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Determination of nitrofurantoin drug in pharmaceutical formulation and biological fluids by square-wave cathodic adsorptive stripping voltammetry

Essam Hammam *,[1]

Department of Chemistry, Faculty of Science, Tanta University, 31527 Tanta, Egypt

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Abstract

Nitrofurnation is an antibacterial drug. It is used in the treatment of initial or recurrent urinary tract infections caused by susceptible organisms. The cyclic voltammogram of the drug in Britton–Robinson buffers (pH 2–11) exhibited a single well-defined cathodic peak at the hanging mercury drop electrode, that due to the reduction of its nitro group to the amine stage. A fully validated, sensitive, and reproducible developed procedure was described for determination of the drug in bulk form, pharmaceutical formulation, human serum and human urine using, square-wave cathodic adsorptive stripping voltammetry. The optimal experimental parameters for the drug assay were: accumulation potential = -0.4 V (vs. Ag/AgCl/ KCl_s), accumulation time = 40 s, frequency = 120 *Hz*, pulse amplitude = 50 mV and scan increment = 10 mV in Britton–Robinson buffer (pH 10). A mean percentage recovery of 100.68 ± 0.17 ($n = 5$) and a detection limit of 1.32×10^{-10} M of bulk drug were achieved. Applicability to assay of the drug in pharmaceutical formulation, human serum and human urine was studied and illustrated. The mean percentage recoveries were found as: 101.49 ± 0.65, 103.94 ± 0.73 and 101.98 ± 0.52 ($n = 5$) in pharmaceutical formulation, human serum and human urine, respectively. Detection limits of 2.86×10^{-10} M and 5.77×10^{-10} M nitrofurantoin were achieved in human serum and urine, respectively.

Keywords: Nitrofurantoin determination; Square-wave cathodic adsorptive stripping voltammetry; Assay of pharmaceutical formulation; Human serum and urine

1. Introduction

Nitrofurantoin {1-(5-nitro-2-furfurylidene amino-hydantoin} is a synthetic, nitrofuran-derivative antibacterial agent. This drug is usually bacteriostatic but may be bactericidal in action, depending on its concentration attained at the site of infection and the susceptibility of the infecting

* Tel.: +2-040-334-3398; fax: +2-040-335-0804
E-mail address: mmghoneim@usa.net (E. Hammam).
[1] Present address: Chemistry Department, The University of Southhampton, Southhampton SO 17 IBJ, UK.

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organism [1]. It is used in the treatment of initial or recurrent urinary tract infections caused by susceptible organisms. The drug is active against many gram-negative and some gram-positive bacteria including: *Citrobacter*, *Corynebacterium*, *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Neisseria*, *Salmonella*, *Shigella*, *Staphylococcus aureus* and *Enterococcus faecalis*. In general, most susceptible bacteria are inhibited in vitro by nitrofurantoin concentrations of 1–32 μg/ml. Higher concentrations of the drug may be required to inhibit some strains of *Enterobacter* and *Klebsiella*. Nitrofurantoin is 20–60% bound to plasma protein, it crosses the placenta and it is distributed into milk and bile. The plasma half-life of nitrofurantoin is approximately 20 min in adults with normal renal function. Plasma concentrations of the drug are higher and the half-life is prolonged in patients with impaired rental function [1].

The drug is partially metabolized, mainly in the liver. A small fraction of the drug is reduced to form amino-nitrofurantoin. Within 24 h, 30–50% of a single oral dose of the drug is excreted intact in urine by glomerular filtration and tubular secretion and about 1% is excreted in urine as aminofurantoin. Therapeutic concentrations of nitrofurantoin are not attained in the urine of patients with creatinine clearances less than 40 ml/min. The drug darkens on prolonged exposure to light and it must be stored in light–resistant nonmetalic containers [1].

Studies of the therapeutic effect of the drug required the use of sensitive methods for its determination at trace levels. Several analytical methods have been described for the assay of nitrofurantoin in pharmaceutical formulation and human urine including colorimetry [2–5], reductive flow injection amperometry [6,7], spectrophotometry [8–14], high performance liquid chromatography [15–22], polarography [23–26], differential pulse polarography [27]. Linear sweep cathodic adsorptive stripping voltammetry [28] has been used in presence of phosphate buffer (pH 5.5) to determine nitrofurantoin in pharmaceutical formulation and urine, with a detection limit of 1×10^{-9} M.

The published, colorimetric, flow injection amperometric, spectrophotometric [2–14] and polarographic methods [23–27] did not offer a sufficient quantification limit for the assay of drug in biological fluids. In addition, the chromatographic methods [15–22] for 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 methods have been published for the assay of nitrofurantoin in human serum. Besides, the square-wave voltammetry technique did not used before for determination of nitrofurantoin either in pharmaceutical formulation or in biological fluids.

Therefore, this paper described a fully validated, simple, rapid and more sensitive developed procedure for the determination of nitrofurantoin in pharmaceutical formulation, human serum and human urine employing square wave cathodic adsorptive stripping voltammetry at the hanging mercury drop electrode. The procedure did not require sample pre-treatment or any time-consuming extraction step prior to the drug assay.

2. Experimental

2.1. Reagents and solutions

(i) A stock standard solution (1×10^{-3} M) of nitrofurantoin (Sigma) in ethanol (in presence of 4% DMF) was prepared, kept in a brown bottle and then stored under refrigeration. Under these conditions, the solution was stable and hence the drug concentration did not change with time. Nitrofurantoin working standard solutions were prepared daily by serial dilutions of the stock standard solution and kept in brown bottles.

(ii) Macrofuran capsule (Kahira Pharm & Chem. Inc.Co., Cairo, Egypt) labeled to contain 50 mg nitrofurantoin in addition to some inactive-ingredients (starch, gelatin, lactose, talc and magnesium stearate). The contents of five capsules were weighed and the average mass per capsule was determined. A quantity of the finely ground sam-

ple equivalent to 50 mg of nitrofurantoin was accurately weighed and then transferred into a 100 ml dark calibrated flask containing 70 ml ethanol. The content of the flask was shaken for about 15 min and then diluted to volume with ethanol. The solution was then filtrated through a 0.45 μm millipore filter (Gelman, Germany), that to separate out the insoluble excipients. The desired concentration for the drug was then obtained by accurate dilution with ethanol.

(iii) Human serum samples spiked with nitrofurantoin were obtained by diluting aliquots of the standard nitrofurantoin solution with human serum in dark test tubes. A 100 μl aliquot of the spiked serum was diluted to 1.0 ml with ethanol in a 5 ml centrifuge tube. The precipitated protein was separated out by centrifugation for 5 min at 5000 rpm. The clear supernatant layer was filtrated through a 0.45 μm milli-pore filter to produce protein free-spiked human serum.

(iv) Human urine samples spiked with nitrofurantoin were obtained by adding aliquots of the standard drug solution to urine samples in brown tubes.

(v) Britton–Robinson buffers (pH 2–11) were prepared [29] (0.04 M of each of acetic, o-phosphoric and boric acids, adjusted to the required pH with 0.2 M sodium hydroxide) and used as supporting electrolytes. All reagents were of analytical grade quality. Deionized water was used throughout to prepare solutions.

2.2. Apparatus

The Electrochemical Analyzers Models 394 and 273A (PAR) were used in the present voltammetry measurements. The electrode assembly Model 303A (PAR), with a dark micro-electrolysis cell and three electrode system comprises of a hanging mercury drop working electrode (area: 0.026 cm^2), a Ag/AgCl (saturated KCl) reference electrode and a platinum wire counter electrode, was used. Stirring of the solution in the electrolysis cell was performed using a magnetic stirrer (Model 305-PAR) and a stirring bar to provide the convective transport during the pre-concentration step. The whole measurements were automated and controlled through the programming capacity of the apparatus.

A Mettler balance (Toledo-AB104) was used for weighing the solid materials. A centrifuge instrument (Eppendorf-5417C) was used for separation of the precipitated protein from human serum solutions. An Orion SA 720 pH- Meter served to carry out the pH measurements. The deionized water was supplied from a Purite-Still Plus Deionizer connected to a Hamilton-Aqua-Matic bidistilled water system. All data were obtained at ambient temperature.

2.3. Assay procedure

A 10 ml volume of the supporting electrolyte was introduced into a dark voltammetric cell, and then de-aerated with pure nitrogen for 10 min (and for 30 s before each cycle). A selected accumulation potential was then applied to a new mercury drop for a selected time period, while the solution was stirred at 400 rpm. The stirring was then stopped, and after 5 s the voltammogram was recorded by applying a negative-going scan. After the background voltammogram had been recorded, aliquot of the analyte solution was introduced into the cell and the adsorptive stripping cycle was then repeated using a new mercury drop. Quantification was performed by means of the calibration curve method and the data were treated through a PC-computer loaded with software (Model 394 Analytical Voltammetry version 2.01–copyright (1994) and interfaced to the Electrochemical Analyzer

3. Results and discussion

The cyclic voltammetric behavior of nitrofurantoin (5×10^{-7} M) in Britton–Robinson buffers (pH 2–11) at the hanging mercury drop electrode (HMDE) showed in the cathodic direction a single well-defined peak due to the reduction of the nitro group to the amine stage. The peak potential shifted to more negative values with the increase of pH indicated the involvements of

hydrogen ions in the proper electrode reaction. No peaks were observed in the anodic direction that indicated the irreversible nature of the electrode reaction.

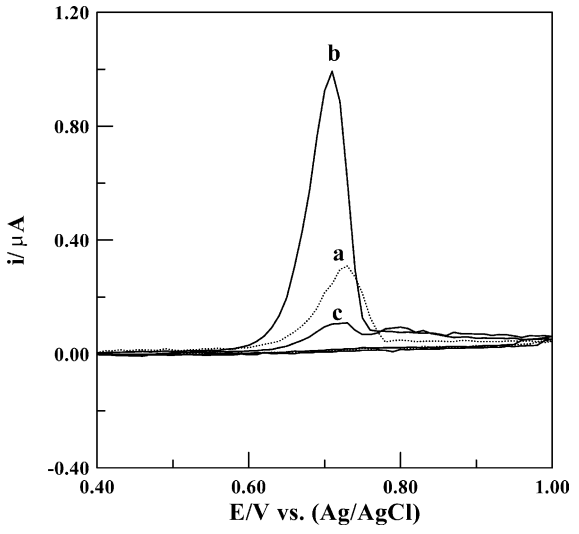

Fig. 1. Cyclic voltammograms for 5×10^{-7} M nitrofurantoin in Britton–Robinson buffer (pH 10) at scan rate of 100 mVs-1; equilibrium time = 5 s, (a) without pre-concentration, (b) after 30 s pre-concentration at -0.40 V and (c) its second cycle at the same mercury drop.

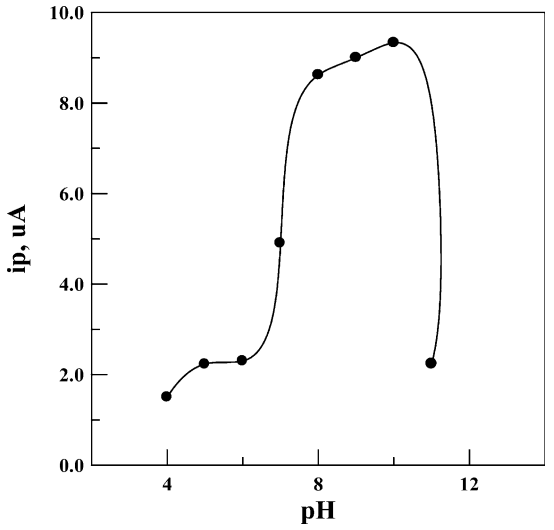

Fig. 2. The dependence of the square-wave cathodic adsorptive stripping peak current (i_p), for 5×10^{-7} M nitrofurantoin, on pH, $E_{acc.} = -0.4$ V, $t_{acc.} = 30$ s, frequency $f = 120$ Hz, pulse amplitude $\Delta E_{sw} = 50$ mV and scan increament $\Delta s = 10$ mV.

Fig. 1 displayed three voltammograms for 5×10^{-7} M nitrofurantoin in B.R. buffer (pH 10) at the HMDE without pre-concentration, $t_{acc.} = 0$ s, (curve a), after 30 s pre-concentration at -0.4 V (curve b) and then its second cycle at the same mercury drop (curve c). A maximum developed peak current (i_p) was achieved after pre-concentration of the drug onto the electrode surface for 30 s, whereas the second cycle exhibited a smaller peak response that may be due to desorption of drug species out of the mercury electrode surface. This behavior confirmed the adsorptive character of the drug at the mercury surface. Adsorptive stripping cycles carried out for increased values of scan rate (v) gave rise to a reduction peak with intensities that showed a linear increase with scan rate between 0.025 and 0.5 Vs-1, followed the relationship: i_p (μA) = 1.083 (v/Vs-1)-2.151; r = 0.992 and $n = 8$. This relation is similar to that expected for an adsorption-controlled process [30]. Moreover, the peak potential shifted linearly to more negative values with increase of scan rate that confirmed the irreversible nature of the electrode reaction.

The cathodic adsorptive stripping voltammetry response of nitrofurantoin was examined in Britton–Robinson buffers (pH 4–11) using square wave waveform. Fig. 2 showed that a maximum developed peak current was achieved over the pH range 8–10. Therefore, Britton–Robinson buffer of pH 10 was chosen throughout the present study.

The square-wave cathodic adsorptive stripping peak height for 2×10^{-7}M nitrofurantoin increased with the increase of accumulation time up to 20 s then decreased (Fig. 3-a); the later behavior may be attributed to the complete coverage of the mercury electrode surface with the drug species. For lower concentrations, at least an accumulation time of 40 s was required for the maximum development of the peak current (Fig. 3-b).

The effect of accumulation potential ($E_{acc.}$) on the cathodic peak current (i_p) was also examined over the potential range -0.3 to -0.5 V. As shown in Fig. 4, maximum development of the peak current was reached over the potential range -0.4 to -0.5 V. Hence, an accumulation poten-

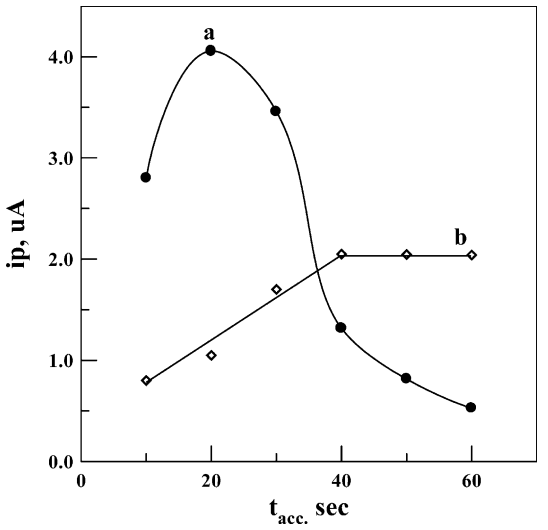

Fig. 3. Effect of the accumulation time ($t_{acc.}$) on the square wave peak current (i_p) for (a) 2×10^{-7} M and (b) 2×10^{-8} M nitrofurantoin in B-R. buffer (pH 10); $E_{acc.} = -0.4$ V, equilibrium time = 5 s, $f = 120$ Hz, $\Delta s = 10$ and pulse amplitude $\Delta E_{sw} = 50$ mV.

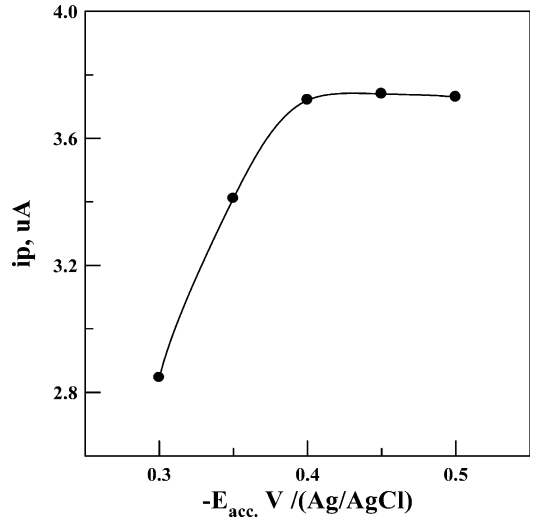

Fig. 4. Effect of accumulation potential ($E_{acc.}$) on the square-wave stripping peak current (i_p) for 2×10^{-7} M nitrofurantoin in B-R. buffer (pH 10), $t_{acc.} = 20$ s. The other conditions as those indicated in Fig. 3.

tial of -0.4 V was used throughout the present study.

The ability to readily assay nanomolar concentrations was attributed not only to the effective accumulation step, but also to the improved sensitivity of the applied waveform for monitoring of the accumulated drug. Fig. 5 showed the cathodic adsorptive stripping voltammograms for 1×10^{-7} M nitrofurantoin in B–R. buffer of pH 10 after 20 s accumulation time at -0.4 V applying the differential pulse (a) and square-wave (b) waveforms. As shown in Fig. 5 the square wave waveform (curve b) offered a 20-fold enhancement of the peak current over that attained by differential pulse one (curve a). Similar advantage of application of square-wave waveform has been documented in connection with the trace analysis of several drugs [31–35]. Therefore, the square-wave waveform was used throughout the present analytical application in order to improve the sensitivity and the rapidity of assay of nitrofurantoin in pharmaceutical formulation and biological fluids.

The square wave response markedly depends on the parameters of the excitement signal. In order to obtain the maximum development of the square-wave cathodic adsorptive stripping peak current, optimization of the frequency (f), pulse amplitude ΔE_{sw} and scan increment (Δs) were

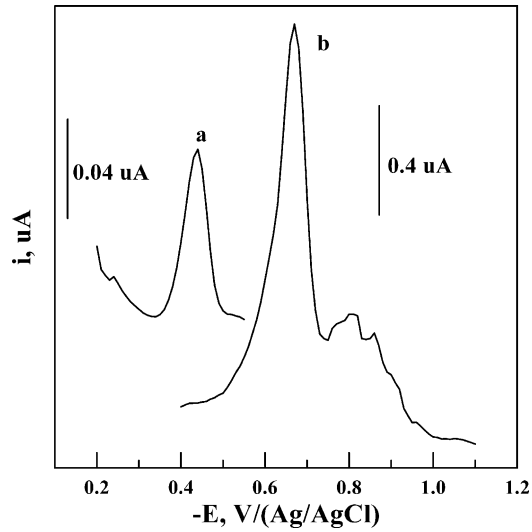

Fig. 5. Comparison between the differential pulse (a) and square wave (b) cathodic adsorptive stripping voltammograms for 1×10^{-7} M nitrofurantoin in B-R. buffer (pH 10) after 20 s pre-concentration at -0.40 V. The other conditions as those indicated in Fig. 3.

Table 1
Characteristics of the calibration plots of nitrofurantoin

Preconcentration time (s)	Linearity range (M)	Regression equation (slope in μA/nM)	Correlation coefficient (r)	LOD (M)	LOQ (M)
0	1×10^{-7}–1×10^{-6}	$y=5.410x+0.087$	0.998	1.08×10^{-7}	3.60×10^{-7}
20	2×10^{-8}–2×10^{-7}	$y=41.859x+0.602$	0.996	4.07×10^{-9}	1.35×10^{-8}
40	1×10^{-8}–2×10^{-7}	$y=65.547x+0.675$	0.996	1.32×10^{-10}	4.40×10^{-10}

attempted. Frequency was varied from 20 to 120 Hz using a scan increment of 10 mV, pulse amplitude of 25 mV and 20 s accumulation time. A linear relationship was obtained between the peak current and the frequency of the signal up to 120 Hz that was chosen to improve the sensitivity without any distortion of the peak or the baseline. At this frequency, the pulse amplitude was varied between 25 and 100 mV. Although the peak current increased linearly with the increase of pulse amplitude; peak distortion that resulted in a poorer resolution was observed only over 50 mV. Thus, pulse amplitude of 50 mV was applied, as it was better for analytical purposes. Also the scan increment of 10 mV was found to develop the highest peak current. Thus, a frequency of 120 Hz, a pulse amplitude of 50 mV and a scan increment of 10 mV were used throughout the present analytical study.

3.1. Analytical applications

3.1.1. Validation of the procedure

Calibration curves for nitrofurantoin were attempted under the optimized procedure conditions followed different accumulation time periods. The regression equations associated with the calibration curves (Table 1) exhibited good linearity ($r=0.996$), that supported the validation of the proposed procedure.

Validation of the procedure for the quantitative assay of the drug was examined via evaluation of the limit of detection (LOD), limit of quantitation (LOQ), repeatability, recovery, specificity, robustness and ruggedness. The LOD and LOQ were calculated from the calibration curves as $kS.D_a/b$ [36] where $k=3$ for LOD and 10 for LOQ, $S.D_a$ is the standard deviation of the intercept and b is the slope of the calibration curve. Both LOD and LOQ values (Table 1) confirmed the sensitivity of the proposed method compared with reported voltammetric method (1×10^{-9} M and 3.33×10^{-9} M, respectively) [28].

Repeatability and recovery were examined by performing five replicate measurements for 8×10^{-8} M bulk nitofurantoin after 40 s pre-concentration. A mean percentage recovery of 100.68 ± 0.17 ($n=5$) was achieved, that indicated high precision of the proposed procedure for the assay of drug.

Specificity of the optimized procedure for assay of nitrofurantoin was examined in presence of some common excipients (starch, gelatin, lactose, talc and magnesium stearate), as in pharmaceutical preparations. The mean percentage recovery for 8×10^{-8} M nitrofurantoin was found as 101.92 ± 0.35 ($n=5$), that showed no significant excipients interference, thus the procedure was able to assays nitrofurantoin in the presence of excepeints and hence it can be considered specific.

Robustness [37] was examined by evaluating the influence of small variation of some of the most important procedure variables including pH, pre-concentration potential ($E_{acc.}$) and pre-concentration time ($t_{acc.}$). The results shown in Table 2 indicated that none of these variables significantly affect the recovery of nitrofurantoin. This provided an indication of the reliability of the

proposed procedure for the assay of drug and it could be considered robust [37].

On the other hand, ruggedness [37] was examined by applying the developed procedure to assay the drug using two potentiostats (PAR- 273A and 394) under the same optimized experimental conditions at different elapsed time. The results obtained due to lab-to-lab and even day-to-day variations were found reproducible since there was no significant difference between the recovery results (Table 2).

3.1.2. Analysis of macrofuran capsules

The developed procedure was applied successfully for determination of nitrofurantoin in its pharmaceutical formulation (Macrofuran). There was no need for any extraction step priors to the drug assay. The percentage recovery of the drug in Macrofuran capsules, based on the average of five replicate measurements was found as 101.49% ± 0.65. The recovery value obtained was compared with that obtained by the reported method (98.53 ± 0.55) [28].

Table 2
Results of the assay of drug at various experimental conditions

Variables	Conditions	(%) R ± S.D. ($n = 3$)
pH		
9.0	$t_{acc.} = 40$s, $E_{acc.} = -0.40$ V.	97.89 ± 0.45
9.5		98.45 ± 0.35
10.0		100.90 ± 0.27
$E_{acc.}$ (V)		
−0.38	pH = 10, $t_{acc.} = 40$ s	96.82 ± 0.65
−0.40		100.90 ± 0.27
−0.42		101.22 ± 0.33
$t_{acc.}$ (s)		
38	pH 10, $E_{acc.} = -0.4$ V	95.70 ± 0.82
40		100.90 ± 0.27
42		101.33 ± 0.49
Laboratory		
Laboratory (1)	pH 10, $t_{acc.} = 40$ s, $E_{acc.} = -0.40$ V, Potentiostat (PAR) 394	100.90 ± 0.27
Laboratory (2)	pH 10, $t_{acc.} = 40$ s, $E_{acc.} = -0.40$ V, Potentiostat (PAR) 273A	101.14 ± 0.32

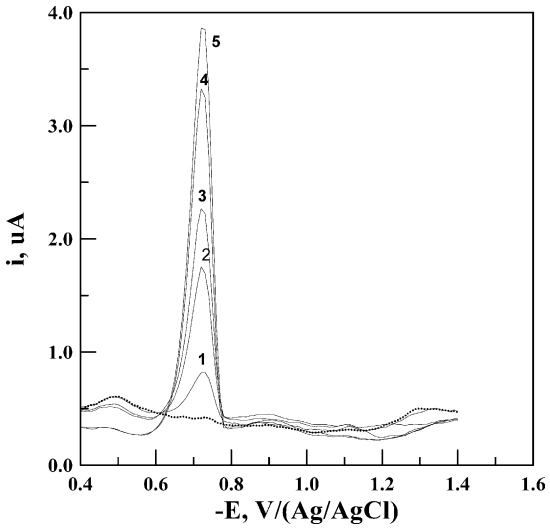

Fig. 6. Square-wave Cathodic adsorptive stripping voltammograms for nitrofurantoin spiked to human serum samples; (1) 1×10^{-8} M, (2) 2×10^{-8} M, (3) 3×10^{-8} M, (4) 4×10^{-8} M and (5) 5×10^{-8} M drug. Dashed line represents the background voltammogram; $E_{acc.} = -0.40$ V and $t_{acc.} = 40$ s. The other conditions as those indicated in Fig. 3.

3.1.3. Analysis of spiked human serum

The optimized procedure was successfully applied for the determination of nitrofurantoin in protein-free spiked human serum samples. No extraction steps other than the centrifugal protein separation were required prior to the assay of drug. Fig. 6 illustrates the response of successive standard additions of nitrofurantoin, each addition effecting a drug concentration of 1×10^{-8} M; 40 s accumulation period was employed. The peak current versus drug concentration was represented by a straight line followed the equation; i_p (μA) = 0.734 C (M/10^{-8}) + 0.586, with a correlation coefficient of 0.996. A detection limit of 2.86×10^{-10} M was achieved. The percentage recovery of nitrofurantoin based on the average of five replicate measurements was found to equal 103.94 ± 0.73, that compared with the value obtained by reported method (97.20 ± 0.54) [18].

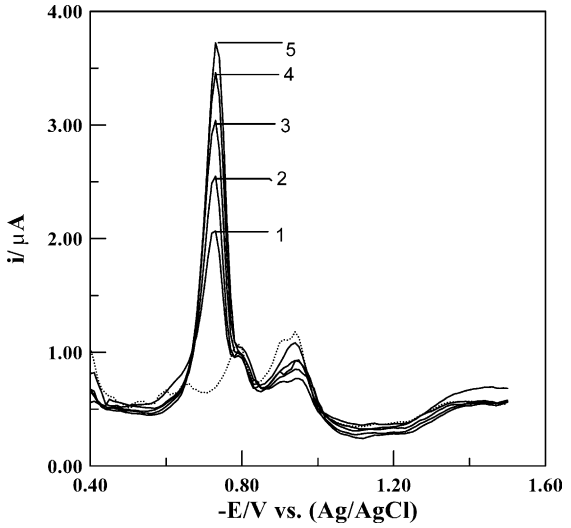

Fig. 7. Square-wave Adsorptive stripping voltammograms for nitrofurantoin spiked to human urine samples; (1) 2×10^{-8} M, (2) 4×10^{-8} M, (3) 6×10^{-8} M, (4) 8×10^{-8} M and (5) 1×10^{-7} M drug. Dashed line represents the background voltammogram, $E_{acc.} = -0.40$ V and $t_{acc.} = 40$ s. The other conditions as those indicated in Fig. 3.

3.1.4. Analysis of spiked human urine

Nitrofurantoin was successfully determined in spiked human urine samples by applying the optimized procedure without any prior extraction steps. Fig. 7 displayed voltammograms of five standard additions of nitrofurantoin in a human urine sample, each addition effecting a drug concentration of 2×10^{-8} M using accumulation time of 40 s. The peak current versus drug concentration gave a linear relationship followed the equation i_p (μA) $= 0.213$ C (M/10^{-8}) $+ 1.1708$, with a correlation coefficient of 0.996. The percentage recovery of nitrofurantoin based on the average of five replicate measurements was found to equal 101.98 ± 0.52, that compared with the value obtained by reported method (97.81 ± 0.74) [28]. The LOD and LOQ values of 5.77×10^{-10} M and 1.92×10^{-9}, respectively, were achieved. Both LOD and LOQ values were compared with those obtained by reported method (1×10^{-9} and 3.33×10^{-9} M, respectively [28]. Accordingly, the proposed method was found to be 10-fold more sensitive than the reported one [28].

4. Conclusion

In the present proposed procedure, square-wave cathodic adsorptive stripping voltammetry was used, for the first time, in the determination of nitrofurantoin in pharmaceutical formulation and human biological fluids. The procedure showed clear advantages such as short period of real time of drug analysis. Besides, no pre-treatment or time-consuming extraction steps were required prior to the drug analysis, other than the centrifugal separation of protein precipitated from serum solutions. In addition the proposed procedure offered lower detection limits for determination of the drug in pharmaceutical formulation and biological fluids compared with those of the reported electroanalytical [23–28], colorimetric [2–5], flow injection amperometric [6,7] and spectrophotometric [8–14] methods. The optimized procedure is simple, rapid, specific, reproducible and sensitive enough for the drug assay at trace analysis and clinical laboratories.

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