The present work was aimed to construct highly sensitive electrochemical biosensor for detection of OP pesticides, based on covalent immobilization of organophosphorus hydrolase (OPH) on to chemically modified PVC beaker surface.

The OPH enzyme was purified from *Brevundimonas diminuta* by 14.15 fold with 19.92 % yield, using Sephadex G-100 gel chromatography and ion exchange chromatography on DEAE-Sepharose column. The purified enzyme showed homogeneity, as confirmed by appearance of a single protein band in SDS-PAGE using Coomassie brilliant blue as protein stain. The molecular weight of purified enzyme was 72 KDa as determined by SDS-PAGE.

In the new reported method OPH enzyme was covalently immobilized onto PVC surface. The OPH enzyme was covalently immobilized by using following steps: (a) chemical modification of PVC surface, (b) activation of PVC surface and (c) immobilization of enzyme onto activated PVC surface. Scanning electron microscopic (SEM) study of PVC beaker surface demonstrated that OPH was immobilized on to the inner surface of PVC beaker.

Carbon nanotubes based working electrode was fabricated using single walled carbon nanotubes. The nanotubes powder and NH$_4$Cl were mixed with paraffin oil to obtain the consistency of paste. This electrode along with reference (Ag/AgCl) electrode and an auxiliary (Pt) electrode were connected through potentiostat and along with PVC beaker with immobilized OPH form the working prototype model of biosensor. The electrode was polarized at different potential (in volts) and current (µA) generated was measured. The cyclic voltammetric response for working electrode showed the typical oxidation peak at +0.8V.

The optimum pH, incubation temperature and time of incubation of the present method was 8.0, 40°C and 10 min respectively. The effect of substrate concentration on the response of OPH biosensor was also studied. $K_m$ and $I_{max}$ were 322.58 µM and 1.1 µA respectively for present method.
The analytical performance of the present biosensor employing PVC immobilized OPH was evaluated. A good linearity was obtained between substrate concentration ranges from 0.1-200 μM. The minimum detection limit of present method was 0.01 μM. The mean analytical recoveries were 98.6% and 99.1% for the present method. The results of within batch and between batch coefficients of variation (CVs) were < 1.58% and < 1.78%.

A good correlation was obtained between methyl parathion values in spiked water samples as measured by a standard HPLC method and the present biosensor with $R^2 = 0.985$ and regression equation: $y = 0.987x + 0.484$.

The possible interfering species such as fructose, glucose and sucrose, had practically no effect on the response of present method. Among the heavy metal ions tested individually such as Zn(II), Cu(II), Cd(II), Ni(II) and Pb(II) on methyl parathion detection by the present biosensor none had practically any effect on response of the present biosensor.

The amperometric response of present method lost 50% of its initial activity during its regular use for 25 times over a period of 50 days, when stored in sodium phosphate buffer (pH-8.0) at 4°C.

The significance of the present study is the use of PVC surface as immobilization support for enzyme. Enzyme was covalently immobilized on to Polyvinyl chloride (PVC) surface rather than working electrode. This provides us an opportunity to change the working electrode on deterioration, without wasting the immobilized enzyme hence economical and convenient. Further more any electrochemical changes taking place at the surface of electrode will not hinder or destroy the activity of enzyme as it is not in direct contact with the electrode.