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Research Article

Synthesis of poly o-anisidine based sensor for amperometric detection of pesticide carbaryl.

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ABSTRACT
A novel poly o-anisidine (POA) based amperometric carbaryl biosensor has been developed for selective and quantitative determination of carbaryl by immobilizing enzyme Acetyl cholinesterase (AChe) onto nano composite graphite (Gr) paste electrode for monitoring the amperometric response caused by the immobilized enzyme. AChe immobilization on electrode was investigated using an amperometric method, and factors affecting its immobilization such as concentration of AChe, pH was discussed in detail. Organized materials were characterized by analytical techniques such as UV-Vis, FTIR and FE-SEM analysis. The performance of the developed carbaryl biosensor was evaluated and obtained carbaryl biosensor exhibited shorter response time (3s), wider linear range, lower detection limit and good stability with about 90% of the original response signal retained after 2 month.

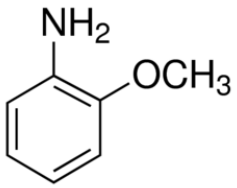
KEYWORDS
Amperometric, Biosensor.

1. INTRODUCTION

The carbamates belong to a group of pesticides that has attained great popularity in recent years due to their broad biological activity [1]. They are used as insecticides, miticides, fungicides and molluscicides [1]. Because of the wide range of uses in the treatment of seeds, soils, or crops [1], the methyl carbamates constitute one of the most important classes in this group and of these; carbaryl (1-naphthyl N-methyl carbamate) has been one of the most used because it has low oral and skin toxicity in spite of its great insecticide capacity [2].

Most of the analytical methods employed for the quantitation of carbaryl have been based on chromatographic techniques. Classical gas chromatography has been shown to be generally unsatisfactory due to the thermal instability of carbaryl, requiring either a chemical derivation step (use of different reagents) or the employment of short columns, specially treated columns or short capillaries [1]. Thus many authors have preferred to make use of liquid chromatography (generally reversed-phase) linked to various detectors, e.g. UV [3], diode-array [4, 5], florescence [5, 6], or electrochemical [7], although some of these methods also require pre-treatment steps to form detectable derivatives. Thin-layer chromatography has occasionally been employed [8]. Other methods for estimation of carbaryl in various matrices are found in the literature. These include: spectrophotometric techniques using UV/vis [9, 10, 11, 12], infrared spectrometry [13], and electrochemistry [1, 2]. All these are founded on the conversion of carbaryl to 1-naphthol by means of alkaline hydrolysis. The spectrophotometric methods using UV/vis detection mention several chromogenic reagents that form coloured complexes with 1-naphthol in order to achieve an appropriate selectivity and sensitivity of the spectrophotometric measurements. The ease with which 1-naphthol is oxidized allows satisfactory detection using electrochemistry based on a differential pulse voltammetric method [1].

The electrochemical cell was assembled in a conventional one compartment three-electrode design the working electrode will modified paste electrode consist of composition of 70:25:5 (graphite: mineral oil: POA) pestle will allow to homogenize for few hours. The paste was then filled in a Teflon micropipette tip. A platinum wire was dissected through the paste, to provide an electrical contact. Smooth and fresh electrode surfaces were obtained by squeezing out 0.5 mm of paste from the syringe, scraping off the excess and polishing it against butter paper until the surface had a shiny appearance and o-anisidine composite in which the enzyme AChe will immobilized; the Ag/AgCl/KCl (3.0 mol L_1) used as the reference electrode; and a graphite/ITO use as the counter electrode. By using this electrochemical amperometric system carbaryl pesticide detected at low range. Below figure shows structure of POA. Following figure shows structure of o-anisidine. Conducting polymer o-anisidine is a derivative of aniline plays a role of conductivity with graphite also it Provide a good platform for enzyme.



Structure of o-anisidine

2. MATERIALS AND METHODS

2.1. Materials and chemicals

Carbaryl (99%), Ache was purchased from Sigma Aldrich, Graphite fine powder extra pure (particle size 240×10^{-6}m) obtained from Loba Chemie Pvt. Ltd. India, Paraffin liquid heavy or mineral oil (viscosity at 37°C is 64cS) purchased from High purity lab, Mumbai, India.

2.2 Synthesis of enzyme based biosensor Gr/POA/Ache (working electrode)

Organization of 65:25:5 graphite: mineral oil: POA this pestle allowed homogenizing for 60 minutes. The glue was then filled in a plastic syringe micropipette tip. A platinum wire was analyzed through the glue, to offer an electrical contact. Delicate and new anode surfaces were gotten by crushing out 0.5 mm of glue from the tip, scratching off the overabundance and cleaning it against spread paper.

AChe immobilization on the composite surface done by dropping of the PBS (pH7) containing the compound on the anode surface, which additionally dried at a controlled temperature. The working cathode was set up by dropping 7.0 µL of refined phosphate oxidase arrangement onto the surface of the Gr/POA/AChe terminal and brooding it at 20°C for 24 h. The terminal was washed with refined water, dried and put away at 4°C before utilize. This constitutes a genuine commitment from the composite surface to the proficiency of the biosensor without the cross-connecting operators to make clinging to the dynamic destinations of catalysts, in this way hindering their action. The undefined material attached to the POA is related to the AChe.

2.3. Arrangement of compound arrangements

The enzyme AChe, about 4.0 U/ml was arranged instantly before use in frosty potassium phosphate support (400 mM) at constant pH.

3. RESULTS AND DISCUSSION

3.1 FTIR study of Gr/POA/AChe

2924 and 2854 cm-1 assigned to aliphatic –CH3 and –CH2, 2825 to 2925 cm-1 are due to C-H stretching, 1654, 1637 and 1637 cm-1 were attributed to C=C stretching , peak at 3150-3550 cm-1 corresponds to N-H stretching, observation clearly indicate that Gr/POA functional group.

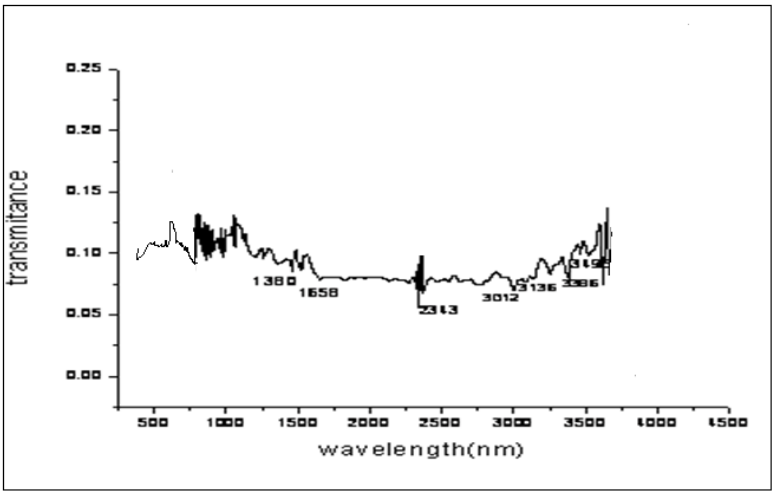


Fig. 1. FTIR- spectrum of sensor Gr/POA/AChe

3.2 UV-Vis study of Gr/POA/AChe

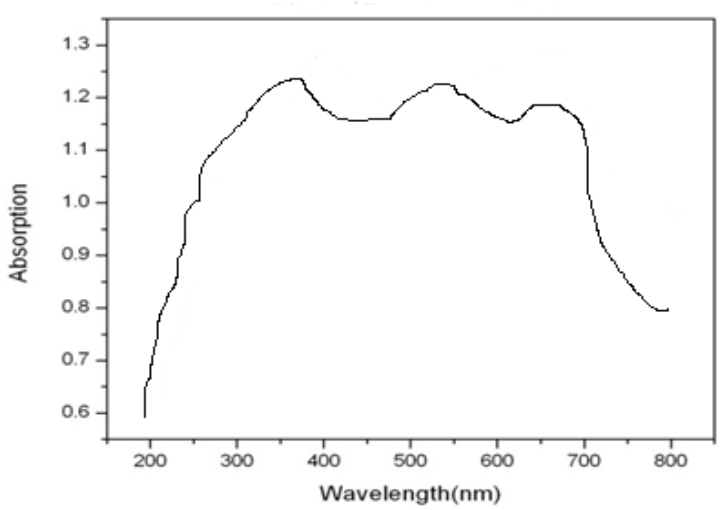


Fig. 2. UV-visible spectrum of sensor Gr/POA/Ache.

The UV-Visible spectrum exhibited an absorption band at around 200-800nm, Gr/POA has resulted in to stronger absorption at 310 & 675 nm, Peak 550-700 indicating NH stretching and 400-500 nm which is a typical Plasmon band Fig A and B. Furthermore, the UV-Vis spectrum revealed that the reaction medium exhibited absorption and around 265nm and 250nm for sample A and B respectively

3.3SEM study of Gr/POA/Ache

In Figure are the morphological components of Gr/POA/Ache anodes utilizing SEM. It unmistakably demonstrates permeable morphology of cathode surface. This nature supportive for effectively immobilization of chemicals for the biosensor application It indicates more uniform in nature and no isolated graphite particles could be watched, which shows the astounding adherence of POA to graphite , Moreover, homogeneous covering of the catalyst demonstrated that the proposed anode before immobilization fills in as a great host-visitor stage for biomolecules immobilization.

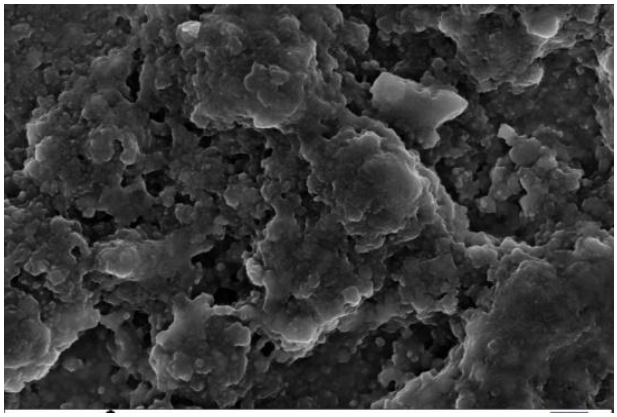


Fig. 3. SEM image of Gr/POA/AChe at 20µM.

3.4. Determination of potential response of Gr/PANI/PNP electrodes

For impact of working potential observed during amperometric reaction of Gr/POA/Ache terminal for 4mM phosphate is appeared in Fig. 4. The electro-chemical effect was recorded by applying diverse inclination potential to the compound cathodes in phosphate cradle 400mM and AChe (4 mM). For Gr/POA/Ache anodes, the reaction current expanded in gradually, after 0.5V which might be because of the likelihood of meddling species getting corroded at higher potential. These outcomes likewise supported 0.5 V as working potential. Keeping these outcomes in examination a capability of 0.5 V as picked as the working potential for further reviews.

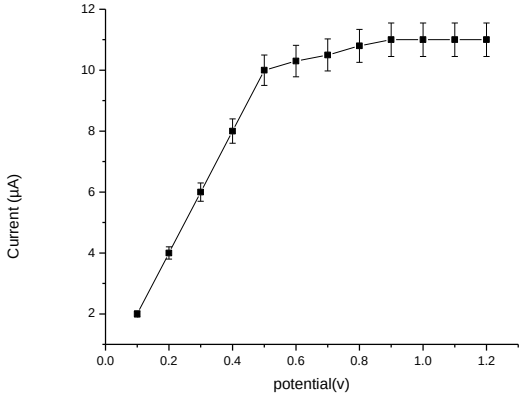


Fig. 4. Effect of potential on Gr/PANI/PNP electrode.

3.5. Effect of pH

The pH study was completed by changing the pH in the scope of 1 to 10. The pH of the test arrangement was balanced utilizing H_2SO_4 and NaOH. It likewise maintains a strategic distance from the loss of the chemical movement. Hence catalyst sensor reaction relies on upon the functioning pH of the examining arrangement. The impact of pH on the conduct of the protein cathode was studied with 0.1 M Phosphate cushion arrangement with 5 mM phosphate. The unfaltering state streams at 0.5 V as a component of pH qualities is appeared in Fig.3 the electrochemical reaction is very great at pH extending from 5 to 8 and the most extreme current.

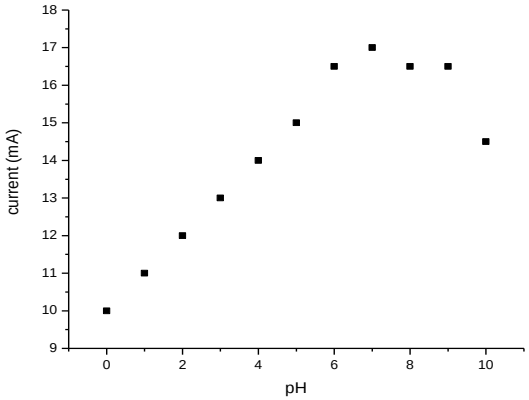


Fig. 5 (a). Effect of pH on Gr/POA/AChe at potential 0.5V.

3.6. Current-time response of Gr/POA/Ache electrodes

The current–time relationship when the ability of the compound terminal was set at 0.5 V is as showed up in Fig.6 It was discovered that the response current of the concoction anode adequately reaches to persevering state. The association between response current and phosphate center in 0.1 M phosphate bolster at pH 7, Current response, current increases with increasing carbaryl concentration in the range of $0.1x10^{-6}$ to $1.2\ x10^{-6}$ M. In the present case, expecting that the protein is reliably appropriated all through the cathode, the reaction happens overwhelmingly on the surface of the anode in the lower center. In any case, the reaction on the surface of the anode and the scattering happening in the meantime at the reaction time. With expanding centralizations of carbaryl, the reaction current additionally expanded lastly come to unfaltering state esteem. Figure demonstrates the relentless state potential reliance adjustment bend for the every individual carbaryl focus in the scope of $0.1x10^{-6}$ to $1.2\ x10^{-6}$ M. The reaction of Gr/POA to carbaryl was observed to be wide straight scope of $0.1x10^{-6}$ to $1.2\ x10^{-6}$ M and as far as possible was observed to be $1x10^{-6}$ M. Linearity range is well concurrence with that acquired in the amperometric reaction of sensor is fitting in extent to phosphate fixation.

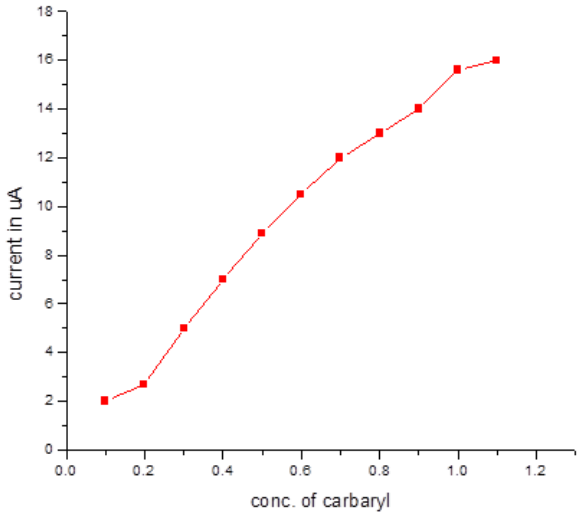


Fig. 6. Amperometric response of Gr/POA/Ache.

3.5 Storage stability

Long term stability is one of the most important features required for the satisfactory application of a biosensor as shown in Fig.7 In order to evaluate the storage stability, the both sensor was tested for 2 month of storage in 0.1 M phosphate buffer pH 7 at 25°C. There is a slight decrease in sensitivity of the sensor (Gr/POA/AChe) of about 15% from the initial value, revealing a very good preservation of the bioactivity.

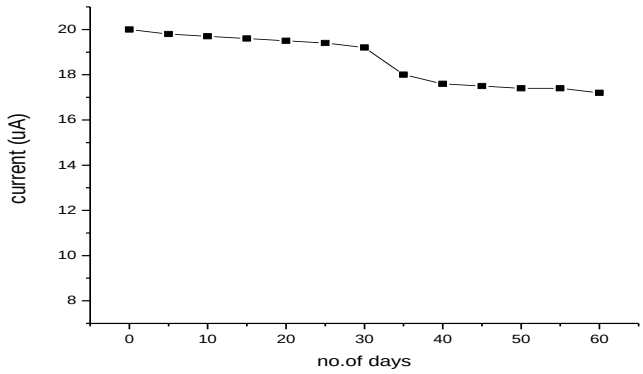


Fig .7. Stability of the Gr/POA/Ache electrode storage for 60 days.

4. CONCLUSION

A Gr/POA/Ache electrode has been developed and success-fully employed for the carbaryl determination laboratory sample. A detection limit of 0.1×10^{-6}M for carbaryl was achieved with the use of the Gr/POA. The present work shows that, POA is better combination with graphite powder; it shows better current response as supporting conducting polymer. Gr/POA electrode also gives the better storage stability for two months, it save the cost of enzyme. This method gives benefits such advantages as high sensitivity, low detection limit, easy handling, resistance against surface fouling, and low cost. Consequently, this method is recommended for the analyses of phosphate, antimony, glucose, creatinine in clinical as well as quality control laboratories.

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