Thesis Abstract

The project undertaken in this study was to develop highly sensitive, fast, reliable, field applicable and coat effective methods for detection of pesticides using immunoassays/ immunobiosensors. We targeted Atrazine; which is one of the most widely used pesticide and most frequently encountered environmental pollutant all over the world. Antibodies were chosen as the biological element for development of biosensor because they allow determination of target analyte with very high degree of specificity and it is usually possible to produce antibodies against any given target molecule. Atrazine being a small molecule do not initiate antibody production on it’s own and has to be coupled with a large carrier protein to make it immunogenic. Three different haptons for the target molecule were prepared, which bear partial structural similarity with the target pesticide and also provided for a carboxyl functional group for conjugation to the carrier protein (BSA/OVA) or tracer labels such as HRP. The proposed hapten structures were characterized in silico using molecular modelling techniques. These haptons were then chemically synthesized and their chemical structure was confirmed using spectroscopic techniques such as NMR, IR and UV-visible spectroscopy. Protein (Carrier or enzymes) and Haptons were chemically conjugated and Hapten-protein/enzyme conjugates were thoroughly characterized for gross structural and activity change of the labels using various biophysical techniques such as CD, IR, Fluorescence and UV-visible spectroscopy, MALDI Mass spectrometry, electrophoresis etc. The course of structural changes in proteins after conjugation was studied using Molecular Modelling and the justification of experimental results using modelling results was done. Using these conjugates antibodies were produced with high degree of specificity for Atrazine as indicated by AFM and SPR measurements. Using these antibodies a highly sensitive ELISA with a sub ppb level detection limit (0.6-0.9 ppb) was optimized. A gold nanoparticle reagent based rapid Lateral Flow Dipstick Immunoassay (LFDA) was developed which showed detection limit in the 25-50 ppb range without the aid of any sophisticated instrument. This same combination of antibody Hapten-HRP gave good sensitivity with sub ppb detection limit (0.386±0.03 ppb) in a fast online Flow Injection Affinity Column (FIAC) Immunosensor device. Overall, Immunosensors were found more suitable when quick results (1 hour) were required for test solutions while ELISA, though taking much more time (8-10 hrs) as compared to immunosensors (1 hr), had their strength in being able to test a large number of samples/multiple samples in one single assay in a 96 well plate format.