

Amperometric Determination of Ascorbic Acid and Riboflavin Using Ferrocene/Thionin Bimediator Modified Electrode

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ABSTRACT

A bimediator modified electrode composed of ferrocene monocarboxylic acid covalently linked to thionin was developed for the amperometric determination of ascorbic acid and riboflavin. The bimediator molecule was characterized with FTIR spectroscopy and the electrochemical behaviour of the bimediator modified electrode was studied using cyclic voltammetry. Ferrocene present in the bimediator favoured the electrocatalytic oxidation of ascorbic acid and thionin favoured the electrocatalytic reduction of the riboflavin both in static and dynamic conditions. From the amperometric studies, a linear range for the oxidation of ascorbic acid from 23 μ M to 138 μ M with a correlation coefficient of 0.9875 and for the reduction of riboflavin from 9.90 μ M to 94.20 μ M with a linear regression coefficient of 0.9864 was observed with the bimediator modified electrode.

Keywords : Amperometry, Bimediator, Ascorbic acid, Riboflavin

I. INTRODUCTION

Ascorbic acid (AA), the Vitamin C is known for its reductive properties, being easily oxidized to dehydroascorbic acid. It acts as a powerful antioxidant and fights against free-radical induced diseases[1]. Ascorbic acid is found in high concentration in some fruits and foods together with vasoactive amines such as dopamine[2]. Also, ascorbic acid is the most common electroactive biological compound, being easily oxidized, and this constitutes the basis of its electrochemical determination. Riboflavin (RF) is one of the components of the flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). It is also an essential nutrients in mammals, closely associated with the metabolism of carbohydrates, fats and amino acids[3]. This vitamin exists both in vegetables and in animals. RF deficiency is partially associated with anaemia, and it causes lips and mouth sores, skin disorders, etc. So it is necessary to develop simple, economic and sensitive methods for ascorbic acid and riboflavin determinations. In this regard,

electrochemical methods have gained attention during the past two decades as they offer many advantages such as high sensitivity, less time-consuming and low costs over other analytical methods[4–6].

Ferrocene is an organometallic compound which acts as an effective redox mediator for the electrocatalytic oxidation[7] of various substrates due to its unique electrochemical behavior and ease of redox reaction at very low potential and fast electron transfer kinetics which helps in lowering overpotential of oxidation. Various forms of surface modification with ferrocene and their derivatives have been used and studies have been carried out for the electrochemical determination of various species due to the extraordinary Fc/Fc^+ reversible redox behavior. Ferrocene derivatives of substituents have attracted substantial interest, owing to their usefulness in the synthetic application of large ferrocene-based assemblies[8–10]. Thionin (TH), a cationic organic redox dye, which can be easily dissolved in water and ethanol[11] is known to show a reversible process

with good stability and reproducibility. As redox mediator, thionin has good conductivity for electron transfer and high electrochemical signal. Hence, it has been widely used in the fabrication of various modified electrode, for the determination of various substrates such as H₂O₂ [12], carcinoembryonic antigen[13] and NADH [14], etc. Amine group of the thionin has been utilized for covalent linkage with functionalized MWCNT, cadmium sulfide nanoparticles[15], 3-aminopropyltrimethoxy silane[16], MWCNT-chitosan[17] to develop CMEs for the determination of H₂O₂ and DNA.

In the present work, ferrocene covalently linked with thionin (TH), was used to modify the electrode to act as amperometric sensor for ascorbic acid and riboflavin. The formation of bimedator compound was confirmed with FTIR spectroscopy. The electrochemical behaviour of this bimedator modified electrode was studied and various parameters were optimized. The results showed that the bimedator modified electrode exhibited a good current response and hence could serve as a novel electrode for both oxidation and reduction of analytes. The electrocatalytic oxidation of ascorbic acid and reduction of riboflavin using Fc-TH bimedator modified electrode is reported here.

II. EXPERIMENTAL

A. Materials

Ascorbic acid was obtained from Himedia laboratories, India. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl) and N-Hydroxy benzotriazole (HOBt) were purchased from SRL chemicals, India. Riboflavin was purchased from s. d. fine chemicals limited, India. Ferrocene monocarboxylic acid (Fc), thionin acetate and graphite rods were purchased from Sigma Aldrich. Other chemicals were of analytical grade and purchased from Merck, India. Double distilled water was used for the preparation of background

electrolytes and pure nitrogen gas was purged to remove dissolved oxygen before every experiment.

B. Instruments

FTIR spectra were obtained using JASCO (FT-IR 460 Plus, Japan) spectrometer. Elico pH meter (model LI 120) was used for the pH measurements of the background electrolytes. A conventional three electrode system consisting of a saturated calomel electrode as reference electrode, platinum wire as counter electrode and bimedator modified electrode as working electrode was used. All the electrochemical experiments were carried out using CHI 400A electrochemical workstation (CH Instruments, USA).

C. Preparation of Fc-TH bimedator compound

The preparation of the bimedator was reported already by us[18]. In short, the ferrocene monocarboxylic acid was covalently linked with thionin (2:1 ratio) using EDC, HOBt as coupling agents as reported [19] with a slight modification. Ferrocene mono carboxylic acid (23 mg, 0.1 mmol) and an equimolar EDC (19 mg, 0.1 mmol) were mixed and stirred for 2 h then HOBt (13.5 mg, 0.1 mmol) was added to the reaction mixture with continuous stirring. To this thionin (14.3 mg, 0.05 mmol) was added and stirred continuously. After 12 h of stirring, the precipitated formed was washed with ethanol several times to remove the unreactive precursors. The bimedator compound was then dried at 80 °C and stored at 4 °C for further use.

D. Fabrication of Fc-TH bimedator modified electrode

The paraffin wax impregnated graphite electrode (PIGE) served as the bare electrode. The PIGE was prepared as reported earlier[20]. Prior to fabrication of modified electrode, the surface of PIGE was cleaned and polished with the use of emery sheets followed by 0.5 μm alumina slurry, washed with double distilled

water and then dried under nitrogen atmosphere. 10 mg of the bimediator compound dispersed in 1 mL of ethanol was used as the stock solution for the modification of electrode. 10 μ L of ethanolic dispersion of bimediator compound was drop casted on the PIGE surface and dried in ambient condition. Then, the electrode was washed thoroughly with water to remove unbound bimediator on the electrode surface and then dried with nitrogen gas.

III. RESULTS AND DISCUSSION

A. FT-IR Spectral characterization

The covalent crosslinking of thionine with ferrocene monocarboxylic acid was confirmed by FT-IR spectral analysis. The Fc-TH bimediator compound showed a shifted in the carboxylic acid group (C=O) stretching vibration from 1689 cm^{-1} to 1654 cm^{-1} . The disappearance of N-H stretching vibrations of primary amine at 3330 cm^{-1} and 3150 cm^{-1} and a peak at 741 cm^{-1} were attributed to twisting vibration of N-H amide bond. This observations confirms the formation of amide bond between Fc and TH [18].

B. Electrochemical characterization of the Fc-TH bimediator modified electrode

The Fc-TH bimediator modified electrode was characterized by electrochemical methods. The cyclic voltammogram of modified electrode showed two pair of redox peaks, where one redox peak was observed in positive potential for ferrocene and other redox peak appeared in the negative potential for thionin which are present in the bimediator. The cyclic voltammograms of bare and bimediator modified electrodes were recorded in 0.1 M PBS of pH 7.0 at a scan rate of 50 mVs^{-1} (Figure 1). These results showed the presence of ferrocene and thionin in the bimediator compound on the electrode surface (curve (ii)). The ferrocene in the bimediator showed an oxidation at a potential of 0.28 V and a reduced reduction at 0.23 while the thionin in the bimediator

was reduced at -0.265 V and oxidized at -0.191 V. The electrochemical behavior of the bimediator modified electrode was studied in various electrolytes including KCl, KNO_3 , NaCl, NaNO_3 , NH_4Cl , NH_4NO_3 and Phosphate buffer solution (PBS). pH of the background medium also varied from 4 to 9. From the voltammetric response, it was observed that the bimediator modified electrode showed a well defined redox peaks in the PBS at a pH 7.0. Hence the PBS of pH 7.0 was chosen as the background electrolyte for further studies.

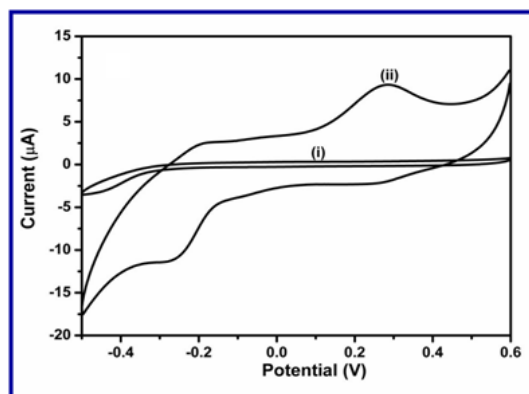


Figure 1. CVs of (i) Bare and (ii) Fc-TH bimediator modified electrode in 0.1 M PBS pH 7.0 in the potential range between -0.5V to 0.6V at a scan rate of 50 mVs^{-1} .

Effect of scan rate for the bimediator modified electrode in PBS (pH 7.0) was studied both for ferrocene in the positive potential range between 0 to 0.6 V and for thionin in the negative potential range between 0 to -0.5 V. The scan rate was varied from 5 to 150 mVs^{-1} . The current was plotted against the scan rate which showed a linear relation confirming that the bimediator modified electrode showed surface confined electrode process.

C. Electrochemical oxidation of ascorbic acid

Figure 2 displays the cyclic voltammogram of the bare and bimediator modified electrodes in absence (curve a & b) and presence (curve c & d) of ascorbic acid. It showed that the peak current at a potential of 0.325 V increased in presence of AA due to the catalytic

oxidation of ascorbic acid by the ferrocene present in the bimediator where as bare electrode showed a peak for the oxidation of ascorbic acid at higher potential with low current. To evaluate the applicability of the bimediator modified electrode towards the oxidation of ascorbic acid in the flow system, amperometry study was carried out. Based on the hydrodynamic voltammetry, a potential of 0.35 V was fixed and a known concentration of ascorbic acid was added to the stirred solution of PBS pH 7.0 at regular intervals of time. The current increased as the concentration of the AA increased in the solution (**figure 3**). The current response of the bimediator modified electrode was plotted against the concentration of the AA (**Figure 4**). A linear relationship was obtained in the concentration range from 23 μM to 138 μM with a correlation coefficient of 0.9875.

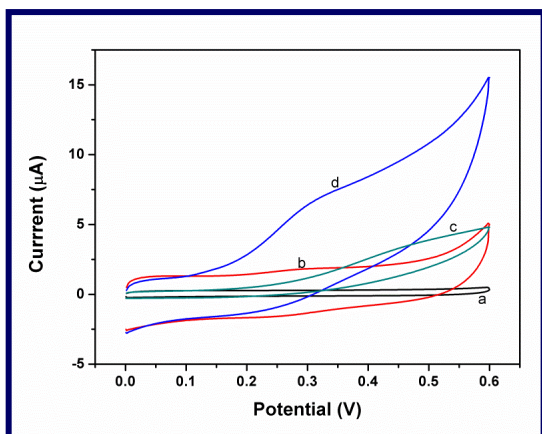


Figure 2. CVs of (a) bare (b) Bimediator modified electrode in the absence and (c), (d) in the presence of 2.91×10^{-5} M ascorbic acid.

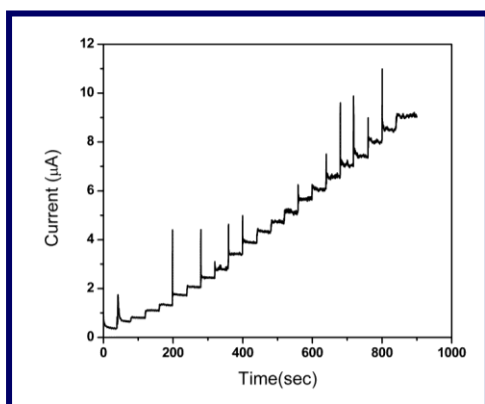


Figure 3. Amperometric response for the Fc-TH bimediator modified electrode under dynamic

condition at 0.35 V for the successive additions of ascorbic acid in 0.1 M PBS.

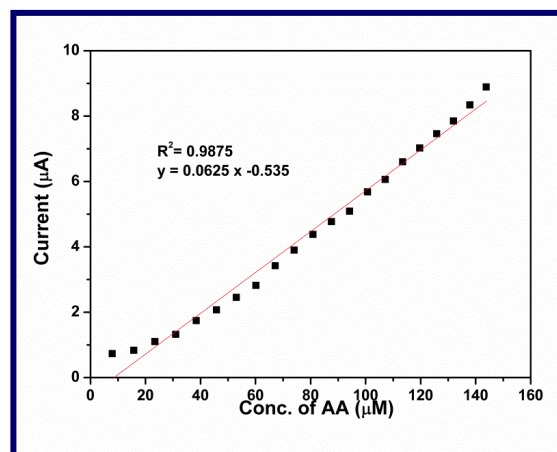


Figure 4. Calibration plot of current with concentration of ascorbic acid.

D. Electrochemical reduction of riboflavin

In order to test the electrocatalytic activity of the bimediator modified electrode towards the reduction of riboflavin, cyclic voltammetry study was carried out. **Figure 5** presents the cyclic voltammetric response of bare and bimediator modified electrode in PBS (pH 7.0) in absence and presence of riboflavin. The bare electrode showed a peak for the reduction of riboflavin at -0.55 V while the Fc-TH bimediator modified electrode showed the reduction peak at -0.49 V. The enhancement in the peak current at the lower potential for the reduction of riboflavin at the bimediator electrode compared to bare electrode confirmed that the thionin present in the bimediator enhanced the electron transfer for the catalytic reduction of riboflavin. To study the applicability of the bimediator modified electrode in the flow system, amperometric study was carried out by applying a fixed potential of -0.5V to the bimediator electrode and the current response of the modified electrode for the successive additions of known concentration of riboflavin is given in **figure 6**. The calibration plot of current against the concentration is shown (**figure 7**). A linear relation confirmed that the current increased with the increase in the concentration of the

riboflavin from 9.90 μM to 94.20 μM with a linear regression coefficient of 0.9864.

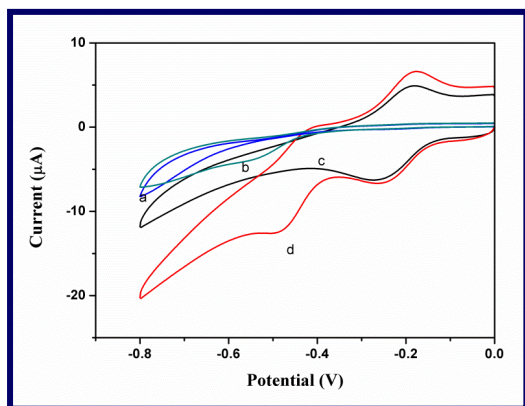


Figure 5. CVs of (a) bare (c) Fc-TH bimedator modified electrode in the absence and (b, d) in the presence of 9.09×10^{-5} M riboflavin.

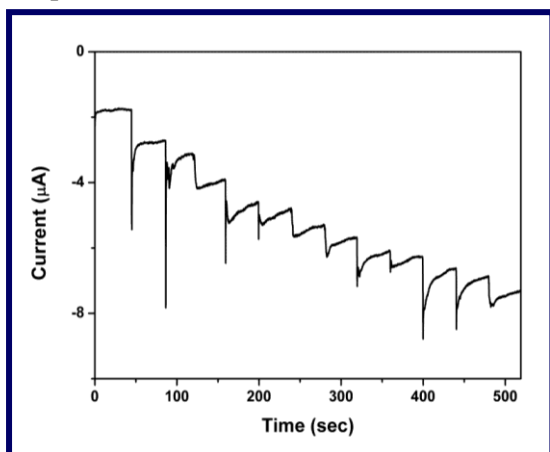


Figure 6. Chronoamperometric response for the Fc-TH bimedator modified electrode under dynamic condition at -0.5 V for the successive additions of riboflavin in 0.1 M PBS.

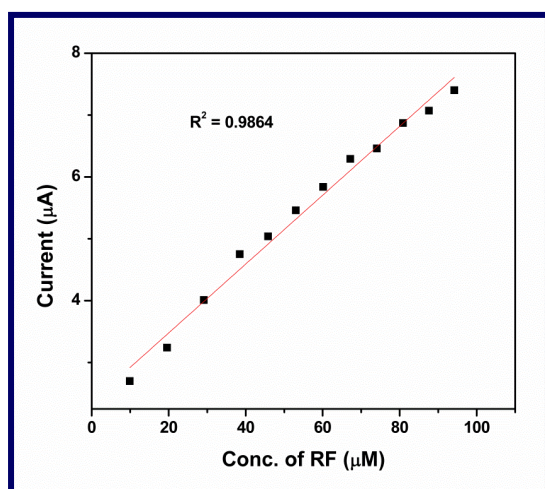


Figure 7. Calibration plot of current with concentration of riboflavin.

IV. CONCLUSION

A bimedator modified electrode containing ferrocene monocarboxylic acid and thionin was characterized. The FTIR study confirmed the formation of bimedator and the cyclic voltammogram confirmed the presence of both mediators on the electrode surface. The electrocatalytic activity of the Fc-TH bimedator modified electrode was studied for the oxidation of AA and reduction of RF. The amperometric response of the bimedator modified electrode under dynamic condition showed a good response for the oxidation of ascorbic acid at 0.35V and reduction of riboflavin at a potential of -0.5 V.

V. REFERENCES

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