CHAPTER I

INTRODUCTION

1.1 Solid-phase extraction

1.1.1 The sample preparation [1-4]

In the last decade, the requirements for higher sensitivity, selectivity, accuracy, precision, and number of sample to be processed has increased dramatically. The corresponding increases in speed and sophistication of data collection and analysis have outpaced improvements in many traditional techniques of sample collection and preparation that are essential to future progress in analytical science.

The sample preparation for most analytical techniques such as high performance liquid chromatography (HPLC), gas chromatography (GC), spectrophotometry and radio immunoassay (RIA) has a threefold objective that to provide the sample component of interest in solution, free from interfering matrix elements and have an appropriate concentration for detection or measurement. Generally, samples are prepared via traditional methods of dissolution, homogenization, extraction (liquid or solid phase), filtration, concentration, evaporation, separation, chemical derivatization or standardization (internal or external). Usually such methods are used in combination of multiple steps, which form a sample preparation protocol. The fewer steps and methods used in any given protocol, the simpler, more convenient, cost effective and less time consuming are required.

One of the most useful tools for isolating a desired component from a mixture is liquid-liquid extraction (LLE). Selective partitioning of the compound of interest into one of two immiscible phases occurs by the proper choice of extraction solvents. Often, however, it is not possible to find the optimum conditions that provide both high recovery and purity of the product in one extraction step. Low recoveries may necessitate further extractions to achieve acceptable yield. Reaching purity may require a second extraction procedure with a different solvent or pH. Each successive extraction increases the time required. Also, the resulting large volume of extraction solvent must be evaporated to recover the product. In summary, this countercurrent LLE method is expensive, cumbersome and time consuming.

1.1.2 Solid-phase extraction [1, 5]

Solid-phase extraction (SPE) is an extraction technique based on the selective partitioning of one or more components between two phases, one of which is a solid sorbent. The second phase typically is a liquid, but it may also be and emulsion, a gas, or a supercritical fluid. The components of interest may either preferentially adsorb to the solid, or they may remain in the second, non-solid phase. Once equilibrium has been reached, the two phases are physically separated by decanting, filtration, configuration or similar process. If the desired analytes are adsorbed on the solid phase, they can be selectively desorbed by washing with an appropriate solvent. If the components of interest remain in a liquid phase, they can be recovered via concentration, evaporation, chromatographic separation and/or recrystallization. When SPE is performed in this single-step equilibrium batch mode, it is similar in practice to LLE, where the solid sorbent simply replaces one of the immiscible liquids.

Using solid sorbent in the traditional batch adsorbtion method just described still can lead to incomplete sample recovery and purification, due to unfavorable partition equilibria, insufficient sample capacity, liquid entrapment within the solid matrix, or incomplete desorption of the adsorbed species after filtration. The real benefits of SPE are gained when the sorbent is packed efficiency into a tube to form a uniform bed with good flow distribution characteristics by passing a liquid (or gas) through this bed, the liquid-solid phase extraction technique becomes a form of column chromatography, now commonly called solid phase extraction (SPE), that is governed by liquid chromatographic principles. However, there are a few common sample preparation objectives and provides a general description of the technique that best achieve these goals;

- To remove sample constituents that elute after the analytes of interest or are strongly adsorbed
- To remove sample constituents that coelute with an analyte of interest
- To enrich sample components present in low concentration
- To desalt samples
- To exchange solvents
- To fractionate classes of compounds
- To derivatize analytes using solid-phase reagents

1.1.3 Advantages of solid phase extraction over liquid-liquid extraction [1, 5, 6]

One of the fundamental advantages of column liquid-solid extraction is that, by choosing suitably selective adsorbents, the partition equilibrium of specific sample components can be driven to effect nearly complete adsorbtion or desorbtion. In batch-mode SPE or in single-stage LLE, each extraction step is single equilibration process, equivalent to one chromatographic "plate". SPE operated in a single equilibration process with promise of higher recoveries and grater enrichment of desired component than could be obtained from a single step LLE or batch SPE. In addition, since number of plates affects resolution of two components, SPE with 50 plates has a good chance of providing a more pure product than LLE with one or two extraction steps. To achieve similar results with LLE, one must perform several manual liquid extractions or perform countercurrent LLE.

Table 1.1 Advantages and disadvantages of LLE and SPE technique

LLE	SPE
• Large solvent consumption • Time/labor intensive • May require an evaporation step prior to analysis to remove excess solvent • Requiring more than one extraction per sample • Low recovery	• Use small amount of solvent • Safe time • Easy to automate • Cleaner extract • Higher recovery

1.1.4 Step of solid-phase extraction procedure [1, 7]

The SPE process provides samples that are in solution, free of interfering matrix components and concentrated enough for detection. This is done in five steps that are pretreatment of sample, conditioning the cartridge, loading sample, washing and elution of the fraction.

1.1.4.1 Pretreatment of the sample

In many cases, the sample is in a solid form. Therefore, the first step in the pretreatment of the sample is either to dissolve or homogenized the solid, and extracts the analyte in an appropriate solvent. Next, the sample has to be brought into a state that facilitates the adsorption of the analytes onto the solid phase extraction column. The pretreatment step may also include the addition of an internal standard for convenient quantitative analysis.

1.1.4.2 Conditioning of the cartridge

It is usually advisable to precondition the sorbent with the solvent used to load the sample. In some cases, this step can be omitted to streamline the process. In the case of reverse phase sorbents, preconditioning of the sorbent with an organic solvent such as methanol, acetonitrile, isopropanol or tetrahydrofuran is usually necessary to obtained reproducible results. Without this step, a highly aqueous solvent could not penetrate the pores and wet the surface. Nevertheless, a complete preconditioning of a reversed-phase cartridge includes the salvation step and an equilibration with a low-strength solvent such as water or buffer.

1.1.4.3 Sample loading step

The amount of the sample that is applied should be controlled even if quantitation is not necessary or if an internal standard is used. Sample sized must be scaled to suit the sized of the cartridge bed and the separation mode and strategy to be employed. The quantity of the cartridge includes every substance that may be strongly retained in any given sample, not just the component of interest. Less strongly held compounds may begin to elute before large volume samples have completely passed into the bed.

1.1.4.4 Washing step

To remove unwanted, weakly retained materials, wash the packing with the solutions that are stronger than the sample matrix but weaker than needed to remove compound of interest. A typical solution may contain less organic and inorganic salt than the final eluant. Pure solvents or mixtures of solvent differing sufficiently in polarity from the final eluant may be useful wash solutions.

1.1.4.5 Eluting step

The analyte of interest are eluted out of the cartridge using a solvent of higher eluting strength while more strongly adsorbed sample constituents are left behind to be discarded with the sorbent. In some cases, it may be desirable to perform a multi-step fractionation. The elution strength of the solvent is increased gradually in several steps. Step-wise elution is actually the procedure recommended during the development of a new SPE method to ensure that all analytes have been recovered quantitatively.

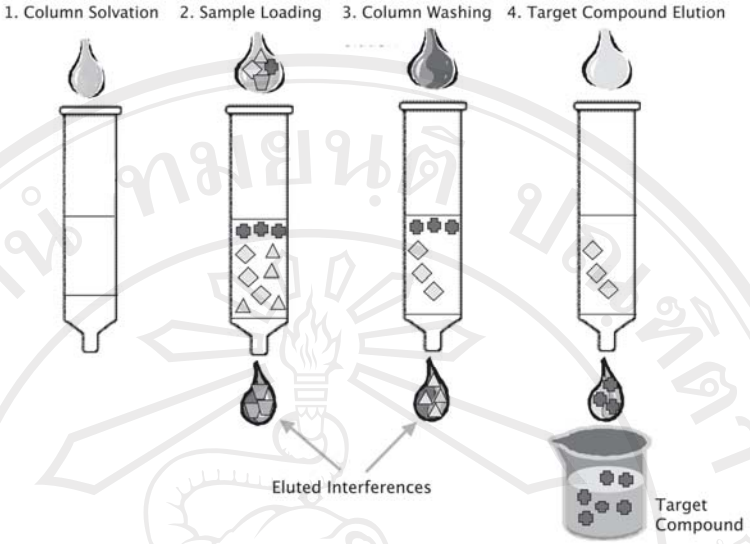


Figure 1.1 The general SPE procedure (http://www.biotage.com/graphics/9223.jpg).

1.2 Molecularly Imprinted Polymers (MIP)

1.2.1 Theory of MIP

Molecular imprinting is a technique in which the highly selective recognition site can be generated in a synthetic polymer, thus these materials are called molecularly imprinted polymers (MIPs). In recent years there has been an almost exponential growth in publication resulting from imprinted polymer researches which both target the fundamental aspects of these materials, their potential and their applications.

The concept underlying molecular imprinting is the assembly of a cross-linked polymer matrix around the template's moieties. Upon removal of the templates, cavities or recognition sites are created which are complementary both in terms of shape and functionality to the original template present in the sites. The binding of functional monomers to the templates, and in the recognition sites following template removal, can be effected by either covalent or non-covalent interactions (or combinations of both), thereby allowing considerable flexibility in the choice of monomers and the types of templates that can be imprinted. To date, polymers have been produced for recognizing templates as diverse as peptides[8], sugars[9, 10], pesticides[11, 12], nucleotides[13], steroid[14] and metal ions[14].

Due to the properties of MIPs, the highly selective recognition, low cost, ease of production and good stability in both of physical and chemical, these materials have been used in many applications such as receptors in sensors[16-19], catalyst[20-22], immuno-like assay[23-26], and solid phase extraction materials[27-32].

1.2.2 Molecular imprinting procedure

In the common approach, the synthesis of MIPs often involved three main steps. First, the imprint is achieved by arranging polymerizable functional monomers around a guidable template, and the complexes are formed through covalent, non-covalent, or semi-covalent interactions between the template and monomers. Then the complexes are assembled in the liquid phase and fixed by a crosslinking polymerization, acting as cavity-creating template. Finally, the template molecules are removed leaving behind vacant recognition sites that exhibit high affinity for the imprint species[33]. (Figure 1.2)

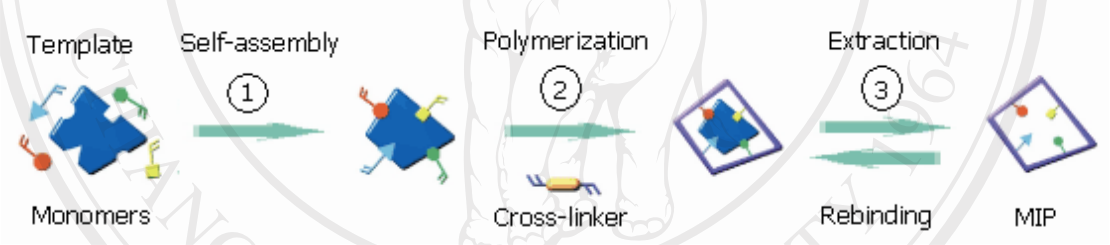


Figure 1.2 The schematic of MIP preparation (1) self-assembly (2) polymerization and (3) extraction of the template (http://www.imego.com/commonfiles/files/172.gif)

The molecular imprinting method is of two types, either covalent or non-covalent, depending on the nature of adducts between functional monomer and template which is responsible for localizing the chemically active moieties of the target molecule during copolymerization. Both have advantages and disadvantages, and thus the choice of the best method strongly depends on various factors [34-37].

1.2.2.1 Covalent Imprinting

In covalent system, a template-monomer complex is formed through reversible covalent bonding involve ester, amide, ketal, Schiff-base and metal coordination. In order to obtained specific and homogeneous binding sites, it is crucial to maintain stable template-monomer complexes during the imprinting process. Therefore, the advantages of the covalent systems are monomer-template conjugate are stable and stoichiometric, and thus the molecular imprinting processes are relatively clear-cut. On the other hand, a wide variety of polymerization conditions (e.g. high temperature, high or low pH and highly polar solvent) can be employed, since the conjugates are formed by covalent linkages and are sufficiently stable. Whereas several disadvantages should be considered such as the synthesis of the monomer-template conjugate is often troublesome and less economical, the number of reversible covalent linkage available is limited and the imprinting effect slow binding kinetics that may not be suitable for chromatographic separations. Also, suitable covalent bonding can hardly be found because the bond should be cleaved easily and re-bond, since they involved the formation and breakdown of the covalent linkage.

Although this covalent bonding strategy provides well-defined cavities, the limited choice of functional monomers and hence of usable template molecule has restricted its use.

1.2.2.2 Non-covalent imprinting

In order to connect a functional monomer with a template, non-covalent interactions are employed. Regarding non-covalent molecular imprinting using hydrogen bonding, electrostatic interactions, hydrophobic interactions, etc. The adducts can be obtained in situ simply by adding the components to reaction mixtures. After polymerization, the template is removed by extracting the polymer with appropriate solvents. The guest binding by the polymer occurs through the corresponding non-covalent interaction. The advantages of non-covalent molecular imprinting are that the procedure is simple and easy to perform because the complexation step can be achieved by mixing the template with functional monomers and polymerization are carried out without isolating the complexes formed. Furthermore, the template can easily be removed under mild conditions. However, non-covalent bonding may not be strong enough to maintain template-monomer complexes; therefore the population of the complex species is governed by equilibrium. Thus, an excess of functional monomers is usually added to the reaction mixture in order to complete the template-monomer complexation and to maintain the complex stability under the polymerization conditions, resulting in the presence of several species of template-monomer complexes in the reaction mixture. This results in a heterogeneous property of the binding sites in terms of affinity.

In general, non-covalent imprinting is easier to achieve and applicable to a wider spectrum of template. With respect to the strictness of imprinting, however, covalent imprinting is usually superior.

Table 1.2 Advantages and disadvantages of covalent and non-covalent imprinting

	Covalent	Non-covalent
Synthesis of monomer-template conjugate	necessary	unnecessary
Polymerization conditions	rather free	restricted
Removal of template after polymerization	difficult	easy
Guest-binding and guest release	slow	fast
Structure of the binding site	clearer	less clear

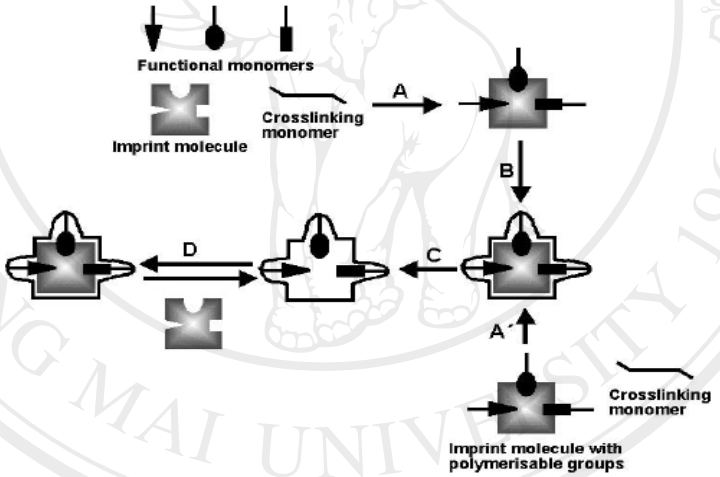


Figure 1.3 Schematics of MIP preparation.
The non-covalent approach:
(A) Functional monomers, cross-linking monomers, radical initiator and imprint molecule are mixed in a proper solvent to allow complexes to form between the functional monomers and the imprint molecule.
(B) The functional monomers are locked in position by the polymerization reaction,
(C) After having extracted the imprint molecule
(D) The MIP is able to recognize the imprint molecule.
The covalent approach:
(A') Imprint molecules with substitutions of polymerisable groups are mixed with crosslinking monomers and radical initiator in a proper solvent and polymerization is initiated.
(C) After completed polymerization the covalent bonds between the imprint molecule and the MIP can be broken and
(D) The MIP is able to rebind the imprint molecule utilizing covalent bonds

1.2.3 Constraints of MIP synthesis [34, 37, 38]

Generally, MIPs are usually synthesized by free radical polymerization reaction which consists of template molecule, functional monomer, cross-linker, initiator and solvent. These parameters have to be considered in order to create the selective site to the target analyte.

1.2.3.1 Templates

In all molecular imprinting processes the template is of central importance in that it directs the organization of the functional groups pendent to the functional monomers. 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 the properties of the suitable template:

- Template should not contain the polymerisable group
- Template should have no functionality that could potentially inhibit or retard a free radical polymerization
- Template should be stable at moderately evaluated temperatures

1.2.3.2 Functional monomers

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 flavor the formation of template, functional monomer self-assemblies. It is clearly very important to match the functionality of the template with the functionality of the functional monomer in a complementary fashion in order to maximize complex formation and thus the imprinting effect. To date, many functional monomers are also commercially available and the several types of monomers are shown in Figure 1.4.

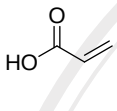
1.2.3.3 Cross-linkers

In the imprinted polymers, the cross-linker fulfills three major functions. The cross-linkers are important to control morphology of the polymer matrix, serve to stabilize the imprinted binding site and impart mechanical stability to the polymer matrix. For efficient imprinting, the reactivity of the crosslinking agent should be similar to that of the functional monomer. By choosing an appropriate cross-linker, random copolymerization occurs successfully, and the functional residues are uniformly distributed in the polymer network.

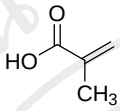
The mole ratios of crosslinking agent to functional monomer are also important. If the ratios are too small, the guest-binding sites are located so closely to each other that they cannot work independently. In extreme cases, the guest binding by one site completely inhibits the guest binding by the neighboring sites. At extremely large mole ratios, however, the imprinting efficiency is damaged,

especially when the cross-linkers show non-covalent interactions with functional monomers and templates.

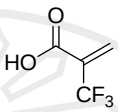
Acrylates



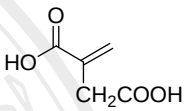
Acrylic acid
(AA)



Methacrylic acid
(MAA)

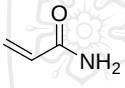


Trifluoromethyl acrylic acid
(MSA)

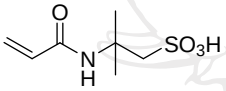


N,N'-dimethylamino-ethyl methacrylate
(DMA)

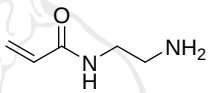
Acrylamides



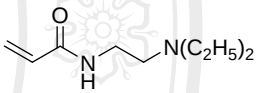
Acrylamide
(AAm)



2-Acryamido-2-methyl-1-propane sulphonique acid
(AMPSA)

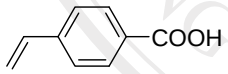


N-(2-aminoethyl)-methacrylamide
(2AEMA)

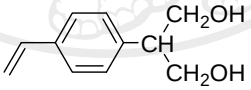


Diethylaminoethyl methacrylate
(DAEMA)

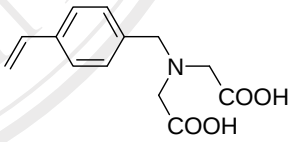
Styrenes



4-vinyl benzoic acid
(4VBA)

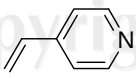


2-(4-vinylphenyl)-1,3-propanediol
(4ES)

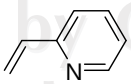


(4-vinylphenyl)methyl amine *N*,*N'* diacetique acid
(VPMADA)

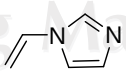
Pyridines and imidazoles



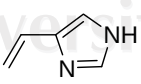
4-vinylpyridine
(4VP)



2-vinylpyridine
(2VP)

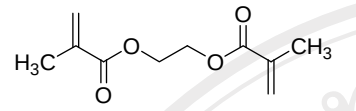


1-vinylimidazole
(1VI)

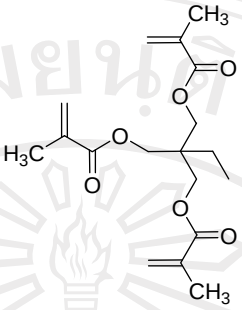


4-vinylimidazole
(4VI)

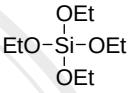
Figure 1.4 The functional monomers most commonly used in MIPs



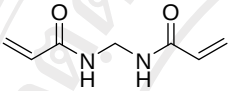
Ethylene glycol dimethacrylate
(EGDMA)



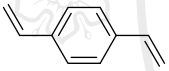
Trimethylopropane trimethacrylate
(TRIM)



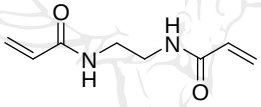
Tetraethoxysilane
(TEOS)



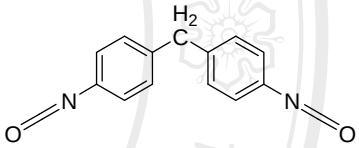
N,*N'*-methylene bisacrylamide
(MBA)



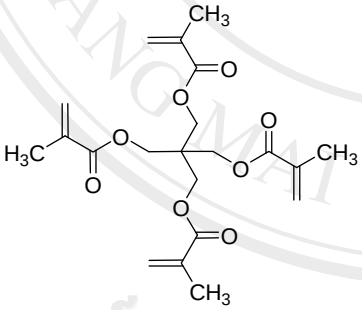
p-Divinylbenzene
(DVB)



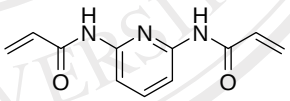
N,*N'*-ethylenebis(prop-2-eneamide)
(EFPA)



p,*p'*-Diisocyanatodiphenylmethane
(DIDM)



Pentaerythritol tetraacrylate
(PETEA)



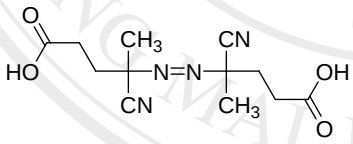
2,6-bisacryloylamidopyridine

Figure 1.5 The cross-linkers most commonly used in MIPs

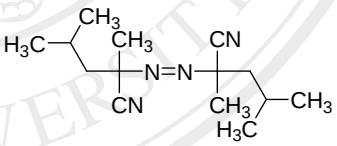
1.2.3.4 Initiators

In principle, any of the methods of initiation describes earlier can be used to initiate free radical polymerizations in the presence of templates. However, there may well be drivers for selecting one over another arising from the system under study. For instance, if the template were photochemically or thermally unstable then initiators that can be trigged photochemically and thermally, respectively, would not be attractive. Where complexation is driven by hydrogen bonding then lower polymerization temperatures are preferred, and under such circumstances photochemically active initiators may well be preferred as these can operate efficiently at low temperature.

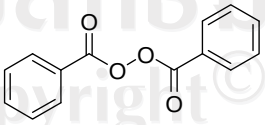
The chemical structures of selected polymerization initiators are shown in Figure 1.6.



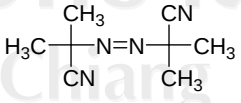
4,4'-azo(4-cyanovaleric acid)



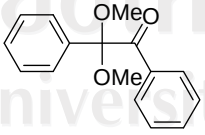
Azobisdimethylvareronitrile (ABDV)



Benzoylperoxide (BPO)



Azobisisobutyronitrile (AIBN)



Dimethylacetal of benzil

Figure 1.6 The initiators most commonly used in MIPs

1.2.3.5 Solvents (Porogens)

The trivial role of solvents is to dissolve the agents for polymerization. However, they have more crucial roles of providing porous structures to imprinted polymers, and promote their rates of guest binding. Release of the bound guest from the polymer is also facilitated by the porosity. In the polymerization, solvent molecules are incorporated inside the polymers and are removed in the post-treatment. During these processes, the space originally occupied by the solvent molecules is left as pores in the polymers. Polymers prepared in the absence of solvents are consistently too firm and dense, and hardly bind guest, another role of solvents is to disperse the heat of reaction generated on polymerization.

Choice of solvents is dependent on the kind of imprinting. The solvent in non-covalent imprinting polymerization must also be judiciously chosen such that it simultaneously maximizes the likelihood of template, functional monomer complex formation. Normally, this implies that a polar and non-protic solvents are preferred as such solvents stabilized hydrogen bonds, however if hydrophobic forces are being used to drive the complexation then water could well be the solvent choice.

1.3 Molecularly imprinted solid-phase extraction (MISPE) [39-51]

Analytical methods for rapid determination of chemicals, biomolecules or cells are growing interest in various fields such as environmental control, drug development, health protection, forensics and biotechnology. The steadily increasing number of organic environmental pollutants is a major health hazard and has therefore created a need for efficient environmental monitoring relying on low-cost, rapid and automated methods of analysis. An equally important challenge is the rapid analysis of low level of drugs, narcotics and their metabolites in human as well as the quality control of food, foodstuff and pharmaceuticals. The development of efficient analytical methods in these fields will have a direct impact on the quality and efficiency of health care, forensic activities and the food industry.

The analysis of target molecules in complex mixtures often requires pretreatment steps. Firstly, if the analyte is presence in low concentration, it needs to be concentrated in order to be detected by standard analytical techniques. Secondly, if it is present in a complex mixture of similar compounds, a clean up step is required. SPE has become a widely used technique for sample pretreatment as it is easily automated, flexible and environmentally friendly.

Highly selective sorbents towards a large number of analytes of environmental and pharmaceutical interest can be prepared by molecular imprinting. The most versatile approach to the synthesis of molecularly imprinted sorbents is based on self-assembly of the template and complementary functionalize monomer prior to polymerization. When using MIPs as the selective sorbent in SPE, the technique is called molecularly imprinted solid-phase extraction (MISPE). Several examples of the successful application of MIPs to SPE have been described in Table 1.3. In some

cases, the protocols have only been preliminarily tested to show the feasibility of the approach. The retentivity and selectivity of the sorbents are generally first assessed by HPLC-UV. The results of these studies reveal whether aqueous samples can be directly applied to the column, as in extraction of hydrophobic analytes from aqueous media, such as biological or environmental samples, or whether the analyte has to be transferred to an organic solvent or lower polarity prior to the MISPE, as for analytes that are not sufficiently retained in aqueous mobile phases.

Table 1.3. Various application of MIP in SPE

Analyte	Sample	Reference
Pentamidine	Urine	52
Flavoniod	Wine, orange juice, tea	53
Chloramphenacol	Honey	54
Antrazine	Beef liver extract	55
Naphthalene sulfonate	Water	56
Mycrophenolic acid	Human plasma	57
Clenbuterol	Calf urine	58
	Bovine liver	59
	Animal feeds	60
Triazine	Grape juice, soil	61
Theophilline	Green tea	62
Caffeine	Green tea	62
	Beverage	63, 64

Table 1.3 Various application of MIP in SPE(continued).

Analyte	Sample	Reference
	Environmental water	65
4-Nitrophenol	Human plasma, serum	66
Cafathiamidine	Human plasma	67
Anaesthetics		

In the last few years, MIPs have been increasingly applied in MISPE. To date, the main application of MISPE has been the extraction of analytes from biological samples such as plasma, serum and urine. The first MIP used for SPE was reported by Sellegren[52] in 1994 for sample pre-treatment of pentamidine, the AIDS-related pneumonia. The high selectivity of the polymer allowed the drug to be sufficiently enriched even when presents in low concentration in diluted human urine sample. In this system the adsorption and desorbtion were switched by changing the pH of loading. It was shown that, electrostatic interaction is the main factor for the specific extraction of pentamidine which resulted in MISPE with a good performance.

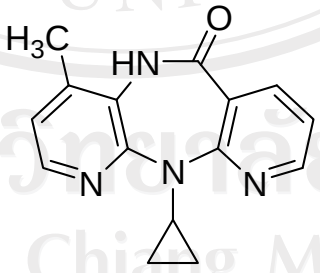
MISPE was as well applied for other biological samples such as human plasma and liver. The rapid screening of mycrophenolic acid (MPA) in human plasma was reported by Yin and co-workers[57]. This assay used MISPE coupled with HPLC method for the analysis of MPA. It was shown that MPA-MIP used as SPE sorbent exhibited a good selectivity for MPA without interference from other related compounds, while a high sample recovery was obtained. Muldon and Stanker[55] applied MISPE for isolation of antrazine from beef liver extract which was then

analyzed by HPLC and enzyme-link immunosorbent assay (ELISA) method. It was shown that the used of MISPE improved the accuracy and precision of the HPLC method.

MISPE for caffeine[62-64] has also been developed by Farrington and co-workers[63]. Caffeine from beverages and human blood were extracted with SPE having caffeine imprinted polymer as selective sorbent. It was shown that high degree of sample clean-up was obtained. Other ingredients presented in sample matrixes were not present as high concentration as caffeine when analyzed by HPLC. In another study by Theodoridis and co-workers[64], influence of loading conditions on the efficiency of MISPE for caffeine was studied. It was shown that loading of aqueous samples such as buffer gave better performance than that with samples in organic solvent.

1.4 Nevirapine

Nevirapine (Viramune), the IUPAC name being 1-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido (3,2-b:2',3'-e) (1,4) diazepin-6-one[1], is the first non nucleoside reverse transcriptase inhibitor (NNRTI) to receive regulatory approval for the treatment of HIV-1 infection and is currently used in combination with nucleoside reverse transcriptase (RT) inhibitors such as azidothymidine (AZT), lamivudine (3TC), stavudine (D4T) and dideoxyinosine (DDI). It has also been shown to have synergistic effects with the protease inhibitor saquinavir in vitro. Clinical trials examining combinations of nevirapine and other HIV-protease inhibitors also show great promise. HIV seems to get resistant to the effects of nevirapine very quickly unless the drug is used as part of an effective anti-HIV drug combination. If a person becomes resistant to nevirapine, he may also be resistant to other NNRTI drugs such as delavirdine (Rescriptor) and efavirenz (Sustiva). The cross-resistance problems could be avoided if the level of nevirapine is seriously monitored [68, 69].



Nevirapine (NVP)

(1-cyclopropyl-5, 11-dihydro-4-methyl-6H-dipyrido [3, 2-b: 2', 3'-e] [1, 4] diazepin-6-one)

Figure 1.7 The structure of Nevirapine

The main side effect of NVP is the appearance of rashes, which are toxic in nature and can be reduced in frequency by initiating therapy with half the daily dose for the first 2 weeks. A few cases of Steven-Johnsons syndrome have been reported. Other side effects include anorexia, nausea and hepatotoxic reactions. Because hepatic failure has been reported, a marked increase in alanine transaminase (ALT) or bilirubin should prompt therapy withdrawal. The bioavailability of NVP is 90% and it is metabolized by the cytochrome P-450 system in the liver. Its concentration in the cerebrospinal fluid is about 45% of the serum level [70].

Currently, HPLC [71-78], GC [79] and Liquid chromatography tandem mass spectrometry (LC-MS-MS) [80] are being used as standard tools for determination of nevirapine in human plasma. However, in the sample pre-treatment normally performed by liquid-liquid extraction, large amount of organic solvent is required and the process is very time-consuming. Therefore, to overcome such limitation, a new method for sample pre-treatment is highly required.

In biological samples, nevirapine is normally present at low concentration and efficient methods are required for their isolation for further analysis. Nowadays, MISPE is extensively used in chemical isolation from complicated matrixes. However, so far, there have been no studies of MISPE for nevirapine. The purpose of this study is to develop MISPE for nevirapine isolation in order to facilitate nevirapine monitoring in HIV infected patients.

1.5 Aim of this research

1.5.1 To synthesize molecular imprinted polymer selective to nevirapine

1.5.2 To optimize imprinting conditions for generating new molecular imprinted polymers suitable for MISPE application

1.5.3 To optimize the extraction conditions to develop MISPE for nevirapine in plasma sample

1.5.4 To develop MISPE for nevirapine for facilitating nevirapine monitoring process