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Biosensor Design Aspects for Pesticide Monitoring

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***Abstract*— The indiscriminate use of pesticides in agricultural field resulted in environmental contamination and posed an incredible risk to human health. Conventional, methods used for pesticide detection have few impediments; thus there is a need to promote the build up such practical sensors that can aptly identify the pesticide residues to check the further absorption of these harmful toxins. This concern pulls our attention towards the development of Biosensors as a conservative device for fast acknowledgement of poisonous chemicals by making the sample preparation, extraction and cleans up methods simpler. Organophosphates and carbamates are broadly used pesticides in the agricultural fields due to their ability to form a stable adduct with enzyme ACHE and inhibit their activity. The change of enzyme activity can provide a peerless measure of target analyte concentration. This paper highlights the essential steps required for the development of biosensor design for monitoring of pesticides based on choice of bioreceptor element, method of immobilization and transducer used.**

***Index Terms*— ACHE, Biosensor, Electrochemical, Immobilization, ISFET.**

I. Introduction

Pesticides are the harmful chemicals that are used to prevent, inactivate or destroy the variety of pests. In accordance with the type of target they inactivate, can be insecticides, fungicides, herbicides, nematacides and rodenticides. These pesticides are permeated into the environment as a result of various agricultural, industrial, medicinal, household activities etc and disrupt the natural ecological balance. Their acute as well as chronic nature of toxicity badly affects human health and cause of several diseases such as asthma, diabetes, cancer, developmental & learning disorders, birth defects etc.

A. Why Comprehensive use of Pesticides?

I. Population blast made a disproportion between yield and utilization.
II. To enhance the subjective and quantitative forms associated with agriculture production.
III. To expand the production of Horticulture &Floriculture.
IV. Due to increased cognizance among the people regarding the use of variety of chemical substances such as pesticides over the agricultural fields to protect the crop from pest attack before and after harvesting.

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B. Types of Pesticides: Chemical and Biopesticides [6]

I. Chemical Pesticides: These are the man made materials used to restrain, suppress or halt the numerous pests. Examples of such pesticides include insecticides, herbicides, acaricide, bactericide, fungicides etc. Since, these substances are not biodegradable; hence can hamper the growth of plants by making supply of essential nutrients such as nitrogen, phosphorous and potassium insufficient.

II. Biopesticides: These pesticides are gotten from raw materials and curb the pest growth by use of microorganisms (viruses and bacteria), genetically modified plants (RNAi) and biochemical means (non toxic substances). Their use in the environment and exposure to human being are very much safe. Still their use is limited due to lower output, improper awareness & less worked research area.

Chemical pesticides can be further classified as organophosphates, organochlorines, carbamates and pyrethroids. Among all, Organophosphates and Carbamates are extensively employed pesticides over agricultural fields. The use of Organochlorines were banned all over the world due to their ability to persist in the environment even after several months of exposure and cause chronic effects even at lower concentrations and pyrethroids are simply household insecticides that are less toxic to human beings at lower concentrations .

II. Organophosphate and Carbamate Detection

Organophosphates and Carbamates are two noteworthy classes of pesticides whose detection are most imperative since they have ability to suppress the activity of various cholinesterase enzymes such as Acetylcholinesterase (ACHE), Butrylcholinesterase (BCHE), Alkaline phosphatase (ALP) and Tyrosinase (Tyr) [19]. Their detection can be best exhibited by the inhibition of enzyme acetylcholinesterase (ACHE) that plays a vital role in handling the passage of nerve signals of an individual during synapsis process [3].

Table 1: Catalogue of organophosphates and carbamates [1]:

Methyl Parathion	Methomyl
Chlorpyrifos	Carbofuran
Dichlorvos	Propoxur
Malathion	Bendiocarb
Paraxon	Terbucarb
Dibrom	Methiocarb
Tetrachorvinphos	Carbaryl
Temephos	Aldicarb
Phosmet	Carbosulfan

A. Inhibition Mechanism of ACHE

An acetylcholinesterase enzyme is mainly present in red blood cells, nerve cells and brain. Major sites present in ACHE enzyme comprises of β anionic, aromatic gorge and an active site. Active site comprises of esteratic and α anionic subsite. Histidine (440), serine (200) and glutamine (327) are the major amino acids present in an active site that facilitate either the breakdown of acetylcholine to produce choline and acetic acid or forming a stable covalent bond with pesticide (phosphorylated ACHE adduct) [9].

Organophosphates and carbamates inhibit the protein ACHE by phosphorylation & carboxylation with serine hydroxyl moiety of an enzyme's active site. When these moieties assemble was obstructed by these pesticides, catalyst ACHE was not any more ready to decompose an essential neurotransmitter acetylcholine to provide choline and acetic acid. Therefore, accumulation of acetyl choline takes place, whose increased concentration provides an excellent measure to the pesticide concentration present in the human blood [10]. Acute exposure to pesticides can cause fatigue, headache, blurred vision, excessive sweating, vomiting, skin problems, fertility disorders etc [1].

Conventional spectrophotometric (SP), chromatographic and elecroanalytical techniques used for pesticide detection were very reliable, sensitive and accurate [16] but require well equipped laboratories with qualified technicians for their analysis, Complex extraction and purification steps [18] and cost factor per analysis was high. Owing to the limitations associated with conventional techniques, there is an exigency to promote the development of such competent biosensors which can keenly identify the pesticide sort and keep us from the admission of these harmful chemicals.

III BIOSENSOR: An Alternative

Thanks to Professor Leyland Clark, renowned as Father of Biosensor, marked history by developing a powerful tool called Biosensor for rapid and real time detection of harmful chemicals in the environment [17]. Thus, Biosensor can be defined as a device that put together its two essential components bioreceptor and transducer to detect toxic chemicals. Biorecognition element such as Enzymes, Antibodies, Aptamers, Molecularly imprinted polymers distinguishes the target analyte from the sample and Transducer such as electrochemical, acoustic, piezoelectric [15] , mechanical etc converts this acknowledgment into measurable electronic signals that can further be amplified, processed and finally displayed [7]. Immobilization of Bioreceptor on physical transducer is key step in biosensor development process.

Excellent examples of Biosensors includes Blood Glucose to monitor sugar level in blood [12], Pregnancy Test Biosensor to detect hcg protein in urine, Biochemical Oxygen Demand for examination of waste water, Metal ion detection based microbial mer-lux biosensor and many more.

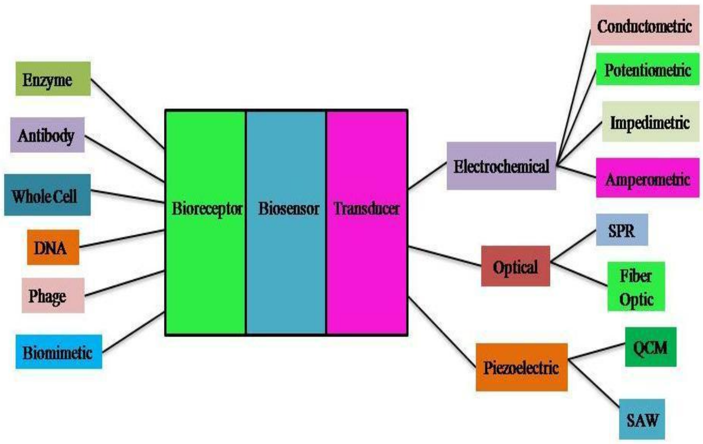

Figure 1: Components of Biosensor

A Design steps in construction of Biosensor [20]:

I. Choice of Biorecognition element
II. Immobilization method
III. Type of physical transducer

Different types of Bioreceptor Elements

I. **Enzymes:** It is a large protein molecule that acts upon substrates to form enzyme substrate complex. Enzyme and substrate have matched shapes that fit into each other just like lock and key.

II. **Antibody:** These are the class of proteins having unique ability to bind with analyte and a molecule that binds to an antibody is called antigen or foreign material. Antibody can be produced by the immune system when a foreign material enters inside our body. Antigens can be disease causing microorganisms such as pathogens, bacteria, viruses or other pollutants [11].

III. **Whole cells:** A group of organisms can be grown on the surface of transducer to produce an electrical measurable signal. Examples are Arxula adeninivorans, Bacillus subtilis etc.

IV. **Aptamers:** These are peptide molecules that bind to specific target molecule. They can a useful replacement to antibodies as sensing molecules because of their unique character.

V. **Molecularly Imprinted Polymers**: These are the polymers that can be prepared by molecular imprinting technique which leaves cavities in polymer matrix with affinity to chosen template molecule. This technique requires a template, functional monomer, cross linker, initiator and extraction solvent for detection of wide variety of analytes [7].

Immobilization Techniques

The technique used for physical or chemical attachment of bioreceptor elements such as enzymes, antibodies, cells etc on to the solid support i.e. transducer in order to increase their stability and make possible of repeated use. Interfacing is a crucial stage in biosensor development since inhibition activity depends directly or indirectly on this [4]. Immobilization of bioreceptor on the transducer surface can be done by the following ways:

Table 2: Comparative study of immobilization techniques

Parameters	**Adsorption**	**Entrapment**	**Crosslinking**	**Covalent Binding**
Method of Immobilization	Physical Method	Physical Method	Chemical Method	Chemical Method
Complexity & cost	Simple & inexpensive	Simple & moderate	Complex and highly expensive	Complicated and highly expensive
Supporting materials used for immobilization	Enzymes are adsorbed on transducer surfaces via substances such as alumina, silica gel, clay, carbon nano tubes etc.	Matrices used for entrapment of biomolecule involve calcium alginate, gelatin polyacrylamide, and silica sol gel etc [4].	glutraldehyde or bifunctional agents (carbodimide, succinimide) are used for binding without the need of any support.	The use of nucleophilic groups such as NH2, COOH, SH, OH etc for coupling.
Methods of bonding	No permanent bond exists between enzyme and supporting material. Only weak van der walls and hydrogen bonds are present.	Covalent or non covalent bonding is employed to bind the bioreceptor inside the matrix.	Enzymes are attached to matrix via covalent bonds (bifunctional or multifunctional reagents)	Enzymes are attached to matrix via covalent bonds (Diazoation, peptide bond , Schiff base formation etc)
Limitations	Adsorbed biomaterials are prone to environmental changes such as pH, temperature etc. Enzyme leakage problem exists.	Leakage of enzyme and microbial contamination of the surfaces takes place.	Enzyme conformation takes places i.e. an active site of an enzyme is not preserved during immobilization process.	Enzyme conformation takes places i.e. an active site of an enzyme are not preserved during immobilization process.

Different types of Transducers

I. **Elecrochemical Biosensors:** Electrochemical analysis is one of the most widely applied detection technique in biosensor systems. The principle of electrochemical biosensors is based on the occurrence of chemical reactions between an enzyme and target analyte, where ions or electrons are produced or consumed. This results in the change of the electrical properties of the solution. According to the measurable property electrochemical sensors are can be potentiometric, or amperometric. Amperometric biosensors are based on measurement of the current due to oxidation or reduction of an electroactive species, when constant potential is applied at working electrode while Potentiometric ones are usually based on the utilization of ion sensitive field effect transistor (ISFET) or electrodes. The pH change caused by the releasing of acetic acid can be determined by the colour change of an indicator [2].

II. **Piezoelectric biosensors:** These biosensors are based on the principle that when a voltage is applied on the crystal surface, crystal becomes deformed. The change in the frequency corresponding to mass change on the electrode surface of piezoelectric biosensor can be determined by Sauerbrey equation. for detection of samples in gaseous phase, while Kanazawa allowed real time liquid solution detection, where frequency change also depends on density and viscosity of liquid [8].

III. **Optical biosensors:** These are based on optical principles [13] such as absorbance, fluorescence, Luminescence, Evanescent wave, surface Plasmon resonance etc to determine the analyte concentration with high specificity [14].

B. Design Considerations during Biosensor development for Organophosphate and Carbamate Monitoring

The working principle of pesticide inhibition is based on the enzymatic (ACHE) hydrolysis of acetyl thiocholine (ATCh) to produce acetic acid and thiocholine (TCh) an electroactive compound. Initial enzyme activity is determined by monitoring the oxidation of thiocholine. After incubation with pesticide, enzyme activity was again measured. Difference in enzyme activity with and without inhibitor gives the actual measure of analyte concentration present in sample [16].

I. Immobilization process must provide biocompatible environment around the enzyme that preserves its activity even in case of harsh environmental changes such as pH, temperature etc.

II. Transducer used for detection must ensure fast electron transport mechanism between the ACHE and electrodes during oxidation or reduction of electroactive species thiocholine with lower required applied potential. Pesticide detection could be achieved satisfactorily, only if the measurable signal obtained current, voltage or impedance etc are as high as possible [5].

III. Generally, an enzyme loses its activity after each measurement. In order to restore the enzyme activity, certain oxime compounds such as pralidoxime and obidoxime can be employed.

IV. CONCLUSION

Biosensors became an indispensable tool because of their unique capabilities of rapid and sensitive detection of pesticides. Organophosphates and carbamates are excellent cholinesterase inhibitors, thus their presence can be actively determined by measurement of change in enzyme activity before and after incubation. While designing a biosensor, choice of biorecognition, element, transducer and immobilization method employed plays a vital role that ultimately affects the performance of biosensor in terms of several parameters such as sensitivity, selectivity, response time and detection limit.

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