EGE UNIVERSITY
GRADUATE SCHOOL OF
NATURAL AND APPLIED SCIENCES

(MASTER THESIS)

AN OPTICAL SENSOR FOR THE DETERMINATION OF SOME ORGANIC COMPOUNDS BASED ON MOLECULARLY IMPRINTED POLYMER MEMBRANE

Burak BERAN

Supervisor: Prof. Dr. Berin YENİGÜL

Department of Chemistry

Code of discipline: 405.03.01 (Analytical Chemistry)
Date of presentation: 17.08.2010

Bornova-İZMİR

Burak Beran tarafından yüksek lisans tezi olarak sunulan "**AN OPTICAL SENSOR FOR THE DETERMINATION OF SOME ORGANIC COMPOUNDS BASED ON MOLECULARLY IMPRINTED POLYMER MEMBRANE(KİMİ ORGANİK BİLEŞİKLERİN TAYİNİ İÇİN MOLEKÜLER BASKILI POLİMER MEMBRANA DAYALI OPTİK SENSÖR GELİŞTİRİLMESİ)**" 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 **17.08.2010** tarihinde yapılan tez savunma sınavında aday oybirliği/~~oyçokluğu~~ ile başarılı bulunmuştur.

Jüri Üyeleri:		**İmza**
Jüri Başkanı	**:** Prof. Dr. Berin Yenigül	**................................**
Raportör Üye	**:** Prof. Dr. Emür Henden	**................................**
Üye	**:** Doç. Dr. Kadriye Ertekin	**................................**

ÖZET

KİMİ ORGANİK BİLEŞİKLERİN TAYİNİ İÇİN MOLEKÜLER BASKILI POLİMER MEMBRANA DAYALI OPTİK SENSÖR GELİŞTİRİLMESİ

BERAN, Burak

Yüksek Lisans Tezi, Kimya Bölümü
Tez Yöneticisi: Prof. Dr. Berin YENİGÜL
Temmuz 2010, 65 sayfa

Çeşitli organik bileşiklerin seçimli ve duyar tayininde son yaklaşımlardan biri moleküler baskılı polimerlerin (MIPs) sentezidir. Moleküler baskılama yapay reseptör bölgeleri oluşturma tekniği olup, fonksiyonel monomer veya monomerlerin çapraz bağlayıcı varlığında tayin edilecek molekül (hedef molekül) etrafında polimerleştirilmesi ile elde edilir. Hedef molekülün özütlenmesinden sonra elde edilen polimerler ayırmada, bağlama denemelerinde, katalizde ve sensör yapımında kullanılabilmektedir.

Mefenamik asit insanlarda ağrı kesici ve ateş düşürücü olarak kullanılmaktadır. Doz aşımı durumunda toksik metabolik birikim hastalık veya ölümle sonuçlanabilen karaciğerde akut doku ölümüne neden olur. Bu nedenle MFA'ya duyar yöntemlerin geliştirilmesi önemlidir.

Bu çalışmada MFA baskılı polimerlerin sentezlendikten sonra MFA'nın geri kazanımı, polimerlerin hedef analiti adsorplama kapasitesi gibi performansının öncelikli saptanması ve heterojen optik sensor geliştirmede uygulanabilmesi amaçlanmıştır. Yapılan literatür taramasında MFA baskılı polimerlere rastlanmaması yapılan çalışmayı orijinal kılmaktadır.

Moleküler baskılamada; metakrilik asit monomer (MAA), etilen glikol dimetakrilat (EDMA) çapraz bağlayıcı ajan ve mefenamik asit (MFA) kalıp molekül olarak kullanılarak; MFA molekülünü seçimli olarak bağlayabilen

moleküler baskılı polimerler hazırlanmıştır. Moleküler baskılama çalışmalarında 4:1 monomer:kalıp molekül oranı kullanılmıştır.

Polimerden MFA' nın geri kazanımı iki şekilde kloroform ortamında sokslet aygıtı ile ve 0.1M NaOH ortamında batch yöntemiyle özütlendiğinde sonuçlar birbirine yakin ve sırasıyla %93.25 ve %93.94 bulunmuştur.

MFA' nın polimere geri bağlanmasında kloroform ortamında en yüksek kapasite 0.1M NaOH ile özütlenmiş olan polimer için 45.2 mg/g olarak elde edilmiştir. Bu değer baskılanmamış polimer (NIP) için 6.37 mg/g dır.

MFA baskılı polimerler ile hazırlanan PVC bazlı heterojen optik sensörler, MFA yalnız kloroform ortamında flüoresans gösterdiğinden kloroform ortamda yapılan ölçümlerde sensor içeriğinde bulunan plastizerin çözünmesi nedeniyle tatmin edici kalibrasyon ergileri elde edilememiştir.

Sentezlediğimiz polimerlerden hazırlanan MFA ya seçimli MI-SPE kullanılmış ve %98.37 bağlama yüzdesi elde edilmiştir.

Bu tezde ilk kez hazırlanan mefenamik asit baskılanmış polimerlerin ayırma ve ön-deriştirme amacıyla katı faz ekstraksiyonunda dolgu maddesi olarak kullanılması denenmiştir.

Anahtar sözcükler: Mefenamik asit, moleküler baskılama, moleküler baskılı polimerler, moleküler tanıma, optik sensör, kati faz ekstraksiyon, SPE, MIP, flüoresans, spektroflorimetrik analiz

ABSTRACT

AN OPTICAL SENSOR FOR THE DETERMINATION OF SOME ORGANIC COMPOUNDS BASED ON MOLECULARLY IMPRINTED POLYMER MEMBRANE

BERAN, Burak

MSc in Chemistry
Supervisor: Prof. Dr. Berin YENİGÜL

July 2010, 65 pages

The syntheses of molecularly imprinted polymers (MIPs) are the most recent approaches for selective and sensitive determination of various organic compounds. Molecular imprinting is a technique that forming artificial receptor sites and obtained by polymerizing functional monomer or monomers with a cross linking agent around analyte (target molecule). These polymers can be used for separation, rebinding experiments, catalysis and sensor materials.

Mefenamic acid is a drug for humans and used as analgesic and anti-inflammatory. Overdoses of MFA produce toxic metabolite accumulation that causes acute hepatic necrosis, inducing morbidity and mortality in humans.

The aim of this thesis as synthesis of MFA imprinted polymers, determination of its performance such as recovery of MFA from polymer, rebinding of MFA on polymer and its application for the preparation of heterogen optical sensor for MFA. As far as we know from the literature survey MFA imprinted polymer was synthesized for the first time.

In molecular imprinting; by simply adding methacrylic acid monomer (MAA), etilen glycol dimethacrylate (EDMA) cross linking agent and mefenamic acid (MFA) template molecule; a polymer that can bind MFA selectively was acquired. The monomer:template ratio was used as 4:1.

The extraction of MFA from polymer in two ways; soxhlet extraction with chloroform and batch extraction with 0.1M NaOH. Both of them gave very close results such as 93.24% and 93.94% respectively.

The maximum capacity of rebinding of MFA from polymer in chloroform with the use of 0.1M NaOH extracted polymer was found 45.2 mg/g. For the non-imprinted polymer (NIP) it was found as 6.37 mg/g.

MFA imprinted polymers were used for the preparation of PVC based heterogen optical sensors and due to the fluorescence property of MFA obtained only in chloroform, measurements carried out in chloroform. However the solubility of plasticizer in chloroform satisfactory results cannot be obtained.

The MI-SPE of the MFA was carried out using the polymer we have synthesized and binding yield of 98.37% was obtained.

In this thesis mefenamic acid imprinted polymer prepared for the first time was examined as solid phase extraction packaging material for separation and pre-concentration purposes.

Keywords: Mefenamic acid, molecular imprinting, molecularly imprinted polymers, molecular recognition, optical sensor, solid phase extraction, SPE, MIP, fluorescence, spectrofluorimetric analysis

TEŞEKKÜR

Tez çalışma konumu öneren, değerli bilgi ve önerileriyle bana yol gösteren danışman hocam Sayın Prof. Dr. Berin Yenigül sonsuz teşekkür ederim.

Çalışmalarımda ve her türlü konuda yardımlarını esirgemeyen Sayın Dr. Fadim Yemiş' e sonsuz teşekkür ederim.

Tez çalışmalarım boyunca denemelerimi yapma olanağı sağlayan Kimya Bölümü ve özellikle Analitik Kimya Anabilim dalı çalışanlarına teşekkür ederim.

Gerekli ilaç etken maddesi tedariki konusunda yardımlarını esirgemeyen Ege Üniversitesi Eczacılık Fakültesi Farmasotik Teknoloji Anabilim Dalı Öğretim üyesi Prof. Dr. Tamer Güneri' ye teşekkür ederim.

Çalışmalarımda değerli katkı ve önerilerinden yararlandığım Sayın Prof. Dr. Emür Henden ve Sayın Prof. Dr. Duygu Toscalı' ya teşekkürlerimi sunarım.

Tez çalışmalarını birlikte yürüttüğümüz sevgili arkadaşım Kimyager Sevilen Yılmaz' a teşekkürlerimi sunarım.

Eğitimimin her aşamasında maddi manevi sonsuz desteklerini gördüğüm, her zaman yanımda olan sevgili babam Timuçin Beran, annem İnci Beran ve abim Dr. Bora Beran' a sonsuz teşekkürler.

CONTENTS

Page

ÖZET V

ABSTRACT VII

TEŞEKKÜR IX

LIST OF FIGURES XVII

LIST OF TABLES XXI

SYMBOLS AND ABBREVIATIONS XXIII

1 INTRODUCTION 1

1.1 What is Molecular Imprinting 1

1.2 Components of MIP 4

1.2.1 Templates 4

1.2.2 Functional monomers 5

1.2.3 Crosslinking agents 5

1.2.4 Solvents 6

1.2.5 Initiators 7

1.3 Classification of Methods 8

1.3.1 In-block imprinted polymers 9

CONTENTS (CONTINUE)

Page

1.3.1.1 Covalent imprinting and non-covalent imprinting 9

1.3.1.1.1 Covalent imprinting 9

1.3.1.1.2 Non-covalent imprinting 10

1.3.1.2 Advantages and disadvantages of covalent and non-covalent imprinting 12

1.3.1.2.1 Advantages of covalent imprinting 12

1.3.1.2.2 Disadvantages of covalent imprinting 12

1.3.1.2.3 Advantages of non-covalent imprinting 12

1.3.1.2.4 Disadvantages of non-covalent imprinting 13

1.3.1.3 Dummy molecular imprinting 13

1.3.2 Other imprinting methods 14

1.3.2.1 In-situ imprinted polymers 14

1.3.2.2 Directly synthesized polymer beads 15

1.4 Stability of the Monomer-Template Assemblies 16

1.4.1 Thermodynamic factors 17

1.4.2 The number of the type of interaction sites on the monomer-template 17

1.4.3 The template shape 18

1.4.4 The monomer-template rigidity 18

CONTENTS (CONTINUE)

Page

1.5 The Stability, Integrity and Accessibility 19

1.6 Application of Imprinted Polymers 20

1.6.1 Sensors 21

1.6.1.1 Quartz crystal microbalance-based sensors 21

1.6.1.2 Electrode-type sensors 22

1.6.1.3 Optical sensors 22

1.6.2 Signaling polymers 22

1.6.3 Molecular imprinted sorbent assays 23

1.6.4 Molecularly imprinted membranes 23

1.6.5 Affinity based solid-phase extraction 23

1.6.6 In-situ preparation of imprinted polymers 24

1.6.7 Molecularly imprinted catalysts 24

1.6.8 Separation 25

1.6.8.1 MI-SPE (Off-line SPE) 25

1.6.8.2 Chromatography (On-line SPE) 27

1.6.8.3 Capillary electrochromatography 27

1.6.9 Application in biological and pharmaceutical fields 28

CONTENTS (CONTINUE)

Page

1.7 Mefenamic Acid 30

1.7.1 Method of analysis 31

1.7.1.1 Identification 31

1.7.1.2 Light absorption 31

1.7.1.3 Assay 31

1.8 The Aim of the Study 32

2 EXPERIMENTAL 33

2.1 Apparatus 33

2.2 Reagents and Solutions 33

2.3 Process of Molecular Imprinting Polymer 34

2.4 Extraction of MFA from MIPs 34

2.4.1 Batch extraction 34

2.4.2 Soxhlet extraction 34

2.5 Batch Rebinding Studies of MFA to MIPs 35

2.6 Preparation of MIP Optical Sensor 35

2.7 Spectrofluorometric measurement for MFA 35

2.8 MI-SPE 36

CONTENTS (CONTINUE)

Page

3 RESULTS AND DISCUSSIONS 37

3.1 Spectrophotometric Behavior of MFA 37

3.2 Spectrofluorometric Behavior of MFA 41

3.3 Extraction of MIP 44

3.3.1 Soxhlet extraction 44

3.3.2 Batch extraction 45

3.4 Rebinding of MFA in Chloroform 46

3.4.1 Rebinding with soxhlet extracted polymers with chloroform 47

3.4.2 Rebinding with batch extracted polymers with 0.1M NaOH 50

3.5 Rebinding of MFA in 0.1M NaOH 52

3.6 Binding capacity 53

3.7 MIP-based Optical Sensor 53

3.7.1 Time for stable signal 54

3.7.2 Calibration of MIP and NIP optical sensors 55

3.8 The studies of MI-SPE 59

4 CONCLUSION 60

REFERANCES 61

CONTENTS (CONTINUE)

Page

5 CIRRICULUM VITAE 65

LIST OF FIGURES

Figure Page

1.1 Schematic diagram of molecularly imprinted polymer preparation.........2

1.2 Typical reagents 8

1.3 Covalent imprinting of mannopyranoside using its 4-vinylphenylboronic acid ester as a functional monomer 10

1.4 Non-covalent imprinting by theophylline (a drug): Step 1: Pre-organization of functional monomers through non-covalent interactions Step 2: Polymerization of pre-organized functional monomers Step 3: Removal of the template..11

1.5 Molecular imprinting by triethylmelamine as a dummy template for atrazine 14

1.6 Factors influencing the formation of the templated binding sites 17

1.7 Types of binding sites in MIPs..20

1.8 Schematic procedure of molecularly imprinted solid phase extraction..26

1.9 Structure of Mefenamic Acid 32

3.1 The absorbance spectra of MFA in chloroform. 37

3.2 Calibration curve of MFA in chloroform at λ_{max}:365 nm. 38

3.3 Calibration curve of MFA in chloroform at λ_{max}:285 nm. 38

3.4 The absorbance spectra of MFA in ethanol..39

3.5 Calibration curve of MFA in ethanol at λ_{max}:348 nm...........................39

LIST OF FIGURES (CONTINUE)

Figure Page

3.6 The absorbance spectra of MFA in 0.1M NaOH. 40

3.7 Calibration curve of MFA in 0.1M NaOH λ_{max}:285 nm. 40

3.8 The emission spectrum for exiticion at 314 nm and excitation spectrumfor emission in 460 nm. 42

3.9 Fluorescence spectra of MFAconcentration range of 0-20 mg/L. 43

3.10 Fluorescence spectra of MFAconcentration range of 0-50 mg/L. 43

3.11 Spectrofluorimetric calibration curve of MFA in chloroform. 44

3.12 Binding rates of MFA in chloroform on MFA imprinted polymer extracted with chloroform (Experiment 1). 48

3.13 Binding rates of MFA in chloroform on MFA imprinted polymer extracted with chloroform (Experiment 2). 48

3.14 Binding rates of MFA in chloroform on MFA imprinted polymer extracted with chloroform (Experiment 3). 49

3.15 Binding rates of MFA in chloroform on MFA imprinted polymer extracted with chloroform (Experiment 4). 49

3.16 Binding rates of MFA in chloroform on MFA imprinted polymer extracted with 0.1M NaOH.. 51

3.17 Binding rates of MFA non-imprinted polymer. 51

3.18 Binding rates of MFA in 0.1M NaOH on MFA imprinted polymer extracted 0.1M NaOH. 53

3.19 Spectrofluorimetric measurements of MIP optical sensor vs. time black: 5 min. blue: 10 min. red: 15 min. green: 20 min. light blue: 25 min. and pink: 30 min. at 0.5 mg/L mefenamic acid solution in chloroform. 54

LIST OF FIGURES (CONTINUE)

Figure Page

3.20 Spectrofluorimetric measurements of NIP optical sensor vs. time blue: 5 min. red: 10 min. green:15 min. and pink: 20 min. at 1 mg/L mefenamic acid solution in chloroform. 55

3.21 Spectrofluorimetric measurements of NIP optical sensor for different concentrations of MFA in ethanol. 56

3.22 Spectrofluorimetric measurements of MIP optical sensor black: blank solution vs. MFA concentration blue: 0.1 mg/L red: 0.5 mg/L green: 1 mg/L pink: 2 mg/L light blue: 3 mg/L. 57

3.23 Calibration graph of MIP optical sensor 0.1 to 3 mg/L. 57

3.24 Spectrofluorimetric measurements of NIP optical sensor vs. MFA concentrations blue: 1 mg/L red: 1.5 mg/L green: 2 mg/L pink: 2.5 mg/L light blue: 3 mg/L and yellow 3.5 mg/L. 58

3.25 Calibration graph for NIP optical sensor 0.5 to 3.5 mg/L 59

LIST OF TABLES

Table Page

3.1 Maximum absorbances and molar absorbance coefficiencies for different solvents. 41

3.2 Recovery of MFA from MIP with soxhlet extraction 45

3.3 Recovery of small amount of MFA from MIP with batch extraction 46

3.4 Recovery of large amount of MFA from MIP with batch extraction 46

3.5 Equilibrium binding time of MFA in chloroform from polymer extracted with chloroform. 47

3.6 Equilibrium binding time of MFA in chloroform from polymer extracted with 0.1M NaOH.. 50

3.7 Equilibrium binding time of MFA in 0.1M NaOH from polymer extracted with 0.1M NaOH. 52

3.8 Binding capacity of MIPs. 53

SYMBOLS AND ABBREVIATIONS

Abbreviations	**Explanations**
ACN	Acetonitrile
ADVN	2,2’-azobis(2,4-dimethylvaleronitrile
AIBN	2,2’azobis(isobutyronitrile)
BA	Benzylamine
BR	Britton-Robinson
CE	Capillary electrophoresis
CEC	Capillary electrochromatography
CSD	Clean screen Dau
DPE	Differential pulsed elution
DOP	Dioctyl phtalate
DON	Deoxynivalenol
EDMA (EGDMA)	Etilenglycoldimethacrylate
FPE	Final pulsed elution
HPLC	High-performance liquid chromatography
MAA	Methacrylic acid
MFA	Mefenamic acid

MIP	Molecularly imprinted polymer
MIPs	Molecularly imprinted polymers
MISPE (MI-SPE)	Molecularly imprinted solid phase extraction
MIPSPE (MIP-SPE)	Molecularly imprinted polymer based solid phase extraction
NIP	Non-imprinted polymer
NIPs	Non-imprinted polymers
NSAIs	Non-steroid anti-inflammatories
L-PA	L-phenylalanine anilide
QCM	Quartz crystal microbalance
PETEA	Pentaerythritol tetraacrylate
PETRA	Pentaerythritol triacrylate
PVC	Polyvinyl chloride
SPE	Solid phase extraction
THF	Tetrahydrofuran
TRIM	Trimethylolpropane trimethacrylate
UV	Ultraviolet
UV-vis	Ultraviolet-visible
VPY	Vinyl pyridine
λ_{max}	Maximum absorbance

1 INTRODUCTION

The most important point in analytical chemistry is selectivity, particularly at low analyte concentrations in the presence of interfering substances. The selective and sensitive determination of a large number of trace compounds find extensive application in many fields such as food, industry, biotechnology, environment, pharmaceutical industry and healthcare for diagnosis of diseases (Yemiş, 2010).

Several analytical methods such as spectrophotometry, fluorometry, gas chromatography and high performance liquid chromatography have been developed for the quantitative determination of these trace compounds, being sensitive and selective. Most of these methodologies require either sophisticated instruments or expensive reagents or involve manipulation and derivatization steps (Yemiş, 2010).

One of the recent approaches for the sensitive and selective determination of several compounds is synthesis of molecularly imprinted polymers. Since the Nobel Prize was awarded to Cram, Lehn, and Pederson in 1987, the term 'molecular recognition' has been recognized all over the world. The concept of molecular recognition and related chemistry can be a powerful tool for the understanding of physiological and pharmacological phenomena, because the generation and maintenance of life is governed by combining many simple but specific chemical reactions based upon molecular recognition. Since molecular recognition is the origin of biological functions, the preparation and combination of synthetic molecules capable of molecular recognition may enable us to regenerate bio-functionalized artificial molecules. Such biomimetic molecules would be extremely useful as substitutes for biomolecules in biotechnological, medical and bioanalytical fields. (Soares da Silva et al., 2009)

1.1 What is Molecular Imprinting?

Molecular recognition technology is a promising alternative way to create highly specific sites for a molecule, within a polymer, via imprinting polymerization. Molecular imprinting technique uses the functionality of the target molecule (template), to assemble its own recognition site by forming specific interactions with the matrix. In the molecular imprinting process, the functional group of the monomer interacts with the template molecule, in the

presence of a porogen and a cross-linker agent that freezes the complex within a porous polymer matrix. The imprinted sites formed, chemical and physically complementary to the template molecule are then made accessible by dissociation of the complex, by template removal (Soares da Silva et al., 2009).

Molecular imprinting processes are composed of the following three steps:

1. Preparation of covalent conjugate or non-covalent adduct between a functional monomer and a template molecule,

2. Polymerization of this monomer-template conjugate (or adduct), and

3. Removal of the template from the polymer (Komiyama et al., 2003).

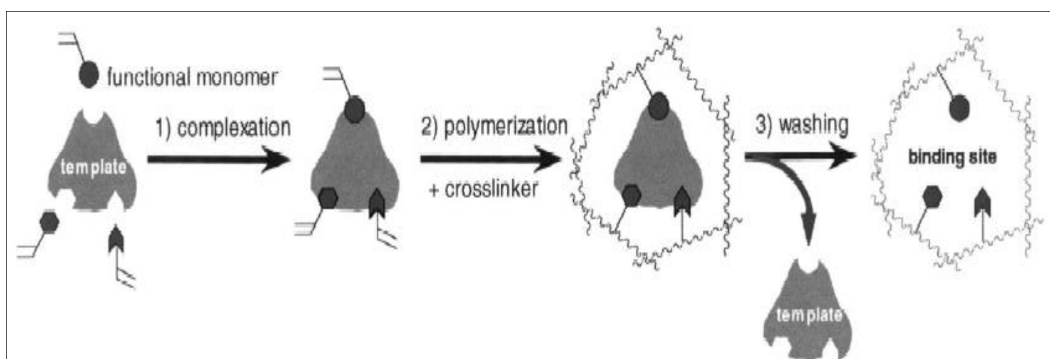

Fig. 1.1 Schematic diagram of molecularly imprinted polymer preparation (Takeuchi and Haginaka, 1999).

In step 1, functional monomer and template are connected by a covalent linkage (in »covalent imprinting«) or they are placed nearby through non-covalent interactions (in »non-covalent imprinting«).

In step 2, the structures of these conjugates (or adducts) are frozen in a three-dimensional network of polymers. The functional residues (derived from the functional monomers) are topographically complementary to the template.

In step 3, the template molecules are removed from the polymer. Here, the space in the polymer originally occupied by the template molecule is left as a cavity. Under appropriate conditions, these cavities satisfactorily remember the

size, structure, and other physicochemical properties of the template, and bind this molecule (or its analog) efficiently and selectively (Komiyama et al., 2003).

In principle the template can be any substance ranging from small molecules such as drug substances, amino acids, steroid hormones or metal ions to larger molecules such as peptides or proteins. Large molecules and molecular assemblies, such as cells and viruses, may also be perceived. However, the difficulty of making the imprinted materials generally increases with the size of the selected key molecule. When preparing molecularly imprinted polymer against large imprint species, the template could be trapped permanently inside the polymer after polymerization. The rebinding process, which relies on the diffusion of the template molecule to the recognition sites, will also be hindered by this size effect (Ramström and Yan, 2005).

The advantages of MIP-based materials include: (i) binding affinities comparable to a biological recognition element, (ii) robustness and stability under a wide range of chemical and physical conditions, and (iii) an ability to easily design recognition sites for analytes that lack suitable biorecognition elements (Holthoff and Bright 2007).

Molecularly imprinted polymers can and do find use in many disciplines (chromatographic separations, catalysis, sensors and materials) and, consequently, are attracting attention in the scientific community. In spite of this interest, only in the late of the 1990's the groups of Guiochon and Sellergren, followed independently by Shimizu, started a systematic research using binding isotherms to characterize MIPs (Garcia-Calzon and Diaz-Garcia, 2006).

A few examples of detection methods using imprinted polymers include: fluorescence (Suarez-Rodriguez and Diaz-Garcia, 2001), field effect transistors (Zayats et al., 2002), surface plasmon resonance (Kugimaya and Takeuchi, 2001), differential pulse voltammetry (Andrea et al., 2001), impedance measurements (Panasyuk-Delaney ae al., 2001), acoustic wave sensor (Peng et al., 2000), optical approaches (Suarez-Rodriguez and Diaz-Garcia 2000) and conductrometric sensors (Kriz et al., 1996).

1.2 Components of the MIP

1.2.1 Templates

One of the many attractive features of the molecular imprinting method is that it can be applied to a diverse range of analytes. The imprinting is of small, organic molecules (e.g., pharmaceuticals, pesticides, amino acids and peptides, nucleotide bases, steroids and sugars) is now well established and considered almost routine. Somewhat larger organic compounds (e.g., peptides) can also be imprinted via similar approaches, whereas the imprinting of much larger structures is still a challenge, although specially adapted protocols have been proposed for e.g., proteins, cells and even mineral crystals. It has also been shown that the porogenic solvent itself can introduce some kind of molecular memory into the polymer, which can be regarded as a molecular imprinting process in a wider sense. The same is true for metal and other ions, which have been used as templates to induce the specific arrangement of functional groups in organic polymers and silica (Haupt and Mosbach, 2000).

A MIP is usually synthesized for a specific analytical use that implies the choice of a given template molecule. The structure and the functionalities of this molecule define the subsequent properties of the binding sites. The criteria to consider when selecting a candidate molecule are its cost, its availability and its chemical functionalities defining its ability to strongly interact with monomers (Yemiş, 2010).

For expensive or otherwise difficult to achieve target analyte, a structural analog can be used as a template for the synthesis in order to decrease the cost of the material. This dummy molecule must resemble the target analyte in terms of shape, size and functionalities. The resulting MIP should give rise to imprints that have the ability to bind the target analyte. The dummy approach is also used to avoid the risk of residual template leaking from the polymer and causing erroneous results, particularly in MIPSPE applied to the trace determination of compounds. Indeed, the complete removal of the template from the MIP is difficult to achieve and necessitates extensive washing steps with various organic solvents in basic or acidic conditions. The use of a dummy molecule constitutes an easy way to circumvent this problem as any leakage will be different from the analyte. At last, the dummy approach can also be used when the too low solubility of the target analyte does not allow its use for the synthesis of the MIP (Pichon and Chapuis-Hugon, 2008).

1.2.2 Functional monomers

All kinds of polymerization (radical, anion, cation, and condensation) can be employed for molecular imprinting. The only requisite is that the polymerization can satisfactorily occur under the conditions where all the components (the templates, the crosslinking agents, non-covalent adducts between the monomer and the template in non-covalent imprinting, and others) remain intact. However, radical polymerization is most commonly used, mainly because of its versatile applicability and experimental easiness (note that radical polymerization is also most widely used in industry, mainly for economic reasons). In covalent imprinting, templates are bound to vinyl moieties by covalent linkages. Esters and amides of acrylic acid or methacrylic acid are most often used. The synthesis is easy in most cases. For non-covalent imprinting, vinyl monomers bearing appropriate functional groups are designed and synthesized. Furthermore, many functional monomers are also commercially available. However, it should be noted that these commercial monomers usually contain inhibitors or stabilizers (e.g., hydroquinones and phenols) to avoid undesired polymerization during their storage. Thus, commercial monomers must be distilled before use in your molecular imprinting experiments (Komiyama et al., 2003).

1.2.3 Crosslinking agents

For molecular imprinting in organic solvents, ethylene glycol dimethacrylate (EDMA) and divinylbenzene are often used. A typical water-soluble crosslinking agent is N,N'-methylenebisacrylamide. The fundamental role of these reagents is to fix the guest-binding sites firmly in the desired structure. They also make the imprinted polymers insoluble in solvents and facilitate their practical applications. By using different kind of crosslinking agent, we can control both the structure of the guest-binding sites and the chemical environments around them (Komiyama et al., 2003).

For efficient imprinting, the reactivity of the crosslinking agent should be similar to that of the functional monomer (otherwise, either the functional monomer or the crosslinking agent polymerizes predominantly, and copolymerization cannot take place sufficiently). By choosing an appropriate crosslinking agent, random copolymerization occurs successfully, and the functional residues (derived from the functional monomers) are uniformly distributed in the polymer network (Komiyama et al., 2003).

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 crosslinking agents show non-covalent interactions with functional monomers and/or templates (Komiyama et al., 2003).

1.2.4 Solvents

The trivial role of solvents is to dissolve the agents for polymerization. However, they have more crucial roles. One of them is to provide 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 guests. Another role of solvents is to disperse the heat of reaction generated on polymerization. Otherwise, the temperature of reaction mixture is locally elevated, and undesired side-reactions occur there. Furthermore, the formation of monomer-template adducts, which is required for efficient non-covalent imprinting, is suppressed (Komiyama et al., 2003).

Choice of solvents is dependent on the kind of imprinting. In covalent imprinting, many kinds of solvents are employable as long as they satisfactorily dissolve all the components. In non-covalent imprinting, the choice of solvent is more critical to the promotion of the formation of non-covalent adducts between the functional monomer and the template and thus to enhancement of the imprinting efficiency. Chloroform is one of the most widely used solvents, since it satisfactorily dissolves many monomers and templates and hardly suppresses hydrogen bonding. However, commercially available chloroform is usually stabilized by ethanol to avoid the formation of poisonous phosgene during the storage. This ethanol is inappropriate for most molecular imprinting (especially for non-covalent imprinting), since it inhibits hydrogen bonding between monomer and template. In order to obtain good results, commercial chloroform must be distilled before use to remove ethanol. Carbon tetrachloride is not appropriate for molecular imprinting (with a few exceptions). In radical

polymerization, it is a chain transfer agent and decreases the molecular weight of polymers (Komiyama et al., 2003).

1.2.5 Initiators

Radical polymerization can be initiated by using thermal decomposition of radical initiators. Typically, 2,2/-azobis(isobutyronitrile) (AIBN) and 2,2'-azobis(2,4-dimethylvaleronitrile) (ADVN) are used. The initiation radicals formed by the decomposition attack the monomer, producing the propagating radicals. The reactions are very simple and economical. However, it is important to remove molecular oxygen from polymerization mixtures, since it traps the radical and retards (or even stops) the polymerization. In order to remove oxygen, degassing with nitrogen or argon, as well as freeze-and-thaw cycles under reduced pressure, is effective (Komiyama et al., 2003).

In some cases, non-covalent adducts between functional monomers and templates are too unstable to be used at higher temperatures, and the polymerization must be carried out at lower temperatures. Under these conditions, the thermal decomposition of initiator cannot be used to initiate the polymerization, and the initiators are decomposed with UV-light irradiation (photo-initiation never requires high temperatures). If the monomers themselves absorb UV-light sufficiently, the polymerization is initiated even in the absence of any radical initiators (Komiyama et al., 2003).

Chemical structures of typical reagents used are presented in Fig. 1.2.

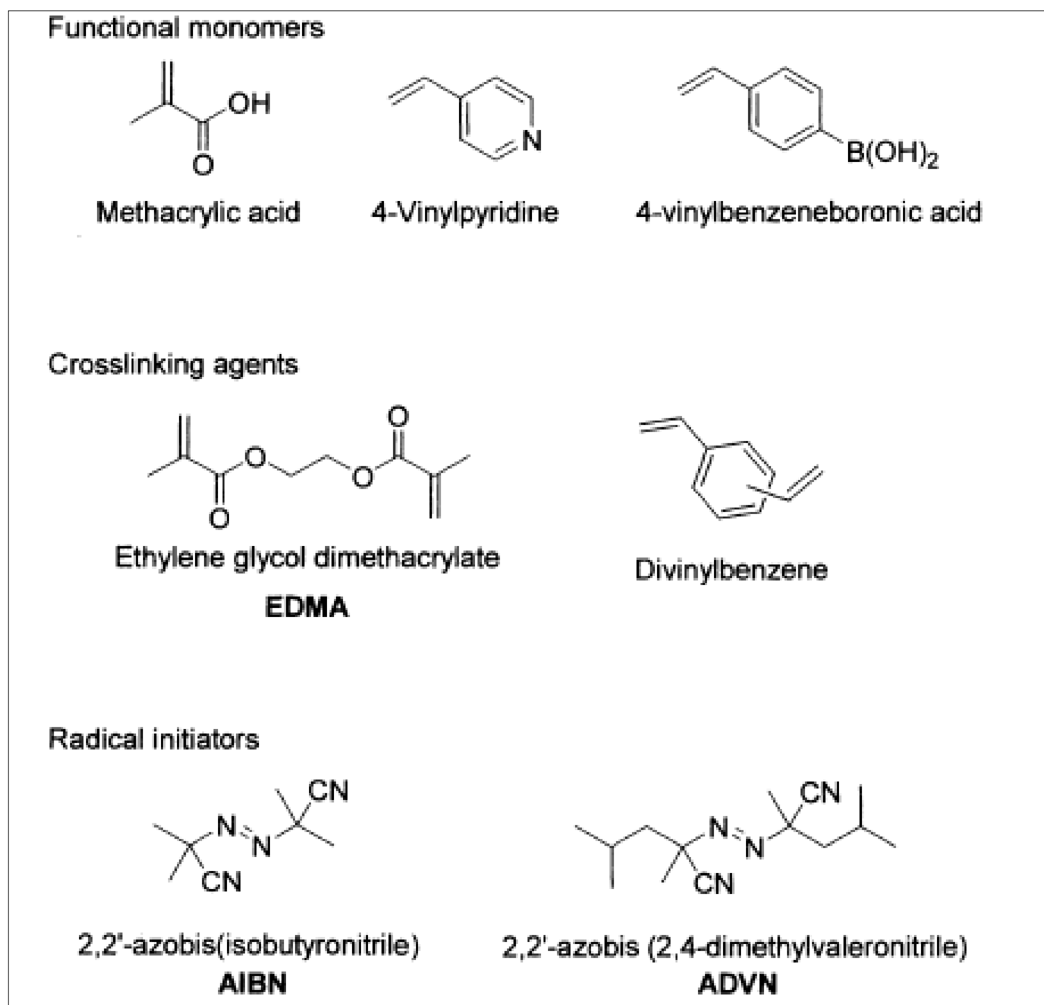

Fig. 1.2 Typical reagents (Komiyama et al., 2003).

1.3 Classification of Methods

The general principle of molecular imprinting is based on such a process where functional and cross-linking monomers are copolymerized in the presence of a target analyte (the imprint molecule) which acts as a molecular template. This procedure can be accomplished via either reversible covalent bonding or non-covalent interactions between monomers and imprint molecules. Other preparation methods of molecular imprinting polymers (MIPs) have been reported including chemical grafting, soft lithography technique, molecular self-assembled approach and electropolymerization (Özcan and Şahin, 2007).

1.3.1 In-block imprinted polymers

At present, one of the most popular methods of producing MIPs is bulk polymerization, in which functional monomers are bound either covalently or non-covalently to a print molecule or template. The correct positioning of these functional groups allows them to converge on the template molecule in a reciprocal fashion. The resulting pre-polymer complex is copolymerized with an excess of cross-linking monomer in the presence of an equal volume of inert solvent and free radical initiator. Polymerization of this mixture results in a highly cross-linked insoluble polymer. Removal of the template, in most cases by extraction or hydrolysis, leaves sites complementary in size and shape to the template molecule, resembling the “lock and key” model of the enzymes. The block of polymer is then ground and sieved in order to produce imprinted receptor particles of appropriate size. This method, usually applied to the preparation of stationary phases for high-performance liquid chromatography (HPLC), can be used to prepare imprinted polymeric membranes or polymer-coated electrodes for electrochemical sensing (Merkoçi and Alegret, 2002).

1.3.1.1 Covalent imprinting and non-covalent imprinting

The molecular imprinting method is of two types, depending on the nature of adducts between functional monomer and template (either covalent or non-covalent). Typical examples of these two kinds of methods are presented in Figs. 1.3 and 1.4. Both have advantages and disadvantages, and thus the choice of the best method strongly depends on various factors (Komiyama et al., 2003).

1.3.1.1.1 Covalent imprinting

In Fig. 1.3 prior to polymerization, functional monomer and template are bound to each other by covalent linkage (step 1). Then, this covalent conjugate is polymerized under the conditions where the covalent linkage is intact (step 2). After the polymerization, the covalent linkage is cleaved and the template is removed from the polymer (step 3). Upon the guest binding by the imprinted polymers, the same covalent linkage is formed (Komiyama et al., 2003).

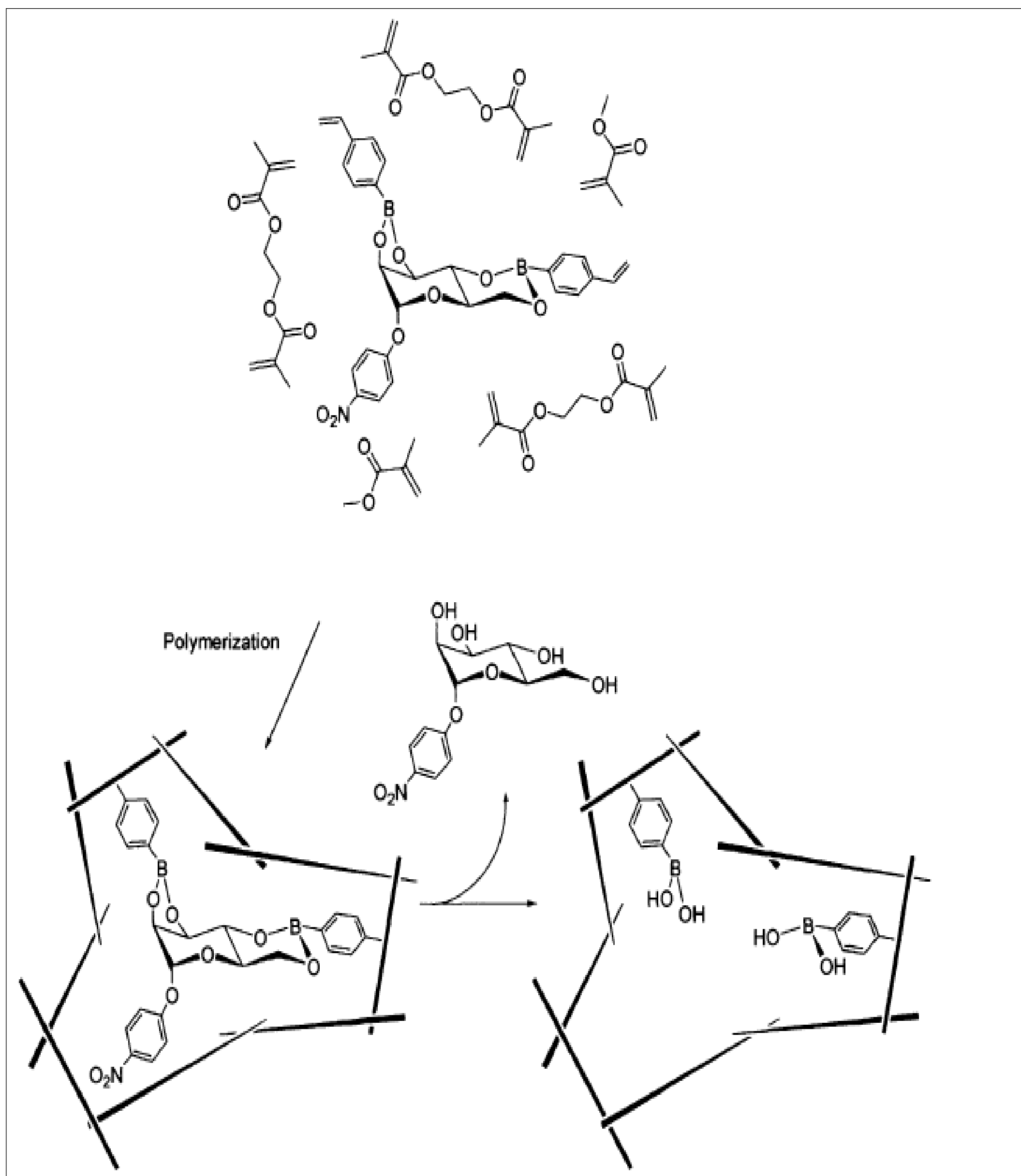

Fig. 1.3 Covalent imprinting of mannopyranoside using its 4-vinylphenylboronic acid ester as a functional monomer (Komiyama et al., 2003).

1.3.1.1.2 Non-covalent imprinting

In order to connect a functional monomer with a template, non-covalent interactions (e.g., hydrogen bonding, electrostatic interaction, and coordination-bond formation) are used here (Fig. 1.4). Thus, the adducts can be obtained *in situ* simply by adding the components to reaction mixtures (step 1). After the polymerization (step 2), the template is removed by extracting the polymer with appropriate solvents (step 3). The guest binding by the polymer occurs through the corresponding non-covalent interactions (Komiyama et al., 2003).

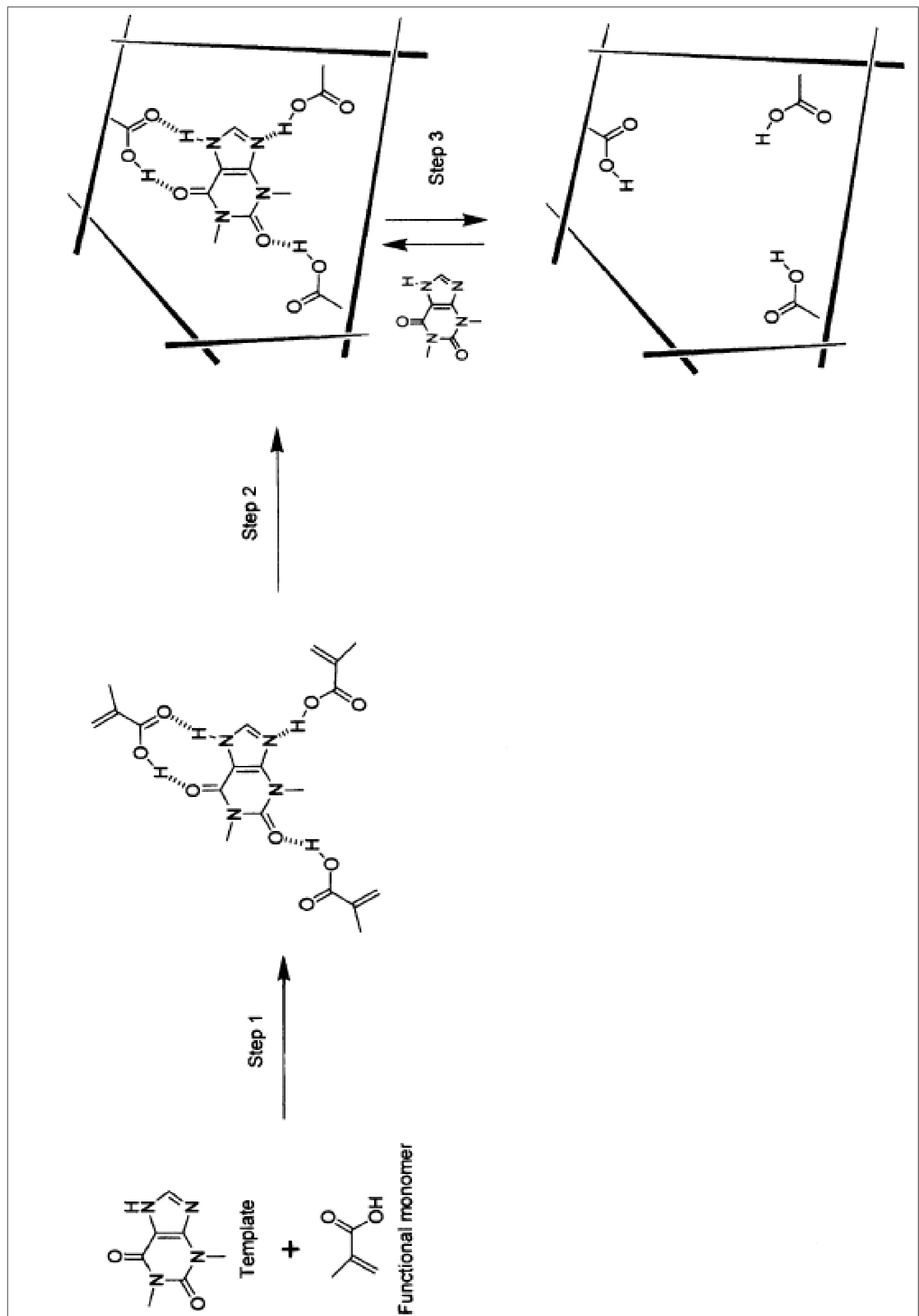

Fig. 1.4 Non-covalent imprinting by theophylline (a drug): Step 1: Pre-organization of functional monomers through non-covalent interactions Step 2: Polymerization of pre-organized functional monomers Step 3: Removal of the template (Komiyama et al., 2003).

1.3.1.2 Advantages and disadvantages of covalent and non-covalent imprinting

In general, non-covalent imprinting is easier to achieve and applicable to a wider spectrum of templates. With respect to the strictness of imprinting, however, covalent imprinting is usually superior. One should choose either of these two methods, depending on the need and situation of their operations (kind of the target guest compound, the guest selectivity required, the cost and time allowable for the preparation, and others) (Komiyama et al., 2003).

1.3.1.2.1 Advantages of covalent imprinting

1. Monomer-template conjugates are stable and stoichiometric, and thus the molecular imprinting processes (as well as the structure of guest-binding sites in the polymer) are relatively clear-cut.

2. 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 (Komiyama et al., 2003).

1.3.1.2.2 Disadvantages of Covalent Imprinting

1. Synthesis of the monomer-template conjugate is often troublesome and less economical.

2. The number of reversible covalent linkages available is limited.

3. The imprinting effect is in some case diminished in step 3 (cleavage of covalent linkages), which requires rather severe conditions.

4. Guest binding and guest release are slow, since they involve the formation and breakdown of a covalent linkage (Komiyama et al., 2003).

1.3.1.2.3 Advantages of non-covalent imprinting

1. Synthesis of covalent monomer-template conjugates is unnecessary.

2. Template is easily removed from the polymer under very mild conditions, since it is only weakly bound by non-covalent interactions.

3. Guest binding and guest release, which take advantage of non-covalent interactions, are fast (Komiyama et al., 2003).

1.3.1.2.4 Disadvantages of non-covalent imprinting

1. The imprinting process is less clear-cut (monomer-template adduct is labile and not strictly stoichiometric).

2. The polymerization conditions must be carefully chosen to maximize the formation of non-covalent adduct in the mixtures.

3. The functional monomers existing in large excess (in order to displace the equilibrium for adduct-formation) often provide nonspecific binding sites, diminishing the binding selectivity (Komiyama et al., 2003).

1.3.1.3 Dummy molecular imprinting

This method is used to prepare artificial receptors toward certain bioactive compounds or environmental hormones (e.g., dioxin). When we wish to achieve molecular imprinting with these templates, it often happens that the template compound is not available in a sufficient amount. Alternatively, the template can be too toxic and dangerous to be used in laboratories. Under these conditions, »direct« molecular imprinting is hard to achieve. In these cases, an appropriate substitute compound which has a similar structure to the real template but is more easily available (or non-toxic) is used as the template. In the following example, an artificial receptor for atrazine (a herbicide) is prepared by using trialkylmelamine (non-toxic) as the template. These two chemicals resemble each other, so that the imprinted polymer obtained with the dummy binds atrazine selectively and effectively. This »dummy molecular imprinting« is an extension of non-covalent imprinting, and enormously widens the spectrum of target compounds (Komiyama et al., 2003).

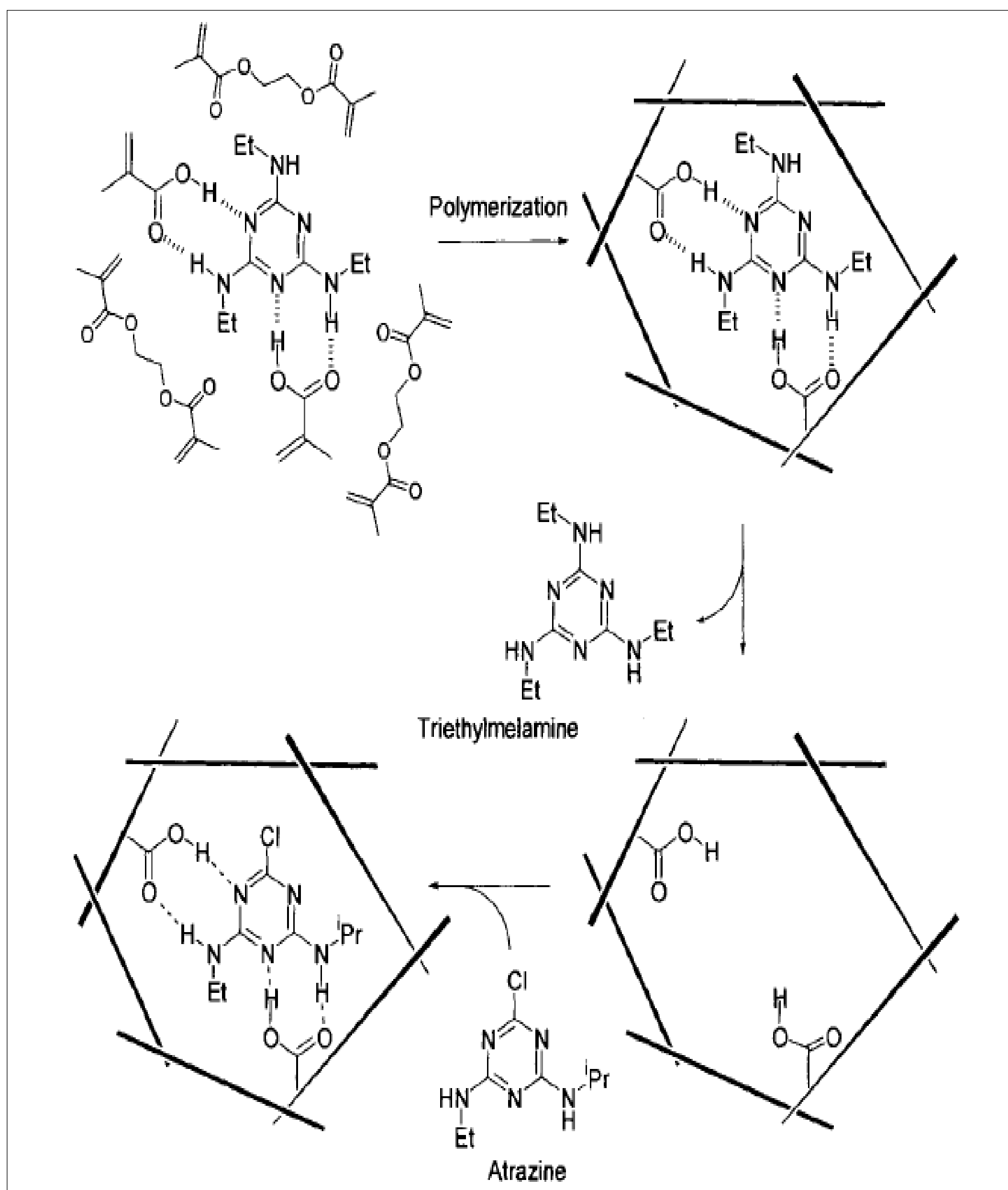

Fig. 1.5 Molecular imprinting by triethylmelamine as a dummy template for atrazine (Komiyama et al., 2003).

1.3.2 Other imprinting methods

1.3.2.1 In-situ imprinted polymers

To avoid the grinding, sieving, and packing process, continuous porous polymer rods inside the separation column were introduced in HPLC by in situ polymerization procedures. To obtain MIP monoliths with suitable porous structures providing acceptable flow conditions for the mobile phase, the type and amount of porogenic solvent requires careful consideration. In particular, toluene–isooctane blends have proven their suitability as porogen for the preparation of

molecularly imprinted super-porous polymer monoliths. Several types of functional and crosslinking monomers are amenable to this preparation route including the crosslinking monomers trimethylolpropane trimethacrylate (TRIM), pentaerythritol triacrylate (PETRA), pentaerythritol tetraacrylate (PETEA), and EGDMA, and the functional monomers MAA and 2-vinyl pyridine (2-Vpy). However, both toluene and isooctane are aprotic and apolar solvents, which provide limited solubility for some templates. Moreover, it was reported that the stability of thus synthesized MIPs is limited, as such monolithic structures are subject to shrinking after repeated use. Recently, silica-based hybrid MIP monoliths with viable chiral recognition properties were reported by using a hydrophobic room temperature ionic liquid for reducing gel shrinkage, which simultaneously acts as a pore template (Wei and Mizaikoff, 2007).

1.3.2.2 Directly synthesized polymer beads

Monodisperse particles with diameters of 3–5 μm suitable for HPLC columns can be obtained by selecting monomers, crosslinkers, and porogens amenable to suspension polymerization strategies. However, suspension polymerization requires the presence of additional constituents (e. g., dispersants) for facilitating the generation of spherical particles. Hence, the complexity of the imprinting strategy further increases providing reduced control on the polymer properties without fundamentally understanding the additional intermolecular interactions given the increased number of involved species. Exemplarily, a comparatively high level of nonspecific binding was observed at imprinted beads synthesized by the perfluorocarbon suspension method, which may be attributed to the introduction of fluorinated surfactants to the system. Furthermore, the cost of synthesized MIP particles may increase with the need for additional components (Wei and Mizaikoff, 2007).

More recently, precipitation polymerization was introduced for the preparation of monodisperse molecular imprints, thereby providing a relatively simple one-step polymerization route toward the formation of spherical particles in a highly diluted solution derived from the conventional bulk polymerization recipe. However, to prepare beads with a diameter of 3–5 μm by the precipitation method, toluene/acetonitrile (ACN) mixtures need to be used as porogen for obtaining sufficient porosity. The reported total pore volume of 0.38 mL/g obtained for beads is still lower compared to irregular shaped MIP particles (0.64 mL/g) prepared with similar formulation. It is evident that the chromatographic

performance of thus prepared spheres strongly depends on the porosity of the particles. An increase in the polymerization temperature from 60 to 70°C could potentially increase the degree of crosslinking resulting in the formation of smaller pores; however, a fine balance between the achievable porosity and the particle size of the resultant polymer beads requires careful optimization by controlling confounded parameters during polymer synthesis. While apparently a straightforward synthetic route, the precipitation polymerization method is not the best method of choice for templates with limited availability, as the yield of microspheres is relatively low (40–60%) in contrast to particles obtained via bulk polymerization (90%; however, a significant amount of particles were not usable after grounding and sieving), which results from the reduced monomer and initiator loading, along with the generation of soluble polymer resulting from the amount of crosslinking monomers required for this method. However, if low-cost dummy templates can be utilized, this synthetic route may provide a viable strategy (Wei and Mizaikoff, 2007).

1.4 Stability of the Monomer-Template Assemblies

The functional monomers must strongly interact with the template prior to and during polymerization to achieve a high yield of imprinted binding sites. Considering one particular binding site, the following factors have been identified that are likely to affect the recognition properties of the site (Figure 1.6) (Sellergernren, 1999).

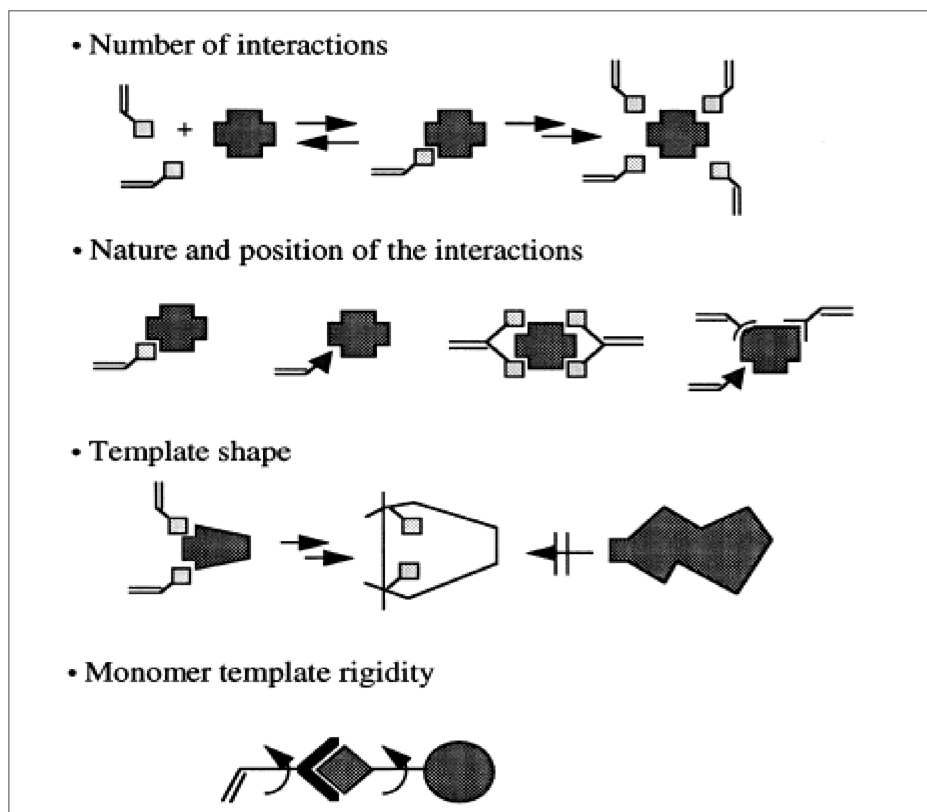

Fig. 1.6 Factors influencing the formation of the templated binding sites (Sellergren, 1999).

1.4.1 Thermodynamic factors

An important part of the optimization process is the stabilization of the monomer-template assemblies by thermodynamic considerations. The enthalpic and entropic contributions to the association will determine how the association will respond to changes in the polymerization temperature. The change in free volume of interaction will determine how the association will respond to changes in polymerization pressure. Finally, the solvent's interaction with the monomer-template assemblies relative to the free species indicates how well it will stabilize the monomer-template assemblies in solution (Sellergren, 1999).

1.4.2 The number of the type of interaction sites on the monomer-template

A larger number of complementary interactions will increase the binding strength and fidelity in the recognition. Thus, templates offering multiple site of interaction for the functional monomer are likely to yield binding sites of higher specificity and affinity for the template. The strength and positioning of these

interactions are equally important. Functional monomers should be chosen that allow a maximum number of complementary interactions to develop. Thus templates containing basic groups or acid groups are usually best imprinted using acidic, i.e.,MAA, and basic, i.e., vinylpyridine (VPY), functional monomers respectively. Furthermore, combinations of two or more functional monomers, giving terpolymers or higher, have in a number of cases given polymers with better recognition ability than the recognition observed using the corresponding copolymers. These systems are particularly complex when the monomers constitute a donor-acceptor pair, since a monomer-monomer association will strongly compete with a template-monomer association if neither of the monomers has a particular preference for the template (Sellergren, 1999).

1.4.3 The template shape

The shape of the template may be sufficient to create the necessary steric complementarity for efficient discrimination between two molecules. The first unambiguous evidence for the influence of template shape on the recognition properties of MIPs was obtained using a covalent template system. Thus diol-containing binding sites for aryl-1,3-diketones with different aryl groups exhibited a memory for the respective aryl group. Shape complementarity was also suggested as one contributing factor in the recognition of MIPs for L-phenylalanine anilide (L-PA) and the corresponding N-methylanilide. A number of other examples suggest that shape complementarity contributes to recognition in MIPs. For instance, polymers imprinted with benzylamine (BA) are capable of recognizing its template compared to structurally related compounds. Pronounced shape complementarity and size exclusion effects were recently observed in the imprinting of amino acids with different N-protecting groups (Sellergren, 1999).

1.4.4 The monomer-template rigidity

The more conformationally defined the monomer and the template, the lower the number of stable conformers that will be imprinted and the more defined the recognition sites will be. The rebinding will also occur without much loss of rotational entropy, further promoting strong binding. The importance of preorganization is well known in the area of host-guest chemistry. Thus the better the fit between the site and the template, the less entropy will be lost due to conformational changes in the site as well as in the template upon binding. This will increase the affinity and selectivity in the recognition (Sellergren, 1999).

1.5 The Stability, Integrity and Accessibility

For the formation of defined recognition sites, the structural integrity of the monomer-template assemblies has to be preserved during polymerization to allow the functional groups to be fixed in space in a stable arrangement complementary to the template. This is achieved by the use of a high level of crosslinking. The lower level of crosslinking for recognition to be seen is about 50%. The role of the polymer matrix, however, is not only to contain the binding sites in a stable form but to provide porosity allowing easy access for the template to all sites. Porosity is achieved by carrying out the polymerization in the presence of a porogen. Most of the crosslinked network polymers used for molecular imprinting have a wide distribution of pore sizes associated with various degrees of diffusional mass transfer limitations and swelling. Based on the above criteria, i.e., site accessibility, integrity, and stability, the sites can be classified into different types (Fig. 1.7). The sites associated with meso- and macro-pores (>20 Å, site A) are expected to be easily accessible compared to sites located in the small ermicropores (<20 Å, site B) where the diffusion is slow. The number of the latter may be higher since the surface area, for a given pore volume, of micropores is higher than that of macropores. One undesirable effect of adding an excess of template is the loss of site integrity due to coalescence of the binding sites (site D), which is related to the extent of template self-association. The optimum amount of template is usually about 5% of the total amount of monomer but can be higher when trivinyl monomers are used as crosslinkers, where a larger fraction of functional monomer is used. The amount of template is of course also limited by the solubility and availability of the template, although recycling is possible (Sellergren, 1999).

For most applications in liquid media, permanent porosity and a large surface area of accessible meso and macro-pores are preferred. This gives materials containing mainly accessible sites of type A, although a significant number of nonspecific sites of type F may also be present. For macroporosity to be achieved, the volume of diluent should be slightly larger than the volume of monomers, the level of crosslinking should be relatively high (>50 mol %) and the swelling and porosity should be controlled by the type of solvent-nonsolvent mixture during polymerization. It is important to keep in mind that the latter also affects the stability of the monomer-template assemblies (Sellergren, 1999).

One problem in molecular imprinting concerns the small amount of template that remains strongly bound to the polymer after extraction (site G in Fig. 1.8). This usually amounts to more than 1% of the amount of template given to the monomer mixture and remains bound even after careful washing of the polymer. This may not constitute a problem in preparative separations or catalysis, but when the materials are used for sample preparation prior to analytical quantification of low levels of analytes, bleeding of this fraction will cause false results. In spite of careful washing, slow leakage of template often occurs upon exchange of solvents. This problem may be reduced by thermal posttreatment of the materials or prevented by the use of a template analog as template. Also more effective washing procedures may lead to less bleeding (Sellergren, 1999).

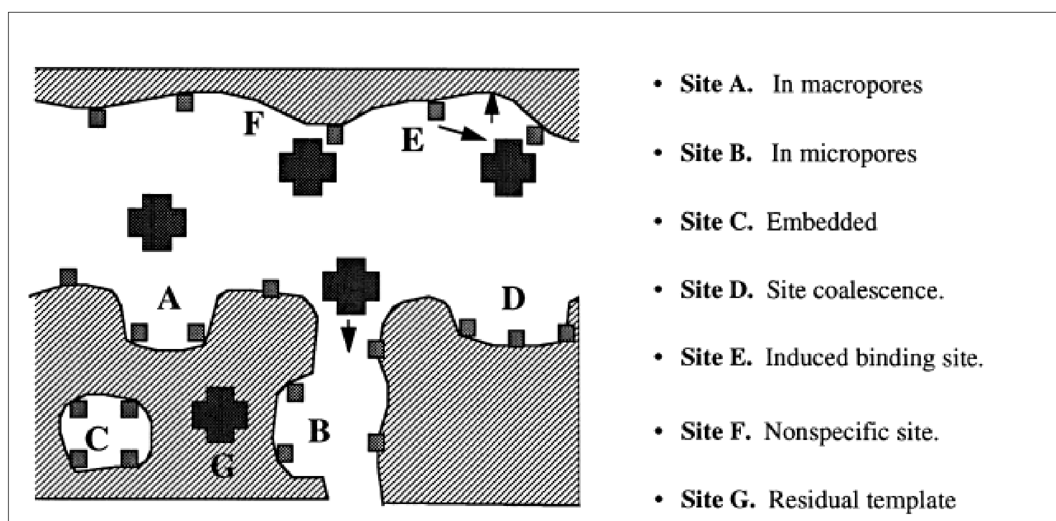

Fig. 1.7 Types of binding sites in MIPs (Sellergren, 1999).

1.6 Application of Imprinted Polymers

MIP technology is now in the maturing after a slow start because of difficulties in resolving problems associated with poor performance of MIPs in aqueous environments and because of the absence of any general procedure for MIP design. As a result little information is available about the commercial application of MIPs, and it should be noted that the following prognosis and analysis involve a degree of speculations (Piletsky et al., 2001).

In past few years, the variety of compounds to which MIPs have been developed has increased dramatically. The larger part of applications of MIPs is their use at solid phase adsorbents for HPLC (Merkoçi and Alegret, 2002).

Subsequently, their use has been extended to other analytical techniques including thin-layer chromatography, capillary electrochromatography, solid phase extraction, immunoassay-type binding assays and chemical sensors (Haupt and Mosbach, 2000).

In addition, the use of MIPs in other chemical applications has also expanded greatly. These new areas with opportunities for the application of MIPs include the development of analytical assays and sensors, membranes for purification of waste and drinking water, capillary electrophoresis (CE), and the production of polymers with special functions, such as drug-release matrices (Merkoçi and Alegret, 2002).

1.6.1 Sensor

1.6.1.1 Quartz crystal microbalance-based sensors

Quartz crystal microbalance (QCM) sensors have been developed using imprinted polymers as molecular recognition elements. There are two categories: one involving the immobilization of polymer particles on the electrode of the QCM and the other in situ polymerization on this electrode (Komiyama et al., 2003).

Ethylene glycol dimethacrylate (EDMA) methacrylic acid (MAA) copolymer-based imprinted polymer particles were mixed with poly (vinyl chloride) in THF, and the solution was then spread on the electrode of the QCM by spin coating. After evaporation of the THF, the polymer particles were immobilized on the surface. A Phenobarbital imprinted QCM sensor prepared in this way worked in ethanol, while epinephrine and caffeine imprinted QCMs worked in buffer solutions (pH 6.0 and pH 8.0, respectively) (Komiyama et al., 2003).

In situ preparation of imprinted polymer films on a QCM was performed using S-propranolol as the template. A pre-polymerization mixture containing MAA, trimethylolpropane trimethacrylate (TRIM, a crosslinker), the template and acetonitrile (porogen) was poured on the electrode of the QCM and immediately covered by glass and polymerized by UV irradiation. A low amount of the crosslinker (about 40% of total monomers) was used to prepare more flexible polymer, allowing the polymer to be stably adhered on the electrode. The sensor

showed enantioselective response with a selectivity factor of 5, and the detectability of S-propranolol was 50 µM in acetonitrile (Komiyama et al., 2003).

1.6.1.2 Electrode-type sensors

A capacitive sensor with a molecularly imprinted polymer film as a sensitive layer has been reported. The layer was prepared by electropolymerization of phenol on a gold electrode with the template molecule, phenylalanine. The sensor capacitance was decreased by the addition of phenylalanine, but there was almost no change with glycine, tryptophan and phenol. The response time was 15 min (time for a half of the stationary value, 60 min), and the dynamic range was given as 0.5 to 8 mg/mL. The authors mentioned that temporal stability and reversibility were poor because there was no cross-linking; however, the sensor has merits such as a good selectivity and reproducibility between sensors and is suitable for a single-use sensor (Komiyama et al., 2003).

1.6.1.3 Optical sensors

A fluorescence optical sensor for dansyl-L-phenylalanine has been reported. In the optical sensor, the imprinted polymer was held in front of a fiber-optic device by a nylon net. Although the system worked well, there are some inherent problems that need to be addressed; the time required for a steady response was 4 h, which seems too long, and only fluorescent analytes could be applied to this system (Komiyama et al., 2003).

1.6.2 Signaling polymers

A metal-complexing glucose-imprinted polymer involving ligand exchange on a triazacyclononane-copper (II) complex was reported. The polymer was prepared by polymerization of the copper (II) complex of l-(4-vinylbenzyl)-l,4,7-triazacyclononane and methyl-ß-D-glucopyranoside. After removal of the methyl-ß-D-glucopyranoside, the resultant polymer bound glucose selectively at alkaline pH, with the release of protons in proportion to the concentration of glucose. By operating an appropriate alkaline pH region where the buffer capacity of biological samples is small, interference with the measurement of protons released in biological samples was minimized. Equilibration of the complexation is very

rapid, which suggested that this system would be suitable for continuous glucose monitoring in clinical and bioprocess applications (Komiyama et al., 2003).

1.6.3 Molecular imprinted sorbent assays

Molecularly imprinted sorbent assays represent one of the most typical applications of biomimetic use, where imprinted polymers are used as substitutes of natural antibodies in immunoassays. The assays usually involve competitive binding of an analyte with a certain quantity of labeled ligands, in which the labeled ligand unbound is proportional to the analyte added. Because dissociation constants of common imprinted polymers are around 10^{-6}-10^{-9} M, competitive binding assays could easily be performed. In practice, many molecularly imprinted sorbent assays have been developed for biologically active compounds, including theophylline, diazepam, S-propranolol, morphine, Leuenkephalin, cyclosporin A, yohimbine, methyl-a-glucoside, corticosteroid, atrazine and 2,4-D (Komiyama et al., 2003).

1.6.4 Molecularly imprinted membranes

A nucleotide base-imprinted polymer membrane has been reported in which methacrylic acid was used as a functional monomer for the imprinting of an adenine derivative, 9-ethyladenine. A free-standing film was prepared by polymerizing a DMF solution containing methacrylic acid and ethylene glycol dimethacrylate on a silanized glass slide at 65-70 °C under nitrogen atmosphere (Komiyama et al., 2003).

1.6.5 Affinity based solid-phase extraction

Molecularly imprinted solid-phase extraction (MI-SPE) of triazine herbicides has been reported using an atrazine-imprinted polymer. This procedure consists of three steps: (1) sample loading where the polymer works as a reversed phase system because the aqueous sample is loaded on the column, (2) washing with dichloromethane, when the system is changed to a hydrogen-bonding-based affinity mode in which triazine herbicides can be selectively retained in the polymer while other structurally unrelated impurities are washed off, (3) recovery of triazine herbicides with methanol, when hydrogen bonding is significantly weakened because of the interference in the hydrogen bond formation by the methanol. By employing such MI-SPE, simazine (0.1 ppm), one of the herbicides

commonly used in golf courses in Japan, was selectively concentrated approximately 60-fold with over 90 % recovery from a mixture of simazine, asulam, mecoprop, propyzamide and iprodione (0.1 ppm each, 500 mL of aqueous solution) (Komiyama et al., 2003).

1.6.6 In-situ preparation of imprinted polymers

In situ molecular imprinting is a convenient way to prepare imprinted polymers. Here, imprinted polymers are prepared in a place where the polymers are subsequently utilized. In general, molecularly imprinted polymers are prepared by bulk polymerization, and block polymers obtained are broken to pieces, ground, sieved and packed in a column. These experimental procedures are extremely tedious and time-consuming. The procedure also results in polymer particles of irregular size and shape, which may have a negative influence on column efficiency (Komiyama et al., 2003).

In order to overcome these problems, the first *in situ* molecular imprinting was performed to prepare a molecularly imprinted chromatographie stationary phase. A column filled with all the reagents necessary for molecular imprinting was heated in a water bath, resulting in a ready-to-use column filled with a continuous imprinted polymer rod, through which the eluent flows because of the porosity of imprinted polymers. An enantioselective polymer rod was obtained by *in situ* molecular imprinting using L-phenylalanine anilide as the template, and exhibited a separation factor of 1.7 (Komiyama et al., 2003).

1.6.7 Molecularly imprinted catalysts

One of the most attractive applications would be molecularly imprinted catalysts. In principle, such catalysts could be prepared if substrate, product or transition-state analogs could be used as template molecules, since to natural catalytic antibodies are produced in a similar way. Since molecularly imprinted polymers are considered to be analogous to antibodies in that binding sites are tailor-made, catalytic antibody-like activity in imprinted polymers could also be conceived, enabling an »artificial catalytic antibody« with the advantageous features of synthetic molecules to be produced (Komiyama et al., 2003).

The first »artificial catalytic antibody« for the hydrolysis of p-nitrophenyl acetate was prepared using a transition state analog, p-nitrophenyl

methylphosphonate as the template. Polyvinylimidazole cross-linked by 1,4-dibromobutane in the presence of the transition state analog exhibited 1.7-fold higher activity for catalyzing the planned hydrolysis reaction than that of a non-imprinted reference polymer. The activity was inhibited by the addition of the template, suggesting that imprinted cavities successfully operated as catalytic sites (Komiyama et al., 2003).

1.6.8 Separation

Predominantly, MIPs are prepared using conventional free radical bulk polymerization followed by grinding and sieving, thereby providing particles between 10 and 25 lm in diameter, which are finally packed as stationary phase into HPLC or SPE columns. A significant body of literature has demonstrated that baseline separation of templates from their structure analogs is achievable with MIP-based HPLC columns. Substantial interest in the development of imprinted polymers for biologically active constituents has provided the impetus for the development of an increasing number of MIP-based stationary phases. For example, selective polymer matrices for the separation/extraction of deoxynivalenol (DON) from beverages have been prepared enabling a detailed analysis of selected mycotoxin contaminations in food. Since the first application of MIPs to SPE (also referred to as "MISPE" (molecular imprinted SPE)) for the extraction of pentamidine in urine, MISPE has been applied to selective clean-up and preconcentration from a wide variety of sample matrices including biological fluids, environmental matrices, and food/beverages. However, more widespread application of MIP-based HPLC stationary phases in commercial products is still limited by the drawbacks of band broadening and tailing resulting from suboptimal particles obtained during conventional bulk polymerization, and to date limit the rational design of the synthetic route for flexibly creating the desired retention properties for a wide range of templates (Wei and Mizaikoff, 2007).

1.6.8.1 MI-SPE (Off-line SPE)

During the last few years, MIPs have appeared as new selective sorbents for SPE of organic compounds in complex materials. The potential value of MIP–SPE lies in the ability of selectively isolating specific compounds or their structural analogs from a complex matrix. The application of these synthetic polymers as sorbents allows not only preconcentration and cleaning of the sample but also selective extraction of the target analyte, which is important, particularly when the

sample is complex and impurities can interfere with quantification. As in conventional SPE procedures, a small amount of imprinted polymer (typically 50–200 mg) is packed into a cartridge. Subsequently, the steps of conditioning, sample loading, washing, and elution are carried out. A schematic procedure for MIP–SPE is shown in Fig. 1.8. The use of MIPs for sample concentration and cleanup by SPE is attractive owing to their high specificity and stability, and also because of their compatibility with both aqueous and organic solvents. Once the polymer has been obtained, it can be used in SPE protocols, where a careful selection of the most appropriate solvents to be used in the different steps is needed in order to extract the target analyte selectively. MIPs also allow the analyte of interest to be pre-concentrated while simultaneously removing interfering compounds from the matrix so that selective enrichment and cleanup are obtained, resulting in a higher accuracy and a lower detection limit in the subsequent analysis. The applicability of this method has been demonstrated with a number of compounds such as herbicides and drugs, which can be selectively extracted even from samples such as beef-liver extract, blood serum, and urine (Qiao et al., 2006).

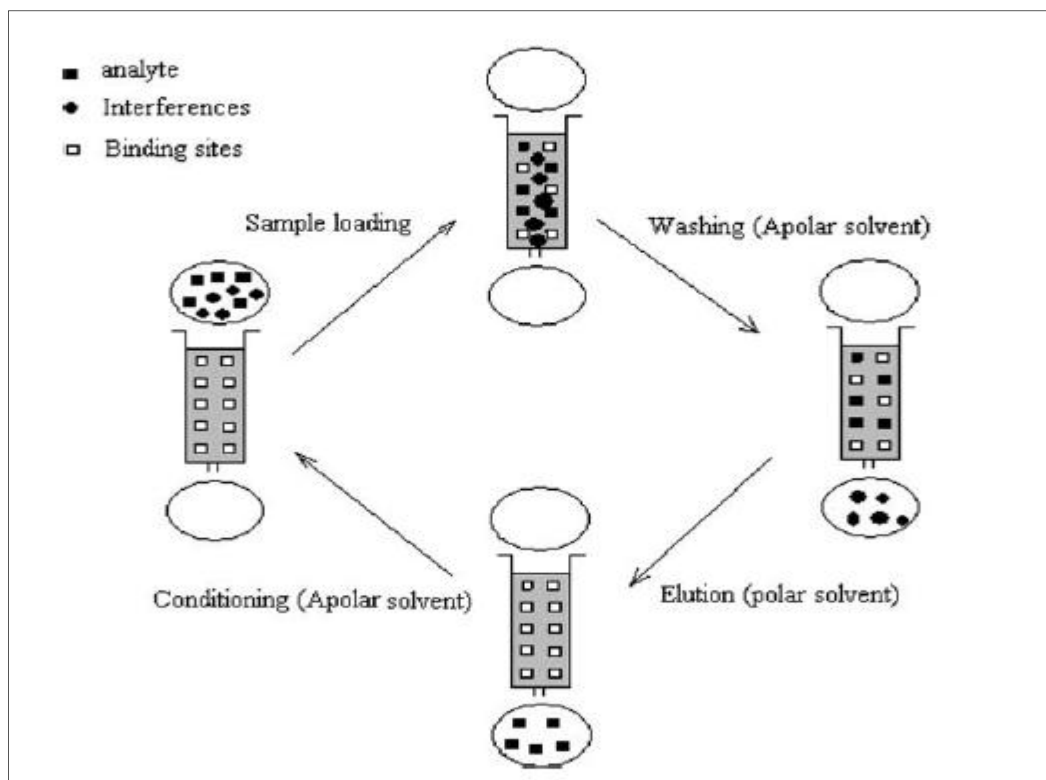

Fig. 1.8 Schematic procedure of molecularly imprinted solid phase extraction (Qiao et al., 2006).

1.6.8.2 Chromatography (On-line SPE)

For on-line coupling, the MIP is packed in a stainless steel cartridge to be used in a column switching arrangement prior to HPLC analysis. On-line SPE has advantage over off-line SPE since there is no sample manipulation between the preconcentration and the analysis steps. Loss of analyte and the risk of contamination are reduced and the detection limits and reproducibility are also improved. Moreover, online systems allow the entire sample passed through the MIP to be injected directly onto the subsequent analytical system, so the sample volume can be smaller, the consumption of organic solvents is lower, and the potential for automation improved. In this format, the MIP cartridge is placed in the loop of a sample injection valve. After preconcentrating the sample on the MIP cartridge and washing out interfering compounds, the analytes are eluted by the mobile phase into the analytical column. This approach is especially appropriate for multianalyte determinations using MIPs capable of recognizing several structurally related compounds. Bjarnason et al. developed on-line MIP–SPE methods for the determination of triazine herbicides in aqueous samples, urine, and apple extracts. The samples were first enriched on a precolumm filled with octadecyl silica and subsequently the analytes (and interfering compounds) were eluted on-line onto a MIP precolumn. Finally, after the usual washing step, the analytes were eluted using the mobile phase. Theodoridis reported the combination of HPLC with sequential injection analysis, which utilized a rapid automated and efficient on-line MIP–SPE procedure. Vicente et al. described the application of bisphenol A MIP for selective preconcentration of bisphenol A from environmental water samples by on-line MIP–SPE process. A 4-nitrophenol imprinted polymer was prepared and evaluated as a selective sorbent in MIP–SPE on-line coupled to a reversed-phase HPLC column. 4-Nitrophenol was selectively extracted and the humic acid interference was reduced considerably. It was shown that the conditions chosen for washing the MIP and for eluting the analyte in the MIP–SPE process are extremely important for ensuring good selectivity and recovery (Qiao et al., 2006).

1.6.8.3 Capillary electrochromatography

Capillary electrochromatography (CEC) might be one of the more promising chromatographic techniques to be used in combination with MIPs, in particular for chiral separations. MIP-based CEC profits from the inherent separation power of this method; compared with MIP-based HPLC, appreciable resolution (>100

000 plates m^{-1}) and separation factors can be achieved. Other possibilities of using MIPs in combination with CEC or capillary electrophoresis is in the form of continuous polymer rods (Lin et al., 1997), particles included in a gel matrix (Lin et al., 1996) and small particles suspended in the carrier electrolyte (Schweitz et al., 2000) (Haupt, 2001).

1.6.9 Application in biological and pharmaceutical fields

The first study of MIP–SPE was made by Sellergren. He prepared a MIP that could selectively extract pentanamide from diluted human urine samples. Andersson et al. investigated the influence of detergents in the buffer during MIP–SPE of local anesthetics from human plasma. They found that three different neutral detergents were able to eliminate non-selective adsorption to the polymer and leave selective MIP-analyte binding essentially unaffected. A modification of this MIP–SPE method in pure aqueous systems has recently been presented by Dirion et al., by modifying the original MIP composition via the incorporation of a hydrophilic co-monomer, 2-hydroxyethyl methacrylate, the non-specific binding was reduced, especially during the loading of the plasma sample. The MIP–SPE cartridge can also be washed with selective solvents that are capable of disrupting the non-selective (but not the selective) interaction with the polymer, when extracting aqueous samples. The selectivity then occurs in the washing rather than the loading step, leading to selective desorption rather than selective extraction (Qiao et al., 2006).

Two different clean-up steps for the multi-residue analysis of b-agonists in urine with respect to minimization of ion suppression using a mixed-phase SPE column, ‘‘clean screen Dau’’ (CSD), and a MIP–SPE column were evaluated by Hoof et al.. Ion suppression experiments revealed that CSD sample cleanup can lead to false negative results for some beta-agonists, and that clean-up using MIP columns is more selective. Moller et al. investigated the matrix effects on a MIP–SPE method for the clean-up of diphenyl phosphate in urine samples. The recovery and repeatability of the MIP–SPE method were affected most by NaCl in the tested concentrations, while urea, creatinine, and hippuric acid had no significant influence. NaCl most likely weakens the binding during the loading of the sample. This effect could be suppressed by diluting the urine sample with a citrate buffer at pH 4.0 (Qiao et al., 2006).

Andersson extracted a homologous series of anesthetics, including bupivacaine; ropivacaine and mepivacaine from human plasma with a MIP prepared using a structural analogue, pentycaine, as the template. Dilution of the plasma prior to extraction with pH 5.0 citrate buffer containing ethanol and Tween 20 was found optimal for selective imprint– analyte binding, and for reduction of non-specific adsorption of lipophilic contaminants to the hydrophobic MIP surface. Elution under basic conditions using triethylamine–water–acetonitrile mixtures recovered bupivacaine in 89% yield with superior selectivity over elution under acidic conditions (Qiao et al., 2006).

Monolithic MIPs prepared by in situ polymerization using ceramide III as print molecule was prepared and applied to the on-line SPE of ceramides from yeast extracts by Zhang et al.. The results showed that using ceramide III as the print molecule significantly affected the pore structure and distribution of the monolith, and greatly improved the retention of ceramide III and its analogues used in cosmetics. The retention of ceramide III on the monolithic MIP could be reduced by increasing the ratio of chloroform to hexane in the eluting buffer (Qiao et al., 2006).

The development of enantioselective MIP–SPEs for the enantiomers of the chiral Tröger's base were evaluated by Adbo. A series of Tröger's base imprinted MAA-EDMA co-polymers have been synthesized and baseline separation of racemic Tröger's base was readily achieved. Enantioseparation factors (a) of up to 4.8 ± 0.2 were obtained, showing clear differences in extraction efficiencies and extraction rates. This level of enantioselectivity corresponds to a difference in Gibbs free energy of binding between the two enantiomers (Qiao et al., 2006).

A robust MIP–SPE–DPE–FPE method specifically developed using a smart material for the recognition of metformin hydrochloride from plasma was reported by Feng et al.. Methanol + 3% trifluoroacetic acid was good for quantitative pulsed elution of the bound metformin. An intermediate step of DPE used acetonitrile with 5% picric acid to remove phenformin and other structural analogues. The FPE of metformin for direct UV detection was achieved using 3% trifluoroacetic acid in methanol (Qiao et al., 2006).

1.7 Mefenamic Acid

Non-steroid anti-inflammatories (NSAIs) are a family of drugs very much used for symptomatic treatment of both acute and chronic diseases; about 20% of people aged above 65 years usually are given these drugs and they are prescribed to 20% of hospital patients. Although they are rather safe drugs when properly dosed to selected patients, adverse effects and dangerous interactions may arise, especially in elderly people, enhancing the symptoms of other pathologies. On delivering a given drug in a particular pharmaceutical way it undergoes several processes (liberation, absorption, distribution, metabolism and excretion) and, especially for the so called class II drugs with low solubility and high permeability (a group to which NSAIs belong), the dissolution rate is the limiting factor for their absorption, and consequently dissolution of the drug is the most important step in the overall process to determine its absorption (Arco et al., 2006).

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed agents worldwide to treat a variety of pain-related conditions, including arthritis and other rheumatic diseases. In addition, epidemiological studies have shown that long-term use of NSAIDs reduces the risk of developing Alzheimer disease and delays its onset. NSAIDs are included in many cold and allergy preparations. Mefenamic acid is used mainly to treat rheumatoid arthritis and osteoarthritis. Mefenamic acid has also been found to produce closure of patient ductus arteriosus in premature neonates (Saghatforoush et al., 2009).

Mefenamic acid is used to relieve the symptoms of many diseases such as rheumatoid arthritis, non-articular rheumatism, and sport injuries. It is used to treat mild to moderate pain, including headache, dental pain, post-operative and post-partum pain, dysmenorrhoea, as well as musculoskeletal disorders and joint disorders such as osteoarthritis. Overdoses of MFA produce toxic metabolite accumulation that causes acute hepatic necrosis, inducing morbidity and mortality in humans. Due to the vital importance of the assay of MFA for pharmaceutical formulations and biological fluids, several analytical methods have been developed for the quantitative determination of this drug in both pharmaceutical and biological samples. Therapeutic mefenamic acid level is 10 μg/mL (Saghatforoush et al., 2009).

Mefenamic (*N*-(2,3-xylil) antranilic acid) is derivative of *N*-phenylanthranilic acid and is widely used clinicallyas non-steroidal anti-inflammatory drugs especially in the treatment of rheumatoid arthritis, osteoarthritis and other muscular–skeletal diseases. Mefenamic acid has also been found effective to produce closure of patent ductus arteriosus in premature neonates. However the use of this drug has been implicated in several cases of nephrotoxicity including acute renal failure and tubulo intestinal nephritis (Capitan-Vallvey et al., 2000).

Mefenamic acid occurs as a white to light yellow powder. It is odorless and tasteless at first but leaves a slightly bitter aftertaste. It is sparingly soluble in diethyl ether, slightly soluble in methanol, in ethanol and chloroform, and practically in soluble in water. It dissolves in NaOH. It melts between 227-232 ^{o}C.

1.7.1 Method of analysis

1.7.1.1 Identification

The infra-red absorption spectrum is concordant with reference spectrum of mefenamic acid.

Dissolve 25 mg in 15 ml of chloroform and examine under UV light 254 nm, the solution exhibit a strong greenish yellow florescence. Carefully add 0.5 ml of trichloro-acetic acid drop wise and examine under UV light 254 nm. The solution doesn’t exhibit florescence.

1.7.1.2 Light absorption

Absorption of a 0.002% W/V solution in a mixture of 1 volume of 1M HCl and 99 volumes of methanol at the maximum at 279 nm 0.69 to 0.74 and the maximum at 350 nm 0.56 to 0.60.

1.7.1.3 Assay

Dissolved 0.6 g in 100 ml of warm absolute ethanol previously neutralized to phenol red solution and titrate it with 0.1M NaOH using phenol red solution as indicator. Each ml of 0.1M NaOH is equivalent to 0.02413 g of mefenamic acid.

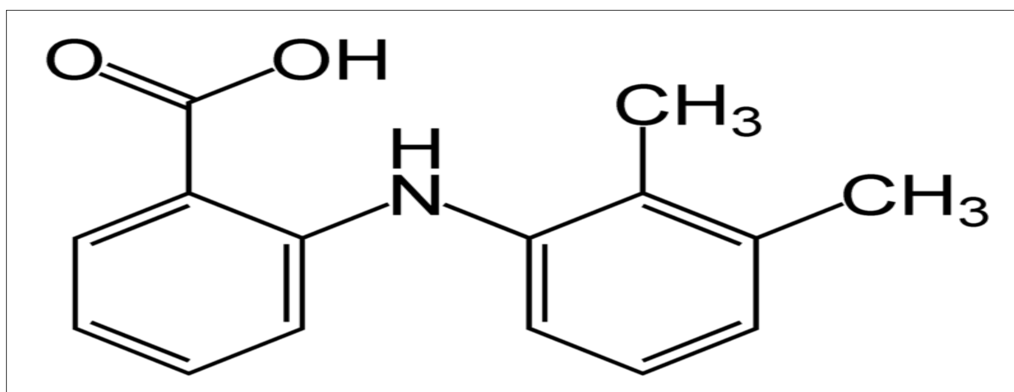

Fig. 1.9 Structure of Mefenamic Acid.

1.8 The Aim of the Study

With increasing need of drugs for human health, rapidly and reliable determination of this drugs are very important in pharmaceutical industry. For most of the drugs there are lots of serious adverse effects so serum and biological fluid analysis are important for these drugs. There is a need for selective separation agents and sensors for drug analysis.

There is a need for developing new artificial molecular recognition systems such as molecular imprinting polymer. Molecularly imprinted polymers (MIPs) are stable synthetic materials that possess selective molecular recognition sites formed by template-assisted arrangements of functional groups within the polymer matrix. To develop specific strategies of integration between these new artificial molecular recognition systems and optical sensing is necessary.

The aim of this work is to selective and sensitive determination of mefenamic acid by MIP-based optical membranes and use of MIPs as a separation media.

2 EXPERİMENTAL

2.1 Apparatus

The photometric measurements were carried out using a Varian Cary 100 Bio UV-vis spectrophotometer. The cuvettes of quartz glass (1-1 cm) were used.

The spectrofluorometric measurements were carried out using a Shimadzu RF-5301 PC spectrofluorophotometer.

pH measurements were performed using a WTW 330i pH meter.

Nüve BM 302 water bath shaker and Biosan Orbital OS-10 shaker were used.

All weight measurements were performed using a Precisa XB 220A (readability: 0.0001g)

75 and 125µm Retsch ASTM E11 sieves were used for sieving polymer particles.

10-100 and 100-1000 µL Brand Transferpette S digital micropipettes were used for all preparations.

2.2 Reagents and Solutions

(i) Chloroform ($CHCl_3$), etilenglycoldimethacrylate (EDMA), methacrylic acid ($C_4H_6O_2$), 2,2'-azobis(isobutyronitrile) (AIBN), tetrahydrofuran (THF) and N,N-dimethylformamid (C_3H_7NO) were obtained from Merck. Sodium hydroxide (NaOH) and ethanol (C_2H_5OH) were obtained from Carlo Erba. Polyvinyl chloride (PVC) was obtained from MP biomedicals. Dioctyl phtalate (DOP) %99 was obtained from Aldrich. Potassium tetrakis(4-chlorophenyl)-borate was obtained from Fluka.

(ii) A stock standard solution (100 mg/L) of mefenamic acid (Pfizer) in chloroform was prepared, kept in a brown bottle and then stored in room temperature. Under these conditions, the solution was stable and hence the drug concentration did not change with time. Mefenamic acid working standard solutions were prepared in chloroform daily by serial dilutions of the stock standard solution and kept in brown bottles.

All reagents were of analytical grade quality.

2.3 Process of Molecular Imprinting Polymer

Mefenamic acid as template molecule (1 mmol), methacrylic acid (MAA) (4 mmol) as functional monomer, ethylenglycoldimethacrylate (EDMA) (20 mmol) as crosslinker and 2,2'-azobis(isobutyromitrile) (AIBN) (40 mg) as initiator were dissolved in chloroform (10 ml). The mixture was degassed with N_2 for 10 min and then the tube was sealed and put into a water bath at 60^0C for 24 hours. Molar ratio of functional monomer to template was 4:1. A control polymer (NIP) was prepared following the same procedure without the template (mefenamic acid). The glass tube was smashed and MIP and NIP were dried at room temperature.

2.4 Extraction of MFA from MIPs

MIPs were crushed and ground in a agat mortar and pestle sieved to a suitable particle size as 75-125 µm. For all the experiments the particle size of 75-125 µm were used. Two different methods, batch and soxhlet extractions were applied for the extraction of MFA from MIPs. The amount of MFA passed to the solution was measured by the absorbance of MFA in 0.1M NaOH at λ_{max}: 285 nm, in chloroform at λ_{max}: 365 nm and in ethanol at λ_{max}: 348 nm.

2.4.1 Batch extraction

0.5 g MIP was weighted and extracted with 100 ml of 0.1M NaOH. The mixtures were stirred continuously at 25^0C for 24 hours then filtrated with filtration paper. The extraction was repeated one more time with the same amount of NaOH solution. Each extraction solution was diluted to a measurable concentration and the absorbance at λ_{max}:285 nm was read. The percentage of MFA extracted from MIP was calculated from the sum of the concentration of extraction solution.

2.4.2 Soxhlet extraction

0.5 g MIP was weight and tightly packed with filtration paper and placed in the soxhlet extractor. 300-350 ml chloroform was used for the extraction. After 24 hours absorbance of the solution was measured at λ_{max}: 365 nm for the calculation of the percentage of extracted MFA. Extracted MIPs were dried at room temperature.

2.5 Batch Rebinding Studies of MFA to MIPs

MIPs obtained from batch and soxhlet extraction were investigated for rebinding. The kinetics of the binding for MFA to MIP and NIP were investigated as follow: 20 mg of the MIP particles were placed in 10 ml bottle and mixed with 2 ml of the 500 mg/L MFA solution. The mixture was incubated at 25^0C under continuous stirring. Samples were taken after 0.5 h, 1 h, 2 h and 3 h then filtrated with filtration paper. Each supernatant solution was diluted to a measurable concentration of MFA and the absorbance was read at λ_{max}: 365 nm. The concentration of MFA in the supernatant was calculated using a standard solution calibration curve. The amount of the analyte bound to the imprinted polymer was calculated by subtracting the concentration of the unbound target molecule from the initial concentration.

The incubation time for the binding to the polymer and binding capacity were investigated.

2.6 Preparation of MIP Optical Sensor

480 mg PVC was dissolved in 6 ml THF and 1 ml DOP was added to this solution. After all ingredients were dissolved 500 mg of extracted MIP was added. The mixture was stirred for 45 min, then it poured on a flat surface covered with a watch glass and left to dry for 48 hours. After 48 hours dried membrane was ready for measurements. A control membrane was prepared following the same procedure, using NIP.

2.7 Spectrofluorometric Measurements for MFA

The membrane was cut into pieces which fit on 1-1 quartz cuvettes hypotenuse forming two triangles. The membrane pieces were dipped in MFA

solutions. After waiting for measure time solutions removed and fluorescence intensities were measured.

2.8 MI-SPE

The MI-SPE studies were carried out by using a syringe as SPE cartage and 0.2 g of extracted MIP was used as column packing material and it was immobilized with glass wool. 3.5 ml of 500 mg/L MFA solution was added and permeate was measured directly.

3 RESULTS AND DISCUSSIONS

3.1 Spectrophotometric Behavior of MFA

UV-vis spectra of standard solutions of MFA in chloroform, ethanol and 0.1M NaOH were recorded. The spectra of MFA in chloroform depending on MFA concentration were shown in Fig. 3.1.

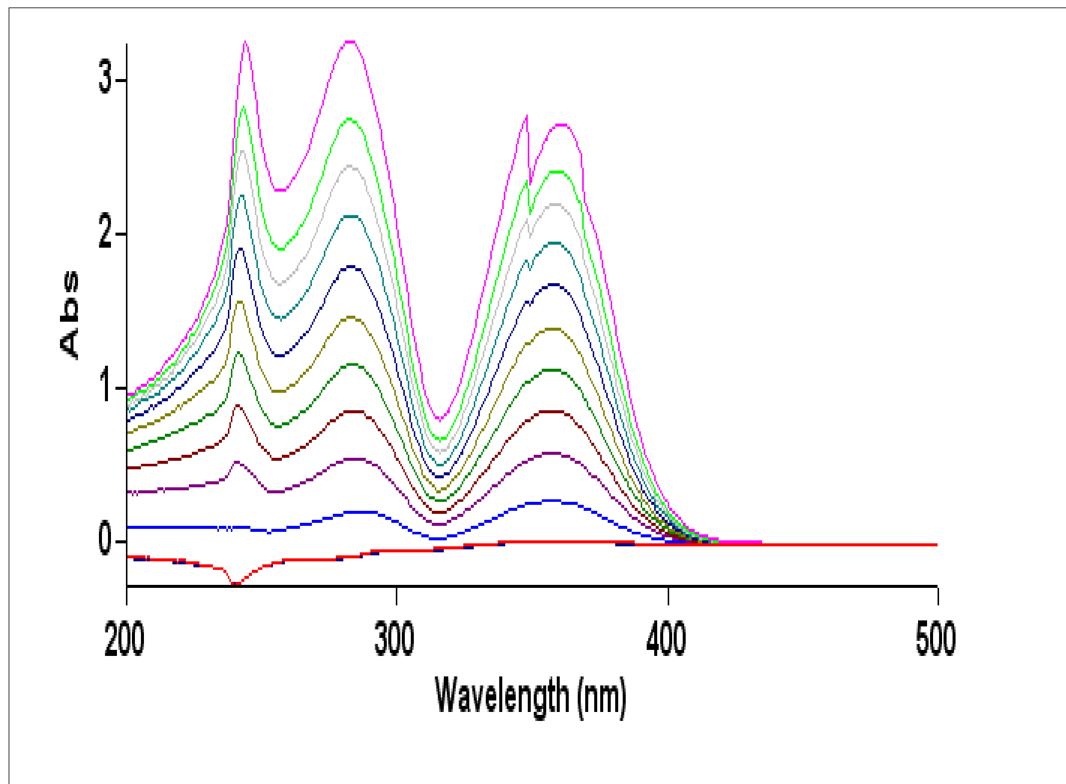

Fig. 3.1 The absorbance spectra of MFA in chloroform

In this spectrum there are three maximum absorbance observed at 365 nm, 285 nm and 250 nm. The dependence of absorbance at 365 nm and 285 nm on MFA concentration in chloroform were given in Fig. 3.2 and 3.3.The experimental datas reported in Figure 3.2 and 3.3 were fitted by a straight line with coefficients (R^2=0.9992) and (R^2=0.9974) . The calibration curves were found as linear in the range of 1-100 mg/L and 10-100 mg/L.

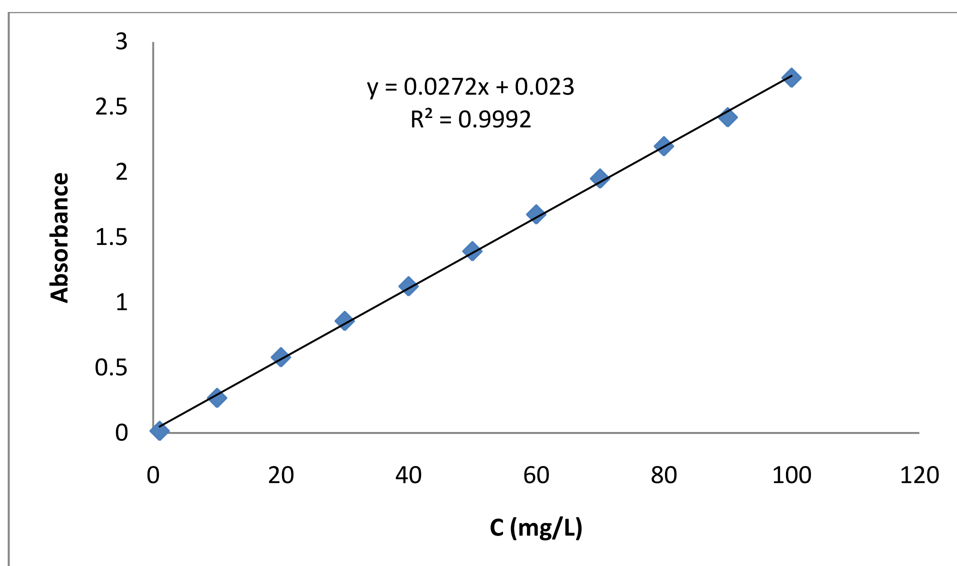

Fig. 3.2 Calibration curve of MFA in chloroform at λ_{max}: 365 nm.

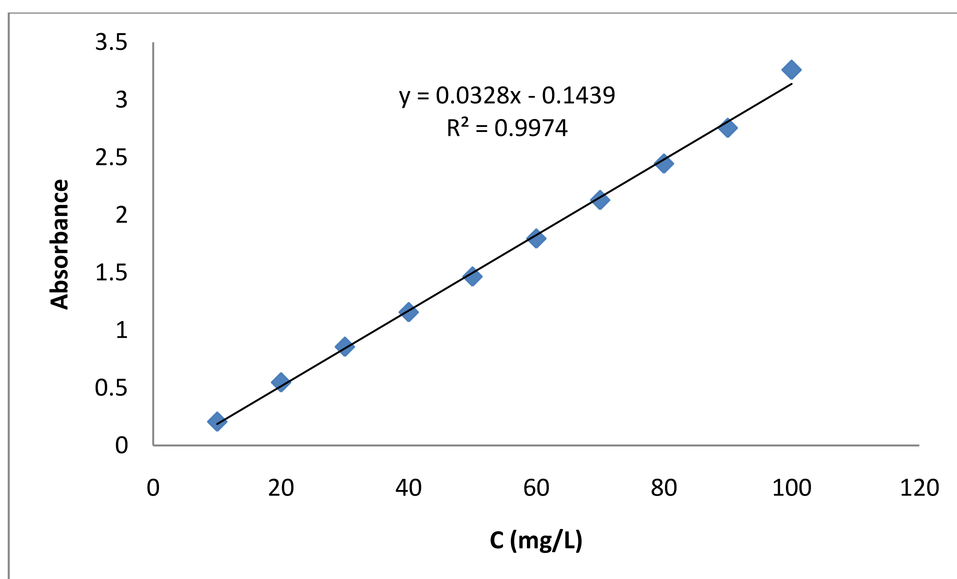

Fig. 3.3 Calibration curve of MFA in chloroform **at** λ_{max}: 285 nm

UV-vis spectra of MFA in ethanol was shown in Fig. 3.4. Three maximum absorbances were obtained at 348 nm, 280 nm and 230 nm.

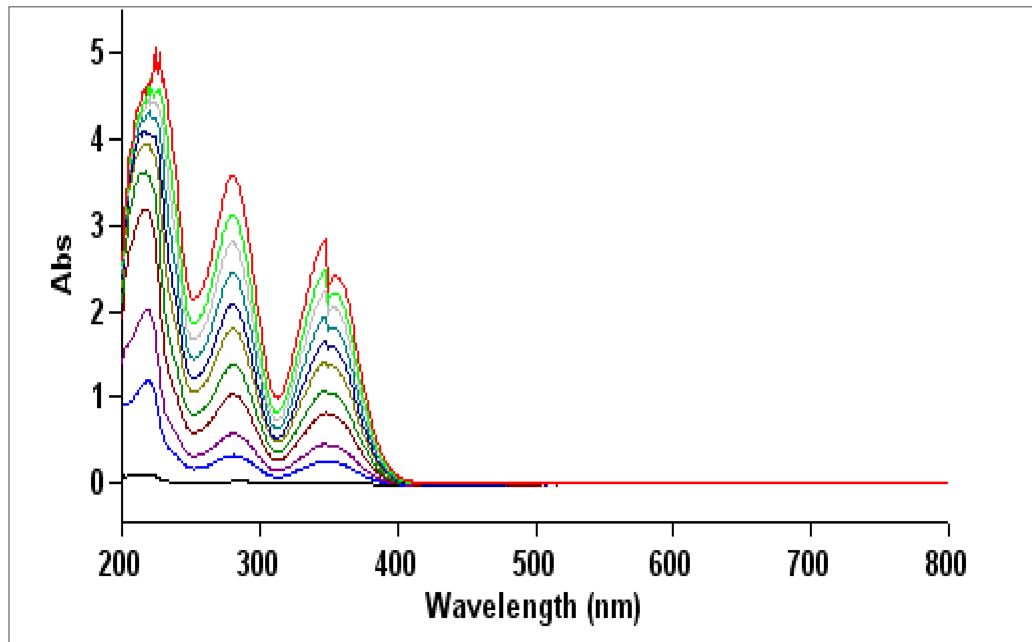

Fig 3.4 The absorbance spectra of MFA in ethanol.

At λ_{max}: 348 nm the dependence of absorbance MFA concentration gave a linear calibration curve Fig. 3.5. The experimental data reported in Figure 3.5 was fitted by a straight line with coefficient (R^2=0.9988). The calibration curve was found as linear in the range of 1-100 mg/L.

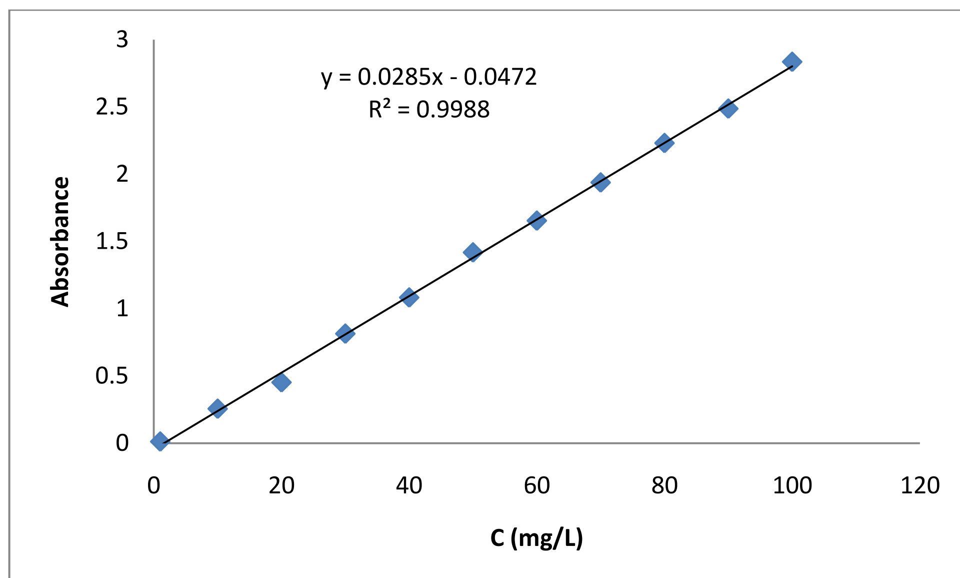

Fig. 3.5 Calibration curve of MFA in ethanol at λ_{max}: 348 nm.

In 0.1M NaOH solution three maximum absorbances were obtained at 333 nm, 285 nm and 225 nm. The spectral change depending on MFA concentration and calibration curve at λ_{max}: 285 nm were shown in Fig. 3.6 and 3.7.

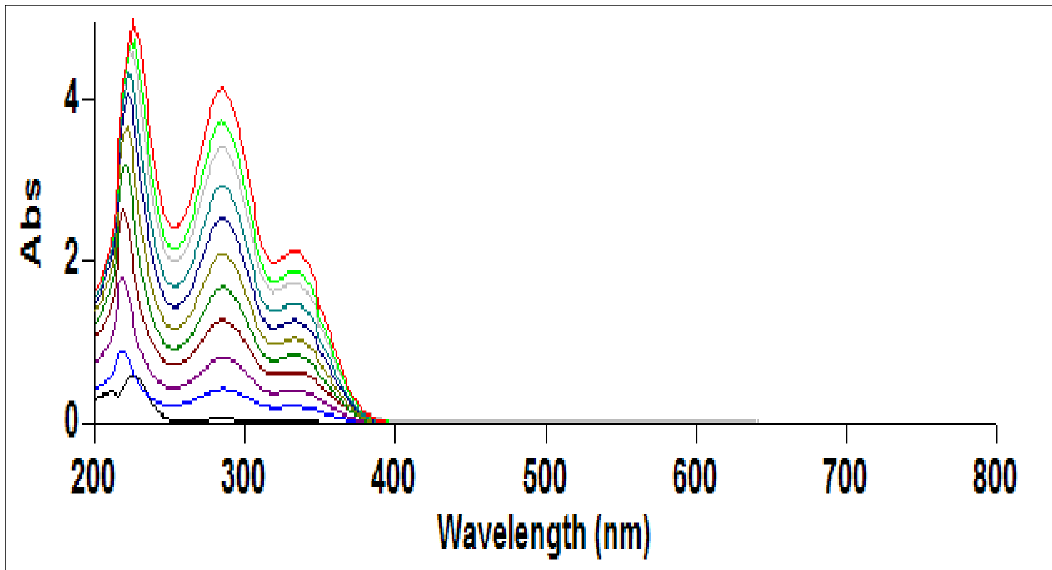

Fig. 3.6 The absorbance spectra of MFA in 0.1M NaOH.

The experimental data reported in Figure 3.7 was fitted by a straight line with coefficient (R^2=0.9994). The calibration curve was found as linear in the range of 1-100 mg/L.

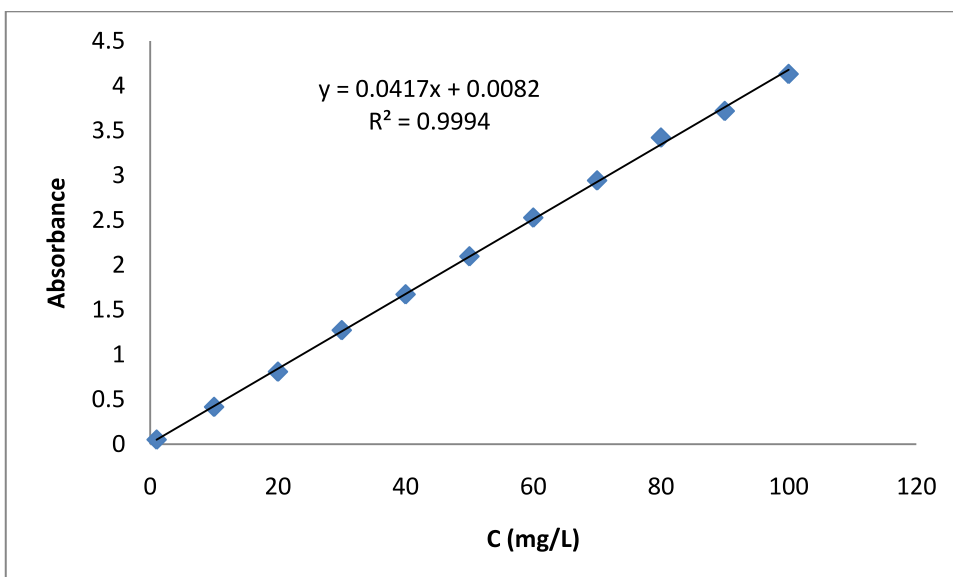

Fig. 3.7 Calibration curve of MFA in 0.1M NaOH at λ_{max}: 285 nm

The linearity of all three calibration curves was not changed even at absorbances of 3-4 at the concentration range of 0-100 mg/L MFA. The maximum absorbances and molar absorbance coefficiencies for different solvents were given in Table 3.1.

Table 3.1 Maximum absorbances and molar absorbance coefficiencies for different solvents.

	λ_{max}	ε $L.cm^{-1}.mol^{-1}$	λ_{max}	ε $L.cm^{-1}.mol^{-1}$	λ_{max}	ε $L.cm^{-1}.mol^{-1}$
Chloroform	365 nm	6563	285 nm	6573	250 nm	7480
Ethanol	348 nm	6877	280 nm	8612	230 nm	12065
0.1M NaOH	333 nm	5118	285 nm	10062	225 nm	11823

3.2 Spectrofluorometric Behavior of MFA

Fluorescence spectra of MFA at excitation 314 nm and fluorescence intensities 460 nm was read in chloroform. It was confirmed by the spectra given in Fig. 3.8. The stokes shift for MFA was found 95 nm from 365 to 460 nm.

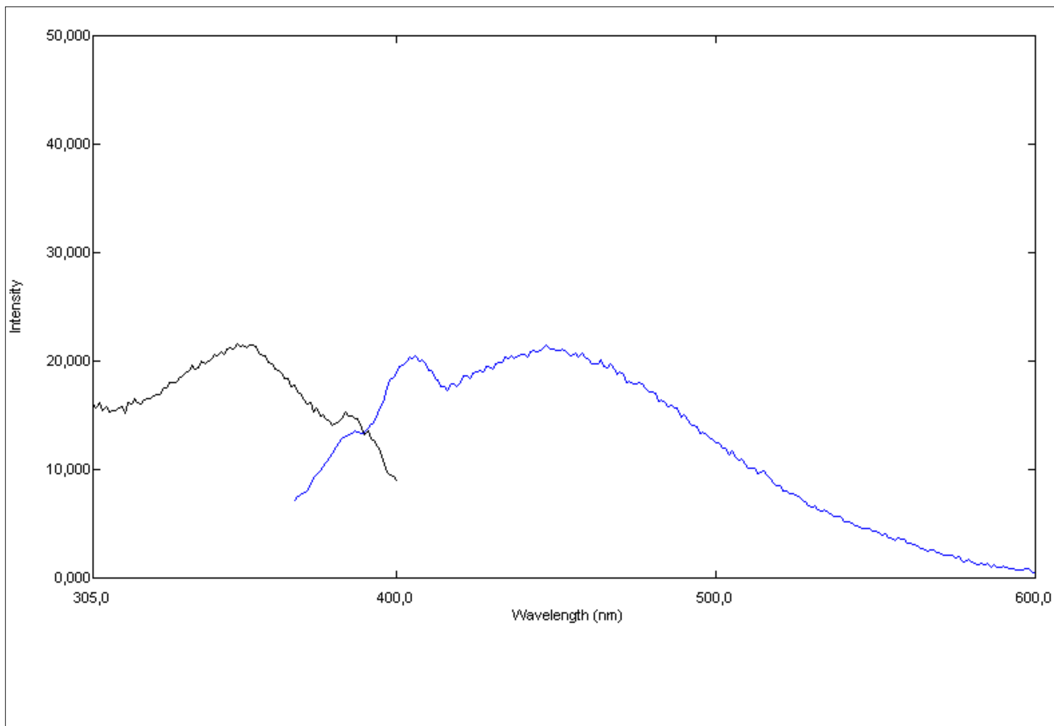

Fig. 3.8 The emission spectrum for exiticion at 314 nm and excitation spectrum for emission in 460 nm.

Two different concentration ranges were used which were 0-20 mg/L and 20-50 mg/L. The fluorescence spectra of 0-20 mg/L and 20-50 mg/L ranges were given in Fig. 3.9 and 3.10.

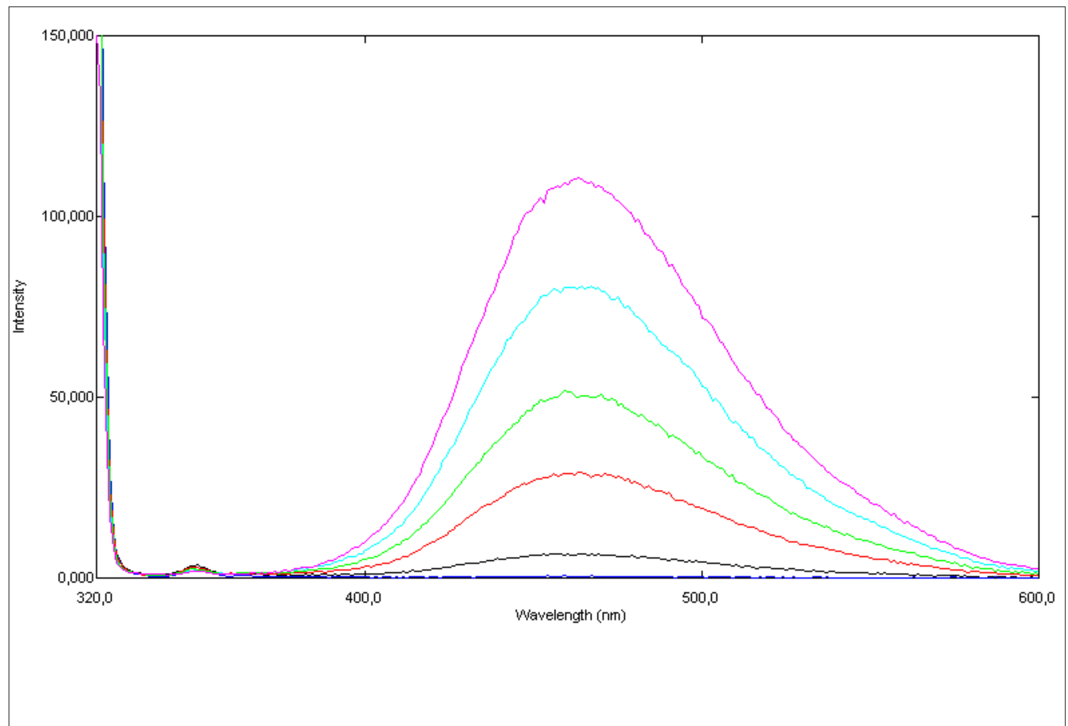

Fig. 3.9 Fluorescence spectra of MFA concentration range of 0-20 mg/L.

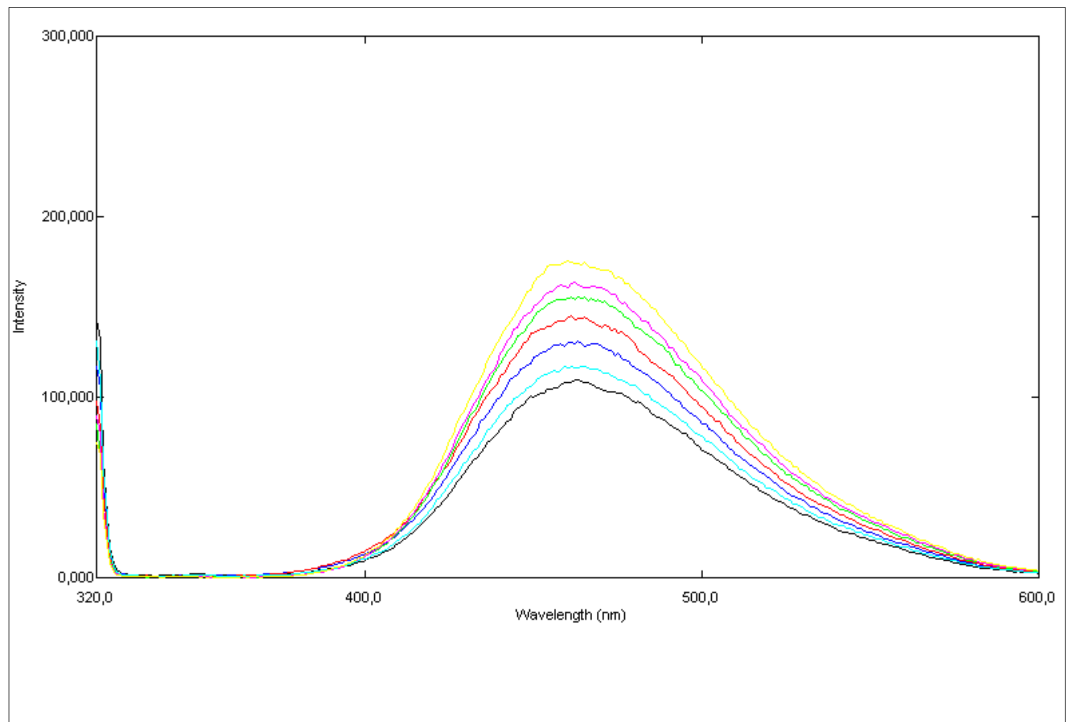

Fig. 3.10 Fluorescence spectra of MFA concentration range of 20-50 mg/L.

The experimental data reported in Figure 3.11 was fitted by two straight lines with coefficients (R^2=0.9987 and R^2=0.9944). The calibration curves were found as linear in the range of 0-20 mg/L and 20-50 mg/L.

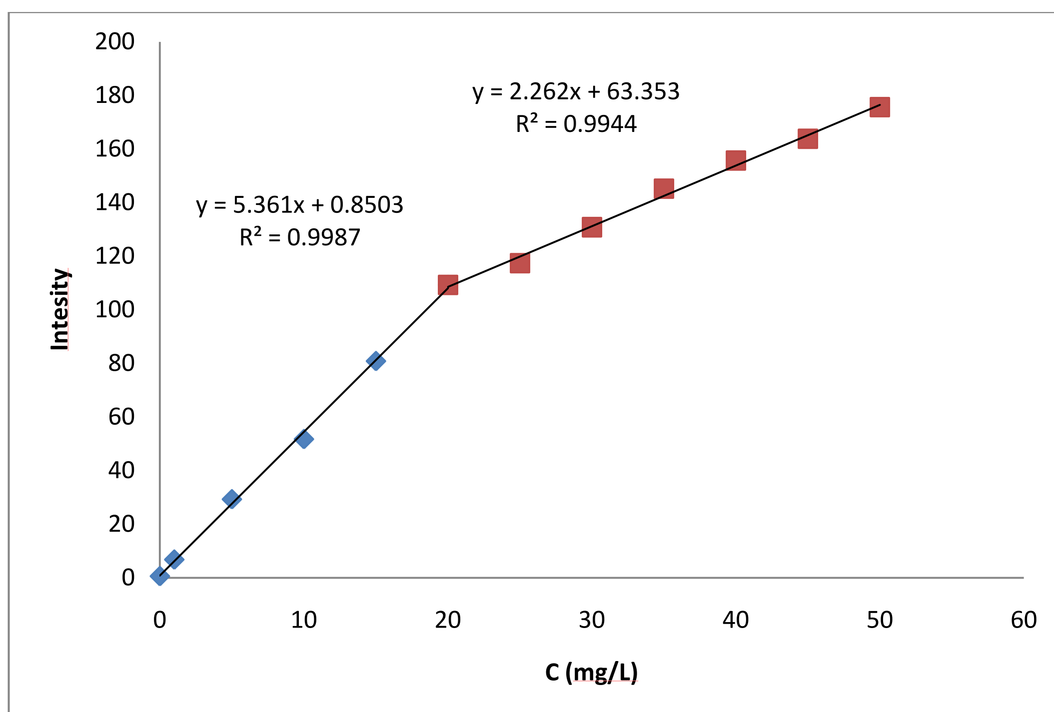

Fig. 3.11 Spectrofluorimetric calibration curve of MFA in chloroform

MFA has not shown any significant fluorescence intensities in other solvents like ethanol and water.

3.3 Extraction of MIP

Extraction of MFA from MIP carried out by two different methods which were soxhlet and batch extractions.

3.3.1 Soxhlet extraction

For the extraction of MFA from MIP six experiments were carried out. We tried two different extraction times which were 12 and 24 hours. The extraction time of 24 hours was found more suitable. Calculation of recovery yield was carried out by using MFA amount in synthesized MIPs, weight of the synthesized polymer, amount of polymer used in extraction and amount of MFA recovered. Average recovery for 24 hours extraction was found to be 92.2% with a standard deviation of ±0.889% (n=4). Results were given in Table 3.2.

Table 3.2 Recovery of MFA from MIP with soxhlet extraction.

Experiment number	Extraction time (hours)	Eluent volume (ml) $CHCl_3$	Amount of polymer (g)	Total amount of MFA in weighted polymer (mg)	Amount of MFA extracted (mg)	Recovery %
1	12	340	0.5000	25.0	22.3	89.3
2	12	320	0.5003	25.0	21.9	87.4
3	24	296	0.5003	23.0	23.3	93.3
4	24	306	0.5002	25.0	23.1	92.4
5	24	323	0.8823	34.8	31.9	91.5
6	24	322	0.6002	29.6	27.1	91.4

3.3.2 Batch extraction

We tried batch extraction because of the well known toxic properties of chloroform environment friendly 0.1M NaOH solution was examined for the batch extraction of MFA due to its good solubility in this media. Two different amounts of polymer which were 0.5 and 1.188 g were used for this experiment. Both of the polymers were extracted two times for 24 hours with 0.1M NaOH. Results were given in Table 3.3 and 3.4. For 0.5 g polymer the recovery found to be ~94% and for a larger amount of (1.188 g) polymer even the eluent volume proportionally increased, recovery found ~89%.

Table 3.3 Recovery of small amount of MFA from MIP with batch extraction.

Extraction number	Extraction time (hours)	Eluent volume (ml)	Amount of polymer (g)	Amount of MFA recovered (mg)	Recovery %
1	24	100	0.5005	20.67	83.75
2	24	100	0.5005	2.201	10.19
Total					93.94

Table 3.4 Recovery of large amount of MFA from MIP with batch extraction.

Extraction number	Extraction time (hours)	Eluent volume (ml)	Amount of polymer (g)	Amount of MFA recovered (mg)	Recovery %
1	24	250	1.1880	44.71	76.32
2	24	250	1.1880	7.34	12.53
Total					88.85

Soxhlet extraction was found much faster and less tiring then batch extraction but the use of toxic solvents and more expensive material the usage of batch extraction is more advisable.

3.4 Rebinding of MFA in Chloroform

For rebinding experiments two different extracted polymers were used. These were soxhlet extracted with chloroform and batch extracted with 0.1M NaOH.

3.4.1 Rebinding with soxhlet extracted polymers with chloroform

For observing rebinding of MFA, the MIPs which were extracted with chloroform were used and stirred in different concentrations of MFA solutions in chloroform for 0.5, 1, 2, 3 and 4 hours. The solutions were filtrated with filtration paper and after dilutions results of the absorbance measurements were given in Table 3.5. The bound MFA yields were calculated from extraction yields and maximum value was obtained as 41% for these experiments.

Table 3.5 Equilibrium binding time of MFA in chloroform from polymer extracted with chloroform.

Experiment No	Experimental Data		Stirring Time (hours)				
			0.5	1	2	3	4
1	0.01 g polymer 4 ml 100 mg/L MFA	Unbound MFA in solution mg/L	74.63	68.75	69.1	68.75	69.7
		Bound MFA mg on to 1g MIP	10.15	12.50	12.35	12.50	12.13
		Bound MFA yield %	20.30	25.00	24.70	25.00	24.26
2	0.02 g polymer 1 ml 1000 mg/L MFA	Unbound MFA in solution mg/L	713.6	622	597	737.5	596.3
		Bound MFA mg on to 1g MIP	14.32	18.90	20.15	13.13	20.18
		Bound MFA yield %	28.64	37.79	40.29	26.25	40.36
3	0.02 g polymer 2.5 ml 400 mg/L MFA	Unbound MFA in solution mg/L	347.8	346.8	346.3	340.7	363.8
		Bound MFA mg on to 1g MIP	6.53	6.65	6.71	7.41	4.52
		Bound MFA yield %	13.05	13.31	13.42	14.82	9.04
4	0.175 g polymer 10 ml 900 mg/L MFA	Unbound MFA in solution mg/L	843.4	806.6	836.8	834.6	812.5
		Bound MFA mg on to 1g MIP	3.24	5.34	3.61	3.74	5.00
		Bound MFA yield %	6.47	10.68	7.23	7.48	10.0

It was determined that in first experiment (Fig. 3.12) rebinding was nearly completed in 30 minutes. But the rebinding yields were too low (reaches maximum at 1 hour and it was 25%).

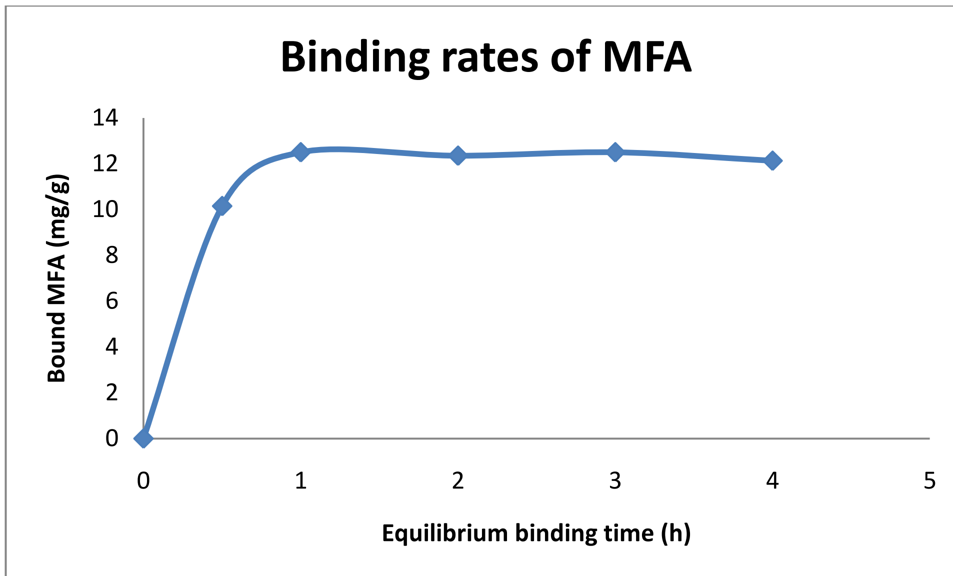

Fig. 3.12 Binding rates of MFA in chloroform on MFA imprinted polymer extracted with chloroform (Experiment 1).

In the second experiment (Fig. 3.13) rebinding was not found as expected. The rebinding continued up to 1.5 hour but after that it released some of its bound MFA between 1.5-3 hours after that rebinding continued. The maximum rebinding yield was found 41.14% at the time of 1.5 hours.

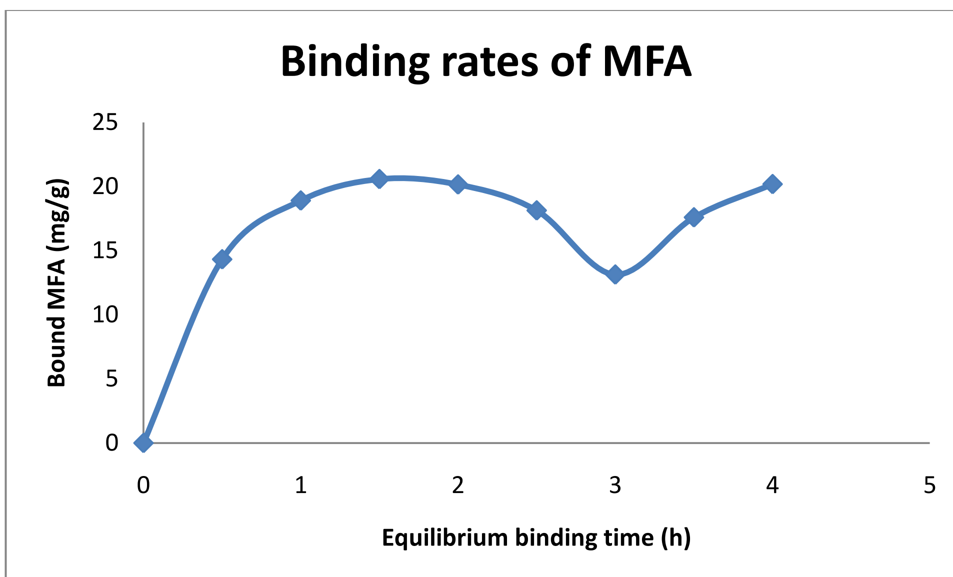

Fig. 3.13 Binding rates of MFA in chloroform on MFA imprinted polymer extracted with chloroform (Experiment 2).

At the third experiment (Fig. 3.14) rebinding was nearly completed in 30 minutes but the rebinding yields were too low as in experiment one (reaches maximum at 3 hours and it was 14.82%).

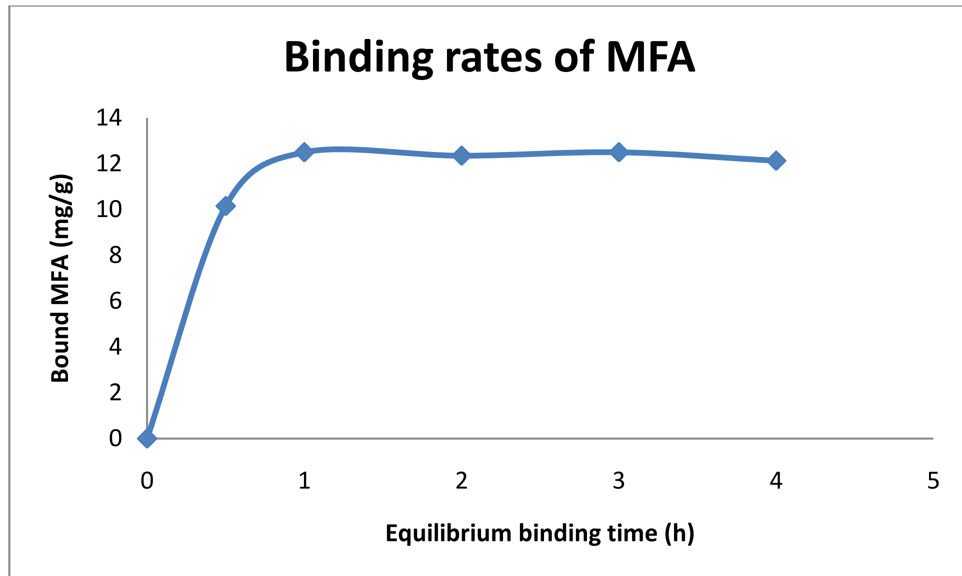

Fig.3.14 Binding rates of MFA in chloroform on MFA imprinted polymer extracted with chloroform (Experiment 3).

Fourth experiment (Fig. 3.15) showed that the binding and releasing continues. It reached its maximum at 1 hour but instantly released some of its bound MFA. The maximum rebinding yield was 10.68% and it reached that in 1 hours.

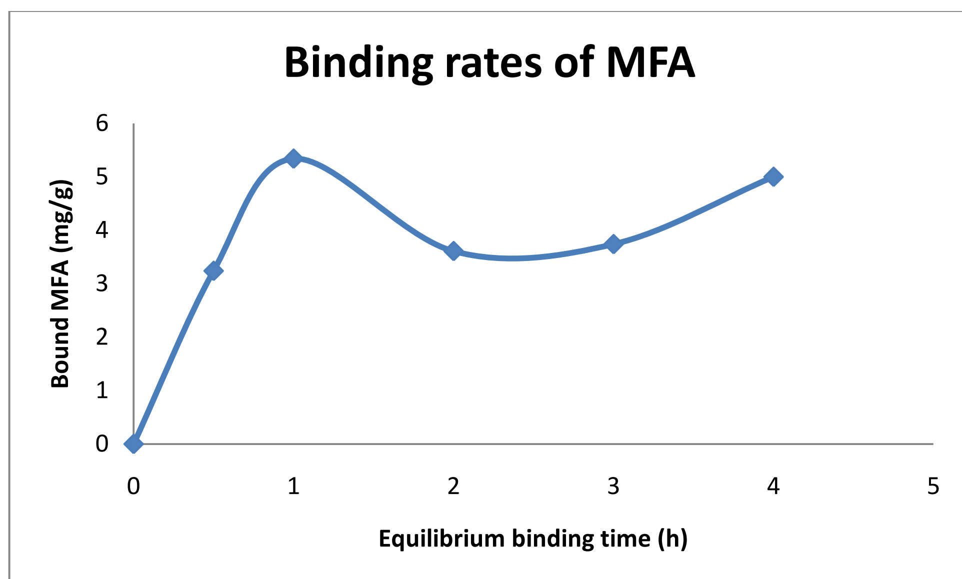

Fig. 3.15 Binding rates of MFA in chloroform on MFA imprinted polymer extracted with chloroform (Experiment 4).

3.4.2 Rebinding with batch extracted polymers with 0.1M NaOH

3 ml 500 mg/L MFA solution in chloroform and 0.02 g MIP were stirred and stirring times for 0.5, 1, 2 and 3 hours were used. After the stirring time the solutions were filtrated with filtration paper. After suitable dilutions results of absorbance measurements were obtained and given in Table 3.6. The bound MFA yield was calculated from extraction yield by calculating amount of MFA (mg) can bound to MIP and it was 93% for this experiment.

Table 3.6 Equilibrium binding time of MFA in chloroform from polymer extracted with 0.1M NaOH.

Stirring time (h)	Bound MFA (mg) onto 1 g MIP	Bound MFA yield %	Bound MFA (mg) onto 1 g NIP
0	0	-	0
0.5	39.15	78.3	0.10
1	41.55	83.1	6.37
2	43.35	86.7	4.06
3	45.2	90.4	-

Time dependence of bound MFA mg to 1 g MIP was given in Fig. 3.16. It was determined that rebinding was nearly completed in 30 minutes.

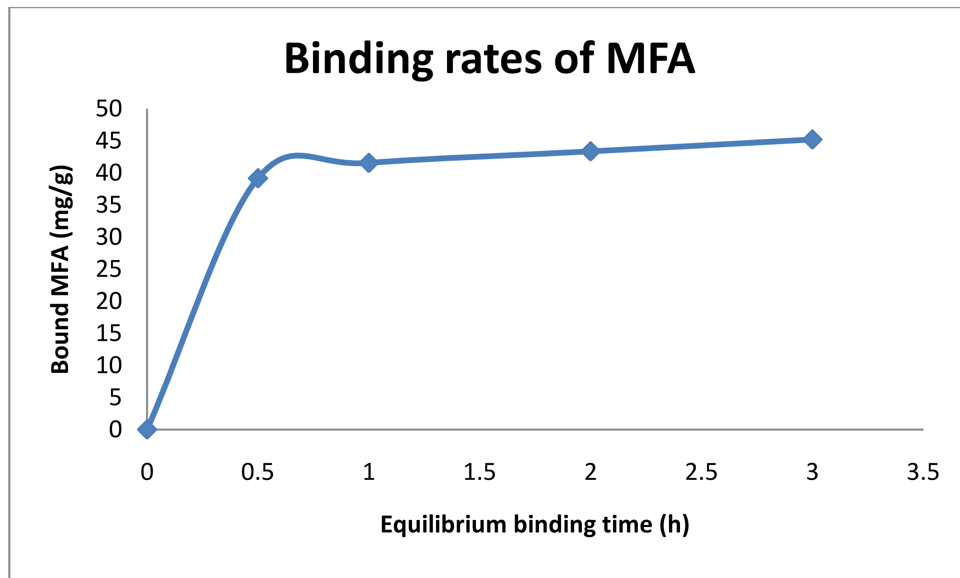

Fig. 3.16 Binding rates of MFA in chloroform on the MFA imprinted polymer extracted with 0.1M NaOH.

Similar studies were repeated for NIP and results were given in Fig. 3.17. After 30 minutes there was not any significant binding of MFA but at 1 hour it reached its maximum but it was 7-fold lower than MIPs. After 1 hour NIP start to release MFA to the solution.

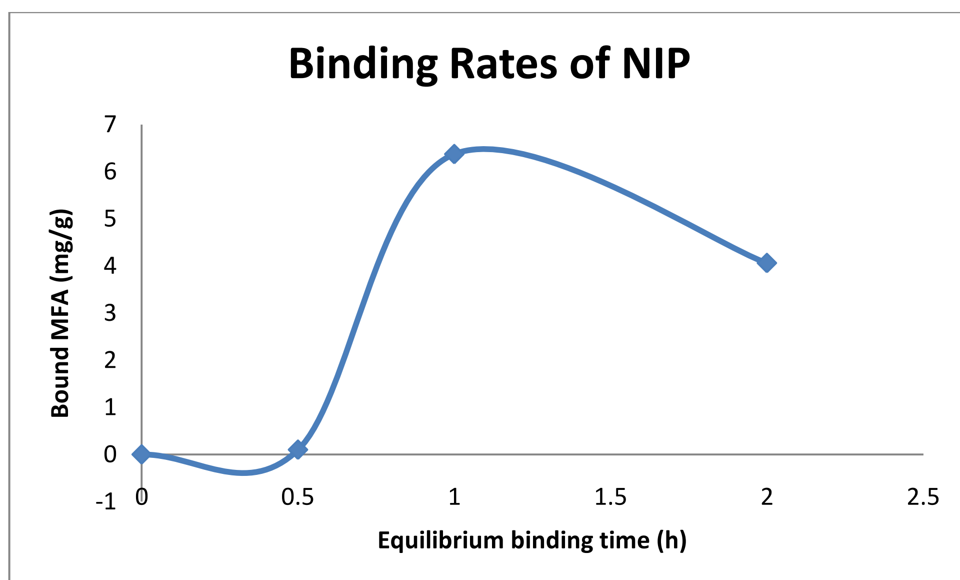

Fig. 3.17 Binding rates of MFA on non-imprinted polymer.

3.5 Rebinding of MFA in 0.1M NaOH

For observing rebinding of MFA the MIPs which were extracted with NaOH were used and stirred in 100 mg/L MFA solutions in 0.1M NaOH for 0.5, 1, 2, 3, and 4 hours. The solutions were filtrated with filtration paper and after dilutions results of the absorbance measurements were obtained and given in Table 3.6. The bound MFA yield was calculated from extraction yield and its maximum value was 12% for this experiment.

Table 3.7 Equilibrium binding time of MFA in 0.1M NaOH from polymer extracted with 0.1M NaOH.

Polymer amount (g)	Solution volume (ml)	MFA concentration (mg/L)	Stirring time (h)	Bound MFA mg on to 1g MIP	B ound MFA yield %
0.02	10	100	0.5	5.34	10.68
			1	5.64	11.28
			2	6.02	12.04
			3	5.83	11.66
			4	5.23	10.46

Time dependence of bound MFA mg to 1 g MIP was given in Fig. 3.18. It was determined that rebinding was near completion in 30 minutes with a small rebinding yield of 12%.

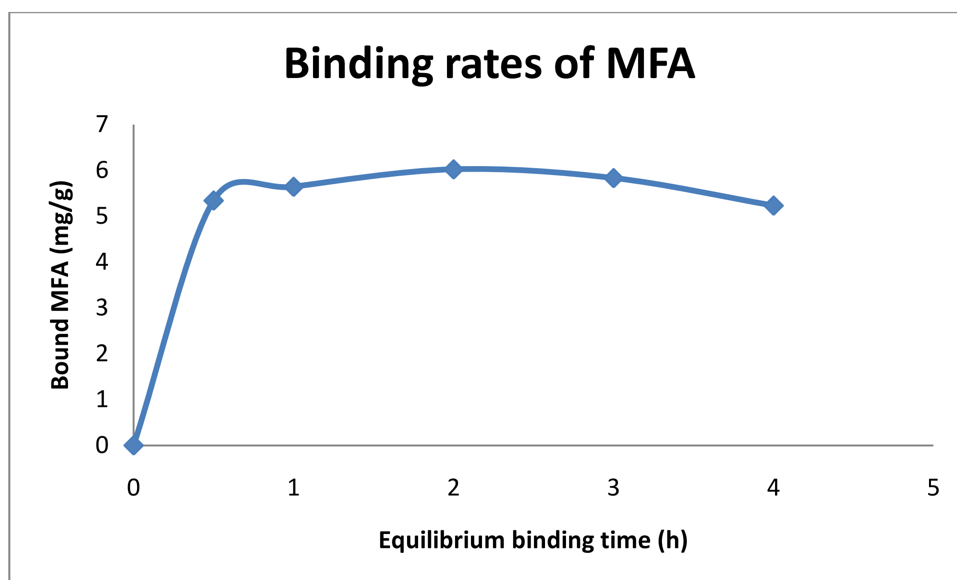

Fig. 3.18 Binding rates of MFA in 0.1M NaOH on MFA imprinted polymer extracted with 0.1M NaOH.

Rebinding of MFA on to MIPs extracted with chloroform in chloroform and MIPs extracted with 0.1M NaOH in NaOH gave too low rebinding yields.

3.6 Binding capacity

2 ml of 500 mg/L MFA solution containing 1 mg MFA was added to 0.02 g polymers. The amount of MFA absorbed per unit mass of imprinted polymer and non-imprinted polymer was calculated. The maximum MFA binding capacities of MIP and NIP were shown in Table 3.8.

Table 3.8 Binding capacity of MIPs.

	MIP	NIP
Maximum bound mg MFA/1 g polymer	45.2	6.37

3.7 MIP-based Optical Sensor

From the results of performance analysis, due to its maximum rebinding results MFA imprinted polymers extracted with 0.1M NaOH were used for the preparation of heterogen optical sensors. For comparison non-imprinted polymer based heterogen optical sensors were prepared in the same way.

3.7.1 Time for stable signal

The prepared sensors were dipped in 0.5 mg/L MFA solutions and at every five minutes the solutions were removed and fluorescence intensities were measured. The fluorescence spectra were given in Fig.3.19 for MIP and Fig. 3.20 for NIP.

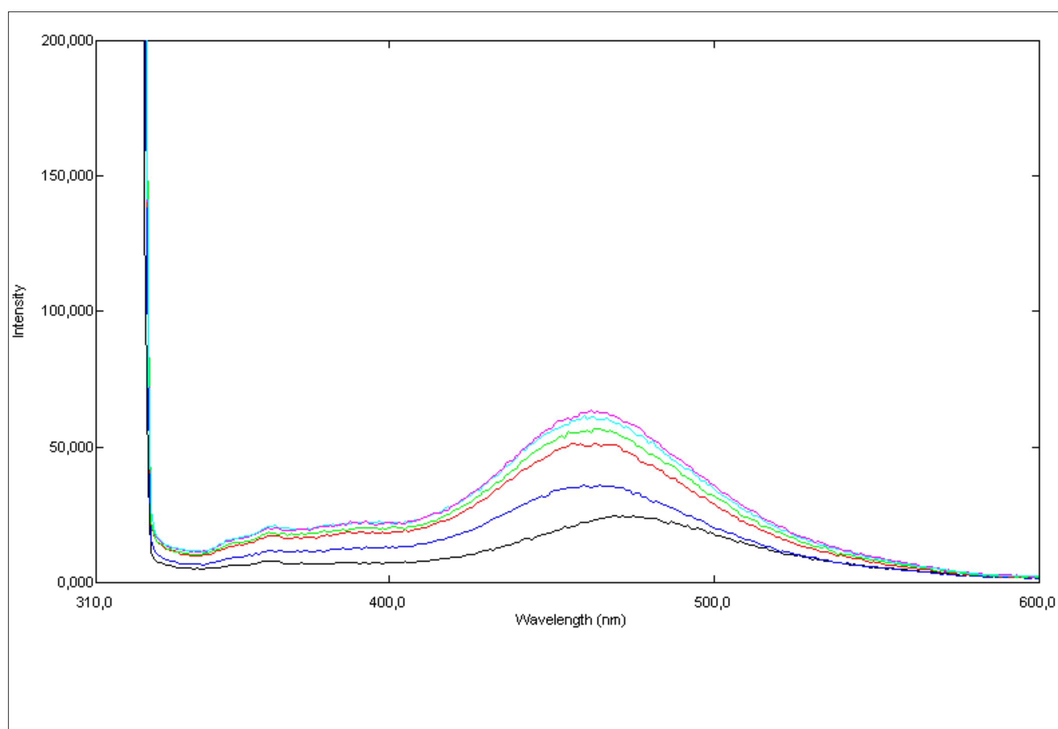

Fig. 3.19 Spectrofluorimetric measurements of MIP optical sensor vs. time black: 5 min. blue: 10 min. red: 15 min. green: 20 min. light blue: 25 min. and pink: 30 min. at 0.5 mg/L mefenamic acid solution in chloroform.

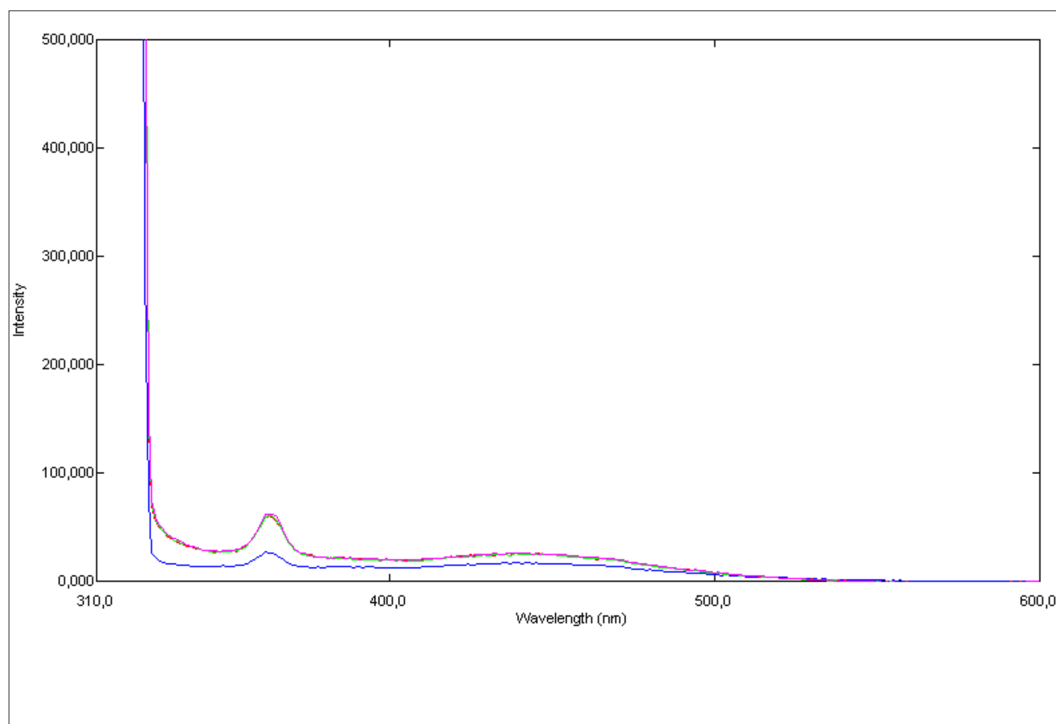

Fig. 3.20 Spectrofluorimetric measurements of NIP optical sensor vs. time blue: 5 min. red: 10 min. green:15 min. and pink: 20 min. at 1 mg/L mefenamic acid solution in chloroform.

The signal of MIP-based optical sensor was stable after 30 min. and it was viable with the rebinding data. But for the same experiments with NIP it has not shown any significant fluorescence at 460 nm. The sensors we have used became more fragile and lost its flexibility during the measurements. The plasticizer we have used was slightly soluble in chloroform and because of the leakage of the plasticizer the sensors structure was changed.

3.7.2 Calibration of MIP and NIP optical sensors

Because of the solubility problem of the plasticizer we changed our solvent and used ethanol but the plasticizer has some solubility as well and the MFA has not shown any significant fluorescence in ethanol (Fig. 3.21) so we tried a mixture of chloroform, ethanol and water for less solubility with high fluorescence intensity.

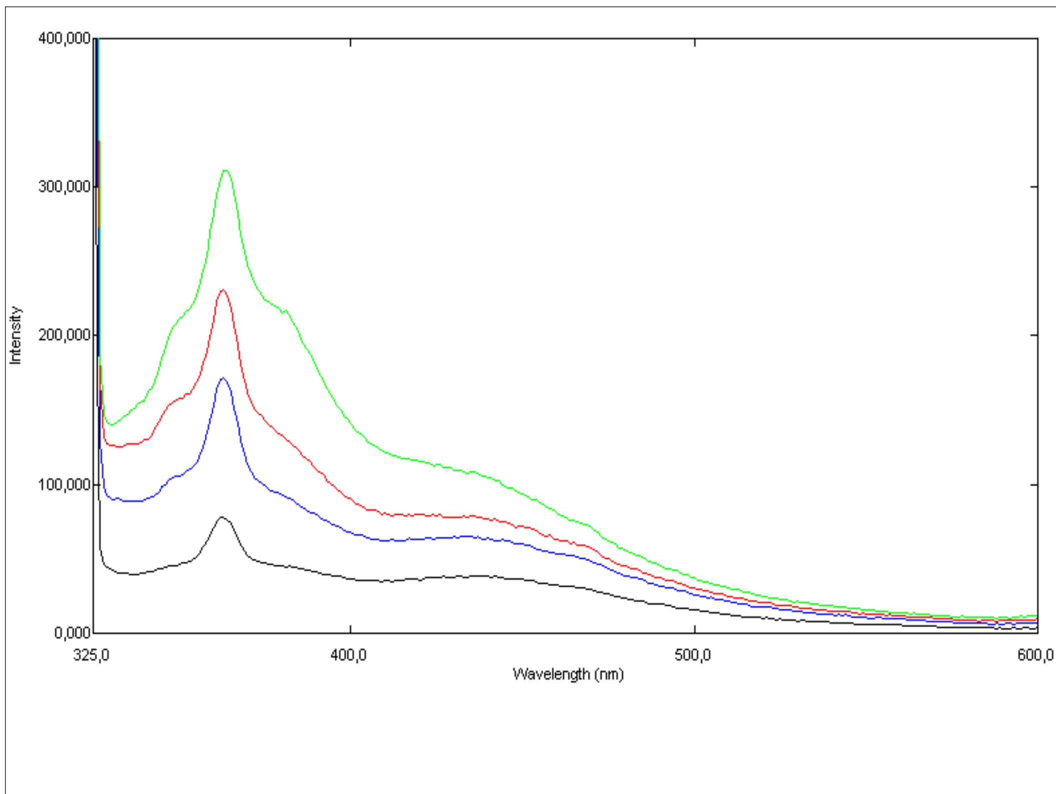

Fig. 3.21 Spectrofluorimetric measurements of NIP optical sensor for different concentrations of MFA in ethanol.

The dilution solution was prepared by mixing 40 ml of ethanol and 60 ml of water and using stock MFA solutions in chloroform.

The measurements for the MIP optical sensor was given below (Fig. 3.22).

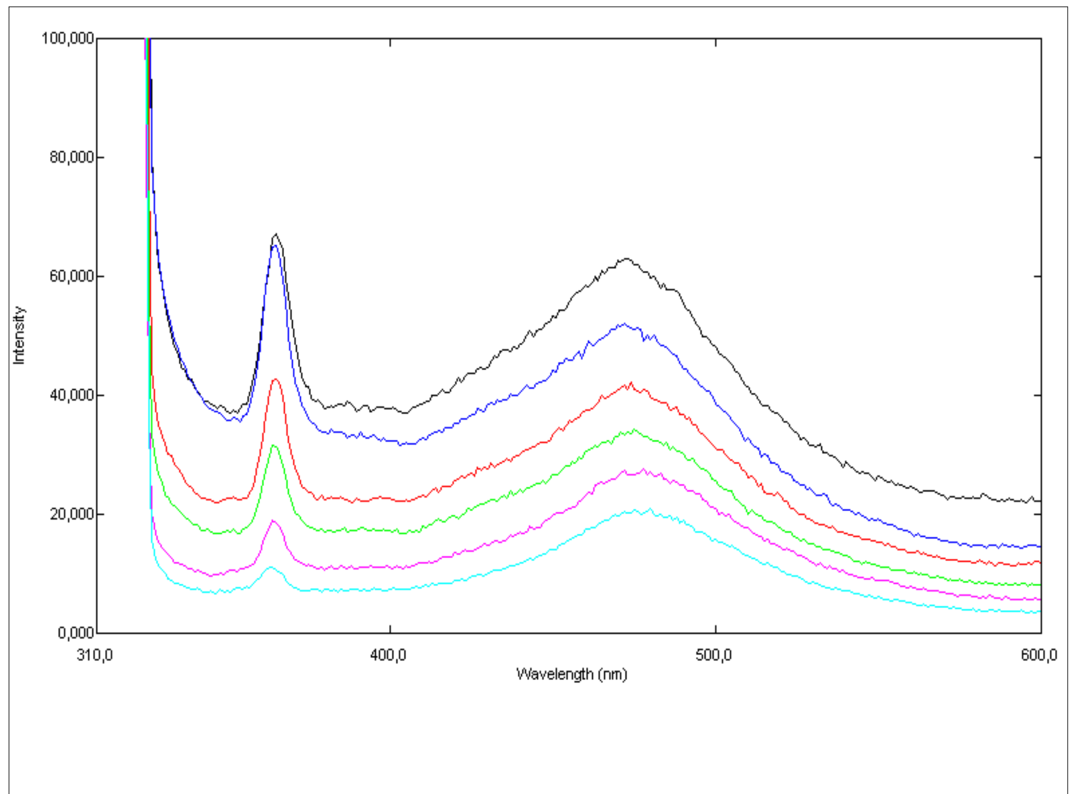

Fig. 3.22 Spectrofluorimetric measurements of MIP optical sensor vs. MFA concentration black: blank solution blue: 0.1 mg/L red: 0.5 mg/L green: 1 mg/L pink: 2 mg/L light blue: 3 mg/L.

The calibration graph of measurements in Fig. 3.22 were given in Fig. 3.23.

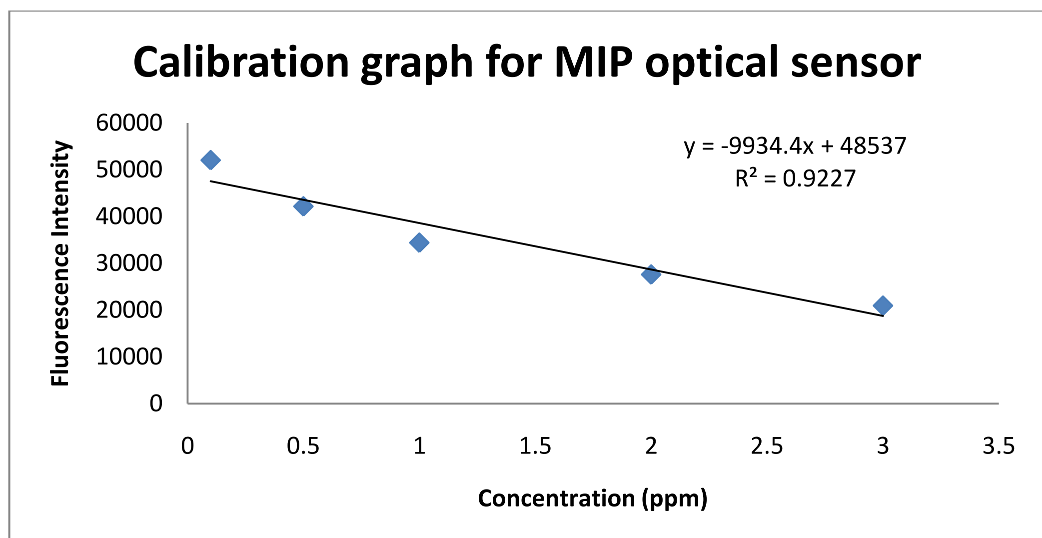

Fig. 3.23 Calibration graph of MIP optical sensor 0.1 to 3 mg/L.

In Fig. 3.24 two different stock MFA solutions (100 mg/L and 200 mg/L) were used for keeping the chloroform level low. For 1, 1.5 and 2 mg/L 100 mg/L and for the others 200 mg/L stock solutions were used.

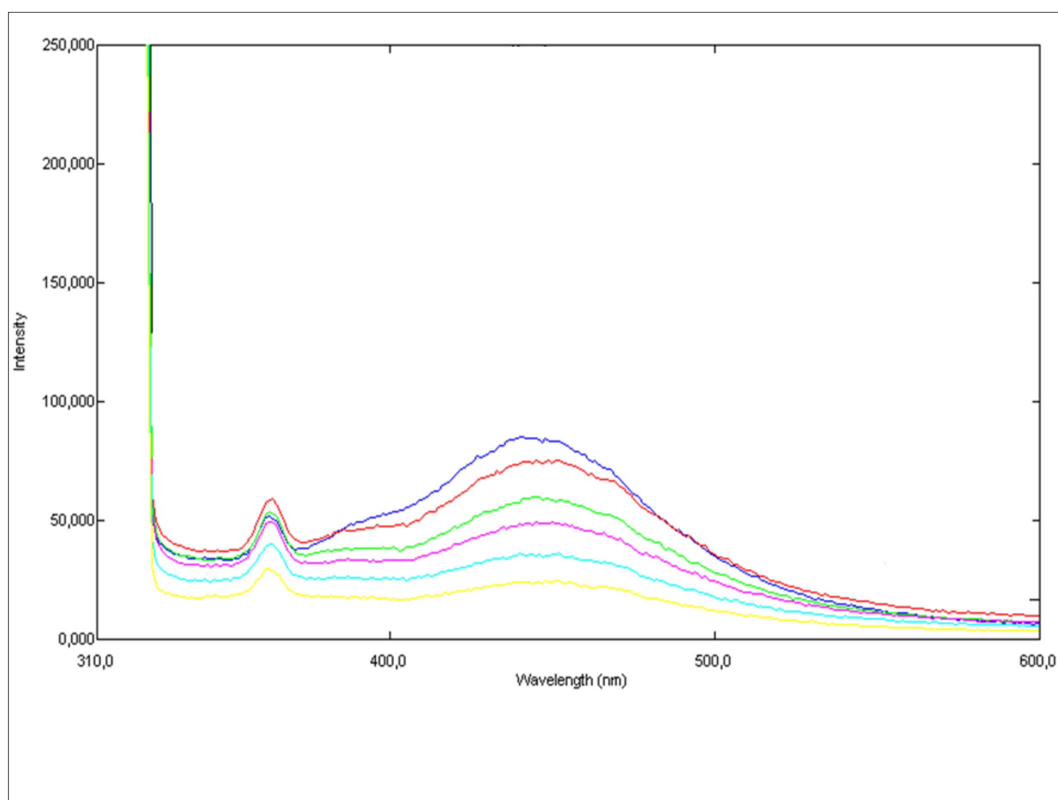

Fig. 3.24 Spectrofluorimetric measurements of NIP optical sensor vs. MFA concentration blue: 1 mg/L red: 1.5 mg/L green: 2 mg/L pink: 2.5 mg/L light blue: 3 mg/L and yellow 3.5 mg/L.

The calibration graphs of measurements in Fig. 3.24 were given in Fig. 3.25. There was a cross relativity of concentration and fluorescence intensity and it was one of the things we could not describe.

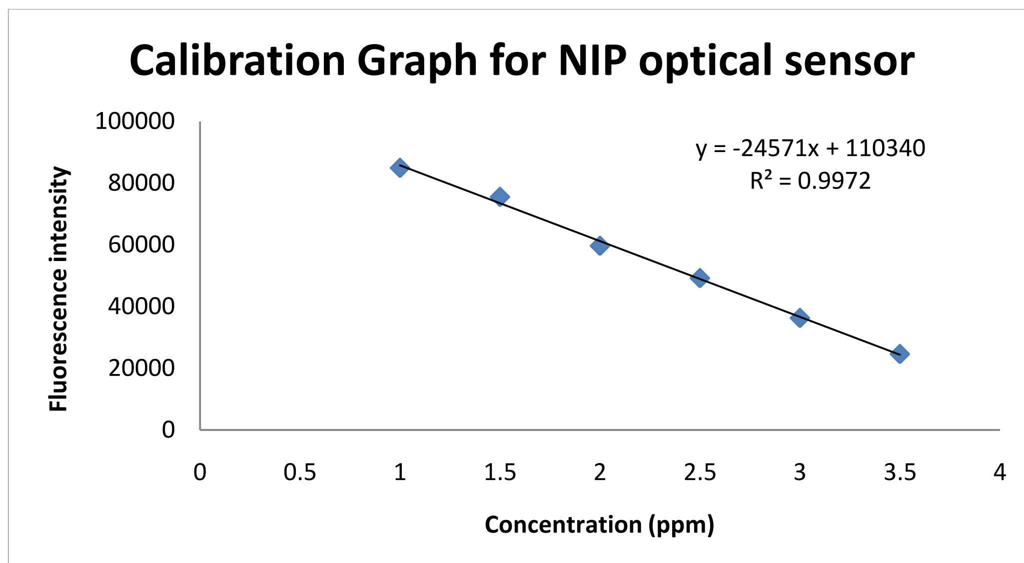

Fig. 3.25 Calibration graph for NIP optical sensor 1 to 3.5 mg/L.

From the limitations such as mefenamic acid not showing any significant fluorescence other than chloroform, sensors durability in chloroform is very low and the calibration graphs of both MIP and NIP optical sensors had a cross relativity for concentration and fluorescence intensity. We decided using MIP for other applications such as SPE.

3.8 Studies of MI-SPE

The usage of MFA imprinted polymers as column packaging material was investigated. For this purpose 0.2 g of MIP was packed in a 5 ml syringe and 3.5 ml of 500 mg/L MFA solution in chloroform was passed through the column. The flow rate of the column was 1 ml per hour. Using the calibration curve depending on absorbance the measurement of eluent was carried out and the binding yield of 98.37% was acquired.

4 CONCLUSION

In this study we successfully prepared artificial receptors, MFA imprinted polymers and its performance studies were investigated.

We synthesized the polymer and extracted it up to 93.25% with chloroform at soxhlet extractions and 93.94% with 0.1M NaOH at batch extractions. But polymers that were extracted with chloroform were not able to rebind MFA properly.

To investigate whether the imprinted polymer was working we carried out rebinding experiments and the MIP showed a 3 hours time for 90% of cavities to be filled. But the NIP showed that there was not a specific binding towards MFA it binds small amount of MFA and then releases it to the solution again. If we look at the maximum binding capacities of MIP and NIP it was 45.2 mg/g for MIP and 6.37 mg/g for NIP. The MIP binds more than seven folds of the NIP and MIP binds MFA stronger then NIP and not releases MFA.

For optical sensor preparation we prepared a membrane with MIPs, PVC and DOP and used the membrane with the solutions of MFA in chloroform. But the solubility of DOP in chloroform caused a deformation at membranes and we had to change the medium but the MFA has a good fluorescence in chloroform and with the other solvents the fluorescence intensities were not good enough to measure. So we tried a mixture of water, ethanol and chloroform. Here water was used for stopping deformation and chloroform to give us a good fluorescence intensity and ethanol to mix these solvents. But even with these the results showed that with the increasing MFA amounts the fluorescence intensities decrease there was a cross relativity. For the NIP the results showed that the binding was linear but for MIP it was not good as NIP so with all these drawbacks optical sensor preparation was not possible.

For separation and pre-concentration the MI-SPE studies were carried out and with the flow velocity of 1ml per hour the binding yield was 98.37%.

5 REFERENCES

Andrea, P., Mirsolav, S., Silvia, S. and Stanislav, M., 2001, A Solid Binding Matrix/Molecularly Imprinted Polymer-Based Sensor System for the Determination of Clenbuterol in Bovine Liver Using Differential-Pulse Voltammetry, *Sensors and Actuators B*, 76: 286-294 pp.

Arco. M., Fernández, A., Martin, C. and Rives, V., 2006, Intercalation of Mefenamic and Meclofenamic Acid Anions in Hydrotalcite-like Matrixes, *Applied Clay Science*, 36: 133-140 pp.

Capitán-Vallvey, L.F., Navas, N., Olmo, N. and Consonni, V., Todeschini, R., 2000, Resolution of Mixtures of Three Nonsteroidal Anti-inflammatory Drugs by Fluorescence Using Partial Least Squares Multivariate Calibration With Previous Wavelength Selection by Kohonen Artificial Neural Networks, *Talanta*, 52: 1069-1079 pp.

Garcia-Calzon, J.A. and Diaz-Garcia, M.E., 2006, Characterization of Binding Sites in Molecularly Imprinted Polymers, *Sensors and Actuators B*, 123: 1180-1194 pp.

Haupt, K., 2001, Molecularly Imprinted Polymers in Analytical Chemistry, *The Analyst*, 126: 747-756 pp.

Haupt, K. and Mosbach, K., 2000, Molecularly Imprinted Polymers and Their Use in Biomimetric Sensors, *Chemical Rewievs*, 100(7): 2495-2504 pp.

Holthoff, E.L. and Bright, F.V., 2007, Molecularly Templated Materials in Chemical Sensing, *Analytica Chimica Acta*, 594: 147-161 pp.

Komiyama, M., Takeuchi, T., Mukawa, T. and Asanuma, H., 2003, Molecular Imprinting, *Wiley-VCH Verlag GmbH & Co. KGaA*, Germany, 147p.

Kriz, D., Kempe, M. and Mosbach, K., 1996, Introduction of Molecularly Imprinted Polymers as Recognition Elements in Conductometric Chemical Sensors, *Sensors and Actuators B: Chemical,* 33: 178-181 pp.

REFERENCES(Continue)

Kugimaya, A. and Takeuchi, T., 2001, Surface Plasmon Resonance Sensor Using Molecularly Imprinted Polymers for Detection of Sialic Acid, *Biosensors and Bioelectronics,* 16: 1059-1062 pp.

Merkoçi, A. and Alegret, S., 2002, New Materials for Electrochemical Sensing IV. Molecular Imprinted Polymers, *Trends in Analytical Chemistry*, 21(11): 717-125 pp.

Özcan, L. and Şahin, Y., 2007, Determination of Paracetamol Based on Electropolymerized-Molecularly Imprinted Polypyrrole Modified Pencil Graphite Electrode, *Sensors and Actuators B*, 127: 362-369 pp.

Panasyuk-Delaney,T., Mirsky, V.M., Ulbricht, M. and Wolfbeis, O.S., 2001, Impedometric Herbicide Chemosensors Based on Molecularly Imprinted Polymers, *Analytica Chimica Acta*, 435: 157-162 pp.

Peng, H., Liang, C., Zhou, A., Zhang, Y., Xie, Q. and Yao, S., 2000, Development of a New Atropine Sulfate Bulk Acoustic Wave Sensor Based on a Molecularly Imprinted Electrosynthesized Copolymer of Aniline with o-phenylenediamine, *Analytica Chimica Acta*, 423: 221-228 pp.

Pichon, V. and Chapuis-Hugon, F., 2008, Role of Molecu;arly Imprinted Polymers for Selective Determination of Environment Pollutants, *Analytica Chimica Acta*, 622: 48-61 pp.

Piletsky, S.A., Alcock, S. and Turner, A.P.F., 2001, Molecularly Imprinting: At The Edge of The Third Millennium, *Trends in Biotechnology,* 19(1): 9-12 pp.

Ramström, O. and Yan, M., 2005, Molecularly Imprinted Materials, *Science and Technology*, 2 pp.

REFERENCES(Continue)

Qiao, F., Sun, H., Yan, H. and Kyung, H.R., 2006, Molecularly Imprinted Polymers for Solid Phase Extraction, *Chromatographia*, 64: 625-634 pp.

Saghatforoush, L., Hasanzadeh, M., Karim-Nezhad, G., Ershad, S., Shadjou, N., Khalilzadeh, B. and Hajjizadeh, M., 2009, Kinetic Study of the Electrooxidation of Mefenamic Acid and Indomethacin Catalysed on Cobalt Hydroxide Modified Glassy Carbon Electrode, *Bull. Korean Chem. Soc.*, 30(6): 1341-1348 pp.

Sellergren, B., 1999, Polymer- and Template-Related Factors Influencing The Efficiency in Molecularly Imprinted Solid-Phase Extractions, *Trends in Analytical Chemistry*, 18(3): 164-174 pp.

Soares da Silva, M., Vão, E., Temtem, M., Mafra, L., Caldeira, J., Aguiar-Ricardo, A. and Casimiro, T., 2009, Clean Synthesis of Molecular Recognition Polymeric Materials with Chiral Sensing Capability Using Supercritical Fluid Technology. Application as HPLC stationary phases, *Biosensors and Bioelectronics*, 25: 1742-1747 pp.

Scweitz, L., Spégel, P. and Nilsson, S., 2000, Molecularly Imprinted Microparticles for Capillary Electrochromatographic Enantiomer Seperation of propranolol, *Analyst*, 125: 1899-1901 pp.

Suarez-Rodriguez, J.L. and Diaz-Garcia, M.E., 2000, Flavonol Fluorescent Flow-Through Sensing Based on a Molecular Imprinted Polymer, *Analytica Chimica Acta*, 405: 67-76 pp.

Suarez-Rodriguez, J.L. and Diaz-Garcia, M.E., 2001, Fluorescent Competitive Flow-Through Assay for Chloramphenicol Using Molecularly Imprinted Polymers, *Biosensors and Bioelectronics,* 16: 955-961 pp.

Takeuchi, T. and Haginaka, J., 1999, Separation and Sensing Based on Molecular Recognition Using Molecularly Imprinted Polymers, *Journal of Chromatography B*, 728: 1-20 pp.

REFERENCES(Continue)

Wei, S. and Mizaikoff, B., 2007, Recent Advances on Noncovalent Molecular Imprints for Affinity Separations, *Wiley-VCH Verlag GmbH & Co. KGaA*, Weinheim 30: 1794-1805 pp.

Yemiş, F., 2010, Voltammetric Determination of Some Organic Compounds By Modified Electrodes With Molecularly Imprinted Polymers 110 pp

Zayats, M., Lahav, M., Kharitonov, A.B. and Willner, I., 2002, Imprinting of Specific Molecular Recognition Sites in Inorganic and Organic Thin Layer Membranes Associated with Ion-Selective Field-Effect Transistors, *Tetrahedron*, 58: 815-824 pp.

CURRICULUM VITAE

Name : Burak

Surname : BERAN

Date of Birth : 27.02.1986

Place of Birth : Hereke/Kocaeli

Nationality : Turkish

Adress : Ege University, Faculty of Science, Department of Chemistry, Division of Analytical Chemistry, 35100 Bornova/Izmir/Turkey

E-mail : burakberan@hotmail.com

EDUCATIONAL BACKGROUND

High School in School, 2008, Selçuk Anatolian High School, Izmir/Turkey

B.S in Chemistry, 2008, Ege University, Izmir/Turkey