Chapter 1

Introduction and Objectives
INTRODUCTION AND OBJECTIVES

1.1. Introduction

Molecular imprinted polymers (MIPs) are highly crosslinked materials polymerised in the presence of a template molecule to form specific binding sites. These sites are complementary in size, shape and functionality to the template molecule, providing tailor-made receptors that can bind the desired molecular target with high affinity and selectivity. In principle, movements of molecules are frozen in polymeric structures so that they are immobilized in a desired fashion.

The molecular imprinting method has recently been developed to provide versatile receptors efficiently and economically. The chemical circumstances in guest-binding sites can be easily regulated by combining appropriate monomers, crosslinking agents, and/or comonomers. The advantages of MIPs over their biological counterparts such as antibodies and enzymes are that they are easy to produce and process, they are less costly, and more stable. Furthermore, the MIPs can be tailored to bind target molecules for which natural receptors do not exist or hard to obtain. These features make molecular imprinting method so unique and challenging that the scope of its future application is beyond prediction.

1.2. Objectives of the present work

The present work aims at the tailoring of some herbicide imprinted polymers with high specificity and selectivity towards the imprinted template molecule. The selected templates include two herbicides such as 2,4-dichloro...
phenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which are commonly used in the agricultural field. 2,4-D is extensively used as a broad leaf weed killer on field crops, turf, and non-crop lands. 2,4,5-T also is a broad range herbicide with the danger of dioxin contamination connected with the commercial product\textsuperscript{5,6}. Current concerns about potential health hazards connected with pesticide use have focused on 2,4-D and 2,4,5-T as suspected cancer-causing agents. The high toxicity for mammals and aquatic organisms, and the long-term persistence in the environment as residues, make them a suitable subject of trace analysis. Phenolic compounds are also toxic for living beings, and affect the taste and odour of water and fish, they are also included in the priority pollutant list of the EEC and US-EPA\textsuperscript{7-11}. Imprinted polymers with non-chlorinated phenoxyacetic acid (POA) and \textit{p}-hydroxybenzoic acid (\textit{p}-HB) as templates were also synthesised and used to investigate the effect of role of chlorine substitution in the aromatic ring, and number of binding sites in the template in the binding process. Non-imprinted polymers were also synthesised without the template. The most common methods of herbicide detection are gas chromatography with mass spectrometry detection (GC-MS) and high performance liquid chromatography (HPLC)\textsuperscript{12-14}. The detection and quantification of these herbicides at trace levels cannot be performed directly using these methods, but it requires one or more preconcentration steps due to the insufficient sensitivity of these methods. Moreover, these methods are mostly laboratory bound and tedious; they require sophisticated instruments and are time consuming. So the development of a sensitive and convenient method for trace level detection and separation of these pollutants are necessary. Molecular imprinting is a promising method in this field and imprinted polymers can be effectively used for many analytical applications\textsuperscript{15-20}. The reported techniques
under this method also require high crosslinking, tedious laboratory bound work and longer analysis time.

Here we introduce our efforts towards the design of imprinted polymers of the selected templates, and a simple UV-vis. spectrophotometric technique for the detection and extraction of these pollutants, using the imprinted polymers. Detailed studies on either the interdependence of the nature and extent of crosslinking on rebinding capacity of molecular imprinted polymers or Optimization of rebinding conditions for maximum specificity and selectivity have not been reported so far. So emphasis is given to the design of imprinted polymers with maximum specificity and selectivity, using different crosslinking agents and by observing the various parameters related to rebinding. In order to investigate the effect of nature of the crosslinking agent, three crosslinking agents such as divinylbenzene (DVB), N,N’-methylene-bis-acrylamide (NNMBA) and ethyleneglycol dimethacrylate (EGDMA) having varying degree of rigidity and flexibility, hydrophilic-hydrophobic balance, and solubility were used. The conditions for maximum specificity and selectivity were optimized by altering certain factors like extent of crosslinking, concentration of template solution, rebinding medium, mass of polymer and time of incubation. Infrared spectroscopy and proton nuclear magnetic resonance were employed for analysing pre-polymerisation complexes, whereas $^{13}$C NMR and scanning electron microscopy methods were applied for the characterisation of imprinted polymers. The specific binding of template by the imprinted polymers was evaluated with respect to template binding by a non-imprinted polymer of same crosslinking. The binding parameters of imprinted polymers were determined using Scatchard equation. The selectivity of the imprinted polymers towards the template were quantified as separation and selectivity factors by assessing the cross-
reactivity with structural analogues. The study can be outlined under the following heads:

A. **2,4-D Specific Polymers**

A. 1. *Synthesis in methanol/water*

(i) Synthesis of DVB-, NN MBA- and EG DMA-crosslinked 2,4-D imprinted and non-imprinted polymers with varying: (a) template-monomer ratio, and (b) extent of crosslinking

(ii) Characterisation of 2,4-D imprinted and non-imprinted polymers

(iii) Swelling studies

(iv) Rebinding studies

(v) Selectivity studies

(vi) Comparison of DVB-, NN MBA- and EG DMA-crosslinked polymers

A. 2. *Synthesis in chloroform*

(i) Synthesis of EG DMA-crosslinked 2,4-D imprinted and non-imprinted polymers with varying extent of crosslinking

(ii) Swelling studies

(iii) Rebinding studies

(iv) Comparison of specificity of imprinted polymers synthesised in methanol/water and chloroform

B. **2,4,5-T Specific Polymers**

(i) Synthesis of DVB-, NN MBA- and EG DMA-crosslinked 2,4,5-T imprinted and non-imprinted polymers with varying: (a) template-monomer ratio, and (b) extent of crosslinking
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(ii) Characterisation of 2,4,5-T imprinted and non-imprinted polymers

(iii) Swelling studies

(iv) Rebinding studies

(v) Selectivity studies

(vi) Comparison of DVB-, NNMB- and EGDMA-crosslinked polymers

C. POA Specific Polymers

(i) Synthesis of DVB-, NNMB- and EGDMA-crosslinked POA imprinted and non-imprinted polymers with varying: (a) template-monomer ratio, and (b) extent of crosslinking

(ii) Characterisation of POA imprinted and non-imprinted polymers

(iii) Swelling studies

(iv) Rebinding studies

(v) Selectivity studies

(vi) Comparison of DVB-, NNMB- and EGDMA-crosslinked polymers

D. p-HB Specific Polymers

(i) Synthesis of EGDMA-crosslinked p-HB imprinted and non-imprinted polymers with varying extent of crosslinking

(ii) Characterisation of p-HB imprinted and non-imprinted polymers

(iii) Swelling studies

(iv) Rebinding studies

(v) Selectivity studies
As a background to the present work, a brief survey of the existing literature on the various aspects of molecular imprinting in general, studies related to the selected herbicide-specific polymers and their applications in various fields are given in the beginning. In the present study, emphasis is given to the design of imprinted polymers of the selected templates with maximum specificity and selectivity by varying the nature and extent of crosslinking agent.

1.3. Organisation of the thesis

This thesis consists of five chapters.

Chapter I is an introduction to the work stating its objectives and importance in the field of specific and selective recognition of target molecules.

Chapter II deals with the previous studies related to molecular imprinting. Researches relating to molecular imprinting technique and its application in synthetic polymers in various fields, especially, in the development of polymeric sensors selective to molecules of biological significance and environmental concern have been discussed in this chapter.

Chapter III is the experimental part of the thesis. It describes the synthesis of 2,4-D, 2,4,5-T, POA and p-HB specific imprinted polymers by molecular imprinting in different template-monomer ratio. In order to investigate the effect of the nature and extent of crosslinking agent on specificity and selectivity, imprinted and non-imprinted polymers were prepared with varying extents of DVB, NNMB and EGDMA crosslinking.

Chapter IV discusses the investigation results of the effects of template-monomer ratio, nature and extent of crosslinking agent and other factors influencing specificity and selectivity of the designed imprinted
systems. Observations and results of certain factors that affect the specific rebinding capacity, such as the effect of concentration of template solution, binding medium, mass of the polymer and time of incubation are outlined here. The swelling and solvation characteristics of the polymers and their relation with specific binding with respect to different crosslinking agents and templates are also described. Selectivity of the imprinted polymers towards the template molecule with respect to structural analogues and its dependence on the extent of crosslinking are also discussed.

Chapter V serves as a conclusion. It summarises the work done, the results of investigation of the designed template selective imprinted polymers and the effect of various factors affecting specificity and selectivity.

References


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