



## ELECTROCHEMICAL SYNTHESIS OF COMPOSITE FILM OF ANILINE AND 2-AMINO PYRIDINE FOR APPLICATION AS BIOSENSORS.

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### ABSTRACT:

The paper covers main applications of conducting polymers in chemical sensors and biosensors. A new carbon-based conducting composite has been developed as electrochemical sensor and biosensor. An amperometric biosensor was prepared by in situ deposition of horseradish peroxidase (HRP) enzyme on a composite aniline-2-amino pyridine film doped ITO electrode. The composite film was electrochemically deposited on the electrode at 100mVs<sup>-1</sup>/Ag-AgCl. Cyclic galvanostatic characterization of the film in 1M H<sub>2</sub>SO<sub>4</sub> showed two distinct redox peaks, which prove that the composite film was electroactive and exhibited fast reversible electrochemistry. The surface concentration and film thickness of the adsorbed electroactive species was estimated to be  $1.85 \times 10^{-7}$  mol cm<sup>-2</sup> and approximately 16 nm, respectively. HRP was electrostatically immobilized onto the surface of the film, and galvanostatic was used to monitor the electrocatalytic reduction of hydrogen peroxide under diffusion-controlled conditions. Linear responses over the concentration range  $2.5 \times 10^{-4}$  to  $5 \times 10^{-3}$ M were observed. The biosensor surface morphology was characterized by scanning electron microscopy (SEM) using composite film-doped screen-printed carbon electrodes (SPCEs) in the presence and absence of peroxide. The SEM images showed clear modifications of the conducting film surface structure when doped with HRP, as well as the effect of hydrogen peroxide on the morphology of biosensor.

**KEYWORDS:** Biosensor; conducting polymers; composite film of aniline and 2 amino pyridine-based biosensor; scanning electron microscopy of biosensor.

### 1. INTRODUCTION :

Organic conducting polymers have attracted interest in recent years because they exhibit a wide range of novel electrochemical properties. Among that we studied composite film of aniline and 2 amino pyridine which has been studied extensively as an important conducting material that possesses interesting electrical, electrochemical, and optical properties. The continuous growing interest in the study of composite film caused by these diverse, unique properties, and its promising potential in commercial applications. The potential applications of composite film of aniline and 2 amino pyridine include anti-corrosive coatings, secondary batteries, electrochromic devices, and electrochemical biosensors. Advantages of utilizing composite film of aniline and 2 amino pyridine-coated electrodes in biosensors are

impressive signal amplification and elimination of electrode fouling. This polymer also provides a suitable environment for immobilization of biomolecules. Polymer films can be deposited on electrode surfaces very readily. Polymerization of the monomer aniline can be achieved either chemically or electrochemically. Electrochemically, the polymer can be grown on the electrode surface by pulse, galvanostatic, potentiostatic or potentiodynamic techniques. The latter being preferred because of the homogeneous film produced and a strong adherence to the electrode surface. An important advantage of the electropolymerization technique in the fabrication of a biosensor is that the film thickness and characteristics can be controlled by monitoring polymerization charge. Determination of hydrogen peroxide and other organic peroxides is of practical importance in

clinical, environmental, and many other fields. Hydrogen peroxide has been determined by volumetric, colorimetric, and chemiluminescence methods that tend to be complex, time-consuming, and suffer from various interferences. The sensitive determination of hydrogen peroxide may be achieved by the use of peroxidase-modified electrodes, since enzymes are known to show excellent selectivity for their substrates. The fabrication of  $H_2O_2$  sensors has attracted great interest as the measurement of  $H_2O_2$  is the basis of detecting many biologically active materials such as glucose and cholesterol. This paper reports the fabrication and characterization of a composite film of aniline and 2 amino pyridine-based biosensor incorporating the enzyme horseradish peroxidase (HRP) for the detection of hydrogen peroxide. Both square wave and cyclic galvanostatic have been used as detection methods for hydrogen peroxide. This sensor was interrogated using electrochemistry, spectroelectrochemistry and scanning electron microscopy (SEM) as characterization techniques.

### 2.1. Reagents :

All reagents were purchased from Sigma–Aldrich and were of analytical grade. The enzyme peroxidase (EC 1.11.1.7 type II from horseradish, 150–250 units  $mg^{-1}$ ) was used for biosensor preparation. One unit of HRP will form 1.0 mg purpurogallin from pyrogallol in 20 s at pH 6 at 20 °C. Aniline and 2 Amino pyridine used for composite film formation but Aniline was distilled before use, and fresh solutions of hydrogen peroxide were prepared daily. Phosphate buffer solution (0.05 M, pH 6.8) was prepared from anhydrous disodium hydrogen phosphate and potassium dihydrogen phosphate monohydrate. The anhydrous salt was dried for 2 h at 110 °C and cooled in desiccators before being used for buffer preparation. Analytical grade argon was used to degas the system.

### 2.2. Apparatus :

All electrochemical experiments were carried out and recorded with a computer interfaced to a BAS/50W integrated automated electrochemical workstation. Cyclic galvanostatic, square wave galvanostatic and differential pulse galvanostatic were carried out in a 20ml electrochemical cell, with Ag/AgCl (3M NaCl type) and graphite reference and counter electrodes, respectively. A Faraday cage (BAS C2) was used for all experiments. A Indium titanium oxide (ITO) used as working electrodes. UV–vis absorbance measurements were recorded at room temperature on a UV/VIS 920 spectrometer using a specially designed quartz cell (designed in-house), incorporating a ITO, Ag/AgCl, and graphite rod as working, reference, and auxiliary electrodes, respectively. Scanning electron microscopy was performed with a Hitachi X650 scanning electron microscope.

### 2.3 Enzyme immobilization

The ITO/composite film of aniline and 2 amino pyridine electrode was first reduced in 10 ml of 0.05M phosphate buffer solution, pH 6.8 at  $-500mV$  for 15 min until a steady state was achieved. Enzyme immobilization was achieved by oxidation of the composite film of aniline and 2 amino pyridine film in the presence of 20  $\mu$ l HRP (4  $mg\ ml^{-1}$ ) at a potential of  $+650mV$  for 20 min. During the oxidation process, HRP became electrostatically attached to the polymer film. The ITO/composite film of aniline and 2 amino pyridine/HRP electrode was rinsed with de-ionized water to remove any loosely bound enzyme, and stored in buffer solution (pH 6.8) at 4 °C when not in use.

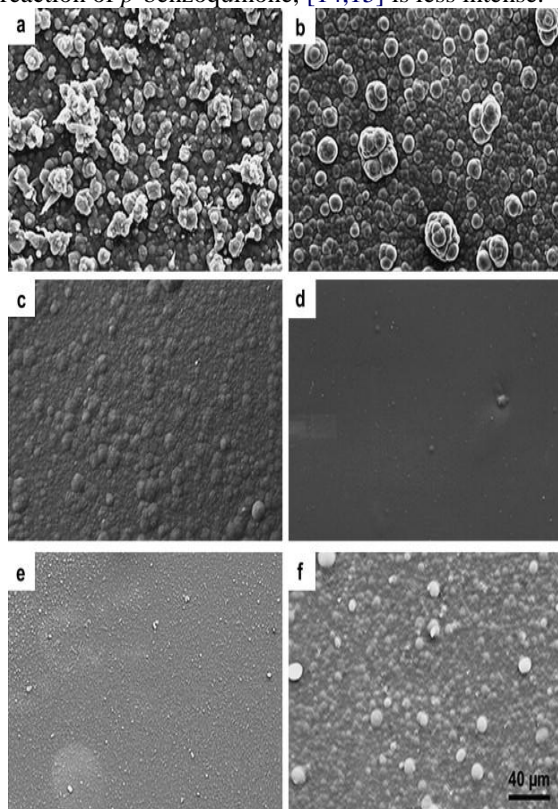
### 2.4 Electrochemical measurements

The cell used for the electrocatalytic reduction of hydrogen peroxide consisted of ITO/composite film of aniline and 2 amino pyridine/HRP electrode, ITO, and Ag/AgCl as the working, counter, and reference electrode, respectively. The 10 ml test solution containing 0.05M phosphate buffer (pH 6.8) was degassed with argon after each addition of small amounts of 0.1M  $H_2O_2$ . The concentration range of peroxide studied was between  $2.5 \times 10^{-4}$  and  $5 \times 10^{-3}$  M. Square wave and cyclic galvanostatic were used to measure the responses of the biosensor to  $H_2O_2$ . Square wave galvanostatic was carried out using a step potential of 4mV, a frequency of 5 Hz, and amplitude of 50mV. All cyclic voltammograms (CVs) were performed at  $5mVs^{-1}$ .

## 3. RESULTS AND DISCUSSION

**3.1. Electrosynthesis of composite film of aniline and 2 amino pyridine on ITO composite film of aniline and 2 amino pyridine** was electrochemically synthesized from an acidic medium of aniline by sweeping the potential from  $-200$  to  $+1100mV$  at  $100mVs^{-1}$ . Fig. 1 shows a typical cyclic voltammogram of ITO in 0.2M aniline per 1M  $H_2SO_4$  solution for this electropolymerization process. The composite film of aniline and 2 amino pyridine layer was seen to be redox active in the potential region studied, exhibiting three sets of redox peaks. Initially, oxidation of aniline occurred at approximately  $+900mV$  resulting in the nucleation of composite film of aniline and 2 amino pyridine. During subsequent scans, the oxidation of composite film occurred at lower potentials due to the catalytic effect of composite film of aniline and 2 amino pyridine, which resulted in deposition of polymer on the electrode surface. Redox couples A/B and E/F were attributed to intrinsic redox processes of the polymer itself. The redox couple A/B occurred at approximately  $+200mV$  and is attributed to the transformation of PP composite film of aniline and 2 amino pyridine Y from the reduced leucoemeraldine (LE) state to the partly oxidized emeraldine state

(EM). The redox couple E/F at approximately +800mV corresponds to transition of the Pcomposite film of aniline and 2 amino pyridenePY from LE to pernigraniline (PE) state, and is accomposite film of aniline and 2 amino pyridene by the oxidation of aniline monomer [15]. The redox couple C/D at approximately +500mV, which is generally attributed to the redox reaction of *p*-benzoquinone, [14,15] is less intense.



### 3.5. Scanning electron microscopy

SEM studies were carried out on screen-printed carbon electrodes. Fig. a shows the surface of a typical SPCE. The surface topography showed good definition of graphite particles. Fig. b shows that the growth of composite film of aniline and 2 amino pyridene from acidic media resulted in a sponge-like, branched, porous-structured, high-surface area polymer film on a SPCE, ideal for inclusion of enzyme. The enzyme immobilization (0.08 g l<sup>-1</sup>) can be observed by a change in topology to a speckled, grainy image (Fig. c), each of the speckles representing clusters of protein on the surface of the polymer. Following the performance of a cyclic galvanostatic in the presence of H<sub>2</sub>O<sub>2</sub> (1 mM), the morphology of the enzyme layer underwent a change (Fig. d). The many clusters of protein observed in Fig. c lessened, and the polymer layer beneath the protein could be seen clearly again. This deterioration in enzyme coverage may be due to the fact that a weak electrostatic immobilization method was employed and leaching of the enzyme into bulk solution upon application of potential. Another reason may be due to HRP inhibition by high concentration of H<sub>2</sub>O<sub>2</sub>.

Further SEM and stability studies must be employed to elucidate the reasons for these results.

### 4. CONCLUSION

Electrochemical characterization of the electroactive polymer, composite film of aniline and 2 amino pyridene, was carried out on the surface of a ITO electrode. This electroactive polymer was then successfully applied in a horseradish peroxidase-based biosensor. It served as an efficient non-diffusional mediator, shuttling electrons between the redox active center of the enzyme and the electrode surface and also acted as a point of attachment for the enzyme. This ITO/composite film of aniline and 2 amino pyridene/HRP electrode effectively catalyzed the reduction of hydrogen peroxide, and both square wave and cyclic galvanostatic were used to monitor the catalytic current. The linear range of this biosensor was from  $2.5 \times 10^{-4}$  to  $5 \times 10^{-3}$  M and *r*<sup>2</sup> was estimated at 0.995. Optimizing the protein loading on the surface of the ITO/composite film of aniline and 2 amino pyridene electrode could potentially increase the sensitivity of this biosensor. Spectroelectrochemistry (results not presented) and SEM studies provided an interesting insight into the behavior of the biosensor. Although the analytical performance of this biosensor is not as good as reported in the literature, this biosensor has several advantages such as control of the polymer film characteristics, good signal amplification, ease of fabrication, and application to screen-printed electrodes for disposable use.

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