PREPARATION OF FE AND FE-NI BASED MOLECULARLY IMPRINTED POLYMER FOR SOLID PHASE EXTRACTION OF SALICYLIC ACID

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Zeynep BİLDİK

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ABSTRACT

PREPARATION OF FE AND FE-NI BASED MOLECULARLY IMPRINTED POLYMER FOR SOLID PHASE EXTRACTION OF SALICYLIC ACID

Aspirin is the most preferred pain reliever among the people. Salicylic acid (SA) is the drug active ingredient of aspirin and has an antipyretic, analgesic, and anti-inflammatory properties. It is also used in the treatment of acne, fungus, varicose veins, and calluses.

The aim of this project is to prepare Fe and Fe-Ni based molecularly imprinted polymers (MIPs) for solid phase extraction of salicylic acid and then determine it with HPLC-DAD. MIPs were prepared by using methanol as porogen, 4- vinyl pyridine as monomer, ethylene glycol dimethacrylate cross linker and the analyte, salicylic acid, as the template. Fe and Fe-Ni is used to prevent the hydroxyl and carboxyl groups in the structure of salicylic acid from forming hydrogen bonds between themselves.

In this study, a magnetic molecular imprinting solid phase extraction (MMISPE) methodology was proposed for determination of salicylic acid to HPLC-DAD analysis. For this goal, critical experimental parameters of MMISPE method were optimized and determined as follows; sorbent amount of 25.0 mg for 10.0 mL of 5.0 mgL^{-1} working solution, sorption time of 30 min and MeOH: Acetic acid (9:1) as desorption solvent. The proposed method was repeated with same sorbent and MIPs show quantitative sorption in three times. For characterization of MIPs and NIPs, EDX and XRD analysis was done. In XRD diffractogram, the peaks are relatively low intensities indicate that the material is mostly amorphous. Also, Fe and Ni peak cannot observe because of the trace amount of Fe and Ni in the polymer in the EDX graph.

ÖZET

SALİSİLİK ASİDİN KATI FAZ EKSTRAKSİYONU İÇİN DEMİR VE DEMİR-NİKEL BAZLI MOLEKÜLER BASKILANMIŞ POLİMERLERİN HAZIRLANMASI

Aspirin, insanlar arasında en çok tercih edilen ağrı kesicidir. Salisilik asit (SA), aspirinin ilaç aktif bileşenidir ve ateş düşürücü, analjezik ve iltihap önleyici özelliklere sahiptir. Akne, mantar, varis ve nasırların tedavisinde de kullanılmaktadır. Öte yandan aşırı kullanımı alerjik reaksiyonlara ve çeşitli kanamalara neden olabilir.

Bu projenin öncelikli amacı, demir ve demir-nikel bazlı moleküler baskılanmış polimer (molecularly imprinted polymers, MIPs) sentezi ile salisilik asitin katı faz ekstraksyionu için hazırlanması ve sonrasında HPLC-DAD ile tayin edilmesidir. MIP'ler monomer olarak 4-vinilpiridin, çapraz bağlayıcı olarak etilen glikol dimetakrilat, porojen olarak methanol ve şablon analit olarak salisilik asit kullanılarak hazırlanmıştır. Salisilik asidin yapısında bulunan karboksil ve hidroksil grubun kendi arasında hidrojen bağı yapmasını engellemek için demir ve demir-nikel kullanılmıştır.

Bu çalışmada, salisilik asitin HPLC-DAD ile tayini öncesinde moleküler baskılanma polimer katı faz ekstraksiyonuna (MISPE) dayanan bir methodoloji önerilmektedir. Bu amaçla MISPE metodunun kritik deneysel parametleri optimize edilmiş; bu değerler, çözelti pH'sı 6.0, 10.0 mL 5 mgL^{-1} çalışma çözeltisi için sorbent miktarı 25.0 mg, sorpsiyon süresi 30 dakika ve desorpsiyon çözeltisi olarak 90:10'luk MeOH:Asetik asit olarak belirlenmiştir. Önerilen yöntem aynı sorbent ile tekrarlandı ve MIPs üç kez nicel sorpsiyon kapasitesi göstermetedir. MIP ve NIP karakterizasyonu için EDX ve XRD analizi yapıldı. XRD difraktogramında, düşük şiddetli spektrumlar gözlenlendi, bu da malzeminin çoğunlukla amorf bir yapıya sahip olduğunu gösterir. Ayrıca, EDX grafiğinde, polimerdeki demir ve nikel miktarının az olmasından dolayı demir ve nikel spekturumları gözlenememiştir.

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CHAPTER 1

INTRODUCTION

1.1. Salicylic Acid

Salicylic acid (SA) can be obtained both naturally and synthetically. Salicylic acid is found in willow tree bark as a natural product. It functions as a plant hormone and is essential for photosynthesis enhancement and transpiration.

Salicylic acid, also known as 2-hydroxoybenzoic acid, is a major metabolite of aspirin in plasma and is generally applied for a variety of dermatological processes to treat skin. Because of the beneficial properties such as keratolytic, bacteriostatic, fungicidal and photo protective, it is the world's most widely utilized analgesic, antipyretic, and anti-inflammatory agent. In dermatology, salicylic acid is primarily used as a keratolytic agent to cure fever and pain (Jafari, Badihi et al. 2012). Salicylic acid reduces sebum release enhancing its therapeutic effect in acne patients. In the treatment of this disease, concentration of 5% (m:v) or less is used. Salicylic acid concentrations ranging from 10% to 40% (m:v) are effective in treating warts and localized hyperkeratosis. Concentrations of 6% or higher are acceptable in creams and lotions (Madan and Levitt 2014).

1.2. Levels Of Salicylic Acid In Dermatological Products

Salicylic acid can be found in a variety of forms in dermatological products. The specified dosage and usage in the therapy is the most significant aspect. It can cause local skin irritation if the amount is not regulated. The dose and the frequency of use are detailed in Table 1.1. For example, the lotions and solutions are applied directly; therefore, the absorption in these cases is fast, so it requires to be used 1 to 3 times per day. The gel form has a longer duration of action since it is released slowly into the skin. Children under the age of two should not be exposed to salicylic acid because the

children's skin absorbs salicylic acid at a faster rate than adults. They may be more susceptible to skin irritation (Frothingham 2019).

Table 1.1. Levels of salicylic acid in various products

Form	Salicylic acid percentage	How frequently it is used
Gel	0.5-5%	Once per day
Lotion	1-2%	1 to 3 times per day
Ointment	3-6%	As needed
Pads	0.5-5%	1 to 3 times per day
Soap	0.5-5%	As needed
Solution	0.5-2%	1 to 3 times per day

1.3. Toxicity Of Salicylic Acid

Positions of the carboxyl or hydroxyl groups found in the structure of salicylic acid alter its potency and toxicity. The ortho position of hydroxyl group affects its action. This structure leads salicylic acid to exert effects on pain, kidneys, hearts, blood, and immunologic systems and also generating local irritation.

Benzene ring in salicylic acid converts ultraviolet sunlight into longer wave radiation, which is radiated from the skin as heat, giving a sunscreen effect. This effect causes dryness, skin irritation and rash (Madan and Levitt 2014).

These harmful effects can be prevented when applied to a small part of the body for a short time (Frothingham 2019).

1.4. Determination Methods Of Salicylic Acid

There are many analytical techniques for salicylic acid determination in different samples. In some of these methods, before the instrumental determination, commercial solid phase extraction (SPE) cartridges are generally employed for pre-concentration of the analyte. For instance, McMahon and Kelly (1998) developed a new column-switching method for the detection of salicylic acid and aspirin. They used a PEEK cartridge (Anachem) in SPE method before HPLC determination. In the reported study, only 300

μL of plasma was needed for analysis and the technique could be easily mechanized using the 10-port switching valve. In another work by Pirker et al. (2004), willow bark extract has been used to produce a method for extracting gentisic, salicyluric, and salicylic acid from human plasma. Oasis HLB (Waters) cartridges were used in the pre-concentration method. A method combining RP-HPLC, electrospray ionization mass spectrometry (ESI-MS), fluorescence detection (FLD) and diode array detection (DAD) has also been optimized. The recovery rates for gentisic acid ranged from 91.3-102.1%, for salicyluric acid from 86.8-100.5%, and for salicylic acid from 75.8-81.4% by the analysis with RP-HPLC-ESI-MS. Rozhon et al. (2005) developed a method to measure free and total soluble salicylic acid (SA) in *Arabidopsis thaliana* using 5-fluorosalicylic acid (5-FSA) as a standard solution since its absorption and fluorescence spectra are similar to those of SA. In this study, phenyl-phase cartridges were used for the SPE process which gave similar recoveries for salicylic acid (SA) and 5-FSA (*ca.* 90%). In another work by Mikami et al. (2002), an HPLC method for direct detection of dehydroacetic acid (DHA), benzoic acid (BA), sorbic acid (SOA), and salicylic acid (SA) was developed for use in cosmetics. The preconcentration technique was carried out using Bond-Elut SI cartridges. The recoveries were 2.5 ng DHA, 4.0 ng BA, 2.0 ng SOA, and 5.5 ng SA, respectively. Fang et al. (2018) determined four salicylic acid related compounds in aloe by using liquid chromatography photodiode array detector (LC-PDAD) with solid phase micro extraction (SPME). A specially designed SPME fiber was proposed in the study. The limit of detections for the final method were 2.8 μgL^{-1} for acetyl salicylic acid, 2.6 μgL^{-1} for salicylic acid, 1.8 μgL^{-1} for 4-SA and 2.2 μgL^{-1} for 3-SA.

Jafari et al. (2012) identified the salicylic acid in human urine and plasma using molecular imprinting method for sample preparation which provides selective separation prior to negative electrospray ionization ion mobility spectrometry (ESI-MS). The molecularly imprinted polymer (MIP) was synthesized using a non-covalent approach with SA as template and 4-vinyl pyridine as monomer. The study of SA yielded a LOD 0.022 $mgmL^{-1}$ and RSD of less than 6%. For the analyzed medicine, the mean recovery was calculated to be around 92 percent. In other work by Ma et al. (2017), molecular imprinting polymers were used to perform a selective electrochemical determination of salicylic acid in wheat. The modified electrodes measured cyclic voltammetry and electrochemical impedance spectroscopy. Precipitation method was used to synthesize the molecular imprinted polymer. Salicylic acid concentrations ranging from $5x10^{-10}$ to

$5x10^{-5}$ $molL^{-1}$ were measured in the study, demonstrating that the proposed sensor was suitable for food analysis. Parham and Rahbar (2009) demonstrated a new approach for determining salicylic acid in blood serum utilizing magnetic iron oxide nanoparticles as an extractor. The solid phase extraction-spectrophotometric technique was quick and easy to apply for the determination of SA with the detection limit of $5.5x10^{-3}$ gmL^{-1}. From the tested samples for salicylic acid, the intra-day precision was less than 10.1% and the inter day RSD was % 3.6.

One of the most time-consuming steps in analytical techniques is sample preparation. The sample preparation step usually consists of separation (and/or pre-concentration) of target chemicals from biological, pharmacological, environmental and food matrices. Extraction is usually required before chromatographic procedures in order to separate the analytes from interfering matrix components and to enrich them prior to analysis (Buszewski and Szultka-Mlynska 2012).

1.5. Solid Phase Extraction (SPE)

For separation and pre-concentration of specified analytes in samples, a variety of extraction techniques are frequently used. Solid phase extraction (SPE), liquid-liquid extraction (LLE), and ion exchange are among them.

Solid phase extraction (SPE) has become an increasingly popular method and is widely considered as an important sample pre-treatment technique. SPE methods are used not only for extraction, but also for eliminating interfering components of complex matrices in order to purify the analytes. SPE sample preparation can improve the stability and reliability of biological, pharmacological, and environmental applications. SPE has recently been used to replace LLE in biological samples prior to analysis due to its simplicity, stability, minimal solvent use, and ease of automation. Figure 1.1 describes the four essential steps involved in SPE. First step is activation of the SPE column/cartridge by using some solvents. Second step is loading the sample onto the SPE column. Third step is washing the SPE column to avoid impurities. Last step is elution of the extracted analyte(s) from the SPE column by using a suitable solvent. This step also ensures the reproducibility of the method (Sun et al. 2011).

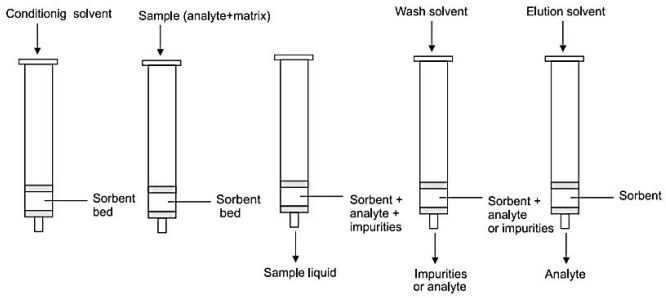


Figure 1.1. The basic procedure of SPE method

(Source: Zwir-Ferenc and Biziuk 2006)

The SPE technique is classified into two types. The first one is column type. The column is initially loaded with sample solution. The solid sorbent absorbs the analyte, which is then recovered using a small quantity of eluent. The percentage of elution is determined by the concentration of analyte in the eluate.

In batch type SPE method (Figure 1.2), in sorption step the sorbent is directly mixed with the sample solution and the resulting mixture is shaken for a predetermined time. After this step, a filtration process is applied, and the sorbent is collected for elution step. In the elution step, the analytes are eluted from the sorbent by a proper eluent in a similar manner to the sorption step.

In general, SPE mechanism is based on the van der Waals forces, hydrogen bonds, polar interactions, or ionic interactions between the analyte and the sorbent. This interaction influences the selection of SPE phase, so it is necessary to understand some sorbent and solute features such as polar and ionogenic characteristics (Zwir-Ferenc and Biziuk 2006). SPE separation modes include normal and reverse phase extraction, and ion exchange extraction.

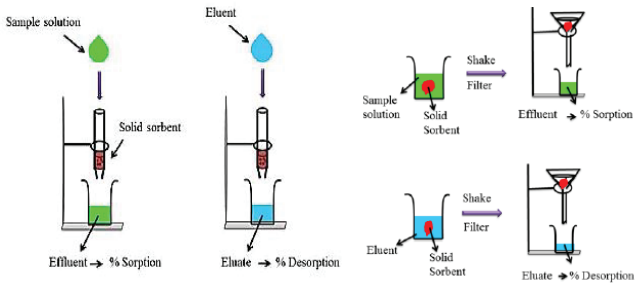


Figure 1.2. Column and batch type of SPE

(Source: Ölçer 2016)

Diatomaceous earth, silica, and alumina are generally used as extraction sorbents. Diatomaceous earth, silica, and alumina are generally used as extraction sorbents. Non-polar or weakly polar compounds can be extracted with silica. Non-polar alkanes, such as C_{18} and C_8, are used in the reverse phase extraction columns, while divinyl benzene polystyrene resin modified with weak/strong acid/base functional groups are used as the ion exchange sorbent. On the other hand, traditional SPE sorbents have low selectivity, which make it difficult to extract specific analytes from complicated environments and biological processes and have low enrichments. There are various forms of solid sorbents that have been produced and employed. Selecting a suitable sorbent with the required functional groups to interact with the analyte molecule is essential for a successful SPE operation. Molecular imprinting is a process that is increasingly being used to create functional compounds with good selectivity for a certain analyte (Sun et al. 2011).

1.5.1 Molecular Imprinted Polymers (MIPs)

The use of MIP has received a great deal of attention in recent years. It has gained popularity in a variety of domains, including chiral separation, sensors, and immunoassay. The molecular imprinting approach allows analyte selectivity due to the presence of special functional sites in functional material. This approach is quite useful for distinguishing between two structurally related compounds. They have several significant advantages including ease of preparation, cost, speed, repeatability, and sample load capacity (Buszewski and Szultka-Mlynska 2012) .

The principle mechanism of MIPs is based on the lock and key idea of protein-guest interaction (Zaidi 2016). The form and location of the functional groups at the recognition sites inside the polymer matrix are complimentary to the analytes (Buszewski and Szultka-Mlynska 2012). The main purpose of MIPs is to create specific cavities for the analyte (template). These cavities provide selectivity or specificity for target molecule.

In Figure 1.3, the basic steps in the synthesis of MIPs are shown. Firstly, the monomer and template are mixed in a suitable solvent to form prepolymerization complexes through covalent or non-covalent interactions. After that, cross linker and initiator are added. The cross linker surrounds the monomer and forms a complex

structure with a three-dimensional polymer network and polymerization occurs. After the synthesis, the polymer is washed with a proper solvent and the template molecules are removed to generate imprinted cavities which are appropriate for the analyte molecule in terms of size, shape, and molecular arrangement. An additional polymer is synthesized under the same conditions, but without the addition of the template. In this case, imprinted sites are not formed, and polymer is known as non-imprinted polymer (NIP). The selectivity can be determined by comparing the performances of two polymers.

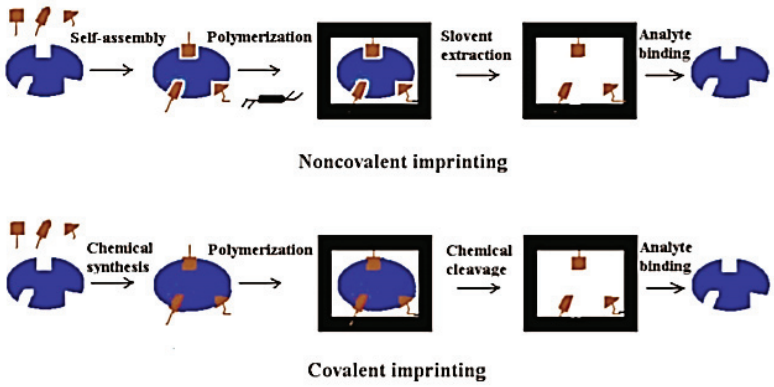


Figure 1.3. Schematic representation of MIPs
(Source: Buszewski and Szultka-Mlynska 2012)

MIPs can be prepared in a variety of ways as shown in Figure 1.4. In most cases, the bulk polymerization process is used. Heat or ultraviolet irradiation is used to polymerize the monomer and crosslinker in the presence of template. The polymer is washed until the templates are removed. The grinding and sieving process can be applied however it may degrade imprinted cavities and reduces its efficiency. Also, if crosslinker is employed in excess, this approach produces non uniform particles with a wide size distribution, making template removal difficult. As a result, the imprinted cavity in the polymer is poor and small. The second approach shown in the figure is in-situ polymerization in which the prepolymerization mixture is filled in tubing/capillary and polymerization reaction takes place in situ. This method usually is the method of choice in the preparation of capillary columns with improved separation in the analysis. The last method shown in the figure is suspension polymerization process in which microsphere materials are created. In this polymerization method, a hydrophobic monomer and a polar

organic solvent or water are used to break the bond between the monomer and template (Sun et al. 2011) .

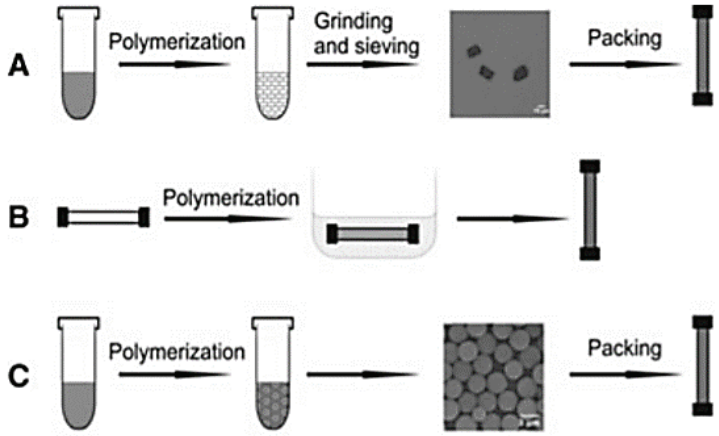


Figure 1.4. (A) Bulk polymerization, (B) In situ polymerization, (C) Suspension polymerization (Source: Cheong et al. 2013)

MIPs have two main disadvantages. The template molecules cannot be cleaned easily, and mass transfer resistance is considerable, so the quantity of analyte that can be adsorbed is significantly reduced. Therefore, strategies to overcome these disadvantages should be developed. In this line magnetic particles appear as promising materials. Magnetic nanoparticles contain the special features of nanocrystals, such as small size effect, super magnetism, and surface effects, and may be functionalized by active functional groups, such as -COOH, -OH, and $–NH_2$, to facilitate the interaction of analyte with molecular imprinted polymers during the sample pretreatment step. Magnetic molecular imprinted polymer provides demonstrated advantages such as easy template removal, high binding capacity, low mass transportation resistance, fast binding kinetics, and large specific surface area (He et al. 2019).

1.5.1.1 Magnetic Molecularly Imprinted Polymers

Magnetic nanoparticles have received a lot of attention in recent years because of their potential uses in magnetic separation, biosensors, biological imaging, and drug administration. Magnetic materials have a high surface to volume ratio, are quick and effective in binding target analytes, and have a high magnetic susceptibility. Also, the

isolation and extraction of target molecules is very easy to manage by imposing an external magnetic field. Combining magnetic separation with molecular imprinting would be a great approach to create a strong analytical tool for separation applications (Liu et al. 2011).

A typical process employing magnetic materials for analyte extraction is demonstrated in Figure 1.5. In this method, magnetic materials are introduced to the solution or suspension containing the target analytes. Then, the analytes are adsorbed onto the magnetic sorbents. In the next step, a suitable magnetic separator is used to separate the sorbent containing the analytes from the sample solution. Subsequently, the analytes are eluted from the sorbents with a suitable solvent and then the eluate is analyzed.

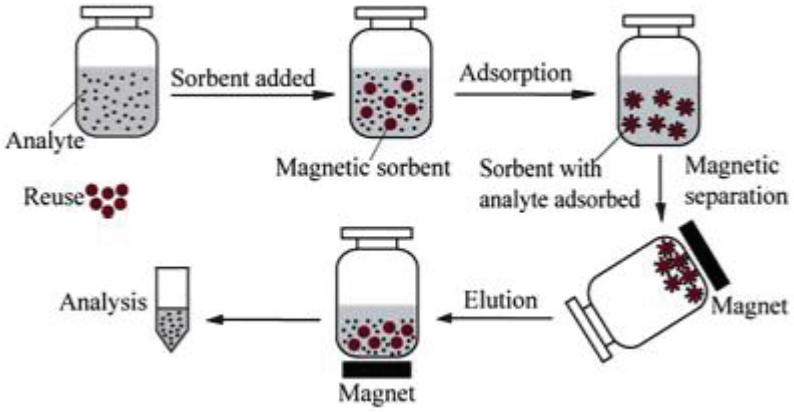


Figure 1.5. Magnetic separation procedure

If such magnetic components are enclosed in MIPs, the resulting composite polymers, magnetic MIPs (MMIPs), will exhibit magnetic susceptibility as well as selectivity for the guest molecules. Different approaches have been used to make the MMIPs. The most widely utilized preparation method is divided into four stages (Figure 1.6). The first step is the production of magnetic nanoparticles, such as Fe_3O_4. The second step is modification or functionalization of the magnetic nanoparticle. The third step is surface imprinted polymerization utilizing a functionalized nanoparticle as a magnetic core. The fourth step is the removal of the template molecules from the polymer. The magnetic nanoparticles are generally modified or functionalized in the second preparation phase using one of two ways. The process of silanization is one of the options. Stober technique is used to coat magnetic nanoparticles with SiO_2 shells, which are subsequently

modified using a silane coupling agent. Surfactants, such as oleic acid or ethylene glycol, are the other options for the modification of the particles (Chen and Li 2012).

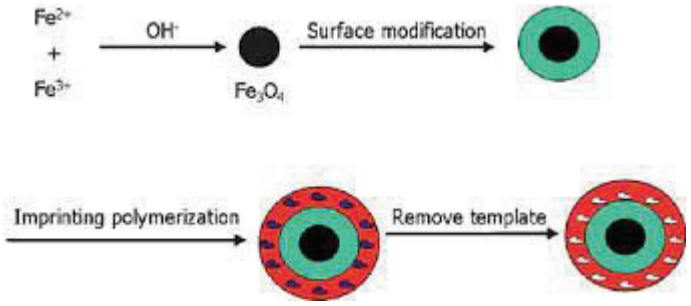


Figure 1.6. The general steps of the preparation of magnetic molecularly imprinted polymer

1.6. Aim Of The Study

The aim of the thesis is to prepare Fe and Fe-Ni based molecularly imprinted polymers (MIPs) for solid phase extraction of salicylic acid prior to its determination by HPLC-DAD.

CHAPTER 2

EXPERIMENTAL

Experimental part is composed of three sections. The first section is about the optimization of operating conditions for determination of salicylic acid with HPLC-DAD. The second part is preparation of bimetallic nanoparticles. The third part is related to the synthesis of Fe and Fe-Ni based molecularly imprinted polymers (MIPs).

2.1. Optimization Of HPLC-DAD Parameters

Firstly, for the determination of salicylic acid, HPLC conditions were optimized. A stock solution of salicylic acid (500 mgL^{-1} in methanol) was prepared in an amber glass bottle and stored in refrigerator at -18 °C.

Agilent 1200 High Performance Liquid Chromatography (HPLC) with Diode Array Detector (DAD) was used in all analyses. The tested optimization conditions of HPLC-DAD are given in Table 2.1. As mobile phase, various solutions and their mixtures were investigated. The mixture of 85:15 Methanol: Water (pH of aqueous phase was adjusted to 2.5 using acetic acid) and the flow rate of 1.0 $mLmin^{-1}$ provided the best results. Also, Limit of Detection (LOD) and Limit of Quantification (LOQ) were found by analyzing the lowest concentration in the standard solutions by 3 and 10 times, respectively.

Table 2.1. Optimization conditions of HPLC-DAD

Column	Sunfire C18 (4.6x250mm) column
Mobile phase	100% Methanol 100% Acetonitrile Methanol/Water (acetic acid, pH:3) (90:10) Methanol/Water (acetic acid, pH:3) (85:15) Methanol/Water (acetic acid, pH:2.5) (85:15) Methanol/Water (acetic acid, pH:2.5) (90:10)
Thermostat temperature	30°C
Sample injection volume	20.0 µL
Flow rates	0.8, 1.0 $mLmin^{-1}$
Standard solutions	0.05, 0.25, 1.0, 5.0 mgL^{1}

2.2. Preparation Of Fe, Fe-Ni And Fe-Cu Nanoparticles

In 12.5 mL of solution made of pure ethanol and distilled water (4:1, v/v), a 2.67 g $FeCl_2.4H_2O$ was dissolved. 1.27 g $NaBH_4$ was dissolved in 35.0 mL distilled water to make a 1 M solution as the reducing agent. Excess borohydride is commonly used to speed up the process and to ensure that iron particles develop uniformly. Drop by drop the $NaBH_4$ solution was added to the Fe^{2+} solution which was continuously stirred. After the first drop of $NaBH_4$ solution was added, black particles of nZVI started to emerge. Following the addition of all $NaBH_4$ solution, the mixture was left to mix for 20 minutes more. Vacuum filtering with blue filter paper was used to separate the black iron particles from the solution. After that, the solid was rinsed three times with ethanol. This washing step proved to be important in protecting nZVI from rapid oxidation when exposed to the environment. Finally, the powder was placed in a watch glass and dried overnight at room temperature (25°C) under N_2 atmosphere in an home-made dryer (Üzüm, Shahwan et al. 2008).

The same procedure was applied for production of Fe-Ni and Fe-Cu nanoparticles. The amount of 2.485 g $FeCl_2.4H_2O$ and 2.46 g $NiCl_2.6H_2O$ was used to synthesize Fe-Ni nanoparticles and 2.485 g $FeCl_2.4H_2O$ and 2.125 g $CuCl_2.2H_2O$ was used to synthesize Fe-Cu nanoparticles.

2.3. Preparation Of Fe Based Magnetic Molecular Imprinted Polymer

For the preparation of MIP and NIP particles the same process should be used, with exception of template molecule (salicylic acid) for NIP synthesis. Experimental procedure used for the MIP synthesis was as follows: Initially, 1.0 mmol salicylic acid (template), 0,013 mmol iron (II) chloride tetra hydrate and 6.0 mmol 4-vinylpyridine (monomer) were dissolved in 10.0 mL methanol and mixed for 1.0 hour. Then, 30.0 mmol ethylene glycol dimethacrylate (EDGMA) was added to the mixture as a crosslinker. Finally, the mixture was purged with Ar to remove the dissolved oxygen and 0.30 mmol of 4,4'-azobis(cyanovalericacid) (AIVN) was added as an initiator. Polymerization reaction was performed in an oil bath at 60°C for 24 hours.

After polymerization, the solid powder of MIP and NIP were obtained. The mixture of methanol: acetic acid (90:10, v: v) was used to remove the template molecule from the MIP structure. After removing process, MIP and NIP were dried in oven at 60°C.

2.4. Preparation Of Fe-Ni Based Magnetic Molecular Imprinted Polymer

The preparation of Fe-Ni based magnetic molecular imprinted polymer consisted of two steps.

a) First step: Synthesis of Fe-Ni@MPS

250.0 mg Fe-Ni nanoparticles was dispersed in 150.0 mL MeOH and mixed for 20 minutes. Then, 10.0 mL of 3-(methacryloyloxy) propyl trimethoxysilane (MPS) was added dropwise to the mixture and left for 48 hours under continuous stirring. Resulting particles were collected with the help of an external magnet, rinsed with methanol thoroughly and dried under vacuum.

b) Second step: Synthesis of Fe-Ni@MPS@MIP and NIP

Initially, 1.0 mmol salicylic acid (template) and 6.0 mmol 4-vinylpyridine (monomer) were dissolved in 10.0 mL of methanol and mixed for 24 hours. After that 250.0 mg Fe-Ni@MPS was added to the mixture and shaken for 4 hours. Then, 30.0 mmol of EDGMA was added to the mixture as a crosslinker. Finally, the mixture was purged with Ar gas and 0.30 mmol of AIVN was added as initiator. The polymerization

step was carried in an oil bath at 60°C for 24 hours. The same procedure was used for preparation of NIP with exception of the template molecule (salicylic acid).

After polymerization, the solid powder of MIP and NIP were obtained. The mixture of methanol: acetic acid (90:10, v: v) was used to remove of the template molecule from the polymer structure. After the removal process, MIP and NIP were dried in oven at 60°C.

2.5. Sorption Of Salicylic Acid With Metallic Nanoparticles

2.5.1. Effect Of The Analyte Concentration On Sorption Of Salicylic Acid

The experimental parameters are summarized in Table 2.2. As a sorbent 10.0 mg of Fe, Fe-Ni and Fe-Cu nanoparticles were weighed and placed in vials. To each vial 10.0 mL of standard solutions was transferred and shaken at 480 rpm for 1 hour. After that the mixture was filtered through cellulose acetate membranes (0.45 μm pore size). The solution was analyzed by HPLC-DAD.

Table 2.2. Parameters of binding experiment

Standard concentrations	0.25, 0.5, 1.0, 5.0 mgL^{-1}
Amount of sorbent	10.0 mg
Sample solution volume	10.0 mL
Sorption time	1 hour

2.5.2. Effect Of Solution pH On Sorption Of Salicylic Acid

The experimental parameters used in this study are summarized in Table 2.3. To investigate the effect of pH on sorption of salicylic acid standard solutions with various pHs were prepared (adjusted with acetic acid) as given in the table. Sorbents, Fe, Fe-Ni and Fe-Cu (10.0 mg) were weighed and placed in vials. Following this step, 10.0 mL of the solutions with various pHs were transferred into the vials and shaken at 480 rpm for 1 hour. After that the solid/liquid mixtures were filtered through cellulose acetate

membranes (0.45 µm pore size). The pHs of the solutions were also measured after the sorption process to check if they have changed during sorption.

Table 2.3. Experimental parameters used for the investigation of the effect of solution pH on the sorption of salicylic acid

Standard concentrations	0.25, 0.5, 1.0, 5.0 mgL^{-1}
pH	2.0, 4.0, 6.0
Amount of sorbent	10.0 mg
Solution volume	10.0 mL
Sorption time	1 hour

2.6. Optimization Of Extraction Parameters For Fe-Ni Nanoparticles

2.6.1. Effect Of Sorbent Amount On Extraction Of Salicylic Acid

To investigate the effect of sorbent amount on extraction of salicylic acid, Fe-Ni was weighed at different amount as given Table 2.4 in vials. 10.0 mL of 5.0 mgL^{-1} salicylic acid solution was prepared and 10.0 mL of the solution was transferred into each vial and shaken at 480 rpm for 1 hour. After that the mixtures were filtered through cellulose acetate membranes (0.45 µm pore size). The samples were analyzed by HPLC-DAD at 240 nm.

Table 2.4. Experimental parameters used for the investigation of the effect of sorbent amount on the sorption of salicylic acid

Standard concentration	5.0 mgL^{-1}
pH	6.0
Amount of sorbent	5.0, 10.0, 25.0, 50.0 mg
Solution volume	10.0 mL
Sorption time	1 hour

2.6.2. Effect Of Sample Volume On Sorption Of Salicylic Acid

To investigate the effect of the sample volume on extraction 25.0 mg Fe-Ni was weighed in vials. Then, different volumes of 5.0 mgL^{-1} salicylic acid solution as indicated

in Table 2.5 were added into the vials with sorbents and shaken at 480 rpm for 1 hour. Finally, the mixtures were filtered through cellulose acetate membranes (0.45 μm pore size) and analyzed with HPLC-DAD at 240 nm.

Table 2.5. Experimental parameters used for the investigation of the effect of sample volume on the sorption of salicylic acid

Standard concentration	5.0 mgL^{-1}
pH	6.0
Amount of sorbent	25.0 mg
Solution volume	5.0, 10.0, 20.0 mL
Sorption time	1 hour

2.6.3. Effect Of Shaking Time On Sorption Of Salicylic Acid

To investigate the effect of shaking time on extraction of salicylic acid, Fe-Ni (25.0 mg) was weighed in vials. 10.0 mL of 5.0 mgL^{-1} salicylic acid solution was added to each vial and the resulting mixture was shaken at 480 rpm for different time intervals as given in Table 2.6. After each time, the solid/liquid mixtures were filtered through cellulose acetate membranes (0.45 μm pore size) and the samples were analyzed by HPLC-DAD at 240 nm.

Table 2.6. Experimental parameters used for the investigation of the effect of shaking time on the sorption of salicylic acid

Standard concentration	5.0 mgL^{-1}
pH	6.0
Amount of sorbent	25.0 mg
Sorption time	1.0, 5.0, 15.0, 60.0, 120.0 min

2.7. Sorption Of Salicylic Acid With Fe And Fe-Ni Based MIPs/NIPs

In order to investigate the extraction characteristics of Fe and Fe-Ni based molecular imprinted polymers the binding experiments described below were conducted.

2.7.1. Effect Of The Analyte Concentration On Sorption Of Salicylic Acid

To conduct the binding experiments standard solutions with two different concentrations were prepared. The experimental parameters are given in Table 2.7. In summary, firstly 10.0 mg of MIP and NIP were weighed and taken in vials. After that 10.0 mL of standard solutions were transferred into these vials and then shaken at 480 rpm for 1 hour. Finally, the mixtures were filtered through cellulose acetate membranes (0.45 μm pore size) and the samples were analyzed by HPLC-DAD at 240 nm.

Table 2.7. Experimental parameters used for the investigation of the binding of salicylic acid on MIP and NIP

Standard concentrations	0.5, 5.0 mgL^{-1}
Amount of sorbent	10.0 mg
Sample solution volume	10.0 mL
Sorption time	1 hour

2.8. Optimization Of Extraction Parameters For Fe And Fe-Ni Based MIPs

2.8.1. Effect Of Sorbent Amount On Extraction Of Salicylic Acid

To investigate the effect of sorbent amount on extraction of salicylic acid, MIPs and NIPs were weighed at different amounts as given Table 2.8. in vials. In a typical experiment, 10.0 mL of 5.0 mgL^{-1} salicylic acid solution was transferred into a vial containing the sorbent and then shaken at 480 rpm for 1 hour. Following the sorption step, the mixture was filtered through cellulose acetate membrane (0.45 μm pore size) and the solution was analyzed by HPLC-DAD.

Table 2.8. Experimental parameters used for the investigation of the effect of MIP and NIP amounts on the sorption of salicylic acid

Standard concentration	5.0 mgL^{-1}
Amount of sorbent	5.0, 10.0, 25.0, 50.0, 100.0 mg
Solution volume	10.0 mL
Sorption time	1 hour

2.8.2. Effect Of Sorption Time On Extraction Of Salicylic Acid

In a typical experiment conducted to investigate the effect of shaking time on extraction of salicylic acid first, MIP or NIP (25.0 mg) was weighed in a vial. Then, 10.0 mL of 5.0 mgL^{-1} salicylic acid solution was added to the vial and resulting mixture was shaken at 480 rpm for different time intervals as given in Table 2.9. Finally, the solid/liquid mixture was filtered through a cellulose acetate membrane (0.45 μm pore size) and analyzed by HPLC-DAD.

Table 2.9. Experimental parameters used for the investigation of the effect of shaking time on the sorption of salicylic acid by MIPs and NIPs

Standard concentration	5.0 mgL^{-1}
Amount of sorbent	25.0 mg
Solution volume	10.0 mL
Sorption and Desorption time	1.0, 5.0, 15.0, 60.0, 120.0 min

2.8.3. Effect Of Eluent Type On Desorption Of Salicylic Acid

In order to find the best eluent that is capable of desorbing salicylic acid from the sorbents quantitatively, various solutions were prepared and tested. In a typical experiment, firstly, 10.0 mL of 5.0 mgL^{-1} of salicylic acid was added into a vial which included 25.0 mg MIP or NIP. The sorption of salicylic acid was performed by shaking the vial at 480 rpm for 1 hour. Following the sorption step, the MIP/NIP was separated from the solution and subjected to a rebinding process using the eluents given in Table 2.10. Finally, the sorbent was separated from the elution solution and the eluent was analyzed with HPLC-DAD.

Table 2.10. Experimental parameters used for the investigation of different solutions on elution of salicylic acid from MIP and NIP

Standard concentration	5.0 mgL^{-1}
Amount of sorbent	25.0 mg
Solution volume	10.0 mL
Sorption time	30 min
Desorption matrix	Methanol Methanol: Water (1:1) Methanol: Acetic acid (9:1 and 1:1) Acetic acid Acetic acid: Water (1:1)

2.8.4. Reusability Of The Sorbents

Reusability studies were carried out to determine the number of times the sorbent can be used in the SPE process. For this process, 10.0 mL of 5.0 mgL^{-1} of salicylic acid solution were added into vials containing 25.0 mg MIP or NIP and shaken at 480 rpm for 30 min. Salicylic acid was then eluted form the sorbent by methanol: acetic acid (9:1, v: v). Sorbent was subjected to the same sorption cycle in four times. Then this process was repeated with the same sorbent. All parameters are given in Table 2.11.

Table 2.11. Experimental parameters used for reusability experiments

Standard concentration	5.0 mgL^{-1}
Amount of sorbent	25.0 mg
Solution volume	10.0 mL
Sorption time	30 min
Desorption matrix	Methanol: Acetic acid (9:1)

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Optimization Of Instrumental Parameters

Instrumental parameters given in Table 2.1 were used during the study. For the chromatographic separation Sunfire C18 (RP 4.6 mm x 250mm) column was used under isocratic run conditions with 1.0 $mLmin^{-1}$ flowrate of mobile phase composition of MeOH: H_2O (85:15, v: v). pH of aqueous phase was adjusted to 2.5 with acetic acid and this condition was used in all related experiments throughout the study. The column temperature was set to 30°C. With the injection volume of 20.0 µL LOD and LOQ were calculated as 0.030 mgL^{-1} and 0.101 mgL^{-1}, respectively. Figure 3.1 shows calibration graph of salicylic acid under the optimized chromatographic conditions.

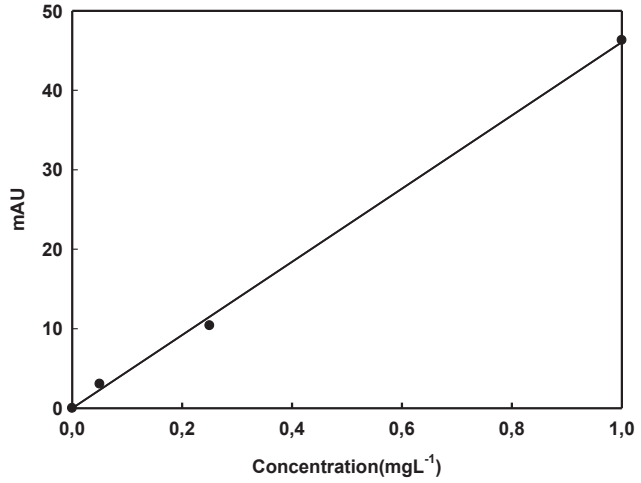


Figure 3.1. Calibration plot for salicylic acid (Agilent 1200 Series HPLC-DAD system, Sunfire C18 (RP 4.6 mm x 250 mm) column, 85:15 MeOH: H_2O (pH of the aqueous phase was adjusted to 2.5 with acetic acid) mobile phase, 1.0$mLmin^{1}$ flow rate, 240 nm).

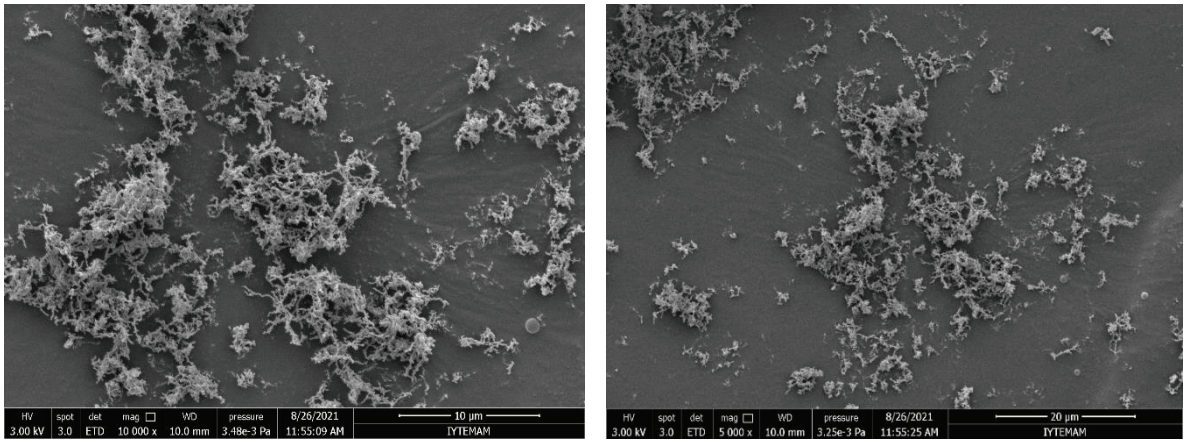
3.2. Characterizations Of The Sorbents

For the characterization of the sorbents different techniques were used; namely, scanning electron microscopy (SEM), energy dispersive X-ray (EDX) spectroscopy, and X-Ray diffraction (XRD). Obtained results for each sorbent are summarized below.

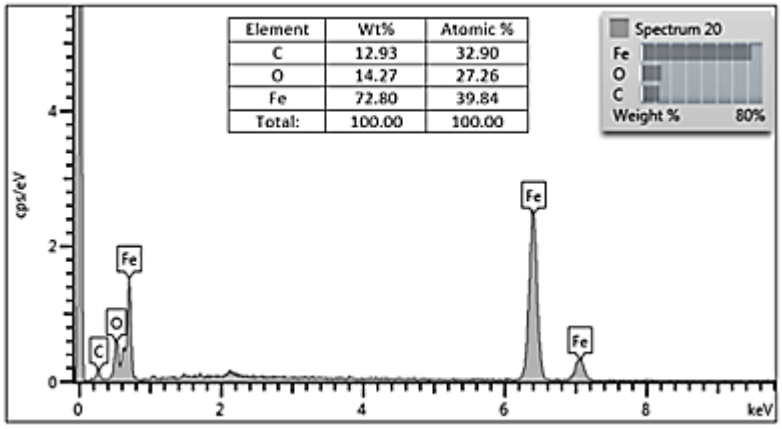
3.2.1. Characterization Of Fe Nanoparticles

The SEM images obtained at the different magnifications for Fe nanoparticles are shown in Figure 3.2(a). The typical chain-like structure of nanosized Fe particles can be seen in the figure (Shahwan et al. 2011). The images also depict that the particle size distribution is homogeneous.

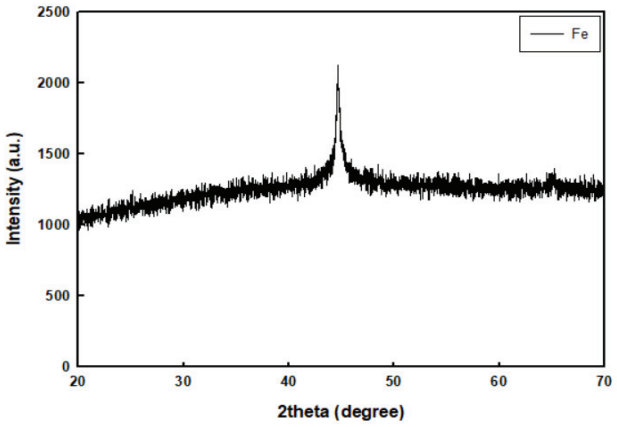
Figure 3.2(b) shows EDX spectrum of the Fe nanoparticles. The elemental composition, shown in the EDX spectrum, confirms that that the material is composed of Fe and O, as expected. The peak of C originates usually from adventitious carbon sources during the analysis. The XRD pattern of Fe nanoparticles (Figure 3.2(c) shows that the synthesized particles are mainly composed of zero-valent iron with its typical reflection at 44.9° (Shahwan et al. 2011). The peak appears to be relatively sharp and intense reflecting a crystalline order in the structure of the material.



(a)



(b)



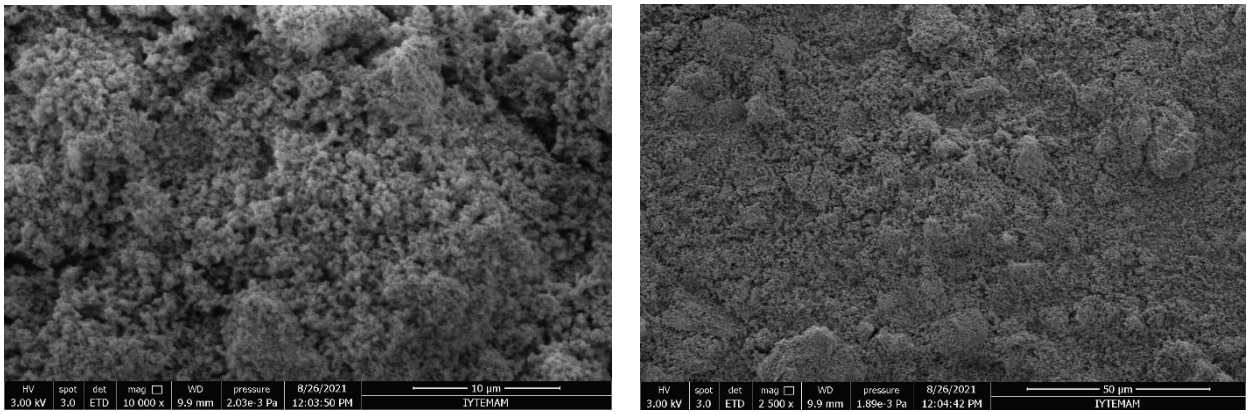
(c)

Figure 3.2. (a) SEM images (b) EDX graph (c) XRD diffractogram of Fe nanoparticles

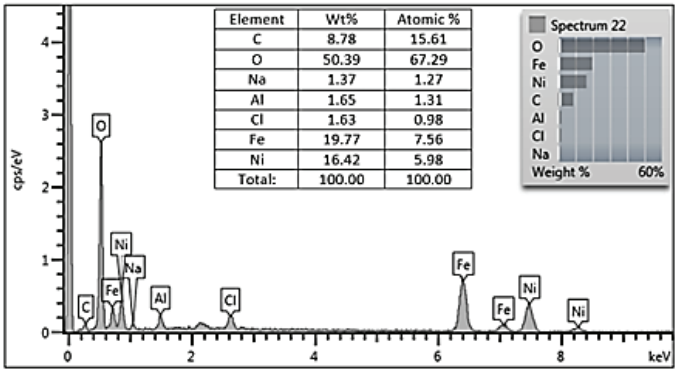
3.2.2. Characterization Of Fe-Ni Nanoparticles

The SEM images obtained at the different magnifications for Fe-Ni nanoparticles are shown in Figure 3.3(a). The SEM image shows aggregate formation due to the magnetic attractive properties of Fe and Ni metals. The images also depict that the particle size distribution is homogeneous.

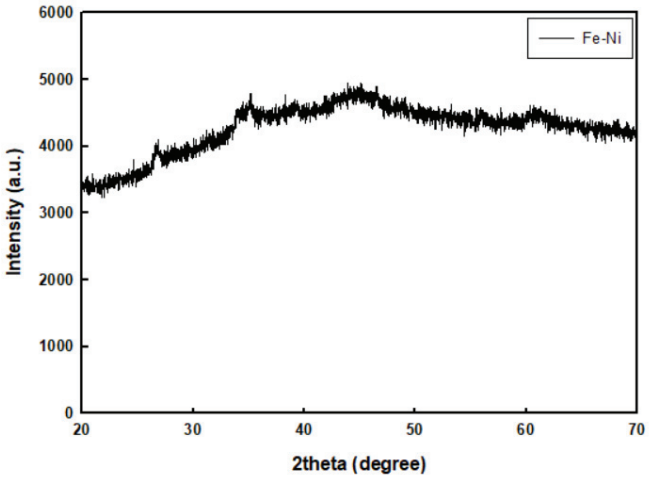
Figure 3.3(b) shows EDX spectrum of Fe-Ni nanoparticles. The spectrum shows the elemental composition of the material. As expected, peaks indicating significant presence of Fe, Ni, and O are observed. However, the spectrum contains signals also of Na and Cl, which probably reflects the precipitation of sodium chloride formed during the preparation of the nanoparticle material from the chemical precursors. The XRD pattern of Fe-Ni nanoparticles (Figure 3.3(c)) shows a broad peak centered at 43-45°, which originates from an overlap between reflection of metallic Ni observed usually around 44.5°, and that of metallic Fe major reflection at 44.9° (Naser and Shahwan 2019). The broad reflections with relatively low intensities indicate that the material is mostly amorphous.



(a)



(b)



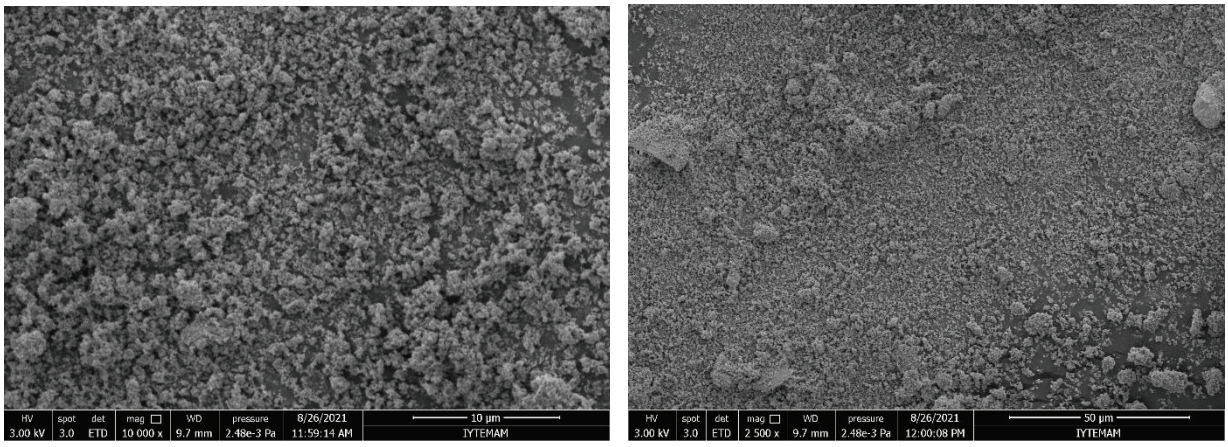
(c)

Figure 3.3. (a) SEM images (b) EDX graph (c) XRD diffractogram of Fe-Ni nanoparticles

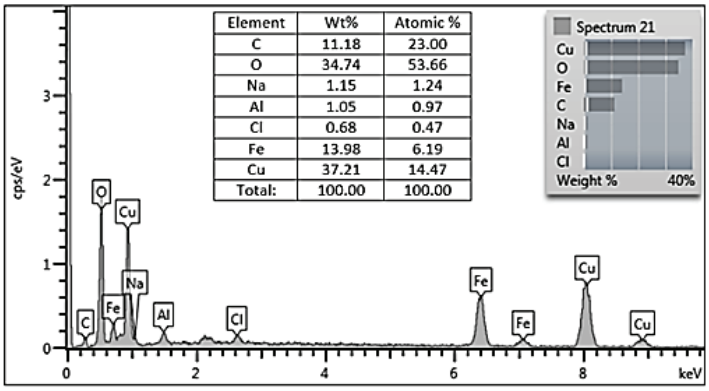
3.2.3. Characterization Of Fe-Cu Nanoparticles

The SEM images obtained at the different magnifications for Fe-Cu nanoparticles are shown in Figure 3.4(a). The SEM images reveal a certain extent of chain-like structure of the material. The images also depict that the particle size distribution is homogeneous.

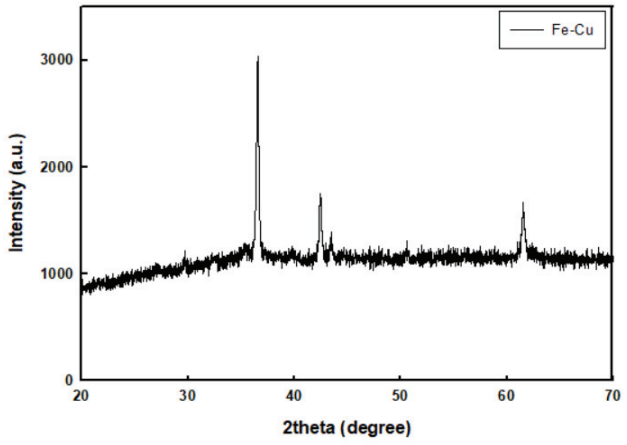
Figure 3.4(b) shows EDX spectrum of Fe-Cu nanoparticles. The elemental composition, shown in the EDX spectrum, indicates that the material is composed of Fe, Cu, and O. However, the spectrum contains signals also of Na and Cl, which probably reflects the precipitation of sodium chloride formed during the preparation of the nanoparticle material from the chemical precursors. The XRD pattern of Fe-Cu nanoparticles (Figure 3.4(c)) shows diffraction signals for metallic Fe at 44.9° and for metallic Cu at 43.4°. The remaining reflections in the diagram originate from the reflections of cuprite (Cu_2O) (Karabelli et al. 2008). The peaks are relatively sharp and intense reflecting the presence of a crystalline order in the structure of oxide material.



(a)



(b)



(c)

Figure 3.4. (a) SEM images (b) EDX graph (c) XRD diffractogram of Fe-Cu nanoparticles

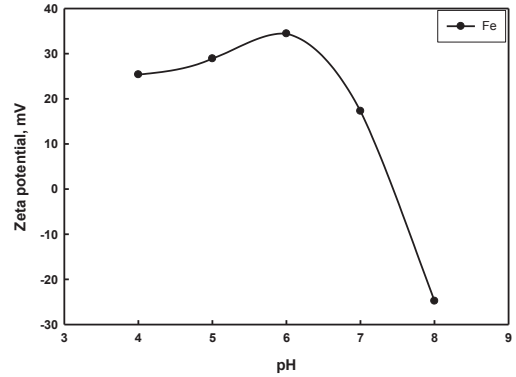
3.2.4. Point Of Zero Charge (PZC)

10.0 mg of Fe, Fe-Ni and Fe-Cu nanoparticles were dispersed in 50.0 mL of distilled water. The pHs of the mixtures were adjusted with different concentrations of hydrochloric acid and sodium hydroxide to the pH values given in Table 3.1. Zeta potential vs pH graphs for Fe, Fe-Ni, and Fe-Cu are given in Figure 3.5.

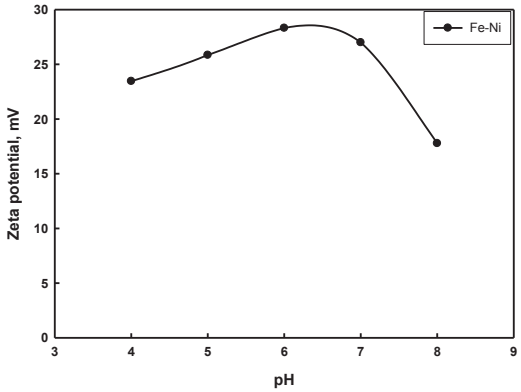
Table 3.1. Parameters used for PZC determination of Fe, Fe-Ni, and Fe-Cu

pH	0, 5.0, 6.0, 7.0, 8.0
Amount of sorbent	10.0 mg
Solution volume	50.0 mL

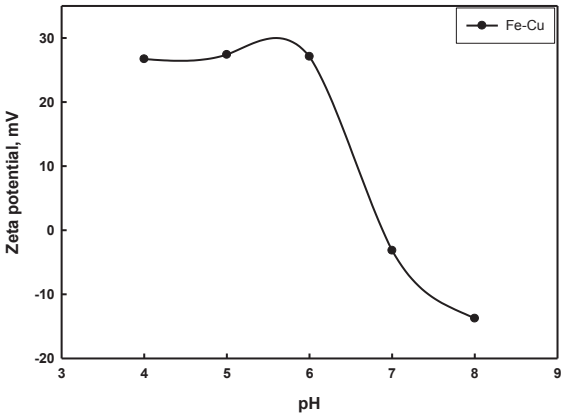
Based on Figure 3.5(a), the PZC of Fe nanoparticles can be estimated as 7.5, which means that the particles charges are about to be eliminated, and aggregation is about to start. In Figure 3.5(b), the value of zeta potential at pH: 8 is around 15 mV, which means that the particles charges are not completely eliminated: Here, it can be speculated that the aggregation can start at pH values higher than 9, rt, and as such the material is coming close to its PZC value. In Figure 3.5(c), the value of zeta potential approaches zero at pH: 6.8 which means that the particles charges are about to be eliminated, and aggregation is about to start, and as such the material is coming close to its PZC value.



(a)



(b)



(c)

Figure 3.5. Zeta potential vs. pH graphs for (a) Fe, (b) Fe-Ni, (c) Fe-Cu

3.2.5. Characterization Of Fe Based MIP/NIP

The SEM images obtained at different magnifications for Fe based MIP and NIP particles are shown in Figure 3.6. The SEM images show aggregate formation due to the magnetic attractive properties of Fe metal.

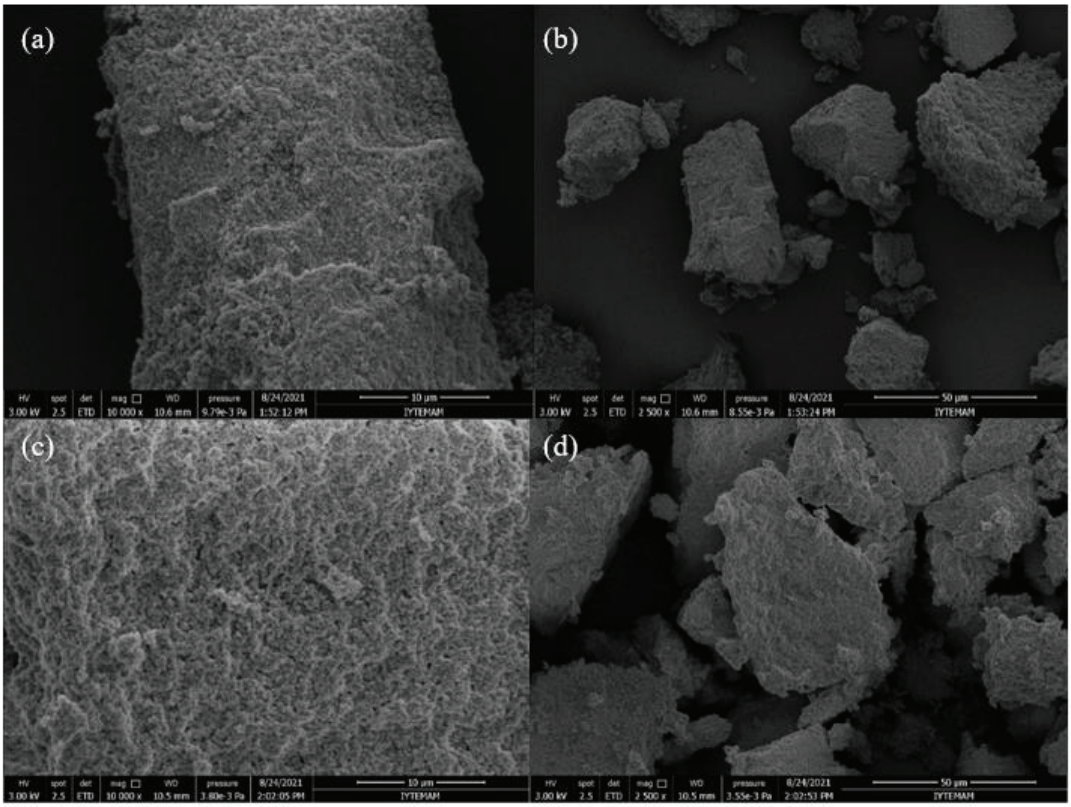
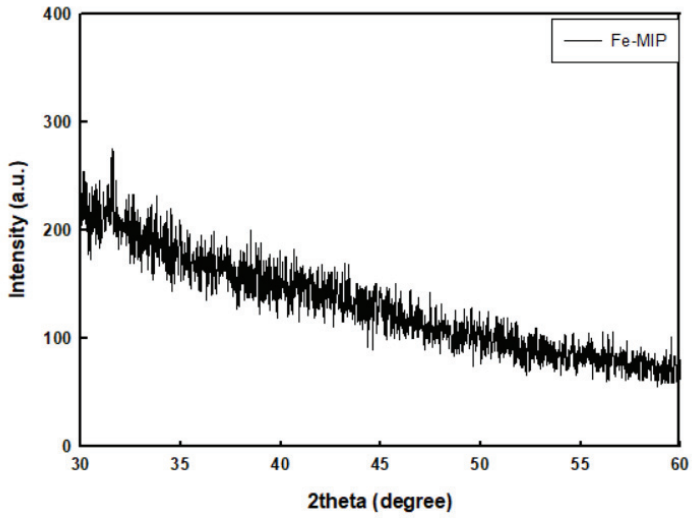
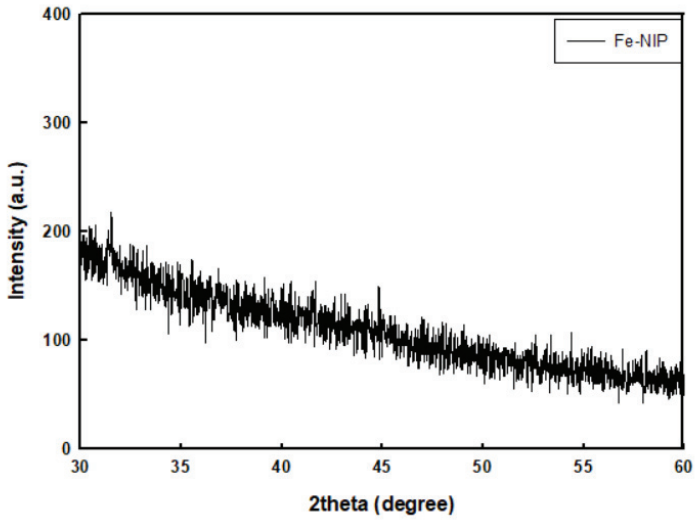


Figure 3.6. SEM images of (a) and (b) Fe based MIP, (c) and (d) Fe based NIP

Figure 3.7 shows XRD diffractograms of Fe based MIP and Fe based NIP. The obtained peaks are relatively low in intensities indicating that the materials are mostly amorphous.



(a)



(b)

Figure 3.7. XRD diffractogram of (a) Fe based MIP and (b) Fe based NIP

3.2.6 Characterization Of Fe-Ni Based MIP/NIP

The SEM images obtained at different magnifications for Fe-Ni based MIP and NIP particles are shown in Figure 3.8. SEM images show aggregate formation due to the magnetic attractive properties of Fe and Ni metals.

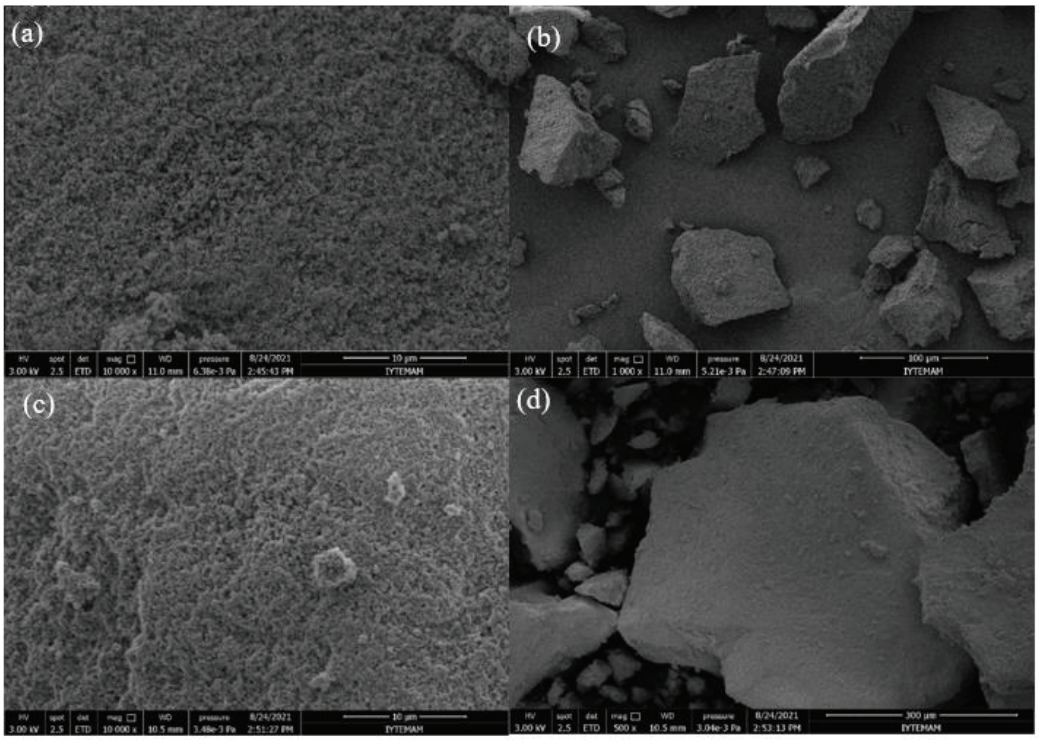
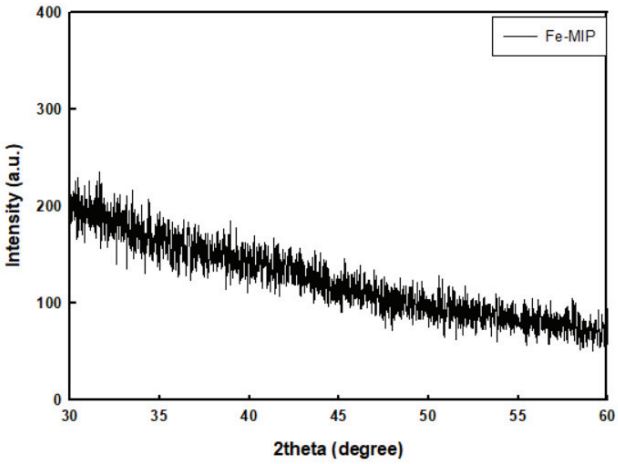
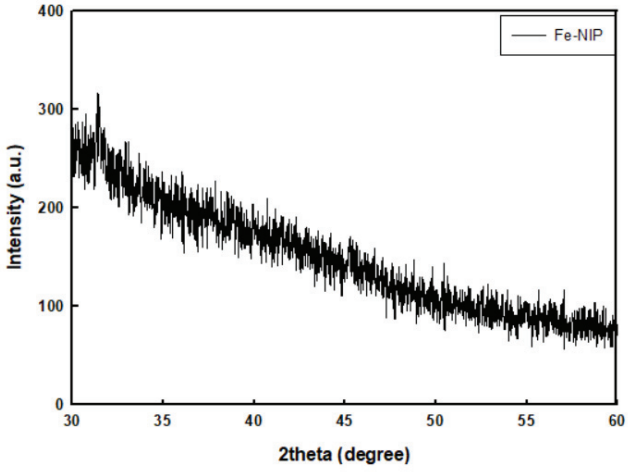


Figure 3.8. SEM images of (a) and (b) Fe-Ni based MIP, (c) and (d) Fe-Ni based polymer

Figure 3.9 shows XRD diffractograms of Fe-Ni based MIP and Fe-Ni based NIP. If the synthesized material is crystalline, metallic Fe major reflection is expected to be seen at 44.9° and metallic Ni around 44.5° (Naser and Shahwan 2019). The peaks with relatively low intensities indicate that the material is mostly amorphous.



(a)



(b)

Figure 3.9. XRD diffractogram of (a) Fe-Ni based MIP, (b) Fe-Ni based NIP

3.3. Sorption Of Salicylic Acid With Metallic Nanoparticles

3.3.1. Effect Of The Analyte Concentration On Sorption Of Salicylic Acid

As given in the experimental section, for this study the sorption was performed with 10.0 mg of sorbent and 10.0 mL of sample solution which was spiked to contain 0.25, 0.5, 1.0 and 5.0 mgL^{-1} salicylic acid. The extraction time and agitation conditions were 60 min and 480 rpm, respectively.

The obtained results for sorption of salicylic acid with Fe, Fe-Ni and Fe-Cu particles are shown in Figure 3.10. Based on these results, the sorption of Fe varied non-linearly with respect to concentration. In case of Fe-Ni particles decrease in sorption percentage was observed as analyte concentration increased. Contrary to the behavior observed for Fe-Ni particles, the percent sorption of salicylic acid increased for Fe-Cu particles as the concentration of the analyte increased. This behavior might be related to the pH of the medium and the PZC values of the particles in the medium. In order to further scrutinize these behaviors, as a next step, the sorption experiments were carried out in solutions with different pHs.

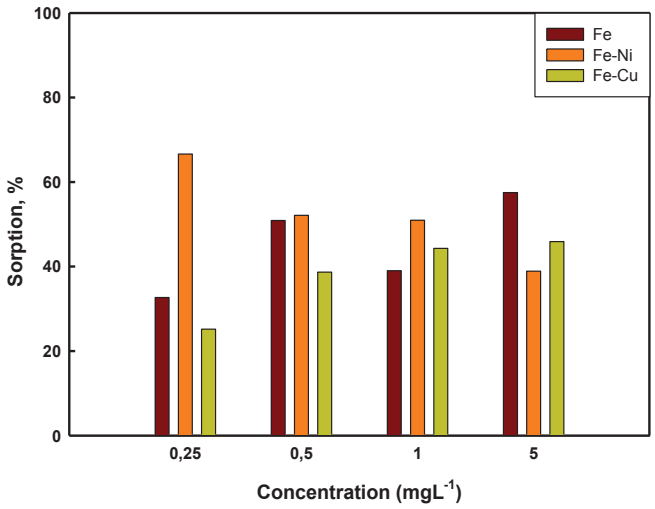


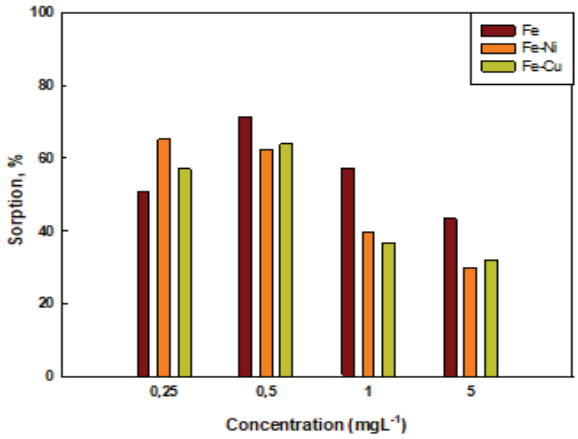
Figure 3.10. Sorption of salicylic acid on Fe, Fe-Ni and Fe-Cu nanoparticles at different analyte concentrations

3.3.2. Effect Of Solution pH On Sorption Of Salicylic Acid

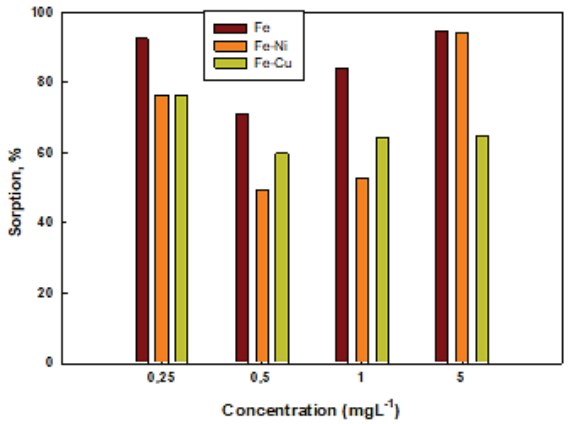
As described in more detail in the experimental part, for this study the sorption was performed with 10.0 mg of sorbent and 10.0 mL of sample solution which was spiked to contain 0.25, 0.5, 1.0 and 5.0 mgL^{-1} salicylic acid under three different pHs (pH 2.0, 4.0 and 6.0). The extraction time and agitation conditions were 60 min and 480 rpm, respectively.

The sorption behavior of the sorbents at different pHs were investigated and results are shown in Figure 3.11. Based on the PZCs given in the previous section, at tested pHs all particles are expected to be positively charged. In case of salicylic acid, a

weak acid with pKa_1 of 2.79 and pKa_2 of 13.82, the analyte is present mainly in its neutral form at pHs below 2.79 and negatively charged above this pH. In light of this information, the obtained sorption behaviors were scrutinized. Based on the above explanations, as would be expected, for all nanoparticles, the highest sorption was observed at pH 4.0. A pH at which analyte is mainly negatively (-1) charged and sorbent particles are positively charged. Therefore, it is possible to say that the ionic interactions between the sorbents and analyte are strong at this pH. At pH 2.0, the analyte is found mainly in its neutral form however at this pH the sorbents must show some dissolution which effects the sorption behavior of the sorbents. In case of higher pH on the other hand, pH 6.0, the analyte is mainly negatively charged but the sorbents have less positive charge as the pH gets closer to PZCs of the sorbents.



(a)



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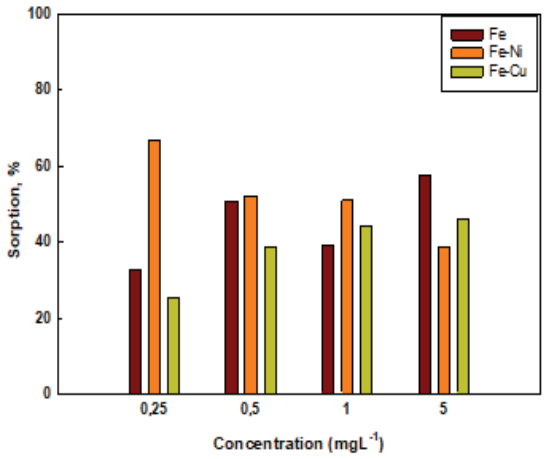


(c)

Figure 3.11. Sorption of salicylic acid on Fe, Fe-Ni and Fe-Cu nanoparticles at (a) pH:2.0, (b) pH:4.0, (c) pH: 6.0

3.4. Optimization Of Extraction Parameters For Nanoparticles

3.4.1. Effect Of Sorbent Amount On Extraction Of Salicylic Acid

As the first parameter of the optimization sorbent amount was tested. As described in the experimental part, for this study the sorption was performed with Fe-Ni nanoparticles as sorbent and 10.0 mL of sample solution which was spiked to contain 5.0 mgL^{-1} salicylic acid and had a pH of 6.0 was used. The sorbent amounts tested were 5.0, 10.0, 25.0 and 50.0 mg. The extraction time and agitation conditions were 60 min and 480 rpm, respectively.

Figure 3.12 demonstrates the obtained results for the effect of sorbent amount on the sorption percentage of salicylic acid. The sorption percentage increased as sorbent amount was increased and reached to its maximum value at 250 mg. This value was considered as optimum sorbent amount under the experimental conditions applied.

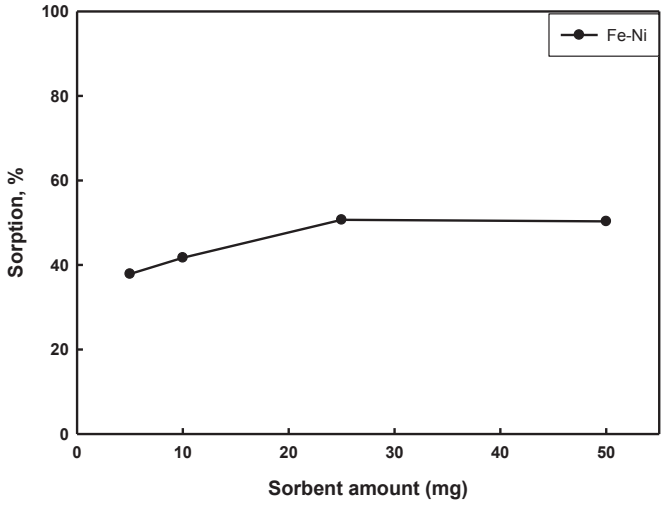


Figure 3.12. Effect of sorbent amount on sorption of salicylic acid by Fe-Ni nanoparticles

3.4.2. Effect Of Sample Volume On Sorption Of Salicylic Acid

In order to investigate the effect of sample volume on sorption of salicylic acid sample volume was varied while the sorbent amount was kept constant. For this study the

sorption was performed with 25.0 mg of sorbent as it has been found as the optimum amount. The sample volume tested were 5.0, 10.0, and 20.0 mL, all spiked to contain 5.0 mgL^{-1} salicylic acid with the final solution pH of 6.0. The extraction time and agitation conditions were 60 min and 480 rpm, respectively.

The obtained sorption results are shown in Figure 3.13 and based on these results can be said that under the tested conditions the sample volume did not significantly change the percent sorption of the analyte. However, it should be kept in mind if the purpose of the sorption study is to load salicylic acid on the sorbent, it can be concluded that higher amount of salicylic acid will be adsorbed on the sorbent when the sample volume is higher. Nevertheless, for the further studies 10.0 mL was chosen as the sample volume.

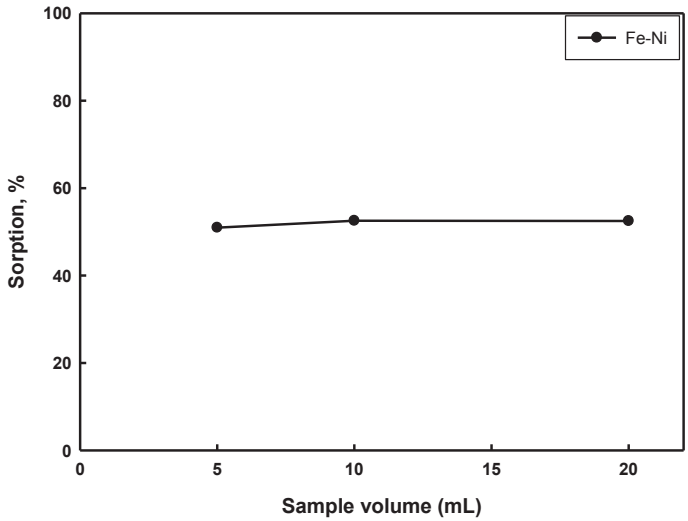


Figure 3.13. Effect of sample volume on sorption of salicylic acid by Fe-Ni nanoparticles

3.4.3. Effect Of Shaking Time On Sorption Of Salicylic Acid

To investigate the effect of shaking time on sorption of salicylic acid the sorption was performed with 25.0 mg of sorbent and 10.0 mL of sample solution which was spiked to contain 5.0 mgL^{-1} salicylic acid with the final solution pH of 6.0 was used. The extraction times tested were 1.0, 5.0, 10.0, 60.0 and 120.0 min with constant agitation of 480 rpm.

The results of this experiment are sown in Figure 3.14. As can be seen from the figure, sorption reaches its equilibrium considerably fast, approximately in 60 min due to mainly intramolecular interactions from the surface. Based on these results, 60 min was selected as optimum sorption time.

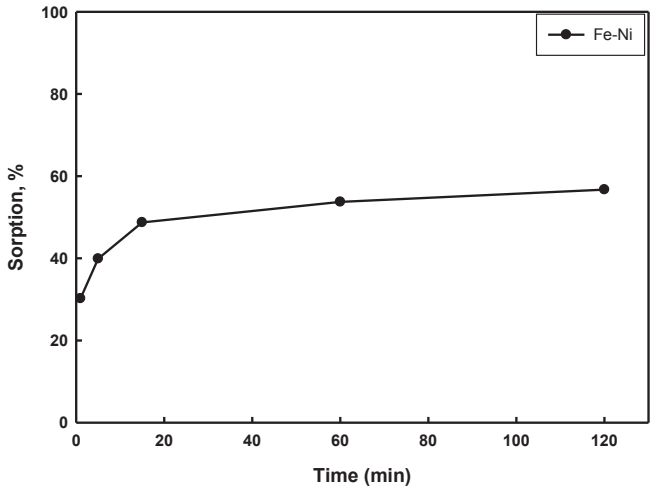


Figure 3.14. Effect of shaking time on the sorption of salicylic acid by Fe-Ni nanoparticles

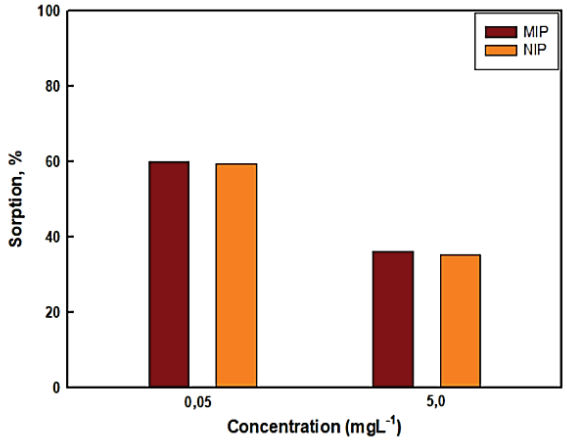
3.5. Sorption Of Salicylic Acid With Fe And Fe-Ni Based MIPs

3.5.1 Effect Of The Analyte Concentration On Sorption Of Salicylic Acid

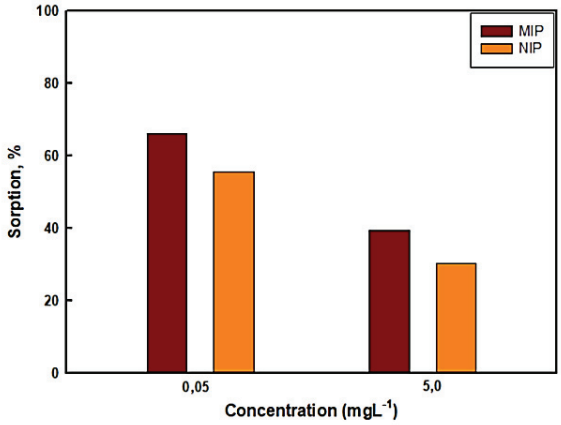
As detailed in the experimental section, for this study the sorption was performed with 10.0 mg of sorbent and 10.0 mL of sample solution which was spiked to contain 0.5, and 5.0 mgL^{-1} salicylic acid. The extraction time and agitation conditions were 60 min and 480 rpm, respectively.

The sorption behavior of Fe and Fe-Ni based molecularly imprinted and non-imprinted polymers at two different salicylic acid concentrations are shown in Figure 3.15. While no significant sorption differences were observed between MIP and NIP for Fe based sorbent, 10% sorption difference was observed between the NIP and MIP sorbents which were based on Fe-Ni. It can be speculated that the presence of Ni in the

Fe-Ni based molecularly imprinted polymer prevents the intramolecular hydrogen bonding in salicylic acid and increases the possibility of interaction between the sorbent cavities and salicylic acid.



(a)



(b)

Figure 3.15. Sorption percentage of (a) Fe based MIP/NIP (b) Fe-Ni based MIP/NIP

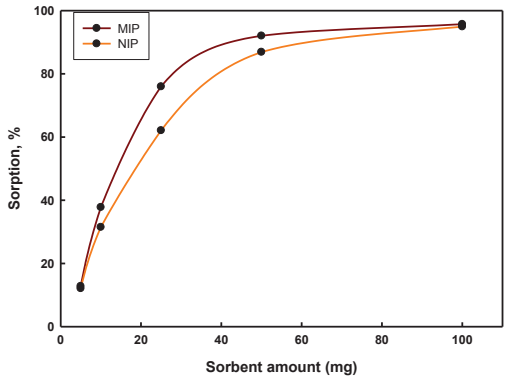
3.6. Optimization Of Extraction Parameters For Fe And Fe-Ni Based MIPs

3.6.1. Effect Of Sorbent Amount On Extraction Of Salicylic Acid

The first optimized parameter was sorbent amount. As described in the experimental part, for this study the sorption was performed with various amounts of

sorbents with fixed volume of (10.0 mL) sample solution which was spiked to contain 5.0 mgL^{-1} salicylic acid. The sorbent amounts tested were 5.0, 10.0, 25.0, 50.0 and 100.0 mg. The extraction time and agitation conditions were 60 min and 480 rpm, respectively.

Figure 3.16 shows the effect of sorbent amount on the sorption percentage of salicylic acid with the prepared metal-based MIP/NIP. Up to 25.0 mg of sorbent amount, sorption increased almost linearly for both metal-based MIP/NIP. After this amount, sorption percentage still continues to increase but with a lower incline until 50.0 mg of sorbent amount which is also the equilibrium value for Fe based MIP. However, in case of Fe based NIP and Fe-Ni based NIP and Fe-Ni based MIP, the maximum sorption was observed at approximately 100.0 mg of sorbent. Also, the most significant sorption capacity difference between MIP and NIP was observed in Fe based molecularly imprinted polymer at 25.0 mg of sorbent amount.



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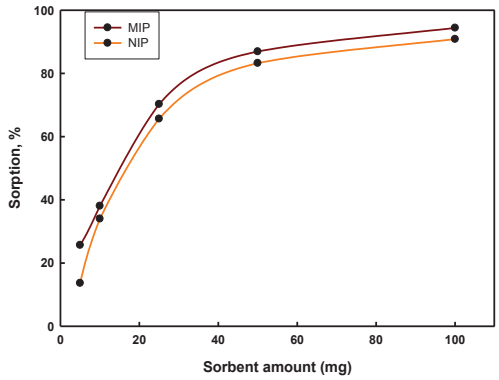


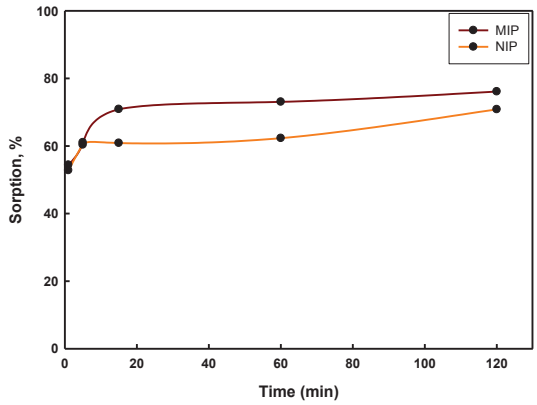
(b)

Figure 3.16. Effect of sorbent amount on extraction of salicylic acid (a) Fe based MIP/NIP (b) Fe-Ni based MIP/NIP

3.6.2. Effect Of Sorption Time On Extraction Of Salicylic Acid

To investigate the effect of shaking time on sorption of salicylic acid on metal-based MIPs and NIPs the sorption was performed with 25.0 mg of sorbent and 10.0 mL of sample solution which was spiked to contain 5.0 mgL^{-1} salicylic acid. The extraction times tested were 1.0, 5.0, 15.0, 60.0 and 120.0 min with constant agitation at 480 rpm.

The results of this study are illustrated in Figure 3.17. As can be seen from the figure the sorption reaches equilibrium in a short time for both of MIP and NIP. This means that nanostructured sorbents have high surface area which might have contributed to rapid sorption kinetics observed in the study. Since the interaction time has little effect on sorption, 30 minutes was chosen for the remaining experiment.



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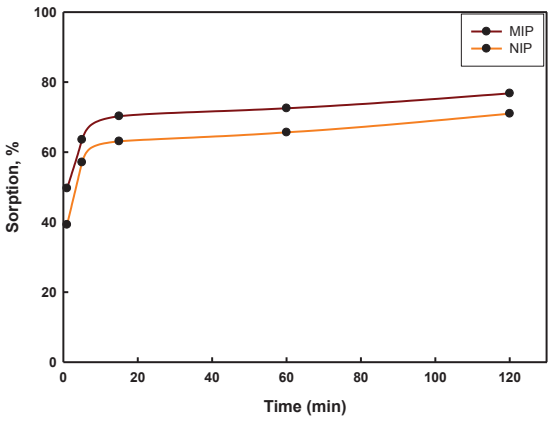


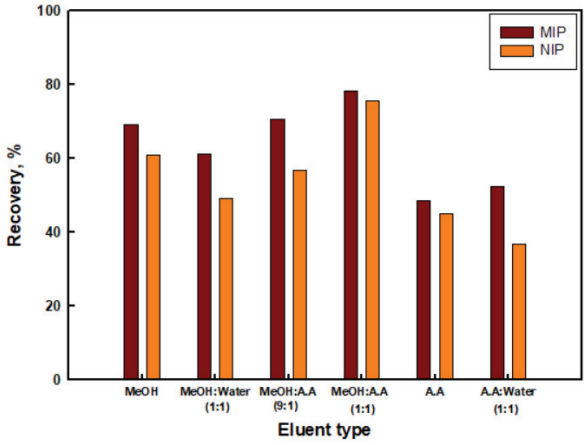
(b)

Figure 3.17. Effect of shaking time on sorption of salicylic acid with (a) Fe based MIP/NIP, (b) Fe-Ni based MIP/NIP

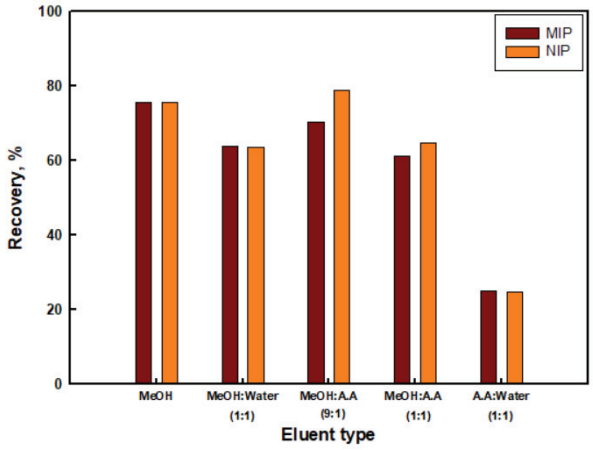
3.6.3. Effect Of Eluent Type On Desorption Of Salicylic Acid

When the sorbent is intended to be used for pre-concentration purpose prior to instrumental analysis, the quantitative elution of extracted analytes from sorbent plays a critical role in the method development. In addition, if the sorbent is intended to be reused, to find an eluent that is capable of desorbing all of the analytes extracted without affecting the integrity and sorption properties of the sorbent is crucial. For these reasons, as a next step, the best eluent was selected. To investigate the effect of various solutions as eluent for the salicylic acid first sorption under optimized conditions was performed with both metallic NIP and MIP sorbents. For this purpose, 25.0 mg of sorbent and 10.0 mL of sample solution which was spiked to contain 5.0 mgL^{-1} salicylic acid was used in the extraction. The sorption time and agitation conditions were 30 min and 480 rpm, respectively. Following the extraction step, the particles were separated from the solution and then fresh desorption solution (10.0 mL) was added into the vial and desorption was performed for 60 min. As desorption solutions MeOH, MeOH: H_2O (1:1, v: v), MeOH: AA (9:1, v: v), and AA: H_2O (1:1, v: v) were tested. (AA stands for acetic acid.)

The results of this experiment are given in Figure 3.18 for Fe based and Fe-Ni based MIPs/NIPs. Among the tested solvents, it was found that MeOH and MeOH: A.A (9:1, v: v) can be considered the best eluents, as they give the highest percent recoveries for salicylic acid from all tested NIP and MIP sorbents. MeOH: A.A (9:1) is already the solvent for removal of template molecules after MIP synthesis. This behavior is due mainly to the disruption of hydrogen bonding between the analyte and the sorbent. Therefore, MeOH: A.A (9:1) solution was decided to be used as the eluent in the further studies.



(a)



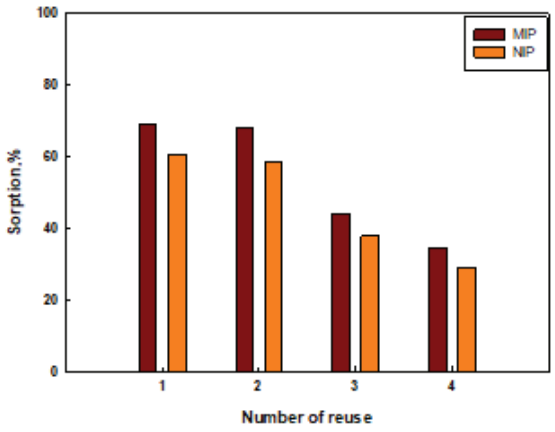
(b)

Figure 3.18. Effect of eluent type on desorption of salicylic acid from (a) Fe based MIP/NIP, (b) Fe-Ni based MIP/NIP

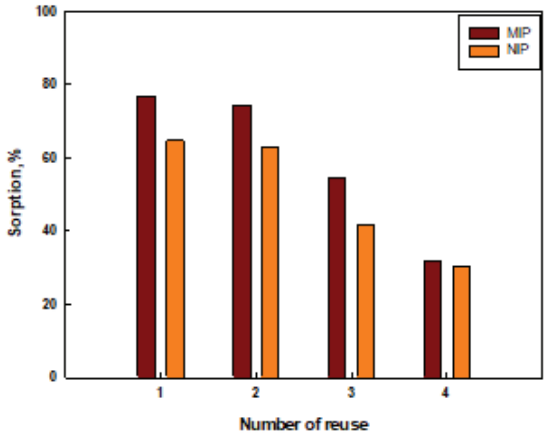
3.6.4. Reusability Of The Sorbents

To investigate the reusability of the sorbents for sorption of salicylic acid 25.0 mg of sorbent and 10.0 mL of sample solution which was spiked to contain 5.0 mgL^{-1} salicylic acid was used in the extraction step. The extraction time and agitation conditions were 30 min and 480 rpm, respectively. Following the extraction step, the particles were separated from the solution, MeOH: AA (9:1, v: v) was added into the vial and desorption was performed for 60 min. This sorption-desorption steps were repeated for 4 consecutive cycles.

To determine the number of times that the sorbent can be used for SPE process, reusability experiments were realized. Figure 3.19 shows that, with the experimental conditions applied, the sorbents can be used only two times without lost in performance and then the sorption percentage starts to decrease significantly. The main reasons of this low reusability can be due to the loss of some of the sorbent during the separation steps and most importantly the acetic acid present in desorption solution might affect the sorbent which eventually results in partial dissolution of the metallic core. As the MIP and NIP were both affected in a similar way in reusability studies, the overall contribution of the last process can be considered relatively insignificant.



(a)



(b)

Figure 3.19. Reusability of (a) Fe based MIP/NIP, (b) Fe-Ni based MIP/NIP

CHAPTER 4

CONCLUSION

In this study, the performance of magnetic molecularly imprinted polymers (MMIP) synthesized by using Fe and Fe-Ni for solid phase extraction of salicylic acid were investigated.

For this purpose, firstly characterization experiments were performed to compare the sorption capacities for Fe, Fe-Ni and Fe-Cu for selectivity of salicylic acid. Fe-Ni has linear and consistent behavior against salicylic acid. Point of zero charge of Fe-Ni nanoparticle was determined. At pH: 6.0, Fe-Ni is positively whereas salicylic acid is negatively charged. In this case, the main mechanism between the sorbent and the analyte is said to be electrostatic attraction. Critical experimental parameters were optimized and determined as follows; sorbent amount 25.0 mg, sample volume 10.0 mL and sorption time 60 min. Characteristic peaks of Fe, Ni and Cu observed in EDX and XRD analysis. Fe and Fe-Cu nanoparticles have crystalline part, but Fe-Ni nanoparticle has amorphous structure.

After that Fe and Fe-Ni based molecularly imprinted polymers were prepared and used in determination of salicylic acid with HPLC-DAD. For this purpose, optimization and characterization parameters were determined. Optimized amount of sorbent, shaking time and desorption matrixes were determined as 25.0 mg for 10.0 mL of 5.0 mgL^{-1} working solution, 30 minutes, and MeOH: A.A (9:1), respectively, for both MIP synthesizes. The proposed method was repeatedly used with the same sorbent and the results show that MIPs can be used for three times without loss in sorption percentage. For characterization of MIPs/NIPs, EDX and XRD were performed. In XRD diffractogram, the peaks which are of relatively low intensity indicate that the materials are mostly amorphous.

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