Chapter 7

Conclusions and future scope

7.1 Overall conclusions

The seventh chapter is the final chapter of the thesis comprises of concluding remarks drawn from all the previous chapters. A summary of each individual chapter is given to recapitulate the main and important findings and conclusions for that chapter. Also, the significance and possible future scope of the work have been highlighted.

7.1.1 Chapter 1

In this chapter introduction to biosensors in general, GST biosensors in particular and the related terminologies have been provided. A literature survey on the various analytical techniques available for pesticides and recent development on GST based biosensor fabrication has been done. It further provided a brief description of the different elements of this thesis and a discussion of the aims and objectives and the layout of the thesis.

Conclusions from this chapter are:

- Development of alternative analytical methods to the conventional methods based on sophisticated instruments like GC-MS, HPLC etc. is the need of the hour.
- GST based biosensors can be promising alternative to those sophisticated methods for different varieties of pesticide classes.
- In recent time, huge focus is devoted towards the development of better efficient biosensors. However, the field still remains as an open area for further development in terms of stability, sensitivity, reproducibility, reusability and selectivity of the biosensors.
- A brief discussion on enzyme immobilization is drawn. Among various immobilization matrices, graphene oxide is considered as versatile enzyme hoisting matrix.

7.1.2 Chapter 2

Chapter 2 describes the reagents and instruments used in the research work. Also the methods and parameters used in the study have been highlighted.

7.1.3 Chapter 3

In this chapter, we have studied the GST catalyzed reaction between GSH and CDNB in two different solvents: methanol and ethanol, through electrochemical cyclic voltammetric method.

- The reaction follows two different pathways in the two solvents. Unlike the case when ethanol is used as the solvent, in methanol GSH get transformed to an electroactive intermediate state under the influence of applied electric potential. This electro active intermediate undergoes oxidation at 0.30 V, sufficiently stable (more than one hour) and reacts with CDNB to form UV active final product.

- The same electroactive intermediate is also formed by non-electrochemical process in presence of CDNB and the formation is catalyzed by GST.

- Influence of different components of the reaction mixture on the electrochemical response has been evaluated. It was observed that methanol, CDNB, GSH and PB do not interfere with the peak at 0.30 V.

- Optimum GST amount, methanol concentration, saturated substrate concentration and apparent Michaelis-Menten constant for the enzymatic reaction have been determined. An amount of 0.12 mg (120 μL) of GST was found to be the maximum enzyme amount for the reaction of GSH and CDNB, optimum methanol concentration for feasibility of the reaction was estimated to be 25%, saturated substrate concentration for both GSH and CDNB was obtained as 2 mM. Apparent Michaelis-Menten constant obtained through the Lineweaver- Burk plots were 0.11 $mmolL^{-1}$ and 0.12 $mmolL^{-1}$ at low concentration and 1.66 $mmolL^{-1}$ and 1.91 $mmolL^{-1}$ at high concentration region respectively for GSH and CDNB.

- We have also studied the influence of typical pyrethroid pesticide, cypermethrin, on the said reaction and found that cypermethrin has negative influence on the reaction. Application of the phenomena for quantifying cypermethrin through cyclic voltammetric method has been demonstrated.

- Cypermethrin was detected down to 2 ppb by using normal cyclic voltammetric method. The quantification method has been validated through spiked sample and using QuEChERS extraction/clean up method.

7.1.4 Chapter 4

In chapter 4, we have used the GST catalyzed GSH-CDNB reaction to other classes of pesticides in order to see the feasibility of the developed method in broad spectrum application. To our surprise, it has successfully paved the way for development of a new type of amperommetric biosensor for detection of wide varieties of pesticides. The major findings of this chapter are:

- Organophosphates and organocarbamates inhibit the GST activity. Thus the GST catalyzed reaction between GSH and CDNB can be used to detect these two classes of pesticides.

- The inhibitory power of fenobucarb, temephos and dimethoate follows the trend fenobucarb › temephos › dimethoate.

- Detection limits of the method for the three pesticides were found to be 2, 4 and 5 ppb respectively for fenobucarb, temephos and dimethoate.

7.1.5 Chapter 5

In this chapter, development of a novel method for fabrication of a GST biosensor using GO support matrix, gluteraldehyde as cross linker and gelatin as enzyme stabilizer is the major focus. Optimization of the fabrication and operational parameters and application to pesticide analysis has been described. The important conclusions from this chapter are:

- GST can be immobilized in GO to fabricate a highly stable and highly sensitive biosensor. The sensor has good interstate precision of 5.73% and intra state precision of 0.70%. The sensor could be reused 8-10 times after reactivating through phosphate buffer (pH 6.5) solution.
- GO amplifies the amperometric signal of GSH-CDNB oxidation because of its high electrical conductivity and biocompatibility.
- Gelatin can provide a biocompatible micro-environment to GST inside GO matrix. Gelatin and gluteraldehyde mixture can enhance the stability of the electro entrapped biosensors by providing a better cross linking**.**
- GSH shows more substrate specificity compared to CDNB towards GST enzyme.
- The developed biosensor is of broad spectrum application. It can be applied to at least six different classes of pesticides namely pyrethroid, organochlorine, organophosphate, carbamate, benzimidazole and phenolic classes.

7.1.6 Chapter 6

Chapter 6 is an approach to assess different mode of interaction of the pesticides with the GST enzyme. The inhibition patterns were studied using Lineweaver-Burk plots. Inhibitor dissociation constant (K_i) values were obtained as indicators of affinity of inhibitors towards the enzyme. Few important findings of this chapter are as follows:

- Fenobucarb, DDT and cypermethrin show competitive type of inhibition, temephos, ethion and chlorpyrifos show non-competitive mode of inhibition whereas dimethoate, dinocap and carbendazim show mixed type of inhibition.
- It is also observed that inhibition of GST activity by different members of the same group differs. This is attributed to structural influence on binding site selectivity and/or on the kinetics.

7.2 Significance of the work

- The present technique is advantageous over the other enzyme (AChE, PPO) biosensing techniques in that the detection process uses a moderately high (25%) concentration of methanol as electrolyte as well as extracting solvent.
- This technique has rendered biosensing of pesticide a more practical and dependable one because pesticides are mostly extracted in organic solvent.
- The method is versatile as it can be used for detection of almost all classes of pesticides.
- The method has made it possible to analyze hydrophobic pesticides through bioanalytical technique.

7.3 Drawbacks

The present method offers slightly higher LODs as compared to other biosensors such as DNA aptamer based biosensors and acetylcholinesterase (AChE) based biosensors. However, the method is able to meet the required sensitivity for residue analysis of the selected pesticides in majority of the agricultural commodities as per the European Union (EU) guidelines (List is available in Appendix V Table A52).

7.4 Future scope

While this research confirms the ability to detect different classes of pesticides using the GST based biosensors, there is still a plethora of research to complete this study.

- Actual mechanism of GSH-CO interaction under electrode polarization needs further study.
- Detail mechanism of interaction between pesticide and GST needs further study. Within the same class, all members do not give positive results to the method.
- The reaction between CDNB and GSH is slow even under catalyzed (by GST) condition. This results in an initial loss of at least 20 minutes before analytic run. On the other hand the reaction between GSH and DTNB is extremely fast even in absence of catalyst.

It will be very significant and important work to find a substrate with an optimized response time for rapid analysis of pesticides using GST enzyme.

- The work has indicated the possibility of developing ELISA test method for pesticides using GST enzyme. The ELISA test method of this type will be more advantageous over the existing GST method because the test can be carried out in organic medium with 25% methanol solution.