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اندازه‌گیری الکتروشیمیایی ایزونیازید در فرمولاسیون‌های دارویی و سرم انسان با استفاده از الکترود مغز مداد اصلاح‌شده با پلی اریوکروم بلاک تی

کریم اسدپور زینالی*، یاسر ارتشی
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Electroanalytical Determination of Isoniazid in Pharmaceutical Formulation and Human Plasma, Using a Poly(Eriochrome Black-T) Modified Pencil Lead Electrode

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چکیده

در این پژوهش با استفاده از الکترود مغز مداد اصلاح‌شده با پلی(اریوکروم بلک-T) یک روش ارزان و مؤثر برای اندازه‌گیری ایزونیازید ارائه شده‌است. توانایی الکترود اصلاح‌شده در اندازه‌گیری الکتروشیمیایی ایزونیازید با استفاده از روش‌های ولتامتری چرخه‌ای و آمپرومتری هیدرودینامیک مورد بررسی قرار گرفته است. تعداد کل الکترون‌های مبادله شده در فرآیند اکسیداسیون ایزونیازید ۴ عدد به دست آمده است. مقدار ثابت نفوذ محاسبه‌شده برای ایزونیازید برابر با $۹/۷۴ \times ۱۰^{-۷}$ سانتی‌مترمربع بر ثانیه می‌باشد. مقادیر حد تشخیص برای الکترود اصلاح‌شده با استفاده از روش‌های ولتامتری چرخه‌ای و آمپرومتری به ترتیب برابر با ۶۶ میکرومولار و ۲۰/۴ میکرومولار به دست آمد. توانایی الکترود تهیه‌شده برای اندازه‌گیری ایزونیازید در نمونه حقیقی با بکارگیری الکترود جهت اندازه‌گیری ایزونیازید موجود در پلاسمای خون انسانی بررسی شده و نتایج حاصل با روش استاندارد اندازه‌گیری ایزونیازید که توسط فارماکوپه ایالات متحده ارائه شده است، مقایسه گردید. روش ارایه شده دقت مناسبی به نمایش می‌گذارد (RSD%=۴/۶۴). روش ارائه شده همبستگی مناسبی با روش استاندارد در سطح اطمینان ۹۵٪ نشان می‌دهد.

واژه‌های کلیدی

ایزونیازید؛ اریوکروم بلک-T؛ الکترود اصلاح‌شده؛ اندازه‌گیری.

Abstract

In this research, an inexpensive and effective method for determination of Isoniazid (INH) is presented by using a poly (Eriochrome black T) modified pencil lead electrode. The potential of modified electrode in electrochemical sensing of INH was evaluated by cyclic voltammetry and hydrodynamic amperometry methods. The overall number of electrons involved in oxidation of INH was fond 4 electrons. The calculated diffusion coefficient for INH was equal to 9.74×10^{-7} cm^2/s. Calculated limit of detection for method was 66.0µM and 20.4µM applying cyclic voltammetry and hydrodynamic amperometry methods, subsequently. The ability of prepared electrode for determination of INH in real sample was evaluated by applying the proposed method to human plasma analysis and the results were compared with the standard method, presented by United State Pharmacopeia. Presented method exhibited a satisfying precision (%RSD=4.64). Also the proposed method showed a good accordance with standard method in confidence level of 95%.

Keywords

Isoniazid; Eriochrome Black-T; Modified Electrode; Determination.

1. INTRODUCTION

There are several benefits in use of modified electrodes for determination of therapeutics. These sensors are generally simple, easy to fabricate and they have emerged as useful substances for many electroanalytical measurements owing to their ability to multi-analyte detection [1]. Coating a carbonic substrates is one of the several ways for modification of electrodes. Modified carbonic electrodes are successfully applied for determination a wide range of therapeutics [2-7].

Based on World Tuberculosis (TB) Report presented by World Health Organization (WHO), in 2015 there were an estimated 10.4 million new TB cases worldwide, mostly have occurred in

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developing countries, because TB is one of the infectious diseases[8]. With this fact in mind, determination of therapeutic agents used for treatment of TB, especially in complex matrixes is of great importance.

Isonicotinic Acid Hydrazide (INH), commonly called Isoniazid, is mainly used in antituberculosis regimen either individually or in combination with some other drugs and is known as the first-line drug against tuberculosis. Preparation of a simple, inexpensive and environmental-friendly sensor with satisfying confidence, accuracy and precision for determination of INH is practically desirable.

Due to its importance in therapeutic programs, there are several analytical methods such as chromatography [9-11], spectroscopy [12-15] and electrochemistry [16-25] that were applied for determination of INH, whether in the form of pharmaceuticals or biologic fluids such as urine and plasma.

Several carbonic substances are modified with a poly (Eriochrome black-T) (EBT) layer and used for determination of some electroactive reagents such as nitric oxide [26] and even some biomolecules such as Epinephrine [27] and Dopamine [28, 29].

The aim of this study is to present a simple, rapid and inexpensive sensor for electroanalytical determination of INH in both pharmaceutical formulation and human plasma. Modified electrode prepared by electro-polymerization of EBT on pencil lead surface. Cyclic voltammetry (CV) method applied for electro-polymerization and investigation of electrochemical behavior of INH. Concentration of INH in real sample as well as spiked solution are determined by modified electrode using CV and amperometry methods. The prepared electrode enabled us to determine INH therapeutic concentration present in human plasma. Comparing results between presented method and standard method presented by United State Pharmacopeia (USP), we can introduce this method as a simple way to determination of INH with satisfying precision and accuracy levels.

2. EXPERIMENTAL

2.1. Reagents and instrumentation

INH and all other analytical grade reagents are obtained from MERCK (Germany). All solutions were prepared in double distillated water. INH tablets were supplied from an Iranian pharmaceutical company with marked value of 100 mg of INH. Britton-Robinson solutions with different pH values were used as the general buffer for investigation of pH values variance on oxidation peak. EBT solutions were preserved in refrigerator and protected from light. All of experiments were performed at room temperature. A piece of commercially available pencil lead, with 2 mm diameter, and a platinum wire were applied as the working electrode and counter electrode, respectively. A saturated calomel electrode (SCE) was employed as the reference electrode and all potential values in the whole text were referred to it. The both counter and reference electrodes were obtained from Azar Electrode Co. (Urmia, Iran). Voltammograms were obtained using a Behpajouh potentiostat from Sama Co. (Isfahan, Iran). A UV-1700 Pharma. Spec. spectrophotometer from SHIMADZU (Japan) was employed to set up perform the experiments related to standard determination method, suggested by United State Pharmacopoeia (USP) [30].

2.2. Preparation of modified electrode

First, pencil lead was polished finely, using a sandy paper (degree of 2500) and washed by distilled water. Polished pencil lead immersed in HCl 0.1M and treated by CV method between -0.4 to 1.5V for 25 cycles. Then, the electrode was washed by distilled water, placed in NaOH 0.1M, and was treated by CV method similar to the previous step. Polymerization of modifier was carried out using CV method between -0.4 to1.5V for 25 cycles, when the treated electrode with HCl and NaOH was immersed in a 0.1M solution of NaOH (as the carrier electrolyte) containing 0.75mM of EBT.

Offering an exact and detailed mechanism for electropolymerization of EBT is so difficult because of complicity in EBT polymerization process. However, a possible mechanism for polymerization process is reported by Geng et al.[26]. In the purposed mechanism, electropolymerization of EBT was realized through 8 pathways in which the role of naphthol structure was highlighted.

Finally, the modified electrode washed with diluted water and treated with ammonia buffer 0.1M between -0.15 to 0.35V for five cycles. In all of the above experiments, scan rate was fixed at $0.1 V.s^{-1}$.

3. RESULT AND DISCUSSION

3.1. Voltammetry behavior of INH

Electrochemical behavior of INH was investigated using the CV method. Cyclic voltammograms obtained in 0.1M of ammonia buffer solution (pH=9) while the potential of system was scanned between -0.15 to 0.35V. Voltammograms related to 1mM of INH in above conditions are shown in Fig. 1.

In Fig. 1, (a) and (b) voltammograms are related to background and unmodified electrode, respectively. Voltammograms (c) and (d) are related to modified electrode in absence and in presense of INH, respectively. Modified electrode, in practically optimum conditions, shows an observable, irreversible oxidative peak at 0.13V.

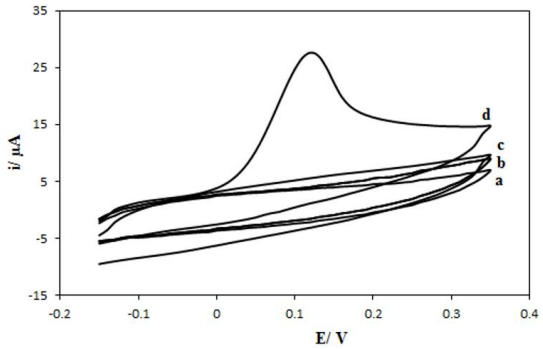
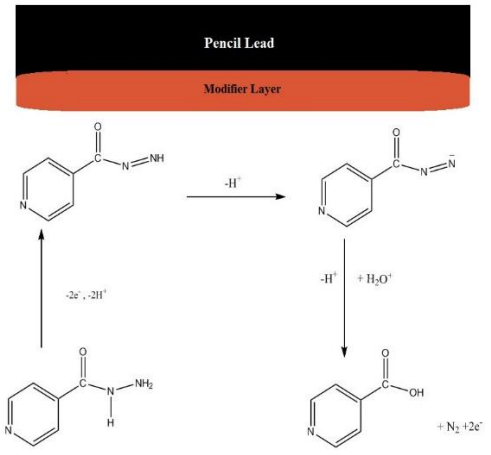


Fig. 1. Cyclic voltammograms in ammonia buffer solution (pH=9) for unmodified electrode in absence (a), and in presence of 1mM of INH (b), also modified electrode in absence (c) and in presence of 1mM of INH (d). Scan rate was 0.1 $V.s^{-1}$.

Comparing results between voltammograms (b) and (c), fortunatly a minimum increas in baseline curve was recognized. These results show that the modifier layer had no detectable peak by itself and presence of INH yields the hole current value exibited in Fig.2.(d). Also, comparing between voltammograms (b) and (d), we can abviously see undubtfull role of the modifier layer in analysis. As shown in Fig.1 there was no response in the absence of the modifier layer.

Based on obtained results (see next sections), the reaction of electrocatalitic oxidation of INH at the surface of modified electrode can be written as shown on Scheme1.



Scheme 1. The electrocatalytic oxidation of INH at the surface of modified electrode.

3.2. Effect of medium pH on oxidation of INH

According to the chemical structure of INH and pK_a values of INH [25], it can be proposed that in pH values almost lower than $pH=pK_a$, INH is positively charged and there are associated four hydrogen existing in the structure. In fact, oxidation peak is affected by separation of the proton consisting in the amine group.

The effect of the medium pH was studied using a 0.1M solution of Britton-Robinson buffer as the general buffer solution. Fig. 2 shows the relationship between the pH and both of the peak potential (E_p) and peak current (i_p) and as expected, the E_p value decreases when the pH rises. This behavior illustrates that the oxidation of INH occurs with taking part a proton transferring and the amine group releases its proton through the oxidation.

In the investigated pH values there is a linear relationship between E_p and pH which is described with the following equation:

E_p= 62.9 pH+741.1 R^2 = 0.9904 (1)

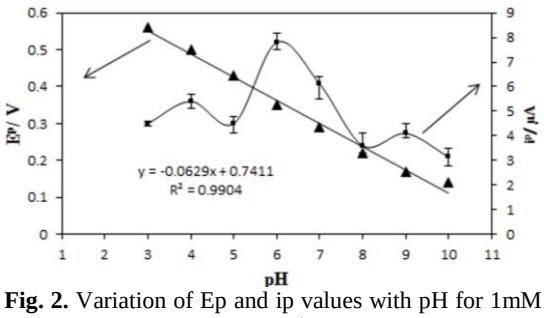


Fig. 2. Variation of Ep and ip values with pH for 1mM of INH. Scan rate was 0.1 $V.s^{-1}$.

According to the slope of the above equation, the number of electrons and protons transferred during the oxidation of INH, are equal; because there is a good agreement between the values of the slope in equation (1) and 59 mV per pH unite as the theoretical value. Therefore, it can be considered that oxidation of INH occurs through a four electron- four proton mechanism. The same behavior is reported in previous researches [25]. Following the same logic, it can be predicted that in pH>11, when the ammonia group proton is separated, oxidation process occurs whereas three protons take part in the process.

The pH 9 was chosen as the optimum value because, as it can be seen in Fig. 2, at pH 9 the E_p has a low value while the i_p shows a high value. Therefore, all of the further experiments were carried out at ammonia buffer solution (pH=9).

3.3. Chronoamprometry studies

Electrocatalytic oxidation of INH was studied by chronoamprometry method. Chronoamprometric responses for a series of INH solutions with

incremental concentrations from 0.086 mM to 3.548 mM are illustrated in Fig. 3.

As it can be seen in Fig. 3, increase in the concentration causes an increase in both of the i_p and the Cottrell level. Using the chronoamprometry technique and as for the Cottrell equation, when the value of electrode area, concentration of INH and the number of electrons participated in the oxidation were given, the diffusion coefficient for analyte can be calculated.

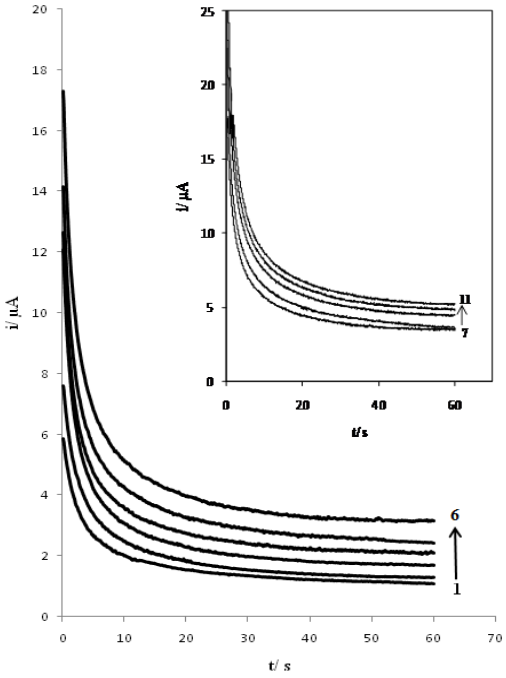


Fig. 3. Chronoamprograms related to different concentrations of INH 1)0.086, 2)0.283, 3)0.496, 4)0.654, 5)0.855, 6)1.094, inset: 7)1.367, 8)1.677, 9)2.211, 10)2.837 and 11)3.548mM. Potential was fixed at 0.4V.

In order to calculate the diffusion coefficient, the plot of current vs. square inverse of time ($t^{-1/2}$) was drawn and is presented as Fig. 4.

Fig. 4A shows the variation of current with $t^{-1/2}$ related to the first six values of concentrations for INH, mentioned in Fig.3. Fig. 4B illustrates the relationship between the slope values in Fig. 4A and concentration of INH from 0.086 mM to 3.548 mM.

Fig. 4A clearly shows the above discussed increase in current and therefore increase in related slope values. According to the Cottrell equation, the slope values in Fig. 4B whereas diagram of slopes of i-$t^{-1/2}$ and concentration of INH is presented, slope values can be applied for the calculation of diffusion coefficient (D). As shown in Fig. 4B there are obviously two distinguishable linear regions in the diagram with different slope values. When INH concentration increases from 0.086 mM to 1.094 mM, linear relationship between two parameters established with higher slope (R^2 = 0.9948) in contrast to the concentration increases from 1.367mM to 3.548mM (R^2 = 0.9635). This behavior defines that when the concentration of INH was more than 1.094 mM, transferring of analyte from solution bulk endures a limitation. This behavior is considered to be depending on generation of N_2 during the oxidation, as the final product of the reaction. Using the data obtained from chronoamperometry studies, the diffusion coefficient value for INH was calculated as 9.74×10^{-7} cm^2/s that shows a good agreement with previous reports [25].

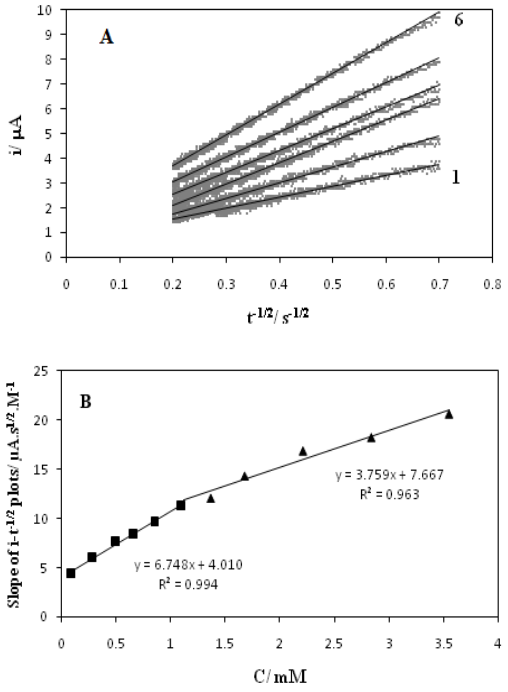


Fig. 4. (A) The variation between the current and $t^{-1/2}$ values. (B) Relationship between the slope of i-$t^{-1/2}$ plots and concentration of INH from 0.086mM to 3.548mM

3.4. Determination of INH by CV method

CV method was applied for determination of INH in ammonia buffer (pH=9) medium. The concentration of INH was increased from 0.086 mM to 4.335 mM and standard curve was drawn using standard addition method. The results are exhibited in Fig. 5.

Fig. 5A and B show the voltammograms of enhancing concentrations of INH from 0.086 mM to 1.367 mM and from 1.677 mM to 4.335 mM, respectively. The standard curve drawn by standard addition method, related to cyclic voltammograms are presented in Fig. 5C.

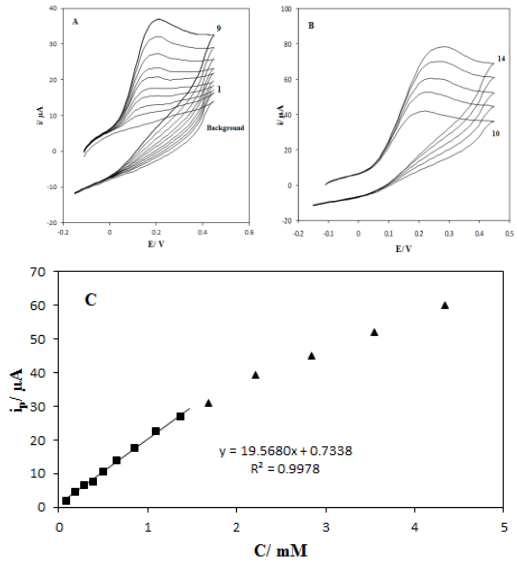


Fig. 5. Cyclic voltammograms related to different concentration of INH: (A) 1)0.086, 2)0.181, 3)0.283, 4)0.381, 5)0.496, 6)0.654, 7)0.855, 8)1.094, 9)1.367mM (B) 10)1.677, 11)2.211, 12)2.837, 13)3.548 and 14)4.335mM. (C) Standard curve based on voltammograms.

Calibration curve shows a linear range between 0.086mM to 1.367mM of INH (R^2 = 0.9978) and when the further values of INH concentration was participated into calibration curve, slope and linearity of curve was low. It can depend on the kinetically limitation during the oxidation of INH for the generation of N_2.

LOD was calculated using the regression equation:

INH: i_p (μA) = 19.568 C_{INH} (mM) + 0.7338 (R^2 = 0.9978) (2)

The detection limit was calculated by means of the equation: $Y_{LOD}=Y_B+3S_{y/x}$ Where Y_B is a signal of the blank (here intercept of the calibration graph) and $S_{y/x}$ is standard deviation of a blank (here standard deviation of the calibration graph) [31]. LOD for the presented method applying CV technique was 66.0μM.

3.5. Determination of INH by amperometry method

Hydrodynamic amperometry is found to be a sensitive electrochemical method in analytical experiments. This ability refers to the diffusion layer, lower in thickness and fresh analyte that is available for sensor during the experiment. Therefore, we employed this method as a sensitive one for electro-catalytic determination of INH in a pH=9, ammonia buffer solution. Results are presented in Fig. 6. Like the chronoamperometry experiments, during these series of studies, potential was fixed at 0.4V.

Fig. 6 illustrates the amperometric response of INH when its concentration successfully was increased from 0.086 mM to 2.211mM and related standard curve, drawn using the standard addition method, as inset.

As it can be seen in Fig. 6, calibration curve drawn using the amperometry data, illustrate two detectable linear ranges. The first range with steeper slope is depended on the concentration increase between 0.086 mM and 0.654 mM. The regression equation in this range was:

INH: i_p (μA) = 6.748 C_{INH} (mM) + 4.010 (R^2 = 0.994) (3)

Second range is related to increase of INH concentration between 0.854 mM to 2.211 mM that becoming with lower slope. The equation of standard curve in this range was:

INH: i_p = 3.759 C_{INH} (μM) + 7.667 (R^2 = 0.963) (4)

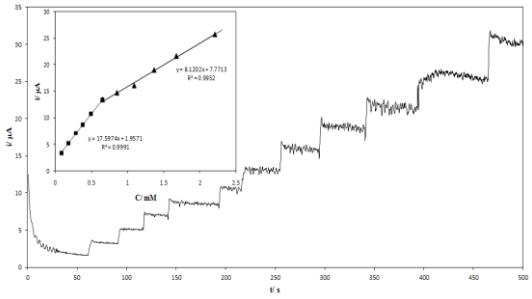


Fig. 6. Amperometric response related to increasing of INH concentration from 0.086mM to 2.211mM. Inset: calibration curve related to amperogram. Potential was fixed at 0.4V.

As it is predictable, comparing with CV method, amperometry method defines the kinetic limitation in INH oxidation, obviously, because of its sensitivity. LOD for our method was calculated using the method illustrated in previous section. The LOD was found to be 20.4 μM using the first linear range of standard curve.

3.6. Determination of INH in real samples

The accuracy of presented method and the ability of obtained sensor were tested by direct determination of INH either containing in pharmaceutical formulation or spiked into human plasma.

A given amount of INH was spiked into human plasma and then modified electrode was employed for the determination of INH. The experiments were repeated for three times and the results were summarized in Table 1. According to the results in Table 1, sensing method shows a satisfying accuracy and precision (%RSD=4.64) and therefore it can be applied for determination of INH in biological mediums, confidently.

Table1. Analytical data related to determination of Isoniazid, spiked into human plasma.

no. of Analyses	m INH (μg)		Recovery (%)	% RSD
	Added	Calculated		
1	86.87	88.49	101.87	4.64
2	86.87	85.67	98.62	4.64
3	86.87	80.69	92.89	4.64

The concentration of INH in heart blood, subclavian blood, urine and bile have been reported and are equal to 43 mg/L (0.093mM), 94 mg/L (0.204mM), 470 mg/L (1.019mM), and 900 mg/L (1.951mM), respectively [32]. According to these values and the linear range of method, the prepared sensor can easily detect the INH in plasma concentration levels, using amperometery technique. As well as using CV technique, the prepared sensor can detect INH in all plasma levels, except that one related to bile.

Also, the amount of INH containing in pharmaceuticals was determined using standard method for the determination of INH; suggested by USP and based on direct determination of INH using UV spectrophotometry technique.

To ensure that there is no significant difference between the standard and the applied method, first F test is done ($F_{3/3}$=3.86<F_c=9.277) and then T test was applied to compare the results coming from each of two methods ($t_{ex.}$=1.76<t_c=2.45). It was shown that there is no significant difference between the results in confidence level of 95%. Results are summarized in Table 2.

Based on these results, this method can be introduced as an inexpensive and confident method with high accuracy; especially to use in drug controlling projects.

Table2. Statistics data for determination of Isoniazid in pharmaceutical formulation, compared with USP method.

no. of Analyses	m_{INH}(mg)		$\overline{m}_{INH}$(mg)		Standard Deviation	
	Standard method	by Sensing	Standard method	by Sensing	Standard method	by Sensing
1	96.27	95.89	96.33	96.36	1.14	0.58
2	95.23	96.20	96.33	96.36	1.14	0.58
3	97.51	97.01	96.33	96.36	1.14	0.58

4. CONCLUSIONS

This study has shown that INH can be a determinate base on its oxidation, using a poly (eriochrome black-T) modified pencil lead electrode using either CV or amperometry techniques. Studies show the facts that oxidation of INH occurs through a four proton transferring mechanism and endures a limitation, considered to be depended on generation of N_2 as the final product of oxidation. Diffusion coefficient was calculated using the chronoamperometry method. Also, LOD of method was calculated by CV and amperometry methods. The method was successfully applied to determination of INH in pharmaceuticals and human plasma and exhibited a good accuracy according to the standard method which suggested by USP.

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