MIP EXTRACTION APPLICATIONS IN QUANTIFICATION OF FEW DRUG MOLECULES IN BIOLOGICAL SAMPLES

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ABSTRACT

Molecularly imprinted polymers are extensively cross-linked synthetic materials with artificially generated recognition sites able to rebind a target molecule specifically even in the presence of other closely related compounds from biological fluids and environmental samples. Molecular recognition between a molecular receptor (host- polymer) and a substrate (guest-template) in a matrix containing structurally related molecules requires discrimination and binding and their approaches in molecular imprinting and their types are studied. Synthesis of molecular imprinted polymers in different polymerizations studied. For MIP making template, monomer, cross-linkers, porogenic solvents, initiator and polymerization temperature. General procedure for synthesis of mip is determined and applications of mip in extraction of drug molecules from Biological Samples.

Keywords: Template, Monomer, Initiator, Porogenic.

1.1 GENERAL INTRODUCTION

Biological samples are commonly considered to be complex matrices, and their analysis requires adequate sample preparation prior to separation, detection and quantification. For example, biological materials, such as blood and urine, often contain matrix components like proteins, inorganic salts, and organic compounds, with many analytes in these samples present in trace amounts. Even though highly developed selective detection techniques provided by mass spectrometry are available direct injections of crude samples are not recommended. Since these matrix components inhibit or enhance the analyte ionization, hampering accurate quantification. Therefore, nowadays sample preparation or pretreatment is considered as a most important step in the analytical process and it is necessary to extract, isolate, fractionate, and/or concentrate the

analytes from complex matrices, and these methods may greatly influence the reliable and accurate analysis of these materials. [1]

Another valuable aspect of using cleaned samples is the reduction in the time needed to maintain instruments, thus reducing the associated costs. The main objectives of sample pretreatment techniques are:

1) They help to retain the analytes of interest and at the same time efficiently remove the endogenous interferences.
2) They convert the analyte into a more suitable form for detection or separation.
3) They help to provide a robust, reproducible method independent of variations in the sample matrix.

This could be achieved by employing a wide range of techniques. Although many traditional sample preparation methods are still in use the trends in recent years have been towards:

1) The ability to use smaller sample sizes even for trace analysis.
2) Greater specificity or greater selectivity in extraction.
3) Increased potential for automation or for on-line methods reducing manual methods.
4) To minimize the amount of glassware and organic solvents to be used
5) The main sample preparation techniques used are:

1) Liquid-liquid extraction (LLE) and
2) Solid-phase extraction (SPE).

Liquid-liquid extraction (LLE): LLE of an analyte is based on its partition between an aqueous phase and an immiscible organic phase. However, Traditional liquid-liquid extraction (LLE) does not fulfill current requirements in sample preparation and it has been displaced from laboratories in recent years by new extraction techniques [e.g., solid-phase extraction (SPE), solid-phase micro extraction (SPME), micro SPE (MSPE), liquid phase micro extraction (LPME)].

1.2 SOLID PHASE EXTRACTION (SPE)

Solid phase extraction (SPE) is an extraction method that uses a solid phase and a liquid phase to isolate one, or one type, of analyte from a solution. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantify the amount of analyte(s) in the sample. [Zwir-Ferenc et al. (2006)]. SPE is the most widely used QuEChERS (standing for quick, easy, cheap, effective, rugged and safe), sample preparation procedure today. With SPE, many of the problems associated with liquid/liquid extraction can be prevented such as

- incomplete phase separations,
- less-than-quantitative recoveries,
- use of expensive, breakable specialty glassware and
- Disposal of large quantities of organic solvents.

SPE is more efficient than liquid/liquid extraction, yields quantitative extractions that are easy to perform, is rapid, and can be automated. Solvent use and lab time are also reduced.

The main principle involved in SPE is the partitioning of solutes between two phases. It uses the affinity of solutes dissolved or suspended in a liquid (known as the mobile phase) for a solid through which the sample is passed (known as the stationary phase) to separate a mixture into desired and undesired components. The result is that either the desired analytes of interest or undesired impurities in the sample are retained on the stationary phase. The portion that passes through the stationary phase is collected or discarded, depending on whether it contains the desired analytes or undesired impurities. If the portion retained on the stationary phase includes the desired analytes, they can then be removed from the stationary phase for collection in an additional step, in which the stationary phase is rinsed with an appropriate eluent. [2]

1.2.1 SORBENT FORMATS

The stationary phase comes in the form of a packed syringe-shaped cartridge, a 96 well plate or a 47- or 90-mm flat disk, each of which can be mounted on its specific type of extraction manifold.

In SPE technique four types of sorbent formats exist.

i. Free disks (which are generally 47 or 90 mm in diameter or the standard filtration size).
ii. Disks in syringe barrels-cartridge (which vary in size from micro sized disks in 1 ml syringes to a 6 ml syringe).
iii. 96-well micro titer plate configuration that uses the 1-ml disk and
iv. The SPE pipette tip.

CARTRIDGES

The main popular format in SPE is the cartridge type. The typical SPE cartridge consists of an open syringe barrel containing a sorbent packed between porous plastic, stainless steel or polyethylene frits. The syringe barrels are constructed of polyethylene, polypropylene, glass, or metal. The open portion above the sorbent packing, generally referred to as the ‘‘sample reservoir,’’ is available with volumes from about 1–20 mL. The standard outlet of the cartridge design is Luer-lock fitting.They are available in a wide range of sizes, with volumes ranging from

1 ml to 50 ml. During selection for the optimum cartridge size for a particular application, the following factors must be considered:

- Ability to retain all analytes
- volume of original sample and
- Final volume of the purified sample after elution.

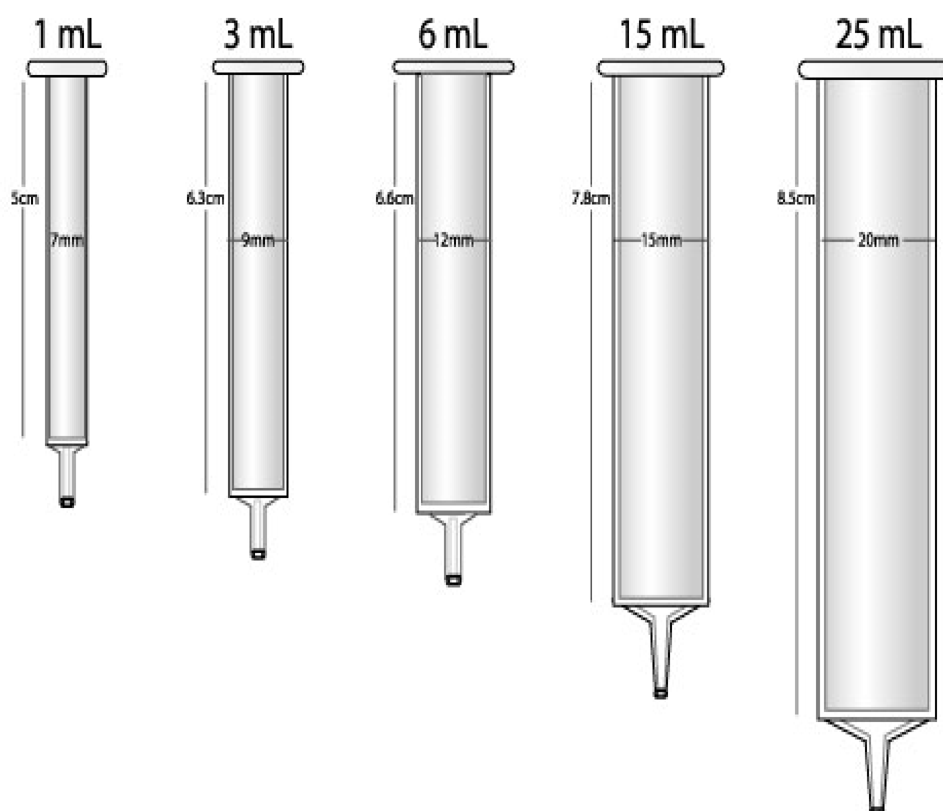

Figure 1.2: Various sizes of SPE cartridges

The limitations of packed SPE conventional cartridges include restricted flow-rates and plugging of the top frit when handling water-containing suspended solids such as surface water or wastewater.

1.2.2 SPE EXTRACTION PROCEDURE

SPE is a sample preparation or sample clean-up technique used to separate compounds of interest from interfering matrix components. The actual process of SPE has been explained in four steps:

1) conditioning
2) sample introduction or load

3) washing and
4) Elution.

1) **Conditioning:** It is required to wet the surface of the sorbent and to remove any impurities that may be present on the unused tube or disk. The amount of conditioning solvent is usually one full column volume if using a tube or cartridge and about 5mL for a 47-mm disk.

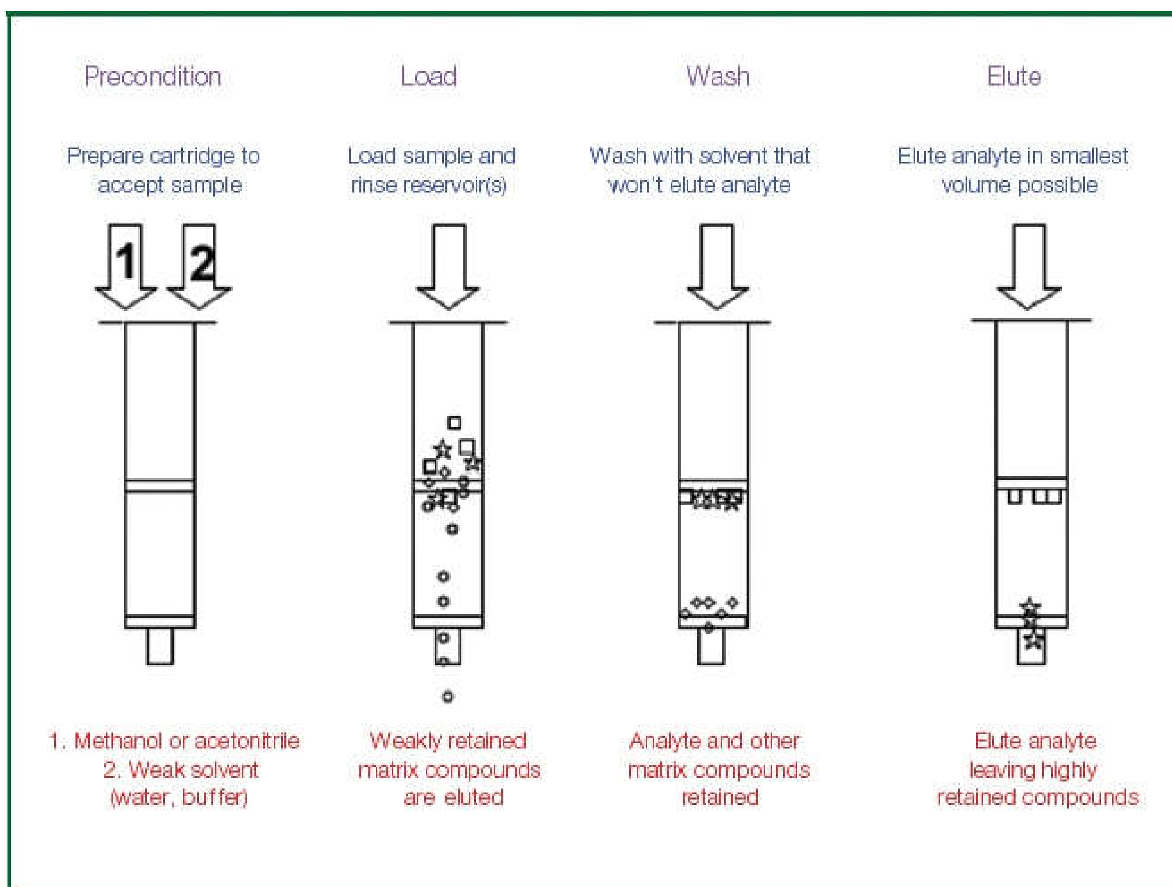

Figure 1.3: SPE extraction procedure

2) **Sample introduction or load:** Tubes can be processed individually by simply pouring the liquid sample on top of the frit or disk and allowing the solvent to drip through the packed bed by gravity. Other methods include the use of syringe plungers and single tube processors fitted for the tube design and attached via a tube adapter or Luer-lock fitting.

3) **Wash**: The washing step typically uses similar volumes as the conditioning step. Elution: Eluting the analytes of interest from the packing is customarily done with as small a volume as necessary to completely remove the analytes while leaving behind any interfering compounds that were retained during the wash step. Several smaller eluting volumes usually remove the analytes better than one larger volume. Depending on disk or tube size, eluting volumes range. [3]

DRAWBACK

Solid phase extraction (SPE) is the most popularly used technique for its advantages like time and cost saving application and minimal consumption of organic solvents. However, one of the major drawbacks associated with SPE is the low selectivity.

1.3 MOLECULARLY IMPRINTED POLYMERS (MIP)

1.3.1 INTRODUCTION

Molecular Imprinting Technology (MIT) is a technique to design artificial receptors with a predetermined selectivity and specificity for a given analyte, which can be used as ideal materials in various application fields. The technique of molecular imprinting, introduced in 1972 by Wulff and Sarhan and much expanded by the work of the group of Arshady and Mosbach in 1980s, has been shown to be capable of producing materials with “antibody-like” selectivity.

Molecularly imprinted polymers are extensively cross-linked synthetic materials with artificially generated recognition sites able to rebind a target molecule specifically even in the presence of other closely-related compounds from biological fluids and environmental samples. The process of molecular imprinting often consists of the following three steps.

1) The imprint is obtained by arranging polymerizable functional monomers around a template as the target molecule, and complexes are then formed through covalent or non-covalent or semi-covalent molecular interactions between the template and monomer precursors.
2) The complexes are assembled in the liquid phase and fixed by cross-linking polymerization.
3) Removal of the template through extraction or hydrolysis with appropriate solvents leaves behind vacant recognition sites exhibiting high affinity for the target molecule. These sites are complementary both sterically and chemically to the template molecule, resembling the “lock and key” paradigm of enzymes postulated by Fischer in 1890s.

MIT is considered a versatile and promising technique which is able to recognize both biological and chemical molecules including amino acids and proteins, nucleotide derivatives, pollutants, drugs and food. Further, application areas include: separation sciences and purification, chemical sensors, catalysis, drug delivery, biological antibodies and receptors system. [4]

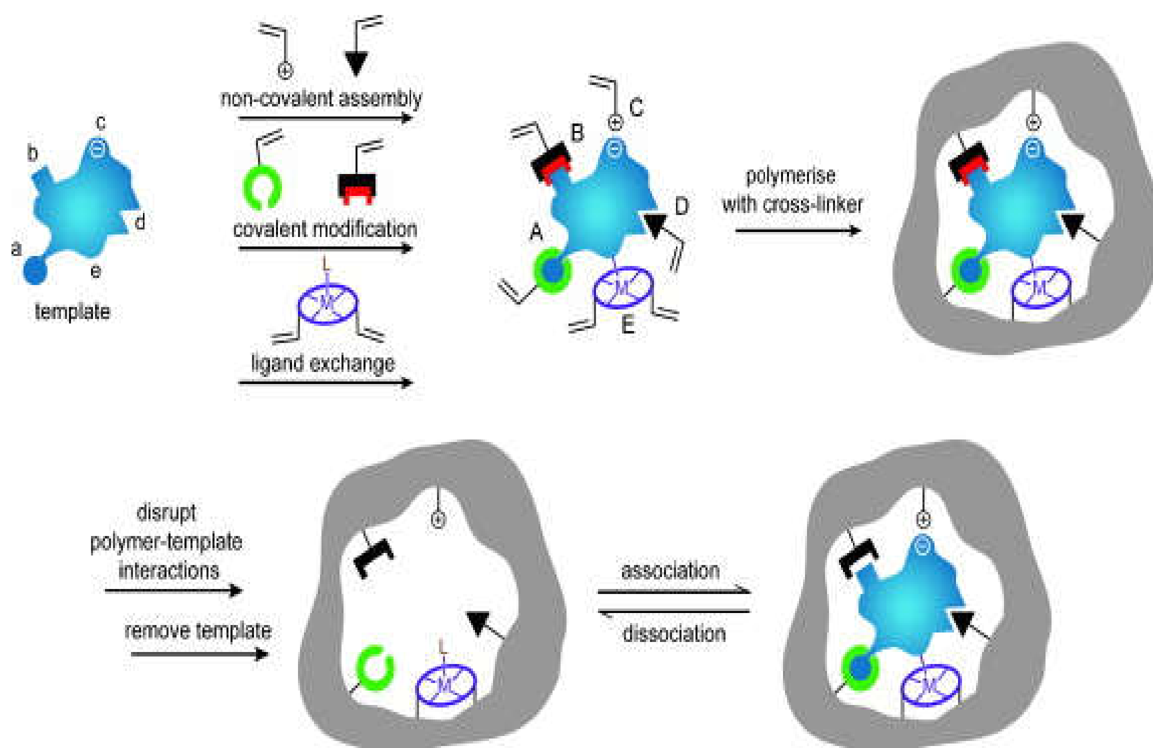

Figure 1.4: Various interactions involved in the formation of molecularly imprinted polymer cavity

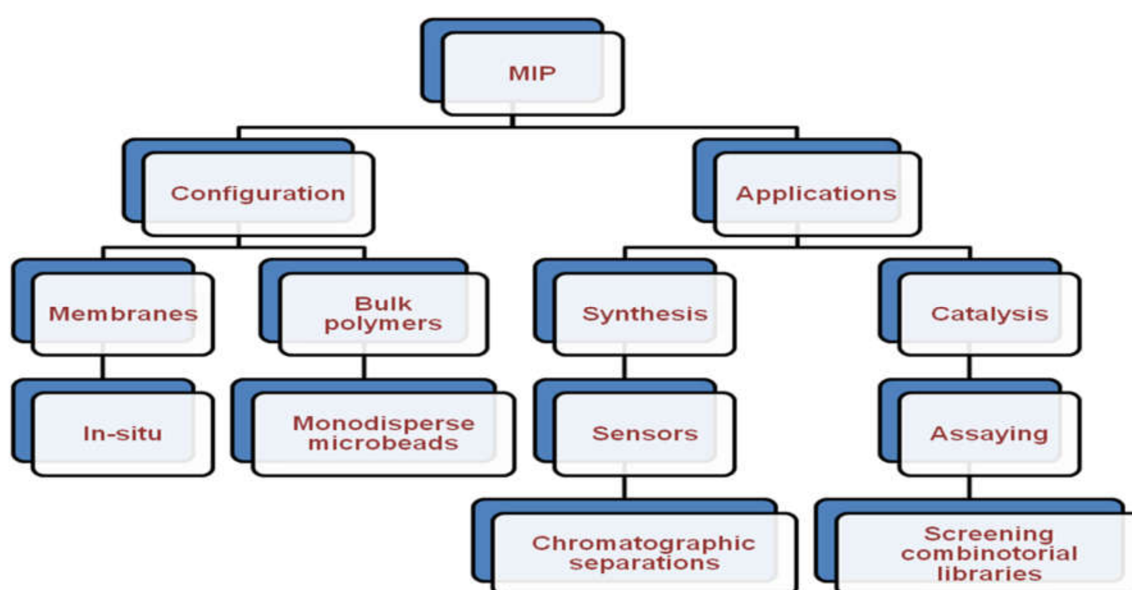

Figure 1.5: Configurations and Applications of MIP

Because of the super-cross linked nature, MIPs are stable to physical and chemical treatment, including heating, acids, bases and organic acids. The imprinted polymers have been applied in an increasing number of applications where molecular binding events are of interest. The stability and low cost of molecular imprinted polymers make them advantageous for use in analysis as well as in industrial scale production and application.

1.3.2 PRINCIPLE OF MOLECULAR IMPRINTING

Molecular recognition between a molecular receptor (host- polymer) and a substrate (guest-template) in a matrix containing structurally related molecules requires discrimination and binding. This can happen only if the binding sites of the host (polymer) and guest template molecules complement each other in size, shape and chemical functionality. The working hypothesis of the binding site structure in molecular imprinted polymers is based on the idea that the pre-polymer complex is locked into place by polymerization. This assumption postulates the formation of a cavity with functional groups in complimentary array for the convergent interactions with the template. The relationship of template to the imprinted cavity corresponds to the lock and key principle proposed by Emil Fisher for enzyme catalysis.

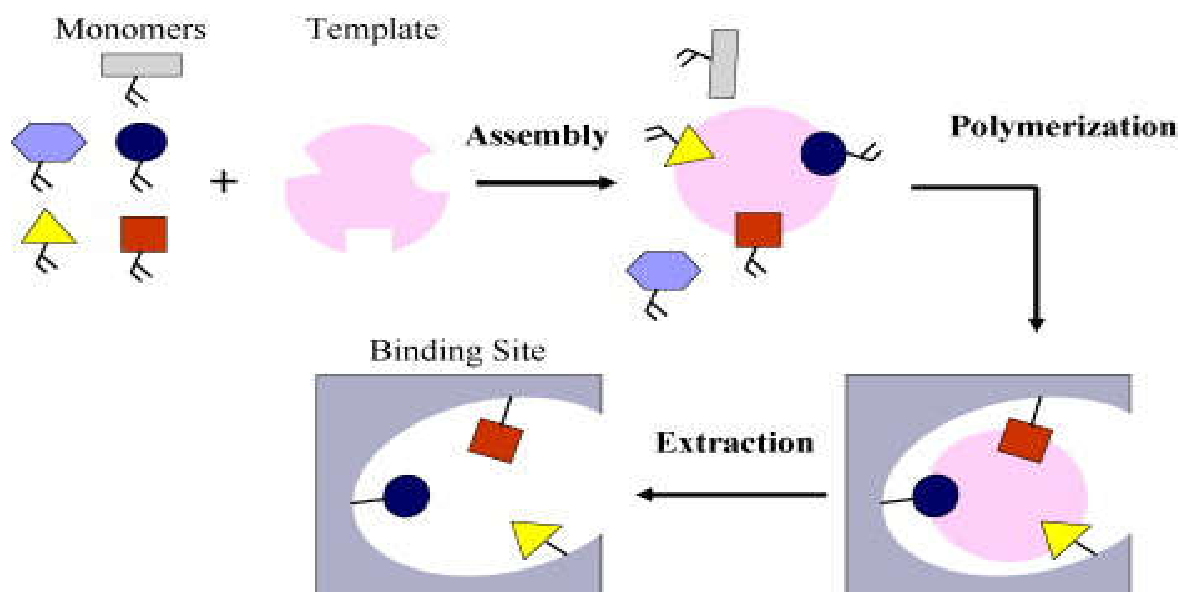

Figure 1.6: Principle of molecular imprinting.

1.3.3 APPROACHES IN MOLECULAR IMPRINTING

Two kinds of molecular imprinting strategies have been established based on covalent bonds or non-covalent interactions between the template and functional monomers. 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. [5]

NONCOVALENT 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[6]. 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. This 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. [7]

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 and another limit is represented by the need of several distinct points of interactions, for eg., some molecules characterized by a single interacting group, such as an isolated carboxyl, generally give imprinted polymers with very limited molecular recognition properties, which have little interest in practical applications.

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 copolymerization with cross linker, the imprint molecule is chemically cleaved from the highly cross linked polymer. However, since the choice of reversible covalent interactions and the number of potential templates are substantially limited, reversible covalent interactions with polymerizable monomers are fewer in number and often require an acid hydrolysis procedure to cleave the covalent bonds between the template and the functional monomer. [8]

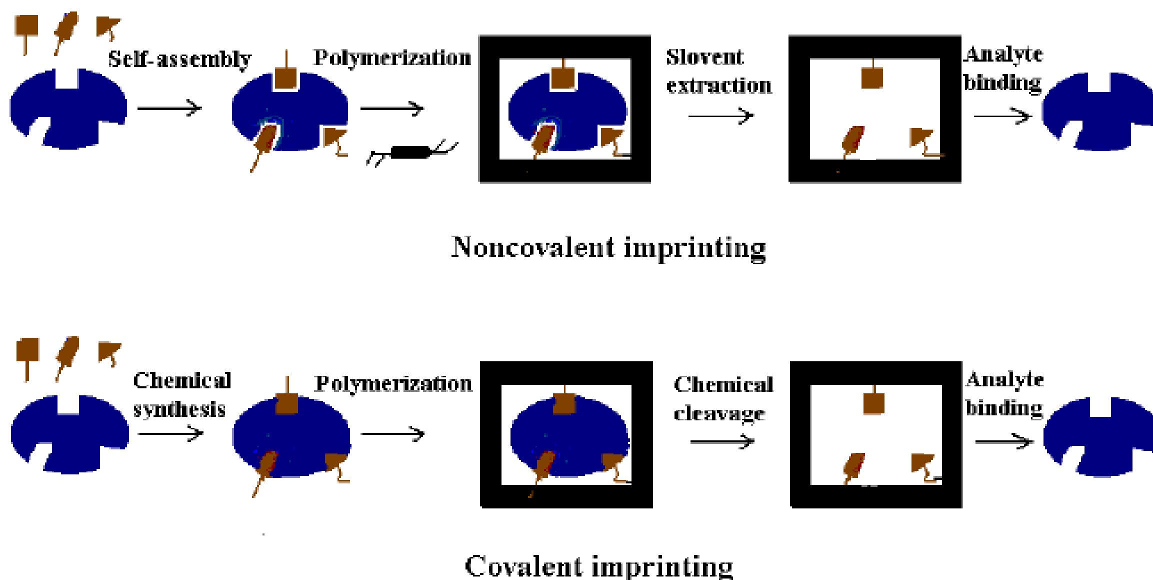

Figure 1.7: Approaches in molecular imprinting.

Of the two strategies, the non-covalent approach has been used more extensively due to three reasons:

1) Non-covalent protocol is easily conducted, avoiding the tedious synthesis of prepolymerization complex.
2) Removal of the template is generally much easier, usually accomplished by continuous extraction.
3) A greater variety of functionality can be introduced into the MIP binding site using non-covalent methods.

1.3.4 SYNTHESIS OF MOLECULARLY IMPRINTED POLYMERS

Majority of imprinted polymers are synthesized by radical polymerization from functional and cross linking monomers having vinyl or acrylic groups. To suit the final application, molecular imprinted polymers can be synthesized in a variety of physical forms. The general mechanism of synthesis involves various reagents like template (desired analyte), monomer, cross-linker, initiator and porogenic solvent.

BULK POLYMERIZATION

The conventional method for preparing MIP is via solution polymerization or bulk 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. Particle sizes <25 µm are usually used in chromatographic studies. 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.

MULTI-STEP SWELLING POLYMERIZATION

Uniformed spherical particles have been obtained by using multi-step swelling method. Particles can be prepared directly in the form of spherical beads of controlled diameter. Beads synthesized in this way can be rendered magnetic through inclusion of iron oxide particles.

SUSPENSION POLYMERIZATION

A rather simple method for the preparation of imprinted supports not requiring mechanical grinding is suspension polymerization, which yields aggregates of spherical particles, if the system is sufficiently dilute, uniformly sized microspheres. In this technique the beads obtained are of diameter 5-50 µm depending on the stirring speed and the amount of surfactant used.

Table 1.1: Various polymerization reactions involved in MIP

MIP format	Benefits	Limitations
Bulk polymerization	Polymerization simplicity and universality, No require particular skills or sophisticated instrumentation	Tedious procedures of grinding, sieving, and column packing, Irregular particle in size and shape, low performance.
Suspension polymerization	spherical particles, Highly reproducible results, Large scale possible	Phase partitioning of complicates system, Water is incompatible with most imprinted procedures, Specialist surfactant polymers required
Multi-step swelling polymerization	Monodisperse beads of controlled diameter, Excellent particle for HPLC	Complicated procedures and reaction conditions, Need for aqueous emulsions,
Precipitation polymerization	Imprinted microspheres, Uniform size and high yields	Large amount of template High dilution factor
Surface polymerization	Monodisperse product, Thin imprinted layers	Complicated system, Time consuming
In-situ polymerization	One-step, in-situ preparation, Cost-efficient, good porosity	Extensive optimization required for each new template system

PRECIPITATION POLYMERIZATION

MIP microspherical shapes with more uniform size (0.3-10 μm) can be obtained by the method of precipitation polymerization, which offers a higher active surface area by manipulating its compositions.

SURFACE IMPRINTING POLYMERIZATION

Surface grafting of MIP layers onto preformed beads has been recently proposed as attractive and apparently general techniques to obtain chromatography-grade imprinted materials. In this method, thin imprinted layers have been successfully used as coatings on chromatography-grade porous silica using several techniques to restrain the radical polymerization at the surface of the beads.

INSITU-POLYMERIZATION OR MONOLITHIC IMPRINTED POLYMERIZATION

Monolithic molecularly imprinted technology combined the advantage of monolithic column and molecular imprinted technology, which was prepared by a simple, one-step, in-situ, free-radical polymerization ‘‘molding’’ process directly within a chromatographic column without the tedious procedures of grinding, sieving, and column packing. Monolithic MIP is expected to improve the separation and enable direct analysis with high-speed and high performance after in–situ polymerization. [9]

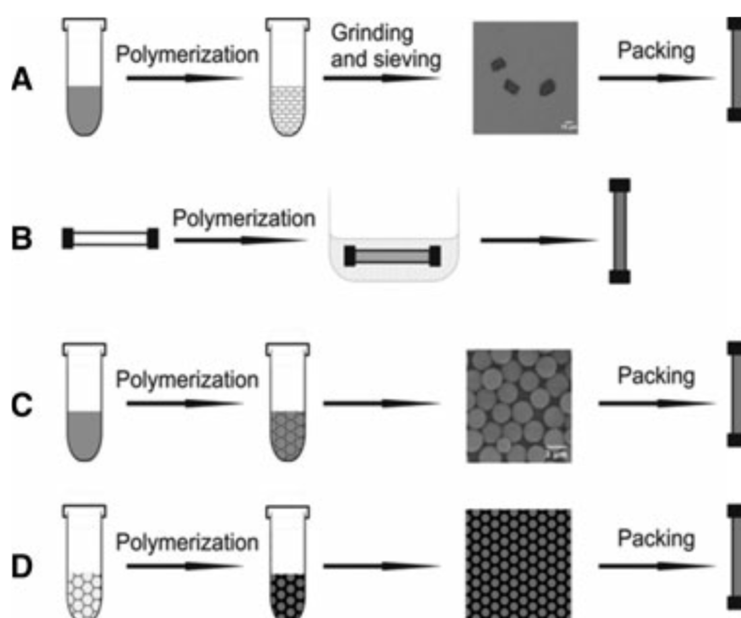

Figure: 1.8 Polymerization approaches for MIP-based HPLC stationary phases: (A) bulk polymerization, (B) *in situ* polymerization, (C) one-step suspension or precipitation polymerization, (D) MIP composite beads.

The synthesis of molecularly imprinted polymers is a chemically complex pursuit and demands a good understanding of chemical equilibrium, molecular recognition theory, thermodynamics and polymer chemistry in order to ensure a high level of molecular recognition. The polymers should be rather rigid to preserve the structure of the cavity after splitting off the template. On the other hand, a high flexibility of the polymers should be present to facilitate a fast equilibrium between release and reuptake of the template in the cavity. These two properties are contradictory to each other, and a careful optimization became necessary.

TEMPLATE

The template is central importance and it directs organization of the functional groups pendent to the functional monomers in all molecular imprinting processes. In terms of compatibility with free radical polymerization, templates should ideally be chemically inert under the polymerization conditions, thus alternative imprinting strategies may have to be sought if the template can participate in radical reactions or is for any other reason unstable under the polymerization conditions.

The following are legitimate questions to ask of a template:

1) Does the template bear any polymerizable groups?
2) Does the template bear functionality that could potentially inhibit or retard a free radical polymerization?
3) Will the template be stable at moderately elevated temperatures or upon exposure to UV irradiation?
4) It should bear good hydrogen bond acceptors or hydrogen bond donors functional groups.

The imprinting of small, organic molecules (e.g., pharmaceuticals, pesticides, amino acids and peptides, nucleotide bases, steroids, and sugars) is now well established and considered almost routine. The binding sites consist of a functional group attached, capable of interacting with the template molecule and ideally the functional groups exists on the surface of the cavity left by the template, readily available for rebinding. The best results are obtained when the templates get attached to more than one binding site. The bond between the template and the binding group should be as strong as possible during the polymerization to enable the binding group to be fixed by the template in a definite orientation on the polymer chain during crosslinking. The template should be able to be removed as completely as possible. The interaction of the binding site with the template should be very fast and reversible [10]

MONOMERS

The careful choice of functional monomer is one of the utmost importance's to provide complementary interactions with the template and substrates. For covalent molecular imprinting, the effects of changing the template to functional monomer ratio is not necessary because the template dictates the number of functional monomers that can be covalently attached, furthermore, the functional monomers are attached in a stoichiometric manner. For non-covalent imprinting, the optimal template /monomer ratio is achieved empirically by evaluating several polymers made with different formulations with increasing template. The underlying reason for this is thought to originate with the solution complex between functional monomers and template, which is governed by Le Chatelier's principle. From the general mechanism of formation of MIP binding sites, functional monomers are responsible for the binding interactions in the imprinted binding sites, and for non-covalent molecular imprinting protocols, are normally used in excess relative to the number of moles of template to favor the formation of template-functional monomer assemblies. It is very important to match the functionality of the template with the functionality of the functional monomer in a complementary fashion (e.g. H-bond donor with H-bond acceptor) in order to maximize complex formation and thus the imprinting effect.

The functional monomers can be basic, acidic, permanently charged, hydrogen bonding, hydrophobic and others. Generally, MIPs prepared in a relatively non-polar solvent employ different functional monomers whereas acrylamide could be a promising functional monomer to form strong hydrogen bonds with template molecule in polar solvents.

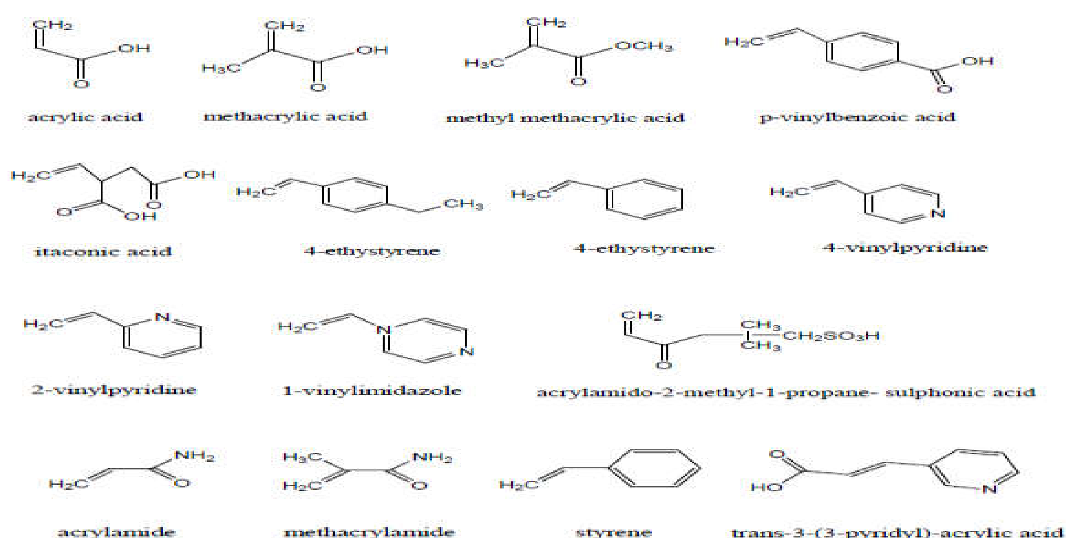

Figure 1.9: Monomers

MONOMER-TEMPLATE RATIO

The molar relationship between the functional monomer and template (M/T) has been found to be important with respect to the number and quality of MIP recognition sites. Low M/T ratios result in less than optimal complexation on account of insufficient functional monomers. Most of the functional monomer will be complexed and only a limited number of functional monomer will be located outside the imprinted cavities scattered on the surface of the polymer particles. During rebinding, the imprinted binding sites may compete successfully with the residual monomers for a limited number of guests, thus increasing selectivity. But when the ratio (M/T) is high, only a limited number of functional monomer is complexed with the template while those located outside the cavities, remain scattered in the bulk of the polymer particles.

CROSS-LINKERS

The selectivity is greatly influenced by the kind and amount of cross-linking agent used in the synthesis of the imprinted polymer. In an imprinted polymer, the cross-linker fulfils three major functions:

1) The cross-linker is important in controlling the morphology of the polymer matrix, whether it is gel-type, macro porous or a micro gel powder.
2) It serves to stabilize the imprinted binding site.
3) It imparts mechanical stability to the polymer matrix.

From a polymerization point of view, high cross-link ratios are generally preferred in order to access permanently porous (macro porous) materials and in order to be able to generate materials with adequate mechanical stability. So the amount of cross-linker should be high enough to maintain the stability of the recognition sites. These may be because the high degree of cross-linking enables the micro cavities to maintain three-dimensional structure complementary in both shape and chemical functionality to that of the template after removal of the template, and thus, the functional groups are held in an optimal configuration for rebinding the template, allowing the receptor to `recognize' the original substrate. Polymers with cross-link ratios in excess of 80% are often be used.

Divinylbenzene isomers are used as crosslinking agents in the synthesis of MIPs based on polystyrene, whereas EGDMA is found to be the most appropriate crosslinking agent for the synthesis of MIPs based on acrylic or methacrylic acid. The MIPs prepared with the use of EGDMA as crosslinking agent found to be more specific, they also exhibit good mechanical strength, thermal stability, porosity and wettability.

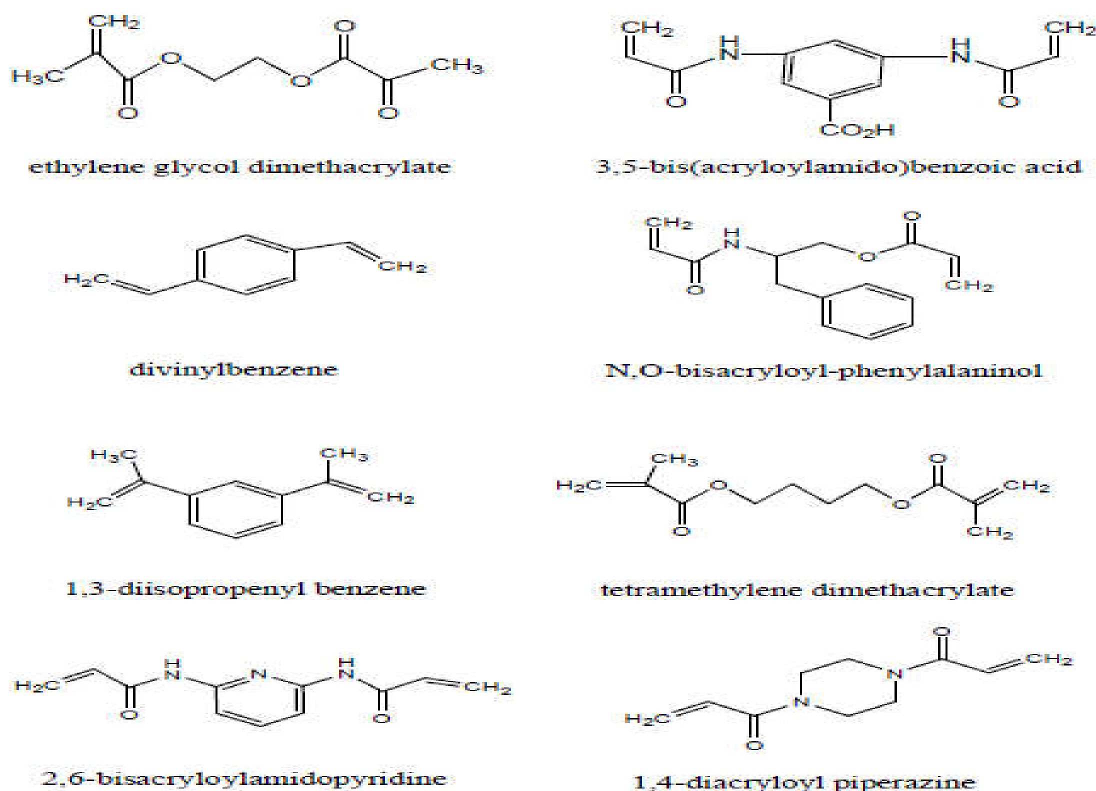

Figure 1.10: Cross-linkers

POROGENIC SOLVENTS

Porogenic solvents play an important role in formation of the porous structure of MIP, which known as macro porous polymers. It is known that the nature and level of porogenic solvents determines the strength of non-covalent interactions and influences polymer morphology which, obviously, directly affects the performance of MIP.

1) Firstly, template molecule, initiator, monomer and cross-linker have to be soluble in the porogenic solvents.
2) Secondly, the porogenic solvents should produce large pores, in order to assure good flow-through properties of the resulting polymer.
3) It provides porosity within the network, which facilitates mass transfer of the analyte to and from the binding sites.
4) Another role of solvents is to disperse the heat of reaction generated on polymerisation. The volume of the porogen used is generally half of the total volume of the mixture, and creates pores by phase separating into channels during polymerisation.

5) Thirdly, the porogenic solvents should be relatively low polarity, in order to reduce the interferences during complex formation between the imprint molecule and the monomer, as the latter is very important to obtain high selectivity MIP.

The porogen parameters such as polarity and hydrogen bonding are important in determining the final morphology of the network structure and porosity. It is believed that solvents with low permittivity's and which are no polar and non-protic (toluene, dichloromethane, and chloroform) are best suited for molecular imprinting. Chloroform is one of the most widely used solvents, since it satisfactorily dissolves many monomers and templates and hardly suppresses hydrogen bonding. In these solvents, the monomer-template non-covalent interactions are stronger than in polar solvents. Increasing the volume of porogenic solvents increases the pore volume. Besides its dual roles as a solvent and as a pore forming agent, the solvent in a non-covalent imprinting polymerization must also be judiciously chosen such that it simultaneously maximizes the likelihood of template, functional monomer complex formation.

INITIATOR

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

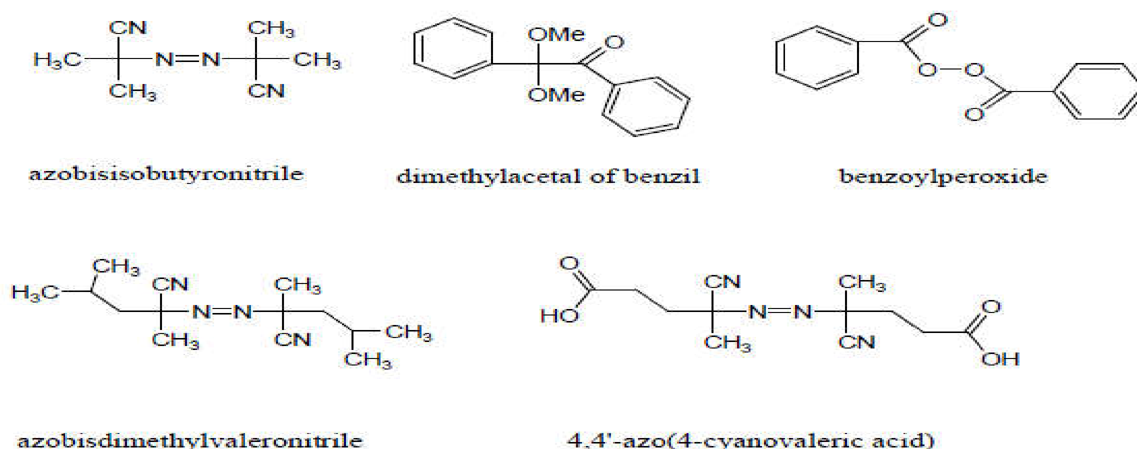

Figure 1.11: Initiators

POLYMERIZATION TEMPERATURE

The position of equilibrium between free templates – monomers and their corresponding complexes is a product of both temperature and pressure. Previous studies found that lower temperature of polymerisation is favorable for the preparation of MIPs based on electrostatic interaction due to the greater strength of electrostatic interaction at lower temperature. At low temperature the polymerization process is slow and the chain formation does not interfere with the template - monomer interactions. An optimal condition of temperature should be found for each combination of template and monomer. pH of the rebinding medium also induces a significant role in molecular imprinting, especially in covalent imprinting.

Oxygen gas retards free radical polymerizations, thus in order to maximize the rates of monomer propagation, ensure good batch-to-batch reproducibility of polymerizations, removal of the dissolved oxygen from monomer solutions immediately prior to proliferation is advisable. Removal of dissolved oxygen can be achieved simply by ultrasonication or by sparging of the monomer solution by an inert gas, e.g. nitrogen or argon. 11

1.3.5 GENERAL PROCEDURE FOR SYNTHESIS OF MIP

The general procedure for synthesis of MIP involves following steps:

- Template (desired analyte) is added in thick walled glass tube and porogenic solvent is added followed by monomer, cross-linker and initiator.
- The entire mixture is subjected to sonication for 5 minutes and nitrogen gas was purged for 3 minutes and tightly sealed the tube with paraffin wax paper.
- The glass tube was kept at U.V cabinet for 24 hours or it is kept at 60^0c for 24 hours to initiate polymerization reaction.
- After 24 hours the formed polymer was carefully removed from the tube and mechanically ground and sieved to obtain uniform size.
- The template was completely removed by washing the polymer using suitable washing solvent.
- After washing the polymer was dried and carefully packed in a SPE cartridge and used for selective extraction.

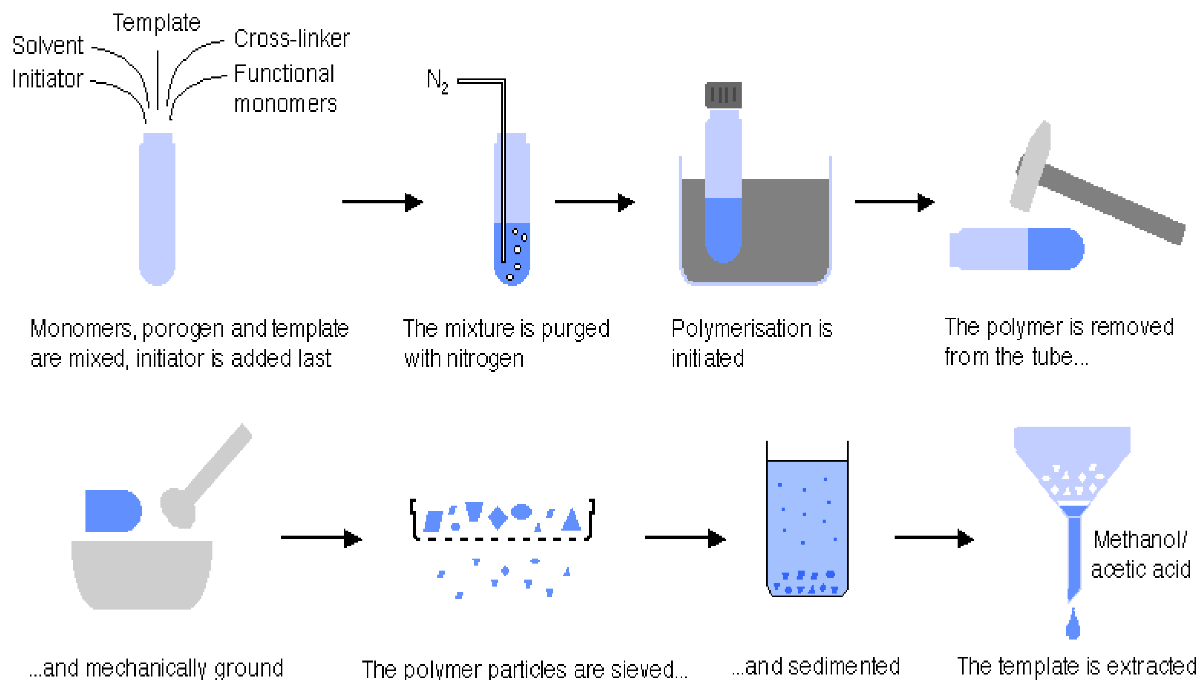

Figure 1.12: Synthesis of MIP

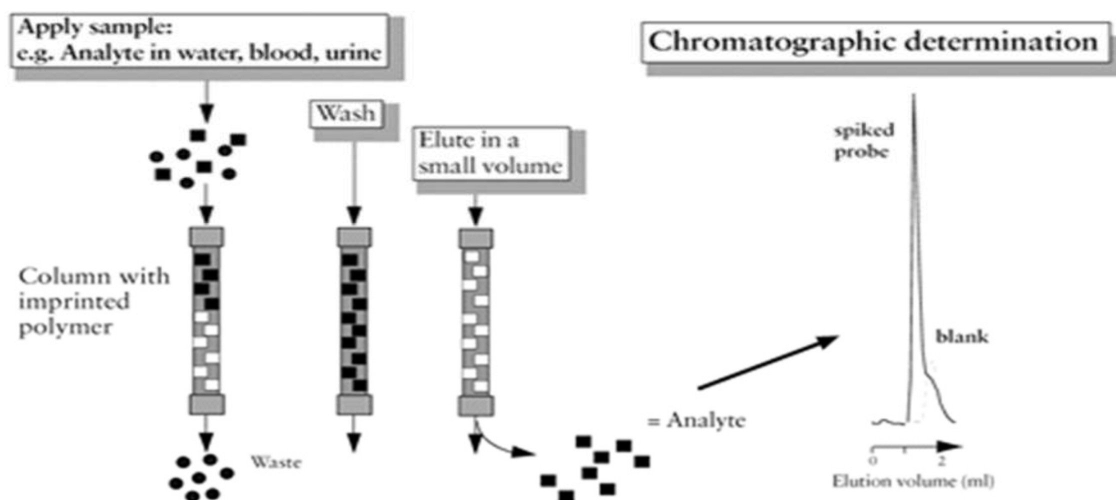

Figure 1.13: MISPE followed by Chromatographic determination

- After proper packing in to SPE cartridge the polymer was conditioned and biological sample was applied followed by washing step.
- Then elution solvent was introduced on the polymer and analyte was collected and subjected to HPLC for quantification.

APPLICATION OF MIP IN EXTRACTION OF DRUG MOLECULES FROM BIOLOGICAL SAMPLES

S.NO	DRUG	REFERENCE
1	Metoclopramide	Javanbakht M, Shaabani N, Akbari-Adergani B. Novel molecularly imprinted polymers for the selective extraction and determination of metoclopramide in human serum and urine samples using high-performance liquid chromatography. Journal of Chromatography B. 2009 Aug 15;877(24):2537-44.
2	3-methylflavone-8-carboxylic acid	Rao RN, Maurya PK, Kuntamukkala R, Vitthal WD, Talluri MV. Molecularly imprinted polymer for selective extraction of 3-methylflavone-8-carboxylic acid from human urine followed by its determination using zwitterionic hydrophilic interaction liquid chromatography. Journal of separation science. 2011 Nov;34(22):3265-71.[2]
3	emtricitabine	Lai JP, Xie L, Sun H, Chen F. Synthesis and evaluation of molecularly imprinted polymeric microspheres for highly selective extraction of an anti-AIDS drug emtricitabine. Analytical and bioanalytical chemistry. 2013 May;405:4269-75.[3]
4	citalopram	Majid Abdouss., Saman Azodi-Deilami, Ebadullah Asadi and Zahra Shariatina (2012) Synthesis of molecularly imprinted polymer extraction of

		citalopram from human serum and urine. *J Mater Sci: Mater Med.*, **23**, 1543-1552.
5	zidovudine and stavudine	Duy SV., I. Lefebvre-Tournier, V. Pinchon, F. Hugon –Chapuis, JY. Puy and C. Periguad (2009) Molecularly imprinted polymer for analysis of zidovudine and stavudine in human serum by liquid-chromatography-mass spectrometry. *J. Chromatogr. B.*, **877**, 1101-1108.
6	Dextromethorphan	Mohammad Mahdi Moeina., Mehran Javanbakht and Behrouz Akbari-adergani (2011) Molecularly imprinted polymer cartridges coupled on-line with high performance liquid chromatography for simple and rapid analysis of dextromethorphan in human plasma samples. *J. Chromatogr. B.*, **879,** 777-782.

REFERENCES

1. Javanbakht M, Shaabani N, Akbari-Adergani B. Novel molecularly imprinted polymers for the selective extraction and determination of metoclopramide in human serum and urine samples using high-performance liquid chromatography. Journal of Chromatography B. 2009 Aug 15;877(24):2537-44.
2. Rao RN, Maurya PK, Kuntamukkala R, Vitthal WD, Talluri MV. Molecularly imprinted polymer for selective extraction of 3-methylflavone-8-carboxylic acid from human urine followed by its determination using zwitterionic hydrophilic interaction liquid chromatography. Journal of separation science. 2011 Nov;34(22):3265-71.

3. Lai JP, Xie L, Sun H, Chen F. Synthesis and evaluation of molecularly imprinted polymeric microspheres for highly selective extraction of an anti-AIDS drug emtricitabine. Analytical and bioanalytical chemistry. 2013 May; 405:4269-75.
4. Majid Abdouss., Saman Azodi-Deilami, Ebadullah Asadi and Zahra Shariatina (2012) Synthesis of molecularly imprinted polymer extraction of citalopram from human serum and urine. *J Mater Sci: Mater Med*., 23, 1543-1552.
5. Duy SV., I. Lefebvre-Tournier, V. Pinchon, F. Hugon –Chapuis, JY. Puy and C. Periguad (2009) Molecularly imprinted polymer for analysis of zidovudine and stavudine in human serum by liquid-chromatography-mass spectrometry. *J. Chromatogr. B*., 877, 1101-1108.
6. Mohammad Mahdi Moeina., Mehran Javanbakht and Behrouz Akbari-adergani (2011) Molecularly imprinted polymer cartridges coupled on-line with high performance liquid chromatography for simple and rapid analysis of dextromethorphan in human plasma samples. *J. Chromatogr. B*., 879, 777-782.
7. Martin'n-Esteban A (2013) Molecularly imprinted polymers as a versatile, highly selective tool in sample preparation', *Trends in Analytical Chemistry*., 45, 169-182
8. Michael, J., Whitecome M, Rodrigue E and Villar P (1995) A new method for the introduction of recognition site functionality into polymers prepared by molecular imprinting: synthesis and characterization of polymeric receptors for cholesterol. *J. Am. Chem. Soc*., 117, 7105-7111.
9. Qianqian He and Binghu Fang (2013) Simultaneous determination of quinoxaline-1, 4-dioxides in feeds using molecularly imprinted solid-phase extraction coupled with HPLC. *J.Sep.Sci*, 36, 301-331.
10. Rao RN., Pawan K. Maurya, Ramakrishna Kuntamukkala, Wani D. Vitthal and Murali V. Talluri (2011) Molecularly imprinted polymer for selective extraction of 3-methylflavone-8-carboxlyic acid from human urine followed by its determination using zwitterionic hydrophilic interaction liquid chromatography. *J. Sep. Sci*., 34, 3265-3271.
11. Scorrano S., Mergola L, Del Sole R and Vasapollo G (2011) Synthesis of molecularly imprinted polymers for amino acid derivatives by using different functional monomers. *Int. J. Mol. Sci*., 12, 1735-1743.