TRANSPORT AND REACTIONS IN MOLECULARLY IMPRINTED POLYMERS

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FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
(IN CHEMISTRY)

BY
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CERTIFICATE

Certified that the work incorporated in the thesis entitled "TRANSPORT AND REACTIONS IN MOLECULARLY IMPRINTED POLYMERS" submitted by Mr. Vivek P. Joshi was carried out by the candidate under my supervision. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

Date: November 10, 1998
Place: NCL, Pune

Research Guide
(Dr. R. A. Mashelkar)

DECLARATION STATEMENT UNDER 0.771

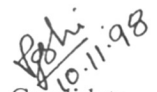
The work presented in the thesis has been carried out by me under the guidance of Dr. R. A. Mashelkar, D.G. CSIR and Secretary DSIR.

The experimental work, observations and interpretation of the data in connection with the studies are entirely my own.

The work reported in this thesis is original and has not been submitted in part or full for any degree or diploma to any other University or Institution.

Research Guide

(Dr. R. A. Mashelkar)



Candidate

(Vivek P Joshi)

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It was a turning point in my life to get an opportunity to work with Dr. R. A Mashelkar, FRS for my Ph.D. degree. His constant enthusiasm for science in spite of his busy schedule and an eye for perfection always kept me on my toes and made me explore newer and newer avenues in research. His constant encouragement and unstinted support at all the times of ups and downs was a moral booster for my work. Other than his scientific achievements, what touched me the most was his warm, caring personality and humility, which always reminded me of a saying in Sanskrit which goes like this. "The more the mango tree is leaden with fruits the more it bends". Words fail me to describe the multifaceted personality of my guide, teacher and mentor, which reminds me of a Kaleidoscope with its dazzling new display at every turn.

I am unable to express my sincere thanks to Dr. M. G. Kulkarni for his constant encouragement and critical comments during entire course of my work. I enjoyed the freedom, which he gave me during course of my research work to explore uncharted areas.

It is my honour to acknowledge Dr. P. Ratnasamy, Director NCL for permitting me to present this work in the form of thesis.

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This work would not have been complete without help and support from the scientific and support staff of NCL. I would specifically like to thank Dr. Sainkar (SIL) and Sanjay Patil for their help in scanning electron microscopy. I would also like to thank Dr. H. S. Soni for his help in determining surface area and porosity of the polymers. It was a great pleasure to discuss some of the work with Dr. Ashish Lele. His suggestions and critical comments helped as a pointer in the right direction.

It gives me an immense pleasure to acknowledge my friends and colleagues in Chemical Engineering Division who made my stay at NCL a memorable experience. My stay away from home in the hostel was made memorable by various friends and room mates from biosciences and catalysis divisions.

Finally I would like to thank my parents for their unstinted support and love, without which this degree would not have been possible.

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Vivek P Joshi

Imagination is more important than knowledge

Knowledge is limited,

Imagination encircles the world

—Albert Einstein

Table of Contents

Abstract of the thesis entitled

"Transport and Reactions in Molecularly Imprinted Polymers"

This thesis deals with studies on molecularly imprinted polymers (MIPs) with special reference to the use of imprinted polymers in separations. MIPs have been hitherto used as stationary phases for racemic resolution, sensor materials and selective catalysts. In this work, we have shown that MIPs can be used as selective sorbents in two new areas, wherein the application potential of MIPs has not been tapped. These applications are, a) purification of organic compounds i.e. removal of trace impurities from organic streams and b) separation of positional isomers. The actual separation systems studied were as follows,

a) Separation of phenol from anisole
b) Separation of phenol from bisphenol-A
c) Separation of 2,4- dihydroxy benzophenone (2,4- DHB) isomers.

The work done in the thesis involves synthesis of imprinted polymers for the separations listed above. We have studied effect of various factors such as imprinting technique, solvents, experimental conditions (equilibrium vs. non-equilibrium) etc. on the selectivity and sorption capacity of MIPs. A predictive mathematical model was developed for predicting the breakthrough behaviour of a packed bed adsorber containing imprinted polymeric adsorbents and thermodynamics of adsorption was studied. For ease of understanding, the thesis is divided in to six main chapters, as follows.

1. Introduction (Literature search)

Molecular recognition is one of the most important phenomena of various life processes such as, enzymatic catalysis, antigen - antibody interactions and interaction between nucleic acid base pairs. There have been various attempts to synthesise receptors which exhibit the recognition phenomena similar to their natural counter parts (cyclodextrin, cryptands, crown ethers etc.).

Since the recognition in the case of enzymes, nucleic acids and antibodies is achieved as a result of three dimensional macromolecular structure, numerous approaches have been used to achieve the same using synthetic polymeric networks. A process for the preparation of polymeric hosts selective for the guest molecules is known as molecular imprinting of polymers (Wulff G., 1995, Steinke et. al., 1995, Mosbach et. al., 1996). Molecularly imprinted polymers, MIPs are prepared by polymerisation of functional monomers and a large excess of crosslinker in the presence of a template molecule. After polymerisation, the template is leached out leaving behind a cavity complementary to the template and having appropriate arrangement of functional groups for rebinding interactions.

The pre-organisation of the monomers and the template can be done using covalent interactions (Wulff and Haarer, 1991) such as carbonate and carboxylate ester, ketal, boronate ester, Schiff base etc. The other method of synthesis of MIPs is through use of non-covalent interactions (Mayes et. al., 1994). Various non-covalent interactions such as hydrogen bonding, hydrophobic interactions, van der Waals interactions, electrostatic interactions have been used in synthesis of MIPs. Metal ion coordination can also be used for organisation of template and the functional monomers (Plunkett and Arnold, 1995). The most commonly used rebinding interactions are non-covalent, as the kinetics of rebinding is faster compared to covalent interactions.

The normal method for preparation of MIPs is precipitation polymerisation (Bulk imprinting), where the functional monomer, template and the crosslinker are polymerised in the presence of a porogen, followed by mechanical crushing and sieving to the desired particle size (Matsui et. al., 1998). Alternatively MIPs can be prepared on a pre-formed polymeric support (Karmalkar et. al., 1996). This is known as surface imprinting. MIPs in the form of microspheres can also be made by seeded emulsion polymerisation, phase inversion polymerisation, suspension polymerisation (Mayes and Mosbach, 1996) etc.

Molecularly imprinted polymers have found applications in areas such as stationary phase for racemic resolution (Andersson et. al., 1990), Sensors

(McNiven et. al., 1998) and selective catalysis (Santora et. al., 1998). Two areas where MIPs can have a potential for application, but unfortunately have not been exploited are a) Removal of trace impurities from organic streams and b) Separation of positional isomers.

This investigation therefore was undertaken to design molecularly imprinted polymers for removal of phenolic impurities from various organic streams. Studies were carried out to elucidate the role of various factors, such as imprinting techniques, solvents, nature of the template molecule etc. which helped in arriving at a rationale for designing MIPs for optimum separation efficacy.

2. Objectives and scope

Phenolics are many a times present as an impurity in an organic stream as a result of the synthetic route (for instance, phenol in anisole and bisphenol-A, nitrophenols in nitrobenzene etc.). The presence of phenolic impurities either leads to problems in down stream processing or deterioration of quality. The normal methods for removal of these impurities are many a times highly energy intensive and cumbersome. The commercially available adsorbents for removal of phenols from aqueous phase (Amberlite XAD series) have low capacity for sorption from organic streams and also lack the selectivity in separation. Therefore the objectives in undertaking this work were following,

- Design and development of molecularly imprinted polymeric adsorbents for selective removal of phenolic compounds from organic streams. The actual systems studied were separation of phenol from anisole and bisphenol-A and separation of 2,4- DHB isomers.
- Study the effect of various parameters such as, imprinting techniques, solvents used in separation studies, experimental conditions for separation studies (equilibrium vs. non-equilibrium) on the sorption capacity and selectivity.
- Mathematical modelling for prediction of breakthrough behaviour of imprinted polymers when packed in an adsorption column.
- Study of the thermodynamics of adsorption

3. Materials and Methods

This chapter describes synthesis of various template monomers such as phenyl methacrylate, 2- (methacryloyl), ethyl phenyl carbonate, phenyl, 4-vinyl benzoate, cumyl, phenyl methacrylate, Allyl, 2,4- DHB carbonate and 2,4- DHB methacrylate. Synthesis of imprinted polymers, imprinted for phenol, p- cumyl phenol and 2,4- DHB using surface and bulk imprinting technique is reported. Experimental procedures for determination of sorption capacity, selectivity for the template under equilibrium and non-equilibrium conditions, generation of adsorption isotherms and breakthrough curves are described.

4. Kinetics and Thermodynamics of Adsorption

Study of kinetics and thermodynamics of adsorption gives an insight in the adsorption process, helps in predicting the breakthrough behaviour of the packed bed adsorber. This enables improvement in the process efficacy and leads to design of the better adsorbents. In this chapter, we have described the mathematical model developed for predicting the breakthrough behaviour of a packed bed adsorber packed with molecularly imprinted polymers. Two different types of the adsorption isotherms viz., empirical adsorption isotherm, obtained by power law fit to the sorption data and dual sorption isotherm, which takes into consideration the adsorption and absorption components of sorption, were used in model development. Mathematical model for prediction of breakthrough curves for following two systems is described, a) separation of phenol from anisole and b) separation of phenol from bisphenol-A.

5. Results and Discussion

We decided to study the design and application of MIPs for separations in the field of impurity removal and isomer separation. Following systems were chosen for the study on imprinted polymers as selective adsorbents.

a) Separation of phenol from anisole
b) Separation of phenol from bisphenol-A
c) Separation of 2,4- dihydroxy benzophenone isomers

Studies were carried out on these systems in order to elucidate the effect of various factors on imprinting efficacy, selectivity and sorption capacity. The results obtained were as follows.

a) Separation of phenol from anisole

Molecularly imprinted polymers selective for phenol were prepared using hydroxy ethyl methacrylate (HEMA), methacrylic acid (MAA) etc. as functional monomers. Surface imprinting was the method of synthesis. Imprinting effect could not be seen when anisole was used as the solvent for adsorption studies, as the polymer showed extensive swelling in anisole, leading to distortion of the imprint cavity. On the other hand a high selectivity for the template, phenol in presence of competing sorbates such as, chlorobenzene, bromobenzene and o-nitrophenol could be seen, when non-swelling solvents such as THF, heptane, 1% acetic acid in methanol were used for selectivity studies. A high selectivity for the template was seen under equilibrium as well as non-equilibrium conditions. Increase in the selectivity as a result of the increase in the crosslink density of the support polymer was observed (Joshi et. al., 1998a).

A predictive mathematical model was developed to predict the breakthrough behaviour. Empirical adsorption isotherm was used in development of the model and a very good agreement between the theory and the experimental data was observed. The use of dual sorption isotherm, which takes into account the adsorption and absorption of the solute on the polymeric adsorbent , led to more accurate model predictions.

b) Separation of phenol from bisphenol-A

MIPs selective for phenol were prepared using MAA and HEMA as functional monomers. Surface and bulk imprinting techniques were used for synthesis of MIPs. Imprinted polymer selective for bisphenol-A was also prepared, using p- cumyl phenyl methacrylate as the template monomer, by bulk imprinting process.

Surface imprinted polymers were found to show lower selectivity for the template as compared to bulk imprinted polymers. The reasons for this were, the presence of non-specific adsorption sites on the support polymer used for surface imprinting , small size of the template (phenol) and single rebinding interaction. The selectivity was found to depend on the hydrogen bond donator factor of the solvent. The selectivity and the trends in selectivity also depended on whether equilibrium was reached or not. The trends in imprinting efficacy with respect to the solvent polarity and hydrogen bond donator factor of the solvent were exactly opposite under the equilibrium and non-equilibrium conditions (Joshi et. al., 1998b and 1998c).

c) Separation of 2,4- dihydroxy benzophenone isomers (2,4- DHB)

Imprinted polymers were prepared using 2,4 -DHB as the template and allyl alcohol (Joshi et. al., 1998d) and MAA as the functional monomers. Surface as well as bulk imprinting was used for the preparation of MIPs. Various positional isomers of 2,4- DHB, such as 2,2'- DHB, 4,4'- DHB, 2- HB etc. were used as the competing sorbates.

The surface imprinted polymers showed better selectivity as well as sorption capacity than the bulk imprinted polymers, in equilibrium experiments. This was found to be mainly due to the limitations on accessibility of the imprint site in bulk imprinted polymers. The effect of low accessibility was accentuated as the template was bulky in nature.

Under non-equilibrium conditions, the sorption capacities for the surface imprinted polymers were lower than their equilibrium sorption capacities. Selectivity values were also lower than the equilibrium values, but they were still high enough for use of MIPs on industrial scale. Solvents and experimental conditions were again shown to influence not only the selectivity but also the sorption capacity.

Role of imprint cavity and rebinding interactions in governing the selectivity was validated by blocking the imprint cavity, when the selectivity values showed a drastic reduction.

6. Conclusions and Directions for future work

Conclusions

In summary, this work highlights synthesis and applications of molecularly imprinted polymers as selective adsorbents for removal of trace impurities from organic streams as well as separation of positional isomers.

Our studies on effect of various factors on selectivity, imprinting efficacy and sorption capacity, led to development of a rationale for designing of separation systems using MIPs with enhanced efficacy.

A mathematical model was developed for predicting the breakthrough behaviour of MIP adsorbents packed in a column. The model being truly predictive can be used for extrapolating the results obtained on a laboratory scale to commercial scale.

Directions for future work

Though we were able to arrive at a rationale for the design of imprinted polymers for enhanced separation efficacy, there still lacks a deeper understanding of the phenomena involved in the process of molecular imprinting. Experimental tools such as Xenon-NMR and theoretical tools such as molecular modelling can give a deeper insight into the imprinting phenomenon.

On the other hand, for development of commercial applications of imprinted polymers in separations, various systems of commercial importance need to be identified and explored. Similarly for use of MIPs on commercial scale, various system parameters need to be studied in great detail.

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Chapter 1

Introduction

1.1 Molecular recognition

Molecular recognition is an important phenomenon in various biological processes, as many of the interactions in biological processes are selective in nature. For instance, the selectivity in enzymatic catalysis is achieved as a result of recognition of a substrate molecule by the enzyme followed by selective binding. This brings the functional groups necessary for catalysis in close proximity of the substrate thereby forming an enzyme-substrate complex and catalysing the reaction. Similar is the case with nucleic acids and antibodies, which show selective recognition phenomenon.

The enzymes can show a wide range of substrate selectivity. For instance, specificity and selectivity of proteolytic enzymes substilisin, trypsin and thrombin increase in that order for hydrolysis of peptide bond. Substilisin does not show any specificity, trypsin cleaves the peptide bond next to lysine or arginine carboxyl, whereas thrombin cleaves only that peptide bond, which is between arginine carboxyl and glycine amine (Stryer L., 1981). The reason for this substrate selectivity was proposed to be the presence of a cavity that can exactly fit only a particular substrate that is specific for the particular enzyme (Lock and Key mechanism), but this was found to be not always true. The fit between the enzyme and substrate is not always as perfect as a lock and key but the presence of substrate can induce changes in enzyme structure thereby leading to an induced fit (Carboxypeptidase A). It is now established that the origin of this selectivity in enzymes is the three dimensional structure of enzymes, which creates certain pockets wherein the substrate can be fitted. Depending on the size, shape and hydrophobicity / hydrophilicity of this pocket, selectivity for the substrate can be achieved. Hence certain enzymes can show a very high selectivity for a particular substrate (Thrombin), whereas certain enzymes only show selectivity for a class of compounds (Substilisin).

Similar to the enzymes, the purine and pyrimidine bases present in nucleic acids also show a very high selectivity for their respective counterparts. For instance the purine base adenine always combines with thiamine as guanine always combines with cytosine (Stryer L., 1981). The reason for this very high

selectivity between the nucleotide bases is the selective hydrogen bonding between these bases. This selectivity leads to perfect replication of DNA and RNA that encode all the vital genetic information about an organism and mismatch of the base pairs can be fatal. The selectivity of proper base pairing in DNA / RNA replication is so high that a mismatch occurs only once in million base pairs. Similarly the antibodies created against an antigen, an external stimulus show a high selectivity for each other.

Over the years it had been the dream of scientists to synthesise the materials, which show the molecular recognition phenomena. The earlier attempts were made with intricate molecular hosts such as macrocyclic polyethers having hetero atoms in the ring i.e. crown ethers (Petranek and Ryba, 1974 and Bochenska and Biernat, 1984), cryptands (Cram D., 1986 and Lehn J., 1988), calixarines, cyclodextrins (D'Souza and Bender, 1987) etc.

All these molecules show a selective host - guest interaction. But almost all of them are monomeric in nature, whereas the molecular recognition phenomenon as seen in nature is shown mainly by macromolecular hosts (enzymes, nucleic acids and antibodies). This recognition stems mainly due to their specific three dimensional structures (e.g. α-helix and β-plate structure of enzymes and double helical structure of DNA and RNA). The origin of selectivity on enzymes can be traced to the three dimensional structure of enzymes, which leads to generation of cavities / pockets lined with hydrophobic / hydrophilic amino acids residues. For instance the binding subsite in α- chymotrypsin is hydrophobic in nature, 10-12 A^0 deep and 4 X 6 A^0 in cross section. This cavity can snugly fit the aromatic side chain in a peptide molecule and bring about the hydrolysis of the neighbouring peptide bond (D'Souza and Bender, 1987). Close to this binding site, α-chymotrypsin contains the serine - aspartic acid - histidine triad crucial for catalysis. Hence the interest to synthesise polymeric hosts having a specific three dimensional structure, lined with functional groups specific for guest molecules is obvious.

A technique for the synthesis of three dimensional polymeric hosts that are selective for a particular template is known as molecular imprinting of polymers.

The polymers formed using molecular imprinting process also contain cavities, which fit only the template molecule snugly and have functional groups that can interact with the template.

1.2 Principle of molecular imprinting

Molecularly imprinted polymers (MIPs) are synthesised by polymerisation of functional monomer / monomer combinations along with a large excess of crosslinker and a porogen in the presence of a template molecule (Mosbach and Ramstrom, 1996, Steinke et. al., 1995 and Wulff G., 1995). After polymerisation, the template is leached out leaving behind a cavity that is complementary to the template molecule and contains appropriately positioned functional groups that help in rebinding interactions.

The earliest references on MIPs are found mainly on imprinted silicas, imprinted for dyes such as methyl orange (Dickey F., 1949), methyl orange and ethyl orange (Bernhard S., 1952), quinine and cinchonidine (Beckette and Anderson, 1957) etc. These were reported to show slightly higher selectivity for the template used during the synthesis, but the cause for this selective uptake was not known. The presence of traces of the template molecules present in these silicas was thought to be responsible for the selective uptake of the template. Similar to imprinted silicas, polymers can also be made selective for a particular substrate (template) by preparing a highly crosslinked polymeric network in the presence of the template and removal of the template after polymerisation. Gunter Wulff and Klaus Mosbach were the pioneers in molecular imprinting of polymers in seventies and eighties (Wulff et. al., 1973, Wulff et. al., 1977, Arshady and Mosbach, 1981). Figure 1.1 gives the schematic of molecular imprinting of polymers for the template (T).

1.3 Methodology of imprinting

Imprinted polymers are prepared mainly by two different methods of synthesis viz., bulk imprinting (precipitation polymerisation) and surface imprinting, though some novel synthesis routes such as phase inversion emulsion

polymerisation, surface template polymerisation, seeded emulsion polymerisation etc. have been explored. Composite MIPs are also synthesised, which comprise of an inorganic support polymer such as silica and a grafted imprint layer that is polymeric in nature.

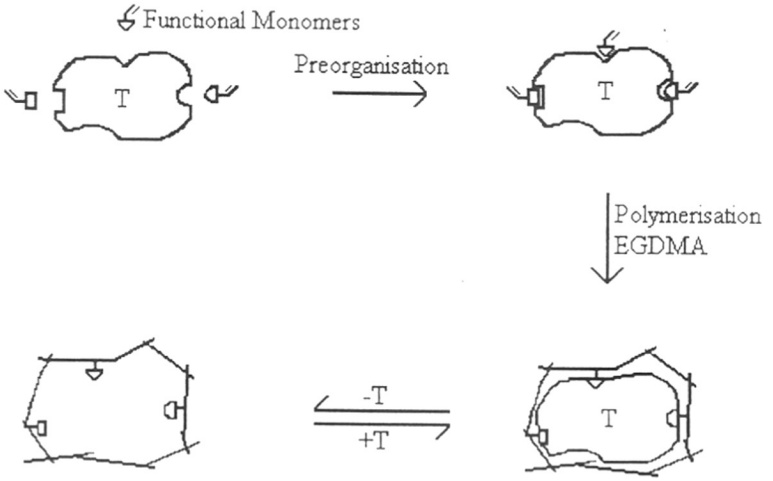


Figure 1.1 : Schematic of molecular imprinting for a template (T) using various functional monomers

1.3.1 Bulk imprinting (precipitation polymerisation)

Normally molecularly imprinted polymers (MIPs) are prepared by precipitation polymerisation technique (Arshady and Mosbach, 1981 and Yu et. al., 1997). Arshady and Mosbach, (1981) reported synthesis of Rhodanile blue and Saffranine-O imprinted polymers using precipitation polymerisation technique. In this method, the template, functional monomers and the crosslinker are mixed with a solvent (porogen) and polymerisation is carried out by any of the standard methods of polymerisation. As polymerisation progresses, the polymer, being insoluble in the porogen, phase separates from it entrapping the solvent along with it. This generates a highly porous polymeric matrix essential for accessibility of the imprint sites. After polymerisation, the polymer formed is washed free of the unreacted monomers, crosslinker and the porogen. Leaching of the template molecule is achieved either by a solvent (Mayes et. al., 1994) or with the help of

chemical treatment (Bystrom et. al., 1993), depending on the method used for imprinting. The polymer is then crushed mechanically and sieved to the desired particle size. The irregularly shaped polymer particles can be packed in a HPLC column using slurry technique, wherein the column is used for selectivity studies.

For use as packing materials in the HPLC columns, MIPs can directly be synthesised in a steel column as a porous monolith. For instance, Matsui et. al., (1993) synthesised L- phenyl alanine anilide imprinted polymers directly in a HPLC column as a porous monolith. After polymerisation, the column was directly connected to the HPLC and with the help of a solvent, unreacted monomers, porogen, crosslinker and the template were removed. The porous monolith was then used for the selectivity studies.

Though, most preferred and simplest method of synthesis of MIPs is precipitation polymerisation, it can lead to problems in accessibility of the imprint site. There are conflicting reports in literature as to the accessibility of imprint sites in bulk imprinted polymers. For instance, Whitcombe et. al., (1995) reported that in cholesterol imprinted polymers prepared by bulk imprinting, the accessibility of the imprint site was high as indicated by the degree of hydrolysis of the template, cholesteryl, (4-vinyl) phenyl carbonate (degree of hydrolysis = 73-106%), whereas Steinke et. al., (1995) reported that some of the imprint sites in bulk imprinted polymers remain inaccessible to the template. To overcome the accessibility problems, surface imprinting for the preparation of MIPs was proposed, wherein the imprint cavity is generated on the surface of a support polymer.

1.3.2 Surface imprinting

In this process, the functional monomers, crosslinker and the template are sorbed on a preformed macroporous support from a solvent and post polymerisation is carried out on this support (Karmalkar et. al., 1996, Dhal et. al., 1995). As the imprint layer is formed only on the surface of the support polymer, accessibility of the imprint sites is very high. The support used can either be polymeric (Karmalkar et. al., 1996) or inorganic in nature (Plunkett and Arnold, 1995 and Wulff et. al., 1986). The imprinted polymer is obtained in the form of

microspheres, which can be easily packed in the HPLC column and used in selectivity studies. As a result of regularity of size and shape of these particles, they also offer an additional advantage of lower back pressure at higher flow rates compared to bulk imprinted polymers. Another advantage of using surface imprinting technique is the ease with which the surface characteristics of the support polymer can be changed (Lele et. al.,1998) without affecting the imprint layer.

A highly porous support polymer for use in surface imprinting can be prepared by various techniques of polymerisation such as, suspension polymerisation (Svec et. al., 1975), emulsion polymerisation or bicontinuous emulsion polymerisation (Burban et. al., 1995). Highly porous support polymers used in perfusion chromatography (for instance POROS^{TM}) can also be used for this purpose. These supports have a highly porous structure, thereby reducing the equilibration time. Similarly highly porous silica supports needed for this application are available commercially (e.g. Lichrosorb Si60, Lichrosorb Si100 etc. from Merck) in a variety of pore sizes and pore size distribution as well as surface chemistries.

Figure 1.2 gives a schematic of surface and bulk imprinting. It also outlines the factors that can affect the kinetics of the rebinding process, selectivity and sorption capacity.

In order to obtain MIPs in the form of regularly sized microspheres, direct suspension polymerisation of the functional monomers, crosslinker and the template can be carried out in an inert medium. For instance, Mayes and Mosbach, (1996) reported direct suspension polymerisation of a mixture of template, functional monomers and the crosslinker in perfluorinated hydrocarbon medium. These polymers showed similar selectivity for the template as that of bulk imprinted polymers and gave lower back pressure when packed in a HPLC column even at higher flow rates thereby, increasing the throughput. There are some reports on novel synthetic routes for MIP synthesis, such as surface template polymerisation (Fujiwara et. al., 1996), seeded emulsion polymerisation (Tsukagashi et. al., 1993) etc. All these methods lead to generation of imprinted

polymers in the form of microspheres and hence show all the advantages of surface imprinted polymers.

The methods of MIP synthesis described above, give MIPs in the form of microparticles, which can be packed in a HPLC column and used in separation studies. But MIPs can also be used for membrane based separations, when imprinted membranes are synthesised. For instance Mathew-Krotz and Shea, (1996) reported synthesis of 9-ethyl adenine imprinted polymeric membranes showing a high selectivity for the template coupled with a high flux. Similarly Wang et. al., (1996) reported polymeric membranes imprinted for theophyllin prepared by phase inversion technique, which showed a high selectivity for template when compared to caffeine (selectivity, $\alpha_{(Theo./Caff.)} = 9.5$).

1.3.3 Bio-imprinting

The earlier two methods of imprinting viz., surface and bulk imprinting discussed in sections 1.3.1 and 1.3.2 make use of synthetic polymers for creating the three dimensional scaffold that contains the selective cavities. But natural macromolecules such as proteins (Braco et. al., 1990, Dabulis and Klibanov, 1992), enzymes (Staahl et. al., 1990, LionDagan and Willner, 1997), polysaccharides (Choi K., 1990) etc. can also be used to create polymeric hosts with unusual selectivities. In many of these reports on bio-imprinting, the protein configuration is frozen with the help of a solvent in the presence of a substrate, though there are some reports on chemical crosslinking. This leads to creation of artificial pockets in the enzyme structure and can give rise to unusual selectivity behaviour. For instance Staahl et. al., (1990) reported precipitation of aqueous solution of α-chymotrypsin-N-acetyl-D-tryptophan complex in anhydrous propanol leading to formation of imprinted enzyme, which showed increased activity for esterification of D-substrate over L-substrate in anhydrous cyclohexane. Similarly Rich and Dordick, (1997) reported synthesis of thymidine imprinted substilisin, by lyophilisation of the enzyme-thymidine complex, for selective acylation of the template over structurally related substrates.

Thus enzymes with unusual selectivities can be prepared by molecular imprinting technique. Various proteins can also be used for preparation of selective bio-adsorbents using the same technique.

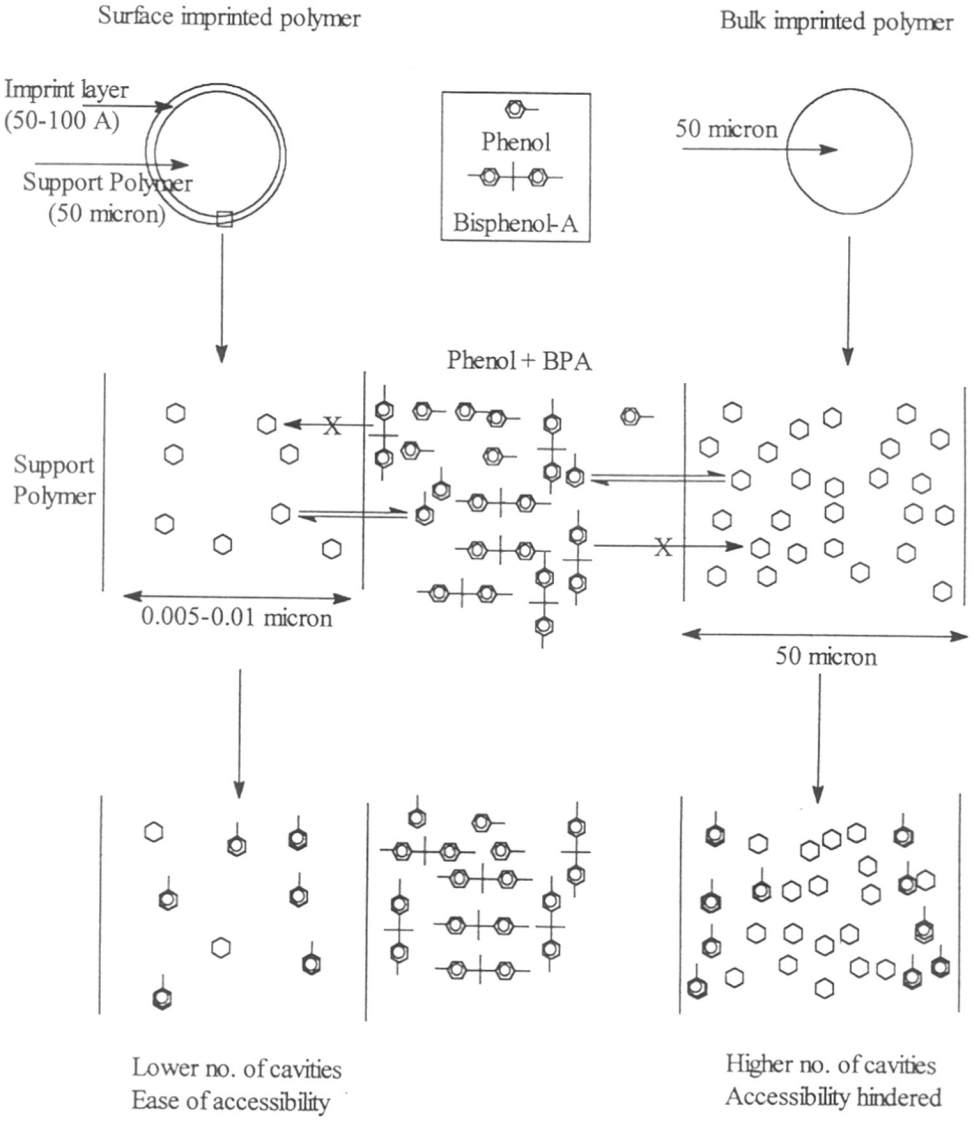


Figure 1.2: Schematic of Surface vs. Bulk imprinting for separation of phenol and Bisphenol-A

1.4 Interactions during MIP synthesis

Synthesis of MIPs relies on organisation of the functional monomers around the template molecule and interactions between the template and the functional monomers. Different classes of functional monomers are used for the synthesis of MIPs for a variety of templates. Some representative monomers commonly used in MIP synthesis are listed in table 1.1.

Table 1.1

Various monomers used for synthesis of MIPs

Sr. No.	Chemical class	Monomer	Reference
1	Acidic	Methacrylic acid	Andersson et. al., 1995
		Itaconic acid	Fischer et. al., 1991
		2-acrylamido, 2-methyl-1-propane sulphonic acid	Dunkin et. al., 1993
		2-(Trifluoromethyl) acrylic acid	Matsui and Takeuchi, 1997
2	Basic	4- vinyl pyridine	Haginaka J. et. al., 1997
		Diethyl amino ethyl methacrylate	Cheong et. al., 1998
		Oleyl amine	Fujiwara et. al., 1996
3	Neutral	Acrylamide	Yu and Mosbach, 1997
		Merocyanine acrylate	Marx-Tibbon and Willner, 1994
		Hydroxy ethyl methacrylate	Sreenivasan K., 1997
		BOC-L- Val- methacrylate	Yano et. al., 1997
		2- (p-vinyl phenyl), 1,3 - propane diol	Shea and Sasaki, 1991
		Oleyl phenyl hydrogen phosphate	Murata et. al., 1996
		β- cyclodextrin	Asanuma et. al., 1997

The efficacy of separations of MIPs depends on this preorganisation and hence various methods are used for this purpose. There are three main methodologies of organisation of the functional monomers around a template. These are a) covalent linkages, b) non-covalent linkages and c) metal ion coordination.

1.4.1 Covalent linkages

As the name suggests, this technique makes use of covalent link between the template and the functional monomer. Thus the template molecule forms a part of the functional monomer, during the synthesis of MIPs. The template monomer is mixed with the crosslinker and polymerised in the presence of a porogen generating a macroporous matrix. After synthesis, the template is removed from the polymer by chemical treatment such as, hydrolysis leaving behind a size and shape selective cavity. The cavity then acts as the selective host for the template and the appropriately positioned functional groups, help in rebinding interactions. The choice of covalent linkages is therefore limited by the ease with which covalent bond can be formed between the template and the functional monomer and more importantly by the ease with which this bond can be cleaved with out affecting the network polymer. These two limitations restrict the use of covalent linkages for synthesis of MIPs to a few classes of compounds, which satisfy these conditions.

The various covalent linkages that can be used for synthesis of MIPs are carboxylate esters (Wulff and Vietmeier, 1989, [4- vinyl benzyl esters of N-protected amino acids]), carbonate esters (Whitcombe et. al., 1995 [cholesteryl, 4-(vinyl), phenyl carbonate]), Schiff base (Belokon et. al., 1980 [N^{α}- 5- methacryloyl amino salicylidene N^{ε}- methacryloyl- (S)- lysinato copper], Wulff et. al., 1986), boronate esters (Wulff and Kirstein, 1990 [4- vinyl phenyl boronate ester of phenyl α-D mannopyranoside], Wulff and Haarer, 1991), mono and bis ketals (Shea and Dougherty, 1986 [mono and bis ketals of 1,3- diacetyl benzene with 2-(p- vinyl phenyl) 1,3- propane diol]) etc. In most of these cases removal of the

template after the polymerisation is carried out using acidic or alkaline hydrolysis under mild conditions.

Though the use of covalent linkages in MIP synthesis is restricted by the ease of formation and cleavage of the template-monomer bond, the cavity complementarity is expected to be superior to non-covalent imprinting approach. As the template forms a part of the functional monomer, all the cavities formed are similar in shape and size as well as the selectivity. The external factors such as temperature of polymerisation and porogen do not affect the interactions between the template and the monomer.

1.4.2 Non-covalent linkages

This method relies on organisation of the functional monomers around the template using secondary non-covalent interactions, such as hydrogen bonding (Cheong et.al., 1998, Tanabe et. al., 1995 and Yano et.al., 1998), van der Waals interactions (Dickert et.al., 1998), hydrophobic interactions (Andersson et. al., 1996, Yu et. al., 1997), electrostatic interactions (Marx-Tibbon and Willner, 1994 and Sellergren B., 1994) etc.

The MIPs are synthesised by mixing the functional monomers, crosslinker and the template in a porogen (solvent). As a result of secondary interactions between the functional monomers and the template, a weakly organised structure is obtained. In this complex, the functional monomers organise themselves around the template in order to maximise the various secondary interactions between the template and the monomers. This organised structure is then fixed spatially by the formation of a rigid three dimensional network as a result of polymerisation. The template can be easily removed using a solvent as no covalent bonds are formed, leaving behind a cavity having appropriately positioned functional groups useful for rebinding. As covalent bond formation is not necessary for the synthesis, this method has a much wider application potential as compared to covalent linkages. But the disadvantage is the interaction between template and the functional monomer is weak and high cavity complementarity is not achieved. Cavities with a

wide range of selectivities are obtained as a result of this. Figure 1.3 gives a comparison between covalent and non-covalent imprinting.

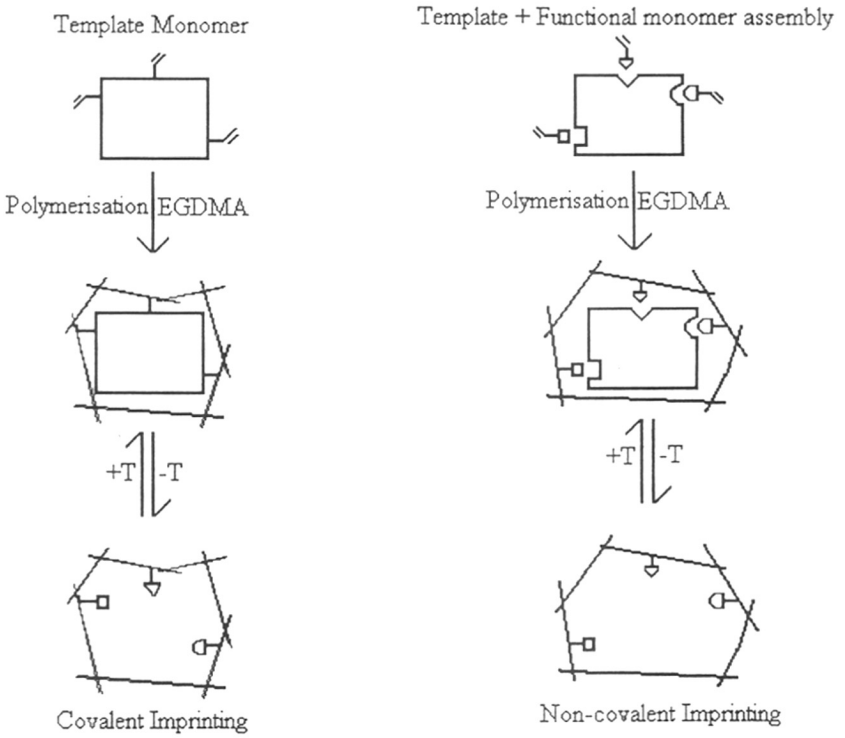


Figure 1.3: Schematic of covalent and non-covalent imprinting technique for the synthesis of MIPs for a template (T)

1.4.3 Metal ion coordination

Metal ion coordination relies on metal - ligand interactions for organisation of the template and the functional monomers and bond between the two is coordinate bond. The metal ion can either be used as a) template or b) functional monomer.

1.4.3.1Metal ion as a template

Use of metal ion as a template generates the imprint cavities, which are selective for the template ion. These materials can be used as selective adsorbents for the template metal ion (Yu et. al., 1992, Gupta and Neckers, 1982 and Zeng and Murray, 1996). Depending on the coordination number of the metal ion and

the free coordination sites available, a complex is formed with the functional monomers. This complex is then polymerised and the metal ion template is removed using either a solvent or a stronger chelating agent such as EDTA. On re-equilibrating with a variety of metal ions, selective complexation can be seen with the template metal ion.

1.4.3.2 Metal ion as the functional monomer

Metal ion can also be used for the organisation of template and the functional monomers. Here metal ion can be used during the synthesis of MIPs for organisation of the template and the functional monomers (Karmalkar et. al., 1996 and Lele et. al., 1998). On the other hand, it can also form a part of the functional monomer and is necessary for the rebinding of the template (Plunkett and Arnold, 1995, Dhal and Arnold, 1991). Similar to the non-covalent imprinting, a complex of functional monomers, template and the metal ion is polymerised with large excess of crosslinker in a porogen. After polymerisation, the template and the metal ion are removed with the help of a suitable solvent. Depending on whether the metal ion is necessary for selective recognition, the imprinted polymer is re-equilibrated with the same metal ion as was used during synthesis of MIPs.

Coordination bond being stronger than the non-covalent interactions, the organisation of the template and the functional monomers is better than non-covalent interactions. But the use of metal ion coordination is limited by the ability of the template and the functional monomers to form a stable complex with the metal ion and hence is restricted mainly to transition metals.

1.5 Rebinding interactions

Similar to the interactions used for synthesis of MIPs, the rebinding interactions that are necessary for the selective uptake of the template also fall under the same three categories, viz., covalent, non-covalent and metal ion coordination.

1.5.1 Covalent interactions

Covalent interactions used during rebinding are the same as those used during synthesis of MIPs. These are boronate ester formation, ketal formation carbonate ester formation etc. The main limitation on the use of covalent linkages for rebinding interactions is the slow kinetics of the process. The usage of covalent interactions during rebinding is also restricted by the types of covalent interactions that can be formed easily and reversibly under the experimental separation conditions such as packed HPLC column.

1.5.2 Non-covalent interactions

Non-covalent interactions are the most commonly used rebinding interactions used for separation studies using MIPs, as the kinetics of interactions is faster than the covalent interactions and are reversible as well. Similar to the synthesis of MIPs, interactions such as hydrogen bonding, hydrophobic interactions, van der Waals interactions and electrostatic interactions are used for rebinding studies.

1.5.3 Metal ion coordination

Metal ion coordination can be used as the rebinding interaction when the metal ion itself acts as the part of the assembly of the functional monomers (Fujiwara et. al., 1996, Matsui et. al., 1996). Advantage of use of metal ion coordination is again the faster kinetics of rebinding as compared to covalent rebinding interactions.

1.6 Factors affecting the binding and rebinding of the template

Various factors such as, the choice of porogen, solvent used for selectivity experiments, crosslinker, polymerisation and rebinding temperature can affect the efficacy of the binding and rebinding interactions between the functional monomers and the template, thereby changing the selectivity behaviour.

1.6.1 Porogen

The effect of porogen in the synthesis of MIPs is two fold. Firstly, porogen is essential for generating a porous imprinted matrix, which is important for the ease of the accessibility of the imprint sites. Thus the porogen chosen should be such that, it should be a good solvent for the monomer, but a poor solvent for the polymer, which leads to a porous structure as a result of phase separation. Secondly, porogen can also affect the organisation of functional monomers around the template. This is most crucial in the case of non-covalent linkages as well as metal ion coordination. In both these cases the organisation of the functional monomers is done with the help of weak secondary interactions which can be adversely affected by improper choice of the porogen (Spivak et. al., 1997).

1.6.2 Solvent used for rebinding studies

The choice of solvent can alter the secondary interactions that are responsible for rebinding of the template and monomers and thereby alter the selectivity pattern. For instance Andersson, (1996) reported that in the case of (S)-propranolol imprinted polymer, synthesised using non-covalent interactions, use of toluene as the solvent, gave a high enantioselectivity (cross reactivity for (R)-propranolol < 1%) whereas the use of water as a solvent, led to high selectivity against structurally similar substrates. This difference was a result of the different rebinding interactions in toluene and water (hydrogen bonding and electrostatic interactions respectively). Effect of hydrogen bond donator factor of the solvent, on selectivity in racemic resolution by MIPs was similarly reported by Allender et. al., (1997).

1.6.3 Temperature

Another factor that can affect the binding and rebinding interactions, is the temperature of polymerisation as well as temperature of rebinding. Effect of polymerisation temperature on selectivity can be seen, mainly in the case of non-covalently imprinted polymers, as the non-covalent interactions are significantly weakened at a high temperature. It was reported by Spivak et. al., (1997) and

Cheong et. al., (1997) that the non-covalently imprinted polymers prepared at a lower temperature, showed a higher selectivity for the template as compared to those prepared at a higher temperature. Thus in order to have a strong binding between the template and functional monomers during synthesis of MIPs, it is advantageous to use low temperature for polymerisation. Though thermal polymerisation is the common method for MIP synthesis, various alternative methods are devised for initiating polymerisation at lower temperature such as, photo initiation (Andersson and Mosbach, 1990 and Schweitz et. al., 1997), gamma ray irradiation (Sreenivasan, 1997, Sreenivasan and Sivakumar, 1997) etc. The adverse effect of higher polymerisation temperature on selectivity is not only observed when MIPs are prepared as microparticles, but also in the preparation of imprinted membranes using phase inversion technique. Thus it was observed that the use of low temperature for phase inversion, led to higher selectivity for the imprinted membranes (Wang et. al., 1997).

Similar to polymerisation temperature, the rebinding temperature also affects the selectivity behaviour of the imprinted polymers. It was reported by Wulff et. al., (1987) and O'Shannessy et. al., (1989) that the use of a high temperature for rebinding, improves the selectivity and peak shape in chromatographic separations using MIPs. The reason for this could be improved kinetics of the rebinding process and the reduction of non specific binding at a higher temperature.

1.6.4 Crosslinker

The chemical type of the crosslinker as well as the degree of crosslinking are known to affect the selectivity for the template, when MIPs are used as selective sorbents. It was reported by Wulff et. al., (1987), that in the case of phenyl, α-D- mannopyranoside imprinted polymers no selectivity for the template was shown by the imprinted polymers when the crosslink density was lower than 10%. The selectivity for the template increased from 1.5 to 3.04 when the crosslink density was increased from 47.5% to 64%. Further increase in the crosslink density to 95% led to a slower rise in the selectivity to 3.66. A further

increase in crosslink density did not increase the selectivity. Wulff et. al., (1987) also reported that the highest selectivity was obtained by using ethylene glycol dimethacrylate (EGDMA) as a crosslinker in comparison to divinyl benzene (DVB), Bisphenyl dimethacrylate, tetramethylene glycol dimethacrylate etc. Similar results were obtained when hexamethylene diisocyanate (HMDI) and toluene diisocyanate (TDI) were used as crosslinkers for preparation of molecularly imprinted polyurethanes i.e. HMDI based polyurethanes showed higher template selectivity than TDI based polymers (Asanuma et. al., 1997). The reason for this behaviour was found to be perfect balance between rigidity and the flexibility of the network polymer obtained using EGDMA / HMDI as crosslinker. Hence EGDMA is the most common crosslinker used in synthesis of MIPs. Recently it was reported that trimethylol propane trimethacrylate (TRIM), having three double bonds gives higher selectivity as compared to EGDMA (Kempe and Mosbach, 1995).

1.7 Applications of MIPs

A very high selectivity for the template molecule in the presence of structurally similar substrates make imprinted polymers an ideal choice for following applications,

a) Separation of optical isomers (racemic resolution)
b) Sensors / receptor mimics / selective sorbents
c) Size and shape selective catalysis

1.7.1 Separation of optical isomers (racemic resolution)

MIPs show a very high selectivity for the template chosen during the synthesis, due to the formation of cavities with appropriately oriented functional groups. Since the optical isomers of a compound differ only in the spatial arrangement of the functional groups, it is possible to use MIPs for racemic resolution. The earliest references for the use of MIPs in racemic resolution could be found for the partial elucidation of stereochemical configuration of some of the natural products such as morphine, levorphanol etc. (Beckett and Anderson, 1960).

In recent times, MIPs have been used for separation of drug molecules (Nilsson et. al., 1994), amino acids and their derivatives (Glad et. al., 1995), oligopeptides (Ramstrom et. al, 1994), carbohydrates (Mayes et. al., 1994), nucleic acid bases (Spivak and Shea, 1998) etc.

1.7.1.1 Racemic resolution of drugs

Many a times the therapeutic activity of a chiral drug molecule is restricted to one of the optical isomer, whereas the other isomer is highly toxic. Table 1.2 gives the differences in the therapeutical properties of some of the common drugs (Nicholls et. al., 1995).

Table 1.2

Therapeutical properties of optical isomers of drugs

Sr. No.	Drug	S- Isomer	R- Isomer
1	Thalidomide	Teratogenic*	Sedative
2	Ethambutol	(SS)- Tuberculostatic	(RR)- Blindness*
3	Penicillamine	Anti arthritic	Toxic*

(* Toxic optical isomer of the drug molecule)

It can be seen from the above table that with many of the optically active drugs sold as racemic mixtures, the therapeutic activity is present only with one isomer whereas the other antipode is highly toxic. Due to this reason, the drug regulatory authorities now have stricter regulations on marketing of asymmetric drugs as racemic mixtures, which requires the manufacturers to carry out toxicological studies on both the optical isomers as well as the racemic mixture of the drug. Therefore it has become imperative to devise methods for resolution of optical isomers of the drugs which are currently sold as racemic mixtures. Though asymmetric synthesis, enzymatic resolution, chiral stationary phases can be resorted to for such separations, MIPs can also be an attractive alternative for the same purpose. In fact, it has been observed (table 1.3) that the MIPs also exhibit

similar selectivity profile for racemic resolution, as has been shown by commercially available chiral stationary phases (CSPs) (Nicholls et. al., 1995).

Table 1.3

Comparison between selectivity (α) of MIPs and commercial CSPs in racemic resolution

Sr. No.	CSP	α	Reference
1	Anti S- naproxen MIP	1.65	Kempe and Mosbach, 1994
2	Pirkle naproxen brush selector CSP1	1.22	Pirkle and Welch, 1991
3	Pirkle brush selector, CSP3	2.26	Pirkle et. al., 1992
4	Human serum albumin	1.32	Noctor et. al., 1991
5	α-1- acid glycoprotein (EnantioPac)	1.71	Kern J., 1991

MIPs have been used for the racemic resolution of drugs such as pentamidine (Sellergren B., 1994 and Nilsson et. al., 1994), (S)- naproxen (Haginaka et. al., 1997), chloramphenicol (Levi et. al., 1997), (S)- ropivacaine (Schweitz et. al., 1997), (S)- timolol (Fischer et. al., 1991), cyclobarbital (Tanabe et. al., 1995), erythromycin A (Siemann et. al., 1997) etc.

1.7.1.2 MIPs in racemic resolution of molecules other than drugs

Though the main thrust of applications of MIPs had been for chiral resolution of drugs, these are also used for separation of amino acids and their derivatives. For instance Sellergren and Shea, (1993), Matsui et. al., (1993) and O'Shannessy et. al., (1989) reported resolution of L- phenyl adenine anilide using MIPs. Damen and Neckers, (1980a) reported separation of t-butyloxy carbonyl-L-Proline using covalently imprinted MIPs. Yoshikawa et. al., (1998) reported separation of isomers of CbZ-glutamic acid using molecularly imprinted carboxylated polysulphone membranes. Some work has also been done in resolution of oligopeptides using MIPs (Yano et. al., 1997, Ye et. al., 1998 and Ramstrom et. al., 1994). Lastly MIPs have been used as selective adsorbents for

the separation of anomers and epimers of carbohydrates and carbohydrate derivatives (Mayes et. al., 1994, Wulff and Haarer, 1991 and Wulff and Kirstein, 1990).

In almost all of these applications reported, the MIPs show a very high enantio and regio selectivity. The MIPs are not only able to distinguish the template from its optical isomer (stereoisomer) but also from structurally similar substrates (diastereoisomers). But these methods have still not found applications on a commercial scale as a result of their low sorption capacity (~ 1.0 - 5.0 mg / g of resin).

1.7.2 MIPs as sensors / receptor mimics / selective adsorbents

Due to their high selectivity for the template, MIPs have also been used as sensors in monitoring the drug levels in biological fluids. They are also used for detection of various pollutants in water, air etc for environmental studies.

Table 1.4

Molecularly imprinted polymeric sensors

Sr. No.	Analyte	Conc. Range, µg / ml	Transducer	Reference
1	Vitamin K1	0-4	Ellipsometry	Andersson et. al., 1988
2	Phenylalanine anilide	33-3300	Potentiometry	Andersson et. al., 1990
3	Dansyl-L-phenylalanine	0-30	Fibre optic fluorescence	Kriz et. al., 1995
4	Atrazine	0-0.5	Conductometry	Piletsky et. al., 1995
5	Morphine	0-10	Amperometry	Kriz and Mosbach, 1994

The chief requirement of MIPs as sensor materials is fast and linear response over the desired concentration range. The response obtained due to selective uptake of the analyte can then be amplified and detected using a variety of techniques such as conductometry, fluorescence, ellipsometry etc. Table 1.4

given above lists various analytes for which molecularly imprinted sensors are prepared and the methods of their detection (Kriz et. al., 1997).

As sensors, MIPs can be used as alternatives to antibodies in immuno sorbent assays. The advantages are a high selectivity for the analyte and a low cross selectivity similar to antibodies. The additional benefits being their higher stability against environmental abuse and ease of preparation. For instance, Vlatakis et. al., (1993) reported theophyllin imprinted polymers which show similar selectivity profile in a radio ligand binding assay when compared with theophyllin antibodies but are much more mechanically stable as compared to them.

Table 1.5

Application of MIPs as selective adsorbents / Receptor mimics

Sr. No.	Template	Application	Reference
1	Morphine and Leu-enkephalin	Opioid receptor mimic	Andersson et. al., 1995
2	Yohimbine / Corynanthine	α_2- adrenoreceptor mimic	Berglund et. al., 1996
3	Cortisol / Corticosteroid	Antibody mimic	Ramstrom et. al., 1996
4	Cyclosporin A	Selective sorbent for conc. determination	Senhaldt et. al., 1997
5	Tamoxifen	Solid phase extraction	Rashid et. al., 1997
6	7- hydroxy coumarin	Solid phase extraction	Walshe et. al., 1997
7	Testosterone	Selective sorbent	Cheong et. al., 1997
8	Creatinine	Selective sorbent	Sreenivasan and Sivakumar, 1997
9	Anthraquinone dyes	Selective sorbent	Kozuka et. al., 1985
10	Glucose oxidase	Selective sorbent	Burrow and Minoura, 1996

Other than monitoring drug levels and as antibody mimics in immuno sorbent assays, MIPs can also be used in detection of pesticide residues in environmental analysis or analysis of waste water streams. For instance Haupt et. al., (1998) reported synthesis of 2,4-dichloro phenoxy acetic acid (24-D) imprinted polymers, which show a high selectivity for the template but a very low cross selectivity for structurally similar substrates. In addition to sensors, MIPs can also be prepared as selective adsorbents / receptor mimics for a variety of substrates such as enzymes, steroids, nucleotide bases etc. Table 1.5 lists a few representative examples of compounds for which MIPs are synthesised.

Other than these applications, MIPs have found applications as selective adsorbents for various metal ions. These are helpful in purification of waste water streams contaminated with metal ions, especially heavy metals, which are highly toxic in nature. Table 1.6 lists a variety of metal ions for which the imprinted polymers have been prepared.

Table 1.6

MIPs as selective adsorbents for metal ions

Sr. No.	Cation / Anion	Monomer used	Reference
1	Ferrocyanide, $[Fe(CN)_6]^{2-}$	Oleyl amine	Fujiwara et. al., 1996
2	Cu^{2+} / Ni^{2+} / Co^{2+}	Methacrylic acid	Tsukagoshi et. al., 1993
3	Cu^{2+} / Cd^{2+} / Zn^{2+}	Oleyl phenyl hydrogen phosphate	Murata et. al., 1996
4	Pb^{2+}	Vinyl benzoic acid	Zeng and Murray, 1996

1.7.3 Selective catalysis

MIPs contain an imprint cavity that is selective for the template molecule and appropriately positioned functional groups for the interactions. As a result, with judicious choice of the functional monomers and the template molecule, MIPs can be used in selective catalysis of a particular reaction. The template used during the synthesis of these polymers can either be the substrate or the transition state

analogue (Heilmann and Maier, 1994) for the reaction that the imprinted polymer catalyses. Morihara was a pioneer in use of imprinted silicas as catalysts, prepared by the technique known as footprint catalysis (Morihara et. al., 1988a and 1988b). Since enzymatic catalysis also relies on the presence of a cavity for the substrate and coordination between various functional groups for efficient catalysis, MIPs have been used as models for the study of enzymatic reactions. For instance, serine protease mimics (Lele et. al., 1998 and Karmalkar et. al., 1996), pyridoxal enzyme mimic (Belokon et. al., 1980), aldolase mimic (Matsui et. al., 1996) etc. have been reported in literature. Other than models for enzymatic reactions, there are also reports on use of MIPs as catalysts in reactions such as 2,4-dinitrophenolysis of benzoic / acetic anhydride (Shimada et. al., 1992), selective reduction of steroid ketones (Bystrom et. al., 1993), dehyro fluorination of 4- fluoro, 4-(p-nitrophenyl) butane-2-one (Beach and Shea, 1994), photodimerisation of trans- cinnamic acid to α-truxillic and β and δ- truxinic acids (Damen and Neckers, 1980b) etc.

1.8 Conclusions

The molecular imprinting technology has come a long way from the imprinted silicas as reported by Dickey F.,(1949), wherein a very low selectivity for the template could be seen. By optimising various system parameters such as the template, functional monomers or combinations thereof, crosslinker, porogen, polymerisation conditions etc. a highly selective matrix can be obtained. These matrices have shown selectivity as close to their natural counterparts viz. antibodies and receptors and hence in true sense can be called as antibody mimics / receptor mimics. The high stereo and regio selectivity of these polymers coupled with a high structural stability, has enabled their usage in racemic resolution of drugs and allied molecules, sensor technology as replacement for immuno sorbent assay and selective catalysis or models for enzymatic reactions.

Though MIPs compare well in terms of their enantioselectivity with the commercially available chiral stationary phases (CSPs) used in racemic resolution, their industrial scale applications are not forthcoming as a result of their lower sorption capacity. This is the area of imprinting technology, which needs to be

paid attention to. Another area of imprinting technology that is not well studied in the literature is the effect of imprinting technique (surface vs. bulk) and the size of template (small template with single point rebinding as against large template with multiple sites for rebinding) on the selectivity and sorption capacity. Though most of the studies in molecular imprinting have been concentrated on use of a bulky template molecule, many a times the desired separation demands separation of smaller molecules. The imprinting strategy to be used for these two different kinds of templates would be different and needs to be studied in greater detail. The selectivity of imprinted polymers also strongly depends on the experimental conditions under which the experiments are carried out i.e., whether equilibrium is attained or not during the experiment. These experiments can give an idea, so as to arrive at the optimal conditions under which experiments need to be carried out to achieve a higher selectivity. Thus overall, a rationale needs to be developed for synthesis of MIPs in order to get enhanced separation efficacy for a desired separation.

The work, which we carried out, has expanded the application repertoire of molecularly imprinted polymers (MIPs) to two new areas of application, viz., a) purification of organic compounds (trace impurity removal) and b) separation of positional isomers using MIPs. We decided to study the various factors, which are known to affect the separation efficacy and sorption capacity of the imprinted polymers. A mathematical treatment was given to the separation achieved by MIPs so that prediction of sorption behaviour can be done under differing experimental conditions. All these studies culminated in development of a rationale for optimising the separation efficacy and sorption capacity of imprinted polymers.

Chapter 2

Objectives and Scope

2.1 Introduction

Molecularly imprinted polymers have found various applications as stationary phases for racemic resolution, sensors and selective catalysts. An extensive literature can be found in these areas. Two areas wherein MIPs can find potential for application, but which have not been explored so far are,

a) Removal of trace impurities from bulk organic streams.

b) Separation of positional isomers.

We decided to limit the scope of this work only to these two applications of MIPs and the systems which we limited ourselves to were, removal of phenol from anisole and bisphenol-A and separation of 2,4- dihydroxy benzophenone (2,4-DHB) isomers.

2.2 Removal of trace impurities

Phenolics are present as impurities in various organic streams such as, phenol in anisole, p- nitrophenol and other nitrophenol derivatives in nitrobenzene, p- tertiary butyl catechol in styrene, phenol and isomers of bisphenol in bisphenol-A etc. Presence of phenolic impurities leads to a problem in down stream processing of these compounds. For instance, presence of phenol in anisole can deactivate the catalyst used in the oxidation of anisole to guiacol. Similarly presence of p- tertiary butyl catechol in styrene affects the polymerisation kinetics. Presence of phenol and o,p'- isomer of bisphenol-A in bisphenol-A leads to deterioration of the performance of polycarbonate resins prepared from it. For all these reasons it is necessary to remove phenolic impurities from various organic streams. Another advantage of the removal of these impurities is improvement in the quality, which is desirable for any compound.

There are various methods for the removal of phenolic impurities from aqueous as well as organic streams. For instance, the process for removal of phenol from anisole involves treatment with alkali, followed by acid and water wash and drying (Kovacs et. al., 1981). Purification of bisphenol-A to remove phenolic impurities is carried out on an industrial scale using distillation, crystallisation, adduct formation etc. (Meurer et. al., 1997 and Sakatani et. al.,

1996). But most of these methods are cumbersome and multi step leading to generation of additional waste streams, which need to be treated further. Development of adsorptive separations for removal of phenols, would be therefore advantageous.

Adsorptive separations are commonly used on large scale for removal of phenolic impurities from waste water streams. Commercial adsorbents such as Amberlite XAD series (Amberlite XAD- 2, 4, 7 and 8 from Rohm and Haas) are well suited for these applications. These adsorbents can effectively remove polar compounds from relatively non-polar streams, as well as non-polar compounds from aqueous streams, based on the differences in polarity. They are well suited for applications, wherein a wide variety of impurities need to be removed from aqueous streams, but do not have any features that can distinguish between different sorbates based on functionality or size and shape. They work mainly, on the basis of polarity differences between the solute and the solvent and have a very high surface area, which leads to higher sorption capacity. They can show selectivity to phenols as a class of compounds, but can not distinguish between two different phenols. Also their sorption capacities are drastically reduced, when used for adsorption from organic streams. Hence selective adsorption using molecularly imprinted polymers, can be an attractive alternative in the separation of structurally similar phenolic trace impurities from organic streams.

2.3 Separation of positional isomers

Due to the stringent regulations imposed by the regulatory authorities in terms of toxicological studies, there is a growing demand for separation of optical isomers of drug molecules, which hitherto were used as racemic mixtures. Molecularly imprinted polymers (MIPs), show a very high selectivity for the template molecule and extensive literature is available on use of MIPs in racemic resolution. Although MIPs can be used in these separations on large scale, their application potential is limited by their low sorption capacity.

Similar to chiral resolution, MIPs can also be used for the separation of non-chiral positional isomers. Many a times organic syntheses involving aromatic

nuclei lead to formation of positional isomers (for instance, synthesis of p-xylene from toluene always leads to a mixture of o, m and p- xylenes, similarly a mixture of o and p- methoxy phenols is obtained in the synthesis of guiacol from anisole). These isomers differ from one another, only in the position of the functional groups and are therefore difficult to separate. The need for their separation is either for value addition (guiacol from p- methoxy phenol) or for the purpose of purification. Selective adsorption using MIPs is therefore an attractive alternative for these separations.

2.4 Objectives

Our first objective therefore was to develop separation systems based on molecularly imprinted polymers. The systems we worked on were as follows,

a) Separation of phenol from anisole
b) Separation of phenol from bisphenol-A
c) Separation of 2,4- dihydroxy benzophenone isomers

The technological importance of the systems is explained in detail in the introduction above. We limited scope of this work to only these three separation systems.

Most of the studies in MIPs have been concentrated on use of various classes of templates for the preparation of MIPs, selectivity for the template, rebinding interactions between the sorbate and the adsorbent etc. But there are various factors such as,

1. imprinting techniques
2. choice of solvent
3. experimental conditions under which the selectivity experiments are done, equilibrium vs. non-equilibrium conditions,

which affect separation efficacy and sorption capacity of MIPs. But many of these factors have not been studied in detail.

Hence, our second objective was to study the effect of all these factors on the separation efficacy of MIPs and to arrive at a rationale for developing

molecularly imprinted adsorbents, with enhanced separation efficacy. We decided to study only effect of these factors, on separation efficacy for MIPs.

For the use of MIPs on large scale as selective adsorbents, it is necessary to know the sorption capacity of these materials, as this gives an idea about useful life of the adsorbent. It is also necessary to have a knowledge, about the active life of the adsorbent under different sets of operating conditions.

Hence our third objective was to study the packed bed adsorber, wherein the MIPs were used as adsorbent and mathematically model the breakthrough behaviour of the same. Prediction of the breakthrough behaviour would be beneficial in extrapolating the results obtained on laboratory scale to the industrial scale separation.

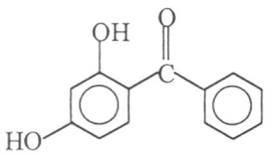
Finally the study of adsorption phenomena is not complete without the study of adsorption isotherms and the thermodynamics. It gives an insight in the adsorption process, which would be immensely helpful for designing better separation systems. Hence our fourth objective in undertaking this work was to study the thermodynamics of adsorption using MIPs as selective adsorbents.

Chapter 3

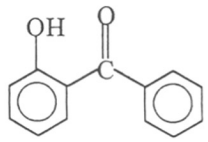
Materials and Methods

3.1 Chemicals and reagents

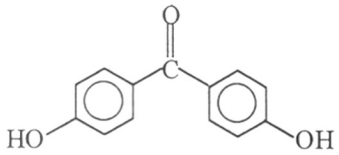
Glycidyl methacrylate (GMA), ethylene glycol dimethacrylate (EGDMA), trimethylol propane trimethacrylate, (TRIM), poly (N-vinyl pyrrolidone) [PNVP, M.W. 360,000], methacrylic acid (MAA), p-toluic acid, 2-hydroxy ethyl methacrylate (HEMA), phenyl chloroformate, allyl chloroformate, dicyclohexyl carbodiimide (DCC), 2,4- dihydroxy benzophenone (2,4- DHB), 2- hydroxy benzophenone (2- HB), 4,4'- dihydroxy benzophenone (4,4'- DHB), 2,2'- dihydroxy benzophenone (2,2'- DHB), Bisphenol-A (BPA) and 4,4'- dimethoxy benzophenone (4,4'- DMB), (see figure 3.1 for structure of sorbates) were obtained from Aldrich Chemical Company, USA and were used as received.



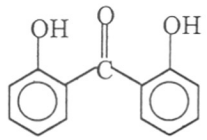
a) 2,4 - dihydroxy benzophenone



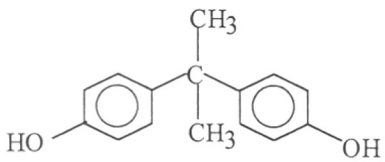
b) 2- hydroxy benzophenone



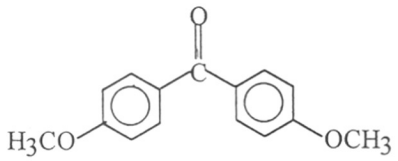
c) 4,4' - dihydroxy benzophenone



d) 2,2' - dihydroxy benzophenone



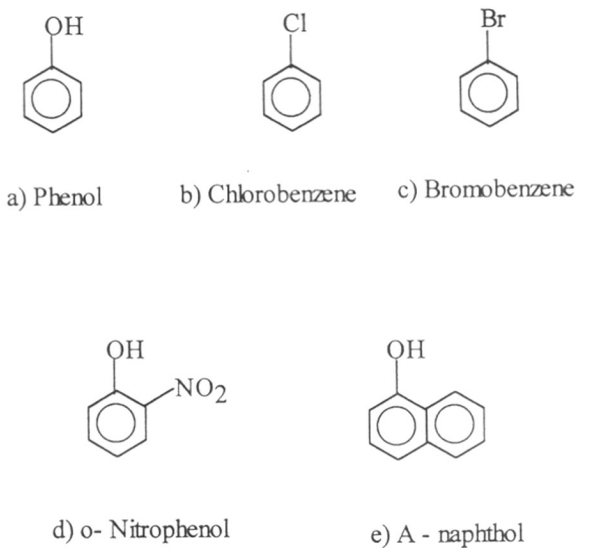
e) Bisphenol - A



f) 4,4' - dimethoxy benzophenone

Figure 3.1: Chemical structures of 2,4- DHB isomers

Phenol, chlorobenzene, bromobenzene, o- nitrophenol, α- naphthol (see figure 3.2 for chemical structures), benzoyl chloride, triethyl amine (TEA), azobis isobutyronitrile (AIBN), sodium lauryl sulphate (SLS), cyclohexanol, dodecanol etc. were obtained from local suppliers and were of the highest quality available. Solvents such as, tetrahydrofuran (THF), ethyl acetate, ethanol, chloroform, dichloromethane (DCM) etc. were of commercial grade and were purified by standard methods before the use. HPLC grade methanol was obtained from Qualigens Ltd. Water used for HPLC was filtered through Milli-Q filtration unit (Millipore Corp.). Amberlite XAD - 7 (Rohm and Haas) was a gift from Ion Exchange India Ltd.



a) Phenol b) Chlorobenzene c) Bromobenzene

d) o- Nitrophenol e) A - naphthol

Figure 3.2: Chemical Structures of phenol and the competing sorbates

3.2 Synthesis of monomers: *(see figure 3.3 for the chemical structures of the template monomers)*

Note: All the NMR spectra were recorded on Bruker MSL200 FT-NMR using TMS as the internal standard and IR spectra were recorded on Shimadzu 8300 FT-IR using KBR windows.

3.2.1 Methacryloyl chloride

Methacryloyl chloride was prepared by condensation reaction between methacrylic acid and benzoyl chloride. 46 g of MAA was taken in a 250 ml round bottom flask and about 1.0 g hydroquinone (polymerisation inhibitor) was added to it. 132 ml benzoyl chloride was added to the mixture and the solution was refluxed for about 60-90 min. The solution was allowed to cool and distilled to obtain methacryloyl chloride as colourless to pale yellow liquid (boiling range: 91-95^{0}C).

3.2.2 Phenyl methacrylate (PHMA)

PHMA was synthesised by the condensation of phenol (9.4 g) with methacryloyl chloride (11.495 g, 10.75 ml) in the presence of TEA (11.11 g, 15.3 ml) using chloroform as a solvent, according to Schotten - Baumann procedure. After the reaction, insoluble by-products were filtered and the organic layer was washed with cold dilute $NaHCO_3$, followed by dilute HCl and finally with chilled brine. The organic layer was dried for 24 hours over anhydrous Na_2SO_4 and then the solvent was evaporated at low pressure to obtain the monomer as a yellow oily liquid.

IR (Neat): 1737 cm^{-1} (ester, C=O)

^{1} H NMR ($CDCl_3$): δ (ppm) 7.0 – 7.5 (M, aromatic, 5H), 5.7 and 6.4 (dd, = CH_2, 2H), 2.2 (s, -CH_3, 3H)

3.2.3 Phenyl, 4 - vinyl benzoate

4 - Carboxy styrene, (4 - CS) was synthesised from p- toluic acid according to the procedure outlined by Broos et al, (1978). Phenyl, 4 - vinyl benzoate was synthesised by DCC coupling reaction between 4 - CS and phenol in DCM as solvent. The suspension was filtered to separate the urea by-product. The organic layer was washed with cold dilute $NaHCO_3$, followed by dilute HCl and chilled brine. The organic layer was dried over anhydrous Na_2SO_4 for 24 hours, filtered and the solvent was evaporated at low pressure to yield the solid monomer.

IR (Nujol): 1738 cm^{-1} (aryl ester, C=O)

^{1}H NMR ($CDCl_3$): δ (ppm) 7.5 – 8.5 (dd, aromatic, 4H), 7.0 – 7.5 (M, aromatic, 5H), 6.4 (Q, = CH, 1H), 5.4 and 5.9 (dd, = CH_2, 2H)

3.2.4 2-(methacryloyl), ethyl, phenyl carbonate

To a cooled solution of 13 g of HEMA in 100 ml of dry THF containing 15.3 ml. TEA, a cooled solution of 17.2 g phenyl chloroformate in 40 ml THF was added. The suspension was stirred for 24 hours at room temperature and filtered to remove triethyl amine salt. The solvent was removed under low pressure to obtain the crude monomer. The crude monomer was dissolved in dry ethyl acetate, washed with chilled brine and dried for 24 hours over anhydrous Na_2SO_4. The pure monomer was obtained by removing ethyl acetate under reduced pressure.

IR (Neat): 1767 cm^{-1} (carbonate, C=O), 1721 cm^{-1} (ester, C=O)

^{1} H NMR ($CDCl_3$): δ (ppm) 7.0 – 7.5 (M, aromatic, 5H), 5.7 and 6.3 (dd, = CH_2, 2H), 2.1 (s, - CH_3, 3H)

3.2.5 p- Cumyl phenyl methacrylate

6.36 g p- cumyl phenol and 5.0 ml TEA were dissolved in about 75 ml dry and distilled THF and cooled to 0-5^0 C. A solution of 3.65 g methacryloyl chloride in about 20 ml THF was added with constant stirring. After addition, the suspension was stirred at room temperature for 24 hours and filtered. The crude monomer was obtained by evaporating the solvent under low pressure. The monomer was purified by dissolving it in ethyl acetate, washing successively with cold dilute $NaHCO_3$, dilute HCl and finally with chilled brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under low pressure to get the pure monomer as an oily liquid.

I.R.(Neat): 1735 cm^{-1} (aryl ester, C=O)

^{1}H NMR($CDCl_3$): δ (ppm) 7-7.5 (M, aromatic, 8 H), 6.3 and 5.7 (dd, $=CH_2$, 2 H), 2.2 (s, $-CH_3$, 3 H), 1.5 (s, $-CH_3$, 6 H)

3.2.6 Allyl 2,4- DHB carbonate

2.14 g of 2,4- DHB and 2.22 g TEA were dissolved in about 50 ml dry and distilled THF and cooled to 0-5^{0}C. To this a solution of 2.65 g allyl chloroformate in 25 ml THF was added slowly under stirring. During the addition (45-60 min) temperature was held constant. After addition, the suspension was allowed to warm to room temperature and stirring was continued for another 24 hours. The suspension was filtered to remove the TEA salt and the crude monomer was obtained after evaporation of the solvent. The crude product was dissolved in ethyl acetate, washed with chilled brine, dried over anhydrous Na_2SO_4 and the solvent was evaporated to obtain the monomer as yellow oily liquid (yield 70%).

I.R.(Neat): 1764.7 cm^{-1} (C=O, carbonate), 1666.4 cm^{-1}(C=O, aryl ketone)

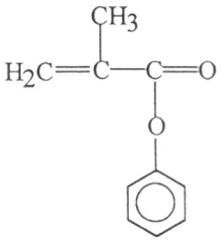
^{1}H NMR ($CDCl_3$): δ (ppm) 7-8 (M, aromatic, 8 H), 5.9 (M, =CH, 2 H), 5.3 (M, =CH_2, 4 H), 4.6 and 4.8 (dd, -CH_2, 4 H)

3.2.7 2,4- DHB methacrylate

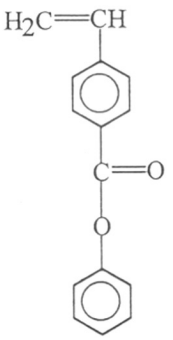
2.14 g 2,4- DHB and 2.22 g TEA were dissolved in 50 ml dry, distilled DCM and cooled to 0-5^{0}C. To this a solution of 2.29 g methacryloyl chloride in DCM was added slowly under stirring. During the addition, temperature was held constant between 0-5^{0}C. After the addition the suspension was allowed to warm to room temperature and stirred for 24 hours. The suspension was filtered to remove TEA salt. The organic layer was washed with cold dilute $NaHCO_3$, followed by cold dilute HCl and finally with chilled brine. It was then dried over anhydrous Na_2SO_4 overnight and filtered. The solvent was evaporated under reduced pressure to get the pure monomer as a yellow oil.

IR (Nujol): 1739 cm^{-1} (C=O, ester), 1629 cm^{-1} (C=O, aryl ketone), 1247.9 and 1147.6 cm^{-1} (C-O stretching, ester)

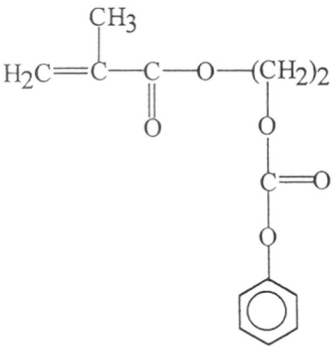
^{1}H NMR ($CDCl_3$): δ (ppm) 7.5-8 (M, aromatic, 8 H), 5.9 and 6.3 (dd, =CH_2, 4 H), 2 (s, -CH_3, 6 H)



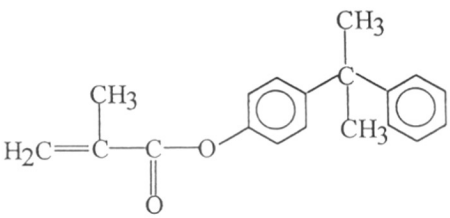
a) Phenyl methacrylate



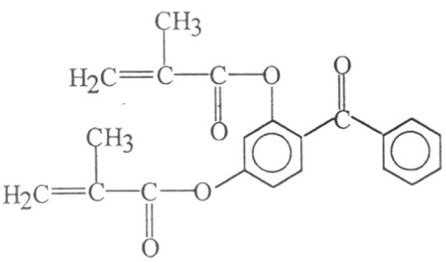
b) Phenyl, 4- vinyl benzoate



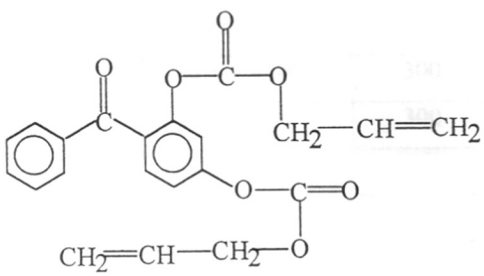
c) 2-(methacryloyl), ethyl, phenyl carbonate



d) p- cumyl phenyl methacrylate



e) 2,4- DHB methacrylate



f) Allyl, 2,4- DHB carbonate

Figure 3.3: Structures of the template monomers

3.3 Synthesis of macroporous support polymer

Macroporous support polymers containing GMA were synthesised according to a modified suspension polymerisation process (Svec et. al., 1975).

In a typical procedure, a mixture of GMA, crosslinker, porogen and initiator, AIBN was degassed by passage of nitrogen. This was added slowly under stirring (500 RPM) to a 1% (w/v) solution of PNVP in water containing 0.1% (w/v) SLS. The PNVP solution was also degassed by passage of nitrogen before addition of the monomer mixture. The suspension was heated to 70^0C. Stirring was continued and temperature was maintained for 2 hours. After 2 hrs. the temperature was raised to 80^0C and maintained for next 6 hours. After polymerisation (8 hours), the suspension was allowed to cool under stirring for further 2 hours. The suspension was then decanted, washed with water, methanol and dried in a vacuum oven at 50^0C. The microspheres were sieved and the particles between 37 -75 μm were chosen for the adsorption studies. The monomer composition for the preparation of support polymer is given below in table 3.1.

Table 3.1

Composition of monomers in macroporous support polymers

Polymer code	Monomer (GMA), g	Crosslinker, g	AIBN, mg	Porogen (cyclohexanol + dodecanol) g	Water, ml
GE60	10.88	16.32(EGDMA)	0.272	36.4 + 4.08	300
GE75	6.8	20.4(EGDMA)	0.272	36.4 + 4.08	300
GE90	2.72	24.48(EGDMA)	0.272	36.4 + 4.08	300
GT90	3.28	29.4(TRIM)	0.326	39 + 4.34	240

3.4 Synthesis of surface imprinted polymers

Surface imprinted polymers were prepared using the macroporous support polymers as synthesised above.

In a typical procedure, 1.0 g of the support polymer was suspended in about 50 ml benzene. The template monomer, crosslinker and AIBN were added to the

suspension and stirred in order to sorb the monomers on the support polymer (18-20 hours). The dry powder, after evaporation of the solvent was then thermally polymerised at 75^0C for 24 hours. After polymerisation, the polymer was washed with methanol to remove unreacted monomers and dried.

3.4.1 Hydrolysis of the template monomer

Hydrolysis of the template monomer was carried out using aqueous NaOH solution. In a typical procedure, the polymer was suspended in 50-100 ml of 1-2 N NaOH and the suspension was refluxed for a period of 6 hours, during which the hydrolysis was found to be complete. After hydrolysis, the suspension was filtered, washed with dilute HCl, followed by water and methanol and the polymer was dried in vacuum oven at 60^0C. The amount of the phenolic template released during hydrolysis was estimated using UV- visible spectrophotometer (Shimadzu UV-240). This gave an idea about the amount of the functional monomer loaded on to the polymer, which in turn gave an idea about the theoretical sorption capacity of the polymer.

In the case of 2,4- DHB imprinted polymers, the alkaline filtrate obtained after hydrolysis of the template was acidified and 2,4- DHB was extracted in ethyl acetate. Solvent was evaporated and 2,4- DHB obtained was weighed. The weight of 2,4- DHB gave an idea about the amount loaded in the polymer. Table 3.2 gives the monomer composition used for preparation of imprinted polymers.

3.4.2 Synthesis of non-imprinted polymers

Following the same procedure as above (section 3.4), non-imprinted polymers were also prepared. Care was taken to ensure that the amount of functional monomers used in both imprinted and non-imprinted polymers was the same. This avoided the differences in the selectivity arising from the differences in loading of the functional monomer. Non-imprinted polymers were used as control for evaluating the imprinting efficacy of the imprinted polymers. Table 3.2 gives the composition of monomers used in the preparation of non-imprinted polymers.

Table 3.2

Monomer composition for synthesis of imprinted and corresponding non-imprinted polymers prepared by surface imprinting technique

Sr. No.	Polymer code	Monomer used	Amount, g
1	GE60-H M	2 -(methacryloyl), ethyl, phenyl carbonate	0.40 g
2	GE60-H B	HEMA	0.19 g
3	GE60-M M	PHMA	0.40 g
4	GE60-M B	MAA	0.22 g
5	GE60-V M	Phenyl, 4 – vinyl benzoate	0.40 g
6	GE60-V B	4 - vinyl benzoic acid	0.26 g
7	GE75-H M	2 - (methacryloyl), ethyl, phenyl, carbonate	0.40 g
8	GE75-H B	HEMA	0.19 g
9	GE90-H M	2 - (methacryloyl), ethyl, phenyl, carbonate	0.40 g
10	GE90-H B	HEMA	0.19 g
11	GT90-PHMA-M	Phenyl methacrylate	0.40 g
12	GT90-MAA-B	Methacrylic acid	0.21 g
13	GT90-HEMA-M	2-(methacryloyl) ethyl, phenyl carbonate	0.40 g
14	GT90-HEMA-B	2-hydroxy ethyl methacrylate	0.22 g
15	GE90-All24DHB-M	Allyl, 2,4-DHB carbonate	0.2 g
16	GE90-All-B	Allyl alcohol	0.06 g
17	GE90-24DHBMA-M	2,4-DHB methacrylate	0.2 g
18	GE90-MAA-B	Methacrylic acid	0.096 g

(Support polymer: 1.0 g, EGDMA: 0.2 ml, AIBN: 25 mg)

3.5 Synthesis of bulk imprinted polymers

Bulk imprinted polymers were prepared by precipitation polymerisation process. In a typical procedure, the template monomer, porogen, AIBN and

crosslinker were mixed thoroughly in a glass test tube and degassed by passage of nitrogen for about 10-15 min. The tube was sealed and polymerisation was carried out at 75^0C for 24 hours after which, the tube was broken to remove the porous polymeric rod. The polymer was crushed and sieved and particles below 75 μm were used in all the adsorption studies. The polymer was then washed thoroughly with methanol to remove unreacted monomers as well as the porogen and dried in an oven.

Table 3.3

Monomer composition for the synthesis of imprinted and corresponding non-imprinted polymers prepared by precipitation polymerisation

Sr. No.	Polymer code	Monomer used	Amount, g	Porogen, g
1	PHMA-M-BU	PHMA	1.0 g	2.5 g (2.0 g + 0.5 g)
2	MAA-BL-BU	MAA	0.53 g	2.0 g (1.5 g + 0.5 g)
3	CUPHMA-M-BU	p- cumyl, phenyl methacrylate	3.2 g	4.7 g (4.2 g + 0.5 g)
4	CMAA-BL-BU	MAA	1.0 g	2.5 g (2.0 g + 0.5 g)
5	ALL24DHBC-MIP-BU	Allyl, 2,4- DHB carbonate	0.60 g	3.0 g
6	ALL-BL-BU	Allyl alcohol	0.18 g	3.0 g
7	24DHBMA-MIP-BU	2,4- DHB methacrylate	0.6 g	3.0 g
8	MAA-BL-BU	MAA	0.29 g	3.0 g

Initiator, AIBN: 0.05 g

(Note: The crosslinker used for polymers 1 - 4 was EGDMA, (1.0 ml) and the porogen used was a mixture of cyclohexanol and dodecanol. For polymers 5 - 8, 2.4 g TRIM was used as the crosslinker and toluene was used as the porogen.)

Hydrolysis of the template was carried out exactly in similar manner as described above (section 3.4.1) and the amount of the template released during hydrolysis was estimated.

Non-imprinted bulk polymers were also prepared containing the same amount of functional monomer as that of the imprinted polymers and were used as control. Monomer composition for the preparation of imprinted and non-imprinted polymers by precipitation polymerisation is given in table 3.3.

Imprinted polymers wherein the imprint cavity was blocked by the template were prepared by surface and bulk imprinting techniques. They were prepared exactly in the same way as described above (section 3.4 and 3.5), the only difference being that the hydrolysis of the template monomer was not carried out leaving behind the covalently linked template in the imprint cavity. These polymers were used to validate the role of imprint cavity and rebinding interactions in conferring selectivity in MIPs.

3.6 Characterisation of the polymers

The polymers being highly crosslinked the only characterisation techniques used were surface area determination, surface morphology and swelling measurement.

3.6.1 Surface area measurement

Surface area and the pore volume measurements for the polymers synthesised, were done using nitrogen adsorption technique(BET method). Omnisorp CX100 (Coulter) was used to determine surface area and porosity of the polymers. Surface area was determined, using the adsorption isotherm of nitrogen adsorption on the polymeric materials at -195^0C, whereas the desorption isotherm was used for determining the pore volume and pore size distribution of the polymers under study. Surface areas and pore volumes for various polymers under study, are given in table 3.4.

Table 3.4

Surface area and pore volume of the imprinted and non-imprinted polymers

Sr. No.	Polymer code	BET surface area, m^2/ g	Pore volume, ml / g
1	GE60-H M	96.1	0.458
2	GE60-H B	129.2	0.414
3	GE60-M M	135.2	0.552
4	GE60-M B	128.7	0.437
5	GE75-H M	128.2	0.376
6	GE75-H B	147.6	0.433
7	GE90-H M	339.9	0.828
8	GE90-H B	310.1	0.734
9	GT90	264.3	0.487
10	GT90-PHMA-M	149.9	0.402
11	GT90-MAA-B	148.6	0.313
12	GT90-HEMA-M	150.7	0.455
13	GT90-HEMA-B	157.3	0.366
14	GE90-All24DHB-M	134.6	0.344
15	GE90-All-B	267.6	0.322
16	GE90-24DHBMA-M	134.9	0.372
17	GE90-MAA-B	49.8	0.333
18	ALL24DHBC-MIP-BU	39.53	0.085
19	ALL-BL-BU	3.0	0.003
20	24DHBMA-MIP-BU	1.7	0.004
21	MAA-BL-BU	75.7	0.492

3.6.2 Surface morphology

In order to get an idea about the porosity of the polymers, the surface morphology of the polymers was studied using scanning electron microscopy. Stereoscan 440, Leica Labs was used for studying the surface morphology. Surface imprinted polymers were found to be highly porous, whereas many of the bulk

imprinted polymers were found to be non-porous. Some of the representative surface morphologies for the imprinted polymers are shown in figure 3.4.

3.6.3 Swelling measurements

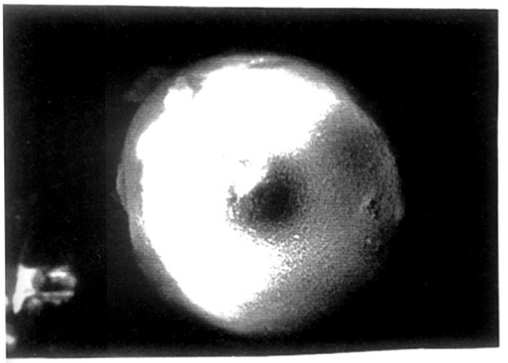
Using anisole as the solvent, equilibrium swelling ratio for phenol imprinted polymers was estimated as follows. In a typical procedure, a weighed quantity of the polymer (50 ± 5.0 mg) was added to an excess of anisole in an eppendorf tube (1.5 ml. capacity) and the suspension was allowed to equilibrate at 31^0C for 4 days, during which the equilibrium was achieved. After equilibrium was attained, the suspension was centrifuged, excess solvent drained off and the polymer was weighed. From the amount of anisole sorbed, the swelling ratio was calculated as, the ratio of the weight of the solvent sorbed to the weight of the dry polymer. Swelling ratios for various polymers under study are listed in table 3.5.

Table 3.5

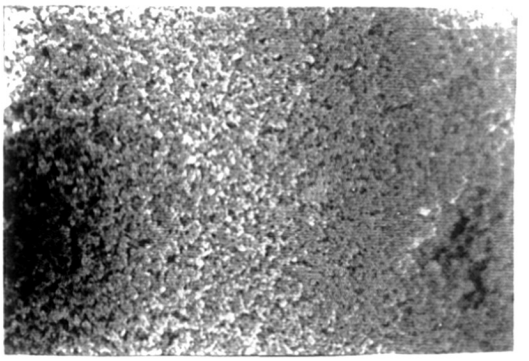
Equilibrium swelling ratio of surface imprinted polymers in anisole

Sr. No.	Polymer code	Swelling ratio*, @ 31^0 C
1	GE60-H M	3.62
2	GE60-H B	3.73
3	GE60-M M	3.64
4	GE60-M B	3.47
5	GE75-H M	4.07
6	GE75-H B	4.08
7	GE90-H M	3.36
8	GE90-H B	3.48

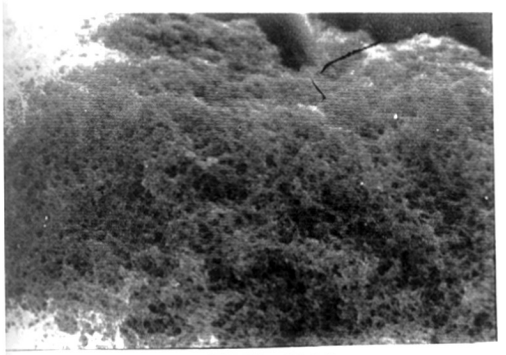
* Swelling ratio = Weight of the solvent (anisole) sorbed / dry weight of the polymer



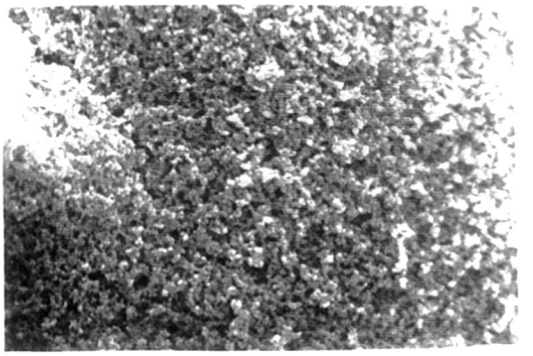
GE60- M M (Low Magnification)



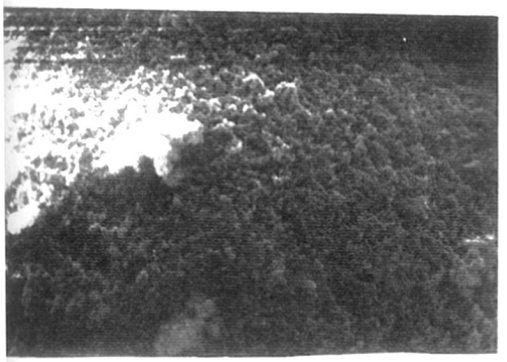
GE60- M M (High Magnification)



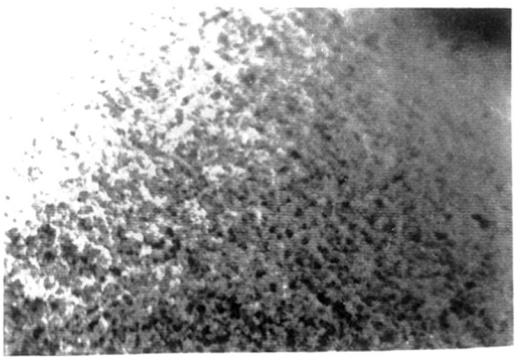
GE60- H M



GE60- H B



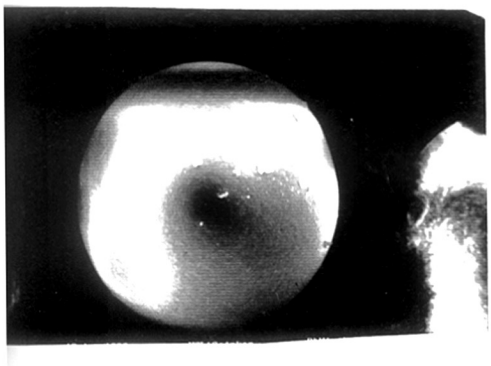
GE75- H M



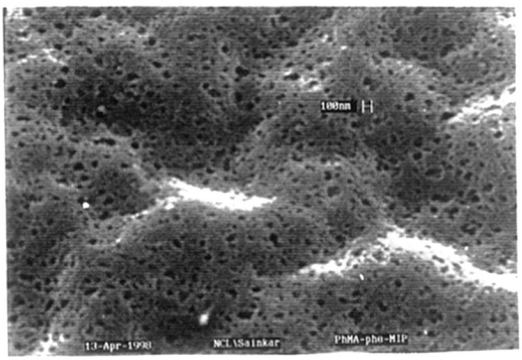
GE90- H M

Figure 3.4 a: Surface morphology of imprinted polymers

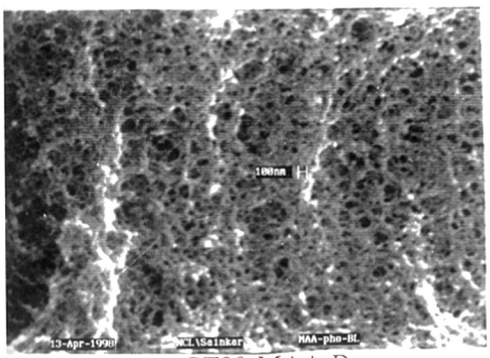
(Separation of phenol from anisole)



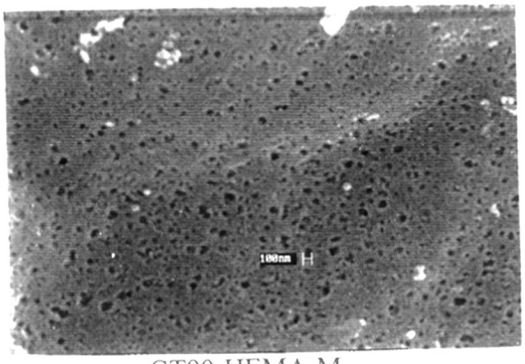
GT90-PHMA-M (Low Magnification)



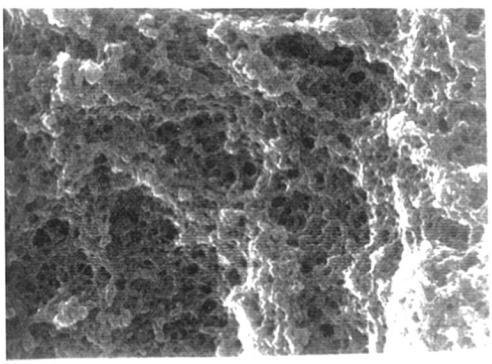
GT90-PHMA-M (High Magnification)



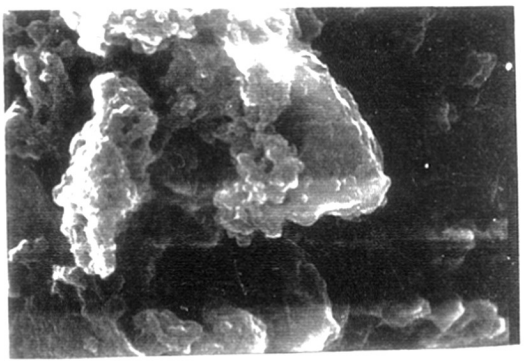
GT90-MAA-B



GT90-HEMA-M



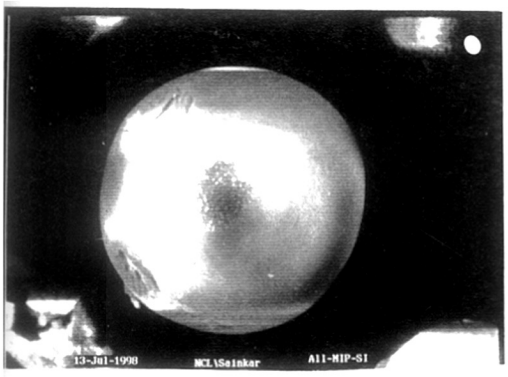
PHMA-M-BU



CUPHMA-M-BU

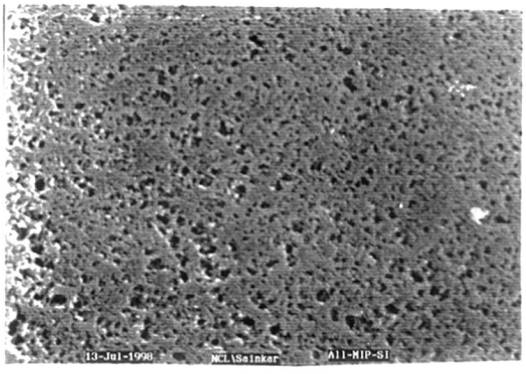
Figure 3.4 b: Surface morphology of imprinted polymers

(Separation of phenol from bisphenol-A)



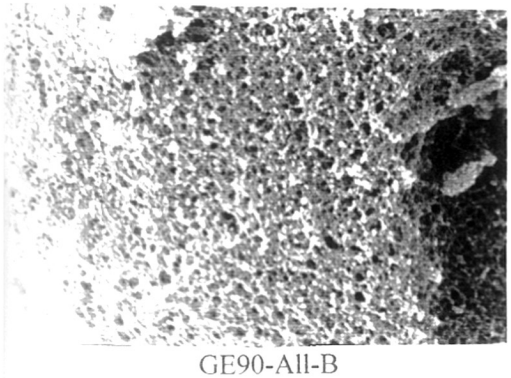
GE90-All24DHB-M

(Low Magnification)

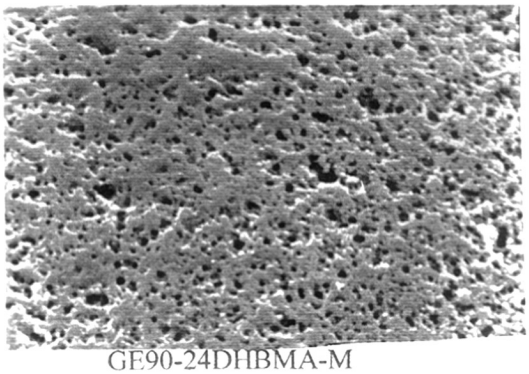


GE90-All24DHBM-M

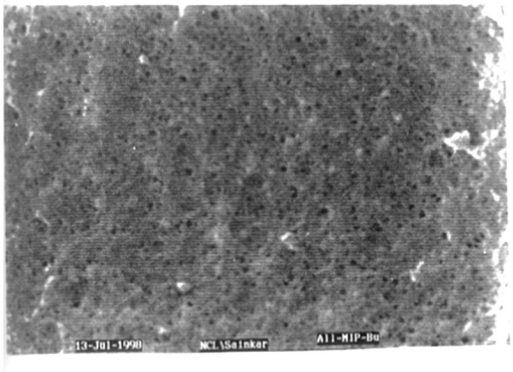
(High Magnification)



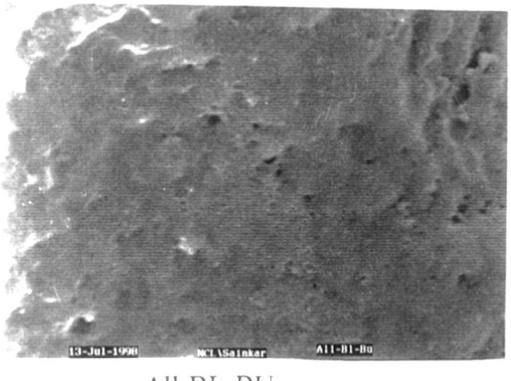
GE90-All-B



GE90-24DHBMA-M



All24DHBC-MIP-BU



All-BL-BU

Figure 3.4 c: Surface morphology of imprinted polymers

(Separation of 2,4- DHB isomers)

3.7 Selectivity studies

Experiments to determine the selectivity of MIPs for the template, in presence of the competing sorbates, were carried out under two different conditions, viz., equilibrium and non-equilibrium conditions.

3.7.1 Equilibrium selectivity measurements

These experiments are useful, as they give an idea about the absolute selectivity of the imprinted polymers when the diffusional and hydrodynamic forces do not affect the selectivity measurements. These experiments were carried out in batch adsorption mode for surface as well as bulk imprinted polymers. Equilibrium adsorption experiments were also used for generation of adsorption isotherms for the sorption of template on the imprinted polymers.

3.7.1.1 Selectivity experiments

In a typical procedure to determine the selectivity under equilibrium conditions, a known weight of the imprinted polymer (20-100 mg) was taken in a screw cap glass tube. To this a known volume of solution of the template sorbate, along with one or more competing sorbates was added. The tubes were sealed and kept in a water bath maintained at 25^0C. The tubes were shaken horizontally at 180 cycles per minute (cpm) for a period ranging from 12 -24 hours during which the equilibrium was attained. After equilibrium was attained, the supernatant was analysed for the concentration of the sorbates using reverse phase HPLC.

The HPLC system comprised of Waters 510 solvent delivery pumps (2 nos.), 680 automatic gradient controller, 486 tuneable absorbance detector and 746 dual channel integrator. The column used was Waters μ- Bondpak C-18 (3.9 mm X 300 mm) packed column and the mobile phase was a mixture of methanol and water (70:30 v/v). The mobile phase flow rate was 1.0 ml / min. and the amount injected was 20 μl. All the phenols were detected by their absorbance at 275 nm and halobenzenes (chlorobenzene and bromobenzene) were detected by their absorbance at 250nm. The retention times of all the sorbates are given in the table 3.6.

Table 3.6

HPLC Retention times of various sorbates at 1.0 ml flow rate

Sr. No.	Sorbate	Retention time, min. (± 0.1 min)
1	Phenol	3.80
2	o- Nitrophenol	4.77
3	Chlorobenzene	6.93
4	Bromobenzene	7.45
5	Bisphenol-A	5.30
6	2,4- dihydroxy benzophenone	6.10
7	4,4'- dihydroxy benzophenone	3.60
8	2,2'- dihydroxy benzophenone	5.70
9	4,4'- dimethoxy benzophenone	7.95
10	2- hydroxy benzophenone	8.20

Concentration of the individual sorbates in the supernatant, was estimated from the peak areas, using the calibration curves plotted using HPLC. From the initial and final concentration of the sorbates, the amount of individual sorbate adsorbed was calculated and then the selectivity, α was defined as the ratio of the amount of the template sorbate adsorbed to that of the competing sorbate adsorbed.

$$\alpha = \frac{\text{Amount of template adsorbed}}{\text{Amount of competing sorbate adsorbed}} \quad (3.1)$$

Similar selectivity experiments were also carried out using non-imprinted polymers and α values were determined. The imprinting efficacy β, was then determined as the ratio of the α value for the imprinted polymer to that of the non-imprinted polymer.

$$\beta = \frac{\alpha \text{ for MIP}}{\alpha \text{ for non - imprinted polymer}} \quad (3.2)$$

Selectivity experiments were also carried out using the imprinted polymers wherein the imprint cavity was blocked with the template molecule and the selectivity, α was estimated as described above.

3.7.1.2 Generation of adsorption isotherm

Experiments to generate adsorption isotherm were carried out in batch mode as outlined earlier in section 3.7.1.1. The amount of polymer used was 20 mg and a solution of the template sorbate in a suitable solvent was added to it. Eight different concentrations of the template sorbate were used to generate the adsorption isotherm. The screw cap tubes were sealed and shaken at 160 cpm at three different temperatures to generate adsorption isotherms. The tubes were allowed to equilibrate for a period ranging from 7 to 14 days and then the supernatant was analysed for the concentration of the template using HPLC as outlined above (section 3.7.1.1).

Adsorption isotherms were plotted by fitting two different types of isotherm equations to the sorption data. They were, a) empirical isotherm obtained by regression of the data and b) dual sorption isotherm obtained by non linear steepest gradient algorithm.

3.7.2 Selectivity under non-equilibrium conditions

These experiments were carried out under conditions such that equilibrium between the sorbate and the adsorbent was not attained. These experiments are useful in determining the sorption capacity, generation of the breakthrough curves and competitive selectivity measurements. For surface imprinted polymers, these experiments were carried out using a packed bed adsorber (un-steady state measurements), where as for the bulk imprinted polymers, these experiments were carried out in a batch mode (non-equilibrium condition).

Selectivity experiments were carried out in a variety of solvents to find out the effect of the solvent on the selectivity, sorption capacity and imprinting efficacy.

3.7.2.1 Surface imprinted polymers

In the case of surface imprinted polymers the non-equilibrium selectivity experiments were carried out in a packed bed adsorber. In a typical experiment, 0.2 to 0.3 g of the adsorbent was slurried in chloroform and packed in a glass burette (25 ml capacity). The adsorbent bed was washed with 15-20 bed volumes of chloroform followed by the solvent, which was used for the adsorption studies. 10.0 ml of the solution of the template sorbate along with one or more competing sorbates was passed over the column at a flow rate of approximately 0.25 ml / min. and the eluate was collected as 1.0 ml fractions. Concentrations of the individual sorbates in the eluate were estimated using reverse phase HPLC as described above (section 3.7.1.1) and the amount adsorbed was calculated knowing the inlet concentration. Selectivity, α was then determined as explained above (equation 3.1).

Similar selectivity experiments were also carried out using non-imprinted polymers and the selectivity, α and imprinting efficacy, β were estimated (see equations 3.1 and 3.2).

The packed bed experiments were also used for generation of the breakthrough curves for the packed bed adsorber.

3.7.2.2 Bulk imprinted polymers

Non-equilibrium selectivity experiments for bulk imprinted polymers were carried out in a batch adsorption experiment. In a typical experiment, about 100 - 200 mg of the polymer was stirred with 10.0 ml solution of the template sorbate along with one or more of the competing sorbates for an hour. At the end of one hour, the supernatant was analysed for the concentrations of the sorbates and from the amounts adsorbed, selectivity, α was calculated. By carrying out similar experiments using non-imprinted polymers, imprinting efficacy, β was calculated.

Chapter 4

Kinetics and Thermodynamics of Adsorption

4.1 Kinetics of adsorption

4.1.1 Introduction

Most of the industrial adsorptive separations are carried out under non-equilibrium conditions, either in a packed bed adsorber (un-steady state operation) or in a batch adsorber (non-equilibrium operation). Packed bed adsorber is the common of the two and is used frequently for large scale adsorptive separations. Hence for use of imprinted polymers as adsorbents on a large scale, it is essential to study packed bed adsorber operation. There are various factors that affect the performance of a packed bed adsorber such as, bed height and diameter, flow rate, concentration of the inlet stream etc. Hence for use of any adsorbent on a large scale it is essential to study the effect of these factors on the sorption behaviour. These studies can be easily carried out on laboratory scale, but are difficult to carry out on a large scale. Thus the need to predict the behaviour of a packed bed adsorber in terms of sorption capacity as well as breakthrough behaviour is imminent. This helps not only in understanding the kinetics of the process, but also in the prediction of breakthrough behaviour of the column. With this aim in mind we decided to develop a mathematical model for predicting breakthrough behaviour of a packed bed adsorber containing molecularly imprinted polymeric adsorbent.

4.1.2 Model development: Separation of phenol from anisole

Adsorption in a packed bed adsorber containing imprinted polymeric adsorbent can be modelled using the framework of a process where convective diffusion is accompanied by adsorption. Three mass balance equations viz.,

a) Mobile phase balance describing the concentration in the bed voids
b) Intraparticle mass balance, (describing the diffusion process within the porous adsorbent)
c) The external film diffusion which couples the first two, need to be used in modelling.

Our framework is analogous to the one used by Huang et. al., (1994) and Horstmann and Chase, (1989). The symbols used in the model development here have been described in the nomenclature and the model parameters are listed in Tables 4.1 and 4.2. The mobile phase mass balance, including axial dispersion is given by,

$$\frac{\partial C_b}{\partial t} + v\frac{\partial C_b}{\partial z} - D_L\frac{\partial^2 C_b}{\partial z^2} = -\left(\frac{1-\varepsilon_b}{\varepsilon_b}\right)\left(\frac{3}{R}\right)D_e\frac{\partial C_p}{\partial r}\bigg|_{r=R} \tag{4.1}$$

The intraparticle mass balance is given by,

$$\varepsilon_p\frac{\partial C_p}{\partial t} + \rho(1-\varepsilon_b)\frac{\partial q}{\partial t} = \varepsilon_p D_e\left(\frac{\partial^2 C_p}{\partial r^2} + \frac{2}{r}\frac{\partial C_p}{\partial r}\right) \tag{4.2}$$

At the surface of the particle, rate of mass transfer through the external film relates the bulk liquid concentration (C_b) to the concentration within pores of the particle (C_p).

$$D_e\frac{\partial C_p}{\partial r} = k_f(C_b - C_p) \tag{4.3}$$

The relevant initial conditions are,

$$\begin{aligned} C_b &= 0 \quad \text{at } t = 0 \\ C_p &= 0 \quad \text{at } t = 0 \\ q &= 0 \quad \text{at } t = 0 \end{aligned} \tag{4.4}$$

and

$$\begin{aligned} C_b &= C_o \quad \text{at } z = 0 \\ \frac{\partial C_b}{\partial z} &= 0 \quad \text{at } z = L \\ \frac{\partial C_p}{\partial r} &= 0 \quad \text{at } r = 0 \end{aligned} \tag{4.5}$$

The amount adsorbed per gram of resin (q) is related to C_p via the adsorption isotherm (see later).

Using the following dimensionless variables,

$$C_b^{\bullet} = \frac{C_b}{C_o}; C_p^{\bullet} = \frac{C_p}{C_o}; q^{\bullet} = \frac{q}{q_o}; z^{\bullet} = \frac{z}{L}; r^{\bullet} = \frac{r}{R'}; t^{\bullet} = \frac{t}{\tau} \tag{4.6}$$

Equations (4.1-4.3) can be non dimensionalised to give,

$$\left(\frac{1}{\tau}\right)\frac{\partial C_b^{\bullet}}{\partial z^{\bullet}} + \left(\frac{v}{L}\right)\frac{\partial C_b^{\bullet}}{\partial z^{\bullet}} - \left(\frac{D_L}{L^2}\right)\frac{\partial^2 C_b^{\bullet}}{\partial z^{\bullet 2}} = -\left(\frac{1-\varepsilon_b}{\varepsilon_b}\right)D_e\left(\frac{3}{R'^2}\right)\frac{\partial C_b^{\bullet}}{\partial r^{\bullet}}\bigg|_{r=R'} \tag{4.7}$$

$$\left(\frac{1}{\tau}\right)\frac{\partial C_p^{\bullet}}{\partial t^{\bullet}} + \left(\frac{1-\varepsilon_b}{\varepsilon_b}\right)\left(\frac{\rho q_0}{C_0}\right)\left(\frac{1}{\tau}\right)\frac{\partial q^{\bullet}}{\partial t^{\bullet}} = \left(\frac{D_e}{R'^2}\right)\left(\frac{\partial^2 C_p^{\bullet}}{\partial r^{\bullet 2}} + \frac{2}{r^{\bullet}}\frac{\partial C_p^{\bullet}}{\partial r^{\bullet}}\right) \tag{4.8}$$

with

$$\left(C_b^{\bullet} - C_p^{\bullet}\big|_{r^{\bullet}=1}\right) = \left(\frac{D_e}{k_f R'}\right)\frac{\partial C_p^{\bullet}}{\partial r^{\bullet}}\bigg|_{r^{\bullet}=1} \tag{4.9}$$

From the system parameters listed in tables 4.1 and 4.2, it can be seen that for reasonable estimates of D_e, the pore diffusion is several orders of magnitude faster than the other rate processes. In the limiting case of pore diffusion being instantaneous, equation 4.8 simplifies to,

$$\left(\frac{1}{\tau}\right)\frac{\partial C_p^{\bullet}}{\partial t^{\bullet}} + \left(\frac{1-\varepsilon_b}{\varepsilon_b}\right)\left(\frac{\rho q_0}{C_0}\right)\left(\frac{1}{\tau}\right)\frac{\partial q^{\bullet}}{\partial t^{\bullet}} = \left(\frac{3k_f}{R'\varepsilon_p}\right)\left(C_b^{\bullet} - C_p^{\bullet}\right) \tag{4.10}$$

The mobile phase governing equation can also be recast as,

$$\left(\frac{1}{\tau}\right)\frac{\partial C_b^{\bullet}}{\partial t^{\bullet}} + \left(\frac{v}{L}\right)\frac{\partial C_b^{\bullet}}{\partial z^{\bullet}} - \left(\frac{D_L}{L^2}\right)\frac{\partial^2 C_b^{\bullet}}{\partial z^{\bullet 2}} = -\left(\frac{1-\varepsilon_b}{\varepsilon_b}\right)\left(\frac{3k_f}{R'}\right)\left(C_b^{\bullet} - C_p^{\bullet}\right) \tag{4.11}$$

The relevant initial conditions are now given by,

$$\begin{aligned} C_p^* &= 0 \qquad \text{at } t^* = 0 \\ C_b^* &= 0 \qquad \text{at } t^* = 0 \\ q^* &= 0 \qquad \text{at } t^* = 0 \end{aligned} \tag{4.12}$$

While the boundary conditions are,

$$\begin{aligned} C_b^* &= 1 \qquad \text{at } z^* = 0 \\ \frac{\partial C_b^*}{\partial z^*} &= 0 \qquad \text{at } z^* = 1 \end{aligned} \tag{4.13}$$

Equations (4.10 - 4.13) along with equations (4.14 - 4.15) can be used to predict the breakthrough curves in a packed bed adsorber, once the adsorption isotherm is determined from equilibrium experiments.

4.1.2.1 Estimation of model parameters

The axial dispersion coefficient (D_L) can be estimated from the following correlation given by Chuang and Wen, (1968).

$$\frac{D_L \rho_L}{\mu_L} = \frac{\text{Re}}{0.2 + 0.011\text{Re}^{0.48}} \quad ,10^{-3} \le \text{Re} \le 10^3 \tag{4.14}$$

The external film coefficient (k_f) can be calculated from the correlation given by Foo and Rice, (1975).

$$\frac{2R' k_f}{D_m} = 2.0 + 1.45\text{Re}^{1/2} Sc^{1/3} \tag{4.15}$$

The molecular diffusivity (D_m) can be estimated from the Wilke - Chang equation, (1955).

The adsorption isotherm used for the model development, in the adsorption of phenol from anisole was of the form,

$$q = A\, C_i^B \tag{4.16}$$

The parameters A and B were estimated by fitting this power law expression to experimental equilibrium sorption data. Using this adsorption isotherm and various parameters determined either experimentally or using empirical correlations, the non-dimensionalised equations were solved using 4^{th} order Runge-Kutta algorithm and the breakthrough data was generated.

4.1.2.2 Dual sorption isotherm

Though the model developed above for adsorption of phenol from anisole predicted breakthrough behaviour of the polymeric systems under study well, there was some deviation from the experimental data in the later parts of the breakthrough curve. This was due to the fact that the model did not take into account the swelling of polymers under experimental conditions, viz., the presence of anisole. During the separation of phenol from anisole it was observed that there was extensive swelling of the polymers (see table 3.5). Under such circumstances, the sorption comprises of two components viz., adsorption and absorption (Gusler et. al., 1993). This is similar to dual sorption of gases in glassy polymers (Vieth et. al., 1976). Though dual sorption theory is mainly applied to sorption of gases in glassy polymers, it can also be extended to sorption of a solute from a solution.

Dual sorption theory was developed for the explanation of the penetrant solution and diffusion in the microheterogeneous media. The theory was mainly evolved to explain anomalous sorption of gases in glassy polymers. The theory postulates two component sorption in a microheterogeneous medium. The non-linear sorption isotherm can be divided into a linear part which accounts for the normal dissolution of the solute in the polymer, whereas the non-linear part of the isotherm, which is similar to the Langmuir adsorption isotherm, accounts for immobilisation of the solute at the microvoids. The two component sorption is observed as a result of the microvoids that are formed when a polymer is cooled through its glass transition temperature. These microvoids being rigid in nature can

then immobilise a part of the solute in addition to normally diffusible solute. The equilibrium equation for such behaviour can be given by,

$$C = C_D + C_H = k_D p + \frac{C_{H'} bp}{1 + bp} \tag{4.17}$$

Where, C = solubility, cc(STP) / cc of polymer atm

k_D = Henry' law dissolution constant, cc(STP) / cc polymer atm

b = hole affinity constant, atm-1

$C_{H'}$ = hole saturation constant, cc(STP) / cc polymer

p = pressure, atm

The first term in this equation C_D, represents that portion of the sorbate that is normally diffusible in the polymer, whereas the second term C_H, represents the portion of the solute that is immobilised in the microvoids in the polymer. The second term was initially applied to immobilisation of the gaseous solute in the microvoids in the glassy polymer, but can be extended to any mode of immobilisation of the solute in a microheterogeneous polymeric system.

Though dual sorption theory was initially developed to account for the anomalously high sorption of gases in the glassy polymers, it could successfully explain diverse phenomena such as, diffusion in glassy polymers and molecular sieves, dye migration in microporous media and textile fibres, reverse osmosis solvent transport etc. In the case of molecularly imprinted adsorbents, there are cavities created during the synthesis, which act as the microvoids for adsorption of the phenol. Also since the polymer swells extensively in anisole, there is normal diffusion of solute in the polymer. Thus the sorption of phenol from anisole has two components viz., dissolution in the polymer and adsorption in the cavities. Hence the usage of dual sorption theory for generating the sorption isotherm is appropriate.

Therefore in the improved model, the dual sorption adsorption isotherm was fitted to equilibrium sorption data. The dual sorption isotherm has the form,

$$q = k_D C_i + \left(\frac{C_{H'} b C_i}{1 + b C_i} \right) \tag{4.18}$$

k_D Henry' law dissolution constant

$C_{H'}$ Hole saturation constant

b Hole affinity constant

The first term in the equation 4.18 represents sorption of normally diffusible species while the second term represents sorption in the microvoids or holes. The second mode of sorption is not limited to hole filling process, but is applicable to any sorption process wherein the sorbate is immobilised in a microheterogeneous medium.

The dual sorption parameters were estimated by a non-linear steepest gradient algorithm (Press et. al., 1990) by fitting equation 4.18 to the equilibrium sorption data.

Both these models were used to predict the breakthrough behaviour for the adsorbent column in the separation of phenol from anisole.

4.1.3 Model development: Separation of phenol from bisphenol-A

For separation of phenol from bisphenol-A, the adsorption isotherm was obtained by applying the linear regression to the equilibrium sorption data. The isotherm equation was of the form, $q = mC_i$.

Since ethanol was used as the solvent for generating the breakthrough data in competitive adsorption experiments for separation of phenol from bisphenol-A, same was used as the solvent for generating the adsorption isotherms. The imprinted polymers, do not swell in ethanol and hence use of dual sorption isotherm equation was not necessary for the accurate model prediction. Hence the earlier model, based on empirical isotherm used in separation of phenol from anisole was used.

The system parameters for both the systems under study are given below in table 4.1 (separation of phenol from anisole) and table 4.2 (separation of phenol from bisphenol-A).

Table 4.1

Operational variables for mathematical modelling of phenol / anisole system

Sr. No.	Parameter	Value used in model
1	Empirical sorption isotherm, GE60 – H M	$q = 59.37 \times (C_i)^{1.103}$
2	Empirical sorption isotherm, GE60 – M M	$q = 67.55 \times (C_i)^{1.189}$
3	Dual Sorption isotherm, GE60-H M	$q = 13.52C_i + (25.43C_i)/(1+18.52C_i)$
4	Dual Sorption isotherm, GE60- M M	$q = 13.27C_i + (23.24C_i)/(1+18.4C_i)$
5	Inlet concentration, C_o, GE60 – H M	$C_o = 4.95 \times 10^{-3}$ g / ml
7	Inlet concentration, C_o, GE60 – M M	$C_o = 5.25 \times 10^{-3}$ g / ml
8	Radius of the particle, R'	2.8×10^{-3} cm
9	Density of the polymer, ρ_L	1.28 g / cm^3
10	Void fraction of the resin, ε_p	0.83
11	Bed void fraction, ε_b	0.26
12	Column diameter, D	0.84 cm
13	Axial dispersion coefficient, D_L	2.11×10^{-4} cm^2 / sec
14	Film mass transfer coefficient, k_f	8.33×10^{-3} cm / sec
15	Superficial velocity, u_0	0.767×10^{-3} cm / sec
16	Adsorber bed height, L	1.35 cm

4.2 Thermodynamics of adsorption

4.2.1 Introduction

For better understanding of the adsorption process, it is essential to understand the thermodynamics of the process. It helps not only in understanding the energetics of the process but also in design of an adsorber, which gives optimum performance in terms of selectivity and sorption capacity. Unfortunately there are very few reports on the study of thermodynamics of adsorption using molecularly imprinted adsorbents (Sellergren and Shea, 1995, Lin et. al., 1997 and Sajonz et. al., 1998).

In this work, we decided to study thermodynamics of adsorption of phenol from bisphenol-A. The adsorption isotherm used for study of thermodynamics of

the process was the same as used earlier for model development. Adsorption isotherms were plotted at three different temperatures viz., 21^0C, 31^0C and 41^0C to see the effect of temperature on sorption capacity.

Table 4.2

Operational variables for mathematical modelling of phenol / bisphenol-A systems

Sr. No.	Parameter	Value used in model
1	Adsorption isotherm, GT-HEMA-M	$q = 23.76\ C_l$
2	Adsorption isotherm, GT-PHMA-M	$q = 24.59\ C_l$
3	Inlet concentration C_o, GT-HEMA-M	2.55×10^{-3} g / ml
4	Inlet concentration C_o, GT-PHMA-M	2.25×10^{-3} g / ml
5	Radius of the particle, R'	2.80×10^{-3} cm
6	Density of the polymer, ρ	1.28 g / cm^3
7	Void fraction of the resin, ε_p	0.83
8	Bed void fraction, ε_b	0.26
9	Column diameter, D	0.84 cm
10	Axial dispersion coefficient, D_L	1.27×10^{-2} cm^2 / sec
11	Film mass transfer coefficient, k_f	0.49 cm / sec
12	Superficial velocity, u_0	0.767×10^{-3} cm / sec
13	Adsorber bed height, L	0.90 cm

4.2.2 Enthalpy of adsorption

The isosteric heat of adsorption at different levels of adsorption (q) was estimated by using an equation derived from Van't Hoff equation (Garcia-Delgado et. al., 1992).

$$\ln\left(\frac{1}{C_i}\right) = \ln(k_0) + \left(\frac{-\Delta H}{RT}\right) \tag{4.19}$$

Here C_i is the inlet concentration, ΔH is isosteric heat of adsorption, R is the universal gas constant and T is absolute temperature.

ln ($1/ C_i$) vs. $1/T$ was plotted for the systems under study and the straight line plots were obtained using linear regression. Using slopes of the plots and the

Van't-Hoff equation the isosteric heat of adsorption, at different values of q (amount adsorbed) can be calculated.

4.2.3 Change in free energy

Free energy change accompanying the adsorption, (ΔG) was estimated using the following expression derived from Gibb's adsorption equation.

$$\Delta G = -RT \ln \frac{C_l}{C_s} \tag{4.20}$$

In this equation, C_l is the concentration of the solute in the solution and C_s is the solute concentration on the adsorbent surface. C_l was determined experimentally using adsorption isotherm, whereas C_s could be determined by assuming a monolayer adsorption of the sorbate, phenol on the adsorbent surface. Knowing surface area of the adsorbent and the thickness of phenol monolayer on the surface (Fu et. al., 1949) C_s was calculated and used for determination of free energy change.

4.2.4 Entropy of adsorption

Entropy change accompanying the adsorption was estimated using the Gibb's equation,

$$\Delta S = \frac{\Delta H - \Delta G}{T} \tag{4.21}$$

Knowing the changes in enthalpy and free energy, entropy change at a particular temperature can be easily calculated.

Notations used for modelling and thermodynamic studies

Operational variables for modelling, as used in the isotherm equation

C	Outlet concentration for breakthrough experiments, g / ml
C_b	Solute concentration in the bulk phase, g /ml
C_b^*	Solute concentration in bulk phase (non-dimensional)
C_o	Inlet concentration for breakthrough experiments, g / ml
C_p	Solute concentration in the pore, g / ml
C_p^*	Solute concentration in the pore (non-dimensional)
D	Column diameter, cm
D_e	Effective diffusivity for pore diffusion, cm^2 / sec
D_L	Axial dispersion coefficient, cm^2 / sec
D_m	Molecular diffusivity, cm^2 / sec
k_f	Film mass transfer coefficient, cm / sec
L	Bed length, cm
L^*	Bed length (non-dimensional)
q	Solute concentration in solid phase, g /g of resin
q^*	Solute concentration in solid phase (non-dimensional)
r	Radial distance from the centre of spherical particle, cm
r^*	Radial distance from the centre of the spherical particle (non-dimensional)
R'	Particle radius, cm
Re	Reynolds number ($2R'u_0\rho_L/\mu_L$) (Dimensionless)
Sc	Schmidt number ($\mu_L/\rho_L D_m$) (Dimensionless)
T	Absolute temperature, ^{0}K
t	Time, seconds
t^*	Non-dimensional time
u_0	Superficial velocity, cm / sec
v	Interstitial velocity, cm / sec
v^*	Interstitial velocity (non-dimensional)
z	Distance in flow direction, cm

Distance in flow direction (non-dimensional)

Greek letters

ε_b Bed void fraction,

ε_p Intraparticle void fraction,

μ_L Liquid viscosity, g / cm sec

ρ_L Density of adsorbent particle, g / cm^3

τ Time constant (non-dimensional)

Variables used in thermodynamic calculations

ΔG Change in free energy

ΔH Change in enthalpy

ΔS Change in entropy

C_i Inlet concentration, μmole / lit

C_l Equilibrium liquid phase concentration of solute, μmole / lit.

C_s Equilibrium solid phase concentration of solute, μmole / lit

R Universal gas constant (8.3144 J / mole ^{0}K)

T Absolute temperature, ^{0}K

Chapter 5

Results and Discussion

5.1 Introduction

Molecularly imprinted polymers have found applications in a variety of areas such as racemic resolution, sensor materials, catalysts etc. In this work we have ventured into hitherto unknown areas of application for MIPs, viz., removal of structurally and functionally similar trace impurities from bulk organic chemicals and separation of positional isomers. The systems which we chose for our study are as follows.

a) Separation of phenol from anisole
b) Separation of phenol from bisphenol-A
c) Separation of 2,4- dihydroxy benzophenone isomers

5.2 Choice of system

Other than catalysis, sensors and racemic resolution, MIPs can be exploited in two new areas of applications, viz., removal of trace impurities from organic streams and separation of positional isomers.

5.2.1 Removal of trace impurities

Removal of structurally and functionally similar impurities from bulk organic streams had been a neglected area in the literature on MIPs. Hence in this study we decided to work on such separations. We decided to study removal of phenol as impurity from two different streams viz., anisole and bisphenol-A.

5.2.1.1 Separation of phenol from anisole: Presence of phenol in anisole leads to deactivation of the catalyst, which is used for oxidation of anisole to guiacol, guiacol being an important intermediate in synthesis of vanillin. The standard method of removing phenolic impurity from anisole involves treatment with alkali, followed by acid and water wash and drying and distilling if necessary (Kovacs et. al., 1981). The process is highly energy intensive and generates waste streams which need to be taken care of. The commercially available adsorbents (Amberlite XAD series, Rohm and Haas), though useful for removal of phenols from aqueous streams are not useful for use in organic streams and they also lack the selectivity

in separation.

5.2.1.2 Separation of phenol from bisphenol-A (BPA): Bisphenol-A is an important monomer used on a very large scale, for preparation of polycarbonate resins. There are two different grades of bisphenol available viz., epoxy and polycarbonate grades that differ mainly in their phenolic content (table 5.1).

Table 5.1

Various grades and specifications of Bisphenol-A

Sr. No.	Specification	Tolerance Limit	
		Epoxy grade BPA	Polycarbonate grade BPA
1	Phenol	0.10% max	0.02% max
2	o,p'- Bisphenol	2.00% max	0.01% max
3	Colour (APHA scale)	100 max	25 max
4	Iron	1.5 PPM max	1.0 PPM max
5	Assay	95-98%	99.7%

Presence of phenolic impurities in BPA (i.e. phenol and o,p'- bisphenol) affects the performance of polycarbonate resins. Hence it is essential to remove these impurities from BPA. Unfortunately, the methods used on large scale purification of bisphenol-A involve highly energy intensive operations such as, distillation (Meurer et. al., 1997), adduct formation (Sakatani et. al., 1996 and Li M., 1981), solvent washing followed by extraction (Mendiratta A., 1984) etc.

These conventional methods are useful on a very large scale, but their efficiency goes down as the concentration of the impurity reduces. The standard purification technique of alkali wash is not useful in the case of removal of phenol from bisphenol as both, the impurity as well as the bulk stream is phenolic in nature. It would be therefore desirable to develop selective adsorbents for such kinds of separations. Use of selective adsorbents will not only make the process single step but will also be advantageous in terms of energy savings.

Molecular imprinting of polymers is a technique that can be used for the

preparation of such selective adsorbents. MIPs are known to have a very high selectivity for the template molecule in the presence of a large excess of structurally similar compounds. Molecularly imprinted polymers are prepared by polymerisation of functional monomers, a large excess of the crosslinker and a porogen in the presence of a template molecule. After polymerisation the template is leached out leaving behind a cavity complementary in shape and size to the template and having appropriately positioned functional groups. The cavity acts as the selective host for the template whereas the functional groups help in rebinding interactions.

5.2.1.3 Separation of hydroxy benzophenone isomers: MIPs are well known for their applications in racemic resolution, but can also be used for separation of non-chiral positional isomers, which differ only in the positions of the functional groups. Unfortunately this area has also not received due attention in literature. Positional isomers are formed during many organic syntheses involving aromatic nuclei. For instance, synthesis of p-xylene from toluene invariably leads to a mixture of o, m and p- xylene which needs to be separated for preparation of p-xylene (intermediate for synthesis of terephthalic acid). Similarly synthesis of guiacol (o-methoxy phenol) from anisole leads to formation of p-methoxy phenol, along with the desired product. Though process optimisation leads to enrichment of one of the isomers, a mixture is still formed. Since these isomers are chemically similar to one another and many a times have very close physical constants, they are very difficult to separate by standard methods of separation (distillation, crystallisation, solvent extraction etc.). Molecularly imprinted polymers can therefore make an impact in this niche area due to their shape selectivity. The model system that we have studied in this work is the separation of 2,4- dihydroxy benzophenone isomers.

5.2.2 Interactions during synthesis of MIPs and rebinding

5.2.2.1 Synthesis of MIPs

The pre organisation of the monomers around the template for the synthesis of MIPs can be brought about by covalent as well as non-covalent techniques. In covalent imprinting, the template is covalently linked to the functional monomer during the polymerisation. After polymerisation, the template is leached out chemically, most commonly by acid / alkali hydrolysis. The other method of imprinting, viz., non-covalent linkage relies on the organisation of the functional monomers around the template with the help of secondary non-covalent interactions such as hydrophobic interactions (Andersson et. al., 1996), hydrogen bonding (Yano et. al., 1998), electrostatic interactions (Marx-Tibbon and Willner, 1994) etc.

Covalent imprinting for synthesis of MIPs is preferred over non-covalent imprinting, as it is known to give better complementarity of the imprint site since the template forms part of the functional monomer. In the case of covalent linkage all the cavities are structurally similar to one another and hence they would show similar rebinding interactions and selectivity for the template. On the other hand non-covalent imprinting leads to a broad spectrum of cavities having dissimilar selectivities. Hence in this study we decided to use covalent imprinting as the method of synthesis of MIPs.

The various covalent linkages used for synthesis of MIPs are boronate esters (Wulff and Kirstein, 1990), Schiff's Base (Wulff et. al., 1986), ketal (Shea and Dougherty, 1986), carbonate ester (Joshi et. al., 1998a) etc. The choice of the covalent interaction depends on the chemical nature of the template molecule and the functional monomer. The choice is also governed by the ease with which the template can be leached out quantitatively from the polymer. The template molecules being phenolic in nature in all the three systems under study, carboxylate and carbonate esters were chosen as the covalent links used for synthesis of MIPs.

5.2.2.2 Rebinding interactions

The rebinding interactions, which are needed for recognition of the template molecule by the imprinted polymer can also be covalent or non-covalent, but in most of the applications the preferred interactions are non-covalent, even though the synthesis of MIPs is done through covalent linkage. The main reason for this being the fast kinetics of non-covalent interactions, which is helpful in attaining desired separation under experimental conditions of a packed column.

5.2.3 Choice of monomers

The various monomers chosen for the synthesis of MIPs were as follows.

a) Allyl alcohol
b) 2- Hydroxy ethyl methacrylate
c) Methacrylic acid
d) 4- vinyl benzoic acid

The choice of monomers was mainly governed by the templates chosen. In all the three systems studied, the templates were phenolic in nature. Phenolic compounds form esters with carboxylic acid, which can be cleaved easily using alkaline hydrolysis. This hydrolysis is nearly quantitative and can be carried out under reasonably mild conditions. The carboxylic acid group generated on hydrolysis acts as the rebinding functionality. It is well known that the carboxylic groups interact with the hydroxyl groups through hydrogen bonding (Mayes et. al., 1994). One more requirement of the monomers was the presence of an unsaturation, which is needed for the polymerisation reaction. All these factors culminated in the use of methacrylic acid (MAA) and 4- vinyl benzoic acid as the functional monomers.

Similar to carboxylate esters, phenols also form carbonate esters with various alcohols and phenols. These undergo hydrolysis easily under alkaline hydrolytic conditions to generate the free template and hydroxyl groups, which are known to form hydrogen bonds with other hydroxyl functionalities (Borman S., 1995). Hence the other two monomers chosen in this study were allyl alcohol and 2- hydroxy ethyl methacrylate (HEMA).

The added advantage in using all these four monomers was the ease of synthesis of the template monomers. The synthesis of the carboxylate ester using the carboxylic acid and the phenol could be done easily and quantitatively through the acid chloride route (Schotten- Baumann synthesis) or the dicyclohexyl carbodiimide (DCC)route. Similarly the carbonate esters of the phenolic templates were prepared quantitatively, using the chloroformate reaction of the template and the functional monomer.

5.2.4 Choice of imprinting technique

Molecularly imprinted polymers are normally prepared by bulk imprinting technique (precipitation polymerisation), wherein the template, functional monomers, crosslinker and polymerisation initiator are mixed together with a porogen and polymerised thermally. After polymerisation, the polymer obtained is crushed mechanically and sieved to the desired particle size (Yu et. al., 1997). The template molecule is leached out either by washing with an appropriate solvent (non-covalent linkage) or by chemical treatment (covalent linkage). But there are conflicting references as to the accessibility of the imprint sites (Steinke et. al., 1995 and Whitcombe et. al., 1995).

In order to overcome accessibility problems, imprinted polymers can also be prepared surface imprinting, wherein the template and the functional monomers are sorbed on a macroporous support polymer and polymerised on the surface (Dhal et. al., 1995, Karmalkar et. al., 1996) [surface imprinting]. MIPs can also be made directly in the form of microspheres using suspension polymerisation technique (Mayes and Mosbach, 1996).

In this study we therefore decided to synthesise MIPs by surface imprinting as well as bulk imprinting in order to arrive at a rationale for the choice of the imprinting technique, for enhanced separation efficacy. The support polymer used for surface imprinting can be organic (Karmalkar et. al., 1996) as well as inorganic in nature (Plunkett and Arnold, 1995), but we decided to use organic macroporous polymeric support synthesised by suspension polymerisation technique.

5.2.5 Polymerisation techniques

Various methods of polymerisation initiation used in the synthesis of MIPs are thermal, photochemical (Spivak et. al., 1997), gamma ray irradiation etc. It has been shown that in the case of non-covalent imprinting technique, use of low temperature favours formation of imprint cavities with a better complementarity with the template (Spivak et. al., 1997). This is because, the interactions used for organisation of the monomers around the template being of secondary type (hydrogen bonding, hydrophobic interactions etc.), are considerably weakened at a higher temperature. In this work, the template was covalently linked to the functional monomer and hence the method of polymerisation initiation does not affect the imprinting efficacy. Hence in all the systems studied, we decided to use thermal initiation for the polymer synthesis.

5.3 Separation of phenol from anisole

Molecularly imprinted polymers, imprinted for phenol were synthesised using surface imprinting technique on a preformed GMA-EGDMA support polymer (GE60, GE75 and GE90). These polymers were used for separation of phenol from anisole. The monomers used were phenyl methacrylate, 2-(methacryloyl), ethyl, phenyl carbonate and phenyl, 4- vinyl benzoate. Selectivity and sorption capacity experiments were carried out under equilibrium (batch mode) and non-equilibrium (packed bed adsorber) conditions. Equilibrium adsorption was characterised by the adsorption isotherm and the kinetics of adsorption was modelled using a suitable mathematical frame work to predict the breakthrough behaviour.

We chose surface imprinting as the method for synthesis of MIPs because of the high porosity and surface area of the support polymers, which leads to better accessibility of the imprint sites. Though this is only one of the advantages of using surface imprinting over precipitation polymerisation, other advantage being that, the degree of crosslinking in the support resin and in the surface graft can be independently varied. Also support polymers with smaller particle size and narrow size distribution can be easily obtained by controlling their synthesis. When

packed in column, such regularly shaped materials give lower back pressure and better flow through characteristics in chromatographic applications. Porous crosslinked resin supports for such applications can be synthesised by suspension polymerisation in the presence of diluents (Svec et. al., 1975), microemulsions, polymerisation in bicontinuous emulsions (Burban et al., 1995) etc. As can be seen from surface area data (table 3.4) as well as SEM photographs (figure 3.4a) all these materials are highly porous.

5.3.1 Sorption capacity (unsteady state operation)

In industrial adsorptive separations, many a times equilibrium is not attained between the solute and the adsorbent. Under such conditions, the sorption capacity of the polymeric adsorbent is likely to be lower than the equilibrium sorption capacity. For use of MIPs on large scale, it is therefore essential to have high sorption capacity, coupled with their high selectivity. Study on the sorption capacity of MIPs under non-equilibrium conditions can therefore be used as a screening technique between various functional monomers so that the functional monomer, which shows highest sorption under non-equilibrium conditions can be used for preparation of MIPs in separation. In these studies Amberlite XAD-7 was used as the standard adsorbent, as it is used industrially for removal of phenolic impurities from aqueous waste streams. In aqueous media Amberlite XAD-7 shows adsorption capacity of about 100 mg phenol / g of the resin. Sorption capacities for various imprinted polymers under study are given in table 5.2. Sorption data for non-imprinted polymers is also given to see the imprinting effect.

From the sorption capacities it is very clear that Amberlite XAD-7, though a very efficient adsorbent for phenol from water, shows low capacity for adsorption of phenol from anisole, in spite of having a high surface area (450 m^2 / g) as compared to the imprinted resins (see table 3.4). The reason for this low sorption capacity can be attributed to extensive swelling of the resin in the solvent used (anisole) as well as lack of functional groups for interaction with the phenol molecules. On the other hand the imprinted polymers show a much higher phenol

uptake (65-70 mg / g) in spite of lower surface areas, as a result of favourable interactions between the template and the functional groups.

Table 5.2

Sorption capacities of imprinted and non-imprinted polymers

Polymer code	Sorption capacity, (mg / g of resin)
Amberlite XAD - 7	47.5
GE60 - H M	72.9
GE60 - H B	69.8
GE60 - M M	66.7
GE60 - M B	70.3
GE60 - V M	33.1
GE60 - V B	36.3

(A 0.5% solution of phenol in anisole was used for the sorption studies and the flow through experiments were carried out in a packed bed adsorber at a flow rate of 0.25 ml / min.)

The imprinted polymer based on 4- vinyl benzoic acid showed much lower sorption capacity as compared to the other polymers and hence no further studies were carried out. It can be seen from the results that the imprinted and non-imprinted polymers show almost similar sorption capacity. The reason for this behaviour was swelling of these polymers in anisole (see table 3.5 for swelling ratios). As a result of the swelling, the imprint cavity was distorted and hence imprinting effect was not seen. In the absence of imprinting effect, both the imprinted and non-imprinted polymers behave as functional polymers and since the loading of the functional monomers is same in both the cases, these polymers show similar sorption capacity.

5.3.2 Equilibrium sorption

The adsorption isotherm gives an idea about sorption capacity of the adsorbent as a function of inlet concentration and is also needed to verify the results of mathematical modelling. From equilibrium sorption studies on various MIPs, it was observed that HEMA based MIP showed slightly higher sorption capacity than MAA based system. The adsorption isotherms for MIPs based on HEMA (GE60- H M) and MAA (GE60- M M) are shown in figure 5.1. The isotherm equations for both the systems, viz., GE60- H M and GE60- M M are give in table 4.1.

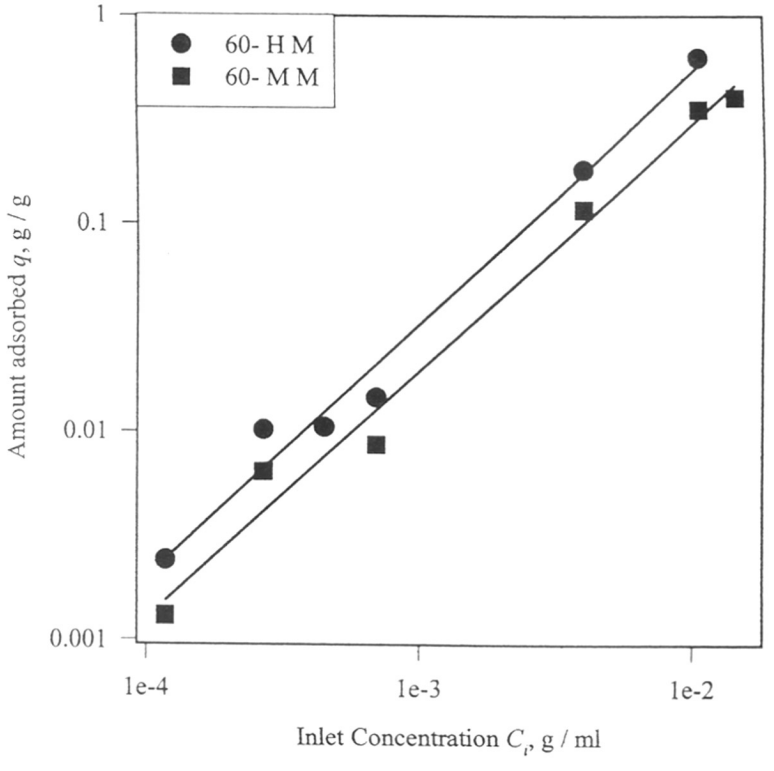


Figure 5.1: Isotherm for sorption of phenol from anisole on MIP GE60- H M and GE60-M M (31^0C)

It was also observed that as the crosslink density of the support polymer was increased from 60 to 90 %, the equilibrium sorption capacity also shows a marginal rise (figure 5.2). This can be attributed to increase in surface area from 96.1 m^2 / g to 339.9 m^2 / g with increasing cross-link density.

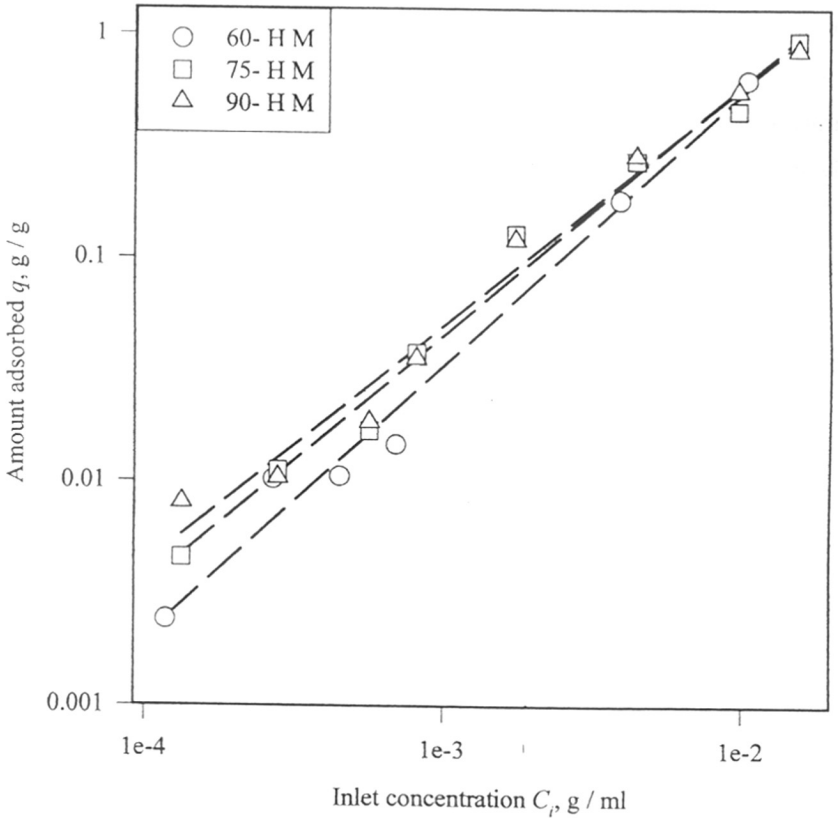


Figure 5.2:Adsorption isotherms for sorption of phenol from anisole on MIPs based on HEMA (GE60-H M, GE75-H M and GE90-H M): Effect of crosslink density

It was observed during equilibrium studies that the resins undergo extensive swelling, and hence the non-imprinted polymers also show similar sorption capacities as the imprinted polymers. It is reported in the literature that, when an adsorbent undergoes swelling during sorption studies, not only adsorption but also absorption of the sorbate as well as the solvent needs to be taken in to account (Gusler et. al., 1993).

5.3.3 Competitive sorption experiments

Since there was no difference in the sorption capacities of imprinted and non-imprinted polymers for sorption of phenol from anisole, competitive experiments were carried out from non-swelling solvents, to demonstrate the imprinting effect. Two types of sorbates were chosen, viz., the sorbates, having the same functionality as phenol but different size than phenol and the sorbates, similar to phenol in size but differing in functionality. These experiments were carried out under equilibrium as well as non-equilibrium (unsteady state) conditions so as to get an idea about absolute selectivity of the polymers along with the selectivity under diffusional constrains.

5.3.3.1 Choice of solvent for competitive adsorption experiments

We have synthesised MIPs by surface grafting technique for use in separation of phenol from anisole. This limits the amount of functional monomer that can be loaded on the macroporous support (about 10-15%). Hence the major part of the adsorbent comprises of the support polymer that can influence the adsorption of various sorbates to different extent. Also the GMA - EGDMA support polymers (GE60, GE75 and GE90) contain epoxy groups which hydrolyse to give hydroxyl groups when the template is hydrolysed with NaOH. These hydroxyl groups can then act as non-specific adsorption sites. Hence various solvents, which can minimise this non-specific adsorption were chosen for different pairs of sorbates.

5.3.4 Equilibrium selectivity experiments

5.3.4.1 Size and shape effect

O – nitrophenol (ONP) was chosen as the competing sorbate to demonstrate the size effect. Though ONP contains a phenolic hydroxyl group, in the solvent chosen (heptane), it can form intramolecular hydrogen bond leading to formation of a fused ring structure and thus would have much larger size then phenol. Selectivity values for imprinted as well as non-imprinted polymers for phenol / ONP pair are summarised in tables 5.3 (GE60- H M) and 5.4 (GE60- M M). The MIPs show higher selectivity for phenol than non-MIPs. These competitive experiments elucidated the role of size and shape of the cavity in governing the selectivity of adsorption.

Table 5.3

Equilibrium selectivity for phenol on the MIP GE60- H M

No	Polymer system	Substrate	Solvent	Selectivity	
				α	β
1	GE60- H M	Phenol + Chlorobenzene	Heptane	5.345	
2	GE60- H B			4.216	1.268
3	GE60- H M	Phenol + Bromobenzene	1% acetic acid in methanol	1.34	1.373
4	GE60- H B			0.976	
5	GE60- H M	Phenol + o-nitrophenol	Heptane	7.902	
6	GE60- H B			5.408	1.46

α= Amount of phenol adsorbed / amount of competing sorbate adsorbed

β = α for MIP / α for non-imprinted polymer

5.3.4.2 Mechanism of rebinding

Another set of competing adsorption experiments was carried out with sorbates that did not form hydrogen bonds or formed weaker hydrogen bonds. These were bromobenzene and chlorobenzene. Both these have a size similar to

phenol, but can only weakly hydrogen bond with hydroxyl or carboxyl groups present in the imprinted as well as non-imprinted polymers. The selectivity values given in tables 5.3 and 5.4 clearly indicate the importance of hydrogen bonding in rebinding, even though the sizes of both these sorbates are quite similar to the template, viz. phenol.

Table 5.4

Equilibrium selectivity for phenol on the MIP, GE60- M M

No	Polymer system	Substrate	Solvent	Selectivity	
				α	β
1	GE60- M M	Phenol + Chlorobenzene	Heptane	7.699	1.296
2	GE60- M B			5.94	
3	GE60- M M	Phenol + Bromobenzene	Heptane	5.85	4.09
4	GE60- M B			1.42	
5	GE60- M M	Phenol + o-nitrophenol	Heptane	16.07	1.423
6	GE60- M B			11.488	

Of the two competing sorbates chlorobenzene and bromobenzene, the latter is larger in size and exhibits weaker hydrogen bonding interaction . As a result a higher imprinting effect β, is observed for the system phenol / bromobenzene as compared to phenol / chlorobenzene. These experiments clearly indicate that not only the size and shape of the cavity, but also the rebinding interactions play a very important role in governing the selectivity.

5.3.5 Selectivity under non-equilibrium conditions

Selectivity studies (competitive sorption studies) were also done under non-equilibrium conditions, as many a times in industrial adsorptive separations, equilibrium may not be achieved. Hence for use of MIPs as adsorbents on a large

scale, it is essential to study their selectivities under similar conditions. The selectivity values obtained are tabulated in tables 5.5 (GE60- H M) and 5.6 (GE60- M M). These results clearly validate effect of cavity size and shape as well as functionality on the selectivity.

Table 5.5

Selectivity for phenol on MIP, GE60- H M (non-equilibrium conditions)

No	Polymer system	Substrate	Solvent	Selectivity	
				α	β
1	GE60- H M	Phenol + Chlorobenzene	1% acetic acid in methanol	1.484	1.773
2	GE60- H B			0.837	
3	GE60- H M	Phenol + Bromobenzene	1% acetic acid in methanol	2.31	2.584
4	GE60- H B			0.894	
5	GE60- H M	Phenol + o-nitrophenol	Heptane	1.992	---

Table 5.6

Selectivity for phenol on MIP, GE60- M M (non-equilibrium conditions)

No	Polymer system	Substrate	Solvent	Selectivity	
				α	β
1	GE60- M M	Phenol + Chlorobenzene	1% acetic acid in methanol	2.224	1.636
2	GE60- M B			1.36	
3	GE60- M M	Phenol + Bromobenzene	1% acetic acid in methanol	4.156	4.822
4	GE60- M B			0.862	
5	GE60- M M	Phenol + o-nitrophenol	Heptane	3.83	1.232
6	GE60- M B			3.107	

It can be seen from the tables 5.5 and 5.6 that the absolute values of selectivity are lower under non-equilibrium conditions than the equilibrium selectivity values. The reasons are diffusional limitations and hydrodynamic effects, which affect the attainment of equilibrium and hence the selectivity. Under these non-equilibrium conditions all the imprint cavities are not filled with the template giving rise to lower selectivities. Though the selectivities are lower than equilibrium conditions, they are high enough for separations to be carried out on a large scale.

5.3.6 Modelling results

Mathematical modelling of the packed bed adsorber, containing the imprinted adsorbents based on HEMA and MAA viz., GE60 -H M and GE60-M M was carried out. The equilibrium sorption plots for both the imprinted polymeric systems are shown in figure 5.1. The adsorption equations for both the systems are given in table 4.1. Using the isotherm equations, equilibrium sorption capacity, q for the inlet concentration, C_i was calculated and used in model computations. These values are also listed in Table 4.1.

Langmuir adsorption isotherm could not be fitted to the equilibrium data and hence adsorption data were fitted in the experimental range using the isotherm equation of the form given by,

$q = AC_i^B$, where A and B are arbitrary constants

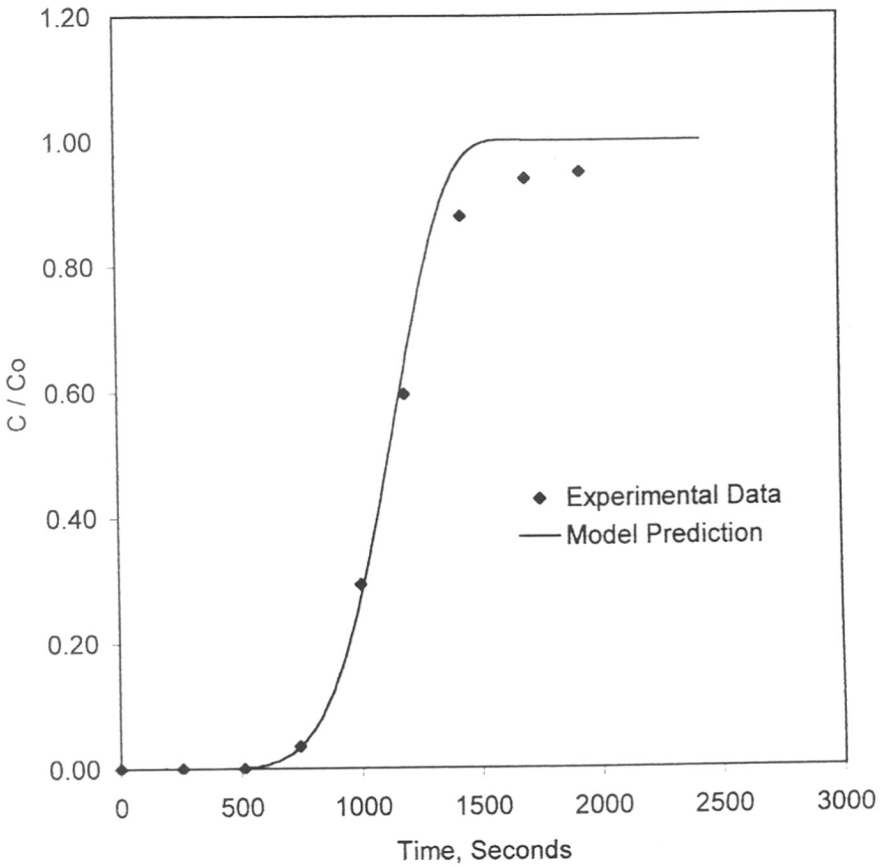


Figure 5.3: Model prediction of breakthrough curve for adsorption of phenol from anisole on MIP based on HEMA (GE60- H M)

The experimental breakthrough curves and model prediction obtained for both the systems under study are plotted in figures 5.3 and 5.4. It can be seen that there is a good agreement between the prediction and the experimental results. As can be seen from the plots, the data and model prediction agree well in the initial stages, whereas in the later stages, the model breakthrough is steeper than the experimental data. This discrepancy could be resolved by taking into account the swelling of the polymeric adsorbent, which was neglected in this model.

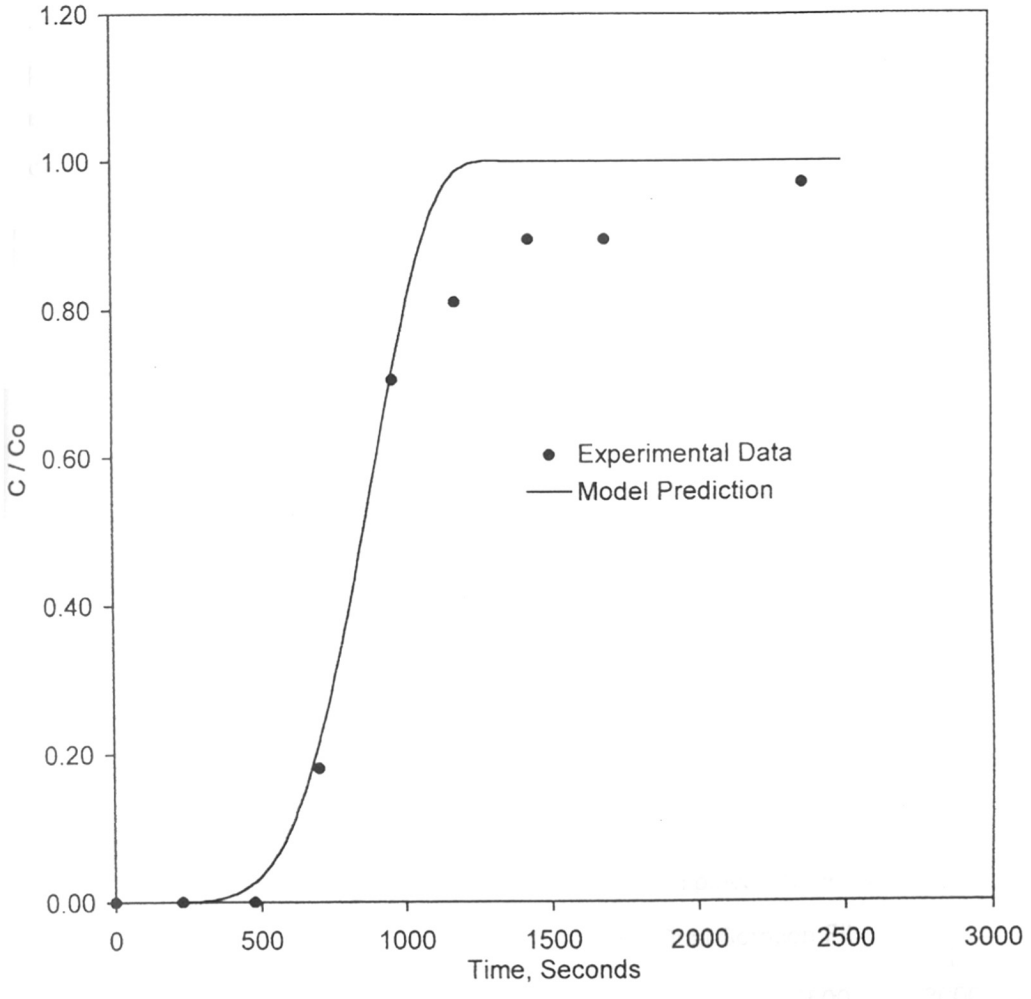


Figure5.4: Model prediction of breakthrough curve for sorption of phenol from anisole on MIP based on MAA (GE60- M M)

The adsorbents that have been used in this study swell extensively in the solvent chosen i.e. anisole. As a result, the sorption of phenol is not only limited to adsorption but there is also absorption of the solute, phenol through out the polymer. Thus the sorption comprises of two components, viz., adsorption at the imprint sites and absorption by the polymer. The adsorption isotherm, which we had used for model prediction above does not take into account these two

components and hence the model fit even though reasonably good in the initial part, diverges considerably in the later stages and the breakthrough is achieved earlier than the experimental data. To overcome this limitation we decided to use dual sorption isotherm (Vieth et. al., 1976).

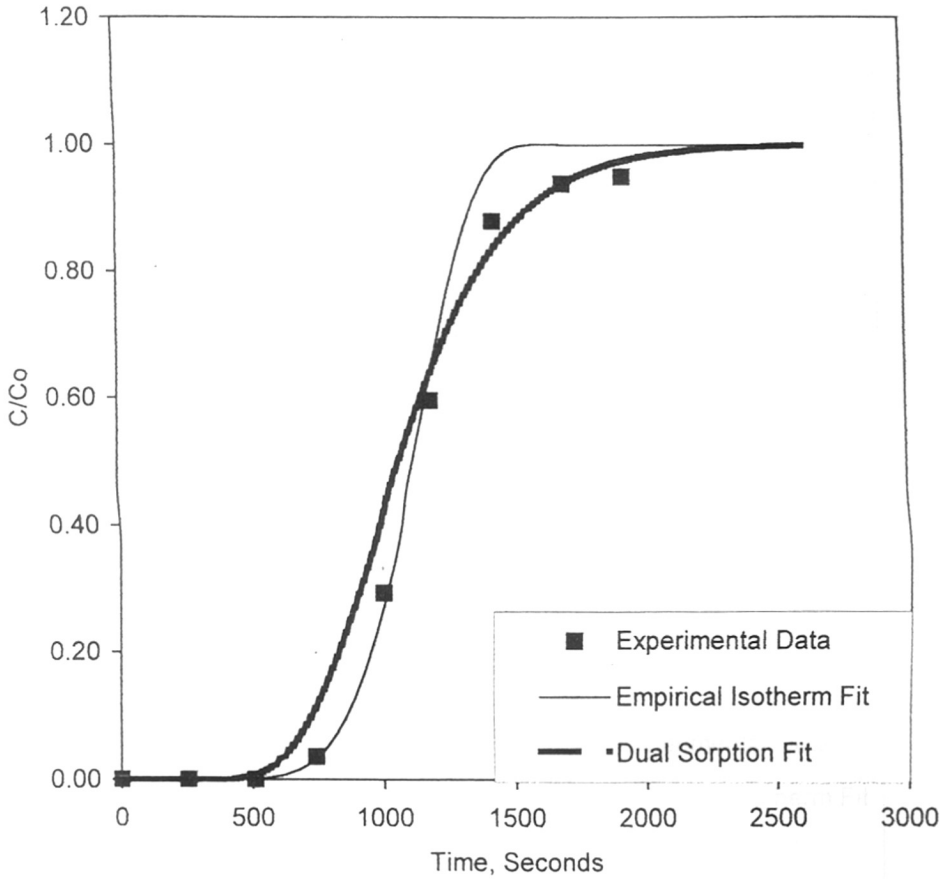


Figure 5.5: Comparison between model fit by dual sorption isotherm and empirical isotherm for sorption of phenol from anisole (GE60- H M)

The mathematical model was modified accordingly and duel sorption isotherm was used instead of empirical isotherm for model prediction. It can be seen clearly from the figures 5.5 and 5.6 that the model prediction and the experimental data agree well with one another. It can be also seen that the fit is substantially improved than the empirical isotherm fit. The reason for this

improvement is the change in isotherm, which now takes into consideration the swelling of the polymer that was neglected in the earlier isotherm.

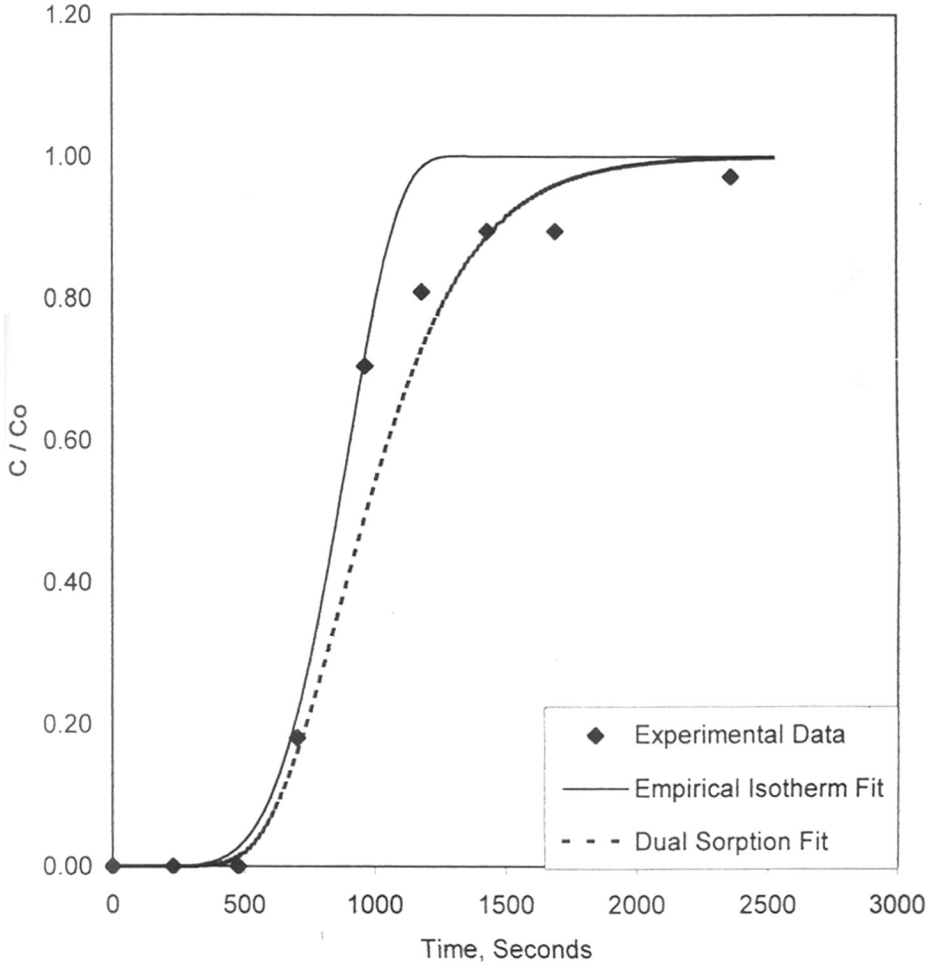


Figure 5.6: Comparison between model fit by dual sorption isotherm and empirical sorption isotherm for sorption of phenol from anisole (GE60- M M)

5.3.7 Effect of crosslink density

Effect of crosslink density on selectivity in MIPs has been studied extensively in the literature (Wulff et. al., 1987). It has been observed that the selectivity is enhanced as degree of crosslinking is increased. Selectivity for the template is shown by MIPs only when the degree of crosslinking is more than 50 % and increases with crosslinking in the range 50 -90 % and then levels off. This enhancement in selectivity is mainly due to the rigidity of imprint cavity.

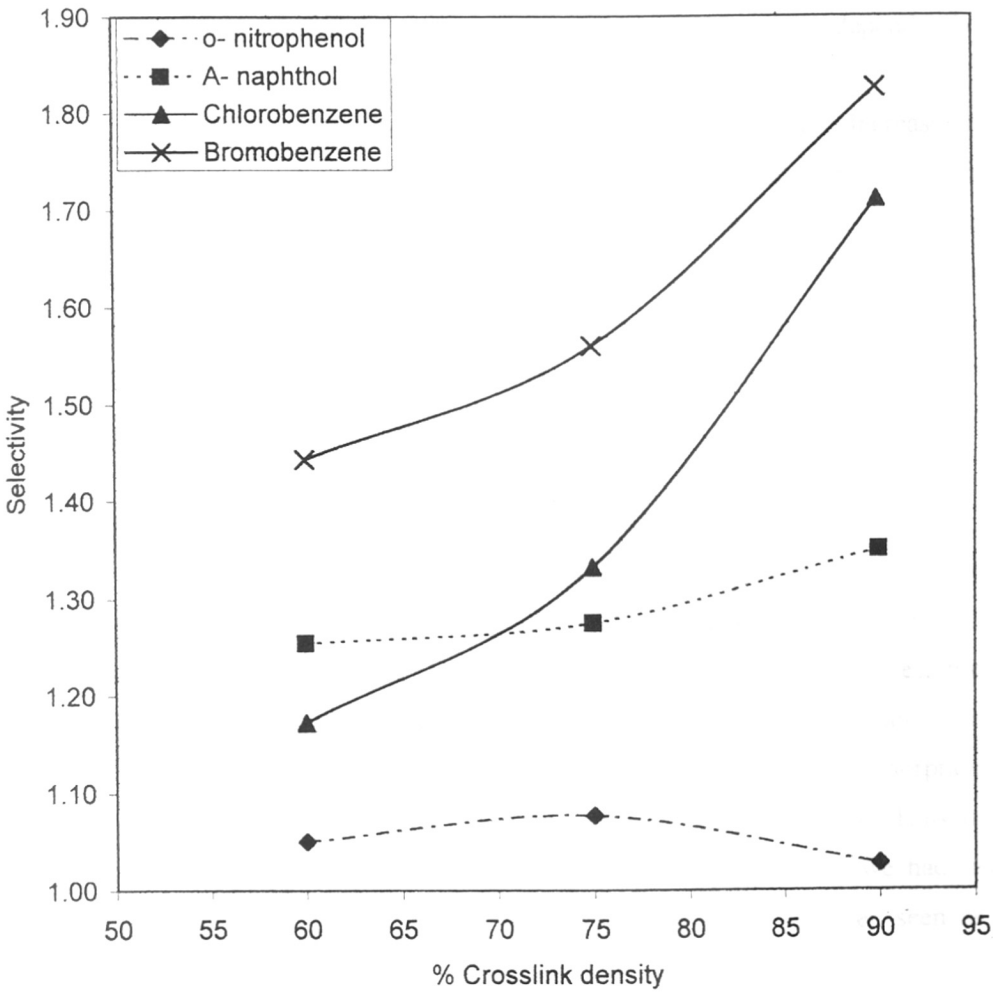


Figure 5.7: Effect of crosslink density of support polymer on selectivity for phenol using MIPs based on HEMA (GE60- H M, GE75- H M, GE90- H M)

Most of these studies are done on polymers synthesised by precipitation polymerisation, wherein by increasing the degree of crosslinking, accessibility to the imprint site can be greatly reduced. Also at higher crosslinking density, changes in porosity and surface area might reduce the sorption capacity. Unfortunately no data are available in literature for validation.

In order to overcome the accessibility problem, we chose method of surface imprinting, wherein even though crosslink density is increased, the accessibility of the imprint site is not impaired. Also the effect of crosslink density of the support polymer as well as the grafted layer on selectivity could then be independently studied.

We observed an enhancement in the selectivity for phenol with increase in crosslink density e.g. the selectivity for phenol / chlorobenzene increased from 1.17 to 1.71, when the crosslink density increased from 60% to 90%. This increase in selectivity can be attributed to the increase in surface area from 96.1 m^2 / g to 339.9 m^2 / g leading to higher loading of the template on the support, which in turn would increase the selectivity. Increase in selectivity (α) with crosslink density for all the systems investigated is depicted in figure 5.7.

5.3.8 Demerits of the system under study

The major disadvantage of the polymeric systems used in separation of phenol from anisole was swelling of the polymers in anisole. Due to swelling of the polymer, the imprint cavity gets distorted. Under these circumstances, the imprinted and non-imprinted polymers behave as functional polymers and since the concentration of functional monomers was same in both of them, their sorption capacities were similar and hence imprinting effect could not be seen. Thus in order to see the imprinting effect and the selectivity for separation, we had to choose non swelling solvents. A very high imprinting effect was then seen as indicated by high α and β values.

Thus for use of molecularly imprinted polymers in the separation of phenol from anisole, the support polymer chosen should be such that it does not swell in anisole. Silica can be one such support. Silicas of controlled porosity and uniform

size are available commercially, wherein they give all the benefits of using macroporous polymeric support polymers with an added advantage of non swelling character.

The other observation that was made during this study was the effect of solvents on selectivity. It was observed that the solvents affect the selectivity of separation substantially and hence we chose only the solvents, which gave best selectivity for the template. A detailed study of the effect of solvent on the selectivity needs to be done as right choice of solvent can substantially enhance separation efficacy of MIPs.

Another observation was made as regards the effect of crosslink density. It was seen that the selectivity increases significantly when the crosslink density was increased from 60% to 90% (figure 5.7). Since all the selectivity studies on phenol imprinted polymers used for separation of phenol from anisole were done with the polymers having 60% crosslink density, the selectivities obtained may be lower than optimum.

All these factors were taken into consideration while synthesising imprinted polymers for use in separation of phenol from bisphenol-A.

5.4 Separation of phenol from bisphenol-A: Surface imprinting

Imprinted polymers were prepared using phenol as template and MAA and HEMA as the functional monomers. Surface imprinting was used as the method of imprinting. GMA-TRIM macroporous support (GT90) was used as the support polymer for the synthesis. TRIM was used as the crosslinker as it has three unsaturations and therefore it gives a more rigid network at the same degree of crosslinking when compared with EGDMA. Phenol and bisphenol-A being solids, various solvents were chosen for the selectivity studies. Studies such as selectivity under equilibrium and non-equilibrium conditions, solvent effect, mathematical modelling and generation of adsorption isotherm were done.

5.4.1 Selectivity studies under equilibrium conditions

The selectivity experiments carried out under equilibrium conditions give

an idea about the absolute selectivity of the MIPs. As the main rebinding interaction between the template and the polymer is hydrogen bonding factors, which affect this interaction affect the separation efficacy as well. The choice of solvent can thus affect the selectivity, as it can compete with the solute for the adsorption sites. Recently Allender et. al., (1997) reported that the solvents used for chromatographic experiments influence the selectivity of MIPs in racemic resolution. It is also reported that the use of non-polar solvents enhances the separation efficacy, as they do not interfere with the solute-polymer interactions.

Table 5.7

Effect of solvent on equilibrium selectivity for phenol imprinted polymers

Sr. No.	MAA based polymer				HEMA based polymer		
	Polymer	Solvent	α	β	Polymer	Solvent	α
1	GT90-PHMA-M	Ethyl acetate + Heptane (20:80,v/v)	0.173	1.559	GT90-HEMA-M	Ethyl acetate + Heptane (20:80,v/v)	0.183
2	GT90-PHMA-M	1,4-dioxan	1.353	2.627	GT90-HEMA-M	1,4-dioxan	0.969
3	GT90-PHMA-M	1% acetic acid in methanol	1.6	1.804	GT90-HEMA-M	Ethyl acetate	1.1
4	---	---			GT90-HEMA-M	Ethanol	1.228

α = Amount of phenol adsorbed / amount of BPA adsorbed

β = α for MIP / α for the non-imprinted polymer

Heptane was therefore considered the ideal solvent for separation of phenol from BPA because of its non-polarity. Since the solubility of BPA in heptane was very poor, 20% ethyl acetate (v/v) was added. The α values, (0.173 and 0.183) (table 5.7) indicate preferential adsorption of BPA, even when the polymer was

imprinted for phenol. This could be attributed to the non-specific adsorption of BPA on the support polymer (GT90). To confirm this hypothesis and suppress the non-specific adsorption, we decided to choose more polar solvents. Although polar solvents suppress non-specific adsorption, they also compete with the solute for the adsorption sites on the polymer and hence can reduce the sorption capacity. As can be seen from table 5.7, the selectivity of MIPs for phenol is significantly enhanced with increasing solvent polarity. The ratio β, which defines the selectivity of MIPs over non-MIPs or the imprinting efficacy, is also high and increases with solvent polarity, indicating clearly that molecular imprinting has enhanced the separation achievable.

In the case of surface imprinted polymers, the template loading is normally low. For instance, the amount of phenyl methacrylate loaded on the support was found to be 34.5 mg / g and that of 2-(methacryloyl) ethyl, phenyl carbonate was 49.8 mg / g of resin. In contrast, when co-polymerised with EGDMA using precipitation polymerisation (bulk imprinting), 95 mg of phenyl methacrylate could be incorporated in the copolymer. The amount of monomer, which can be sorbed and polymerised on a crosslinked polymeric support depends on and can be influenced by the relative hydrophilicity / hydrophobicity of the monomers being sorbed and the polymeric support. The amount sorbed could be enhanced by the appropriate choice of the support and the sorbate (Lele et. al, 1998). However, no attempt was made in this work, to enhance the loading of the template monomer.

In order to demonstrate the non-specific adsorption, we investigated the uptake of phenol and BPA on the GT90 support polymer. As can be seen from table 5.8, the GMA-TRIM support polymer exhibits an inherent selectivity for BPA, indicated by α values less than unity, irrespective of the solvent chosen. This inherent selectivity for BPA can be attributed to the hydrophobicity of the support polymer as well as to the presence of hydroxyl groups generated during template hydrolysis due to the simultaneous hydrolysis of GMA. We have observed in our studies with Amberlite adsorbents that Amberlite XAD-7, an acrylate ester based adsorbent, shows selective adsorption of BPA over phenol, whereas Amberlite XAD-2, a styrenic adsorbent shows selectivity for phenol over BPA. Since the

support polymer used by us was methacrylate ester based, its inherent selectivity for BPA is only to be expected.

Table 5.8

Effect of solvent on selectivity of the support polymer (GT90) for phenol (Non-equilibrium conditions)

Sr. No.	Solvent	Selectivity, α
1	Ethyl acetate + Heptane (20:80 v/v)	0.414
2	1,4 – dioxan	0.874
3	Chloroform	0.575
4	Ethyl acetate	0.815
5	Ethanol	0.843
6	Methanol	1.076

Another factor limiting the performance of the surface imprinted polymers for separation of phenol from BPA could be the small size of phenol and the fact that it offers only a single site for rebinding. To confirm if this was indeed so, we selected a series of dihydroxy benzophenone isomers. Surface imprinted polymers were prepared using allyl, 2,4- DHB carbonate on a GMA-EGDMA support (GE90). The selectivity values are improved substantially as can be seen from table 5.9. The role of imprinting is evident from higher α values for the imprinted polymer *vis-à-vis* non-imprinted polymers, which leads to high β values.

The absolute values of α are in the following order 2-HB ~ 4,4'-DHB < 2,2'-DHB < BPA < 4,4'-DMB (see figure 3.1 for the chemical structures). As we move from 2-HB to 4,4'-DMB, the position of the functional groups on the molecule as well as the size changes with respect to 2,4-DHB. This leads to increase in selectivity. These results clearly indicate that surface imprinted MIPs can conveniently be used for substrates, which are bulky in size and have multiple binding sites. Also in the case of bulky substrates, the use of surface imprinting

circumvents the problem of accessibility of the imprint sites because the imprint sites are located on the surface of the matrix.

Table 5.9

Equilibrium selectivity for 2,4- DHB in methanol for MIP (GE90-All24DHB-M)

Sr. No.	Polymer	Substrate	α	β
1	GE90-All24DHB-M	2- HB	1.556	---
2	GE90-All-B		---	
3	GE90-All24DHB-M	4,4- DHB	1.687	1.402
4	GE90-All-B		1.203	
5	GE90-All24DHB-M	2,2'- DHB	1.948	1.315
6	GE90-All-B			
7	GE90-All24DHB-M	BPA	4.865	13.043
8	GE90-All-B		0.373	
9	GE90-All24DHB-M	4,4'- DMB	8.159	19.519
10	GE90-All-B		0.418	

α = Amount of 2,4 –DHB adsorbed / amount of the competing sorbate adsorbed

5.4.2 Thermodynamics of adsorption

Study of thermodynamics of adsorption is essential in order to get an insight in the adsorption process. It also helps in optimising the experimental conditions under which a high selectivity could be obtained.

5.4.2.1 Change in enthalpy (ΔH): Using Van't Hoff equation (equation 4.19) isosteric heat of adsorption was calculated. Plots of ln (1/ C_i) vs. 1/T for various values of phenol adsorption (q values) on molecularly imprinted polymers based on MAA and HEMA are shown in figures 5.8 and 5.9. Using the slope of these straight line graphs, change in enthalpy was calculated. ΔH values were positive (table 5.10) and decreased with the increase in temperature. These results are in agreement with the literature reports (Wulff et. al., 1987 and O'Shannessy et. al.,

1989). Thus an endothermic process is consistent with the observations made in the literature.

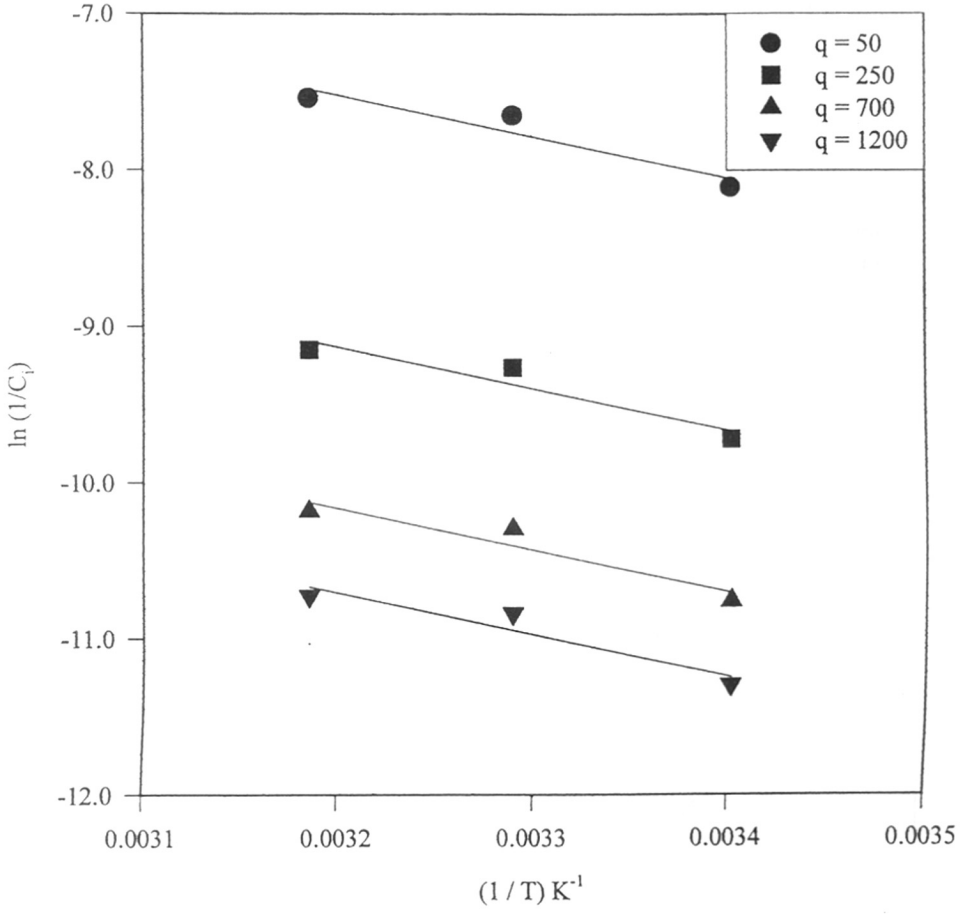


Figure 5.8: Van't Hoff plots for enthalpy at different q values (GT90-PHMA-M)

5.4.2.2 Change in free energy (ΔG): Free energy of adsorption (ΔG) was calculated using expression derived from Gibb's adsorption equation (equation 4.20).

In this equation, C_l is the concentration of the solute in the solution and C_s is the solute concentration on the adsorbent surface. C_l was determined

experimentally using adsorption isotherm, whereas C_s was determined assuming a monolayer adsorption on the adsorbent surface. Knowing the surface area of the adsorbent and the thickness of the adsorbate (phenol) monolayer on the surface (Fu et. al., 1949), C_s was calculated and used for determination of free energy change. The free energy change was negative as expected and independent of the amount adsorbed as can be inferred from table 5.10.

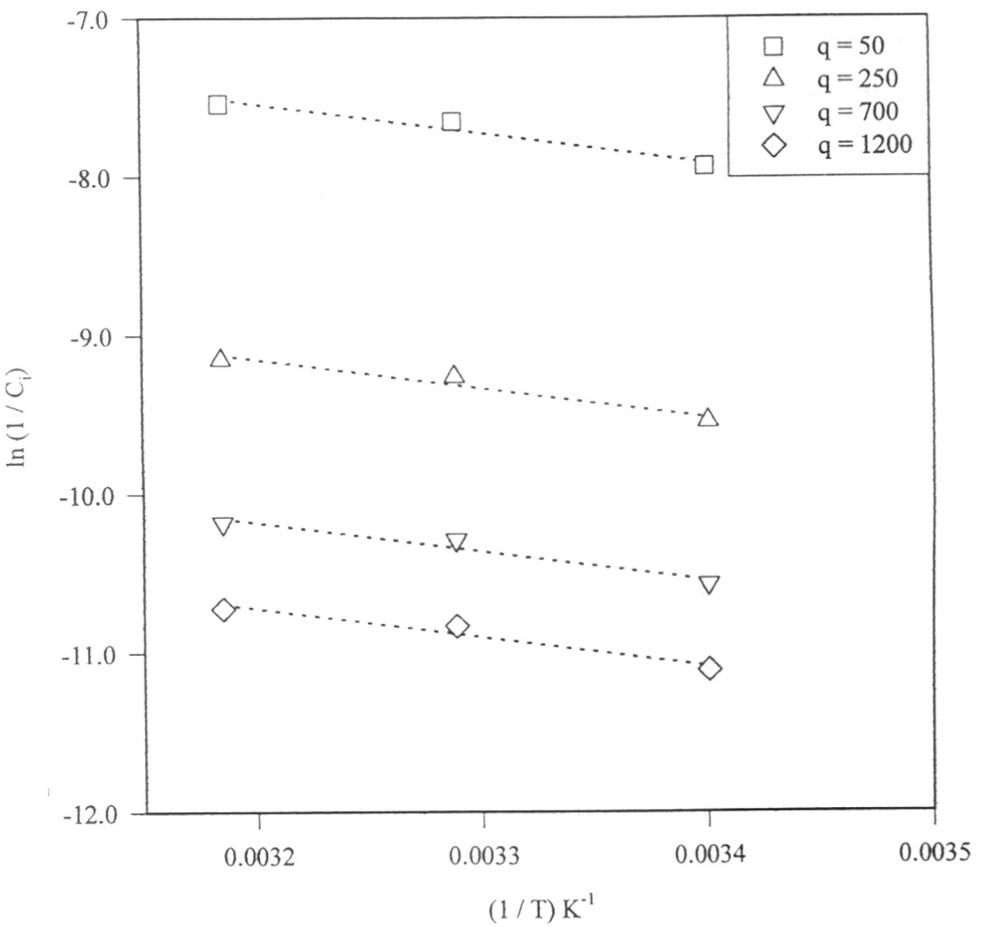


Figure 5.9: Van't Hoff plots for enthalpy at different q values (GT90-HEMA-M)

5.4.2.3 Change in entropy (ΔS): The entropy of adsorption (ΔS) was estimated from the Gibb's equation (equation 4.21).

A linear adsorption isotherm of the type $q = m\ C_i$ was used to fit the sorption data. Strictly speaking the adsorption on a molecularly imprinted adsorbent will comprise two components viz., the sorption at the imprint sites and the non-specific adsorption. The sorption at the imprint sites would result in decrease in entropy, as a strong solute-sorbent complex is formed, while the non-specific adsorption will result in increase in entropy (Jacobson et. al., 1990). The net magnitude of the change of entropy will depend on the sum of the two. Since surface imprinting is limited by lower template loading, the non-specific adsorption component will dominate over the specific adsorption. The overall entropy change would therefore slightly positive as was the case (table 5.10). The positive entropy change is also consistent with the lower selectivities for phenol (tables 5.7 and 5.8) indicating dominance of non-specific adsorption.

Table 5.10

Thermodynamic parameters for adsorption of phenol

No.	Polymer	'q' µmole / lit.	ΔH, KJ / mole	ΔG, KJ / mole		ΔS, KJ / mole	
				21^{0}C	41^{0}C	21^{0}C	41^{0}C
1	GT90-PHMA-M	50	29.9	-4.14	-2.77	0.115	0.104
		250	21.9	-4.14	-2.77	0.088	0.078
		700	21.9	-4.14	-2.77	0.088	0.078
		1200	21.86	-4.14	-2.77	0.088	0.078
2	GT90-HEMA-M	50	15.28	-3.69	-2.66	0.064	0.057
		250	15.2	-3.69	-2.66	0.064	0.057
		700	15.0	-3.69	-2.66	0.063	0.056
		1200	15.0	-3.69	-2.66	0.063	0.056

5.4.4 Selectivity under non-equilibrium conditions

Large-scale adsorptive separations are carried out in industry with the help of packed bed adsorbers. For MIPs to be used as commercial adsorbents, it is necessary to evaluate the selectivity under kinetically controlled conditions. Hence we carried out selectivity experiments with MIPs packed in a column. Various

solvents were used as in the case of equilibrium experiments to study the effect of solvents on selectivity.

Table 5.11

Effect of solvent on selectivity for phenol on MIP based on MAA

(Non-equilibrium conditions)

Sr. No.	Polymer	Solvent	α	β
1	GT90-PHMA-M	Ethyl acetate and	0.489	
2	GT90-MAA-B	Heptane (20:80 v/v)	0.401	1.219
3	GT90-PHMA-M	Ethyl acetate	0.886	
4	GT90-MAA-B		0.873	1.015
5	GT90-PHMA-M	1,4-dioxan	1.011	
6	GT90-MAA-B		0.988	1.023
7	GT90-PHMA-M	Methanol	1.016	
8	GT90-MAA-B		0.786	1.294

Similar to the trends in the selectivity experiments done under equilibrium conditions, the selectivity under non-equilibrium conditions increased with an increase in the solvent polarity (table 5.11), but the absolute values were very low. Under these conditions too, it was the non-specific component of adsorption that influenced the selectivity and efficacy of separation. We demonstrated in the preceding section (section 5.4.2) that surface imprinted polymers were not particularly suited for the sorption of small molecules, which offer only single rebinding site.

In order to enhance the separation efficacy of phenol from BPA, we prepared the imprinted polymer using phenyl methacrylate by precipitation polymerisation technique. The results in table 5.12 clearly show that the imprinted polymers prepared by precipitation polymerisation exhibit higher selectivity for phenol as compared to the surface imprinted polymers. Also, there was an increases in selectivity with an increase in solvent polarity as this suppressed non-specific adsorption. The β values show a decrease with an increase in the solvent

polarity, which is mainly due to the reduction in imprinting effect with increase in solvent polarity.

Table 5.12

Selectivity for phenol on system based on MAA (Bulk imprinted polymer)

(Non-equilibrium conditions)

Sr. No.	Polymer Code	Solvent Used	α	β
1	PHMA-M-BU	Ethyl acetate + Heptane (20:80 v/v)	0.562	2.083
2	PHMA-M-BU	1,4- dioxan	1.333	---
3	PHMA-M-BU	Ethyl acetate	1.502	1.493
4	PHMA-M-BU	Ethanol	2.016	0.951
5	PHMA-M-BU	Methanol	1.058	0.844

The increased selectivity in the case of bulk imprinted polymers as against surface imprinted polymers was due to two factors. Firstly the imprinted polymers could be now prepared with higher template loading (95 mg phenyl methacrylate / g of polymer as against 34.5 mg / g in surface imprinted polymer). Secondly, the non-specific adsorption was suppressed due to absence of GMA in the bulk imprinted polymer.

It can be seen from table 5.12 that in non-polar solvent such as heptane the phenol selectivity due to imprinting was higher as indicated by higher β value, but the actual selectivity α, was low due to non-specific adsorption. In a polar solvent like ethanol, suppression of the non-specific adsorption resulted in high value of α. But the imprinting effect on selectivity was suppressed resulting in lower value of β. Finally in a solvent like ethyl acetate, the uptake of phenol was higher than that of BPA and also the imprinted polymer showed higher selectivity towards phenol as against the non-imprinted polymer. Thus higher selectivity for phenol over BPA and the contribution of imprinting to the selectivity was seen when ethyl acetate was used as the solvent. These results clearly indicated the importance of the

choice of the solvent in enhancing the separation efficacy by MIPs. Further these results also demonstrated that for a substrate like phenol that has only a single point of binding, it would be beneficial to use bulk imprinting technique to prepare MIPs rather than surface imprinting.

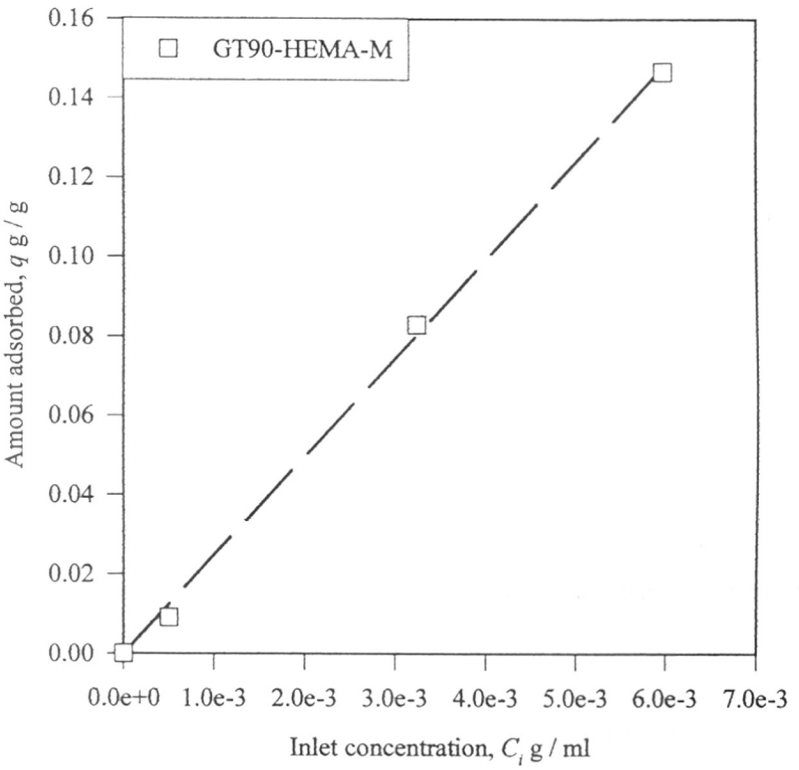


Figure 5.10: Adsorption isotherm for sorption of phenol from ethanol on GT90-HEMA-M

5.4.5 Mathematical model

The adsorption isotherm for GT90-HEMA-M is shown in figure 5.10. Adsorption isotherm for GT90-PHMA-M being similar in nature to that based on HEMA, is not shown. The isotherms were data fitted using linear regression in the

form $q = m\ C_i$. The isotherm equations and various parameters used in molecular modelling are summarised in table 4.2 (Chapter 4).

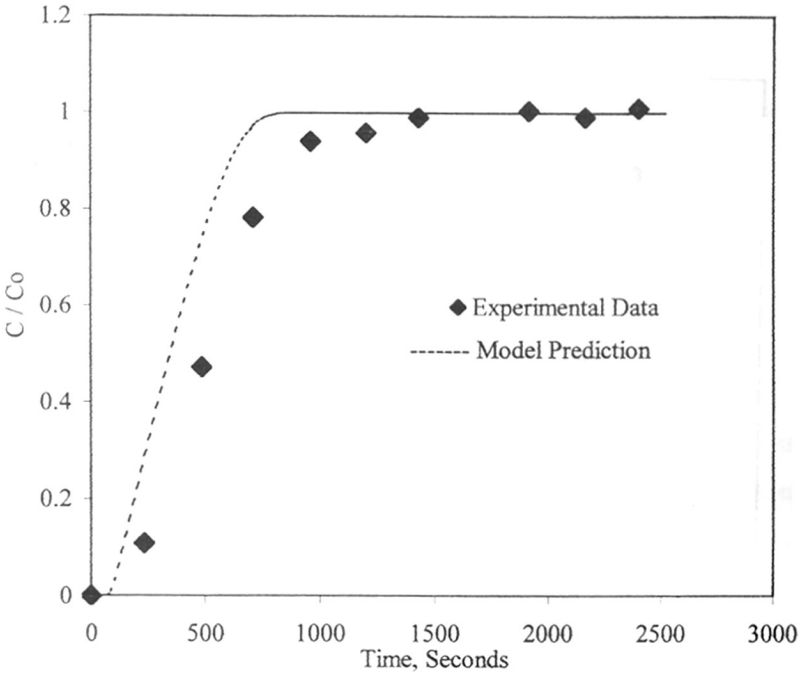


Figure 5.11: Model prediction of breakthrough curve for adsorption of phenol from ethanol on GT90-PHMA-M

Mathematical modelling of MIP based packed bed adsorber helps in predicting the breakthrough curves under differing conditions such as the column characteristics, flow rate etc. It helps in scaling up the adsorber from laboratory scale to commercial scale and gives an idea of the useful life after which regeneration would be necessary.

Recently we developed a mathematical model, which predicts the breakthrough behaviour of a packed bed adsorber containing molecularly imprinted polymeric adsorbents (Joshi et. al., 1998a). The same model can be extended to the current system under study. For both the systems under study, viz., GT90-PHMA-M (Figure 5.11) and GT90-HEMA-M (Figure 5.12), the model

prediction of the breakthrough behaviour matches well with the experimental data. As all the parameters in the model were determined experimentally or with the help of correlations, the model is very close to being predictive.

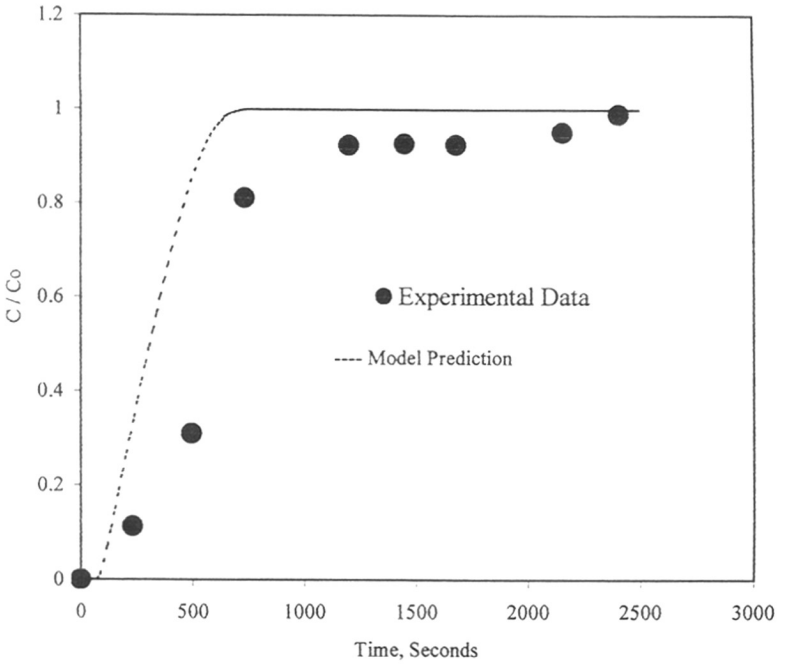


Figure 5.12: Model prediction of breakthrough curve for adsorption of phenol from ethanol on GT90-HEMA-M

5.4.6 Limitations of surface imprinting for separation of phenol and bisphenol-A

All the results obtained for the surface imprinted polymers used in separation of phenol from bisphenol-A, clearly indicated that when the template molecule is small in size and has only a single point of rebinding interaction, surface imprinting is not the right choice for preparation of MIPs. Due to presence of a large excess of non-specific adsorption sites, the overall selectivity for the template is reduced. This was the reason why we decided to synthesise phenol imprinted polymers using phenyl methacrylate, as the template monomer by bulk imprinting technique. We also synthesised polymers imprinted for p-cumyl phenol,

using cumyl phenyl methacrylate by bulk imprinting technique. This polymer was used as a selective adsorbent for bisphenol-A. The comparison between these two polymers gives an idea as to the effect of the template on selectivity as in both these cases the functional monomer was the same viz., methacrylic acid. Various solvents were used to study the effect of solvent on selectivity.

5.4.7 Selectivity under equilibrium conditions : Bulk imprinting

Equilibrium selectivity experiments were carried out in order to get an idea about the absolute selectivity of the MIPs.

5.4.7.1 Phenyl methacrylate system

As can be seen from table 5.13, equilibrium selectivity shown by polymer imprinted for phenol using phenyl methacrylate increases with an increase in the solvent polarity. The imprinting efficacy, β also shows a significant increase with an increase in the solvent polarity / hydrogen bond donator factor. As can be seen the selectivities are much higher than the surface imprinted polymers (table 5.7). This can be explained as follows.

Table 5.13

Equilibrium selectivity values for phenol imprinted polymer based on MAA

Sr. No.	Polymer code	Solvent	α
1	PHMA-M-BU	Ethyl acetate	0.627
2	MAA-BL-BU		0.647
3	PHMA-M-BU	Ethanol	3.469
4	MAA-BL-BU		1.236
5	PHMA-M-BU	Methanol	1.642
6	MAA-BL-BU		0.524

In the case of MIPs, there are two types of sites, where adsorption can take place. These are specific adsorption sites (cavities created by imprinting) and non-

specific adsorption sites, whose presence is attributed to the functional groups on the backbone polymer. During the equilibrium experiments, sufficient time is given for the sorbate to attain equilibrium with the polymer. Under such conditions, due to strong rebinding interactions between the sorbate and functional groups in the cavity, most of the accessible cavities present in the imprinted polymer would be filled with the template as against the non-equilibrium batch experiments.

The solvent competes with the sorbate for the non-specific adsorption sites. As the solvent is in large excess as compared to the sorbate, it will be preferentially adsorbed at the non-specific adsorption sites. Thus with an increase in the solvent polarity the solvent- polymer interaction becomes stronger and the non-specific adsorption of the sorbate is reduced due to preferential sorption of solvent at the non-specific adsorption sites.

Table 5.14

Solvent properties

Solvent	R_2	π^H_2	$\Sigma\alpha^H_2$	$\Sigma\beta^H_2$	Log *L*
n-Heptane			0.00	0.00	3.173
1,4-dioxane	0.329	0.75	0.00	0.64	2.892
Ethyl acetate	0.106	0.62	0.00	0.45	2.314
Ethanol	0.246	0.42	0.37	0.48	1.485
Methanol	0.278	0.44	0.43	0.47	0.97
Acetic acid	0.265	0.65	0.61	0.44	1.750
Phenol	0.805	0.89	0.60	0.30	3.766

Due to a synergistic effect, there is an increase in the selectivity as well as imprinting efficacy with increase in the solvent polarity (figure 5.13). The same trend is also observed in figure 5.14 where β is plotted against the hydrogen bond donator factor, $\Sigma\alpha^H_2$ (see table 5.14 for various solvent properties, Abraham, M., (1993)). With an increase in the solvent polarity, hydrogen bond donator factor of

the solvent also increases and hence the imprinting efficacy also increases. This increase in imprinting efficacy with increase in $\Sigma\alpha^{H}_{2}$ is exactly opposite to that reported by Allender et. al.,(1997) which may be due to attainment of equilibrium in our case as against the HPLC separations reported by them.

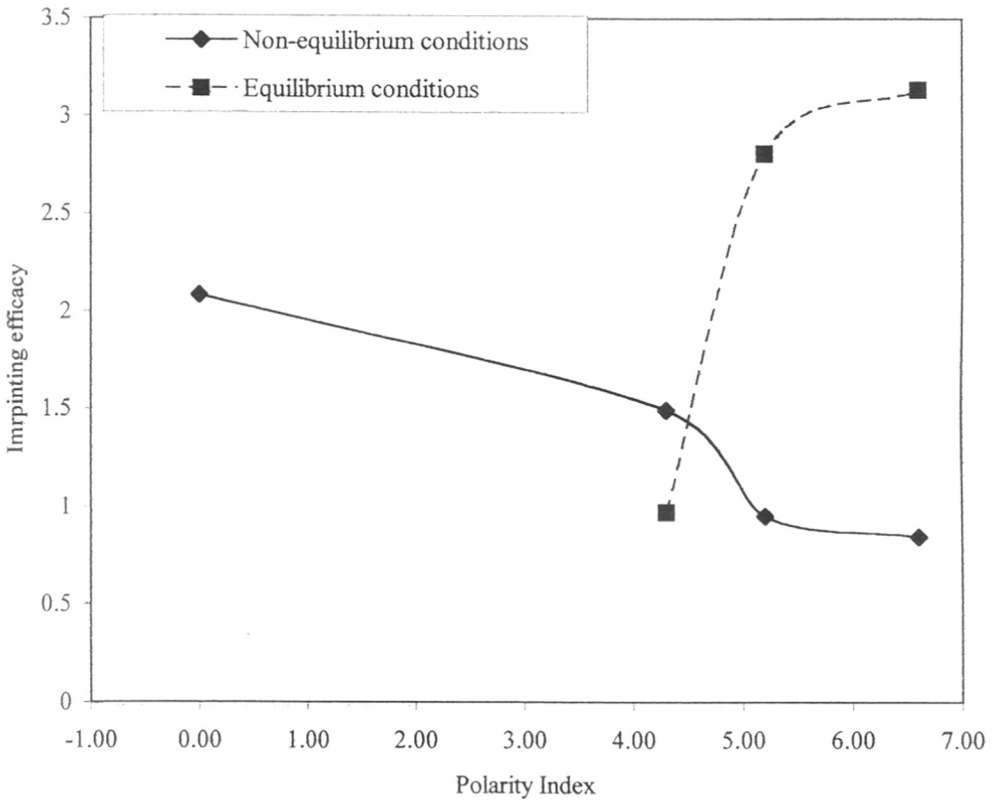


Figure 5.13: Effect of solvent polarity on imprinting efficacy (PHMA-M-BU)

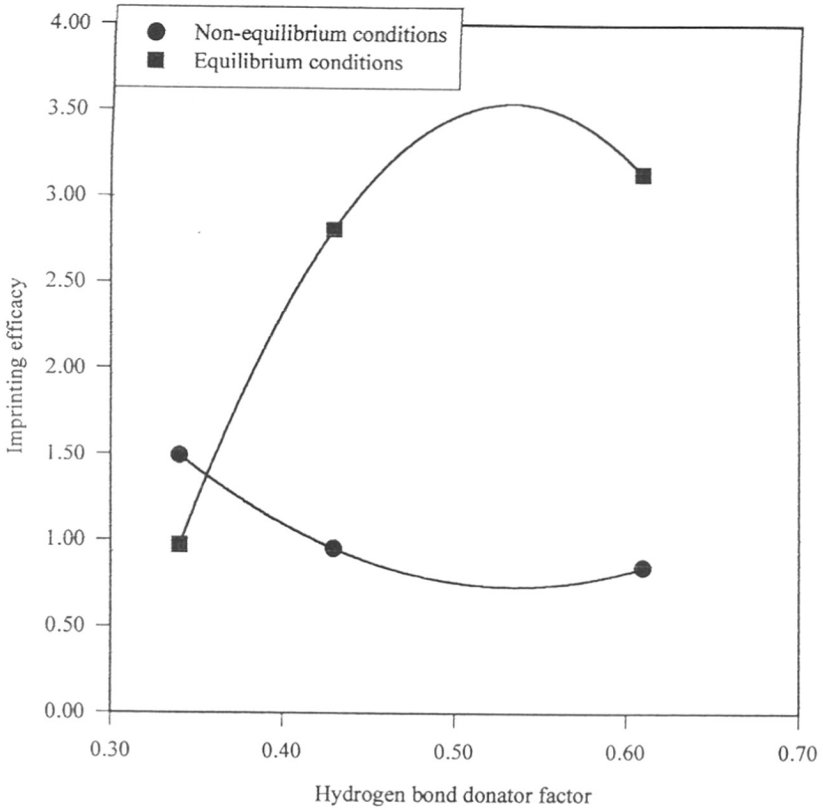


Figure5.14: Effect of hydorgen bond donator factor of the solvent on imprinting efficacy (PHMA-M-BU)

5.4.7.2 Cumyl phenyl methacrylate based system

As can be seen from table 5.15, the cumyl phenol imprinted polymer shows a decrease in the selectivity for BPA with an increase in the solvent polarity, reaches a minimum and then increases with further increase in the solvent polarity.

A very high selectivity in the case of ethyl acetate/heptane is mainly due to preferential partitioning of BPA on the polymer phase due to its low solubility in heptane. Hence this solvent shows anomalously high selectivity value. It shows a very high value for imprinting efficacy as well, as the non-polar solvent (heptane)

does not interfere with the rebinding interactions between the polymer and template.

Table 5.15

Equilibrium selectivity for BPA using cumyl phenol imprinted polymer based on MAA

Sr. No.	Polymer code	Solvent	α
1	CUPHMA-M-BU	Ethyl acetate + Heptane (20:80, v/v)	2.568
2	CMAA-BL-BU		1.435
3	CUPHMA-M-BU	Ethyl acetate	0.793
4	CMAA-BL-BU		1.470
5	CUPHMA-M-BU	Ethanol	1.207
6	CMAA-BL-BU		0.792
7	CUPHMA-M-BU	Methanol	1.497
8	CMAA-BL-BU		0.810
9	CUPHMA-M-BU	1% Acetic acid in methanol	2.731
10	CMAA-BL-BU		1.397

With an increase in the solvent polarity, when the solubility of BPA is enhanced, the partitioning effect is significantly reduced (ethyl acetate), and the selectivity drops down significantly. Hence ethyl acetate shows a low value for α as well as β. With further increase in solvent polarity and the hydrogen bond donating capacity, the non-specific adsorption is significantly reduced and hence the selectivity and imprinting efficacy are substantially increased. This effect is similar to that seen in the case of phenol imprinted polymer and hence can be explained in the same manner. The trends in the imprinting efficacy, (β) as a function of polarity index of the solvent and the hydrogen bond donator factor, ($\Sigma\alpha^{H}_{2}$) can be seen from figures 5.15 and 5.16 respectively.

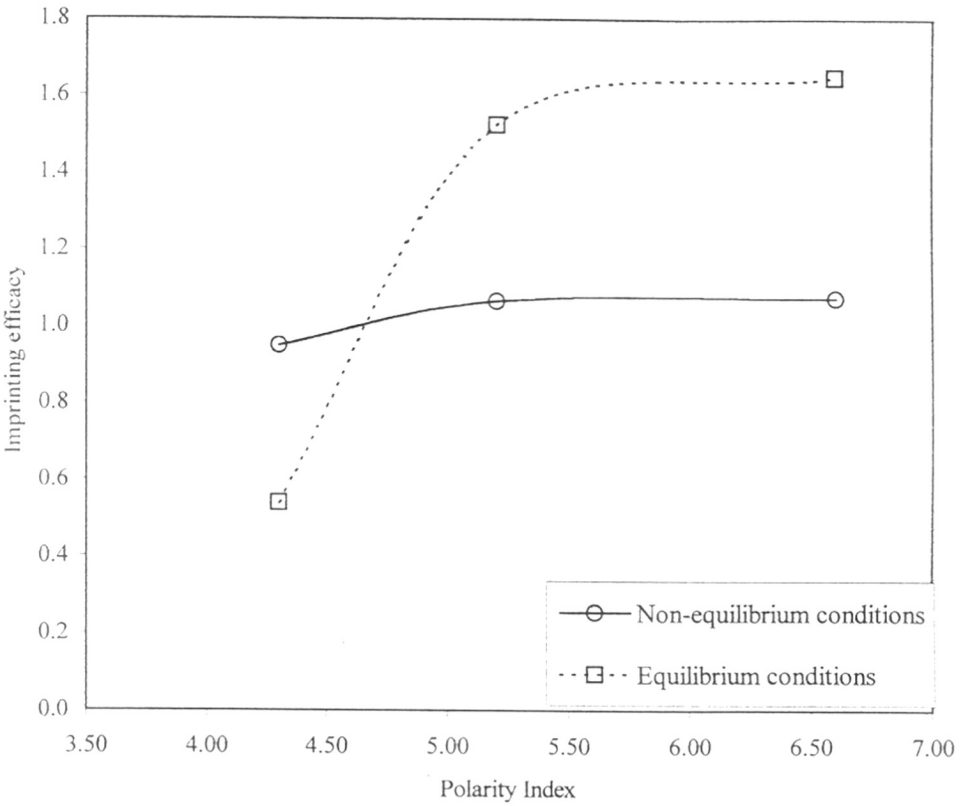


Figure 5.15: Effect of solvent polarity on imprinting efficacy (CUPHMA-M-BU)

5.4.8 Selectivity under non-equilibrium conditions : Bulk imprinting

It is essential to study selectivity of MIPs under non-equilibrium conditions as in most of the industrial adsorptive separations, equilibrium is not attained between the solute and sorbent. It is a well known fact in the literature on MIPs that the use of a non-polar solvent gives high selectivity in separation as it does not interfere with the rebinding interactions between the template and the polymer. Hence in our study on separation of phenol and BPA using MIPs, we started with a low polarity solvent such as heptane. Due to the poor solubility of BPA in heptane we modified it with 20% ethyl acetate. To study the effect of solvent polarity on

separation we successively chose solvents with increasing polarity and the results obtained are described now.

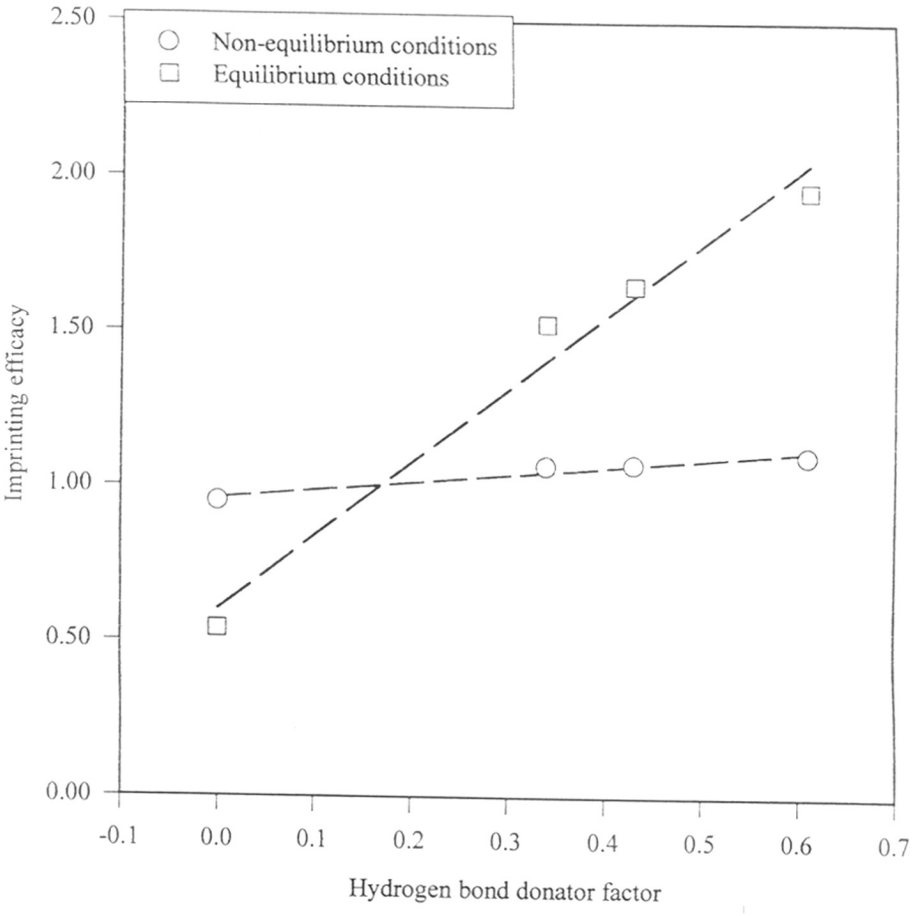


Figure 5.16: Effect of hydrogen bond donator factor of the solvent on imprinting efficacy (CUPHMA-M-BU)

5.4.8.1 Phenyl methacrylate system

In case of phenol imprinted polymer, using phenyl methacrylate as the template monomer selectivity α, is low in low polarity solvent such as mixture of ethyl acetate and heptane (table 5.16) and the selectivity increases with an increase in the solvent polarity. Low selectivity in non-polar solvents results mainly due to the non-specific adsorption of BPA on the polymer. Especially in the case of heptane based solvent, solubility of BPA being very poor, BPA is preferentially partitioned on to the polymer phase. Thus the polymer, even though imprinted for phenol, shows selective uptake of BPA. As the solvent polarity increases, solubility of BPA is substantially improved, thereby reducing the partitioning effect. The non-specific component of adsorption is also suppressed, since the solvent competes for the non-specific adsorption sites on the polymer. Solvent being in large excess as compared to the sorbate, is preferentially adsorbed on non-specific sites. Both these effects synergistically enhance the selectivity for phenol as can be evidenced from higher α values in table 5.16 for high polarity solvents. This trend is exactly similar to that seen in the equilibrium sorption experiments and can be explained similarly.

Although the selectivity increases with increased solvent polarity, the imprinting efficacy, β is suppressed. The reason for this suppression of imprinting effect is the competing hydrogen bonding between the solute, solvent and the polymer. Figure 5.13 shows this decrease in β with increase in the polarity index of the solvent.

The most commonly used rebinding interaction in MIPs is the hydrogen bonding. Therefore, the factors which affect this interaction, can affect the selectivity of separation. Hydrogen bond donator factor, ($\Sigma\alpha^{H}_{2}$) of the solvent is one such factor. With an increase in the hydrogen bond donator factor of the solvent, competing hydrogen bonding between the solvent and polymer becomes strong and the solvent being in a large excess this bond is easy to form.

When the selectivity experiments are done under non-equilibrium conditions, due to the diffusional limitations as well as the lower contact time between the sorbate and sorbent, only the easily accessible imprint cavities will be

filled with the template. Thus the imprinted and non-imprinted polymers then behave as functional adsorbents and since the functional group concentration in both these polymers is the same the imprinting effect is difficult to see. Due to this, the β values are low and these reduce further with an increase in hydrogen bond donator factor of the solvent. This trend can be clearly seen from figure 5.14. With an increase in $\Sigma\alpha^{H}_{2}$ there is an increase in the competing hydrogen bonding between the solvent and the polymer which effectively reduces the polymer-sorbate interaction and hence shows a decrease in β.

Table 5.16

Selectivity values for phenol imprinted polymer based on MAA

(non-equilibrium conditions)

Sr. No.	Polymer code	Solvent	α
1	PHMA-M-BU	Ethyl acetate + Heptane	0.562
2	MAA-BL-BU	(20:80, v/v)	0.2698
3	PHMA-M-BU	Ethyl acetate	1.502
4	MAA-BL-BU		1.006
5	PHMA-M-BU	Ethanol	2.016
6	MAA-BL-BU		2.12
7	PHMA-M-BU	Methanol	1.058
8	MAA-BL-BU		1.253

Thus for separation of phenol from BPA, a low polarity solvent (ethyl acetate + heptane) gives low α and high β. The low selectivity is mainly due to partitioning of BPA on the polymer, whereas the high β value is due to the fact that a non-polar solvent such as heptane does not interfere with the rebinding interactions between polymer and the template. On the other hand, a high polarity solvent like ethanol gives high selectivity, α as the partitioning effect is reduced and the non-specific adsorption is suppressed, but imprinting efficacy, β is low as the solvent can now interfere with the solute-polymer interactions. An intermediate

polarity solvent such as ethyl acetate gives the optimum performance in terms of selectivity, α and imprinting efficacy, β.

5.4.8.2 Cumyl phenyl methacrylate based system

In the case of the polymer imprinted for cumyl phenol, the polymer shows high selectivity for BPA in a low polarity solvent as heptane / ethyl acetate (table 5.17). It also shows a very high imprinting effect as evidenced by high β value (α = 4.565 and β = 2.66). The main reason for this high selectivity for BPA is its preferential partitioning on the polymer as a result of poor solubility of BPA in heptane. A higher imprinting effect is observed as the solvent does not interfere with the solute-polymer interactions and does not compete for the adsorption sites.

Table 5.17

Selectivity for BPA using cumyl phenol imprinted polymer based on MAA (non-equilibrium conditions)

Sr. No.	Polymer code	Solvent	α
1	CUPHMA-M-BU	Ethyl acetate + Heptane (20:80 v /v)	4.565
2	CMAA-BL-BU		1.716
3	CUPHMA-M-BU	Ethyl acetate	1.192
4	CMAA-BL-BU		1.256
5	CUPHMA-M-BU	Ethanol	1.377
6	CMAA-BL-BU		1.293
7	CUPHMA-M-BU	Methanol	1.377
8	CMAA-BL-BU		1.285
9	CUPHMA-M-BU	1% Acetic acid in methanol	1.199
10	CMAA-BL-BU		1.091

With an increase in the solvent polarity, solubility of BPA is enhanced and the partitioning effect is drastically reduced as evidenced by reduction in selectivity (table 5.17). The absolute selectivity values of the MIP for BPA are still lower than those for the phenol imprinted polymer. These lower selectivity values

can be attributed to the diffusional constraints on a bulky BPA molecule. Imprinting efficacy β is also significantly reduced with an increase in the solvent polarity. There are two reasons for this. Firstly the solvent competes with the solute for the adsorption sites, which reduces the adsorption of the template at the imprint site. Secondly due to diffusional limitations, it is difficult for the bulky template molecule (BPA) to reach the imprint site and fill all the cavities, which further reduces the imprinting efficacy. Thus if one neglects high α and β values in ethyl acetate / heptane, which is mainly due to partitioning of BPA, in all the other solvents, increase in the solvent polarity leads to a marginal change in the imprinting efficacy (figure 5.15). A similar trend in imprinting efficacy is also seen with an increase in ($\Sigma\alpha^{H}_{2}$), the hydrogen bond donator factor of the solvent (figure 5.16).

5.4.9 Shortcomings of the system

Some of the limitations faced in separation of phenol from anisole viz., the swelling of the polymer in the solvent, lower crosslink density were taken care of by appropriate choice of solvent for sorption studies and use of highly crosslinked network polymers. But it was found out that there were certain additional factors such as the choice of solvent, experimental conditions (equilibrium vs. non-equilibrium), imprinting techniques (surface vs. bulk), which affect the separation efficacy. Especially the solvents were found to affect the selectivity and separation efficacy significantly. For better understanding of the separation efficacy it was therefore necessary to study the solvent effect in detail for another system. It was also observed that in case of a smaller template molecule having single point rebinding interaction, bulk imprinting gave better selectivity as compared to surface imprinting. Whether this was true in the case of bulky substrates also needed to be ascertained.

5.5 Separation of 2,4- dihydroxy benzophenone isomers

We decided to work in another area wherein MIPs can find potential applications i.e. the separation of positional isomers, the importance of these

separations being explained earlier. With this application in mind we decided to study separation of positional isomers of 2,4 - dihydroxy benzophenone (2,4-DHB) as a model system. In our earlier work on separation of phenol and bisphenol we observed that when the template molecule is small and has only a single point rebinding interaction it is advantageous to use bulk imprinting technique as the method of preparation of imprinted polymers. On the other hand we also observed that even surface imprinted polymers can give a very high selectivity for the template if the template molecule is bulky and has multiple sites for rebinding (2,4- DHB). Hence we expected that in the case of 2,4- DHB the bulk imprinted polymers would be able to give high selectivity coupled with higher sorption capacity, as they can be prepared with a higher template loading compared to the surface imprinted polymers. Therefore we decided to synthesise MIPs imprinted for 2,4- DHB using surface as well as bulk imprinting. We chose two different monomers viz., allyl alcohol and methacrylic acid as the functional monomers as both of them are known to show rebinding interaction through hydrogen bonding. Also in our earlier studies on separation of phenol and anisole, we had seen that, the imprinted polymers containing HEMA showed higher sorption capacity as compared to methacrylic acid based polymers. Various solvents and experimental conditions were chosen to study the selectivity as they were found to affect the separation efficacy.

5.5.1 Allyl, 2,4- DHB carbonate based system

Imprinted polymers were prepared using allyl alcohol as the functional monomer and 2,4- DHB as the template. Surface and bulk imprinting were chosen as the methods of polymer synthesis. Surface imprinting was carried out on a preformed GE90 macroporous polymeric support and bulk imprinting was carried out using TRIM as the crosslinker (see tables 3.2 and 3.3 for monomer composition used in synthesis of polymers). Various positional isomers of 2,4-DHB such as 2- HB, 4,4'- DHB, 2,2'- DHB and 4,4'- DMB were chosen as the competing sorbates. BPA was also chosen as the competing sorbate as it also has a similar structure.

5.5.1.1 Equilibrium experiments

Measurement of the absolute selectivity and capacity of the imprinted polymers, under equilibrium conditions is the first step towards the characterisation of MIPs. Since diffusional and hydrodynamic influences are eliminated, the data can be used to correlate the structure with the performance of the adsorbents.

5.5.1.1.1 Equilibrium sorption capacity

Equilibrium sorption capacity measurements on MIPs give an idea about the efficacy of utilisation of the theoretical capacity of the adsorbent. Table 5.18 lists the sorption capacities for both the surface as well as bulk imprinted polymers.

Table 5.18

Equilibrium sorption capacity of the allyl alcohol imprinted polymers

Sr. No.	Solvent	Adsorption capacity, mg / g	
		All24DHBC-MIP-BU	GE90-All24DHB-M
--	Template loading	140- 150	40-50
1	THF	2.58	43.34
2	Ethanol	3.27	40.50
3	Methanol	4.05	21.50
4	1% acetic acid in methanol	2.25	19.05

Depending on the solvent used, the percentage efficiency for the surface imprinted polymers was 40% to 95% while that for the bulk imprinted polymers it was only 2% to 5%. The low sorption capacity of bulk imprinted polymers in spite of high template loading can be attributed to non-accessibility of the imprint sites (Steinke et. al., 1995). The surface area and pore volume data (table 3.4) as well as surface morphology as seen by SEM (figure 3.4c) shows lower porosity for bulk imprinted polymers as compared to surface imprinted polymers. Especially in the

case of bulk imprinted polymers the pores were found to be in microporous region (pore radius < 20 A^0), which imposes restrictions on the accessibility of the imprint sites, crucial for a bulky substrate such as 2,4- DHB.

Surface imprinted polymers on the other hand showed higher sorption capacity and better utilisation of the theoretical sorption capacity (40% to 95%). This is mainly due to high accessibility of the imprint site. Higher surface area and pore volume (table 3.4) as well as highly porous morphology as seen in the SEM study (figure 3.4c) clearly validates this point. It was also observed from the pore size distribution data that the pores in surface imprinted polymers were in the mesoporous region (pore radius, 20 - 200 A^0) thereby increasing accessibility of the sites.

It is pertinent to note here that the polymerisation conditions and recipes for the synthesis of bulk imprinted polymers and the support used in the synthesis of surface imprinted polymers are similar except for the presence of the template monomer in the synthesis of the former. This was responsible for the low pore volume as well as pore sizes which limits the accessibility of the imprint site. As a result, the sorption capacity in the case of bulk imprinted polymers was low and hence for separation of isomers, where the sorbate molecule is bulky and has multiple rebinding interactions, surface imprinting should be the preferred method of synthesis.

Equilibrium sorption capacity was determined in various solvents as solvents are known to affect the sorption capacity. As expected the solvents did not have any effect on sorption capacity of bulk imprinted polymers because the capacity was already severely reduced due to non-accessibility of the sites. In the case of surface imprinted polymers there was a decrease in the sorption capacity as the solvent polarity increases which was expected.

5.5.1.1.2 Equilibrium selectivity

Tables 5.19 and 5.20 summarise the selectivity data for the bulk as well as the surface imprinted polymers respectively. The bulk imprinted polymers show a high selectivity for the template in a low polarity solvent as THF (table 5.19)

whereas in other solvents the selectivity is poor. The selectivity in THF is high since the solvent does not interfere with the solute-sorbent interactions. Solvents with increasing polarity compete with the imprint sites for adsorption. Also since the accessibility of the imprint sites is limited, the solvent molecules which are smaller in size find it much easier to reach the imprint cavity. As a result there is a decrease in the selectivity as the solvent polarity increases from THF to methanol.

Table 5.19

Equilibrium selectivity for 2,4- dihydroxy benzophenone from various solvents

For MIP, All24DHBC-MIP-BU

Sr. No.	Solute	Selectivity, α			
		THF	Ethanol	Methanol	1% Acetic acid in methanol
1	2- HB	1.055	0.699	0.748	0.645
2	4,4'- DHB	5.285	2.389	1.427	0.542
3	2,2'- DHB	4.741	1.08	1.102	0.461
4	BPA	0.757	1.918	1.699	0.678
5	4,4'- DMB	3.407	0.928	1.173	0.431

On the other hand, the surface imprinted polymers exhibit a very high selectivity for the template in all the solvents under study (table 5.20) and the selectivity increases with the polarity of the solvent. This increase in selectivity with the increase in the solvent polarity, results from reduction in non-specific adsorption. As the accessibility of the imprint site is very high, in case of surface imprinted polymers, even in the polar solvents a high selectivity is seen for the template.

The selectivity of the MIPs for the template increases as the structural difference between the template and the competing sorbate increases. In the series of competing sorbates used in this study, the structural difference between 2,4-DHB and the competing sorbates increases in the following order, 2- HB ~ 2,2'-DHB ~ 4,4'- DHB < BPA < 4,4'- DMB (see figure 3.1 for chemical structures of

the sorbates). The difference between 2,4- DHB and 2- HB, 2,2'- DHB and 4,4'- DHB is only the positioning of one of the hydroxyl functional groups. BPA differs from the template in not only the positioning of the functional groups but also in the bridging C atom which is attached to two methyl groups, instead of a carbonyl group. 4,4'- DMB differs from the template the most, as it lacks both the functional groups and positioning of the methyl groups is also different. This trend in selectivity can be seen in the case of surface imprinted polymers when THF was used as the solvent. In other solvents, the high polarity of the solvent interferes with the solute-sorbate and solute-solvent interactions and hence no distinct trend emerges. In the case of bulk imprinted polymers, the amounts sorbed are so small that no discernible trend emerges.

Table 5.20

Equilibrium selectivity for 2,4- dihydroxy benzophenone from various solvents for MIP, GE90-All24DHB-M

Sr. No.	Solute	Selectivity, α			
		THF	Ethanol	Methanol	1% acetic acid in methanol
1	2- HB	2.454	2.162	1.556	2.792
2	4,4'- DHB	2.396	4.303	1.687	12.383
3	2,2'- DHB	2.643	3.566	1.948	2.019
4	BPA	3.043	1.867	4.865	3.965
5	4,4'- DMB	3.197	1.818	8.159	1.034

The rebinding interactions in the case of MIPs are hydrogen bonding and hence any factor which affects it can affect the selectivity as well as imprinting efficacy. The intrinsic capacity of the solvent molecule to form hydrogen bond, is reflected in the hydrogen bond donator factor of the solvent ($\Sigma\alpha^{H}_{2}$). As $\Sigma\alpha^{H}_{2}$ of the solvent increases, the solvent becomes a stronger hydrogen bond donor and can form hydrogen bonds with the solute and sorbate. This can lead to change in selectivity behaviour of MIPs.

When $\Sigma\alpha^{H}_{2}$ of the solvent was plotted against imprinting efficacy β, two distinct trends were observed. The surface imprinted polymer showed an increase in the imprinting efficacy with increase in the hydrogen bond donator factor, (figure 5.17) whereas the bulk imprinted polymer did not show any trend (figure 5.18).

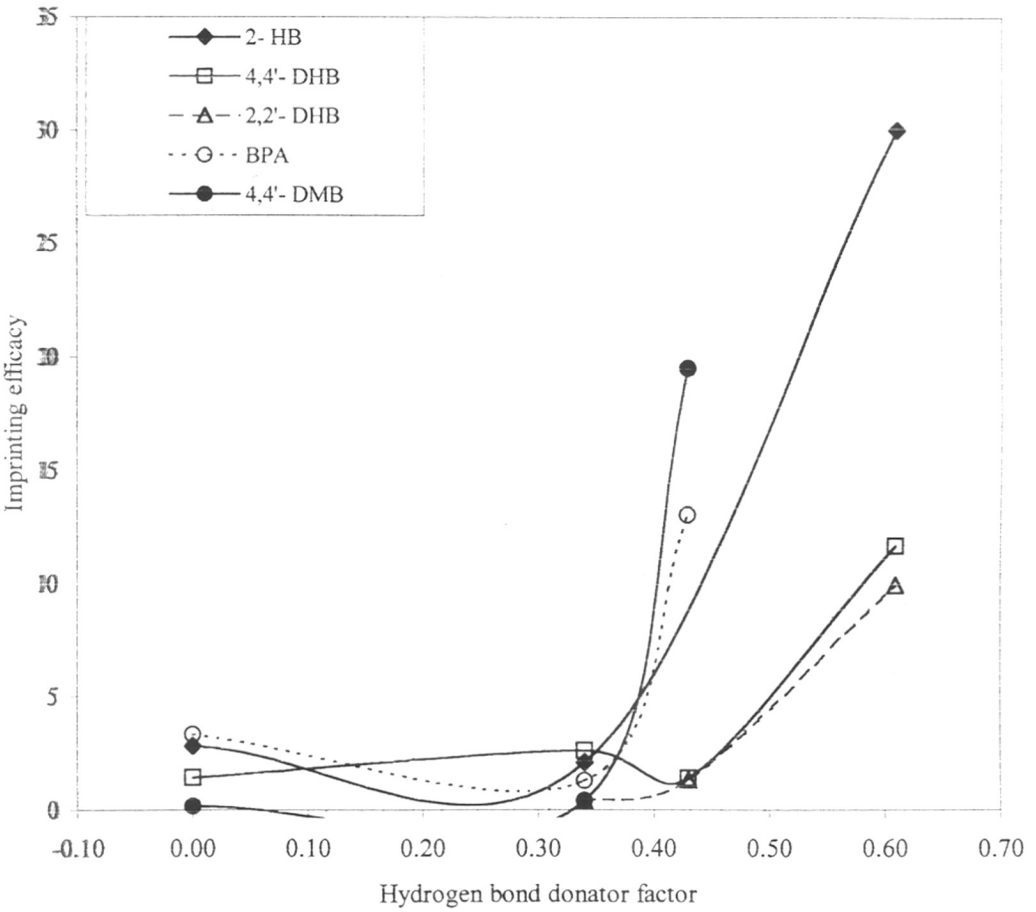


Figure5.17: Effect of hydrogen bond donator factor on imprinting efficacy for MIP GE90-All24DHB-M: Equilibrium conditions

The surface imprinted polymers show an increase in β as a function of hydrogen bond donator factor, even though it does not contain hydrogen bond acceptor functionality needed for competing interactions between sorbent and the solvent. As the accessibility of the imprint sites is good in surface imprinted polymers, a decrease in the non-specific adsorption leads to increase in the selectivity and a corresponding increase in the imprinting efficacy. This increase in the imprinting efficacy is opposite to that reported by Allender et. al., (1997). This could be because, in the HPLC separations reported by them equilibrium was not reached. We conducted similar selectivity experiments on two different systems viz., separation of phenol and bisphenol-A (figure 5.14) and separation of hydroxy benzophenone isomers (figures 5.20 and 5.21) under equilibrium and non-equilibrium conditions (figure 5.22). In both these cases, the same trend in imprinting efficacy as reported here were observed for equilibrium experiments, whereas under non-equilibrium conditions a trend similar to that reported by Allender et. al. (1997) was observed.

The reason why imprinting efficacy does not depend on hydrogen bond donator factor in the case of bulk imprinted polymers is the lack of hydrogen bond acceptor in the functional monomer viz. allyl alcohol.

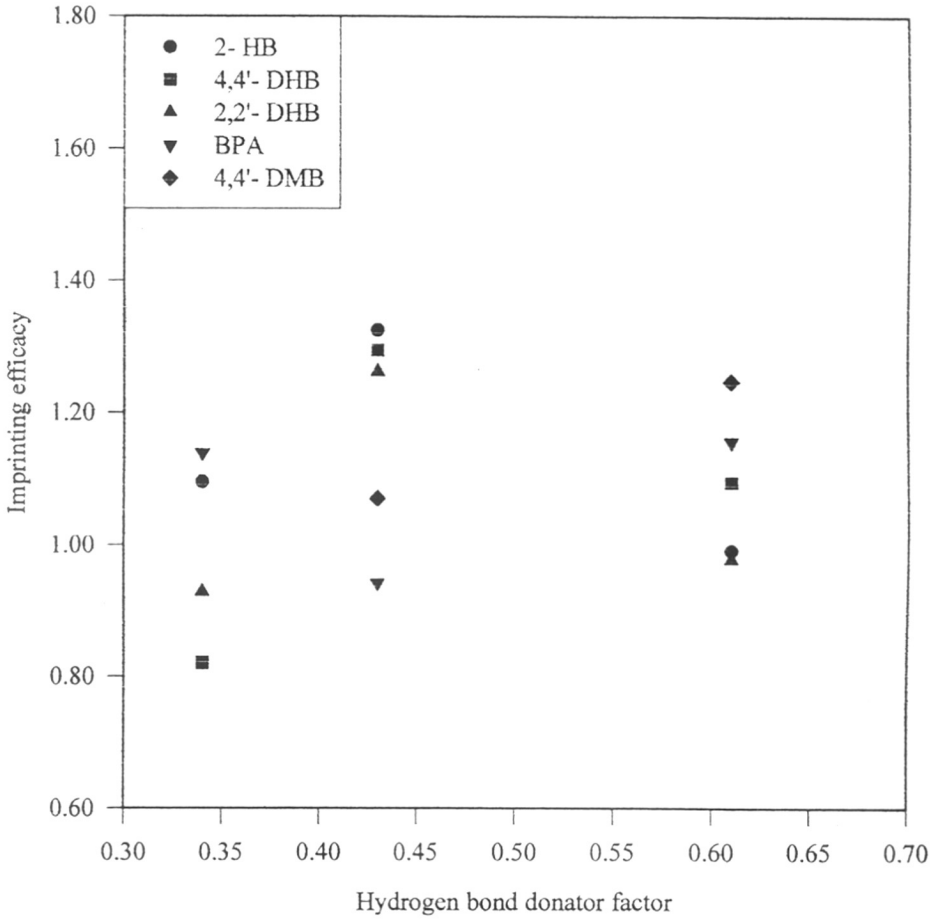


Figure 5.18: Effect of hydrogen bond donator factor of the solvent on imprinting efficacy for MIP All24DHBC-MIP-BU: Equilibrium conditions

The functional group involved in rebinding interactions when the polymer is imprinted with allyl alcohol, is alcoholic -OH group, which itself is a strong

hydrogen bond donor. The solvents also being strong hydrogen bond donors, the competing hydrogen bonding between the polymer and the solvent is weakened and hence the solvent hydrogen bond donator factor does not affect the imprinting efficacy and hence no trend could be seen. When similar experiments were done using methacrylic acid as the imprinting monomer (Joshi et. al, 1998b), even in the case of bulk imprinted polymers an increase in the imprinting efficacy with the increase in the hydrogen bond donator factor was observed. This was because, the functional monomer (methacrylic acid) contains hydrogen bond acceptor functionality (-C=O), which can interact with the solvent.

5.5.1.1.3 Validating imprinting effect

To prove that the selectivity for template results from the imprinting effect and the rebinding interactions, experiments were carried out on MIPs wherein the imprint cavity was blocked with the template molecule.

Table 5.21

Equilibrium selectivity for sorption of 2,4-dihydroxy benzophenone from THF on MIP All24DHBC-MIP-BU (Blocked imprint site)

Sr. No.	Solute	Imprinted polymer (Free cavity)	Imprinted polymer (Blocked cavity)
		α	α
1	2- HB	1.055	0.511
2	4,4'- DHB	5.285	1.992
3	2,2'- DHB	4.741	2.638
4	BPA	0.757	1.125
5	4,4'- DMB	3.407	0.788

The values for α for the bulk and surface imprinted polymers are listed in tables 5.21 and 5.22 respectively. The selectivity reduced drastically when the imprint sites were blocked. This clearly demonstrates that the selectivity in MIPs is a result of the imprinting effect and rebinding interactions. These experiments

were done in solvents in which the imprinted polymer gave highest selectivity for the template as compared to the competing sorbates. The decrease in the selectivity as a result of the blockade of the cavity is lowest in the case of 2- HB, the substrate which is structurally closest to the template (see figure 3.1). For rest of the sorbates there is a substantial decrease in the selectivity. Again the decrease in selectivity is more significant in the case of surface imprinted polymers than bulk imprinted polymers. This might be due to additional limitations in accessibility of the imprint sites in bulk polymers, resulting from the blockade of the cavity.

Table 5.22

Equilibrium selectivity for sorption of 2,4-dihydroxy benzophenone from 1% acetic acid in methanol on MIP, GE90-All24DHB-M (Blocked imprint site)

Sr. No.	Solute	Imprinted polymer (Free cavity)	Imprinted polymer (Blocked cavity)
		α	α
1	2- HB	2.792	1.468
2	4,4'- DHB	12.383	0.394
3	2,2'- DHB	2.019	0.418
4	BPA	3.965	1.037
5	4,4'- DMB	1.034	1.073

5.5.1.2 Selectivity under non-equilibrium conditions

Many a times, in industrial scale adsorptive separations, equilibrium between the sorbate and the adsorbent may not be attained. In this regard surface imprinted polymers are expected to attain equilibrium in a much shorter time than bulk imprinted polymers. To verify this assumption, the selectivity and sorption capacity of surface imprinted polymers were studied under non-equilibrium conditions.

5.5.1.2.1 Sorption capacity

Data for the sorption capacity of the surface imprinted adsorbent under non-equilibrium condition is summarised in table 5.23 It is clear from the lower sorption capacity values, that the equilibrium is not achieved even though the accessibility of the imprint site is very high. As the equilibrium is not attained even in the surface imprinted polymers, it is highly unlikely that it would be achieved in bulk imprinted polymers and hence experiments with bulk imprinted polymers under non-equilibrium conditions were not carried out. As the solvent polarity increases from THF to methanol, the sorption capacity decreases. This trend is similar to that observed under equilibrium conditions. It was observed that as the solvent polarity was increased, though the sorption capacity decreased, the difference between equilibrium and non-equilibrium sorption also decreased. This may be due to competing sorption by high polarity solvent.

Table 5.23

Non-equilibrium sorption capacity for adsorption of 2,4- DHB on MIP, GE90-All24DHB-M

Sr. No.	Solvent	Amount adsorbed, mg / g
1	THF	29.60
2	Ethanol	21.13
3	Methanol	15.30
4	1% acetic acid in methanol	22.04

As the imprinted polymers show higher sorption capacity coupled with a high selectivity in THF, a non-polar solvent, it should be the solvent of choice for this separation. The only limitation of THF seems that the equilibrium is not readily attained. In order to enhance the kinetics of the process, highly porous polymers used in perfusion chromatography (e.g. POROS^{TM}) can be used as support polymers for the preparation of MIPs.

5.5.1.2.2 Selectivity under non-equilibrium conditions

Selectivities of the surface imprinted polymers for the template, 2,4- DHB under non-equilibrium conditions are listed in table 5.24. The selectivity values are lower than the values obtained under equilibrium conditions. This is because, under the experimental conditions equilibrium is not reached. Under the non-equilibrium conditions, the selectivity is highest in the non-polar solvent THF. As the solvent polarity is increased, the solvent competes for the imprint site and this leads to reduction in sorption and hence in selectivity. This behaviour is exactly opposite to that shown by the imprinted polymers under equilibrium conditions. When hydrogen bond donator factor of the solvent was plotted against the imprinting efficacy β, a decrease in β with the increase in hydrogen bond donator factor of the solvent was observed.(see figure 5.19). This trend is consistent with the literature reports (Allender et. al., 1997) as well as our own results (Joshi et. al., 1998b and 1998c). This decrease in β results, since the sorbent and the solvent compete for the binding site and also because equilibrium is not attained.

Table 5.24

Selectivity for sorption of 2,4- DHB from various solvents for MIP GE90-All24DHB-M (non-equilibrium conditions)

Sr. No.	Solute	Selectivity, α			
		THF	Ethanol	Methanol	1% acetic acid in methanol
1	2- HB	1.341	0.99	1.306	1.245
2	4,4'- DHB	1.885	0.812	0.856	1.006
3	2,2'- DHB	2.089	1.172	0.731	1.394
4	BPA	1.694	2.076	1.041	1.66
5	4,4'- DMB	1.689	1.259	0.907	1.252

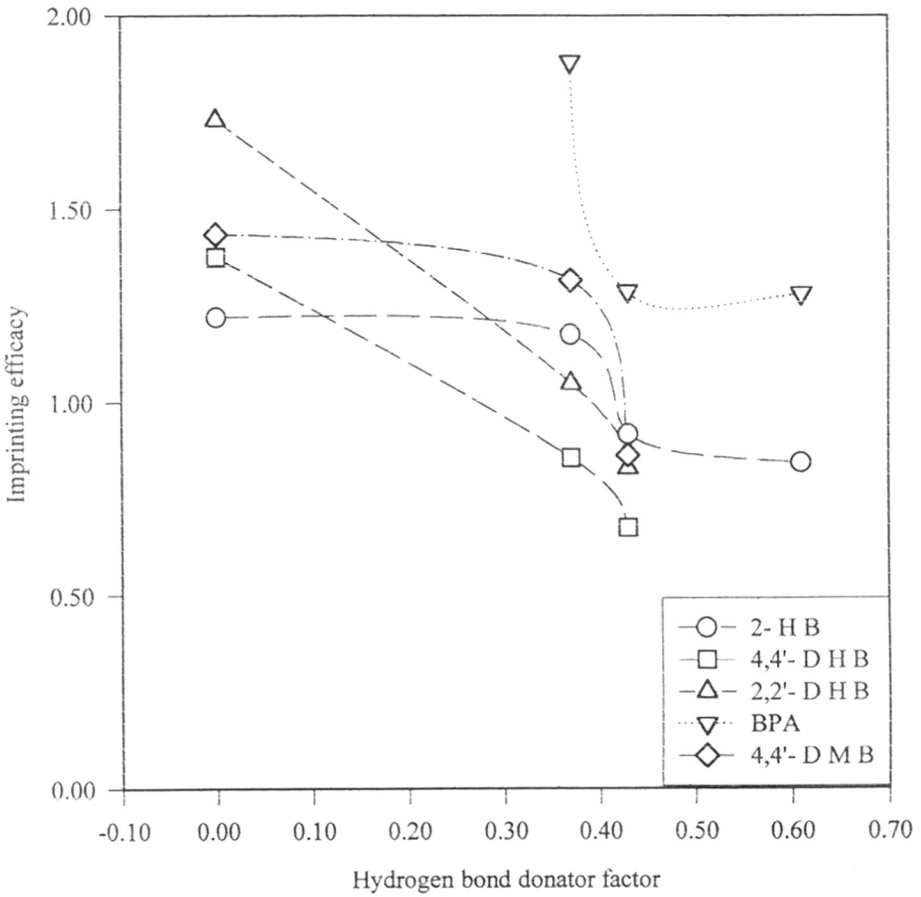


Figure 5.19: Effect of hydrogen bond donator factor on imprinting efficacy for MIP GE90-All24DHB-M: Non-equilibrium conditions

5.5.2 2,4- DHB methacrylate based system

Imprinted polymers templated for 2,4- DHB were also prepared using methacrylic acid as the functional monomer. It was seen in the preceding section that allyl alcohol based MIPs showed higher selectivity and sorption capacity when prepared by surface imprinting. But when bulk imprinting was used, these polymers showed lower sorption capacity in spite of their high template loading. For use of MIPs on a large scale, it is necessary to have materials which show high

sorption capacity coupled with the selectivity. We therefore prepared imprinted polymers using surface and bulk imprinting techniques and MAA as the functional monomer. Bulk imprinted polymers can always be prepared with a higher template loading than the surface imprinted polymers, but due to accessibility problems allyl alcohol based MIPs showed lower sorption in spite of higher template loading. Since the main cause of poor porosity of bulk imprinted polymers was presence of the template monomer (Allyl, 2,4- DHB carbonate), we decided to use another template monomer based on MAA, viz., 2, 4- DHB methacrylate. The monomer being different in terms of rebinding interaction than allyl alcohol, we expected that it might show a different behaviour in terms of selectivity and sorption capacity. Selectivity and sorption capacity studies similar to allyl alcohol based system were carried out. All the competing sorbates used for allyl alcohol based system were used in this study.

5.5.2.1 Equilibrium sorption capacity

Equilibrium sorption capacity measurements on MIPs give an idea about the efficacy of utilisation of the theoretical capacity of the adsorbent. Table 5.25 lists the sorption capacities for both the surface as well as bulk imprinted polymers. The utilisation of the sorption capacity in the case of bulk imprinted polymers is very poor (1%-5%), as can be seen from the low sorption capacities and the solvent was seen to have no effect on the sorption capacity. On the other hand there was a strong dependence of solvent on sorption capacity in the case of surface imprinted polymers, but no clear trend emerged. The polymer showed low sorption capacity (20%-30% of the theoretical capacity) in THF and methanol. In ethanol the capacity utilisation was almost 100%, whereas in acetic acid in methanol, there was an anomalously high adsorption, overshooting the theoretical sorption capacity. The reason for this erratic behaviour is not known.

The low sorption capacity of bulk imprinted polymers in spite of high template loading can be attributed to non-accessibility of the imprint sites and since the sorption capacities were low, the effect of solvent could not be discerned. The surface area and pore volume data (table 3.5), as well as surface morphology

as seen by SEM (figure 3.4c), shows lower porosity for bulk imprinted polymers. Especially in the case of bulk imprinted polymers the pores were found to be in microporous region (pore radius < 20 A^0) from pore size distribution data, which imposes restrictions on the accessibility of the imprint sites, crucial for a bulky substrate such as 2,4- DHB.

Table 5.25

Equilibrium sorption capacity for adsorption of 2,4- DHB from various solvents on MIPs based on methacrylic acid

Sr. No.	Solvent	Adsorption capacity, mg / g	
		24DHBMA-MIP-BU	GE90-24DHBMA-M
	Template loading	140-150	40-50
1	THF	0.64	12.9
2	Ethanol	4.98	39.61
3	Methanol	5.38	13.38
4	1% acetic acid in methanol	4.24	101.96

Surface imprinted polymers on the other hand showed higher sorption capacity and better utilisation of the theoretical sorption capacity (20%- 200%) than bulk imprinted polymers. But unlike in case of allyl alcohol, (Joshi et. al., 1998d) no trend emerged with regards to solvent effect and hence it would be difficult to predict the sorption behaviour with the changes in solvent. The sorption capacity of surface imprinted polymers was still higher than the bulk imprinted polymers as a result of high accessibility of the imprint site that is crucial for the bulky substrate such as 2,4- DHB. Pore size distribution data for surface imprinted polymers also shows that the pores in surface imprinted polymers are in the mesoporous region (pore radius, 20 - 200 A^0) thus increasing accessibility of the sites.

It is pertinent to note here that the polymerisation conditions and recipes for the synthesis of bulk imprinted polymers and the support used in the synthesis of

surface imprinted polymer were similar except for the presence of template monomer in the former and are known to give porous matrices. This was responsible for the low pore volume as well as pore size which limits the accessibility of the imprint site. As a result of this, the sorption capacity in the case of bulk imprinted polymers was low and hence for separation of isomers, where the sorbate molecule is bulky and has multiple rebinding interactions surface imprinting should be the preferred method of synthesis, even though the synthesis involves a two step process. Other way to improve porosity of bulk imprinted polymers would be proper choice of porogen as we saw that even after changing the template monomer, the porosity of these polymers was low. Thus by proper choice of the porogen it would be possible to get highly porous matrices as that of surface imprinted polymers, which would also have high template loading thus giving higher sorption capacity.

5.5.2.2 Equilibrium selectivity

Tables 5.26 and 5.27 summarise the selectivity data for the bulk as well as the surface imprinted polymers. In both the cases the selectivity is high in a high polarity solvent such as, methanol for bulk imprinted polymers and acetic acid in methanol for surface imprinted polymers. The selectivity for the template in other solvents is poor in the case of bulk imprinted polymers but same was found to be true for the surface imprinted polymers contrary to our earlier observations (table 5.20, Joshi et. al., 1998d). This deviation from the expected may be due to the differences in rebinding interactions with the functional monomer which were different in both the cases.

The low selectivity of bulk imprinted polymers in the non-polar solvents can be attributed to limitations on accessibility of the imprint site, whereas in the surface imprinted polymers, even though the accessibility is high it does not reflect in the selectivity, the reason for which is not known.

Table 5.26

Equilibrium selectivity for adsorption of 2,4 DHB from various solvents on MIP based on 2,4-DHB methacrylate (24DHBMA-MIP-BU)

Sr. No.	Solute	Selectivity, α			
		THF	Ethanol	Methanol	1% Acetic acid in methanol
1	2- HB	0.384	0.72	1.313	0.98
2	4,4'- DHB	1.221	1.674	6.647	1.974
3	2,2'- DHB	1.809	1.191	5.098	1.521
4	BPA	0.289	2.76	4.213	1.176
5	4,4'- DMB	0.293	0.906	1.403	0.914

Table 5.27

Equilibrium selectivity for adsorption of 2,4 DHB from various solvents on MIP based on 2,4-DHB methacrylate (GE90-24DHBMA-M)

Sr. No.	Solute	Selectivity, α			
		THF	Ethanol	Methanol	1% acetic acid in methanol
1	2- HB	0.592	1.109	0.715	1.722
2	4,4'- DHB	1.093	---	1.155	8.335
3	2,2'- DHB	0.792	3.889	1.656	7.009
4	BPA	0.343	1.198	---	5.106
5	4,4'- DMB	0.41	1.52	0.307	4.644

The selectivity of the MIPs for the template increases as the structural difference between the template and the competing sorbate increases. In series of competing sorbates used in this study, the structural difference between 2,4- DHB and the competing sorbates increases in the following order, 2- HB ~ 2,2'- DHB ~ 4,4'- DHB < BPA < 4,4'- DMB. The difference between 2,4- DHB and 2- HB, 2,2'- DHB and 4,4'- DHB is only the positioning of one of the hydroxyl functional

groups. BPA differs from the template in not only the positioning of the functional groups but also in the bridging C atom which is attached to two methyl groups, instead of a carbonyl functionality. 4,4'- DMB differs from the template the most, as it lacks both the functional groups and positioning of the methyl groups is also different. Normally this increasing trend in selectivity would be seen in a non-polar (THF) solvent, which does not interfere with the solute-sorbent interactions This trend in selectivity could not be seen in both the surface and bulk imprinted polymers. The reason in the case of bulk imprinted polymers would be low accessibility of the imprint site coupled with low sorption capacity. In the case of surface imprinted polymers, inherently the selectivity in THF was very low because of which this trend did not emerge. The high polarity solvents like methanol or acetic acid in methanol interfere with the solute-sorbent interactions and hence the trend is not seen in those solvents.

The rebinding interaction in the case of MIPs is hydrogen bonding and hence any factor which affects it, can affect the selectivity as well as the imprinting efficacy. The intrinsic capacity of the solvent molecule to form hydrogen bond is reflected in the hydrogen bond donator factor of the solvent. As $\Sigma\alpha^{H}_{2}$ of the solvent increases, the solvent becomes a stronger hydrogen bond donor and can form hydrogen bonds with the solute and sorbate. This can lead to change in the selectivity behaviour of MIPs.

In both the cases studied i.e. bulk and surface imprinting, a clear trend emerges in terms of selectivity as well as imprinting efficacy. Tables 5.26 and 5.27 show that there is an increase in the selectivity with the increase in the solvent polarity. Also when the hydrogen bond donator factor, $\Sigma\alpha^{H}_{2}$ of the solvent was plotted against the imprinting efficacy β, an increase in imprinting efficacy could be seen in bulk (figure 5.20) as well as surface imprinted polymers (figure 5.21).

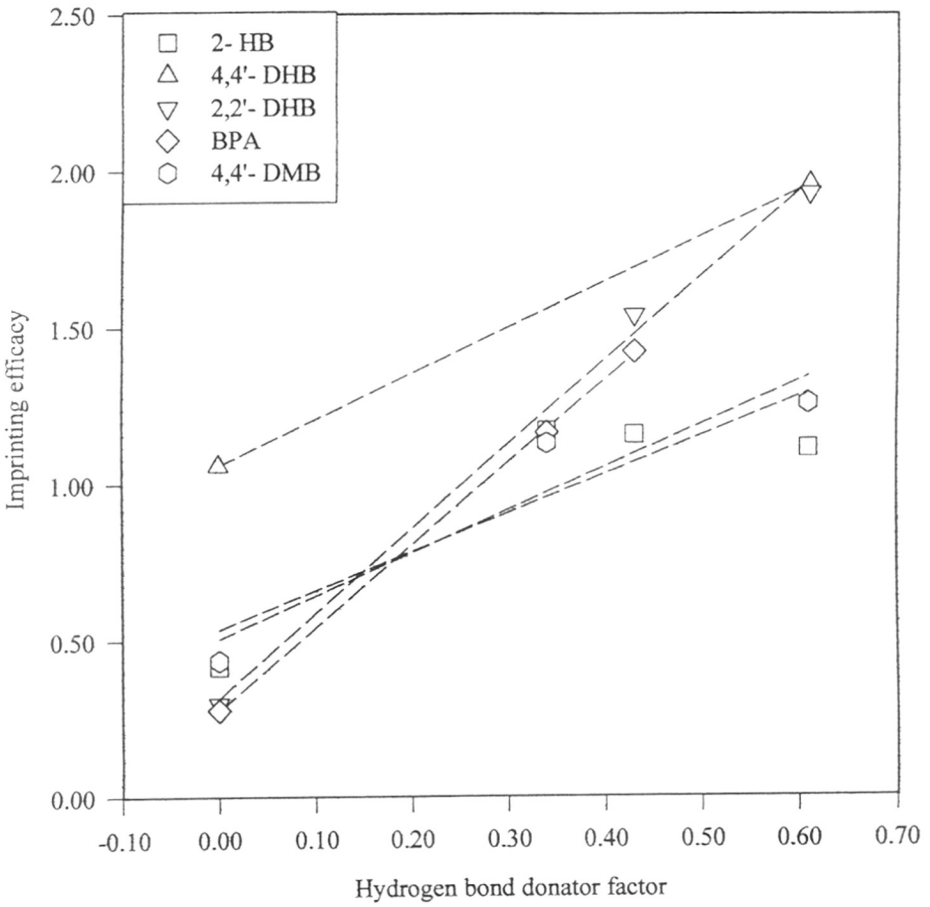


Figure 5.20: Effect of hydrogen bond donator factor on imprinting efficacy under equilibrium conditions (24DHBMA-MIP-BU)

The uniform increase in the imprinting efficacy can be explained in the following way. As the hydrogen bond donator factor of the solvent increases, the donor potential of the solvent increases. This leads to the competing hydrogen bonding between the solute and solvent for the imprint sites. In this case especially, as the functional monomer (methacrylic acid) contains hydrogen bond acceptor functionality (-C=O), this interaction would be stronger. But the rebinding interaction between the template and the functional monomer is even stronger compared with the interaction between the solvent and the polymer.

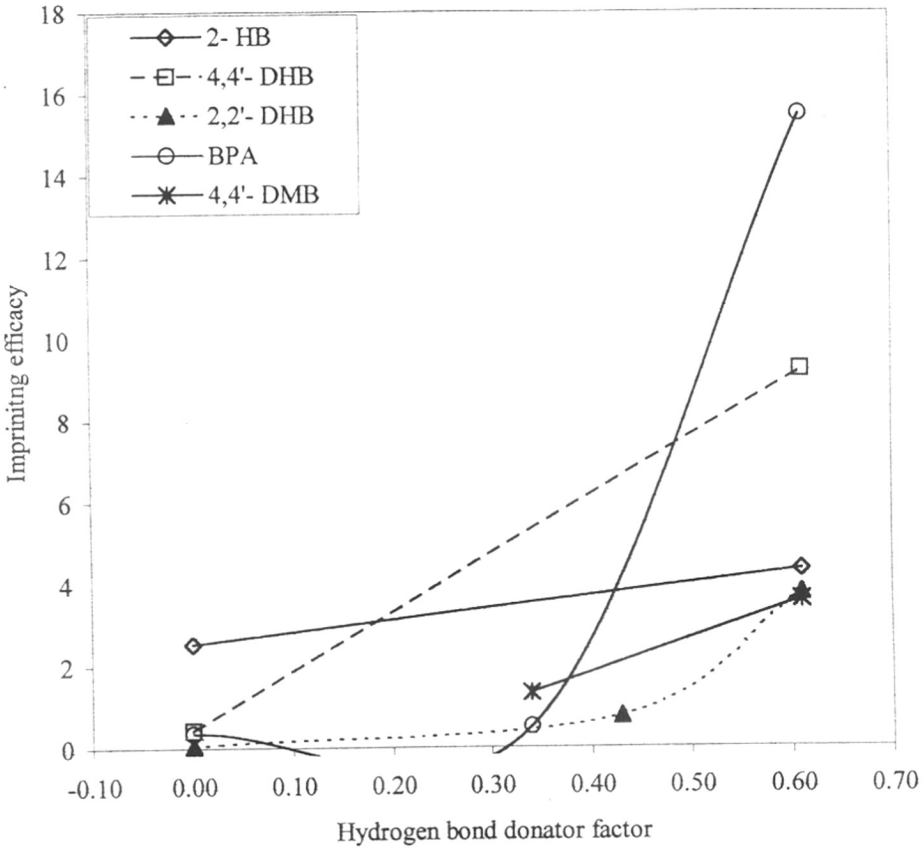


Figure 5.21: Effect of hydrogen bond donator factor on imprinting efficacy under equilibrium conditions (GE90-24DHBMA-M)

Hence under equilibrium conditions the template would be preferentially sorbed at the imprint site, whereas the non-specific adsorption sites would be filled with the solvent. With the increasing hydrogen bond donator factor of the solvent, the non-specific adsorption would go down leading to an increase in the imprinting efficacy, which is expected and could be seen in both the systems under study.

This increase in imprinting efficacy as a function of the hydrogen bond donator factor is exactly opposite to the trend reported by Allender et. al., (1997). This could be because, in the HPLC separations reported by them, true equilibrium

may not have been attained. We conducted similar selectivity experiments on two different systems viz., separation of phenol and bisphenol-A (Joshi et. al., 1998c) and separation of hydroxy benzophenone isomers, (Joshi et. al., 1998d) under equilibrium and non-equilibrium conditions. In both these cases, the same trend in imprinting efficacy as reported here was observed for equilibrium experiments, whereas under non-equilibrium conditions a trend similar to that reported by Allender et. al., (1997) was observed.

5.5.2.3 Validating imprinting effect

To prove that the selectivity for template results from the imprinting effect and the rebinding interactions, experiments were carried out on MIPs, wherein the imprint cavity was blocked with the template molecule.

Table 5.28

Equilibrium selectivity for adsorption of 2,4- DHB from methanol on MIP, 24DHBMA-MIP-BU (Blocked imprint cavity)

Sr. No.	Solute	Imprinted polymer (Free cavity)	Imprinted polymer (Blocked cavity)
		α	α
1	2- HB	1.313	0.614
2	4,4'- DHB	6.647	1.774
3	2,2'- DHB	5.098	0.994
4	BPA	4.213	1.026
5	4,4'- DMB	1.403	0.665

For the bulk and surface imprinted polymers, α values are summarised in tables 5.28 and 5.29 respectively. The selectivity is drastically reduced when the active sites are blocked. This clearly demonstrates MIPs show selectivity as a result of the imprinting effect and rebinding interactions. These experiments were done in solvents in which the imprinted polymer gave highest selectivity for the template as compared to the competing sorbates. The decrease in the selectivity as

a result of the blockade of the cavity was lowest in the case of 2- HB, the substrate which is structurally closest to the template (see figure 3.1). For the rest of the sorbates there is a substantial decrease in the selectivity. Again the decrease in selectivity is more significant in the case of surface imprinted polymers than bulk imprinted polymers, due to additional limitations in accessibility of the imprint sites in bulk polymers, resulting from the blockade of the cavity.

Table 5.29

Equilibrium selectivity for adsorption of 2,4- DHB from 1% acetic acid in methanol on MIP, GE90-24DHBMA-M (Blocked imprint cavity)

Sr. No.	Solute	Imprinted polymer (Free cavity)	Imprinted polymer (Blocked cavity)
		α	α
1	2- HB	1.722	0.118
2	4,4'- DHB	8.335	0.613
3	2,2'- DHB	7.009	0.869
4	BPA	5.106	0.339
5	4,4'- DMB	4.644	0.2

5.5.2.4 Selectivity under non-equilibrium conditions

Many a times in industrial scale adsorptive separations equilibrium between the sorbate and the adsorbent may not be attained. In this regard surface imprinted polymers are expected to attain equilibrium in a much shorter time than bulk imprinted polymers. To verify this assumption, the selectivity and sorption capacity of surface imprinted polymers under non-equilibrium conditions were studied.

5.5.2.4.1 Sorption capacity

Sorption data for the adsorbent under non-equilibrium conditions is summarised in table 5.30. It is clear from the lower sorption capacity values, that the equilibrium is not achieved even though the accessibility of the imprint site is

very high. As the equilibrium was not attained even in the surface imprinted polymers, it was highly unlikely that it would be achieved in bulk imprinted polymers and hence experiments with bulk imprinted polymers were not carried out under non-equilibrium conditions. Similar to the results under equilibrium conditions, no trend emerges as far as the relationship between the solvent polarity and sorption capacity is concerned. There is a significant reduction in sorption capacity, when acetic acid in methanol was used as the solvent, whereas in all the other solvents the sorption capacity more or less remains the same under both conditions.

Table 5.30

Sorption capacity for 2,4- DHB on MIP, GE90-24DHBMA-M

(non-equilibrium conditions)

Sr. No.	Solvent	Amount adsorbed, mg / g
1	THF	22.10
2	Ethanol	31.24
3	Methanol	13.71
4	1% acetic acid in methanol	21.76

As the imprinted polymer shows higher sorption capacity coupled with a high selectivity in ethanol, it should be the solvent of choice for this separation. The limitation of ethanol is that the equilibrium kinetics appears to be slow. In order to enhance the kinetics of the process, highly porous support polymers used in perfusion chromatography can be used as support polymers for the preparation of MIPs.

5.5.2.4.2 Selectivity under non-equilibrium conditions

Selectivities of the surface imprinted polymers, for the template, 2,4- DHB under non-equilibrium conditions are listed in table 5.31. The selectivity values are lower as compared to the values obtained under equilibrium conditions. This was because under the experimental conditions equilibrium was not reached. Under

the non-equilibrium conditions, the selectivity is highest when ethanol was used as the solvent. As the solvent polarity is increased, the solvent competes for the imprint site and this leads to reduction in sorption and hence in selectivity. This behaviour is exactly opposite to that shown by the imprinted polymers under equilibrium conditions.

Table 5.31

Selectivity for adsorption of 2,4- DHB from various solvents on MIP, GE90-24DHBMA-M (non-equilibrium conditions)

Sr. No.	Solute	Selectivity, α			
		THF	Ethanol	Methanol	1% acetic acid in methanol
1	2- HB	0.993	1.377	0.604	1.121
2	4,4'- DHB	1.092	1.266	0.87	0.969
3	2,2'- DHB	1.128	1.609	0.756	1.06
4	BPA	1.526	2.116	0.635	1.231
5	4,4'- DMB	1.102	2.108	0.513	1.137

When hydrogen bond donator factor of the solvent was plotted against the imprinting efficacy β, it shows a decrease with the increase in hydrogen bond donator factor of the solvent (figure 5.22). This trend is consistent with the literature reports (Allender et. al., 1997) as well as our own results (Joshi et. al., 1998c and 1998d). There is a decrease in β, since the sorbent and the solvent compete for the binding site and also because equilibrium between solute and sorbent was not attained.

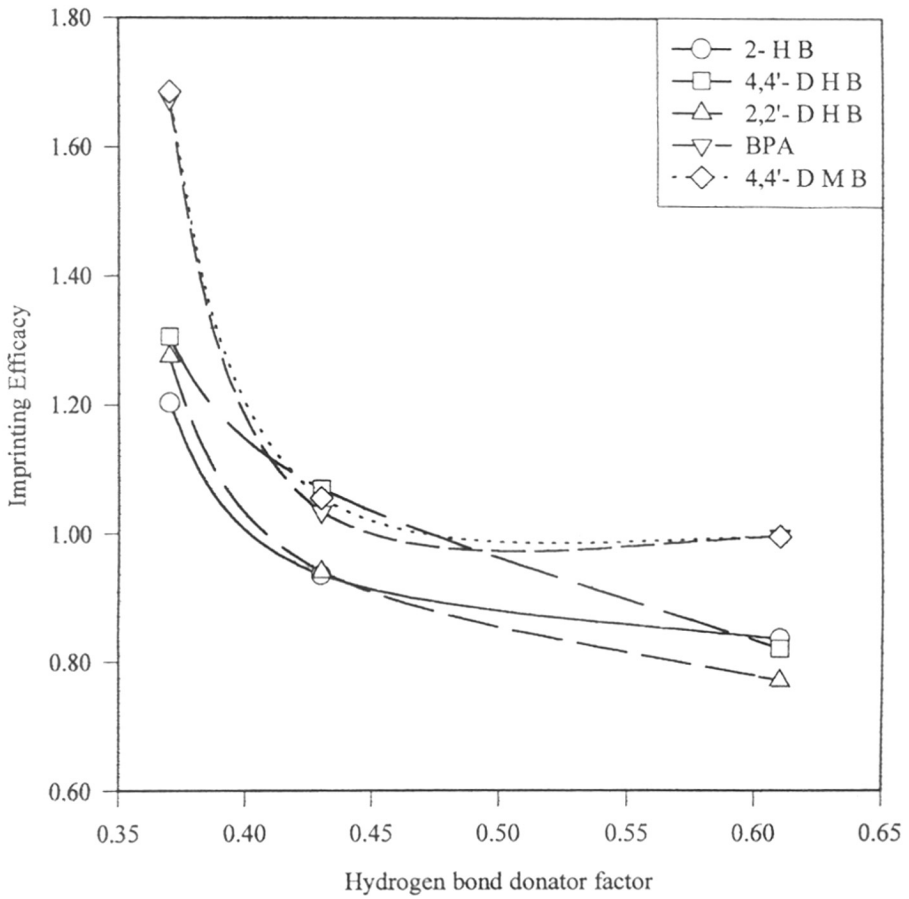


Figure 5.22: Effect of hydrogen bond donator factor on imprinting efficacy for MIP (GE90- 24DHBMA-M), Non-equilibrium conditions

Chapter 6

Conclusions and Directions for future work

6.1 Conclusions

6.1.1 Introduction

Molecularly imprinted polymers have been hitherto used in racemic resolution, sensors, as selective catalysts and as artificial antibodies in bio-analytical assays. Their application potential in the following two areas is untapped.

a) Removal of structurally and functionally similar trace impurities from bulk chemicals.

b) Separation of positional isomers, which are many a times formed in organic reactions involving aromatic nuclei.

This work was aimed at tapping the untapped potential of MIPs for these two applications. To achieve this aim, we synthesised molecularly imprinted polymers for separation of, a) phenol from anisole, b) phenol from bisphenol-A and c) 2,4- dihydroxy benzophenone isomers. The various results obtained can be summarised as follow.

6.1.2 Separation of phenol from anisole

- Use of molecularly imprinted polymeric adsorbents is particularly suitable for the removal of trace impurities from organic streams. MIPs can find an application in this niche area, since adsorbent resins that are commercially available lack a mechanism for separation of structurally similar sorbates.
- The polymers showed high selectivity for the template in competitive sorption experiments, under equilibrium as well as non-equilibrium conditions thereby allowing their use on commercial scale adsorptive separations.
- From the competitive sorption experiments, it could be unequivocally proved that the selectivity for the template in case of MIPs is a result of presence of the imprint cavity and the rebinding interactions.

- Cross link density of the support polymer was shown to influence the selectivity and higher selectivity could be obtained just by increasing the crosslink density of the polymer. This increase could be mainly attributed to increased surface area of polymers with increase in crosslink density and increased rigidity of the cavity.
- Predictive mathematical models were developed using empirical and dual sorption adsorption isotherms for packed bed adsorber. Use of dual sorption theory, which takes into consideration two component sorption behaviour helped to achieve better fit between the experimental data and the model prediction. The model being truly predictive in nature, would be useful in predicting breakthrough behaviour of a large scale adsorber for phenol sorption from laboratory data.
- Finally it was observed that the resins synthesised in this separation showed extensive swelling in anisole leading to distortion of the imprint site and therefore reduction in the selectivity for the template molecule. To overcome this difficulty, the chemical nature of the support polymer needs to be changed or a support with a very high crosslink density could be synthesised using a multifunctional crosslinker such as trimethylol propane trimethacrylate.

6.1.3 Separation of phenol from bisphenol-A

- The choice of a solvent was found to substantially alter the selectivity in separations using MIPs. Though this has been reported for racemic resolutions (Allender et. al., 1997), it was equally true, when MIPs were used as selective adsorbents for trace impurity removal.
- Not only the solvent, but also the conditions under which the selectivity experiments were done, (equilibrium vs. non-equilibrium conditions) influenced the selectivity.
- Therefore a judicious choice of support polymers, imprinting technique and system parameters help in enhancing the adsorptive separations using molecularly imprinted adsorbents.

- The right choice of imprinting technique for enhanced separation efficacy was found to depend on the template size, shape and functionality. In the case of a small template molecule such as, phenol having only a single point rebinding interaction, the right choice of imprinting technique was bulk imprinting and not surface imprinting as it led to non-specific adsorption.
- The separation efficacy was significantly influenced by the choice of the solvent. Although the enhancement in separation efficacy due to imprinting was most clearly seen when non-polar solvents were used, the dominant non-specific adsorption led to lower overall separation efficacy. While the polar solvents suppressed non-specific adsorption and enhanced separation efficacy α, the contribution due to imprinting effect was suppressed, since the solvent can also compete with the template for the sorption sites. Only in the case of moderately polar solvent both the effects could be clearly seen.

6.1.4 Separation of 2,4- dihydroxy benzophenone isomers

- Use of surface imprinted polymers, under equilibrium conditions led not only to higher selectivity, but also to higher sorption capacity as was observed in the case of imprinted polymers synthesised using allyl alcohol and methacrylic acid as the functional monomers. The capacity utilisation in the case of allyl alcohol was about 40%-95%, whereas in the case of methacrylic acid based system it was between 20%-200%.
- On the other hand, even though bulk imprinted polymers could be synthesised with a higher template loading, they invariably gave a lower selectivity as well as sorption capacity (1%-5% capacity utilisation) under equilibrium conditions. The low sorption capacity and selectivity could be attributed to their low porosity.
- Both the sorption capacity and selectivity were seen to depend strongly on the solvent used. While the sorption capacity decreased with the

increase in the solvent polarity, the selectivity (imprinting efficacy) increased with the solvent polarity.

- The reduction in the selectivity, on blocking the imprint site in the case of surface as well as bulk imprinted polymers, validated the role played by the imprint cavity and the rebinding interactions in deciding the selectivity.
- For surface imprinted polymers, under non-equilibrium conditions, the sorption capacity and selectivities were lower than those obtained under equilibrium conditions. But the selectivities were still high enough for the industrial separations. The proper choice of solvent under non-equilibrium conditions again led to better selectivity and higher sorption capacities. As equilibrium was not reached under these conditions, full utilisation of the sorption capacity was not achieved even in the case of surface imprinted polymers, which had a very high porosity. This limitation could be overcome by selecting resins used in perfusion chromatography (POROS) as support polymers for surface imprinting.

6.1.5 Summary of the entire work

- Our work gives an insight into the subtle interactions between the MIP adsorbent, sorbate (template) and the solvent and hence it will be of immense help in designing better adsorbents for a particular separation system.
- Proper choice of imprinting technique can lead to enhancement in selectivity for the template and the choice of imprinting technique depended on the size, shape and functionality of the template. Thus for a bulky template having multiple rebinding sites surface imprinting was found to be advantageous, as it improves accessibility of the site. On the other hand, use of bulk imprinting was preferred when the template was a small molecule having single rebinding site, as it reduced the non-specific adsorption.

- The choice of solvent is critical in attaining a desired separation as also the experimental conditions (equilibrium vs. non-equilibrium) under which selectivity is measured.
- The selectivity of MIPs under equilibrium conditions gives an idea as to the absolute selectivity of MIPs, wherein the diffusional and hydrodynamic limitations are nullified. On the other hand a high selectivity under non-equilibrium conditions is desirable from the point of view of application of these polymers on industrial scale.
- Thus overall this study led to a rationale for design of efficient separations using molecularly imprinted polymers.

6.2 Directions for future work

6.2.1 Introduction

During our studies on molecularly imprinted polymers as selective adsorbents, we observed that various factors play a key role in deciding the selectivity and sorption capacity of these polymers. We studied effect of some of these factors such as, solvent, imprinting technique and experimental conditions (equilibrium vs. non-equilibrium). These studies led to a better understanding of the imprinting process and could provide us with a rationale, for development of better separation systems based on MIPs. But there is still a lack of complete theoretical understanding particularly with respect to the processes that are involved in the formation of the imprint cavities and the rebinding interactions.

6.2.2 Influence of imprinting technique (surface vs. bulk)

During our study, we observed that the support polymer on which surface imprinting was done, affects the template loading and in turn can affect the sorption capacity. However, no attempt was made in this study to optimise the support characteristics for enhancing the sorption capacities. Also it is well known that using bulk imprinting technique, MIPs can be prepared with a higher template loading as compared to surface imprinted polymers and their synthesis procedure

is much simpler than surface imprinting. However, the bulk imprinted polymers used in this study showed poor sorption capacity in spite of their high template loading, as a result of poor accessibility of the imprint site. Similar to surface imprinted polymers, no attempt was made to modify synthesis procedure to give highly porous polymers, which could have avoided the accessibility problems.

Hence in order to enhance the separation efficacy and sorption capacities of imprinted polymers, optimisation of polymerisation process is necessary. Thus new classes of support polymers can be prepared using suspension, emulsion, and bicontinuous emulsion polymerisation techniques, the main aim being synthesis of a highly porous matrix having a high surface area. The monomers used for synthesis of these polymers can be changed in order to modify the hydrophobicity / hydrophilicity of these polymers, which would favourably improve the template loading. Highly porous polymeric matrices used in perfusion chromatography can also be used to prepare surface imprinted polymers, which would improve kinetics of the rebinding and help in attaining equilibrium faster. Not only polymeric supports, but also the inorganic support matrices such as silica can be used for synthesis of MIPs. Again, we have not done any work on these supports. Advantages in the use of silica are, its easy availability on commercial scale and the ease with which its porosity can be controlled, both of which make it an attractive adsorbent on large scale. The problem of swelling of the matrix would also be avoided by the use of silica.

As against modification of the support polymers, modification of bulk imprinting process to obtain porous matrices would be much easier as the variables involved are few. The main factor responsible for porosity in these polymers is the porogen used. Hence by judicious choice of porogen, a highly porous matrix with a high surface area can be obtained. Such matrices will provide easier access to the imprint site and improve the sorption capacities.

6.2.3 Commercial importance of the systems

The systems studied by us in this work were of commercial importance (phenol from anisole and phenol from bisphenol-A). Since the results obtained

were encouraging, it means that these polymers can be used on commercial scale for such separations. The transition from the laboratory scale to commercial scale would involve a lot of process optimisation and study of various parameters. This was again out of scope of this thesis work. In future, this optimisation can therefore lead to use of molecularly imprinted polymers as selective adsorbents, on commercial scale. Some work in this direction is already going on in our group and a lot needs to be done.

The use of MIPs in removal of trace impurities and separation of positional isomers, had been a neglected area in the literature and hence a large number of separation systems can be explored to develop efficient separation systems. Some representative examples of commercial importance are,

(a) ***Separation of xylene isomers:*** p-xylene is used as an intermediate in synthesis of terephthalic acid. Synthesis of xylenes by alkylation of toluene always leads to a mixture of o, m and p- xylene, which needs to be separated to purify p-xylene.

(b) ***Separation of H-acid isomers:*** H acid is an important intermediate in the synthesis of azo dyes which is sold as the mixture of various isomers. One of the isomers present in this mixture needs to be isolated for value addition. Use of MIPs for this separation can be a worthwhile effort.

(c) ***Removal of terephthalaldehyde from terephthalic acid:*** This separation is critical for purity of terephthalic acid as the aldehyde impurity can act as chain terminator leading to lower molecular weight of the polyesters

(d) ***Removal of polymerisation inhibitors from monomers:*** Presence of these inhibitors affects the polymerisation kinetics. For instance, presence of p-tertiary butyl catechol in styrene affects the kinetics of styrene polymerisation.

6.2.4 Elucidation of rebinding mechanism

The nature of rebinding interactions in MIPs has been studied in literature. It was shown by Shea and Sasaki (1991), by IR and NMR spectroscopy that the rebinding interactions are hydrogen bonding when the functional group involved is an amide. But in our case the functional groups involved in rebinding being

hydroxyl and carboxyl, it was extremely difficult to experimentally validate the nature of rebinding interactions. A study on a technique which can give insights into these interactions such as, Raman spectroscopy would be desirable for better understanding of the process.

6.2.5 Elucidation of imprinting interactions

Molecular imprinting relies on the implicit assumption that shape and size selective cavities are created during imprinting process. But there is no unequivocal proof on existence of these. The selectivity obtained for the template gives an indirect idea as to the existence of the cavities, as also the experiments done with the polymers in which the imprint cavity is blocked.

An experimental technique, which can give an idea about the existence of the imprint cavities is Xenon- NMR, wherein Xenon gas is adsorbed on the polymeric adsorbent. The chemical shifts obtained for Xenon are strongly dependent on the electronic environment of the Xenon atoms. Assuming the presence of cavities there would be two different kinds of Xenon atoms, the ones that are adsorbed inside the cavity and hence are in a different electronic environment and the others which are occupying any other non-specific adsorption sites. The chemical shifts in both these cases might be different. Thus these studies might be able to shed some light as to the nature of imprint cavities.

Though there may not be many experimental tools to verify the existence of the imprint cavities, theoretical tools such as molecular modelling can be of immense importance. Assuming the existence of the cavities, it can be easily conjectured that the cavities are tailor made for the template molecule and also have appropriately positioned functional groups useful in rebinding. Under such conditions, it can be assumed that the template would preferably occupy the imprint cavities. Hence, there is expected to be a drop in the free energy of the system (sorbate + sorbent). Such energy minimisation studies need to be done using molecular modelling techniques for molecularly imprinted polymers. These studies can be of immense importance in understanding of the process and will also help in better designing of the imprinted polymers. These studies can be used

for making proper choice of functional monomer for a particular separation, as it can give an idea about the changes in the strength of rebinding interactions by changing the functional monomer.

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List of Publications

1. **Joshi V. P.**, Karode, S. K., Kulkarni, M. G. and Mashelkar, R. A., 1998. Novel separation strategies based on molecularly imprinted adsorbents, Chem. Engng. Sci., **53**, 2271-2284.
2. **Joshi, V. P.**, Kulkarni, M. G. and Mashelkar, R. A., 1998. Effect of solvents on selectivity in separation using molecularly imprinted adsorbents: Separation of phenol and bisphenol-A.
   Polymer (Communicated)
3. **Joshi, V. P.**, Kulkarni, M. G. and Mashelkar, R. A., 1998. Enhancing adsorptive separations using molecularly imprinted polymers: Role of imprinting techniques and system parameters.
   Chem. Engng. Sci. (Communicated)
4. **Joshi, V. P.**, Kulkarni, M. G. and Mashelkar, R. A., 1998. Molecularly imprinted adsorbents for positional isomer separation
   J. Chromatography (Communicated)
5. **Joshi, V. P.**, Kulkarni, M. G. and Mashelkar, R. A., 1998. Separation of hydroxy benzophenone isomers using molecularly imprinted adsorbents II: Surface imprinted polymers
   Manuscript under preparation
6. **Joshi, V. P.**, Kulkarni, M. G. and Mashelkar, R. A., 1998. Separation of hydroxy benzophenone isomers using molecularly imprinted adsorbents III: Thermodynamics of adsorption
   Manuscript under preparation

List of Patents

- A process for the preparation of polymeric adsorbents: NF - 247 / 97
- A process for the preparation of polymeric adsorbents by precipitation polymerization: NF - 96 / 98
- A process for the preparation of organic compounds free from phenolic impurities: NF - 95 / 98