A Highly Sensitive Electrochemical Determination of Isoniazid in Pharmaceutical Preparation by Differential Pulse Polarography

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Abstract

A simple strategy and highly sensitive electrochemical method was developed and described in Britton – Robinson buffer for isoniazid determination by Differential Pulse Polarography (DPP). A dropping mercury electrode (DME) was used to characterize the performance of the sensor. The reduction process on the (DME) gave a rise with one peak over E0.17 v (vs. Ag/AgCl/), within the studied pH range (2 – 9), 3M KNO3 as a supporting electrolyte and 4nm2 mercury drop size at 25°C. A standard calibration plot was conducted in the range between 0.05- 0.40μg.ml-1. The monitored differential pulse current was directly proportional to the concentration of Isoniazid and it showed a linear response in the studied range (correlation coefficient (r) = 0.9997) and the detection limit was 0.05μg. ml-1. This method effectively applied in commercial Isoniazid pharmaceuticals.

Keywords: Isoniazid, pharmaceutical preparation, Differential pulse polarography.

I. INTRODUCTION

Isoniazid, also known as isonicotinylhydrazide (INH) (Fig. 1), is an antibiotic used as a first-line agent for the prevention and treatment of both latent and active tuberculosis.[1] It is effective against mycobacteria, particularly Mycobacterium tuberculosis. It is also active against some atypical types of mycobacteria, such as M. kansasii and M. xenopi.[2] Isoniazid is an organic compound that is available in tablet, syrup, and injectable forms.[3][4][5]. Isoniazid was first made in 1952. [6] [7] Three pharmaceutical companies unsuccessfully attempted to patent the drug at the same time. [8] The most prominent among them was Roche, which launched its version, Rimifon, in 1952. [9] With the introduction of isoniazid, a cure for tuberculosis was first considered possible. It is available worldwide, inexpensive, and is generally well tolerated. Isoniazid is on the World Health Organization's List of Essential Medicines, a list of medicines that constitute the bare minimum for a basic health system.[10][11][12]

Polarography is the study of the electrolysis of solutions of electrooxidizable and or electro reducible substances between a dropping mercury electrode (DME) and some reference electrode (RE)[13][14]. The potential between these electrodes is varied and the consequent changes in the flow of current are measured. On plotting the changes in current flow versus the potential variation, one obtains an i - E curve known as polarograms [15][16]. The first scientist who discovered the use of the DME in electrolysis is Jaroslave Heyrovsky in 1922 who then received the Nobel Prize in Chemistry in 1959[17][18].

In differential pulse polarography, fixed magnitude pulses superimposed on a linear potential ramp - are applied to the working electrode at a time just before the end of the drop [19]. The current is measured twice at each mercury drop, before each pulse and at the end of the pulse time. The difference between the measurements Δi is plotted against the direct potential and produced peak - shaped polarograms, as Δi is largest for potential alterations in the region of the half - wave potential[20][21]. The formation of this difference also leads to a further reduction of the capacitive current contribution and therefore to an increase in sensitivity, even when compared with determinations by normal pulse polarography. The detection limit for determinations by differential pulse polarography is similar to that for square wave polarography at about (10^-7 - 10^-8 M) [22][23].

Many analytical methods have been developed for the determination of isoniazid,
including titrimetry [24], spectrophotometry [25][26], high performance liquid chromatography (HPLC) [27], chemiluminescence [28], fluorimetry [29], capillary electrophoresis [30] and electroanalytical methods [31]. Among these approaches, electroanalytical techniques are of particular advantage because of their practicality, simplicity, low-cost, good sensitivity, precision and rapidity for real-time detection.

II. EXPERIMENTAL

A. Solutions and Reagents

Isoniazid (analytical purity, Samarra drugs factory) was used without further purification. A stock solution of isoniazid was prepared with deionized water and kept in a refrigerator at about 4 °C. Isoniazid tablets (100 mg/tablet) were taken from Markets. Aqueous solutions were prepared with deionized water and stored in the shade. The 0.1M Britton – Robinson buffer(BR), within the pH range studied (2 – 9), and 3M KNO₃ as a supporting electrolyte also 4mm mercury drop size at room temperature. A standard stock solution (100 µg. ml⁻¹) of isoniazid was prepared by dissolving an accurate mass of bulk drug in an appropriate volume of deionized water. More dilute solutions (0.05/-0.40 µg. ml⁻¹) 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 2-9 [32] were prepared in deionized water. All the chemicals used were of analytical-reagent grade and were used without further purification.

B. Apparatus

All the electrochemical experiments were conducted in a three-electrode single compartment glass cell, including a DME electrode as working electrode, a platinum plate as auxiliary electrode, and an Ag/AgCl (3.0 mol L⁻¹KCl) as reference electrode. The polarographic measurements were carried out using a797 AV computrace (Metrohm, Herisau, Switzerland). The sample solutions were purged with high-purity nitrogen gas for at least 5 min at the beginning of the experiments to remove oxygen. All measurements were carried out at room temperature.

C. Preparation of Tablet Sample

Ten tablets, each containing 100mg of isoniazid, were finely powdered respectively. The white powder was accurately weighed to 0.01 g and dissolved into 10mL deionized water. The mixture was shaken for 30 min and filtered into a 100mL volumetric flask. The residue was several times washed with deionized water and the solution was diluted to the mark.

III. RESULTS AND DISCUSSION

A. Differential Pulse Polarography

The DPP-polarograms for 0.4 µg. ml⁻¹ isoniazid in B-R. buffers of pH 2/-9 exhibited one irreversible cathodic peak. In B-R. buffers of pH7, the height of the peaks shown in (Fig. 2).

![Fig. 2. DPP-Polarograms for 0.4 µg. ml⁻¹ Isoniazid in B.R. Buffers of Different pH Values: (1) 2, (2) 4, (3) 7, (4) 9.](image)

The peaks of isoniazid solution at different pH 2, 4, 7 and 9 showed a great effect on the diffusion current and half wave voltage, (Fig. 3). A weak cathode peak has been reached in the acid levels.

It has been found that pH 7 is the best level for isoniazid analysis. The reduction peak voltage resulting from pH showed that an electrochemical reaction exists consuming the hydrogen ions. A relationship between pH value and E₁/₂ has been noticed, as values turn to be more negative when acidity increases. This proves that isoniazid electrochemical behavior depends on the pH of its solution.

These results suggested that the two-electron irreversible cathodic wave pH 7 may be attributed to the reduction of the C=O of the amide group.
B. Effect of Buffer Solutions

The effects of buffer solutions have been studied by choosing Britton–Robinson buffer, phosphate buffer and carbonate buffers solution at different pH levels. The results of the rise of the electric current value and the peak shape have been interpreted that Britton–Robinson buffer solution gives the best peak in comparison to the other buffer solutions when changing isoniazid buffer solutions, (Fig. 4). Moreover, results showed that B-R buffer solution at pH 7 gives the highest peak waves in comparison to other pH levels; therefore, it has been selected for the analysis, (Fig. 5).

A 1–10.5 ml volume of the isoniazid solutions were analysis using Polarography device 797VA Computrace-Metrohm under the optimal experimental conditions found during this work. A standard calibration graph for isoniazid (Fig. 7) in the concentration range 0.05 to 0.40μg. ml⁻¹ were prepared using the Method of Least Squares, M.L.S [33], and used to determine the amounts of isoniazid. The Proposed reduction mechanism of isoniazid showed in (Fig. 8).
Fig. 6. Polarogram of Different Solution of Isoniazid Drug by Using Britton–Robinson Buffer pH 7.

Fig. 7. Standard Calibration Graph for Isoniazid Drug at Concentration Range 0.05-0.4 µg. ml⁻¹.

![Reduction Mechanism of Isoniazid](image)

Fig. 8. Proposed Reduction Mechanism of Isoniazid.
Table 1. Optimum Analytical Conditions for Isoniazid Analysis.

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Variable</th>
<th>Optimum conditions</th>
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<tbody>
<tr>
<td>Solvent</td>
<td>Water, Ethanol, Methanol, Acetonitrile</td>
<td>Water</td>
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<tr>
<td>pH</td>
<td>2, 4, 7, 9</td>
<td>7</td>
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<tr>
<td>Buffer Solution</td>
<td>Britton – Robinson (B-R) buffer, Phosphate buffer, Carbonate buffer</td>
<td>(B-R) buffer</td>
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<td>Supporting Electrolyte</td>
<td>KNO₃, KCl, LiCl</td>
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<td>Drop Size mm</td>
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<td>Temperature (°C)</td>
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Table 2. Analysis of Synthetic Made Isoniazid Sample.

<table>
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<tr>
<th>Primary Conc. µg. ml⁻¹</th>
<th>Measured Current (nA)</th>
<th>Measured Conc. µg. ml⁻¹</th>
<th>Rec. %</th>
<th>AE</th>
<th>RE %</th>
<th>SD</th>
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<td>0.4</td>
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<td>AV=22.73</td>
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Table 3. Analysis of Commercial Pharmaceuticals Isoniazid.

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<th>Primary Conc. µg. ml⁻¹</th>
<th>Measured Current (nA)</th>
<th>Measured Conc. µg. ml⁻¹</th>
<th>Measurement of Drug</th>
<th>Rec. %</th>
<th>SD</th>
<th>RSD %</th>
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<td>AV=0.3993</td>
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</table>
C. Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) for the isoniazid was calculated using signal to noise ratio (S/N) of 3.3 and 10 respectively. The results were equal to 0.05 and 0.12 μg ml⁻¹ ,respectively.

D. Accuracy and Precision

The accuracy and precision of the method for the determination of isoniazidwas tested. A 0.4 μg·ml⁻¹ synthetic isoniazid sample was prepared and analysis, the results show absolute errors between -0.0016 to 0.0007 and relative errors ranging from -0.00 to 0.70 % with 0.001 SD and %RSD not exceed ± 0.250, Table.2.

IV. CONCLUSION

In the practical applications of this method several advantages were found, first; using DPP which gives sensitive and selective determination of isoniazid, and second, the developed methods proved to be rapid since the analysis of each sample required few minutes.

ACKNOWLEDGMENTS

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REFERENCES

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