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Detection of Diethyl 4-Nitrophenyl Phosphate (Paraoxon) by Modified Polyaniline based Nanocomposite Graphite Paste Electrode

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Abstract: ***New graphite and conducting polyaniline based nanocomposite has been developed as electrochemical biosensor for the amperometric detection of paraoxon(D4-NPP) an organ phosphorous pesticide. The sensor being highly sensitive, selective with a low detection limit was successfully applied for paraoxon determination which occurs in commercial fruit, vegetables samples. An interference free pesticide biosensor has been developed, based on the immobilization of acetyl cholinesterase(AChE) on surface of modified electrodes. Effect of influence of pH and effect of potential on response of biosensor was investigated for optimization the process parameter for good operational stability of sensor. Organized materials were characterized by analytical techniques such as FT-IR, UV-Vis and FE-SEM analyses. The sensor responds for paraoxon in 0.2M phosphate buffer solution (PBS) (pH7) at the range $1x10^{-6}$ to $9x10^{-6}$M and the detection limit were found to be $1x10^{-6}$. About 90% of the enzyme activity is retained for about 40days.***

Keywords: Sensors; nanocomposite; FT-IR.

I. INTRODUCTION

In present work the electrochemical cell was assembled in a conventional one compartment three electrode system design, the working electrode was modified paste electrode consist of composition of 70:25 (graphite: mineral oil)and polyaniline, this paste allowed to homogenize for few hours.

The paste was then filled in a teflon tip. A platinum wire was dissected inside the paste, to provide an electrical contact. Instead of platinum wire steel wire also used and sensing action recorded. Smooth and fresh electrode surfaces were obtained by pressing out 0.5mm of paste from the syringe, scraping off the excess and polishing it by butter paper until the surface has a shiny look and polyaniline composite in which the acetyl cholinesterase (AChE)immobilized; the Ag/AgCl used as the reference electrode; and a graphite use as the counter electrode.

Concentration of AChE immobilization on the composite surface carried out by dropping of the buffer solution containing the enzyme on the electrode surface, which further dried at a controlled temperature. No cross-linking agent (gluteraldehyde or di-amide) was necessary as use in earlier work[1-3]. This constitutes a real contribution from the composite surface to the productivity of the biosensor since the cross-linking mediators frequently bond themselves to the active sites of enzymes, thus inhibiting their movement.

II. EXPERIMENTAL

Paraoxon (99%), Acetyl cholinesterase was purchased from Sigma Aldrich, Graphite fine powder extra pure (particle size 240×10^{-6}m) obtained from Lobachemie Pvt. Ltd. India, Paraffin liquid heavy or mineral oil (viscosity at 37°C is 64cS) purchased from High purity lab, Mumbai, India. UV–Visible spectra (UV–Vis) were recorded in air at room temperature in the wavelength range of 200–800 nm using a Jena specord 210 spectrophotometer.

FT-IR spectra were recorded on aOcean optics HPX-2000 (Fiber coupled) spectrometer in the range of 4000-500 cm^{-1}. FE-SEM carried by JEOL JSM-7500F.

All pH measurements were carried out on a Systronic (model μ pH system 362) pH meter. Amperometric response characteristics were studied with a $4^{1/2}$ Digit True RMS Multi meter (MODEL 1085).

III. RESULTS AND DISCUSSION

A. UV–Visible study

The optical absorption spectrum of synthesized PANI and Graphite/PANI/AChE electrode shown in Fig.1(a and b). The spectrum recorded in directly without any solution on it. All spectra were recorded in the wave lengthrangeof300-800nm.The shoulder is appearing at 491nmforH_2SO_4 corresponds to the formation of ES (Emeraldine salt) phase irrespective of their organic supporting electrolyte. It shows very good resemblance with earlier reported work[4-5]. The optical absorption spectrum of synthesized electrode is shown in Fig.1(b).The peak is appearing at310-380 nm for$H_2SO_4$and at 480 nm. It shows resemblance with earlier reported work[6-7].

B. FT-IR study

The FT-IR spectrum of freshly synthesized PANI shows in Fig.2(a)peak at 3150-3550 cm^{-1} corresponds to N-H stretching. The incorporation of the counter anion in the polymer is evidenced by the peaks. Further evidence of the presence of this anion in the polymer electrode is revealed by peaks at 1000-1500 and 1600-1640 cm^{-1} which may be assigned to SO_2 stretch in sulphonates. The vibration bands are observed at 1728- 1784 cm^{-1} (C=O), 1527-1548 cm^{-1} (N-H bending). These bands correspond to the characteristic bands for aniline; it shows very good agreement with earlier reported work [8-9]. Thus, the FT-IR spectral results confirm the presence of PANI. Fig. 2(b) shows FT-IR spectrum of synthesized PANI based electrode. A peak at 3351 cm^{-1} corresponds to N-H stretching. Electrode is revealed by peaks at 1219 and 1638 cm^{-1} which shows SO_2 stretch in sulphonates.

C. SEM study

Fig. 3(a-b)shows FE-SEM images of surface of modified electrode with and without enzyme at 50 KX.Fig. 3(a) clearly shows porous morphology of electrode surface. This nature helpful for easily immobilization of enzymes for the biosensor application, The physical morphology of the native PANI and graphite embedded surface modified with and without enzyme (AChE) immobilization were characterized by scanning electron microscopy. When the enzyme was covalently immobilized over the surface of the PANI/graphite electrode the porous structure was disappeared and instead bright rectangular shape particles have been seen on the surface of the electrode Fig.3 (b) at the magnification of 50 KX, which may be assigned to enzyme (AChE) molecules. When AChE was immobilized onto the modified electrode, a morphology change in the electrode surface can be easily observed.

3.5Optimization of experimental parameter

D. Effect of potential

The response current increases rapidly with increase in potential, which indicates that the response of the AChE electrode was controlled by the electrochemical methods as shown in Fig. 4. It is well known that the velocity of an electrode reaction is related to the concentration of electro active species, the pH value of solution and applied potential[10-13].Above the potential 0.5V, the response was almost steady, which could be explained by the rate-limiting process of enzyme kinetics, diffusion-control of H_2O_2 and substrate[14-15]. Considering the decrease in response of the Graphite/PANI/AChE electrode at higher potential, which also has affected the electrochemical response of the enzyme electrode, we preferred to set the potential at 0.5 V for the further studies of Graphite/PANI/Ach Electrode.

D. Current response of Graphite/PANI/AChE electrodes

The change in response current of the active device AChE is the parameter of interest for sensor applications. The response current of the device depends on several factors such as (1) the contact resistance between the metal electrodes and the Graphite/PANI/AChE electrodes (2) the geometric factor of the electrode and (3) the electrode conductivity. The electrode activity is dependent on several factors such as analyte pH, temperature, substrate concentration and enzyme loading.

The current-time relationship when the potential of the enzyme electrode was set at 0.5 V is as shown inFig.5(a). It was found that the response current of the enzyme electrode easily reaches to steady state. The relationship between response current and paraoxon concentration in 0.1 M phosphate buffer pH 7 is shown. It was found that, current increases with increasing paraoxon concentration in the range of$1x10^{-6}$ to $9x10^{-6}$M. In the present case, assuming that the enzyme is uniformly distributed throughout the electrode, the reaction takes place predominantly on the surface of the electrode in the lower concentration. However, the reaction on the surface of the electrode and the diffusion occurring simultaneously at higher concentrations delays the response time. With increasing concentrations of paraoxon, the response current also increased and finally reached to steady state value. The response of biosensor to paraoxon was found to be linear in the range of $1x10^{-6}$ to $3.9x10^{-6}$ M. This linearity range is in well conformity with

that obtained in the amperometric response studies. Long term stability is one of the most important features required for the satisfactory application of a biosensor as shown in Fig.5(b). In order to evaluate the storage stability, the sensor was tested for 6 weeks of storage in 0.1 M phosphate buffer pH 7 at 25°C. There is a slight decrease in sensitivity of the sensor of about 10% from the initial value, revealing a very good preservation of the bioactivity than other work[16-17].

IV. CONCLUSION

Graphite/PANI/AChE biosensor showed higher sensitivity, dynamic range of detection, good fabrication reproducibility, short response time and acceptable stability toward organ phosphorous pesticide detection. The cost effectiveness and simple method of fabrication of Graphite/PANI/AChE biosensor is an additional advantage as compared with conventional electrodes. The method not only can be used to immobilize enzymes to construct a range of biosensors but also may be extended to develop other biological molecules, such as antibody and DNA for biosensor.

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Figure caption

1) Fig.1 (a-b): UV–Visible study of(a) freshly prepared Polyaniline, (b) Graphite/PANI/AChE.
2) Fig. 2 (a-b): FT-IR studies (a) freshly prepared PANI; (b) PANI based Electrode.
3) Fig. 3 (a-b): (a) Surface of modified electrode without enzyme Mag. 50 KX (Graphite/PANI), (b) Surface of modified electrode with enzyme Mag. 50 KX (Graphite/PANI/AChE).
4) Fig.4. Current–potential curves for the Graphite/PANI/AChE electrode in 0.1 M PBS (pH 7).
5) Fig. 5(a-b): (a)Current–time curve during the Graphite/PANI/AChE electrode at 0.5 V and pH 7 in 0.1 M PBS for different paraoxon solution of $1x10^{-6}$ to $9x10^{-6}$M.(b)Stability of the Graphite/PANI/AChE electrode on storage in 0.1 M PBS (pH 7).

Fig 1(a-b)

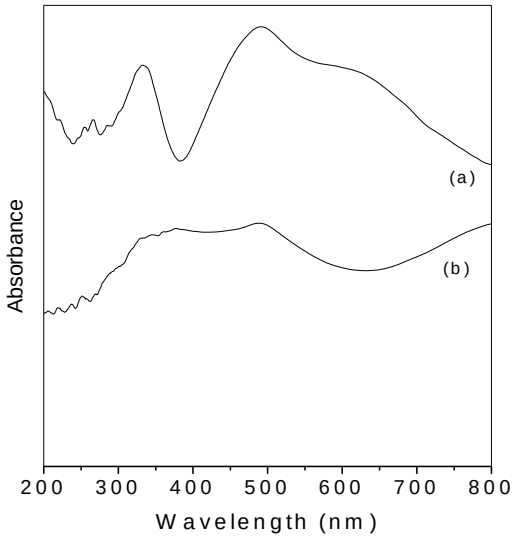

Fig 2(a-b)

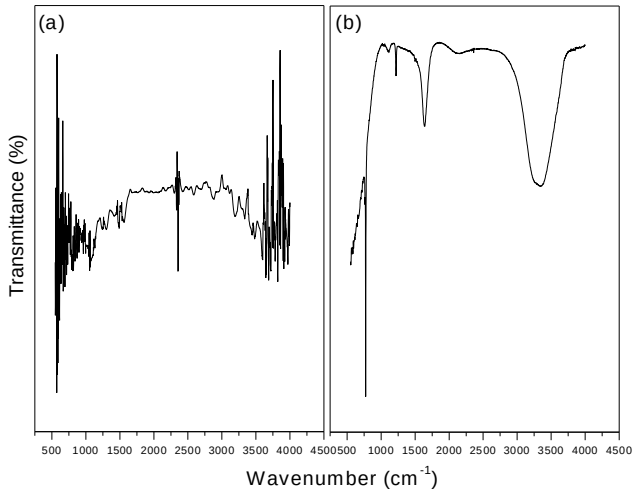

Fig. 3 (a-b)

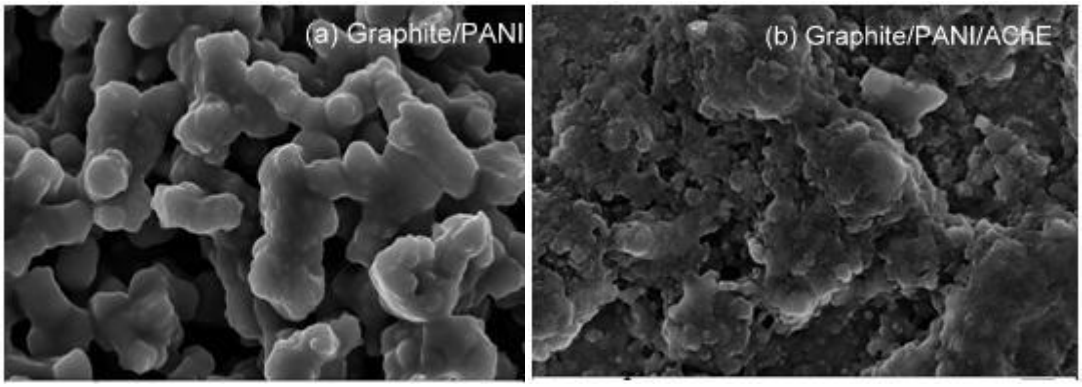

Fig. 4

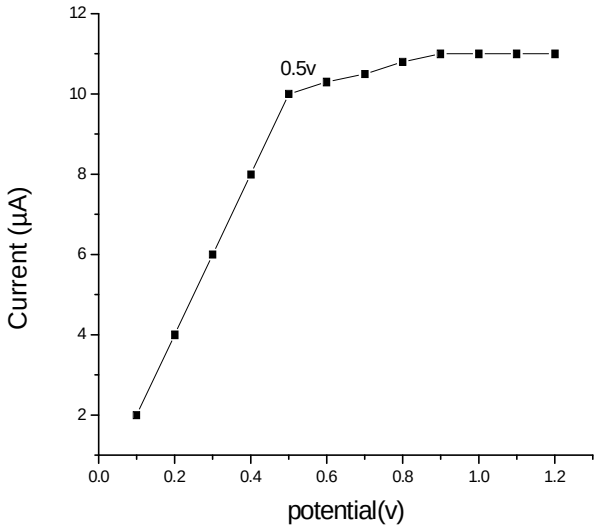

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Fig. 5 (a-b)

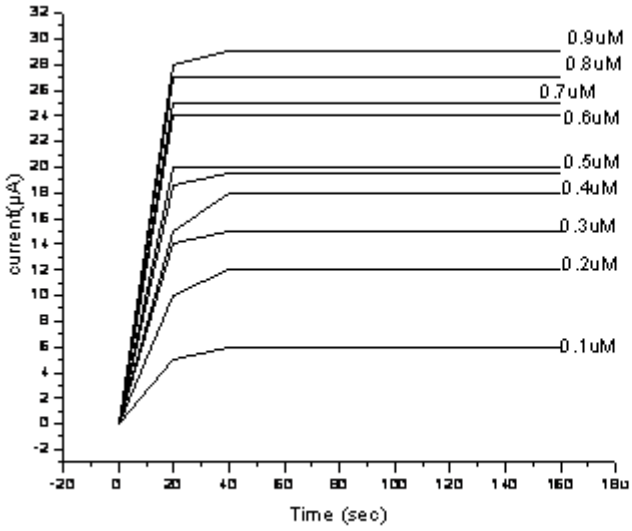


Fig 5(a)

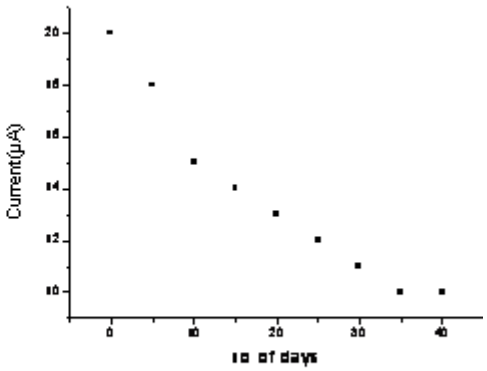
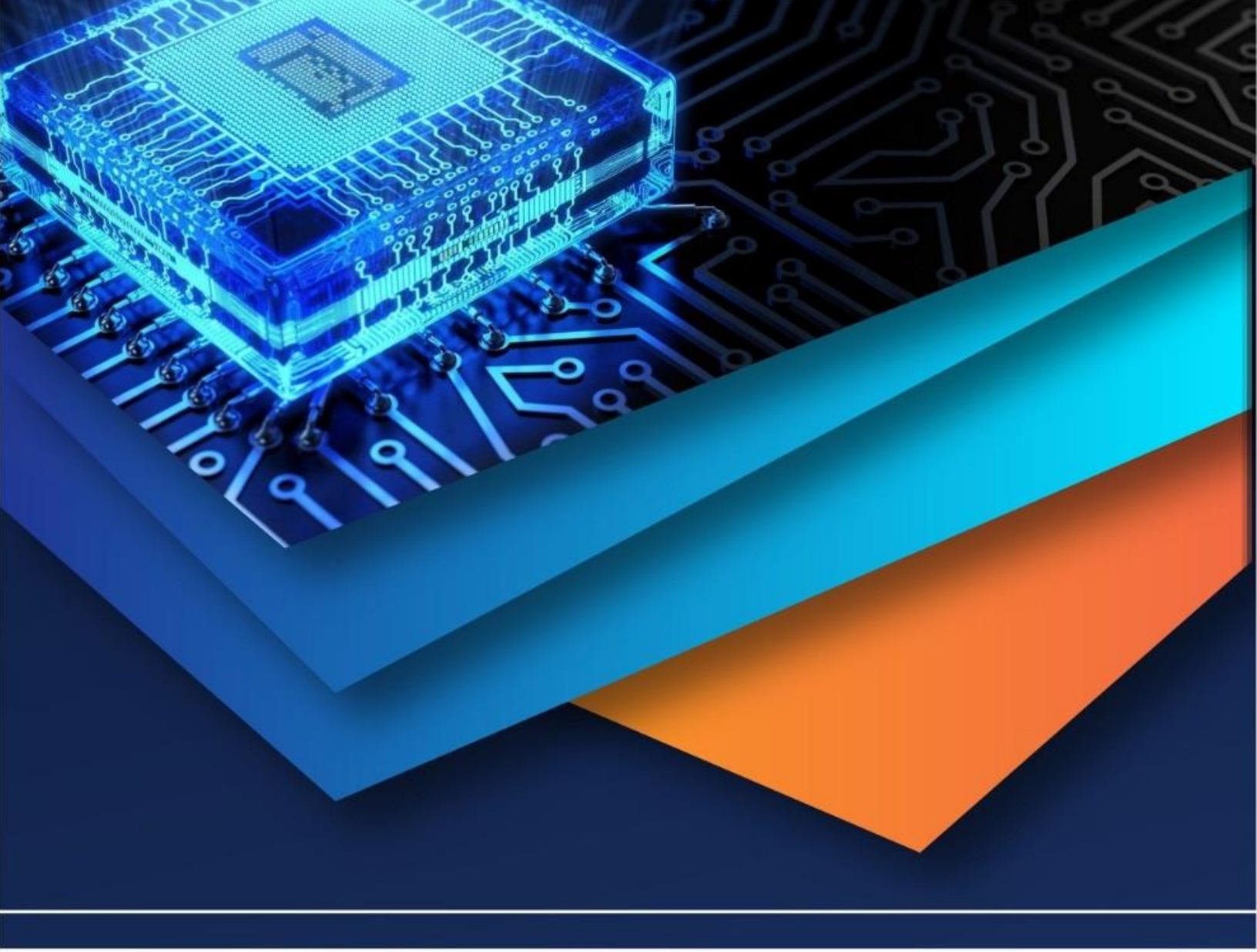


Fig. 5(b)



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