Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Review Article

Molecular Imprinting: An Emerging Technology

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Received on: 14-03-2015
Accepted on: 26-03-2015
Published on: 15-04-2015

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ABSTRACT

This article gives the recent developments in molecular imprinting for proteins. Currently bio-macromolecules such as antibodies and enzymes are mainly employed for protein recognition purposes. However, such bio-macromolecules are sometimes difficult to find and/or produce, therefore, receptor-like synthetic materials such as protein-imprinted polymers have been intensively studied as substitutes for natural receptors. Recent advances in protein imprinting shown here demonstrate the possibility of this technique as a future technology of protein recognition.

This review summarizes the previous and current literature regarding the analytical tools employed for characterization of synthesized MIPs. It is our expectation that this will facilitate researchers to plan their own sophisticated analytical pathway for characterization of MIPs in a more logical and structured fashion, and to begin to appreciate the limitations of the present approaches in this molecularly complex area.

Key-words: Molecular imprinted polymer; characterization; analytical

Cite this article as:
Jesika Rane Priyanka Adhikar, Dr. R.L. Bakal , Molecular Imprinting: An Emerging Technology , Asian Journal of Pharmaceutical Technology & Innovation, 03 (11); 2015. www.asianpharmtech.com*

INTRODUCTION:

Molecular Imprinting is a technique to create template-shaped cavities in polymer matrices with memory of the template molecules to be used in molecular recognition.[1][2] This technique is based on the system used by enzymes for substrate recognition, which is called the "lock and key" model. The active binding site of an enzyme has a unique geometric structure that is particularly suitable for a substrate. A substrate that has a corresponding shape to the site is recognized by selectively binding to the enzyme, while an incorrectly shaped molecule that does not fit the binding site is not recognized.

In a similar way, molecularly imprinted materials are prepared using a template molecule and functional monomers that assemble around the template and subsequently get cross linked to each other. The functional monomers, which are self-assembled around the template molecule by interaction between functional groups on both the template and monomers, are polymerized to form an imprinted matrix (commonly known in the scientific community as a molecular imprinted polymer i.e. MIP). Then the template molecule is removed from the matrix under certain conditions, leaving behind a cavity complementary in size and shape to the template. The obtained cavity can work as a selective binding site for a specific template molecule.

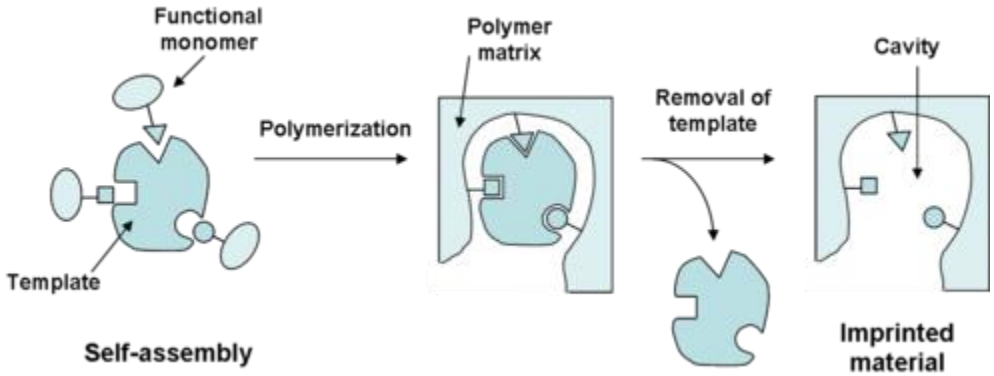


Fig.1 ; Schematic representation of molecular imprinting (a) formation of a pre-polymerization complex between template and functional monomers; (b) polymerization step; and (c) template extraction, [3]

In recent decades, the molecular imprinting technique has been developed for use in receptors, chromatographic separations, fine chemical sensing, etc. Taking advantage of the shape selectivity of the cavity, use in catalysis for certain reactions has also been facilitated. Klaus Mosbach was the pioneer of the Non-covalent approach of Molecular Imprinting.

Advantages of molecular imprinted polymers:

Molecular imprinted polymers (MIPs) possess two of the most important features of biological receptors- the ability to recognize and bind specific target molecules. In comparison to their biological counterparts other than possessing antibody-like molecular selectivity the major advantages of using molecularly imprinted polymers are,

a) Because of the three dimensional polymeric structure they exhibit high physical resistance against external degrading factors and are stable against mechanical stress, high temperature and pressure, resistant against treatment with acids, bases or metal ions and are stable in a wide range of solvents.[17]

b) They can be stored in the dry state at ambient temperature for several years and can be regenerated and reused many times without loss of their molecular memory,

c) polymers can be imprinted with substances against which natural antibodies are difficult to raise. Therefore, artificial receptors prepared by molecular imprinting can provide an attractive alternative or complement to natural antibodies and receptors in many applications.

Disadvantages of molecular imprinted polymers:

MIPs possess many disadvantages. Traditional polymer monoliths tend to be relatively dense leading to difficulty in the accessibility of the binding site sculpt in the three dimensional polymer networks. Such poor mass transport

and permanent entrapment result in inadequate recognition properties. The heterogeneity in binding affinities, slow mass transfer in and out of the polymer matrix, overall low binding affinity, lack of a read out for complexation and trapped template slowly leaching out [18] are the drawbacks most often mentioned for these synthetic polymers.

METHODOLOGIES:

Essentially, two kinds of molecular imprinting strategies have been established based on covalent bonds or non-covalent interactions between the template and functional monomers (Figure 1). In both cases, the functional monomers, chosen so as to allow interactions with the functional groups of the imprinted molecule, are polymerized in the presence of the imprinted molecule. The special binding sites are formed by covalent or, more commonly, non-covalent interaction between the functional group of imprint template and the monomer, followed by a cross-linked co-polymerization [19]. These three methods are described as follow:

A. NON COVALENT IMPRNINTIG:
B. COVALENT IMPRINTING
C. SACRIFICIAL SPACER(SEMI COVALENT)

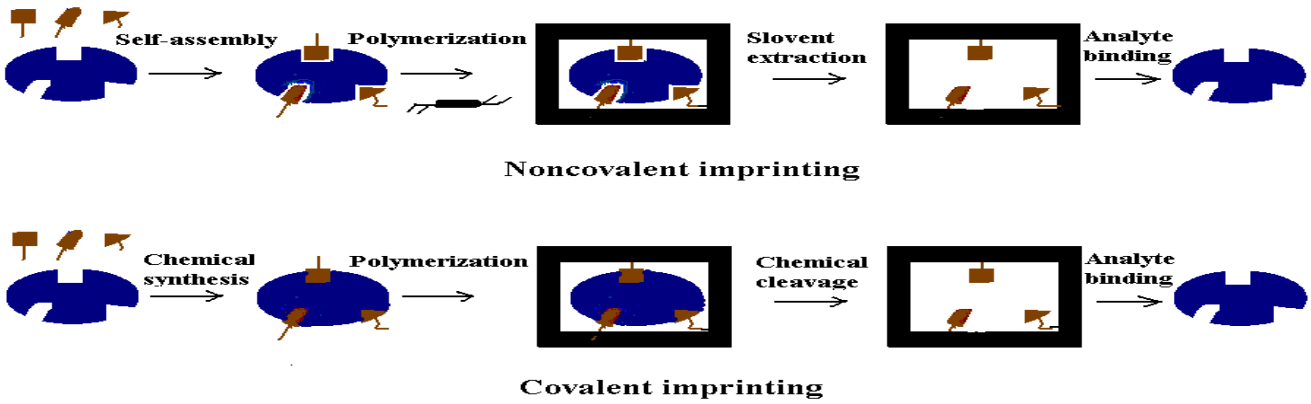
A. NON COVALENT IMPRINTING:

Non-covalent approach is the most frequently used method to prepare MIP due to its simplicity. During the non-covalent approach, the special binding sites are formed by the self-assembly between the template and monomer, followed by a cross-linked co-polymerization [20-21]. The imprint molecules interact, during both the imprinting procedure and the rebinding, with the polymer via non-covalent interactions, e.g. ionic, hydrophobic and hydrogen bonding. 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 [22-23]. Limits to the non-covalent molecular imprinting are set by the peculiar molecular recognition conditions. Most of fact, the formation of interactions between monomers and the template are stabilized under hydrophobic environments, while polar environments disrupt them easily.

ADVANTAGES AND DISADVANTAGES OF NON-COVALENT IMPRINTING:

The advantages of the non-covalent strategies are many and include the myriad templates that may be imprinted, the vast array of functional monomer that may be employed and the fast rebinding kinectics possible. The potential to use a wide range of solvents and a number of initiation methods as well as the low risk of damage to the template during the polymerization have also been cited as advantages of the non-covalent method [24] Despite these obvious advantages, there are also a number of disadvantages inherent to this strategy, including, as stated in the preceding section, a lack of homogeneity of the binding sites. This is due in part because of the need to use an excess of functional monomer to drive template-monomer complex formation as the interactions between monomer and template are generally weak [25].

Fig.1 SCHEMATIC REPRESENTATION OF NON COVALENT AND COVALENT IMPRINTING



B. COVALENT IMPRINTING:

In covalent approach, the imprinted molecule is covalently coupled to a polymerizable molecule. The binding of this type of polymer-relies on reversible covalent bonds. After co polymerization with cross-linker, the imprint molecule is chemically cleaved from the highly cross-linked polymer. Wulff [27-28] and co-workers first produced MIP by synthesizing specific sugar or amino acid derivatives which contained a polymerisable function such as vinylphenylboronate by covalent imprinting methods. After polymerization they hydrolyzed the sugar moiety and used the polymer for selective binding and result shown that for covalent molecular imprinting, selectivity of MIP increases with maximization of cross linker. Moreover, the requirements of covalent imprinting are different than those for non-covalent imprinting, particularly with respect to ratios of functional monomer, cross-linker, and template.

ADVANTAGES AND DISADVANTAGES OF COVALENT IMPRINTING:

Dispite the ability to prepare binding sites using the exact stiochiometric ratio of functional monomer to template, thereby reducing non-specific binding, as well as the increasing stability of the template-monomer complex during polymerization, a factor that lends itself to increased homogenicity of the binding sites, a number of problems arises in case of covalent molecular imprinting. These problems include the relatively low number of templates and corresponding functional monomers that can be used to synthesis appropriate template-monomer complexes. Template removal has also been cite as a problem [26] as well as the slow kinectics of template release or rebinding, which would make this approach less desirable for application requiring fast binding kinectics, such a chromatography. The impact of some of these negative factors can often be overcome, or at the very least decreased, by the use of non-covalent or semi-covalent binding strategies.

C. SACRIFICIAL SPACERS (SEMI-COVALENT):

Semi-covalent molecular imprinting attempts to couple the homogenous binding sites created by covalent imprinting with the fast rebinding kinectics achieved using non-covalent methods by utilizilng covalent linkages to create the binding site but employing non-covalent interaction to effect rebinding [29].

The first truly semi-covalent approach has been cited [30] as that used by Anderson and Sellergren, for the imprinting of p-aminophenylalanine ethyl ester [31]. In this instances, a structural the analogue that had two covalently attached polymerisable group containing ester linkages was used. Upon completion polymerization and removal of the imprint molecule two carboxylic acid groups remained in binding sites, each having the capacity to rebind the amino acid via non-covalent interaction. This method is exemplified by Cacho et al. during the preparation of an MIP prepared for the herbicide, propazine, by semi covalent methods [32].An outline of the procedure used is given in Fig.2

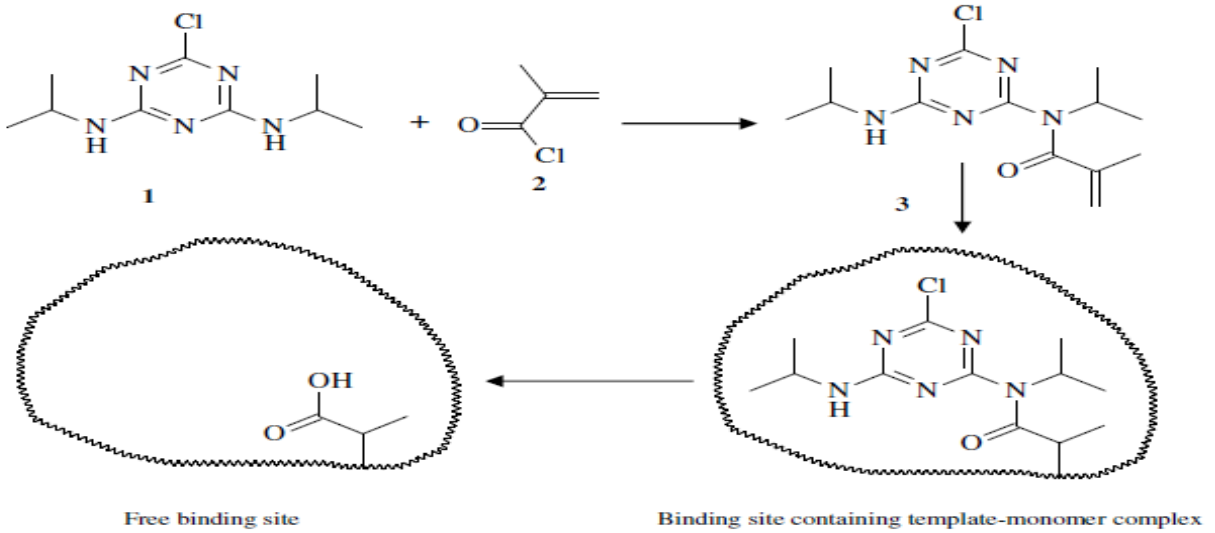


Fig 2: Schematic for the procedure used by Cacho et al. for the semi-covalent imprinting of propazine,1:propazine;2: Methacryloyl chloride; 3:propazine methacrylate.

Fig. 2: depicts the procedure used semi-covalent imprinting of propazine.The template (1) was reacted with methacryloyl chloride (2) in dicholoromethane, to prepare the vinyl amide template template-monomer(3). After polymeristion,basic hydrolysis was used to remove the template, leaving the binding site free non-covalently rebind propazine.

The method shown in Fig.2 was compared to earlier work carried out by Cochoetal. Where propazine was imprinted using a non-covalent strategy [33]. It is interesting to note that semi-covalent method produced a polymer containing 52% more binding sites (though this was still considerablely lower than that expected by such methods) and binding isotherms revealed a heterogeneity index almost 20% higher than that seen in non-covalent approach. However, the binding constant obtained from semi-covalent polymer using isotherm data, at 151.7Mm^{-1}, was considerably lower than that observed for the non-covalent method, which had a value of 1484 mM^{-1} .This was attributed to the high affinity binding sites prepared in non-covalent to the 1:1 stoichiometry used for this method. A variation of this method has been developed, which also uses covalently bound template-monomer complexes and rebind the target via non-covalent means, and is known as the sacrificial spacer method

The sacrificial spacer method was introduced by Whitcombe et al. a method to effectively imprint cholesterol, a molecule considered to have poor functionality for imprinting by non-covalent means [34]. Here the template used was a 4-vinylphenyl carbonate ester of cholesterol and upon removal of the template the 'sacrificial spacer', CO_2 was lost and a recognition site beating a phenolic residue remained , as shown in fig. 3;

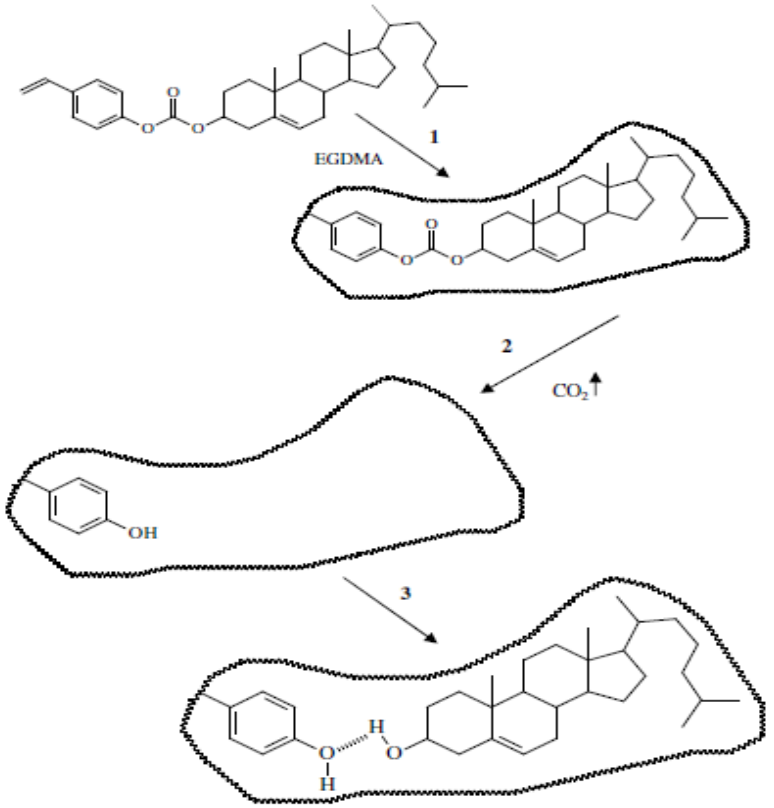


Fig. 3; Sacrificial spacer method of imprinting for cholesterol 1: Polymerisation of template monomer, cholesteryl (4-vinyl) phenyl carbonate to form a polymer containing a template; 2: Removal of template leaving phenolic residue in the binding site; 3: Rebinding of template via. Non-covalent interaction. [34]

After polymerisation of the template-monomer with EGDMA, a polymer is formed containing the template-monomer within the binding sites. Upon removal of the 'sacrificial' CO2 group by hydrolysis, the site is free to non-covalently bind the target. The loss of the spacer reduces problems caused by steric crowding during the non-covalent rebinding step. The binding affinities of these polymers were studied using Scatchard analysis, a

method for quantifying the binding interactions between ligand and receptors, and revealed affinities as high as Kdiss=0.59+/- 0.12 mM at a capacity of 114+/-6µmol/g [34].

Cholesterol has also been the target for chitin-based molecularly imprinted polymers prepared using the semi-covalent method [35].Here, Tong et al. prepared cholesteryl chitin carbonate, a novel chitin derivative, and cross-linked the polymer using toluene-2,4-diisocyanate.This resulted in a polymer, which was able to bind up to 30% more cholesterol than either the non-imprinted polymer or an imprinted polymer prepared via non-covalent means.

FACTORS AFFECTING THE MOLECULAR IMPRINTING:

1. **Template**
2. **Functional monomer**
3. **Cross-linking monomers**
4. **Solvent/ Porogen**
5. **Initiator**
6. **Temperature**

1. **Template:**

The shape, size and chemical functionality of the template species has significant bearing on the imprinting approach utilized. At present, imprints of several hundred template species have been accomplished, including a vast range of molecular shapes and functionality. Generally, with regard to choice of template, factors should be considered such as: solubility in organic solvents, possession of electrostatic functionalities, being chemically inert under polymerization conditions in order to be compatible with free radical polymerization. For larger molecules imprinting can be difficult to achieve.

While the features of the template species are the most important aspects of the imprinting process, it is inclined to be the most restricted in terms of potential variations. Removal of the template provides a cavity, which matches the physical and chemical characterized of the template species.

2. **Functional monomer:**

The chemical characteristics of the functional monomer are of primary importance to the imprinting process. The functional monomer is selected to assist the formation of strong non-covalent interactions between it and the template.

A detailed review of the different approaches employed in optimizing MIP design has been put forward by Karim et al. [41]. Interactions occurring in the pre- and post- polymerization media and the different techniques used to access them were reviewed.

The choice of functional monomers is very important to preserve stable monomer-template complexes during the imprinting process. The functional group is chosen as to complement the chemical functionality of the template molecule. In general, for template with acid groups, monomers with basic functionality are preferred. For instances, MAA is commonly used for basic template [42].for template carrying carboxylic acid moieties, vinylpyridine is the monomer of preference.

3. **Cross-linking monomers:**

Creating a recognition site and deciding on an appropriate imprinting strategy is just the first step in making an imprint. The next step is to prepare the polymer. Free radical vinyl polymerization is the most common method of forming imprinted polymers. The main features of polymers prepared in this way is their high degree of cross-linking due to a substantial proportion (typically between 70 and 95 percent). This high level of cross-linking serves an important function: it provides an element of rigidity in the recognition sites by forming the supporting matrix.

4. **Solvent/ Process:**

Both the imprinting process and physical state (morphology, pore size distribution, pore structure, swellability and toughness) is shaped by the choice and amount of the porogenic solvent used in the polymerization recipe for the MIP as reported by Sellergen and Shea [44]. The influence of the polymerization solvent has multiple roles:

a) It solubilizes all the monomers in the pre-polymerization mixture before polymerization
b) It stabilizes template monomer pre-polymerization complexes
c) It acts as a 'porogen' helping to control the porosity of the resulting polymer.

The most considerable influence of the porogen in non-covalent imprinting is its ability to stabilize template-monomer complexes. The properties of the solvent (porogen) used in the imprinting step in terms of hydrogen bond capacity and polarity is likely to influence the strength of interactions. In a study by Sellergren and Shea [44], it was observed there was no apparent link between selectivity and polymer morphology, but there was a connection between the hydrogen bonding capacity of porogen and polymer selectivity.

5. Initiator:

The most common method of initiation in the synthesis of MIPs is by the formation of free redicals. Free radical formation has generally been performed thermally or photolytically.

The polymerization mechanism involves three distinct phases: initiation, propagation and termination. **Initiation** , where a free radical resulting from the decomposition of the initiator (AIBN) attacks the double bond of the vinyl monomer molecule, forming a heterodimer, possessing a free radical functionality**;** **Polymerization**, where the reaction continues, a chain transfer is formed from the reaction of the free radical and a new monomer unit and propagation occurs rapidly by addition of new monomers under the formation of a growing radical chain; **Termination**, can occur in many ways, the most important, is by transfer of an active centre to another molecule where the radical of two growing polymers chains are coupled, resulting in the removal of radical from the polymerization process. This can occur by two different mechanisms (1) combination or (2) disproportonation.

6. Temperature:

The formation of monomer-template complexes are equilibrium based and thus sensitive to their thermal environment. The effect of polymerization temperature on MIP performance has been the subject of several studies [45; 46 ;47]. MIPs polymerized at lower temperatures from polymers with greater selectivity in contrast to polymer prepared at higher temperatures. To polymerized at colder temperatures it is necessary to use photochemical polymerization.

PREPARATION OF MOLECULAR IMPRINTING POLYMERS BY POLYMRISATION:

a) PRECIPITATION POLYMERIZATION
b) IN SITU POLYMERIZATION
c) BULK POLYMERIZATION
d) MULTI-STEP POLYMERIZATION
e) CORE SHELL POLYMERIZATION

a) PRECIPITATION POLYMERISTION:

In the precipitation polymerization, the polymer was synthesized in the present of a larger amount of solution than that used in the traditional polymerization. Molecular imprinting using a covalent approach was reported to be more efficient than the non-covalent approach. Nevertheless, imprinting using a non-covalent approach presents the advantage that guest binding and release are very fast [48]. Therefore, the present imprinted polymers were synthesized using a non-covalent approach. MIP aggregates or microspheres of a few tenths micrometer in diameter were obtained in precipitation polymerization. Matching the solubility parameter of the resulting polymer to that of the porogen (solvent) was found important in precipitation polymerization. For example; Mono-dispersed microspheres were obtained by copolymerization with divinylbenzene(DVB) in a mixture of toluene and ACN. Thus, mono-dispersed theophylline MIP of ca. 5 _m was prepared by this approach employing MAA as a functional monomer, and HPLC separation of theophylline by the MIP was well carried out.

Precipitation polymerization method was developed by Ye and co-workers (Ye and Mosbach (2001) [49] which can provide 0.3-10 μm size of particles. It is based on the precipitation of the polymeric chains out of the solvent in the form of particles as they grow more and more insoluble in an organic continuous medium (Pérez-Moral and Mayes,

2004) [50]. This method is found to be able to obtain uniform size and high yields of resultant polymers. Yet, it requires large amount of template and high dilution factor.

b) IN-SITU POLYMERISTION:

In situ polymerization is a very simple method for preparing MIPs as it is a one-step for HPLC or SPE separation (Hosoya *et al*., 1996 [52]; Zhang *et al*., 2003 [53]; Lin *et al*., 2006 [54]) where the polymerization is carried out directly in a chromatographic column. Matsui and his co-workers first used the in-situ polymerization technique for preparation of molecularly imprinted monoliths (Matsui *et al*., 1993 [55]; Matsui *et al*., 1995[56]). Its good porosity and permeability makes it a favorable method in preparing stationary phases for chromatography and SPE (Liu *et al*., 2005[57])

c) BULK POLYMERISATION:

Molecularly imprinted polymers can be prepared in a variety of physical forms to suit the final application desired. The conventional method for preparing MIP is via solution polymerization followed by mechanical grinding of the resulting bulk polymer generated to give small particles and sieve the particles into the desired size ranges, which diameters usually in the micrometer range [58-59]. This method, by far the most popular, presents many attractive properties, especially to newcomers. In fact, it is fast and simple in its practical execution and it does not require particular operator skills or sophisticated instrumentation. Particle sizes <25 μm are usually used in chromatographic studies [60]. Such ground and sieved particles have been packed into conventional HPLC columns, immobilized on TLC plates, and entrapped in capillary columns using acrylamide gels or silicate matrices.

Although bulk polymerization is simple, and optimization of imprinting conditions is relatively straightforward, however, bulk polymerization method presents many drawbacks anyway. First of all, the particles obtained after the last sieving step have a highly irregular in size and shape, some interaction sites are destroyed during grinding, and thus lead to a negative impact on chromatographic performance and lower MIP loading capacity with respect to theoretical values. Moreover, the procedure of grinding and sieving is cumbersome, and it causes a substantial loss of useful polymer, that can be estimated between 50 and 75% of the initial amount of bulk material. Since a portion of polymer can only be used as packing material, this method suffered high consumption of the template molecules. Last, but not least, due to its exo-thermical nature, bulk polymerization cannot be scaled-up without danger of sample overheating.

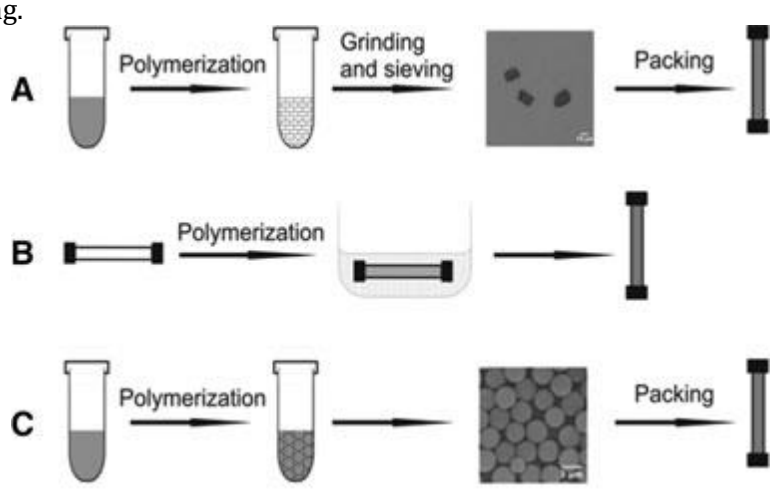


Fig 4: Polymerization approaches for MIPs 1) Bulk polymerization 2) In-situ polymerization 3) one step or precipitation polymerization [62]

d) MULTI-STEP SWELLING POLYMERISTION:

Multi-step swelling polymerization used in molecular imprinting was first described by Hosoya et al. [63]. A series of uniformity sized diaminonaphthalene imprinted particle beads were was prepared in an aqueous two step swelling and polymeristion method for HPLC. The process was based on the non-aqueous technique employed

by Matsui et al. [64] for the formation of imprinted rod materials. The ability of applying the method in aqueous media was shown by the selective recognition of templates in both producers.

Mono-disperse particle were produced by the step-wise swelling of a polystyrene seed particle with a mixture of fresh monomer and an activating solvent (dibutylphthalate). In the second stage, non cross-linked seed particles prepared by suspension polymerization can be swollen with porogen, template, functional monomer and cross-linked for the preparation of uniform-sized MIP particles by photo or thermal polymerization.

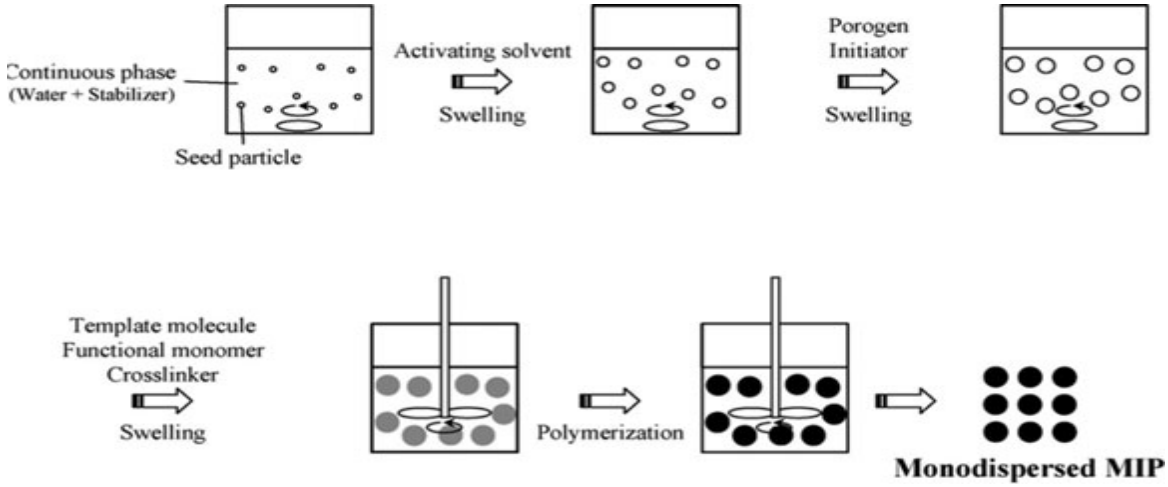


Fig 5: Preparation of mono-dispersed MIPs through multi-step swelling and polymerization [65]

Mono-dispersed MIPs are easy to prepare. However, a disadvantages of the method is that template molecule and functional monomer interactions could be disrupted as water is used as a continuous phase.

The group of Hosoya and Haginaka [66, 63] have utilized the multi-step swelling technique for the imprinting and application of polymeric beads with different templates and functional monomers. MIPs for β-estradiol and bisphenol A (BPA) [67], catechingallate [68] were prepared using 4-vpy and 2-vpy as functional monomers respectively. A hydro-organic mobile phase (for example – a mixture of phosphate buffer and organic modifier such as acetonitrile, ethanol or 2-propanol as an eluent) was employed for the specific recognition of each template molecule. Hydrogen bonding interactions between a template molecule and pyridinyl groups on the polymer mainly worked for its template recognition and its shape recognition. Furthermore, MIPs for each template molecule were prepared and evaluated using a hydro-organic mobile phase. Cinchona alkaloids [69] and atropine [70] were prepared using MAA or acrylamide as a functional monomer. Ionic and hydrophobic interaction worked for template recognition in both studies.

Additionally, MIPs for chiral templates such as (s)-naproxen [66;71], (s)-ibuprofen [72], (s)-propranolol [73], d-chlorpheniramine (d-CP) [74], d-brompheniramine (d-BP) [75, 76], N-protected L-amino acids [77] and (-)-ephedrine [78] were prepared by multi-step swelling and polymerization. A hydro-organic mobile phase or organic mobile phase was used to get baseline or near baseline resolution of enantiomers. A high degree of chiral duicrimination was exhibited by ibuprofen and partial resolution of structural analogue was attained [72]. Chromatographic conditions were optimized by elevating the colume temperature and reducing the flow of rates applied to naproxen imprinted beads [66].

Multi-step swelling and polymerization was also applied to the imprinting of the β-blokers propranolol [79]. The imprinted beads demonstrated ability to enantio-selectively discriminate between enantiomers of structurally realetd compounds. This strategy employed for (s)-propranolol was then applied for imprinting of the chiral template d-CP [74] which was optimized in a cross-selectivity study [75]. The retentive and enantio-selectivity properties of d-CP, d-BP and their structurally related compounds using the MIPs were studied using hydro-organic mobile phases. Ion exchange and hydrophobic interactions work mostly for the retention and enantiosepartion of d-CP and d-BP on the both MAA-co-EGDMA and TFMAA-co-EDMA polymers in hydro-organic mobile phases. MIPs gave the highest enantioselectivity for the template molecule. In addition, MIPs for d-CP and d-BP gave very similar enantioselectivity and resolution of chlorpheniramine (CP) and

brompheniramine (BP). Therefore, CP or BP could be used as a structural analogue to each other to prevent the leakage of a template molecule.

e) CORE SHELL POLYMERIZATION:

Core shell particles are formed in two stage process from a seed latex. This is in contrast to the multi-stage swelling polymerization described above. The seed particle which may be cross-linked is surrounded by a shell of new polymer in a second emulsion polymerization, which allows the imprinted sites to be positioned near or at the surface of the beads. Perez et al. [80] prepared sub-micrometer surface imprinted particles for cholesterol by two stage aqueous emulsion polymerization with a poly (divinylbenzene) shell over a cross-linked poly(styrene) core. Particles were produced with hydro-phobic recognition cavities in the surface of hydrophilic bead by using a specially designed template-surfactant. Both a template surfactant (TS) pyridinium 12-(cholesteryloxycarbonyloxy)dodecanesulfate and a polymerisable surfactant (PS) (pyridinium 12-(4-vinylbenezyloxycarbonyl) dodecanesulfate were synthesized in the second polymerization step and used in the preparation of surface imprinted core shell particles. A distinguishing aspect of this type of surface imprinting is that the template is positioned at a predefined distance from the polymer surface at the same time stabilizing the particles during the second polymerization step.

APPLICATION OF IMPRINTED POLYMERS:

The process of molecular imprinting is a simple concept that creates macromolecular matrices that exhibit selectivity molecular recognition behavior. Owing to the ease of preparation and realatively low cost, stability against high temperatures and pressures, chemical resistance to harsh environments, and the ability to act as synthetic biological receptors, imprinted polymers have lead to useful applications in several areas. Novel using different preparation methods. As a result advances in this direction have brought better use of MIPs in –

(a) Separation
- Chromatography
- Capillary electro-chromatography
- Solid-phase extraction

(b) Sensor
(c) Catalysis Enzyme-mimic
(d) Template assisted synthesis
(e) Molecular imprinted sorbent assay-antibody mimic

a) SEPARTION:

Molecular Imprinting allows the molding of complementary binding sites for target molecules into synthetic polymers (MIPs). The recognition properties displayed by MIPs can be on a par with their biological counterparts and they have therefore gained ground as a robust selector phase in chromatography or SPE or as receptors in chemical sensors. This issue is devoted to recent developments within this burgoning field in separation science.

- **CHROMATOGRAPHY:**

Molecularly Imprinted Chromatography is one of the most traditional applications of molecularly imprinted polymers [81, 82, 83] especially for Liquid Chromatography (LC) [84, 85] with MIPs usually synthesized by bulk polymerization, ground and sieved mechanically and subsequently packed in a chromatographic column [86]. However, the mechanical processing leads to irregular particles with relatively broad size distribution, resulting in packing of irreproducible quality. For this reason monolithic molecular imprinting columns have been recently prepared directly inside stainless steel columns or capillary columns [87, 88]. The monolithic MIPs have fewer nonselective sites than the conventional bulk MIPs particles, even if the polar porogen used for MIPs synthesis

can give poorer enantiomeric separation. Many efforts to decrease heterogeneous size distribution have been made also by preparing spherical and mono-dispersed beads as HPLC stationary phases [89].

- **CAPILLARY ELECTRO-CHROMATOGRAPHY:**

MIPs have also been used as media for Capillary Electro-chromatography (CEC). CEC is a hybrid separation technique that combines the stationary phase of LC with the electrosmotically driven mobile-phase transport of electrophoresis. MIP-based micro-columns for CEC are recently realized for separation of several compounds [83, 97, 98].

- **SOLID-PHASE EXTRACTION:**

Solid Phase Extraction (SPE) is another important area of application of MIPs in analytical chemistry [99, 90, 100–103]. MIP for Solid-phase extraction (MISPE) has been applied both in on-line and off-line procedures. MIPs particles, used as selective sorbent materials, can be packed in an HPLC pre-column for the on-line mode and in a cartridge between two frits for the off-line mode [104].The on-line MISPE procedure, is directly coupled with specific analytical systems such as HPLC, minimizing samples manipulation and reducing the loss of analytes and the risk of contamination [102, 105]. Furthermore, this method considerably reduces the time for pretreatment of the samples.
The principle of MISPE is based on the same main four steps as classical SPE: conditioning of the sorbent, loading of the sample, washing away interferences and elution of the target analytes (Figure 6). In the loading step, the sample is percolated through the MIP sorbents. Generally, this solvent must have a polarity similar to that used in the polymerization process, since it increases the number of interactions between analytes and specific binding sites in the MIP sorbents [106].

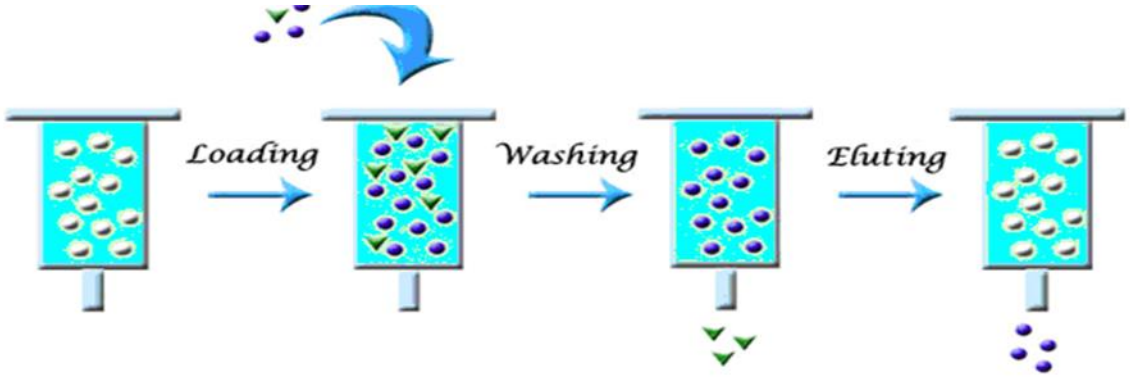


Fig 6: The four steps of Molecularly imprinted solid phase extraction.

b) Sensors :

One of the areas where specific recognition phenomena play a key role is in sensor technology. A sensor is characterized by two key components a recognition element, which has a specific interaction with an analyte or environmental condition, and a transducing element, which converts this interaction into a measurable effect. Many sensors for environmental monitoring, biomedical and food analysis etc. rely on natural receptors, such as antibody/antigen, enzymes, nucleic acids/DNA, cellular structures/cells, as the specific recognition elements due to their evolved high affinity and specificity [127]. Molecular imprinting technology (MIT) offers an alternative means to produce materials that are able to mimic natural binding entities. 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 toconstruct chemical sensors based on these materials as the recognition elements [128], 1a. The analytical techniques by which binding is transduced into a readout are varied and include measuring changes in the MIP'soptical (*e.g.*, fluorescence) [129], electrochemical properties [130], mass (QCMand SAW) [131] and refractive index (SPR) [132].

c) Catalysis - Enzyme mimic :

One strong driving force in the development of MIPs has been them mimicking of enzyme action. Creation of enzyme mimics or artificial enzymes as novel catalysts [137] has been a dream of chemists for a long time. Imprinted polymer catalysts are yet to match enzymes or even catalytic anti-bodies in terms of rate enhancements [138]. This is partly due to the involvement of just a single functional monomer in the catalytic sites of polymers prepared so far, compared with the highly cooperative interactions of numerous amino acid residues in the active sites of biological catalysts. However the true potential of 'printzymes' lies not in their ability to compete with proteins but to complement them as robust catalysts made for particular reactions for which no enzymes can be found. As an example of this new kind of catalyst, researchers have used arhodium-containing polyurethane imprinted with a chiral ligand to catalyze the reduction of ketones to alcohols yielding an enantiomer ratioconsistent with a 'memory' for the chiral template [139].

d) Template assisted synthesis :

MIPs have also been used for synthetic applications [140] in controlling or inducing certain chemical reactions. Even though the polymers are not catalytic in this approach, they facilitate the assembly of the reactants through their specific binding properties. By using a specific reaction component as a print molecule, whether it is a product or a reactant, the reaction equilibrium can be shifted in a desired direction using molecularly imprinted polymers. Thus imprinted polymers are used to shift thermodynamically unfavorable equilibrium of enzymatic reactions.

The principle is that reaction product is constantly removed through adsorption on an imprinted polymer which has been prepared using reaction product as a template. As model system evaluated the enzymatic synthesis of aspartame [141] from *Z*-L aspartic acid and L-phenylalaninemethyl ester. Addition of polymer imprinted against *Z*-aspartame resulted in considerable increase (40%) in product yield. Continuous isolation of the product would also be possible in the same way simply by physically separating the adsorbent. In view of the attractive physical features displayed by molecularly imprinted polymers, such as high pressure and temperature stability, allowing MIPs to withstand sterilization conditions, this methodology may find use in various synthetic applications.

e) Molecular imprinted sorbent assay - antibody mimics :

A very attractive application of molecularly imprinted polymers is in immunoassay-type binding assays [147] instead of antibodies. Molecularly imprinted sorbent assay [148] (MIA) means radio gland assay using molecularly imprinted polymers as recognition elements. In clinical and research laboratories the most common applications of antibodies are in sandwich-type or competitive immune assays [149] and immuno- affinity chromatography. They are also used in immune-sensors in combination with some form of transducer that detects the binding of antigen and antibody directly [150]. The binding utilises the recognition properties of an antibody for the antigen, in which the antigen fits exactly into the antibody's binding site. Molecularly imprinted materials appear to offer a potential recognition element alternative to natural antibodies. MIPs have the following advantages:

They are more stable than their biological counterparts; they are applicable to both aqueous and non-aqueous assays; there is no need for conjugation of the template to an immunogenic carrier as haptens for antibody production and the need to use laboratory animals for antibody production is avoided. Molecularly imprinted polymers cannot compete intheir present forms with natural antibodies for use in techniques in whichthey are used in their soluble form (e.g., in immunodiffusion, immunoelectrophoresis, immunoblotting, and tissue immunofluorescence).However for techniques such as immunoassay, immunoaffinity chromatography, and immunosensors, which utilize antibodies bound to a solid support molecularly imprinted polymers appear to offer a potential recognition element alternative to natural antibodies [151].

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