SOLID PHASE EXTRACTION OF IBUPROFEN IN WATERS WITH MOLECULARLY IMPRINTED POLYMERS PRIOR TO HPLC-DAD DETERMINATION

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ABSTRACT

SOLID PHASE EXTRACTION OF IBUPROFEN IN WATERS WITH MOLECULARLY IMPRINTED POLYMERS PRIOR TO HPLC-DAD DETERMINATION

Endocrine disrupting compounds (EDCs) attract great attention worldwide due to their undesired effects on human health. Ibuprofen, an example of endocrine disrupters, is a nonsteroidal anti-inflammatory drug (NSAID). In this study, highly selective molecularly imprinted polymers (MIPs) with different morphologies (as monolith and microspherical beads) were synthesized by bulk and precipitation polymerization strategies. MIPs were prepared by using acetonitrile as porogen, methacrylic acid (MAA) as monomer, trimethylolpropane trimethacrylate (TRIM) as crosslinker and the analyte, ibuprofen, as the template.

MIPs revealed higher affinity to the template molecule as compared with non-imprinted polymers (NIPs). The MIP prepared by precipitation polymerization was decided to be used as the primary solid phase extraction (SPE) sorbent due to its higher binding capacity towards ibuprofen compared to the MIP prepared by bulk polymerization. Selectivity of MIP to ibuprofen was examined in the presence of structurally related compounds.

In this study, a molecular imprinting solid phase extraction (MISPE) methodology was proposed for determination of ibuprofen prior to HPLC-DAD analysis. For this purpose, critical experimental parameters of MISPE method were optimized and determined as follows; solution pH of 8.0, sorbent amount of 25.0 mg for 10.0 mL of 1.0 mgL^{-1} working solution, sorption time of 30 min and MeOH:H2O (acetic acid, pH 3.0) ratio of 80:20 as desorption solution.

The accuracy of the proposed methodology was verified with spike recovery tests for tap and drinking waters and overall recovery was found as 97.4 (± 0.3) for n=3.

ÖZET

SULARDA BULUNAN İBUPROFEN'İN HPLC-DAD İLE TAYİNİ ÖNCESİ MOLEKÜLER BASKILANMIŞ POLİMERLERLE KATI FAZ EKSTRAKSİYONU

Endokrin Sistem Bozucu Maddeler (EDC) insan sağlığı üzerindeki olumsuz etkilerinden dolayı dünya çapında büyük bir ilgi toplamaya başlamıştır. Endokrin sistem bozucu maddelerden olan ibuprofen, non steroidal antienflamatuvar (NSAID) ilaç grubunda yer alan bir ağrı kesicidir. Bu çalışmada farklı morfolojilere sahip moleküler baskılanmış polimerler (MIPs) (yekpare ve mikroküresel parçacıklar) kütle ve çöktürme polimerizasyonu ile sentezlenmiştir. MIP'ler monomer olarak metakrilik asit (MAA), çağraz bağlayıcı olarak trimetilolpropan trimetakrilat (TRIM), porojen olarak asetonitril ve şablon analit olarak ibuprofen kullanılarak hazırlanmıştır.

Moleküler baskılanmamış polimerlere (NIPs) kıyasla MIP'ler ibuprofene daha yüksek bir eğilim göstermişlerdir. Çöktürme yöntemi ile sentezlenen MIP seçici katı faz ekstraksiyon sorbenti olarak seçilmiştir; çünkü MIP ve NIP arasındaki ibuprofene karşı bağlanma kapasite farkının çöktürme polimerizasyon yöntemi ile sentezlenmiş MIP ve NIP'den daha yüksek olduğu görülmüştür. Sentezlenen maddenin seçiciliği benzer yapıdaki maddelerin varlığında da kanıtlanmıştır.

Bu çalışmada, ibuprofenin HPLC-DAD ile tayini öncesinde moleküler baskılama polimer katı faz ekstraksiyonuna (MISPE) dayanan bir metodoloji önerilmektedir. Bu amaçla MISPE metodunun kritik deneysel parametreleri optimize edilmiş; bu değerler, çözelti pH'sı 8.0, 10.0 mL 1.0 mgL^{-1} çalışma çözeltisi için sorbent miktarı 25.0 mg, sorpsiyon süresi 30 dakika ve desorpsiyon çözeltisi olarak 80:20'lik MeOH:H2O (asetik asit, pH 3) olarak belirlenmiştir.

Önerilen metodun kesinliği çeşme ve içme sularına standart katma/geri alma testleriyle doğrulanmış ve n=3 için toplam geri kazanma yüzdesi 97.4 (± 0.3) olarak bulunmuştur.

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CHAPTER 1

INTRODUCTION

1.1. Endocrine Disrupting Compounds (EDCs)

Endocrine disrupting compounds (EDCs) are one of the current issues taking great attraction of worldwide organizations, such as WHO and EPA, dealing with protecting human health and environment. Endocrine disrupting compounds are both man-made and natural exogenous chemicals which cause some disorders in human body by interfering normal hormone functions. Hormones in endocrine system control many of the processes in body, such as early processes (cell differentiation and organ formation), and normal functioning of tissues during adulthood. These exogenous substances when included in endocrine system during early, mid, or late prenatal or postnatal period can actualize its own disruption by two ways: It can directly affect hormone-receptor protein complex or specific protein which is responsible for hormone delivery to a specific place at an exact time (WHO/UNEP 2012).

As all of the chemicals, endocrine disrupters have dose response relationship. However, unlike other chemicals, EDCs have non-monotonic dose response curves (Fig.1.1) that completely different from the view of ‘the dose make poison’ (in monotonic curves). Even if at the quite low doses, EDCs may be toxic for both humankind and wildlife. The response may be huge at lower doses when compared to higher doses (EPA 2015).

Endocrine disrupters are classified into some groups: household product ingredients, personal care products (cosmetic ingredients), food additives, flame retardants, plastics, pesticides ingredients, antimicrobials, biogenic compounds, industrial additives, solvents, metals, byproducts, medicals, metabolites (TEDX 2015). In this study, especially the medicals, which are classified as Non-Steroidal Anti-Inflammatory Drugs (NSAID) and used widely as painkillers in certain cases, were investigated.

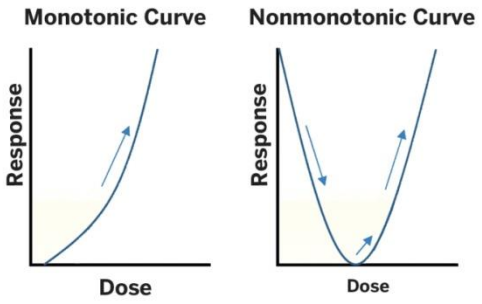


Figure 1.1. Examples of monotonic and non-monotonic curves.
(Source: EPA 2015)

1.1.1 Diseases Caused by EDCs

Endocrine disruptors can affect reproductive function of humankind adversely during preconception or pregnancy, or during childhood and puberty of offspring. Considering reproduction system, chronic exposure from pregnancy to adulthood is more critical than acute exposure to endocrine disruptors for humankind (Palioura, Kandaraki, and Diamanti-Kandarakis 2011).

First disruption has been seen from a synthetic oestrogen called diethylstilbestrol, which is used for prevention of miscarriage of pregnant women. Chronic exposure of the women taking these oestrogen pills during pregnancy has caused the daughters of these women to have unexpectedly gynecologic neoplasm and vaginal adenocarcinoma (Herbst A.L., Ulfelder H., and C. 1971). The later studies have shown that these two are not the only disorders that could be observed due to disruption; the others are characteristic T-shaped uterus, infertility, preterm birth, menstrual irregularity, ectopic pregnancy and poor pregnancy outcome concerned to spontaneous abortion (Kaufman et al. 2000, R.H. 1982, Palmer et al. 2001). In addition to these, puberty timing in girls has changed and caused the larche and menarche in an earlier time due to estrogen mimics and antiandrogens, which are considerably related to environmental factors (Euling, Herman-Giddens, et al. 2008, Euling, Selevan, et al. 2008, Jacobson-Dickman and Lee 2009).

Male reproductive system is also affected from endocrine disrupters. There are three major diseases; poor semen quality and infertility, urogenital tract abnormalities

and testicular germ cancer. Some endocrine disrupting chemicals are related with these illnesses. For example, polychlorinated biphenyls, phthalates, and non-persistent pesticides have an inverse effect on sperm parameters (Dallinga et al. 2002, Hauser et al. 2003, Hauser et al. 2006, Juhler R.K. et al. 1999).

Endocrine disrupting chemicals also act as thyroid hormones. These hormones have many functions in both vertebrates and invertebrates as regulation of development, tissue growth, and metabolism (Heyland and Moroz 2005). Especially during pregnancy, fetus' growth and its brain development are greatly affected by disruption of thyroid hormones with a result of neurological and cognitive deficiencies (Boas et al. 2006).

One of the most important public health problems in these days is obesity epidemic. In addition to change in food intake, physical activity, and genetic predisposition; endocrine disrupters can also have an effect on obesity as an exogenous factor by impairing body's natural weight control system (Baillie-Hamilton 2002). 'Obesogens', that have reversely effect on regulation of lipid metabolism, are responsible for overweight and obesity in human life if any exposure has occurred during development (Grun et al. 2006).

Endocrine disrupting compounds are also the reason of some metabolic disorders. Even in the low doses, bisphenol A can decrease the glycose level that is responsible for a rise in insulin in blood (Alonso-Magdalena et al. 2005). The other metabolic disorders because of endocrine disruption are some hearth diseases, diabetes, insulin resistance, and disturbed glycogen secretion.

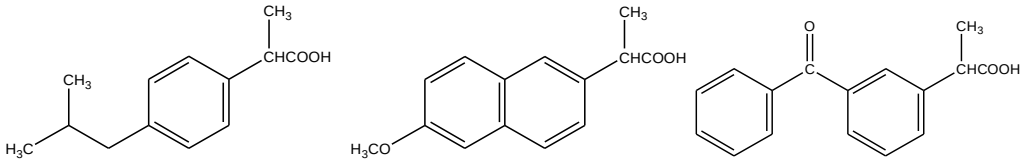
1.2. Ibuprofen and Its Derivatives

Ibuprofen (IBU), naproxen (NAP), and ketoprofen (KET) are all propionic acid derivatives of nonsteroidal anti-inflammatory drug (NSAID) group in mild analgesic drugs. They are used for the same purposes (analgesic, antipyretic, and anti-inflammatory) and work in the same principle. NSAIDs reduce the pain, which is induced by local inflammantion, by inhibiting the group of enzymes called cyclooxygenases (COXs) in the body. COXs catalyze the synthesis of prostaglandin (PG) which brings pain and inflammation at local area as a product of the inflamed white blood cells (Bloom 2015). There are two different types of COXs enzymes

responsible for this synthesis: COX-1 and COX-2. COX-1, as constitutive enzyme, is produced in constant amounts in almost every sides of the body. However, COX-2, as an adaptive enzyme, is synthesized in the case of inflammantion.

When ibuprofen is taken to get rid of pain, it inhibits both COX-1 and COX-2 because of its non-selective behaviour. Inhibition of COX-2 supply therapeutic effect (Haworth 2015) whereas the inhibition of COX-1 which is useful for body, can cause endocrine disrupting effects by taking ibuprofen as painkiller. Table 1.1 shows Structures, molecular weights and pKa values of related NSAIDs.

Table 1.1. Structures, molecular weights and pKa values of related NSAIDs.



IBUPROFEN	**NAPROXEN**	**KETOPROFEN**
Mw=206.3 gmol^{-1}	**Mw=230.6 gmol^{-1}**	**Mw=254.2 gmol^{-1}**
pKa=4.85	**pKa=4.15**	**pKa=5.94**

1.2.1. Possible Diseases in Case of Exposure to Ibuprofen

In the NSAIDs ibuprofen is an example of endocrine disruptors. It causes congenital cryptorchidism (deficiency of one or both testicles) to male-child of impregnate by interfering in testis development (dysgenesis). In later life of the descent, he would have the risk of poor semen quality and testicular germ cancer due to cryptorchidism. The risk is increased when ibuprofen is used during the second trimester of gestation period and further increased by the simultaneous usage of more than one type endocrine disrupting mild analgesics (Kristensen et al. 2011). The real pathogenesis is the inhibition of the synthesis of PG by COXs, because it has great effect on semen motility and functional capacity of sperms (Bygdeman M et al. 1987).

1.2.2. Mix Ibuprofen and Its Derivatives in to Water

Possible pollution of water from these compounds can come true via household wastes, agricultural lands, and wastes from pharmaceutical production industries, livestocks and landfills (Fig.1.2). Through these ways, drinking waters can be affected if any proper wastewater treatment or drinking water treatment is not applied.

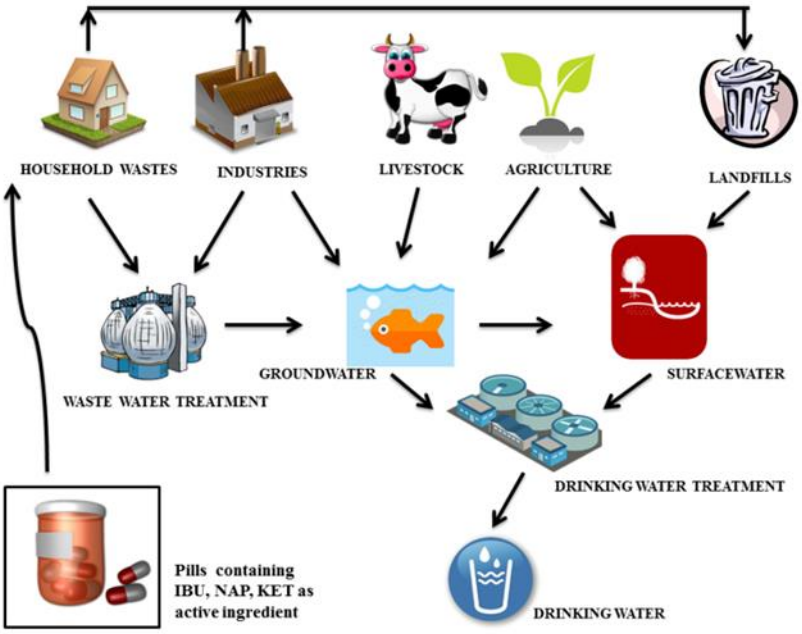


Figure 1.2. The way of IBU, NAP, and KET into drinking water.

1.2.3. Determination of Ibuprofen

There are many analytical methods used for the determination of ibuprofen in many samples. In some of these, commercial cartridges were used as the pre-concentration process prior to instrumental determination. Gomez et al. (2006) developed an analytical method for the detection of 19 pharmaceuticals including ibuprofen in hospital effluent wastewaters. They used Oasis HLB cartridges (Waters) for SPE process before LC-MS/MS determination of pharmaceuticals. The reported recoveries of pharmaceuticals were greater than 75.0%. In another work by Lee, Peart, and Svoboda (2005), sewage water was analyzed to show the existence of endocrine

disrupting phenols, acidic pharmaceuticals and personal care products. Oasis MAX SPE commercial cartridges were used for the pre-concentration process. Ibuprofen was found in both influent and effluent (6.77 and 0.31 μgL^{-1}, respectively) by the analysis with gas chromatography-mass spectrometry (GC-MS). Gros, Petrovic, and Barcelo (2006) determined trace level pharmaceuticals in surface and wastewaters by LC-MS/MS. At the end of the comparison of four different commercial cartridges, Oasis HLB cartridge gave the higher recovery percentage for ibuprofen. In another work belongs to Santos et al. (2005), high pressure liquid chromatography with diode array and fluorescence detector (HPLC-DAD-FL) was used as an inexpensive analytical technique over HPLC-MS for determination of ibuprofen. After the pre-concentration with SPE method, pharmaceuticals including ibuprofen in wastewater samples of Seville city (Italy) were analyzed. Kot-Wasik et al. (2006) demonstrated two different methods (HPLC-DAD and LC-MS) for the detection of NSAIDs in natural waters. Online and offline SPE procedures were applied by using commercial SPE cartridges. The highest recoveries were reported for in the range of 96-109% with online coupling systems.

Synthesized sorbents are much more preferred if selective recognition to a molecule group or to only a molecule is desired. In the work of Abd Rahim et al. (2016), a sol-gel hybrid material synthesized by using methyltrimethoxysilane-mercaptopropyltrimethoxysilane was used as a SPE sorbent for the determination of ibuprofen and other NSAIDs. All analyses were performed with HPLC-DAD. All the results were compared with a commercial cyano sorbent and the selectivity of hybrid material was proven. Poly(ethylene-glycol) (PEG) grafted multi-wallet carbon nanotubes (PEG-g-MWCNTs) was prepared by applying sol-gel method and used as solid phase microextraction (SPME) fiber coating. This fiber was combined with gas chromatography-flame ionization detector (GC-FID) for the detection of ibuprofen, naproxen and diclofenac in real water samples. Recoveries were reported from 84-107% (Sarafraz-Yazdi et al. 2012). In another research, for determination of NSAIDs including ibuprofen, different functionalized alkyl chains were synthesized and were covered with magnetite in order to use in magnetic solid phase dispersion. All analyses were done with HPLC-DAD. From the synthesized materials, octyl chains provided the highest recovery at pH 3 in wastewaters (>90%) (Aguilar-Arteaga et al. 2010). Hung et al. (2006) synthesized MIP by using MAA as functional monomer and ethylene glycoldimethacrylate as crosslinker with bulk polymerization. This polymer was used

for the detection of ibuprofen. After the grounding and sieving of the polymers, the synthesized polymers were packed into analytical columns. Performance of MIP was investigated by HPLC-DAD determinations.

Capillary zone electrophoresis was used for the detection of seven pharmaceuticals including ibuprofen by Chen and Wu (2005). After the optimization of conditions, they obtained recoveries over 95%.

Another research group used UPCL-MS/MS for determination of salycilic acid, ibuprofen, naproxen and diclofenac in real samples. They coupled two phase hollow fiber liquid-phase microextraction with UPCL-MS/MS. This method was reported to given recoveries from 98 to 115 % (Zhang et al. 2013).

Gibbons, Wang, and Ma (2011) developed a method, based on capillary electrophoresis coupled with UV detector (CE-UV). The method was fast and more economical than HPLC-MS/MS. In addition to these detection limits were between 1.6-68.7 ppb.

1.3. Solid Phase Extraction (SPE)

There are many extraction methods, which are used for separation and pre-concentration of selected analytes form samples. Liquid-liquid extraction (solvent extraction), electrodeposition, ion exchange and membrane filtration are some of the examples that are generally used for this purpose.

Solid phase extraction is an enrichment and purifying method, and has some advantages over other methods. It supplies fast and easy manipulation, does not require large amounts of solvent and has high pre-concentration factors. Figure 1.3 shows four basic steps of SPE. First step is conditioning in which the sorbent is wetted and rinsed by the eluting solvent. Second step is sample loading. In this step, a liquid sample is passed through a short column of a solid sorbent, where the desired compounds are sorbed. In rinsing, unwanted compounds are rinsed with a suitable solvent. Last step is elution, in which a proper solvent elutes the analytes. This step is also improves the reproducibility of the method.

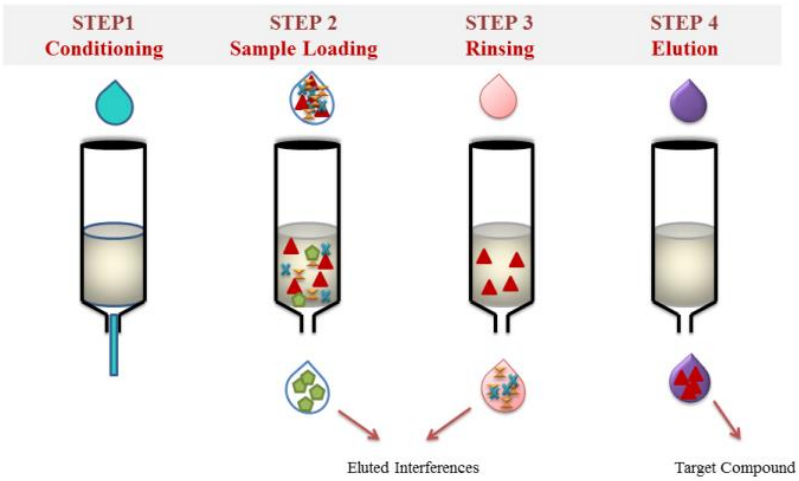


Figure 1.3. Basic steps of solid phase extraction

There are two types of solid phase extraction method. First one is column type SPE (Figure 1.4 (a)). At first, the column is loaded with sample solution. Analyte is sorbed by the solid sorbent and tis recovered with a little amount of eluent. The concentration of the analyte in the eluate gives the percentage of elution. Finally, total recovery can be calculated by an equation given as 1.1

$$Total\ Recovery = \frac{[analyte]eluate}{[analyte]sample} \times 100 \quad (1.1)$$

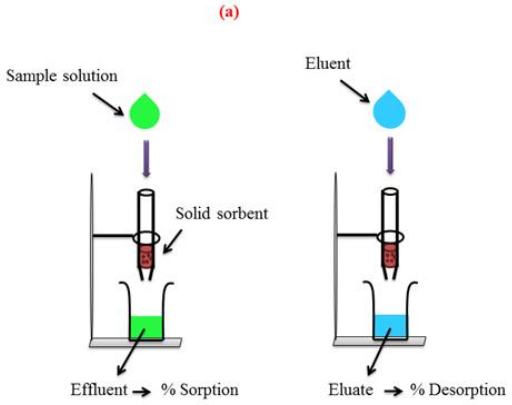


Figure 1.4. Solid Phase Extraction Types (a) Column type SPE, (b) Batch type SPE **(continue on next page)**.

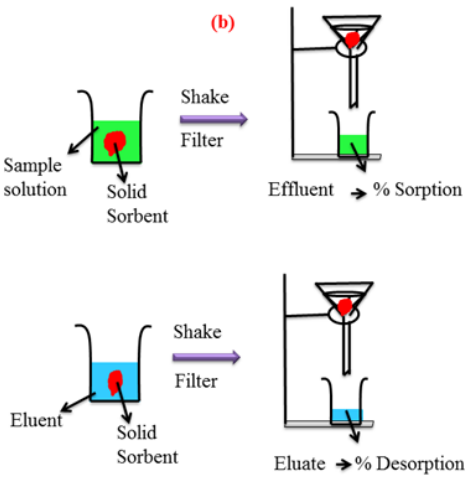


Figure 1.4. Solid Phase Extraction Types (a) Column type SPE, (b) Batch type SPE **(continue)**.

Another one is batch type (Figure 1.4 (b)). In this process solid sorbent is directly put into the sample solution and shaken. After filtration is applied, the analytes are eluted with a solvent and total recovery is calculated as in 1.1.

Many types of solid sorbents have been synthesized and used. Some of them will be explained in the following sections. The key to have a successful SPE process is to choose a proper sorbent, which should have the necessary functional groups to interact with the anayte molecule.

1.3.1. Hypercrosslinked Sorbents

Hypercrosslinked sorbents are classified in two groups: non-polar and polar. Non-polar extractions can be considered as hydrophobic or reversed phase attractions. In this type, the sorption capacity of sorbent is increased by increasing the specific surface area, which supplies an increase in the number of interaction points between analyte and sorbent. Between the linear polymer chains bridges are created with crosslinking agents, so specific area is increased. In addition, crosslinking supplies robustness.

Polar extractions are classified in two groups in itself: hydrophobic crosslinked sorbents and small and monodispersed particle size hypercrosslinked sorbents. These

ones can be used for the online enrichment of organic pollutants from aqueous samples as in guard columns (Nuria Fontanals, Rosa Maria Marce, and Borrull 2010).

1.3.2. Hydrophilic Sorbents

Hypercrosslinked sorbents are generally of hydrophobic nature that restricts the extraction of polar compounds. In order to overcome this problem, some polarity is introduced to the polymers resins by two ways: modifying of PS-DVB based polymers or copolymerization of hydrophilic monomers (Fritz 1999, Masque N. et al. 1999).

1.3.3. Mixed Mode Polymeric Sorbents

Mixed mode polymeric sorbents supply the combination of polymeric skeleton and ionic groups. Thus, two types of interaction (both non-polar and ionic) occur during the extraction process. In addition to their selectivity, these sorbents supply clean extracts which is important especially if sensitive detectors like tandem mass spectrometry are used for detection (Nuria Fontanals, Rosa Maria Marce, and Borrull 2010).

1.3.4. Molecular Imprinted Polymers (MIPs)

Complexes of molecules in solutions or gases have relatively small lifetimes that they have nearly zero concentration in solutions. Some types of molecules, namely receptors, can recognize only one type of substances among other species. In the crowd of solutions, receptors can distinguish their own partner molecule and make stable complexes with quite high concentrations.

There are lots of naturally occurring receptors in body. These receptors are responsible for many processes, which are essential for existence. Without these molecules, living being cannot survive. The superiority of these substances makes scientists to create new molecules by imitating natural receptors. These artificial receptors have advances among the natural ones and do not only deal with proteins. For a variety of compounds, a specific molecule can be created. In addition, humankind can determine stability, flexibility and activity in different conditions. In addition to all these, by a proper chemical design, someone can create a substance that has the sites

completely suit to an analyte. In general, this process can be named as ‘molecular recognition’, which is the key idea of ‘molecular imprinted polymers’.

For the treatment of waste water, the usage of the receptor is a common method. However, its cost is quite high. For industries dealing with the production of NSAIDs, separation and purification of the product and clarification of their waste water cost more than the production process. Here MIP can be put into use as an inexpensive method with quite high yield. Being cheap is not only the advantage of MIP, also they are very easy to prepare in a short time and have high stability and activity during a wide range of conditions in addition to robustness. Selective and strong binding sites against analyte in water conditions with the other qualities of MIP make it a favorable method for water treatments.

Synthesis of MIP is achieved by the polymerization of monomer(s) and crosslinking agent around the template molecule (analyte or its analog) by starting with the usage of an initiator. Template molecule has an important role for the synthesis of MIP. It should not have any functional group that stop or retard the inhibition of polymerization and should be stable in a wide range of temperature and UV-radiation.

Figure 1.5 shows three basic steps of molecular imprinting: pre-polymerization, polymerization, and removal of template. In pre-polymerization step, monomer and template molecule let to connect to each other via covalent or non-covalent interaction (Makoto Komiyama et al. 2003).

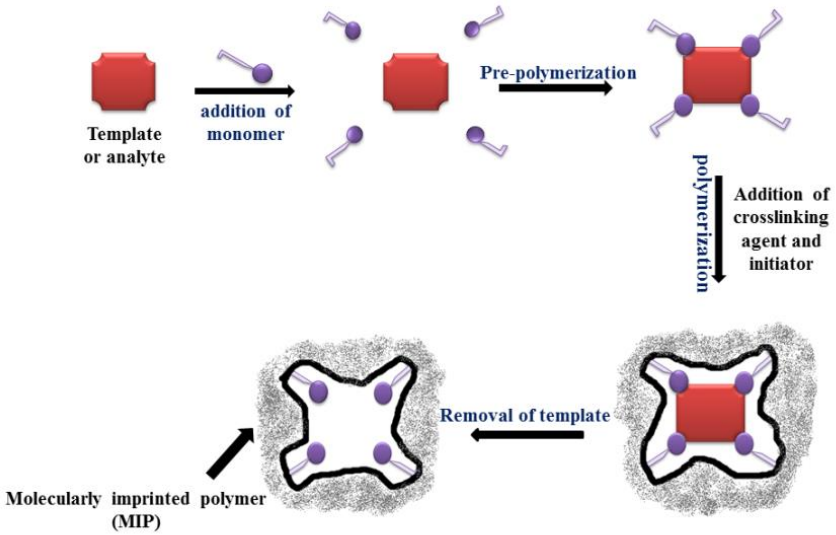


Figure 1.5. Schematic illustration of molecular imprinting.

These interactions give specific name of the total process, semi-covalent imprinting or non-covalent imprinting. Non-covalent approach is based on polar interactions such as H-bonding and electrostatic interactions, whereas semi-covalent imprinting occur from covalent bonding. In semi-covalent approach, covalent bonds are formed during the pre-polymerization step, but rebinding is achieved by non-covalent approach (Nuria Fontanals, Rosa Maria Marce, and Borrull 2010). Even if the clearer structure of cavities and free polymerization conditions are supplied by covalent imprinting, generally non-covalent imprinting is preferred. The reason originates from the fast removal, rebinding, and release of template in non-covalent imprinting. However, restricted conditions during polymerization make non-covalent imprinting not an easy process at the beginning (Makoto Komiyama et al. 2003).

In addition, the type of the solvent that is chosen as reaction medium determines the type of imprinting process. There are two types of solvents used during polymerization: non-to moderately polar/aprotic solvents/porogens (DCM, toluene, chloroform, acetonitrile) and polar protic solvents (methanol, ethanol, water). Polar protic solvents decrease the polarity of the interactions between the template and monomer, so they are used for covalent imprinting. In contrast to protic solvents, aprotic solvents (porogens) support and stabilize the H-bonding during non-covalent interaction (Nuria Fontanals, Rosa Maria Marce, and Borrull 2010).

In the polymerization step, crosslinking agent and initiator are added into the reaction mixture with a fixed ratio. The mixture is left to polymerization. Crosslinking agent controls the morphology of MIP and supply robustness to the polymer while stabilizing the specific binding sites (imprinted region). The addition of initiator under proper conditions changes the destiny of polymerization step if radical copolymerization method is chosen. Free radical copolymerization is initiated by the thermal decomposition of radical initiator and molecular oxygen is taken away from the reaction mixture in order to prevent from trapping of radical. Removal of oxygen is supplied by degassing with argon or nitrogen gas or freeze-and-thaw cycle. In some cases, application of high temperatures for initiation can be harmful to non-covalent interaction between monomer and template. This time photo-initiation by UV-light can be applied under low temperatures. In addition to these, the usage of UV-absorbable monomer supplies the initiation in the absence of initiator. If these processes are not applied, the polymerization cannot be started (Makoto Komiyama et al. 2003).

After the achievement of polymerization, the solid particles, now called MIPs, are filtered, then washed until no template molecule is observed at chromatogram. The most remarkable problem during template removal is template bleeding in trace analysis. When analyte molecule is used as template and is not removed completely from MIP, higher amount of analyte can be observed than expected at real samples. In order to overcome this problem, a dummy molecule can be used as template. Dummy molecules resemble the target analyte in terms of size, shape and functionality, but gives different chromatographic separation than template. Thereby, template and analyte molecule can be discriminated during chromatographic separation (Nuria Fontanals, Rosa Maria Marce, and Borrull 2010).

Before starting the synthesis of this special polymer, physical nature of the product must be determined according to needs for the experimental procedures and conditions. Some important parameters should be taken into account. The crosslink ratio with respect to the total number of monomer and the volume of the solvent determines and changes all the physical morphology of MIP (Figure 1.6) (Peter A.G Cormack and Elorza 2004).

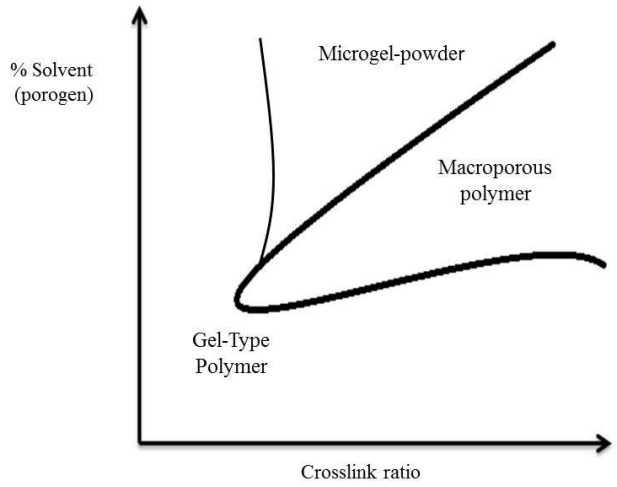


Figure 1.6. Polymer pseudo diagram.

High crosslink ratio at low percentages of solvent or vice versa produce gel-type polymer. This type of MIP can swell in solvents that have thermodynamically good properties. However, it supplies low specific surface area, which is not useful for the experiments that deal with the great amount of analyte in working solution. In addition

to that, when crosslink ratio is low, the resulting poor mechanical strength may restrict the experimental conditions. Macroporous polymer synthesis is the most widely used method for the preparation of MIP. When compared to gel-type polymers, it has higher specific surface area and more robustness due to the high crosslink ratio. When solvent ratio is increased, microgel powders can be synthesized via precipitation polymerization. This method supplies spherical particles that have radius around micrometers. In general, the reagents used in MIP synthesis should have a ratio to have polymer according to the needs for the chosen process. Finding exact ratio is the most important part and the most time-consuming process that may continue several weeks. This can be achieved only by trial-and-error experiments (Cormack and Elorza 2004).

The polymerization techniques generally used in MIP synthesis are one-step or multi-step swelling, suspension, grafting, bulk and precipitation polymerization. Bulk and precipitation techniques, which were used in this thesis, will be briefly explained.

Bulk polymerization is a simple technique, which is widely used in MIP synthesis. The resulting polymer in monolithic form is needed to be crushed, grounded and then sieved in order to obtain definite sized particles before placing into disposable cartridges for SPE process or other applications. These processes require extra time. In addition to that, due to the separation of only definite sized particles, high amounts of waste are produced. Precipitation polymerization is used to have micro and nano-sized spherical particles. Crosslink ratio and solvent amount must be carefully determined in order to avoid agglomerated polymer particles. The usage of these particles does not require any crushing, grounding and sieving processes. Compared to monoliths the spheres supply higher surface area. However, for column type SPE process, these micro and nano-spheres are not large enough to be used (Nuria Fontanals, Rosa Maria Marce, and Borrull 2010).

Addition of the template to the polymerization medium supplies specificity and selectivity to polymer by creating imprinted sites against the analyte or analyte group. To understand the existence of imprinted sites, an extra polymer is synthesized under the same conditions as MIP. However, this time template or dummy molecule is not added into the reaction medium. Thereby, imprinted sites are not created. This second substance is called Non Imprinted Polymer (NIP). By comparing the results of two polymers, selectivity can be clarified.

1.4. AIM OF STUDY

The purpose of this study is to prepare a novel solid phase extraction sorbent for the specific recognition of ibuprofen prior to chromatographic determination. For this purpose, molecular imprinted polymers were synthesized against ibuprofen with different morphologies. The sorption performances of MIPs with different morphologies were investigated in terms of binding capacity. According to characteristic sorption performances, one of the MIPs was chosen. Afterwards, optimization parameters of the proposed MISPE procedure were examined in terms solution pH, amount of sorbent, amount of working solution, sorption time, desorption matrix through batch type SPE studies. Validation studies were realized by spiking ibuprofen in different water samples.

CHAPTER 2

EXPERIMENTAL STUDY

2.1. Optimization of Instrumental Parameters

For the HPLC-DAD optimization process, firstly 500.0 mgL^{-1} of ibuprofen stock solution was prepared in methanol. The solution was stored in an amber glass bottle at 4.0 ºC in refrigerator. Standards and sample solutions were prepared daily with proper dilutions.

All analyses were performed with Agilent 1200 series HPLC with Diode Array Detector. The tested parameters were shown in Table 2.1. After optimization of the experimental parameters, limit of detection (LOD) and limit of quantification (LOQ) were calculated by analyzing the least concentrated standard 20 times with HPLC-DAD.

Table 2.1. HPLC-DAD optimization parameters.

Column	Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column
Mobile phase	85:15 methanol:water 80:20 methanol:water 70:30 methanol:water 60:40 methanol:water 85:15 methanol:water (acetic acid, pH:3.0) 80:20 methanol:water (acetic acid, pH:3.0) 70:30 methanol:water (acetic acid, pH:3.0) 60:40 methanol:water (acetic acid, pH:3.0)
Thermostat temperature	25.0, 30.0 ºC
Sample injection volume	20 μL
Flow rate	0.7, 0.8, 0.9, 1.0 $mLmin^{-1}$
Standard solutions	0.025, 0.050, 0.10, 0.25, 0.50, 1.0, 5.0 mgL^{-1}

2.2. Synthesis of Molecularly Imprinted (and Non-Imprinted) Polymers (MIPs and NIPs)

MIP and NIP should be prepared parallel and identical. During the synthesis of MIP100 by using precipitation polymerization strategy, the experimental steps were as follows: Firstly, 0.33 mmol ibuprofen (template), 2.66 mmol methacrylic acid (MAA, monomer) and 100.0 mL acetonitrile (ACN, porogen) were added into a 100.0 mL amber reaction vessel and stirred 1.0 hour for pre-polymerization; then, 6.66 mmol trimethylolpropane trimethacrylate (TRIM, cross-linker) was added. 4,4'-azobis(4-cyanovalericacid) (AIVN, initiator) was used % 2 mole (0.27 mmol) of all reagents except ibuprofen in reaction mixture and was added under Ar gas carefully to remove dissolved oxygen. Polymerization reaction was performed in an oil bath at 60°C, 8 hours.

After polymerization, solid MIP100 was obtained. Removal of template molecule was performed by using two different solutions; namely, methanol and methanol:water (acedic acid pH:3) (80:20) mixture. After complete removal of ibuprofen, MIPs were dried in an oven at 60.0 °C and the sorbent was ready for the experiments. The preparation of NIP100 was the same as in MIP100, except the addition of ibuprofen. The schematic illustration of MIP synthesis is given in Figure 2.1.

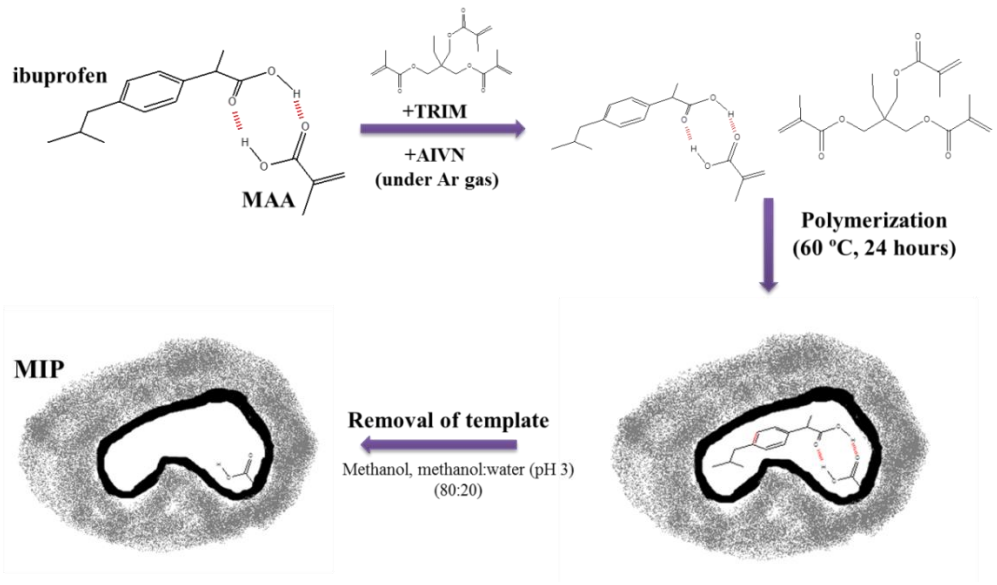


Figure 2.1. Synthesis of MIP by copolymerization of MAA and TRIM.

MIP10 and NIP10 were synthesized as in the synthesis of MIP100 and NIP100 mentioned above, but this time 10 mL acetonitrile was used instead of 100 mL. Identity, mole ratios of reagents and particle sizes of all synthesized polymers are given in Table 2.2.

Table 2.2. MIP and NIP compositions.

	identity	ibuprofen (mmol)	MAA (mmol)	TRIM (mmol)	AIVN (mmol)	ACN (mL)	Particle size (μm)
Molecularly imprinted monolith	MIP10	0.33	2.66	6.66	0.19	10	×
Molecularly non-imprinted monolith	NIP10	0.0	2.66	6.66	0.19	10	×
Molecularly imprinted microspherical particles	MIP100	0.33	2.66	6.66	0.19	100	1.1
Molecularly non-imprinted microspherical particles	NIP100	0.0	2.66	6.66	0.19	100	1.1

In order to understand the physical nature of MIPs and NIPs, scanning electron microscopy (SEM) was applied.

2.3. Characterization Experiments

Characterization experiments were made to prove the presence of cavities in MIPs before the comparison of sorption capacities of all NIPs and MIPs. For this purpose, binding characteristic assay was applied. After this step, the study was carried out using only MIP100 and NIP100. To understand the selectivity of MIP100 against ibuprofen, the experiment was performed in the presence of structurally related compounds.

2.3.1. Binding Characteristic Assay

Sample solutions were prepared as in Table 2.3 and 10.0 mL of these solutions were added into amber vials, which already had 25.0 mg of the sorbent MIP orNIP. After that, mixtures were shaken at 480 rpm, 8 hours. The solid/liquid mixture was filtered through cellulose acetate membranes (0.2 μm pore size) to separate MIP100 from solutions. Effluents were analyzed with HPLC-DAD at 220 nm. The same procedure was applied for each type of the MIPs/NIPs

Table 2.3. Parameters of binding characteristic assay.

Standard concentrations	1.0, 5.0, 10.0, 20.0, 50.0, 100.0, 250.0 mgL^{-1}
Amount of sorbent	25.0 mg
Sample solution volume	10.0 mL
Sorption time	8 hours
Shaking speed	480 rpm
Ambient temperature	25.0 ºC

2.3.2. Cross Sensitivity

50.0 mgL^{-1} mixtures of ibuprofen, naproxen and ketoprofen were prepared. 10.0 mL of this mixture was added into 25.0 mg of MIP100 and/or NIP100 containing vials. Sorption was realized on an orbital shaker at 480 rpm, 8 hours. Cellulose acetate membranes (0.2 μm pore size) were used to filter the mixtures. Effluents were analyzed with HPLC-DAD at 220 nm. The sorption parameters are given in Table 2.4.

Table 2.4. Studied parameters during sorption.

Standard concentration	50.0 mgL^{-1}
Amount of sorbent	25.0 mg
Sample solution volume	10.0 mL
Sorption time	8 hours
Shaking speed	480 rpm
Ambient temperature	25.0 ºC

2.4. Optimization Parameters

2.4.1. Effect of pH on Sorption

To understand the effect of pH on the sorption of ibuprofen, 1.0 mgL^{-1} ibuprofen solutions (in UPW) were prepared at 7.0, 8.0, 9.0, and 10.0 pHs (adjusted with nitric acid and sodium hydroxide). 10.0 mL of these solutions were added into amber vials, which already had 25.0 mg of MIP100 or NIP100. Vials were shaken at 480 rpm, 8 hours. pHs of mixtures were also checked after the sorption process and they were filtered with membrane filtration system by using cellulose acetate membranes (0.2 μm pore size). Effluents were analyzed with HPLC-DAD at 220 nm. Parameters used in the pH study are given in Table 2.5.

Table 2.5. Parameters used for the pH determination.

Standard concentration	1.0 mgL^{-1}
pH	7.0, 8.0, 9.0, 10.0
Amount of sorbent	25.0 mg
Solution volume	10.0 mL
Sorption time	8 hours
Shaking speed	480 rpm
Ambient temperature	25.0 ºC

2.4.2. Effect of Sorbent Amount

Effect of sorbent amount was investigated as follows; MIP100 sorbents were weighed as given in Table 2.6 and taken into amber vials. 10.0 mL of 1.0 mgL^{-1} ibuprofen solution at pH 8.0 (adjusted with nitric acid and sodium hydroxide) was added. Sorption was realized on the orbital shaker at 480 rpm, 8 hours. Filtration was made with membrane filtration system by cellulose acetate membranes (0.2 μm pore size). Effluents were analyzed with HPLC-DAD at 220 nm.

Table 2.6. Studied parameters in sorbent amount determination.

Standard concentration	1.0 mgL^{-1}
pH	8.0
Amount of sorbent	5.0, 10.0, 25.0, 50.0, 100.0 mg
Solution volume	10.0 mL
Sorption time	8 hours
Shaking speed	480 rpm
Ambient temperature	25.0 ºC

2.4.3 Effect of Sample Volume

Solution volumes of ibuprofen were prepared as in Table 2.7. The pH of solutions was adjusted to 8.0 (using nitric acid and sodium hydroxide) and 1.0 mgL^{-1} ibuprofen solutions with different volumes were added into the sample vials containing 25.0 mg MIP100. Sorption was carried out on the orbital shaker at 480 rpm, 8 hours. Membrane filtration system by cellulose acetate membranes (0.2 μm pore size) was used for filtration of the mixtures. Effluents were analyzed with HPLC-DAD at 220 nm.

Table 2.7. Optimization parameters for determination of sample volume.

Standard concentration	1.0 mgL^{-1}
pH	8.0
Amount of sorbent	25.0 mg
Solution volume	5.0, 10.0, 20.0, 50.0 mL
Sorption time	8 hours
Shaking speed	480 rpm
Ambient temperature	25.0 ºC

2.4.4. Effect of Sorption Time

10.0 mL of 1.0 mgL^{-1} ibuprofen solution at pH 8.0 (adjusted with nitric acid and sodium hydroxide) was added into sample vials containing 25.0 mg MIP100. Sorption was achieved on the orbital shaker at 480 rpm. Effluents were taken at specific intervals as written in Table 2.8 and the mixtures filtered. Samples were analyzed with HPLC-DAD at 220 nm.

Table 2.8. Studied parameters used in determination of sorption time.

Standard concentration	1.0 mg/L
pH	8.0
Amount of sorbent	25.0 mg
Sample solution volume	10.0 mL
Shaking time	5.0, 10.0, 15.0, 30.0, 60, 90, 120, 180, 240, 300, 360 min
Shaking speed	480 rpm
Ambient temperature	25.0 ºC

2.4.5. Effect of Eluent Type

For the elution of ibuprofen from sorbent, two different solutions were tried. 10.0 mL of 1.0 mgL^{-1} of ibuprofen at pH 8.0 (adjusted with nitric acid and sodium hydroxide) were added into sample vials containing 25.0 mg MIP100. Sorption was carried out on the orbital shaker at 480 rpm. After the filtration, methanol and methanol:water (MeOH:H_2O) (acetic acid, pH 3.0) (80:20) were used as eluents. Eluates were analyzed with HPLC-DAD at 220 nm. Parameters used in the determination of eluent are given in Table 2.9.

Table 2.9. Parameters used in the determination of eluent.

Standard concentration	1.0 mg/L
pH	8.0
Amount of sorbent	25.0 mg
Solution volume	10.0 mL
Shaking time	30.0 min
Shaking speed	480 rpm
Desorption matrix	Methanol, MeOH:H_2O (acetic acid, pH 3.0) (80:20)
Ambient temperature	25.0 ºC

2.4.6. Reusability of the Sorbent

To determine the number of times that the sorbent can be used for MISPE process, reusability experiments were realized. For this purpose, 10.0 mL of 1.0 mgL^{-1} of ibuprofen solution at pH 8.0 (adjusted with nitric acid and sodium hydroxide) were added into sample vials containing 25.0 mg MIP100. Sorption was achieved on the orbital shaker at 480 rpm. The analyte molecule was eluted from sorbent by methanol:water (MeOH:H_2O) (acetic acid, pH 3.0) (80:20). Then this process was repeated 10 times with the same sorbent. All parameters are given in Table 2.10.

Table 2.10. Parameters for reusability experiments.

Standard concentration	1.0 mgL^{-1}
pH	8.0
Amount of sorbent	25.0 mg
Sample solution volume	10.0 mL
Sorption time	30.0 min
Shaking speed	480 rpm
Ambient temperature	25.0 ºC
Number of reuse	10

2.5. Method Validation

Sorption efficiency of the sorbents was investigated by using the spiked samples of ultrapure, drinking and tap water. This was realized by spiking 10.0 mL aliquots of ultrapure, drinking, and tap water samples with 100.0 μgL^{-1} ibuprofen using the parameters of the batch type MISPE process. Method validation parameters are given in Table 2.11.

Table 2.11. Method validation parameters.

Sample type	ultrapure water, tap water, drinking water
Analyte concentration	0.1 mgL^{-1}
pH	8.0
Amount of sorbent	25.0 mg
Solution volume	10.0 mL
Shaking time	30.0 min
Shaking speed	480 rpm
Eluent type	Methanol:water (acetic acid, pH:3) (80:20)
Temperature	25.0 ºC

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Optimization of Instrumental Parameters

Instrumental parameters given in Table 2.1 were used throughout the study. Optimum parameters for mobile phase composition, flow rate, and column temperature were determined as 80:20 MeOH:H_2O (acetic acid, pH 3.0), 0.8 mL.min^{-1} and 30ºC, respectively. LOD value was calculated as 0.023 mgL^{-1} and LOQ was found as 0.075 mgL^{-1}. Calibration graph for ibuprofen with the use of optimum instrumental parameters is shown in Fig. 3.1.

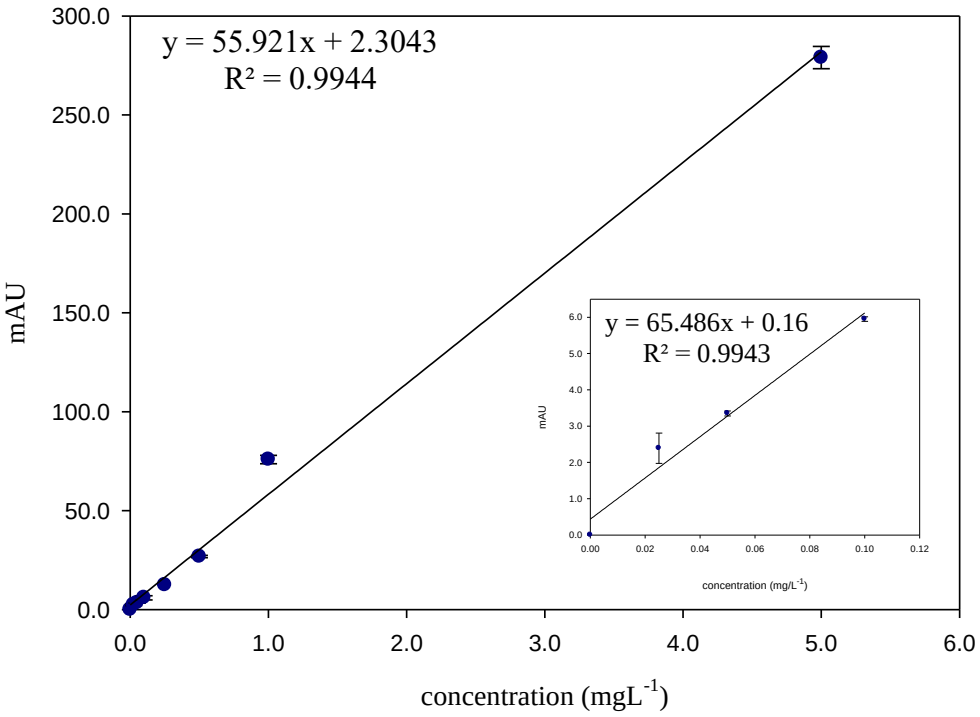


Figure 3.1. Calibration plot for ibuprofen. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column, 80:20 MeOH:H_2O (pH 3.0) mobile phase, 0.8 mLmin^{-1} flow rate, 220 nm).

3.2. Synthesis of Molecularly Imprinted (and Non-Imprinted) Polymers (MIPs and NIPs)

Various types of MIPs were synthesized using the methods given in Section 2.2. Scanning electron microscope (SEM) images for both types of the MIPs and NIPs are shown in Figure 3.2.

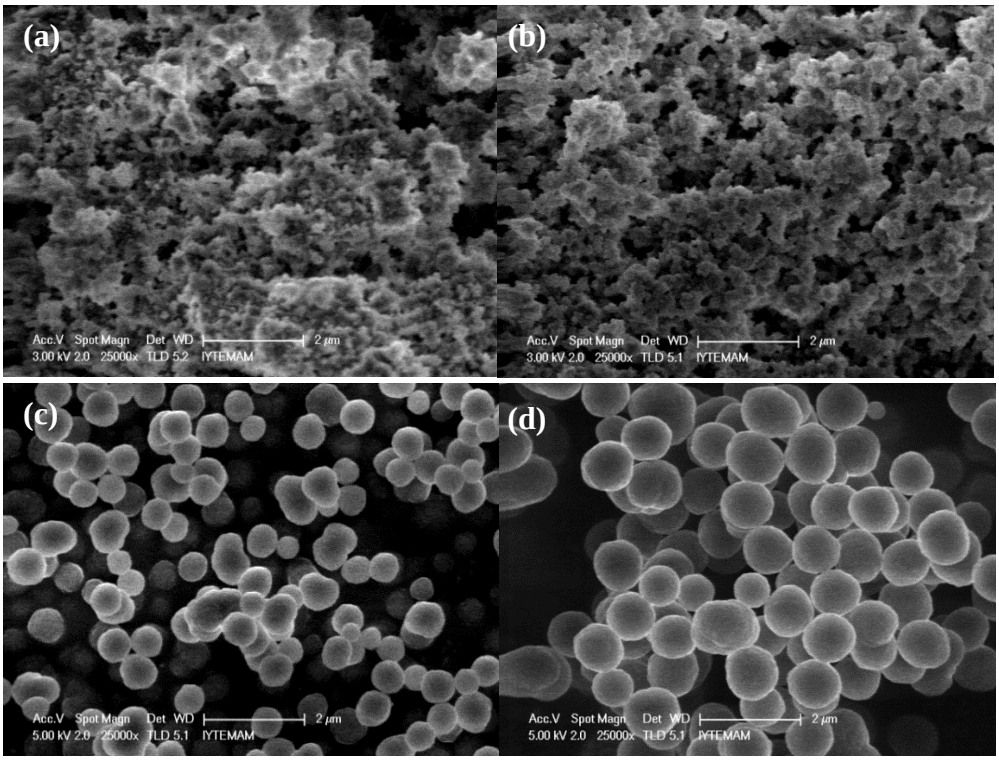


Figure 3.2. SEM images of MIPs: (a) MIP10, (b) NIP10, (c) MIP100, (d) NIP100.

Physical morphologies of MIPs are only the result of use of different amounts of reaction solvents during the synthesis. As mentioned before, amount of solvent versus crosslink ratio determines the physical nature of synthesized polymer (Peter A.G Cormack and Elorza 2004).

The ratio of total monomer to porogen (w/v %, total monomer/porogen) gives information about the method of polymerization. Smaller ratios than 5 % make the polymers to be synthesized with precipitation polymerization method, which resulted in spherical polymer particles with homogenous binding site distribution. For MIP100 and NIP100 particles, the ratio was calculated as 2.5 %. This number explains the spherical shape of the synthesized polymer. (Figure 3.2 (c) and (d)) For the values bigger than 5

%, MIPs are synthesized with bulk polymerization. For MIP10 and NIP10 this ratio is 24.5 % (Figure 3.2 (a) and (b)), which also explains the monolithic morphology of the synthesized sorbents. For bulk polymerization, it is said that the polymers need to be crushed and sieved before use in SPE process. (Nune et al 2010). However, for the MIP particles synthesized there was no need any crushing and sieving.

SEM images have also shown that there was no difference between MIP and NIP particles (Figure 3.2) due to the small ibuprofen cavities created in the MIPs

After the synthesis of polymers, removal processes were applied to wash out ibuprofen, the template, from MIPs as mentioned in Section 1.3.4. The chromatogram of ibuprofen in Figure 3.3 shows that after 10. wash, ibuprofen is completely removed from MIPs.

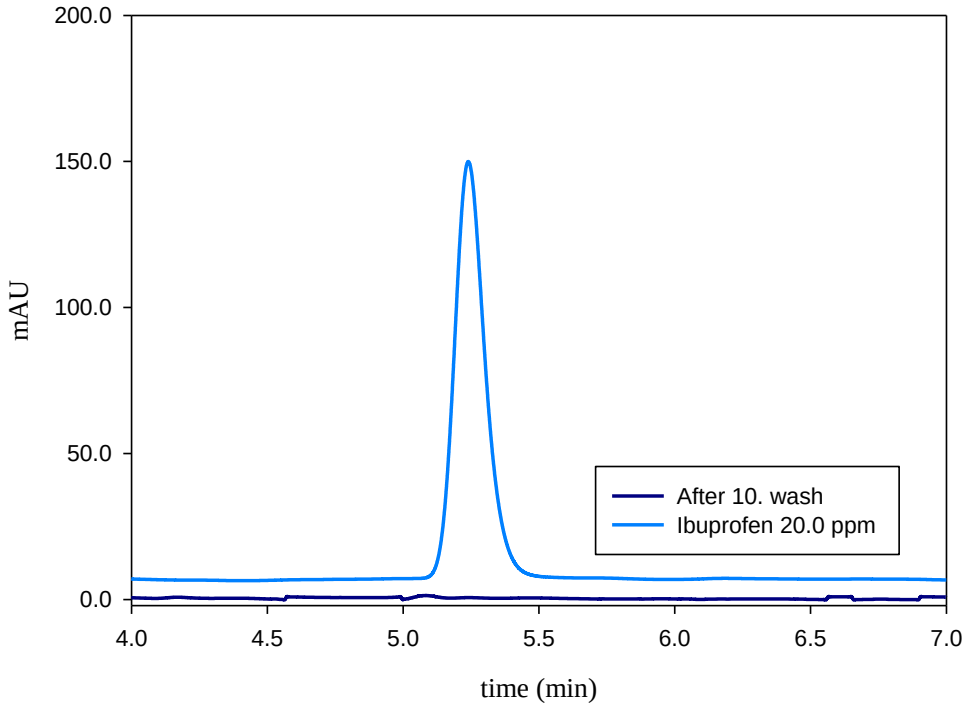


Figure 3.3. Chromatogram of ibuprofen before and after the washing steps. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column, 80:20 MeOH:H_2O (pH 3.0) mobile phase, 0.8 $mLmin^{-1}$ flow rate, 220 nm)

3.3. Characterization Experiments

3.3.1. Binding Characteristic Assay

Sorption capacities of MIP100/NIP100 and MIP10/NIP10 particles are shown in Figure 3.4. The ordinate, Q, shows the ratio of mmol of ibuprofen to 1g of MIP or NIP.

MIP100 and NIP100 particles have shown maximum sorption at 50 mgL^{-1} (Figure 3.4 (a)). The difference in the sorption capacities of MIP100 and NIP100 can clearly be seen after 20 mgL^{-1}. However, MIP10 and NIP10 difference in capacity have seen at 100 mgL^{-1} (Figure 3.4 (b)). This result, the increase in the sorption, must be due to the presence of ibuprofen cavities in MIP100 and MIP10.

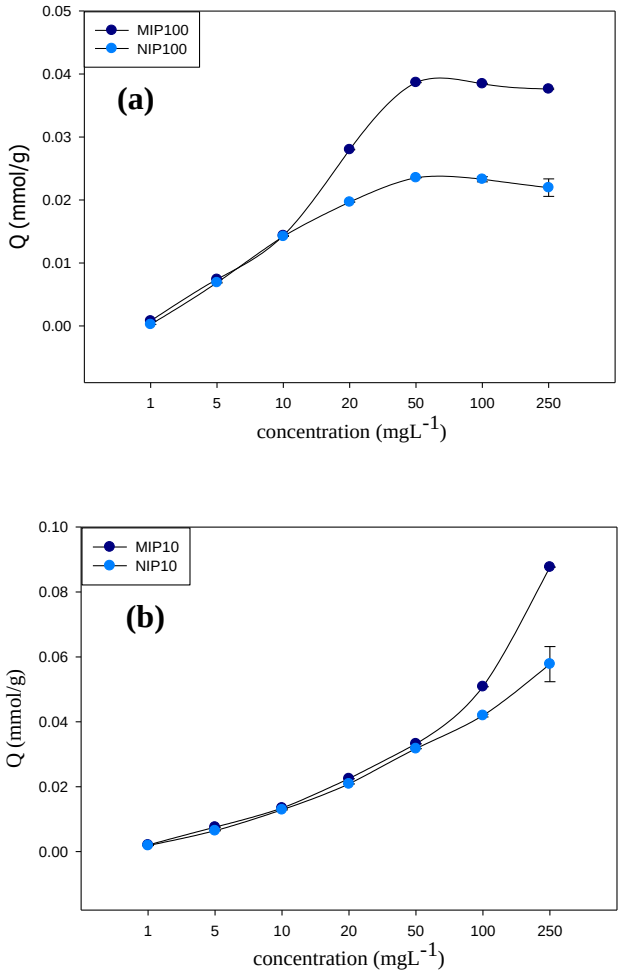


Figure 3.4. Sorption capacities of (a) MIP100/NIP100 and (b) MIP10/NIP10. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column, 80:20 MeOH:H_2O (pH 3.0) mobile phase, 0.8 $mLmin^{-1}$ flow rate, 220 nm)

Rebinding experiments demonstrated that the difference in the sorption capacities of MIP100 and NIP100 was higher than the difference in the sorption capacities of MIP10 and NIP10. Spherical nature with advantage of homogenous distribution of cavities make MIP100 a more favorable sorbent for batch type SPE method. Thence, the rest of experiments were realized by using MIP100 as the SPE sorbent.

3.3.2. Cross Sensitivity

Figure 3.5 shows the sorption capacities of MIP100 and NIP100 obtained by applying the conditions mentioned in Section 2.3.2.

In presence of structurally related compounds, MIP100 shows better sorption capacity against ibuprofen than NIP100. This is again the proof of the presence of the analyte cavities in MIP100. For naproxen and ketoprofen, both of the polymers, showed the same sorption. These two compounds must have been sorbed only on the surface of the polymers, not cavities.

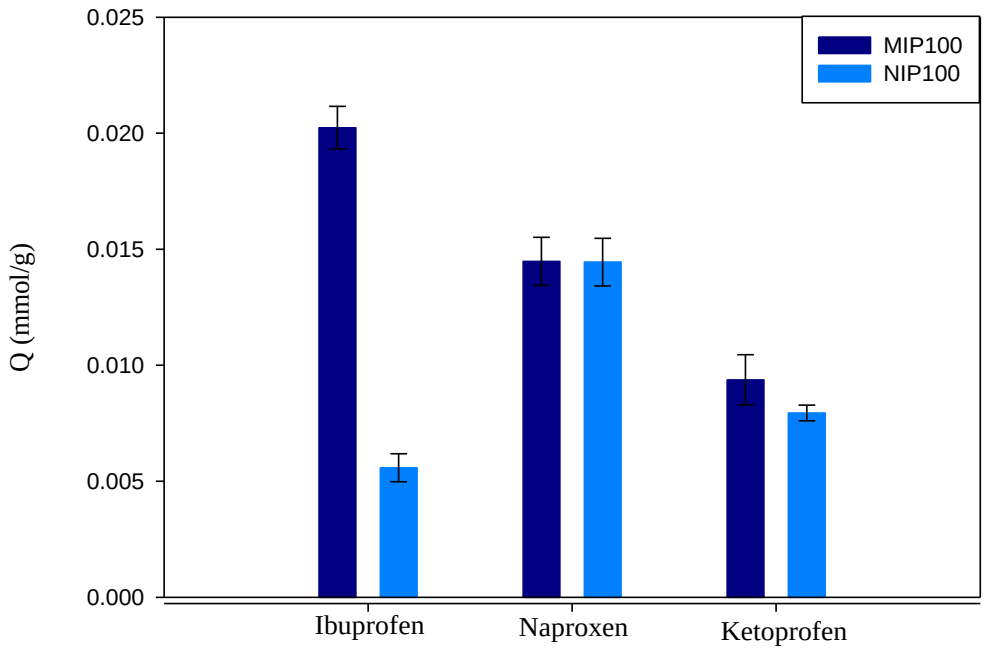


Figure 3.5. Sorption capacity of MIP100 and NIP100 in the presence of structurally related compounds. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column, 80:20 MeOH:H_2O (pH 3.0) mobile phase, 0.8 $mLmin^{-1}$ flow rate, 220 nm)

3.4 Optimization Parameters

3.4.1 Effect of pH on Sorption

Effect of pH on sorption was investigated as described in Section 2.4.1. MIP100 has shown quantitative sorption (99.0%, ± 0.3, n=3) at pH 7.0 and 8.0, and then sorption capacity decreased by increasing pH (Figure 3.6). At pH 8.0, NIP100 has 78.2% (±0.4. n=3) sorption. This decreasing in sorption of ibuprofen can be explained by the increasing –OH concentration which blocks the active sites on the surface. So, the most significant difference between the sorption of MIP100 and NIP100 for ibuprofen was obtained at pH 8.0 and the remaining experiments were carried out at this pH. Here, it should be mentioned that, independent of the initial pH, the pH of the mixtures had changed to 7.0 after having been shaken with the sorbents. This situation can be arising from the zero point charge of the synthesized MIPs.

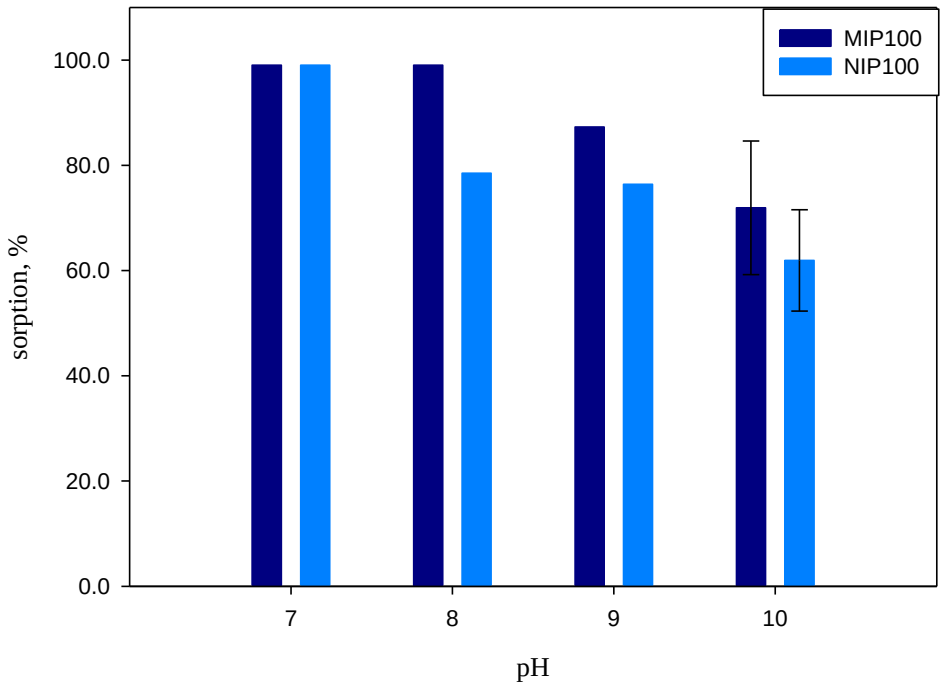


Figure 3.6. Effect of pH on sorption. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column, 80:20 MeOH:H_2O (pH 3.0) mobile phase, 0.8 $mLmin^{-1}$ flow rate, 220 nm)

At first, 78.2 % sorption can be seen sufficient for some purposes and the necessity of MIP100 can be arguable. However, as it has usually been accepted, if any NIP shows good sorption capacity, MIP can be expected to give better results (Baggiani et al. 2012). This explanation is valid here with a sorption percentage of 99.0% with MIP100.

3.4.2 Effect of Sorbent Amount

For each amount of MIP100 sorbent given in Table 2.6., batch type SPE procedure was applied as described in Section 2.4.2. Figure 3.7 shows the effect of sorbent amount to sorption percentage. Up to 20.0 mg of MIP100, sorption increased almost linearly. After this amount, however, sorption percentage did not change (98.5 %, ±0.7251, n=3). To be on the safe side, a sorbent amount of 25.0 mg was applied in the remaining experiments.

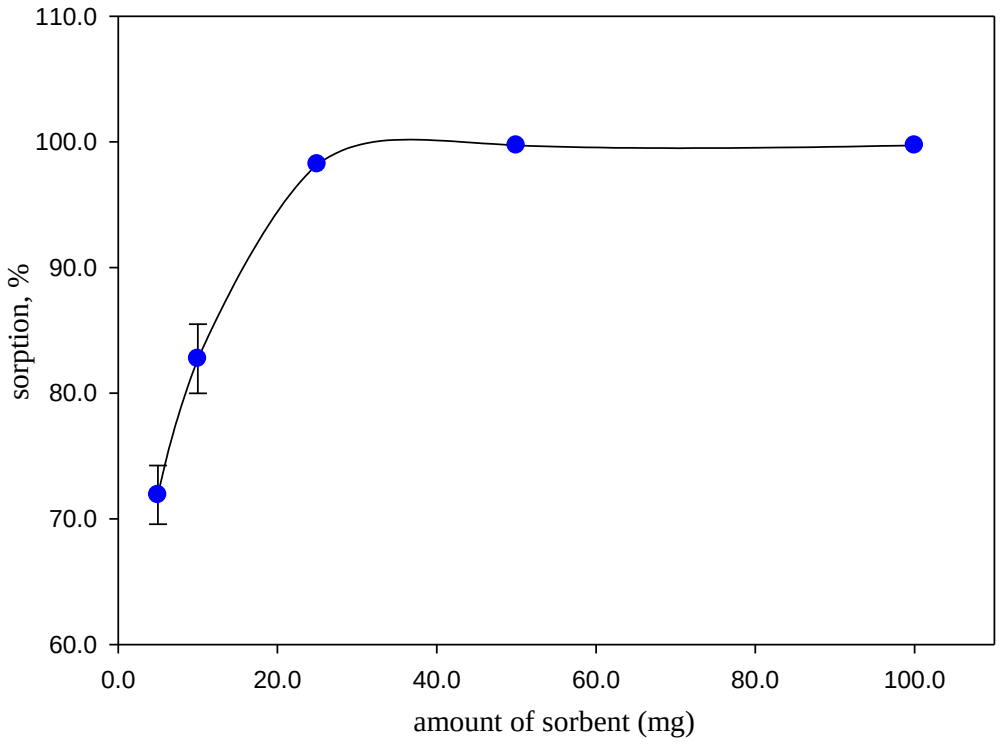


Figure 3.7. Effect of sorbent amount for 10 mL, 1.0 mgL^{-1} of ibuprofen. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column, 80:20 MeOH:H_2O (pH 3.0) mobile phase, 0.8 $mLmin^{-1}$ flow rate, 220 nm)

3.4.3. Effect of Sample Volume

The effect of sample volume in sorption percentage was investigated as described in Section 2.4.3. Figure 3.8 shows that, with the optimized conditions, 5.0 and 10.0 mL of ibuprofen solutions give higher sorption capacity (98.6%, ±3.5, n=3 and 98.5%, ±3.3, n=3, respectively) than the other volumes. In order to guarantee the quantitative sorption, 10.0 mL of 1.0 mgL^{-1} ibuprofen solution was used in the remaining experiments.

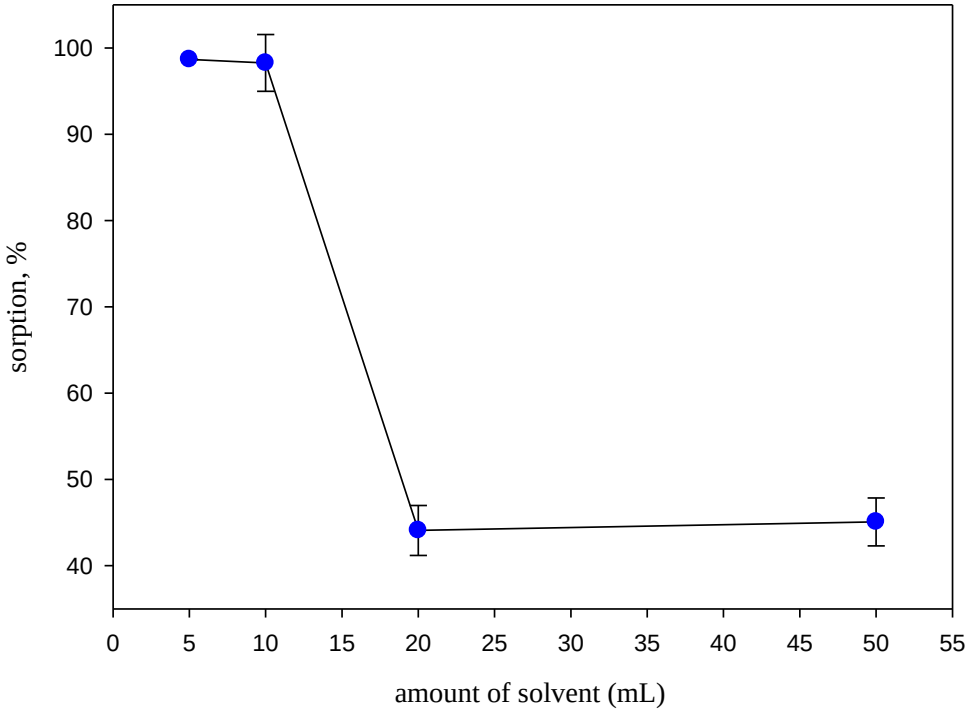


Figure 3.8. Effect of sample volume for 1.0 mgL^{-1} ibuprofen and 25.0 mg MIP100 sorbent. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column, 80:20 MeOH:H_2O (pH 3.0) mobile phase, 0.8 $mLmin^{-1}$ flow rate, 220 nm)

3.4.4. Effect of Sorption Time

By using the predetermined parameters, effect of time on sorption was examined as described in Section 2.4.4. As seen in Figure 3.9, the interaction time was not critical on sorption and 30 min was chosen in order to guarantee the sorption in the remaining experiments.

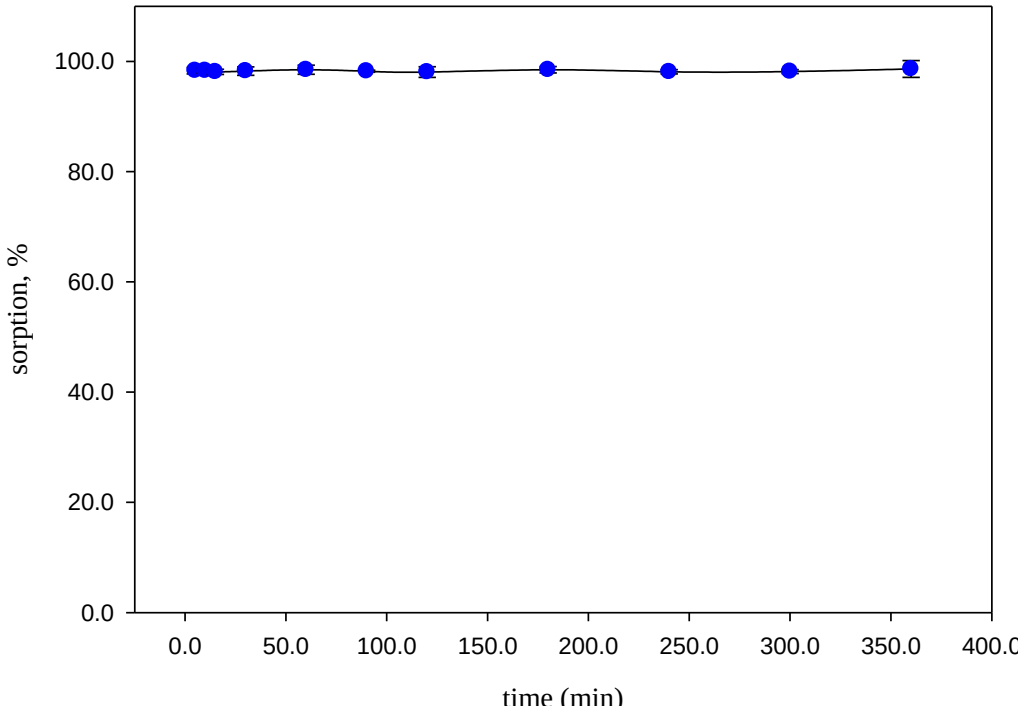


Figure 3.9. Effect of shaking time on the sorption of 10 mL 1.0 mgL^{-1} of ibuprofen and 25 mg MIP100 sorbent. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column, 80:20 MeOH:H_2O (pH 3.0) mobile phase, 0.8 $mLmin^{-1}$ flow rate, 220 nm)

3.4.5 Effect of Eluent Type

Desorption is equally important part of the SPE process as the sorption. Therefore, The analyte sorbed should be recovered from the sorbent with a proper eluent and desorption percentage should be calculated. Two different eluents were tried for the desorption process.

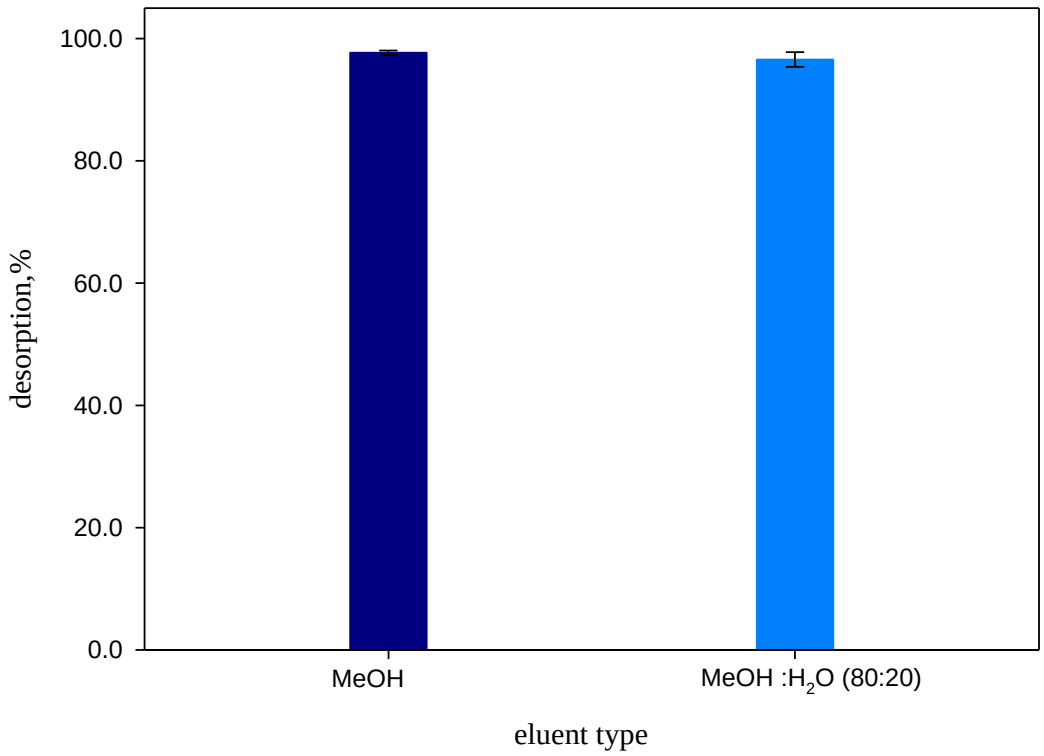


Figure 3.10. Effect of desorption matrix. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column, 80:20 MeOH:H_2O (pH 3.0) mobile phase, 0.8 $mLmin^{-1}$ flow rate, 220 nm)

Methanol (MeOH) and MeOH:H_2O (acetic acid, pH 3) (80:20) were tried as eluents. These solvents disturb the hydrogen bonding between the analyte and the solid sorbent. Although both of the eluents gave >97 % (97.2 %, ±0.8, n=3) desorption as seen in Figure 3.10, MeOH:H_2O (80.20) solution was decided to be used in the remaining experiments since it was the mobile phase employed in HPLC-DAD determinations.

3.4.6. Reusability of Sorbent

To determine the number of times that the sorbent can be used for MISPE process, reusability experiments were realized as described in Section 2.4.6.

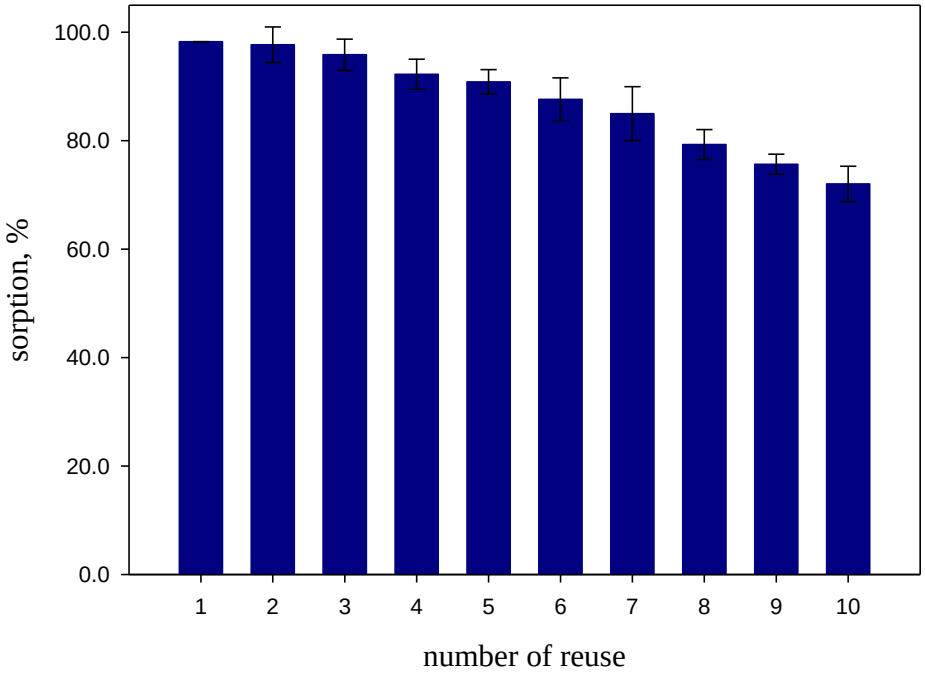


Figure 3.11. Regeneration for reuse. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column, 80:20 MeOH:H2O (pH 3.0) mobile phase, 0.8 $mLmin^{-1}$ flow rate, 220 nm)

Figure 3.11 shows that up to fifth sorption MIP100 shows quantitative sorption (>90.0 %). The decrease in sorption capacity can be explained by the disruption of specific cavities during extraction process. Thus, it can be concluded that the sorbent MIP100 can be used only up to five times with the experimental conditions applied. This is obviously an important point to study on if a competitive sorbent is going to be synthesized.

3.5. Method Validation

MIP100 was used in sorption of ibuprofen in different type of spiked water samples as described in Section 2.5. For all spiked water samples sorption percentages were greater than 97 % for n=3, (97.4 % (±0.3), 97.2% (±0.3), and 97.7 % (±0.2) for ultrapure, drinking and tap water, respectively (Figure 3.12). The identical results of spiked drinking and tap water samples with ultrapure water, clearly shows that the proposed methodology can be applied in real samples. These results indicate that sorption by the MIP was not affected from the possible heavy matrixes of the two water types.

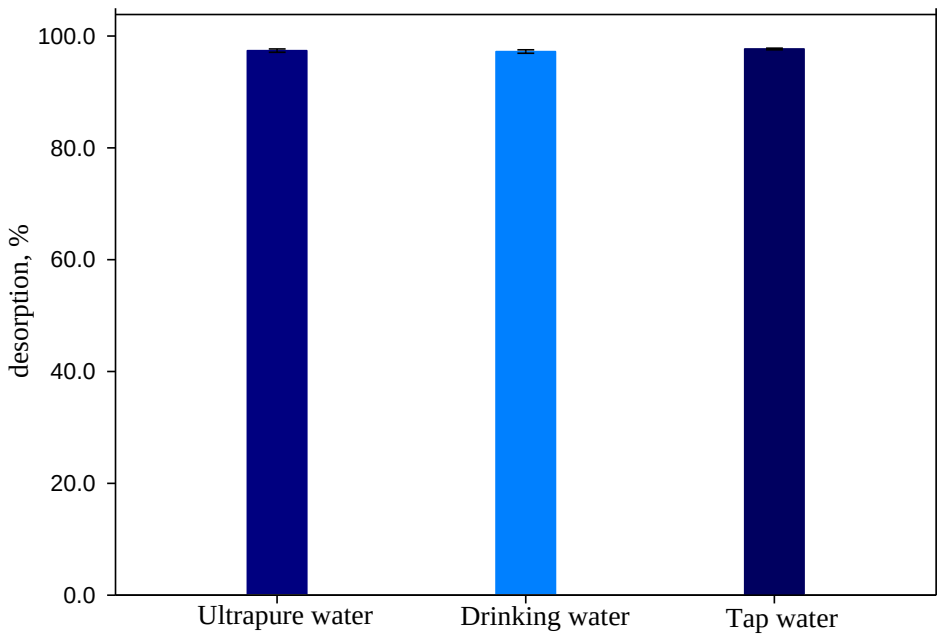


Figure 3.12. Validation of the proposed method with spiked water samples. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (Lichrosphere RP 18-5, 25cm×4.6mm) column, 80:20 MeOH:H_2O (pH 3.0) mobile phase, 0.8 $mLmin^{-1}$ flow rate, 220 nm)

CHAPTER 4

CONCLUSION

In this study, two different types of molecularly imprinted polymers were prepared by precipitation polymerization and bulk polymerization. The so-called MIP100 and MIP10 synthesized by precipitation polymerization for the specific recognition of ibuprofen prior to determination by HPLC-DAD. After characterization steps, MIP100 was chosen for the rest of MISPE optimization steps. The specificity of MIP100 to ibuprofen was also proven in the presence of structurally related compounds, namely naproxen and ketoprofen.

MIP100 showed quantitative sorption (>99 %, ± 0.3, n=3) at pH 7.0 and 8.0. The maximum difference in the sorption of MIP100 and NIP100 was obtained at pH 8.0. Optimized amount of sorbent, amount of solvent and shaking time were determined as 25.0 mg, 10.0 mL, and 30.0 minutes, respectively. Desorption was realized with MeOH and MeOH:H_2O, 80:20 (acetic acid, pH 3.0). Both of the matrixes showed quantitative desorption (>97%, ±0.8, n=3) and MeOH:H_2O, 80:20 (acetic acid, pH 3.0) was employed throughout the study since it was also the mobile phase in HPLC. The proposed method was repeated 10 times with the same MIP100 sorbent. Results show that up to fifth sorption MIP100 shows quantitative sorption (>90%, n=3). The validity of the method was checked via spike recovery experiments with different types of water (drinking and tap). The method worked efficiently for drinking and tap water (97.2% (±0.3, n=3) and 97.7% (±0.2, n=3), respectively).

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