

Short Communication

Selective Potentiometric Sensor for Isoniazid Ultra-Trace Determination Based on Fe₃O₄ Nanoparticles Modified Carbon Paste Electrode (Fe₃O₄/CPE)

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Abstract

Significant effects of magnetic iron oxide (Fe₃O₄) nanoparticles on the potentiometric behavior of carbon paste electrode for bio determination of Isoniazid aromatic drug (INH) were studied. The electrochemical performance of modified Fe₃O₄/CPE was investigated. The constructed Fe₃O₄/CPE showed high precision, sensitivity and selectivity, for successfully determination of ultra-trace concentration of INH samples. In this research, pH effects, optimal condition for electrode and interfering Ions, on electrode response were investigated. The best potential response for Fe₃O₄/CPE was obtained with electrode composition of 0.012 g magnetic NPs, 0.006 g silicon oil and 0.060 g graphite powder at pH 6.5 and showed such a low detection limit as 3.09×10^{-13} mol L⁻¹ INH.

Keywords: Isoniazid, Magnetic nanoparticles, Fe₃O₄/CPE, Potentiometric sensor.

1. INTRODUCTION

Isoniazid (INH) is a first-line antituberculosis drug. It has been widely used since 1952 alone in the prophylaxis, and also in combination with other antituberculars [1-3]. Isoniazid (pyridine-4-carboxylic acid hydrazide or isonicotinic acid hydrazide) (Fig. 1), is a white, odorless, crystalline powder that is soluble in water (14%) and has a melting point of 171.4 °C [4].

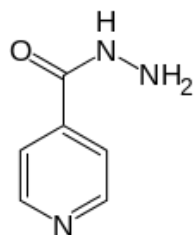


Figure 1. Chemical structures of Isoniazid.

Due to its wide usage and side effects, many HPLC methods for quantitation of INH have been reported [5-13]. Several other methods for the analysis of Isoniazid are also available in the literature such as titrimetry [14, 15], spectrophotometry [16-20], chemiluminescence [21-23], fluorimetry [24], capillary electrophoresis [25-27] and electroanalytical methods [28-34]. Although each method has certain advantages, some defects still exist. Most of the reported methods did not achieve low enough detection limits or required sample pretreatment and time-consuming extraction due to the small weight of INH, as well as expensive apparatus and costly maintenance [9, 31].

Unfortunately, hydrophilic compounds such as INH show inherent challenges for

separation under conventional chromatographic conditions. That's because basic functional groups interact with residual silanol groups of the stationary phase to give a long chromatographic peak tailing [35]. The titrimetry method is only suitable for macroanalysis [14, 15].

Among the electroanalytical mentioned techniques potentiometric detection offers several advantages such as speed and ease of preparation and procedures, simple instrumentation, fast responsibility in a wide dynamic range, reasonable selectivity, good sensitivity, and low cost. On the other hand, applications of potentiometric sensors in the field of pharmaceutical and biomedical analysis have been advocated [36]. The approach provides simple, fast, and selective technique for determination of various drugs [37-41]. In this case we reported the first potentiometric sensor for INH with such a highly suitable detection limit and linear range as compared to the other reported works [30-34].

Carbon paste electrode (CPE) is a special kind of heterogeneous carbon electrode that is a mixture of carbon powder (as graphite, glassy carbon and others carbonaceous materials) and a suitable water-immiscible or non-conducting binder [42-44]. The use of carbon paste as an electrode was initially reported in 1958 by Adams [45]. Afterward a wide variety of modifiers have been used with these versatile electrodes including enzymes [46-49], polymers (nafion, chitosan, etc) [50-54] and nanomaterials [55-57]. Modified carbon paste electrodes (MCPE) are widely applicable in both electrochemical studies and electroanalysis thank to their advantages such as very low background current (compared to solid graphite or noble metal electrodes), facility of construction, low cost, chemical inertness, large potential window, simple surface renewal process, stability and easiness of miniaturization [58-61]. Moreover the feasibility of incorporation of different materials during the paste

preparation, are the advantageous that allow the fabrication of electrodes with desired composition, and hence, with predetermined properties [62, 63]. Furthermore CPE in the form of chemically modified carbon paste electrode (CME) have been widely used due to its enhancement in sensitivity of the electrochemical sensing [64-67]. Nanoparticles based CME's have been the spotlight because of their increased sensitivity, amplified response signal, and well reproducibility [68-74].

Magnetic nanomaterial applications have bench marked versatility in science and technology because of its exceptional properties [75-80]. An exclusive nanoparticle for theranostic applications has also been laid by these magnetic nanoparticles and therefore its bioscience applications are of immense interest to researchers [81-82]. Magnetite nanoparticles are good choices as modifiers in carbon paste electrodes due to their interesting properties, such as high electron transfer ability, electrocatalytic activity, high surface area, the cheap and ease of their construction, low toxicity, high conductivity and good electrochemical and thermal stability. Magnetic field effects on electrochemical systems have been interested in electron transfer kinetics and increasing system efficiency and electrochemical flux [74, 83, 84]. All in all result in enhancing the electrode or electrochemical-sensor sensitivity and causing a large useful linear concentration range. The present study is aimed on the synthesis of Fe_3O_4 nanoparticles and construction of modified carbon paste electrode (Fe_3O_4/CPE) for rapid, ultra-trace and sensitive determination of INH by the use of potentiometric method. As compared to the other reported results, the improvement in sensitivity, linear range and response time was achieved successfully [30-34].

2. EXPERIMENTAL

2.1. Materials

Graphite powder, mineral oil (Silicon oil), Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 99%) and ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 99%), Phosphoric acid, Ammonia solution 28 %, all were obtained from Merck, Germany. The Graphite powder (Aldrich, Milwaukee, WI, US 1–2 mm) was used as well. A stock solution of INH (1.0×10^{-3} mol L^{-1}) was prepared with double-distilled water, deoxygenated by purging with pure nitrogen (99.999% from Roham Gas Company). Nitrogen gas was also passed over the surface of the test solutions during the measurements.

2.2. Instrument

All potentiometric measurements performed with potentiometer model PTR 79. Ag/AgCl electrode was used as reference and the Fe_3O_4 nanoparticles modified carbon paste electrode ($\text{Fe}_3\text{O}_4/\text{CPE}$) was used as working electrodes. The pH measurements were performed using an EIL 744MetrohompH meter. All experiments were performed at ambient temperature (25 ± 2 °C).

2.3. Preparation of Magnetic Fe_3O_4 Nanoparticles

During synthetic process, ideal Fe_3O_4 NPs were obtained under the molar ratio 2:1 of $\text{Fe}^{3+}:\text{Fe}^{2+}$. First, 0.04 mol of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.02 mol of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ were dissolved in 25 mL of water solution, at room temperature under nitrogen gas flow (40 mL min^{-1}) with vigorous stirring. After completing ion exchange, 28% ammonia solution was added drop by drop (with dropping rate of 10 d min^{-1}) to bring up the pH solution up to 10 for synthesis of magnetite Fe_3O_4 NPs. Then the solution was heated to 80 °C. Stirring under 80 °C was carried out for 30 min to ensure the complete growth of the nanoparticle crystals. The resulting suspension was cooled to the room temperature and was repeatedly washed with double distilled water and ethyl alcohol to remove unreacted chemicals. Finally the wet magnetic particles were obtained using

magnet. The wet solid particles obtained were dried by freeze drying and then characterized.

2.4. Preparation of Magnetic Nanoparticles Modified Carbon Paste Electrode

The MCPE was prepared by mixing mineral oil, graphite powder and Fe_3O_4 nanoparticles with various weight ratios. $\text{Fe}_3\text{O}_4/\text{CPE}$ was prepared by thoroughly hand mixing 0.6 g of graphite powder and 0.006 g of Silicon Oil with significant amount of synthesized nanoparticles in an agate mortar with pestle. The pastes were then packed into a teflon micro tip (diameter 0.5 mm) and a copper wire inserted into the paste established an electrical contact. A new surface was regenerated by pressing out an excess of paste out of the tip and manually smoothed by polishing it on clean paper. The MCPE was immersed in the supporting electrolyte placed in the cell and several sweeps were applied to obtain a low background current.

3. RESULTS AND DISCUSSION

The obtained results from the experimental works revealed that the performance of INH carbon paste potentiometric sensor can be highly improved by addition of magnetic NPs to the paste.

3.1. Characterization of the Nanomodifier

Synthesized magnetic nanoparticles (Fe_3O_4 NPs) were characterized by scanning electron microscopy (SEM) and X-ray diffractometer (XRD) before utilizing in the carbon paste electrode (Fig. 2). SEM images of the particles, as shown in Fig. 2, revealed the average diameter of synthesized nanoparticles around 70 nm. The XRD-pattern of magnetite nanoparticles shows the characteristic peaks of Fe_3O_4 (Powder Diffraction file, JCPDS card no. 25-1402) [74].

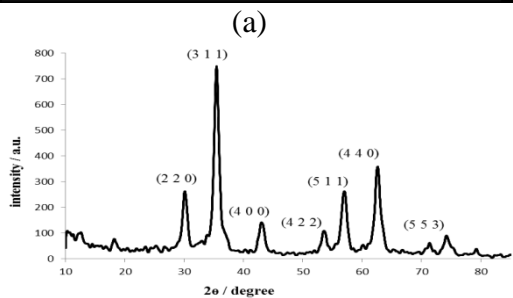


Figure 2. SEM image (a) and XRD-pattern (b) of synthesized Fe_3O_4 nanoparticles.

3.2. Electrode Composition and Modification

The effect of electrode composition and the modifier amount has been studied by potentiometric method to obtain the optimal conditions. As said before a modified paste was prepared in a similar fashion with unmodified CPE consist of silicon oil and graphite powder, except that the graphite powder was mixed with a desired weight of nano modifier, to get different composition as given in Table 1.

According to Table 1, the electrode composed of 0.012 g magnetic NPs, 0.006 g silicon oil and 0.06 g graphite powder (No. 6), was found to be optimal for the INH determination. This new nano-composition was selected for further examinations.

Using magnetic NPs in the carbon paste improves the conductivity and therefore, conversion of the chemical signal to an electrical response. This is due to the loading of Fe_3O_4 nanoparticles acting as a promoter by increasing the electro-active surface area. Decreasing the potential slowly at higher percentages is due to the fact that at higher amounts of modifier, the

catalytic properties of electron transfer and thus the conductivity is reduced [73]. Also, the existence of the NPs enhances the mechanical resistance of the electrode [73].

Table 1. The optimization of the carbon paste compositions, $pH=6.5$.

CPE	Fe_3O_4 (g)	Graphite (g)	Silicon Oil (g)	Slope	R^2
1	0.006	0.05	0.006	11.3	0.917
2	0.012	0.05	0.006	10.0	0.997
3	0.018	0.05	0.006	9.37	0.959
4	0.024	0.05	0.006	6.85	0.968
5	0.012	0.05	0.006	11.3	0.944
6	0.012	0.06	0.006	10.1	0.999
7	0.012	0.08	0.006	9.28	0.992
8	0.012	0.06	0.005	11.5	0.947
9	0.012	0.06	0.007	7.17	0.983
10	0.012	0.06	0.008	9.08	0.920

3.3. Effect of pH

In order to investigate the pH effect on the potential response of the electrode, the potentials were measured for standard solutions of INH (1×10^{-5} mol L^{-1}) in PBS buffer at different pH values by addition of dilute solutions of H_3PO_4 (0.1 mol L^{-1}) or NaOH (0.1 mol L^{-1}). The potential variations as a function of pH are given in Table 2. The composition of the electrode was kept constant during all experiments. The results showed the potential response of the electrode is the best at pH 6.5. The decrease in potential values above the pH values might be justified by the competition between INH and hydroxyl groups in the solution. Also, the decrease in potential at too acidic media could be attributed to the changing in the drug structure and also dissolution of the NPs.

Table 2. Investigation of pH Effect on cell potential of the modified electrode.

pH	Slope mV/decade	R^2
5.0	10.00	0.846
5.5	7.71	0.988
6.0	7.34	0.924
6.5	10.02	0.998
7.0	8.03	0.990
7.5	12.31	0.976
8.0	8.46	0.893

3.4. Analytical Characteristics

Here, the analytical characteristics of the electrode were investigated. The precision of the proposed electrode was evaluated by carrying out an analysis using standard working solutions with the same electrodes, at the same conditions and also in different conditions, for various INH concentrations to determine the stability of the constructed electrode. The low R.S.D.s for three measurements are shown in Table 3. Limit of detection was obtained according to $3S_b/m$, where S_b is the standard deviation of the blank (by 20 times determination in PBS buffer solution, pH=6.5) and m is the slope of calibration curve, as 3.09×10^{-13} mol L⁻¹. Also calibration curve of the proposed electrode is given in Fig. 3. The electrode exhibited a liner response in concentration rang of 1.0×10^{-5} to 1.0×10^{-10} (mol L⁻¹).

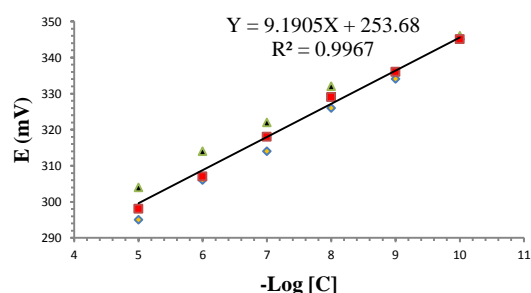


Figure 3. Calibration curves of Fe_3O_4/CPE at optimum conditions, repeating for 3 times.

3.5. Effect of Interfering Ions

For investigating the selectivity of the prepared electrode for determination of INH, the common blood electrolyte interferences such as Cd^{2+} , Cu^{2+} , Fe^{2+} , Zn^{2+} , Co^{2+} and K^+ cations were checked as potential interfering species in the analysis. The tolerance limit was taken as the maximum concentration of foreign species that caused a relative error of below 5.0% for the determination of INH at optimum conditions. It was found that the common existing cations in real samples did not interfere even up to 100-fold excess over the analyte. This implies no interference occurred due to these species. Therefore the direct application of the new electrode in real samples with interfering cations is possible.

4- CONCLUSION

Fe_3O_4 nanoparticles modified carbon paste electrode was developed as an effective and highly sensitive electrode for determination of an aromatic drug Isoniazid potentiometric method. On the best of our knowledge, there is no previous report for determination of INH based on magnetic nanoparticles modified CPE. Also INH electrochemical status by used of potentiometric technique was surveyed for the first time. We check the ability of

Table 3. Repeatability of the modified CPE at optimal conditions.

Concentration (mol L ⁻¹)	E ₁ (mV)	E ₂ (mV)	E ₃ (mv)	E _{mean} (mv)	RSD%
1×10^{-10}	345	345	346	345.66	0.16
1×10^{-9}	334	336	336	333	0.33
1×10^{-8}	326	329	332	325	0.91
1×10^{-7}	314	318	322	315	1.25
1×10^{-6}	306	307	314	309	1.29
1×10^{-5}	306	307	314	309	1.29

the magnetic nanoparticles as an accelerator for electron transfer in carbon paste electrode for INH sensing. This new electrode showed a considerable enhancement in sensitivity, improved detection limit, high reproducibility and repeatability, and thereby has provided *promising* sensor that is available for detection of trace amounts of INH in pharmaceutical and clinical preparations. The proposed electrode exhibits excellent performance compared many existing sensing methods for INH and has two additional advantages of simplicity of operations and low-cost instrument as well

[5-34]. It provided a wide working activity range in such a low concentrations of INH (1.0×10^{-5} to 1.0×10^{-10} mol L⁻¹), Nernstian slope (9.51 mV decade⁻¹ of activity), very low detection limit (3.09×10^{-13} mol L⁻¹) and good selectivity. The fabricated sensor strongly shows outstanding potential performance towards INH, with significant increasing in selectivity and sensitivity.

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