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Poly(3,4 –ethylenedioxythiophene) (PEDOT) Modified Glassy Carbon Electrode for the Voltammetric Determination of Diazinon

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List of Symbols or Abbreviation

AChE	AcetylCholinesterase
AdsSV	Adsorptive stripping voltammetry
ADI	Acceptable daily intake
ARfD	Acute reference dose
bw	body weight
CP	Conducting polymer
CPE	Carbon paste electrode
CV	Cyclic voltammetry
CuC_1TPP	Copper complex meso-tri-(4-sulfonatophenyl)mono (4-Carboxyl phenyl propyrin)
DETP	Diethylthiophosphate
DEP	Diethylphosphate
DLLM	Dispersive liquid-liquid microexteraction
DPV	Differencial puls voltammetry
DT_{50}	Time required for 50% of a chemical to degradation
DZN	Diazinon
ELISA	Enzyme- linked immunosorbent assay
EU	European union
EVA	Ethiopian veterinary association
EDOT	3,4-ethylenedioxythiophene
FAO	Food and agricultural organization
FPD	The flam photometric detector
GCE	Glassy carbon electrode
GC-ECD	Gas chromatography, electron capture detector
GC/FPD	Gas chromatography/Flame photometric detector

IMHP	2-Isoprophy-4-methyl-6-hydroxypyrimidine
IC_{50}	Concentration required to inhibit activity by 50%
HPLC	High performance liquid chromatography
HS-SPME	A head space solid phase microexteraction
JMPR	Joint meeting on pesticide residue
LC-TMS	Liquid chromatography-tandem mass spectrometry
LC_{50}	Concentration that kills 50% of the test population of organisms
LD_{50}	Dosage of chemical that kills 50% of the test population of organisms
LOD	Limit of detection
ME	Modified electrod
M_2GCE	PEDOT modified GCE
MWCNT	Multi wall carbon nanotubes
MRLs	Minimal risk levels
MAC	The maximum acceptable concentration
NGCE	Nafion –coated glassy electrode
NOAEL	No-observed-adverse-effect- level
SSD	Solid state detection
O,S-TEPP	O,O,O,O –tetraethyl-thiopyrophosphate
OPH	Organophosphorus hydrolase
OP	Organophosphate
PEDOT	Poly(3,4-ethylenedioxythiophene)
S,S-TEPP	O,O,O,O –tetraethyl-dithiopyrophosphate
TEPP	Tetraethyl pyrophosphate
TLS	Thermal lens spectrometry
Tyr	Tyrosinase
PAn MBT	Polyaniline mercaptobenzothiazol
WHO	World health organization

Abstract

A glassy carbon electrode (GCE) is modified with PEDOT and its application for differential pulse voltammetry (DPV) detection of diazinon ,DZN (organophosphate pesticide) is observed. The PEDOT shows a strong affinity toward diazinon molecule which provides sensitivity for the modified GCE. Instrumental and Chemical parameters influencing the voltammetric response are optimized for diazinon determination . Under Optimum experimental condition the peak current is linear to diazinon concentration over the range of 20μM to 80μM with a correlation coefficient of 0.99866. The limit of detection based on three times the standard deviation of the blank ($3S_b$) was 4.245x 10^{-6}M.

The recovery values for sample tap-water solution containing low ,middle and high concentration of diazinon (50μM, 60 μM & 80 μM) were 95.24%,88.4% & 81.29%; respectively. Thus this modified electrode is considerably applied for the determination of DZN in tap-water sample.

Key words : Diazinon , differential pulse voltammetry, PEDOT , GCE

1.Introduction

1.1 Pesticides

Modern agricultural practices often include the extensive use of a wide range of pesticides for increased crop production as well as for greater yield by controlling pests [1, 2]. The use of pesticides, therefore, continues to exist as world population and the demand for food production continues to grow.

In spite of the undeniable advantages that pesticides have brought to modern agricultural economy for controlling pests, they can generate a series of problems for untargeted organisms, if the necessary precautions are not taken during applications and storages [3]. Large fractions of the pesticides used in agricultural settings end up moving with surface run–off into streams, rivers and lakes, leaching to the ground water systems, or volatilize to the atmosphere [4]. As a result, residues of such compounds can be the main source of environmental pollution [5], they may be found in the soil in which the crop was grown, may also appear in the atmosphere, in run-off water following heavy rain, irrigation, in ground water or in surface water and consequently, they can directly or indirectly pollute food and food products and biological systems.

In general, agricultural chemicals, while often benefiting farming productivity, can have determinable environmental effects when applied improperly [6]. Therefore, pesticides and their potentially undesirable effects on the environment, aquatic organisms and human health has been one of the major concerns of recent research [7]. Among all the hazardous environmental compounds, pesticides are the most abundant in soil, water, the atmosphere and agricultural products. Due to their widespread presence, great environmental concerns have recently appeared around this type of pollution [8].

The purposes of pesticide use are to increase the production of food and fiber and to promote public health; in practice they are also used for aesthetic reasons. A pesticide that kills insects is an insecticide. One killing plants is a herbicide. Among the pests attacking agricultural crops are insects, weeds, rodents, birds, and disease-causing organisms including fungi and bacteria. Weeds are undesired plants of any kind that, if left uncontrolled, crowd out the desired crop plant. The amount of manual labor necessary to control weeds without help from herbicides or machines may be difficult to imagine. Well-to-do societies with their abounded food supply also don't ordinarily think of the total lose of a food crop. For thousands of years People looked for means to rid their crops of the insects eating them, the weeds eating them or the fungi making them uneatable [9]. There are many types of pests, and , not surprisingly, many types of pesticides developed to fight them. Pesticides are grouped or classified according to the pests they control ,their chemical structure, how/when they work or their mode of action (site of action). Many pesticides can be grouped in to chemical family; prominent insecticide families include **Polychlorinated insecticides** , **Organophosphate insecticides** , **carbamate insecticides** [7].

Organophosphates (OP) are a group of compounds that have historically been used as pesticides as well as chemical warfare agents. OPs are phosphorus-containing insecticides whose insecticidal qualities were first observed in Germany during World War II in the study of the extremely toxic OP nerve gases sarin, soman, and tabun. This group of chemicals includes insecticides such as malathion, diazinon, chlorpyrifos, azamethiphos ,dichlorvos, parathion and methyl parathion [10].Organophosphorus (OP) insecticides are of serious environmental concern. As a consequence of their widespread use, they are present in

the environment, food, and drinking water. Although OP compounds show selective toxicity to insects with respect to mammals [6].
Organophosphate insecticides came into wide use as highly chlorinated pesticides were being banned. Organophosphates are also toxic to the nervous system of insects, inhibiting the action of the enzyme that breaks down the neurotransmitter acetylcholine; as acetylcholine levels build up, nerves fire uncontrollably [9,11]. Organophosphates are more acutely toxic than organochlorine pesticides, sometimes much more so. Most acute, human pesticide poisonings are caused by organophosphate pesticides. In fact, these insecticides are chemical relatives of the exceedingly toxic organophosphate nerve gases. Despite their toxicity, organophosphates were more attractive than organochlorines because they have much shorter lives in the environment and, as they are not fat soluble, they don't bioaccumulation to high levels in fat. However, this implies increased water solubility; thus, after application to a field, rainwater can carry the organophosphates into water bodies or they can percolate down into groundwater.

Organic phosphates, were widely used as pesticides because of their rapid decomposition and not likely to be bioaccumulated. They are still of great concern to contaminate surface water and ground water because of their solubility in water [9,11]. Various treatment technologies such as adsorption and decomposition processes have been developed to remove or destroy organic compounds from waters. Among these processes, ozonation is an attractive extensive attention as a potential treatment for various organic pollutants in water[9 ,12] .

Diazinon(DZN) is the International Organization for Standarrdization (ISO) approved name for the contact organothiophosphate insecticide, O,O-diethyl-O-(-2-isopropyl-6-methylpyriamidin-4-yl)phosphorothioate.
Diazoxon, the biologically active metabolite of diazinon, inhibits the activity of cholinesterase. Diazinon has been reviewed by the Joint

Meeting on Pesticide Residue (JMPR) on several occsions since the first evaluation in 1963. In 1966, an acceptable daily intake (ADI) of 0–0.002 mg/kg bw per day was established based on a no-observed-adverse level (NOAEL) of 0.02 mg/kg bw per day for inhibition of plasma cholinesterase activity in a 37–43 day study in humans. In 2001,the JMPR established an acute reference dose (ARfD) for diazinon. Although a new study of acute toxicity in humans was submitted, the ARfD of 0.03 mg/kg bw was based on a NOAEL of 2.5 mg/kg bw observed in a study of acute neurotoxicity in rats [3].The maximum acceptable concentration (MAC) for diazinon has been derived by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) which is 0.02mg/L (20μg/L)[6]. The European Union(EU) allow a maximum concentration of 0.1μg/L of each individual pesticide and 0.5 μg/L of the all pesticide in drinking water (European Union,1998). Diazinon is one of the most widely used organophosphate insecticide in agriculture.

Diazinon is an insecticide ,which is often used in field crops and in household [14,12]. Diazinon and its substituted derivative are widely used as agricultural insecticide and known to be toxic in nature. In spite of it low volatibility (125^{O}C) and low solubility in water ; leaching may occur especially in sandy soils. Qualitative and quantitative study of these materials is thus is very important for environmental control. The objective of this study to carryout a simple and easy electrochemical method for sensitive determination of diazinon using PEDOT modified glassy electrode. Differential pulse voltammetry was used for quantitative determination. Of the various electrode materials, glassy carbon (GC) is particularly is useful because of its high electrical conductivity ,impermeability to gas ,high chemical resistance, reasonable mechanical and dimensional stability and widest potential range of all carboneous electrodes. Although GC serves as very good electrode material, many attempts have been made to improve its electrochemical properties by chemical modification. Surface modification compresses several methodologies including 1)the addition of a variety of molecular catalyst and mediators to the electrode surface by adsorption 2) covalent bonding of electro active catalysts.3) entrapment of metals with in affixed polymer film and 4)vapor or electrode position of metals on to electrode. It is well known that GC is a convenient material for surface modification metals [15].

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) on bare or differently modified GC electrodes have to be proved to be suitable for sensitive and selective determination of many pesticide compounds[15].

1.2. **Physical and Chemical Properties of Diazinon**

Diazinon (O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidin-4-yl) Phosphorothionate is colorless liquid organophosphate pesticide, However , the commercial product is brown. Diazinon is relatively soluble in water and most organic solvents [16]. DZN has a chemical formula $C_{12}H_{21}N_2O_3PS$,represent by chemical structure (Figure 1)

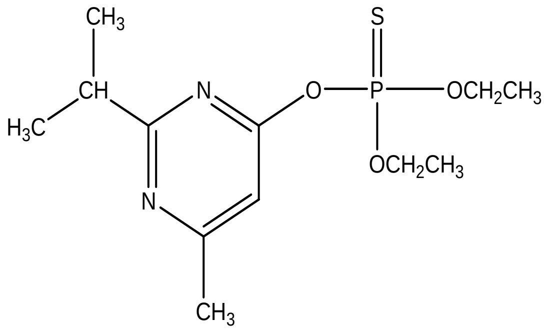


Figure 1 Structure of Diazinon ($C_{12}H_{21}N_2O_3PS$; O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl)

Diazinon (DZN) may be absorbed by all routes of exposure; May be absorbed by the intact skin as well as by inhalation from gastrointestinal tract. Its solubility in water is 40mg/L,at 20^{o}C(Novartis);60mg/L at 20^{O}c (WHO,1998) in other solvent completely miscible in acetone , methanol, ethanol, chloroform, acetonitrite, cyclohexane, dichloromethane, hexane, benzene ,petroleum ether, and carbon disulfide. DZN decompose at temperatures above 120^{O}C. It is stable in alkaline media, but is slowly hydrolyzed by water and by dilute acids; it is quite stable in pH range of to 6.0 to 8.0,although the hydrolysis process is quit rapid under acidic (pH >3.1)or alkaline condition (pH<10.4). The presence of a small amount of water in acid medium promote decomposition to highly toxic by products and hydrolyze at pH 5,$t_{1/2}$ =38 days; at pH 7,$t_{1/2}$=78 days; at PH 9 ,$t_{1/2}$=40. Moreover when aqueous solution of 34mg/L diazinon was exposed to artificial sunlight ,53% decomposition has occur after 97 hours (Novartis,1979). Acidic natural and alkaline pH values of 4, 7, and 10 were selected to study the effect of pH on the oxidation rate of diazinon using a medium pressure mercury UV lamp. The photolysis of diazinon by UV irradiation was found to be sensitive to pH. Direct photolysis of DZN was slower at acidic pH of 4, while no significant effect was observed between neutral and alkaline pH values of 7 and 10 [17]. Diazinon considered non-corrosive .

To determine the nature of by-products, which may be formed, the pathways for the degradation of the compound are helpful. In this part the pathways of the degradation of DZN solutions were explained [18]. The degradation of diazinon insecticide include the formation of several by-products that were identified using GC/quadruple mass spectrometry with EI^{+} ionization mode[18].

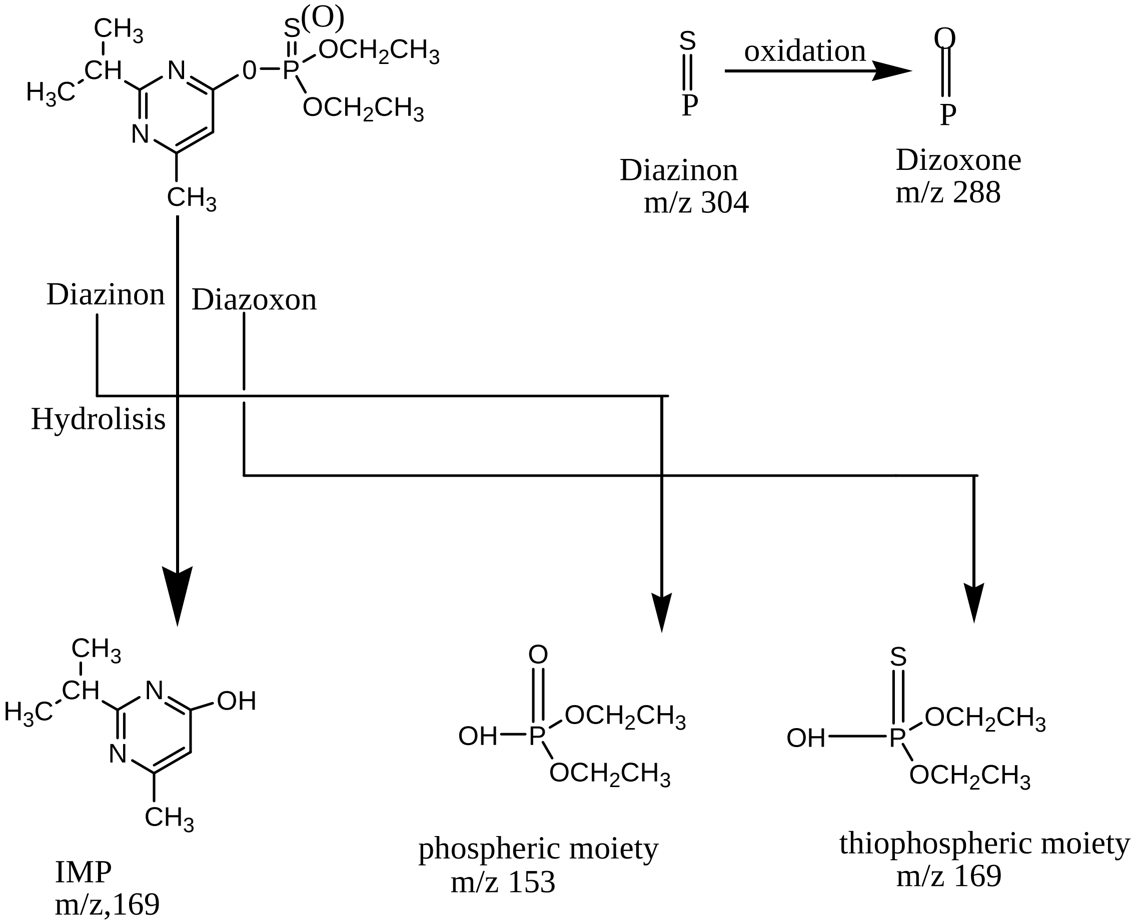


Figure 2. Chemical structure and potential degradation path of the main diazinon by ionization radiation.

Diazinon is fairly resistant to degradation by sunlight, high temperature and moisture. Half –lives are in the order of 140 days ,100 days for soil , 30 days for biota and < 1 day in air . Diazinon persistence in soil combine with its relatively high solubility (log K_{ow} =3.81) posses potential concerns for transport to watercourses during run-off and is believed to be why is one of the most commonly observed OP insecticide at a frequency of 18 %[19].

Table -1- Some properties of Diazinon

Color	Clear yellow to brown
Odor	Faint ester-like odor
Physical state	Slightly viscous liquid
Vapor pressure	The vapor pressure of the active ingredient is $1.4x10^{-4}$mmHg at 20^{o}C
Water solubility	Approximately40 to 60 mg/L(based on the pure active substance.
Viscosity ((At 20oC)	13 to14 mPa.s (Novaris)
Ignition temperature	360^{o}C
Flammability	The flash point is over 170^{o}Cby open cup
Explosiveness	Not explosive
Hazard rate	Health hazard-high; occupation toxicity-high; Fire hazard fumes would be expected to be moderately to highly toxic

1.3 Environmental fate and toxicological effect of diazinon

Organophosphates (OP) are a group of compounds that have historically been used as pesticides as well as chemical warfare agents. These compounds are potent irreversible acetylcholinesterase inhibitors that have a profound effect on the nervous system of exposed organisms[20,21] .

Populations living within or very near areas of heavy agricultural DZN use would have an increased risk to exposure to relatively large amount of DZN through dermal contact with contaminated plants, soils, surface-waters, or artificial surfaces such as playground equipment and pavements; by inhalation of the mist formed from the applied insecticides or by ingestion of water or food-borne residues. Those likely to receive the highest levels of exposure are those who are involved in the production, formulation, handling, and application of DZN, farm workers who enter treated fields prior to the passage of the appropriate restricted entry intervals, and workers involved in the disposal of DZN or diazinon-containing wastes. Dermal contact appears to be the major route of exposure for workers. Inhalation of DZN in occupational settings depends on its volatility, the type of formulation used, and the application technique employed [11].

Diazinon as of organophosphates share a common mechanism of action, namely inhibition of acetylcholinesterase (AChE) [12]. Diazinon classified by the world Health Organization (WHO) as " moderately hazardous" Class II . It was associated with toxicity to aquatic organisms at concentration of 350ng/L,with an LC_{50} in killing (48h) of 4.4mg/L. Fatal human dose were found to be in the rang from 90 to 444mg/kg bw. Although the Toxicological Profile for DZN presents minimal risk level**s** **(**MRLs) derived for DZN in particular, human exposure scenarios may include simultaneous exposure to multiple organophosphate AChE inhibitors[11]. A number of diazinon-related deaths have been reported in companion animals and cattle. The main concern with diazinon products is the formation of breakdown products, particularly S,S-TEPP and O,S-TEPP (figure 4). These degradation products (O,S-TEPP and S,S-TEPP) are much more toxic than diazinon, and are formed from DZN in the presence of a small amount of moisture, air, ultraviolet radiation. Therefore, the exclusion of water in DZN source material as well as in the hydrocarbon-based /emulsifiable concentrate formulated products is absolutely necessary to guarantee the stability of diazinon and its formulations.

Diazinon, an anticholinesterase inhibits AChE in the central and peripheral nervous system. Inhibition of AChE results in accumulation of acetylcholine at muscarinic and nicotinic receptors leading to peripheral and central nervous system effects. These effects usually appear within a few minutes to 24 hours after exposure, depending on the extent of exposure. Most of the located reports of incidents of human exposure to diazinon involved occupational exposure via the inhalation route, although it is possible that significant exposure also took place via the dermal route[11].

The rate of degradation of diazinon in water and soil is strongly influenced by pH. DZN undergoes only slight photolysis in water, with reported half-life estimates ranging from 42 to 88 days [22]. Corroded metal containers and metal ions also contribute to the catalytic decomposition of diazinon to the toxic degradation products. The breakdown rate is dependent on the acidity of water. At highly acidic levels, one half of the compound disappeared within 12 hours while in a neutral solution, the pesticide took 6 months to degrade to one half of the original concentration. In excess water, the principal products of hydrolysis of diazinon are diethylthiophosphoric acid and 2-isopropyl-4-methyl-6-hydroxypyrimidine [17,22]; (Figure 3). Under the reaction conditions, diethylthiophosphoric acid is further hydrolysed to ethylthiophosphoric acid, thiophosphoric acid and ultimately phosphoric acid, which is non-toxic.

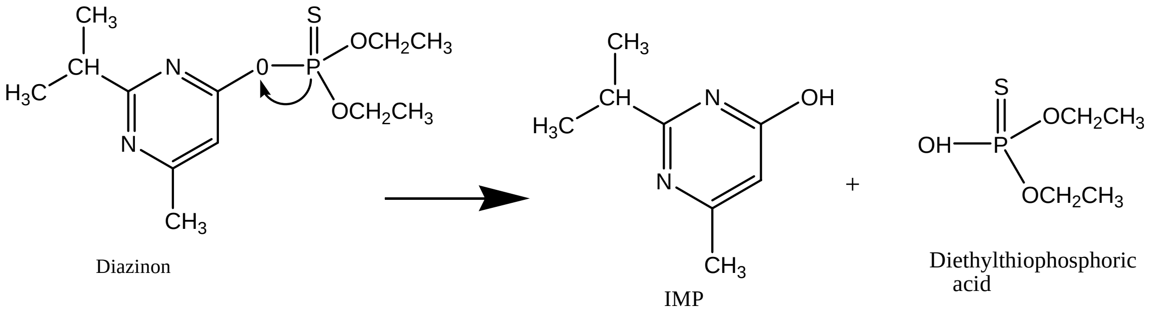


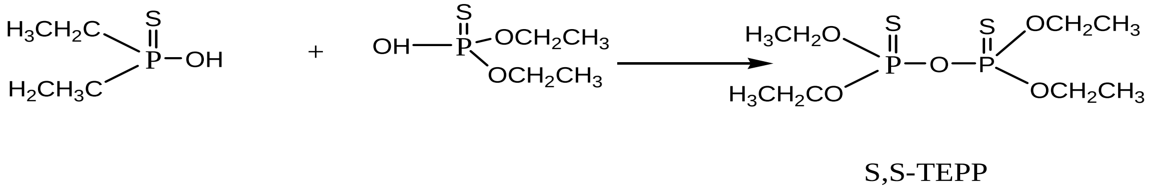
Figure 3.Degradation of diazinon to form IMP and diethylthiophospheric acid

On the other hand, the presence of only trace amounts of water leads to the formation of the S,S-TEPP and, O,S-TEPP. The initial hydrolysis of diazinon gives diethylthio phosphoric acid and 2-isopropyl-4-methyl-6-hydroxypyrimidine (see above). Two molecules of diethylthiophosphoric acid can combine in an acid catalyzed or radical initiated dimerization (radical ions are formed by UV radiation) to form the S,S-TEPP [23,17].

The extent of poisoning is directly related to the quantity ingested, inhaled or in contact with the skin. The degrade product of DZN: S,S-TEPP, O,O-TEPP and O,S-TEPP are more toxic,which are approximately 300-, 1700- and 2500-fold more toxic, respectively, than the parent compound. It is this property of DZN that causes particular concerns for safety of people and animals exposed to products containing diazinon [18,23].

The formation of O,S-TEPP occurs in a similar manner: the diazinon undergoes oxidation and hydrolysis to diethylphosphoric acid, which subsequently combines with diethylthio phosphoric acid to form the O,S-TEPP.

(a)



(b)

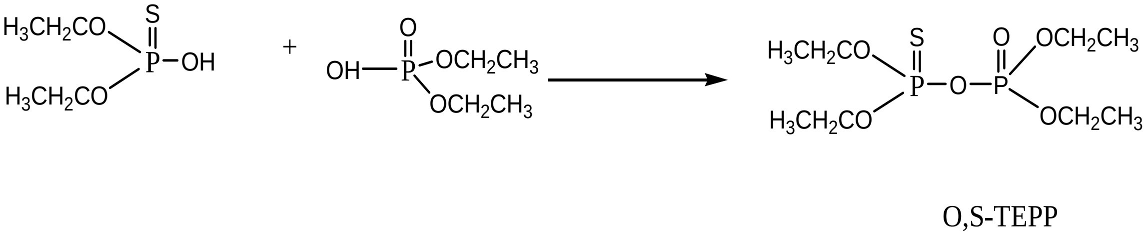


Figure 4. The degradation products of Diazinon (a) S,S –TEPP and (b) O, S-TEPP

Similarly, simultaneous oxidation and hydrolysis of diazinon in the presence of a small amount of water lead to the formation of TEPP, which is formed by the combination of two molecules of diethylphosphoric acid. However, TEPP is rapidly hydrolyzed by water even at room temperature [DT_{50} (50% decomposition) of TEPP and S,S-TEPP is 6.8 hours and 8.2 days respectively, in neutral aqueous solution at 25^0C] forming diethylphosphoric acid, which is non-toxic to both insects and animals. TEPP is therefore is not present in decomposed samples (O,S-TEPP and S,S-TEPP are more stable)[23,17].

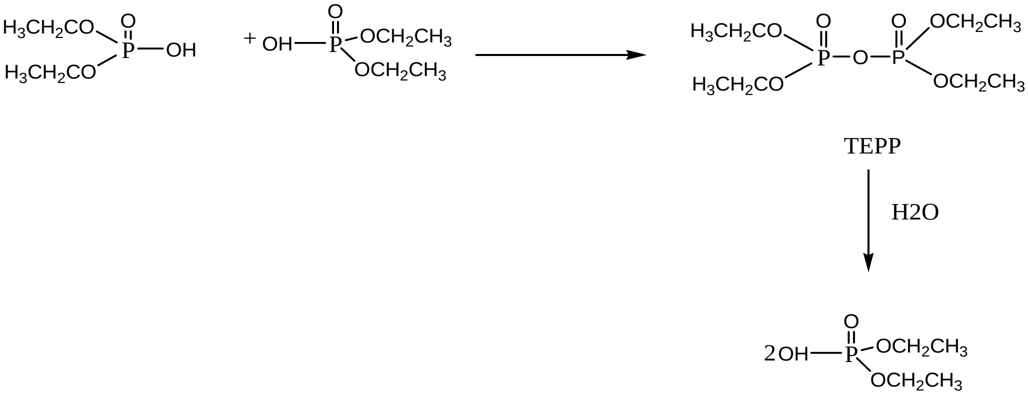
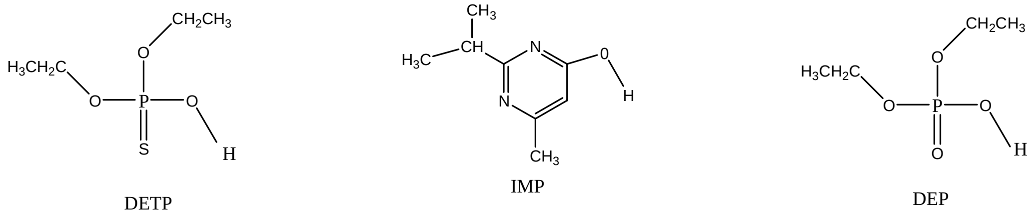


Figure 5 .Hydrolysis of TEPP to form diethylphospheric acid

Organophosphate pesticides (OPs) are generally regarded as safe for use on crops and animals due to their relatively fast degradation rates. Their degradation varies as a function of microbial composition, pH, temperature, and availability of sunlight. Under laboratory conditions (25°C and pH 7) biodegradation is about one order of magnitude faster than chemical hydrolysis, which in turn is roughly ten times faster than photolysis [24].

Inside living things, diazinon is transformed into a molecule called diazoxon; humans can still be potentially exposed to diazinon through the inhalation, ingestion, and/or dermal routes of exposure at their homes. Once diazinon is absorbed into the body, it is rapidly metabolized into several more polar metabolites including diethylphosphate (DEP), diethylthiophosphate(DETP),and2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP). IMHP is commonly used as the specific urinary biomarker of exposure to diazinon and has been detected at low levels (>5 ng/mL) in urine samples of both children and adults [20]. Normally, impulses are transmitted chemically from the end of one nerve cell to the beginning of another; one of the chemical transmitters used in animal nervous systems is called acetylcholine. After transmitting the nerve impulse, acetylcholine is destroyed by an enzyme called acetylcholinesterase (AChE) in order to clear the way for another transmission. Organophosphates attach to AChE and prevent it from destroying acetylcholine causing over stimulation of the nerves[25].



Figur6. Metabolitesofdiazinon in to Diethylthiophosphate (DETP), Diethylphosphate (DEP) and 2-Isopropyl-4-methyl-6-hydroxypyrimedine.

Mammal and insect nervous systems are similar enough that effects of organophosphates are similar. It is worth noting that not all of diazinon's toxicological effects stem from its inhibition of AChE. Diazinon and other organophosphates inhibit numerous enzymes with molecular structures that are similar to AChE. For example, an enzyme involved in the metabolism of the amino acid tryptophan is strongly inhibited by diazinon and diazoxon [25].

1.4 The pesticides Use, Practice and Hazard in Ethiopia

Over 85 percent of Ethiopia's population; currently estimated at 81 million, live in rural areas and depend on agriculture for food and other basic necessities. Although chemical pesticide use in Ethiopia was historically low, recent developments in increased food production and expansion in floriculture industry have resulted in higher consumption of chemical pesticides. The impacts of pesticides in Ethiopia are much more aggravated by the limited knowledge among users on safe practice, toxicological and chemical properties of these substances. Even worse, less is known about the long term and indirect effects of pesticides on rural and urban communities as well as on local and national food production systems [26].

Traditionally, agriculture in Ethiopia is diverse and complex. Inter-cropping is a common practice. Different crops are often grown side by side, like cereals and vegetables or cereals and trees. The production technologies are primarily traditional. A large number of insect pests have been recorded on major crops in Ethiopia, but only a few of these are considered to be of economic importance. Examples of these pests are migratory pests like locusts and the African armyworm. crop yield losses due to pests and diseases in Ethiopia and in Africa are about 30-40 %. However, these great yield losses are seen mostly in commercial agriculture, with monoculture[27].

The state sector, with the former Ministry of State Farms Development, has been the major user of pesticides in Ethiopia. research on pesticides has been going on in Ethiopia since the 70s. Almost all pesticides are imported. Some come as donations from industrialized countries. Between 1983 and 1993 the import was approximately 3.800 tonnes annually and about 203 tonnes annually was donated. Of the imported pesticides 72 % were insecticides, 25% herbicides, 2.6 % fungicides and 1.3 % others. (Abate T. 1996.)[27].

Pesticide use in Ethiopia state farms is estimated at 7.76kg/ha/yr,and less than 0.1kg/ha/yr in smallholder farms. cotton on commercial farms uses 90% of imported insecticides. inventories made in Ethiopia (Ministry of Agriculture ,2000) showed over stores with 1,500tonnes of obsolete pesticides and 1,000 tones of contaminated equipments (containers, sprayers, etc). The storage conditions were poor, with leaking drums ,burst open sacks, and the stores themselves in a bad state ,posing great threat to human health and the environment, Overall Ethiopia was considered to be one of the worst contaminated countries in Africa, Over the last 10 years, the Ministry of Agriculture and Rular Development has removed 1,507 tones of pesticides from eight states namely (1)Mekele, (2) Kombolcha, (3) Bahir Dare,(4) Nekemt, (5)Jimma, (6) Awassa (7) Dire Dewa and (8) Addis Ababa [28].

The most commonly used pesticides in Ethiopia are organophosphates, carbamates and to some extent organochlorides. They are usually applied as an aerosol produced from knapsacks and from simple hand sprayers. (Mekonnen Y and Agonafir T, 2002.[3].In particular more than 50 percent of processed skins are rejected in Ethiopia because of the “Ekek” ectoparasite disease spread by lice and keds. The disease was identified in the early 1990s by a FAO project that also recommended diazinon as the most cost effective way to control the disease. Under a partnership with the Ethiopian Veterinarian Association (EVA), the team started a pilot project to demonstrate the efficacy of diazinon in controlling Ekek and to establish a pre-harvest traceability program. The project was launched in partnership with the Ethiopian Tannery, Pittards and Debre Birhan Research Institute with an estimated budget of 800,000 birr through cost sharing. The trials will be conducted on 1,220 sheep by the Dibre Birhan Research Institute. This project is designed to demonstrate the positive effect of diazinon treatment on the parasites causing Ekek. The skins of Menz and Washera sheep breeds will be analyzed to determine their respective resistance to Ekek under the treatment. The trials will also demonstrate that parasite-free sheep from birth should have no Ekek defects when compared to those that had the parasite and were latter treated[29].

1.5 Some Methods for Analysis Dazinon

Considerable interest in the development of highly sensitive, selective, rapid and reliable analytical methods of detection for toxic chemical compounds in the environmental matrix have been grown it is important to develop methods for control of specific amounts of this compound due to its toxicity for living organisms. There are several analytical methods for establishing level of concentration for diazinon [30]. The available methods for DZN analysis include enzyme linked immunoassay, gas chromatography ,high-performance liquid chromatography (HPLC) with mass spectrometric detection (LC-MS), capillary electrophoresis and HPLC with ultraviolet(UV) [16, 31].

Many applications are based on chromatographic determination, both GC and HPLC, using different detection systems, Chromatography method offer great advantages because they can discriminate individually the pesticides belonging to the same family. In spite of the advantage of chromatographic techniques, these methods do not easily allow continuous on-site monitoring and usually require a previous exhaustive step of sample preparation, which includes an extraction step and a clean-up procedure in order to obtain a final extract fully with the chromatographic determination[32]. In addition ,chromatography usually generate waste containing organic solvents, which requires their treatment after analysis.

Due to a growing interest in rapid and accurate determination the standard procedure based, on liquid chromatography(LC) or gas chromatography (GC), are very reliable but there is a need for fast and innovative methods, especially in field and on-line applications. Electrochemical enzyme sensors are considered as an alternative to the conventional spectrometric techniques for pollutant determination due to their simplified sample treatment, portable, economical, fast and sensitive analysis[32].

Quantitative detection of some pesticides and insecticides using electrochemical biosensor based on enzyme activities have emerged as the past few years as the most promising alternative to detect pesticides. Among these electrochemical methods on AChE have shown satisfactory results(low detection limits) for pesticide analysis. But this methods suffer from extensive pre-treatment steps and lengthy time of analysis [34].Electrochemical techniques are more environmentally friendly than the chemical methods in polluted reaction[31]. Most of the method mentioned above had the major drawback and not suitable for suitable analysis. Electrochemical method provide quantitative detection of pesticides with a low coast short analysis time and can be easily mineralized. Voltammetric detection methods have been widely used in many field. However there are many reports on the direct voltammetric pesticides determination because of their simplicity. So this methods have drawn more and more attention for OP residue analysis [34].Generally some of the electroanlytical methods applied for the detection of diazinon in different environmental(water,soil etc…) as well as in samples (blood, urine etc…) summarized in Table -2- below.

Table 2: Summery for the determination of Diazinon in different analytical Methods

Method	The sample in which DZN analyzed	Linear range	Limit of detection	Recovery %	Reference
GC-FPD	Onion &Welsh onion	0.002-0.01mg/kg	0.003 mg/kg	61 to 105	35
LC-tandom MS	Human blood	0.5 to 100ng/ml	0.5ng/ml		30
GC/ECD/FPD using SPME	Human fluids	1 to 0.4ng/ml		Over 70	46
DLLME	Tea	0.01-20.0ng/g	0.1µg/kg	85.6	47
ELISA	Olive oil		46ng/ml	Between 94 &122	63
Column adsorption. MWCNT-HPLC	Tap-water	0.3 to 10,000ng/ml	0.06 ng/ml		48
Voltammetric using NGCE .		0 to 5µM	75nM	99.4	49
SPE &GC/MS	Postmortem blood	0.4 -277 mg/L	0.04mg/L	87±2.5 79±7.5	64
SPE &HPLC with UV detector	Rat urine	50 and 150 ng/mL		81.3 ±7.6	50
	Rat plasma			86.3± 8.6	50

DPP& adsSV	Water/methanol solution		2.65×10^{-6}M & $4.01 \mathrm{X} 10^{-9}$M		51
SPME&GC/ECD/FDP	Urine	0.1-0.4 ng/ml		Over 70	52
LC-UV	Standard solution	25-2500 ng/ml	0.47ng/ml	66.2 ± 1.6	53
HPLC-UV	Plasma&urin of rat	20-200 ng/ml	80-200 ng/ml		54
Amperometric biosensor/Screen –print Tyr ME	Natural river	19-50ppb		92.5-98.5	55
CV and SWA &SWC stripping volt.		6 to 62 ng/L	0.5ng/L (1.64PM)		56
CV at CPE modified with $Co(en)_3I_2.3H_2O$		0.05 to 25ppm	0.0075ppm		50
Conductometric tyrosnas biosensor	Water solution		5.0×10^{-8}M		57
Acetylcholinesterase-polyaniline biosensore			0.147ppb		58
Optical SSD CuC_1TPP		700ppt to 40ppm	800ppt		45

1.6 Chemically Modified Electrode

The nature of the working electrode, particularly its bulk composition and surface treatment, is critical to the performance of the redox reaction. Carbon paste, glassy carbon, mercury, and metal electrodes are the common electrodes used in electrochemical reactions. There is active research in the preparation of chemically modified electrodes [8,21].

Chemical and/or electrochemical surface pretreatments are always needed in order to get reproducible data on solid electrodes ; they are mostly focused on the removal of surface impurities (oxides). The 'activation' of solid electrodes by (electro) chemical pretreatment is, however, a complex and not well understood process. A qualitatively new approach to the surface pretreatment of solid electrodes is their chemical modification, which means a controlled attachment of suitable redox-active molecules to the electrode surface. The anchored surface molecules act as charge mediators between the electrode and a substance in the electrolyte[36].

In an ideal case the electroactive mediator is attached in a monolayer coverage to a flat surface. The immobilized redox couple shows a significantly different electrochemical behavior in comparison with that transported to the electrode by diffusion from the electrolyte. In general, a complex structure is designed toward a particular end, perhaps to facilitate an electrode process , or to inhibit a reaction , or to produce selectivity toward a particular process [36]

1.6.1.Electrocatallycally Modified Electrode: often the desired redox reactions at the bare electrode involves slow electron transfer kinetics and therefore occur at an appreciable rate only at potentials substantially higher than its thermodynamics redox potential. Such reaction can catalysed by attaching a suitable electron transfer mediator. Electrocatallytic reaction play a central role in electrochemistry and a vital role in sensing and energy related application. The function of the mediator is to facilitate the charge transfer between the analyte and the electrode. In most cases the mediated reaction sequence (for reduction process) can be described by [8,49]

$$M_{ox} + ne^{-} \longrightarrow M_{red}$$

$$M_{red} + A_{ox} \longrightarrow M_{ox} + A_{red} \qquad (1)$$

Where M represent the mediator and A, the analyte. Hence the electron transfer takes place between the electrode and mediator and not directly between the analyte and the electrode. The active form of the catalyst electrochemically regenerated. The net result of electron shutting are a lowering of the overvoltage to the formal potential of the mediator and an increase in current density[8,37].

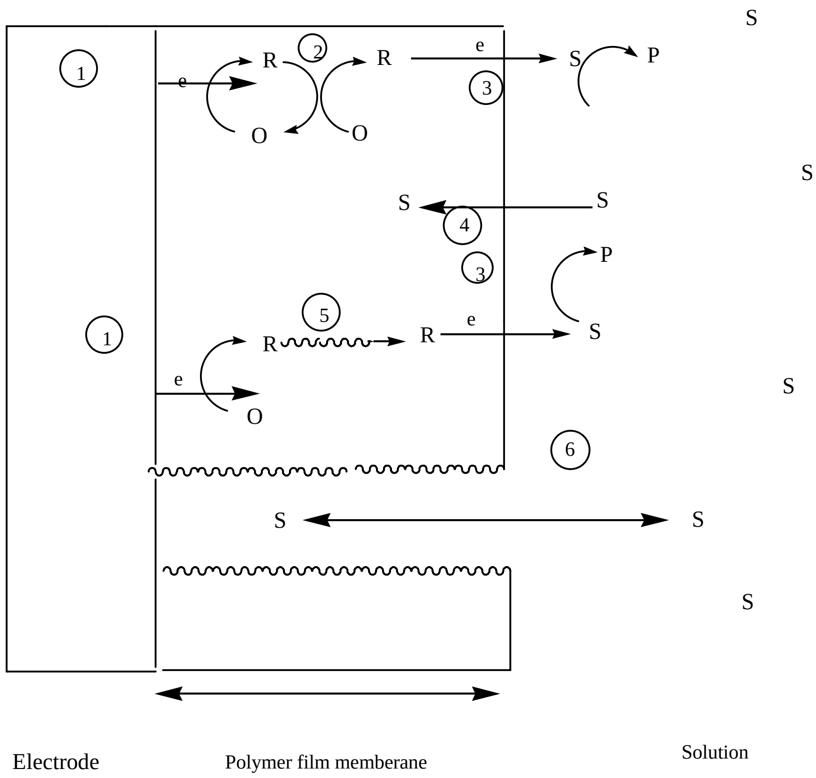


Figure 7. Schematic diagram of processes that can occur at a modified electrode. O represents a reducible substance in a film on the electrode surface and S, a species in solution. Processes shown are: (1) heterogeneous electron transfer to O to produce the reduced form, R; (2) electron transfer from R to another O in the film (electron diffusion or electron hopping in the film); (3) electron transfer from O to S at the film/solution interface; (4) penetration of S into the film (where it can also react with O or at the substrate-film interface); (5) movement (mass transfer) of O within the film; (6) movement of S through a pinhole or channel in the film to the substrate, where it can be reduced [21].

The end is achieved by developing within the structure a favorable interplay of the dynamics by which electrons are conveyed between the electrode and the species whose oxidation or reduction is ultimately required to achieve the goal. It may be necessary to employ a selective catalyst, or to restrict access to the interior of the structure, or to allow for the ready flow of electrons to more remote parts of an assembly. Many modified electrodes are based on films thicker than the monolayer, and the theoretical treatments are more complex, because mass transfer and reaction kinetics within the film must be considered. A schematic picture of the typical situation is given in Figure 7, wherein a primary reactant, S, in the external solution is converted to a product, P. This can occur by mass transport of S moving through the film to the underlying electrode or by cross-reaction with a catalyst, O, contained in the film and renewed electrochemically. Species S might react with O either within the film or at the film/solution interface.2[21]. In figure 7 the substrates, which is irreversibly (or quasi-reversibly) reduced at the bare electrode ,is transported across the polymer film-solution interface and diffuse in to the polymer film membrane. The electrocatalyst mediator, R/O, undergoes heterogeneous electron transfer at the electrode surface and charge propagation through the polymer film is described by a rate of given by the charge-transport diffusion coefficient. The mediator undergoes homogeneous electron transfer with the substrate in the polymer film[38].

The transport of charged through the polymer is though to take place via an electron hopping mechanism . The electron hop from one redox active site to the next . Depending upon the nature of polymer coating ,three processes can potentially control the charge transport process within the layer. These are 1/.Polymer movement 2/. Electron movement 3/.Ion movement. Electrocatalysis accelerates the electron transfer of the target analyte which is slow at the same potential at a bar electrode induced by an immobilized charge mediator i.e., catalyst. Catalyzed electrode reactions usually occur near the formal potential of the mediator.

Characterization of modified electrodes can be carried out by electrochemical, spectroscopic, and microscopic methods. Of the electrochemical methods stress cyclic voltammetry which combined together measure the number of redox centers, film conductivity, kinetics of the electrode processes, etc. Modified electrodes often give rise to currents that are higher than in the absence of the modifier. Sometimes, on placing the modified electrode in a solution that contains supporting electrolyte only, the voltammetric characteristics of the immobilized species are observed. This is extremely useful for diverse applications such as, for example, in electroanalysis [6].

There are several ways of preparing different type of modified electrodes:Chemisorption, Covalent bond formation, coating electrode film with polymer film and Langmuir –Budgett film (LB) method. Of these polymer coated electrodes may be further sub-divided by the process used to apply the film. These are Dip-coating, solvent evaporation, spin coating, Electrochemical deposition ,Electrochemical polymerization and crossing –linking[22].

1.6.2 Electronically Conducting Polymers. The world-wide interest in electronically conducting polymers has been triggered in 1977/8,[8]. Electronic conducting polymers (such as polypyrol, polythiophen and polyaniline) have attracted considerable attention because of their ability to reversibility switch between the positively charged conductive state and a neutral, essentially insulating form ,and to incorporate and expel anionic species (from and to the surrounding solution),on oxidation or reduction:

$$P^{o} + A^{-} \rightarrow P^{+}A^{-} + e^{-} \qquad (2)$$

Where P and A^- represent the polymer and the” dopant” anion, respectively. The latter serve to maintain the electrical neutrality, counter balancing the positive charge of the polymer backbone [6].

The redox changes(2) are not localized at a specific center, but rather localized over a number of conducting polymer groups. The conjugation of the π-electron system creates molecular orbital which extend through out the polymer chain. The electrical conductivity of this film ,which originated from the electronic structure their polymeric backbone (i.e electron hopping involving the delocalization of π-electrons) can vary with the applied potential. The conductivity values depend on the amount of carriers (electrons or holes) created in the polymer chain and the carriers mobility through the polymer. These polymers are readily prepared by in situ polymerization (from monomer solution).Oxidation of monomer proceed according to (Figure 8)

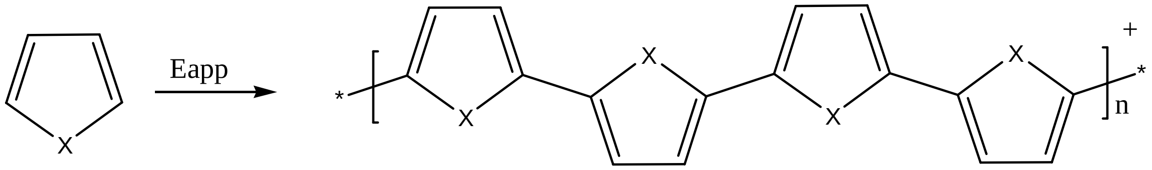


Figure 8. Oxidation of X=NH(polypyrol),X=S(polythiophene) ,X=O(polyfuran)

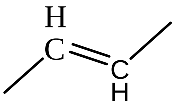
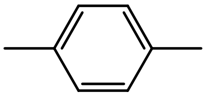
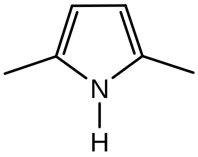
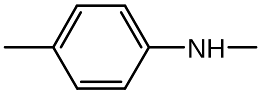
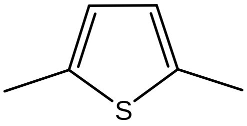
Often the first step in the electropolymerization process is the electroxidative formation of a radical cation from the starting monomer. This step commonly followed by a diamerization process, followed by further oxidation and coupling reactions. The behavior of electropolymerizaed film can be controlled by polymerization conditions including the electrolyte (particularly the nature and level of anion serving as dopant),solvent monomer concentration, applied potential or current, and duration.

Oxidation or reduction of polymer can be performed either chemically or electrochemically; this process is often termed p- or n-doping . A polymer can partly be oxidized or reduced with considerable increase in conductivity. In both cases, the polymer backbone is virtually intact except for accumulating positive (p-doping) or negative (n-doping) charge. This charge is compensated by ions of opposite sign, which are inserted into the polymer backbone either from a chemical dopant or from a supporting electrolyte.

Doping of the conjugated polymers means oxidation (p-doping) or reduction (n-doping) of the polymer skeleton associated with insertion of a corresponding number of anions (cations) into the polymer matrix. The electron hopping take place in the conduction band between localized states within the energy gap. This can be described by Mott's model of variable range electron. The charge transport in a conjugated chain and the inter chain hopping is explained in terms of conjugation defects (radical or ionic sites), called solitons and polarons

A soliton is an electroneutral radical site with a spin equal to 1/2 connected to the rest of the chain by somewhat longer bonds; the charge and the bond lengths are however ,distributed in the close surroundings of the defect. Two neutral solitons can recombine and the chain defect is cancelled. On the other hand, charged and neutral solitons in neighboring sites form a stable pair called polaron. Both the solitons and polarons are mobile along the chain, but a soliton, unlike a polaron, moves virtually without overcoming energy barriers. All important electronically conducting polymers, except perhaps for polyacetylene, can be prepared electrochemically by anodic oxidation of the monomers[36].

Table 3 Examples of electronically conducting polymers, y is the level of electrochemical doping and κ is the maximum electricalconductivity. except for polyacetylene and polyparaphenylene, only p-doping is considered

Polymer	Repeated unit	y	κ(S/cm
Polyacetylene			
n-doped		0.09	100
p-doped		0.05	1000
Polyparaphenylene			
n-doped		0.44	100
p-doped		0.1	100
Polypyrrol		0.4	500
Polyaniline		0.2	5
Polythiophene		0.2	190

A mediator facilitates electron transfer and minimizes attraction and repulsion effects between the biological molecule and the electrode. However, a careful choice of substrate may lead to the development of methods that do not need mediator and allow direct immobilization. In technology, conducting polymers will probably have an important role, as they can be successively oxidized or reduced[36].

$$\begin{array}{c}\textbf{Insulating polymer}\\ \textbf{neutral}\end{array} \rightleftarrows \begin{array}{c}\textbf{Oxidized polymer}\\ \textbf{conducting}\end{array} \qquad (2)$$

In specific cases, electrode bulk as opposed to surface modification can be employed. The modifier, a substance that reacts preferentially in some way with a species to be determined. The mode of action is either by catalyzing the analyte reaction or pre-concentrating the analyte on the surface before determination.

Poly(3,4-ethylenedioxythiophene) (PEDOT), a polythiophene derivative with high electrical conductivity, transparency, structural stability suitable morphology and fast doping–undoping mechanism, has also attracted much attention. This material, which was originally described and is currently commercialized by the Bayer company, exhibits higher thermal and environmental stabilities, and easier processability than common conducting polymers, including PAni and PPy . Consequently, it has been proposed for several technological applications, e.g. as sensor , molecular condenser and anticorrosive additive of conventional organic coatings [39,40]. One derivative of thiophene that has become immensely popular is 3,4-ethylenedioxy thiophene (EDOT). Recently, poly(3,4-ethylenedioxythiophene) (PEDOT) has emerged as a novel polymer with enhanced electrochemical stability when compare to polypyrol(Ppy)[53]. PEDOT, is exceptionally stable in its doped state and highly conducting. It also exhibits a low oxidation potential with respect to classical polythiophene derivatives. Wudl and co-workers reported that PEDOT have promising applications in the field of “plastic electronics”.[42,43].

3,4-ethylenedioxythiophene (EDOT) can be polymerized electrochemically to form the semiconducting polymer poly(3,4-ethylenedioxythiophene) (PEDOT)[39,42]. To avoid the undesired β couplings within the conjugated polymer backbone and increase the electron-rich character while lowering the band gap, scientists at Bayer AG developed poly(3,4-ethylenedioxythiophene) (PEDOT) in the 1990s .

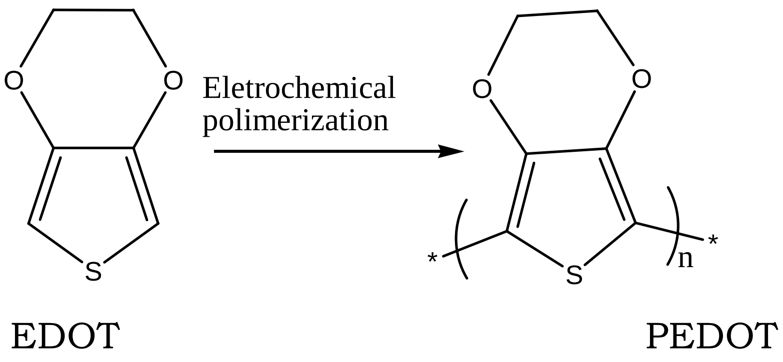


Figure 9. Electropolymerization of EDOT (monomer) to PEDOT (polymer)

Combining a relatively high conductivity with optical transparency in the doped state, this water-processable polymer has become one of the most important commercial conducting polymers for electronics [44]. Poly(3,4-ethylenedioxythiophene) (PEDOT) is a highly conductive, low band gap (1.6 eV) polymer. In the oxidized state, thin films of PEDOT are stable and nearly transparent[57].

1.7 Objective of the Study

At a modified electrode the modified surface is deliberately altered by adsorption ,by the physical coverage ,or by bonding of specific species. The result is to block direct access to the electrode , inhabiting some electrode processes and promoting others. Modification can therefore be an important aid in obtaining great selectivity, and thence importance in analysis. This modification can be done on the working electrode array to produce tiny specific chemical sensors. Normally the modifier layer is electroactive acting as mediator between the solution and the electrode-substrate in electron transfer [6].

In this work, it is planned to combine the interesting concepts mentioned above for the electrochemical sensitive determination of diazinon (an organophosphate pesticide) by using PEDOT modified glassy carbon electrode in voltammetric method.

The specific objective is :

i / To study electrochemical behavior of Diazinon at PEDOT modified glassy carbon electrode (GCE)

ii/ To establish the optimal parameters for the sensitive determination of diazinon (DZN) i.e to study the basic electroanalytical parameters required to determine DZN at the specified condition

iii/ To study the analytical application of the developed method for the determination of Diazinon on portable water sample

2. Background

2.1 Practical Consideration

2.1.1 Electrochemical Cells

The basic instrumentation required for controlled-potential experiment readily available commercially. The basic necessities include a cell (with the three electrode cell) a voltammetric analyzer ,and a plotter. The three –electrode cell, Figure 10; are commonly used in controlled-potential experiments. The cell is usually a covered beaker of 5-50mL volume, and contains the three electrode (working, reference, and auxiliary),which are immersed in the sample solution. While the working electrode is the electrode at which the reaction of interest occurs ,the reference electrode provide a stable and reproducible potential(independent of the sample composition), against which the potential of the working electrode compared. Commonly used reference electrodes are Ag/AgCl and Hg/Hg_2Cl_2. As inert conducting material, such as platinum wire or graphite wire ,is Usually used the current-carrying auxiliary electrode[8,58].

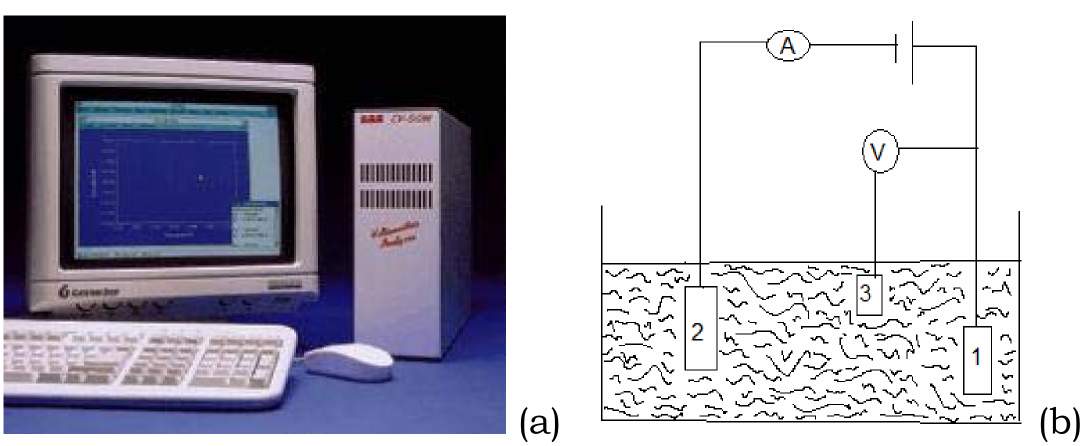


Figure 10. (a) BAS model CV-50W Voltammetric Analyzer ,(b) Schematic diagram of a cell for voltammetric measurements:1/Working electrode 2/ Reference electrode 3/ Auxiliary electrode

Electrochemical measurements are commonly carried out in a medium that consists of solvents containing a supporting electrolyte. The choice of solvent is dictated by the solubility of the analyte and its redox activity, and by the solvent properties[8,6,33].

2.1.2 Working Electrode

The performance of the voltammetric procedure is strongly influenced by the working electrode material. The Working Electrode should provide high signal-to-noise characteristics ,as well as reproducible response. Thus its selection primarily depends on two factors: the redox behavior of the target analyte and the background current over the potential region required for the measurement. Other consideration include the potential window , electrical conductivity ,mechanical properties ,cost, availability and toxicity. The most popular are those involving mercury, carbon or noble metals. Mercury is a very attractive choice for the electrode materials because it has a high hydrogen overvoltage that greatly extends the cathodic potential window (compare to solid electrode materials) and possesses a highly reproducible, readily renewable , and smooth surface. Disadvantage of the mercury are its limited anodic range (due to oxidation of mercury) and toxicity. The most common popular carbon electrode materials are those involving glassy carbon, carbon paste ,carbon fiber ,screen- print carbon strip carbon film and other carbon compositions.

Glassy Carbon Electrode: Glassy carbon has been very popular because of its excellent mechanical and electrical properties , wide potential window, chemical inertness (solvent resistance),relatively reproducible performance. The structure of glassy carbon involves thin, tangled ribbons of cross-linked graphite- like sheets. Because of its high density and small pore size, no impregnating procedure is required. However surface treatment is usually employed to create active and reproducible glassy carbon electrodes and to enhance their analytical performance such pretreatment is usually achieved by polishing (to the “mirror-like “ appearance)with successively smaller alumina particles (down to 0.05μm). The electrode should them rinsed with deionizer water before use. Additional activation steps, such as electrochemical, chemical, heat or laser treatments, have also been used to enhance the performance

2.2 Cyclic Voltammetry

Cyclic voltammetry is the most widely used technique for qualitative information about electrochemical reactions. The power of cyclic voltammetry result from its ability to provide considerable information on the thermodynamics of redox processes and the kinetics of heterogeneous electron transfer reactions and on coupled chemical reactions or adsorption processes. Cyclic voltammatry is the often the first experiment performed on the electeroanalytical study. In ‘particular it offer a rapid location of redox potential of the electroactive species, and convenient evaluation of the effect of media on the redox process.

Cyclic voltammatry consists of scanning linearly the potential of a stationary working electrode (in an stirred solution), using a triangular potential waveform (Figure11). Depending on the information sought, single or multiple cycle can be used. During the potential sweep ,the potentiost measure the current resulting from the applied potential. The resulting current-potential plot is termed a cyclic voltammogram. The cyclic voltammogram is a complicated, time-dependent function of a large number of physical and chemical parameters.

Figure 12-illusteraes the expected response of a reversible redox couple during a single potential cycle. It is assumed that only the oxidized form O is present initially. Thus ,a negative-going potential scan is chosen for the first half cycle ,starting from a value where no reduction occurs. As the applied potential approaches the characteristic E^o for the redox process, a cathodic current begins to increase, until a peak is reached. After traversing the potential region in which the reduction process takes place (at least 90/n mV beyond the peak), the direction of the potential sweep is reversed. During the reverse scan, R molecules (generated in the forward half-cycle ,and accumulated near the surface) , are reoxidized back to O, resulting in an anodic peak [8,7].

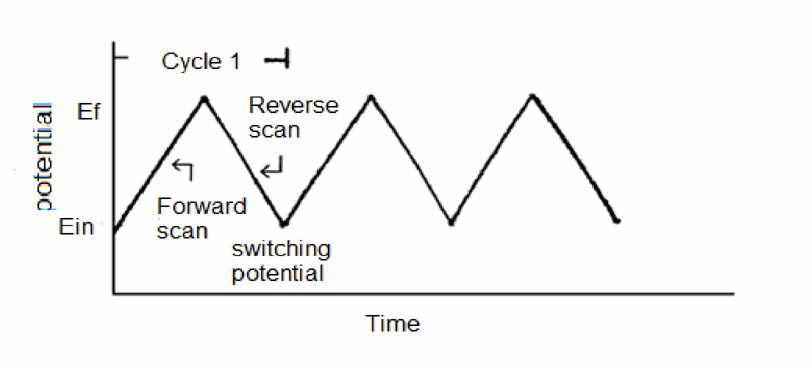


Figure 11. Potential-time exitation signal in cyclic voltammetric experiment

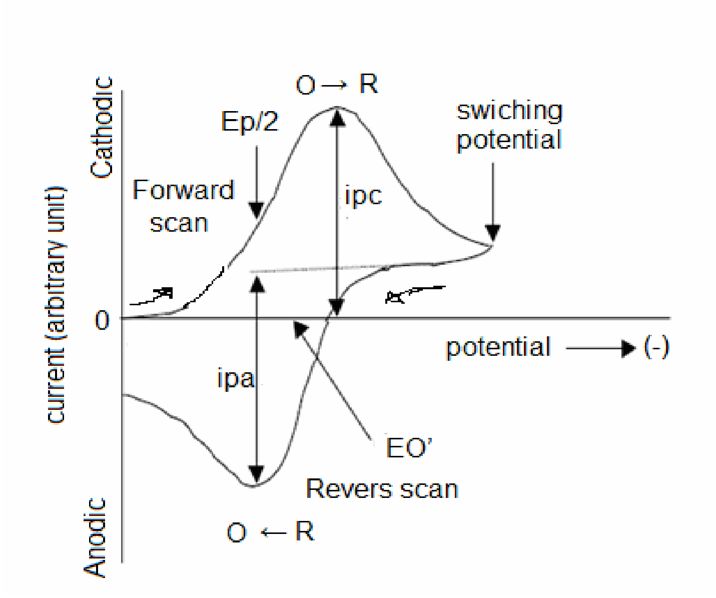


Figure 12. Typica cyclic voltammogram for reversible O + ne = R redox process: where $E_{p/2}$= half peak potential ,E^{0}'= formal reduction potential,, i_{pa} and i_{pc} are peak current for anodic & cathodic current at which the peak potentials Epa & E_{pc} occur ;respectively

2.3 Pulse Voltammetric Technique

Pulse voltammetric technique introduced by Bark and Jenkin, are aimed at lowering the detection limits of the voltammetric measurements. By substantial increasing the ratio between the faradic and non-faradic currents, such techniques permit convenient quantization down to the

10^{-8}M concentration level. Because of their greatly improved performance , modern pulse techniques have largely supplanted classical polarography in the analytical laboratory. The various pulse techniques are all based on a voltammetric techniques is the excitation waveform and the current sampling technique.

Differential –Pulse Voltammetry: Differential–Pulse Voltammetry is an extremely useful technique for measuring trace level of organic and inorganic species. In differential pulse voltammetry, fixed magnitude pulse-superimposed on a linear potential ramp-are applied to the working electrode at a time just before the end of the drop(Figure 13) The current is sampled twice, just before the pulse application (at 1) and again late in the pulse life (at 2). The first current is instrumentally subtracted from the second, and this current difference ($\Delta i = i(t_2) - i(t_1)$) is plotted against the applied potential. The resulting differential-pulse voltammogram is consists of current peak, the height of which is directly proportional to the concentrations of the corresponding analyst

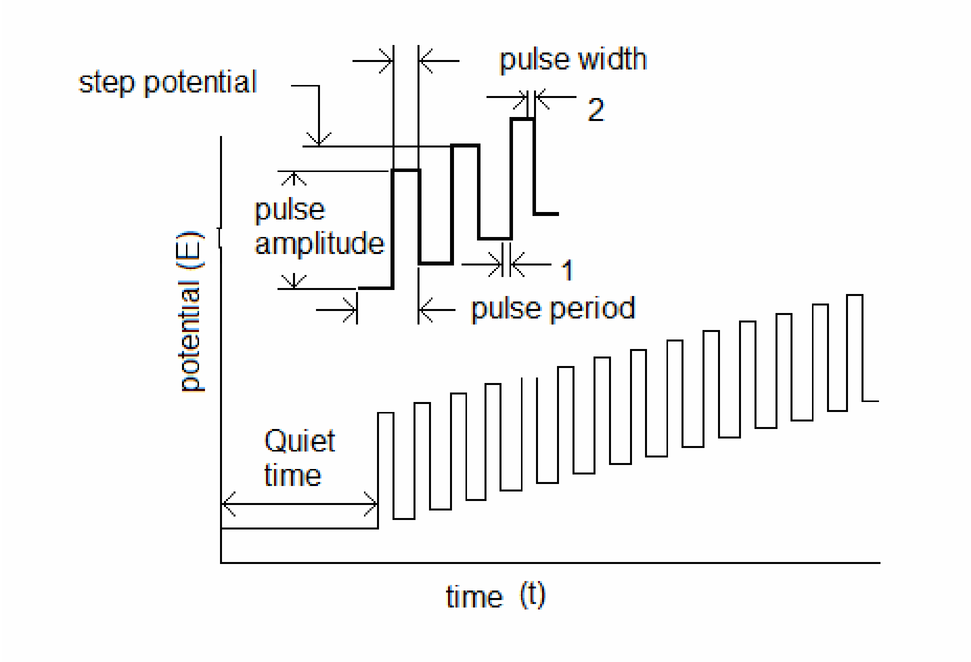


Figure 13. Excitation signal for the differential –pulse voltammetry

2.4 Preconcenterating Electrode

Preconcentrating CMEs, with surfaces designed for binding of target analyst ,hold greater promise for chemical sensing. The concept is analogous to striping voltammetric schemes, where the target analyte is preferentially partitioned from dilute sample into preconcenterating surface layer, and is subsequently reduce or oxidized during a potential scan. Unlike conventional stripping procedures, the preconcenterating step is nonelectrolytic. Most preconcenterating CMEs employ electrostatic binding or coordinating reactions, for collecting the analyte. The preconcenterating agents may be incorporated within the interior of carbon paste matrix or via functionalized films. The major requirements for a successful analytical use of preconcentrating electrodes are strong and selective binding, prevention of saturation ,and a convenient surface regeneration. Following the accumulation, the electrode can be transferred to more suitable solutions that facilitate the measurement and “cleaning”steps.

3.Experimental Part

3.1 Reagent and Chemicals

The reagent and chemicals used were Diazinon(5mM),sodium citrate (0.1mol/L),citric acid(0.1mol/L),sulfuric acid(0.5mol/L) ,alumina (0.05μm) ,distill water ,0.01M EDOT (monomer),Concentrated HCl and 2M NaOH.

3.2 Apparatus

This work was performed using a three electrode system ,Glassy carbon electrode as working electrode, platinum foil as auxiliary electrode and Ag/AgCl as reference electrode. The electrochemical behaviors of diazinon at glassy carbon electrode were studied by using voltammetric methods. The voltammetric experiment were performed by using BAS CV 50W electrochemical analyzer which is connected with personal computer. A magnetic stirrer with a hot plate form was used for stirring in pH adjustments. The pH of the buffer solution was measured with a digital pH meter with combination glassy electrode

3.3 Procedure

Before each electrochemical test the surface of working electrode (glassy carbon) was polished subsequently with alumina(0.05µm) slurry to a mirror finish, treated for several cycles in 0.5M sulfuric acid solution by CV to activate it and finally rinsed with distilled water. Several buffer solutions were prepared in distilled water and their pH s were prepared in distilled water and their pHs were adjusted by pH/Ion meter .Before the electrochemical polymerizations; the appropriate working windows were selected using the supporting electrolyte solutions. Finally characterization & quantitative study were carried out by using the modified electrode under the specified conditions.

4. **Result and Discussion**

4.1 Modification of the Glassy Carbon Electrode

The polymer film was deposited electrochemically from 0.01 mol/L 3,4-ethylenedioxythiophene (EDOT) monomer solution The polymer film were made by applying eight cycles in the range of 0.0 to +1.30V at scan rate of 50mv/s.The film thickness was controlled by the number of cycles delivered at constant modifier concentration given above.

Once the electropolymerization complete, the polymer film deposited on the electrode surface was stabilized in 0.1mol/L citrate buffer solution by applying potential between -500mv to 500mv at scan rate of 50mv/s for 40 segments in pH 1 citrate buffer solution.

4.2 Cyclic Voltammetric Investigation

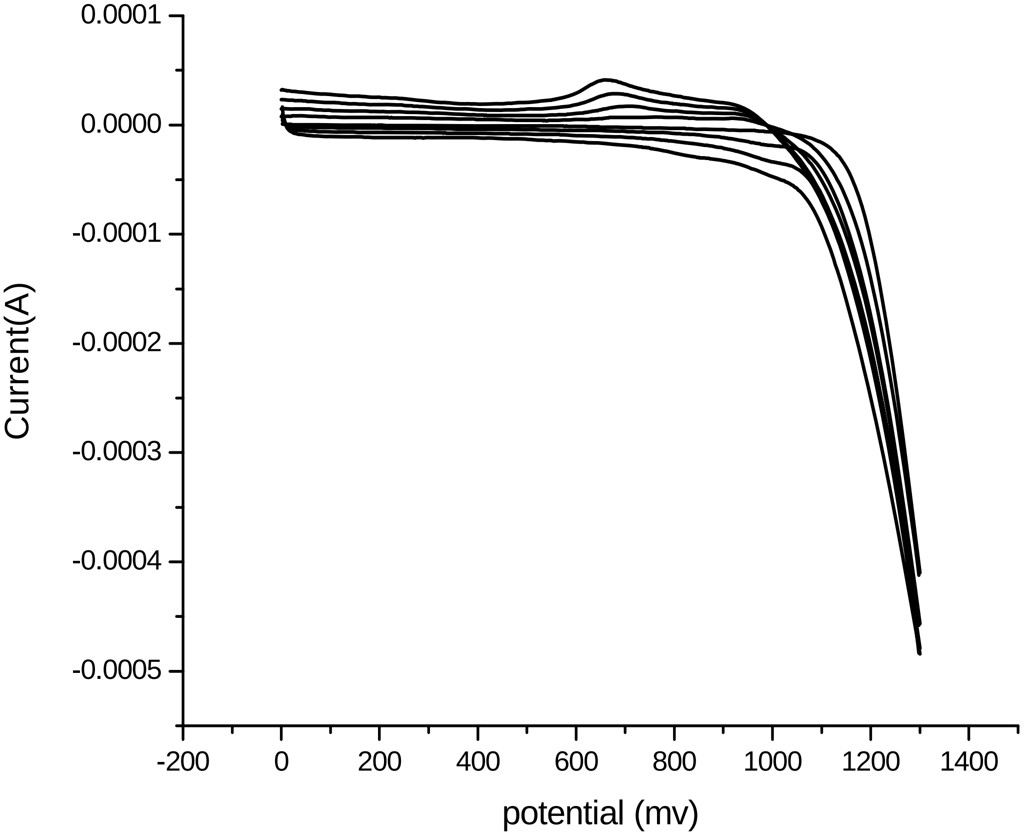


Fig 14. Repeated Cyclic Voltammograms for the electropolymerization of 0.01M monomer at glassy carbon electrode ,scan rate 50mv/s, number of segment 8

Figure 14 shows the repetitive cyclic scans corresponds to the polymerization of 0.01M of 3,4-ethylenedioxythiophene,EDOT measured by cyclic voltammetry. It has been well documented that multiple ring monomers with extended conjugation undergo facile electrochemical polymerization to form stable electroactive, electrically conducting polymers. These monomers polymerize at low enough potentials to avoid detrimental side reactions such as overoxidation of the polymer[41].

As showed in figure 14, current intensity becomes higher as the number of scan is increases ; this fact demonstrate the formation of polymer(PEDOT) films in the successive scan. When PEDOT synthesized through electrochemical method, the electropolymerization condition such as solvent the electrode, supporting electrolyte, polymerization condition and applied electropolymeriation method, have important effect on the properties of the PEDOT film. The polymer film deposited at the given potential range was stabilized by running cyclic voltammetry between -500mV to 500mV thus no more decrease in current intensity observed for about 40 number of cycles; this may be due to the formation of stable PEDOT film on the GC electrode(figure 15).The monomer expected to polymerized trough the external open position of the EDOT to form stable polymers. PEDOT emerge as a novel polymer with enhanced electrochemical stability[41].

The cyclic voltammetric studies on the electrochemical behavior of 1mM diazinon(DZN) have been carried out on both modified and unmodified glassy carbon electrodes. As the result below indicate that the voltammogram for the voltammetric response of diazinon on these two electrodes surface in citrate buffer pH 1 media differ significantly(Figure 16). The advantage of PEDOT modified electrode as signal enhancement for electrochemical sensing of diazinon in comparison to the bar glassy carbon electrode. The PEDOT modified GC electrode exhibited good activity (mediator) toward the oxidation-reduction of diazinon. This shows good voltammetric response to the diazinon.

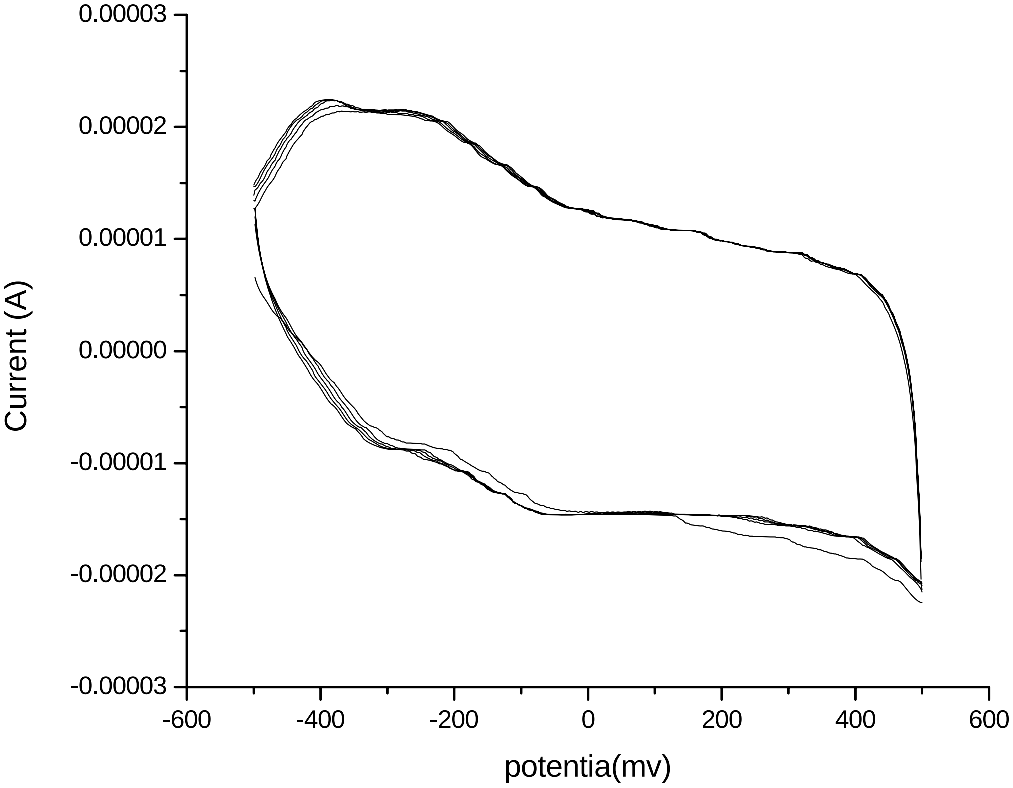


Figure 15 .Cyclic voltammetric response stabilization of the PEDOT film on the glassy carbon electrode in 0.1M citrate buffer(pH 1) at 50 mV/s scan rate & 40 number of segments.

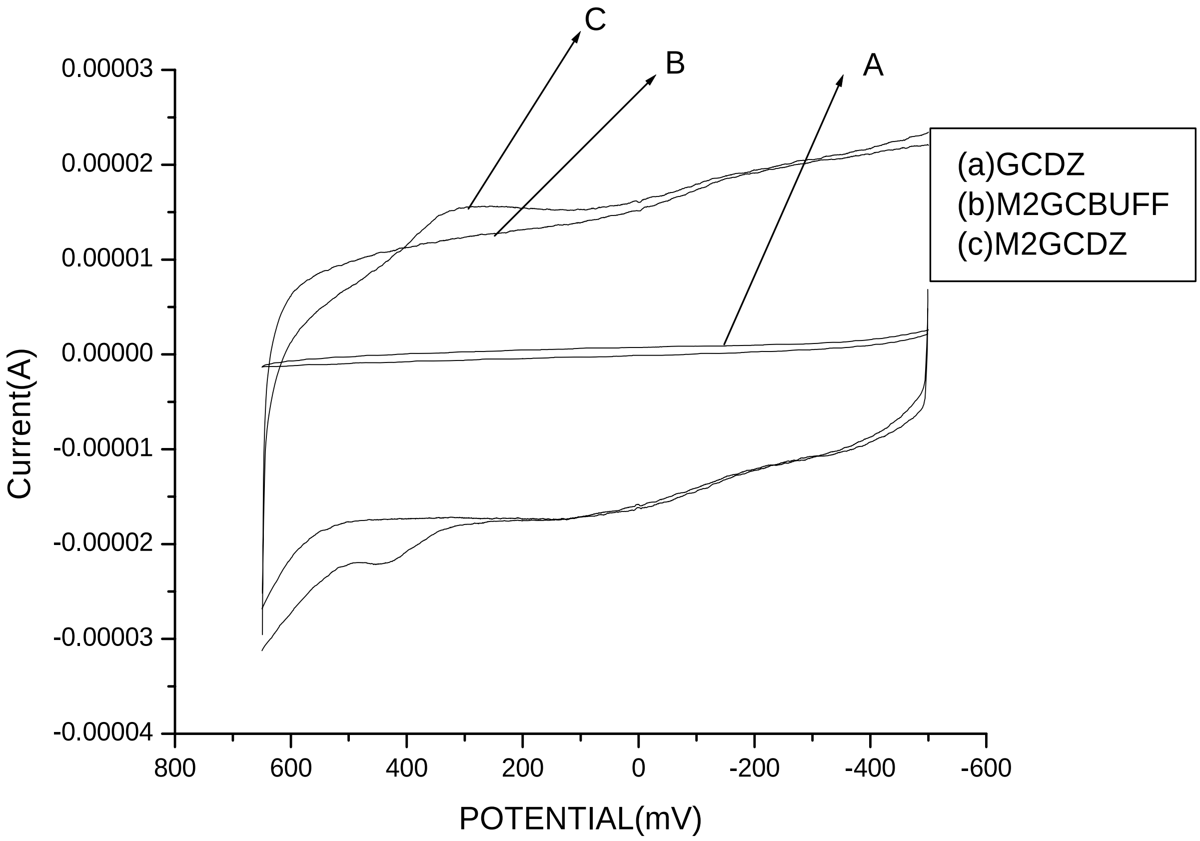


Figure 16 The Cyclic voltammograme response of 1 mM diazinon at PEDOT modified GCE in 0.1M citrate buffer (pH=1) at scan rate of 100mv/s ; switching potential at -500mv and 650mv

The advantage of CP modified electrodes over conventional metal or GCE electrodes is provided by the electrocatalytic ability of some CP. This often leads to better separation of oxidation (or reduction) peaks of the different analytes in voltammograms. The compounds are oxidized via an electron transfer from the analytes to the oxidized polymer units[57]

In Figure 16 curve (a) shows the response of bare glassy carbon electrode in the 1mM analyte and, curve (b) for the modified glassy carbon electrode in the citrate buffer solution ,while curve (c) represent the cyclic voltammogramme for the response of diazinon to ward the modified glassy carbon(M_2GC) electrode. From these the following conclusion can be made under the specified potential range i.e for curve (a) & curve (b) no more significant peak observed, but in case of curve (c) well defined cathodic and anodic peak depicted, although the background current is not subtracted. This will provide information about the importance modifying the substrate (bare glassy carbon electrode) by PEDOT in order to facilitate as a mediator the electron transfer between the GCE & the solution under the given conditions in order to detect the analyte (diazinon).

4.3. Differential Pulse Voltammetry Investigation

As can be see in Figure 16, the redox peaks obtained for diazinon with PEDOT modified electrode; the evaluation of the analytical performance of PEDOT modified GCE for diazinon analysis required optimization of many aspects associated to preconcenteration procedure .These includes accumulation potential, preconcenteration time and pH .According to this study, a series of experiment was conducted to attain suitable experimental conditions for the determination of diazinon. The effect of particular variable was studied under identified conditions by keeping all variables constant except one under study.

4.3.1. **Effect of Accumulation Time**

The deposition time a decisive factor in any technique dealing with preconcenteration step. The dependence of peak current on the preconcenteration time was studied for $1.0x10^{-3}M$ concentration of diazinon under careful controlled condition.

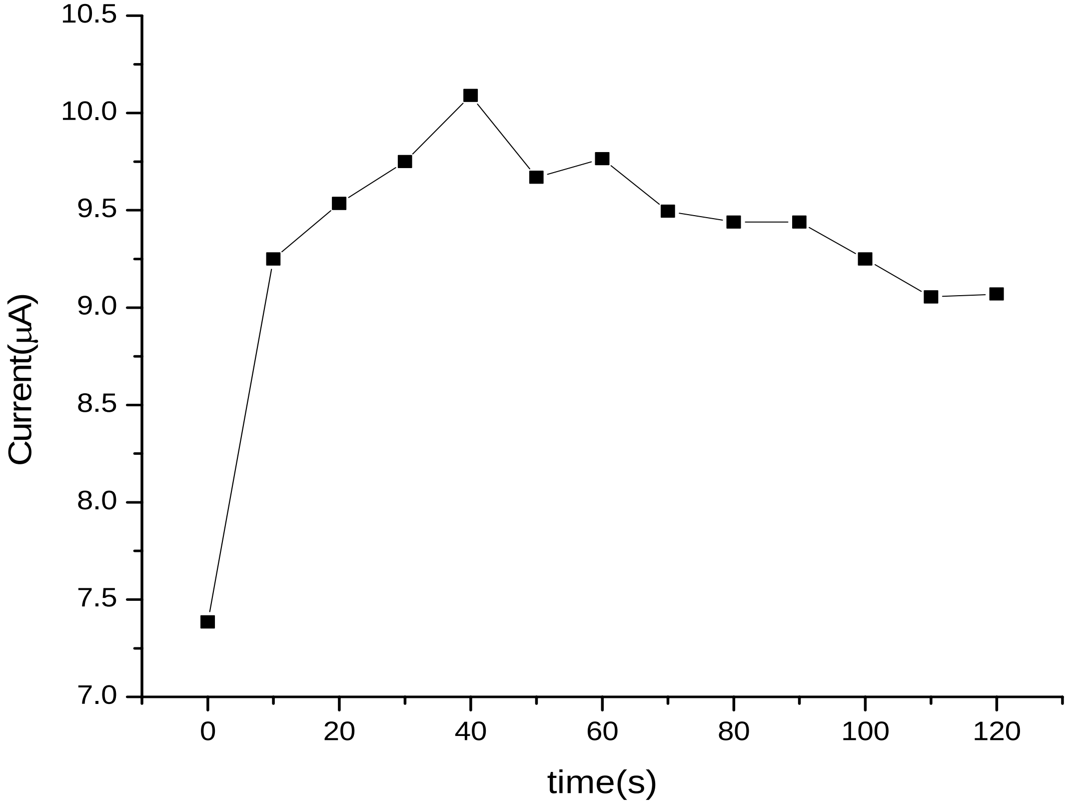


Fig.17. The relative response of PEDOT modified GCE as a function of the deposition potential. Experimental conditions:1Mm of diazinon in 0.1M citrate buffer ,Eapp=-300Mv.Each point was the mean value from three measurement.

Figure 17: Shows the dependence of the peak current on the accumulation time. The peak current increase with increasing accumulation time indicating enhancement of the diazinon concentration on the surface of PEDOT modified glassy carbon electrode. The dependence of peak heights on the accumulation time is an evidence for deposition current. Short preconcentration periods(up to 40 s) yield nearly a linear and rapid increment in peak current with an increase with time. Beyond 40s deposition periods the increase portion of curve decline ,the curve soon become nearly level off approaching a limit value. The manifestation of this limiting value the current at longer

accumulation periods could arise from the attainment equilibrium between at the modified electrode surface and in the solution or saturation of the deposition site.

4.3.2 Effect of Preconcentration Potential

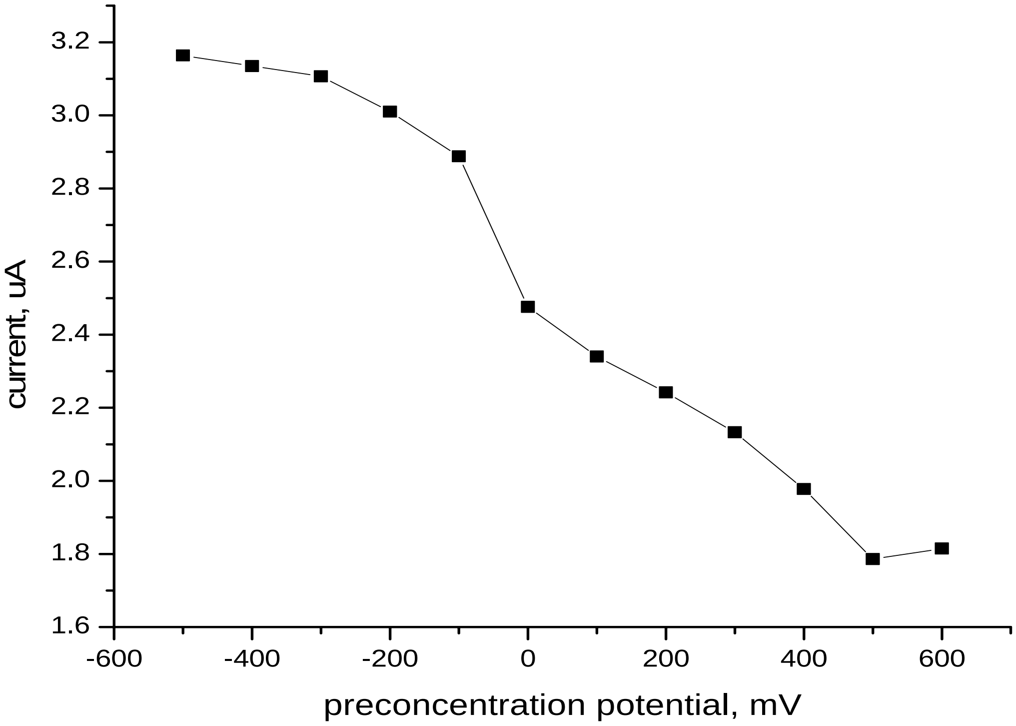


Fig.18. Effect of preconcentration potential on of Differential pulse Voltammetric response for 1mM diazinon in pH 1 citrate buffer at the PEDOT GC electrode

The effect of accumulation potential (E_{acc})on the peak current (i_p) of the differential pulse voltammetry were studied for the range of accumulation potential from -500 to 600mv; Figure 18 shows the effect of the accumulation potential on the peak current. At low and high accumulation potential values the peak current were relatively independent of accumulation potential where as at intermediate accumulation potential the values there was significant variation of the peak current. The peak current change is small in the indicated potential range of -0.5 to -0.3V. The reason is obvious that DZN cannot be oxidized in this potential range as shown in Figure 16.

The curve i_p-E_{acc} in the intermediate accumulation potential value can be explained by considering the sharp increment in DPV response. The peak current increase for the preconcenteration potential, up to -300mV; beyond -300mV accumulation potential the increasing portion of the curves become almost constant.

4.3.3 Effect of pH

The pH of the media used for the purpose of the accumulation has profound effect on the voltammetric response. It affect the rate and equilibrium state of the accumulation process and the rate of the electrode reaction. Therefore, throughout the process of accumulation pH of solution play crucial role . For that reason this variable was studied for diazinon at PEDOT modified GCE by differential pulse voltammetry in the pH range 1.0 to 8.0. The diazinon peak current as a function of pH is show in Figure 19. Diazinon show different voltammetric behavior in the pH range studied, This may due to the influence of pH on the accumulation potential. Generally on the modified glassy carbon the amount of accumulated diazinon is larger at pH 3.0. The observed behavior can be explained on the basis of the protonation /deprotonation character of diazinon. According to the observed result, PEDOT modified GC electrode at pH =3.0 has been selected to improve detection.

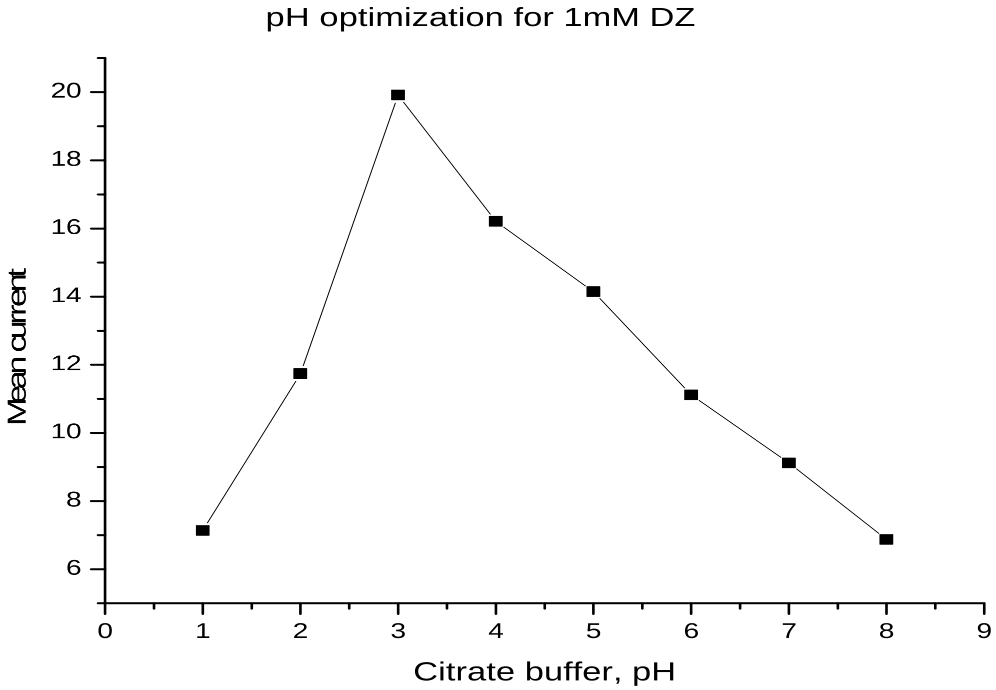


Fig. 19 .Influence of the pH value on the response of the modified GC electrode for 1mM diazinon ;measurement made by DPV employing 0.1M citrate buffer (pH 1) solution

4.3.4 Optimized Experimental Conditions

The effect of preconcentration time, deposition potential and pH were studied to obtain the optimized the experimental conditions for differential pulse voltammetric determination of diazinon at PEDOT modified glassy carbon electrode(M_2GCE). The optimum parameters identified for the determination of the analyte in order to plot the calibration curve are summarized as follow.

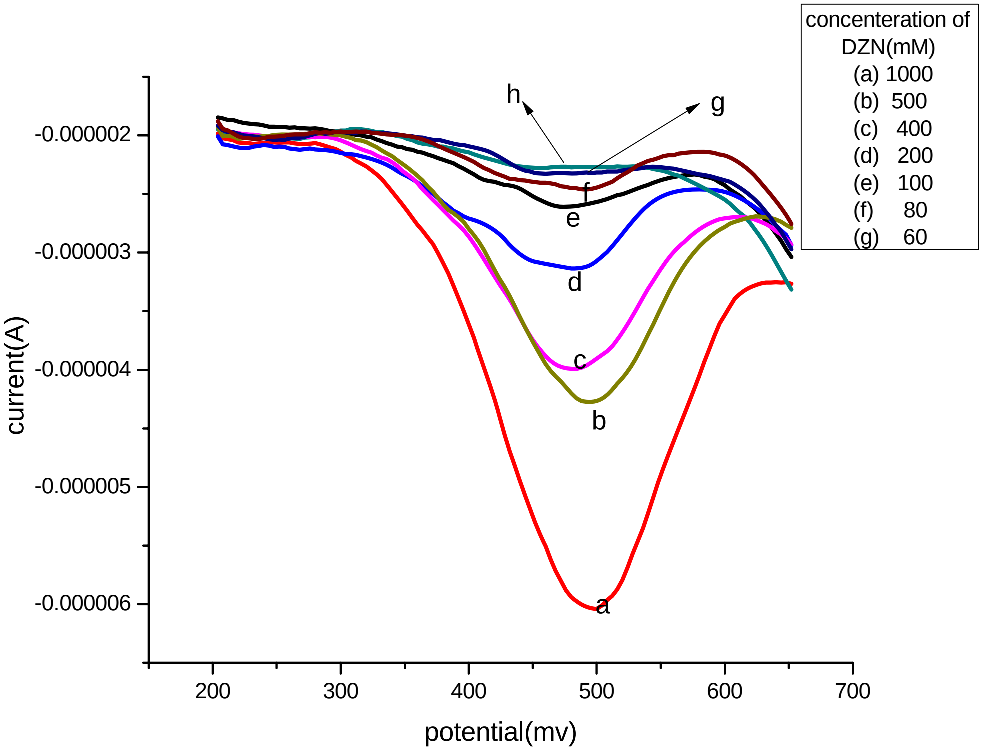
Table4: Optimum experimental conditions for the determination of Diazinon by DPV at PEDOT modified glassy carbon electrode.

Parameters	Optimum values
Ph of supporting electrolyte	3
Preconcentration time	40 s
Deposition potential	-300 mv

4.3.5. Linear Range and Detection Limit

The analytical utility of a given procedure depends on achieving well defined concentration dependence. According to the optimal experimental conditions and the procedures described above; the dependence of voltammetric signal on the concentration of diazinon and the inherited sensitivity of the method are illustrated by differential pulse voltammetry at different concentration of diazinon(Figure 20). The peak current was found to be directly proportional to the bulk concentration of diazinon.

The calibration data were obtained for Diazinon solutions under optimum experimental conditions described in table -4-. Figure (20) show some of the typical DPV voltammograme recorded on the PEDOT modified glassy carbon electrode (M_2GCE) after being in contact with different diazinon concentrations.



Figure(20) Differential pulse voltammograms for Diazinon solution of different concentrations from (a) to (h); where(a) 1x 10^{-3} M, (b) 5x 10^{-4} M, (c) 4x10^{-4}M, (d) 2x10^{-4}M, (e) 1x10^{-4}M, (f) 8x10^{-5} M,(g) 6x10^{-5}M ,(h) 4x 10^{-5}M

It is seen (Figure 20) that DZN starts to oxidized at approximately 0.45V and oxidation peak potential shift slightly to the higher value with the increase of concentration. This voltammetric behavior may be typical for electrochemically electroactive diazinon. Based on the DPV voltammograms taken for about three trials; the calibration data was collected and the corresponding calibration curves were plotted; the calibrations curve after the three trials are given below in Figure 21 by considering the mean current of the three trials under similar experimental conditions.

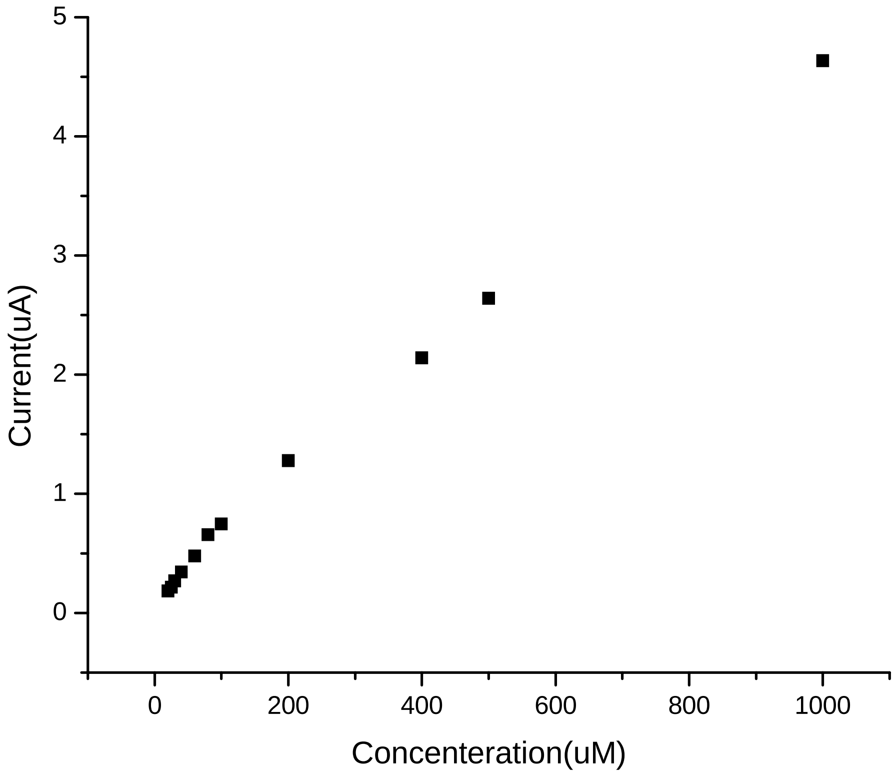


Fig21. Calibration curve corresponding to the response of M2GC electrode for different concentration of diazinon, the mean current value taken for the three trials

The limit of detection(LOD) is the lowest concentration of the analyte in a sample which can be detected but not necessarily quantified. The limit of quantification is the lowest concentration of the analyte in a sample which can be quantified with an acceptable degree of accuracy and precision[45].

The calibration curve in figure 21 and its linearity treated into two portions one of which lie in the rang of concentration between 100μM to 1000 μM and the second one is between 20 μM and 80 μM. However the more preferential one is most likely the one which show such linear range in smallest concentrations rang; if the linear range near to the small concentration rang expanded the portion of this calibration curve is plotted as indicated in Figure 22 below.

The linearity of an analytical method is its ability to produce test results that correspond directly to the concentration of the analyte in the samples within a given range. For the linearity studies, a minimum of five different concentrations are recommended[38].

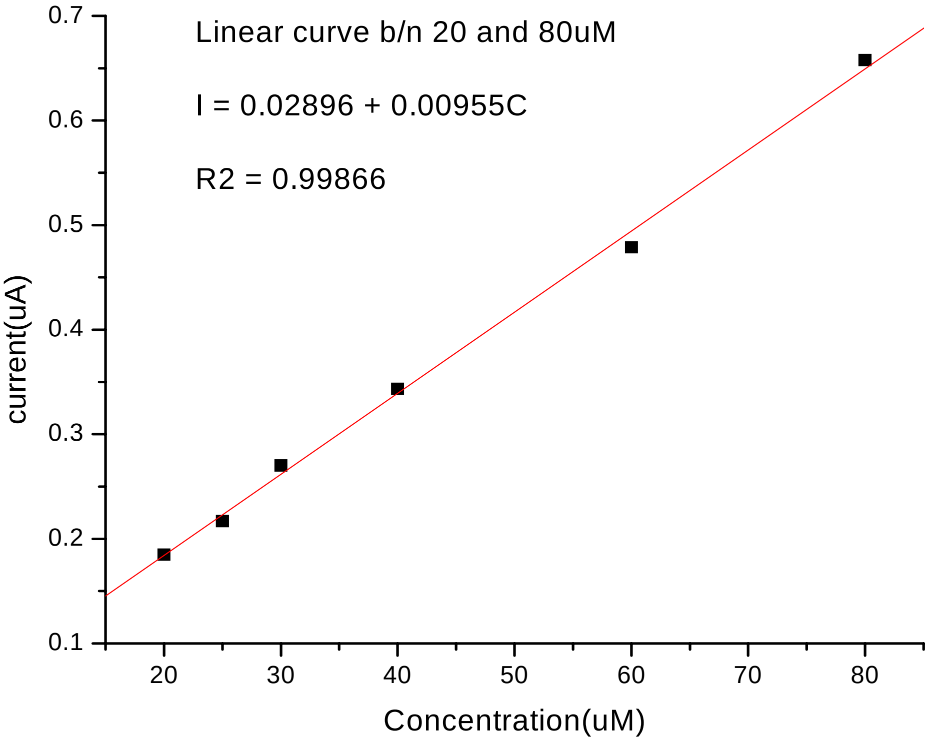


Fig.22 . Calibration curve correspond to the repose of M2GCE after expanding the linear detection limit in the indicated concentration range from figure 21.

The linear calibration rang (LCR) or rang of linearity is a region of a calibration curve in which a plot of the concentration of the analyte versus the response of the particular analyte remains linear [32]. A linear response was obtained in the range of concentration between 20µM and 80µM diazinon. The linear response equation for the dependence of peak current (**i_p**) and diazinon concentration(µM) in this concentration rang is given as:

$$Ip\ (\mu A) = 0.02896 + 0.00955C\ (\mu M) \qquad (4)$$

Where I_p peak current and C concentration of the diazinon with a slop(sensitivity) and a correlation coefficient of 0.00955 and 0.99866; respectively. The limit of detection based on three times of the standard deviation of the blank ($3S_b$) was $4.245x10^{-6}$ M for DZN

5. Recovery

The analytical utility of the method was assessed by applying it to the determination of diazinon in tap-water. None of the natural water samples analysed contained any diazinon, so it has to be spiked with the analyte at the certain concentration.

In order to taste the validity of the method, the proposed procedure was applied for determination of diazinon in water sample; recovery test were used to examine the reliability and accuracy of the method, for that reason tap-water sample & its diazinon content after deliberate contamination with different concentration were evaluated.

The diazinon solution of 50µM, 60µM and 80 µM were prepared by using the near by tap-water then the concentration of diazinon in each case were determined by standard addition method. The differential pulse voltammograms were recorded for each addition then the corresponding peak current reading used to calculate the equivalent concentration by considering the calibration curve equation in (4). The DZN content of each sample was determine at optimum conditions. The DZN content of different samples and recoveries of added analyte were evaluated; the result shows that it is possible to determine the DZN concentration in the real sample solutions using the propose method outlined in this investigation. The result summarized in table 5.

Table 5. Recovery of diazinon in spiked tap water sample by DPV using PEDOT modified glassy carbon electrode.

Sample	Amount added(µM)	Amount Found(µM)	Recovery %
Tap-water	60	57.15	95.24
	70	61.89	88.4
	80	65.03	81.29

6. Conclusion

The developed method of this study was simple, fast, sensitive and cheap especially, if no more sophisticated techniques such as chromatography is not available. The main feature of this work is to prepare PEDOT modified GC electrode simple electrochemical method. In addition, it is noticeable that up to my knowledge this may be the first modified glassy carbon electrode(M2GCE) used for the detection of diazinon. The detection of DZN using enzyme/mediator based on different types of modifiers have reported. Here the electrochemical behavior of PEDOT modified electrode has characterized using cyclic voltammeterically. LOD of the method (4.245μM) is comparable to some of the previously reported methods. The developed method was successfully applied for the determination of DZN in tap-water sample. Table 2 shows a comparison of some of the proposed method. Some reported LOD values (biosensor methods) are better than that of the developed method, but these methods suffer from extensive and expensive pre-treatment steps and lengthy time of analysis. The developed method shows a comparable & appreciable recovery with respect some of other the reported methods.

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