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Electrochemical behavior of the antituberculosis drug isoniazid and its square-wave adsorptive stripping voltammetric estimation in bulk form, tablets and biological fluids at a mercury electrode

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Abstract

Isoniazid, pyridine-4-carboxylic acid hydrazide, is an antituberculosis-agent, which is used to prevent the development of clinical tuberculosis. A validated square-wave adsorptive cathodic stripping voltammetric procedure for the trace determination of the bulk drug at the hanging mercury drop electrode (HMDE) has been developed. Under the optimized conditions, (accumulation potential = -0.9 V, accumulation time = 50–300 s, scan increment = 8 mV, pulse-amplitude = 25 mV, frequency = 120 Hz and acetate buffer at pH 5.5) isoniazed generated two irreversible cathodic peaks. The first peak current showed a linear dependence with the drug concentration over the range 5×10^{-10}–2×10^{-6} M. The mean percentage recoveries, based on the average of five replicate measurements, for 7×10^{-9} and 5×10^{-8} M isoniazid were 97.71 ± 2.93 and 99.76 ± 0.77, respectively. The achieved limits of detection (LOD) and quantitation (LOQ) were 1.18×10^{-10} and 3.93×10^{-10} M isoniazid, respectively. The procedure was applied to the assay of the drug in tablets (Isocid and T.B. Zide), spiked human serum and urine with mean percentage recoveries of 97.81 ± 1.49, 97.45 ± 2.09, and 97.08 ± 1.06, respectively. The limits of detection of 1.47×10^{-9} and 2.4×10^{-8} M, and quantitation of 4.9×10^{-9} and 8×10^{-8} M drug in human serum and urine, respectively, were achieved. The mean values of the various pharmackinetic parameters of isoniazid (C_{max}, T_{max}, $t_{1/2}$, AUC, and K_e), estimated from analysis of plasma of two volunteers by means of the proposed procedure were similar to literature values.
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Keywords: Isoniazid; DC-polarography; Adsorptive stripping; Square-wave voltammetry; Estimation in bulk drug; Tablets; Human serum; Urine; Plasma phrmacokinetic parameters

1. Introduction

Isoniazid (Scheme 1), pyridine-4-carboxylic acid hydrazide, is an antituberculosis-agent, which is usually used alone to prevent the development of clinical tuberculosis. Isoniazid may have bacterisotatic or bacteriocidal action, depending on the

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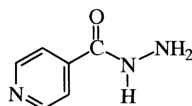

Scheme 1.

concentration of the drug attained at the site of infection and the susceptibility of the infecting organism [1].

Several mechanisms of drug action have been proposed including interference with metabolism of bacterial proteins, nucleic acids, carbohydrates and lipids. One of the principle actions of the drug appears to be inhibition of mycolic acid synthesis in susceptible bacteria, which results in loss of acid-fastness and disruption of the bacterial cell wall. Isoniazid is active against susceptible bacteria only when they are undergoing cell division. Susceptible bacteria may undergo one or two divisions before multiplication is arrested. The drug is readily absorbed from the GI tract and from IM injection sites. When administrated with food, the extent of absorption and peak plasma concentrations of the drug may be reduced. Following oral administration, plasma concentrations are attained within 1–2 h. Isoniazid is distributed into all body tissues and fluids. CSF concentrations of the drug are reported to be 90–100% of concurrent plasma concentrations. The drug is not substantially bound to plasma proteins. Isoniazed is also used in conjunction with other antituberculosis agents in the treatment of diseases that caused by other mycobacteria [1].

In a study using an HPLC method [2], the various pharmacokinetic parameters for isoniazed were estimated from the plasma concentration–time profile of fourteen volunteers. Following an oral administration of a 300-mg isoniazid single dose, the mean values of the pharmacokinetic parameters were found to be $C_{max} = 4.29$ μg ml^{-1}, $T_{max} = 1.54$ h, AUC = 23.7 μg h ml^{-1}, $t_{1/2} = 3.66$ h, and $K_e = 0.22$ h [2].

The electrochemical oxidation of isoniazid were studied by means of DC- and differential-pulse polarography, linear sweep and cyclic voltammetry in the pH range 6–13 [3]. The oxidation processes were found to be of the ECE type, where the rate-determining step was the release of an H^+ ion from the intermediate formed after two reversible one-electron transfers. The electroreduction of isoniazid was studied in acidic media of pH < 6 [4] and in neutral and basic media [5]. On the basis of polarographic and voltammetric measurements and by regarding the Tafel slopes and reaction orders, it was concluded that in a strongly acidic medium (pH < 2) the rate-determining step of the process was the loss of an ammonia molecule to yield isonicotinamide. Whereas at pH > 2 the process was controlled by the second one-electron irreversible transfer.

The determination of isoniazid in pharmaceutical preparations and biological fluids were studied using different methods. These include, capillary electrophoresis [6–8], spectrophotometry [9–17], flow injection chemiluminescence [18–26], colorimetry [27–30], potentiometry [31], titrimetry [32,33], oxidimetry [34,35], liquid chromatography [36–38], high performance liquid chromatography [39–46], gas chromatography-mass spectrometry [47], high-performance thin-layer chromatography [48] polarography [49,50], differential-pulse polarography [51,52] and voltammetry at a glassy carbon electrode [53].

Most of the reported methods for the drug assays did not achieve low enough detection limits, or required sample pretreatment and time-consuming extraction or evaporation steps prior to the analysis. To date, no attempt was made to assay the drug using adsorptive stripping voltammetry technique.

Here a square-wave adsorptive cathodic stripping voltammetric procedure to assay the drug in bulk, pharmaceutical formulations and biological fluids is described, without the need for sample pretreatment, or any time-consuming extraction or evaporation steps prior to the analysis. The electro-reduction reaction pathway of the drug at the dropping mercury electrode also is reconsidered and discussed.

2. Experimental

2.1. Materials

Bulk isoniazid drug was supplied from AMSAL-CHEM PVT. LTD. Pharmaceutical formu-

lations Isocid® (Chemical Industries Development, S.A.A) and T.B. Zide® (ADWIC) were purchased from the local market of Egypt. Isocid® and T.B. Zide® tablets labeled to contain 50 and 100 mg isoniazid per tablet, as an individual drug, respectively.

2.2. Reagents and solutions:

A standard stock solution (1×10^{-3} M) of isoniazid was prepared by dissolving an accurate mass of the bulk drug in an appropriate volume of de-ionized water, and then stored in the dark at 4 °C. More dilute solutions (10^{-6}–10^{-4} M) were prepared daily by accurate dilution just before use. Isoniazid solutions were stable and their concentrations did not change with time. Britton–Robinson (B–R). buffers of pH 2–12 [54] and acetate buffers of pH 3.8–6.3 [55] were prepared in de-ionized water. All the chemicals used were of analytical-reagent grade and were used without further purification.

2.2.1. Tablets solution

Five tablets (Isocid® or T.B. Zide®) were weighed and the average mass per tablet was determined. A portion of the finely ground material equivalent to 100 mg of isoniazid was accurately weighed and transferred into a 100 ml calibrated flask contained 70 ml de-ionized water. The content of the flask was sonicated for about 15 min and then made up to the volume with de-ionized water. The solution was next filtrated through a 0.45 μm Milli-pore filter (Gelman, Germany). The desired concentrations of the drug were obtained by accurate dilutions with de-ionized water. The solution was directly analyzed, according to the proposed procedure, without the need for any pretreatment or extraction steps.

2.2.2. Serum samples

Drug free-serum samples were obtained from healthy male volunteers and stored frozen until the assay. An aliquot of the standard stock isoniazid solution was fortified with the human serum sample. A 100 μl aliquot of this solution was diluted to a 1.0 ml volume with ethanol in a 2 ml volume centrifuge tube. The precipitated proteins were separated by centrifugation for 3 min at 14 000 rpm. The clear supernatant layer was filtrated through a 0.45 μm Milli-pore filter to produce a protein free-spiked human serum solution. Then the protein-free spiked serum solution was directly analyzed, according to the proposed procedure, without any pretreatment or extraction steps.

For pharmacokinetic studies, blood samples (3 ml) were collected from two male volunteers (of age 40–45 years) shortly before the administration of the drug dose (0 h) and at 1, 1.5, 2, 3, 4, 6, 8, 10, 15, 20 and 24 h after an oral administration of a 100 mg T.B. Zide® single dose. The blood samples were immediately centrifuged at 14 000 rpm for 10 min., then plasma was separated into tubes and stored frozen till the analysis by means of the proposed procedure.

2.2.3. Urine samples

Urine samples, 1 ml each, spiked with 10–100 μl aliquots of 1000 μg ml^{-1} isoniazid working solution were made up to 10 ml with de-ionized water to obtain urinary concentrations of 10–100 μg ml^{-1} of urine. Then the solution was directly analyzed, according to the proposed procedure, without any pretreatment or extraction steps.

2.3. Instrumentation

Polarograph Model 4001 (Sargent-Welch) was used for study of the polarographic behavior of isoniazid solution. A dark polarographic cell contained a dropping mercury electrode as a working electrode ($m = 1.03$ mg s^{-1}, $t = 3.3$ s at mercury height = 60 cm) and a saturated calomel electrode (SCE) as a reference electrode was used. The recorded polarograms were redrawn, by taking the middle of the oscillations to represent the character of the polarograms, after making the necessary corrections for residual current.

Voltammetric measurements were carried out employing the Electrochemical Analyzers (Models 394 and 263A-PAR). The electrode assembly (303A-PAR) incorporated with a dark micro-electrolysis cell comprising of a hanging mercury drop electrode (HMDE) as a working electrode

(surface area = 0.026 cm^2), an Ag/AgCl/KCls reference electrode and a platinum wire auxiliary electrode, was used. A magnetic stirrer (305-PAR) and a stirring bar were used to provide the convective transport during the preconcentration step. The peak heights were automatically measured using the “tangent fit” capability of the instrument.

The potentiostat/Galvanostat Model 173-PAR incorporated with digital coulometer Model 179-PAR was used for the controlled-potential coulometric measurements. A dark coulometric cell, incorporated with a Pt wire sealed through the cell bottom for contact with a mercury pool as a working electrode, a SCE as a reference electrode and a platinum gauze immersed in a bridge tube as a counter electrode, was used. The potential of the mercury pool-working electrode was maintained constant with respect to that of the reference electrode (potentiostatic control). The potential selected was adjusted to be equal the $E_{1/2}$ of the polarographic wave of the drug plus 0.1 V or at the beginning of the limiting current of the wave (i.e. at the plateau). The total charge passed during the exhaustive electrolysis was obtained by integrating the current electronically. The charge due to the residual current was subtracted from the total measured charge in order to obtain the faradic charge for the electrode reaction of interest. Faraday’s law relates the measured net charge Q (Coulombs) transferred to the amount of material electrolyzed as: $N = Q/nF$, where N is the number of moles of substance being electrolyzed, and F is Faraday’s constant (96 485 C g^{-1} eq). Accordingly, the number of electrons (n) transferred per reactant molecule was found to equal 6 or 4 in acidic (pH < 5) or alkaline (pH > 8) solutions, respectively.

An Eppendorf centrifuge 5417C was used for separation of the precipitated protein from the human serum samples before assay of the drug. A digital micropippetter (Volac) was used for solution transfer during the present electrochemical measurements. A Mettler balance (Toledo-AB104) was used for weighing the solid materials. Deionized water was obtained from a Purite Still Plus HP de-ionizer attached to a Hamilton AquaMatic bidistillation water system.

2.4. Procedures

For polarographic measurements, a known volume of isoniazid solution was pipetted into a 10 ml calibrated flask then made up to the volume with B–R buffer. The solution was transferred into the dark electrolysis cell and deoxygenated with nitrogen for 10 min, then the polarograms were recorded.

For stripping voltammetric analysis, 10 ml of an acetate buffer of pH 5.5 and an appropriate volume of the drug solution (bulk, tablet or spiked serum solution) were introduced into the dark micro-electrolysis cell, through which a pure nitrogen stream was passed for 5 min before recording the voltammogram. An accumulation potential of −0.9 V (vs. Ag/AgCl, KCls) was applied to the HMDE for a selected time while the solution was stirred. At the end of the accumulation time period the stirring was stopped and 5 s were allowed for the solution to become quiescent. Then the voltammograms were recorded by scanning the potential toward the negative direction using the square-wave waveform. All data were obtained at room temperature.

3. Results and discussion

3.1. DC-polarography

The DC-polarograms for 2.5×10^{-4} M isoniazid in B–R. buffers of pH 2–8 exhibited two irreversible cathodic waves. In B–R. buffers of pH < 5, the height of the second wave was almost double that of the first one (Fig. 1). On the increase of pH (2–8) the height of the first wave was pH-independent, while that of the second wave was gradually decreased over the pH range 5–8 till reached its half height at pH ≅ 8. At pH values > 8, the first and second waves coalesced to form a single wave; its limiting current was pH-independent and equivalent to 2/3 of the total limiting current obtained in solutions of pH < 5 (Figs. 1 and 2). As shown in Fig. 2, the pH-dependent limiting current of the second wave (pH 5–8) may be attributed to the change of its electrode reduction from four-electron process to

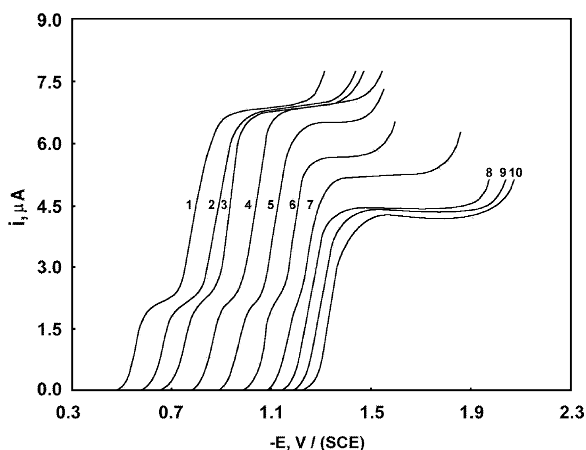

Fig. 1. DC-polarograms for 2.5×10^{-4} M isoniazid in B.R. buffers of different pH values: (1) 1.9, (2) 3.0, (3) 4.1, (4) 5.2, (5) 6.4, (6) 7.4, (7) 8.3, (8) 9.2, and (9) 10.8.

two-electron one. This behavior was confirmed by controlled-potential complete electrolysis of isoniazid in B–R buffers of different pH values which revealed that the total number of electrons involved in the reduction process of isoniazid was 6 or 4 per reactant molecule in acidic (pH < 5) or alkaline (pH > 8) solutions, respectively. Two electrons were consumed via the 1st reduction step in solutions of pH < 8, while four or two electrons were consumed via the 2nd wave in

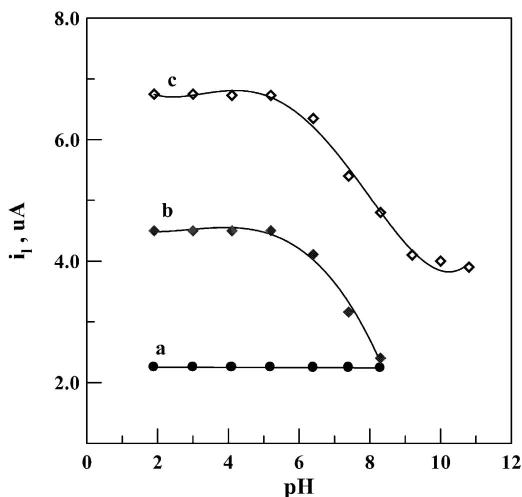

Fig. 2. The i_l–pH plots for the polarographic waves of 2.5×10^{-4} M isoniazid in B–R buffers: (a) 1st wave, (b) 2nd wave and (c) total limiting current.

solutions of pH < 5 or pH ≅ 8, respectively. At pH values > 8, four electrons were consumed via a single wave. These results suggested that the first two-electron irreversible cathodic wave (pH < 8) may be attributed to the reduction of the N=C site of the pyridine ring. While the second four-electron wave (pH < 5) may be due to the two merged reduction processes: saturation of the C=O of the amide group and cleavage of the –HN–NH_2 linkage with the release of NH_3. The latter was confirmed by adding the Nessler reagent to the completely electrolyzed isoniazid solution (pH < 5) where a reddish brown precipitate was observed. In alkaline solutions of pH > 8, the single four-electron irreversible wave may be attributed to the two merged reduction processes: saturation of both the C=N of pyridine ring and C=O of the amide group.

With an increase of pH, the $E_{1/2}$ of the three reduction waves shifted toward more negative values. According to Zuman [56], the $E_{1/2}$–pH-dependent behavior indicated the involvement of protons in the reduction rate-determining step and the proton transfer reaction precedes the electrode process proper. The $E_{1/2}$–pH plots for the 1st, 2nd, and the single waves were straight lines (Fig. 3), with the slope values S_1, $[S_1 = (0.0591/\alpha n_a)Z_{H^+}]$ reported in Table 1. Logarithmic analysis [57] of the polarographic waves of isoniazid was performed at different pH values by plotting $E_{d.e}$ versus $\log(i/i_d - i)$. The plots were straight lines (Fig. 4) with the slope values, S_2, $[S_2 = 0.0591/\alpha n_a]$ reported in Table 1. From which αn_a values were evaluated at different pH values. Values of αn_a reported in Table 1 indicated that the symmetry coefficient α value at different pH varied between 0.55 and 0.74 and the number of electrons n_a involved in the rate-determining step was found to be 2. The number of hydrogen ions (Z_{H^+}) participated in the rate-determining step was calculated [57,58] as:

$$\partial E_{1/2}/\partial \mathrm{pH} = (0.0591/\alpha n_a)\, Z_{H^+}$$

$$\text{i.e.}\quad Z_{H^+} = (\partial E_{1/2}/\partial \mathrm{pH})/(0.0591/\alpha n_a) = S_1/S_2$$

Values of n_a and Z_{H^+} reported in Table 1 indicated that the rate-determining step of the electrode

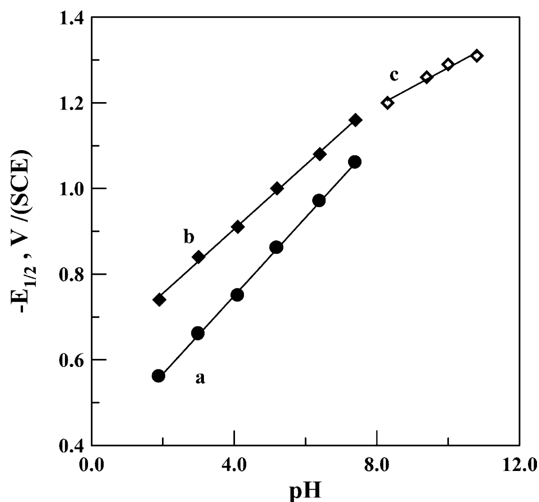

Fig. 3. The $E_{1/2}$–pH plots for: (a) 1st wave, (b) 2nd wave and (c) single wave.

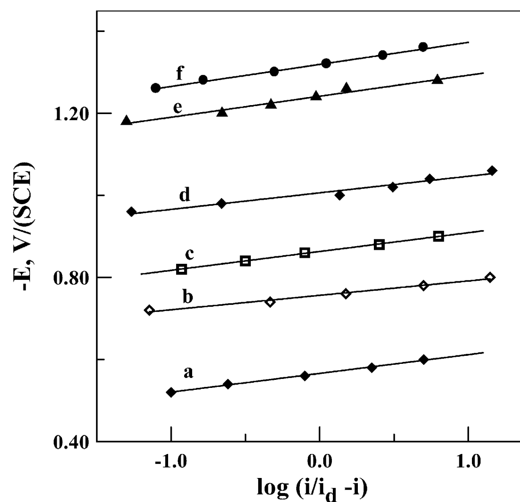

Fig. 4. The $E_{d.e}$–log$(i/i_d - i)$ plots at different pH values for: the 1st wave: (a) pH 1.9, (c) pH 5.2; the 2nd wave: (b) pH 1.9, (d) pH 5.2; and the single wave: (e) pH 9.2, (f) pH 10.8.

process involved the transfer of two protons and two electrons in solutions of pH < 5 while in solutions of pH > 8, one proton and two electrons were transferred.

3.2. *Cathodic adsorptive stripping voltammetry*

3.2.1. *Effect of pH and type of supporting electrolyte*

The adsorptive cathodic stripping voltammetric response for 2×10^{-6} M bulk isoniazid was examined in both B–R. buffers (pH 2–11) and acetate buffers (pH 3.8–6.3) using the square wave waveform, under the operational conditions: $E_{acc.} = -0.6$ V, $t_{acc.} = 30$ s, pulse-amplitude = 50 mV, frequency = 120 Hz and scan increment = 10 mV. The voltammograms in both buffer series showed two irreversible cathodic peaks. The first peak in acetate buffer of pH 5–6 was well defined and showed a much developed peak current intensity (Figs. 5 and 6). Therefore, acetate buffer at pH 5.5 was considered in the rest of the present analytical study.

Table 1
Data obtained from the DC-polarographic measurements for 2.5×10^{-4} M isoniazid in B.R. buffers of different pH values, at 25 °C

pH	$-E_{1/2}$ (V)	S_1 (mV)	S_2 (mV)	αn_a	α ($n_a = 2$)	$Z_H^+ (S_1/S_2)$
1st wave						
1.9	0.56	91.20	47.59	1.24	0.62	1.92
4.1	0.75		49.23	1.20	0.60	1.85
5.2	0.97		47.14	1.25	0.62	1.93
2nd wave						
1.9	0.74	74.96	41.04	1.44	0.72	1.82
4.1	0.91		40.87	1.44	0.72	1.83
5.2	1.08		39.90	1.48	0.74	1.87
Single wave						
9.2	1.26	45.03	52.245	1.12	0.56	0.86
10.0	1.28		49.12	1.20	0.61	0.91
10.8	1.31		53.85	1.10	0.55	0.84

S_1, slope of $E_{1/2}$–pH plot; S_2, slope of $E_{d.e}$–log$(i/i_d - i)$ plot.

3.2.2. Effect of accumulation time

The dependence of the peak current intensity of the first peak (i_{p1}), in pH 5.5 acetate buffer, on the accumulation time period at $E_{acc.} = -0.6$ V, was investigated for the drug concentrations: (a) 2×10^{-9}, (b) 2×10^{-7}, and (c) 2×10^{-6} M isoniazid. The plots of i_p versus t_{acc} for 2×10^{-9} and 2×10^{-7} M bulk drug solutions were linear up to the time periods 250 and 150 s, respectively (Fig. 7 curves a and b). For a 2×10^{-6} M drug solution the response was only linear up to 50 s, then leveled off and decreased (Fig. 7 curve c); the latter behavior may be attributed to the complete coverage of the mercury electrode surface with the drug species. Thus, the accumulation time of choice will be dictated by the sensitivity needed.

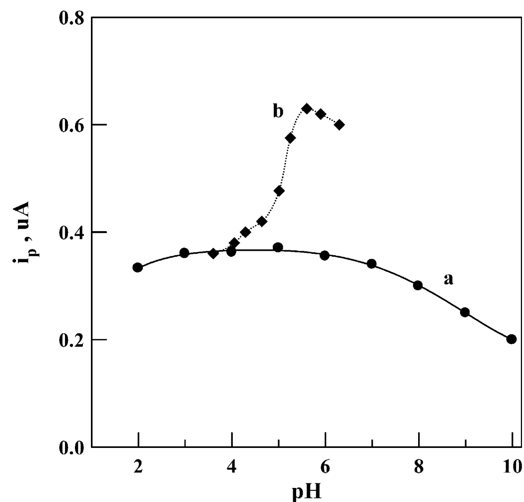

Fig. 6. The i_p–pH plots for 2×10^{-6} M isoniazid in: (a) B–R buffer of pH 5.5 and (b) acetate buffer of pH 5.5. The operational parameters are as those indicated in Fig. 5.

3.2.3. Effect of accumulation potential

To determine the optimal accumulation potential, the potential range of 0.0 to -1.0 V was examined for 2×10^{-6} M isoniazid in acetate buffer at pH 5.5, followed preconcentration for 30 s. Isoniazid generated a much developed peak current intensity (i_{p1}) over the potential range -0.7 to -0.9 V (Fig. 8). Thus an accumulation

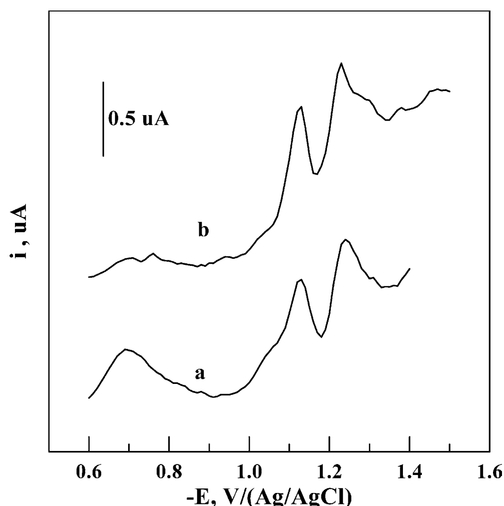

Fig. 5. SWAdCS voltammograms for 2×10^{-6} M isoniazid in B–R buffer of pH 5.5 (curve a), and acetate buffer of pH 5.5 (curve b); $E_{acc.} = -0.6$ V, $t_{acc.} = 30$ s, pulse-amplitude = 50 mV, frequency = 120 Hz, scan increment = 10 mV, rest period 5 s and purge time 10 min.

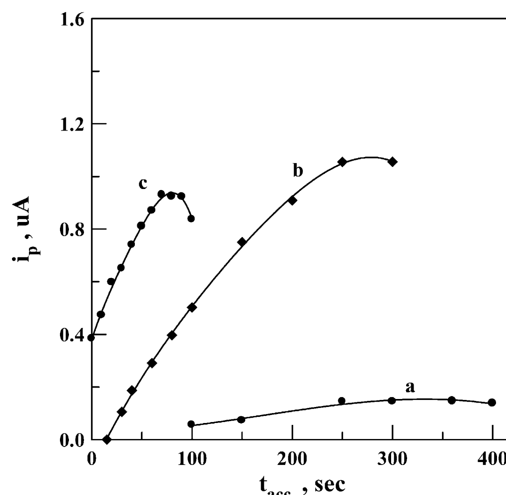

Fig. 7. Effect of accumulation time period ($t_{acc.}$), on the 1st peak current for: (a) 2×10^{-9} M, (b), 2×10^{-7} M and (c) 2×10^{-6} M isoniazid in acetate buffer of pH 5.5. The operational parameters are as those illustrated in Fig. 5.

potential of -0.9 V (vs. Ag/AgCl/KCls) was chosen for all subsequent measurements.

3.2.4. Square-wave operational parameters

The square-wave parameters depended strongly on those of the excitation signal. Therefore, optimization of frequency (*f*), pulse-amplitude

and scan increment were attempted. The dependence of the first peak current (i_{p1}) on frequency (20–120 Hz) was a linear relation. Thus, a frequency of 120 Hz was chosen to improve the sensitivity without any distortion of the peak or the baseline. At a frequency of 120 Hz, the pulse-amplitude was varied between 20 and 100 mV. Although, the peak current increased linearly with the increase of pulse-amplitude, a value of 25 mV was applied, since it generated good resolution, which was better for analytical purposes. Also a scan increment of 8 mV was found to develop a sharper peak and a higher current intensity.

The influence of the surface area of the working electrode on the peak current was also studied. As expected, an increase of the electrode surface area generated a higher peak current, so a mercury drop of a large area (0.026 cm^2) was considered in the present study. The influence of the rest time was also considered and a time period of 5 s was chosen. The optimal experimental and instrumental operational conditions of the proposed stripping procedure are reported in Table 2.

3.2.5. *Validation of the analytical procedure*

The proposed square-wave stripping procedure was applied to the determination of different concentrations of standard solutions of the drug,

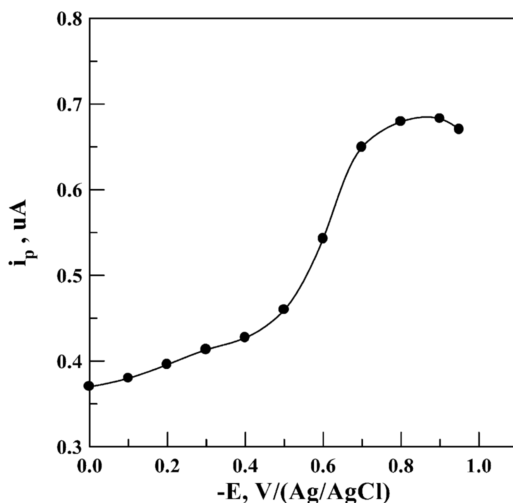

Fig. 8. Effect of accumulation potential ($E_{acc.}$) on the 1st peak current for 2×10^{-6} M isoniazid in acetate buffer of pH 5.5. The operational parameters are as those indicated in Fig. 5.

followed preconcentration for different time periods. Linear calibration graphs over different concentration ranges between 5×10^{-10} and 2×10^{-6} M, depending on the preconcentration time period, were obtained. Characteristics of these graphs are reported in Table 3. As the analyte concentration was extended above 2×10^{-6} M a more or less pronounced deviation from the linearity was appeared, which may be attributed to the complete coverage of the mercury electrode surface with the adsorbed drug species.

Validation of the optimized procedure for the quantitative assay of the drug was examined via evaluation of the limit of detection (LOD), limit of quantitation (LOQ), repeatability, recovery, selectivity, robustness and ruggedness. The LOD and LOQ were calculated from the calibration graphs, obtained after preconcentration of the drug onto the HMDE for four different time periods, using the equations [59]:

$$\mathrm{LOD} = 3\ \mathrm{S.D.}/b \qquad (1)$$

and

$$\mathrm{LOQ} = 10\ \mathrm{S.D.}/b \qquad (2)$$

where S.D. is the standard deviation of the intercept and b is the slope of the calibration graph. The obtained results are reported in Table 3.

Repeatability [60] and recovery were examined by performing five replicate measurements for 5 ×

Table 2
The optimal operational parameters of the proposed procedure for determination of isoniazid in different samples

Parameters	Optimal value
pH	5.5
Electrolyte	Acetate buffer
Temperature (°C)	25
Preconcentration potential (V)	− 0.9 V
Preconcentration time (s)	50–300
Mercury drop size (cm^2)	0.026
Stirring rate (rpm)	400
Purge time (min)	5
Rest time (s)	10
Frequency (Hz)	120
Scan increment (mV)	8
Pulse-amplitude (mV)	25

Table 3
Characteristics of the calibration graphs of bulk isoniazid drug in acetate buffer of pH 5.5 under the optimized operational conditions of the proposed procedure

$t_{acc.}$ (s)	Regression equation i_p(nA) = bC(nM)+a	Linearity range (M)	(r)	LOD (M)	LOQ (M)
50	$i_{p1} = 0.89C+10$	1×10^{-7}–2×10^{-6}	0.998	1.35×10^{-9}	2.96×10^{9}
100	$i_{p1} = 1.35C+20$	5×10^{-8}–5×10^{-7}	0.997	8.88×10^{-10}	4.50×10^{-9}
250	$i_{p1} = 2.48C+30$	2×10^{-9}–2×10^{-7}	0.996	5.43×10^{-10}	1.81×10^{-9}
300	$i_{p1} = 1.60C+90$	5×10^{-10}–5×10^{-9}	0.996	1.18×10^{-10}	3.93×10^{-10}

r, Correlation coefficient.

10^{-8} M isoniazid followed preconcentration for 250 s. A mean percentage recovery of 99.76±0.77 was achieved, which indicated high precision of the proposed procedure.

The selectivity [60] of the optimized procedure for the assay of 5×10^{-8} M isoniazid was examined in the presence of some common excepients in the same ratio usually used in pharmaceutical preparations (e.g. starch, gelatin, lactose, talc and magnesium stearate). The mean percentage recovery based on the average of three replicate measurements (97.41±1.21) showed no significant interference from excepients. Thus the procedure was able to assay isoniazid in the presence of excepients and it can be considered selective.

The robustness [60] of the measurements was examined by a study of the effect of small variations in some important operational parameters such as pH (pH 5–5.5), accumulation potential ($E_{acc.} = -0.8$ to -0.9 V), and preconcentration time period ($t_{acc.} = 250$–300 s) on the recovery of 5×10^{-8} M isoniazid. The obtained mean percentage recoveries (Table 4) were not significantly affected within the studied range of variation of the operational parameters, and consequently the proposed procedure can be considered robust.

The ruggedness [60] of the measurements was examined by assay of 5×10^{-8} M isoniazid when using two potentiostats, models 263A and 394-PAR, under the same optimized operational conditions at different elapsed time. The mean percentage recoveries obtained due to lab-to-lab (Table 4) and even day-to-day variation were found reproducible, since there was no significant difference between the mean percentage recovery and S.D. values.

Table 4
Influence of variations of some of the operational conditions of the proposed procedure on the mean percentage recovery of 5×10^{-8} M isoniazid; frequency = 120 pulse-amplitude = 25 and scan increment = 8 mV

Variables	Experimental conditions	R±S.D.%
pH of the medium		
5	$E_{acc} = -0.9$ V, $t_{acc} = 250$ s,	98.10±0.91
5.5		99.78±0.78
Preconcentration potential (E_{acc})		
−0.8 V	pH 5.5, $t_{acc} = 250$ s	98.13±0.82
−0.9 V		99.78±0.78
Preconcentration time (t_{acc})		
250	pH 5.5, $E_{acc} = -0.9$ V	99.78±0.78
300		101.22±1.11
Potentiostat (*PAR*)		
Lab (1) model 394	pH 5.5, $E_{acc} = -0.9$ V	99.78±0.78
Lab (2) model 263A	$t_{acc} = 250$ s	100.98±1.23

3.3. Application

3.3.1. Assay of isoniazid in tablets

The proposed procedure was successfully applied to the analysis of Isocid® and T.B. Zide® tablets without the need for any pretreatment or extraction steps prior to the analysis. The mean percentage recovery of isoniazid, based on the average of five replicate measurements was favorably compared (Table 5) with that obtained by assay of the same tablet solution by means of a reported spectrophotometric method [14].

3.3.2. Assay of isoniazed in human urine

Isoniazid was also successfully determined in spiked human urine samples by applying the proposed procedure without any sample pretreatment or any time-consuming extraction or evaporation steps prior to the analysis. The peak current versus drug concentration gave a linear relationship over the range 1×10^{-8}–1×10^{-6} M izoniazed, which followed the equation: i_{p1} (nA) = 7.5C (nM) + 148, with a correlation coefficient, r = 0.995. The mean percentage recovery of isoniazid based on the average of five replicate measurements was found to equal 97.08 ± 1.06. The LOD and LOQ of 2.4×10^{-8} and 8.0×10^{-8} M isoniazid, respectively, were achieved. No application to real samples was performed in this study.

3.3.3. Assay of isoniazid in human serum

The proposed procedure was also applied to the determination of isoniazid in protein-free spiked human serum samples. No sample pretreatment or any time-consuming extraction or evaporation steps, other than the centrifugal separation of proteins, were required prior to the drug assays. Fig. 9 illustrates the response of successive additions of isoniazid spiked in human serum, following preconcentration of the drug onto the HMDE for 250 s. Three calibration graphs were constructed over the drug concentration range 5×10^{-9}–8×10^{-8} M. The calibration graph followed the equation: i_{p1} (nA) = 4.1C (nM) + 69, with a correlation coefficient, r = 0.996. The mean percentage recovery of isoniazid based on the average of five replicate measurements for 5×10^{-8} M was found to equal 97.45 ± 2.09. The LOD and LOQ of 1.47×10^{-9} and 4.9×10^{-9} M were achieved, utilizing the equations [59]: LOD = 3 S.D./b and LOQ = 10 S.D./b, respectively. The results were favorably compared with those obtained by assay of the same spiked human serum sample by means of a reported HPLC method [41].

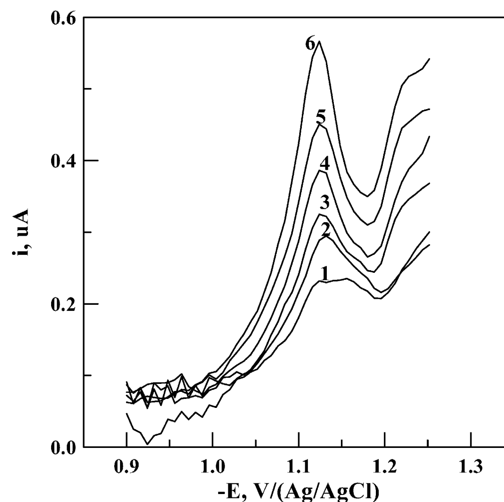

Fig. 9. SWAdCS voltammograms for different concentrations of isoniazid spiked in human serum. (1) Background; (2) 5×10^{-9}; (3) 1×10^{-8}; (4) 1.5×10^{-8}; (5) 2×10^{-8}; and (6) 2.5×10^{-8} M isoniazid; $E_{acc.} = -0.9$ V, $t_{acc.} = 250$ s and scan increment = 8 mV. The other optimized operational parameter are as those indicated in Fig. 5.

In a trial to demonstrate the utility and selectivity of the proposed square-wave stripping procedure, the various pharmacokinetic parameters of isoniazid in human plasma samples collected from two volunteers, followed an oral administration of a 100 mg T.B. Zide® single dose, were estimated. The plasma concentration–time profiles obtained by means of the proposed procedure for two volunteers are shown in Fig. 10. From the plasma concentration–time profiles, the following pharmacokinetic parameters were calculated for the two volunteer.

Table 5
Results of assay of isoniazid in tablets by means of the proposed procedure compared with a reported spectrophotometric method [14]

Brand name	Labeled concentration [drug]/tablet	% Recovery ± S.D.	
		Proposed method	Reported method [14]
Isocid®	50 mg	97.45 ± 1.41	96.21 ± 1.13
T.B. Zide®	100 mg	97.81 ± 1.49	96.24 ± 1.25

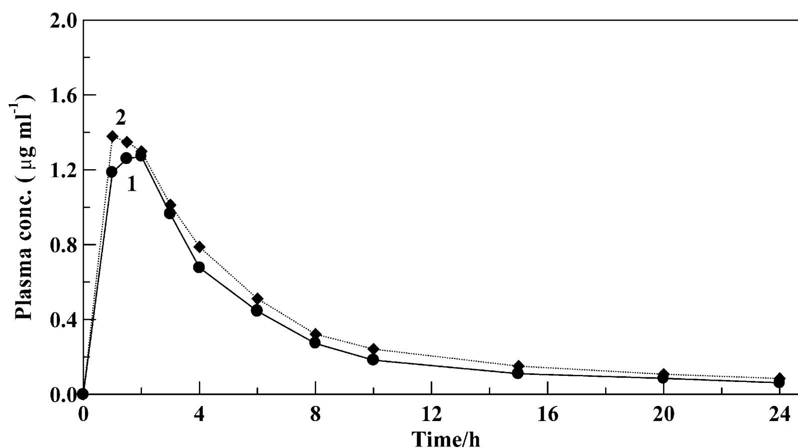

Fig. 10. The plasma concentration–time profile for two volunteers after an oral administration of a 100 mg T.B.Zide® single dose.

Table 6
Pharmacokinetic parameters of isoniazid estimated from the plasma concentration–time profiles of two volunteers (after an oral administration of a 100 mg T.B. Zide® single dose) applying the proposed procedure, compared with those estimated by a reported HPLC method [2]

Parameter*/unit	Volunteer (1)	Volunteer (2)	Average	
			SWAdSV method	HPLC [2] method
C_{max} (μg ml^{-1})	1.27	1.38	1.33	1.43
T_{max} (h)	2.12	1.18	1.65	1.54
AUC_{0-24} (μg h ml^{-1})	7.52	8.23	7.88	7.70
$t_{1/2}$ (h)	4.88	5.72	5.30	3.66
K_e (1/h)	0.20	0.18	0.19	0.22

C_{max} (μg ml^{-1}) = peak plasma concentration, which equals to the plasma concentration at the peak point of the plasma concentration–time profile,
T_{max} (h) = time of peak plasma concentration, which equals to the time period corresponding to C_{max} of the plasma concentration–time profile,
AUC_{0-24} (μg h ml^{-1}) = area under the plasma concentration–time profile, which can be calculated utilizing a suitable software,
$t_{1/2}$ (h) = elimination half-life of the drug, which equals to the time period corresponding to half of the peak plasma concentration C_{max} of the plasma concentration–time profile, and,
K_e (1/h) = elimination rate constant of the drug, which equals to the reciprocal of the elimination half-life of the drug ($1/t_{1/2}$).

These parameters were calculated from the plasma concentration–time profiles utilizing the PK SOLUTIONS 2.0, Noncompartmental Pharmacokinetics Data Analysis software (Summit, Research Services, UK), Table 6. The results were favorably compared with values of the pharmacokinetic parameters of isoniazid determined by a reported HPLC method [2] for volunteers after similar oral administration single dose (Table 6).

4. Conclusion

The polarographic reduction reaction of isoniazid drug in B–R buffers of pH 2–11 has been elucidated.. A validated square-wave adsorptive cathodic stripping voltammetric procedure was developed and successfully applied to the estima-

tion of isoniazid in bulk drug, pharmaceutical formulations, and human biological fluids. The limits of detection and quantitation of the drug by means of the proposed procedure are lower than those obtained by most of the reported analytical methods [6–35,49–53].

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