Current Topics in Pharmacology
Vol. 19, 2015

Original Communication

Voltammetric detection and quantification of pentacyano(isoniazid)ferrate(II), a metal-based drug candidate for the treatment of tuberculosis

Adilio da S. Dadda[a,b], Valnês S. Rodrigues-Junior[a,b], Mariane Rotta[a,c], Leonardo K. B. Martinelli[a,b], Maria M. Campos[a,c,d], Eduardo H. S. Sousa[e], Antônio F. M. Pinto[a], Pablo Machado[a,b], Luiz A. Basso[a,b,c,*,#] and Diógenes S. Santos[a,b,*,$]

[a]Centro de Pesquisas em Biologia Molecular e Funcional (CPBMF, Instituto Nacional de Ciência e Tecnologia em Tuberculose (INCT-TB), Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), 6681/92A Av. Ipiranga, 90619-900; [b]Programa de Pós-Graduação em Biologia Celular e Molecular, 6681/12A Av. Ipiranga, 90619-900; [c]Programa de Pós-Graduação em Medicina e Ciências da Saúde, 6690/60 Av. Ipiranga, 90610-000; [d]Instituto de Toxicologia e Farmacologia, 6681/12C Av. Ipiranga, 90619-900, Porto Alegre, RS, PUCRS; [e]Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Cx. Postal 6021, 60440-900, Fortaleza, CE, Brazil.

ABSTRACT

A voltammetric method was employed to detect and quantify pentacyano(isoniazid)ferrate(II) compound (IQG-607), which is an inorganic complex with anti-tuberculosis (TB) activity. The technique used was adsorptive stripping voltammetry (AdSV) and application of a differential pulse scan in the stripping step (DPAdSV). The McIlvaine buffer was used as the base electrolyte. The compound presented three peaks at -0.70 V, -0.95 V and -1.1 V. Optimization of the method to improve the peak current was carried out by varying different factors such as pulse amplitude, pulse time, deposition time, deposition potential, and pH of the base electrolyte. The calibration curve was linear for IQG-607 concentrations ranging from 1 to 20 μg mL^{-1}, and the limit of detection (LOD) and limit of quantification (LOQ) were 0.5 μg mL^{-1} and 1 μg mL^{-1}, respectively. The stability of IQG-607 stock solution at two temperatures was investigated by the DPAdSV method, and concomitant *M. tuberculosis* 2-*trans*-enoyl-ACP(CoA) reductase (*Mt*InhA) enzyme activity measurements were carried out. These results suggest the formation of isonicotinic acid (INA) at -1.2 V as the degradation product, and revealed that the compound is stable for at least 1 week at 4 ºC in solution. In addition, the correlation between reduction in the value of the first-order rate constant of *Mt*InhA enzyme inhibition and IQG-607 chemical stability provides evidence that the latter, and not its metabolite(s), is the active chemical compound. The optimization of experimental conditions to detect and quantify the IQG-607 compound by the DPAdSV method represents an experimental approach on which to base further pharmacokinetic studies in complex biological fluids, before embarking on costly clinical assays. The IQG-607 compound may be an example of a promising metal-based antimicrobial that inhibits the activity of a validated and druggable target (*Mt*InhA) aiming at the development of an anti-TB agent.

KEYWORDS: pentacyanoferrate(II), inorganic complex, anti-tuberculosis activity, adsorptive stripping voltammetry, 2-*trans*-enoyl-ACP(CoA) reductase

*Corresponding authors
[#]luiz.basso@pucrs.br
[$]diogenes@pucrs.br

INTRODUCTION

Tuberculosis (TB) is an infectious disease, whose main pathogen is *Mycobacterium tuberculosis*. Isoniazid (INH) (Fig. 1A) is a first-line antibiotic used in TB therapy [1]. INH is a prodrug that is activated by a catalase-peroxidase enzyme (KatG) forming a covalent adduct with the nicotinamide adenine dinucleotide, reduced form (NADH) cofactor. The INH-NAD adduct has been shown to inhibit the *M. tuberculosis* 2-*trans*-enoyl-ACP(CoA) reductase (*Mt*InhA) enzyme, which belongs to the mycobacterial type II fatty acid synthase (FAS-II) system [2-4]. However, the emergence of *M. tuberculosis* strains resistant to both INH and to other drugs currently used for the treatment of TB has reinforced the need for the development of new chemotherapeutic agents to treat this disease [5-7].

The pentacyano(isoniazid)ferrate(II) compound (IQG-607) is an inorganic complex designed to act against the INH-resistant strains of *M. tuberculosis* harboring mutations in the catalase-peroxidase gene, *katG* [8, 9]. This compound (Fig. 2) is a slow-binding inhibitor of *Mt*InhA enzyme, with a true overall dissociation constant value of 70 x 10^{-9} mol L^{-1} [10]. The presence of penta-cyanoferrate(II) moiety is thought to mimic the *in vitro* activation of INH by (KatG) enzyme, indicating that IQG-607 would not require the mycobacterial catalase-peroxidase enzyme to exert its action, and thus might overcome this mechanism of INH resistance [10, 11].

The *in vitro* effectiveness of IQG-607 was observed in the macrophage model of *M. tuberculosis* infection, showing a similar effect to that displayed by INH and rifampicin (RIF) [12]. The *in vivo* efficacy of IQG-607 was also observed in the murine model following oral administration of *M. tuberculosis* with an effect comparable to INH [13]. Interestingly, high doses of IQG-607 (10-fold larger than INH) were administered in mice, and no signs of toxicity were observed [13]. Accordingly, the IQG-607 compound appears to be a promising candidate for the anti-TB drug development efforts. However, a number of development steps should be taken before embarking on costly clinical assays [14, 15]. A critical step is the development of an analytical method to detect the primary compound and, hopefully, its likely metabolites.

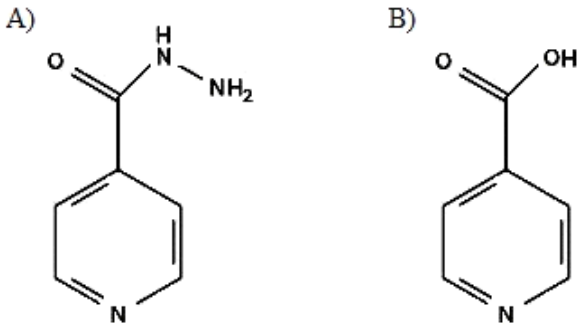

Fig. 1. Chemical structures of INH (A) and INA (B).

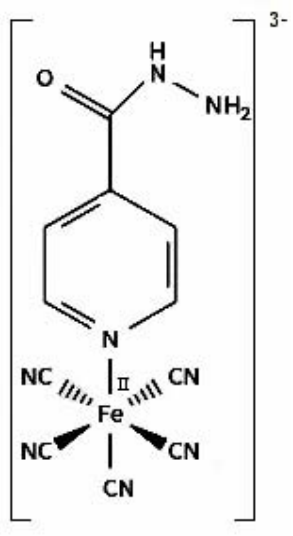

Fig. 2. Chemical structure of IQG-607.

To the best of our knowledge, there has been no analytical method described in the literature to detect and quantify the IQG-607 compound. We have tried a number of experimental protocols to detect the IQG-607 compound by high performance liquid chromatography (HPLC) using a variety of mobile phases and column types, including normal and reverse phases. However, no attempt was successful, probably due to the high polarity of IQG-607, which could lead to a strong retention in chromatographic columns [8, 16]. Issues associated with the analysis of pentacyanoferrate complexes by mass spectrometry, such as loss of cyanide and changes in oxidation state, have also been reported [17]. However, it needs to be shown whether or not there is any cyanide release or change in the oxidation state for IQG-607 in solution. Voltammetry

is an alternative technique that should be investigated, as several reports have shown the usefulness of electrochemical methods in the analysis of INH [18-24] and pentacyanoferrate complexes [25-28]. Stripping voltammetry methods are amongst the most efficient electrochemical techniques for trace analysis and species analysis as the analyte accumulates before its detection, resulting in increased sensitivity and selectivity. Adsorptive stripping voltammetry (AdSV) is suitable for the analysis of organic compounds having surface-active properties that may allow them to be adsorbed at the electrode surface, and differential pulse as a valuable scan mode (DPAdSV) to decrease detection limit.

The aim of the present study was to apply and optimize a voltammetric method for the determination of IQG-607 by adsorptive stripping voltammetry (AdSV) using differential pulse voltammetry as the scan mode (DPAdSV). Chemical stability of IQG-607 was also evaluated by the DPAdSV method under various experimental conditions, and the results were compared with the changes in the *Mt*InhA enzyme inhibition by IQG-607. It is hoped that the analytical method here described will provide a foundation on which to base pharmacokinetic studies in biological fluids (Absorption, Distribution, Metabolism, and Excretion) of IQG-607, a metal-based compound with potential anti-TB activity.

MATERIALS AND METHODS

Measuring apparatus

757 VA Computrace (Metrohm, Switzerland) was used for AdSV measurements, applying a differential pulse (DP) in the stripping step (DPAdSV). Multimode electrode (MME) as the working electrode operating in the hanging mercury drop electrode (HMDE) mode, platinum (Pt) auxiliary electrode, and silver/silver chloride (Ag/AgCl) reference electrode containing 3 mol L^{-1} of potassium chloride (KCl) were used. All experiments were carried out at room temperature (25 ºC). For *Mt*InhA enzyme inhibition assays, a UV-2550 UV/Visible spectrophotometer (Shimadzu, Japan) was employed.

Reagents and solutions

The IQG-607 was synthesized with purity higher than 99%, and its chemical structure was characterized according to Oliveira *et al*. [10]. INH 99% (Acros Organics, Belgium) and Isonicotinic acid (INA) 99% (Sigma-Aldrich, USA) were used as controls. The supporting electrolyte McIlvaine buffer was prepared by dissolving appropriate amounts of sodium phosphate dibasic (Na_2HPO_4) ≥99.0% (Sigma-Aldrich, USA), 0.2 mol L^{-1} and citric acid anhydrous ≥99.5% (Sigma-Aldrich, USA), 0.1 mol L^{-1}. Milli-Q water (Millipore) was used in the preparation of all solutions. The 2-*trans*-dodecenoyl-CoA (DD-CoA) substrate was synthesized according to the protocol described in "*Mt*InhA enzyme inhibition assay" section. The Na_2HPO_4 buffer pH 7.5 (Synth, Brazil), NADH (Acros Organics, Belgium), and PIPES (Sigma-Aldrich, USA) were the chemicals used in *Mt*InhA enzyme inhibition assays.

Voltammetric measurements

For voltammetric measurements, 5 mL of McIlvaine buffer electrolyte (pH 7.5) was added to a voltammetric vessel of 5 mL, and 100 µL of Milli-Q water was added. The 'measure blank' analysis option (757 VA Computrace Software 2.0) was activated to subtract the signal of the electrolyte, for obtaining a straight baseline. IQG-607 detection method was optimized by varying the following parameters: pulse amplitude in the range 0.01-0.1 V, pulse time (0.02-0.1 s), deposition time (0-120 s) and deposition potential (-0.3 – -1.4 V), to obtain the best sensitivity. The effect of pH of the electrolyte on the peak potential (V) and peak current (nA) of the IQG-607 was also evaluated in the range 2.5-9. Hence, the analysis was performed under the following optimized parameters: 1 kgf cm^{-2} of nitrogen purge gas for 150 s, -0.3 V deposition potential, 90 s deposition time, 10 s equilibration time, 0.012 V voltage step, 75 mV pulse amplitude, 20 ms pulse time, 0.4 V voltage step time, -0.3 V starting potential and -1.4 V end potential (cathodic direction). After the reading of the signal electrolyte was recorded, the electrolyte was discarded from the voltammetric vessel. A volume of 5 mL of the electrolyte was again added to the vessel, and 100 µL of the standard solution of IQG-607 with a concentration of 15 µg mL^{-1} was also added, and AdSV voltammogram was recorded after applying a differential pulse scan in the stripping step (DPAdSV). The voltammetric analysis of INH

(Fig. 1A) and INA (Fig. 1B), both at a concentration of 15 µg mL^{-1}, were performed under the same conditions as described for IQG-607. The limit of detection and the limit of quantification (LOD and LOQ, respectively) for IQG-607 were evaluated by employing the optimized method. The LOD was determined from the current obtained with the lowest concentration of the compound that could be detected, as compared with the blank, to distinguish the signal of IQG-607 from any background signal. The LOQ was performed in triplicate by the manual standard addition method, and the accuracy and precision values were obtained using statistical software, evaluating the acceptable values (precision of 20% and accuracy of 80-120%). The analytical curve was constructed in the range of 1-20 µg mL^{-1}, after successive additions of a standard solution of the analyte. The stability of two stock solutions of IQG-607 (15 µg mL^{-1}) was evaluated. The first stock solution was stored at 4 ºC, and the second was incubated at 37 ºC, both in amber bottles. Changes in the electrochemical behavior were evaluated at time zero, seven (7) days, and thirty (30) days after the date of preparation.

*Mt*InhA enzyme inhibition assay

An IQG-607 time-dependent inhibition assay of *Mt*InhA was carried out to determine if the changes in the electrochemical behavior have any effect on its inhibitory activity, corroborating the voltammetric analysis of the compound. Recombinant *Mt*InhA was expressed and purified as described elsewhere [29, 30]. The substrate DD-CoA was synthetized from coenzyme A and 2-*trans*-dodecenoic acid as previously reported [29], and purified by reverse-phase high performance liquid chromatography (HPLC) (GE Health Care Life Sciences, UK) using a 19 x 300 mm C_{18} µBondapak column (Waters Associates, USA) as described elsewhere [31]. The concentration of *Mt*InhA was determined spectrophotometrically by diluting 10 µL of purified enzyme to 500 µL using 100 mM PIPES (pH 7.0), and using a molar absorption coefficient of 29,220 M^{-1} cm^{-1} at 282 nm. The enzyme activity assay of *Mt*InhA was carried out in the presence of substrates DD-CoA and NADH by monitoring the oxidation of NADH to NAD^+ at 340 nm (ε_{NADH} = 6220 M^{-1} cm^{-1}) for 60 s at 25 °C using a UV-2550 UV/Visible spectrophotometer. *Mt*InhA time-dependent inactivation by the compound IQG-607 was performed as described by Oliveira *et al*. [10]. In brief, 3 µM of *Mt*InhA was incubated with 500 µM of IQG-607 (final concentration in a cuvette of 17 µg mL^{-1}) and 100 mM Na_2HPO_4 buffer. Aliquots of this mixture were taken at different times and added to the cuvette containing 100 µM of DD-CoA and 200 µM of NADH to make up the final volume to 500 µL and measure the remaining enzymatic activity. The following equations were employed to determine the k_{obs} and the $t_{1/2}$ of inhibitor IQG-607 in each condition, respectively:

$$v = v_0 e^{-k_{obs} t} \quad (1)$$

$$t_{1/2} = \frac{\ln 2}{k_{obs}} \quad (2)$$

where v is the remaining *Mt*InhA enzyme activity at time t, v_0 is the enzyme activity at time zero, k_{obs} is the observed first-order rate constant for *Mt*InhA inhibition, t is the time in seconds, and $t_{1/2}$ is the half-life of the first-order rate constant for enzyme inhibition.

RESULTS AND DISCUSSION

Voltammetric characteristics of IQG-607

DPAdSV voltammograms of blank solution and IQG-607 in McIlvaine buffer electrolyte are presented in fig. 3. No interfering peaks were observed in the blank solution (Fig. 3A). The IQG-607 compound is characterized by the formation of three peaks after the cathodic sweeping (Fig. 3B), according to the instrumental parameters already optimized (the optimization of the method is presented in the next section). The presence of three peaks indicates that the molecule has three electroactive sites, with corresponding peaks at -0.70V, -0.95 V, and -1.10V (Fig. 3B). Previous studies demonstrated that INH has two electroactive sites when analyzed by differential pulse voltammetry [19, 21, 23]. Furthermore, analyzing INH using the McIlvaine buffer electrolyte, Leandro *et al.* also found one of the peaks in the potential of approximately -1.10 V [23]. Sulaiman *et al.* [19] and Asadpour-Zeynali *et al.* [21] reported a peak at approximately -1.08 V by differential pulse voltammetry. Analysis of INH (15 µg mL^{-1}) in the experimental conditions here described showed a

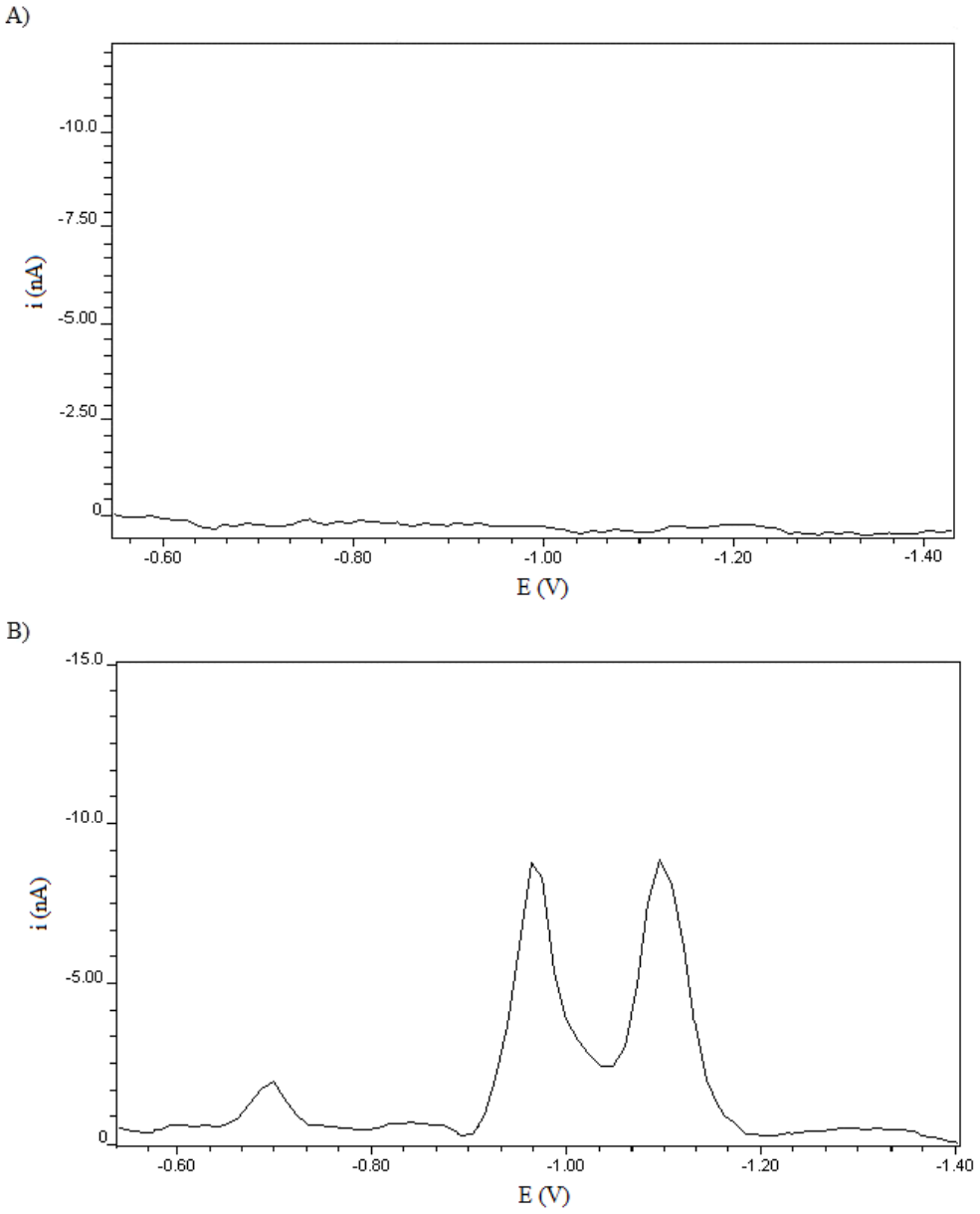

Fig. 3. Differential pulse adsorptive stripping voltammogram for: (A) McIlvaine buffer pH 7.5 (blank), and (B) 15 µg mL^{-1} of IQG-607 in McIlvaine buffer electrolyte (pH 7.5). The analysis was performed under optimized conditions.

peak at -1.10 V (Fig. 4). These results suggest that INH and IQG-607 have the same electrochemically active site with a corresponding peak at -1.10 V. As IQG-607 can be regarded as an INH analog, this finding is not surprising. Accordingly, it is tempting to suggest that this peak corresponds to the same reduction process, namely, hydrazide group reduction and ammonia release, as reported by Angulo *et al.* [32].

The peak at -0.95 V can be attributed to the reduction of the carbon-nitrogen double bond of the pyridine ring of IQG-607, which is likely to be the same reduction process that occurs with INH [32]. However, comparing figs. 3B and 4, the peak for INH appears at a more negative potential than for IQG-607. As IQG-607 has a different electronic configuration due to the presence of the metal center, the reduction occurs at a different potential.

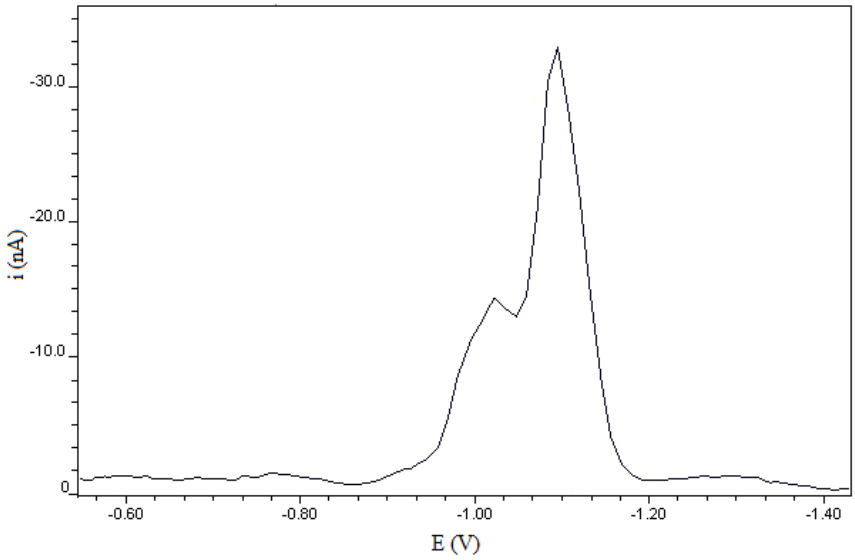

Fig. 4. Differential pulse adsorptive stripping (DPAdSV) voltammogram for 15 µg mL^{-1} of INH in McIlvaine buffer electrolyte (pH 7.5). The analysis was performed under optimized conditions.

The peak at -0.70 V may correspond to a reduction process similar to the one which occurs with sodium nitroprusside [28, 33, 34]. However, further investigation is required as a different reduction process may be involved in the metal center considering the electronic configuration of IQG-607. INH (Fig. 4) presents peak current values larger than IQG-607 (Fig. 3B). Furthermore, the peaks that differentiate IQG-607 from INH are those that appear at -0.70 V and -0.95 V (Fig. 3B), and between these two peaks, the peak at -0.95 V has a larger current intensity value. Accordingly, the peak at -0.95 V was deemed more appropriate to build an analytical method for IQG-607 detection.

Optimization of the voltammetric method

The influences of different voltammetric parameters such as pulse amplitude, pulse time, deposition time, and deposition potential on IQG-607 peak at -0.95 V were evaluated. The peak current of IQG-607 augmented with increasing pulse amplitude in the range that was evaluated (Fig. 5A). Although the highest peak was obtained at 100 mV, the peak at 75 mV was chosen due to its high sensitivity without loss of peak resolution, which is preferable for analytical purposes. In the pulse-time parameter, it is possible to observe a decrease in the peak current with increasing time (Fig. 5B). A pulse time of 20 ms was thus subsequently employed. The dependence of the peak current was also evaluated by varying the deposition time (Fig. 5C). There was a gradual increase in the current up to 90 s, followed by a decrease in longer deposition time (Fig. 5C). Accordingly, a deposition time of 90 s was chosen for improved sensitivity. The influence of deposition potential on the peak current was studied by varying potentials between -0.3 V and -1.4 V (Fig. 5D) at the deposition time of 90 s. As lower peak current values were observed for larger deposition potential values (Fig. 5D), a value of -0.3 V for deposition potential was deemed more appropriate. Thus, according to the results here presented, the parameters employed in IQG-607 analysis were 75 mV pulse amplitude, 20 ms pulse time, 90 s deposition time and -0.3 V deposition potential. The other voltammetric parameters were used as recommended by the equipment manufacturer. The dependence of peak current and peak potential on the pH of the electrolyte was also evaluated (Fig. 6). No peak could be observed at pH values lower than 2.5, whereas there was an increase in peak current for pH values ranging from 4.5 to 7.5 (Fig. 6A). A decrease in the peak current at pH value of 9 was observed (Fig. 6A). Therefore, pH 7.5 was chosen as optimal for the analysis in terms of sensitivity. In addition, the influence of pH on the peak potential

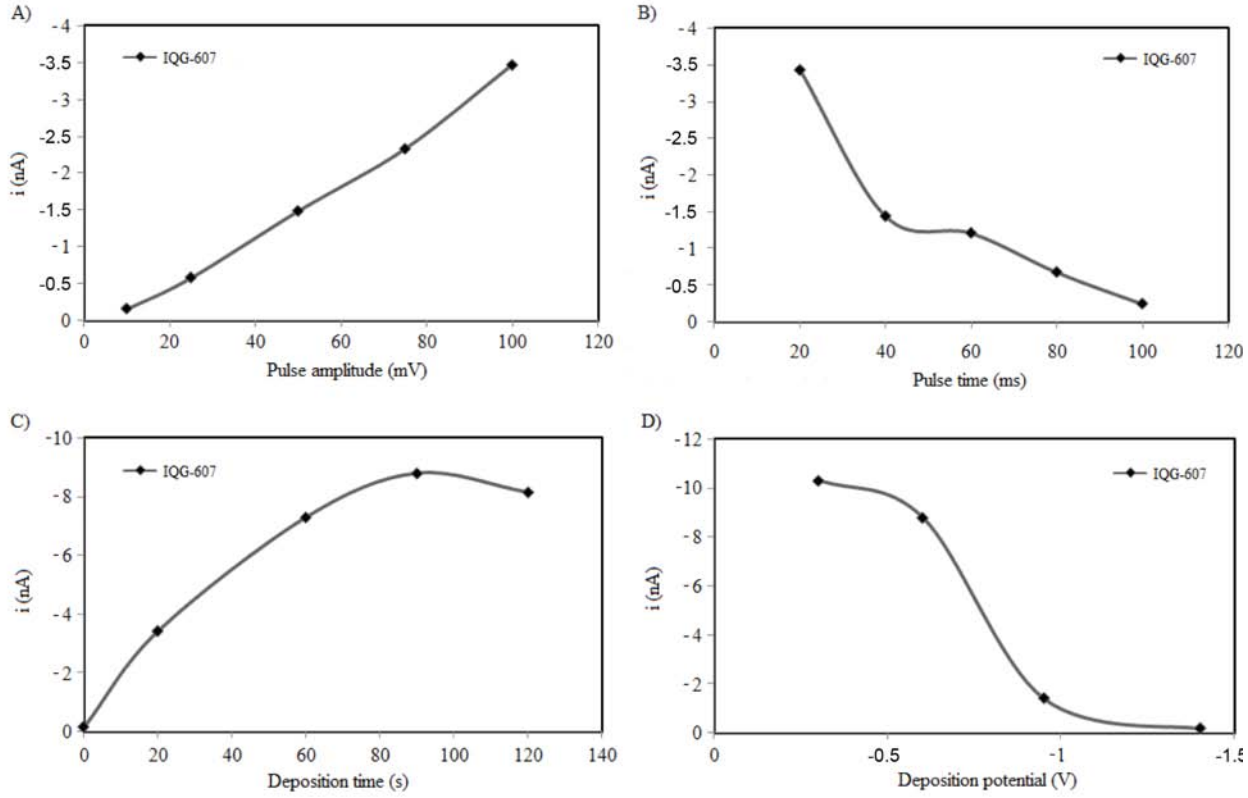

Fig. 5. Effect of different voltammetric parameters on the peak current for 15 µg mL^{-1} of IQG-607 in McIlvaine buffer electrolyte. (A) Pulse amplitude: 10, 25, 50, 75, and 100 mV. (B) Pulse time: 20, 40, 60, 80, and 100 ms. (C) Deposition time: 0, 20, 60, 90, and 120 s. (D) Deposition potential: -0.3, -0.6, 0.95, and -1.4 V.

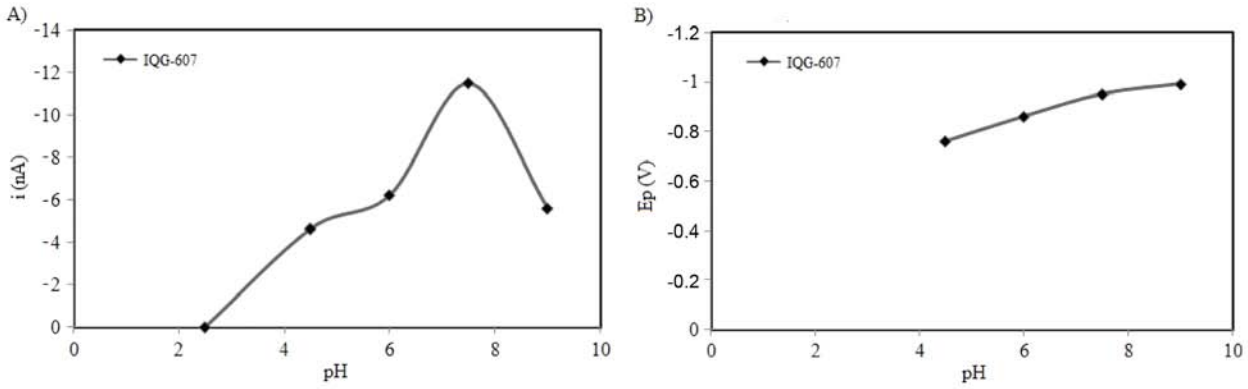

Fig. 6. Effect of different pH values (2.5, 4.5, 6, 7.5, and 9) of the McIlvaine buffer electrolyte on the peak current (A) and peak potential (B) for 15 µg mL^{-1} of IQG-607. The analysis was performed under optimized voltammetric parameters.

is illustrated in Fig. 6B. Accordingly, these results show that a pH value of 7.5 is appropriate to detect IQG-607 compound by AdSV, employing a differential pulse scan in the stripping step (DPAdSV) and recording the peak at -0.95 V.

Linearity, limit of detection (LOD), and limit of quantification (LOQ)

The peak current showed a linear dependence on IQG-607 concentrations ranging from 1 to 20 µg mL^{-1} (Fig. 7), under optimal experimental conditions

(pulse amplitude: 75 mV, pulse time: 20 ms, deposition time: 90 s and deposition potential: -0.3 V). The correlation coefficient (r) value of 0.9991 indicates good linearity. The LOD was 0.5 µg mL^{-1} and the LOQ was 1 µg mL^{-1}, with a relative standard deviation (RSD) of 1.64 %.

Evaluation of stability

The stability of a stock solution of IQG-607 (15 µg mL^{-1}) stored at 4 ºC was estimated by DPAdSV (Fig. 8A). After 7 days, the peaks at -0.7 V and -0.95 V had a slight increase, the peak at -1.1 V decreased, and a new peak appeared at -1.2 V. Measurements of *Mt*InhA activity were carried out to enable to correlate these changes with the reduction of enzyme inhibition by IQG-607. The kinetic results showed no reduction in the enzyme inhibition rate constant after 7 days (Table 1). After 30 days, the DPAdSV peaks at -0.7 V, -0.95 V, and -1.2 V showed a further increase, and the peak at -1.1 V showed a further decrease. An approximately 8-fold reduction in *Mt*InhA inhibition rate constant was observed after 30 days of incubation at 4 ºC (Table 1). The new peak at -1.2 V observed in the DPAdSV probably indicates isonicotinic acid (INA) as a degradation product of IQG-607, which is consistent with the decrease in enzyme inhibition rate constant after 1 month at 4 ºC (Table 1). The peak at -1.2 V showing the formation of INA was confirmed when the INA standard was analyzed by DPAdSV, under the same experimental conditions (Fig. 9), which is in agreement with the previous reports [35, 36]. Interestingly, it has been shown that INA is the main product generated upon oxidation of IQG-607 by H_2O_2 [8, 16]. It is thus tempting to suggest that the formation of INA could account for the decrease of the peak at -1.1 V, which is likely due to a lower concentration of the

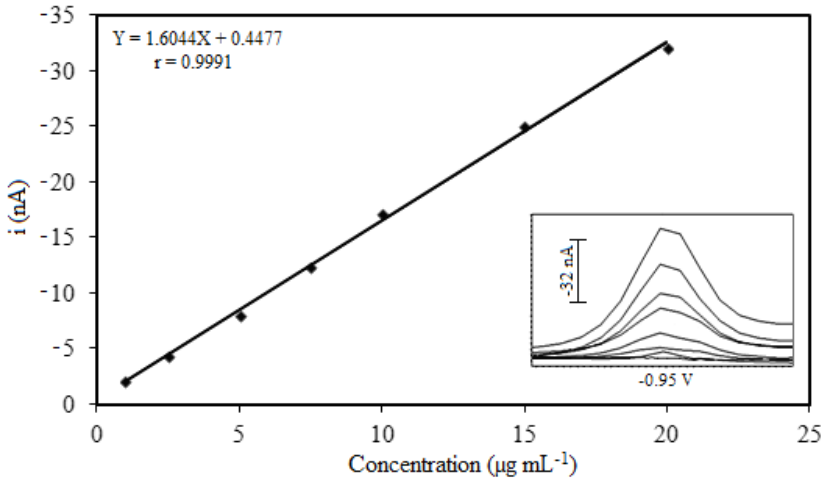

Fig. 7. Linearity of the analytical curve of DPAdSV optimized method for the determination of IQG-607 in McIlvaine buffer electrolyte. The curve was obtained using the following IQG-607 concentrations: 1, 2.5, 5, 7.5, 10, 15, and 20 µg mL^{-1}. The analysis was performed under optimized conditions. The inset shows the influence of increasing IQG-607 concentrations on the peak current (current versus potential).

Table 1. Inhibition parameters of IQG-607 after storage at 4 ºC and 37 ºC over different periods of days.

Temp. (°C)	Time (days)	k_{obs} (min^{-1})	$t_{1/2}$ (min)
-	Zero	0.87 (± 0.01)	0.79 (± 0.01)
4	7	0.80 (± 0.07)	0.86 (± 0.08)
4	30	0.11 (± 0.01)	6.07 (± 0.56)
37	7	0.048 (± 0.004)	14.4 (± 1.2)

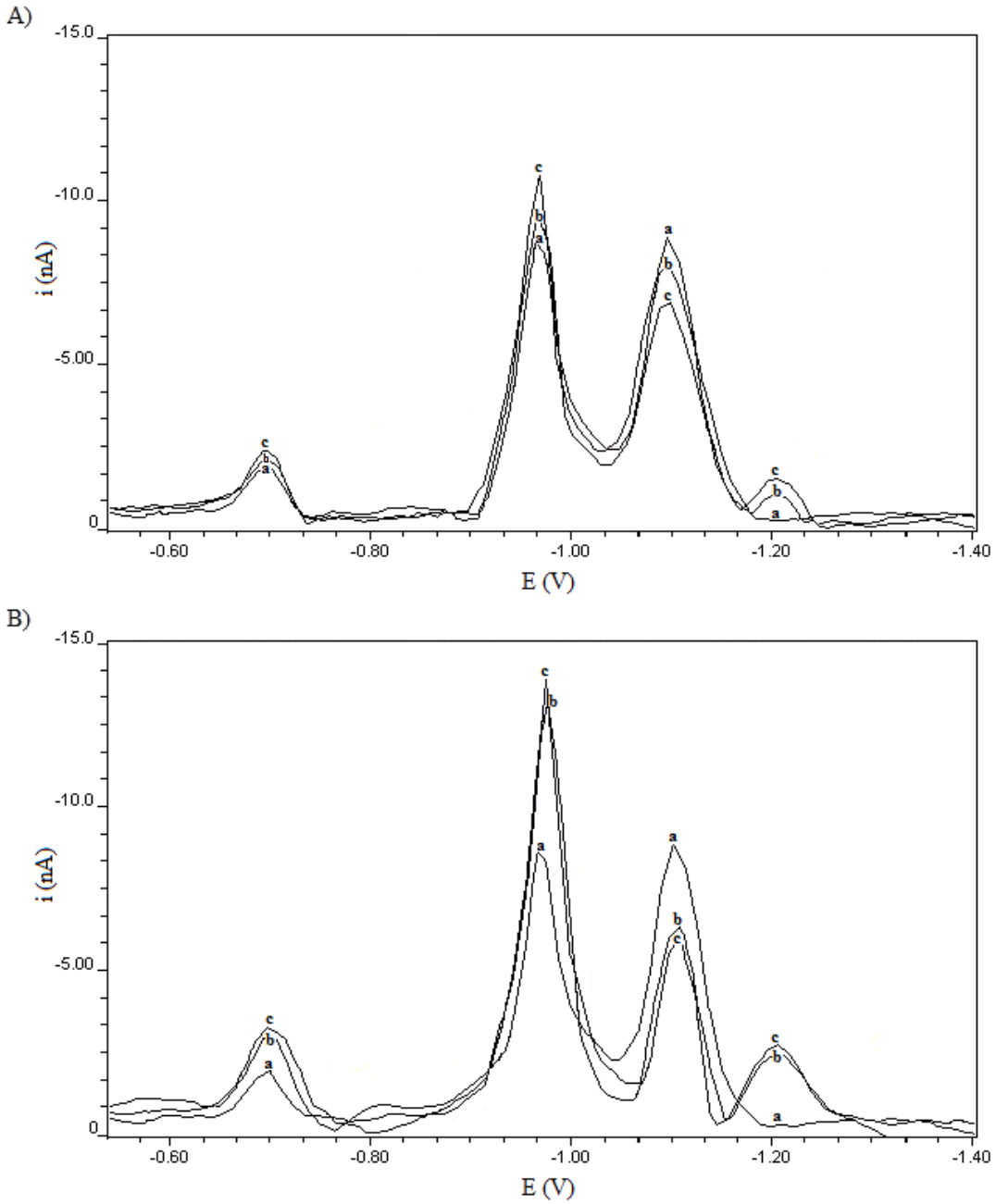

Fig. 8. DPAdSV voltammogram for 15 µg mL^{-1} of IQG-607 before and after incubation at (A) 4 ºC and (B) 37 ºC. (a) Freshly prepared solution; (b) after 1 week; (c) after 1 month. The electrolyte was McIlvaine buffer (pH 7.5). The analysis was performed under optimized conditions.

hydrazide chemical group of the INH moiety of IQG-607 (Fig. 8A). In addition, with the loss of hydrazide, the peaks at -0.7 V and -0.95 V exhibit an increase in the current.

The stock solution of the IQG-607 analyzed by DPAdSV upon storage at 37 ºC showed similar overall changes in the peaks as occurred with the stock solution stored at 4 ºC (Fig. 8B). However, the increase in the peaks that appear at -0.7 V and -0.95 V, and the decrease at -1.1 V are more pronounced. Besides, the intensity of the peak at -1.2 V was greater, indicating that a greater

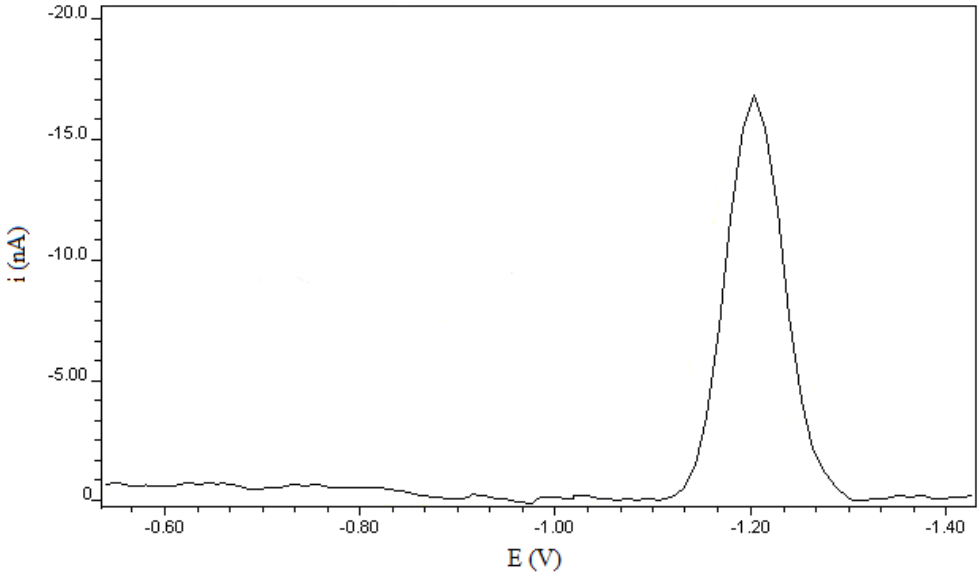

Fig. 9. DPAdSV voltammogram for 15 µg mL^{-1} of INA in McIlvaine buffer electrolyte (pH 7.5). The analysis was performed under optimized conditions.

amount of INA is already formed during the first 7 days of storage. Moreover, the compound has shown a faster loss of enzyme inhibitory activity, since there was an approximately 18-fold decrease in the first-order inhibition rate constant after 7 days of incubation (Table 1). On the thirtieth day of storage, the DPAdSV peaks showed the same trend, but with smaller intensity (Fig. 8B). No enzyme activity measurements were carried out on the thirtieth day as the inhibitory activity of IQG-607 was already rather low at day seven (Table 1). Taken together, these results also show that intact IQG-607 is the inhibitor of *Mt*InhA enzyme activity, and not its degradation products.

The results here reported show that the observed electrochemical changes of IQG-607 upon storage are associated with the loss of enzyme inhibitory activity. Accordingly, it can be proposed that the IQG-607 compound is stable for at least 1week at 4 ºC. The only degradation product observed by voltammetry was INA. However, it should be pointed out that other degradation products may have been formed but not detected by voltammetry, as this experimental approach detects electroactive chemical groups.

CONCLUSION

As far as we are aware, the results here presented are the first report on the use of the DPAdSV to detect and quantify IQG-607 chemical compound. This method has the advantages of being simple, fast, cost-effective, and having good sensitivity (LOD = 0.5 µg mL^{-1}; LOQ = 1 µg mL^{-1}). Moreover, IQG-607 chemical stability was monitored by the voltammetric measurements and associated with the loss of its inhibitory activity of *Mt*InhA enzyme. These data provide evidence that IQG-607 (not its metabolites) is the inhibitor of *Mt*InhA activity. Information on appropriate temperature of storage should aid further studies to be pursued, including stability in drug formulations. It is hoped that the voltammetric method here described may be employed for detection, stability and quality control studies on IQG-607 chemical compound. The method here described represents a fundamental step towards the development of an analytical method to be employed in biological fluids for pharmacokinetic studies, which is required before embarking on costly clinical phases of anti-TB drug development. Lastly, IQG-607 may be an example of a metal-based drug with antimicrobial activity [37], that inhibits the activity of a validated and druggable target (*Mt*InhA), for the development of a chemotherapeutic agent to treat TB.

ACKNOWLEDGEMENTS

This work was supported by funds from Decit/SCTIE/MS-MCT-CNPq-FNDCT-CAPES to

the National Institute of Science and Technology on Tuberculosis (INCT-TB) to D. S. S and L. A. B. L. A. B. and D. S. S. also acknowledge the financial support awarded by FAPERGS-CNPq-PRONEX-2009. L. A. B. (CNPq, 520182/99-5) and D. S. S. (CNPq, 304051/1975-06) are Research Career Awardees of the National Research Council of Brazil (CNPq). V. S. R. J. and L. K. B. M. acknowledge scholarships awarded by FAPERGS-CAPES (DOCFIX). A. F. M. P. acknowledges a post-doc scholarship of CNPq. M. R. is the recipient of a doctoral-degree scholarship awarded by CNPq.

CONFLICT OF INTEREST STATEMENT

The authors hereby declare that there are no conflicts of interest.

ABBREVIATIONS

ACP : acyl carrier protein
AdSV : adsorptive stripping voltammetry
DD-CoA : 2-*trans*-dodecenoyl-CoA
DP : differential pulse
DPAdSV : applying a differential pulse scan in the stripping step of AdSV method
FAS-II : mycobacterial type II fatty acid synthase system
HMDE : hanging mercury drop electrode
INA : isonicotinic acid
INH : isoniazid
IQG-607 : pentacyano(isoniazid)ferrate(II) compound
KatG : *Mycobacterium tuberculosis* catalase-peroxidase
LOD : limit of detection
LOQ : limit of quantification
MME : multimode electrode
*Mt*InhA : *Mycobacterium tuberculosis* 2-*trans*-enoyl-ACP(CoA) reductase
RIF : rifampicin
TB : tuberculosis

REFERENCES

1. World Health Organization. 2014, Global Tuberculosis Report 2013, WHO Press, Geneva.
2. Banerjee, A., Dubnau, E., Quémard, A., Balasubramanian, V., Um, K. S., Wilson, T., Collins, D., de Lisle, G. and Jacobs, W. R. Jr. 1994, Science, 263, 227.
3. Rozwarski, D. A., Grant, G. A., Barton, D. H., Jacobs, W. R. Jr. and Sacchettini, J. 1998, Science, 279, 98.
4. Vilchèze, C., Wang, F., Arai, M., Hazbón, M. H., Colangeli, R., Kremer, L., Weisbrod, T. R., Alland, D., Sacchettini, J. C. and Jacobs, W. R. Jr. 2006, Nat. Med., 12, 1027.
5. Gandhi, N. R., Nunn, P., Dheda, K., Schaaf, H. S., Zignol, M., van Soolingen, D., Jensen, P. and Bayona, J. 2010, Lancet, 375, 1830.
6. Koul, A., Arnoult, E., Lounis, N., Guillemont, J. and Andries, K. 2011, Nature, 469, 483.
7. Udwadia, Z. F., Amale, R. A., Ajbani, K. K. and Rodrigues, C. 2012, Clin. Infect. Dis., 54, 579.
8. Sousa, E. H. S., Basso, L. A., Santos, D. S., Diógenes, I. C. N., Longhinotti, E., Lopes, L. G. F. and Moreira, I. S. 2012, J. Biol. Inorg. Chem., 17, 275.
9. Basso, L. A., Schneider, C. Z., dos Santos, A. J. A. B., dos Santos, Jr. A. A., Campos, M. M., Souto, A. A. and Santos, D. S. 2010, J. Braz. Chem. Soc., 21, 1384.
10. Oliveira, J. S., Sousa, E. H. S., Souza, O. N., Moreira, I. S., Santos, D. S. and Basso, L. A. 2006, Curr. Pharm. Des., 12, 2409.
11. Oliveira, J. S., Sousa, E. H. S., Basso, L. A., Palaci, M., Dietze, R., Santos, D. S. and Moreira, I. S. 2004, Chem. Commun., 7, 312.
12. Rodrigues-Junior, V. S., Junior, A. A. S., Villela, A. D., Belardinelli, J. M., Morbidoni, H. R., Basso, L. A., Campos, M. M. and Santos, D. S. 2014, Int. J. Antimicrob. Agents, 43, 82.
13. Rodrigues-Junior, V. S., Junior, A. A. S., dos Santos, A. J., Schneider, C. Z., Calixto, J. B., Sousa, E. H. S., Lopes, L. G. F., Souto, A. A., Basso, L. A., Santos, D. S. and Campos, M. M. 2012, Int. J. Antimicrob. Agents, 40, 182.
14. Hughes, J. P., Rees, S., Kalindjian, S. B. and Philpott, K. L. 2011, Br. J. Pharmacol., 162, 1239.
15. Turner, J. R. 2007, New Drug Development: Design, Methodology, and Analysis, Hoboken, N.J., Wiley-Interscience.
16. Sousa, E. H. S., Vieira, F. G. M., Butler, J. S., Basso, L. A., Santos, D. S., Diógenes, I. C. N., Lopes, L. G. F. and Sadler, P. J. 2014, J. Inorg. Biochem., 140, 236.
17. Henderson, W. and McIndoe, J. S. 2005, Mass Spectrometry of Inorganic and Organometallic Compounds, Wiley-Interscience, Chichester.

18. Lund, H. 1963, Acta Chem. Scand., 17, 1077.
19. Sulaiman, S. T. and Hameed, Y. O. 1988, Anal. Chim. Acta, 206, 385.
20. Mellado, J. M. R. and Galvin, R. M. 1992, Electrochim. Acta, 37, 1147.
21. Asadpour-Zeynali, K. and Soheili-Azad, P. 2010, Electrochim. Acta, 55, 6570.
22. Ghoneim, M. M., El-Baradie, K. Y. and Tawfik, A. 2003, J. Pharmaceut. Biomed., 33, 673.
23. Leandro, K. C., de Carvalho, J. M., Giovanelli, L. F. and Moreira, J. C. 2009, Braz. J. Pharm. Sci., 45, 331.
24. Szlósarczyk, M., Piech, R., Bator, B. P., Maślanka, A., Opoka, W. and Krzek, J. 2012, Pharm. Anal. Acta, 3, 1.
25. Toma, H. E. and Creutz, C. 1977, Inorg. Chem., 16, 545.
26. Leeuwenkamp, O. R., van Der Mark, E. J., Jousma, H., van Bennekom, W. P. and Bult, A. 1984, Anal. Chim. Acta, 166, 129.
27. Pirzad, R., Moreira, J. C., Rangel, A. O. S. S., Alonso, R. M., Edmons, T. E. and Fogg, A. G. 1994, Analyst, 119, 963.
28. Ishibashi, K., Tsujimura, S. and Kano, K. 2008, Electrochemistry, 76, 594.
29. Quemard, A., Sacchettini, J. C., Dessen, A., Vilcheze, C., Bittman, R., Jacobs, W. R. Jr. and Blanchard, J. S. 1995, Biochemistry, 34, 8235.
30. Basso, L. A., Zheng, R., Musser, J. M., Jacobs, W. R. Jr. and Blanchard, J. S. 1998, J. Infec. Dis., 178, 769.
31. Parikh, S., Moynihan, D. P., Xiao, G. and Tonge, P. J. 1999, Biochemistry, 38, 13623.
32. Angulo, M., Galvin, R. M., Montoya, M. R. and Mellado, J. M. R. 1993, J. Electroanal. Chem., 348, 303.
33. Carapuça, H. M., Simao, J. E. J. and Fogg, A. G. 1998, J. Electroanal. Chem., 455, 93.
34. Glidewell, C. and Johnson, I. L. 1987, Inorg. Chim. Acta, 132, 145.
35. Mathieu, E., Meunier-Prest, R. and Laviron, E. 1997, Electrochim. Acta, 42, 331.
36. Rodriguez-Amaro, R., Perez, R., Camacho, L. and Ruiz, J. J. 1992, J. Electroanal. Chem., 324, 269.
37. Lemire, J. A., Harrison, J. J. and Turner, R. J. 2013, Nat. Rev. Microbiol., 11, 371.