INTRODUCTION AND OBJECTIVES

1.1 Introduction

There are some kinds of molecules called molecular receptors that can pick out their partners from a number of molecules in a system and form a complex with this molecule by non-covalent or covalent bonding. If necessary, predetermined reactions take place in these complexes as observed in enzymatic reactions. This discrimination between molecules, which is named 'molecular recognition', is one of the essential keys to the existence of living things.

Molecular imprinting method has recently been developed to provide versatile receptors efficiently and economically. A molecular imprinted polymer can selectively recognize the template molecule even in the presence of compounds with structure and functionality similar to that of the template\(^\text{1-5}\). In principle, the movements of molecules are frozen in polymeric structures so that they are immobilized in a desired fashion. This method is so unique and challenging that the scope of future applications is high and hard to predict. Recently there has been increased interest in studies related to molecular imprinted polymers and their applications.

1.2 Objectives of the present work

The present work involves the development of imprinted and non-imprinted polymers of few toxicants which we frequently come across. The toxicants selected for the study are nicotine, caffeine and theophylline, the continuous intake of which may cause damage to the health of individuals. Imprinted polymers of these toxicants for various applications have already been reported\(^\text{6-11}\), all requiring sophisticated instruments, high degree of crosslinking and longer analysis time. Here we present an alternative simple UV-vis. spectrophotometric technique, using molecular imprinted polymer for the detection as well as solid phase extraction of nicotine, theophylline and caffeine. Detailed studies on either the interdependence of the nature and degree of crosslinking on the rebinding capacity of molecular imprinted polymers or optimization of rebinding conditions using the above toxicants have not been reported so far. Emphasis is given on the designing of imprinted polymers with maximum specificity and selectivity, by altering the nature and degree of crosslinking in the imprinted system as well as by observing the various parameters related to rebinding. In order to investigate the effect of the nature of crosslinking agent on the selectivity and specificity in rebinding, divinylbenzene (DVB), ethylene glycol dimethacrylate (EGDMA) and triethylene glycol dimethacrylate (TEGDMA) with varying degree of rigidity and flexibility,
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hydrophilic-hydrophobic balance and polarity have been used. Infrared spectroscopy and proton nuclear magnetic resonance were employed for analyzing pre-polymerisation solutions\textsuperscript{12-15} whereas \textsuperscript{13}C NMR, FT-IR and scanning electron microscopy methods were applied for the molecular imprinted polymer characterization\textsuperscript{16-19}. With the help of Scatchard equation\textsuperscript{20-23}, the binding parameters of template imprinted polymer were evaluated. The effectiveness of the imprinted polymer is demonstrated by comparing the binding of the target molecule and molecules with similar structures. By studying molecules with similar structures, the cross-reactivity of the polymer can be assessed to quantify the selectivity of binding. The study can be outlined under the following heads:

A. Nicotine specific polymers

1. Synthesis of DVB-, EGDMA- and TEGDMA-crosslinked nicotine imprinted and non-imprinted polymers: (a) with varying nicotine - functional monomer ratio, and (b) with varying degree of crosslinking.

2. Characterisation of nicotine imprinted and non-imprinted polymers.

3. Desorption of nicotine from the imprinted polymers.

4. Specificity studies of nicotine desorbed imprinted polymers.

5. Investigation of factors influencing nicotine binding.


7. Correlation of the specificity and selectivity characteristics with the nature and extent of the crosslinking agent.

B. Theophylline specific polymers

1. Preparation of DVB-, EGDMA- and TEGDMA-crosslinked theophylline imprinted and non-imprinted polymers: (a) with varying theophylline-functional monomer ratio, and (b) with varying degree of crosslinking.

2. Characterisation of theophylline imprinted and non-imprinted polymers.

3. Desorption of theophylline from the imprinted polymers.

4. Specificity studies of theophylline desorbed imprinted polymers.

5. Investigation of factors influencing theophylline binding.


7. Correlation of the specificity and selectivity characteristics with the nature and extent of the crosslinking.
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2. Characterisation of nicotine imprinted and non-imprinted polymers.
3. Desorption of nicotine from the imprinted polymers.
4. Specificity studies of nicotine desorbed imprinted polymers.

5. Investigation of factors influencing nicotine binding.
7. Correlation of the specificity and selectivity characteristics with the nature and extent of the crosslinking agent.

B. Theophylline specific polymers
1. Preparation of DVB-, EGDMA- and TEGDMA-crosslinked theophylline imprinted and non-imprinted polymers: (a) with varying theophylline-functional monomer ratio, and (b) with varying degree of crosslinking.
2. Characterisation of theophylline imprinted and non-imprinted polymers.
3. Desorption of theophylline from the imprinted polymers.
4. Specificity studies of theophylline desorbed imprinted polymers.
5. Investigation of factors influencing theophylline binding.
7. Correlation of the specificity and selectivity characteristics with the nature and extent of the crosslinking.
Caffeine specific polymers

1. Preparation of DVB-, EGDMA- and TEGDMA-crosslinked caffeine imprinted and non-imprinted polymers: (a) with varying caffeine–functional monomer ratio, and (b) with varying degree of crosslinking.

2. Characterisation of caffeine imprinted and non-imprinted polymers.

3. Desorption of caffeine from the imprinted polymers.

4. Specificity studies of caffeine desorbed imprinted polymers.

5. Investigation of factors influencing caffeine binding.


7. Correlation of the specificity and selectivity characteristics with the nature and extent of the crosslinking.

As a background to the present work, a brief survey of the existing literature on the various aspects of molecular imprinting in general, the studies related to the three selected toxicant-specific polymers and the application of the tailored systems for specific and selective concentration of the above-mentioned toxicants are given in the beginning. In the present study the emphasis is given on the designing of the imprinted polymer with maximum selectivity. Much more importance is given to the difference in the nature of the crosslinking agent on the difference in selectivity.

1.3 Organisation of the thesis

The thesis is divided into five chapters.

Chapter I provides an introduction to the work stating its importance in the selective and specific identification of the target molecule.

Chapter II gives an account of the previous studies related to the molecular imprinting. Studies relating to molecular imprinting technique and its application in the synthetic polymers especially in the development of polymers selective to biologically harmful substances have been focused in the chapter.

Chapter III forms the experimental part of the thesis. It describes the designing of toxicants (nicotine, theophylline and caffeine) specific polymers by molecular imprinting. In order to investigate the effect of the nature and extent of crosslinking agent in the selectivity and specificity in rebinding, imprinted and non-imprinted polymers with varying degree of DVB, EGDMA and TEGDMA crosslinking were prepared.

Chapter IV describes the investigation results of the effect of the nature and the degree of crosslinking and other related factors impinging on specificity and selectivity of the developed molecular imprinted systems.
C. Caffeine specific polymers

1. Preparation of DVB-, EGDMA- and TEGDMA-crosslinked caffeine imprinted and non-imprinted polymers: (a) with varying caffeine–functional monomer ratio, and (b) with varying degree of crosslinking.

2. Characterisation of caffeine imprinted and non-imprinted polymers.

3. Desorption of caffeine from the imprinted polymers.

4. Specificity studies of caffeine desorbed imprinted polymers.

5. Investigation of factors influencing caffeine binding.


7. Correlation of the specificity and selectivity characteristics with the nature and extent of the crosslinking.

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Chapter IV describes the investigation results of the effect of the nature and the degree of crosslinking and other related factors impinging on specificity and selectivity of the developed molecular imprinted systems.
Various conditions which affect the rebinding capacity were investigated and the effects of mass of the polymer, time of incubation and concentration of toxicant stock solution on the specificity in rebinding are outlined here. The swelling and solvation characteristics of the polymers and their relation with the selectivity in the case of different crosslinking agents and toxicants are also described.

The final chapter summarizes the work done, the results of investigation of the toxicant selective molecular imprinted polymers and the effect of the various factors affecting specificity and selectivity.

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