Design, synthesis and characterization of enzyme-analogue-built polymer catalysts as artificial hydrolases

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Abstract

In this review, the concept and various strategies in molecular imprinting is discussed briefly. How the concept of transition state analogue can be used to design a template to prepare catalytic imprinted polymers is described in detail. The use of the “bait and switch” approach and alternative covalent template strategies show how functional groups which assist in the catalytic properties can be assembled within the imprint. Thus, there are so many reports on P catalyzed reactions. Owing to their advantageous properties over natural biological recognition agents, molecularly imprinted polymers (MIPs) therefore offer great potential for various applications.

Introduction

Bio-recognition through molecular imprinting technique has been a time-honoured goal in the biochemistry and materials science in recent years [1]. The intriguing and exacting guest-host chemistry that drives bio-recognition (enzyme-substrate and antibody-antigen specificities) has inspired the researchers. The molecularly imprinted macromolecular polymers exhibit high specificity to bind target molecules in the 3D memory cavity through “lock and key” type fit [2]. Molecular imprinting is a microscopic moulding process in which the print molecule fabricates substrate-selective 3D-recognition sites in the macromolecular polymer matrix [3]. The template molecule directs the molecular positioning and orientation of the catalytic functionalities and the crosslinks ensure polymer rigidity that freezes the 3D molecular architecture of the substrate binding cavity by the subsequent removal of the template. A large number of molecularly imprinted polymers (MIPs) have been investigated and reported over the last few decades with potential applications. Beyond catalysis, the potential of MIPs continues to impact and revolutionize separation [5], adsorption [6], sensing [7], etc.

The esterolytic and amidolytic reactions are debatably two major classes of reactions among the most common reactions found in nature and vital to the degradation of many biochemical substances. Enzymes play the important role as highly specific biological catalysts in biotechnology as well as in chemical reaction engineering. However, the drawbacks of these biomaterials are poor durability, relatively high costs of production, heat and pH sensitivity and incompatibility with organic solvents. Thus, molecular imprinting provides a prevailing, generic, superficial and cost-effective substitute for the preparation of artificial enzymes via the fabrication of specific recognition sites for a pre-determined template in a polymer matrix. Based on the theory of transition state stabilization, phosphonate monoester as a stable transition state analogue (TSA) is generally used as a template for hydrolytic reactions in molecular imprinting [8]. The imprint of the TSA acts like a catalytically active centre. This binding site shows its catalytic effect by reducing the activation energy of the specific reaction.

Since the geometry of the catalytic cavity designed by the print molecule alone does not provide a strong catalytic effect, it is necessary to introduce key catalytic entities to mimic natural enzymes. A variety of functional polymers containing one or more catalytically active groups have often been synthesized and studied as efficient enzyme models. Among them, imidazole-containing polymers have been extensively examined, because imidazolyl groups are known to exist at the active sites of various proteolytic enzymes such as p-chymotrypsin, carboxypeptidase and so on. Catalytic activity in biological systems is controlled by the spatial arrangement of specific functional groups in the active sites of enzymes. The proximity of these groups and their spatial orientation both contribute to the enzyme’s activity and specificity. The intriguing and exacting guest-
host chemistry that drives bio-recognition in enzymatic catalysis has been a major source of inspiration for researchers. The esterolytic and amidolytic reactions are arguably one of the most widespread reactions found in nature and essential to the degradation of many biochemical substances. Enzymes like serine proteases, lipases, cholesterol esterases etc. contribute to the same catalytic machinery and mechanism. Even though the enzymes are highly specific and sensitive, they are labile, expensive and have a low density of binding sites. Hence, there is a significant demand for robust and stable substitute that can mimic bio-recognition elements in enzymes. Molecular imprinting facilitates a powerful, generic, facile and cost-effective alternative for enzyme catalysts. In particular, the “catalytic triad” motif of histidine, aspartic acid and serine present in the serine protease family has served as a model for MIP catalysis.

Enzyme mechanism of serine proteases family

Functional groups responsible for the catalytic action are located at rather distant points from each other along the peptide chain in natural enzymes. Close spatial arrangement of these functional groups due to folding of the peptide chain is responsible for their specificity. Here, both the primary sequence of amino acids with functional groups and the peptide’s tertiary structure are crucial. This “discontinuate word” of arrangement leads to a complex, 3D, steric arrangement of the functional groups [9] (Figure 1).

Bovine chymotrypsin, an important serine protease, displays the amino acid triad – histidine, aspartic acid and serine – at positions 57, 102 and 195, respectively. This catalytic triad forms a pocket in the active site so as to set up proton relay systems for cleaving peptide bond adjacent to a hydrophobic residue. The hydrophobic pocket and oxyanion hole found in the active site of native chymotrypsin and the H-bonding interactions among the catalytic entities in the catalytic triad are depicted in Figure 2. Chymotrypsin is specific to esters/amides comprising of phenylalanyl or tyrosyl residues as a part of the carbonyl group which could be accommodated in its hydrophobic pocket [10,11]. They accelerate the rate of peptide bond hydrolysis by \(\sim 10^{10}\) compared to the uncatalyzed reaction. A variety of structural features are accountable for the catalytic efficacy of these enzymes. The various steps in the catalytic process are depicted in Figure 3.

The catalytic process begins with shape selective recognition and binding of the substrate in the active site. The proper binding of the side-chain of the amino acid residue to the recognition site on the enzyme is essential. Then nucleophilic attack by the hydroxyl group of serine leads to the formation of an acyl intermediate. The nucleophilic action of serine is initiated by the proton abstraction by Histidine-57, which is enhanced by the aspartic acid moiety. A covalent bond is formed between the Ser-195 side-chain oxygen and the substrate. The negative charge developed on the peptide carbonyl oxygen is stabilized by hydrogen bonding with amide protons of protease backbone. This region of the protein is called the "oxyanion hole", because it stabilizes the negative charge on the oxygen; the oxyanion hole is critical for catalysis. His-57 donates a proton to the amide nitrogen of the substrate, leading to the release of the C-terminal part of the substrate as a free peptide. The final step of the catalytic proteolysis is the nucleophilic attack of water molecule on the ester bond between the peptide and the Ser-195 oxygen. A second peptide with one amino acid less gets formed and regenerates the serine hydroxyl for further nucleophilic attack. The second peptide then dissociates from the enzyme to allow another catalytic cycle to begin.

In a simple enzyme-catalyzed reaction scheme (Figure 4), the reaction coordinate diagram shows that in order for the enzyme to catalyze the reaction, \(\Delta G_{ETS}\) must be greater than...
Enzymes are efficient catalysts because they exhibit rapid catalytic turnovers. That is, enzymes typically have lower affinity for product than substrate, and many enzymes undergo conformational changes that favour the release of product. In a simple case, the kinetics can be described by the Michaelis–Menten equation.

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\frac{d[P]}{dt} = - \frac{d[S]}{dt} = \frac{k_{\text{cat}}[E][S]}{1 + \frac{[S]}{K_m}} = \frac{k_{\text{cat}}[E][S]}{[E][S] + K_m}
\]

Where, \( E \) is enzyme, \( S \) is substrate, \( P \) is product, \( ES \) is enzyme-substrate complex, \( EP \) is enzyme-product complex, \( k_{\text{cat}} \) is the rate constant of the catalyzed reaction, and \( K_m \) is the Michaelis constant. The ratio \( k_{\text{cat}}/k_{\text{uncat}} \) is used to express the catalytic activity of the catalysts similarly as it is done with antibodies and natural enzymes. Under standard conditions, the rate of the catalytic reaction is proportional to \( k_{\text{cat}}/K_m \).

Figure 5(a) shows the typical Michaelis–Menten kinetics of enzyme action. The substrate is bound to the enzyme to form enzyme-substrate complex in a pre-equilibrium step. The bound substrate is converted to products, which are subsequently released. If the reaction is carried out with large excess of substrate, the rate of the reaction first increases with increasing substrate but then levels off, and at higher substrate concentration, when all active sites are occupied, it becomes zero-order with respect to substrate concentration (saturation kinetics) \[12\].

The Michaelis constant \( K_m \) reflects the affinity of the enzyme for its substrate; formally it is derived from dissociation constant. The lower the value of \( K_m \) (usually given in
millimoles) the more tightly the substance is bound. An ordinary uncatalyzed reaction or a chemically catalyzed reaction (e.g. by acid) shows, under the same conditions in nearly all cases, a straight line relative to substrate concentration (Figure 5(b)).

α-Chymotrypsin rapidly catalyzes the release of p-nitrophenol [13] from N-carbobenzoxy-L-tyrosine p-nitrophenyl ester, nitrophenyl esters of acetic acid, hydrocinnamic acid and carbobenzoxyglycine. Even though biocatalysts increase the rate to several orders and are highly specific, they suffer from several limitations, for instance, incompatibility with organic solvents, extreme pH, and elevated temperatures. The developing technique of molecular imprinting advances a promising and advantageous alternative to overcome the problems associated with biomolecules. The most archaic and extensive efforts toward MIP catalysts have used the “catalytic triad” motif of serine, histidine, and aspartic acid found in the family of serine proteases to serve as a model [14]. Chymotrypsin, an enzyme with a well-recognized catalytic mechanism has long been a model of choice for MIP catalysts. Rate enhancements by MIPs, however, have yet to reach the catalytic rate of this enzyme, which enhances the rate of the hydrolysis of peptide bonds by a factor of \( \frac{1}{10^{10}} \).

Based on the mechanism of enzymatic catalysis, several requisites must be fulfilled in order to obtain a synthetic material showing enzyme-like behavior [15]. These requisites comprise the presence of a cavity corresponds to the shape of the substrate or the transition state of the reaction, and functional groups that act as binding sites, coenzyme analogues or catalytic groups within the cavity in a defined stereochemistry. Two different imprinting methodologies have been used to fulfill these prerequisites and to synthesize “enzyme-analogue-built polymers”: (i) imprinting of the substrate or product analogue and (ii) imprinting with a transition state analogue (TSA). For this, a crosslinked polymer network is formed around a molecule that acts as a template. The monomer mixture contains functional monomers that can interact with the template through covalent or non-covalent interactions. After the removal of the template, an imprint containing functional groups in a certain orientation remains in the highly crosslinked polymer. The shape of the imprint and the arrangement of the functional groups are complementary to the structure of the template (Figure 6) and precisely arranged to interact with the template on rebinding.

The molecular imprinting process comprises four steps: (1) the template is mixed with the vinyl monomers selected to interact with specific functionalities of the template, (2) the template-monomer complex may be formed by covalent or non-covalent associations (or a mixture of both), (3) the complex is co-polymerized with an excess of crosslinking monomer; and (4) the polymer is usually ground to a powder for ease of handling and the template is removed by solvent extraction or chemical treatment. Advantages of molecularly imprinted polymers lie in the durability, relatively inexpensive cost, ease of formation of network polymers, their stability against heat, chemicals, and solvents. In addition, imprinted polymers are formed as network polymer solids.

### Concept of molecular imprinting and design of molecularly imprinted polymers

MIPs are highly crosslinked polymeric porous material with specific recognition sites in terms of shape, size and functional groups to the target molecule and capable of mimicking antibodies and receptors. Molecular imprinting versatile, facile and generic technology for mimicking natural recognition sites of enzymes or antibodies in synthetic polymer networks [16]. Functional monomers are refluxed in an inert atmosphere in presence of a template/target analyte which establishes suitable bonding interactions with polymerizable

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**Figure 4.** Reaction coordinate diagram for an enzyme-catalyzed reaction.

**Figure 5.** Michaelis–Menten kinetics: (a) enzyme catalyzed reaction and (b) an ordinary non-catalyzed reaction or a chemically catalyzed reaction.
The resulting pre-polymer complex subsequently co-polymerized with the crosslinker into a rigid polymer. The template molecules are then leached out from the polymer by using suitable solvent systems by Soxhlet extraction. The resultant 3D-imprint possesses a steric -size and shape- and chemical -spatial arrangement/complementary functionality- memory for the template. These enable MIPs to rebind the template molecule from a mixture provided that the binding sites of the molecular receptors and the guest molecules complement each other in size, shape and chemical functionalities.

The template molecule directs the organization of the functional groups pendent to the functional monomers. Hence selection of the template molecule is the heart of molecular imprinting. Functional monomer, the polymerizable entity is responsible for the binding interactions in the imprinted binding sites. The complementarity between the functionality of the template and that of the functional monomer, say, H-bond donor and H-bond acceptor stabilizes the pre-polymerization complex [17]. The most commonly used functional monomers are methacrylic acid (MAA) and 4-vinylpyridine (4-VP) [18]. The other components that can be varied in the system are the choice of the monomers, the crosslinkers, and to some extent the initiators.

The crosslinking monomer locks and freezes the position of the template - monomer pre-polymerization complex to a rigid three-dimensional polymer network and preserves the exact geometry of the imprint after its removal. The physicochemical properties and rigidity of MIPs, and hence the substrate selectivity of the polymer depends on the nature and extent of the crosslinking agent to a greater extent [19]. Ethylene glycol dimethacrylate (EGDMA) has proven to be the most efficient crosslinking monomer for methacrylic polymers in molecular recognition. For water compatible systems, N,N-methylene-bis-acrylamide (NNMBA) is found to be the most acceptable one. When the non-polar crosslinker divinylbenzene (DVB) is used, it imparts rigidity and hydrophobicity to the polymer support which increase with increase in crosslinking.

Porogens plays a decisive role in molecular imprinting bringing all the components- template, functional monomers, crosslinker and initiator- in the polymerization mixture into one phase and create the essential pores in macroporous polymers. They affect the physical properties like porosity, the specific surface area, and the swelling behaviour of a polymer. Thus, porogens governs the stability of the pre-polymerization complex through intermolecular interactions between the template and the functional monomers in solution and decisively determines the recognition properties of the MIP [20]. In non-covalent imprinting, the porogen offers low hydrogen bond donor and acceptor capabilities, which is a typical feature of less polar aprotic porogens like acetonitrile and chloroform.

Most of the functional monomers and the crosslinking monomers contain one or more polymerizable functionalities. The molecular imprinting traditionally performed as a free-radical polymerization, initiated via either homolytic thermolysis or photolysis of an initiator. One of the most commonly used free radical initiators is 2,2'-azobis(isobutyronitrile) (AIBN).

**Strategies in molecular imprinting**

The imprinting strategy may be covalent, non-covalent, semi-covalent or metal ion mediated as per the nature of monomer-template interactions during polymerization. In covalent imprinting, one or more polymerizable groups of the monomers are coupled covalently to the functionalities on the template.
template molecule to form a polymerizable template-monomer complex. After polymerization in presence of a suitable crosslinker, the template is leached out from the resulting polymer leaving behind the imprints having steric and chemical memory of the template capable of re-forming the covalent bonds under favourable conditions. Even though the kinetics of rebinding is often quite slow, this approach will give more homogenous recognition sites for the template throughout the polymer.

Weak interactions like hydrogen bonding, π-π stacking, ionic or van der Waals interactions are common in biological recognition systems [21]. In non-covalent imprinting, weak electrostatic interactions and hydrophobic interactions hold together the monomers and the print molecules in the pre-polymer complex. Metal ion mediated molecular imprinting is one of the most applicable non-covalent imprinting. The metal ion can act either as a template or as a component of the polymer-template interaction (Figure 7).

Semi-covalent molecular imprinting comprises covalent interactions during imprinting and non-covalent interactions during rebinding. The semi-covalent approach has successfully been used for the preparation of MIPs selective for p-aminophenylalanine ethyl ester [22] and triazines [23]. Sacrificial spacer approach, a type of semi-covalent molecular imprinting was first developed by Whitcombe et al., in which part of the monomer is also cleaved out along with the template molecule [23].

**Characterization methods of molecularly imprinted polymers**

Crosslinked and macroscopic chain polymers are practically difficult to characterize largely on account of their intractable and insoluble nature. A degree of characterization is possible; however, we can distinguish between three levels of characterization: (i) chemical characterization, (ii) morphological characterization, and (iii) characterization of the molecular recognition properties (Table 1).

**Applications of molecularly imprinted polymers**

Molecularly imprinted polymers have been used as tailor-made separation materials [4,33–35], antibody and receptor mimics in assay systems [36,37] biomimetic recognition elements in biosensors [38,39] and artificial enzymes for catalytic applications [17,40–42] and hence they have broad range of
potential applications [43–45]. In this review, we focused only on the efforts in the synthesis and application of organic MIPs as artificial hydrolases, and the rate enhancements achieved by them in hydrolytic reactions.

### Applications of MIPs as artificial hydrolases

Till now, the analogues of the substrate, transition state, or product are used as the template in the imprinting protocol to achieve catalytically active imprinted materials.

#### Imprinting with substrate analogue of the reaction

Imprinting with substrate analogue involves the use of compounds that mimic the reaction complex between the substrate and the matrix. Thus, the catalytic groups are introduced in the site by “baiting” them with the print species and will subsequently act catalytically upon binding of the true substrate. Substrate inhibition can be avoided since the bait molecule may bear little resemblance to either reaction species.

The first report on substrate analogue imprinted chymotrypsin mimic was announced by Leonhardt and Mosbach in 1987 [46]. Imidazole residues were employed to hydrolyze amino acid p-nitrophenyl esters. A pyridine derivative of the amino acid- picolinyl-N-Boc protected amino acid- was used as the substrate analogue template molecule. The functional monomer 5-vinylimidazole and the template picolinyl-N-Boc protected amino acid were coordinated by Co(II) ions using CoCl₂.6H₂O in the pre-polymerization step. Then on copolymerization in presence of the crosslinker divinylbenzene (DVB), followed by removal of the template and Co (II) ions resulted in an esterase MIP (Figure 8). The incorporation of catalytic groups is responsible for the substrate specificity and catalytic activity within the imprinted cavities. The esterase MIP exhibited a 5- to 7-fold rate enhancement in the hydrolysis of Boc-Met (or Leu)-p-nitrophenyl ester over the control polymer esterase CP with statistically distributed imidazole groups. Nevertheless, the template molecule was not a TSA but an analogue of the substrate, which was likely the cause of the polymers rather low rate enhancement.

#### Imprinting with a transition state analogue (TSA)

TSA imprinting is a superior and well-studied approach for the synthesis of artificial esterases. Here, the substrate recognition site of the polymer matrix is designed so as to stabilize the formation of the reaction transition state, thereby lowering the activation energy requirement and leading to an enhanced reaction rate. The transition state of ester
hydrolysis can be mimicked by phosphonate derivatives which are the most widely used TSAs (Figure 9).

Some important phosphonate TSAs used for the synthesis of artificial esterases are (1) p-nitrophenylmethylphosphonate [47] (2) methyl hydrogen p-nitrobenzylphosphonate [48,49] (3) phenyl-1-benzyloxycarbonylamino-3-methylpentylphosphonate [49] (4) phenyl-1-undecylcarbonylamino-3-methylbutylphosphonate [50] (5) p-nitrophenyl phosphonate [51].

The first artificial esterase with the imprints of TSA was reported by the same group of Mosbach [47]. In 1989, D.K. Robinson and K. Mosbach detailed molecular imprinting of p-nitrophenyl methylphosphonate, a transition state analogue of hydrolytic reactions, through Co(II) ion mediated polymerization utilizing poly [4(5)-vinylimidazole] as the functional monomer and 1,4-dibromobutane, as the bifunctional crosslinker. This polymer catalyst was an efficient esterase for p-nitrophenyl acetate (Figure 10). The print TSA molecule, p-nitrophenyl methylphosphonate indicated structural resemblance with the substrate molecule p-nitrophenyl acetate, but contains a tetrahedral phosphoryl group in place of the carboxyl group. The artificial esterase MIP exhibited 60-fold catalytic competence over the control polymer CP. Further, they announced the print TSA molecules as a competitive inhibitor for p-nitrophenyl acetate.

In 1994, Ohkubo et al. reported the first example of imidazole containing “homogeneous and heterogeneous esterase MIPs” with the imprints of “methyl hydrogen p-nitrobenzylphosphonate” TSA for the hydrolysis of p-nitrophenyl acetate [52]. The TSA-imprinted esterases were prepared by Co(II) ion mediated polymerization of the functional monomer 4(5)-vinylimidazole and the TSA. The TSA was obtained by the hydrolysis of dimethyl p-nitrobenzylphosphonate, prepared by the Arbuzov reaction between trimethyl phosphate and p-nitrobenzyl bromide. They employed N,N’-1,2-ethylenbis-(2-propenamide) as the crosslinker for homogeneous esterase MIP and dibromobutane for heterogeneous esterase MIP (Figure 11).

The homogeneous esterase MIP substantially promoted the hydrolytic reaction with \( k_{\text{MIP}} / k_{\text{uncat}} \) ratio of 6.7 in the framework of Michaelis–Menten kinetics. The esterase MIP with ethylene-bis-acrylamide crosslinks was found to be a more efficient catalyst under certain conditions than the dibromobutane crosslinked esterase MIP, but strong pH dependence was also distinguished (Figure 12). For this esterase MIP, the rate acceleration \( k_{\text{obs}} / k_{\text{uncat}} \) values were 1.3 and 2.5 at pH 7.0 and 8.0 respectively, whereas the rate acceleration observed for ethylene-bis-acrylamide crosslinked esterase MIP were 6.7 and 1.9 at the same pH values.

In 1994, Ohkubo et al. reported the synthesis of water-soluble homogeneous esterase MIP possessing N\(^2\)-carbobenzoxy-L-hystidyl group as catalytic entity [48]. The crosslinked poly(ethyleneimine) esterase MIP was utilized for the hydrolysis of p-nitrophenyl N-carbobenzoxy-L-leucinate (Z-L-Leu-PNP). The esterase MIP was derived from poly (2-methyl-2-oxazoline) to control frame polymer chain length and hydrophobicity. They used a transition-state analogue, phenyl 1-benzyloxycarbonylamino-3-methylpentylphosphonate, specific for the substrate Z-L-Leu-PNP for the synthesis of both alkylated and non-alkylated MIPs. The synthesis of esterase MIPs are depicted in Figures 13 and 14. The cavity formed in the polymer matrix showed substrate recognition in the hydrolysis of esters. The non-alkylated esterase MIP enhanced
the catalytic hydrolysis of Z-L-Leu-PNP, compared to Z-L-Phe-PNP and Z-L-Ala-PNP esters. The alkylated esterase MIP did not exhibit catalytic enhancement for hydrolysis of Z-L-Phe-PNP; but equally promoted the rate of hydrolysis of Z-L-Leu/Ala-PNP. Presumably, the hydrophobicity was increased by the C_{10} part in the catalyst which made the substrate inclusion fast. The mimic recognized the difference between phenyl and isopropyl groups of the substrate, but cannot effectively differentiate Z-L-Ala-PNP from Z-L-Leu-PNP. The steric hindrance of the decyl group protected the catalytic
site from the approaching bulky Z-L-Phe-PNP substrate. Probably, the flexibility of the polymer frame of the catalyst led to incomplete substrate specificity.

In 1994, Sellergren and Shea et al. designed highly cross-linked polymer catalysts for the hydrolysis of N-tert-butoxycarbonyl phenylalanine p-nitrophenyl ester [6,53] (Figure 15), which is the first report on the enantioselective hydrolysis of a non-activated ester. They successfully combined covalent and non-covalent binding forces between the template and the functionalized monomers by the simultaneous introduction of different functional groups into the active sites. They utilized a stable transition state analogue for ester hydrolysis...
in the form of a chiral phosphonate analogue of phenylalanine. Catalytically active imidazole and phenol-containing vinyl monomers were attached to this phosphonate TSA through a labile ester linkage. After polymerization in the presence of methacrylic acid and removal of the template, a catalytically active enantioselective binding site – a site complementary to the transition state structure, and the catalytic triad – hydroxyl, imidazole, and carboxylic acid – group similar to chymotrypsin was fabricated. They reported the hydrogen bonding between carboxylic and imidazole as the main driving force in the stereoselective binding step and imidazole group associated with the binding site as the entity responsible for the catalytic action. Non-imprinted control polymers were also carefully designed. The esterase MIP exhibited 2.5 times imprinting efficiency compared with the control polymer. Further, 10-fold rate enhancements were noticed with the esterase MIP in comparison with the phenol imidazole monomer. Enantioselectivity for the hydrolysis of Boc-L/D-Phe-PNP was surprisingly high, with a $k_L/k_D$ value of 1.85. The CPs showed no preference of one isomer over the other which was explained as due to the lack of imprints with specific configuration. The polymer catalysts were found to be catalytically active and retained the enantioselectivity over a period of about 10 years.

In 1995, Ohkubo et al. examined the esterase activities of polymer catalysts imprinted with a transition-state analogue (TSA) or ground-state analogue (GSA) [54]. They employed phenyl-1-N-benzyloxycarbonylamino-3-methylpentylphosphonate as TSA or N-(N-benzyloxycarbonyl-L-leucinoyl) anthranilic acid as GSA for molecular imprinting. The polymers were synthesized using methyl N-acryloyl-L-histidinate and acrylamide as the functional monomers, N,N’-1,2-ethylenbis-(2-propenamide) as the crosslinker. The L-histidyl group-introduced, crosslinked polymers were utilized for the esterolysis of p-nitrophenyl amino acid esters (Figures 16 and 17). A series of polymer catalysts with varying crosslink densities were synthesized and examined. Further, they carried out the esterolytic reaction using the L-histidine monomer. They observed the catalytic activities of the template imprinted polymers in the order of (None) < (His) < GP1 < TPl/C28 < TP2. The polymer catalyst with the imprints of TSA and with low crosslinker content (8.7%) exhibited efficient substrate recognition and the highest esterase activity towards the marked substrate Z-L-Leu-PNP with positively largest activation entropy and lowest activation free energy. The temperature dependency of catalytic parameters was examined. The results implied that the reaction of the polymer catalysts with the substrate in their template-shape recorded cavities was energetically impaired by the restricted proximity between the catalytically active L-histidine imidazole group of the polymers and the susceptible carbonyl group of the substrate. The catalyst with the imprints of phenyl-1-N-benzyloxycarbonylamino-3-methylpentylphosphonate exhibited lower substrate affinity towards amino acid p-nitrophenyl esters like Z-L-Phe-PNP possessing a more hydrophobic and sterically-hindered side chain and Z-L-Ala-PNP having a more sterically facilitated side chain.

In 1995, Ohkubo et al. reported the catalytic activities of a water-soluble polymer and a water-insoluble one, both of which were imprinted using phenyl
1-benzyloxycarbonylamino-3-methylpentyl phosphonate towards \( p \)-nitrophenyl N-(benzyloxycarbonyl)-L-leucinate [55]. Both catalysts possessing L-histidyl group as a catalytic site were prepared by radical polymerization (Figure 18). The template and binding site in 1:1 molar ratio gave a high yield of hydrogen bonded complex. Catalytic activity and substrate selectivity for the hydrolysis of amino acid \( p \)-nitrophenyl ester were claimed. Enantioselectivity of the polymer catalyst was explained as due to the L-histidine residues in the polymer. Both the polymer catalysts exhibited higher catalytic activities than His monomer in the esterolysis of Z-L-Leu-PNP, but the order of relative catalytic activities was reported as, water soluble MIP > water insoluble MIP > His monomer > blank.

Ohkubo et al. in 1996 reported the synthesis of another “water soluble esterase MIP” with the imprints of a racemic transition state analogue phenyl-1-benzyloxycarbonyl-3-methylpentyl phosphonate for the hydrolysis of \( p \)-nitrophenyl N-(benzyloxycarbonyl)-L/D-Leucinate (Z-L/D-Leu-PNP) [56] (Figure 19). The esterase mimic with L-histidine and quaternary trimethyl ammonium groups was synthesized by radical initiated polymerization of methyl-N-acryloyl-L-histidinate, acrylamide and N-(3-trimethylaminopropyl)acrylamide in presence of N,N-ethylene-bis-(2-propeneamide) as the crosslinker. The water-soluble esterase MIP exhibited 4.3 times rate enhancement over the solution reaction containing same concentration of histidine monomer. The esterase MIP accommodated Z-L-Leu-PNP in the substrate recognition site to form catalyst-substrate complex with \( K_m = 2.24 \times 10^{-4} \) mol\(^{-1}\)dm\(^3\). Thus, the reaction cavity was seemed to be predominantly recorded the shape of L-TSA and exhibited 3-fold substrate stereospecificity. Additionally, the water-soluble esterase MIP exhibited substrate shape-selectivity by...
recognizing both the skeletons of the N-benzyloxy (Z) group and the L-Leu side chain of the substrates. Further they presented the rac-TSA as the competitive inhibitor for Z-L-Leu-PNP.

Using stoichiometric non-covalent imprinting strategy, in 1997, Wulff et al. prepared MIPs for esterolysis [57,58]. Monoaryl phosphonate was imprinted with stoichiometric amounts of amidine monomer having high association constants for both carboxylic and phosphonic acid groups (Figure 20). The strong ionic, double-bridged interaction between the amidine moiety and the phosphonate TSA created a well-defined catalytic site in which the ester carbonyl group gets activated. Moreover, the “oxyanion hole” of the cavity stabilized the transition state intermediate of the subsequent hydrolytic reaction. The esterase MIP accelerated the rate of hydrolysis of non-activated esters by more than 100-fold compared to the blank reaction. On the addition of an equivalent amount of monomeric amidine instead of esterase MIP to the buffer solution, only slight rate increase was observed. Polymerization of the amidinium benzoate gave a somewhat stronger enhancement in rate compared to the solution. In conclusion, these results supported the strong catalytic effect of the esterase MIPs. The amidine groups activate the binding sites and act as key function for catalysis in the alkaline hydrolysis of ester. One of the two amidine binding sites inside the cavity provided anchor for the reactant at the carboxylic acid site, while the other promoted the base-catalyzed ester hydrolysis.

In 1999, Lele et al. have prepared chymotrypsin mimics by a novel surface-grafting procedure, utilizing poly (glycidyl methacrylate-co-ethylene glycol dimethacrylate) beads as the carrier support [40–42] (Figure 21). N²-Methacryloyl derivatives of L-amino acids serine, aspartic acid and histidine – were used as functional monomers. N-Nicotinoyltyrosylbenzyl ester was used as the template molecule. The cobalt ion mediated polymerization was carried out in presence of EGDMA crosslinks. The Co (II) ion co-ordinated assembly was then adsorbed onto the preformed poly (GMA-co-EGDMA) beads. The kinetics of the esterolysis of N-benzyloxy carbonyl-tyrosine p-nitrophenyl ester was carried out in presence of imprinted and non-imprinted catalysts. A comparison of the co-operative catalysis of functional monomers, effect of Co (II) complexation during imprinting and the influence of surface grafting were investigated and reported in detail. The highest catalytic activity was observed for trifunctional mimic prepared through Co (II) ion mediated surface grafting and the substrate specificity for N-CBz-Tyr-PNP was 3-fold higher for TSA imprinted esterase than that of trifunctional CP. The mimic synthesized in the absence of Co (II) was reported to be catalytically inactive. The chymotrypsin mimic synthesized by the surface grafting procedure recognized the substrate specifically and its catalytic efficiency was almost 2-fold that of the CP. They examined the individual role of HEMA, MAA, 4-vinylphenol, cysteine and β-alanine in the catalytic efficiency of the polymer catalysts. They observed higher reactivity of the phenolic –OH group from 4-vinyl phenol over the aliphatic –OH group of HEMA. Further, they noticed that the sulfhydryl group of cysteine displayed more nucleophilicity, cooperativity and substrate affinity in the esterolytic reaction than the –OH group of serine. More efficient co-operative action was observed amongst β-alanine, imidazole and hydroxyl groups and the reason was explained as due to the presence of two methylene groups in β-alanine.

Further they synthesized surface grafted trifunctional polymers by varying weight ratio of poly(GMA-EGDMA) to monomers. They observed a strong dependence of hydrolytic activity of the polymers on the surface area of the polymer catalysts. They mimics exhibited higher hydrolytic rates with increase in the concentration of catalytic functionalities of the support as in the case of immobilized enzymes.
Additionally, they examined the role of hydrophilicity and hydrophobicity of the carrier support in catalytic efficiency of the mimics (Figure 22). The mimic grafted on hydrophilic poly(GMA-EGDMA) support exhibited higher substrate affinity with low $K_m$ values, but showed lower reaction rate with low $k_{cat}$ values. Thus, these polymers are consistent with the non-productive binding exhibited by natural chymotrypsin for hydrophobic substrate. But the mimic grafted on hydrophobic support poly(PHMA-EGDMA) exhibited Michaelis–Menten kinetics showing lower $K_m$ values and higher catalytic activity. Thus, non-productive substrate binding could be eliminated by the choice of appropriate support. This discretion is not possible in the case of native enzyme which have pre-determined catalytic clefts.

In 1999, Kawanami et al. reported the hydrolysis of $p$-nitrophenyl acetate catalyzed by the esterase MIP possessing the imprints of $p$-nitrophenyl phosphate TSA and imidazole as the binding site [51] (Figure 23). Network polymers with TSA imprints were prepared by co-polymerization of vinylimidazole assembled around $p$-nitrophenyl phosphate TSA with divinylbenzene as a crosslinker without cobalt ion complexation. The imidazole-containing esterase MIP showed a 2-fold enhancement in rate compared to the CP and an 85-fold enhancement compared to blank uncatalyzed hydrolytic reaction. They examined the effect of nature and content of crosslinking agent in the catalytic activity. The esterase MIPs networks crosslinked with ethylene glycol methacrylate and N,N’-methylenebisacrylamide showed relatively low catalytic activity. Further, they demonstrated the template TSA as a potential inhibitor for the polymer, providing evidence for the creation of the imidazole-containing active site and or oxyanion hole within the network polymer.

In 2001, Ohkubo et al. reported the dependence of substrate-stereospecific properties of esterase MIPs on the alkyl chain length and the content of the crosslinker [59,60]. Esterase mimics containing L-histidine and
trimethylammonium groups were imprinted with a racemic transition state analogue of phenyl 1-benzyloxy carbonyl-3-methylpentylphosphonate for the hydrolysis of \( p \)-nitrophenyl N-benzyloxy carbonyl-L/D-leucinate using crosslinking with varying alkyl chain length (Figure 24). They used N,N\(_2\)-ethyl ene (C\(_2\)), butylene (C\(_4\)), hexamethylene (C\(_6\)), and decamethylene (C\(_{10}\))-bisacrylamide as the crosslinking agents (Figure 25). Reduced catalytic activity was observed with increasing length of alkyl chain of the crosslinker from C\(_2\) to C\(_4\). Greater stereoselectivity was observed with 20 mol\% C\(_4\)-crosslinked esterase MIP compared to C\(_2\)-crosslinked esterase MIP. Further increase of the crosslinker content resulted in a decrease of the catalytic activities; plenty of crosslinker causes the decrease of the "fluctuations" of the cavity. Thus, substrate-stereospecific properties of crosslinked esterase MIPs could be improved by changing the length and the content of the crosslinker. They reported the effect of styrene comonomer in enhancing the stereoselectivities of the esterase MIPs through better cooperativity with the hydrophobic substrate. Maximal stereoselectivity was obtained for 11 mol\% N,N\(_2\)-C\(_{10}\)-bisacrylamide crosslinked MIP, which was copolymerized with hydrophobic styrene monomer.

J.-M. Kim et al. in 2001 reported the synthesis and application of a monomer as template molecule capable of providing a cavity with the shape of the transition state of the reaction as well as binding sites for the substrate and catalytic functionalities [58]. Template monomer was prepared by coupling of diphenyl chlorophosphate and N-hydroxymaleimide. The diphenyl phosphate moiety of the template monomer could mimic the transition state of the reaction. The maleimide group was hydrolyzed to N-hydroxysuccinimide moieties and act as a nucleophilic catalyst for the hydrolysis of the substrate (Figure 26). The polymer was found to be less effective in hydrolysis than catalytic antibodies. These experiments provide the first example of an imprinted polymer-catalyzed hydrolysis of carbonate. Further, the imprinted polymer with labile covalent interactions demonstrate the feasibility of using this technology to the design of new polymer catalysts.

Ohkubo et al. in 2004 reported the esterolytic reaction of amino acid \( p \)-nitophenyl esters with long hydrocarbon chain as N-protecting group [50]. They investigated the acceleration properties of the esterase MIPs in the hydrolysis of long-chain ester–substrate N-dodecanoyl leucine \( p \)-nitrophenyl ester (Figure 27). They designed and synthesized an esterase MIP from long-chain TSA phenyl-1-undecylcarbonylamino-3-methylbutyl phosphonate and 4-[3-methacryloylamino]ethyl imidazole as the nucleophilic binding site. Imidazole containing monomer was synthesized by the amide linkage reaction of histamine dihydrochloride and methacyrloyl chloride. The pre-polymerization complex was copolymerized with hydrophobic styrene monomer and 10\% divinylbenzene crosslinker. The origin of the enhancement of the hydrolytic activity of the imprinted polymer catalyst was the substrate binding process owing to the hydrophobic effect and electrostatic forces within the memorized cavity.

Imidazole group of histidine residue is reported to be the essential catalytic group in the active site of hydrolase proteins. In 2004, J.T. Huang et al. replaced the imidazole moiety with more nucleophilic 4-(N,N-dimethylamino) pyridines in

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**Figure 21.** First reported chymotrypsin mimic [41,42].

**Figure 22.** Hydrophilic and hydrophobic carrier supports designed by Lee et al.
In order to introduce the super nucleophilic 4-dialkylaminopyridines into a catalytic site, they synthesized a polymeric catalyst containing pyrrolidinopyridine moiety by molecular imprinting with \( p \)-nitrophenol methylphosphonate, a TSA, very similar to the transition state of the substrate \( p \)-nitrophenyl acetate in size and shape.

The TSA-esterase MIP containing 4-alkylaminopyridine groups was found to be better than imidazole-appended polymer in artificial enzyme activity (Figure 28). Although 4-alkylaminopyridine supernucleophilic groups do not exist in any proteins, the catalyst containing both imprinted cavities and supernucleophilic moieties, from the point of view of their biochemical nature, was observed to be efficient enzyme mimic.
**Imprinting with product analogue**

Product analogues have also been used as the print molecule in a number of cases, but not in esterolytic reactions. Once again, this strategy is likely to be sensitive to inhibition, but if the polymer is prepared against an intermediate product that will spontaneously undergo further reaction, this problem can be overcome.

N. Kirsch et al. in 2009, reported product analogue imprinted polymer catalyzed Diels-Alder cycloaddition reaction [63] reaction of 1, 3-butadiene carbamic acid benzyl ester and N,N-dimethylacrylamide (Figure 29). Recognized transition state analogues for the endo- and exo-reaction pathways were used as templates for the synthesis of molecularly imprinted methacrylic acid–divinylbenzene copolymers. The recognized transition state is also shown.

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**Figure 26.** Molecular imprinting using template monomer [58].

**Figure 27.** Esterolysis of long chain ester [50].

**Figure 28.** Introduction of nucleophilic 4-(N,N-dimethylamino)pyridines as functional monomer in imprinting [61].
Batch binding studies revealed that the imprinted polymers were selective for the TSA corresponding to the template used in the polymer synthesis. Studies on the influence of the polymers on the catalysis of the reaction of 1, 3-butadiene carbamic acid benzyl ester and N,N-dimethylacrylamide demonstrated a 20-fold enhancement of the rate of the reaction relative to the solution phase reaction. A surprising temperature dependence of the reaction of 1, 3-butadiene carbamic acid benzyl ester and N,N-dimethylacrylamide in the presence of the polymers was observed, which provides a promising route for the development of new catalytic systems.
support for the role of template-functional monomer complexes in the catalysis of the Diels-Alder reaction.

**Transition state analogue imprinted amidases**

Thus, there are so many reports on the hydrolysis of amino acid \( p \)-nitrophenyl esters using TSA imprinted polymer catalysts; but, the amidolysis using artificial enzymes were not reported yet. In our lab, we carried out the hydrolysis of the amide bonds in amino acid \( p \)-nitroanilides and dipeptides using MIPs. We synthesized EGDMA crosslinked chymotrypsin mimic polymer catalysts by incorporating the imprints of stable phosphonate TSA, phenyl-1-benzyloxy carbonylaminoethylphosphonate and the catalytic triad-His, Ser, Asp-using methacrylated amino acid monomers (Figure 30) [63]. The individual and combined effects of imidazole, carboxyl and hydroxyl groups in the polymer matrix on the catalytic amidolysis of chymotrypsin specific and non-specific amino acid \( p \)-nitroanilides were investigated in detail by synthesizing mono, bi and trifunctional polymer catalysts. The trifunctional mimic exhibited a rate acceleration of 75 and an imprinting efficiency of 43 towards the substrate benzoyloxy carbonyl-L-phenylalanine \( p \)-nitroanilide (Z-L-Phe-PNA). The mimic was found to be enantioselective and substrate specific like natural chymotrypsin (Figure 31). Further, imidazole is noticed to be the key catalytic entity for amidase activity.

A series of artificial amidases with varying crosslink densities (60, 75 and 90%) were synthesized using hydrophilic flexible dimethacrylate crosslinkers of varying alkyl chain length (ethylene glycol dimethacrylate/1,4-butanediol dimethacrylate/1,6-hexanediol dimethacrylate) and hydrophobic rigid crosslinker DVB (Figure 32) [64]. The catalyst with 90% EGDMA crosslinks was found to be most substrate specific among the polymers with methacrylic crosslinks. Remarkable reduction in amidase activity was observed as the alkyl chain length of the hydrophilic flexible methacrylic crosslinker was increased. Poor swelling of the polymer networks and lower rate of diffusion of the substrate molecules into its reaction cavities is thought to be responsible for the lower catalytic efficiency of DVB crosslinked polymer catalysts. Among the DVB crosslinked polymer catalysts, 75% DVB-crosslinked polymer exhibited highest amidase activity with efficient substrate recognition, size, shape, stereoselectivity characteristics. 75% DVB crosslinking was observed to be enough to freeze the reactive functionalities in the polymer networks to preserve the geometry of the print molecule as a substrate recognition site for the selective binding of the \( p \)-nitroanilide molecule. Thus, the methacrylate crosslinker was found to be more effective for the methacyralted amino acid monomers to preserve the 3D-memory sites in the polymer matrix.

We extended the investigation with the organic analogues -4-vinylimidazole, allyl alcohol, methacrylic acid - of
methacrylated amino acid monomers [65]. The achiral monomers formed pre-polymer complex with both L- and D-TSAs from the rac-TSA and fabricated cavities with both L- and D configuration in the polymer matrix. The mimic exhibited reduced catalytic activity which may be expected due to the more rigid nature of organic monomers compared to the flexible amino acid monomers and the presence of both type of cavities. Due to the presence of both L- and D-cavities, these mimics exhibited selectivity towards L- and D-substrates and hence did not exhibit any enantioselectivity. The effect of crosslink density on the catalytic efficiencies of the polymer catalysts was also investigated. For the polymers derived from organic monomers, the rate enhancement and enzyme-like behaviour were observed, in the lower range of crosslink density (20 mol %). Replacement of allyl alcohol from the monomer triad by vinylpyridine afforded a catalyst with better enzymatic activity. The pyridine moiety was found to be capable of exerting H-bonding interaction with carboxyl group of methacrylic acid and π-stacking interaction with aromatic side chain of amino acid residue of the substrate p-nitroanilide (Figure 33) [66].

The first peptidolysis reaction utilizing transition state analogue imprinted polymer was demonstrated utterly in the perspective of size and shape-selective substrate recognition (Figure 34) [67]. The peptidase activity of the enzyme mimic polymer catalyst was investigated by following the hydrolysis of dipeptides spectrophotometrically at 207 nm and the kinetic parameters, rate acceleration $k_{acc}$ and imprinting efficiency $k_{im}$, were evaluated. The imprinted peptidase displayed a rate acceleration of $1.67 \times 10^3$ contrasted with the uncatalyzed peptidolysis and an imprinting efficiency of 45 over the non-imprinted control polymer. The artificial peptidase amazingly promoted the hydrolysis of dipeptides having Phe/Tyr amino acid as the C-terminal residues discriminating chymotrypsin specific and non-specific substrates. The mimic exhibited higher rate acceleration (22 times) and substrate specificity towards peptides compared to amino acid p-nitroanilides.

**Advantages of MIPs over native enzymes**

Natural recognition agents are highly substrate specific, but are highly delicate and unstable [68]. The production and purification of homogeneous biomolecules is laborious, complicated, expensive and often require special handling techniques. Owing to several advantageous properties over natural recognition agents, MIPs offer great potential for various applications in catalysis, diagnostics, separation, purification, etc (Table 2).

**Regeneration and reusability**

Regeneration of biomolecules is difficult and limited; they lose their activity within few reuse cycles, which leads to discrepancy in accuracy and increases cost per analysis. But, the molecular imprints – the artificial receptors have high physico-chemical stability towards various external degrading factors. The functional groups MIPs – the highly appealing alternatives – can easily be regenerated without loss of affinity, and hence are highly useful for continuous use. The reusability of the spent amidase MIP was investigated by our group by carrying out the amidolytic reaction after regenerating the polymer [63]. The rate acceleration of the reaction was evaluated for each cycle. The activity of the fresh mimic was considered as the control with 100% activity. It was observed that mimic regenerated could be used for five cycles of amidolysis without much loss in catalytic activity.
After the fifth cycle, a reduction in catalytic activity of only 5% was observed which can be explained as mainly due to the deformation of some of the substrate recognition sites. Reusability makes the enzyme mimic more economic.

**Solvent stability**

Natural enzymes get denatured in organic solvents. But, the MIPs are stable towards a wide range of solvents. The effect of the morphology of the polymer network – porous/less porous – in the amidase activities of the polymer catalysts in the hydrolytic reactions of amino acid p-nitroanilides have been reported [69]. Molecular imprinting was carried out in thermodynamically stable porogen dimethyl sulfoxide and unstable porogen chloroform, to investigate the morphological effects of polymers on catalytic amidolysis. It was found that the medium of polymerization has vital influence in the amidase activities of the enzyme mimics. The polymers prepared in DMSO and acetonitrile (ACN) were found to be macroporous with pore volume of 0.898 cm$^3$/g and 0.876 cm$^3$/g; while that prepared in CHCl$_3$ was observed to be less porous with a pore volume of 0.354 cm$^3$/g. The morphology of the polymer catalysts and their amidase activities were found to be dependent on the composition of reaction medium. The polymer catalyst prepared in dimethyl sulfoxide is observed to be efficient in 1:9 ACN-Tris HCl buffer and that prepared in chloroform was noticed to be stereospecifically and shape-selectively effective in 9:1 ACN-Tris HCl buffer. As the acetonitrile content in the solvent mixture was increased, the amidase activity of the mimics prepared in DMSO and ACN became very low similar to that of uncatalyzed reaction due to the swelling of the macroporous polymer matrix in the reaction medium. The molecular imprint with specific reactive functionalities gets deformed which limits the correct positioning of the T.S of the amidolysis in the cavity. In the case of the mimic prepared in CHCl$_3$, the rate acceleration was found to be increasing as the ACN in the reaction medium increases. The recognition sites of the rigid polymer catalyst synthesized in chloroform were expected to be less deformed even though the ACN content in the solvent mixture was increased. The accessibility of the catalytic sites increases leading to remarkably higher rate acceleration as the acetonitrile content in the solvent changes from 1:9 (5.87) to 9:1 (52.32). The polymer exhibited higher swelling capacity in 9:1 ACN-Tris HCl buffer and then only the polymer can enfold well-defined imprinted sites and hence increased catalytic activity.

**Thermal stability**

Natural chymotrypsin is denatured in the temperature range around 50 °C. But it was found that, the mimics were stable up to 130 °C and at higher temperatures they showed diminished amidase activity [63].
**Shelf-life**

The polymer catalysts after the shelf life of one, two and three years were employed in amidolysis. The amidase MIP did not exhibit considerable loss in enzymatic activity in amidolysis even after three years of shelf life. The mimic showed only 6% decrease after three years of shelf-life [63].

**Effect of temperature**

It is reported that, as temperature increases, the catalytic sites become more accessible and the enzyme mimic exhibit higher catalytic efficiency. Hydrolysis of esters or amides using natural chymotrypsin is usually carried out at room temperatures because of the denaturation of the enzyme at higher temperatures. The optimum temperature for catalytic temperatures because of the denaturation of the enzyme at higher catalytic efficiency. Hydrolysis of esters or amides using natural chymotrypsin is usually carried out at room temperatures because of the denaturation of the enzyme at higher temperatures. The optimum temperature for catalytic amidolysis using MIPs was found to be 45–50 °C [63–67].

**Effect of pH**

Natural chymotrypsin exhibited its optimum amidase activity in pH range of 7.25–7.89 and displayed a Gaussian type pH profile. In acidic and highly alkaline medium, chymotrypsin is found to be denatured. The enzyme mimic polymer catalyst exhibited bell-shaped pH profile in catalytic amidolysis like natural chymotrypsin. The optimum rate was observed at pH 7.75 in acidic medium protonation of the imidazole moiety is thought to be the retarding force of catalytic rate [63–67].

However, MIPs possess many disadvantageous: it is hard to completely remove the print molecule from MIPs; the imprinted polymer is insoluble; and the polymer contains heterogeneous binding sites. Even though MIPs are effective in catalysis, separation and sensing, there has been no real rationality in the design and synthesis of MIPs. There is no ideal or effective procedure for the design of MIPs against macromolecules. To overcome these limitations new functional monomers and polymerization techniques like hierarchical imprinting and grafting techniques are being investigated. These improvements would be highly beneficial for nearly all potential applications. More recently, combinatorial and computational approaches have also been developed for highly specific MIPs.

Molecular imprinting is a prevailing, generic, superficial and cost-effective substitute for the preparation of artificial enzymes via the fabrication of specific selective recognition sites for a pre-determined template in a polymer matrix. The template directs the molecular positioning and orientation of the catalytic functionalities. Crosslinking ensures polymer rigidity that freezes the 3-D molecular architecture of the substrate binding cavity by the subsequent removal of the template. Beyond catalysis, the MIPs potential continues to impact and revolutionize sensor development, drug delivery, solid phase separation etc. The stability, ease of preparation and low cost of these materials make them particularly attractive.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

The authors gratefully acknowledge the support from Council of Scientific and Industrial Research (CSIR), India for awarding junior and senior research fellowships to Divya Mathew.

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