# Table of contents

## CHAPTER-1: INTRODUCTION AND REVIEW

1.1. PESTICIDES
   1.1.1 Pesticides

1.2. PESTICIDE POLLUTION AND PUBLIC HEALTH
   1.2.1 Pesticide Pollution: A Global Concern
   1.2.2. Pesticide Pollution: Indian perspective

1.3. CURRENT METHODS FOR PESTICIDE DETECTION
   1.3.1. Chromatographic Techniques
   1.3.2. Electrophoretic Techniques
   1.3.3. Mass spectrometry

1.4. ALTERNATIVE TECHNIQUES
   1.4.1. Biosensors
   1.4.2 Imunosensor for Pesticides Analysis
   1.4.3 Types of Immunosensor
      1.4.3.1. Optical Immunosensors
      1.4.3.2. Piezoelectric Crystal Immunosensor
      1.4.3.3. Electrochemical Immunosensors

1.5. IMMUNOSENSOR BASED ASSAY DEVELOPMENT

1.6. PROBLEM DEFINITION: OVERALL AIMS AND OBJECTIVES
   1.6.1 Parathion
   1.6.2. Atrazine
   1.6.3. Methodology
CHAPTER 2: HAPTEN DESIGN AND SYNTHESIS

2.1 INTRODUCTION

2.1.1 Haptens

2.1.2 Hapten design

2.2 AIMS AND OBJECTIVES

2.3 MATERIALS AND METHODS

2.3.1 Chemical Synthesis

2.3.2 Reagents

2.3 RESULTS AND DISCUSSION

2.4.1 Hapten selection and synthesis

2.4.2 Infrared Spectroscopy

2.4.3 'H-NMR spectroscopy'

CHAPTER 3: BIOCONJUGATION

3.1 PREFACE

3.1.1 Conjugated Reagents

3.1.2 Methods for preparation of conjugated reagents

3.1.3 Approaches for preparing Protein conjugates

3.2 AIMS AND OBJECTIVES

3.3 MATERIALS and Methods

3.3.1 Chemicals and Reagents

3.3.2 Preparation of BSA- Amino Parathion conjugates

3.3.3 Preparation of BSA-Maleimido ethyl parathion conjugate

3.3.4 Characterization of Hapten-Protein conjugates

3.3.4.1 Estimation of Protein concentration by Bicinchoninic Acid Method (BCA)

3.3.4.2 Mass spectrometric methods (MALDI-TOF)

3.3.4.3 Electrophoresis

3.4 RESULTS AND DISCUSSION
3.4.1. Preparation of immunoconjugates 57
3.4.2. Mass spectrometric methods (MALDI-TOF) 59
3.4.3. Electrophoretic methods 63
   3.4.3.1. Native PAGE (Electrophoretic Mobility Shift Assay) 64
   3.4.3.2. Fluorescence Spectroscopy 64

CHAPTER-4: ANTIBODY PRODUCTION, PURIFICATION AND CHARACTERIZATION 67

4.1. PREFACE 67
   4.1.1. An Immune system 67
      4.1.1.1. Cellular Immunity 67
      4.1.1.2. Humoral Immunity 68
      4.1.1.3. Immune response to haptenated protein 69
   4.1.2. Antibody Production 69
      4.1.2.1. Antibodies (Abs) 69
      4.1.2.2. Antigens (Ags) 72
   4.1.3. Antibody purification methods 72
      4.1.4. Antibody characterization 74

4.2. AIMS AND OBJECTIVES 74

4.3 MATERIALS AND METHODS 75
   4.3.1. Immunization and sera collection in rabbits 75
   4.3.2. Purification of antibodies using affinity chromatography 75
      4.3.2.1. Saturated Ammonium sulfate (SAS) precipitation 75
      4.3.2.2. Protein-A affinity purification 76
      4.3.2.3. Purification of Hapten specific IgG 76
      4.3.2.4. Purification of Hapten specific antibodies 76
   4.3.3. Characterization of Antibodies 77
      4.3.3.1. Enzyme Linked Immunosorbent Assay (ELISA) 77
4.3.3.2. Determination of Affinity constant of antibodies  77
4.3.3.3. Competitive Inhibition Immunoassay  78

4.4 RESULTS AND DISCUSSION  78
   4.4.1. Immunological characterization of Conjugates  78
      4.4.1.1. Immune response to hapten structure in rabbit  78
      4.4.1.2. Cross reactivity Studies  80
   4.4.2. Characterization of Purified Antibody  82
      4.4.2.1. Affinity constant of antibodies  82

CHAPTER-5: Development and Characterization of Immunosensor  83

5.1. PREFACE  83
   5.1.1. Immunoassays  83
      5.1.1.1. Immunoassays: Basic Principles  83
      5.1.1.2. Immunosensors  84

5.2. AIMS AND OBJECTIVES  84
5.3. MATERIALS AND METHODS  85
   5.3.1. Standardization of ELISA for parathion  85
      5.3.1.1. Direct ELISA  85
      5.3.1.2. Indirect ELISA  85
      5.3.1.3. Competitive Inhibition ELISA  86
   5.4. RESULTS AND DISCUSSION  87
      5.4.1. Cross reactivity Studies  87

CHAPTER-6: SUMMARY AND CONCLUSIONS  93

6.1 IMMUNOBIOSENSOR DEVELOPMENT AND CHARACTERIZATION  93
   6.1.1. Hapten Design And Synthesis  93
   6.1.2. Preparation and characterization of immunogens  94
6.1.3. Preparation and Characterization of Immunoreagents 95
6.1.4. Antibody production and characterization 95

6.2. IMMUNOASSAY DEVELOPMENT 96