

Efficient electrocatalytic oxidation and selective determination of isoniazid by $\text{Fe}(\text{tmphen})_3^{2+}$ -exchanged Nafion®-modified electrode

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Abstract Cyclic voltammetry and electrochemical impedance studies of $\text{Fe}(\text{tmphen})_3^{2+}$ (where tmphen=3,4,7,8-tetramethyl-1,10-phenanthroline)-immobilized Nafion®-modified glassy carbon electrode ($\text{GC/Nf/Fe}(\text{tmphen})_3^{2+}$) are carried out in 0.1 M Na_2SO_4 solution. Nafion– $\text{Fe}(\text{tmphen})_3^{2+}$ complex exhibits efficient electrocatalytic oxidation of isoniazid. The linear double reciprocal plot of current and concentration of isoniazid shows a Michaelis–Menten-type catalytic process. The catalytic oxidation currents are proportional to the concentration of isoniazid and show a wide linear calibration range for the quantitative determination of isoniazid. Detection limit and sensitivity are found to be 13 μM and 2.5 $\mu\text{A mM}^{-1}$, respectively.

Keywords Nafion® · Electrocatalysis · Isoniazid oxidation · $\text{Fe}(\text{tmphen})_3^{2+}$

Introduction

Isoniazid (IZ) is an important bacteriostatic drug which is frequently used for the treatment of pulmonary tuberculosis and leprosy [1]. It is used alone or in combination with some other drugs, such as rifampicin and pyrazinamide [2, 3]. The

regular use of IZ can produce hepatitis, so a patient taking IZ should be carefully monitored and intervened at regular intervals. Therefore, various methods [1–7] have been used for the determination of IZ, and among them, electrochemical method is more superior because of its high sensitivity, simplicity, and reproducibility. However, at the bare unmodified electrodes large overpotential is required for IZ oxidation. In general, to overcome this problem, different types of modified electrodes are used [8–13]. However, there are only very few electrochemical studies reported for the oxidation of IZ at such modified electrodes [8]. Also, it is reported that IZ is oxidized to free radicals by the enzymes like catalase–peroxidase of *Mycobacterium tuberculosis* [14, 15] and horseradish peroxidase [15]. In this work, we used an inorganic metal complex for the oxidation and quantitative determination of IZ. Here, we are reporting for the first time a new and efficient catalyst for the oxidation of IZ at the modified electrodes. $\text{Fe}(\text{tmphen})_3^{2+}$ (where tmphen=3,4,7,8-tetramethyl-1,10-phenanthroline)-immobilized Nafion® (Nf)-modified glassy carbon (GC) electrodes (represented as $\text{GC/Nf/Fe}(\text{tmphen})_3^{2+}$) show efficient electrocatalytic activity toward the oxidation of IZ in 0.1 M Na_2SO_4 , which is successfully used for the sensitive amperometric determination of IZ. Nf is a cation exchange polymer having fluorocarbon backbone [16]. The presence of sulfonate head group accounts for its cation exchange property, and this property has been utilized advantageously to immobilize $\text{Fe}(\text{tmphen})_3^{2+}$ on the GC electrode surface.

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Experimental

Chemicals

Nf (5 wt %) in lower aliphatic alcohols and 3,4,7,8-tetramethyl-1,10-phenanthroline (tmphen) were purchased from

Aldrich. Na_2SO_4 , IZ, and all other chemicals (SRL or Qualigens or S.D.Fine or Himedia, India) were of analytical grade and used without further purification. Triple distilled water was used throughout the studies. $[\text{Fe}(\text{tmphen})_3]\text{SO}_4$ is prepared and characterized by a reported procedure [17].

Instrumentation

CHI-660C (CH Instruments, USA) was used for electrochemical experiments. Glassy carbon electrodes (GC) (CH Instruments, area=0.07 cm^2) or modified GC as working electrode, platinum wire counter electrode, and Ag/AgCl (saturated with KCl) reference electrode were used for the electrochemical study. UV–vis absorbance spectra were measured using 2802 PC UV–vis spectrophotometer (Unico, USA). All experiments are carried out at room temperature (25 °C) under N_2 -saturated conditions unless otherwise mentioned.

Result and discussion

Preparation and characterization of modified electrode

GC/Nf/Fe(tmphen) $_3^{2+}$ electrodes are prepared according to our previous reports [18, 19]. Typically, 0.1 % Nf solution is coated on GC electrode and allowed it to dry at room temperature. The thickness of the film is calculated to be 0.9 μm using the density of Nf as 1.58 g cm^{-3} . Then, GC/Nf is dipped in 0.5 mM Fe(tmphen) $_3^{2+}$ solution for 30 min and rinsed with triple distilled water. Before electrocatalytic studies, the electrodes are cycled continuously between 0.2 and 1.3 V in 0.1 M Na_2SO_4 for 15–20 cycles until a constant current is observed. An absorption maxima at 500 nm is observed for Fe(tmphen) $_3^{2+}$ in aqueous solution (insert (i) a of Fig. 1). Similarly, an absorption band at 504 nm is observed for Fe(tmphen) $_3^{2+}$ ion exchanged into Nf film (insert (i) a' of Fig. 1). This insignificant change in the absorption band position indicates that the electronic properties of Fe(tmphen) $_3^{2+}$ ion exchanged into Nf film are not affected. Figure 1 shows the cyclic voltammograms (CVs) of GC/Nf/Fe(tmphen) $_3^{2+}$ electrode in 0.1 M Na_2SO_4 at different scan rates (10–500 mV s^{-1}). It shows a well-defined peak with an $E_{1/2}$ value of 0.64 V, anodic to cathodic peak current ($I_{\text{pa}}/I_{\text{pc}}$) ratio of 1.24, and ΔE_{p} value of 170 mV. The high ΔE_{p} value could be due to the hydrophobic interaction between Nf film and Fe(tmphen) $_3^{2+}$. The slightly high $I_{\text{pa}}/I_{\text{pc}}$ ratio indicates that the oxidized metal complex Fe(tmphen) $_3^{3+}$ binds strongly than Fe(tmphen) $_3^{2+}$ with Nf film. Thus, the observed CV (Fig. 1) is attributed to the Fe(tmphen) $_3^{2+/3+}$ redox couple. At low scan rates (below 50 mV s^{-1}), the I_{pa} and I_{pc} linearly vary with scan rate (Fig. is not shown) indicating the thin-film behavior at the

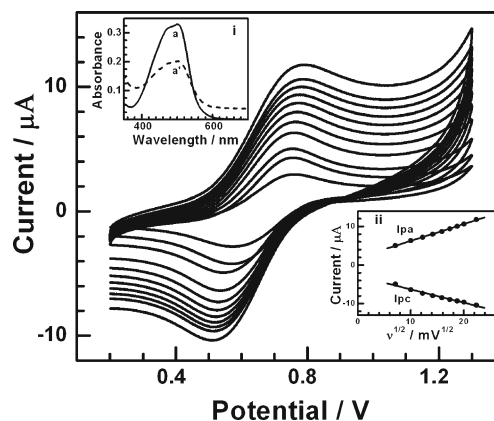


Fig. 1 Cyclic voltammograms of GC/Nf/Fe(tmphen) $_3^{2+}$ electrode with different scan rates (10, 20, 50, 100, 150, 200, 250, 300, 350, 400, and 500 mV s^{-1}) in 0.1 M Na_2SO_4 . *Insert (i)*: UV–vis absorption spectra of Fe(tmphen) $_3^{2+}$ aqueous solution (a) and Fe(tmphen) $_3^{2+}$ adsorbed on a Nf-coated glass plate (a'). *Insert (ii)*: Dependence of anodic and cathodic peak currents on the square root of scan rates

modified electrodes [20]. At high scan rates (above 50 mV s^{-1}), the I_{pa} and I_{pc} are found to be linearly proportional to the square root of scan rate (insert (ii) of Fig. 1) with small intercept, which is expected when semi-infinite diffusion conditions are prevailed [20]. These properties are very similar to the results reported for Ru(bpy) $_3^{2+}$ (where bpy=2, 2' bipyridyl) at Nf-modified electrodes [20].

Electrocatalytic oxidation of isoniazid

The CVs of GC/Nf/Fe(tmphen) $_3^{2+}$ and GC/Nf electrodes in the absence and the presence of 1.0 mM IZ are shown in Fig. 2. At GC/Nf/Fe(tmphen) $_3^{2+}$, a pair of redox peaks corresponded to the Fe(tmphen) $_3^{2+/3+}$ redox process is observed in the absence of IZ (a of Fig. 2), and no characteristic redox peak is observed at GC/Nf (b of Fig. 2). In the presence of 1.0 mM IZ, it is oxidized at about 1.0 V at GC/Nf

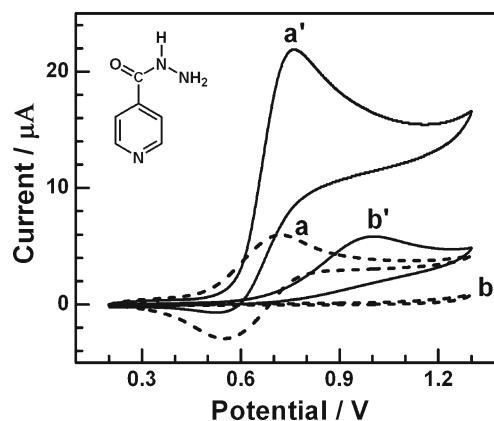


Fig. 2 Cyclic voltammograms of GC/Nf/Fe(tmphen) $_3^{2+}$ (a, a') and GC/Nf (b, b') electrodes in the absence (a, b) and presence (a', b') of 1.0 mM IZ with 0.1 M Na_2SO_4 . Scan rate=20 mV s^{-1} . *Insert* shows the structure of IZ

(b' of Fig. 2), whereas at GC/Nf/Fe(tmphen)₃²⁺, it is oxidized at 0.76 V with high anodic peak current. Thus, IZ is efficiently oxidized with 240 mV less positive potential in the presence of Fe(tmphen)₃²⁺. Marked enhancement in anodic peak current and decrease in cathodic peak current (a' of Fig. 2) in the presence of IZ at GC/Nf/Fe(tmphen)₃²⁺ suggest that Fe(tmphen)₃²⁺ shows strong electrocatalytic effect for IZ oxidation. The CVs of GC/Nf/Fe(tmphen)₃²⁺ are recorded (Fig. 3) with successive addition of IZ (from 50.0 μM to 100.0 mM), and it is observed that anodic current is linearly proportional to IZ concentration from 50.0 μM to 20.0 mM (insert (i) of Fig. 3). The equation for the analytical curve is given as I_p (microamps)=2.41 [IZ] (millimolars)+1.29 ($r=0.995$ and $n=9$). Table 1 indicates the linear calibration range reported for several IZ sensors [8–12, 21–24] together with other properties. This clearly explains that the present work reports the broad linear calibration range with sufficient sensitivity and reasonable detection limit for IZ determination. It is noteworthy to mention that this is the broadest linear calibration range for IZ reported so far. The broad linear range could be due to the slow diffusion of IZ into Nf film which results in the small variation in the oxidation current with IZ concentrations. At higher IZ concentrations (i.e., above 20.0 mM), there is a leveling off of the current response as would be expected for a Michaelis–Menten-type catalytic process [25, 26]. Michaelis–Menten constant can be calculated based on Lineweaver–Burk Eq. (1) [26].

$$1/I_{ss} = (K_M^{app}/I_{max})(1/C_{IZ}) + 1/I_{max} \quad (1)$$

Where K_M^{app} , I_{ss} , C_{IZ} , and I_{max} are apparent Michaelis–Menten constant, steady-state catalytic current, bulk concentration of IZ, and maximum current under substrate (IZ)-saturated conditions, respectively. From the slope and the intercept of a double reciprocal plot of the current and concentration of IZ (insert (ii) of Fig. 3), K_M^{app} is calculated

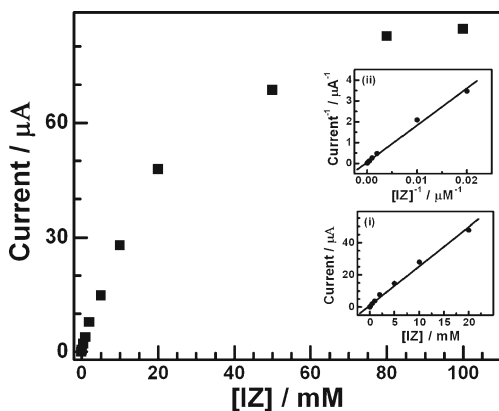


Fig. 3 Variation of anodic oxidation peak current (I_{pa}) with different concentrations of IZ at GC/Nf/Fe(tmphen)₃²⁺ in 0.1 M Na₂SO₄. *Insert (i)* shows linear dependence of I_{pa} with the concentration of IZ in the range of 50.0 μM to 20.0 mM. Scan rate, 20 mV s⁻¹. *Insert (ii)* shows the double reciprocal plot for the calculation of K_M^{app}

and found to be 3.5 mM, which is higher or comparable to the values reported for the oxidation of IZ [14, 15]. It is important to mention that the observed Michaelis–Menten behavior could be due to the competition between charge transport and the catalytic reaction in the film. Thus, the mechanism of electrocatalytic reaction requires further study. However, based on the literature [14, 27], it is believed that the IZ is oxidized to py-CO₂H and N₂ through intermediates (For the structure of IZ, refer to insert of Fig. 2).

Chronoamperometry is used for the estimation of the diffusion coefficient of IZ. For an electroactive material with diffusion coefficient D , the current corresponding to the electrochemical reaction (under diffusion control) is described by Cottrell’s equation (Eq. 2) [9].

$$I = nFAD^{1/2}c\pi^{-1/2}t^{-1/2} \quad (2)$$

Where D and c is the diffusion coefficient (square centimeters per second) and bulk concentration (moles per cubic centimeter) of IZ, respectively, and the other terms have their usual meaning. The plot of I vs. $t^{-1/2}$ is linear (Fig. not shown), and from the slopes of the resulting straight lines with various IZ concentrations [Fig. S1 in the Electronic supplementary material (ESM)], the average value of D is calculated to be 5.2×10^{-6} cm² s⁻¹, which is comparable to the reported value [9].

Electrochemical impedance analysis

Electrochemical impedance measurements at the modified electrodes, GC/Nf/Fe(tmphen)₃²⁺, are performed in the absence and presence of IZ. Figure 4 shows the Nyquist diagrams of GC/Nf/Fe(tmphen)₃²⁺ in the absence (curve a) and presence (3.0, 5.0, 10.0, 15.0, 20.0, and 30.0 mM, curve b–g) of IZ at 0.7 V in 0.1 M Na₂SO₄ analyzed in the frequency range of 1 to 100 kHz. The equivalent circuit compatible with the corresponding Nyquist diagrams is given in the insert of Fig. 4. With increasing concentration of IZ, a steady decrease in the diameter of the semi-circle is observed, which confirms the efficient electrocatalytic oxidation of IZ at GC/Nf/Fe(tmphen)₃²⁺ electrode [11, 28, 29].

Determination of isoniazid in pharmaceutical formulation

Commercially available tablet of IZ [composition of the tablet: rifampin (450 mg) and IZ (300 mg)] is completely dissolved in 10 mL of distilled water. IZ present in 2.0 mL of this tablet solution is analyzed by CV at GC/Nf/Fe(tmphen)₃²⁺ on the basis of oxidation current. IZ concentration estimated by this method is in excellent agreement with the IZ concentration originally present in the tablet indicating the negligible interference due to rifampin. Further, the tablet

Table 1 The reported linear calibration range and detection limit for isoniazid at various electrodes and the references

Method	Electrode	pH, medium	Detection limit (μM)	Linear calibration range (μM)	References
CV	(FcM)TMA-modified Pt	0.1 M, sodium sulfate	–	50–600	[8]
CV	OPPy-modified GC	pH 9.0, ammonium buffer	3.15	3.99–126	[9]
CV	PASA-modified GC	pH 6.5, phosphate buffer	0.01	0.05–10	[10]
LSV	Poly-L-histidine-modified SPCE	pH 7.0, phosphate buffer	0.5	1.5–210	[11]
DPV	Poly-L-histidine-modified SPCE	pH 7.0, phosphate buffer	0.17	0.5–110	[11]
SWV	Poly-L-histidine-modified SPCE	pH 7.0, phosphate buffer	0.25	0.15–11	[11]
Amperometry	Au NPs sol–gel-modified polycrystalline Au electrode	pH 9.2, phosphate buffer	0.0001	0.0001–1000	[12]
DPP	DME electrode	pH 7.0, Britton–Robinson buffer	–	0.06–100	[21]
DPV	MWCNT-Th-modified CPE	pH 4.0, acetate buffer	0.05	1–100	[22]
LSV	ARS-modified GC	pH 6.0, phosphate buffer	3.94	10–800	[23]
DPV	ARS-modified GC	pH 6.0, phosphate buffer	0.016	0.05–0.85	[23]
Amperometry	Nf/OMC-modified GC	pH 7.0, phosphate buffer	0.084	0.1–370	[24]
CV	Nf/Fe(tmphen) ₃ ²⁺ -modified GC	0.1 M sodium sulfate	13	50.0–20,000	This work

CV cyclic voltammetry, LSV linear sweep voltammetry, DPV differential pulse voltammetry, SWV square wave voltammetry, DPP differential pulse polarography, GC glassy carbon, Pt platinum, SPCE screen-printed carbon electrode, OPpy overoxidized polypyrrole, NPs nanoparticles, PASA poly(amidosulfonic acid), MWCNT multiwalled carbon nanotubes, Th thionine, (Fc)TMA (ferrocenylmethyl)trimethylammonium, DME dropping mercury electrode, ARS alizarin red S, OMC ordered mesoporous carbon, Nf Nafion

solution is spiked with different amounts of standard IZ, and the amounts of IZ present in the mixtures are estimated. The results of the recovery (99 to 107 %) and RSD (1.8 to 4.5 %) showed that the present electrode can be conveniently used for the determination of IZ present in the pharmaceutical formulations (Table S1 in the ESM).

Hydrazine, one of the usual interference in the amperometric determination of IZ, is not interfered (even in the presence of high excess concentration) with the determination of IZ by the present method. Detection

limit (based on 3S of the blank) and sensitivity are found to be 13 μM and 2.5 $\mu\text{A mM}^{-1}$, respectively. Operational and long-term stability (for 10 days) studies are also performed with these electrodes. They lost nearly about 6 % of the initial signal on continuous IZ measurements over 50 times. GC/Nf/Fe(tmphen)₃²⁺ stored in distilled water and used for five separate determinations of IZ every day did not show any considerable loss in its current response for 10 days.

Conclusion

GC/Nf/Fe(tmphen)₃²⁺ electrode shows efficient electrocatalytic oxidation for IZ in 0.1 Na₂SO₄ solution. It showed a wide linear calibration range (50.0 μM to 20.0 mM) for amperometric IZ determination. In electrochemical impedance analysis, a steady decrease in the charge transfer resistance with increasing IZ concentration confirmed that the GC/Nf/Fe(tmphen)₃²⁺ exhibits efficient electrocatalytic activity for IZ oxidation. It is successfully applied to determine the concentration of IZ present in a pharmaceutical formulation. Hydrazine is not interfering in the amperometric determination of IZ by the present method.

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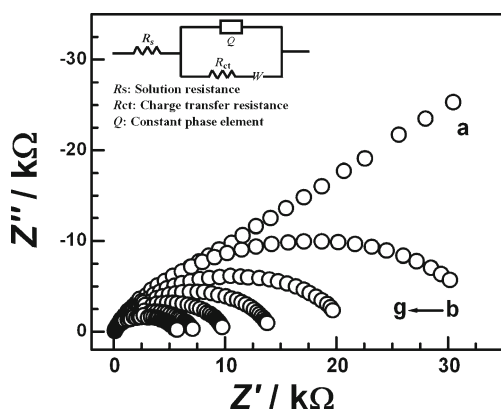


Fig. 4 Nyquist diagrams for GC/Nf/Fe(tmphen)₃²⁺ in 0.1 M Na₂SO₄ in the absence (a) and presence (b–g) of different concentrations (3.0, 5.0, 10.0, 15.0, 20.0, and 30.0 mM, respectively) of IZ. Frequency range, 1 Hz to 100 kHz. Applied potential, 0.7 V (vs SCE). *Insert* circuit shows the most compatible equivalent circuit for the Nyquist plots

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