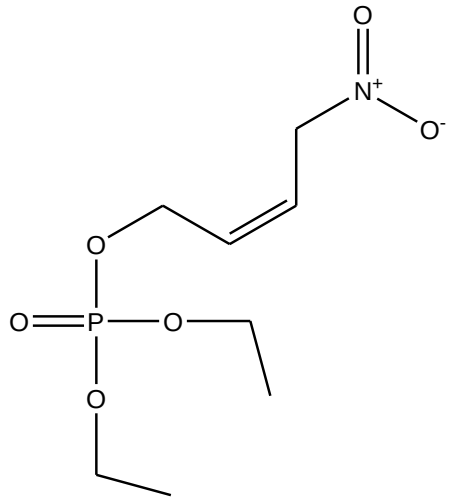
CHAPTER 2: Materials and methods

2.1 Reagents and Materials

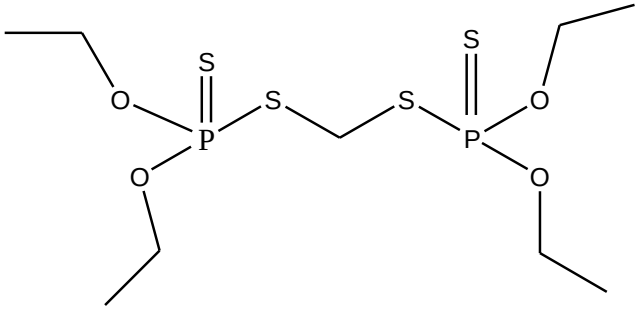
Acetylcholinesterase, Type VI-S (500U/mg) EEL, acetylthiocholine chloride (ATChCl) (99%), Bovine Serum Albumin (BSA) (96%), Pyrrole (98%), gelatin powder (Type A, from porcine skin), 5,5′-dithiobis (2-nitro benzoic acid) (DTNB) (99%), and gluteraldehyde (50 wt % in H_2O) were all procured from Sigma-Aldrich, USA. The organophosphate pesticide used in this study paraoxon-ethyl (90%), Ethion, Profenofos and Dimethoate. The carbamate pesticide used in this study were Carbofuran(98%). All these pesticides were of analytical standard obtained from Pestanal, Sigma-Aldrich. L-Serine (99%) reagent plus, Lipase porcine pancreatic Type III 100-400 units/mg protein were all purchased from Sigma-Aldrich, USA. Ethyl acetate, acetonitrile, Sodium fluoride (NaF) (99%), KH_2PO_4 and K_2HPO_4 were of analytical reagent grade and purchased from Merck chemicals, Germany. Doubled distilled water was used throughout in the experiments. Platinum working electrode used was from CH Instrument, USA. Alumina powder and polishing pad used for the polishing of working electrode were obtained from USA. Magnesium sulphate monohydrate ($MgSO_4.H_2O$) (97%), Sodium chloride (NaCl), tri-sodium citrate dihydrade ($C_6H_5Na_3O_7.2H_2O$) sodium hydrogen citrate sesquihydrate, (Reagent plus, 99%) Primary secondary amine (PSA) used in dispersive solid phase extraction were all purchased from Agilent technology, USA.

2.2 Chemical structure of some reagents used in this study



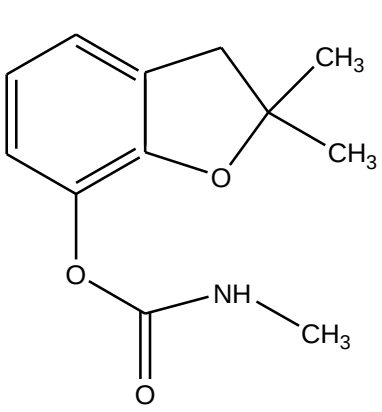
Ethyl paraoxon

diethyl 4-nitrophenyl phosphate

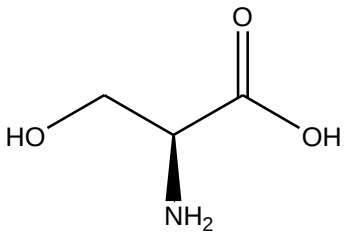


Ethion

O,O,O′,O′-TetraethylS,S'-methylenebis(phosphorodithioate)



Carbofuran
2,2-Dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate



L-Serine
(S)-2-Amino-3-hydroxypropanoic acid

Fig. 2.1 Chemical structure of some reagents used in this study

2.3 Instrument

PAR 273-A Potentiostate/Galvanostate was used both for polypyrrole (PPy) film deposition and electrochemical measurements. UV-VIS spectrophotometer used was UV-2550, Shimadzu, Japan. FT-IR analysis was carried out in Perkin Elmer Frontier FIR-MIR. For Gas Chromatographic analysis Trace GC ultra from Thermo Scientific was used.

2.4 Methods

All electrochemical measurements were performed and recorded in a computer connected to PAR 273-A Potentiostate/Galvanostate analyser with PAR software using either chronoamperometry (CA) or cyclic voltammetry(CV) methods. Quantitative estimation of sensor response was done mostly through chronoamperometric (CA) method; in few cases cyclic voltammetry was also applied. The electrochemical cell set up comprised of three electrodes, the sensor probe as working, Pt coil as auxiliary and Ag/AgCl saturated with 3M NaCl as reference electrodes in PBS (pH 7.2) electrolyte. Details of the chronoamperometric parameters have been discussed in respective chapters. Conventional cyclic voltammetry (CV) was used for qualitative study of sensor response at high and low scan rates throughout the potential range from -0.1 to +0.1 V.

2.5 Pesticide Inhibition study

Time dependence of the inhibitory action of the pesticides was studied by evaluating the percent residual activity ($\%A_r$) with time and the concentration dependence of the same was studied by evaluating the relative inhibition percentage (I %) with concentrations, using equations 2.1 and 2.2 respectively.

$$\%A_r = \frac{I_2}{I_1} \times 100 \qquad (2.1)^1$$

$$I\% = \frac{I_1 - I_2}{I_1} \times 100 \qquad (2.2)^1$$

Here I_1 is the initial CA response of the biosensor to saturated concentration of substrate (ATCh) before incubation, and I_2 is the same after incubation in inhibitor solution.

Limit of detection (LOD) of the PPy-AChE-Geltn-Glu biosensor is defined as the concentration of pesticide resulting in inhibition of 10% (I_{10}).[1]

Reference

1. Valde´s-Rami´rez, G., et al. *Anal. Bioanal. Chem*. **392**, 699--707, 2008.