SOLID PHASE EXTRACTION OF ASCORBIC ACID WITH ZERO-VALENT IRON NANOPARTICLES

A Thesis Submitted to the
Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

in Chemistry

by
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July 2021
İZMİR

ACKNOWLEDGMENTS

I would like to thank a lot of people for their support during my thesis research. Firstly, I would like to express my gratitude to my advisor, Prof. Dr. Ahmet E. EROĞLU, for his patience, support, and encouragement throughout this thesis. I am grateful for his experience, advice, and kindness.

I am thankful to other members of the thesis committee, Prof. Dr. Talal SHAHWAN and Prof. Dr. Mustafa M. DEMİR for their valuable comments and suggestions. I also would like to thank to Assoc. Prof. Ezel Boyacı for her support and help.

I would like to express my gratitude to the research experts at the Centre for Materials Research (IZTECH) for their assistance with the SEM, EDX and XRD facilities. I would like to express my gratitude to BAP for their financial support.

Thank you to my colleagues and lab mates in the Analytical Chemistry Research Group for their encouragement and positive comments. I am thankful to my lab mates Yekta Arya ÖLÇER, Zeynep BİLDİK, Hazal TOSUN and Simge KOŞAR for their friendship and support.

I would like to express my special thanks to Sadettin Eren AYBAR for his understanding, patience, and support.

Finally, without my family's unwavering support, this thesis would not have been accomplished. I am especially grateful to the world's most devoted father, Abdullah YILDIZ, and my mother, Hüsna YILDIZ, for supported me anytime and anywhere in this world. I would also like to thank especially to my sister İrem YILDIZ ÇİLENGİROĞLU who helped me at all the stages of my study and supported me all the time. Finally, to my little sister Melike YILDIZ for always being there for me and her moral support.

ABSTRACT

SOLID PHASE EXTRACTION OF ASCORBIC ACID WITH ZERO-VALENT IRON NANOPARTICLES

Vitamin C (ascorbic acid, AA) is an essential food for humans. It is a water-soluble vitamin found naturally in some foods. With its reducing and chelating capabilities, ascorbic acid is the most effective enhancer of non-heme iron absorption. Sensitive and selective determination of ascorbic acid is necessary in a variety of samples.

In this study, molecularly imprinted polymers (MIPs) were synthesized by polymerization strategies and magnetic imprinting technology. Four different synthesis routes were employed, and the magnetic property of the MIPs was brought in with the use of nanosized zero-valent iron (nZVI). Nano zero-valent iron was synthesized using $NaBH_4$ reduction of aqueous Fe^{+2} ions and used in the solid phase extraction of ascorbic acid prior to HPLC determination. The sorption percentage of the sorbent increased with icreasing pH and reached its maximum level between pH 6.0 and 8.0. Iron oxidation occurs very slowly if the pH is less than 6.0. As a result, when pH 8.0 and higher were tested, there was no effect. The precipitation of iron at pH 8.0 and above could have caused this. The pH level was fixed to 6.0, and the rest of the studies were carried out at this level. The optimal sorbent concentration, solvent concentration, and shaking time were determined to be 10.0 mg, 10.0 mL, and 1 hour, respectively. MeOH and MeOH: H_2O, 85:15, were used to achieve desorption (pH of the eluent was adjusted to 3.0 using acetic acid).

Finally, the characterization of synthesized nanosized zero-valent iron and magnetic MIP/NIP was carried out through the sorption studies and with the use of XRD, SEM and EDX.

ÖZET

SIFIR DEĞERLİKLİ DEMİR NANOPARÇACIKLARI İLE ASKORBİK ASİDİN KATI FAZ EKSTRAKSİYONU

C vitamini (askorbik asid), insanlar için zorunlu bir besindir. Bazı gıdalarda doğal olarak mevcut olan, suda eriyen bir vitamindir. Askorbik asit (AA), indirgeme ve şelatlama yetenekleri ile demir emiliminin en etkili arttırıcısıdır. Çeşitli numunelerde askorbik asidin hassas ve seçici tayini gereklidir.

Bu çalışmada, polimerizasyon stratejileri ve manyetik baskılama teknolojisi ile oldukça seçici moleküler baskılanmış polimerler (MIP'ler) sentezlendi. Dört farklı sentez yolu kullanıldı ve MIP lerin manyetik özelliği, nano boyutlu sıfır değerlikli demir kullanımıyla getirildi. Nano sıfır değerli demir, sulu Fe+2 iyonlarının NaBH4 indirgenmesi kullanılarak sentezlendi ve HPLC tayininden önce askorbik asidin katı faz ekstraksiyonunda kullanıldı. Sorbentin sorpsiyon yüzdesi artan pH ile artmış ve pH 6.0 ile 8.0 arasında maksimum seviyesine ulaşmıştır. pH 6.0'ın altındaysa demir oksidasyonu çok yavaş gerçekleşir. Sonuç olarak, pH 8.0 ve üzeri test edildiğinde herhangi bir etki görülmedi. pH 8.0 ve üzerinde demirin çökelmesi buna neden olmuş olabilir. pH seviyesi 6.0'a sabitlendi ve çalışmaların geri kalanı bu seviyede gerçekleştirildi. Optimum sorbent konsantrasyonu, solvent konsantrasyonu ve çalkalama süresi sırasıyla 10.0 mg, 10.0 mL ve 1 saat olarak belirlendi. MeOH ve MeOH: H_2O, 85:15, desorpsiyon elde etmek için kullanıldı (asetik asit, pH 3.0).

Son olarak sentezlenen nano boyutlu sıfır değerlikli demir ve manyetik MIP/NIP'nin karakterizasyonu, sorpsiyon çalışmaları, XRD, SEM ve EDX kullanımı ile gerçekleştirilmiştir.

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CHAPTER 1

INTRODUCTION

1.1. Ascorbic Acid

In the past, seafarers could not consume fresh fruits and vegetables on the ship, resulting in scurvy due to vitamin C deficiency. The name meaning of ascorbic acid also comes from scurvy (Varvara et al. 2016). In the late 1800s, it was determined that scurvy was caused by vitamin C deficiency (Johnston, Steinberg, and Rucker 2007).

Ascorbic acid or l-ascorbic acid, also known as vitamin C, is a water-soluble vitamin. Ascorbic acid is a derivative of monosaccharide, and its smell is sour and is white in color. Ascorbic acid has a melting point of 193°C and a molecular weight of 176 g/mol, melting point 189 °C and boiling point 192 °C. (Bradshaw et al. 2011). Its chemical formula is $C_6H_8O_6$ with a molecular structure given in Figure 1.1. Its solubility in water is 33 g /100 g, in ethanol 2 g %100 g and in glycerin 1 g /100 g. It is very difficult to dissolve in acetone; is insoluble in ether, petroleum ether, benzene, chloroform, and oils. Ascorbic acid is a water-soluble keto lactone that has two ionizable hydroxyl groups. It has two pK_a values ; pK_1 is 4.2 and pK_2 is 11.6 (Du, Cullen, and Buettner 2012).

Vitamin C is the enantiomer of ascorbic acid that turns light to the left. Commercial vitamin C is usually composed of ascorbic acid crystals or calcium or sodium salts of ascorbic acid.

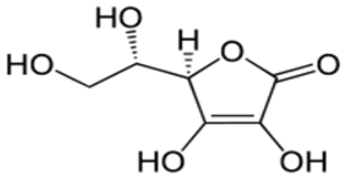


Figure 1.1. Structure of ascorbic acid

Since human body cannot produce vitamin C, it must be taken from foods. Ascorbic acid prevents the harmful effects of free radicals in the body (Fernandes et al. 2018). Some bats, primates, as well as humans, cannot produce vitamin C. Most animals and plants can produce their own vitamin C from glucose (Varvara et al. 2016).

Vitamin C is found in different amounts in vegetables and fruits. It plays an important role in protecting against diseases and acts as an antioxidant in our body. It has a reducing effect on chronic, bone and cancer diseases (Sarkar, Srivastava, and Dubey 2009). It has been observed that consuming fruits rich in antioxidants facilitates uric acid excretion (Zuo et al. 2011). Some epidemiological studies have shown that high vitamin C intake reduces chronic diseases (Valente et al. 2011).

Degradation of ascorbic acid in aqueous solutions; depends on the pH, temperature, and the presence of metal ions in the environment. Ascorbic acid is more stable in the blood and under conditions below -20°C (Johnston, Steinberg, and Rucker 2007).

Ascorbic acid can be easily oxidized in plasma by high temperature, light, oxygen, iron, and copper. The most important points in ascorbic acid determination are sample preparation and freezing. Usually metaphosphoric acid (MPA), trichloroacetic acid (TCA) or perchloric acid are used to keep ascorbic acid stable (Salminen and Alfthan 2008).

1.1.1. Levels of Ascorbic Acid in Foods

According to the World Health Organization (WHO), fruit and vegetable consumption is between 20 and 50 percent and the minimum daily intake is 400 grams. In addition, deaths due to insufficient intake of fruits and vegetables rank 6th among 20 risk factor deaths in the world (Rickman, Barrett, and Bruhn 2007). Again, according to WHO, if enough vitamin C is taken, 2.7 million lives could be saved a year. Table 1.1. shows the vitamin C levels in some foods (The United States Food and Drug Administration, FDA).

Table1.1. Richest food sources of vitamin C (FDA)

Food	Serving size	Milligrams (mg) per serving
Guava,raw	1 cup,raw	377
Sweet red pepper, raw	1 cup,raw	190
Tomato juice	1 cup,canned	170
Orange juice	1 cup	124
Sweet green pepper	1 cup,raw	120
Hot green chili pepper,raw	1 pepper,raw	109
Strawberries	1 cup,sliced	98

1.1.2. Importance of Ascorbic Acid

Vitamin C has an important physiological role in the body. It acts as an electron donor in the body. Thus, it is a powerful antioxidant that protects biomolecules in the body from oxidation. In addition, it has been shown that the level of vitamin C in plasma is low in critically ill patients and the amount of vitamin C needed by people with a standard diet is insufficient for these patients (Carr et al. 2017).

Ascorbic acid is a used for the treatment of diseases such as colds and cancer and also has been shown to have those new biological functions. Some of these are reprogramming and differentiation in stem cells (Li et al. 2021). Ascorbic acid radical plays an important role in many biological reactions such as scavenging, cancer preventing, and immunity improving. Ascorbic acid is a known antioxidant and free radical scavenger. It is a compound that regulates catabolism. High amounts of ascorbic acid are known to regulate blood sugar and lower cholesterol levels (Rafipour et al. 2016). It plays an important role in detecting food spoilage and product quality. It is also known to be effective against aging in the cosmetics industry (Lilly et al. 2014).

1.1.3. Diseases Caused by Ascorbic Acid Deficiency

Ascorbic acid is necessary for the healing of wounds in our body, the formation of collagen, and the maintenance and repair of teeth, bones, and cartilage. Ascorbic acid deficiency may cause scurvy, dry and splitting hair, gingivitis and bleeding gums, dry skin, decreased wound healing, easy bruising, nosebleeds, weakened tooth enamel, swollen and painful joints, anemia, decreased ability to ward off infection, and possibly weight gain due to a slowed metabolism. The need for ascorbic acid may increase in conditions such as chronic diseases, pregnancy, infection, and trauma. It is known to be used in the treatment of common cold (Sanseverino 2005).

1.2. Ascorbic Acid and its Derivatives

Ascorbic acid has two chemical forms, ascorbic acid and its oxidized form dehydroascorbic acid (Cisternas et al. 2014) ; both of which are unstable. Factors such as light, temperature, and pH cause these compounds to be unstable. These factors should be considered in the determination of ascorbic acid by analytical methods. Brown flasks should be used in sample preparation due to its degradation from light. The temperature of the environment must also be controlled in sample preparation. The pH of the solutions must be acidic in order to keep ascorbic acid stable. (Nováková, Solich, and Solichová 2008).

1.3. Determination Methods of Ascorbic Acid

There are many analytical methods for ascorbic acid determination. Some of these methods are titrimetry, fluorimetry, UV-VIS spectrometry, capillary electrophoresis, electrochemical methods, amperometric methods and liquid chromatography (HPLC) (Škrovánková et al. 2015).

Vitamin C concentrations in fresh and freeze-dried herbal juices were determined using direct iodine titration. Fresh juice was found to have more vitamin C than freeze-samples which demonstrated the degradation of vitamin C during the freeze-drying process. After four weeks of storage, stability of ascorbic acid in freeze-dried samples was found to be higher than in fresh juice. However, after 8 weeks, the content had

significantly decreased, making direct titration analysis impossible (Suntornsuk et al. 2002).

(Llamas, Di Nezio, and Fernandez-Band 2011) ascorbic acid using, a flow-injection spectrophotometry with a photodegradation step. Ascorbic acid concentration was measured at 300 nm: after a photodegradation step by UV irradiation. Various commercial and natural fruit juice samples were analyzed, and it was reported that filtration and dilution were required for sample preparation. The authors also mentioned that their approach was quick, has reduced contamination risk, and use low amounts of reagents.

(Costa et al. 2019) Capillary Electrophoresis (CE) applied capillary electrophoresis (CE) for ascorbic acid determination. This technique uses the differential migration of ionic or ionizable substances in the presence of an electric field for separation of the compounds of interests. Differential migration is usually caused by a combination of electromigration and electroosmotic flow.

In an article by (Dong et al. 2007), electrochemical methods were used to detect sugar and ascorbic acid simultaneously. A chemically modified electrode was formed by mixing polyethylene glycol (PEG) and Cu2O as modifiers into conventional carbon paste, and sugars and ascorbic acid were generated in this electrode to generate current responses. Sugar and ascorbic acid were determined in the same run and separated in 22 minutes. Furthermore, the authors reported that four analytes can be determined simultaneously using this method and the method requires low-volume sample, a simple equipment, and gives good reproducibility.

The most used method for the determination of ascorbic acid is HPLC because of its high selectivity, speed of analysis, and high resolution capability (Romeu-Nadal et al. 2006).

In HPLC, the molecules to be determined are passed through a specific column to separate molecules with high pressure and each compound is measured by the detector. Separation depends on the flow rate, pH, sample type, temperature, detector type and polarity (Sahu et al. 2018). HPLC is the most preferred method for analyzing biological samples (Khan et al. 2011).

For the determination of water-soluble vitamins B and C, a procedure combining reverse phase HPLC with biologically active supplements and sodium heptane sulfonate as an ion pair reagent was developed (Rudenko and Kartsova 2010). A method for solid

phase purification of the combined food extract has been proposed. The stability of ascorbic acid and riboflavin in aqueous solutions with various pH levels was investigated and was shown to increase with decreasing pH. The best medium had a pH of 2.5-3.0

(Heydari and Darabi Bazvand 2019) an ultrasound-assisted matrix solid phase dispersion (UA-MSPD) and reversed phase dispersive liquid liquid microextraction (RP-DLLME) were combined to determine vitamin C in a variety of matrices without the need for filtration of sample. HPLC with an UV absorption detector was used to analyze the samples. Keeping in mind that the extraction of polar organic compounds is difficult, the proposed method provided an acceptable recovery and preconcentration factor for vitamin C. In the elution step, the ultrasonic probe improves analyte extraction from the solid matrix into the liquid phase and accelerates analyte desorption from the sorbent into the liquid phase. On the sample surface, the final extracting phase is collected. As a result, solid sample was not removed from the sample vessel after UA-MSPD, and RP-DLLME was done in the presence of solid sample. This method is reported to be simple, low-cost, quick, and working with analytical instrument.

1.4. Bimetallic Nanoparticles

Particles with diameters smaller than 100 nanometers are usually called nanoparticles (NPs). The chemical and physical properties of these materials differ from individual atoms or macro-sized material (bulk). They are used in materials science fields, including catalysis, electronics, and biomaterials (Pinkas et al. 2019).

Bimetallic nanoparticles are made up two metals. The properties of bimetallic nanoparticles are determined by the constituent metals and their nanometric size. These are made by combining distinct architectural structures of metallic nanoparticles. These characteristics may differ from those of pure elemental particles, and may include size dependent optical, electrical, thermal, and catalytic effects. Various approaches have been proposed for their preparation and characterization. Nowadays, the researchers focus on preparing novel bimetallic nanoparticles in a variety of configurations, including alloys, core-shell, and contact aggregate. Physical and spectroscopic measurements have been used to develop various approaches. The structure and miscibility of the two metals in the bimetallic nanoparticle are determined by the preparation procedures. Bimetallic

nanoparticles are often made by reducing two metal ions simultaneously in the presence of a suitable stabilization approach, such as steric hindrance or static electrostatics.

Chemical reduction procedures are frequently used for the synthesis of nanoparticles. The size of the material is affected from the sample size, shape, and composition. Chemical reduction produces metal nanoparticle in the zero valent state. Different reducing agents such as sodium are being used. The most promising methodology of producing core-shell structured bimetallic nanoparticles is true successive reduction. It entails the deposition of a metal onto monometallic nanoparticles of another metal that have been manufactured. The deposited metal atom should chemically surround the pre-synthesized monometallic nanoparticles (Sharma et al. 2019).

1.4.1. Bimetallic Nanoparticles in Various Forms

Special bimetallic nanoparticles are created to achieve the required qualities. There are several types of bimetallic nanoparticles.

1.4.1.1. Nickel Based Bimetallic Nanoparticles

Metal nanoparticles have attracted attention in terms of synthesis and application because of their unique features. Nanoparticles made up of two metals are extremely important from a scientific and technological standpoint for improving the catalytic activities of monometallic nanoparticles in chemical manufacturing.

Nickel nanoparticles are an important class of metal nanoparticles because of their catalytic and magnetic properties. Due to their magnetic character, nickel nanoparticles can easily be isolated from the reaction mixture using an external magnetic field which is a big advantage in sampling step removing the necessity for filtration (Shah, Guo, and Fu 2015).

1.5. Nano Zero Valent Iron (nZVI)

Nanosized zero-valent iron nanoparticles (nZVI) are widely used because of their versatility and the assumption that they are less harmful and bioavailable. The nZVI is

widely used in reduction–oxidation (redox) processes, with a characteristic role of increasing reactivity. It was developed as an extension of micro-scale zero-valent iron technology. Depending on the synthesis procedures, the structural features of nZVI, such as size and surface area, may vary. The size of bare nZVI particles are less than 100 nm, usually in the range of 10 to 100 nm, and the form of nZVI is predominantly spherical iron. The smaller the particle, the greater the surface area to volume ratio, and thus the higher the surface reactivity. Rapid changes in surface property, which generate core-shell morphology, are practically inescapable occurrences during the synthesis process of nZVI or following its reactivity with water or air. The particle's size, size distribution, degree of crystallinity, reactivity, and aging could all be affected by the shell composition (Yirsaw et al. 2016).

1.6. Solid Phase Extraction

Sample preparation is an important step in analysis. There are various extraction methods after sample preparation. Liquid-liquid extraction and solid phase extraction (SPE) are the most used methods. The SPE technique is advantageous compared to other techniques due to its low organic solvent consumption, simplicity, and speed. These advantages of SPE have made this technique preferable in many analytical areas.(Boyacı et al. 2015). Pharmaceutical, clinical, food and industrial chemistry are among the most widely applied areas (Poole 2003).

Extraction is the process of separating a substance in a solution with the help of a suitable solvent. There may be limited effects on highly polarized analytes such as low transition volumes. To overcome this, the sorbent type is changed. Generally, the SPE technique occurs in four steps (Figure 1.2.).

- Column preparation,
- Sample loading,
- Column post-wash and
- Sample desorption (Buszewski and Szultka 2012).

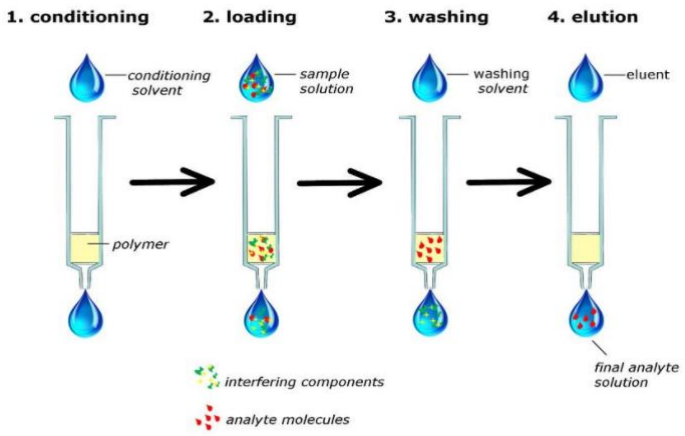


Figure 1.2. General steps for solid phase extraction

(Source: Sandoval R 2017).

In the first stage, the sorbent is wetted with the elution solvent. Then the sample is loaded and allowed to pass through the sorbent column. Compounds that are not to be detected are washed out until they are removed from the analyte. Finally, the analytes are eluted with the use of an appropriate solvent.

SPE technique has found significant applications in biological, environmental, pharmaceutical and food analysis (Płotka-Wasylka et al. 2016).

1.6.1. Molecularly Imprinted Polymers (MIPs)

The molecular imprinting technique (MIT) appeared in the early 1930s for the first time. Selectivity effects were explained by Polyakov (1931) in terms of a template effect. Pauling (1940) was the first to propose the concept of molecular imprinting, which involved a protein antibody self-assembly using an antigen as a template. Wulff and Sarhan (1972), reported the use of organic polymers prepared by reversible chemical bonds. As a result of these pioneering experiments, molecular imprinting technology has grown (Huang et al. 2015).

Molecular imprinted polymers are materials with molecular recognition capability that provide specific selectivity for the target analyte. Polymerization is realized by crosslinking the monomer molecules around the target analyte and the final material is obtained. Whether the monomer is associated with that target analyte or not is crucial since this property affects the chemical structure of the final material (Turiel and Martín-Esteban 2010). The intermolecular interactions such as hydrogen bonds, dipole-dipole

and ionic interactions between the target molecule and the monomer groups are necessary to enable the polymerization. Molecularly imprinted polymers have some advantages:

- High selectivity for target molecule
- Lower production costs
- Long term stability for storage (Vasapollo, Sole, et al. 2011).

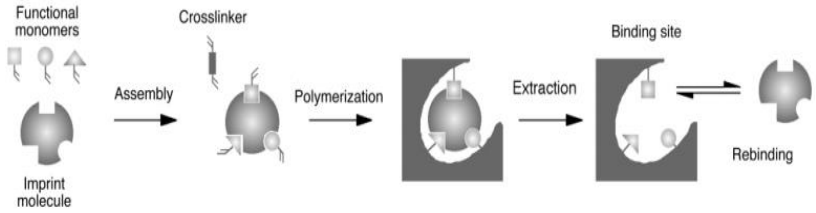


Figure 1.3. Schematic representation of the molecular imprinting principle (Source: Haupt 2001).

A general outline of the molecular imprinting methodology is given in Figure 1.3. The choice of template molecule is important as it relates to the bonds it will make with the functional monomer. Template molecule (the analyte or dummy molecule) must comply with polymerization conditions and must have a suitable group for polymerization. Target molecule must remain stable with the increase in temperature (Cormack and Elorza 2004). The monomers are used to make the material unique by creating selective cavities in the polymers which makes, the monomer selection very important for obtaining high selectivity. A complex structure is obtained having selective/specific recognition sites in the target molecule (Li et al. 2018). Mechanical strength of the material is provided by the crosslinker. The monomer forms a complex by molecular interactions around the template molecule. Polymerization takes place with the addition of the crosslinker. Finally, the template molecule is removed from the polymer.(Fig.1) The resulting polymer is expected to recognize the target molecule (Vasapollo, Del Sole, et al. 2011). Porogenic solvents are also important for MIP synthesis and often play a role in the polymerization process. 2-methoxyethanol, methanol, tetrahydrofuran (THF), acetonitrile, dichloroethane, chloroform, N, N-dimethylformamide (DMF), and toluene are some of the solvents used in MIP synthesis. The polarity of porogens may have an impact on their activity. Acetonitrile, and other less polar organic solvents non-covalent imprinting techniques, such as chloroform, are often used. Because of the adsorption properties, strong imprinting efficiency can be achieved (Chen et al. 2016).

Molecular imprinting technique is utilized to prepare selective materials for target molecules/ions like pharmaceuticals, steroids, pesticides, amino acids, sugars. Use of them in sensors and biomedical studies is gaining importance in many scientific disciplines (Haupt, Medina Rangel, and Bui 2020).

Magnetic MIP is another magnetic material-based separation method. Magnetic MIPs have recognition sites on their surface and are magnetically sensitive. Their sorption capacity is high and their application is easier than the other extraction methods because separation of the solid material from the sample solution can easily be carried out by an external magnet (Dong et al. 2021).

Magnetic materials are introduced to the solution or suspension containing the target analytes as outlined in Figure 1.4. After stirring or standing, the analytes are adsorbed onto the magnetic sorbents and the sorbents with the collected analytes are separated from the solution by suitable magnetic separator. The analytes are then eluted from the sorbents and detected with a proper analytical instrument. Magnetic materials provide various advantages over conventional solid supports, including a high surface-to-volume ratio, rapid and effective binding of target analytes, and high magnetic sensitivity (Chen and Li 2012).

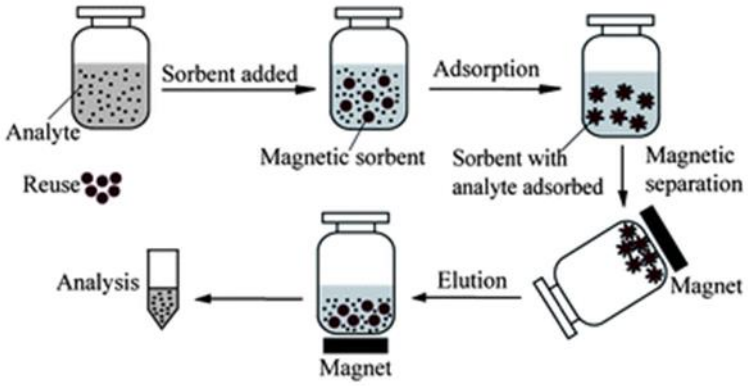


Figure 1.4. Solid phase extraction methodology with the use of magnetic MIPs (Source: Chen and Li 2012)

1.7. The Aim of the Study

In this study, solid phase extraction of ascorbic acid with zero-valent iron nanoparticles was aimed. Optimization parameters of the SPE procedure were investigated in terms of solution pH, sorbent amount, working solution amount, sorption time and desorption matrix.

CHAPTER 2

EXPERIMENTAL

2.1. Optimization of Instrumental Parameters

Initial experiments were about the optimization conditions for the determination of ascorbic acid by HPLC. For this purpose, a stock solution of 100 mgL-1 ascorbic acid in methanol was prepared. Standards and sample solutions were prepared on a daily basis.

All analyses were performed with Agilent 1200 HPLC with Diode Array Detector (DAD). The optimization parameters of HPLC-DAD are given in Table 2.1. The limit of detection (LOD) and limit of quantification (LOQ) were calculated after the experimental parameters were optimized.

Table 2.1. HPLC-DAD optimization parameters

Column	Sunfire C18 (4.6 x 250 mm) column
Mobile phase	90:10 methanol:water 85:15 methanol:water 100% methanol 90:10 methanol:water (acetic acid, pH:3.0) 85:15 methanol:water (acetic acid, pH:3.0) 85:15 methanol:water (phosphoric acid, pH:3.0) 85:15 water:methanol (phosphoric acid, pH:3.0)
Thermostat temperature	25.0ºC
Sample injection volume	20.0 µL
Flow rate	0.8, 0.9, 1.0 $mLmin^{-1}$
Standard solutions	0.010, 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 MIPs)

MIP and NIP should be made at the same time and in the same way. The following were the experimental steps used during the synthesis of MIP utilizing a precipitation

polymerization strategy: First, a 30.0 mL amber reaction vessel are with ascorbic acid as a template, acrylamide as a monomer, and acetonitrile as a porogen was added and agitated for 1.0 hour for pre-polymerization; next, EGDMA (Ethylene glycol dimethacrylate) as a cross-linker was added. As an initiator, 4,4'-azobis(4-cyanovalericacid) was utilized. In the reaction mixture, all reagents except ascorbic acid were carefully added under Ar gas to eliminate dissolved oxygen. The polymerization reaction was carried out for 24 hours in an oil bath at 60°C.

Solid MIP was achieved after polymerization. methanol was used to remove the template molecule. MIPs were dried in an oven at 60.0 °C after the ascorbic acid was completely removed, and the sorbent was ready for the studies. NIP was made in the same way as MIP, but with the exclusion of ascorbic acid. Figure 2.1. shows a schematic representation of MIP synthesis.

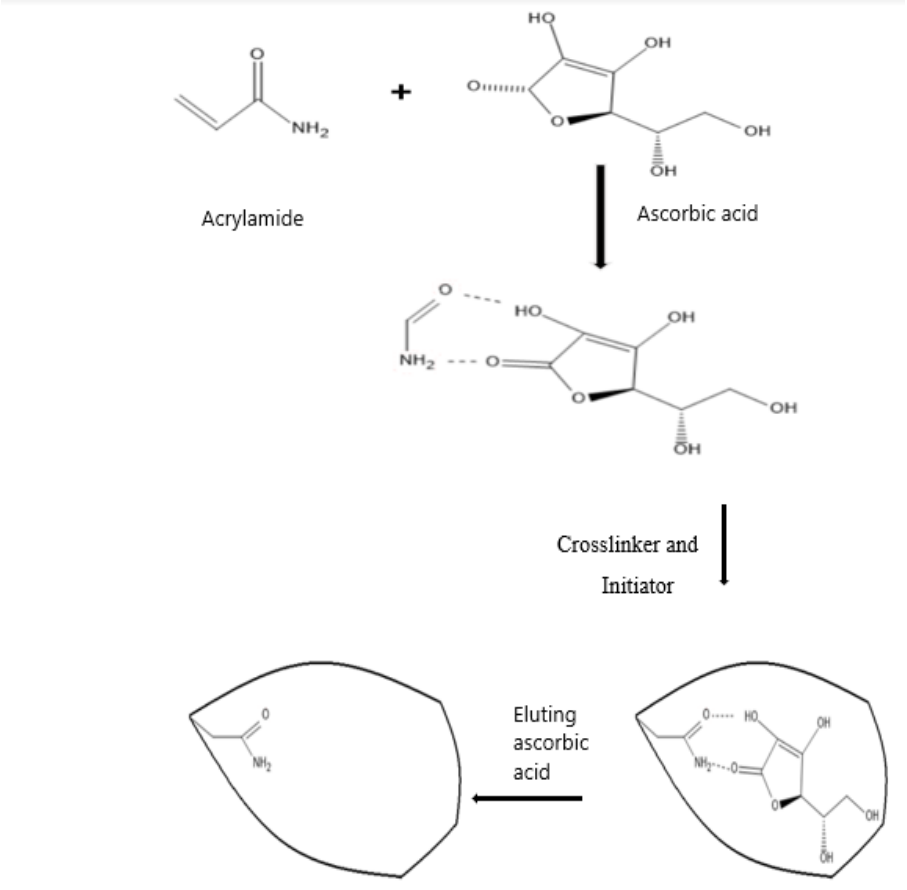


Figure 2.1. Synthesis of MIP by copolymerization of AA and EGDMA

After the synthesis, sorption experiments were started, and it was seen that the tried synthesis did not work. As a result, 4 different syntheses were tried. Finally, these 4

syntheses gave negative results. At this stage, the strategy was changed, and a new stage was started. Magnetic MIP synthesis was tried by using the reducing agent property of ascorbic acid and the magnetic property of iron. 4 syntheses in Table 2.2. were tried and no sorption was observed in the material. The synthesis can be developed in future studies. For this reason, magnetic MIP synthesis was made by taking advantage of the magnetic property of iron.

Table 2.2. Synthesis of ascorbic acid

	Template	Monomer	Crosslinker
Synthesis 1	Vitamin C (0.4 mmol)	Acrylamide (2 mmol)	EGDMA (10 mmol)
Synthesis 2	Vitamin C (1 mmol)	Acrylamide (4 mmol)	EGDMA (20 mmol)
Synthesis 3	Vitamin C (1 mmol)	Methacrylic acid (8 mmol)	EGDMA (20 mmol)
Synthesis 4	Vitamin C (1 mmol)	4 vinylpyridine (6 mmol)	EGDMA (30 mol)

2.3. Synthesis of Nano Zero-valent Iron

Firstly, 17.8 g Iron dichloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) was dissolved in 50 mL of ethanol and purified water (4:1, v/v). 8.47 g $NaBH_4$ was dissolved in 220 mL of deionized water a 1 M solution as the reducing agent. The NaBH4 solution was added directly to the Fe^{2+} solution. At this time, the reaction mixture was thoroughly mixed. The reason it is added directly is because it has to be nano-sized. The black particles of zerovalent iron appeared immediately. Iron powder was separated from solution using vacuum filtration. After that, the solid was rinsed three times with ethanol. the powder dried under nitrogen gas for overnight. During step oven without air evacuation.

2.4. Synthesis of Magnetic Molecularly Imprinting Polymers

First step, synthesis of Fe@MPS was performed. 250 mg of Fe NPs was dissolved in 150 ml of methanol with sonication for 20 minutes. 10.0 ml of MPS (3-

(methacryloyloxy) propyl trimethoxysilane) was added dropwise. The mixture was leaved for 24 h under a continuous stirring. The product was collected with the help an external magnet. The powder was rinsed with methanol and dried in a vacuum or inert atmosphere. Second step, synthesis of Fe@MPS@MIP was carried out. 1.0 mM of the ascorbic acid (template) and 2.0 mM of acrylamide (the functional monomer) was dissolved in 25.0 mL of the methanol. The mixture was shook in a water bath at 25°C for 24 h. 250 mg of Fe@MPS was added and shook for 4 h. Then EGDMA, (Ethylene glycol dimethacrylate) (crosslinker) and 4,4'-azobis (4- cyanovalericacid) (initiator) were added. The synthesis was kept in the oil bath for 24 h. The magnetic molecularly imprinted nanoparticles (MMINPs) can be dried at 40°C under vacuum or inert atmosphere. It can also prepare Fe@MPS@NIP similarly.

2.5. Characterization

Characterization experiments were performed to compare the sorption capacities for Fe, Fe-Cu and Fe-Ni. To better understand the selectivity of Fe, Fe-Ni and Fe-Cu against ascorbic acid, the Cross Sensitivity test was performed in the presence of structurally similar compounds. Following this stage, experimental studies were continued by choosing Fe.

2.5.1. Binding Characteristic Assay

Sample solutions were prepared as in Table 2.3. 10 mg of Fe, Fe-Ni and Fe-Cu were weighed. After that 10 mL these solutions added into these vials and shaken at 480 rpm, 1 hours. The mixture was filtered cellulose acetate membranes (0.45 µm pore size). Analysis was observed with HPLC-DAD at 265 nm.

Table 2.3. Parameters of binding characteristic assay

Standard concentration	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 hours
Shaking speed	480 rpm
Temperature	25.0 ºC

2.5.2. Cross Sensitivity

Ascorbic acid and vitamin B mixtures of 1.0 mgL^{-1} were prepared. 10.0 ml of this mixtures was added to vials containing 10.0 mg of Fe, Fe-Ni and Fe-Cu. Sorption was achieved using an orbital shaker at 480 rpm for 1 hour. The mixture was filtered cellulose acetate membranes (0.45 µm pore size). The mixture was analyzed with HPLC-DAD at 265 nm. Table 2.4. shows the sorption parameters.

Table 2.4. Studied parameters during sorption

Standard concentration	1.0 mgL^{-1}
Amount of sorbent	10.0 mg
Sample solution volume	10.0 mL
Sorption time	1 hours
Shaking speed	480 rpm
Temperature	25.0ºC

2.6. Optimization Parameters

2.6.1. Effect of pH on Sorption

To investigate the effect on pH on ascorbic acid and iron sorption,1.0 mgL^{-1} ascorbic acid solutions at pHs 3.0, 4.0, 6.0 and 8.0 (adjusted with phosphoric acid and sodium hydroxide) were prepared. 10.0 mL of these solutions were added to amber vials

already containing 10.0 mg iron. Vials were shaken for 1 hour at 480 rpm. After the sorption process, the pHs of the mixtures were checked, and they were filtered using a membrane filtration system with cellulose acetate membranes. (0.45 µm pore size). The mixture was analyzed with HPLC-DAD at 265 nm. The parameters tested in the pH study are shown in Table 2.5.

Table 2.5. Parameters used for the pH determination

Standard concentration	1.0 mgL^{-1}
Amount of sorbent	10.0 mg
Solution volume	10.0 mL
Sorption time	1 hours
Shaking speed	480 rpm
Temperature	25.0 ºC
pH	3.0, 4.0, 6.0, 8.0

2.6.2. Effect of Sorbent Amount

The effect of sorbent amount was investigated; iron sorbents were weighed and placed in amber vials as shown in Table 2.6. A solution of 1.0 mgL^{-1} ascorbic acid in 10.0 mL was added. Sorption was occurred on the orbital shaker for 1 hour at 480 rpm. Filtration was accomplished using a membrane filtration system and cellulose acetate membranes. (0.45 µm pore size). Solutions were tested with HPLC-DAD at 265 nm.

Table 2.6. Studied parameters in sorbent amount determination

Standard concentration	1.0 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 hours
Shaking speed	480 rpm
Temperature	25.0 ºC

2.6.3. Effect of Sample Volume

Ascorbic acid solution volumes were prepared as shown in Table 2.7. The solutions pH was adjusted to 6.0. Different volumes of 1.0 mgL^{-1} ascorbic acid solutions were added to sample vials containing 10.0 mg iron. Sorption was performed for 1 hour on the orbital shaker at 480 rpm. For filtration of the mixtures, a membrane filtration system with cellulose acetate membranes (0.45 m pore size) was used. Solutions were analyzed with HPLC-DAD at 265 nm.

Table 2.7. Optimization parameters for determination of sample volume

Standard concentration	1.0 mgL^{-1}
pH	6.0
Amount of sorbent	10.0 mg
Solution volume	5.0, 10.0, 20.0, 50.0 mL
Sorption time	1 hours
Shaking speed	480 rpm
Temperature	25.0 ºC

2.6.4. Effect of Sorption Time

In sample vials containing 10.0 mg iron, 10.0 mL of 1.0 mgL^{-1} ascorbic acid solution at pH 6.0 was added. At 480 rpm, the orbital shaker was used to achieve sorption. HPLC-DAD at 265 nm was used to analyze the samples.

Table 2.8. Studied parameters used in determination sorption time

Standard concentration	1.0 mgL^{-1}
pH	6.0
Amount of sorbent	10.0 mg
Sample solution volume	10.0 mL
Shaking time	1.0, 5.0, 10.0, 15.0, 30.0, 60, 90, 120 min.
Shaking speed	480 rpm
Temperature	25.0 ºC

2.6.5. Effect of Eluent Type

Two different solutions were tried for elution of ascorbic acid from sorbent. In sample vials containing, 10.0 mL of 1.0 mgL^{-1} ascorbic acid at pH 6.0 were added. Sorption was performed at 480 rpm on the orbital shaker. Methanol and methanol:water (MeOH:H2O) (acetic acid, pH 3.0) (85:15) were used as eluents after filtration. HPLC-DAD was used to analyze the solutions at 265 nm.

Table 2.9. Parameters used in determination of eluent

Standard concentration	1.0 mgL^{-1}
Amount of sorbent	10.0 mg
Sample solution volume	10.0 mL
Sorption time	1 hours
Shaking speed	480 rpm
Desorption matrix	Methanol Methanol/Water (85:15) pH:3
Temperature	25.0 ºC

2.6.6. Reusability of Sorbent

Reusability experiments were carried out to determine the number of times the sorbent may be used. 10.0 mL of 1.0 mgL^{-1} ascorbic acid solution at pH 6.0 was added to sample vials containing 10.0 mg nano zero valent iron for this purpose. Sorption was produced using a 480 rpm orbital shaker. Methanol: water (MeOH: H_2O) (acetic acid, pH:3.0) was used to elute the analyte molecule from the sorbent (85:15). The technique was done 6 times with the same sorbent each time. Every parameter is listed in Table 2.10.

Table 2.10. Parameters for reusability of sorbent

Standard concentration	1.0 mgL^{-1}
pH	6.0
Amount of sorbent	10.0 mg
Solution volume	10.0 mL
Sorption time	1 hours
Shaking speed	480 rpm
Temperature	25.0 ºC
Number of reuses	6

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1. Optimization of Instrumental Parameters

The parameters used along the study are shown in the Table 3. As a result of optimization, parameters were determined as mobile phase 85:15 (H_2O: MeOH), flow rate 0.9 ml / min. and column temperature were set to 25 ºC. Limit of detection (LOD) and limit of quantitation (LOQ) values were calculated as 0.017 and 0.057, respectively. The calibration graph of ascorbic acid is shown in Fig.3.1.

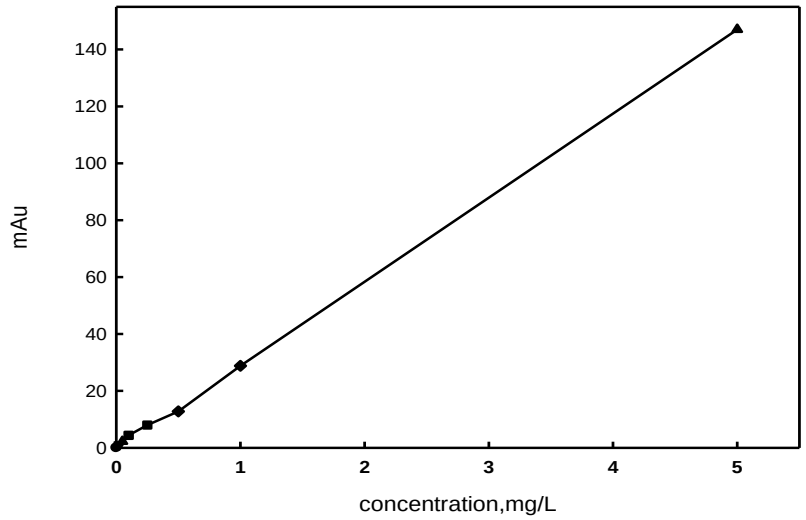


Figure 3.1. Calibration plot for ascorbic acid. (Agilent 1200 Series HPLC-DAD system, Supelco C18 (25cm×4.6mm) column, 15:85 MeOH: H_2O (pH 3.0) mobile phase, 0.9 mLmin-1 flow rate, 265 nm)

3.2. Characterization Experiments

3.2.1. Binding Characteristic Assay

Characterization experiments were performed to compare the sorption capacities for Fe, Fe-Cu and Fe-Ni. To learn more about these bimetallic nanoparticles' selectivity for ascorbic acid. Table shows how to prepared the sample solutions. 10 mg of Fe, Fe-Ni and Fe-Cu were weighed. Following that, 10 mL of these solutions were added to these vials and shaken for one hour. Using cellulose acetate membranes, the mixture was filtered. Following this stage, experimental studies were continued by choosing Fe.

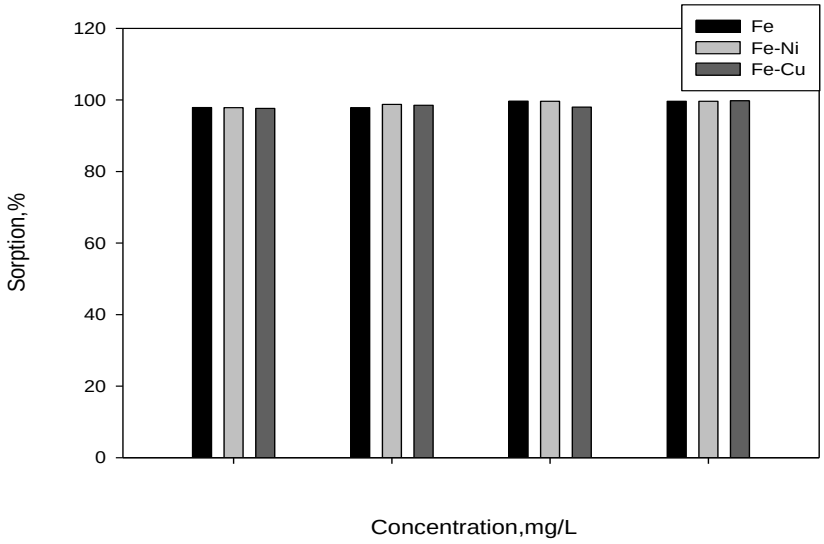


Figure 3.2. Sorption capacities of Fe, Fe-Ni and Fe-Cu

3.2.2. Cross Sensitivity

Figure 3.3. demonstrates the sorption capacities of nZVI as determined by the conditions described in section 2.3.2.

The selectivity and sorption capacity of vitamin C against vitamin B and these bimetallic nanoparticles were investigated. It has been observed that vitamin C has high selectivity. The bimetallic nanoparticles showed the same sorption for both vitamins.

Vitamin B6 was chosen for this experiment because vitamin B6, like vitamin C, is water-soluble. There is a structural difference between vitamin B6 and vitamin C.

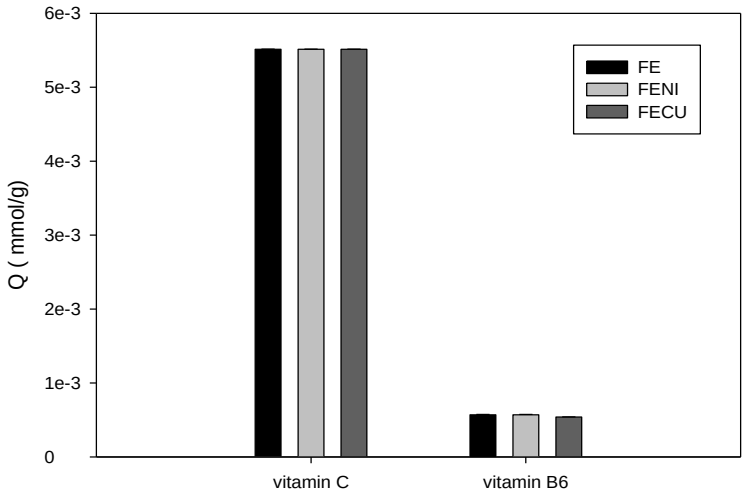


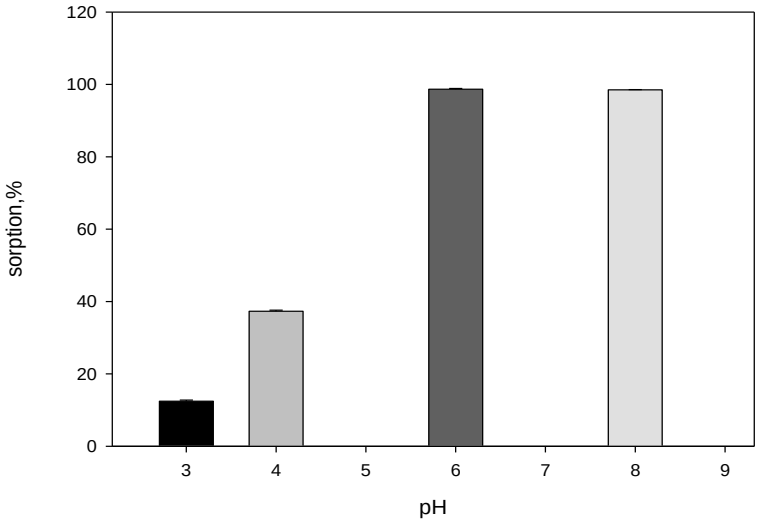
Figure 3.3. Sorption capacity of Fe, Fe-Ni and Fe-Cu in the presence of vitamin B

3.3. Optimization Parameters

3.3.1. Effect of pH on Sorption

As described in Section 2.4.1, the effect of pH on sorption was examined. The sorption capacity increased as the pH increased. At pH 6.0 and 8.0, the percentage of sorption capacity is high. Values of pH 8.0 and above are the most difficult values for iron to be taken. Iron oxidation occurs very slowly if the pH is less than 6.0. As seen in Figure 3.4. (B) nanoparticles are most unstable in the region where the zeta potential is close to zero and it can be said that the isoelectric point for the nZVI nanoparticle is about 8.0-8.5. As a result, no effect was observed when examined at pH 8.0 and above. This may have been caused by the precipitation of iron at pH 8.0 and above. The pH was set to 6.0, and the remaining experiments were performed at this level.

A)



B)

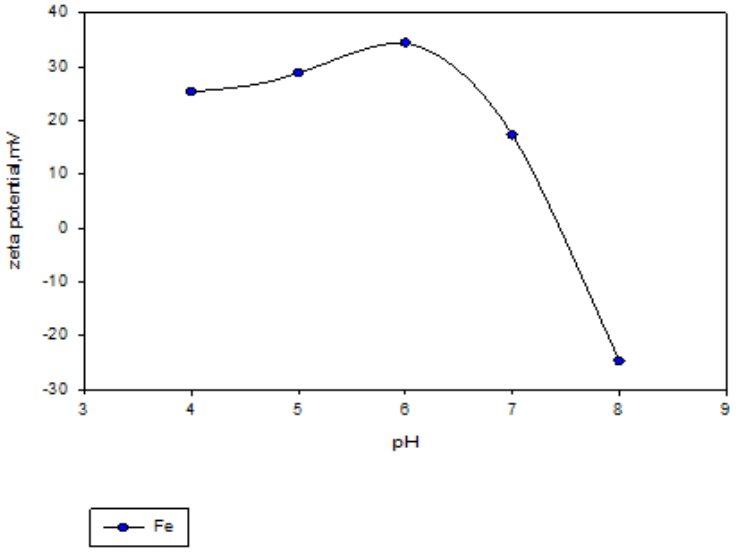


Figure 3.4. (A) Effect of pH on sorption. (B) Determination of the isoelectric point for nZVI particles

3.3.2. Effect of Sorbent Amount

The batch type SPE procedure was applied for the amount of iron sorbent given in Table 2.6., as described in Section 2.4.2. The Figure 3.5. demonstrates the effect of sorbent amount on sorption percentage. The sorption percentage increased up to 10.0 mg, after which there was no change in the sorption percentage. In the remaining experiments, sorbent amount of 10.0 mg was used.

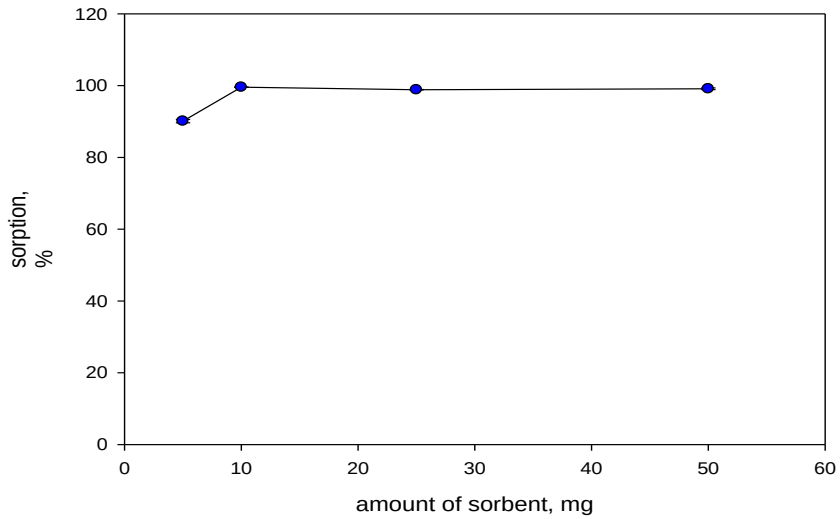


Figure 3.5. Effect of sorbent amount for 10 mL, 1.0 mgL-1 of ascorbic acid

3.3.3. Effect of Sample Volume

As described in section 2.4.3, the effect of sample volume on percent sorption was examined. Figure 3.6. shows that, under optimal conditions, 5.0 and 10.0 mL solutions have greater sorption capacity than the other volumes. Since a decrease in the sorption percentage was observed after 10.0 ml, the sample volume was determined as 10.0 ml in the remaining experiments.

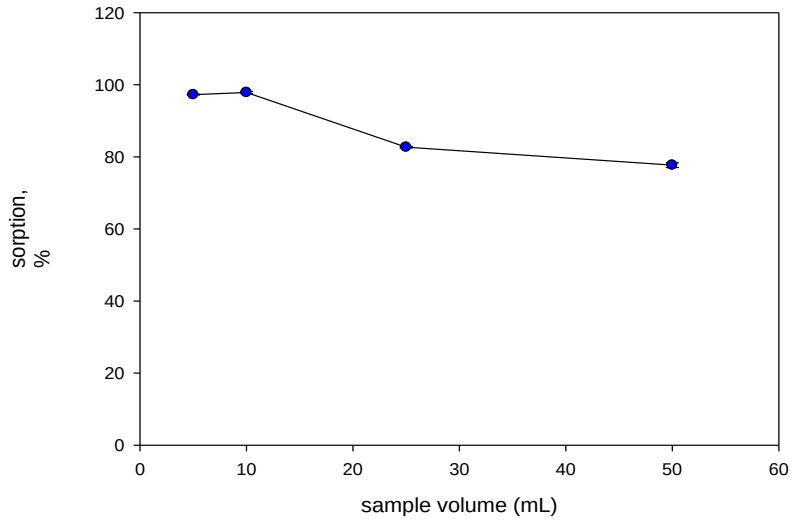


Figure 3.6. Effect of sample volume for 1.0 mgL-1 ascorbic acid and 10.0 mg iron sorbent

3.3.4. Effect of Sorption Time

The effect of time on sorption was investigated, as described in Section 2.4.4. As seen, the interaction time has little effect on sorption, and 60 minutes was chosen to ensure sorption in the remaining experiments.

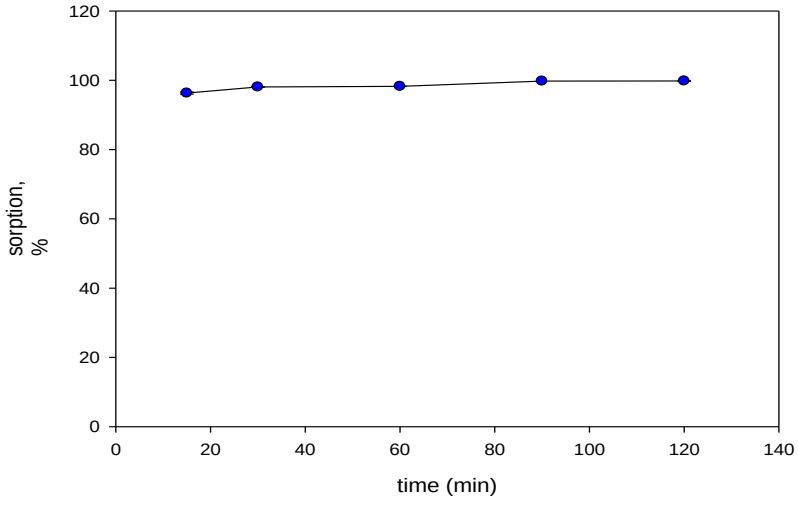


Figure 3.7. Effect of shaking time on the sorption of 10 mL 1.0 mgL-1 of ascorbic acid and 10 mg iron sorbent

3.3.5. Effect of Eluent Type

Desorption is just as crucial as sorption in the solid phase extraction process. As a result, the sorbed analyte should be recovered from the sorbent using the correct eluent, and the desorption percentage computed. For elution of ascorbic acid from sorbent, two different solutions were explored. Methanol has a high binding capacity and tends to form hydrogen bonds. Since sorption does not occur in an acidic environment, high recovery is expected in MeOH: Water (85:15) (pH adjusted with acetic acid) solution.

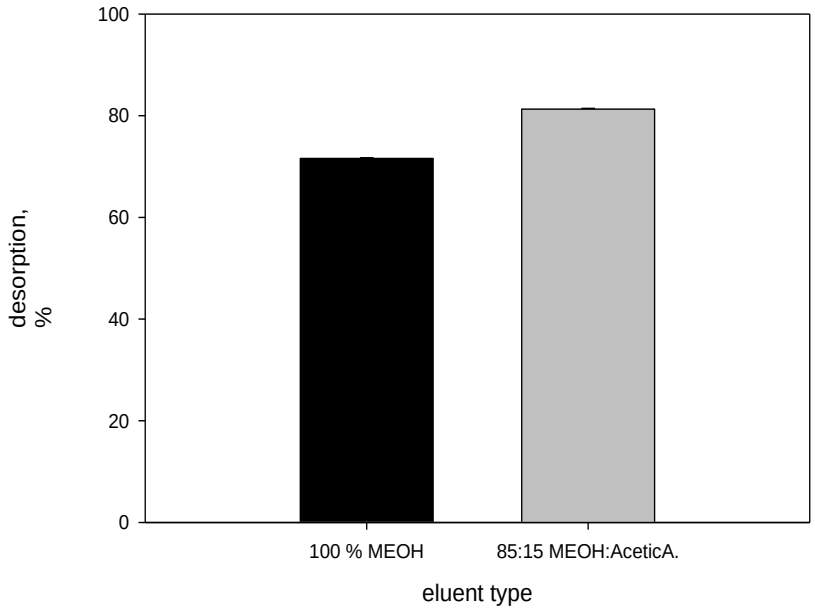


Figure 3.8. Effect of desorption matrix

3.3.6. Reusability of Sorbent

Reusability experiments were carried out to determine the number of times the sorbent may be used.

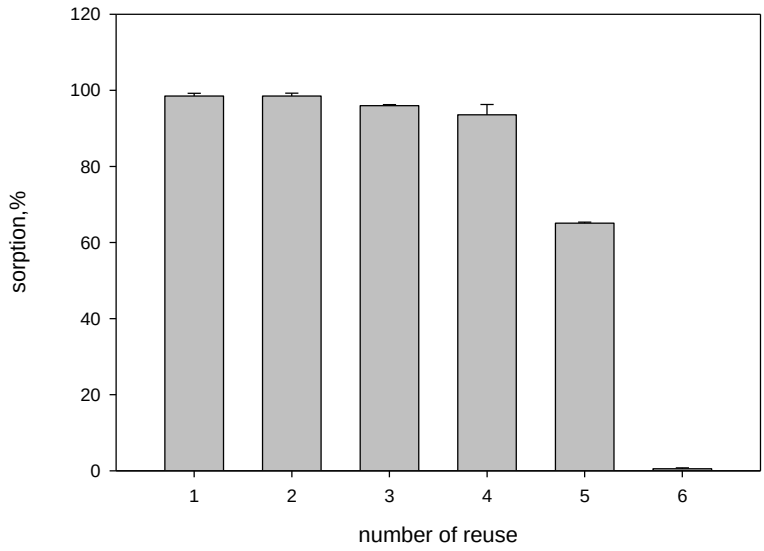


Figure 3.9. Regeneration for reuse.

Figure 3.9. indicates that up to the fourth sorption level, there is quantitative sorption. (>90.0%). As a result, it may be stated that the sorbent can only be utilized four times under the experimental conditions. The reason why the sorption decreased to zero in the 6th trial may be due to the damage to the adhesion areas of the material. In addition, this decrease may have been observed since there was a loss in the amount of substance due to washing during the experiment.

3.4. Determination of the Isoelectric Point for Nano Zero-valent Nanoparticles

The isoelectric point was determined for the synthesized nZVI nanoparticles. 10 mg of iron was dissolved in 50 ml of water and pH values were prepared as 4.0 ,5.0 ,6.0 ,7.0 and 8.0, respectively. Due to instrumental restrictions, pH of the solutions could not have been prepared at pHs higher than 8.0. As seen in Figure 3.10. nanoparticles are most unstable in the region where the zeta potential is close to zero and it can be said that the isoelectric point for the nZVI nanoparticle is about 8.0-8.5.

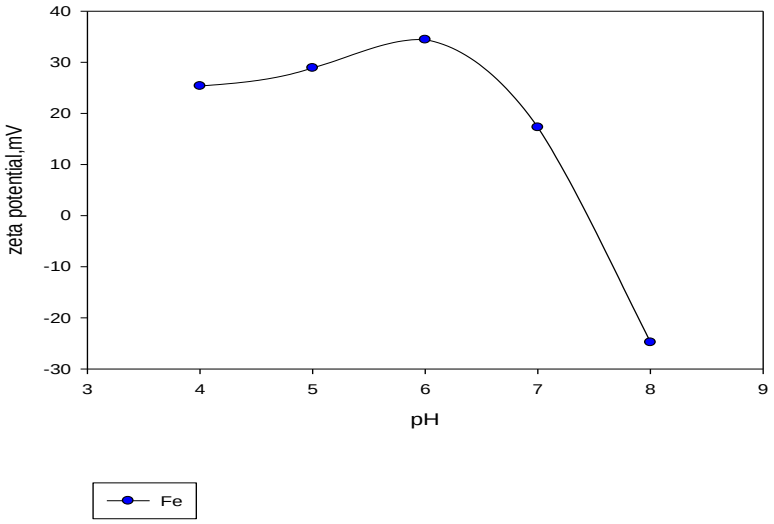


Figure 3.10. Determination of the isoelectric point for nZVI particles

3.5. Characterization of the Sorbents by Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Spectrometry (EDX) and X-ray Diffraction (XRD)

The first technique employed for the characterization of the sorbents was (EDX). SEM images of nZVI particles are shown in Figure 3.11. The typical chain-like structure of the material is attributed to the attractive magnetic forces among the individual iron particles as observed in previous studies (Shahwan et al. 2011). The elemental composition shown in the Figure 3.12. in the EDX spectrum reflects that the material is composed of Fe and O, as expected. The peak of C is usually caused by incidental carbon sources at the time of analysis.

Figure 3.13. shows the XRD pattern of the nZVI particles. The pattern shows that the sorbent is composed of zero-valent iron with its typical reflection at 2θ value of 44.9°. No peaks are observed for iron oxide or iron oxyhydroxide, but this does not eliminate the possibility of their presence in the shell-part of the core-shell nanoparticles structure (Shahwan et al. 2010). The peak appears to be relatively sharp and intense reflecting a crystalline order in the structure of the material.

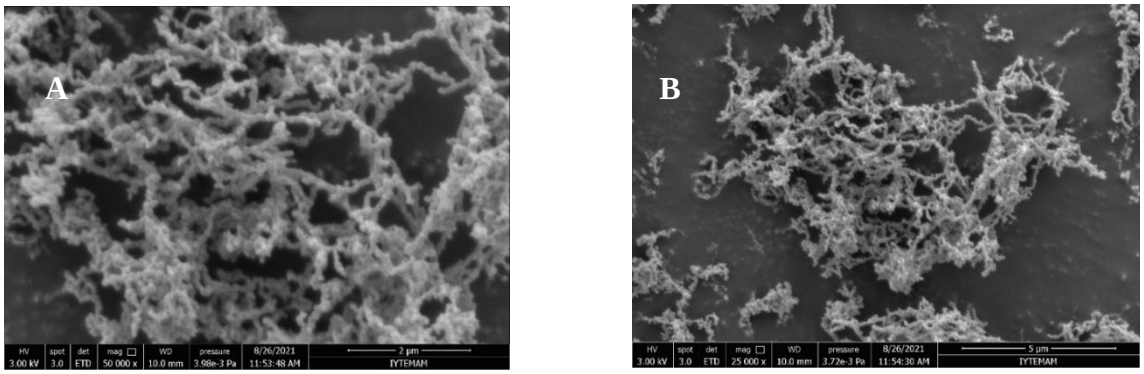


Figure 3.11. SEM images of nZVI particles
(A) 50000x magnification, (B) 25000x magnification

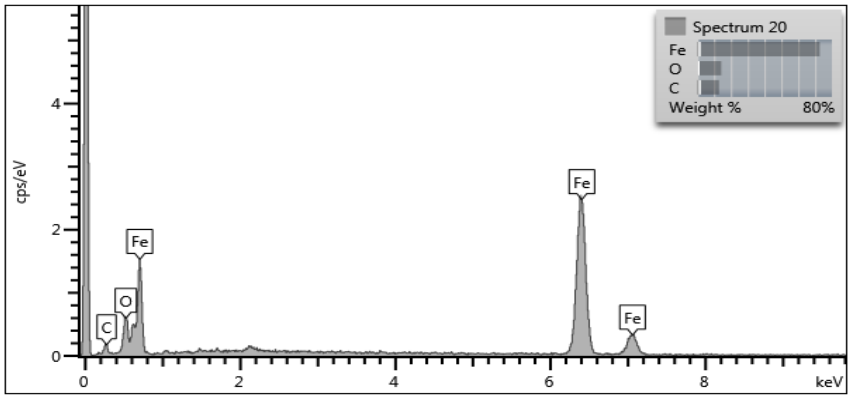


Figure 3.12. EDX spectrum of nZVI particles

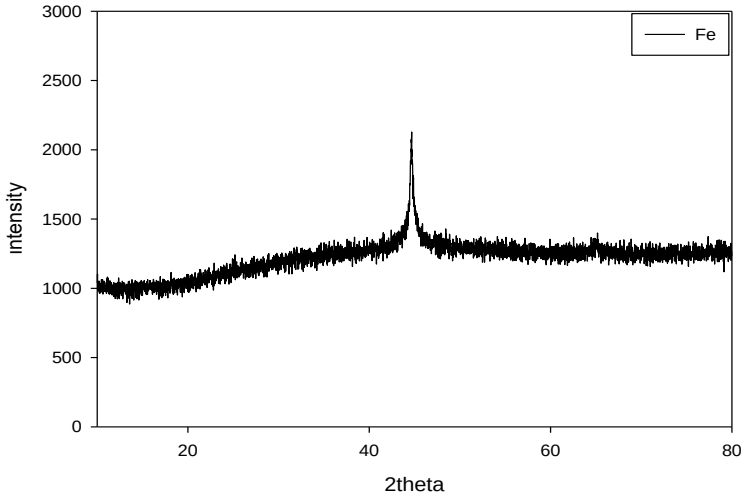


Figure 3.13. XRD spectrum for nZVI

Figure 3.14. shows typical SEM images of magnetic MIP particles. The MMIPs nanoparticles have a spherical shape. These nanoparticles clump together quickly due to their small size. For magnetic MIPs, the EDX spectrum revealed the presence of C and O (Fig.3.15, A), but no characteristic signal.

The adsorption capability of the sorbent is affected by its shape and size (MNIPs). SEM pictures of magnetic MIPs reveal that the material is made up of distinct, spherical particles as shown in Figure 3.14. Figure 3.15. shows the EDX spectrum of magnetic NIPs. For magnetic NIPs, the EDX spectrum revealed the presence of C, O, and Fe.

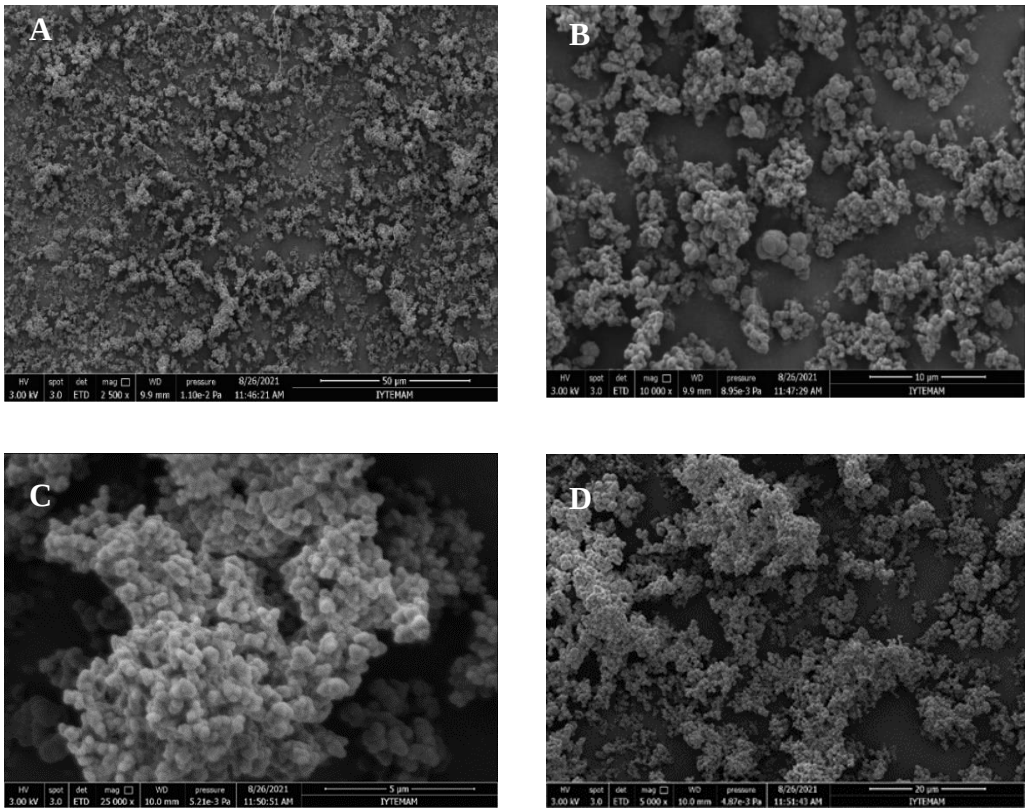


Figure 3.14. SEM images of MMIP (A,B) and MNIP(C,D)

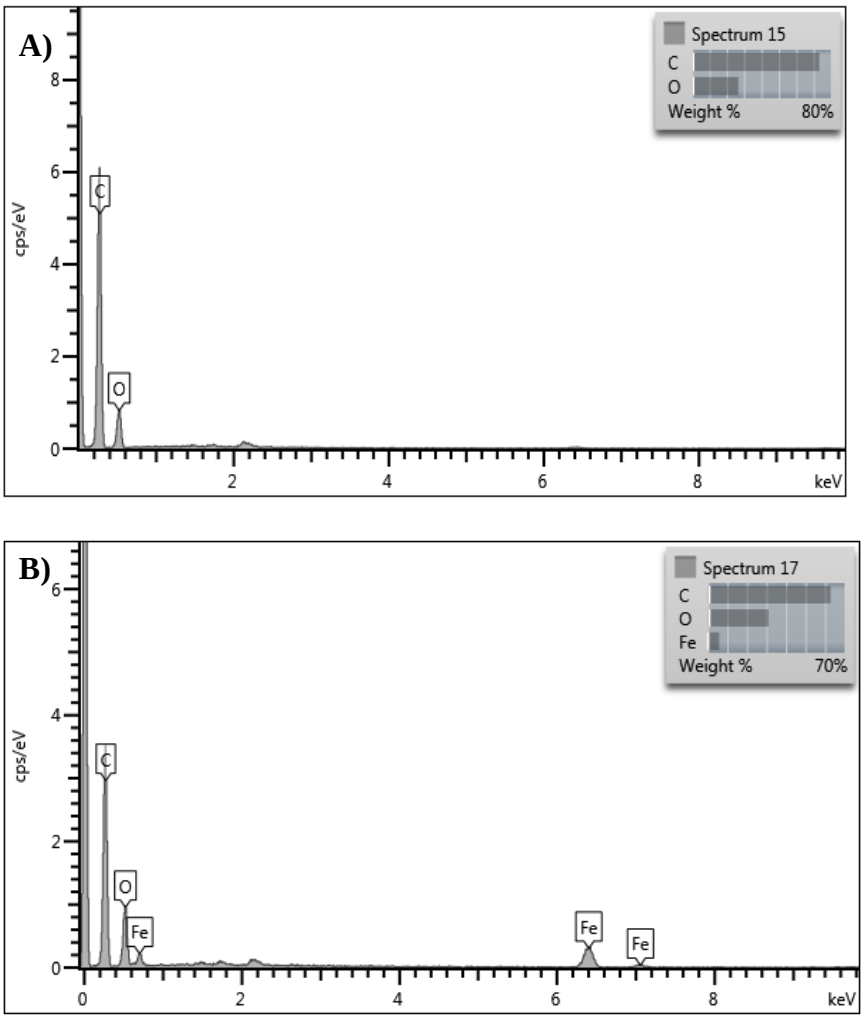


Figure 3.15. EDX spectrum of MMIPs (A) and NMIPs (B)

CHAPTER 4

CONCLUSION

In this study, solid phase extraction studies of ascorbic acid with nano zero valent iron were carried out.

The sorption capacity increased depending on the pH. This capacity is high at pH 6.0 and 8.0. Iron oxidation occurs very slowly if the pH is less than 6.0. As a result, when pH 8.0 and higher were tested, there was no effect. The precipitation of iron at pH 8.0 and above could have caused this. The pH level was fixed to 6.0, and the rest of the studies were carried out at this level. The optimal sorbent concentration, solvent concentration, and shaking time were determined to be 10.0 mg, 10.0 mL, and 1 hour, respectively. MeOH and MeOH: H_2O, 85:15, were used to achieve desorption (acetic acid, pH 3.0). Both matrixes demonstrated quantitative desorption, and MeOH: H_2O, used as the mobile phase in HPLC throughout investigation. With the same nano zero valent iron sorbent, the proposed approach was replicated 6 times. Up to fourth sorption iron demonstrates quantitative sorption, according to the findings.

Characterization experiments (SEM, EDX and XRD) were performed for nano zero-valent iron.

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