Addis Ababa University
Faculty of Science
School of Graduate Studies

Chemistry Department

Graduate Project (Chem. 774)

Electrochemical Detection of Fenitrothion Using Poly-(4-amino-3-hydroxynaphthalene-1-sulfonic acid) Modified Glassy Carbon Electrode

By

Samuel Abicho

June, 2009

Electrochemical Detection of Fenitrothion Using Poly-(4-amino-3-hydroxynaphthalene-1-sulfonic acid) Modified Glassy Carbon Electrode

By

Samuel Abicho

Advisor: Shimelis Admassie (PhD)

A Graduate Project Submitted to School of
Graduate Studies Addis Ababa University in Partial Fulfillment
of the Requirements for the Degree of
Master of Science in Chemistry

I, the undersigned, declare that this project is my original work; it has not been presented for degree in this and any other university. All sources of materials used in this work have been dually acknowledged.

Name Samuel Abicho

Signature ___________

This project work has been submitted for the examination with approval as the university advisor.

____

Advisor: Dr. Shimelis Admassie

Place and date of submission:

Office of Research and Graduate program.

Department of Chemistry

July, 2009

Table of Contents

Contents Pages

List of figures

Figures **Pages**

List of Tables

Acknowledgement

Above all and forever, my very first breath of thanks goes to the **Majesty God in the Heaven, His mercy and mighty hands** did this and made me Successful."…but his bow remained in strength. Gen 49:26"

The persistent commitment of my advisor, **Shimelis Admassie (PhD)**, to help me get on the completion of this project work. I am grateful to his unreserved advices and friendly approach.

I also acknowledge my indebtedness to all my instructors/teachers/, and the uneducated educators: my mam, W/ro **Hanna Helsabo**, my dad, Ato **Abicho Kebeto**. Moreover, my warmest thanks also goes to W/ro Amerech Chefessa, W/ro Aberash Markos, Ato Fekadu Woldesenbet, Ato Dessealegn Abicho, and their Children.

My warmest and special thanks also goes to dear **Brukit Markos**, her continuous cooperation made me fruitful in my long journey. Besides, my thanks is a lot to my friends Mr. Meareg Amare, Mr. Teketel Abrham, Mr. Teketel Abuto, Mr. Anteneh Fikadu, Mr. Taye Gebremeriam, Mr. Yoseph Bereket, Mr. Fekadu Chekol, Mr. Deneke Delikeso, whose friendly support and encouragement has been with me all along the study period.

I am also grateful to my sisters and brothers for all their help while I have been learning in Addis Ababa University. Specifically, the contribution of **Selamu Tegesse** is Unforgettable

Abstract

A glassy carbon electrode (GCE) was modified with electropolymerized film of 4-amino-3-hydroxynaphthalene-1-sulfonic acid (AHNSA) in pH 5.0 acetate buffer solution (ABS). Cyclic voltammetry and square voltammetry were used as the analytical technique to compare the sensitivity of the modified glassy carbon electrode with bare glassy carbon electrode. Fenitrothion detection for different concentrations was performed by square wave voltammetry in pH 5.0 acetae buffer solution (ABS). The irreversible reduction peak current which is at -594 mV is linear with the fenitrothion concentration in the range of $4.0 \times 10^{-6} - 2.0 \times 10^{-5}$ M, and the detection limit is 8.0×10^{-8} M. As a result, the square wave voltammetry at modified glassy carbon electrode provides a better sensitivity and it is faster and less expensive than traditional ones, such as, high performance liquid chromatography or gas chromatography, which is time consuming, expensive and can not be carried out in field [34-36].

Keywords: Electropolymerization; Cyclic voltammetry; Fenitrothion; Square wave
voltammetry; Organophosphorus pesticides (OPPs);
4-Amino-3-hydroxynaphtalene-1-sulfonic acid.

July 06, 2009

To: Dr. Ashebir Fiseha
Head, Department of Chemistry
Subject: *Samuel Abicho's Final M.Sc. Project*

This is to confirm that Samuel Abicho has incorporated the comments of the examining board in the final version of his .Sc. Project.

Sincerely Yours,

Dr. Shimelis Admassie

Addis Ababa University
School of Graduate Studies
Department of Chemistry
Graduate Project (Chem. 774)

Electrochemical Detection of Fenitrothion Using Poly-(4-amino-3-hydroxynaphthalene-1-sulfonic acid) Modified Glassy Carbon Electrode

Submitted by: Signature

Samuel Abicho ____________________

Approved by:

1. Dr. Shimelis Admassie ________________________
   Advisor

2. Dr. Teketel Yohannis ________________________
   Examiner

3. Dr. Mesfin Redi ________________________
   Examiner

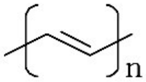
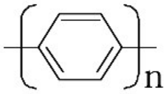
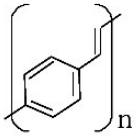
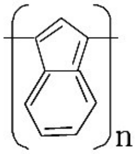
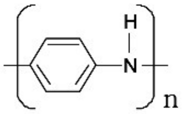
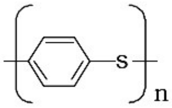
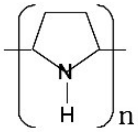
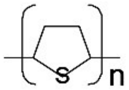
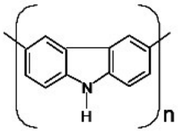
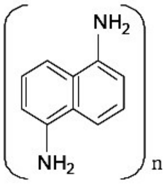
1. INTRODUCTION

Organic conjugated polymers (conducting polymers) [1] are mainly organic compounds that have an extended Π-orbital system, through which electrons can move from one end of the polymer to the other. The conducting polymers are known to have considerable flexibility in chemical structures that can be modified. By chemical modeling and synthesis, it is possible to modulate the required electronic and mechanical properties of conducting polymers. Common classes of organic conductive polymers include poly(acetylene)s, poly(pyrrole)s, poly(thiophene)s, poly(terthiophene)s, poly(aniline)s, poly(fluorine)s, poly(3-alkylthiophene)s, polytetrathiafulvalenes, polynapthalenes, poly(p-phenylene sulfide), poly(para-phenylene vinylene)s etc.. The names, structures, and conductivities of some of these conducting polymers are listed in Table 1.

Conjugated polymers are organic semiconductors, which with respect to electronic energy levels hardly differ from inorganic semiconductors. Both have their electrons organized in bands rather than in discrete levels and both have their ground state energy bands either completely filled or completely empty. The band structure of a conjugated polymer originates from the interaction of the p-orbitals of the repeating units throughout the chain [2, 3].

Conducting polymers (their conducting forms) are usually classified as the cation salts of highly conjugated polymers. The cation salts are obtained by electrochemical oxidation and electrochemical polymerization or chemical oxidation (removal of an electron). It is also possible to obtain the anion salts of the same highly conjugated polymers (which are also conducting but much less stable than the cation counter parts) by either electrochemical reduction or by treatment with reagents such as solutions of sodium naphthalide [4].

Table 1. Names, structures, and conductivities of some common conducting polymers.

Conducting Polymer	Structure	Conductivity (S/cm)
Polyacetylene		~ 1000
Polyparaphenylene		100 ~ 500
Polyparaphenylene vinylene		~ 3
Polyazulene		~ 0.1
Polyaniline		1 ~ 100
Polyparaphenylene sulfide		1 ~ 100
Polypyrrole		40 ~ 100
Polythiophene		10 ~ 100
Polycarbazole		10 ~ 100
Polydiaminonaphthalene		10^{-3}

Chemically modified electrodes (CMEs) comprise a relatively current approach to electrode systems [5] that finds utility in a wide spectrum of basic electrochemical investigations, including the relationship of heterogeneous electron transfer and chemical reactivity to electrode surface chemistry, electrostatic phenomena at electrode surfaces, and electron and ionic transport phenomena in polymers, and the design of electrochemical devices and systems for applications in chemical sensing, energy conversion and storage, molecular electronics, electrochromic displays, corrosion protection, and electro-organic syntheses.

Compared with other electrode concepts in electrochemistry, the distinguishing feature of a chemically modified electrode (CME) is that a generally quite thin film (from a molecular monolayer to perhaps a few micrometers-thick multilayer) of a selected chemical is bonded to or coated on the electrode surface to endow the electrode with the chemical, electrochemical, optical, electrical, transport, and other desirable properties of the film in a rational, chemically designed manner. Chemically modified electrodes (CMEs) can operate both amperometrically (voltammetrically) and potentiometrically, they are generally used amperometrically, a faradic (charge transfer) reaction being the basis of experimental measurement or study [6].

The rapidly growing applications of conducting polymers reflect involvement of the sensor that provides direct information about the chemical composition of its environment. It consists of a physical transducer and a selective layer. In any sensor the sensing process can be divided into two parts, recognition which results in selectivity and amplification which increases the power of the usually weak signals to the level at which it can be conveniently manipulated by electrochemical methods [7].

2. LITERATURE REVIEW

2.1 Conducting Polymers

Manju Gerard et al: explained that polymers are being discarded for their traditional roles as electric insulators to literally take charge as conductors with a range of novel applications. Scientists from many disciplines are now combining expertise to study organic solids that exhibit remarkable conducting properties. A large number of organic compounds, which effectively transport charge, are roughly divided into three groups i.e. charge transfer complexes/ ion radical salts, organometallic species and conjugated organic polymers [8].

2.1.1. The Story of Conducting Polymers

Polymers were considered as insulators (plastics) somehow the opposite of metals i.e. they insulate, they do not conduct electricity. However, electric wires are coated with polymers to protect them and us from short-circuits; the foundation of conducting polymers absolutely changed this properties of conjugated polymers. The conductivity of these materials was increased by either oxidizing agents or reducing agents which are considered as dopant. At conducting polymer modified electrodes, at least three processes should be considered, taking place during electrocatalytic conversion of solution species. One of them is a heterogeneous electron transfer between the electrode and a conducting polymer layer, and electron transfer within the polymer film. As usual, this process is accompanied by the movement of charge compensating anions and solvent molecules within the conducting polymer film, and possible conformational changes of polymer structure as well. The rate of this process is determined by many factors. Among these, electric conductivity of a polymer layer, electron self-exchange rate between the chains and/or clusters of polymer, and anion movement within polymer film seem to be of great importance. The second process is the diffusion of solution species to the reaction zone, where the electrocatalytic conversion occurs. As compared to simple electrode reactions, this process can be more complicated in cases where the electrocatalytic conversion occurs within the polymer film. Then, the diffusion of species within the film, as well as the possible electrostatic interaction of this species with the polymer film should be taken into account. Last, a chemical heterogeneous reaction takes place between solution species and conducting polymer. As a result of these complex processes, the kinetic behavior and voltammetric responses are difficult to interpret, and a great deal of attention has been paid to consider some simplified models. Some models concerning quantitative treatment of electrocatalytic reactions and charge transfer processes within polymer films were a subject of a few reviews [9, 10].

Chemically modified electrode (CME): an electrode made of a conducting or semiconducting material that is coated with a selected monomolecular, multimolecular, ionic, or polymeric film of a chemical modifier and that by means of faradaic (charge-transfer) reactions or interfacial potential differences (no net charge transfer) exhibits chemical, electrochemical, and/or optical properties of the film.

Chemoreceptor: a selective receiving site for analyte recognition and reaction. In the case of a biologically derived receptor, the more specific term biochemical receptor or bioreceptor may be used.

Polymer film-coated electrodes may be further subdivided by the process used to apply the film:

(1) Dip-coating-this procedure consists of immersing the electrode material in a solution of the polymer for a period sufficient for spontaneous film formation to occur by adsorption. The film quantity in this procedure may be augmented by withdrawing the electrode from the solution and allowing the film of polymer solution to dry on the electrode.

(2) Solvent evaporation-a droplet of a solution of the polymer is applied to the electrode surface and the solvent is allowed to evaporate. A major advantage of this approach is that the polymer coverage is immediately known from the original polymer solution concentration and droplet volume.

(3) Spin coating-also called spin casting, a droplet of a dilute solution of the polymer is applied to the surface of a rotating electrode. Excess solution is spun off the surface and the remaining thin polymer film is allowed to dry. Multiple layers are applied in the same way until the desired thickness is obtained.

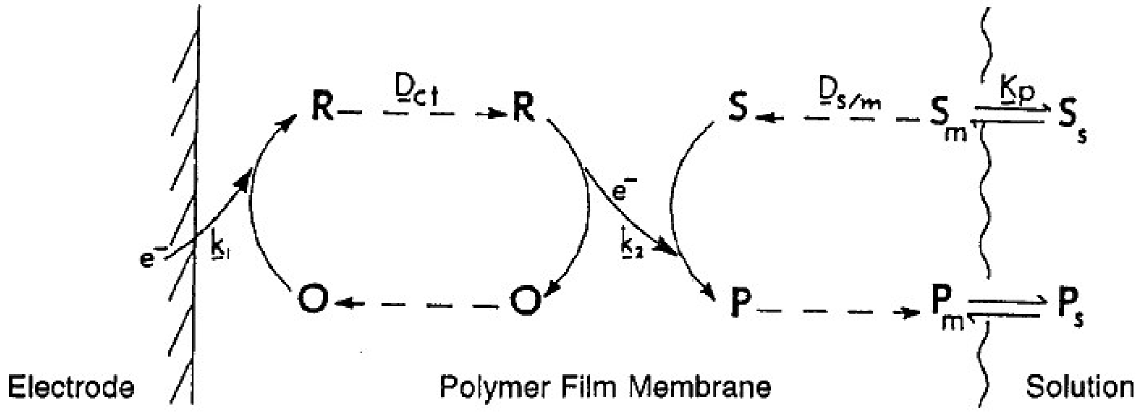
(4) Electrochemical deposition- also called redox deposition polymer solubility with oxidation (and ionic) state, so that film formation will occur, often irreversibly, when a polymer is oxidized or reduced to its less soluble state.

(5) Electrochemical polymerization-a solution of monomer is oxidized or reduced to an activated form that polymerizes to form a polymer film directly on the electrode surface. This procedure results in few pinholes since polymerization would be accentuated at exposed (pinhole) sites at the electrode surface. Unless the polymer film itself is redox active, electrode passivation occurs and further film growth is prevented.

(6) Cross-linking-a chemical step designed to couple chemical components of a film on an electrode to impart some desired property to the film such as increased stability, decreased permeability, or altered electron transport characteristics. Cross-linked films are often formed by copolymerization of bifunctional and polyfunctional monomers. Cross-linking may be activated chemically, electrochemically, photolytically, radiolytically, or thermally.

There are several reasons for the appeal of polymer modification: immobilization is technically easier than working with monolayers; the films are usually more stable; and, because of the multiple layers of redox sites, the electrochemical responses are larger. Questions remain, however, as to how the electrochemical reactions of multimolecular layers of electroactive sites in a polymer matrix occur, e.g., mass transport and electron transfer processes by which the multilayers exchange electrons with the electrode and with reactive species: molecules and ions in the contacting solution [11]. Much of this uncertainty results from a lack of sufficient knowledge about the structure and properties of polymer films and the morphological changes that they undergo when subjected to various chemical, electrochemical and physical processes during use.

Electrocatalysis at a modified electrode is usually an electron transfer reaction between the electrode and some solution substrate which, when mediated by an immobilized redox couple (i.e., the mediator), proceeds at a lower overpotential than would otherwise occur at the bare electrode. This type of mediated electrocatalysis process can be represented by the scheme 1.



Scheme 1. General representation of electrocatalysis process on electrode surface.

In this scheme, the subscripts s and m designate the solution and membrane phases, respectively. The substrate, S, which is irreversibly (or quasi-reversibly) reduced at the bare electrode, is transported across the polymer film-solution interface (partition coefficient, Kp) and diffuses into the polymer film membrane (diffusion coefficient, Ds/m). The electrocatalyst or mediator, undergoes heterogeneous electron transfer (rate constant = k_l) at the electrode surface and charge propagation through the polymer film is described by a rate given by the charge-transport diffusion coefficient, Dct (the symbol De is not recommended). The mediator undergoes homogeneous electron transfer (rate constant = k_2) with the substrate in the polymer film [12].

Preconcentration (preferential uptake) of substrate or analyte ions/molecules is another area of utility of chemically modified electrodes (CMEs) [13]. In practice, the electrode surface is modified by the judicious choice of a reagent capable of selectively, and nonelectrolytically, accumulating the analyte of interest. The mechanism of this process is determined by nature of the reactivity of the modifying moiety, and the most common preconcentration mechanisms are usually based on solubility (e.g., hydrophobic interactions), coordination, or electrostatic phenomena (e.g., ligand- or ion-exchange interactions). In electroanalysis, accumulation of the analyte in the chemical modifier leads to higher sensitivity and may also provide improved selectivity.

Polymer-film should posses the following relevant properties:

Stability-resistance to degradation or dissolution in aqueous and nonaqueous solvents and in other hostile media, and the strength of the bonding between (a) the redox sites and the polymer matrix and (b) the polymer film and the electrode surface.

Permeability-the ease with which various species penetrate the polymer matrix. This includes not only the permeability of the polymer to the reaction substrate, noted above, but also the permeability of the polymer to the reaction product(s) and to ionic components, such as the supporting electrolyte. These parameters, among others, determine the uncompensated resistance within the film during the passage of current, as well as the time for electrochemical charging of sites within the film. The permeability of the film to solvent is also important as this affects transport properties of the other components. Lack of permeability to solvent or ionic species may cause a film to act as a passive barrier even though it contains electrochemically reactive sites.

Site population-this is the concentration of some designated chemical sites within the polymer film and is the ratio of coverage to film physical thickness. Concentration is often difficult to specify accurately since the solvent-swollen thickness of very thin films is difficult to assess, and because the total coverage may in some cases be larger than the electrochemically reactive coverage. The site concentration may nonetheless have important effects on the charge transport rates and consequently the magnitude of the faradic current.

Conductivity- the polymer film may transport electrons (propagate charge) by localized site-site hopping (electron self-exchange) or by an electron delocalization mechanism when highly conjugated polymer chains are present. These conduction modes are termed redox conduction and electronic conduction, respectively, and the latter types of polymers are often termed (electronically) conducting polymers.

2.1.2. The Synthesis and Applications of Conducting Polymers

Most of conducting polymers are based on the ‘electroactivity’ of these materials prepared in the form of a film at the electrode surface, i.e. on the possibility to charge and discharge them electronically within a very short time scale by varying the electrode potential, and this process can be repeated in a cyclic manner. Alternatively, this charging (mostly, by the film oxidation) can also be achieved by a chemical agent but the charging level of the polymer cannot be varied in a continuous and cyclic way [14]. The conduction property of organic polymers has been tested by electropolymerization of their monomers in appropriate solvent. Electropolymerization of the monomers at electrodes, performed either in aqueous, or in organic solutions. Perhaps much of the knowledge on the polymerization mechanism and on the redox transformations taking place during reversible oxidation and reduction of conducting elecroactive polymer was obtained by the use of the electropolymerization procedure. M.A. Vorotyntsev and J. Heinze: stated that the peaks during electropolymeriztion of monomer on the surface of conventional electrodes in cyclic voltammogram curves indicates the 'faradic' process (charge transfer) while the plateaus of the current to the 'capacitive' term [15, 16].

Synthesis of polymeric films by electrochemical polymerization shows a number of advantages over the method of chemical polymerisation. First, the films are formed directly on the electrode surface, and the reaction product, viz., an electroactive film, exhibits a high conductivity. Second, the electrosynthesis provides a good current efficiency and a strict stoichiometry of the process; hence, the films of desired masses and thicknesses can be obtained. Third, the properties of the polymeric film can be controlled in the course of the synthesis [17].

The oxidation or reduction of the polymer can be achieved electrochemically by subjecting the neutral polymer to the appropriate oxidizing or reducing voltage in an electrochemical cell. The charge appearing on the polymer chain is then neutralized by a counter ion from the electrolyte solution. An interesting group of conducting polymers consists of those prepared by the electrochemical oxidation and simultaneous polymerisation of some monomers which react at the anode of an electrochemical cell. This group includes polyaniline, polypyrrole, polythiophene, poly p-(phenylene vinylene), poly p-phenylene and their derivatives. A variety of applications towards technology of these materials has been proposed and demonstrated, viz. rechargeable batteries, electrochromic displays and smart windows, light emitting diodes (LEDs), toxic waste cleanup, sensors, corrosion inhibitors, field effect transistors (FETs), electromagnetic interference (EMI) shielding etc.[18]. The preparation, characterization and application of electrochemically active, electronically conducting polymeric systems are still in the foreground of research activity in electrochemistry. There are at least two major reasons for this intense interest. First is the intellectual curiosity of scientists that focuses on understanding the behavior of these systems, in particular on the mechanism of charge transfer and charge transport processes occurring in the course of redox reactions of conducting polymeric materials. Second is the wide range of promising applications in the field of energy storage, electrocatalysis, organic electrochemistry, bioelectrochemistry, photoelectrochemistry, electroanalysis, sensors, electrochromic displays, microsystem technologies, electronic devices, microwave screening and corrosion protection etc. [19].

Various methods are available for the synthesis of conducting polymers. However, the most widely used technique is the oxidative coupling involving the oxidation of monomers to form a cation radical followed by coupling to form di-cations and the repetition leads to the polymer. Electrochemical synthesis is rapidly becoming the preferred general method for preparing electrically conducting polymers because of its simplicity and reproducibility. The advantage of electrochemical polymerization is that the reactions can be carried out at room temperature. By varying either the potential or current with time the thickness of the film can be controlled. Electrochemical polymerization of conducting polymers is generally employed by: (1) constant current or galvanostatic; (2) constant potential or potentiostatic; (3) potential scanning/cycling or sweeping methods. Standard electrochemical technique which employs a divided cell containing a working electrode, a counter electrode and a reference electrode generally produces the best films. The commonly used anodes are chromium, gold, nickel, palladium, titanium, platinum and indium-tin oxide coated glass plates. Electrochemical synthesis can be used to prepare free standing, homogeneous and self doped films. Besides this, it is possible to obtain copolymers and graft copolymers. Polythiophene, polyaniline, polycarbazole and several other polymers have been synthesized using this approach [20].

Conducting polymers are easily synthesized and deposited onto the conductive surface of a given substrate from monomer solutions by electrochemical polymerization with precise electrochemical control of their formation rate and thickness. Coating electrodes with conducting polymers under mild conditions opens up enormous possibilities for the immobilization of biomolecules and bioaffinity or biorecognizing reagents, the improvement of their electrocatalytic properties, rapid electron transfer and direct communication to produce a range of analytical signals and new analytical applications [21].

M.C. Blanco-Lopez et al: reported that electropolymerization provides another way of obtaining very reproducible, easily prepared sensors. Conducting polymers possess the unique properties over defined areas of electrodes. These properties of conducting polymers have been used for the fabrication of electrochemical sensors and biosensors. Amongst many analytical techniques available, the development of chemical sensors has made significant progress in the electroanalysis of an analyte [22-23].

2.2. Pesticides

Agricultural production currently, and increasingly, depends on the use of pesticides. Pesticide is a term used in a broad sense for chemicals, synthetic, or natural, that are used for the control of insects, fungi, bacteria, weeds, nematodes, rodents, and other pests [24]. Depending on what a compound is designed to do, pesticides have been sub classified into a number of categories. The primary classes of pesticides in use today are fumigants, fungicides, herbicides, and insecticides Depending on the toxicity, formulation concentration, and use patterns, pesticides can be classified as “general” or “restricted” use. A general use pesticide will cause no unreasonable, adverse effects when used according to the label and can be purchased and applied by anyone. A restricted use pesticide, defined as generally causing undesirable effects on the environment, applicator, or workers can only be purchased and applied by an individual who is licensed by the authority. These compounds and the products derived from them by degradation or metabolism give rise to residues that may spread through the environment and are particularly frequent contaminants in superficial and ground waters, in soil and in agricultural and food products.

Organophosphorus pesticides: are phosphoric acid esters or thiophosphoric acid esters and are among the most widely used pesticides for insect control. They realized that the insecticidal properties of these compounds and by the end of the World War II had made many of the insecticidal organophosphorus pesticides (OPPs) in use today, such as ethyl parathion [O,O-diethyl O-(4-nitrophenyl)phosphorothioate]. The first organophosphorus insecticide to find wide spread use was tetraethylpyrophosphate (TEPP), approved in Germany in 1944 and marketed as a substitute for nicotine to control aphids. Because of its high mammalian toxicity and rapid hydrolysis in water, TEPP was replaced by other OP insecticides. The pesticides belonging to this family may be divided into six groups schematically represented in Fig.1.

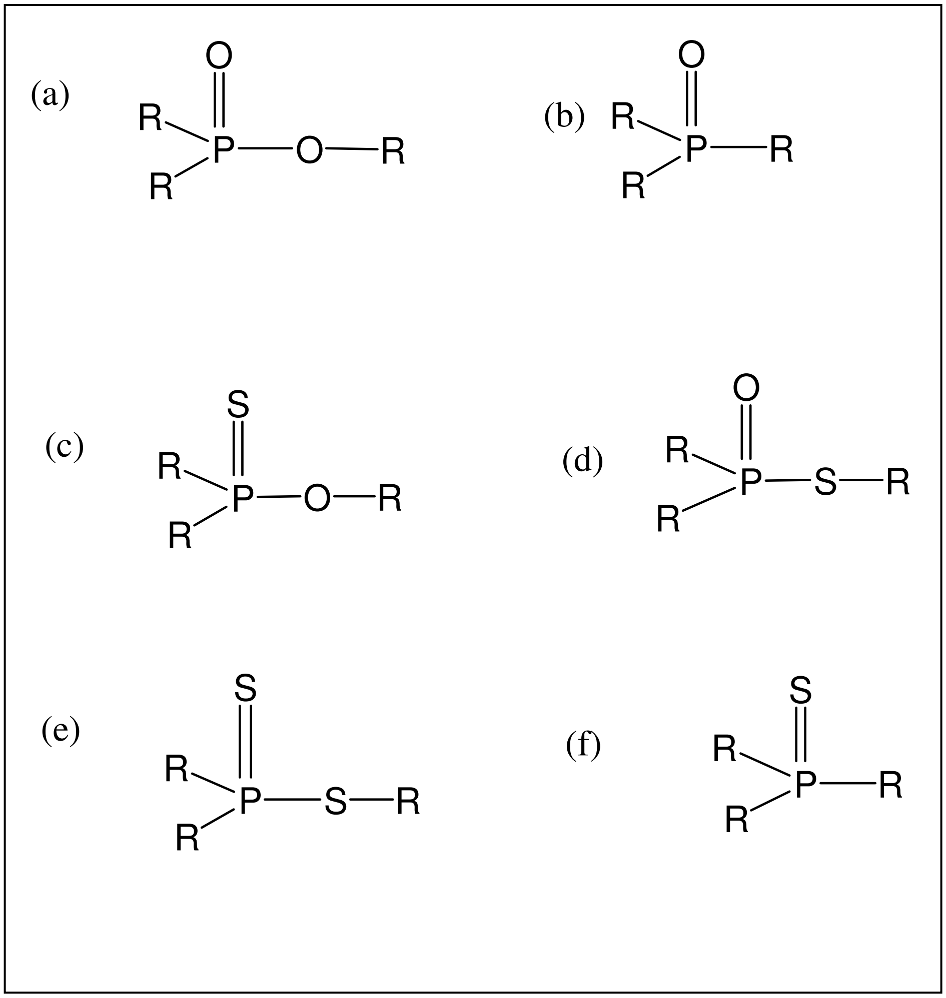


Fig.1. Structures of the six organophosphate pesticide groups.

It is known that the electrochemical activity of a compound is intimately related to its chemical structure. The oscillopolarographic behavior of a group of organophosphoric esters reinforces this fact. Thus the compounds belonging to this group and which possess bonds of the type –P=S– and –S– P= [groups (c), (d), (e), and (f)], show intense adsorption peaks, which allow the estimation of concentrations lower than 1mM. The toxic effects of organophophorus can be adequately explained in terms of their primary mechanism of action as acetylcholinesterase inhibitors. i.e. the presence of low concentrations of inhibitors—especially organophosphorus compounds (OPCs)—strongly and specifically affects enzyme activity. At nerve endings, the release of acetylcholine (ACh) triggers constriction of muscle fibers, but also activates certain parts of the autonomous nervous system. To prevent prolonged and excessive action, the enzyme acetylcholinestrase present at the nerve endings cleaves acetylcholine into inactive acetic acid and choline. Therefore, by measuring enzyme activity in the presence and absence of inhibitors, the concentration of the inhibitor compounds can be assayed [25]. Parathion was another widely used insecticide due to its stability in aqueous solutions and its broad range of insecticidal activity. However, its high mammalian toxicity through all routes of exposure led to the development of less hazardous compounds. Malathion [diethyl (dimethoxythiophosphorylthio) succinate], in particular, has low mammalian toxicity because mammals possess certain enzymes, the carboxylesterases, that readily hydrolyze the carboxyester link, detoxifying the compound. Insects, by contrast, do not readily hydrolyze this ester, and the result is its selective insecticidal action. Organophosphorus pesticides (OPPs) are toxic because of their inhibition of the enzyme acetylcholinesterase. This enzyme inhibition results in the accumulation of acetylcholine in nerve tissue and effector organs, with the principal site of action being the peripheral nervous system (PNS) [26].

2.3. Analytical Methods in pesticides detection.

As many organic compounds used as pesticides contain electroactive groups, voltammetry can be used for their mechanistic and analytical studies. Electrochemical techniques have been very helpful in the elucidation of processes and mechanisms of oxidation and reduction of pesticides. Moreover, the use of electrochemical data combined with spectroscopic studies could provide important information useful to the understanding of the degradation path ways of pesticides in aqueous solutions and in this way to mimicking the environmental processes.

There is a wide range of studies concerned with analytical methods for monitoring the pesticides in environmental samples. Most applications of chemical analysis to pesticide control involve methods with high sensitivity accompanied by sufficient selectivity, precision, and accuracy. Easy sample pre-treatment and rapid analytical procedures are also desirable. When selecting the method, the cost of the instrumentation and the possibility of performing measurements in the field are also important factors to be considered. Since electrochemical methods satisfy all the above criteria, they were a good choose for the analysis and control of environmental pesticides.

Unfortunately, the determination of pesticides in most samples requires their extraction into organic solvents. The well-known practical difficulties of using organic solvents in electroanalysis to determine scarcely water soluble compounds can be overcome by working in oil–water emulsions as these are predominantly aqueous. The principal electrochemical methods are voltammetry, amperometry, potentiometry, and conductimetry. Since electrochemical biosensors for pesticides analysis have been recently reviewed and, special emphasis will be given to focus on the developments concerning the voltammetric and amperometric analyses of pesticides [24]. Different researchers used different methods for analysis of various pesticides from their environment. Some of these methods which are highly exploited in determination of different pesticides are cited below.

Gerald T. Brooks and Terry R. Roberts: Suggested that the use of oxidation –adsorption stages can give better results than coagulation-flocculation and sand filtration during the removal of pesticides [27].

E. M. Garrido et al: mentioned the application of ultramicroelectrodes with dimensions less than 10µM for analysis of pesticides. At these tiny electrodes, voltammetric waves are obtained, rather than conventional peaks even at high voltage scan rates. Because of the low current, the voltage drop in solution is negligible and the supporting electrolyte is not essential in the solution. Thus even organic solvents can be employed without the necessity of using a potentiostat. The use of microelectrodes clearly opens the way for studies in numerous systems of environmental concern [23].

G. S. Nunes and D. Barceló: presented that the electrochemical biosensors are used for the analysis of carbamates and organophosphorus pesticides in food samples [28]. Other methods that have been used widely in the detection of pesticides namely, liquid chromatography and gas chromatography techniques are reported by some researchers. Even if this methods are very sensitive and reliable, but cannot be carried out infield. More over these techniques are time consuming, expensive and have to be performed by highly trained technicians [29].

P. Manisankar, et al: studied the electrochemical reduction of three common insecticides such as cypermethrin (CYP), deltamethrin (DEL) and fenvalerate (FEN) at glassy carbon electrode (GCE), multiwalled carbon nanotubes modified GCE (MWCNT-GCE), polyaniline and polypyrrole deposited MWCNT/GCE using cyclic voltammetry [30].

Luciana B.O. et al: developed a sequential injection-square wave voltammetry (SI-SWV) method for determination of atrazine using a hanging mercury drop electrode [31].

Biological methods such as, immunoassays and inhibition of cholinesterase activity, for organophosphorus pesticide (OP) determination have also been reported. Despite the promise of immunoassay techniques, since these methods require long analysis time (1–2 h) and extensive sample handling (large number of washing steps), they are unsuitable for on-line monitoring of detoxification processes [32].

The determination of pesticides in human urine has been accomplished by means of solid-phase extraction (SPE) followed high-performance liquid chromatography (HPLC) and solid phase microextraction (SPME) coupled with gas chromatography (GC) with nitrogen phosphorus detection (NPD), flame photometry detection (FPD) or mass spectrometer (MS). Though SPE and SPME avoid the use of large volumes of organic solvents, they have the disadvantages of relatively expensive special instruments and columns. Lately, a novel technique named cloud point extraction (CPE) has been used to extract pesticides from water samples prior to high-performance liquid chromatography (HPLC), but extraction of pesticides from urine was scarce. Some researchers have applied CPE to extract ions from urine prior to atomic absorption spectrometry, capillary zone electrophoresis, and flow injection inductively coupled with plasma-optical emission spectrometry. These imply that it is possible to apply CPE to extract pesticides from urine [33].

Generally chromatographic techniques are the most commonly used methods for determination of organophosphorus compounds (OPCs). These techniques allow selective and quantitative determination. However, they have a number of disadvantages: (i) the currently available equipment is complex and expensive, which prohibits use for rapid analyses under field conditions; (ii) the pretreatment and assay procedures are lengthy, hence fast analyses are impossible; (iii) the techniques are expensive and can only be performed by highly trained technicians. Environmental issues require far more sensitive, selective, and quantitative methods, capable of low-level organophosphorus compounds (OPCs) detection under field conditions in streams, ground, and waste waters, soils, and plants as well as in food. The most common approach to enzymatic determination of organophosphorus compounds (OPCs) is based on their inhibition of the activity of choline esterase enzymes. Choline esterase enzymes catalyze the reaction or hydrolysis of a particular choline ester (butyryl choline, acetyl choline, etc.) to the corresponding carboxylic acid with the release of choline. The presence of low concentrations of inhibitors—especially organophosphorus compounds (OPCs) strongly and specifically affect enzyme activity. Therefore, by measuring enzyme activity in the presence and absence of inhibitors, the concentration of the inhibitor compounds can be assayed. The application of spectrometric, GC, HPLC, and polarographic techniques for determination of a number of organophosphorus pesticides have been reported by different researchers [34-36].

2.4. Properties, Application, and Analysis of Fenitrothion

Fenitrothion (O, O-dimethyl O-4-nitro-m-tolyl phosphorothionate – IUPAC) is a contact insecticide and selective acaricide from the organophosphate family. It is widely used in the control of penetrating, chewing and sucking insect pests (coffee leafminers, locusts, rice stem borers, wheat bugs, flour beetles, grain beetles, grain weevils) on cereals, cotton, orchard fruits, rice, vegetables and forests. It is also used as fly, mosquito and cockroach residual contact spray for farms and public health programs. It is also effective against household insects and all of the nuisance insects listed by the World Health Organization. It is a non-systemic and non-persistent pesticide. Fenitrothion is far less toxic than parathion with a range of insecticidal activity.

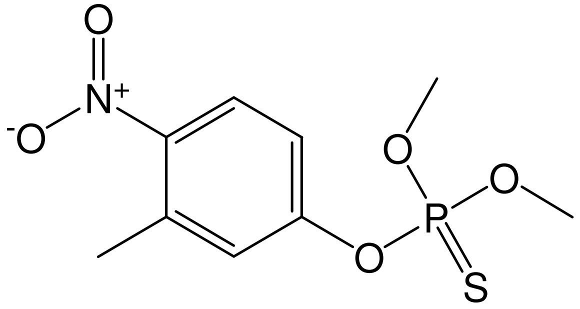
K.H. B□chel: stated that the introduction of a methyl group into the phenyl ring reduces the toxicity without fundamentally changing the activity. [O, O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate] is a contact and stomach insecticide. Substituents in the meta position to the oxygen function (e.g., CH_3, Cl) reduce the toxicity drastically. In many cases the activity remains unaffected [34, 37]. Moreover, the World Health organization classified fenitrothion under moderately hazardous pesticides [38].

Organophosphate pesticide, fenitrothion is widely used for agricultural purpose in many countries, such as China, Brazil, Japan and Australia, due to its low cost and broad spectrum activity. Like other organophosphates, it is neurotoxic, inhibiting the activity of cholinesterase and overstimulating the nervous system causing nausea at lower level exposure, and even death at higher and prolonged exposure. Because of increasing concerns over agricultural worker health and its potential environmental impacts, there is a growing interest to develop novel analytical methods capable of performing rapid detection of these compounds in the field. Many analytical methods, including gas and liquid chromatographic technologies, immunoassays, and biosensors based on cholinesterase or alkaline phosphatase inhibition, have been reported for the determination of organophosphorus pesticides. Although very sensitive, these methods are not satisfactory for rapid and on-line and in-field monitoring [39].

Fenitrothion presents hazardous effects to human health as it promotes inhibition of cholinesterase. In animals the Fenitrothion molecule is oxidized to derivatives that contain P=O groups, which are more powerful inhibitors of cholinesterase than thiophosphate itself [40].

To overcome the residue problems due to fenitrothion, which is moderately toxic for living organisms, analytical methods are vital. Currently, electrochemical methods receive a great attention instead of conventional ones, such as chromatography, solid extraction phase (SEP), spectrometric methods, which are time consuming, expensive and difficult to apply in field [34-35]

Physical and chemical properties of Fenitrothion



o,o-dimethyl O-(3-methyl-4-nitrophenyl)-phosphorothioate

Fig .2. Chemical structure of Fenitrothion

Table 2. Some physical and chemical properties of fenitrothion

Physical state	liquid
Color (liquid)	Yellow- brown
Melting point	0.3 ^{0}C
Boiling point	140-145 ^{0}C
Density	1.32 – 1.34 at 20 ^{0}C
Solubility in water	14mg/litre at 30 ^{0}C
Solubility in organic solvents	Freely soluble in alcohols, esters, ketones, and aromatic hydrocarbons
Stability	Hydrolyzed by alkali: half-life 4.5 h in 0.01M NaOH at 30 ^{0}C, decomposed by heat: 145 ^{0}C. Iron promotes decomposition of fenitrothion.
Volatility	0.09 mg/m^{3}

3. Electrochemical Studies

3.1. Voltammetric Instruments

A standard three electrode system comprises of a working electrode, counter electrode and reference electrode dipped in a single dual compartment cell. The working electrode acts as a substrate for electro-deposition of polymers. Since the polymeric films are deposited by an oxidative process, it is necessary that the electrode should not oxidise concurrently with the aromatic monomer [41].

Working Electrode: The performance of the voltammetric procedure is strongly influenced by the working electrode material. The electrode should provide high signal-to-noise characteristics, as well as a reproducible response. Thus, its selection depends primarily on two factors: the redox behavior of the target analyte and the back ground current over the potential region required for the measurement. The most popular are those involving mercury, carbon or nobel metals (particularly platinum and gold) [42].

Reference Electrode: is the electrode that its potential remains constant throughout the experiment. It is used as a reference point against which the potential of other electrodes (typically that of the working electrode or measuring electrode) can be measured in an electrochemical cell [43].

Counter Electrode: non-reactive high surface area electrode, which completes the circuit in the electrochemical cell, commonly platinum or a gold wire. Even if it is coupled to the working electrode, it plays no part in determining the magnitude of the potential being measured [44].

Supporting electrolyte: an electrolyte added to the solution for the exclusive purpose to increase the solution conductivity, while the electrolyte does not take part in any reactions. Also called "inert," "indifferent," or "swamping" electrolyte [44].

3.2. Voltammetric Techniques

There are several types of voltammetric techniques, depending on the shape of the applied potential function. For linear sweep voltammetry (LSV) and cyclic voltammetry (CV), the potential applied changes linearly with time. When the potential sweep is not a linear function but comprises constant increments on a linear rise (differential pulse voltammetry, DPV) or a square wave function (square wave voltammetry, SWV), these techniques could offer better sensitivity, because they offer better signal-to noise ratios [22].

The common characteristic of all voltammetric techniques is that they involve the application of a potential (E) to an electrode and the monitoring of the resulting current (i) flowing through the electrochemical cell. In many cases the applied potential is varied or the current is monitored over a period of time (t). Thus, all voltammetric techniques can be described as some function of E, i, and t. They are considered active techniques (as opposed to passive techniques such as potentiometry) because the applied potential forces a change in the concentration of an electroactive species at the electrode surface by electrochemically reducing or oxidizing it. Cyclic voltammetry (CV) has become an important and widely used electroanalytical technique in many areas of chemistry. It is rarely used for quantitative determinations, but it is widely used for the study of redox processes, for understanding reaction intermediates, and for obtaining stability of reaction products. This technique is based on varying the applied potential at a working electrode in both forward and reverse directions (at some scan rate) while monitoring the current. For example, the initial scan could be in the negative direction to the switching potential. At that point the scan would be reversed and run in the positive direction [45].

Square Wave Voltammetry: The major advantage of square voltammetry is its speed. The effective scan rate is given by fΔEs. The term f is the square-wave frequency (in Hz) and ΔEs is the step height. Frequencies of 1-100 cycles per second permit the use of extremely fast potential scan rates. For example, if ΔEs = 10mV and f =50 Hz, then the effective scan rate is 0.5mV/s. As a result the analysis time is drastically reduced; complete voltmmogram can be recorded with a few seconds, as compared with about 2-3 min in deferential-pulse-voltammetry [47]. Moreover, the method has some other advantages viz. lower consumption of electroactive species in relation to DPV, and reduced problems with blocking of the electrode surface. Since the current is sampled in both the positive and the negative going pulses, peaks corresponding to the oxidation or reduction of the electroactive species at the electrode surface can be obtained in the same experiment, and by subtraction their difference. Subtraction also means that the difference current is zero for a species at a potential corresponding to the region of mass-transport limited current. In analysis, this can be very useful, particularly for removing the current due to reduction of dissolved oxygen [46].

4. Experimental Section

4.1. Chemicals and Apparatus: Electrochemical measurements were carried out on an electrochemical analyzer [Bass CV-50 W]. A conventional three-electrode system was employed with a bare GCE or poly(AHNSA) film modified GCE (3.0 mm in diameter) as the working electrode, a silver-silver chloride electrode (Ag/AgCl) as the reference electrode, and a platinum electrode as the counter electrode. All the potentials given in this paper were referred to the silver-silver chloride electrode. The pH value of acetate buffer solutions (ABS) was adjusted by pH meter. The solution of AHNSA was prepared by dissolving in HNO_3. All chemicals were of analytical reagent grade and were used without further purification. Acetate buffer solutions (ABS) were prepared from $CH_3CO_2Na.3H_2O$and CH_3CO_2H, and the pH was adjusted with $CH3CO_2Na.3H_2O$ and CH_3CO_2H. Stock solution of fenitrothion (10µM) was prepared by dissolving fenitrothion in pH 5.0 acetate buffer solution (ABS). All aqueous solutions were prepared in distilled water.

4.2. Preparation of Modified Glassy Carbon Electrode: A glassy carbon electrode was polished to a mirror finished with polish paper and 0.05 µm alumina slurry. 2.0×10^{-3} M of monomer (AHNSA) was prepared by dissolving 0.04759 g in 100mL of 0.1M HNO_3 and it was electrochemically polymerized onto the glassy carbon electrode by voltammetric scanning at 100 mV s^{-1}. The polymerization process was carried out under the working potential window between −0.8 and 2.00 V for 50 segments scanning. After that, the modified electrode was electroactivated by cyclic voltammetry from -0.8 to 0.8 V at 100 mVs^{-1} in pH 5.0 acetate buffer solution (ABS).

4.3. Analytical Procedure: Electrochemical experiments were carried out in a conventional electrochemical cell containing 0.1 M acetate buffer solution and a certain concentration of fenitrothion. After accumulation for 500 s under 0.10 V, square wave voltammograms were recorded in the potential range from -0.1 V to -.8 V at a scan rate of 100 mV s^{-1}.

5. Objectives of the Study

The general objective of this study is to electropolymerize 4-amino-3-hydroxynaphthalene -1-sulfonic acid on the surface of glassy carbon electrode. Specifically, the aim is to compare the sensitivity of the poly-4-amino-3-hydroxynaphthalene -1- sulfonic acid modified glassy carbon electrode (GCE) and bare glassy carbon electrode (GCE) for the voltammetric detection of fenitrothion.

6. Results and Discussion

6.1. Electropolymerization of 4-Amino-3-hydroxynaphthalene-1-sulfonic acid (AHNSA) at a Glassy Carbon Electrode (GCE)

Cyclic voltammetry was used to form polymerization film on the surface of GCE. The selected potential range for the formation polymerization film was between -0.8 V and 2.0 V. Voltammograms of 2.0 x 10^{-3} M AHNSA in pH 5.0 acetate buffer solution (ABS) at the GCE is shown in Fig. 3. In the first scan anodic peak 1 and cathodic peak 2 were observed with peak potential value at 0.456 V and -0.137 V, respectively. From the second cycle, anodic peak 3 was appeared at the potential value 0.043 V. Then larger peaks were observed up on continuous scanning, reflecting the continuous growth of the film and this is shown in Fig.4. These facts indicated that AHNSA was deposited on the surface of GCE by electropolymerization. After modification, the poly AHNSA film electrode was carefully rinsed with distilled water and was prepared for the determination of fenitrothion.

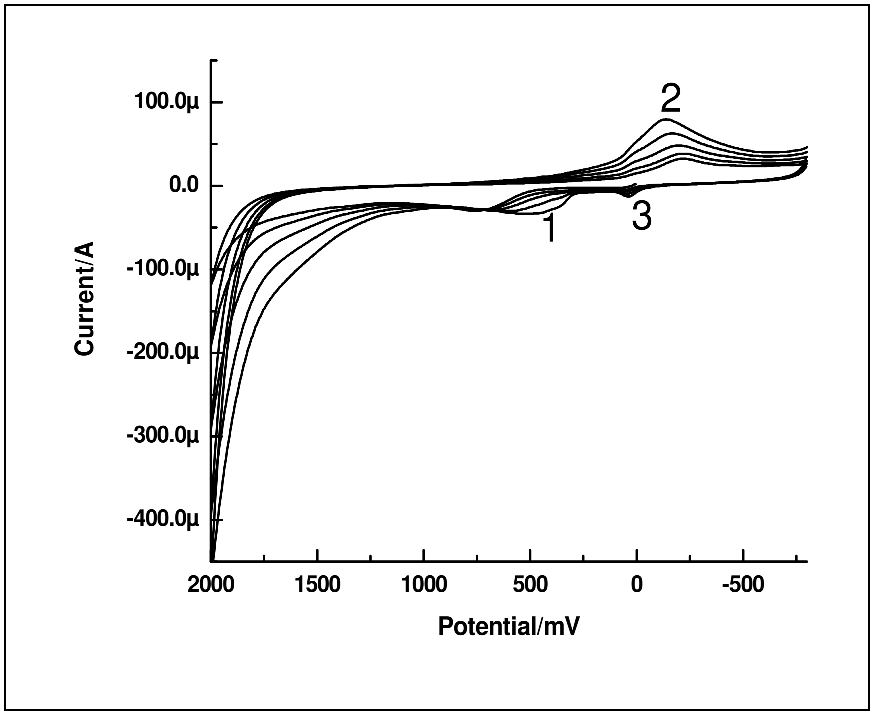


Fig.3. Cyclic Voltammograms of 2.0 x 10^{-3} M AHNSA/GCE in 0.1 M HNO_3 solution. Terminal potential: +2.0 V; initial potential: -0.8 V; sensitivity: 100µAV^{-1}; scan rate: 100 mVs^{-1}.

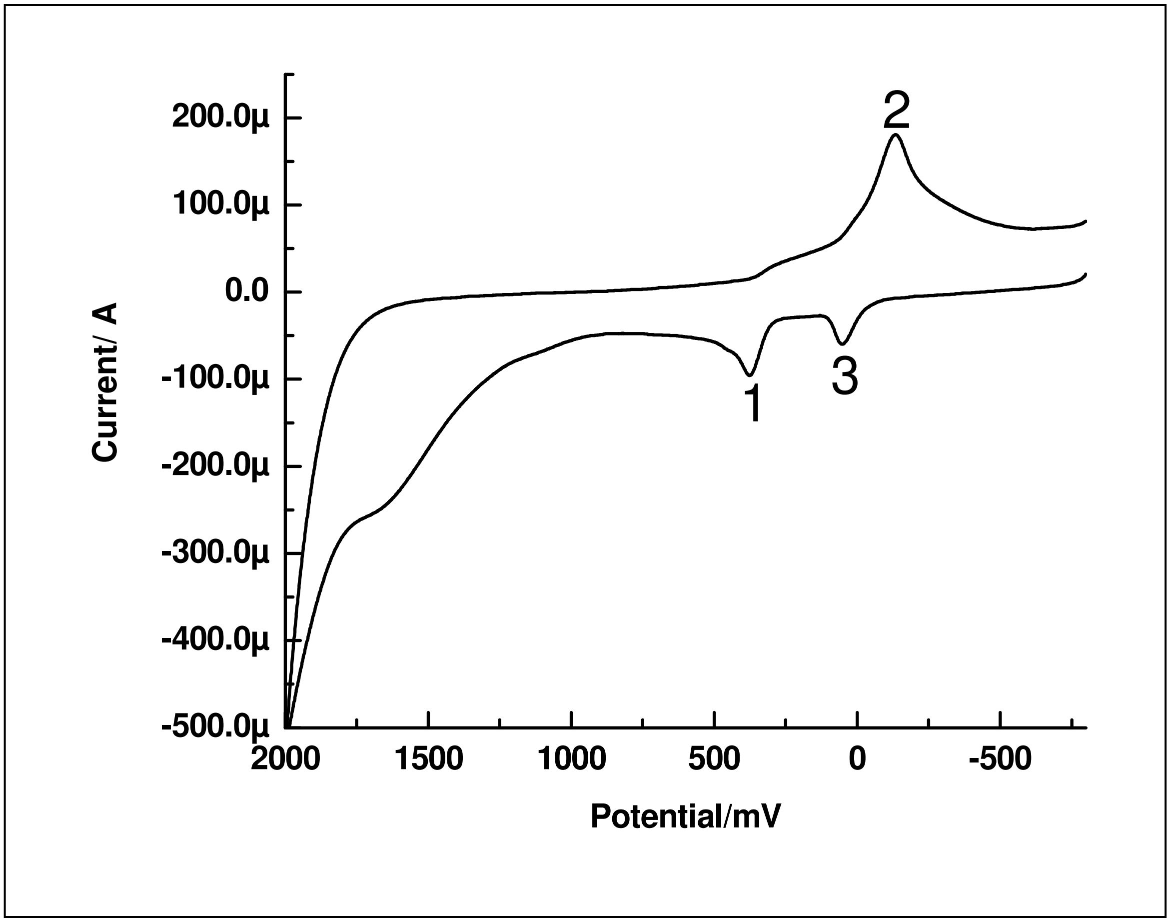


Fig.4. 32nd cyclovolltammogram of 2.0 x 10^{-3} M AHNSA/GCE in 0.1M HNO_3 solution. Terminal potential: +2.0 V; initial potential: -0.8 V; sensitivity: 100μAV^{-1}; scan rate: 100 mVs^{-1}

6.2. Cyclic Voltammograms of Fenitrothion at Bare and Poly (AHNSA) Modified Glassy Carbon Electrodes (GCEs)

Fig.5. shows cyclic voltammetry (CV) recordings of 10μM fenitrothion at bare GCE (▼) and at modified GCE (▲); and pH 5.0 acetate buffer solution at bare GCE (♦) and modified GCE (●). At the bare GCE, fenitrothion showed a weak electrochemical response. But the peak current response at the modified electrode was increased greatly and it provides a better sensitivity and

selectivity for the detection of fenitrothion. The electrochemical behavior of fenitrothion at bare and polymer modified glassy carbon electrodes was similar to the reference reported [47]. The reaction mechanism may be indicated as follows:

$$\varphi\text{-NO}_2 + 4e^- + 4H^+ \rightarrow \varphi\text{-NHOH} + H_2O \qquad \text{(a)}$$

$$\varphi\text{-NHOH} \leftrightarrow \varphi\text{-NO} + 2e^- + 2H^+ \qquad \text{(b)}$$

In the first cycle, only an irreversible cathodic peak 3 at -0.594 V and anodic peak 1 at 0.0345 V were observed on the surface of modified GCE. In successive potential sweeps, another reductive peak 2 was appeared at 0.018 V. The cathodic peak 3 at -0.594 V decreases in subsequent potential sweeps and it is responsible for the increment of the redox peaks 2 and 1. The peak potential of the reduction of fenitrothion was observed at -0.654 V on bare GCE, while anodic potential was appeared at 0.78 V. Another reduction peak was appeared at -0.0355 V with poor electrochemical response. This reflects that low concentration of fenitrothion is difficult to be reduced on a bare glassy carbon electrode (GCE). The oxidation peak 1, which is observed at 0.0345 V on the surface of modified glassy carbon electrode (GCE) is apparently more negative, and the reduction peak 2 located at -0.594 V is more positive than that on bare glassy carbon electrode (GCE). The peak-to-peak potential separation for the redox couple in modified glassy carbon electrode (GCE) is 0.032 V which is less than that of bare glassy carbon electrode (GCE). This indicates that the redox reaction can be catalyzed by poly (AHNSA) film, and a more reversible reaction occurred on the polymer film electrode.

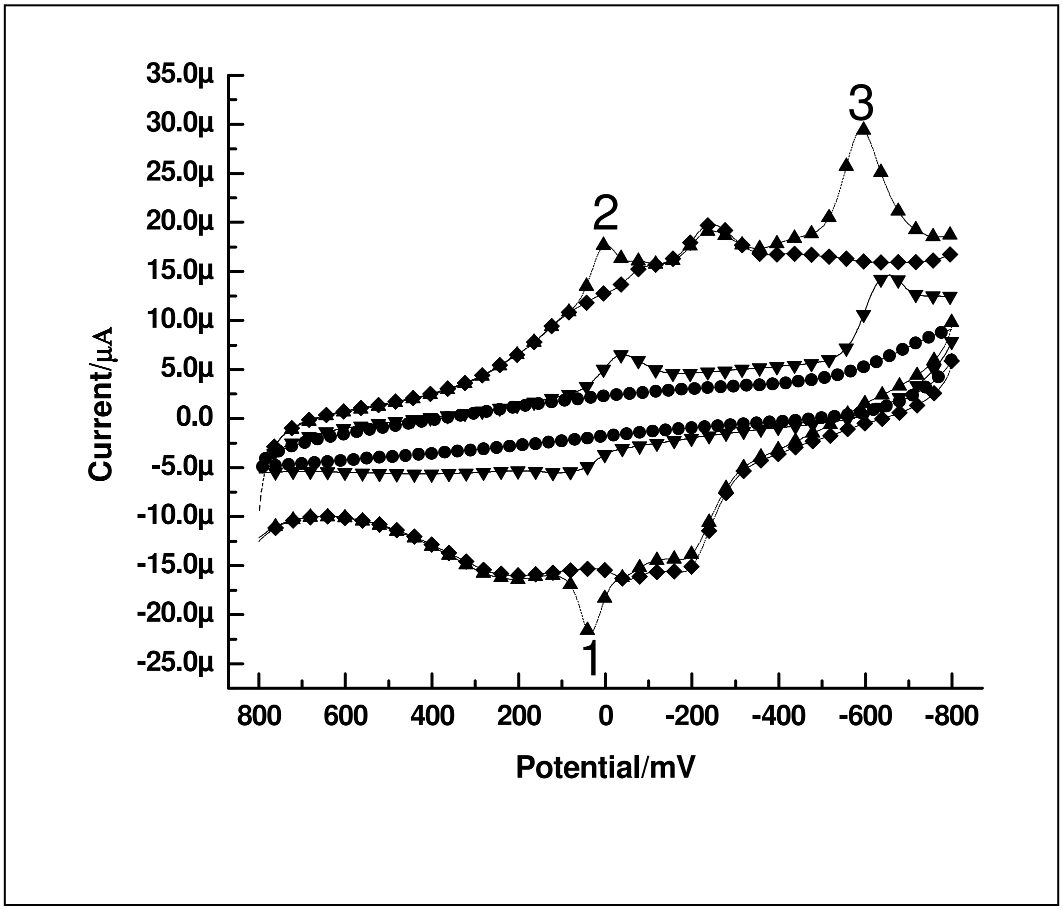


Fig.5. Cyclic voltammograms of 10 µM fenitrothion at bare GCE (▼) and modified GCE (▲); and pH 5.0 acetate buffer solution (ABS) at bare GCE (●) and modified GCE (♦). Sensitivity: 100µAV^{-1}; scan rate: 100 mVs^{-1}.

6.3. Comparison of Square Wave Voltammograms of Bare and Modified Glassy Carbon Electrodes (GCEs).

Accordingly to the voltammetric behavior of fenitrothion in acetate buffer solution (ABS), the fenitrothion has been transformed from an irreversible form into a well- reversible form. Thus, a square wave voltammetry can be used to detect fenitrothion sensitively, and it permits the rapid determination of fenitrothion with relatively inexpensive instrumentation. A typical square wave voltammogram for 10 µM fenitrothion at a bare GCE (a) and the modified GCE (b) in pH 5.0

ABS are shown in Fig. 6. This clearly shows that the poly (AHNSA) film electrode was more sensitive than the bare electrode for the current response of □-NHOH. The higher peak current was due to the adsorption of □-NHOH on the surface of the poly-(AHNSA) modified GCE in the process of accumulation.

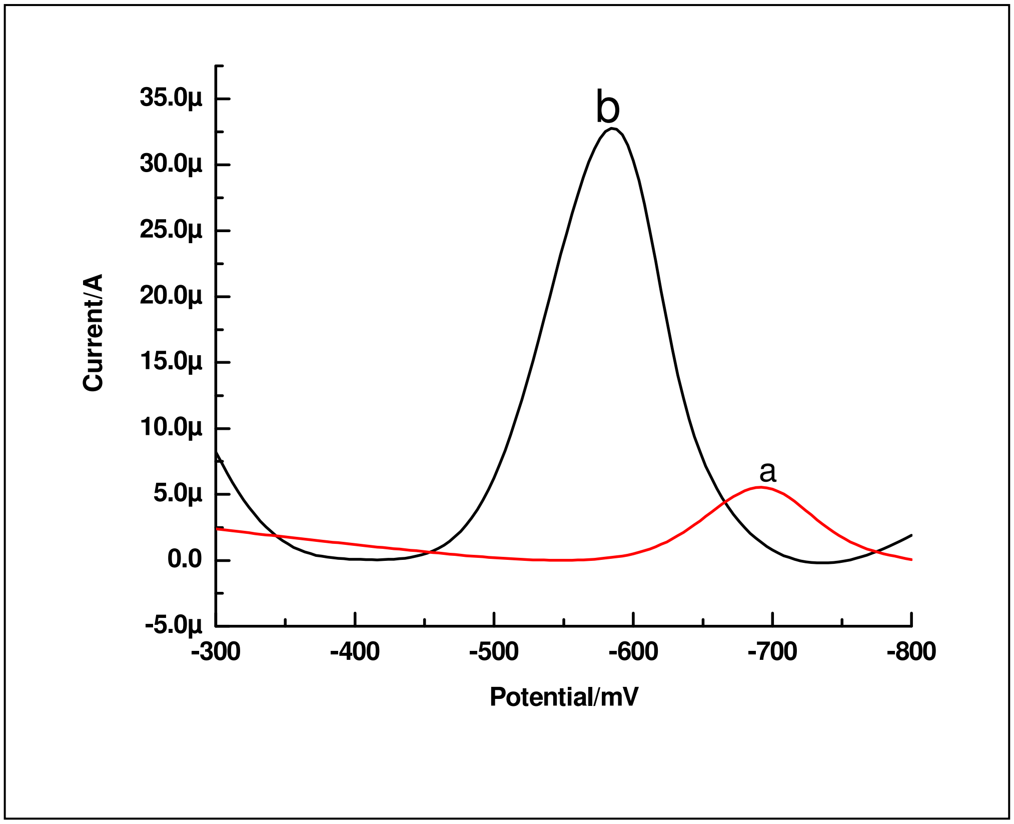


Fig.6. Square wave voltammograms of 10 µM fenitrothion at (a) Bare GCE (b) Modified GCE in pH 5.0 acetate buffer solution (ABS).

6.4. Optimization of Parameters for Fenitrothion Detection

6.4.1. Effect of Accumulation Potential and Time.

It is known that the accumulation potential is usually a major factor affecting voltammetric response. The effect of the accumulation potential on the reduction of peak current of 10 µM fenitrothion for accumulation time 100 s was assayed.

The effect of the accumulation potential on the response of fenitrothion in acetate buffer solution (ABS) pH 5.0 is shown in Fig .7 (a). The figure indicates that between -0.40 V and o.40 V the reduction peak current clearly increases as accumulation becomes more positive and it remains almost constant on further increase of accumulation potential. Thus, an accumulation potential of 0.10 V is used for effective studies to achieve optimum results of fenitrothion during detection. The effect of accumulation time on reduction peak current is illustrated in Fig.7 (b). The reduction peak current increases upon extending the accumulation time and the maximum reduction peak current is attained at 500 s and the cathodic current of fenitrothion is almost constant after 500 s.

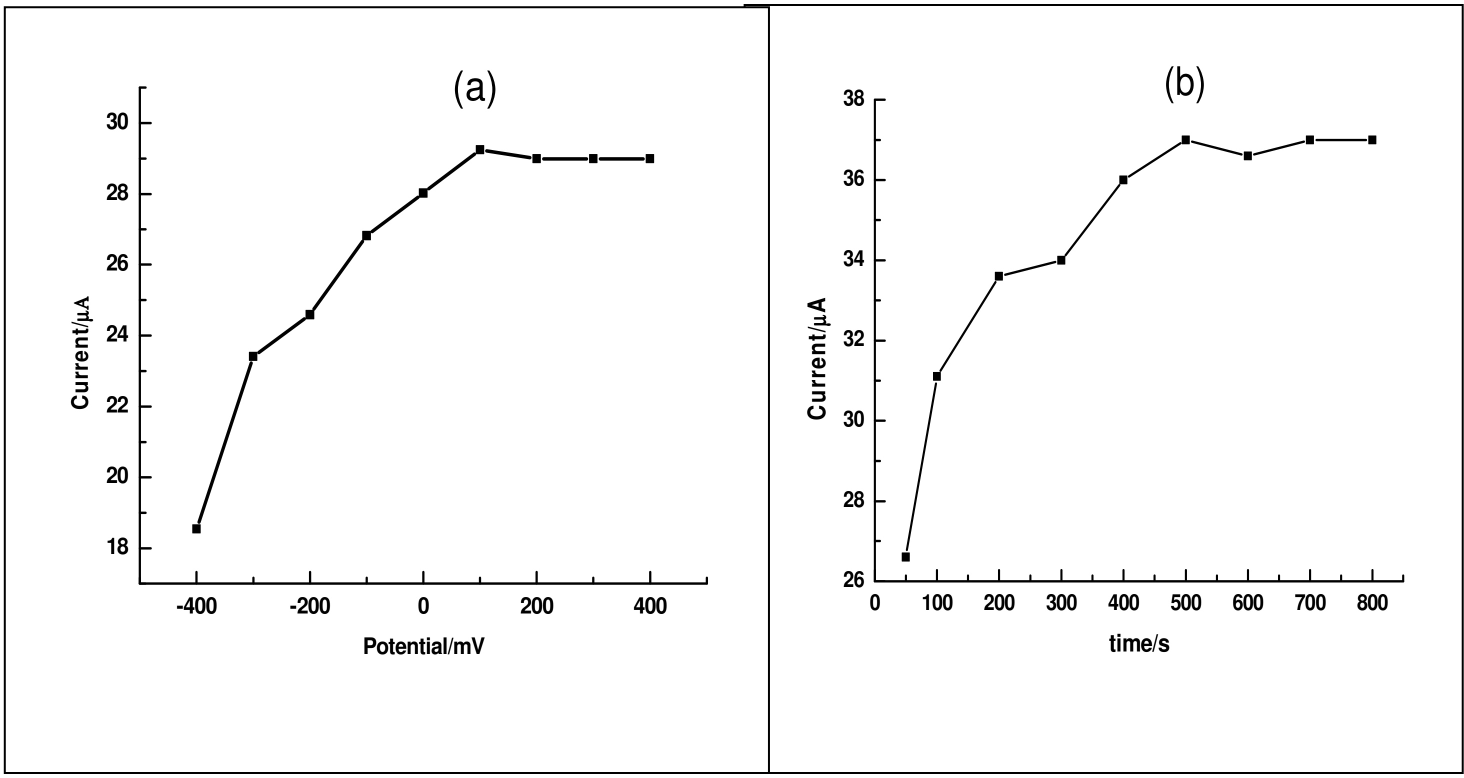


Fig.7. Effect of (a) accumulation potential (p_a) and (b) accumulation time (t_a) on SW voltammetric response for 10 μM fenitrothion in pH 5.0 ABS at the modified GCE. Conditions: (a) t_a = 500 s, (b) p_a = 0.1 V; SWV amplitude: 25 mV; frequency: 15 Hz, scan rate: 100 mV s^{-1}.

This clearly indicates that the accumulation time of 500 s is sufficient to achieve 10 μM fenitrothion saturation of the modified GCE. A larger accumulation time is due to lower concentration of fenitrothion.

6.4.2. Effect of pH on the Detection of Fenitrothion

Effect of pH on the response of fenitrothion was obtained over the range 3-7 on the modified GCE. Figure 8. shows that the peak current increased with increasing solution pH until it reached pH 5.0. The peak current decreases when the pH is greater than 5.0. The reduction peak current significantly increases with increasing the solution pH when the pH of the solution is less than 5.0. A maximum response is observed at pH 5.0 and the best results with respect to the current enhancement are obtained at pH 5.0 of acetate buffer solution (ABS).

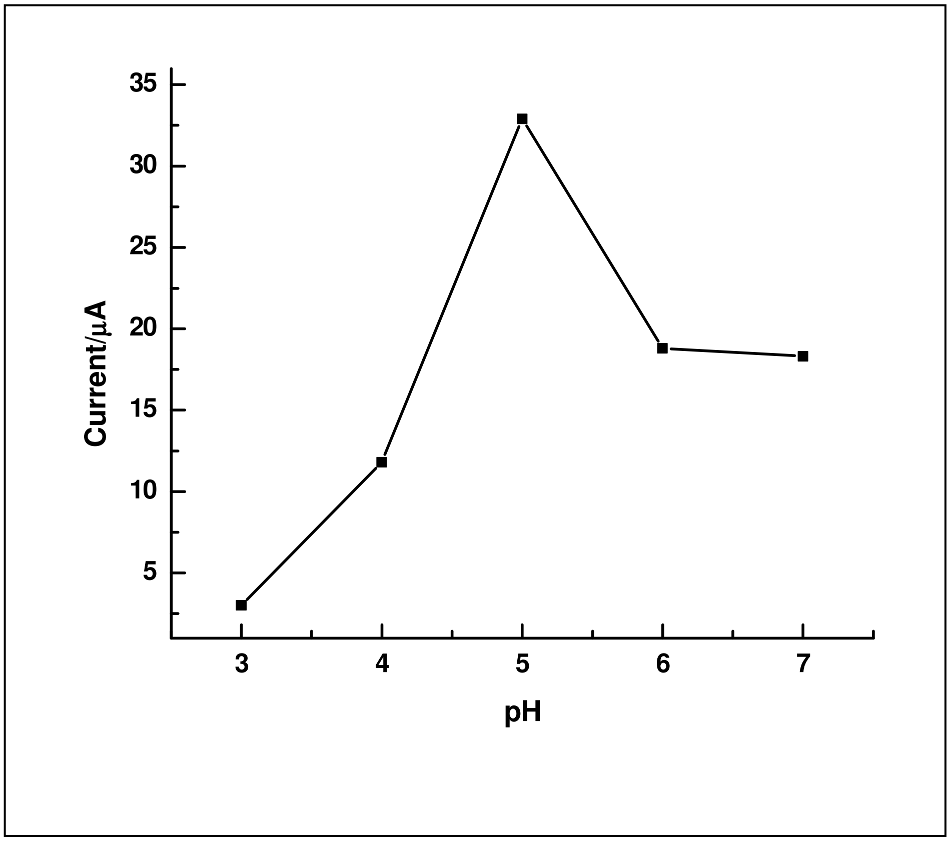


Fig.8. Effect of pH at the modified GCE on SW voltammetric response of 10 μM fenitrothion: Sensitivity: 100 μAV^{-1}; scan rate: 100 mVs^{-1}.

6.4.3. Analytical Characterization of Fenitrothion.

Figure 9. shows the square wave voltammograms of reduction peak current (A) against peak potential (mV) of fenitrothion at modified GCE in 0 to 20 μM range. It increases linearly up to 20 μM. Figure 10. shows the calibration curve of the modified glassy carbon electrode (GCE) sensor for the detection of fenitrothion. Using the optimized conditions, a linear calibration curves are obtained up to 20 μM range in pH 5.0 acetate buffer solution (ABS). The linear regression equation was i_p (μA) = 1.155C (μM) + 5.96. In 0 to 20 μM range, the slope (A/M) and correlation coefficient are 1.155μA/μM and 0.990, respectively.

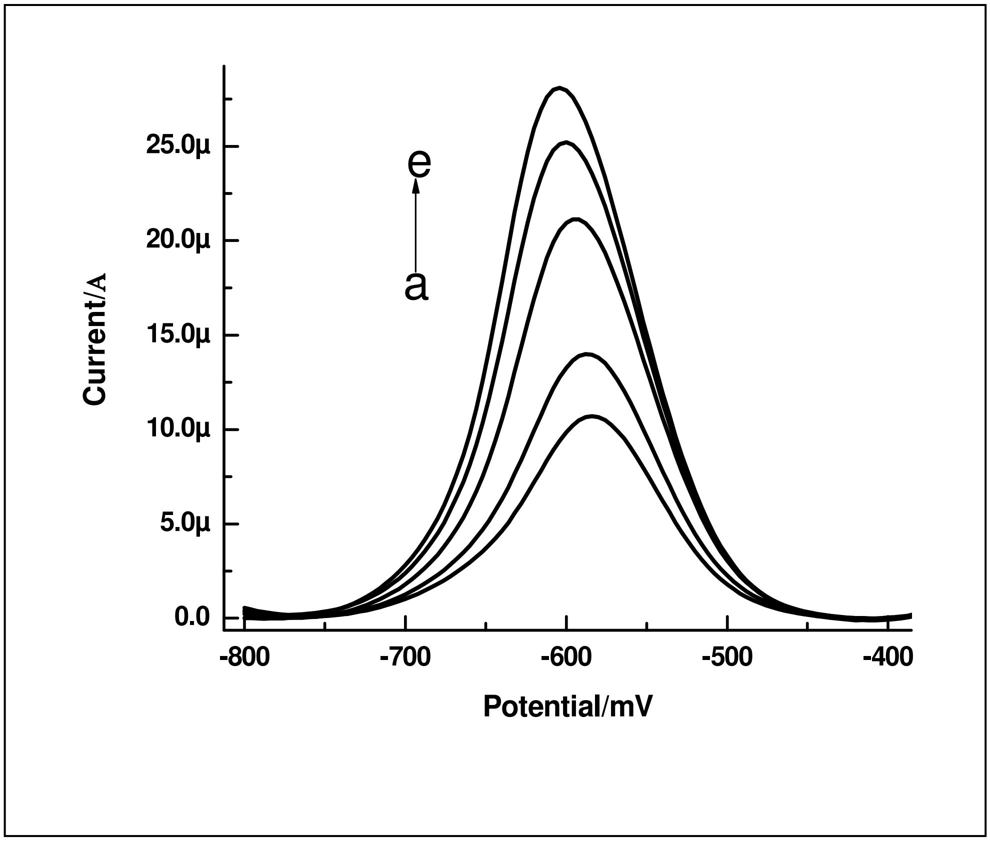


Fig.9. Square wave voltammograms of the reduction peak current of fenitrothion on modified GCE in pH 5.0 acetate buffer solution (ABS) (a) 4 μM, (b) 8 μM, (c) 12 μM, (d) 16 μM, (e) 20 μM

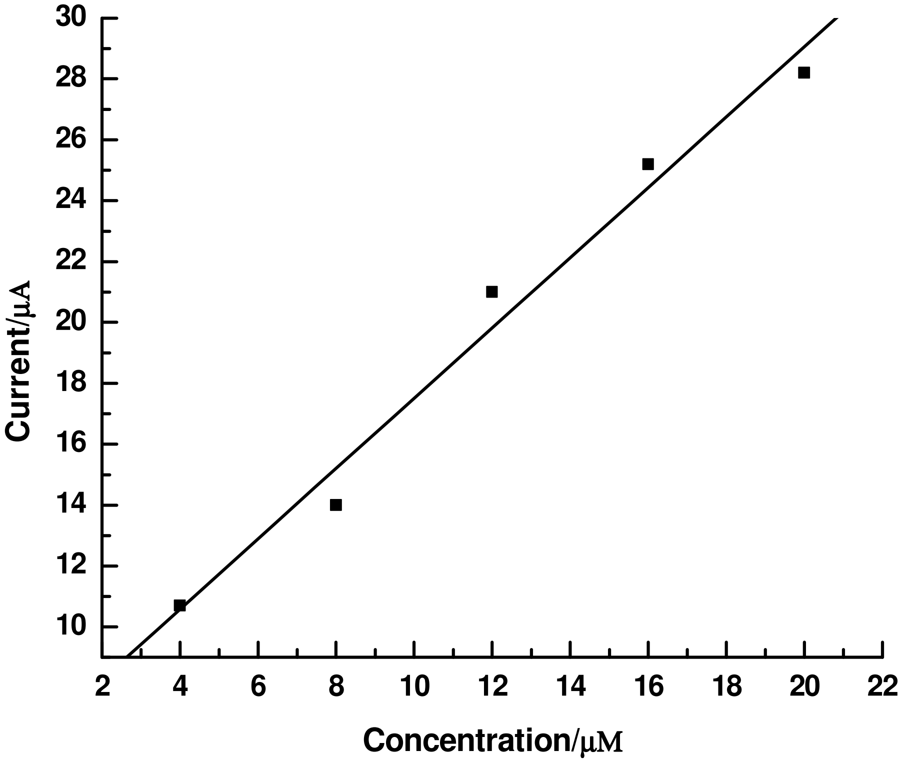


Fig.10. Calibration curve for the detection of fenitrothion on the surface of modified GC electrode in pH 5.0 acetate buffer solution (ABS).

7. Conclusion

This study demonstrated the electropolymerization and application of the AHNSA chemically modified glassy carbon electrode. The analysis in to the electrochemical behavior of fenitrothion proved that the modified glassy carbon electrode could be used for the detection of fenitrothion. Moreover, the modified glassy carbon electrode showed higher sensitivity than bare glassy carbon electrode during the determination of the fenitrothion.

8. Reference

1. M. A. Rahman, P. Kumar, D. S. Park and Y. B. *Shim, Sensors*, 8, **2008**, 118-141.
2. H. A. M. van Mullekoma, J. A. J. M. Vekemansb, E.E. Havingab, E.W. Meijerb, *Mater. Sci. Engin.*, 32, **2001**, 1-40.
3. A. Pron, P. Rannou, *Prog. Polym. SCi.*, 27, **2002**, 135-190.

4. J. D. S. Smith, *Prog. Polym. SCi.*, 23, **1998**, 57-79.
5. R. W. Murray, “Chemically Modified Electrodes,” in Electroanalytical Chemistry, Vol. 13, A. J. Bard, ed., Marcel Dekker Inc., NY, **1984**.
6. R. A. Durst, A. J. Baumner, R. W. Murray, R. P. Buck, and C. P. Andrieux, *Pure & App. Chem.*, 69, **1997**, 1317-1323.
7. V. Saxena, B.D. Malhotra, *Curr. App. Phys.*, 3, **2003**, 293–305.
8. M. Gerard, A. Chaubey, B.D. Malhotra, *Biosens. Bioelectron.,* 17, **2002**, 345–359.
9. J. Volke, F. Liška, Electrochemistry in Organic Synthesis, Springer- Verlag, Berlin Heidelberg, **1994**, pp 68.
10. A. Malinauskas, Synth. Met., 107, 1999, 75–83.
11. G. Inzelt, “Mechanism of Charge Transport in Polymer-Modified Electrodes” in Electroanalytical Chemistry, Vol. 18, A. J. Bard, ed., Marcel Dekker Inc., NY, 1994.
12. M. A. Rahman, P. Kumar, D. Park and Y. Shim, Sensors, 8, 2008, 118-141.
13. J. Wang, “Voltammetry Following Nonelectrolytic Preconcentration” in Electroanalytical Chemistry, Vol. 16, A. J. Bard, ed., Marcel Dekker Inc., NY, 1989.
14. M.A. Vorotyntsev, J. Heinze, Electrochim. Acta., 46, 2001, 3309–3324.
15. A. Malinauskas, Polymer, 42, 2001, 3957-3972.
16. J. J. Grodzinski, Polym. Adv.Technol, 13, 2002, 615-625.
17. B. I. Podlovchenko, V. N. Andreev, Rus. Chem. Rev., 71, 2002, 837 – 851.
18. K. Gurunathan, A. Vadivel Murugan, R. Marimuthu, U.P. Mulik, D.P. Amalnerkar, *Mater. Chem. Phys.*, 61, **1999**, 173-191.
19. G. Inzelt, M. Pineri, J.W. Schultze, M.A. Vorotyntsev, *Electrochim. Acta.*, 45, **2000**, 2403–2421.
20. A. K. Wanekaya, Y. Lei, E. Bekyarova W. Chen, R. Haddon,, A. Mulchandani, N.V.Myung, *Electroanalysis*, 18, **2006**, 1047 – 1054.
21. J.C. Vidal, E. G.Ruiz, J.R. Castillo, Microchim. Acta., 143, 2003, 93-111.
22. M. C. Blanco-Lopez, M. J. Lobo-Castan˜ o´ n, A.J. Miranda-Ordieres, P. Tun˜ o´ n-Blanco, *Tre. Anal. Chem.*, 23, **2004**, 36-48.
23. S. Ruth, H. Bleier, W. Pukacki, *Faraday Discuss. Chem. Soc.*, 88, **1989**, 223-233.
24. E. M. Garrido, C. Delerue-Matos, J. L. F. C. Lima, and A. M. O. Brett, *Anal. Lett.*, 37, **2004**, 1755-1791.

25. M. D. Lagrega, P. L. Buckingham, J. C. Evans, Hazardous Waste management, 2nd ed., NY, **2001**.
26. B. Blake " Toxicology of the Nervous System", in A Text Book of Modern Toxicology, E. Hodgson, ed., John Wiley & Sons, NY, **2004**.
27. G. T. Brooks, T. R. Roberts. Pesticide Chemitry and Bioscience, RS.C. **1999**.
28. G.S. Nunes, D. Barceló, *Anal. Magaz.*, 26, **1998**, 156-159.
29. W. A. Collier, M. Clear, A. L. Hart, *Biosens. Bioelectron.*, 17, **2002**, 815-819.
30. P. Manisankar, P. L .A.Sundari, R. Sasikumar, D. J. Roy, *Electroanalysis*, 20, **2008**, 2076 – 2083.
31. L. B.O. dos Santos, M. S.P. Silva, J. C. Masini, *Anal. Chim. Acta,* 528, **2005**, 21-27.
32. A. Mulchandani, W. Chen, P. Mulchandani, J. Wang, K. R. Rogers, *Biosensor. Bioelectron.*, 16, **2001**, 225–230.
33. G. Jia, C. G. Lv, W. T. Zhu, J. Qiu, X. Q. Wang, Z. Q. Zhou, *J. Hazard.Mater.*, 159, **2008**, 300–305.
34. G Liu, Y. Lin, *Anal. Chem.*, 77, **2005**, 5894-5901.
35. L. B.O. dos Santos, G. Abate, J. C. Masini, *Talanta*, 62, **2004**, 667-674.
36. M. K. Pawlak, *Pol. J. Environ. Stud.*, 13, **2004**, 411-414.
37. K. H. B□chel, Chemistry of Pesticides, John Wiley & Sons, NY. **1983**, pp 153.
38. G. A. Matthews, Pesticide application methods, 2nd ed., Longman Scientific & Technical press, NY, **1992**, pp 18.
39. Y. Lei, P. Mulchandani , W. Chen, A. Mulchandani,, *Sensors*, 6, **2006**, 466-472.
40. L. Z. Melgar, S. A. S. Machado, *J. Braz. Chem. Soc.*, 16, **2005**, 743 - 748.
41. K. Gurunathan, A. V. Murugan, R. Marimuthu, U. P. Mulik, D. P. Amalnerkar, *Mater. Chem. Phys.,* 61, **1999**, 173-191.
42. J. Wang, Analytical Electrochemistry, 3rd ed., John Wiley& Sons, NY, **2006**.
43. D. A. Skoog, J. N. Karen, E. T. Alyea, Electrochemical Systems, John Wiley & Sons, NY, **2004**.
44. D. A. Skoog, D. M. West, F. J. Holler, fundamentals of Analytical Chemistry, 7th ed., NY, **1997**.
45. C. M. A. Brett n and A. M. O. Brett, Electrochemistry Principles, Methods, and Applications, Oxford University press, **1993**, pp 129.

46. S. P. Kounaves, “Voltammetric techniques”, in Handbook of Instrumental Techniques for Analytical Chemistry, F. A. Settle; ed. NJ, **1997**, pp 717-722.
47. C. Li, C. Wang, Y. Ma, S. Hu, *Microchim. Acta,* 148, **2004**, 27-33.