In present study, Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au working electrode was fabricated for the detection of organophosphorus compounds in different samples. Acetylcholinesterase (AChE) was purified from the hypocotyls of eight day old germinated seedlings. The gel filtration methods were used for purification which included Sephadex G-100 followed by DEAE-Sephacel column chromatography. With Sephadex G-100, 18.58% yield was obtained with 33.08 fold purification and specific activity of 26.50 U/mg. While, DEAE Sephacel column chromatography resulted in 39.37 fold purification with 17.93% yield and specific activity of 31.54 U/mg. The molecular weight of purified enzyme was found to be 65 KDa as revealed by Native-PAGE. While SDS-PAGE showed the presence of two bands one of 32 KDa and other of 33 KDa confirming that the purified enzyme is a dimer. The kinetic properties were optimized for maximum activity of the purified enzyme and were found to be pH 7.5, temperature 30ºC, time of incubation 8 min and substrate concentration 550 µM.

The gold nanoparticles were synthesized and were characterized to confirm the synthesis of nano scale particles. UV-Vis spectroscopy showed absorbance peak at 522 nm, which confirms the synthesis of AuNPs. XRD analysis of AuNPs showed diffraction peaks appearing at 38.4°, 44.6° and 64.7° which were designated to (111), (200) and (220) respectively. The above XRD peaks are specific diffraction peaks of gold crystalline plane. The diffraction peaks appeared are in agreement with JCPDS card no. 089-3697. The TEM analysis confirmed spherical shape of gold nanoparticles with average size in range of 20 to 30 nm.

For fabrication of working electrode, multi-walled carbon nanotubes (MWCNT) 50% and AuNPs 30% were mixed with paraffin oil 20% to obtain the consistency of paste. The paste (MWCNT/AuNPs) was filled in plastic tube (1 cm long and 4 mm wide) and fine Au wire was inserted to obtain electrical contact. After solidification of the paste, plastic tube was carefully removed and this formed the core of working electrode. Subsequently, MWCNT/AuNPs-Au core electrode was washed with double distilled water for removing unbound material and stored at 4ºC. Carboxylated single walled carbon nanotubes (cSWCNTs) suspension (2 µL) was mixed with 2 µL of AChE. The mixture was casted onto the surface of electrode core MWCNT/AuNPs-Au using microsyringe and allowed to dry at room temperature. The characterization by FTIR confirmed successful covalent bonding of enzyme with carboxylated nanomaterial. Finally, 1 µL Nafion (5%) was casted onto the body

surface which acts as binder to hold the AChE-SWCNT. Morphological characterization of bare gold wire and electrode with enzyme was carried out by Scanning Electron Microscopy. The SEM image of bare gold (Au) wire showed uniform morphology. While, SEM image of MWCNT/AuNPs-Au and working electrode (Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au) showed mesh like morphology which confirmed successful deposition of nanomaterial on bare Au electrode and enzyme immobilization. The use of CNTs in the fabrication process has enhanced the electrocatalytic properties of the developed electrode. The linear working range of biosensor was found to be 0.1µM-130 µM with a detection limit of 0.01 µM which is better than earlier reported methods. The Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au based biosensor showed a response time of <10 s. Use of Nafion protected enzyme from leaching which leads to high stability (60 days) and reusability (>55 times). This method used for detection of OP compounds showed a good correlation (R^2 = 0.987) with the standard method such as HPLC. The developed sensor was used for determination of various OP compounds in different samples such as soil, water, milk and vegetables.

Conclusion

The new fabrication strategy for development of working electrode for on site monitoring of organophosphorus compounds was tested successfully. The desirable properties of an OP monitoring device were achieved through Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au based biosensor. The use of CNTs in combination with Au nanoparticles resulted in enhanced electrocatalytic properties of the developed electrode and reduces the working potential upto +0.360mV and hence the possibility of false positivity and additional response due to other electrocatalytic species in the sample could be prevented. The linear working range of biosensor was found to be 0.1µM-130 µM with a detection limit of 0.01 µM which is better than earlier reported electrodes. The Nafion/AChE-cSWCNT/MWCNT/AuNPs-Au based biosensor showed a response time of <10 s after incubation of 10 minutes. Use of Nafion protects the covalently bound enzyme from leaching which leads to high stability (60 days) and reusability (>55 times). The nafion layering also protects the electrode from fouling. This method showed a good correlation (R^2 = 0.987) with other standard methods such as HPLC. The developed sensor can be used for determination of various OP compounds in different samples on site.