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Electrochemical biosensor based on silica sol–gel entrapment of horseradish peroxidase onto the carbon paste electrode toward the determination of 2-aminophenol in non-aqueous solvents: A voltammetric study

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ABSTRACT

A novel and effective electrochemical biosensor was developed towards the rapid determination of 2-aminophenol (2-AP) based on carbon paste electrode modified with horseradish peroxidase (HRP) through silica sol–gel entrapment. The developed HRP silica sol–gel modified carbon paste electrode (HRP-SiSG/CPE) retained its bioelectrocatalytic activity towards the oxidation of 2-AP in the presence of organic solvents. The HRP-SiSG/CPE showed high sensitivity and rapid electrocatalytic activity in acetate buffer solution (ABS) of pH 5.0 in combination with 2-propanol as a solvent. All the experiments were carried out in combination with these two media. The proposed electrochemical biosensor exhibited a rapid response, low detection limit (0.92×10^{-5} mol/dm^3) and good linearity range (1×10^{-5} to 1×10^{-3} mol/dm^3) as well as good stability and repeatability. The HRP-SiSG/CPE biosensor was successfully applied for the simultaneous determination of 2-AP in the presence of paracetamol and real samples. The electrochemical impedance spectrum (EIS) has confirmed that the electron transfer rate at HRP-SiSG/CPE was faster.

1. Introduction

Amino phenols are amphoteric, chemically reactive and good reducing agents, which involve aromatic amino group, phenolic hydroxyl moiety as well as substitution on the benzene ring. Amino phenols and their derivatives are of intermediates in the photographic, pharmaceutical and chemical dye industries [1]. At room temperature amino phenols are solid crystalline compounds and among them 2-aminophenol (2-AP) can behave anomalously, because of intramolecular hydrogen bonding [2,3]. 2-AP forms white orthorambic bipyramidal needles having eight molecules to the elementary cell. The molecules are hydrogen bonded from OH to N to form chain [4].

Amino phenols are oxidized easily and they tend to remove oxygen from solutions, 2-AP undergoes multiple reactions, i.e. alkylation, acylation, diazonium salt formation, condensation and cyclization. 2-AP is particularly susceptible to cyclization and condensation reactions and this is due to the adjacency of the amino and hydroxyl groups attached to the benzene ring to give 2-aminophenoxazine-3-one, the mechanism was shown in Scheme 1 [5]. Amino phenols and its derivatives are useful starting materials for the synthesis of oxizines and oxazoles.

In general amino phenols are irritants and their toxic hazard rating ranges from slight to moderate. The repeated contamination of amino phenols may cause itching, skin sensitization, dermatitis and allergy [6]. 2-AP also causes oxidative DNA damage in the presence of metal ions [7]. 2-AP acts as corrosion inhibitor in paints [8] and anticorrosion-lubricating agent in motor cycles.

HRP is an important heme-containing metallo enzyme, which was widely used in the development of enzyme based voltammetric biosensor [9]. The peroxidases are a class of Fe (III) phorphyrins containing proteins that catalyze the oxidation of substrates [10]. During the HRP reaction cycle, the active heme site losses its native conformation and increases the co-ordination number of heme group [11,12]. The use of biosensor based immobilization of enzyme on to the electrode surface has many advantages for chemical analysis [13], however it is very difficult for the confinement of HRP on the electrode surface, hence it requires some specific binding techniques. In the present and past a lot of beneficial immobilization strategies have been employed based on physical adsorption [14], encapsulation [15], layer by layer assembling [16], electropolymerization [17,18], and silica sol–gel (SiSG) method [19]. Among these methods, SiSG method has been used for the immobilization of ample assortment of enzymes and biomolecules onto the electrode surface, due to its inertness, negligible swelling effect, tunable porosity, thermal stability, enabling spectroscopic monitoring, biodegradation and optical transparency [20,21].

The enzyme based biosensors have been found to have numerous applications in chemical synthesis, catalysis and recognition of elements in the presence of organic solvents containing with or without water

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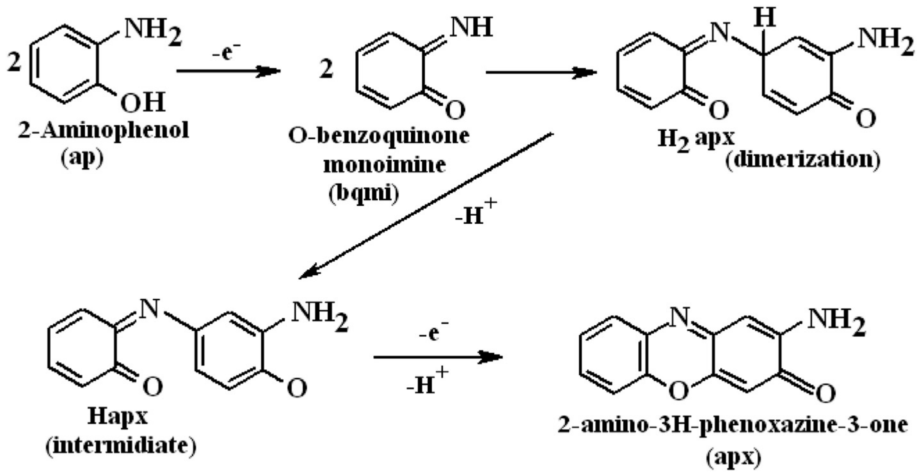

Scheme 1. Electron transfer oxidation mechanism of 2-aminophenol.

[22]. In our previous work, we have developed a biosensor based on the CPE immobilized with HRP, through SiSG entrapment for the determination of hydroquinone (HQ) in non-aqueous solvents [23] and also we have developed biosensor based on HRP immobilized on Ag-nanoparticles/poly(L-arginine) modified carbon paste electrode towards the determination of pyrogallol/hydroquinone [24]. The utilization of enzyme in non-aqueous solvents has some advantages, including the high solubility of hydrophobic substrates, suppression of various side reactions and insolubility of enzymes. The enzyme based biosensor in the presence of non-aqueous solvents gains the activity of enzyme properties, such as solvent hydrophobicity [25], solvent polarity and substrate hydrophobicity.

The above-cited references describe the various important properties with reference to amino phenols, such as chemical, structural, spectral, pharmaceutical, toxic and hazardous nature to the human beings and also biological significance, catalytic oxidation behavior and metabolism of enzymes in biological or living systems. The above references also cite various analytical techniques and binding techniques being used towards the determination of amino phenols. However these methods or techniques suffer from disadvantages such as high costs, tedious extraction process, long time analysis, etc., hence a simple method was needed. The electrochemical techniques and fabrication of working electrodes with novel biosensors have many advantages such as low-priced instruments, simple operation, fast response, time saving, good sensitivity, and good selectivity towards the determination of different analytes. To our knowledge voltammetric determination of 2-aminophenol based on HRP-SiSG film electrode in the presence of non-aqueous solvents was not reported.

In this present manuscript, we have fabricated HRP SiSG biosensor on the carbon paste electrode. The HRP-SiSG/CPE promotes the electrocatalytic response towards the oxidation of 2-AP which was studied through the Cyclic voltammetry (CV), and Differential pulse voltammetry (DPV). The reported HRP-SiSG/CPE biosensor expressed good sensitivity and more stability towards the determination of 2-AP in the presence of non-aqueous solvents.

2. Experimental

2.1. Reagents

All chemicals were obtained from commercial sources and used without further purification. Horseradish peroxidase (E.C. 1.11.1.7 type – VI-A - S/5 mg, *Amoracia rusticana* source, 1840 U/mg) was purchased from Sigma Aldrich pvt limited, India, 2-aminophenol was purchased from Merck pvt limited, Mumbai, Paracetamol from Sigma-Aldrich Chemicals, India, Tetraethyl orthosilicate (TEOS), and Triton X-100 were obtained from Sigma-Aldrich Chemicals Co. USA. Methanol, Ethanol, 2-propanol, 1-butanol, and iso-butanol were purchased from Merck Specialties Pvt. Limited, Mumbai, India. The graphite fine powder was procured from Loba Chemie Pvt. Ltd., Mumbai (India), silicon oil and Acetone from Thermo Fisher Scientific India Pvt. Ltd., Mumbai (India). Acetate buffer solution (ABS) was prepared by assimilating 0.1 mol/dm^3 sodium acetate and 0.1 mol/dm^3 acetic acid. All the aqueous solutions were prepared with double distilled water. The enzyme stock solution and working solutions of chemicals were stored in refrigerator.

2.2. Apparatus

The electrochemical measurements were recorded in an electrolytic cell containing three electrodes at a room temperature of 25 ± 1 °C. The working electrode was an enzyme immobilized carbon paste electrode (HRP-SiSG/CPE). The reference electrode was a saturated calomel electrode (SCE) and glassy carbon rod electrode was used as an auxiliary electrode. Electrochemical measurements were carried out by using CH – Electrochemical Analyzer (Model CHI – 660D, CH Instruments, USA). Elico U 120 pH meter combined with pH CL 51 B electrode was used for measuring the pH values.

2.3. Preparation of carbon paste electrode

The preparation of carbon paste electrode was done by hand mixing of 85% graphite power and 15% of silicon oil in an agate mortar for 40–45 min to get homogeneous paste. This carbon paste was incorporated into 2 mm diameter of Teflon tube and electrical contact was made through a copper wire. The electrode was smoothed on a piece of soft filter paper for obtaining smooth surface before the measurements [26,27].

2.4. Fabrication of biosensor (HRP-SiSG/CPE)

A homogenous TEOS silica sol–gel was made by mixing 2 ml of TEOS, 1 ml of H_2O, 50 µl of 0.1 mol/dm^3 HCl, 25 µl of 10% Triton X-100. The mixture was stirred for 1 h to obtain clear sol. The sol can be stored for about 1 month when it was kept in a refrigerator.

The 10 µl of 5 mg/ml HRP enzyme stock solution was added to the mixture of 10 µl of stock SiSG solution and 40 µl of 0.1 mol/dm^3 ABS. A drop of this dispersion with a volume of 5 µl was cast onto the surface of the CPE, and then it was allowed to dry at room temperature for 3–5 min. The electrode was gently washed with ABS and was used for further experimental procedure [28].