Chapter 1

Introduction and Objectives

INTRODUCTION AND OBJECTIVES

1.1 Introduction

Molecular imprinting technology which originates from the molecular recognition phenomenon in biological systems has been receiving much attention and already rapid developments have been achieved in recent years[1]. Analogous to natural recognition units such as enzymes, antibodies, and receptors, various synthetic mimics of these bioactive components have been produced by artificial means[2]. One of the most promising examples of artificially generated recognition materials is molecular imprinted polymers (MIP). They have become well established as a means of producing biomimetic recognition sites[3]. This technique is based on creating cavities which correspond to the shape of the target molecule in a highly crosslinked polymer matrix. Thus during the molecular imprinting process highly crosslinked copolymers are formed around analyte molecules acting as cavity-creating templates. The template molecules are then removed providing binding sites ideally complementary in size, shape and functionality to the templated analyte. Upon reintroduction of the template preferential binding within the cavity should occur. MIPs are inherently stable and capable of high selectivities[4] approaching that of their natural counterparts. Various organic molecules, transition-state analogs, chiral species, and inorganic ions have been successfully imprinted[5]. Added advantages such as ease of preparation, chemical robustness, low-cost production, and the possibility of shaping molecularly imprinted polymers (MIPs) in various self-supporting formats makes them very attractive materials[6]. These molecularly imprinted

polymers have been widely employed for diverse applications in chromatographic separation[7], drug screening, chemosensors[8], catalysis[9], immunoassays[10] owing to their specificity towards the target molecules and high stability against physicochemical perturbations.

1.2 Objectives of the present work

Chirality plays a crucial role in biochemical systems. The majority of organic substances from which all living creatures are built are chiral. Enantiomers often show very different physiological behaviors[11]. Therefore, chiral recognition is an important topic in chemistry and biochemistry. A number of approaches[12] have been used for the chiral recognition of organic compounds, including polarimetry, circular dichroism, nuclear magnetic resonance, chromatography, and capillary electrophoresis. Amino acids are a class of compounds of great biochemical importance, especially the 20 common amino acids that are the fundamental units of proteins. All common amino acids except glycine are chiral. In addition, although living organisms are composed predominantly of L-amino acids it is now well established that D-amino acids found in higher order organisms in the form of free amino acids, peptides and proteins, play a preponderant role in biochemistry and physio-pathology. For example, free D-serine is known to be an important endogenous synaptic regulator while free D-aspartate seems to play a role as a messenger in the maturation and differentiation of Leydig cells. Therefore, it is of great interest to develop efficient analytical methodologies that are able to detect traces of one enantiomer in the presence of a high excess of the other enantiomer.

Molecular imprinting technology offers the unique opportunity to tailor chiral stationary phases with predefined chiral recognition properties by employing the enantiomer of interest as binding-site-forming template[13]. Mosbach et al.[14] suggested that chiral recognition was observed in molecular imprinted polymers because some chiral information was memorized in the polymer matrix during polymerization procedures. The selectivity observed in racemic resolution is a result of the combination of the exact cavity-shape fitting and the exactness of the arrangement of the functional groups.

The work presented in this thesis aims at the tailoring of imprinted polymers for the specific and selective recognition of N-protected amino acid and to exploit the specificity and selectivity of the successful system for the selective binding of the enantiomers of amino acid derivatives. Many studies aimed at optimizing noncovalent imprinting have been reported in the literature[15]. The majority of these have been chromatographic studies, the goal of which has been to produce stationary phase with enhanced performance. Here we present an alternative simple UV-vis spectrometric technique for the designing of molecular imprints for the specific and selective recognition of amino acid derivatives. The templates selected for this study are N-tert-butoxycarbonyl-L-tryptophan (Boc-L-Trp-OH), N-tert-butoxycarbonyl-L-phenylalanine (Boc-L-Phe-OH) and a chiral compound D-mandelic acid (D-MDA). In order to gain more insight into the origin of the recognition properties of the imprinted polymers, a series of experiments were conducted concerning the influence of the crosslinkers, the functional monomer and the apparent degree of crosslinking on the recognition site integrity, and the subsequent

recognition properties of the imprinted polymers. Investigations were done to determine whether the specificity and selectivity could be enhanced by changing the polymerization conditions, chemical nature and degree of crosslinking of the three dimensional polymer. Here an attempt is made to design imprinted polymers of Boc-L-Trp-OH, Boc-L-Phe-OH D-MDA with optimum specificity and selectivity. L-MDA and its derivatives are multifunctional precursors for synthesis of many optically pure amino acids, angiotensin converting enzyme inhibitors, and coenzyme A[16]. For this reason, a facile and effective method for control of optical purity of this class of compounds was highly desirable. Imprinted polymers of these materials have already been reported. But most of the work done in the design of the imprinted polymers of these compounds uses sophisticated instruments and are time consuming. But this method is a simple UV-vis spectrophotometric technique. Divinylbenzene (DVB) and ethylene glycol dimethacrylate (EGDMA) with varying degree of rigidity, flexibility and polarity were selected as crosslinking agents for evaluating the nature and degree of crosslinking and for optimizing the conditions for maximum specificity and selectivity of the imprinted polymers. The interactions between the templates and the functional monomers 4-vinylpyridine on complex formation, and the polymer characterization were investigated by FT-IR, ^{1}H-NMR, fluorescence measurements and Scanning Electron Microscopy. The polymer preparations were evaluated and compared with non-imprinted polymers for their ability to rebind the print molecule which is a measure of the specificity of the imprinted system. The binding of other substrates similar in structure to the print molecule was also analyzed to monitor the selectivity of the imprinted system and the selectivity of the imprinted

polymers was quantified as separation and selectivity factors[17]. For this the template desorbed polymer was treated with solution of the print molecule and the structural analogues and the extent of binding was followed by UV-vis spectroscopy.

The study can be outlined under the following heads:

A. N-tert-butoxycarbonyl-L-tryptophan (Boc-L-Trp-OH) specific polymers

1. Synthesis of EGDMA- and DVB-crosslinked Boc-L-Trp-OH imprinted and non-imprinted polymers with varying
   a. Composition of template and functional monomer
   b. Crosslink density
2. Characterization of Boc-L-Trp-OH imprinted and non-imprinted polymers
3. Swelling studies
4. Specificity studies
5. Optimisation of the conditions of Boc-L-Trp-OH binding by the EGDMA- and DVB-crosslinked 1: 4 system
6. Selectivity studies
7. Correlation of the specificity and selectivity with the nature and extent of crosslinking
8. Binding studies of DVB-crosslinked Boc-L-Trp-OH imprinted and non-imprinted polymers with 1:2 T/FM ratio

B. N-tert-butoxycarbonyl-L-phenylalanine (Boc-L-Phe-OH) specific polymers

1. Synthesis of EGDMA-, DVB-, and NNMBA-crosslinked Boc-L-Phe-OH imprinted and non-imprinted polymers with varying
   a. Composition of template and functional monomer
   b. Crosslink density
2. Characterization of Boc-L-Phe-OH imprinted and non-imprinted polymers
3. Swelling studies
4. Specificity studies
5. Investigation of the factors affecting Boc-L-Phe-OH binding on the imprinted polymers
6. Selectivity studies
7. Correlation of the specificity and selectivity with the nature and extent of crosslinking
8. Synthesis and binding studies of Boc-L-Phe-OH imprinted EGDMA- crosslinked 4-VP-co-acrylamide polymer.

C. D-Mandelic acid Specific polymers

1. Synthesis of EGDMA- and DVB- crosslinked D-mandelic acid imprinted and non-imprinted polymers with varying
   a Composition of template and functional monomer
   b Crosslink density

2. Characterization of D-mandelic acid imprinted polymers

3. Specificity studies

4 Investigation of the factors affecting D-mandelic acid binding on the imprinted polymers

5 Selectivity studies

6 Correlation of the specificity and selectivity with the nature and extent of crosslinking

1.3. Organisation of the thesis

This thesis consists of five chapters.

The first chapter is an introductory part stating the objectives and importance of the present work.

Chapter II is a review of the literature which deals with the previous studies on molecular imprinting. A brief report on the application of the imprinted polymers with special reference to the use of imprinted polymers for chiral separation is included.

The experimental part is given in the third chapter. The composition of the template, functional monomer (4-VP) and crosslinking agent (EGDMA/DVB) used for the designing of Boc-L-Trp-OH, Boc-L-Phe-OH and D-mandelic acid specific polymers are given in this chapter.

Results and discussion is summarized in the fourth chapter. The results of the effect of template-functional monomer ratio, nature and extent of crosslinking and dependence of other parameters like concentration of template solution, mass of the polymer, solvent and time

on the binding and discrimination of these imprinted polymers towards the print molecule are illustrated in this chapter. Swelling studies and characterization of the imprinted polymers by FT-IR, ^{1}H-NMR spectroscopy, and fluorescence studies are discussed. Selectivity of the imprinted polymers towards template molecule with respect to structural analogues and enantioselectivity are also described.

Chapter V is the concluding chapter. It summaries the work done, the results of the investigation on the designed template selective imprinted polymers, and the effect of various factors affecting specificity and selectivity.

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