurantoin in 1.48×10^{-3} mol L^{-1} cetrimide. The linear response of peak current vs. concentration can be expressed by the equation [y (μA) = (3.1458×10^4) C (mol L^{-1}) + 1.0506], r^2 = 0.995.

Analytical parameters for voltammetric determination of nitrofurantoin using SWCAdSV and DPCAdSV modes are tabulated in Table 3.

Table 3
Analytical parameters for voltammetric determination of nitrofurantoin with cetrimide using SWCAdSV and DPCAdSV modes.

Parameters	SWCAdSV	DPCAdSV
Concentration range (μg mL^{-1})	20–0.1	200–40
Measured potential (mV)	−227	−172
LOD (ng mL^{-1})	0.06	0.12
LOQ (ng mL^{-1})	0.27	0.38
Correlation coefficient (r^2)	0.990	0.995
Intercept (μA)	1.2426	1.0506
Slope × 10^4 (μA μg^{-1} mL^{-1})	2.74	3.14
% Recovery	99.32	99.87
Applications	Tablets	Tablets

(I) *Sensitivity/detection limit*: The detection limit was calculated by the equation LOD = 3 S.D./b, where S.D. is the standard deviation of the intercept and b is the slope of the regression line. The calculated detection limit for the standard solution was 6.9×10^{-11} mol L^{-1} or 0.06 ng mL^{-1}.

(II) *Quantitation limit*: The quantitation limit was examined by the equation LOQ = 10 S.D./b. The lower limit of quantitation for the standard solution was found to be 2.73×10^{-10} mol L^{-1} or 0.27 ng mL^{-1}.

(III) *Specificity*: Specificity is the ability of the method to measure the analytical response in the presence of all potential impurities. For the specificity test, voltammograms of the standard solutions of tablet excipients (starch, gelatin, lactose, and magnesium stearate) were recorded under selected conditions. The response of the analyte in this mixture was compared with the response of pure nitrofurantoin. It was found that assay results were not changed.

(IV) *Stability*: In this study, nitrofurantoin stock solutions were kept in the dark at +4 °C for 1 month and were analyzed at different times (every day). It has been seen that repeatable peak currents of nitrofurantoin stock solution occurred up to 12 days and after that the peak current decreased significantly. So the solutions were found to be stable for 12 days.

3.5. Application of the drug in pharmaceutical formulations

The optimized procedure was successfully applied in the determination of nitrofurantoin in tablets. There was no need of filtration of tablets extracts from undissolved excipients; only dilution of aliquot from the supernatant layer with the supporting electrolyte was required before the measurement. The percentage recovery of nitrofurantoin based on the average of five replicate measurements was 99 ± 0.82% for CAdSV and DPV, respectively. The procedure did not require any time-consuming extraction steps prior to the assay of the drug. The performance data of the proposed method have been tabulated in Table 4.

3.6. Cyclic voltammetric behaviour

The reversibility of the reduction process was investigated by using cyclic voltammetry. The cyclic voltammograms of nitrofurantoin with cetrimide (1×10^{-4} mol L^{-1}) in phosphate buffers (pH 2.5–10) at hanging mercury drop electrode (HMDE) exhibits a single well-defined peak in the potential range −0.21 to −0.31 V, at all concentrations due to the reduction of –NO_2 group. The peak potential shifted to a more negative value on the increase of the scan rate, confirming the irreversible nature of the reduction process. For a totally irreversible electrode reaction the relationship between the peak potential (E_p) and the scan rate (ν) is expressed as E_p = (2.303 $RT\alpha_nF$) log (RT/α_nF)–(2.303 $RT\alpha_nF$) log ν. A straight line is observed when E_p is plotted against log ν at a particular concentration in pH 6 and can be expressed by the equation:

$$y(E_p) = 17.432(\log \nu) - 2.62 \text{ (V)}, \quad r^2 = 0.999$$

From the slope of the straight line ($\Delta E/\log \nu$), the α_n value is calculated by using the expression $\Delta E/\log \nu = -30/\alpha_n$. The α_n value is found to be 1.72. Fractional α values confirm the irreversible reduction of nitrofurantoin.

For finding the adsorptive character of the drug at HMDE a cyclic voltammogram (Fig. 4; curve 1) was recorded after 150 s preconcentration at −1.0 V and with zero second (Fig. 4; curve 2) preconcentration time. The peak current (i_p) increases after preconcentration of the drug on the electrode surface for 150 s.

The effect of scan rate ($\nu^{1/2}$) on stripping peak current (i_p) was examined under the above experimental conditions. As the sweep rate was increased from 50 mV to 500 mV s^{-1} at a fixed concentra-

Table 4
Statistics and performance characteristics of the analytical method from the calibration data.

Tablet				Standard			
I (μA)	$(x-\bar{x})^2$	E (mV)	$(x-\bar{x})^2$	I (μA)	$(x-\bar{x})^2$	E (mV)	$(x-\bar{x})^2$
2.03	0.0004	227	1.44	2.08	0.0001	234	2.56
2.05	0.0000	227	1.44	2.06	0.0001	237	1.96
2.08	0.0009	230	3.24	2.09	0.0004	239	11.56
2.05	0.0000	227	1.44	2.06	0.0001	234	2.56
2.06	0.0001	230	3.24	2.07	0.0000	234	2.56
$\sum x$	$\sum(x-\bar{x})^2$	$\sum x$	$\sum(x-\bar{x})^2$	$\sum x$	$\sum(x-\bar{x})^2$	$\sum x$	$\sum(x-\bar{x})^2$
10.27	0.0014	1141	10.8	10.36	0.0007	1178	21.2
S.D. = 0.0187		S.D. = 1.6431		S.D. = 0.0132		S.D. = 2.302	
C.V. = 0.91		C.V. = 0.72		C.V. = 0.64		C.V. = 0.97	

tion of nitrofurantoin, (i) the peak potential shifted cathodically (ii) the peak current increased steadily, and (iii) the peak current function, $i/AC\ \nu^{1/2}$, exhibited near constancy.A straight line is observed when i_p is plotted against $\nu^{1/2}$, which may be expressed by the equation

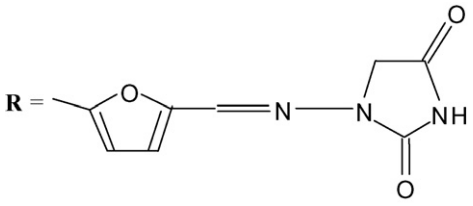
$$y(i_p) = 1.5013\nu^{1/2}\,(\text{mV/s}) + 6.9 \times 10^{-7}, \quad r^2 = 0.9945$$

3.7. Controlled potential electrolysis and coulometry

By using controlled potential coulometry, the number of electrons transferred, n values were calculated from the charge consumed by the desired concentration of nitrofurantoin. The charge consumed was determined in acidic medium. For this purpose 2 mL of 5 mg mL^{-1} solution of the electroactive species was placed in the cell and electrolysis was carried out at a potential of −1.2 to −1.8 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 charge in coulombs, F is Faradays constant and N is number of moles of the substrate. Millicoulometry was also employed to find the number of electrons involved in the electrode process using method of De Vries and Kroon and were found to be four for the cathodic peak of nitrofurantoin.

3.8. Reduction mechanism

On the basis of CAdSV, DPV, cpe, coulometry and spectral studies following mechanism has been postulated for the reduction of nitrofurantoin (Scheme 2).



Scheme 2. Reduction mechanism of nitrofurantoin.

$$R\text{–}NO_2 + 4e^- + 4H^+ \rightarrow R\text{–}NHOH + H_2O$$

The single reduction peak of nitrofurantoin is attributed to the four electron reduction of nitrofurantoin to the corresponding hydroxylamine.

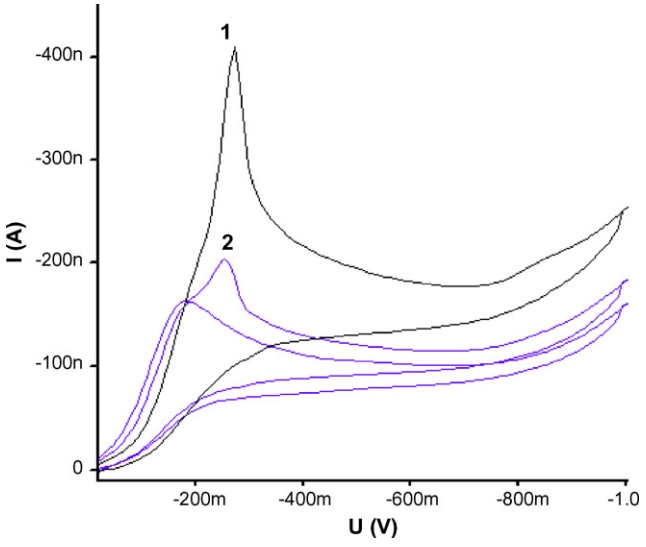


Fig. 4. Cyclic voltammograms of 1×10^{-4} mol L^{-1} nitrofurantoin in 1.48×10^{-3} mol L^{-1} cetrimide in phosphate buffer (pH 6) at a scan rate of 100 mV s^{-1}, equilibrium time = 10 s. (1) After preconcentration and (2) curve shows 0 s preconcentration.

4. Conclusions

The proposed voltammetric method provides a very sensitive and selective technique for the analysis of nitrofurantoin in solubilized systems. In addition this technique also eliminates time-consuming solvent extraction steps. The reduction peak potential and current values are function of pH of electrolyte. The use of surface active agent cetrimide to the nitrofurantoin containing electrolyte was found to enhance the reduction current signal. The developed method with detection limit of 6.9×10^{-11} mol L^{-1} or 0.06 ng mL^{-1} is more sensitive to already reported different spectroscopic and chromatographic methods. Results obtained from the above study showed that the proposed method can be recommended for the determination of nitrofurantoin in pharmaceutical formulation and wastewater.

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