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Fabrication of acetylcholinesterase sensor based on polyaniline/$K_2Cr_2O_7$ composite film modified electrode for amperiometric detection of carbaryl

V.B. Deshmukh[1*], K.S. Paithankar[2], U.N. Shelke[3] N.R. Dhumane[4], V.K. Gade*

[1]Department of Physics Hutatma Rajguru Mahavidyalaya,Rajgurunagar, (M.S.) India
[1,2]Department of Physics Research Center,Ahmednagar College Ahmednagar (M.S.) India
[*]Department of Physics, Shri Anand College, Pathardi (M.S.) India

*vbdeshmukhsir@gmail.com vikas_gade@yahoo.com

In electrochemical synthesis a new responsive and selective acetylcholinesterase (AChE) sensor has been fabricated. The sensor was constructed by deposition of polyaniline/potassium dichromate ($K_2Cr_2O_7$) on indium tin oxide (ITO) substrate and immobilized by AChE enzyme. The morphology and optical properties of the AChE sensor were investigated by using scanning electron microscopy, ultraviolet visible, Fourier transformation infrared and x-ray spectroscopy. The mechanism of the fabrication process and other parameters controlling the preparation process and then amperiometric response of sensor were measured and analytical performance of the sensor was optimized. Under optimized condition, a linear range selected from 1µM to 20µM for the detection of carbaryl and the sensor shows low detection limit and high stability in wide range as compared to previous reviews.

Keywords: Acetylcholinesterase, polyaniline, sensor, amperiometric, carbaryl

1. INTRODUCTION

Carbaryl is a pesticide in carbamate family which is widely used in agricultural activity for controlling insects but due to toxicity it is risky to human being [1-2]. Farmers are used various pesticides for killing insects and pests so it is important to analyze pesticides in agricultural products, for that purpose analytical chromatographic methods are used for low detection limit of pesticides. These methods are expensive, it required technical experts for analysis and it is time consuming so these methods are not suitable for large scale analysis [3]. The enzymatic sensor has been developed for detection of pesticide residues because it has various advantages such as fast response, short time consuming, low cost and sensitive [4-5]. Amperiometric responses of electrode for inhibition action of pesticides on AChE have shown good results. The performance of biosensor depends on the enzyme immobilization on the surface of electrode, immobilization is better on good supporting dopants materials used for synthesis. Amperiometric biosensor realizes the pesticide detection by measuring the change of current induced by the specific reactions between biological recognition element and analytes such as enzyme and substrates. Enzymatic sensor is an analytical device which is combines an enzyme with a transducer to produce signal proportional to target and concentration of analyte.

1.1. Material and Reagents

In pure form, 99% of Carbaryl, Acetylcholinesterase, aniline and other chemicals were purchased from Sigma Aldrich and indium tin oxide (ITO, Resistance 50 Ω/cm) sheet purchased from Lobachemie Pvt. Ltd. Mumbai, India All chemicals were used in analytical reagent grade and double distilled water used for overall synthesis process.

1.2. Apparatus

For synthesis process, galvonostatic electrochemical polymerization set up was performed with three electrodes. The working electrode was a modified indium tin oxide (1cm x 3cm), the counter electrode and reference electrodes were a platinum foil and silver/silver chloride respectively. Scanning electron microscope (SEM) images with EDS as well as UV and FTIR were obtained at Central Instrumentation Facilities (CIF) Savitribai Phule Pune University (SPPU). All experimental process was carried out at ambient temperature.

1.3. Electrochemical synthesis of PANI/$K_2Cr_2O_7$

The aqueous electrochemical polymerization was carried out in one compartment of cell. A 1cm x 3cm ITO coated plate was used as the working electrode (anode). The platinum foil was used as the counter electrode (cathode) and Ag/AgCl as a reference electrode. The digital power supply (0-5V) was used as the source. Electrolytic solution was made by 0.5M H_2SO_4, 0.1N potassium dichromate ($K_2Cr_2O_7$) and freshly prepared 0.1N aqueous solution of aniline (99%) in double distilled water in electrochemical cell [6].The pH of aqueous medium was maintained by using buffer solution. The electrochemical polymerization of aniline and $K_2Cr_2O_7$ was synthesized by galvanostatic method. Then PANI/ $K_2Cr_2O_7$ film was prepared on ITO substrate.

1.4. Synthesis of PANI/ $K_2Cr_2O_7$/ AChE/ ITO

By dropping 0.8 μL pure AChE solution on the surface of the PANI/ $K_2Cr_2O_7$ polymer film and incubating it at 20^0C for 24 hrs. The polymer film is cleaned with distilled water, dried and stored at 10^0 C prior to use. Then the polymer film was prepared for electrochemical measurement, it is a real contribution from the composite surface to the efficiency of the polymer film without cross-linking agents to make bonding to the active sites of enzymes [7].

1.5. Measurement procedure

The PANI/$K_2Cr_2O_7$/AChE biosensor was worked for the determination of carbaryl using potentiostatic method. After that the biosensor performance was measured by its current response for maintaining pH 7.0 PBS solutions. Then the electrodes was cleaned with distilled water and incubated in pH 7.0 PBS solution containing desired concentration of carbaryl for 10 min. at last it was transferred into the 1.0 mM acetylcholine (ATCl) solution for potentiostatic measurement in same condition. Figure 1 shows principle working of pesticide sensor.

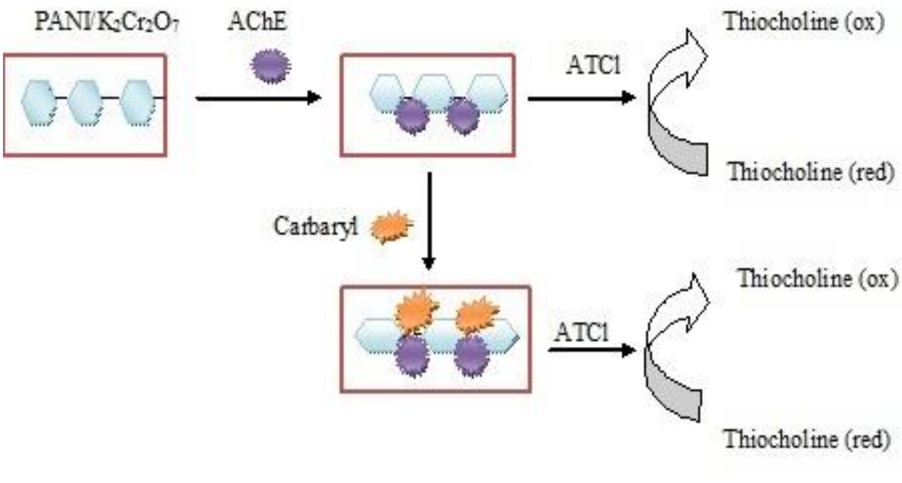


Figure1.Fabrication and principle of the electrochemical AChE sensor

. The inhibition rate of carbaryl was calculated by following formula,

$$Inhibition(\%) = \frac{I_{P(control)} - I_{P(exp)}}{I_{P(control)}} \text{ X } 100$$

Where, $I_{p(control)}$ was the peak current of ATCl on PANI/$K_2Cr_2O_7$/AChE without carbaryl inhibition and $I_{p(exp)}$ was peak current of ATCl on PANI/$K_2Cr_2O_7$/AChE with carbaryl inhibition. After inhibited by carbaryl, the sensor was reactivated by immerging into 5.0 mM pralidoxime iodide for 10 min. then transferred into pH 7.0 PBS containing1mM ATCl for potentiostatic analysis of the electrochemical response. The reactivation efficiency was measured as above,

$$R(\%) = \frac{I_r - I_{P(exp)}}{I_{p(control)} - I_{P(exp)}} \text{ X } 100$$

Where, I_r was the peak current of 1mM ATCl on PANI/$K_2Cr_2O_7$/AChE after 0.5 mM pralidoxime iodide reactivation.

2. RESULT AND DISCUSSION

The electrochemically synthesized composite polymer PANI/K2Cr2O7 on ITO substrate was successfully immobilized by AChE enzyme and following characterization was study.

2.1. Scanning Electron Microscopy and Energy dispersive X-ray spectroscopy

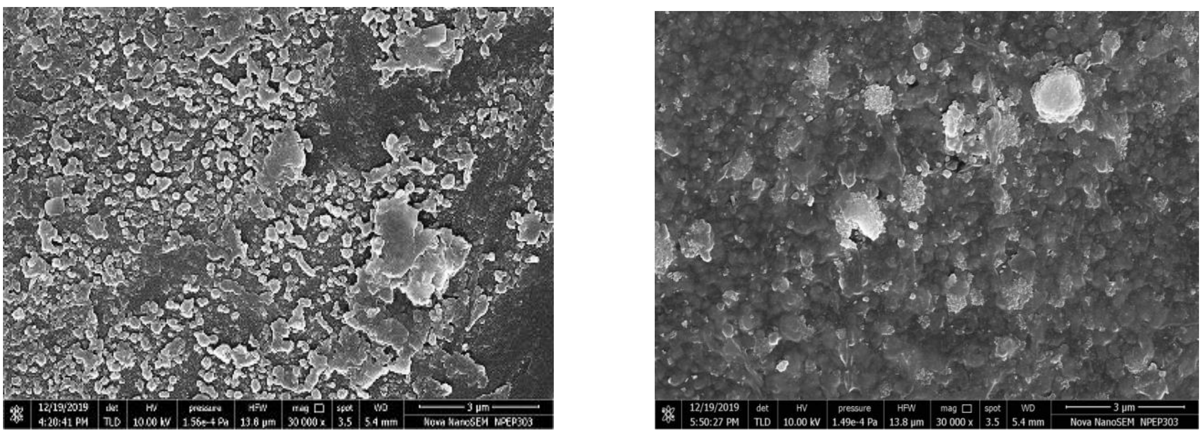
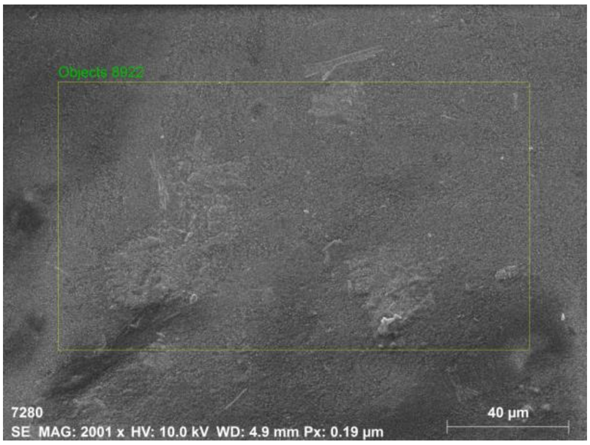
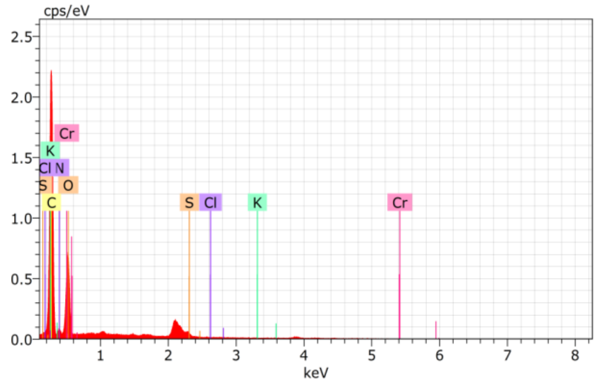


Figure 2.a) SEM of PANI/$K_2Cr_2O_7$ **b) SEM of PANI/$K_2Cr_2O_7$/AChE**





c) EDS of PANI//$K_2Cr_2O_7$/AChE

Figure 2.a.b shows SEM image of PANI/ $K_2Cr_2O_7$ of synthesized thin films without and with immobilization of enzyme having magnifications 30KX. Image 2.a shows porous and granular morphology of composite film. Such nature is easily caring for immobilization of enzymes for the biosensor application. Image b show morphology changed with enzyme immobilized onto the modified electrode whose surface can be easily observed further, homogeneous coating of the enzyme proved that the proposed electrode before immobilization serves as an excellent host-guest platform for bio-molecules immobilization. Fig. 2.c shows E-DAX spectrum and graphical representation of electrode. It indicates percentage of chemical compounds on composed film.

2.2. UV–Visible spectroscopy

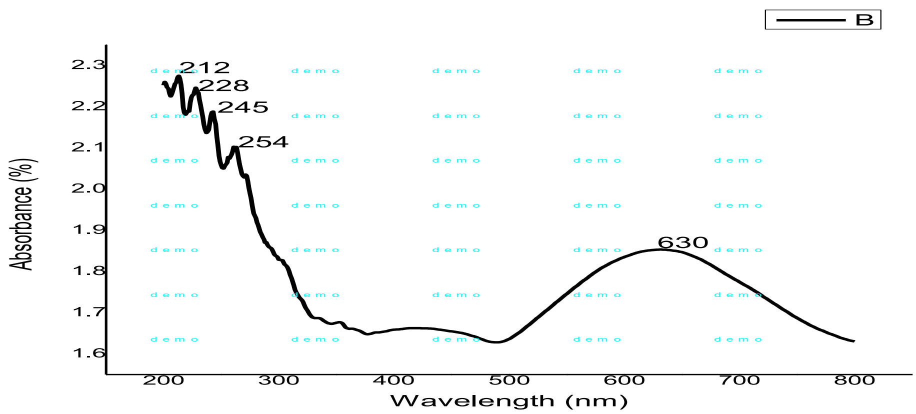


Figure 3.UV-Visible absorption spectra of PANI/$K_2Cr_2O_7$

The UV-Visible absorption spectra of PANI/$K_2Cr_2O_7$ film was recorded at room temperature by Analytic Jena specord210 plus spectrophotometer with wavelength range 200 -800 nm as shown in above figure3. The spectra show various peaks at around 212nm, 226nm, 245nm and 254nm. The peak at around 254 nm shows $\pi \rightarrow \pi^*$ transitions which is available in compounds with unsaturated centers and aromatics compounds. The absorption peak 630nm is responsible for bi-polaron state and it shows charge transfer bands indicates conductivity of thin film having band gap energy is 1.968eV. More doped aniline has resulted in better absorption at 630 nm. The absorption spectra observed for synthesized composite electrode gives good agreement with the earlier reported work [8-9]. This shows very good resemblance with the polymerization potential.

2.3. FTIR analysis

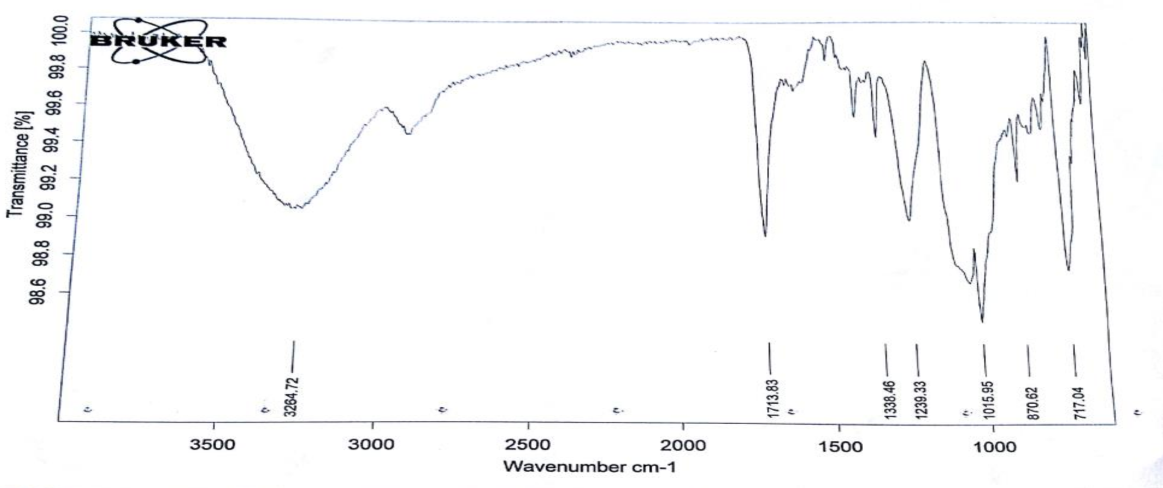


Figure 4.Aniline 0.1M, $K_2Cr_2O_7$, 0.025N, H_2SO_4 0.5M) pH 2.5

The FTIR spectrum of synthesized PANI/$K_2Cr_2O_7$ composite film with immobilization of AChE enzyme is shown in fig.4 the spectra show various peaks, the peak at 3325.33 cm^{-1} corresponds to N-H stretching. The peaks at 1230.38 and 1338.19 cm^{-1} which is assigned to evidence of the presence of anion in the polymer film i.e. S=O stretching in sulfonate. The vibration bands are observed at 1709.33 cm^{-1} (C=O) these peaks correspond to the characteristic for aniline it shows very good agreement with earlier reported work [10]. Thus the FTIR spectral results confirm the presence of polyaniline.

2.4. X-ray diffraction

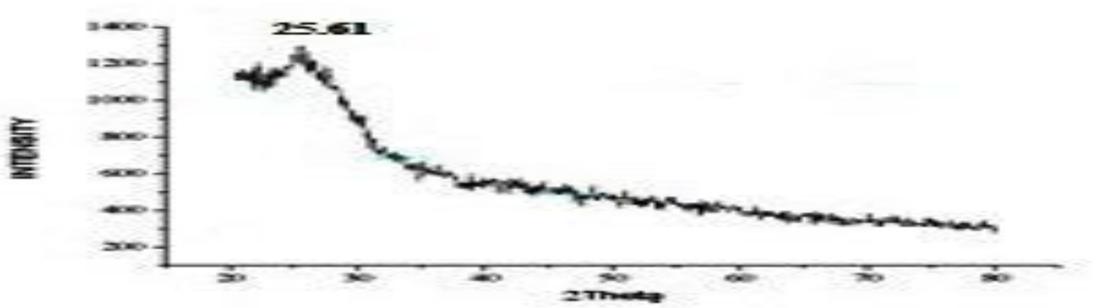


Figure 5.X-Ray spectroscopy of PANI

X-ray spectroscopy of PANI/$K_2Cr_2O_7$ film is shown in fig5. The XRD pattern of PANI/$K_2Cr_2O_7$ film clearly indicates that the intensity of observed peaks is better developed on the composites prepared using di and tri basic acid solutions compared with the monobasic acid. The profile of the characteristic peak of PANI/$K_2Cr_2O_7$ at $2\Theta = 25.31^0$ the obtained patter shows a mostly amorphous material with few crystalline phases. Thus the fraction of crystalline phase found to be increased with voltages, the average crystalline size of polymer film is 63.15 nm and polymer chain separation is 42.52nm. The conductivity of polymer film is measured by four probe method and it was observed as 0.162 S/cm.

3. Detection of carbaryl in standard solution

In optimum condition, inhibition measurements are performed as shown in figure 5. The inhibition of carbaryl is proportional to its concentration between 1µM to 20µM and measured current response. The detection limit of carbaryl has observed about 0.8µM. The result summarized in table 1which displayed that the pesticide sensor exhibited lower detection limit than some of previous studies [11-15].

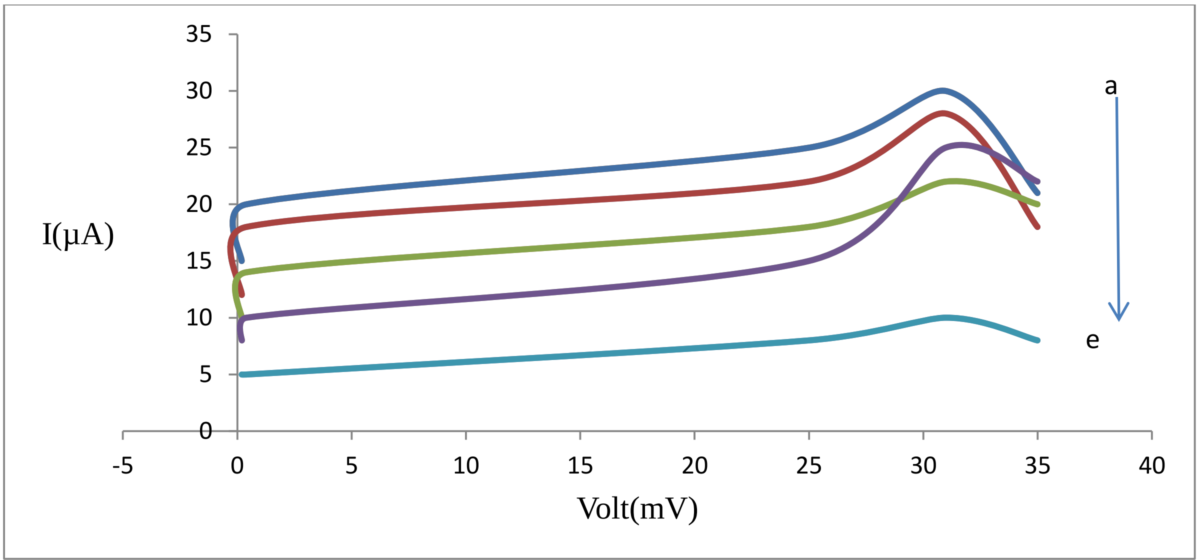


Figure 6.Carbaryl concentration from a to e 1 µM, 5 µM, 10 µM, 15 µM and 20 µM.

Electrode	Analyte	Linear Range	Limit of detection	Reference
CS-AuPtNPs/GCE	Carbaryl	0.030 µM to 6.0 µM	8.0 nM	11
AChE/e-GON–MWCNTs/GCE	Carbofuran	0.03–0.81ng/ml	0.015 ng/ml	12
AChE/carbon paste electrodes	Carbaryl	1µg/ml-15 µg/ml	0.4 µg/ml	13
GC/PANI/MWCNT/AChE	Carbaryl	1.98 µg/ml-9.92 µg/ml	0.28 µg/ml	14
AChE-CdS-G-CHIT	Carbaryl	2 ng/ml-2000 ng/ml	0.7 ng/ml	15
PANI/$K_2Cr_2O_7$/ITO	Carbaryl	1 µM -20 µM	0.8µM	This work

Table 1.Comparison of the proposed AChE biosensor for Carbaryl detection

4. Conclusion

The result shows synthesized H_2SO_4 polyaniline is soluble in dopant $K_2Cr_2O_7$, which increased the conductivity of polymer. The synthesized polymer is used for fabrication of biosensor due to porous structure and easy immobilization of enzyme.

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