Chapter 2

MOLECULARLY IMPRINTED POLYMERS: A REVIEW

2.1. Introduction

Molecular imprinting is an easy and effective method to prepare polymeric separation media having specific molecular recognition towards the template molecule\(^1\). In this method, the molecule is admixed with an appropriate host monomer and a large amount of crosslinking agent and solvent. Possible assembly from the template molecule and the host monomer can be achieved through non-covalent molecular interactions\(^2\), and this assembly can be polymerized and crosslinked to result in molecular imprinted separation media.

The molecule, for which selectivity is desired, called template or target molecule, is allowed to prearrange prior to polymerization in presence of monomer(s) with different functional groups that can interact with the template. After polymerization the target molecules are removed, leaving well-defined three dimensional cavities with spatially oriented functional groups in the highly crosslinked polymer network\(^3\). Covalent and non-covalent interactions can be employed in pre-polymerization rearrangement between template and functional monomer. Covalent bonds are enough strong, but non-covalent interactions require use of functional monomer which forms stable self-assembled complexes. In the later approach the interactions are involved to give pre-polymerization complexes, such as hydrogen bonding, electrostatic and hydrophobic interactions\(^4\). Traditionally, free radical bulk polymerization is utilized to prepare rigid monolith following grinding and sieving. Simplicity and availability are the major
advantages of this method. Unfortunately, particles obtained during grinding and sieving process are heterogeneous due to size, shape and internal morphology. Recently, several other methods were established to give more homogenous products like nano beads or thin imprint films, but they have some disadvantages, i.e. use of large amount of templates.

Design and synthesis of artificial receptor molecules have been an area of focal research for understanding the molecular recognition phenomena in biological systems and developing novel materials mimicking biological functions usable in analytical applications. Molecular imprinting is recognized as a powerful technique to synthesize polymer-type artificial receptors. In this technique, functional monomers and crosslinkers are polymerized in presence of a template molecule, which is followed by the template removal from the resultant polymer network to leave a template-fitted cavity. The functional monomers used are expected to be laid out in the cavity as complementary to the chemical functionality of the template molecule. This is because the functional monomers are bound with the template molecule during the polymerization. The selection of appropriate functional monomers and the determination of their stoichiometry applied are most important in the design of a molecularly imprinted system for a given target molecules. To date, one of the most successful molecular imprinting protocols employed is bulk polymerization to obtain glassy polymer blocks which used as powder after being crushed, ground, and sieved. Due to these tedious and time-consuming experimental steps, it normally takes several days to complete the whole procedure to prepare and evaluate molecular imprinted polymers, though a polymer preparation itself is simple and prompt. Therefore, it has not been easy to find an optimal functional monomer system by examining a number of molecular imprinted
polymers prepared under different conditions. Accordingly, a method involving a combinatorial chemistry based approach has been desired, which can readily perform the preparation and evaluation of molecular imprinted polymers, to establish an optimal functional monomer system in a short time.

Molecular recognition between a molecular receptor (host) and a substrate (guest) in a matrix containing structurally related molecules requires discrimination and binding. This can happen only if the binding sites of the host and guest molecules complement each other in size, shape and chemical functionality. Biological systems such as enzyme-substrate, antigen-antibody and hormone-receptor systems demonstrate molecular recognition properties originating from natural selection\(^7\). The working hypothesis of the binding site structure in molecular imprinted polymers is based on the idea that the pre-polymer complex is locked into place by polymerization. This assumption postulates the formation of a cavity with functional groups in complimentary array for the convergent interactions with the template.

Molecular imprinting seemed like an ideal platform for the synthesis of transition metal catalysts located within the interior of an imprinted active site. The imprinting process consists of the copolymerization of organic or inorganic templates into highly crosslinked organic polymers. Their modification provided molecularly imprinted polymers that retain chemical information related to the original template. While most of the MIP efforts have been directed to analytical applications, these techniques have more recently been applied to problems in synthesis and catalysis\(^8\).
2.2. Molecular imprinting strategy

A seminal study by Wulff and coworkers illustrated an important factor that increasing the amount of crosslinking matrix increases the specific recognition by covalently imprinted polymers\textsuperscript{9}. A new strategy for monomer design has been investigated that combines interactive monomer functionality with a crosslinking format, giving as a result non-covalent molecular imprinted polymers with improved performance. Quantitative structure-selective relationship studies have verified a key improvement to monomer design and in the template interactive functional group in a crosslinking monomer format\textsuperscript{10}. Molecular recognition is crucial for the functioning of living systems, where biological macromolecules including proteins (which may serve as enzymes, antibodies, and receptors), nucleic acids, and saccharides play decisive roles in biological activities. Since the recognition should be based on simple interactions between chemical units, the challenge of synthesizing artificial molecules which are capable of molecular recognition has drawn special attention to this field of chemistry. In the effort to obtain host molecules with precise recognition of guest species, the design, synthesis, and evaluation of supramolecules are being intensely investigated in laboratories throughout the world. However, the requisite multi-step preparative routes to the molecular receptors are frequently tedious and often provide only a low overall yield of the final product. An alternative approach to the synthesis of host molecules which can recognize target guest species is a much simpler template polymerization technique called "molecular imprinting". In this method, precise molecular design is not necessary since a crosslinked polymer is prepared in the presence of a template. The template molecule or ionic species associates with the functional monomer to form a covalent or non-covalent-bonded
complex. This complex is then polymerized with a matrix-forming crosslinker to give a three dimensional crosslink network (Scheme II.1). Upon removal of the template species, cavities are formed in the polymer matrix. These cavities have memorized the spatial features and bonding preference of the template, so the imprinted polymer will selectively rebind the template from a mixture of chemical species.

**Scheme II. 1.** Schematic representation of molecular imprinting

For large templates like viruses, we refer to the term “molecular imprinting” only as a method to produce high-affinity polymers for these specific viruses and do not imply that imprinting has been achieved at the molecular level. If the individual reagents (such as polymer and template) aggregate when mixed together, then the resulting three-dimensional polymer will contain cavities complementary to such aggregate formations, leading to increased nonspecific binding to the target molecule, and loss in selectivity\(^\text{11}\). Another factor is the ease of release of the template to create a complementary cavity in the crosslinked polymer. The ability of molecular imprinted polymers to selectively bind to the target molecule is derived from the vacated complementary cavities. If the wash solution is not successful in removing the template from the crosslinked hydrogel, then a recognition site will not be created to rebind the target molecule\(^\text{12}\). Methods must be developed to prevent aggregate formation prior to polymerization and
crosslinking of the imprinted polymer and to maximize the removal of the template to complete the molecular imprinting process.

2.3. Various approaches of molecular imprinting

Essentially, two kinds of molecular imprinting strategies have been established based on covalent bonds or non-covalent interactions between the template and functional monomers (Scheme II. 2). In both cases, the functional monomers are chosen so as to allow interactions with the functional groups of the template molecule and are polymerized in presence of the template. The spatial binding sites are formed by covalent or, more commonly, non-covalent interaction between the functional group of the template and the monomer, followed by a crosslinked co-polymerization. Of the two strategies, the non-covalent approach has been used more extensively due to following reasons: (i) non-covalent protocol is easy to conduct, avoiding the tedious synthesis of pre-polymerization complex, (ii) removal of the template is much easier, usually accomplished by continuous extraction, and (iii) a high variety of functionality can be introduced into the imprinted binding site using non-covalent methods.

(i) Covalent approach

In covalent approach, the imprinted molecule is covalently coupled to a polymerisable monomer. The binding of this type of polymer relies on reversible covalent bonds. After copolymerization with crosslinker, the imprint molecule is chemically cleaved from the highly crosslinked polymer. Wulff and co-workers first produced molecular imprinted polymers by synthesizing specific sugar or amino acid derivatives which contained a polymerisable function using covalent imprinting methods. After polymerization they hydrolyzed the sugar moiety and used the polymer for
selective binding. For covalent molecular imprinting, selectivity of imprinted polymer increases with increasing amount of crosslinker\textsuperscript{15}. Moreover, the requirements of covalent imprinting are different than those for non-covalent imprinting, particularly with respect to ratios of functional monomer, crosslinker, and template. However, since the choice of reversible covalent interactions and the number of potential templates are substantially limited, reversible covalent interactions with polymerizable monomers are fewer in number and often require an acid hydrolysis procedure to cleave the covalent bonds between the template and the functional monomer.

\begin{center}
\textbf{Scheme II. 2.} Schematic representation of non-covalent and covalent molecular imprinting
\end{center}

(ii) **Non-covalent approach**

Non-covalent approach is the most frequently used method to prepare molecular imprinted polymers due to its simplicity. During the non-covalent approach, the spatial binding sites are formed by the self-assembly between the template and monomer, followed by a crosslinking co-polymerization \textsuperscript{16}. The imprint molecules can interact via non-covalent interactions like ionic,
hydrophobic and hydrogen bonding\textsuperscript{17}. The non-covalent imprinting approach seems to hold more potential for the future of molecular imprinting due to the vast number of compounds, including biological compounds, which are capable of non-covalent interactions with functional monomers\textsuperscript{18}. Limits to the non-covalent molecular imprinting are set by the peculiar molecular recognition conditions. The interactions between the monomer and template are stabilized under hydrophobic environments, while polar environments disrupt them easily. Another limit is represented by the need of several distinct points of interactions: some molecules characterized by a single interacting group such as an isolated carboxyl, generally give imprinted polymers with very limited molecular recognition properties, which have little interest in practical applications. Understanding the basic optimization of non-covalent methods is important for two reasons: the methodology is far easier than covalent methods, and it produces higher affinity binding sites, versus covalent methods. The trends in binding and selectivity in non-covalently imprinted polymers are explained best by incorporating multiple functional monomers for the highest affinity binding sites.

The increased number of binding interactions in the polymer binding site may account for greater strict accuracy of the site, and thus impart greater affinity and selectivity to the site. This would suggest that the number of functional groups in the polymer binding site is not determined directly by the solution phase prepolymer complex rather it is determined during polymerization. Because of the difficulty to characterize the binding site structures the actual events determining the final binding site structure are still a main challenge\textsuperscript{19}. 
2.4. Molecular recognition of imprinted polymers

Despite the wealth of literature on molecular imprinting technology that has been published within past decades, the mechanism of recognition and their rational control appear not entirely understood, thus inhibiting optimization of the imprinting strategy. Molecular recognition ability is dependent on several factors, such as shape and functional complementarities, contributions from the surrounding environment. As for the functional complementarities, even though all non-covalent interactions are applicable to the molecular recognition between a target molecule and a molecular recognition site formed during molecular imprinting, the nature of the template, monomers and the polymerization reaction itself determine the quality and performance of the product polymer.

The recognition of the polymer constitutes an induced molecular memory, which makes the recognition sites capable of selectively recognizing the imprinted species. Hydrogen bond is most often applied as a molecular recognition interaction in molecularly imprinted polymers. From this, acrylic acid and methacrylic acid have usually been adopted as functional monomers since carboxyl group functions as a hydrogen bond donor and acceptor at the same time\textsuperscript{20}. These non-covalent interactions are easily reversed, usually by a wash in aqueous solution of an acid, base, or methanol, thus facilitating the removal of the template molecule from the network after polymerization. In addition to the better versatility of this more general approach, it allows fast and reversible binding of the template.
2.5. Parameters affecting spatial molecular recognition in imprinted polymers

The synthesis of molecularly imprinted polymers is a chemically complex pursuit and demands a good understanding of chemical equilibrium, molecular recognition theory, thermodynamics and polymer chemistry in order to ensure a high level of molecular recognition\textsuperscript{21}. The polymers should be rather rigid to preserve the structure of the cavity after splitting off the template. On the other hand, a high flexibility of the polymers should be present to facilitate a fast equilibrium between release and reuptake of the template in the cavity\textsuperscript{22}. These two properties are contradictory to each other, and a careful optimization became necessary. The challenge of designing and synthesizing a molecularly imprinted polymer can be a discouraging prospect to the uninitiated practitioner, not least because of the sheer number of experimental variables involved, e.g. the nature and levels of template, functional monomer, crosslinker, solvent and initiator, the method of initiation and the duration of polymerization\textsuperscript{23}. Moreover, optimization of the imprinted products is made more difficult due to the fact that there are many variables to consider, some or all of which can potentially impact upon the chemical, morphological and molecular recognition properties of the imprinted materials. Fortunately, in some instances it is possible to predict how changing any one such variable like the crosslink ratio likely to impact upon these properties\textsuperscript{24}.

\textbf{(i) Templates}

The template is of central importance and it directs the organization of functional groups pendent to the functional monomers in all molecular imprinting processes. In terms of compatibility with free radical polymerization, templates should ideally be chemically inert under the
polymerization conditions, thus alternative imprinting strategies may have to be sought if the template can participate in radical reactions or is for any other reason unstable under the polymerization conditions\textsuperscript{25}. The imprinting of small organic molecules like pharmaceuticals, pesticides, amino acids and peptides, nucleotide bases, steroids, and sugars is now well established and considered almost routine. Optically active templates have been used in most cases during optimization. In these cases the accuracy of the structure of the imprint could be measured by its ability for racemic separation, which was tested either in a batch procedure or by using the polymeric materials as chromatographic supports. One of the many attractive features of the molecular imprinting method is that it can be applied to a diverse range of analytes, however, not all templates are directly amenable to molecular imprinting processes\textsuperscript{26}. Most routine imprinted polymers were using small organic molecules as template. Specially adapted protocols have been proposed for large organic compounds such as proteins. Imprinting of such large structure is still a challenge. The primary reason is the fact that such templates are less rigid and thus do not facilitate creation of well-defined binding cavities during the imprinting process. Furthermore, the secondary and tertiary structures of large biomolecules such as proteins may be affected when exposed to the thermal or photolytic treatment involved in the synthesis of imprinted polymers. Rebinding is also more difficult, since large molecules such as peptides and proteins do not readily penetrate the polymer network for reoccupation of pockets left by them\textsuperscript{27 (a)}.

A convenient imprinting method for the preparation of magnetic molecularly imprinted nanowires within the pores of nanoporous alumina membrane is described\textsuperscript{27 (b)}. The template molecule is immobilized on the pore walls of a nanoporous alumina membrane. The nanopores were then
filled with a pre-polymerization mixture containing the super paramagnetic MnFe₂O₄ nano crystallites. After polymerization, the alumina membrane was subsequently removed by chemical dissolution, leaving behind magnetic polymer nano wires that contain template binding sites uniquely residing at the surface and have a saturated magnetization. The resulting magnetic imprinted polymer nanowires were capable of binding the template more strongly than the non-imprinted nanowires. The super paramagnetic nano crystallites can be entrapped in polymer nanowires using alumina membrane as template. Magnetic nano particles immobilized with biological receptors have been extensively used in biomedical and biotechnological areas. Magnetic nanowires with artificial receptors could have potential applications in drug delivery, as biochemical sensors, and for trace enrichment of specific targets.

(ii) Functional monomers

The careful choice of functional monomer is the most important factor to provide complementary interactions with the template and substrates. For covalent molecular imprinting, the effects of changing template to functional monomer ratio are not necessary because the template dictates the number of functional monomers that can be covalently attached; furthermore, the functional monomers are attached in a stoichiometric manner. For non-covalent imprinting, the optimal template to monomer ratio is achieved empirically by evaluating several polymers made with different formulations with increasing template. There is an increase in the number of final binding sites in the imprinted polymer, resulting in an increased binding or selectivity per gram of polymer. From the general mechanism of formation of molecular imprinted binding sites, functional monomers are responsible for binding interactions in the imprinted binding sites. Non-
covalent molecular imprinting protocols are normally used in excess relative to the number of moles of template to favor the formation of template-functional monomer assemblies\textsuperscript{29}. It is very important to match the functionality of the template with the functionality of the functional monomer in a complementary fashion (H-bond donor with H-bond acceptor) in order to maximize complex formation and thus the imprinting effect. High retention and resolution were observed in imprinted polymers with two co-monomers than a single monomer, which indicated an increase in the affinity of the imprinted polymer by the co-operative effect of the binding sites in both monomers. However, it is important to note that the reactivity ratios of the monomers should be verified to ensure whether co-polymerization is feasible\textsuperscript{30}.

The functional monomers used are expected to be laid out in the cavity as complementary to the chemical functionality of the template molecule, because the functional monomers are bound with the template molecule during polymerization. Consequently, resultant polymers exhibit the template-selective binding capacity. The most critical point in the molecular imprinting concept is, especially when the functional monomers are bound to a template by non-covalent bonding, whether functional monomers form a steady complex with a template molecule. Therefore, the selection of appropriate functional monomers and the determination of their stoichiometry applied are most important in the design of a molecular imprinted system for specific target molecules. A new strategy of monomer design for non-covalently imprinted polymers has been shown to be successful for improving the selectivity of molecularly imprinted materials\textsuperscript{31} \textsuperscript{(a)}. The origin of the improved selectivity was attributed to two factors of the crosslinking agents. First the degree of crosslinking is maximized without
imposing restrictions on functional group concentrations. Second, covalent togethering of the functional group to the binding site matrix reduces conformational entropy that would otherwise interfere with specific binding. Last, a strong influence of diastereomeric complexes on imprinted polymer selectivity was discovered (Fig. II. 1).

![Chemical structures](image)

**Fig. II. 1.** Commonly used functional monomers in molecular imprinting

Novel organo phosphorus functional monomers were synthesized, and zinc-imprinted polymers were prepared with the functional monomers by a surface molecular imprinting technique. The competitive adsorption behavior of zinc and copper ions on the surface-imprinted polymers was examined, and the template effect was characterized. It was found that the presence of aromatic rings and a suitable straight alkyl chain in the
functional monomer in addition to a high binding affinity to the target metal ions renders molecular recognition on the surface of the imprinted polymers effective. The presence of three essential factors such as (i) longer alkyl chain yielding a high interfacial activity, (ii) aromatic rings leading to rigidity of the recognition sites, and (iii) organophosphorus groups producing a high binding affinity ensure strong adsorption and high selectivity for target metal ions. The surface template polymers are easy to prepare and possess excellent chemical and physical stability. Importance of the position of vinyl group in the functional monomers for the effective imprinting of amino acid derivatives and oligopeptides in water were also studied.

A method for the synthesis and evaluation of molecularly imprinted polymers on a semi automated miniature scale is reported. This technique combines molecular imprinting with the combinatorial chemistry approach, allowing rapid screening and optimizations of libraries of imprinted polymers. Molecular imprinted polymers were prepared by a combinatorial approach using methacrylic acid (MAA), 4-vinylpyridine (4-VP), acrylamide, and styrene as functional monomers, and acetonitrile and toluene as porogenic solvents. A drug substance having aromatic, hydroxyl and amide functional groups was selected as the template molecule for this study. The results demonstrated that the polymer prepared with MAA as functional monomer shows the strongest binding affinity, and therefore, is preferred for the preparation of this particular template molecule. Due to the low consumption of reagents, and more importantly, the demonstrated ability of this method to effectively identify optimal imprinting conditions, this small scale combinatorial protocol is well suited for fast and efficient screening and optimization of imprinted polymers.
(iii) **Crosslinkers**

The selectivity is greatly influenced by the kind and amount of crosslinking agent used in the synthesis of the imprinted polymer. In an imprinted polymer, the crosslinker fulfills three major functions: first of all the crosslinker is important in controlling the morphology of the polymer matrix, whether it is gel-type, macro porous or a micro gel powder. Secondly, it serves to stabilize the imprinted binding sites. Finally it imparts mechanical stability to the polymer matrix\(^{33}\).

From a polymerization point of view, high crosslink ratios are generally preferred in order to access permanently porous (macro porous) materials and be able to generate materials with adequate mechanical stability. So the amount of crosslinker should be high enough to maintain the stability of the recognition sites. These may be because the high degree of crosslinking enables the micro cavities to maintain three-dimensional structure complementary in both shape and chemical functionality to that of the template after its removal. Thus the functional groups are held in an optimal configuration for rebinding the template, allowing the receptor to ‘recognize’ the original substrate\(^{34}\). Polymers with crosslink ratios in excess of 80% are often be used. But recent results suggest that a moderate degree of crosslinking (40-50%) is only required for the development of imprinted system with enhanced selectivity and specificity. Quite a number of crosslinkers compatible with molecular imprinting are known and a few of which are capable of simultaneously complexing with the template and thus acting as functional monomers (Fig. II.2). The catalytic activity of the EGDMA-crosslinked copolymer with high imidazole content showed higher activity. When DVB used as the crosslinking agent, the catalytic activity of
the imprinted copolymer increased due to the hydrophobic interaction along with the cooperative effect.

Fig. II. 2. Commonly used crosslinking agents in molecular imprinting
(iv) Porogenic solvents

Porogenic solvents play an important role in the formation of porous structure of imprinted polymers, which are known as macroporous polymers. It is known that the nature and level of porogenic solvents determines the strength of non-covalent interactions and influences polymer morphology which obviously directly affects the performance of imprinted polymers. The template molecule, initiator, monomer and crosslinker have to be soluble in the porogenic solvents. The porogenic solvents should produce large pores, in order to assure good flow-through the pores of the resulting polymer. Also the porogenic solvents should be relatively less polar, in order to reduce the interferences during complex formation between the imprint molecule and the monomer, as the latter is very important to obtain molecular imprinted polymers with high selectivity\textsuperscript{35}.

Porogenic solvents with low solubility phase separate early and tend to form larger pores and materials with lower surface areas. Conversely, porogenic solvents with higher solubility phase separate later in the polymerization. This provides materials with small pore size distributions and high surface area. More specifically use of a thermodynamically good solvent lead to polymers with well developed pore structures and high specific surface areas. Use of a thermodynamically poor solvent leads to polymers with poorly developed pore structures and low specific surface areas\textsuperscript{36}. However, the binding and selectivity in imprinted polymers is not appeared to dependent on a particular porosity. Molecular imprinting is an effective method for the synthesis of an enantioselective TSA imprinted polymer catalyst for hydrolytic reaction of amino acid esters. Correlation of catalytic activities of TSA imprinted polymers prepared in two porogens
revealed that, the catalyst prepared in DMSO is more effective than those prepared in chloroform.

Although the results of molecular recognition weaken with increasing polarity of the solvents, it is important to stress that in some cases sufficiently strong template-monomer interactions have been observed in rather polar solvents. Increasing the volume of porogenic solvents increases the pore volume. Besides its dual roles as a solvent and as a pore forming agent, the solvent in a non-covalent imprinting polymerization must also be judiciously chosen such that it simultaneously maximizes the likelihood of template-functional monomer complex formation. Normally, this implies that a polar, non-protic solvents like toluene are preferred, as such solvent stabilize hydrogen bonds. If hydrophobic forces are being used to drive the complexation then water could be the solvent of choice.37

(v) Initiators

Many chemical initiators with different chemical properties can be used as free radical source in free radical polymerization. Normally they are used at low levels compared to the monomer, 1 wt. %, or 1 mol. % with respect to the total number of moles of polymerisable double bonds. The rate and mode of decomposition of an initiator to radicals can be triggered and controlled in a number of ways, including heat, light and by chemical or electrochemical means depending upon its chemical nature. For example the azo initiator, azo-bis-iso-butyronitrile (AIBN) can be conveniently decomposed by photolysis or thermolysis to give stabilized, carbon-centered radicals capable of initiating the growth of a number of vinyl monomers.38
(vi) Polymerization conditions

Polymerization temperature affects the affinity and specificity of molecular imprinted polymers, both of which could be significantly improved by selecting a lower polymerization temperature. The optimum temperature with respect to polymer performance depends on the temperature used in polymer preparation. Thus, polymers synthesized at high temperature will perform better at higher temperature and similarly polymers prepared at low temperature perform better at low temperature. Because of the exothermic nature of free radical polymerization, the practical limit for bulk polymerization is around 50 mL. The reaction volume should not exceed this figure. It was found that photo initiated polymerization resulted in polymers with high degree of cross-linking and specificity. This is also related to the lower temperature achieved during polymerization. Usually most studies used 60°C as the polymerization temperature. However, the initiation of the polymerization reaction was very fast and therefore hard to control at this temperature and resulted in low reproducibility of imprinted polymer formed. Furthermore, the relatively high temperatures have a negative impact on the complex stability, which reduced the reproducibility of the monolithic stationary phases and produced high column pressure drops. Thus, the relatively low temperatures with a prolonged reaction time were selected in order to yield a more reproducible polymerization. Where complexation is driven by hydrogen bonding then lower polymerization temperatures are preferred, and under such circumstances photochemically active initiators may well be preferred as these can operate efficiently at low temperature. For example, Mosbach et al. presented a study on enantioselectivity of L-Ph-NH-Ph imprinted polymers polymerized at 60°C, and 0°C. The results showed that better selectivity is obtained at the lower
temperature versus the identical polymers thermally polymerized at 60°C. The reason for this has again been postulated on the basis of Le Chatelier’s principle, which predicts that lower temperatures will drive the pre-polymer complex toward complex formation, thus increasing the number and possibly the quality of the binding sites formed\textsuperscript{20}.

Moderate pressure applied during polymerization affects the performance of the polymer indirectly through the prevention of solvent boiling in the monomer mixture. Polymerization at high pressure generates more rigid polymers with better defined shapes of imprinting cavities and therefore improved specificity. Increasing the polymerization time leads to more rigid polymers and consequently improves the specificity of imprinted materials. The specificity of the polymer could also be improved by the introduction of a magnetic field. The effect of the magnetic field is related to its influence on the initiation efficiency, reaction rate, and yield of the polymerization reaction brought about by extending the lifetime of radicals in the system. The influence of the orientation effect of the magnetic field on the molecules and polymeric chains in the polymerization mixture is evident in the imprinted polymers which have more uniform binding sites with enhanced affinity and specificity. In order to enhance the performance of imprinted polymers that rely on ionic interactions, the dielectric constant of the solvent used for polymer preparation should be lower than the dielectric constant of the crosslinker. Of all the parameters listed, the polymerization temperature and polymerization rate are the most important to control. These factors are primarily influenced by the nature of initiation and initiator concentration; however, other polymer properties, in particular morphological factors such as porosity, are also affected. The effect of other parameters like pressure can also be linked to temperature effects, emphasizing that
temperature control is the most important factor which influence over other polymer properties. In selecting the best conditions for preparing materials suited to a particular application, some compromises may have to be made between performance and the desired property. It should be noted that the polymerization reaction is very complex process which could be affected by many physical factors. To produce high-performance molecular imprinted polymers, all factors should be thoroughly considered and controlled appropriately\textsuperscript{39}.

2.6. Characterization of molecular imprinted polymers

Analytical characterization of the molecular mechanisms occurring in the pre-polymerization solution will govern the resulting binding site distribution and the recognition properties of the imprinted polymer matrix. In the non-covalent approach the stability of the template-functional monomer complex formed in the pre-polymerisation mixture will govern the resulting binding site distribution and the distribution properties of the imprinted polymer matrix. Close analysis of the pre-polymerisation solution can provide fundamental insights to the various interactions occurring during imprinting. Consequently, spectroscopic studies of the pre-polymerisation mixture provide prevalent information on the imprinting process. Since reorganisation of functional groups at the binding sites is required during rebinding the spectral studies before and after rebinding can also put light into rebinding\textsuperscript{40,41}.
(i) **$^1$H NMR spectra**

Proton NMR titration experiments facilitate observation of hydrogen bond formation between bases and carboxylic acid through hydrogen bonding. These studies have been introduced in molecular imprinting for investigating the extent of complex formation in pre-polymerisation solutions. Thus evaluating the shift of a proton signal due to participation in hydrogen bond was used as the selection criterion for complex formation between the functional monomer and templates\(^4\).

(ii) **FT-IR spectra**

In addition to $^1$H NMR, FT-IR spectra provide the fundamental analytical basis for rationalising the mechanism of recognition during the imprinting process probing the governing interactions for selective binding site formation at the molecular level. The interaction between the functional monomer and template during pre-polymerisation complex formation and the template incorporation into the imprinted polymer during rebinding can be confirmed by the characteristic FT-IR absorption analysis\(^5\).

(iii) **Scanning electron microscopy (SEM)**

SEM can be used in a variety of distinct ways to probe imprinted polymers on a variety of length scales. Scanning electron microscopy is the most widely used technique to study the shape, size, morphology and porosity of polymers\(^6\).

(iv) **Pore analysis**

It is possible to probe the morphology of imprinted polymers in the same way as one is able to do with most porous solids. Depending on the method of analysis, useful information may be acquired on the specific pore volumes, pore sizes, pore size distributions and specific surface area of
materials. Pore size, pore volume and surface area analyses were usually performed by nitrogen adsorption. The surface area was determined using the BET model, the t-plot using Harkin-Jura average thickness equation and the pore volume and pore size distribution using the BJH model\textsuperscript{45}.

2.7. Swelling studies

The efficiency of a functional polymer is governed by the accessibility of the reactive functional groups anchored on it, which in turn depends upon the extent of swelling and solvation\textsuperscript{46}. The rate of diffusion of a reagent into the polymer matrix mainly depends on the extent of swelling\textsuperscript{47}. Thus swelling is an important parameter, which controls the success of rebinding. The most effective solvent can carry out the percentage rebinding reaction very effectively. The extent of swelling can be determined in terms of change in weight on swelling\textsuperscript{48, 49}. Alternatively by packing a definite weight of the polymer in a capillary tube and measuring the volume before and after incubation in the solvent, the swelling ratio can be determined in terms of change in volume\textsuperscript{50}.

The swelling ratio ($S_R$) of the polymer can also be calculated from the dry and swollen weights of the polymer using the equation

$$S_R = \frac{m_s - m_o}{m_o},$$

where $m_s$ is weight of the swollen polymer and $m_o$ is the weight of the dry polymer.

2.8. Imprinting efficiency of molecularly imprinted polymers

Molecular imprinting is an emerging tool for the design of structured porous materials having a precise arrangement of functional groups within the pores of controlled size and shape. Such controlled specificity in
principle can offer opportunities for specific recognition applications. In practice, molecular recognition is often not fully realized, either due to distortion during the imprinting process or due to incomplete imprinting. Using a mean-field lattice model, we study imprinting efficiency of tetra functional monomers using imprinting agents of various sizes and for various preparation conditions. Neglecting imperfections and distortions during gelation and post-treatment, we know that high imprinting efficiencies are hard to achieve. However, monomer-template interactions and preparation conditions can be optimized for a given template size to yield a higher population of high affinity sites\textsuperscript{51}. A notable difficulty with recognition based applications of molecular imprinting is the low yields of high affinity sites. The quality and performance of the imprinted gel is clearly affected by the physical and chemical nature of the monomers and templates and the interactions between them. Therefore, an understanding of the physics governing the formation of monomer-template complexes is fundamental to a strategic design of imprinted polymers. Nevertheless, although it is a rapidly growing field, few efforts have focused on characterizing and understanding the mechanisms underlying formation and recognition, and even fewer theoretical efforts have emerged that investigated the interplay of the various parameters influencing the molecular imprinting process.

In the current paradigm for molecular imprinting, the imprinted binding sites exist as a consequence of the polymerization process around templates, and the properties of non-imprinted polymers have largely been overlooked. Thus nothing can be affirmed a priority concerning the binding properties of non-imprinted polymers. The imprinting effect is due to the presence of a template molecule that enhances the pre-existing binding properties of a polymer. If a non-imprinted polymer shows no binding
towards a target molecule, the corresponding imprinted polymer may show a weak imprinting effect. On the other hand, if a non-imprinted polymer shows binding properties toward a target molecule, the corresponding imprinted polymer will show a significant imprinting effect. For closely related molecules, it is observed that selectivity is an emergent property derived from the imprinting process and not a property of non-imprinted systems\textsuperscript{52}.

2.9. Selectivity parameters of molecular imprinted polymers

The binding parameters of the molecular imprinted polymers are usually estimated from adsorption isotherms using mathematical models\textsuperscript{53-54}. One strategy to perform binding performance is based on saturation experiments and subsequent Scatchard analysis\textsuperscript{55-58}. The obtained binding data were transformed into linear form and analysed to create Scatchard plots based on Scatchard equation

$$\frac{[S]_b}{[S]_f} = \frac{(S_{\text{max}} - [S]_b)}{K_D}$$

where, $K_D$ is an equilibrium dissociation constant, $S_{\text{max}}$ an apparent maximum number of binding sites and $[S]_b$ is the amount of template bound to molecular imprinted polymer at equilibrium. In Scatchard plot bonded concentration is plotted against the ratio between bonded and free ligand concentration. It is possible to estimate the $S_{\text{max}}$ and $K_D$ from a Scatchard plot where $S_{\text{max}}$ is the X intercept; $K_D$ is the negative reciprocal of the slope. For non-covalently synthesised molecular imprinted polymers the Scatchard plots result in a curve with the degree of curvature containing the information on the heterogeneity of the binding sites within the imprinted polymer matrix. The random arrangement of the templates at the binding sites and the incomplete complexation between the template and the
functional monomer led to the heterogeneity in binding sites typical for non-covalent imprinting.

(i) Separation factor

The effectiveness of imprinting was verified by the comparison of the binding of template versus molecules with similar structure\(^\text{59-62}\). A complete secondary screen for binding and selectivity was performed for all the polymers in terms of separation factor\(^\text{63}\).

Separation factor \((\alpha_{\text{template}}) = K_{\text{MIP}}/K_{\text{NIP}}\)

Molecular imprinted polymers possessing high separation factors should be capable of completely recovering the target molecule by the simple process of stirring the imprinted polymers with template solution. In general, the values of separation factor are high when ionic interactions are utilised between the template and the functional monomers. However when non-covalent interactions are employed comparatively less values in the range one to two is obtained.

(ii) Selectivity factor

The selectivity factor is defined as the ratio of separation factors of the two species, template and analogue respectively and is the index of polymer selectivity towards analogues of the template molecule\(^\text{64}\). It is calculated as

\[
\text{Selectivity factor} = \frac{\alpha_{\text{Template}}}{\alpha_{\text{Analogue}}}
\]

It is a measure of the selectivity characteristics of the developed imprinted system.
2.10. Applications of molecularly imprinted polymers

Molecular imprinting technique has become a well known means for the preparation of biomimetic recognition matrices. The resultant materials have good binding affinity, stability and selectivity towards the target molecule, and thus have been widely applied in separation science, recognition of proteins, biosensing, catalysis and drug delivery. Molecular imprinted polymers with tailor made cavities and engineered chemical functionalities are increasingly appreciated as highly target specific polymeric molecular recognition materials with a broad range of potential applications such as chromatographic stationary phase for the separation and isolation, antibody and receptor mimicking, enzyme mimetic catalysis, organic synthesis and drug delivery.

(i) Synthetic materials as substitutes for biological antibodies and receptors

Molecular imprinted polymers can more properly be characterized as a ‘rational design’ approach allowing research and application problems to be solved. Using simple molecular building blocks, material chemist can now produce tailored synthetic materials of much improved stabilities, able to replace or complement natural receptors. The problem of the recognition provided by single imprinted polymer is relatively weak and can be solved using an array of different molecular imprinted polymers simultaneously. The combination of imprinted arrays with appropriate chemometrics would considerably simplify the analysis of more difficult samples.

Molecular imprinted polymers found useful in the field of agriculture. With significant advantages in easy preparation, low cost, predictable specific recognition and high stability, the synthesized molecularly imprinted polymers have the capability of specific adsorption and recognition of the
template molecule. Several herbicides such as phenoxy acetic acid, 2, 4-dichloro phenoxy acetic acid and 2, 4, 5-trichloro phenoxy acetic acid could be successfully imprinted on polymeric system with optimum rebinding.\(^7\)

(ii) **Solid phase extraction**

Molecular imprinting is an emerging technique for the design of polymeric materials possessing selective affinity towards target analytes. The method involves complexation in the solution of a target compound with appropriate functional monomers. Molecular imprinted polymers offer distinct advantages compared to natural receptors; ease in preparation, low cost, tolerance to extreme chemical and thermal condition, long shelf life, and enhanced versatility in experimental design. Separation and solid phase extraction are the major application areas for the imprinted polymers. Molecular imprinted polymers provide tailor-made media that can solve complex separation problems and improve the generic selectivity of the conventional chromatographic media by increasing the separation of the target analytes.\(^7\) To achieve increased selectivity, higher extraction yields and analyte purification in solid phase extraction, several methods were investigated for the production of molecular imprinted polymers for flavonoids.\(^7\)

(iii) **Binding assays**

It was first demonstrated by Mosbach, who developed molecular imprinted polymers based assay for the bronchodilator theophylline and the tranquilizer diazepam due to the capacity of molecular imprinted polymers share with antibodies one of their most important features. Due to the ability to bind a target molecule selectively, they could conceivably be employed in immunoassay type binding assays in place of antibodies.\(^7\) The assay yielded
a cross reactivity profile very similar to that of the natural monoclonal antibodies.

(iv) Artificial catalysts

Molecular imprinting based on non-covalent interactions can also be used to produce catalytic polymers. Polymers that mimic the properties of amino acid esters have been prepared from imidazole monomers. Recently other approaches have been explored to improve enzyme mimicking polymers. Thus antibodies prepared by imprinting the transition state analogue, p-nitrophenyl methyl phosphonate against a phosphonic ester for alkaline ester hydrolysis, enhanced the rate of ester hydrolysis by $10^3$ - $10^4$ fold. The enhancement is due to the preferred binding of the transition state of the reaction. Molecular imprinting seemed like an ideal platform for the synthesis of transition metal catalysts located within the interior of a polymer-imprinted active site. Molecular imprinted polymers have raised considerable interest in the potential to develop new specific catalysts for particular chemical reactions. This involves the use of transition state analogue to create binding sites which can reduce the activation energy for a chemical reaction.

Molecular imprinting is an effective method for the synthesis of enantioselective TSA imprinted polymer catalyst for hydrolytic reaction of amino acid esters. Correlation of catalytic activity with the nature and extent of crosslinking revealed that 90% EGDMA-crosslinked system exhibited better enantioselectivity. The catalytic activity of the EGDMA-crosslinked copolymer with high imidazole content showed higher activity. When DVB was used as the crosslinking agent, the catalytic activity of the imprinted copolymer increased due to the hydrophobic interaction along with the
cooperative effect. Co(II) ion coordination during the prepolymerisation stage has a great influence on the recognition property of molecular imprinted polymers and its higher catalytic activity than non coordinated system.

(v) Polymeric sensors

The ability to develop chemical sensors with high selectivity characteristics of biological enzymes or antibodies, while the robustness to operate in harsh environments such as in high temperature and in organic solvents is a long term aim in sensor research. Molecular imprinted polymers are having the advantage that the recognition sites are tailor made, and at the same time incorporated into a solid polymeric support. Taking into account the very high specificity that can be obtained as well as the chemical and physical stability of imprinted polymers there have been a number of attempts to construct chemical sensors based on these materials as the recognition elements. The challenge currently facing those wishing to exploit the recognition properties of molecular imprinted polymers in such devices is to develop a transducing mechanism to translate the binding event into a measurable signal. Several molecular imprinted polymers based sensing systems have been proposed including sensors utilising field effect devices, conductometric measurements, amperometric measurements, and fluorescence measurements.

The incorporation of magnetic properties allows the formation of sensor spots by magnetic separation. Well organized structures must be obtained for designing magnetic molecular imprinted polymers which can be used as optical sensor phases. This strategy may be further extended for implementing optical sensing phases in portable devices that can control a
broad variety of analytes in different matrices such as water, organic solvents etc, and may be used to improve sensitivity in other magnetic optical sensors.

(vi) Chromatography

Finely ground imprinted polymer, mixed with binders has been investigated for use in chiral TLC\(^9\). The inherent advantages of TLC, such as multiple parallel samples and simplicity, are still attractive. If new preparative methods for imprinted polymers successfully address the problem of binding site heterogeneity, chiral TLC with imprinted polymers may prove useful when large numbers of samples have to be semi-quantitatively screened\(^9\).

(vii) Chiral recognition

The molecular imprinting technique was first introduced into the microchannel of a microfluidic device to form the imprinted polymer for fast enantioseparation of chiral compounds. The molecularly imprinted polymer was chemically polymerized on the microchannel wall using acrylamide as the functional monomer and ethylene glycol dimethacrylate as the crosslinker, and characterized by scanning electron microscopy, atomic force microscopy, and infrared spectroscopy. Under the optimized conditions, such as optimal preparation of molecular imprinted polymers, composition and pH of mobile phase, and separation voltage, enantiomers can be separated\(^9\). The first and the most important application of molecular imprinted polymers is in the enantioseparation of racemic mixtures of chiral compounds. This provides a convenient method for quantitative assessment of the quality of imprints produced by a particular recipe or strategy. Most work has concentrated on the resolution of chiral compounds. This reflects both the importance of this procedure in analytical and synthetic chemistry.
and also the ease of distinguishing between specific and non-specific recognition in this case. Molecular imprinted polymers have more applications in the screening and confirming of appropriate resolving agents for chiral enantiomers in chiral resolution. Specialized interactions between imprinting molecules and molecular imprinted polymers are caused by both the complementary functional groups and the suited structures of molecular imprinted polymers.

In the early 1950’s chiral selectivity for mandelic acid and camphor sulphonic acid enantiomers had been demonstrated by Curti et al. using imprinted silica as stationary phase in column chromatography. Optical resolution of amino acids or amino acid derivatives, direct enantioseparation of drugs, and enatioseparation of sugar and sugar derivatives has been reported. Conditions for the direct resolution of the drugs by TLC were identified using chiral stationary phases based on molecular imprinted polymers. The use of molecular imprinted polymers as chiral stationary phases in TLC demonstrated that they may provide a potentially powerful tool for resolving chiral compounds and this is a useful method for quality control of optically active compounds. A highly enantioselective polymer was reported for the separation of optically active tryptophan methyl ester by a surface molecular imprinting technique. A synthetic host molecule phenyl phosphonic acid mono dodecyl ester was proved to be effective for recognizing the chirality of amino acid esters. The L- or D-tryptophan methyl ester imprinted polymer containing the functional host molecule revealed high enantioselectivity towards the corresponding imprinted isomer. These enantioselectivities were quantitatively supported by high binding constants for the corresponding imprinted isomer.
Polymers prepared by molecular imprinting utilizing non-covalent interactions exhibit enantioselectivity and substrate selectivity for their original print molecules. Molecular imprinted polymers prepared with L-phenyl alanine derivatives as the print molecule and acrylamide as the functional monomers shows high enantioselectivity and substrate selectivity in the chromatographic mode\textsuperscript{100}. The selectivity was shown to be governed by the number, nature and interactions between the substrate and polymeric stationary phase as well as the shape and rigidity of the print molecule. These studies should facilitate further utilization of the concept of molecular imprinting allowing tailor made chiral stationary phases for a particular chromatographic application to be designed. The ligand cross-reactivities of molecular imprinted polymers can be beneficially employed for the simultaneous separation of different structures\textsuperscript{101}. Chiral recognition ability and ligand specificity of the imprinted polymer were demonstrated by several batch wise test using different amino acid derivatives. The surface imprinted polymer could recognize the chirality of amino acids; therefore it preferentially adsorbed the corresponding enantiomer that was imprinted in the preparation. The pH and buffer concentration in the aqueous solution are the key factors enhancing enantioselectivity. The high interfacial activity of the functional molecule and the low swelling property of the imprinted polymer is important in ensuring high imprinting effect\textsuperscript{102}.

There is an increasing demand, especially in the agricultural chemicals and pharmaceutical industries, for better and more efficient means to prepare, purify, and analyze chiral compounds. This demand is driven sometimes markedly by different biological activities of the enantiomers of a given compound and increasing regulations regarding optically active molecules. Despite dramatic improvements in asymmetric synthesis,
chromatographic methods are still indispensable for analyzing and purifying chiral compounds. Commercial chiral stationary phases use immobilized chiral functionalities ranging from small organic compounds to entire proteins. For separating a specific enantiomeric pair, several chiral stationary phases and mobile-phase conditions may have to be evaluated to obtain satisfactory results; furthermore the elution order of each enantiomer is often difficult to predict. Molecular imprinted polymers in contrast, have predetermined selectivity and can be custom-made. When molecular imprinted polymers are used for chromatographic separations the isomer used in the preparation of the molecular imprinted polymers is always the one that is more strongly retained; therefore, the elution order is predictable. This is especially convenient for chiral separations, because further measurement would otherwise be necessary to determine the chirality of the eluted analytes. It is unlikely that molecular imprinted polymers will take the place of the handful of CSPs that currently dominate the market and accommodate the majority of current chiral separation needs. Molecular imprinted polymers offer unique advantages that will keep them at the focus of an expanding research field.

In the first example of molecularly imprinted chiral stationary phase prepared $N$-(3, 5-dinitrobenzoyl)-3-methylbenzylamine (DNB), was chirally discriminated on a stationary phase prepared using racemic DNB as the template. ($S$)-(-)$N$-methacryloyl-1-naphthylethylamine, was utilized as the functional monomer toward the racemic template, and its chiral recognition ability was interestingly found to be enhanced through racemic molecular imprinting. A thermodynamic discussion briefly suggests that the observed chiral recognition ability of the racemic imprinting was proper value.
Two kinds of enantioselective molecular imprinted polymers N-tert-butoxycarbonyl-L-tryptophan (N-Boc-L-Trp) and N-tert-butoxycarbonyl-L-tyramine (N-Boc-L-Tyr) were synthesized by photo-induced and thermal-induced polymerization and were employed as the stationary phase in liquid chromatography. A stoichiometric displacement model for retention was successfully constructed and applied to evaluate the chiral separation of molecular imprinted polymers. D, L-lactic acid and D, L-alanine solutes resolution was studied in supported liquid membrane using a polypropylene hollow-fiber module. The enantioselective transport of solutes was facilitated by N-3, 5-dinitrobenzoyl-L-alanine ester chiral selector, which was dissolved in toluene organic solvent. The maximum D, L-lactic acid separation factor achieved was two and that for the D, L-alanine was 1.75. In both cases the D-enantiomer flux was preferred.

The success of using molecular imprinting technology for tailoring imprinted polymers with predetermined enantioselective binding properties by employing the enantiomers of interest as binding-site-forming templates is evident from various studies. Using EGDMA as the crosslinking agent and 4-vinyl pyridine as functional monomer the selected amino acids having chiral centre L-tryptophan, Boc-L-phenyl alanine and phenyl alanine could be successfully imprinted in the synthetic polymer. EGDMA exhibited superior selectivity compared to the rigid DVB-crosslinked system. With the systematic and careful optimization of the condition it is possible to design imprinted polymers with maximum selectivity and specificity.

Separation of L-glutamic acid from dilute aqueous solution by solid-phase extraction based on molecular imprinting technique using chitosan crosslinked with glutaraldehyde was investigated. L-Glutamic acid imprinted crosslinked chitosan (LGIC) was prepared by crosslinking of
chitosan by glutaraldehyde crosslinker, in the presence of L-glutamic acid. Non-imprinted crosslinked chitosan (NIC) was also prepared by the same procedure in the absence of template molecules. The morphological structures of both LGIC and NIC were examined by scanning electron microscope. LGIC particles were applied to determine the optimum operational condition for L-glutamic acid separation from dilute aqueous solution. In adsorption step, optimum pH and retention time were 5.5 and 100 min, while corresponding values in extraction step were 2.5 and 60 min respectively. The adsorption isotherms confirmed that the molecular imprinting technique creates an enantioselectivity of LGIC toward L-glutamic acid. In addition, chiral resolution of L-, D-glutamic acid racemic mixture was carried out using a column of LGIC.

The Boc-L-Trp imprinted polymeric membranes obtained showed adsorption selectivity toward Ac-L-Trp from its racemic mixtures. From adsorption isotherms, the chiral recognition site that had been formed by the presence of print molecule in the membrane preparation process exclusively recognized Ac-L-Trp which possessed the same configuration of the print molecule.

Molecularly imprinted polymeric membranes were prepared from various oligopeptide tweezers by the use of N-tert-butoxycarbonyl-D-tryptophan (Boc-D-Trp-OH) or N-tert-butoxycarbonyl-L-tryptophan (Boc-L-Trp-OH) as a print molecule. The chiral recognition ability of the formed molecular recognition sites was dependent on the absolute configuration of the print molecule adopted in the membrane preparation process. In other words, the membranes imprinted by the D-isomer recognized the D-isomer in preference to the corresponding L-isomer.
Molecular imprinting is a versatile technique providing functional materials able to recognize and in some cases respond to biological and chemical agents of interest. In contrast to biological antibodies, the best known receptors derived from biological combinatorial processes, molecularly imprinted polymers are obtained by template-directed synthesis. Thus, molecular imprinting can more properly be characterized as a “rational design” approach, allowing research and application problems to be solved. Using simple molecular building blocks, material chemists can now produce tailored synthetic materials of much improved stabilities able to replace or complement natural receptors. A novel approach to the introduction of recognition site functionality into highly crosslinked polymeric matrices via molecular imprinting has been developed for compounds with single or multiple spatially separated hydroxyl groups. The present work is aimed on the preparation of molecular imprints for two \( \alpha \)-hydroxy chiral acids such as D-mandelic acid (D-MDA) and L-phenyl lactic acid (L-Ph LA) and one chiral amino acid derivative D-phenyl glycine (D-Ph Gly) with optimum specificity and selectivity employing simple UV-vis. spectroscopic technique (Fig. II.3).

\[ \text{D-MDA} \quad \text{L-Ph LA} \quad \text{D-Ph Gly} \]

**Fig. II. 3. Structure of three templates**

Mandelic acid is an \( \alpha \)-hydroxy carboxylic acid which has a hydroxyl group on the carbon atom next to the acid group. It is the simplest alpha
hydroxyl acid which has a dual functionality of acid and alcohol in a low molecular weight structure. Its structure provides bactereostatic property. It is excreted well in the urine. It is used as antiseptic ingredient particularly against urinary tract infections. Naturally occurring mandelic acid is found when amygladin (a cyanogenetic glycoside found in bitter almond, apricot and wild cherry) is split by hydrolysis with hydrochloric acid\(^{110}\). L-Phenyl lactic acid has been recently found in cultures of lactobacillus plantarun which shows antifungal activity. It is an organic acid produced from lactic acid and its concentration \(> 7.5\) mg/ml inhibit growth of yeast. D-phenyl glycine is an amino acid derivative which can be used to prepare semi synthetic antibiotics.

Three particular features have made molecular imprinted polymer the target of intense investigation. Their high affinity and selectivity, which are similar to that of natural receptors, their unique stability which is superior to that demonstrated by natural biomolecules and the simplicity of their preparation and ease of adaptation to different practical applications. Molecular imprinting is particularly useful for enantiomeric separation of carboxylic acid and their derivatives. The molecularly imprinted polymers have the ability to bind the print molecule selectively in presence of molecules of like structure. The present study focuses on the separation of alpha hydroxyl carboxylic acid acid derivatives of varying structure and their separation making use of the memory of the molecular imprinted polymers.
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