EU GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

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MASTER'S THESIS

INVESTIGATION OF PERFORMANCE FOR MOLECULARLY THIAMETHOXAM IMPRINTED POLYMERS BY VARIOUS POLYMERIZATION METHODS

Beyza YILDIRIM

Supervisor : Prof. Dr. Berin YENİGÜL

Department of Chemistry

Date of presentation : 04.08.2015

Bornova-İZMİR
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Beyza YILDIRIM tarafından **Yüksek Lisans Tezi** olarak sunulan **"Investigation of Performance for Molecularly Thiamethoxam Imprinted Polymers by Various Polymerization Methods (Farklı Polimerizasyon Yöntemleriyle Hazırlanan Moleküler Thiamethoxam Baskılı Polimerlerin Performansının İncelenmesi)"** başlıklı bu çalışma E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi' nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve 04 / 08 / 2015 tarihinde yapılan tez savunma sınavında aday oybirliği/oyçokluğu ile başarılı bulunmuştur.

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04 / 08 / 2015

Beyza YILDIRIM

ÖZET

FARKLI POLİMERİZASYON YÖNTEMLERİYLE HAZIRLANAN MOLEKÜLER THIAMETHOXAM BASKILI POLİMERLERİN PERFORMANSININ İNCELENMESİ

YILDIRIM, Beyza

Yüksek Lisans Tezi, Kimya Anabilim Dalı
Tez Danışmanı: Prof. Dr. Berin YENİGÜL
Ağustos 2015, 56 sayfa

Thiamethoxam, dünyada zararlılarla mücadele için tarımda geniş çapta kullanılan neonikotinoid grubu bir insektisittir. Yaygın kullanıma, suda yüksek çözünürlüğe ve kararlılığa sahip olması nedeniyle Thiamethoxam kalıntıları çevrede olumsuz etkilere yol açmaktadır. Tüm bu durumlar ticari formulasyonlarda ve çevreden alınan örneklerde Thiamethoxam tayini için yeni, basit ve düşük maliyetli analitik yöntemlere ihtiyaç duyulduğunu göstermektedir. Moleküler baskılı polimerler de pestisit kalıntılarının belirtilmesi ve örnekten ayrılması aşamasında büyük bir potansiyele sahiptir.

Yaptığımız literatür taraması sonucunda, moleküler Thiamethoxam baskılı polimer ile ilgili herhangi bir çalışma bulunamamıştır. Bu tezin amacı farklı polimerizasyon yöntemleriyle moleküler Thiamethoxam baskılı polimerlerin hazırlanması ve performanslarının incelenmesidir. Moleküler baskılı polimerlerin hazırlanması için, geleneksel (yığın polimerizasyonu ile baskılama) ve yeni geliştirilmiş (reçine şişirme ile baskılama) olmak üzere iki yöntem kullanılmıştır. Geleneksel yöntem zahmetli, zaman alıcı ve toksik monomerler kullanımından dolayı yeni yöntem ile moleküler baskılama önerilmiştir.

Yığın polimerizasyonunda, Thiamethoxam (TMX) kalıp molekül, metakrilik asit (MAA) monomer ve etilen glikol dimetakrilat (EGDMA) çapraz bağlayıcı olarak kullanılarak TMX molekülünü bağlayabilen moleküler baskılı polimerler hazırlanmıştır. Kalıp molekül:monomer:çapraz bağlayıcı mol oranı 1:4:20 olarak kullanılmıştır.

Yeni geliştirilmiş baskılama yönteminde, reçineyi şişirme özelliğine sahip uygun bir çözgendeki (Asetonitril) kalıp molekül (TMX) çözeltisine, reçinenin (stiren-divinilbenzen polimer) eklenmesiyle basitçe hazırlanmıştır.

İki farklı yöntemle hazırlanan baskılı polimerlerden Thiamethoxam' ın sökülmesi metanol ortamında sokslet ekstraksiyonu ile gerçekleştirilmiştir. Thiamethoxam' ın geri bağlama performansı kesikli ve kolonda geri bağlama yöntemleriyle araştırılmıştır. İlgili tüm sonuçlar UV-VIS Spektrofotometresi ile elde edilmiştir.

Bu tezde ilk kez hazırlanan Thiamethoxam baskılanmış polimerlerin ayırma ve ön-deriştirme amacıyla katı faz ekstraksiyonu (SPE) dolgu maddesi olarak kullanılması denenmiştir. SPE kolondan Thiamethoxam' ın ayrılması için koşullandırma, yükleme ve elüsyon basamaklarının optimizasyonu gerçekleştirilmiştir. Elüentler UV-VIS Spektrofotometresi ile analizlenmiş ve geri kazanım değerleri hesaplanmıştır.

Anahtar sözcükler: Thiamethoxam, moleküler baskılama, moleküler baskılı polimer (MIP), katı faz ekstraksiyonu (SPE), moleküler baskılı katı faz ekstraksiyonu (MISPE), spektrofotometrik analiz.

ABSTRACT

INVESTIGATION OF PERFORMANCE FOR MOLECULARLY THIAMETHOXAM IMPRINTED POLYMERS BY VARIOUS POLYMERIZATION METHODS

YILDIRIM, Beyza

MSc in Department of Chemistry
Supervisor: Prof. Dr. Berin YENİGÜL
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Thiamethoxam is a broad-spectrum neonicotinoid insecticide and it is used in the world for pest control in agriculture. Due to its extensive usage, high water solubility and stability, the residues of the Thiamethoxam persistent in the environment produce adverse effects. All above facts indicate the need for new, simple and low cost analytical methods for the determination of Thiamethoxam, both in commercial formulations and samples from the environment. Molecular imprinted polymers have a big potential in clean-up stage and sensing of the pesticide residues.

According to our literature survey, there is not any published paper dealing with molecularly Thiamethoxam imprinted polymers. The aim of this study is preparation of Thiamethoxam imprinted polymers by different polymerization methods and investigation of their performance. Two methods were used for the preparation of molecular imprinted polymers: Traditionally method (imprinting by bulk polymerization) and newly improved method (imprinting by resin swelling). Because traditional method was troublesome, time consuming and using toxic monomers, molecularly imprinting by the newly method was suggested.

In bulk polymerization, molecularly imprinted polymers that can bind TMX was acquired using Thiamethoxam (TMX) template molecule, methacrylic acid (MAA) monomer and ethylene glycol dimethacrylate (EGDMA) cross-linker. The mol ratio of template:monomer:crosslinker was used as 1:4:20.

In newly improved imprinting method, molecularly imprinted polymers was prepared by simply inserting the resin (styrene-divinylbenzene polymer) into the solution of template molecule (TMX) in a suitable porogen (Acetonitrile) that swells the resin.

The extraction of Thiamethoxam from imprinted polymers that prepared with two different methods was performed by soxhlet extraction with methanol. Rebinding performance of Thiamethoxam was investigated by batch-rebinding and column-rebinding methods. Related all results was obtained by using UV-VIS Spectrophotometer.

In this thesis, Thiamethoxam imprinted polymer which prepared for the first time was examined as solid phase extraction (SPE) packaging material for separation and pre-concentration purposes. An optimization of conditioning, loading and elution stages for Thiamethoxam separation from SPE column was performed. The eluents were analysed by UV-VIS Spectrophotometer and the recovery values were calculated.

Keywords: Thiamethoxam, molecular imprinting, molecularly imprinted polymer (MIP), solid phase extraction (SPE), molecularly imprinted solid phase extraction (MISPE), spectrophotometric analysis.

TEŞEKKÜR

Tez çalışma konumu öneren, değerli bilgi ve önerileriyle bana yol gösteren, her an ilgi, hoşgörü ve sabırla olumsuzluklar karşısında beni sürekli cesaretlendiren, benim için bir hocadan çok daha fazla anlam taşıyan sevgili danışman hocam Prof. Dr. Berin Yenigül' e sonsuz teşekkür eder, sevgi ve saygılarımı sunarım.

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SYMBOLS AND ABBREVIATIONS

Abbreviations	Explanations
MIP	: Molecularly imprinted polymer
MIPs	: Molecularly imprinted polymers
NIP	: Non-imprinted polymer
MIPresin	: Molecularly imprinted resin polymer
NIPresin	: Non-imprinted resin polymer
SPE	: Solid phase extraction
MISPE	: Molecularly imprinted solid phase extraction
TMX	: Thiamethoxam
MAA	: Methacrylic acid
EGDMA	: Ethylene glycol dimethacrylate
AIBN	: 2,2’-azobis (isobutyronitrile)
MeCN	: Acetonitrile
MeOH	: Methanol
UV-VIS	: Ultraviolet-visible
$W_{final\ (f)}$	: Weight of particles after uptake of water
$W_{initial\ (i)}$	: Weight of particles before uptake of water
n	: Repeatability

1. INTRODUCTION

Molecular imprinting can be employed in a variety of applications, such as separation, sensors or catalysis. The latest developments in the techniques of molecular imprinting have made available polymers that can be used in the detection of drugs, toxins, pesticides, food components, and other molecules that would be difficult to isolate otherwise. Considering the versatility and high level of specificity-recognition capacity that can be achieved, the future of these materials is very promising (Perez- Moral and Mayes, 2004).

Recently, molecular imprinting or template polymerization, has gained much attention as a convenient method for the construction of binding sites for different analytes (Gao et al., 2001). This technique has been used for the preperation of selective recognition sites for a wide variety of molecules (Alexander et al. 2003). Among the alternatives to natural recognition systems, Molecularly Imprinted Polymers (MIPs) have shown high promise. The increasing amount of papers and patents published per annum in this area, which has almost tripled over the last twenty years (Figure 1.1) reflects the potential and broad interest in these materials (Whitcombe, 2012).

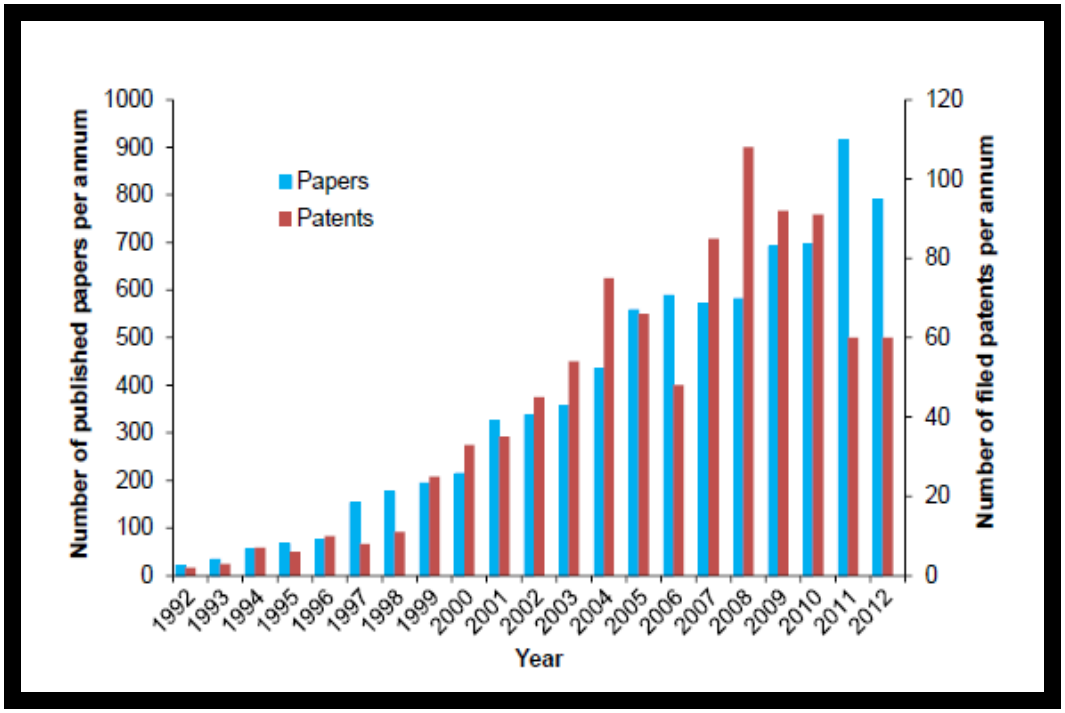

Figure 1.1 Number of published papers and filed patents in the area of molecularly imprinted polymers for the period 1992-2012 (Whitcombe, 2012).

1.1 Molecular Imprinting

A molecularly imprinted polymer (MIP) is a synthetic polymer possessing selective molecular recognition properties because of recognition sites within the polymer matrix which are complementary to the analyte molecule in the size, shape, positioning of functional groups (Komiyama et al., 2003).

Molecular imprinting can be basically described as a way of making artificial locks for molecular keys (Mosbach and Ramström, 1996). In the first step, the selected key molecule is mixed with a variety of lock building blocks. We can imagine key molecules as template molecules and the building blocks as monomers and cross-linkers. In the second step, building blocks and the key is allowed to, either firmly or loosely, attach to each other. Then as a third step, the formed complexes between the key and the building blocks are subsequently "glued" together in order to fix the building block positions around the key as a puzzle. Finally, by removing the molecular key, the leaves a construction which, if everything works properly, is selective for the original key and will not recognise any other key (Ramström, 1996).

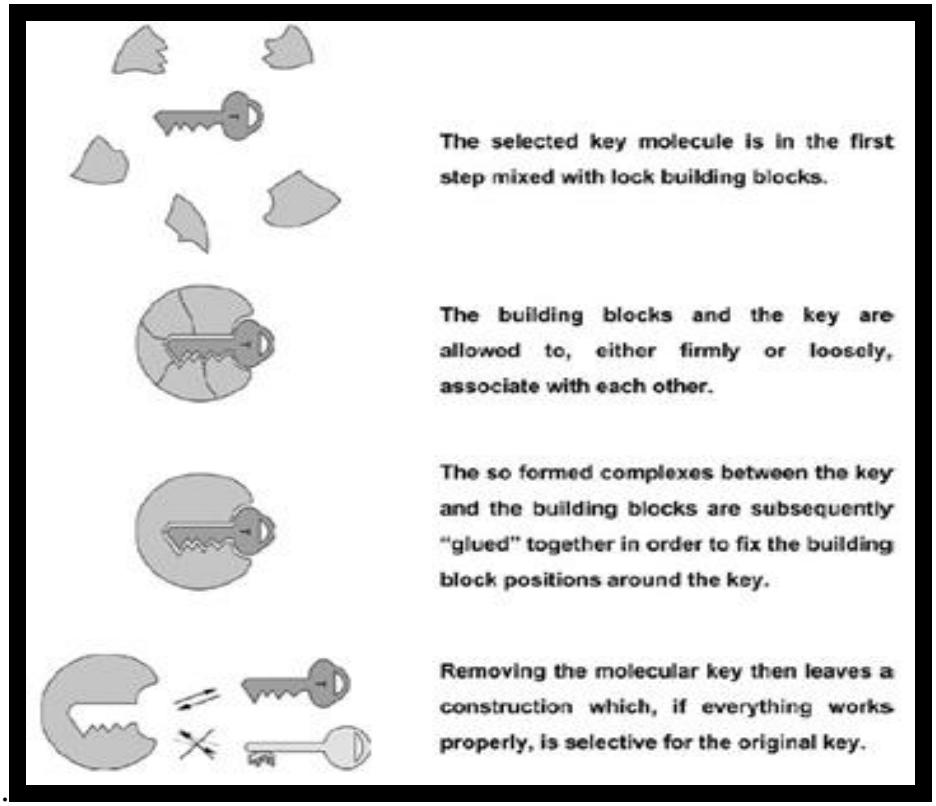

Figure 1.2 Principle of molecular imprinting inspried by Fischer's lock and key metaphor (Ramström and Yan, 2005).

The general procedure for imprinting consists of three steps. Step one involves the pre-association of one or more functional monomers with the template molecule. The second step of the process is the polymerisation of the monomer-template adduct. This is achieved through addition of a cross-linking agent, forming a polymer backbone that holds the functional monomers in place. Polymerisation is performed in a porogen that all constituents are soluble in and is initiated by a thermal or photochemical radical initiator. The final step in MIP preparation involves the removal of the template from the polymer by grinding (in the case of monolith production) followed by washing with solvent, or by combined chemical treatment and washing. This yields a porous material that has cavities complementary in shape and functionality to the template. A diagram of the imprinting process is presented in Figure 1.3 (Kathleen, 2010).

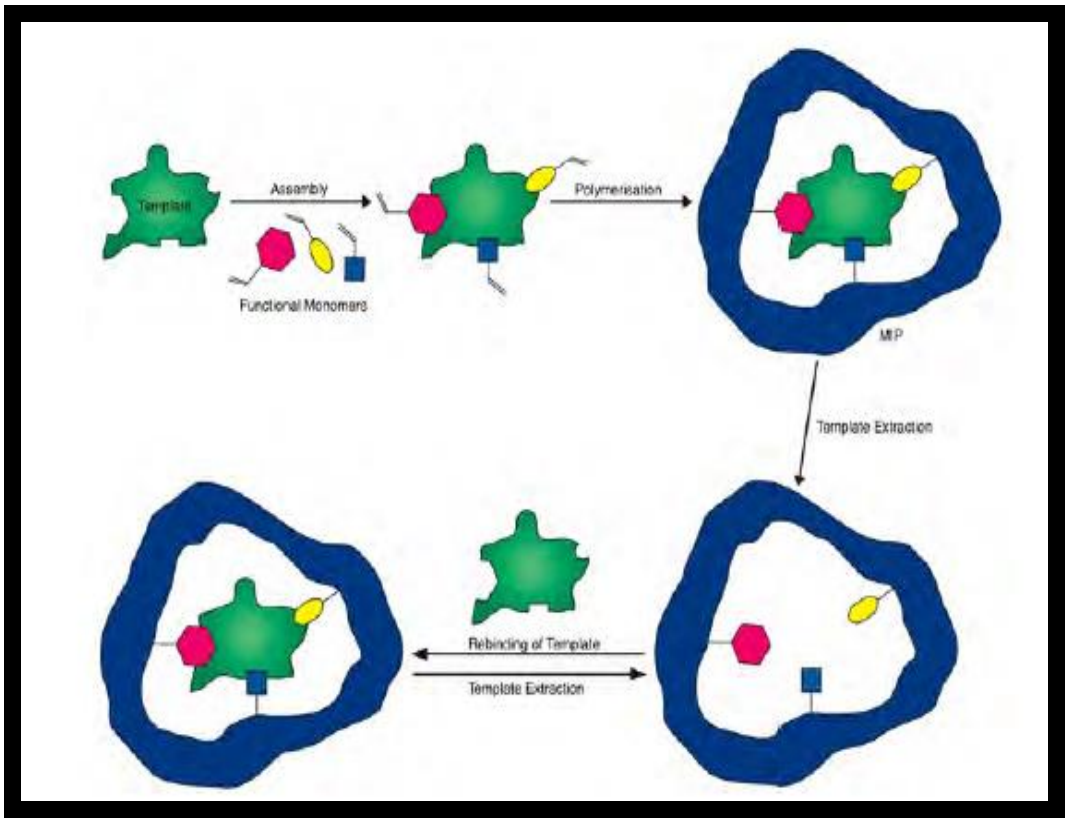

Figure 1.3 Schematic of the imprinting process showing the interaction of one template unit with monomer units (Kathleen, 2010).

As MIPs have outstanding advantages such as predetermined selectivity, simple and convenient preparation, robustness in organic solvents and acidic or basic reagents, and durability to high temperature, molecular imprinting has drawn extensive attention in recent years (Zhang et al. 2001).

The advantages of the MIPs are their ease of preperation, stability, high selectivity and high affinity constants. To date, MIPs have been exploited in a number of applications including their use as seperation materials, as antibody mimics in binding assay systems, and as recognition elements in biosensors for assay of various analytes. Recently, beacuse of their compatibility with organic solvents MIPs have been utilized as solid-phase extraction (SPE) sorbents for environmental applications is rapidly increasing (Zhu et al. 2002**;** Yan and Kapua 2001).

A summary of most important advantages and disadvantages of imprinted polymers are shown below (Sennaroğlu, 2006).

Advantages

- Target defines own recognition site,
- Stability of synthetic materials,
- Specificity of natural systems,
- Adaptability/flexibility in use,
- Facile, "one-pot" synthesis,
- Use in non-aqueous media/aggressive environments.

Disadvantages

- Diversity of binding sites,
- Poor processability.

1.2 Components of the MIP

1.2.1 Templates

The template molecule ideally should contain at least one functional group through that it can interact with the functional monomer as well a distinctive three-dimensional structure. The type of functional group controls the imprinting approach that can be utilised. Not all templates will readily form a covalent bond with a functional monomer that is easily cleaved. On the other hand the number of functional groups affects the affinity of the template for the MIP. Increasing the

number of interactions between the template and functional monomer may increase the affinity with that the MIP rebinds the template (Sellergren, B., 2001).

A selection of different compounds is showed in Table 1.1. Template analytes such as drugs, herbicide, toxins, hormones, amino acids, carbohydrates, co-enzymes, nucleotide bases and proteins have been successfully used for the preparation of selective recognition materials (Sun, 2009).

Table 1.1 List of a few of compounds used as template molecules in imprinting (Sun, 2009).

Compound Class	Example	Compound Class	Example
Drugs	Theophylline	**Amino acids**	Phenylalanine
	Diazepam		Tryptophan
	Morphine		Tyrosine
	Ibuprofen		Aspartic acid
	Chloramphenicol		
	Naproxen	**Carbohydrates**	Galactose
			Glucose
Herbicides	Atrazine		Fucose
	Metsulfuron-methyl		
	2,4-dichlorophenoxyacetic acid	**Co-enzymes**	Pyridoxal
Mycotoxins	Ochratoxin A	**Nucleotide bases**	Adenine
Hormones	Cortisol	**Proteins**	RNase A
	Enkephalin		Urease

In a covalent MIP the template forms stoichiometric bonds with the functional monomer. Covalent bonds that have been used include the boronic ester, Schiff base and carbonate bonds. For a non-covalent MIP the mole ratio of template to functional monomer is usually 1:4; this is often specific to the template and functional monomer that is used because the template could have more than one functional group and so can interact with more than one functional monomer moiety in the active sites. In some cases a slight excess of functional monomer is added to enable complex formation. This means that some of the

functional monomer will not be located within imprinted cavities; these sites can non-specifically rebind the template. A few examples of non-covalent interactions which have been commonly used include hydrogen bond, electrostatic, hydrophobic and π-π electron interactions (Kueh, 2008).

1.2.2 Functional monomers

The functional monomer contains at least a vinyl group and another functional group, through that it can interact with the template. Non-covalent imprinting requires the selection of an suitable functional monomer that will form strong inter-molecular interactions with the template (Sellergren, B., 2001).

To help in the selection process the strength of association between the template and functional monomer can be studied by spectroscopy, as the spectra changes (FT-IR, ^{1}H-NMR or UV-VIS) are related to the change in the interaction between the template and functional monomer. The selection of functional monomers for covalent and semi-covalent imprinting is more restricted because of the antagonistic requirements of polymerization and template extraction. The functional monomer must form a covalent bond with the template which is stable under polymerisation conditions. However, the extraction of the template requires which the bond is readily cleaved once the polymer has been formed. In the case of covalent imprinting the template and functional group in the imprinted cavity must readily re-form the covalent bond to be useful (Kueh, 2008).

Figure 1.4 lists some of the most commonly used functional monomers since the introduction of molecularly imprinting technique. An ideal situation in MIP technology is that, a functional monomer should be able to form strong interactions with the template either covalent or non-covalent bonding. The strength of these interactions has a significant factor on the performance of MIPs including selectivity (Zhang et al., 2008a) and binding capacity for the target compound (Koohpaei et al., 2008; Shamsipur et al., 2007). It is believed that, the stability of the monomer-template complex during pre-polymerization depends on how strong the monomer-template interactions are, and this has a direct effect on the binding capacity of the MIPs (Pakade, 2012).

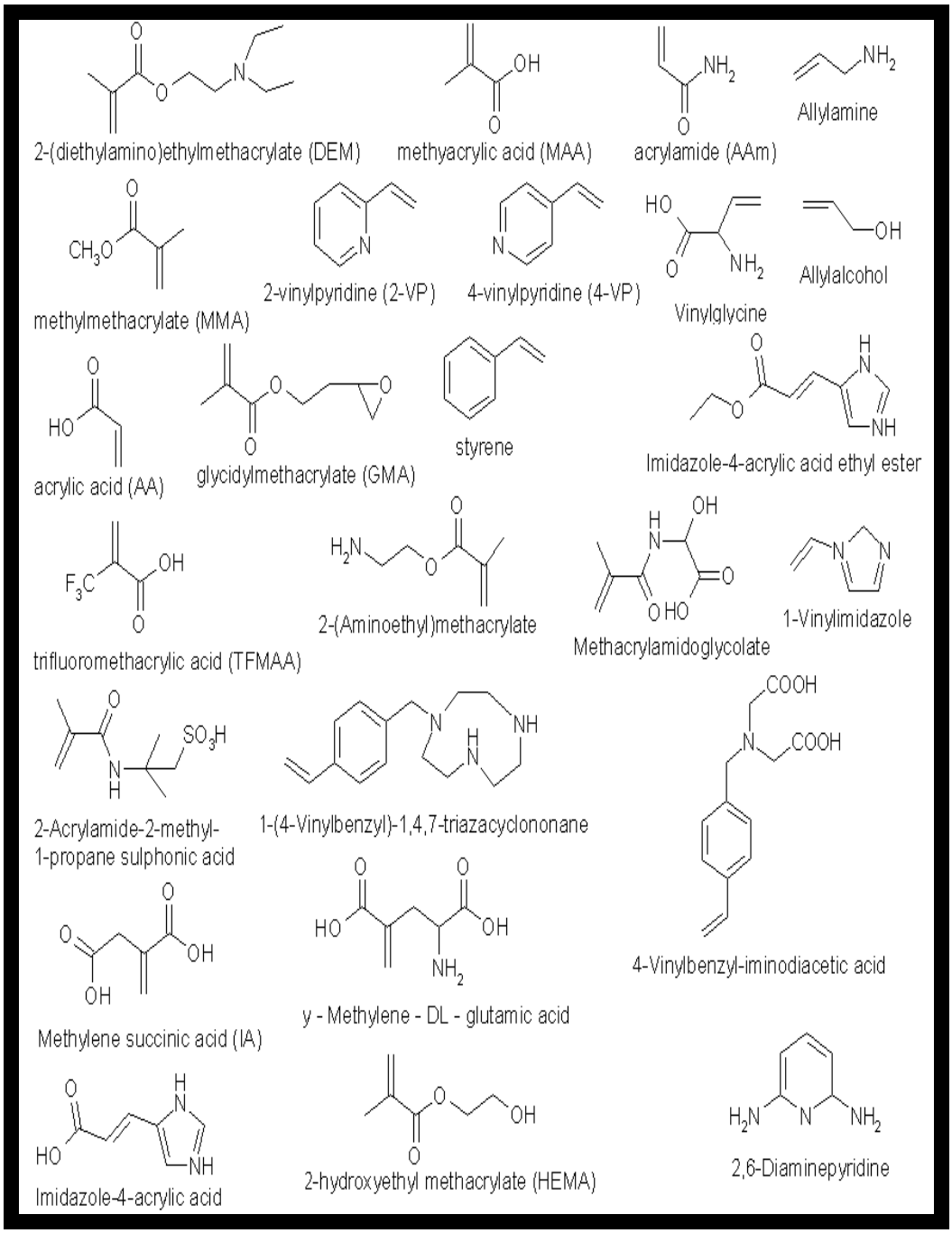

Figure 1.4 Selection of the most commonly used monomers (Pakade, 2012).

1.2.3 Cross-linkers

Choosing appropriate cross-linkers is equally significant as choosing functional monomers. This is because of the role these cross-linkers play in MIP synthesis. Generally, 80% of the amount of cross-linker to functional monomer is used. Cross-linkers bring about the mechanical stability of the polymer, control the morphology of the MIP matrix and stabilize the imprinted binding sites to retain the recognized binding sites (Sellergren, 1999). However, too much of the

cross-linker used can impede the binding sites because it could be difficult to remove the template completely, resulting in polymers with low binding capacity. In addition, the cross-linker should have less interactions with the template to prevent formation of non-specific binding sites. Amongst the many cross-linkers used, ethylene glycol dimethacrylate (EDMA) and trimethalolpropane trimethacrylate (TRIM) are the most commonly utilized and TRIM is viewed to give more rigid and effective binding sites than EDMA (Vasapollo et al., 2011). Nevertheless, EDMA is mostly used in bulk polymerization. Figure 1.5 lists some of those common cross-linkers used (Pakade, 2012).

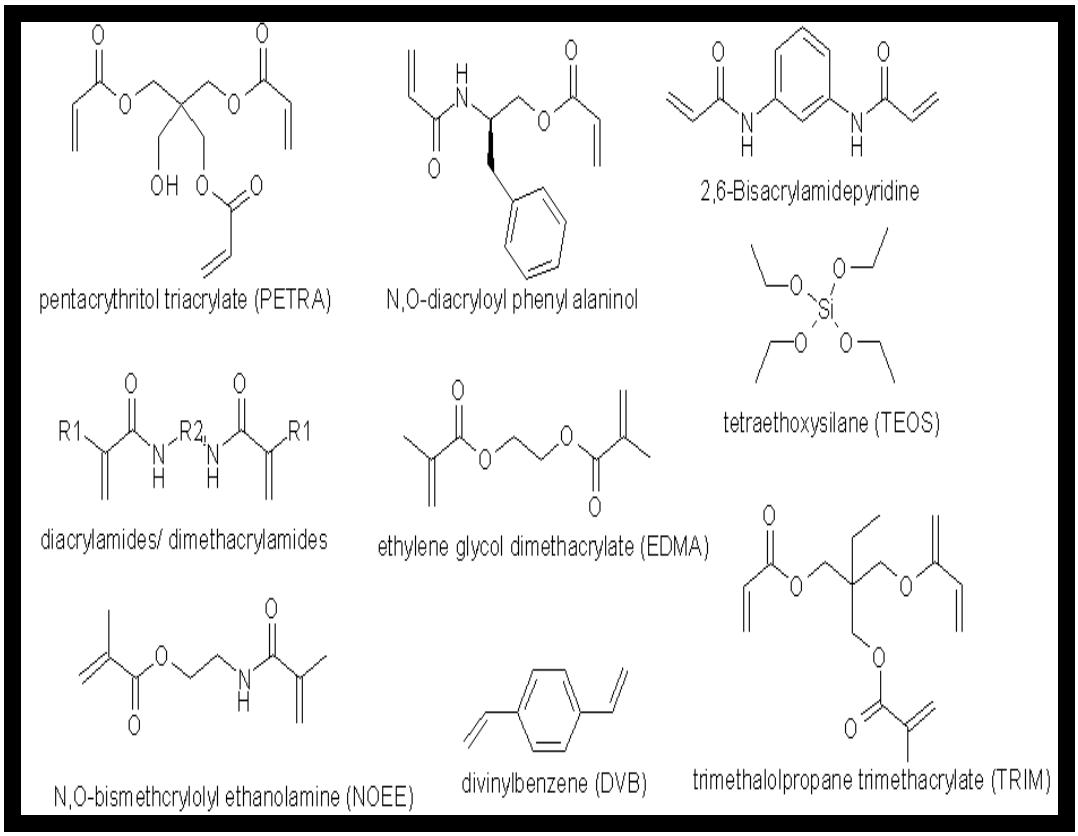

Figure 1.5 Typical cross-linkers used in MIP synthesis (Pakade, 2012).

MIPs are solid and porous as the multiple vinyl groups in the crosslinking monomer can co-polymerise with the functional monomer and so interconnect two radical centres from different polymer chains. Their porous nature allows the template molecules to diffuse into and from the imprinted cavities; their solid state allows the MIP to sustain structural integrity of the imprinted cavities. The degree of cross-linking determines the rigidity of the polymer and may affect the selectivity of a MIP (Wulff et al.,1987).

1.2.4 Initiators

In principle, any of the methods of initiation can be used to initiate free radical polymerisations in the presence of templates. However, there can well be drivers for selecting one over another arising from the system under study. For example, if the template were photochemically or thermally unstable then intiators that can be launched photochemically or thermally. When complexation is driven by hydrogen-bonding then lower polymerization temperatures are preferred and under such circumstances photochemically active initiators can well be preferred as these may operate efficiently at low temperature (Cormack and Elorza 2004).

Many initiators with different chemical properties can be used as the radical source in free radical polymerization (Figure 1.6). Generally they are used at low levels compared to the monomer by 1 mol. % with respect to the total number of moles of polymerisable double bonds. The rate and mode of decomposition of an initiator to radicals can be launched and controlled in a number of ways, including heat, light and by chemical/electrochemical means, depending upon its chemical nature (Yan and Ho Row, 2006).

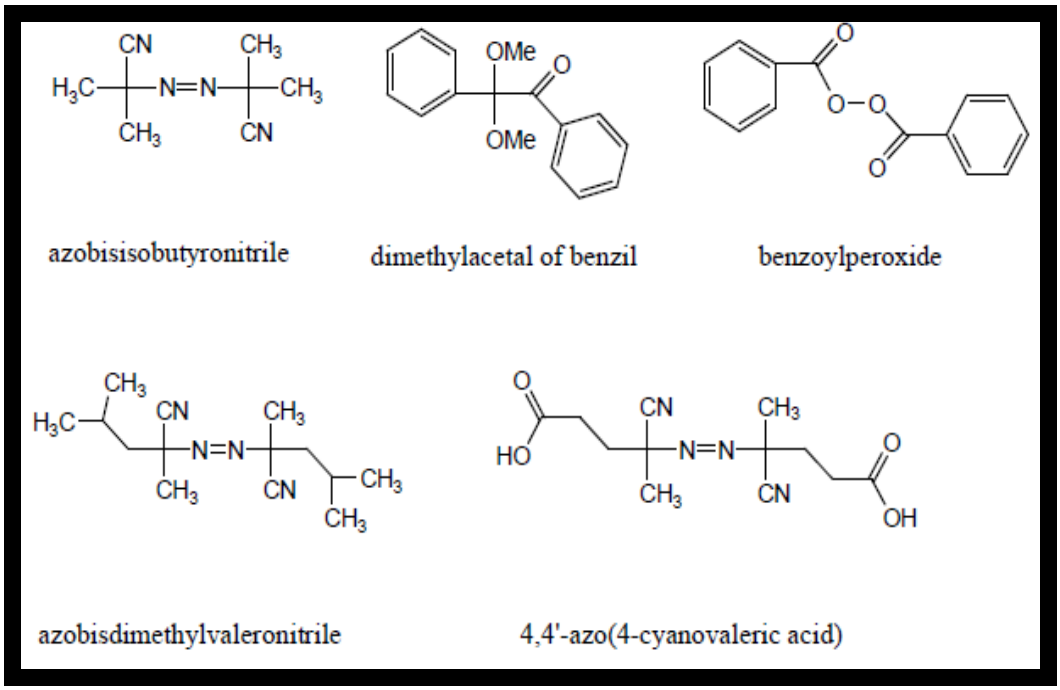

Figure 1.6 Chemical structure of common initiators (Yan and Ho Row, 2006).

Oxygen gas retards free radical polymerizations, thus so as to maximize the rates of monomer propagation, enable 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 (Yan and Ho Row, 2006).

1.2.5 Solvents

The solvent (porogen) plays a significant role in the outcome of a molecular imprinting process, a role that is particularly pronounced in self-assembly systems. To be a porogen in the imprinted polymerization, the solvent governs the strength of non-covalent interactions in addition to its influence on the polymer morphology. Normally, the more polar the porogen, the weaker the resulting recognition effect becomes, as a consequence of the influence of the porogen polarity on non-covalent interactions. The best imprinting porogens, for accentuating the binding strengths, are solvents of very low dielectric constant, such as toluene and dichloromethane. The use of more polar solvents will inevitably weaken the interaction forces formed between the print species and the functional monomers, resulting in poorer recognition. Thus, acetonitrile (ACN) as a quite polar solvent leads to more macroporous polymers than chloroform (Table 1.2). A lower surface area and a lower macroporosity can lead to diminished recognition, because of lower accessibility to the sites (Sun, 2009).

Table 1.2 Polar and non-polar solvents as porogens in molecular imprinting (Sun, 2009).

	Name	Bp (°C)	Dielectric constant
High polar solvent	Water	100	80
	N,N-dimethylformamide	153	38.3
	Methanol	68	33
	Acetonitrile	81	36.6
Low polar solvent	Dichloromethane	39.8-40	9.1
	Tetrahydrofuran	66	7.52
	Chloroform	60.5-61.5	4.8
	Hexane	69	2.02
	Toluene	111	2.4

In the recognition step, similar questions about the choice of solvent rise. Because all non-covalent forces are influenced by the properties of the solvent, non-polar solvents normally cause the best recognition. When applying the polymers to gradually more polar solvents, the recognition is decreased. On the other hand, the morphology is affected since the swelling of the polymers is dependent on the surrounding medium. Thus, swelling is most pronounced in chlorinated solvents, such as chloroform and dichloromethane (DCM), as compared to, e.g. acetonitrile (MeCN) and tetrahydrofuran (THF). This swelling behavior could cause changes in the three-dimensional configuration of the functional groups taking part in the recognition in the polymer binding sites resulting in poorer binding capability. As a rule of thumb, the best choice of recognition solvent should be more or less similar to the imprinting porogen so as to avoid any swelling problems, although this is not certainly a prerequisite. The polymer swelling taking place when polymers are prepared in organic porogens and subsequently used in aqueous phase, is not certainly a gross obstacle, however. The swelling in water is nearly the same as for many other solvents, such as acetonitrile (Sun, 2009).

1.3 Molecular Imprinting Approaches

In terms of imprinting strategies, three fundamental kinds of approaches can be distinguished. They are all classified according to the nature of the bonds established between the template and the functional monomers, and particularly they are predicated as covalent, semi-covalent and non-covalent approaches (Poma, 2012).

1.3.1 Covalent imprinting

Covalent imprinting approach was pioneered by Wulff and colleagues (1972, 1982) and it involves the covalent modification of the template, that is chemically bound to the functional monomers in a reversible way. This modification is then followed by the polymerisation step, during which the template is still covalently connected to the monomer, now polymerised. Finally, the template is cleaved through a mild chemical reaction (e.g. hydrolysis or reduction), leaving behind the binding cavities. This approach indicates very significant advantages, such as that thanks to the strong interaction which occurs between the template and the functional monomer, it usually leads to binding sites that are quite homogeneous (Umpleby II et al., 2000). However, both the removal

of the chemically bound template and its subsequent rebinding are not simple, because they involve the disruption and the re-establishment of covalent interactions, which in turn make the kinetics of these processes really slow. This latter thing has to be considered, especially in the case of separation applications, in which the presence of a fast rebinding kinetics is important (Zhu et al., 2010). Moreover, the prior derivatisation of the template is not easy with this approach, and is dependent on the chemical nature and the available functional groups of the template (Ye and Mosbach, 2002).

1.3.2 Semi-covalent imprinting

In this approach, part of the monomer is also cleaved when the template is cleaved from the polymer (Figure 1.7) (Whitcombe et al., 1995). A variation of the above-mentioned method has been developed by Sellergren and Andersson (1990), and consists in carrying out the imprinting step using the polymerisation of the functional monomer bound to the template, while the rebinding process takes place only thanks to non-covalent interactions. The template is removed by hydrolysis, allowing the subsequent rebinding step to take place through the establishment of mainly hydrogen and electrostatic bonds (Mayes and Whitcombe, 2005). In order to overcome these problems the cleavage of the template has been carried out through the reduction of the covalent bond using $LiAlH_4$. With this technique, for example, a hydroxyl group was obtained instead of a carboxylic one (Byström et al., 1993; Ikegami et al., 2004).

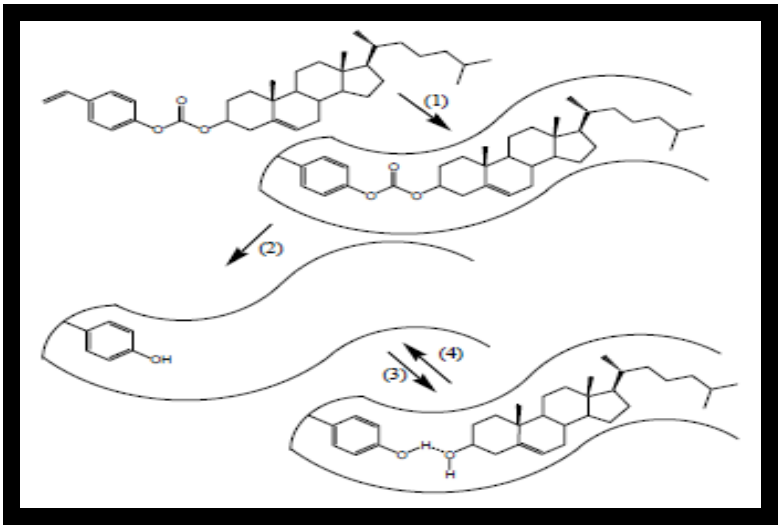

Figure 1.7 Semi-covalent molecular imprinting of cholesterol using the sacrificial spacer approach. Polymerization (1), cleavage and extraction (2), rebinding (association) (3), and dissociation (4) (Whitcombe et al., 1995).

1.3.3 Non-covalent imprinting

The third approach is the so-called non-covalent imprinting, which was pioneered by Mosbach and co-workers (1981, 1984, 1990). It exploits several kinds of non-covalent bonds between the template and the monomers, such as hydrogen, electrostatic and hydrophobic interactions like van der Waals forces. Because all these types of interactions are not really strong, a way to get more stable template-functional monomer complexes is to use an excess of monomers. This choice is far from being free from drawbacks, because it often causes a broad distribution of heterogeneous binding sites (Umpleby II et al., 2000; Haupt, 2003). The coupling of this approach with semi-covalent imprinting has been recently found to rise the imprinting effect of poorly functionalised molecules (Rosengren-Holmberg et al., 2009). However, the prior chemical derivatisation of the template still remains a drawback of these combined approaches. Despite the stated disadvantages, because of its simplicity and the faster template removal and rebinding steps, the non-covalent imprinting approach is probably the most widely exploited method to prepare MIPs (Alexander et al., 2006).

Table 1.3 Advantages and disadvantages of covalent and non-covalent imprinting (Komiyama et al., 2003).

	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 guest-binding site	clearer	less clear

In the pre-polymerization mixture, the dissolved target analyte interacts by covalent, noncovalent, or metal coordination interactions with the functional monomer responsible for localizing the chemically active moieties of the target molecules during copolymerization. Consequently, molecular imprinting is classified into covalent imprinting (pre-organized approach), non-covalent imprinting (self-assembly approach), and semicovalent imprinting according to

the type of interactions between functional monomer building blocks and target molecules in the pre-polymerization mixture and during rebinding (Figure 1.8) (Molinelli, 2004).

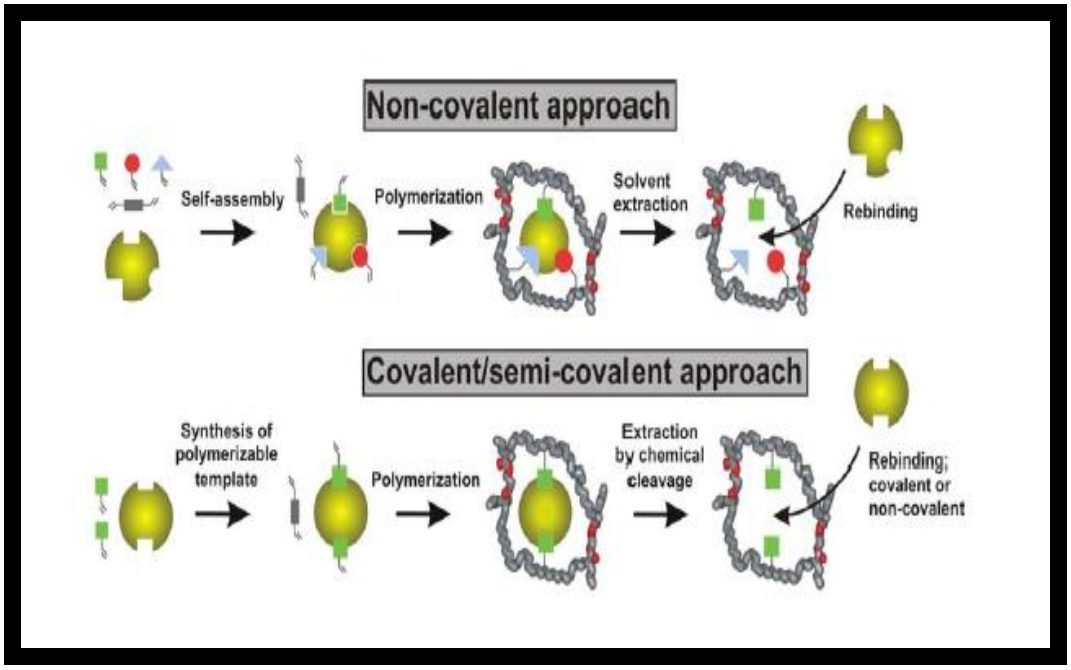

Figure 1.8 Principle of molecular imprinting (Molinelli, 2004).

1.4 MIP Formats

1.4.1 Bulk polymerization

Bulk polymerization is the most widely used method of polymerization in MIP synthesis. Simplicity in preparation, compatibility with many types of templates and the fact that only limited organic chemistry is required give this technique the edge over others. This method is sometimes referred to as traditional polymerization (TP) (Gallego-Gallegos et al., 2009; Chen et al., 2010; Beltran et al., 2010). Although MIP synthesis started much earlier but it is the work in early 1970s that really introduced bulk polymerization (Wulff and Sarhan 1972).

MIP synthesis using bulk polymerization often is carried out in a single reaction vessel where a functional monomer, template, cross-linker and initiator are dissolved in an appropriate porogenic solvent. This solution mixture is usually cooled to 0°C to prevent unwanted polymerization followed with purging with nitrogen to remove dissolved air. Several methods can be used to initiate polymerization. These include, use of heat as in free radical polymerization, by

means of UV radiations or photochemically to produce a bulk monolith (Pakade, 2012). The bulk polymer monolith is then crushed, ground and sieved to obtain particles mainly in the 25–50 μm size range. The last step consists in sedimentation to remove fine particles (Figure 1.9). The amount of fines can be minimised after sieving; this is done by the process called sedimentation, which involves numerous cycles of thoroughly stirring the particles in a solvent and discarding the supernatant after waiting for a specified time. In spite of its easiness of preparation, the bulk polymerization is timeconsuming, labour-intensive and wasteful since only 30–40% of the ground polymer is recovered as useable material. Moreover, the non-regular shape of the particles (Figure 1.10) obtained by bulk polymerization (Pichon and Chapuis-Hugon, 2008).

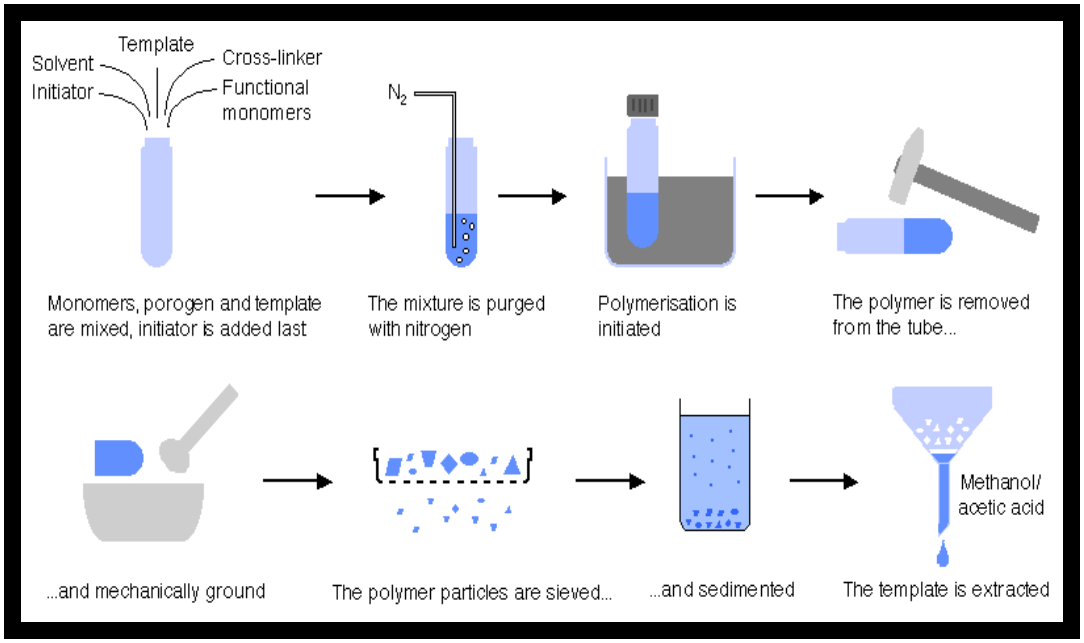

Figure 1.9 Steps of bulk polymerization (Pichon and Chapuis-Hugon, 2008).

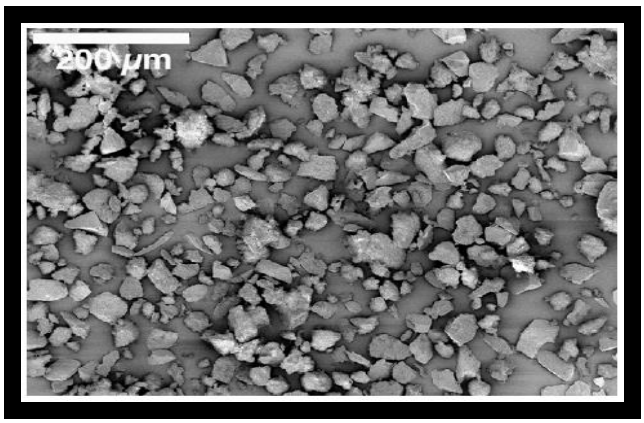

Figure 1.10 Scanning electron micrograph of polymers prepared by bulk polymerization, accelerating voltage: 5kV, working magnification 150x (Pichon and Chapuis-Hugon, 2008).

1.4.2 Precipitation polymerization

After bulk polymerization, precipitation polymerization (PP) is the second mostly used polymerization technique. PP method produces pre-polymerization mixtures that are similar to the ones used in bulk polymerization, except that higher amount of solvent is employed in precipitation polymerization resulting in diluted system (Gallego-Gallegos et al., 2009). Nevertheless, PP method is regarded as a more simpler and better method than emulsion, multistep swelling or grafting as it involves the no use of stabilizers and surfactants which can contaminate the final product (Pakade, 2012).

1.4.3 Suspension polymerization

The suspension polymerization method proposed by Mayes and Mosbach consisted in a heterogeneous polymerization based on the suspension of droplets of prepolymerization mixture in a continuous phase (water, mineral oil or perfluorocarbon), each droplet acting like a mini bulk reactor that allows the production of spherical beads in a broad size range starting at a few micrometers and reaching up to millimeter size (Pichon and Chapuis-Hugon, 2008). The major aim in suspension polymerization is the formation of an as uniform as possible dispersion of monomer droplets in the aqueous phase with controlled coalescence of these droplets during the polymerization process. (Vivaldo-Lima et.al., 1997).

1.4.4 Emulsion polymerization / Surface graft polymerization

Although rarely used, emulsion polymerization is employed when ‘’surface imprinting’’ is used more especially for protein imprinting (Tan and Tong, 2007; Tan et al., 2008). In this case, microsphere particles are produced by emulsion polymerization using water/oil medium. This method requires an oil-soluble functional host molecule, a water-soluble template, an emulsion stabilizer, and a polymer matrix forming monomer. Molecules self-assemble at the oil–water interface forming recognition sites on the polymer surface (Yu et al., 1992). Contrary to suspension and precipitation, particles with monodisperse particle size range are produced (Tan and Tong, 2007).

1.4.5 Dispersion polymerization

Dispersion polymerization is viewed as a modified precipitation polymerization where a monomer is soluble in the dispersion medium but not the polymer and where well-defined polymer particles are formed. The required use of stabilizer differentiates this method from the precipitation polymerization (Winnik et al., 1987; Shen et al., 1993). Similar to emulsion polymerization, dispersion polymerization may be used to address some of the shortfalls of the bulk polymerization method (Li et al., 2010).

1.4.6 Seed polymerization / Multi-step swelling polymerization

In multi-step swelling polymerization procedure, preformed seed particles of identical size are suspended in water and, after several additions of suitable organic solvents, the initial particles swell to a final size in the range 5–10 μm (Beltran et al., 2010). Similarly to dispersion method, particles of monodispersed size range are produced but the method is elaborate and time consuming as it involves multi-step swelling and polymerization (Hoshina et al., 2009; Hantash et al., 2006).

1.4.7 Molecularly imprinting by resin swelling method

A variety of techniques, which use a range of strategies to synthesize molecularly imprinted particles more easily with a better structure than the traditional crushed material, have arised during the last few years. In the thesis of Yemis F., a new method was offered for molecularly imprinting based on resin (styrene-divinylbenzene polymer) swelling (Yemiş F., 2010).

Molecularly imprinted polymer by resin swelling (MIPresin) synthesis is prepared by using template molecule, a suitable solvent and resin. Functional monomer, cross-linker and initiator are not required for imprinting because resin is provided already their functions. Because traditional method was troublesome, time consuming and using toxic monomers, molecularly imprinting by resin swelling method was suggested.

1.5 Characterization Techniques

The most relevant analytical techniques available to characterize molecular imprinting processes are summarized in Figure 1.11 (Molinelli, 2004).

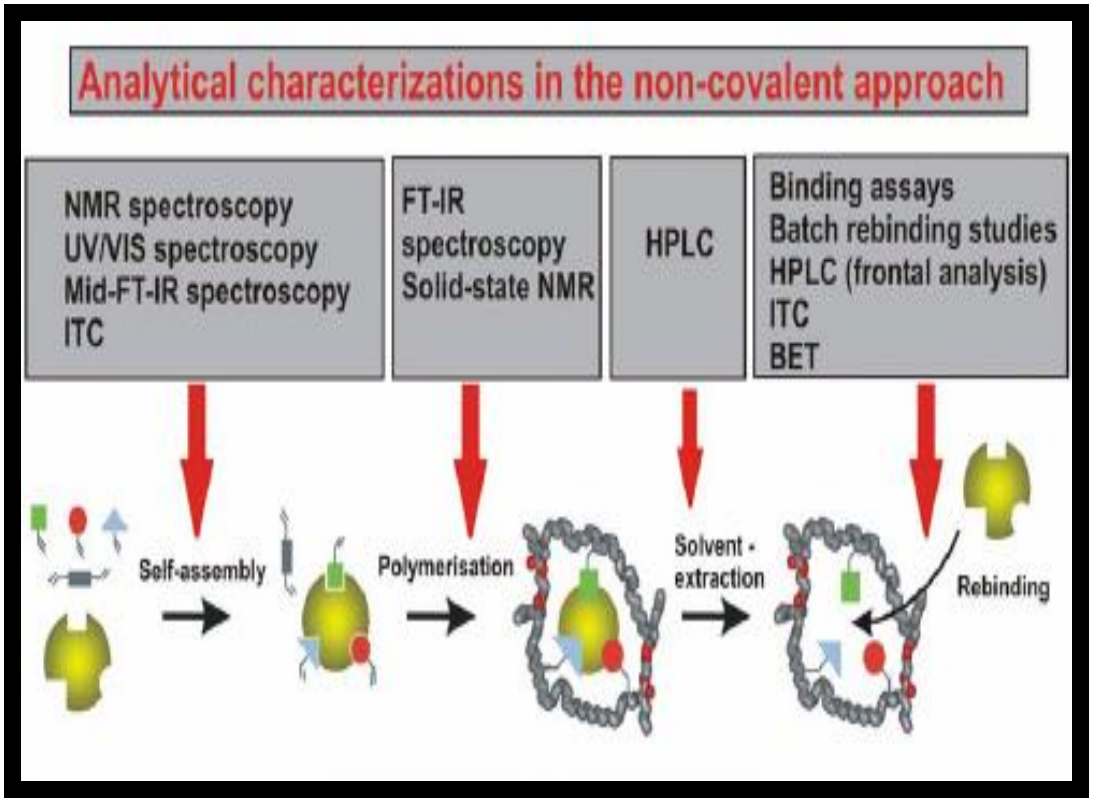

Figure 1.11 Analytical methodology amenable to the characterization of different processing steps during the preparation of non-covalently imprinted polymers (Molinelli, 2004).

1.5.1 Physical methods

Morphological information about the polymers can be acquired by scanning electron microscopy (SEM) measurements. The size and shape of the particles can be studied and particle size distribution analysis can be performed on the obtained SEM micrographs.

N_2 sorption porosimetry allows a detailed surface characterization of the imprinted polymers. Sorbed gases and solvents are removed from the polymer in vacuum then the adsorbed amount of nitrogen is measured at different relative pressures. Also the desorption isotherms can be recorded. Several parameters such as specific surface area, average pore size, pore volume and pore size distribution can be calculated based on different adsorption models.

Swelling tests can also reveal information about the polymers. The dry polymers with a known volume are equilibrated with a solvent and then the volume change is registered. The volume swelling ratio is the volume of the solvent swollen polymer divided by the volume of the dry polymer.

An interesting study combining all three abovementioned characterization methods studied zearalenone selective polymers in terms of the effect of different functional monomers and porogens (Urraca, 2008).

1.5.2 Chemical methods

Most of the chemical measurements aim to reveal the selectivity, and the binding capacity of the developed polymer.

1.5.2.1 Equilibrium batch rebinding measurement

The equilibrium batch rebinding measurement is probably the most straightforward way to characterize MIPs. We can obtain either entire binding isotherms or just measure at one concentration point for example in order to rapidly screen a polymer library for evaluation and selection of an optimal polymer composition. The binding isotherm plots the adsorbed amount on the polymer as a function of the equilibium concentration. The dry polymer is weighted into a glass vial or a plastic sample container, and a certain amount of the template solution in a given concentration is pipetted onto the particles. After homogenization the suspension is left to reach equilibrium with constant agitation. Following centrifugation the concentration of the unbound analyte in the supernatant is quantified by HPLC. Polymers have to be washed carefully before the experiment because the residual template inside the polymer can cause false results by changing the equilibrium between the liquid and the solid phase. If the template relatively weakly adsorbs onto the polymer, very small phase ratio (solvent volume/polymer mass) is required otherwise the concentration change in the supernatant will be very low and can be quantified only with a large experimental error. Nevertheless, this method allows one to thoroughly investigate the binding properties (adsorption isotherms) and the selectivity of MIPs in different solvents, at different temperatures even from solutions containing multiple analytes (Renkecz, 2013).

1.5.2.2 Characterization as liquid chromatographic stationary phase

Frontal chromatography is a well-established method for the characterization of MIPs. In this case the imprinted and nonimprinted polymer is packed into an empty HPLC column. The mobile phase contains the template in varying concentrations and breakthrough curves are recorded. One can calculate the breakthrough volume from the first derivative of the chromatogram and from this the bound amount of template can be readily obtained. The method is suitable for the precise measurement of the adsorption isotherm. The fitting of the binding isotherm can provide further characteristics of the imprinted polymer, for instance binding affinity constant, binding site heterogeneity and binding site concentration. A pioneering work in MIP frontal chromatography was done by Sajonz et al. (Sajonz et al., 1998).

Elution chromatography is also a frequently used technique for the characterization of MIPs. Here, the template is injected onto a column filled with the MIP or the nonimprinted polymer (NIP) and the retention time serves as the primary data from which the retention factor is calculated. The imprinting factor (IF), which is the ratio of the retention factors (k) on the MIP and the NIP, is widely used to give information about the imprinting effect albeit it is not suitable for accurate comparison of the results (Renkecz, 2013).

1.5.2.3 Interpretation of the results

Extensive efforts are made to produce imprinted polymers that mainly possess specific binding sites by minimizing the nonspecific binding. Hence, it is always a requirement to characterize the nonimprinted polymers to justify the imprinting efficiency on the MIP. Unfortunately, we can still find papers in the literature which do not investigate the template binding on NIPs and do not confirm the imprinting effect which significantly reduces the value of the work. It is also an important aspect to investigate the binding of structurally nonrelated compounds on the polymer. Here again, it is necessary to carry out the tests also on the NIP. If we see disparate binding of the non-related compound on the imprinted and nonimprinted polymer, it can signal a difference in the specific surface areas of the polymers, which, in turn, means a different number of non-specific binding sites, as well. Hence, an increased template binding on the MIP compared to the NIP is not necessarily the consequence of the imprinting (Renkecz, 2013).

1.6 Application Areas of MIPs

Application of molecular imprinting has become attractive in many fields of chemistry, biology and engineering, particularly as an affinity material for sensors, binding assays, artificial antibodies, adsorbents for solid phase extraction, chromatographic stationary phase, catalysis, drug development and screening. Among these applications, the one most widely used is SPE, for which MIPs are commercialized, where new applications of MIPs in SPE keep coming out constantly (Lok and Son, 2009).

1.6.1 Solid phase extraction (SPE)

Sample preparation is a significant step of a variety of analytical techniques and often the most time consuming work step in an analytical task. The sample preparation is required for the removal of potential matrix components, to rise the concentration of an analyte and to transform the analyte into a suitable form for separation and detection. Improving the sample preparation step is often attractive in order to optimize and to accelerate analytical techniques (Keçili, 2012).

Nowadays, solid phase extraction (SPE) is the most commonly technique for sample clean-up and enrichment. It has many advantages such as lower solvent consumption, smaller sample volumes and less exposure to organic solvents compared to traditional liquid-liquid extraction (LLE). Besides, SPE materials ensure more reproducible results and give cleaner analyte extracts prior to analysis. Now, there are a large number of SPE materials commercially available. On the other hand, the selectivity of traditional SPE sorbents is often not very high and furthermore low recoveries are often obtained. These problems can be overcome by using selective separation resins such as molecularly imprinted polymers (MIPs) (Keçili, 2012).

In 1994, the first example of SPE using MIPs. In that work, extraction of pentamidine, used for treatment of HIV/AIDS related infection, from urine was successfully achieved (Sellergren, 1994). Since then, several reviews and studies have been reported on the application of SPE using MIPs. SPE using MIPs are currently being successfully employed in bioanalysis, environmental analysis, food analysis (Keçili, 2012).

Solid sorbents used on SPE can be grouped into three categories (Figure 1.12); (i) inorganic based ones, these include silica gel (SiO_2), alumina (Al_2O_3), magnesia (MgO) and other oxide species, (ii) organic based ones these are natural polymers, unnatural polymers such as ion-imprinted polymers, and (iii) inorganic–organic hybrid materials e.g., C_{18}-silica (Prasada Rao et al., 2006).

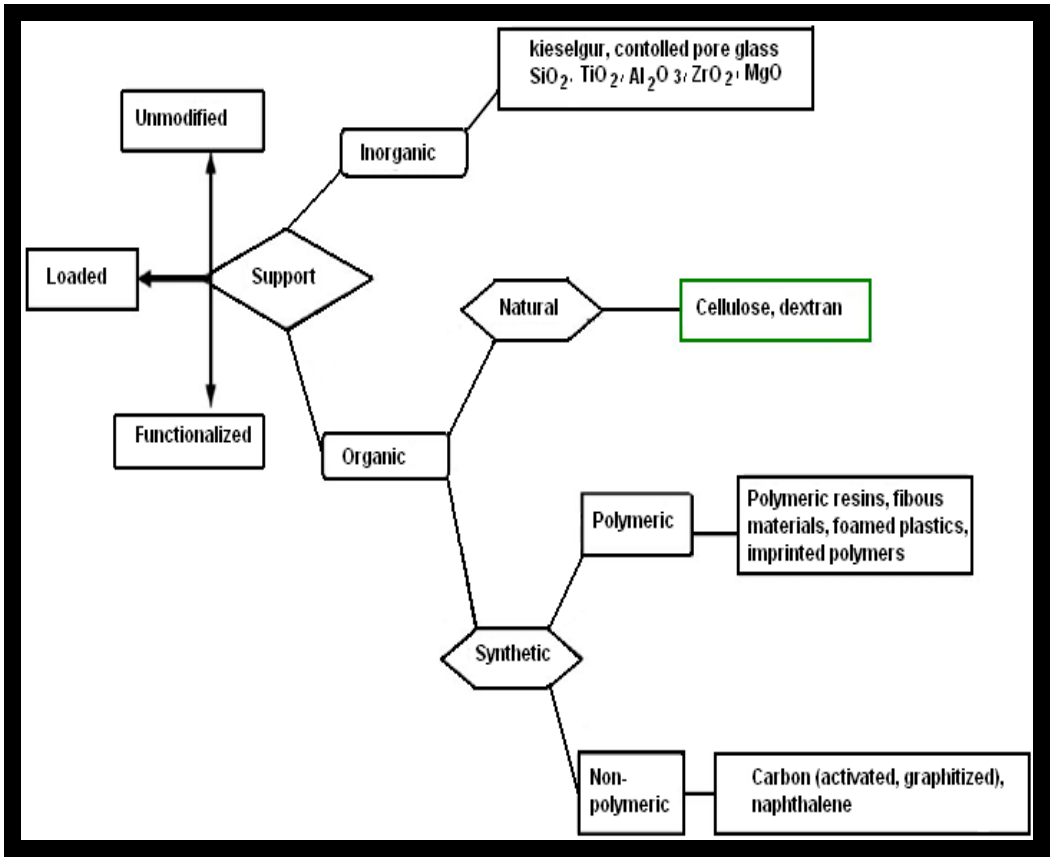

Figure 1.12 Types of solid-phase extraction sorbents (Prasada Rao et al., 2006).

MIPs function as a modern type of SPE sorbents with improved selectivity and efficiency compared to traditional SPE materials such as silica or non-imprinted polymers. MIPs can be selective for a particular analyte and allow pre-concentration of this analyte in complex matrices. Compared to many other selective separation materials, MIPs are mechanically and chemically stable under hard conditions such as extreme pHs, high temperatures and organic solvents. Ultimately, a selective wash removing interfering components from the biological sample, is usually possible when the analytical method involves a molecular imprinted polymer (Keçili, 2012).

Many different types of SPE sorbents are commercially available, for various applications. SPE with tailored MIP sorbents (MISPE) is currently a rapidly growing field. Molecular-imprint based SPE is the most advance application area of MIPs. MIP particles can be packed in a cartridge between two frits and be used off-line. They can also be packed in a small column (20–50mm×2–4mm i.d.) to be coupled on-line with liquid-chromatography (LC). The off-line and on-line coupling of MIP with LC has been recently largely described in recent reviews. The principle of the extraction on a MIP is the same as when using traditional SPE sorbents (Pichon and Chapuis-Hugon, 2008).

A general SPE procedure for aqueous samples using MIPs is demonstrated in Figure 1.13 (Keçili, 2012).

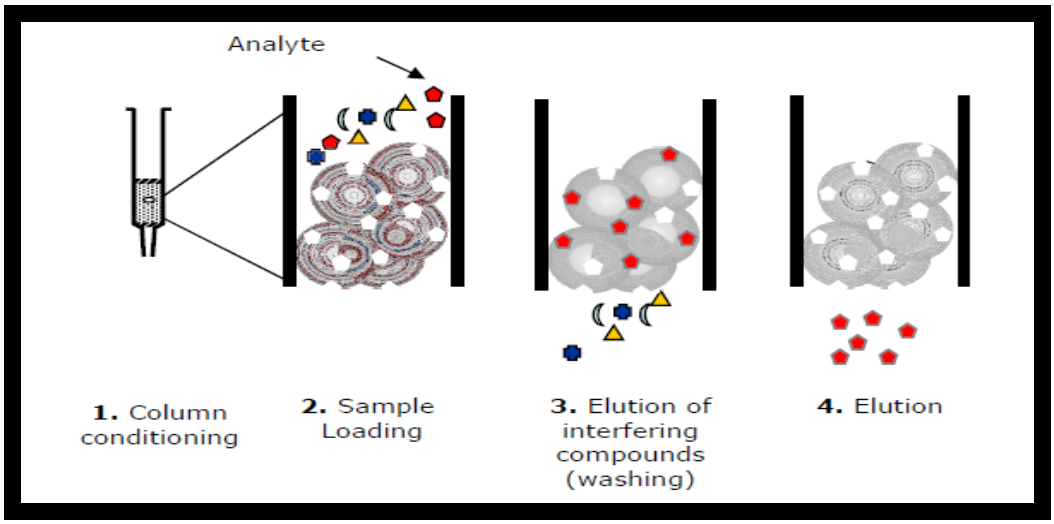

Figure 1.13 Principle of solid phase extraction (Keçili, 2012).

In an initial step called the conditioning step, the MIP is conditioned by a solvent to swell and to wet the polymer. After the solvent conditioning, the analyte is loaded under aqueous conditions and the analyte and other interfering components present in the sample are usually retained on the MIP mainly but for non-selective hydrophobic interactions with the polymer backbone.

After sample loading, the next step of the SPE procedure is one or more washing step. The goal of the washing step is to remove undesired interfering components which are not selectively bound to the MIP without eluting the analytes under conditions that maximize the selective interactions between the analyte and the MIP. Water or buffer is generally used as the first wash to remove hydrophilic interferences such as salts. In order to remove non-selectively bound compounds from the MIP, a second washing step with an organic solvent may be

needed. As a general thing, analytes exhibit the highest selectivity and binding towards the MIPs in an environment that corresponds to the solvent that was used as a porogen during the polymerization.

The washing step is followed by one or more elution steps, where the interactions between analyte and MIP are disrupted. Polar organic solvents e.g. methanol or acetonitrile, or an organic solvent with water or a so called modifier, e.g. acetic acid, formic acid, pyridine etc. are prevalently used as elution solutions depending on the chemistry of the MIP and the bound analyte. The samples obtained from the elution step are collected, the solvent evaporated and the dry residue re-dissolved and analyzed through HPLC, LC-MS, GC or other HPLC or GC techniques (Keçili, 2012).

The term of MISPE is based on the same main four steps as classical SPE. In addition to this, the principle of adsorption is based on a different mechanism, so a different method development strategy is required. Because the advantage of MISPE is its selectivity, it is crucial to optimize the selective retention of the target analyte(s) to the imprints and to suppress non-selective interactions to the polymer surface. Two different approaches can be used to obtain this selectivity; selective adsorption or non-selective adsorption followed by a selective desorption step. These two approaches are shown in Figure 1.14 (Möller, 2006).

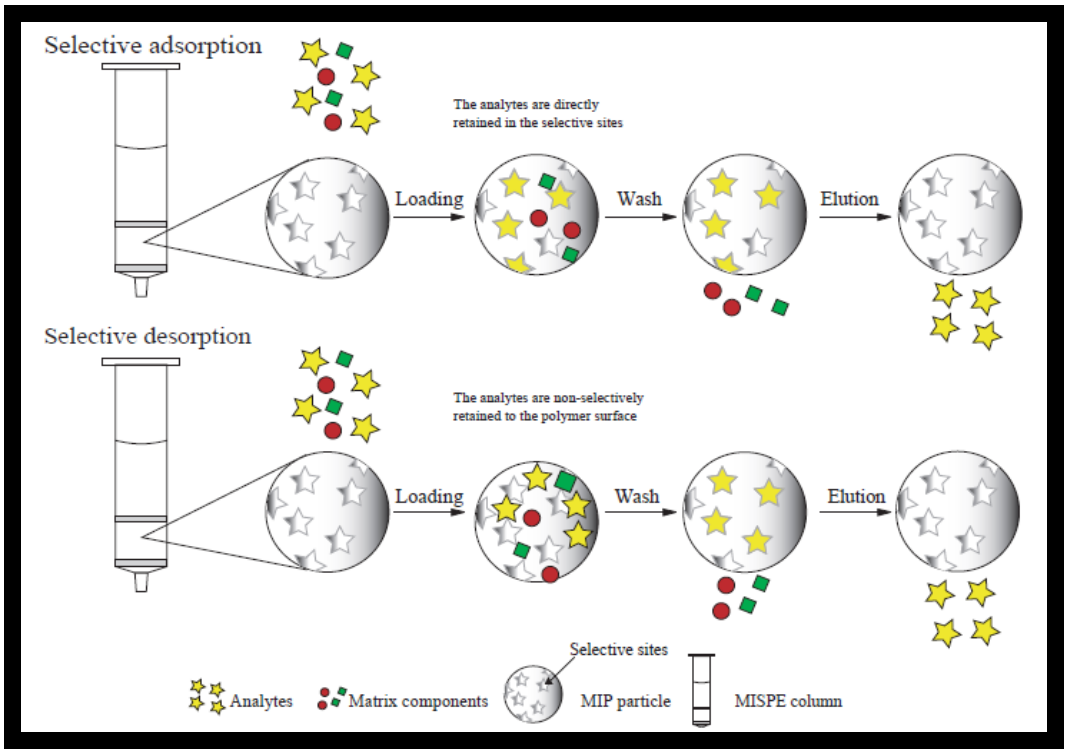

Figure 1.14 Illustration of the two different approaches used for MISPE (Möller, 2006).

1.6.2 Chromatography

The use of MIPs as stationary phases for chromatography is by far the most intensively studied application of imprinted polymers, especially for liquid chromatography (Sellergren, 2001; Ansell, 2005). The pioneering work by the Mosbach group on non-covalent imprinted polymers included separation of amino acid derivatives by LC using an MIP as the stationary phase (Sellergren et al., 1985; Kempe et al., 1993). The material was found to have enantio-selective properties, retaining the enantiomer used as template more strongly than the other enantiomer. Since then, several chiral compounds have been separated by MIP-based LC , including peptides (Kempe, 1996) and commercial drugs (Haginaka et al., 1999; Fu et al., 2003; Yin et al., 2005).

The recognition properties of MIPs ultimately depend on a variety of factors, including the diffusion/permeation properties of the synthesized polymer structure, binding to selective binding pockets and nonselective retention. These effects are schematically summarized in Figure 1.15 considering as an example the application of MIPs as stationary phase material in a separation column for liquid chromatography (Molinelli, 2004).

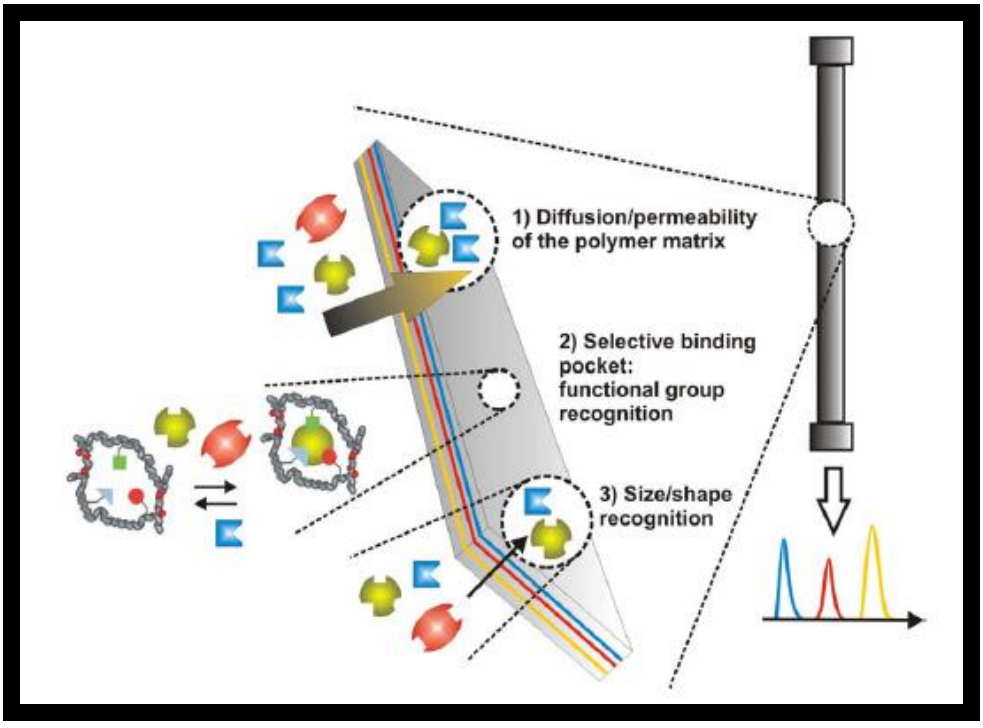

Figure 1.15 Schematic overview on factors affecting the recognition properties of a molecularly imprinted polymer for the example of a chromatographic separation phase (Molinelli, 2004).

1.6.3 Membranes

Incorporation of MIPs in membranes provides further applications, in which they introduce selectivity into the transport through the membrane barrier (Piletsky et al., 1999; Ulbricht, 2004). MIPs have been grafted onto the surface of a microfiltration membrane to introduce specific binding sites in the porous matrix (Piletsky, 2000; Sergeyeva, 2001). These MIP membranes were used in solid-phase extractions of triazine herbicides from water. Another application, involving surface-grafted membranes, is a screening method for identifying optimal conditions to generate a MIP protocol for a given template (El-Toufaili et al., 2004). MIP membranes have also been prepared by the incorporation of synthesized MIP particles as solid adsorption phases in a polyvinyl chloride/dibutyl phthalate membrane matrix (Suedee, 2004).

1.6.4 Catalysis

Since MIP materials contain spatially and functionally selective cavities for the template they can be applied in catalytic processes (Wulff, 2002; Alexander et al., 2003). Catalytically active imprinted polymers have been prepared using transition-state analogues as templates for ester hydrolysis (Wulff, 1997; Strikovsky, 2003; Ohkubo, 2001), ester hydrolysis including a metal-ion coordination (Yamazaki, 2001) and for a Diels-Alder cycloaddition reaction (Visnjevski, 2005).

1.6.5 Binding assays

Due to the similar binding characteristics as antibodies, MIPs have been used as alternatives or substitutes to antibodies in sorbent assays (Sellergren and Andersson, 2000; Lavignac et al., 2004; Ansell, 2004). The advantage of this alternative approach is that the chemical and mechanical stability of MIPs allows them to be used in conditions that are not suitable for antibodies. The production of MIPs is also generally cheaper and faster. Binding assays based on the rebinding of radio-labelled ligands have been developed for the enantioselective rebinding of (S)-propanolol (Andersson, 1996), the selective rebinding of a herbicide (2,4-dichlorophenoxyacetic acid) (Haupt et al., 1998) and the screening of a propanolol-imprinted library (Kempe and Kempe, 2004). Assay-analogues to enzyme-linked immunosorbent assays (ELISAs) have also been developed with MIPs used as antibody substitutes (Surugiu, et al., 2001; Piletsky et al., 2001).

1.6.6 Sensors

One of the most promising applications of imprinted networks is their use as recognition elements in sensors. A reminder of the principle of action of these sensors is given in Figure 1.16. The MIP is in contact with a transducer that converts the chemical or physical signal obtained on adsorption of the analyte into an easily quantifiable signal. Several principles of transduction have been used: We can mention ellipsometry, electric capacity, conductimetry, piezoelectric microgravimetry, evanescent wave IR, fluorescence, amperometry, voltammetry and pH. The imprinted materials allow the highly specific detection of even very dilute molecules in mixtures that can be complex and in extreme conditions (e.g. high temperature). They are therefore used or have been considered for use on ions, molecules with therapeutic properties, combat gases (sarin and soman), etc. Here again, their great stability gives them a real advantage over the biomolecules commonly used (Marty and Mauzac, 2005).

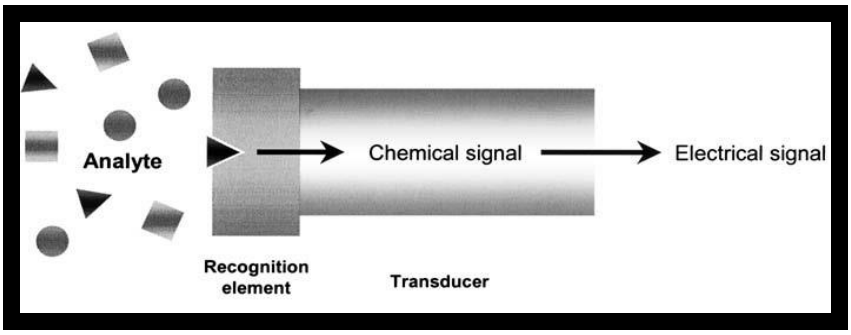

Figure 1.16 Schematic representation of a sensor (Marty and Mauzac, 2005).

1.7 Thiamethoxam

Thiamethoxam [(EZ)-3-(2-chloro-1,3-thiazol-5-yl-methyl)-5-methyl-1,3,5 oxadiazinan-4-ylidene(nitro)amine] is classified according to the pharmacophore as an N-nitroguanidine neonicotinoid (Elbert et al., 2008). The 4-nitroimino-1,3,5-oxadiazinan attached to the halogenated thianicotinyl moiety in thiamethoxam exhibits high insecticidal activity. Thiamethoxam is presently one of the most effective chemicals for the control of sucking pests such as aphids, whiteflies, thrips, somemicro lepidoptera and a number of coleopteran species. The compound shows contact as well as exceptional systemic activity and is recommended for soil, foliar and seed treatments in most agricultural crops all over the world (Karmakar and Kulshrestha, 2009).

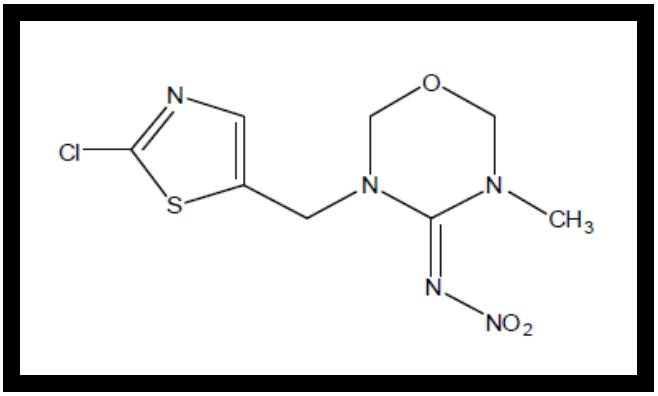

Figure 1.17 Molecular structure of Thiamethoxam (APVMA, 2001).

Thiamethoxam was the first second-generation neonicotinoid. Synthesized in 1991, Thiamethoxam is effective against both chewing and sucking insects, and was introduced to market in 1998 by Novartis Agribusiness, a precursor to the Swiss company, Syngenta Crop Protection. Thiamethoxam is highly stable, with an "estimated half-life at room temperature of 200-300 days" at pH 7. It also exhibits a "relatively high water solubility" of 4.1 g/L at room temperature, and is relatively non-toxic to birds, fish, and mammals (Maienfisch et al., 2001).

Thiamethoxam interferes with a specific receptor site in the insect's nervous system. Once insects feed on the plant or come into contact with Thiamethoxam, feeding is irreversibly stopped and insect damage halts. Thiamethoxam offers growers additional benefits beyond broad-spectrum insect protection. Thiamethoxam positively affects the plant through its ability to increase the biosynthesis of functional proteins resulting in more effective defense against numerous environmental stress conditions. More specifically, Thiamethoxam allows the plant to better cope under stressful growing conditions such as:

- Drought
- Low pH
- Protein degradation resulting from heat stress
- High soil salinity
- Wounding from pests, wind, hail, etc.
- Toxic levels of aluminum.

As Thiamethoxam helps the plant defend itself against these factors, it increases the crops' ability to reach much more of its genetic yield potential under a broad range of field conditions (Syngenta, 2015).

Advantages of neonicotinoids including a broad spectrum of insecticide activity and low acute mammalian toxicity have led to replacement of the organophosphates, carbamates, synthetic pyrethroids that previously dominated the world market. The extensive usage has led to concern about leaching of neonicotinoids into surface, ground water by run-off or wind drift, since these compounds show high toxicity to some aquatic organisms (Kim et al., 2006).

1.8 The Aim of the Study

Thiamethoxam is a broad-spectrum neonicotinoid insecticide in the world for pest control in agriculture. Due to extensive usage, high water solubility and stability, the residues of this insecticide is caused the persistent yield which have adverse effects on honeybees, birds, mammals and aquatic organisms. All above facts indicate that the need for new, simple and low cost analytical methods for the determination of Thiamethoxam in the environment.

Many methods have been described for the determination of pesticides at trace levels. However more specific, sensitive, rapid and economical analytical methods for the dedection of pesticide residues are needed. Molecular imprinted polymers (MIPs) have high stability and specifity towards the target molecules.

The aim of this study is investigation of performance for molecularly Thiamethoxam imprinted polymers by various polymerization methods. Traditionally (imprinting by bulk polymerization) and newly improved (imprinting by resin swelling) two methods are going to be used for the preparation of MIPs. Because traditional method was troublesome, time consuming and using toxic monomers, molecularly imprinting by resin swelling method was suggested.

The solid phase extraction (SPE) technique was applied for preconcentration of Thiamethoxam in water and performance of seperation of Thiamethoxam from MIPs was investigated. For this purpose, MIPs which were prepared by two different methods was used as a sorbent in SPE column. Then the recovery of bound Thiamethoxam in this sorbent was measured by UV-VIS Spectrophotometer.

2. EXPERIMENTAL

2.1 Apparatus, Reagents and Solutions

The photometric measurements were carried out using a PG instruments T80+ UV-VIS spectrophotometer. The cuvettes of quartz glass (1-1 cm) were used. The Waterbath NÜVE BM 302 was used in the polymerization stage. 25-53-75-125 μm Retsch model sieves were used for sieving polymer particles. Elektro-mag Heating Mantles and Soxhlet Extraction Glassware System were used in the extraction stage. Varian solid phase extraction (SPE) columns (3mL) were used for SPE studies. The Magnetic Stirrer and Hotplate CHILTERN were used. 10-100 and 100-1000 μL Brand Transferpette S digital micropipettes were used for all preparations. All weight measurements were performed using a Precisa XB 220A (readability: 0.0001g).

Thiamethoxam (TMX) (>98) is obtained from Syngenta (İzmir, Turkey). Resin (gel polystyrene crosslinked with divinylbenzene and gel strong acid cation hydrogen form Purolite C100MBH) was obtained from Purolite. Methacrylic acid (MAA), Ethyleneglycoldimethacrylate (EGDMA), 2,2'-azobis(isobutyronitrile) (AIBN), Acetonitrile (MeCN) and Methanol (MeOH) were purchased from Merck. All reagents were of analytical grade quality.

The standard solutions ($2x10^{-3}$ M, $4x10^{-3}$ M, $5x10^{-5}$ M) of Thiamethoxam in water were prepared daily.

2.2 Process of Molecular Imprinting Polymer

2.2.1 Bulk polymerization method

Template (Thiamethoxam, 0.297 g, 1 mmol) was dissolved in a suitable solvent (MeCN, 6.0 mL) and placed in a sealed glass tube. Then the functional monomer (MAA, 0.35 mL, 4 mmol) was added and allowed one hour for pre-arrangement. The cross-linking monomer (EGDMA, 3.8 mL, 20 mmol) and the initiator (AIBN, 47.0 mg) were added to above solution. The polymerization mixture was degassed with nitrogen for 10 minutes, and placed at 60 °C. The reaction was allowed to proceed for 24 hours. A reference non-imprinted polymer which did not contain any template was prepared simultaneously using the same method. Finally the tubes were crushed, the polymers were ground and sieved (the

particle size fraction of 25-53 μm, 53-75 μm and 75-125 μm). Then all polymers were extracted with a suitable solvent and dried at 50 °C.

2.2.2 Molecularly imprinting by resin swelling method

First of all, resin (styrene-divinylbenzene polymer) was dried at 50 °C and then the half of dried resin was ground and sieved (53-75 μm) and the other half of dried resin was remained in the original particle size range (425-1200 μm). Template (Thiamethoxam, 0.03 g) was dissolved in solvent (MeCN, 1.5 mL). After adding dried resin (styrene-divinylbenzene polymer, 1.0 g) this mixture was left overnight and resin is subjected to swelling. During the swelling process template molecules are expected to be located themselves in the swelled resin mass. Molecularly imprinted swelled resin polymer (MIPresin) was dried for 3 hours at 50 °C. Lastly both of them were extracted with an appropriate solvent and dried at 50 °C. Non-imprinted swelled resin polymer (NIPresin), that did not contain the template, was obtained with using the same procedure.

2.3 Extraction of Thiamethoxam from MIP and MIPresin

All the polymers, which have different particle size range, were weighted (1.0 g) and tightly packed into filter paper and placed in soxhlet apparatus. After polymers were extracted with methanol (350 mL) via soxhlet extraction and after 48 hours, absorbance of the methanol solution was measured at λ_{max}: 254 nm by UV-VIS spectrophotometer for the calculation of the percentage of extracted Thiamethoxam. Extracted polymers were dried at 50 °C.

2.4 Rebinding Studies of Thiamethoxam to Polymers

Rebinding studies of Thiamethoxam have been done by batch-rebinding and column-rebinding (SPE) methods.

2.4.1 Batch-rebinding studies

50.0 mg of extracted polymers and 5.0 mL of 2.0×10^{-3} M Thiamethoxam solution in water were mixed for 1, 2, 3, 4, 5 minutes at magnetic stirrer. The solutions were centrifuged for 5 minutes and then the absorbance values of supernatants measured at λ_{max}: 250 nm by UV-VIS spectrophotometer.

2.4.2 Column-rebinding studies

100.0 mg of dried MIP and MIPresin, which have different particle size range, packed into solid phase extraction (SPE) cartridge. 5.0 mL of standard solution containing $4.0x10^{-3}$ M Thiamethoxam in water was passed through the preconditioned (with 5.0 mL pure water) MIP and MIPresin columns with flow rate 0.8 mL/min. Rebinding rates to the each polymer of Thiamethoxam that passed through the SPE column was determined by UV-VIS Spectrophotometer.

2.5 Molecularly Imprinted Solid Phase Extraction: MISPE

100.0 mg of dried imprinted (MISPE) and non-imprinted polymer (NISPE) packed into the SPE cartridges of 3 mL with two original frits at each end. The cartridges were washed with 5.0 mL methanol and preconditioned with 5.0 mL pure water before sample loading. 5.0 mL standard solutions of Thiamethoxam in water ($4x10^{-3}$ M or $5x10^{-5}$ M) were passed through the preconditioned SPE columns with flow rate 0.8 mL/min. SPE columns were then dried by passing nitrogen through the column for 10 minutes before the adsorbed Thiamethoxam was eluted. For the elution step, 5.0 mL methanol was used. The absorbance values observed by UV-VIS spectrophotometer in the sample loading and elution steps.

2.6 Swelling Test

Water uptake ratio of MIPresin and NIPresin particles were determined in pure water. Initially dry particles were carefully weighted (0.5 g) before being placed in 5 mL vials containing 2 mL pure water. The vials were left overnight. Then the swelled resin samples were taken out from the water, wiped using a filter paper and weighted. The weight ratio of dry and wet samples was recorded. The water content of the imprinted and non-imprinted polymer particles were calculated by using the following expression:

$$\textbf{Water uptake ratio \% } = [(W_f - W_i) / W_i] \times 100$$

Where $W_{final\ (f)}$ and $W_{initial\ (i)}$ are the weights of particles after and before uptake of water, respectively.

3. RESULTS AND DISCUSSION

3.1 UV Spectrophotometric Behavior of Thiamethoxam

The absorbances of standard solution of Thiamethoxam in methanol were measured and linear calibration curve was obtained. The calibration curve was found linear in the range of $7.0x10^{-6}$ M to $1.0x10^{-4}$ M at 254 nm with a regression coefficient (R^2=0,9999) (Figure 3.1. and Figure 3.2).

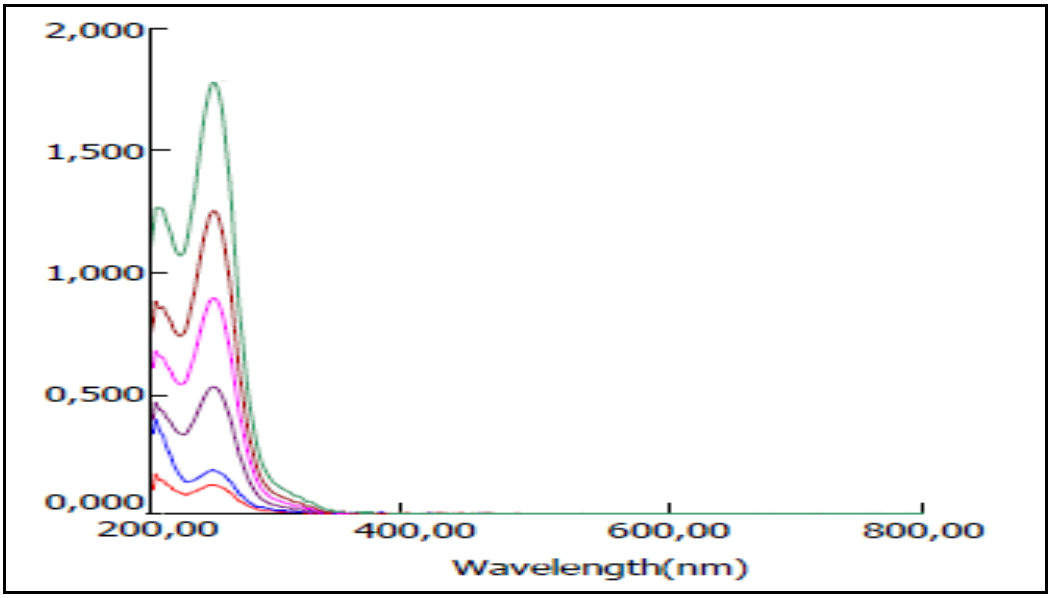

Figure 3.1 UV-VIS spectrum of TMX in methanol in the concentration range of $7.0x10^{-6}$ M to $1.0x10^{-4}$ M

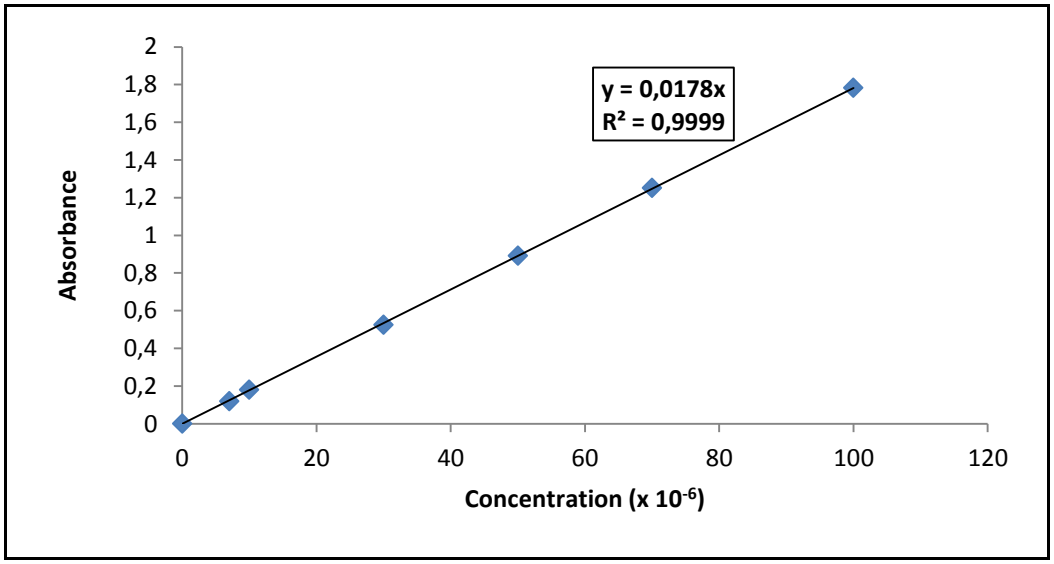

Figure 3.2 Calibration Graph of TMX in methanol by UV Spectroscopy

The absorbances of standard solution of Thiamethoxam in water were measured and linear calibration curve was obtained. The calibration curve was found linear in the range of $7.0x10^{-6}$ M to $1.0x10^{-4}$ M at 250 nm with a regression coefficient (R^2=0,9995) (Figure 3.3 and Figure 3.4).

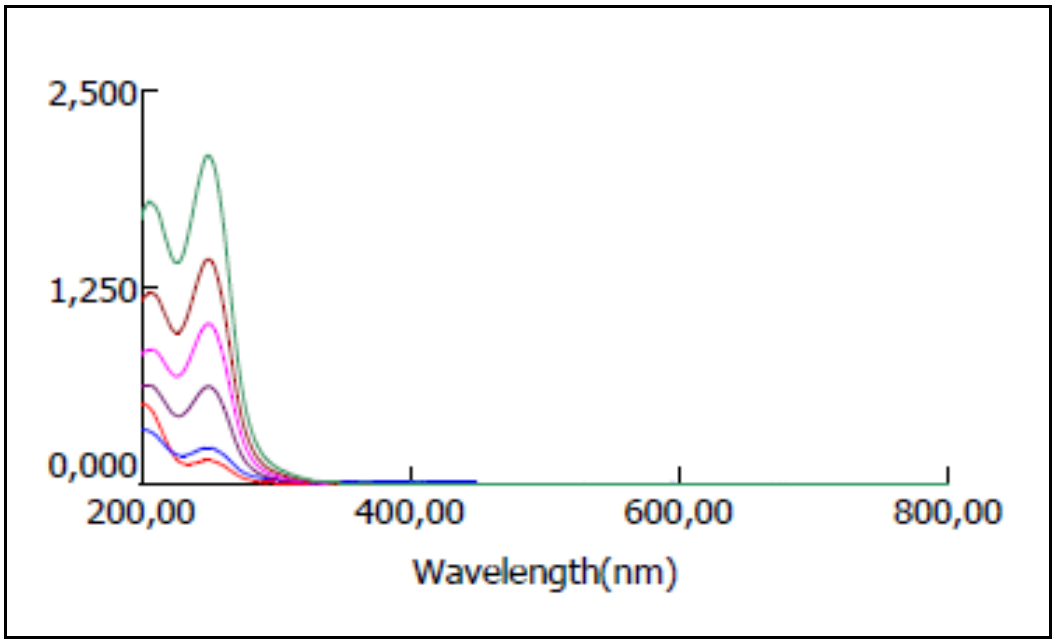

Figure 3.3 UV-VIS Spectrum of TMX in water in the concentration range of $7.0x10^{-6}$ M to $1.0x10^{-4}$ M

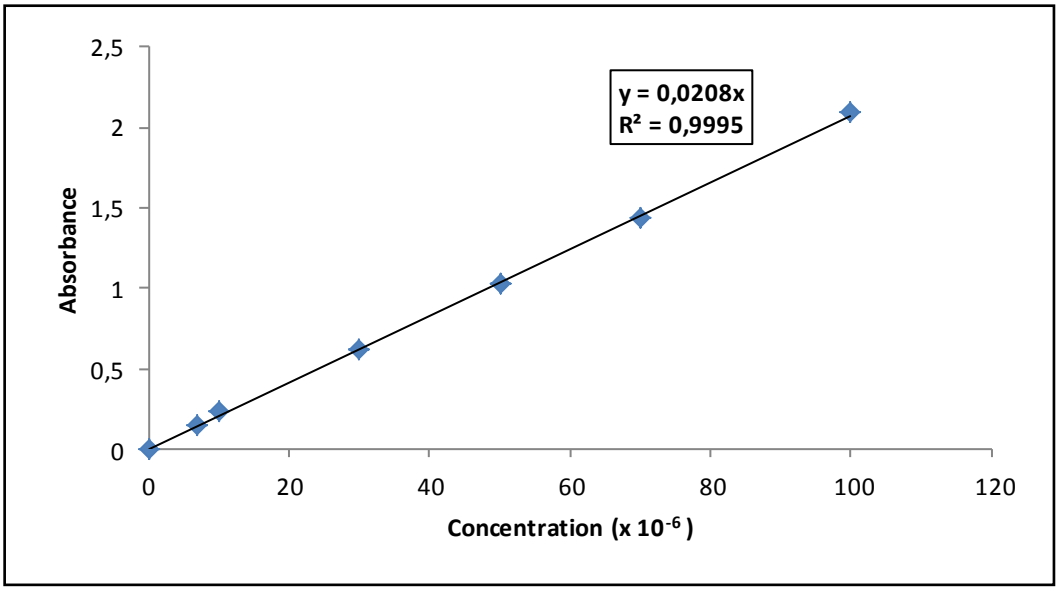

Figure 3.4 Calibration Graph of TMX in water by UV Spectroscopy

3.2 Extraction of Thiamethoxam from MIP and MIPresin

The obtained recoveries of Thiamethoxam from MIP and MIPresin with the solvent methanol for 48 hours by soxhlet extraction were found as 80% (Figure 3.5) and 50% (Figure 3.6), respectively as seen at Table 3.1. To improve the extraction efficiency, extracted and then dried polymers were mixed with the solvent methanol for three hours (until no Thiamethoxam could be detected by UV-VIS analysis) at magnetic stirrer. The solutions were centrifuged for 5 minutes and then the absorbance values of supernatants measured at λ_{max}: 254 nm by UV-VIS spectrophotometer. It can be assumed that the template was nearly completely removed from MIP (Figure 3.7) and MIPresin (Figure 3.8).

Table 3.1 Recovery of Thiamethoxam from MIP and MIPresin with methanol

Polymer	MIP	MIPresin
Recovery (%)	80	50

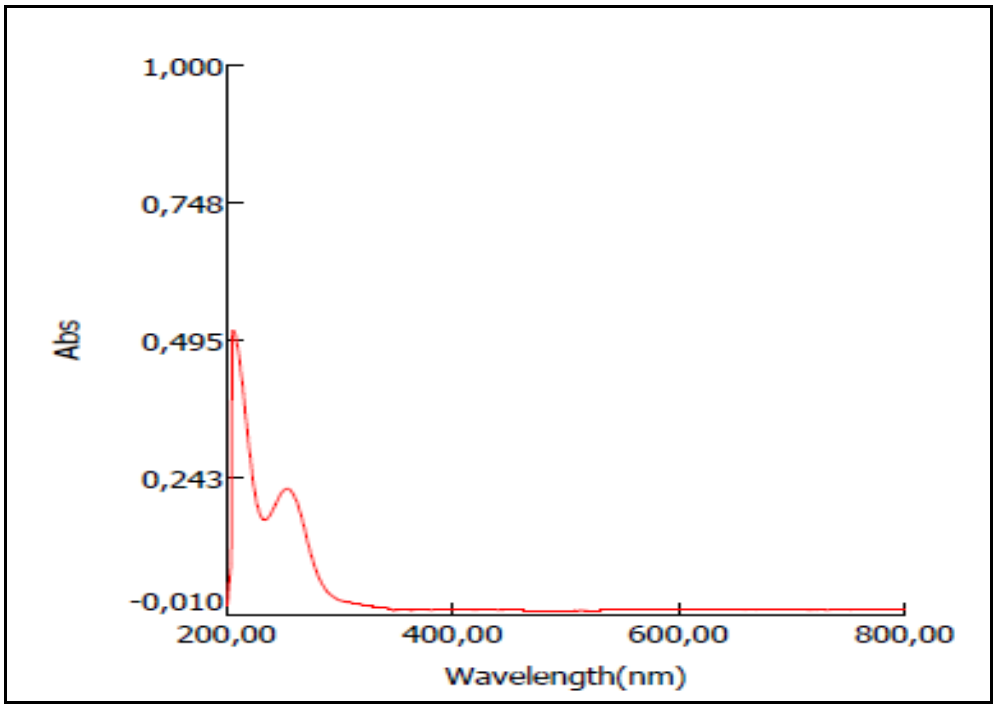

Figure 3.5 The spectrum of extracted MIP with methanol for 48 hours

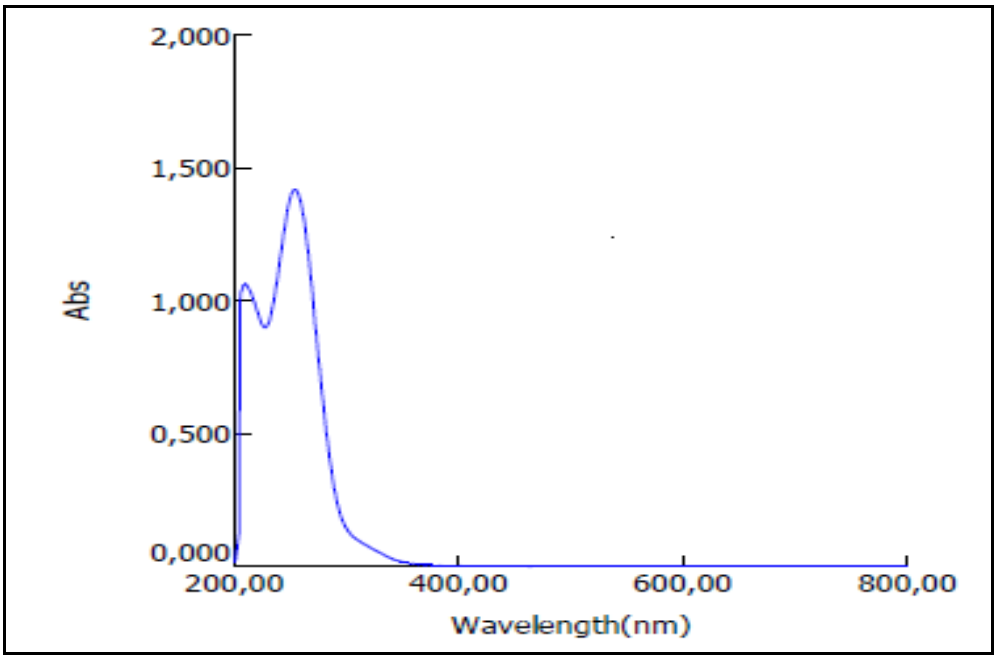

Figure 3.6 The spectrum of extracted MIPresin with methanol for 48 hours

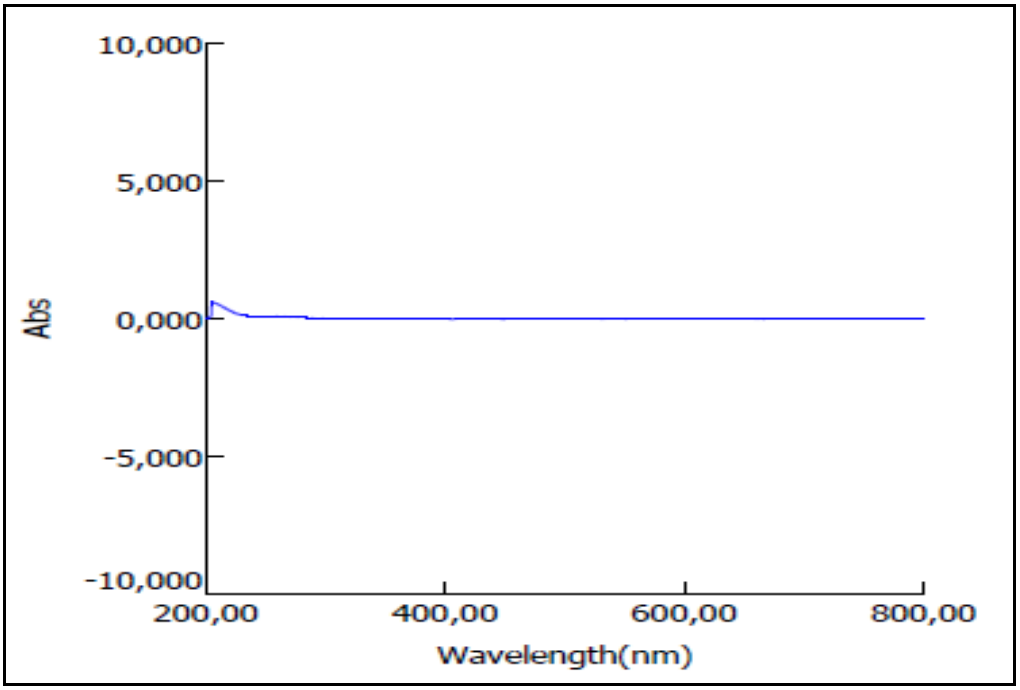

Figure 3.7 The spectrum of washed MIP with methanol for 3 hours

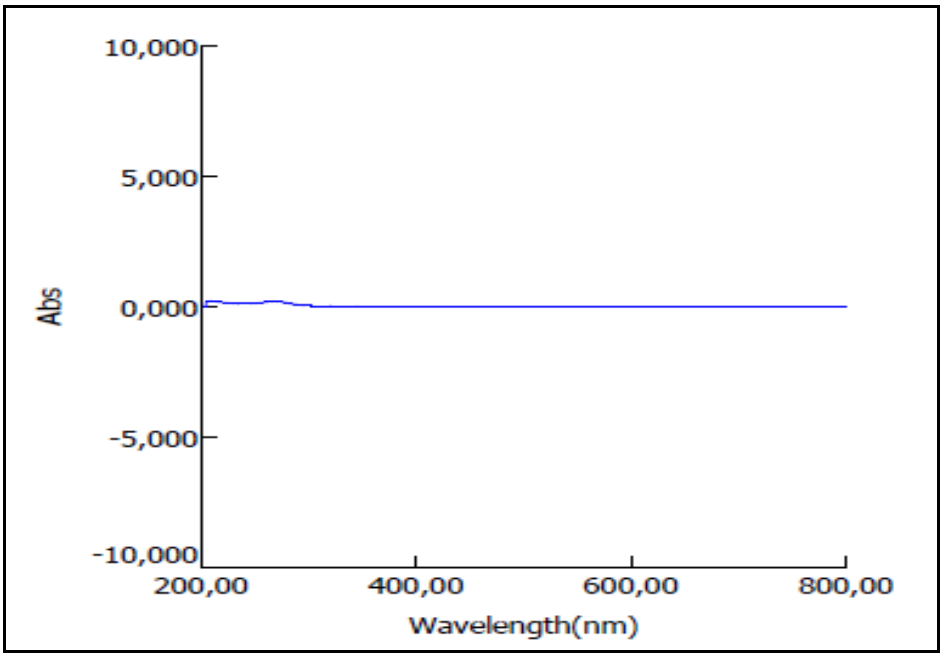

Figure 3.8 The spectrum of washed MIPresin with methanol for 3 hours

The non-imprinted polymer (NIP) and non-imprinted resin polymer (NIPresin) were prepared and washed similarly to the MIP and MIPresin, except that the template was not present in the polymerization media.

3.3 Rebinding Studies of Thiamethoxam to Polymers

3.3.1 Batch-rebinding studies

The binding kinetics of $2.0x10^{-3}$ M Thiamethoxam solutions to 50.0 mg MIPs in 5.0 mL water were determined by measuring the bound quantity at time intervals of 1, 2, 3, 4, 5 minutes for MIP1 (25-53 µm), MIP2 (53-75 µm), MIP3 (75-125 µm), MIPresin1 (425-1200 µm) and MIPresin2 (53-75 µm). The obtained all results are shown in Figure 3.9, Figure 3.10, Figure 3.11, Figure 3.12 and Figure 3.13.

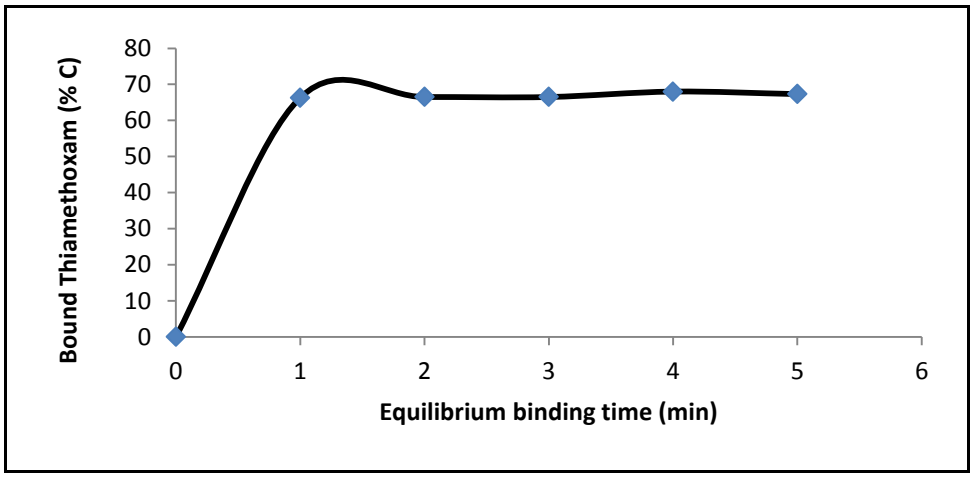

Figure 3.9 Binding rates of TMX to the MIP1 (particle size range of 25-53 µm)

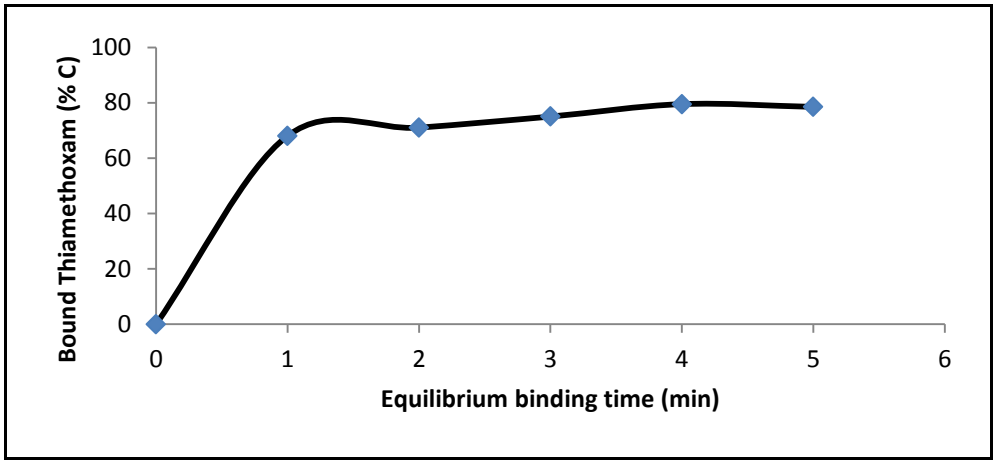

Figure 3.10 Binding rates of TMX to the MIP2 (particle size range of 53-75 µm)

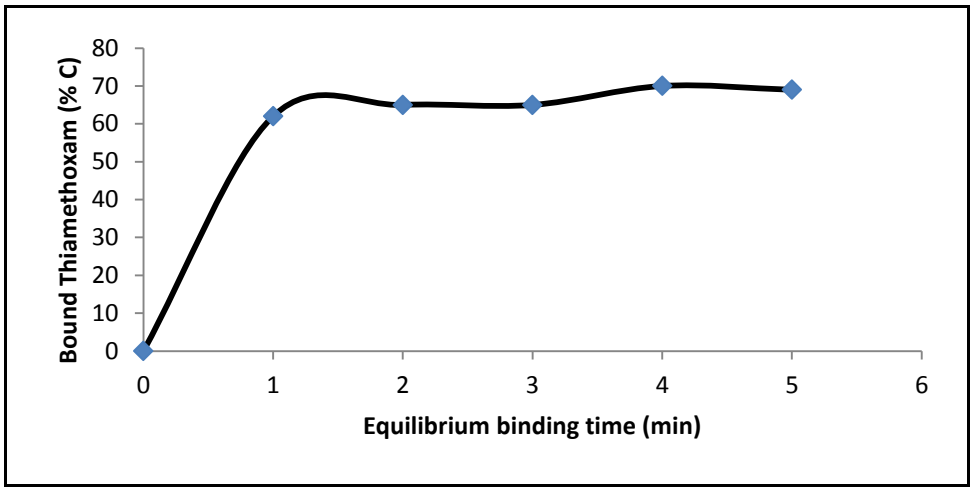

Figure 3.11 Binding rates of TMX to the MIP3 (particle size range of 75-125 µm)

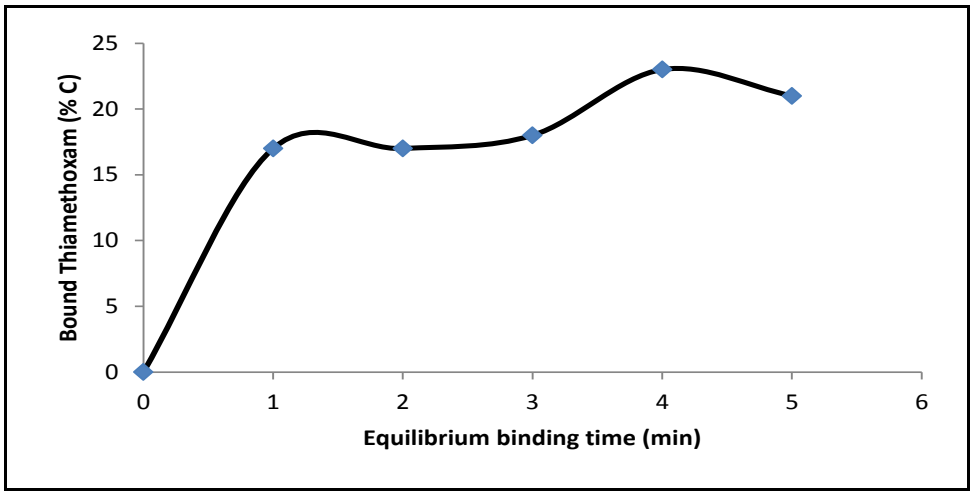

Figure 3.12 Binding rates of TMX to the MIPresin1 (particle size range of 425-1200µm)

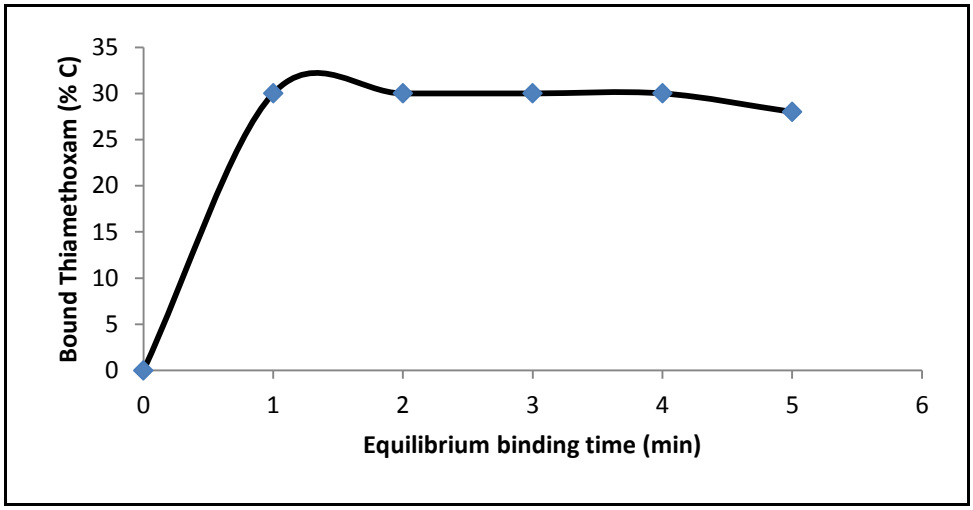

Figure 3.13 Binding rates of TMX to the MIPresin2 (particle size range of 53-75µm)

Rapidly batch-rebinding rates were observed at the beginning of adsorption process, and then saturation values are gradually reached within four minutes for MIPs. The obtained results are shown in Table 3.2.

Table 3.2 The effect of binding time on the bound Thiamethoxam for different MIPs

Stirring Time (min)	Bound Thiamethoxam (%C)				
	MIP1 (25-53 µm)	MIP2 (53-75 µm)	MIP3 (75-125 µm)	MIPresin1 (425-1200 µm)	MIPresin2 (53-75 µm)
0	0	0	0	0	0
1	66.3	68.0	62.0	17.0	30.0
2	66.5	71.0	65.0	17.0	30.0
3	66.5	75.0	65.0	18.0	30.0
4	68.0	79.5	70.0	23.0	30.0
5	67.3	78.5	69.0	21.0	28.0

3.3.2 Column-rebinding studies

100 mg of dried MIP and MIPresin, which have different particle size range, packed into SPE column. 5 mL of standard solutions containing $4x10^{-3}$ M Thiamethoxam in water were passed through the preconditioned MIP and MIPresin columns (flow rate of 0.8 mL/min). Rebinding rates of Thiamethoxam was measured by UV-VIS Spectrophotometer in the solution that passed through the SPE column (Table 3.3).

Table 3.3 Column-rebinding rates of Thiamethoxam with different MIPs

Polymer	MIP1 (25-53 µm)	MIP2 (53-75 µm)	MIP3 (75-125 µm)	MIPresin1 (425-1200µm)	MIPresin2 (53-75 µm)
Bound Thiamethoxam (%)	99	100	90	26	50

3.4 Molecularly Imprinted Solid Phase Extraction: MISPE

100 mg of dried MIP (53-75 μm) packed into the SPE cartridges with two original frits at each end (MISPE). The cartridges were washed with 5 mL methanol and preconditioned with 5 mL pure water before sample loading. In the sample loading step, 5 mL standard solutions ($4x10^{-3}$ M or $5x10^{-5}$ M) of Thiamethoxam in water was passed through the preconditioned MIP column with flow rate of 0.8 mL/min (Figure 3.14, Figure 3.15). MISPE columns were then dried by passing nitrogen through the column for 10 minutes before the adsorbed Thiamethoxam was eluted. For the elution step, 5 mL methanol was used. Absorbance of TMX in the eluent solution that passed through the SPE column was determined by UV-VIS Spectrophotometer (Figure 3.16).

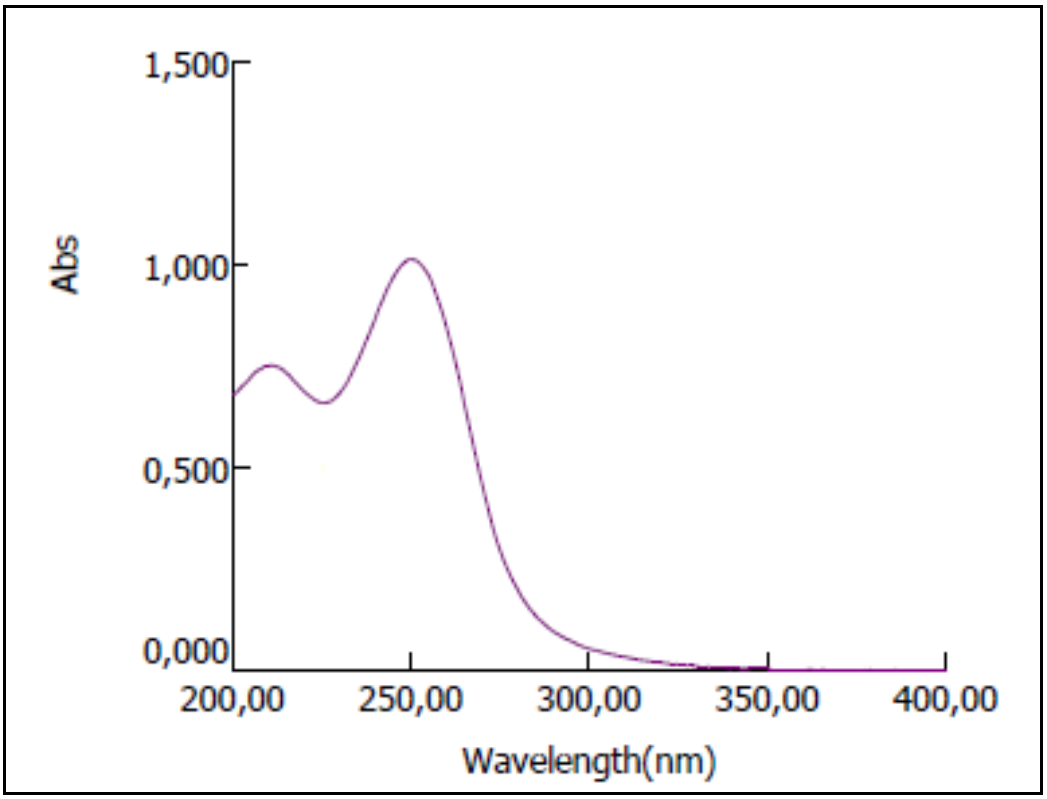

Figure 3.14 The spectrum of $5x10^{-5}$ M TMX in water before SPE

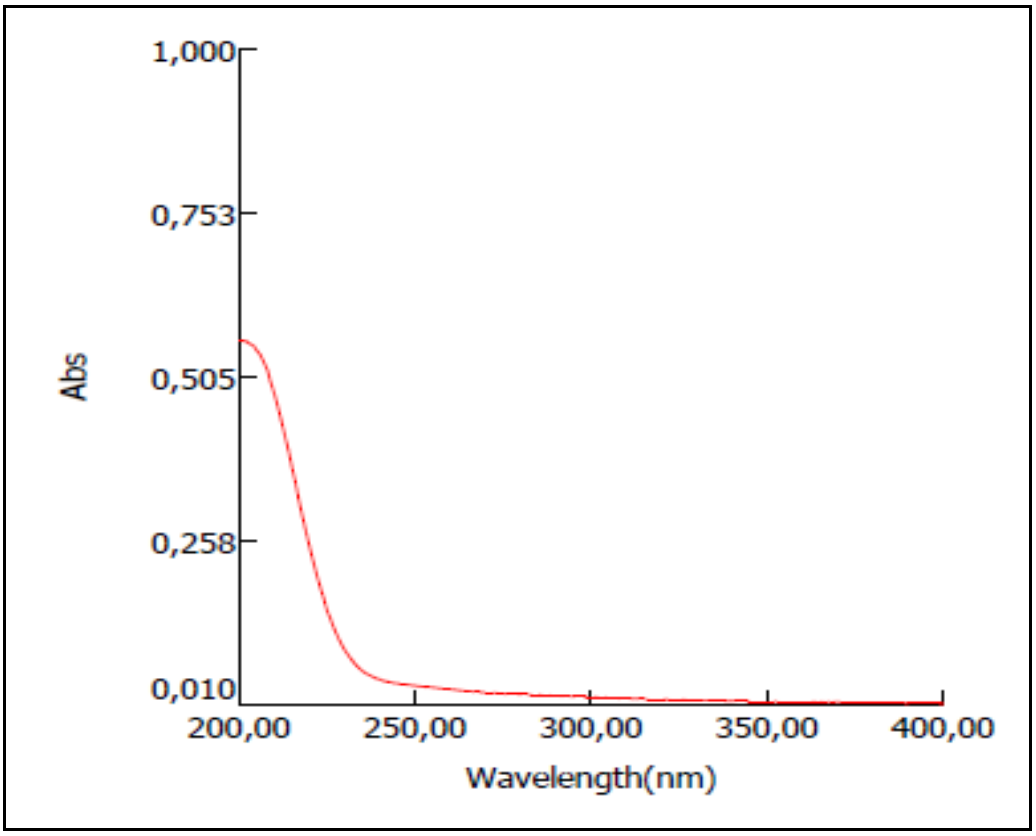

Figure 3.15 The spectrum of $5x10^{-5}$ M TMX in water after passing through SPE column

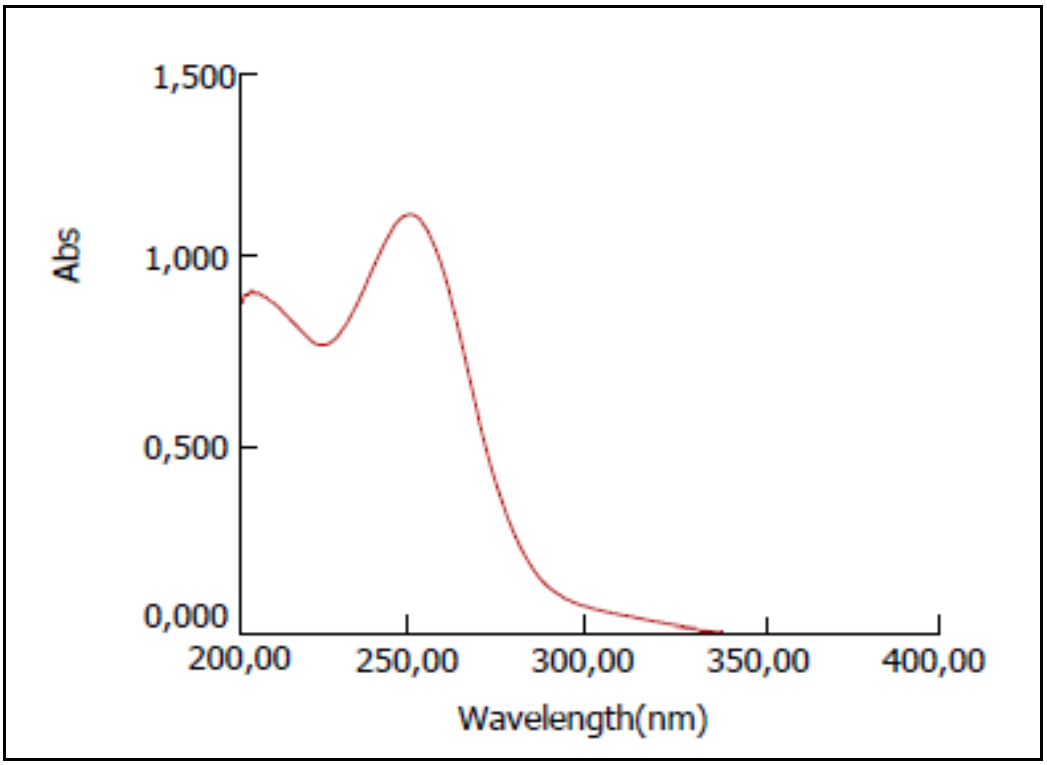

Figure 3.16 The spectrum of eluted TMX with methanol

3.4.1 Repeatability studies

For the determination of repeatability of Thiamethoxam in the MISPE method, experiments were performed five times with $5x10^{-5}$ M standard solution of Thiamethoxam in water. Average rebinding and recovery were found to be 99.2% and 129.2% with a standard deviation of ±0.84% (n=5) and ±0.89% (n=5), respectively. Related all results are shown in Table 3.4.

Table 3.4 Results of repeatability for Thiamethoxam with MISPE method for $5x10^{-5}$ M

Repeatability (n)	Rebinding (%)	Recovery (%)
1	100	128
2	100	130
3	99	130
4	99	129
5	98	130

3.4.2 Comparison of the MISPE and NISPE responses to Thiamethoxam in water

In order to study the Thiamethoxam recognition ability of MIP in water, the MISPE and NISPE were prepared. Recovery experiments in water were carried out, at two concentration levels ($4x10^{-3}$ M, $5x10^{-5}$ M). The obtained results are shown in Table 3.5.

Table 3.5 Recovery of Thiamethoxam from MISPE and NISPE at two spiking levels

Spiked Thiamethoxam (mol/L)	Found at MISPE (mol/L)	Recovery (%)	Found at NISPE (mol/L)	Recovery (%)
$5.0x10^{-5}$	$6.4x10^{-5}$	128	$5.7x10^{-5}$	114
$4.0x10^{-3}$	$4.3x10^{-3}$	109	$4.0x10^{-3}$	100

3.5 Swelling Test

Water uptake ratio of MIPresin and NIPresin particles were determined in water. Initially dry particles were weighted (0.5 g) before being placed in 5 mL vials containing 2 mL pure water. The vials were left overnight. Then the swelled resin samples were taken out from the water, wiped using a filter paper and weighted. The weight ratio of dry and wet samples was recorded. The water content of the imprinted and non-imprinted resin polymer particles were calculated. In order to this study, the equilibrium swelling ratios of MIPresin and NIPresin particles were found to be 5.38% and 4.76%, respectively.

4. CONCLUSION

In this study, the molecularly imprinted polymers for Thiamethoxam were prepared with traditionally (imprinting by bulk polymerization) and newly improved (imprinting by resin swelling) methods. Performance of MIPs that were prepared with two different methods was investigated through batch and SPE methods.

For the extraction of Thiamethoxam from MIP and MIPresin was worked with methanol and consequently the template was nearly completely removed from imprinted polymers.

According to the related results, MIP have more rebinding performance for Thiamethoxam than MIPresin. Furthermore off all imprinted polymers, MIPs of the particle size range of 53-75 μm have greatest efficiency (Table 3.2, Table 3.3).

Solid phase extraction was performed for determination of Thiamethoxam using molecularly imprinted polymers. In the comparison of the MISPE and NISPE responses to Thiamethoxam in water, MISPE has higher recovery percentage for template molecule than NISPE in regard to obtained results by UV-VIS Spectrophotometer. Also diluted solutions have more difference value between MISPE and NISPE than concentrated solutions in the recovery of Thiamethoxam in water (Table 3.5). In most of the experiments, including the results obtained from references, recoveries were found above hundred percent and it seems to be it is quite diffucult to give an appropriate explanation to these results.

All above facts indicate that the development and analysis of molecularly Thiamethoxam imprinted polymers in this study could provide a strategy for development of pesticide MIPs in water samples.

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CURRICULUM VITAE

Name Surname: Beyza YILDIRIM

Profession: Chemist

Date/Place of Birth: 1990/ANKARA

Nationality: Turkish

Sex: Female

Marital Statue: Single

Address: Ege University, Faculty of Science, Department of Chemistry, Division of Analytical Chemistry, 35100 Bornova-İzmir/Turkey

E-mail: beyzayildirimm@gmail.com

EDUCATIONAL BACKGROUND

BSc In Chemistry Education, 2013, Ege University, İzmir/Turkey

MSc In Analytical Chemistry, 2015, Ege University, İzmir/Turkey