Abstract

Preamble

This thesis expounds the development of an acetylcholinesterase based electrochemical biosensor for quantification of organophosphorous and organocarbamate pesticides using polypyrrole as support matrix, its characterization and application in both aqueous and non aqueous solvents.

Background

Controlling of the quality of food, drinking water and the environment is vital for the sustainability of the humans on the globe. Pure, uncontaminated food and drinking water are becoming scarce nowadays due to several reasons such as disproportionate increase of population cum food demand as compared to food production, fast industrial development, natural disasters like drought and flood, and most importantly the use of manmade toxic chemicals, called the pesticides, for agricultural and household pest control. The pesticide residues may enter into the food chain through air, water and soil, apart from the intentional contamination caused during pest control. Pesticide exposure can cause a variety of neurological health effects such as memory loss, loss of coordination, reduced speed of response to stimuli, reduced visual ability, altered or uncontrollable mood and general behavior, and reduced motor skills. These symptoms are very subtle and often may not be recognized by the medical community as a clinical effect. Other possible health effects are asthma, allergies hypersensitivity and liver and kidney malfunctioning. Pesticide exposure is also linked with cancer, hormone disruption, and problems with reproduction and fetal development. Due to their harmful effects on the human health and the environment, it is highly necessary to device efficient detection mechanism for pesticide residues in our food and drinking water for quality control.

The successful use of pesticides around the world has been due to their outstanding control of pests and vector borne diseases. Function wise pesticides are parted in to herbicides (protection against weeds), insecticides (against insects), fungicides (against fungi) and others. Among the various classes of pesticides organophosphorus and organocarbamates are extensively used ones. These two classes, though relatively less persistent than the organochlorine class, they have higher acute and chronic toxicity than the organochlorines.

Abstract

Many methods are accessible for pesticide detection. The techniques of gas chromatography' liquid chromatography and thin film chromatography coupled with mass spectrometry are commonly used methods. However, they present major drawbacks such as complex and time-consuming treatment of the samples including extraction of pesticides, extract cleaning, solvent substitution etc. Additionally, the analysis usually has to be performed in a specialized laboratory by qualified and experienced staff, cannot be used for continuous monitoring and is not suitable for in-situ application. Biosensors are considered as substitute to the expensive, time consuming and sophisticated chromatographic techniques currently used for pesticide detection. They are potentially useful as suitable balancing tools for the real time detection of pesticides residues in food, water and soil, have been an active research area for past few years. Biosensors provide important advantages such as simplicity, sensitivity, selectivity, easy use, high efficiency, miniaturization possibility and fast response time. Among the biosensor technologies available for detection of pesticides, enzyme based electrochemical biosensor are able to draw the attention of the researchers owing to their reliability, high sensitivity and selectivity. It is an established fact that acetylcholinesterase (AChE) inhibition based electrochemical biosensors have emerged as simple, rapid, and ultra-sensitive tools for pesticide analysis in environmental monitoring, food safety, and quality control. In all such AChE based biosensors, the enzyme is immobilized in suitable support matrices that are conductive as well as capable of providing a biocompatible microenvironment for the enzyme inside the matrix.

Conducting polymers have been attracting interest over the past few years as suitable enzyme hoisting matrix. Due to their excellent electrical and electrochemical properties they are finding increasing use in enzyme based electrochemical biosensor construction. Among the other conducting polymers, polypyrrole (PPy) as a biocompatible macromolecule is the best candidate for electrochemical biosensor design. From the perspective of electrochemical biosensor, PPy has a number of attractive features: (i) it can be synthesized electrochemically and can be modified by enzymes in several different ways that gives different analytical characteristics for constructed biosensors; (ii) it protects electrodes from fouling and interfering materials such as electroactive anions; (iii) since it is biocompatible it causes minimal and reversible disturbance to the bio-receptor; (iv) it can be exploited as redox mediator able to transfer electrons from the redox enzymes towards electrodes. PPy film is not easily degraded by organic solvents which indicates the possibilities of developing organic phase enzyme electrodes using PPy.

One important limitation of the biosensors is the non-compatibility of the bio-receptor in organic solvents. Pesticide residues from food items are normally extracted in organic solvents at the time of analysis, because, pesticides are highly soluble in organic solvents but feebly soluble in water or phosphate buffer. Therefore, pesticide analysis in real samples using biosensors necessitates the development of protocols for biosensor application in organic extracts/solvents.

Though a significant amount of research efforts have been devoted in the last two decades towards the development of better efficient biosensors for pesticide quantification, the subject is still open for further improvement in terms of stability, reproducibility, reusability and specificity. Sensor sustainability in organic media is another aspect that needs further improvement.

So, the thesis work was aimed at development of a biosensor for organophosphorous and organocarbamate pesticides through electro entrapment of AChE in polypyrrole matrix, optimization of parameters for stability, longevity and real samples analysis, so as to contribute some efforts towards food safety and quality control.

Contents and layout of the thesis

The thesis entitled "Development of a Polypyrrole Supported Electrochemical Biosensor for Organophosphorous and Organocarbamate Pesticides" comprises of six chapters.

Chapter 1 deals with the general introduction of biosensor, conducting polymers, enzymes and role of pesticides. A brief review on electrochemical biosensors, conducting polymer polypyrrole and role of AChE in biosensor preparation, mechanism of AChE inhibition by organophosphorous and organocarbamates, and the problems associated with application of biosensor in organic solvent have been done in this chapter. This chapter also highlights the background of the problem, objectives in conjunction with plans and methodologies of the present investigation.

Chapter 2 describes the reagents and instruments used in the investigation. The methods and parameters used in the analysis have also been described in this chapter.

Abstract

Our experimental work begins from chapter 3. The content of this chapter includes- a method for easy and effective fabrication of an AChE biosensor probe through electro entrapment in PPy followed by use of gluteraldehyde and gelatin for stability enhancement, Scanning Electron Microscopic (SEM) study to verify the immobilization, study of the electrochemical behavior of AChE entrapped polypyrrole, study of optimum operational conditions for maximum signal output of the sensor, suitable reactivation mechanism and lastly the validation checking of the sensor operation through application to analysis of typical organophosphorous pesticide ethyl paraoxon and organocarbamate carbofuran in laboratory test samples prepared in phosphate buffer and in 5% acetonitrile.

Chapter 4 describes a novel experiment to provide chronoamperometric evidence of low potential thiocholine oxidation on PPy surface, experiments to optimize the conditions for low potential application of the sensor, effect of low potential operation and preconcentration on the sensor stability and on the LODs of thiocholine as well as of the pesticides and the results thereof. Application of the sensor to pesticide analysis at low potential has been demonstrated taking sample organothiophosphate pesticide ethion. A novel correlation procedure has been described through which results obtained by application of the low potential method can be converted to the corresponding values of the conventional high potential method.

Research focus of chapter 5 was to make feasible AChE biosensor application for pesticide analysis in QuECHERS extract. A novel method termed as QET method (QuECHERS tandem ethyl acetate transformation method) has been developed in which QuECHERS extract at the end of the cleanup steps reconstituted in ethyl acetate and then ethyl acetate is transformed to an enzyme(AChE) friendly composition by mixing lipase and L-serine. The AChE biosensor activity remains intact in the transformed mixture (‘TM_{17} with L-serine’) in which the ethyl acetate gets 5 times diluted but the target pesticide get one third diluted. Validation of the method was tested using both gas chromatographic (GC) and biosensor method. The new method gives lower estimate of true value of pesticide content. However, correlation procedure as described in chapter 4 can be applied in this case also to convert results of this novel method to the true value.

Chapter 6 is the conclusion chapter that highlights of the findings of the research work, significance of the work and future scope. This is followed by appendix.