การตรวจวัดครีเอทินินด้วยสเปกโทรโฟโตเมตรีหลังการสกัดด้วยอนุภาคแม่เหล็กที่เคลือบด้วยเอ็มซีเอ็ม-41 ที่ถูกดัดแปรพื้นผิว

นายศักรินทร์ บุญธรรม

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คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2555

DETERMINATION OF CREATININE BY SPECTROPHOTOMETRY AFTER EXTRACTION BY FUNCTIONALIZED MCM-41-COATED MAGNETIC PARTICLES

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ศักรินทร์ บุญธรรม : การตรวจวัดครีเอทินินด้วยสเปกโทรโฟโตเมตรีหลังการสกัดด้วยอนุภาคแม่เหล็กที่เคลือบด้วยเอ็มซีเอ็ม-41 ที่ถูกดัดแปรพื้นผิว. (DETERMINATION OF CREATININE BY SPECTROPHOTOMETRY AFTER EXTRACTION BY FUNCTIONALIZED MCM-41-COATED MAGNETIC PARTICLES) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร.เฟื่องฟ้า อุ่นอบ, 72 หน้า.

งานวิจัยนี้จะสังเคราะห์อนุภาคแม่เหล็กที่เคลือบด้วยเอ็มซีเอ็ม-41และดัดแปรหมู่ฟังก์ชันบนพื้นผิวด้วยหมู่ซัลโฟนิก (Fe_3O_4/MCM-41/SO_3H) ทำให้ได้ตัวดูดซับที่มีสมบัติแม่เหล็กซึ่งสามารถแยกออกได้ง่ายหลังจากใช้งาน ได้พิสูจน์เอกลักษณ์เพื่อยืนยันตัวดูดซับที่สังเคราะห์ได้โดยอาศัยเครื่องเอ็กซเรย์ดิฟแฟรกโทรมิเตอร์ ฟูเรียร์ทรานสฟอร์มอินฟราเรดสเปกโทรโฟโตมิเตอร์ กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด กล้องจุลทรรศน์อิเล็กตรอนแบบส่องผ่าน เครื่องวิเคราะห์เทอร์โมกราวิเมตริก และเครื่องวัดพื้นผิว ตัวดูดซับ Fe_3O_4/MCM-41/SO_3H ถูกใช้ในการสกัดและแยกครีเอทินินออกจากเมทริกซ์ของตัวอย่าง ก่อนจะนำครีเอทินินที่ถูกสกัดแล้วไปวิเคราะห์ด้วยวิธีจาฟเฟ่ จากนั้นจึงทำการศึกษาตัวแปรที่มีผลต่อประสิทธิภาพการสกัด เช่น พีเอชของตัวอย่าง ปริมาณของตัวดูดซับ ปริมาตรของตัวอย่าง ความเข้มข้นของตัวชะ เวลาที่ใช้ในการสกัด และเวลาที่ใช้ในการชะ นอกจากนี้ได้ทำการศึกษาความสามารถในการนำมาใช้ซ้ำของตัวดูดซับอีกด้วย สำหรับประสิทธิภาพของวิธีการวิเคราะห์ที่นำเสนอมาถูกตรวจสอบภายใต้สภาวะที่เหมาะสม โดยจะทดสอบด้วยการตรวจวัดสารละลายมาตรฐานครีเอทินินที่ความเข้มข้น 10 และ 50 มิลลิกรัมต่อลิตร จากผลการทดลองพบว่าค่าการได้กลับคืน (%Recovery) อยู่ในช่วง 95.3 – 98.8 % และค่าความเที่ยงในการวิเคราะห์ (%RSD) อยู่ในช่วง 0.5 – 1.8 % นอกจากนี้พบว่าค่าความเข้มข้นต่ำสุดของการตรวจวัดครีเอทินินอยู่ที่ 1.5 มิลลิกรัมต่อลิตร และมีความสัมพันธ์เชิงเส้นตรงของค่าการดูดกลืนแสงสูงถึงความเข้มข้นอย่างต่ำเท่ากับ 60 มิลลิกรัมต่อลิตร จากการวิเคราะห์ครีเอทินินในตัวอย่างปัสสาวะมนุษย์ที่ได้เติมสารละลายมาตรฐานครีเอทินินที่ทราบปริมาณที่แน่นอนลงไป พบว่าค่าการได้กลับคืน (%Recovery) อยู่ในช่วง 96.7 – 99.5 % และค่าความเที่ยงในการวิเคราะห์ (%RSD) อยู่ในช่วง 0.2 – 1.0 % จากผลการทดลองแสดงให้เห็นว่าวิธีการวิเคราะห์ที่นำเสนอมาสามารถนำไปประยุกต์ใช้ในการวิเคราะห์ครีเอทินินในตัวอย่างปัสสาวะของมนุษย์ได้ด้วยความแม่นและความเที่ยงที่ยอมรับได้

ภาควิชา เคมี ลายมือชื่อนิสิต .....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2555

5372338723 : MAJOR CHEMISTRY
KEYWORDS : SULFONIC ACID / MCM-41 / MAGNETIC PARTICLES / JAFFE METHOD / CREATININE / EXTRACTION

SAKKARIN BOONTHAM : DETERMINATION OF CREATININE BY SPECTROPHOTOMETRY AFTER EXTRACTION BY FUNCTIONALIZED MCM-41-COATED MAGNETIC PARTICLES. ADVISOR: ASST. PROF. FUANGFA UNOB, Ph.D., 72 pp.

Sulfonic acid functionalized MCM-41-coated magnetic particles (Fe_3O_4/MCM-41/SO_3H) which have magnetic properties for easy separation from sample media was synthesized. X-ray diffractometer (XRD), Fourier transforms infrared spectrophotometer (FT-IR), scanning electron microscope (SEM), transmission electron microscope (TEM), thermogravimetric analyzer (TGA) and surface area analyzer were employed for characterization of the synthesized solid. Fe_3O_4/MCM-41/SO_3H adsorbent was used for extraction and isolation of creatinine from samples matrix prior to the analysis by Jaffé method. The influence of extraction parameters on the extraction efficiency were studied including sample pH, amount of adsorbent, sample volume, eluent concentration, extraction time and elution time. Moreover, the feasibility of reusing the adsorbent was also studied. The performance of the proposed method was investigated under chosen condition using the standard solution containing 10 and 50 mg/L of creatinine. The percent recoveries (%Recovery) and the percent relative standard deviations (%RSD) of the method were in the range of 95.3 - 98.8 % and 0.5 - 1.8 %, respectively. Furthermore, the method limit of detection (LOD) was 1.5 mg/L and the linear range could be extended up to 60 mg/L. From the analysis of spiked urine samples, the recoveries and relative standard deviation in the range of 96.7 - 99.5 and 0.2 - 1.0, respectively, were observed. The results show that the proposed method could apply for analysis of creatinine in human urine samples with acceptable accuracy and precision.

Department : Chemistry ..... Student's Signature .....

Field of Study : Chemistry ..... Advisor's signature .....

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LIST OF SCHEMES

LIST OF ABBREVIATIONS

GFR	=	Glomerular filtration rate
SBA	=	Santa Barbara Amorphous
MCM	=	Mobil Crystalline Materials
FTIR	=	Fourier transform infrared spectrometer
XRD	=	X-ray diffractometer
SEM	=	Scanning electron microscope
TEM	=	Transmission electron microscope
TGA	=	Thermogravimetric analyzer
g	=	Gram
mg	=	Milligram
L	=	Liter
mL	=	Milliliter
HSAB	=	Hard-soft-acid-base principle
RSD	=	Relative standard deviation
SD	=	Standard deviation
Min	=	Minute
MPs	=	Magnetic particles
Fe_3O_4/MCM-41	=	MCM-41-coated magnetic particles
Fe_3O_4/MCM-41/SO_3H	=	Sulfonic acid functionalized MCM-41-coated magnetic particles

CHAPTER I

INTRODUCTION

1.1 Statement of propose

Creatinine is a final waste product from metabolism of existing creatine and phosphocreatine in skeletal muscle. The generated creatinine diffuses into the bloodstream [1] and the elimination of creatinine in plasma is mainly achieved by glomerular filtration and excretion with urine [2]. Creatinine is one of an important indicator of renal health; if the filtration of the kidney is deficient, high levels of creatinine in plasma and low levels of creatinine in urine are observed [3]. In addition, amounts of creatinine in 24-hour urine samples are useful information as an indicator of the glomerular filtration rate (GFR) which is roughly constant for an individual person [4]. The alteration of urinary creatinine content can indicate renal problems [5]. Moreover, urinary creatinine concentration can be used as indicator of urine dilution and it was often used as a normalization factor in the determination of other substances in urine samples [5].

Nowadays, there are several methods for determination of creatinine in blood and urine such as enzymatic methods [6-7] and chromatographic methods e.g. capillary electrophoresis [8-9], liquid chromatography [10-11] and liquid chromatography-tandem mass spectrometry [12-13]. However, the most popular method for determination of creatinine in clinical laboratory is colorimetric method called “Jaffé method” due to its simplicity, easy procedure and low operating cost [14]. The method is based on the reaction of creatinine and picric acid under alkaline condition to give orange-red complex which can be analyzed by UV-visible spectrometer. Nevertheless, sample matrix in biological samples such as glucose [15],

cycloketone [16] proteins [17] and ascorbic acid [18] would interfere the method sensitivity and accuracy. Therefore, sample preparation is required to overcome this shortcoming of Jaffé method.

Sample preparation by solid phase extraction has been developed and used to improve specificity of Jaffé method. Clay minerals such as kaolin and Lloyd's reagent were used for creatinine extraction due to its high surface area and low cost [19-20]. Nevertheless, it is difficult to separate the solid from aqueous media and it also extracts other substances such as keto acids, indole, glycocyamidine, various steroids and pigments which can cause false positive results from Jaffé method [21]. Cation exchange resin has also been used for extraction of creatinine. The counter ions of sulfonic acid groups on the resin were exchanged to sodium ions before use to prevent interfering problem in Jaffé method caused by hydrogen ions [22]. This method was successfully applied for extraction of creatinine in biological samples [23]. However, the poor recoveries of creatinine were observed [23].

At present, mesoporous silica materials gain popularity in many applications including as solid phase for extraction of biomolecules due to its durability and high surface area. Sulfonic acid functionalized mesoporous silica such as SBA-15/SO_3H was utilized as strong cation exchanger for extraction of some proteins such as bovine serum albumin, cytochrome C and polypeptides e.g. insulin and glucagon. However, separation of the solid after extraction can be difficult because of its small particle size and hydrophilicity. In this work, MCM-41 mesoporous silica was coated on Fe_3O_4 magnetic particles to give Fe_3O_4/MCM-41 adsorbent which has high surface area and can be easily separated from sample solution using external magnetic field. The solid was functionalized with 3-mercaptopropyltrimethoxysilane and then the thiol groups on Fe_3O_4/MCM-41 surface were converted to sulfonic acid groups by oxidation. The obtained adsorbent was employed in the extraction of creatinine in human urine samples to isolate it from matrix interferences prior to the determination by conventional Jaffé method.

1.2 Research objectives

1.2.1 To prepare and characterize the magnetic adsorbent; sulfonic acid functionalized MCM-41-coated magnetic particles (Fe_3O_4/MCM-41/SO_3H)

1.2.2 To study the influence of extraction parameters on the extraction efficiency to obtain the suitable condition for the extraction of creatinine.

1.2.3 To apply the prepared adsorbent for extraction of creatinine in human urine samples for the analysis by Jaffé method.

1.3 Scopes of the research

Fe_3O_4 magnetic particles were synthesized via co-precipitation method and then coated with MCM-41. The obtained solid was functionalized with 3-mercaptopropyltrimethoxysilane and the existing thiol groups on Fe_3O_4/MCM-41 surface were subsequently oxidized by H_2O_2 to sulfonic acid groups. The obtained Fe_3O_4/MCM-41/SO_3H was characterized by Fourier transform infrared spectrometer (FTIR), X-ray diffractometer (XRD), scanning electron microscope (SEM), transmission electron microscope (TEM), thermogravimetric analyzer (TGA) and surface area analyzer. Finally, the resultant adsorbent was used for extraction of creatinine in aqueous solution and human urine samples

In the extraction process, the influence of extraction parameters such as amount of adsorbent, sample pH, sample volume and eluent concentration were investigated. Moreover, the reusability of the adsorbent was also studied. Finally, the proposed method was validated and applied in the determination of creatinine in human urine samples.

1.4 The benefit of this research

A simple and accurate method for determination of creatinine in human urine samples was obtained.

CHAPTER II

THEORY AND LITERETURE REVIEW

2.1 Creatinine and method for determination of creatinine

2.1.1 Creatinine

As mentioned in chapter 1, creatinine (2-amino-1-methyl-2-imidazoline-4-one, Figure 2.1) is one of the waste products from metabolic processes of human. Creatinine in blood can be removed by glomerular filtration and excretion with urine. Creatinine concentration in urine is one of important indicators of renal health. Furthermore, it is often used as a normalization factor in determination of drugs and other substances in urine samples.

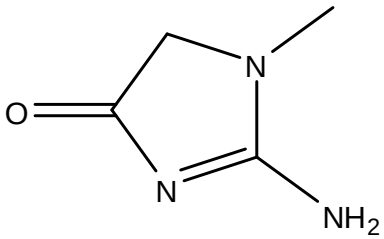


Figure 2.1 Structure of creatinine

2.1.2 Method for determination of creatinine

2.1.2.1 Enzymatic method [6-7]

In this method, three enzymes, creatinine amidohydrolase, creatine amidinohydrolase and sarcosine oxidase, were employed to convert creatinine in urine to H_2O_2 which can be detected by electrochemical methods (Equation 2.1-2.3). This method has high selectivity for creatinine and can be used for determination of creatinine with high accuracy. However, it is not a popular method because the operating cost is high due to the use of enzymes. Moreover, the enzymes are unstable and cannot be stored for a long time.

$$\text{Creatinine} + H_2O_2 \xrightarrow{\text{creatinine amidohydrolase}} \text{Creatine} \qquad (2.1)$$

$$\text{Creatine} + H_2O_2 \xrightarrow{\text{Creatine amidinohydrolase}} \text{Sarcosine} + \text{Urea} \qquad (2.2)$$

$$\text{Sarcosine} + H_2O_2 \xrightarrow{\text{Sarcosine oxidase}} \text{Glycine} + \text{Formaldehyde} + H_2O_2 \qquad (2.3)$$

2.1.2.2 Chromatographic method

Chromatographic method is one of alternative choices for creatinine determination. The method is based on the separation of components in extracted urine by different affinity to stationary phase and the individual species is detected by various types of detector. Some of researchers successfully applied the

chromatographic techniques such as capillary electrophoresis [8-9], liquid chromatography [10-11] and liquid chromatography-tandem mass spectrometry [12-13] for isolation and determination of creatinine with satisfactory results. Nevertheless, these methods are complicated and time consuming. Therefore, it is not widespread method for determination of creatinine.

2.1.2.3 Jaffé method [21]

Jaffé method, a colorimetric method used in clinical laboratory for determination of creatinine in urine and serum, was developed in 1886 by Max Jaffé, a German biochemist. This method is based on the reaction of creatinine and picric acid in basic condition to give orange-red complex called "Janovsky complex" which can be measured by UV-visible spectrometer (Figure 2.2). Nowaday, Jaffé method has been widely used for creatinine analysis due to its simplicity, rapidity and low operating cost.

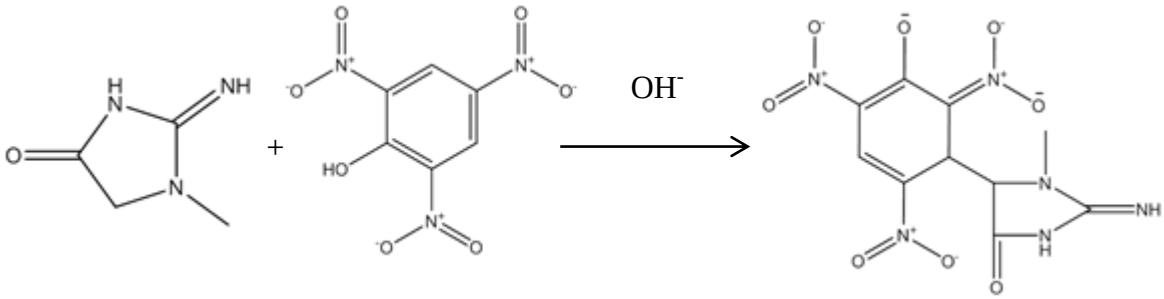


Figure 2.2 Schematic represent the Jaffé method.

However, numbers of works have reported about interferences problems of Jaffé method caused by matrix in biological samples such as glucose [15], ketone compounds [16] proteins [17] and ascorbic acid [18]. These interferences can react with alkaline picrate and form the complexes which are in the same color as Janovsky complex. This phenomenon causes false positive creatinine concentration results. Therefore, sample preparation is required to overcome this drawback of Jaffé method.

2.2 Sample preparation by solid phase extraction for creatinine determination

Various sample preparation methods have been developed for solving the matrix interferences problem in urine samples prior to determination by Jaffé method. Solid phase extraction is one of the effective sample preparation methods to improve the specificity of the method. Urine creatinine is extracted onto the solid phase and isolated from interferences before determination. The extraction of creatinine can be achieved in acidic solution through ion-exchange process. Clay minerals such as kaolin and Lloyd's reagent or conventional ion-exchange resins are used as adsorbent.

2.2.1 Extraction using kaolin and Lloyd's reagent as adsorbent [19-20].

Clay minerals such as kaolin and Lloyd's reagent which are low cost and have high surface area were employed for extraction of creatinine. Urine samples were acidified before extraction. After that the supernatants was discarded and alkaline picrate solution was employed for elution of creatinine from adsorbent. Finally, the orange-red color solutions were analyzed by UV-visible spectrometry. This method was successfully used in the determination of creatinine. Unfortunately the clay

minerals used as adsorbent is difficult to separate from aqueous media. Moreover, it also extracts other substances such as keto acids, indole, glycocyamidine, various steroids and pigments [21] which also interfere creatinine determination by Jaffé method.

2.2.2 Extraction using ion-exchange resins as adsorbent [22].

Ion exchange chromatography [24]

Ion-exchange chromatography (or ion chromatography) is type of chromatographic method which allows the separation of ions and polar molecules based on their charge. At present, it has been widely used in many fields including determination of inorganic ions, analysis and purification of charged proteins, nucleotides, amino acids and organic molecules. In this method, charged analyte molecules are retained on the ion exchangers by electrostatic forces between the surface of charged molecules and the cluster of the ionic functional groups on the exchangers. This type of chromatography can be classified into 2 categories, cation exchange chromatography and anion exchange chromatography. However, only the former will be described here.

Cation exchange chromatography [24]

In cation exchange process (Figure 2.6), a sample containing proteins, molecules or ions that bear a net positive charge at a certain pH is added into a column. The positively charged molecules are attracted to negatively charged functional groups which are chemically bonded to solid support. Neutral and negative charge molecules are not retained. Sulfonic acid group (in strong cation exchanger)

and carboxylic acid group (in weak cation exchanger) are commonly employed ionic functional groups as cation exchanger site. Cation exchange chromatography has been applied in many applications due to its advantages as listed below.

- It is a non-denaturing technique, so it is compatible for extraction or purification of proteins, amino acids or other biomolecules.
- Separation can be controlled by changing pH, salt concentration and the ion exchange media.
- It can serve as a preconcentration step.
- It can resolve molecules with small differences in charge.

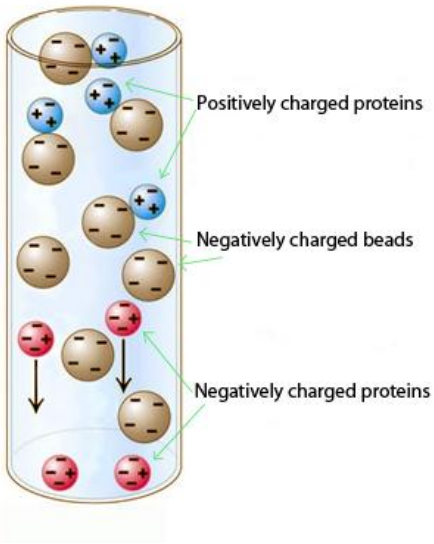


Figure 2.3 Cation exchange process; brown spheres represent negatively charged functional groups on exchanger, blue and red spheres symbolize positively and negatively charged proteins or other molecules, respectively [25].

Cation exchange resin has also been used as adsorbent for extraction of creatinine in urine. The counter ions of sulfonic acid groups on the resin are exchanged to sodium ions to reduce the number of hydronuim ions which would interfere the analysis by Jaffé method. After extraction, creatinine cations extracted onto the resins are eluted by sodium hydroxide solution and the eluted solutions are analyzed by Jaffé method. This method was successfully applied for extraction of creatinine in biological samples to overcome interferences problem in Jaffé method. Nevertheless, the poor recoveries of creatinine were observed [23].

In this research, sulfonic acid-functionalized MCM-41-coated magnetic particles (Fe_3O_4/MCM-41/SO_3H) which have magnetic properties for easy separation from sample media and high surface area were synthesized and used as adsorbent to extract creatinine in urine samples.

2.3 Ferrite magnetic materials

Ferrites are materials with magnetic properties which composed of Fe_2O_3 and other metals as the main components. Materials of this class have a structure pattern called “spinel structure” which is described below.

2.3.1 Spinel structure [26]

Spinel is a major structure pattern of mixed-metal oxides, which has the general chemical composition of AB_2O_4. Generally, A is a divalent cation such as Mg, Fe, Mn, Zn and Cu and B is a smaller trivalent cation e.g., Ti, Fe, Al, and Co. The structure comprises a cubic closed-packed array of 32 oxide ions which forms 64 tetrahedral holes and 32 octahedral holes in one unit cell (containing eight formula

units $(AB_2O_4)_8$). There are two types of sub-cells commonly described for the spinel structure; sub-cell 1 (Figure2.1a) and sub-cell 2 (Figure 2.1b) where cations are filled in two tetrahedral sites and one octahedral site, respectively. The arrangement of these two cubic sub-cells in one unit cell is shown in Figure 2.2. There are 12 octahedral holes filled with cations which are not centered in the sub-cells. Spinel structure can be divided into two types, spinel and inverse spinel. In spinel structure of formula AB_2O_4, all of the divalent cations are filled in 1/8 of the tetrahedral holes and all of the trivalent cations occupy half of the octahedral holes. On the other hand, the inverse spinel structure of formula $B(AB)O_4$ has an alternative arrangement where all of the divalent cations and half of the trivalent cations are located in octahedral holes. The remaining trivalent cations are filled in tetrahedral holes.

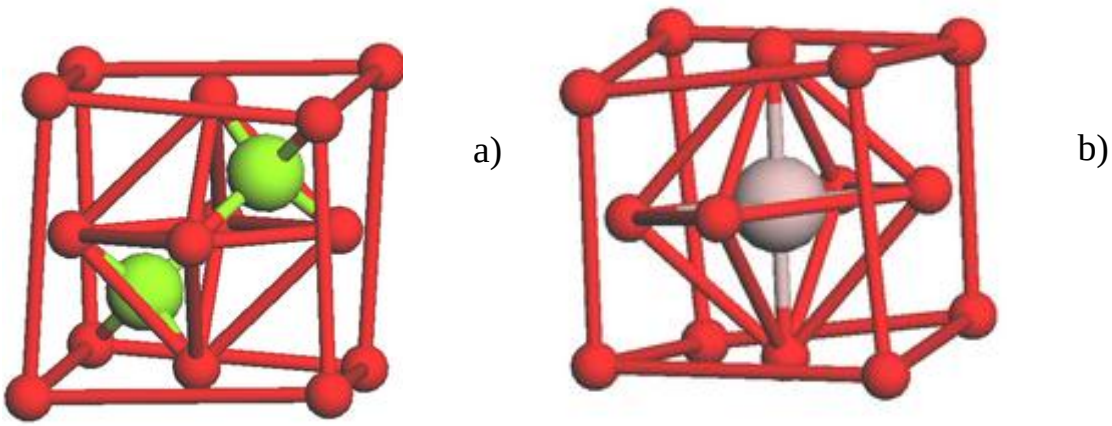


Figure 2.4 Sub-cell 1 (a) displays the occupied tetrahedral holes and sub-cell 2 (b) shows the filled octahedral hole [27].

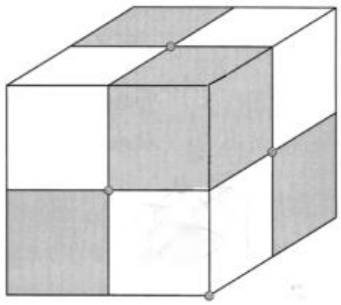


Figure 2.5 Arrangement of sub-cell 1 and 2 in one unit cell. The shaded one represents sub-cell 1, while the white one represents sub-cell 2 [27].

2.3.2 Magnetite magnetic particles (Fe_3O_4-MNPs) [28]

Magnetite (Fe_3O_4 or $FeO \cdot Fe_2O_3$), lodestones, is a ceramic-like material which composed of ferric oxide called hematite (Fe_2O_3) and ferrous oxide called wüstite (FeO). Its IUPAC name is iron(II,III) oxide and the common name is ferrous-ferric oxide. Fe_3O_4 has an inverse spinel structure (Figure 2.6) where tetrahedral holes are filled by half of ferric ions. The other half of ferric ions and ferrous ions occupy octahedral holes. There are two sub-cells containing tetrahedral and octahedral sites, A and B, respectively, which are used to explain about magnetic properties of magnetite (Figure 2.7). Electrons spin of the cations on the A and B sub-cells are antiparallel to the each other. It causes the opposite direction of the magnetic moments from those sub-cells. The magnetic moments of two crystal sites are not equal resulting in the net magnetic moment which depends on the B sub-cell. In addition, the interesting property and the merit of magnetic particles are summarized below.

- It can be easily collected after used by applying external magnetic field.
- It is compatible to use with aqueous and organic solutions.
- Because of its metal oxide nature, the particles have hydroxyl groups on the surface which are the sites for further surface functionalization.

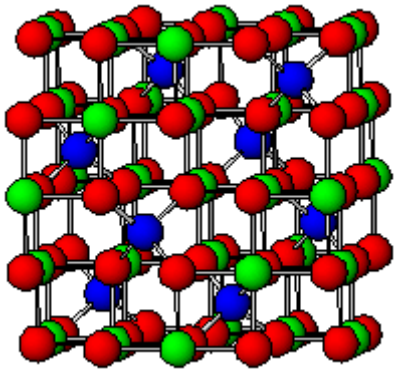


Figure 2.6 Inverse spinel structure of Fe_3O_4 unit cell; the red spheres represent oxide ions, the blue spheres represent ferric ions which occupy tetrahedral holes and the green spheres represent ferric ions and ferrous ions which occupy octahedral holes. [27].

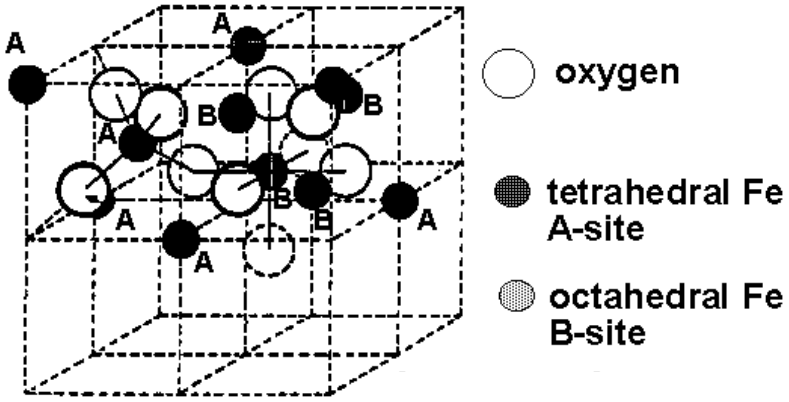


Figure 2.7 The two magnetic sub-cells, A and B, of magnetic materials [28].

2.4 Silica materials

Silica is an oxide of silicon with the chemical formula of SiO_2. It is most commonly found in nature as sand or quartz. It can be classified into 2 types, amorphous silica and mesoporous silica. Mesoporous silica is the silicon dioxide which has a characteristic repeating pattern in the structure, for example, MCM-41 and MCM-48, while amorphous silica has a disorder structure. In this research, the composite of Fe_3O_4 and MCM-41 (Fe_3O_4/MCM-41) was synthesized and it was employed to prepare an adsorbent for creatinine extraction.

2.4.1 Mobil Crystalline Material-41 (MCM-41) [29]

In 1992, a new family of mesoporous molecular sieves, the M41S family, was synthesized by researchers from Mobil Corporation. One of them is Mobil Crystalline Material-41 (MCM-41) which is a mesoporous silica containing arrays of uniform size hexagonal pores. The pore diameters are in the range of 1.5-10 nm depending on the length of alkyl chain of surfactant used as template, the addition of organic compounds and the other synthesis parameters. There are two possible pathway proposed to describe the formation of this mesoporous material (Figure 2.8). The first pathway is that the added surfactant forms micelle rods that further aggregate into hexagonal micelle arrays. After that the inorganic silicates are crystalize around these arrays to generate an inorganic structure. In the second pathway, the anionic silicates are attracted to the cationic surface of the hexagonal micelle arrays due to electrostatic interaction. Therefore, the anionic silicates are encased in hexagonal structure and further undergo condensation to form silica network of the desired MCM-41 material. After MCM-41 is synthesized, surfactant templates are removed by calcination. Due to its high surface area (about 700 m^2/g), high thermal stability, acid resistance, uniform pore distribution and controllable pore size, MCM-41 has been widely applied in the field of catalysis and adsorption/separation.

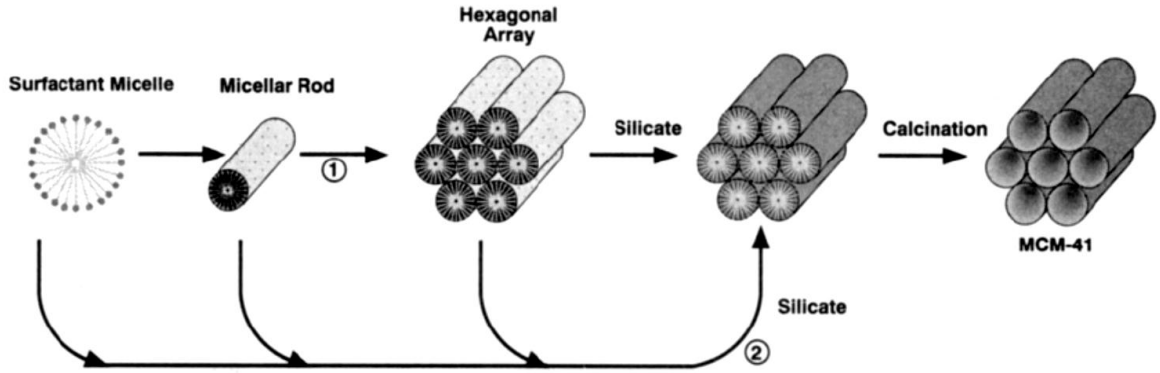


Figure 2.8 Possible pathways for the formation of MCM-41: (1) the first pathway and (2) the second pathway [30].

2.5 Literature review

Because of the attractive properties of silica materials such as durability and large surface area, many works have been reported about the use of it, especially mesoporous molecular sieves, as solid support to prepare the adsorbent for target analytes. Yoshitake *et al*. [31] prepared MCM-41 and SBA-1 mesoporous silica functionalized with 3-aminopropyltrimethoxysilane, [1-(2-aminoethyl)-3-amino propyl]trimethoxysilane and (trimethoxysilyl) propyldiethylenetriamine through grafting method. These synthesized materials were used for adsorption of oxyanions such as chromate and arsenate. According to the results, the triaminosilane-grafted mesoporous silica showed the highest efficiency in adsorption of chromate and arsenate, compared to the other adsorbents.

Thiol functionalized mesoporous silica was often employed as adsorbent for extraction of soft metal ions, classified by HSAB theory. Zheng *et al*. [32] prepared a

thiol functionalized MCM-41 via co-condensation of tetraethyl orthosilicate and 3-mercaptopropyltrimethoxysilane. It was used for selective adsorption of precious metal ions, for example, Pt^{4+} or Au^{3+} ions. The adsorbent showed high adsorption capacity (706.76 mg Pt^{4+}/g, 752.40 mg Au^{3+}/g) and could be completely regenerated by treatment with 5 M HCl or 0.7 M thiourea in 2 M HCl. In the work of Idris *et al*., [33] 3-mercaptopropyltrimethoxysilane (MP-TMS) and N-(3-trimethoxysilylpropyl)diethylenetriamine (DETA-TMS) modified MCM-41 was obtained by means of grafting. MP-TMS adsorbent exhibited selective adsorption of Hg^{2+} ions from samples of distilled water doped with heavy metal ions, tap water and river water, while The DETA-TMS adsorbent preferred to adsorb hard metal ions. Under optimum extraction condition, a maximum adsorption capacity of 1245 µmol/g was achieved for MP-TMS adsorbent. Use of nitric acid (0.2 M) and low energy microwave assisted digestion could successfully remove Hg^{2+} from loaded sorbents without the loss of functionality from MP-TMS adsorbent.

In many past works, mesoporous silica was functionalized with carboxyl or sulfonic acid groups which act as ion-exchanger site. The obtained solids were used as stationary phase for ion chromatography and adsorbent for extraction of ions or charged molecules. These works are described below.

Bruzzoniti *et al*. [34] prepared SBA-15 mesoporous silica functionalized with carboxyl groups. The synthesis was performed by co-condensation of tetraethylorthosilicate and 4-(triethoxysilyl)butyronitrile. Cyano groups existing on the solid was subsequently hydrolyzed to carboxyl groups. The obtained solid was successfully used in the ion chromatography for separation of cationic species, for example, Li+, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, NH^{4+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Pb^{2+} and Fe^{3+}. Moreover, many works have been published on preparation and use of sulfonic acid-functionalized mesoporous silica. In the work of Ganesan *et al*. [35], sulfonic acid-functionalized MCM-41 was prepared by means of co-condensation of tetraethylorthosilicate and 3-mercaptopropyl trimethoxysilane followed by oxidation of the solid in a mixture of H_2O_2, ethanol and water. The content of SO_3H groups on the solid determined by titration method was 0.7 mmol/g. Ion exchange capacities observed by cyclic voltammetry were 0.4, 0.7, 0.4, 0.7 and 4.1 meq/g for Na^{+}, K^{+},

Ca^{2+}, Cu^{2+} and Al^{3+}, respectively. Liu *et al*., [36] prepared sulfonic acid-fuctionalized SBA-15 (SO_3H/SBA-15) via grafting of 3-mercaptopropyltrimethoxysilane on SBA-15 and then oxidation with H_2O_2. The functionalized SBA-15 was utilized as strong cation exchanger and its capability for extraction of some proteins such as bovine serum albumin (BSA), cytochrome C (cyt c) and polypeptides e.g. insulin and glucagon were examined. The results showed that the obtained SO_3H/SBA-15 could not adsorb BSA and cyt c because these molecules have diameter larger than the size of the pores of this mesoporous material. The adsorption capacities were investigated for insulin and glucagon by batch method. Under optimum extraction conditions, this adsorbent showed a maximum capacity for insulin of 430 mg/g and for glucagon of 1303 mg/g.

From the above mentioned works, researchers have successfully prepared adsorbents from mesoporous silica. The synthesized materials could be applied for extraction of various target analytes with satisfactory results. However, it is difficult to separate these materials after extraction process due to their small particles size. To overcome such problem, many researchers reported about the synthesis and use of magnetic nanoparticles-silica composites. Its attribute is similar to original silica materials and it could be easily removed by use of external magnetic field. In the work of Girginova *et al*. [37], magnetite particles were synthesized by hydrolysis of $FeSO_4$ and coated with silica through the base catalyzed hydrolysis of tetraethylorthosilicate. It was subsequently modified with dithiocarbamate groups. Under the optimum extraction condition, the adsorbent showed high efficiency for Hg^{2+} extraction (74%) even at low levels (50 µg/L).

Hakami *et al*. [38] prepared Fe_3O_4 magnetic nanoparticles via co-precipitation method and then coated the particles with mesoporous silica. From the TEM images, the particles were covered by silica component with a well-defined hexagonal arrangement of uniform pores with pore diameter of 2.1 nm. The synthesized materials showed strong response to an external magnetic field, therefore, solid separation from solutions was achieved in less than a minute. Under the optimum extraction condition, the adsorption capacity for Hg^{2+} was calculated to be 207.7 mg/g. The adsorbent could be regenerated using thiourea in a 3 M HCl solution

without loss of their activity in repetitive adsorption tests. In the work of Gill *et al.* [39], sulfonic acid-functionalized silica-coated cobalt ferrite was prepared by grafting 3-mercaptopropyltrimethoxysilane on silica-coated $CoFe_2O_4$ and then the obtained solid was oxidized in a mixture of H_2O_2, methanol and water. The synthesized material was employed as catalyst for acid catalyzed reaction. It showed similar activities to commercial sulfonic acid catalysts.

In this research, sulfonic acid-functionalized MCM-41-coated magnetic particles (Fe_3O_4/MCM-41/SO_3H) which have magnetic properties for easy separation from sample media and high surface area was synthesized. First, Fe_3O_4 particles were synthesized by means of co-precipitation. The magnetite particles were subsequently coated with MCM-41 mesoporous silica. Finally, the solid was modified with 3-mercaptopropyltrimethoxysilane thought grafting method and then the existing thiol groups on Fe_3O_4/MCM-41 surface were further oxidized to sulfonic acid groups using H_2O_2 as oxidizing agent. The obtained adsorbent was used as cation exchanger for extraction and isolation of creatinine from sample matrix prior to the determination by conventional Jaffé method. The parameters affecting extraction efficiency were optimized and then the obtained method was validated. The adsorbent reusability and application for creatinine determination in human urine samples were also investigated.

CHAPTER III

EXPERIMENTAL

3.1 Instruments

The instruments used in this research are listed in Table 3.1

Table 3.1 List of instruments

Instruments	Manufacture/ Model	Purpose
UV-visible spectrometer	HP/8453	Determination of creatinine by Jaffé method
Inductively couple plasma atomic emission spectrometer (ICP-AES)	Thermo/iCAP 6000 Series	Determination of ferric ions leached from adsorbent
Fourier transforms infrared spectrometer (FT-IR)	Nicolet/6700	Functional group identification
X-ray diffractometer (XRD)	Rigaku/1200+	Identification of adsorbent crystallinity
Scanning electron microscope (SEM)	JEOL/JSM-5410 LV	Analysis of adsorbent surface
Transmission electron microscope (TEM)	JEOL/JEM-2100	Photography of adsorbent
Surface area analyzer	BEL Japan/ BELSORP-mini	Adsorbent surface area analysis

Instruments	Manufacture/ Model	Purpose
Thermogravimetric analyzer (TGA)	Perkin-Elmer/Pyris 1	Determination of adsorbent thermal stability
pH meter	Hanna instruments/pH 211	Measurement of solution pH
Stirrer	Gem/MS 101	Agitation of solution in adsorbent preparation and extraction experiments
Centrifuge	MSE/ CENTAUR 2	Isolation of the adsorbent after the extraction process

3.2 Chemicals

The chemicals used in this research are listed in Table 3.2. The reagents were used as obtained without further purification.

Table 3.2 List of chemicals

Chemicals	Supplier / Grade
Ferric chloride	Aldrich/reagent grade
Ferrous chloride	Aldrich/reagent grade
Hydrochloric acid (37 % w/w)	Merck/for analysis
Nitric acid (65% w/w)	Merck/for analysis
Sodium hydroxide	Merck/for analysis
Hexadecyltrimethylammonium bromide	Aldrich/reagent grade

Chemicals	Supplier / Grade
Concentrated ammonia	Merck/for analysis
Ethanol	Merck/for analysis
Tetraethylorthosilicate 98%	Aldrich/reagent grade
Hydrogen peroxide 30%	Merck/AR grade
3-mercaptopropyltrimethoxysilane 95%	Aldrich/reagent grade
Sodium chloride	Merck/for analysis
Picric acid	Sigma-aldrich/reagent grade
Creatinine	Aldrich/reagent grade
Sodium thiocyanate	Aldrich/reagent grade
Ethylenediaminetetraacetic acid disodium salt	Aldrich/reagent grade

3.3 Solution preparation

Creatinine solutions

Creatinine stock solution (10,000 mg/L) was prepared by dissolving creatinine anhydrous powder with deionized water. The obtained solution was further used to prepare creatinine solutions of desired concentrations by dilution with deionized water. The standard creatinine solutions in the concentration range of 8-60 mg/L were extracted by the studied solid phase. Then the eluted solutions were used as working standard solutions and analyzed by Jaffé method for the construction of calibration curve for the determination of creatinine. Moreover, creatinine solution at the concentration of 60 mg/L was used as creatinine sample for parameter optimization.

Sodium hydroxide solutions

Sodium hydroxide solution (0.75 mol/L) was prepared by dissolving approximate amount of sodium hydroxide pellets with deionized water. The obtained solution was used in the determination of creatinine by Jaffé method.

Picric acid solutions

Saturated stock solution of picric acid was prepared by dissolving picric acid powder (1.5 g) with deionized water (50 mL). The insoluble picric acid powder was filtered out of the solution and the yellow solution was kept for further use. The stock solution was used to prepare a 0.04 mol/L picric acid solution by dilution with deionized water. The obtained picric acid solution was used in the determination of creatinine by Jaffé method.

Sodium chloride solution

Sodium chloride solutions (0.5, 1.0 and 1.5 mol/L) were prepared by dissolving appropriate amount of sodium chloride powder with deionized water. The obtained solutions were used in the elution of creatinine from solid phase.

Ethylenediaminetetraacetic acid (EDTA) solution

EDTA solution at the concentration of 0.1 mol/L was prepared by dissolving approximate amount of EDTA powder with deionized water. The obtained solution was used to remove ferric ions from adsorbent surface in washing step.

Sodium thiocyanate solution

Sodium thiocyanate solution at the concentration of 0.5 mol/L was prepared by dissolving approximate amount of sodium thiocyanate powder with deionized water. The obtained solution was used as ferric ions tester.

3.4 Adsorbent preparation

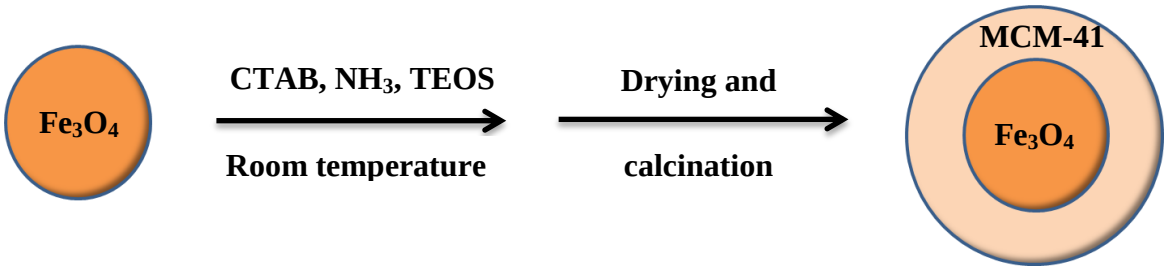
3.4.1 Preparation of Fe_3O_4 magnetic particles

Fe_3O_4 magnetic particles (Fe_3O_4-MPs) were synthesized via co-precipitation method. Firstly, 17.6 g of $FeCl_3$ and 10.5 g of $FeCl_2$ were dissolved in 140 mL of 0.5 M HCl. After that 1.4 L of 1.5 mol/L NaOH was added to the solution dropwise and the mixture was further stirred for 2 hours. The obtained solid was separated by means of centrifugation and washed with deionized water until neutral pH of the washing solution (using pH papers) was observed. Finally, it was dried in an oven at 120 ºC overnight and the obtained solid (Fe_3O_4-MPs) was kept in closed bottle for further use.

3.4.2 Preparation of MCM-41-coated magnetic particles (Fe_3O_4/MCM-41)

Five grams of Fe_3O_4-MPs were dispersed in a solution containing 10.5 g of CTAB, 261.0 mL of deionized water and 121.5 mL of 25% NH_3 solution and then 50.0 mL of 98% TEOS solution was added dropwise. The mixture was stirred at 35 ºC for 3 hours and then left at room temperature for 24 hours. The solid was filtered

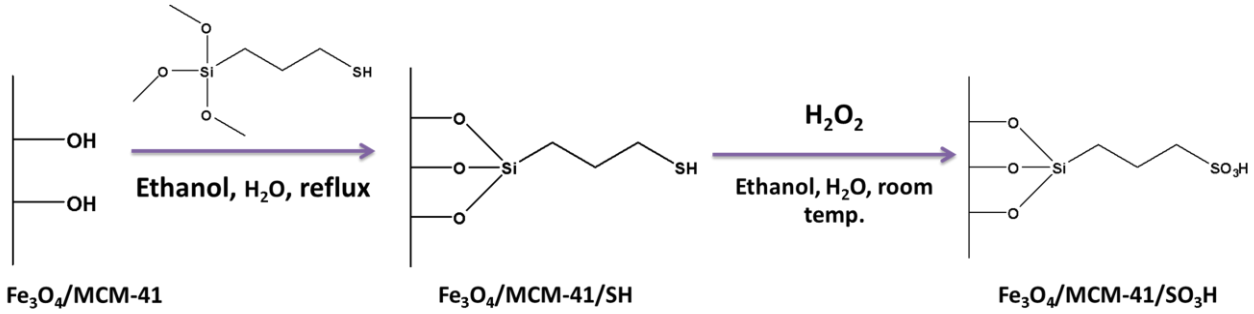
and washed with deionized water until neutral pH of the washing solution was observed by using pH papers. The resultant solid was dried in an oven at 120 ºC overnight and then calcined at 550 ºC for 8 hours.



Scheme 3.1 Preparation of MCM-41-coated magnetic particles (Fe_3O_4/MCM-41)

3.4.3 Surface modification of Fe_3O_4/MCM-41

Four grams of the obtained Fe_3O_4/MCM-41 was dispersed in a solution containing 15.9 mL of 3-mercaptopropyl trimethoxysilane, 160 mL of deionized water and 160 mL of ethanol. The mixture was sonicated using sonication bath and then refluxed under nitrogen atmosphere for 5 hours. The solid was filtered and washed with deionized water. The existing thiol groups on Fe_3O_4/MCM-41 surface were oxidized to sulfonic acid group by H_2O_2. The resultant solid was dispersed in a solution containing 160 mL of 30% H_2O_2, 160 mL of deionized water and 160 mL of ethanol. The mixture was stirred at room temperature for 24 hours. After that the solid was filtered and washed with plenty of deionized water. It was further washed with 1 mol/L NaCl until colorless washing solution was obtained when tested with sodium thiocyanate solution. It was washed again with deionized water and then dried in an oven at 120 ºC overnight.



Scheme 3.2 Surface modification of Fe_3O_4/MCM-41

3.5 Adsorbent characterization

Fourier transforms infrared spectrometer (FT-IR), X-ray diffractometer (XRD), scanning electron microscope (SEM), transmission electron microscope (TEM), surface area analyzer and thermogravimetric analyzer (TGA) were employed to confirm the successful synthesis of Fe_3O_4, Fe_3O_4/MCM-41 and Fe_3O_4/MCM-41/SO_3H.

Functional groups of Fe_3O_4, Fe_3O_4/MCM-41 and Fe_3O_4/MCM-41/SO_3H were identified by using FT-IR with KBr pellet technique. The spectra were recorded in the wavelength range of 450-4000 cm^{-1} in transmittance mode.

XRD was employed to confirm the crystal structure of the synthesized magnetic particles and also the mesoporous structure of MCM-41 which co-existed with magnetic particles. The diffractogram of Fe_3O_4 and MCM-41 were recorded in the range of 20º - 70º and 1.5º - 8.0º, respectively.

The size, shape and pore structure of Fe_3O_4/MCM-41 and Fe_3O_4/MCM-41/SO_3H were investigated by the use of SEM and TEM.

The surface area, total pore volume and average pore diameter of Fe_3O_4/MCM-41/SO_3H were analyzed by surface area analyzer.

Thermal stability of Fe_3O_4/MCM-41 and Fe_3O_4/MCM-41/SO_3H was examined by using TGA. The change in weight of the solid in function of temperature was used as information to identify the difference in chemical composition of these materials. The thermogravimetric analysis was carried out by heating the adsorbent in the temperature range of 30 to 800 ºC at a heating rate of 20 ºC/min under nitrogen atmosphere.

3.6 Extraction and determination of creatinine

3.6.1 Extraction procedure

Firstly, Fe_3O_4/MCM-41/SO_3H adsorbent of desired amount was added into polyethylene test tubes. It was washed twice with 5 mL of 1 M NaCl and 5 mL of deionized water. After that creatinine standard solutions of various concentrations or 50 times diluted human urine samples of appropriate volume were added and HNO_3 solution was added subsequently to adjust the final volume to 5.00 mL. The solution pH was adjusted to the desired value with HNO_3 solution. The mixtures were stirred for specified times and then the solids were separated by external magnetic force or centrifugation. Supernatants were kept for the analysis of residual creatinine. The solids were then washed with 5 mL of 2 mmol/L EDTA solution. Then 5.00 mL of NaCl solution was added as eluent. The mixtures were stirred for a certain period of time. Finally, the solids were separated and supernatants were kept for the analysis of eluted creatinine.

3.6.2 Influence of extraction parameters

The efficiency in creatinine extraction and elution of the proposed method was observed under various experimental conditions including different sample pH, amount of adsorbent, sample volume, eluent concentration, extraction time and elution time to find suitable condition for the extraction process. Moreover, the leaching of Fe from adsorbent into solutions of various pH was also studied by using of ICP-OES. The parameters and studied values are shown in table 3.3.

Due to high concentration of creatinine in human urine (400 – 3000 mg/L), it needs to be diluted prior to determination by Jaffé method. From the extraction method described previously, urine samples were 50 times diluted before extraction process. Creatinine standard solution at concentration of 60 mg/L which is the highest creatinine concentration possibly found in diluted urine sample, was chosen as creatinine sample in this study.

Table 3.3 List of parameters and studied values.

Parameters	Studied value
pH	3, 4, 5
Amount of adsorbent	50, 100 mg
Sample volume	0.5, 0.75, 1.00 mL
Eluent concentration (NaCl)	0.5, 1.0, 1.5 mol/L
Extraction time	10, 20, 40, 60 minutes
Elution time	10, 20, 40, 60 minutes

The creatinine extraction and elution efficiency were presented in the term of percent extraction (%extraction) and percent elution (%elution), respectively. These values can be calculated by using equation 3.1 and 3.2;

$$\%\text{Extraction} = \frac{M_i - M_f}{M_i} \times 100 \quad (3.1)$$

$$\%\text{Elution} = \frac{M_{Eluted}}{M_i - M_f} \times 100 \quad (3.2)$$

where M_i = initial amount of creatinine in the solution before extraction (mg)

M_f = final amount of creatinine in the solution after extraction (mg)

M_{Eluted} = amount of creatinine in eluted solution (mg)

When the proposed method was employed for determination of creatinine in samples, the percent recovery of creatinine can be calculated by using equation 3.3;

$$\%\text{Recovery} = \frac{M_{found}}{M_i} \times 100 \quad (3.3)$$

where M_{found} = amount of creatinine found in eluted solution (mg)

M_i = initial amount of creatinine sample solution (mg)

Furthermore, the leaching of Fe in extraction process were presented in the term of percent ferric ions leaching (%Fe leaching) which can be calculated by using equation 3.3.

$$\%\text{Fe leaching} = \frac{M_{Fe}}{m_{adsorbent}} \times 100 \qquad (3.3)$$

where M_{Fe} = amount of leached Fe in the extracted and eluted solutions (mg)

$m_{adsorbent}$ = amount of the adsorbent used in the extraction process (mg)

3.6.3 Adsorbent reusability

The feasibility of reusing the adsorbent was evaluated. The adsorbent was used for creatinine extraction following the proposed method under chosen condition for 3 cycles. The supernatants from extraction and elution steps were collected for creatinine determination and then the extraction and elution efficiency (%extraction and %elution) were calculated in every cycle to examine the reusability of the adsorbent

3.6.4 Creatinine determination by Jaffé method

Three milliliters of extracted creatinine standard solutions or extracted urine samples were added into test tubes. Then 1 mL of 0.04 M picric acid and 1 mL of 0.75 M NaOH were added subsequently. The solutions were stirred for 15 minutes and their absorbance was measured at 500 nm by UV-visible spectrometer.

3.7 Influence of interfering species

Influence of interfering species on the determination of creatinine by proposed method was investigated. Solutions of 60 mg/L creatinine containing various types of interfering species at several concentrations (Table 3.4) as binary mixtures were analyzed by the method. The level of interfering species concentrations used in this study were the maximum concentrations level that can be found in human urine and ten times of those maximum concentrations. Finally, the obtained results were reported in the term of %recovery of the method.

Table 3.4 List of interfering species and studied values.

Interfering species	Studied value
Bovine serum albumin	6, 60 mg/L
Glucose	3, 30 mg/L
Ascorbic acid	6, 60 mg/L
Urea	2, 20 g/L

3.8 Method validation

The proposed method was validated under the chosen extraction condition. The accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), linear range and repeatability of the method were determined and reported.

3.8.1 Accuracy and precision

Creatinine standard solutions at the concentration of 10 and 50 mg/L were extracted by described extraction method under chosen condition. The eluted solutions were analyzed by Jaffé method. The obtained results were employed for calculation of percent recovery (%recovery) and percent relative standard deviation which represent accuracy and precision of the proposed method. The intra-day accuracy and precision were determined from 6 replicate measurements within a day while the inter-day results were obtained from 12 experiments within 6 days.

3.8.2 Limit of detection (LOD) and limit of quantification (LOQ)

1 mmol/L HNO_3 solution was analyzed by the proposed method in 10 replicates. The obtained eluted solutions were employed as the method blanks which were determined by Jaffé method. The obtained results were used to calculated limit of detection (LOD) and limit of quantification (LOQ) of the method. In this work, the LOD concentration was calculated from the signal obtained by using mean of absorbances of the method blanks plus 3 times of the standard deviations while the LOQ was defined as the concentration that gave the signal of mean of absorbances of the method blanks plus 10 times of the standard deviations.

3.8.3 Linearity and repeatability

In this section, the linearity and repeatability of the method were investigated in the working range of the calibration curve (8-60 mg/L). Standard solutions containing creatinine in the concentration range of 8 to 60 mg/L were extracted by proposed method and then the absorbances of eluted solutions were measured. The

results from 6 replicate measurements within a day were used for calibration curve construction. The linearity of the method was reported in term of coefficient of determination (R^2) while the repeatability was reported in the term of standard deviation (SD) of intercept and slope of the calibration curves.

3.9 Determination of creatinine in human urine samples

Three urine samples collected from our volunteers were determined by the proposed method. Accuracy and precision of the method were determined by using spiked samples and reported in the term of percent recovery (%recovery) and percent relative standard deviation (%RSD). The analysis was done following the described extraction and determination procedure under chosen condition. Creatinine standard solutions in the concentration range of 8 to 60 mg/L were extracted. The eluted solutions were analyzed by Jaffé method and then the obtained results were used for calibration curve construction. Fresh urine samples from a healthy 24 years old male, a healthy 28 years old female and a healthy 54 years old female were diluted 50 times with 1 mmol/L HNO_3 solution. The obtained solution was employed as urine non-spiked sample. The creatinine standard solution was added into the urine non-spiked sample at concentrations of 500 and 1000 mg/L. The obtained solution was employed as urine spiked samples. These solutions were analyzed and the obtained information was employed for calculation of %recovery and %RSD.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter is divided into 4 sections which are adsorbent synthesis and characterization, influence of extraction parameters, method validation and application to human urine samples analysis.

4.1 Adsorbent synthesis and characterization

4.1.1 Synthesis and characterization of Fe_3O_4 magnetic particles

Ferrous-ferric oxide magnetic particles (Fe_3O_4-MPs) were prepared by co-precipitation of ferric ($Fe^{3+)}$ and ferrous ions (Fe^{2+}) in basic condition to give the mixed oxides of iron, as shown in Equation 4.1.

$$Fe^{2+} + Fe^{3+} + 8OH^- \longrightarrow Fe_2O_3 + FeO + 4H_2O \qquad (4.1)$$

The obtained solid was further incubated in an oven at 120 ºC. In this step, Fe_3O_4 was formed by thermal catalyzed condensation of ferric oxide (Fe_2O_3) and ferrous oxide (FeO). The resultant solid showed a strong response to external magnetic field.

The prepared Fe_3O_4 was characterized by Fourier transforms infrared spectrometer (FT-IR) and X-ray diffractometer (XRD). XRD diffractogram of Fe_3O_4-MPs (Figure 4.1) displays a group of peaks at 2theta about 30.5º, 36.0º, 43.0º, 53.7º, 57.0º and 62.8º which are characteristic peaks of Fe_3O_4 according to JSPDS No. 19-0629.

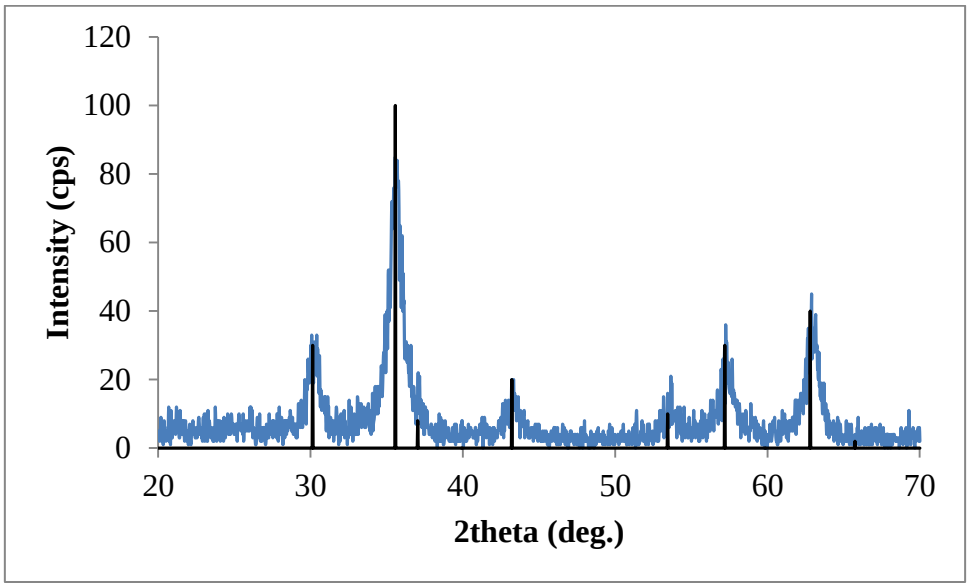


Figure 4.1 XRD diffractogram of the prepared Fe_3O_4-MPs at 2theta in a range of 20º to 70º.

Moreover, IR spectrum of the prepared Fe_3O_4-MPs (Figure 4.2) show a broad peak at about 3300 cm^{-1} and intense peaks at about 550 cm^{-1} corresponding to O–H stretching and Fe–O stretching, respectively. These results indicate the existence of hydroxyl group and Fe–O bond of Fe_3O_4. The absorption band near 1600 cm^{-1} is attributed to the vibration of H_2O molecules revealing the presence of the residual moisture in the synthesized Fe_3O_4. The above mentioned data confirm that the synthesized solid was Fe_3O_4.

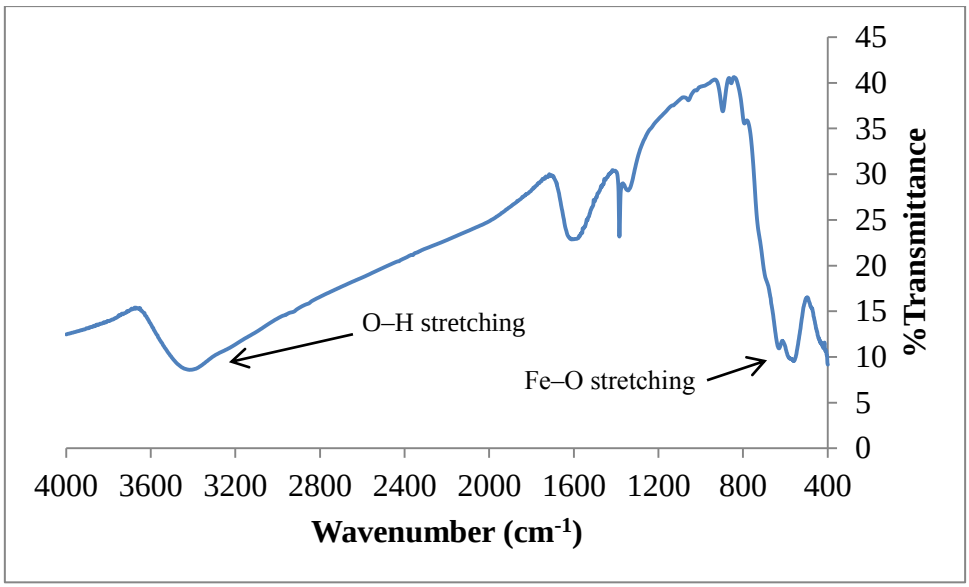


Figure 4.2 IR spectra of the prepared Fe_3O_4-MPs.

4.1.2 Synthesis and characterization of MCM-41-coated Fe_3O_4 magnetic particles (Fe_3O_4/MCM-41)

In this section, the synthesized Fe_3O_4 was further coated with MCM-41 mesoporous silica, to obtain the adsorbent with high surface area and magnetic properties. The coated MCM-41 also protect Fe_3O_4 core from being dissolved when the composite is used in strong acid solution. After coating, the obtained solid was characterized with IR and XRD to demonstrate that the solid was the composite of Fe_3O_4 and MCM-41, while SEM and TEM were employed to confirm the coating of MCM-41 on Fe_3O_4 surface.

MCM-41-coated Fe_3O_4 (Fe_3O_4/MCM-41) was prepared by dispersion of the synthesized Fe_3O_4 particles in a solution containing proper amount of CTAB, deionized water, NH_3 solution and tetraethylorthosilicate (TEOS). Under basic condition, hydroxyl groups on magnetite surface were ionized to generate negatively charged surface. The ethoxy groups of TEOS were hydrolyzed to silanol groups and further ionized to yield negativedly charged inorganic precursor under alkaline circumstance. CTAB also formed hexagonal shape micelle rods at optimum

concentration. It is believed that the negatively charged Fe_3O_4 surface induced the self-assembly of cationic surfactant micelle rods on Fe_3O_4 surface via electrostatic interaction (Figure 4.3). Because of the positive charge at outer sphere of rod-shaped micelles, negatively charged inorganic precursors were attracted and condensation occurred. This phenomenon resulted in the formation of the three-dimensional continuous silica network which contained uniform hexagonal channel, through sol-gel process (hydrolysis and condensation). The obtained solid was washed with deionized water to remove unreacted precursors. Finally, the solid was dried and calcined at 550 ºC for 8 hours to remove the surfactant templates. The synthesized Fe_3O_4/MCM-41 was characterized by XRD, FT-IR, SEM and TEM as follows.

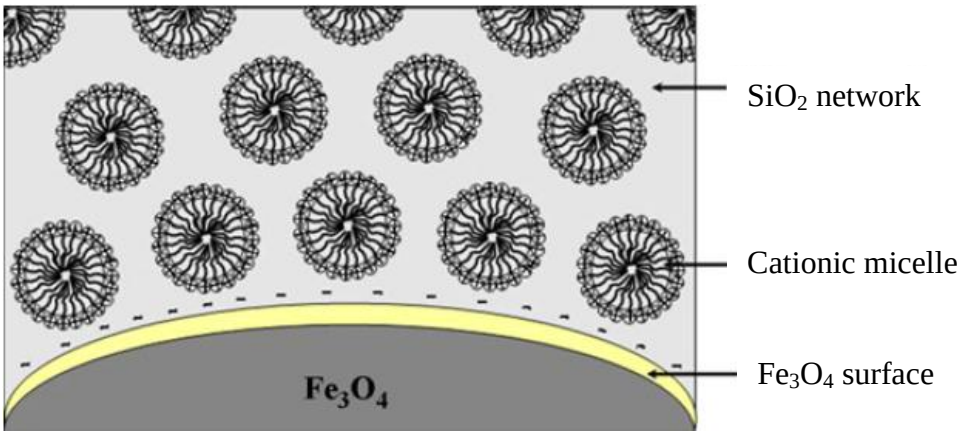


Figure 4.3 Silica coated magnetic particle with self-assembled cationic micelle rods [40].

In the observed XRD diffractogram of the prepared Fe_3O_4/MCM-41 at 2theta in a range of 20º to 70º (Figure 4.4), characteristic peaks of Fe_3O_4 at 2theta about 30.5º, 36.0º, 43.0º, 53.7º, 57.0º and 62.8º were observed. Moreover, broad peak at 2theta about 20º indicate the presence of amorphous silica which was formed due to fluctuation of temperature in the synthesis process of MCM-41. The diffractogram at 2theta in a range of 1.5º to 8.0º (Figure 4.5) displays signal at 2theta about 2.3º, 4.0º

and 4.7º which are characteristic peaks of MCM-41 according to JSPDS No. 49-1712. These results indicate the existence of Fe_3O_4 and MCM-41 in the synthesized Fe_3O_4/MCM-41.

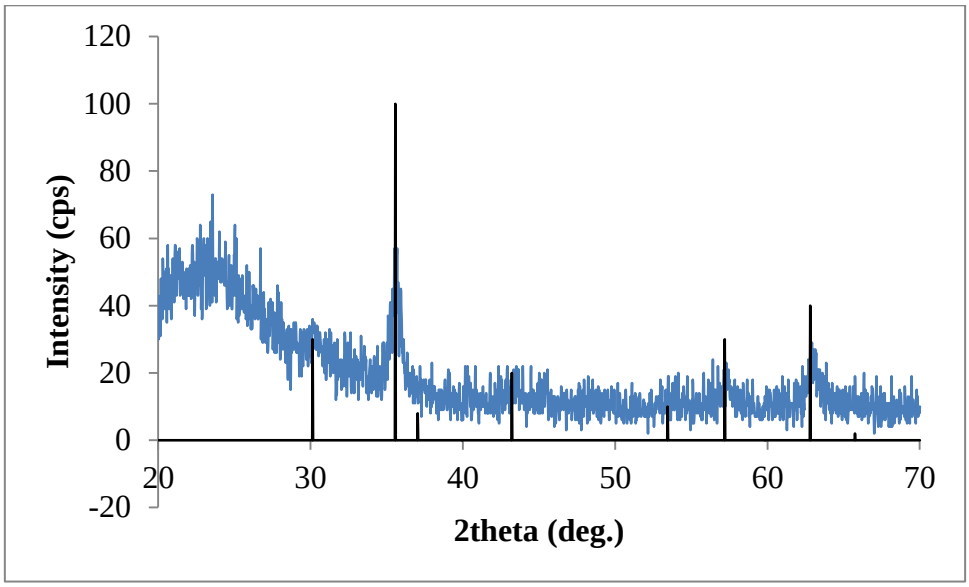


Figure 4.4 XRD diffractogram of the prepared Fe_3O_4/MCM-41 at 2theta in a range of 20º to 70º.

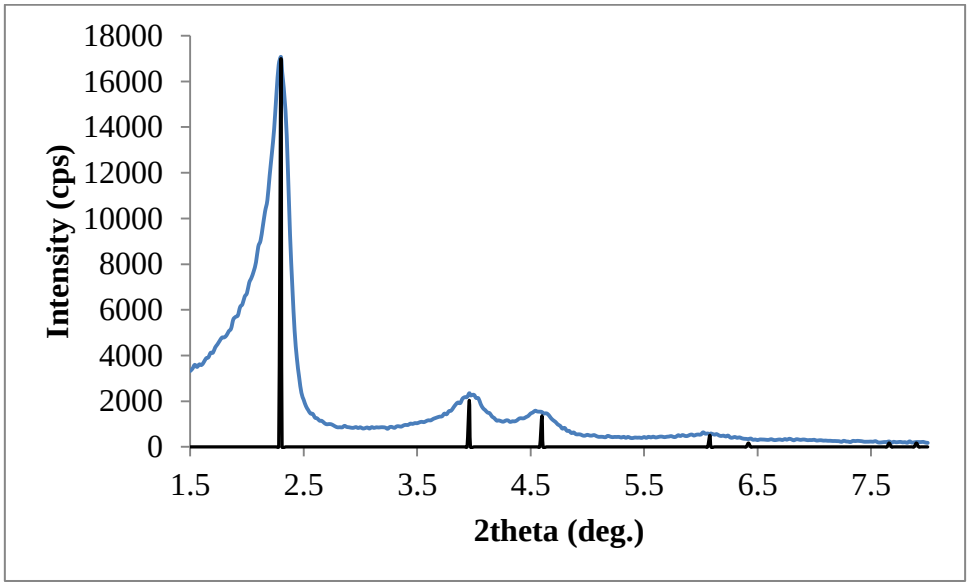


Figure 4.5 XRD diffractogram of the prepared Fe_3O_4/MCM-41 at 2theta in a range of 1.5º to 8.0º.

IR spectra of Fe_3O_4/MCM-41 (Figure 4.6) display a broad peak at about 3300 cm^{-1} and a strong peak at about 450 cm^{-1} relating to O–H stretching and Fe–O stretching, respectively. These results indicate the presence of hydroxyl group and Fe–O bond of Fe_3O_4. Furthermore, the spectra also show Si–O–Si stretching at 1042 and 1200 cm^{-1}. This result implied that the obtained solid contained silica framework of MCM-41.

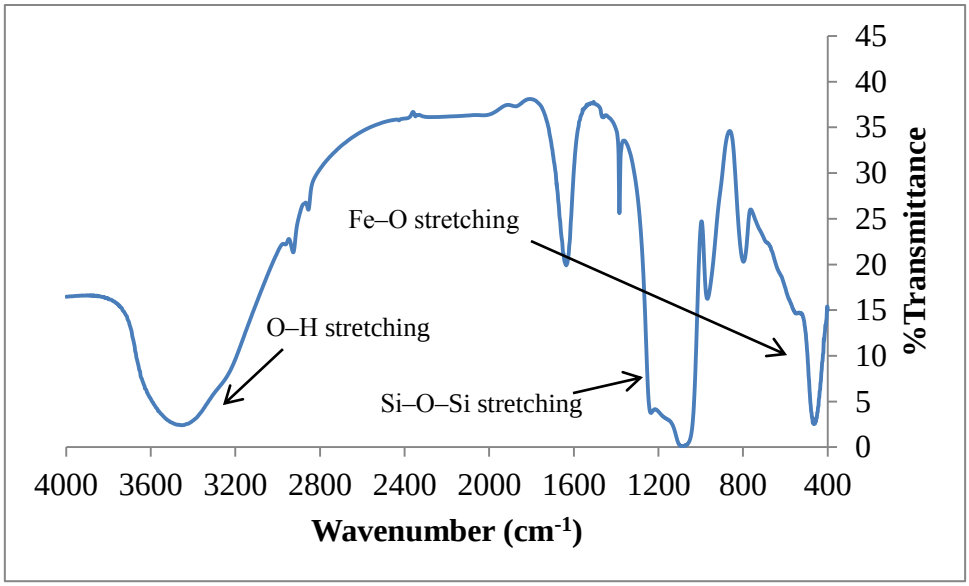


Figure 4.6 IR spectra of the prepared Fe_3O_4/MCM-41.

The SEM image of Fe_3O_4/MCM-41 (Figure 4.7) shows aggregates of Fe_3O_4/MCM-41 particles of various shapes. Difference in size and shape of magnetic particles core obtained by co-precipitation method and difference in thickness of MCM-41 coating may be the reason of several sizes and shapes of the obtained composite particles.

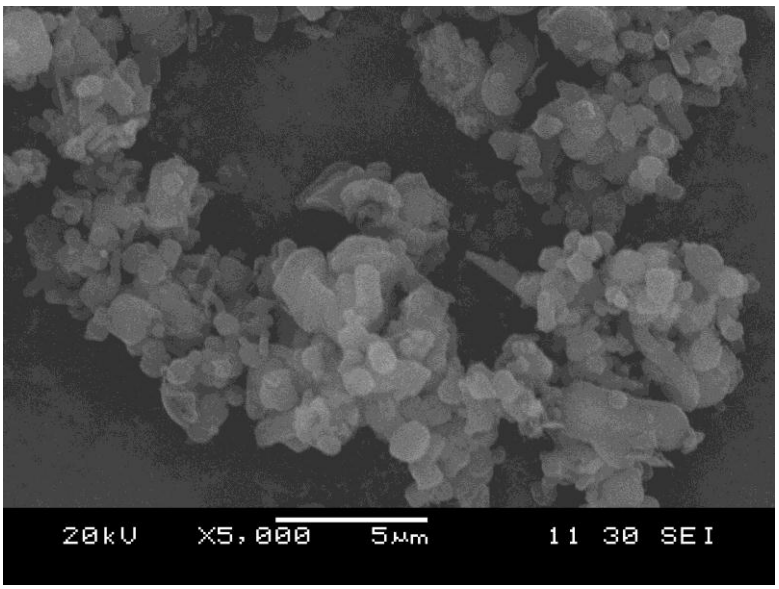


Figure 4.7 SEM image of the prepared Fe_3O_4/MCM-41.

Figure 4.8A and 4.8B show the TEM images of an independent particle and aggregated Fe_3O_4/MCM-41 particles, respectively. The particles size is in a range of 400-700 nm. The outer mesoporous shell of the particles is brighter than the dense inner magnetic cores. Moreover, uniformly distributed hexagonal pores were also observed when using the higher magnifying power. These images indicate the successful coating of magnetite particles with MCM-41 mesoporous silica.

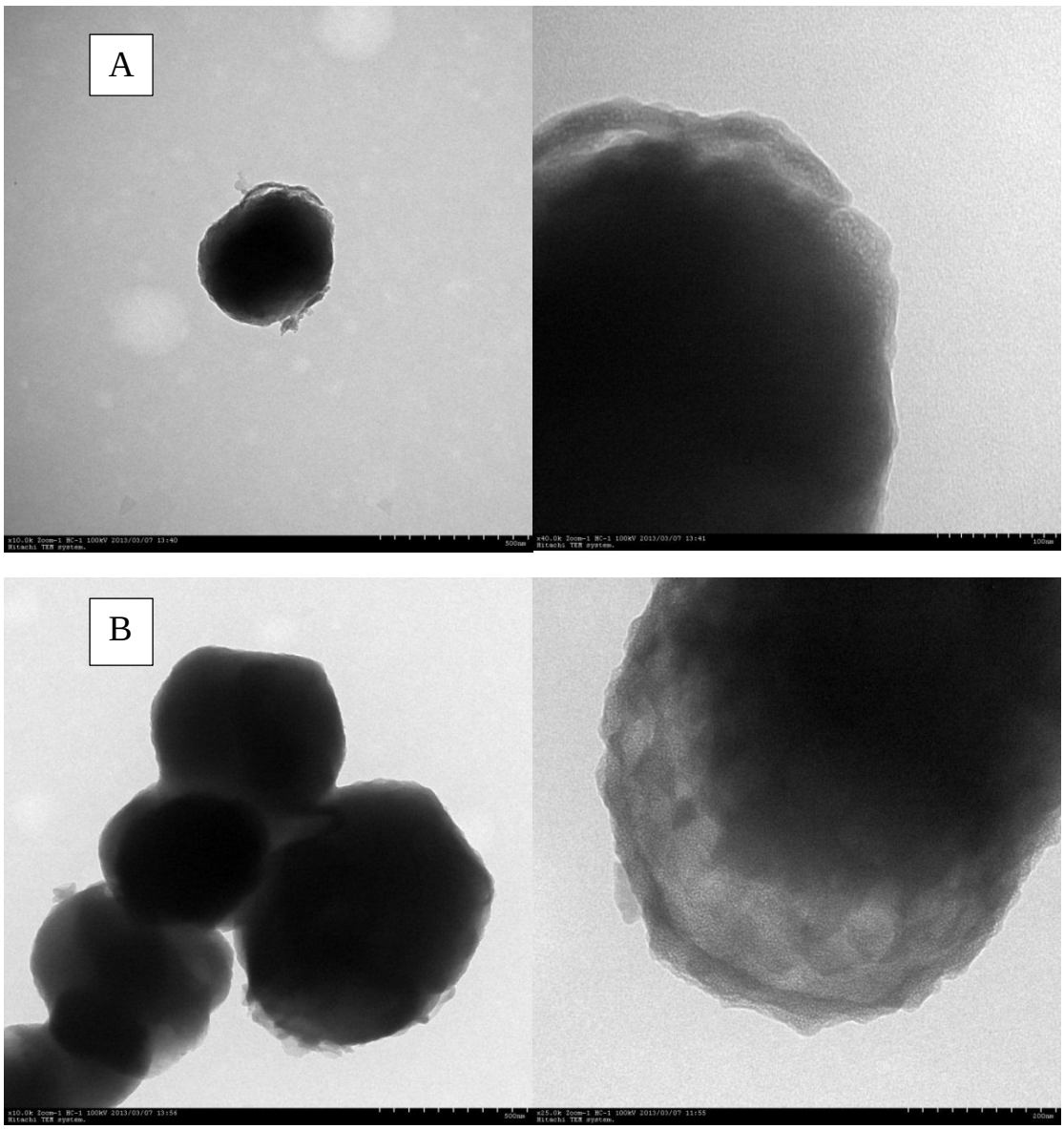
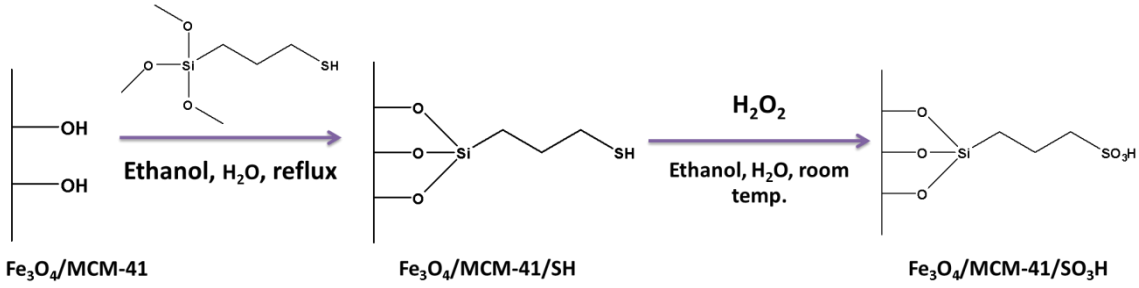


Figure 4.8 TEM images of Fe_3O_4/MCM-41 particles, an independent particle (A) and aggregated particles (B).

4.1.3 Synthesis and characterization of sulfonic acid functionalized MCM-41-coated Fe_3O_4 magnetic particles (Fe_3O_4/MCM-41/SO_3H)

Sulfonic acid functionalized MCM-41-coated Fe_3O_4 magnetic particles (Fe_3O_4/MCM-41/SO_3H) was synthesized by grafting method (Scheme 4.1). The prepared Fe_3O_4/MCM-41 was dispersed in a solution containing 3-mercaptopropyl trimethoxysilane, ethanol and deionized water. The mixture was sonicated before reflux to disperse Fe_3O_4/MCM-41 particles and let the solution penetrate into mesoporous channels. Therefore, its surface could be thoroughly modified by the silane coupling agent. Hydrolysis and condensation reaction played important roles in the functionalization process. The functionalized thiol groups were further oxidized by H_2O_2. The obtained solid was washed with deionized water to remove unreacted precursor or unwanted by-products. Then, it was soaked with NaCl solution. Sodium ions were exchanged with ferric ions that might be leached from magnetic particles during oxidation process and attached to the ion-exchange site on the solid.



Scheme 4.1 Surface modification of Fe_3O_4/MCM-41

The synthesized Fe_3O_4/MCM-41/SO_3H was characterized by FT-IR and XRD to confirm the presence of Fe_3O_4 and MCM-41 in the composite and to investigate the cystralline structure of the composite after functionalization process. Furthermore, TGA was employed to confirm that the organic functional groups truly present in the synthesized Fe_3O_4/MCM-41/SO_3H. Finally, the surface area, total pore volume and

average pore diameter of Fe_3O_4/MCM-41/SO_3H were determined by surface area analyzer.

In the XRD diffractogram of the prepared Fe_3O_4/MCM-41/SO_3H at 2theta in a range of 20º to 70º (Figure 4.9), characteristic peaks of Fe_3O_4 at 2theta about 30.5º, 36.0º, 43.0º, 53.7º, 57.0º and 62.8º were observed. Furthermore, broad peak at 2theta about 20º corresponding to amorphous silica was also detected. The diffractogram at 2theta in a range of 1.5º to 8.0º (Figure 4.10) show the signals at 2theta about 2.3º, 4.0º and 4.7º which are characteristic peaks of MCM-41. These results indicate the presence of Fe_3O_4 and MCM-41 in the synthesized Fe_3O_4/MCM-41/SO_3H and that the crystalline structure of MCM-41 was preserved after functionalization.

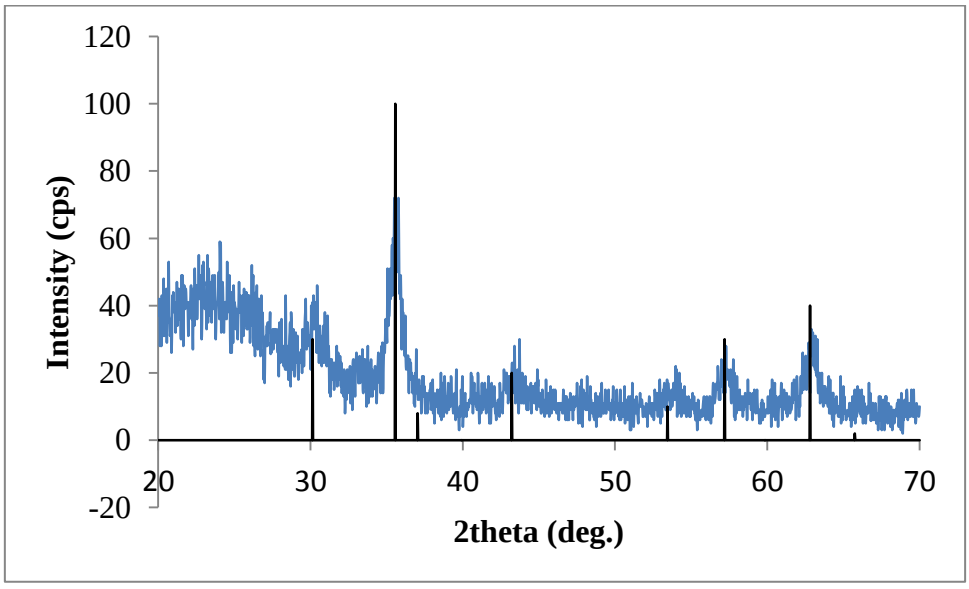


Figure 4.9 XRD diffractogram of the prepared Fe_3O_4/MCM-41/SO_3H at 2theta in a range of 20º to 70º.

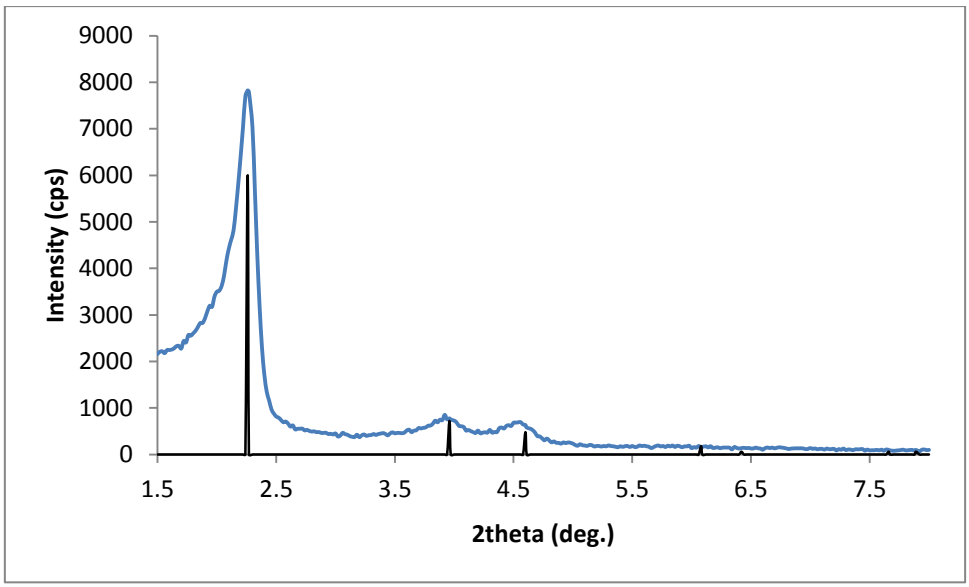


Figure 4.10 XRD diffractogram of the prepared Fe_3O_4/MCM-41/SO_3H at 2theta in a range of 1.5º to 8.0º.

IR spectra of the obtained Fe_3O_4/MCM-41/SO_3H (Figure 4.11) display a broad peak at about 3300 cm^{-1} and a peak at about 450 cm^{-1} corresponding to O–H stretching and Fe–O stretching, respectively. These results indicate the presence of hydroxyl groups and Fe_3O_4 on the solid. The spectra also show a group of overlapped intense peaks at 1050 and 1210 cm-1 which refer to Si–O–Si stretching of silica materials. Unfortunately a peak at about 1345 cm^{-1} corresponding to S=O stretching of the sulfonic acid groups cannot be seen in this spectra because it was concealed by the strong peaks of Si–O–Si stretching.

Thermal stability of the synthesized Fe_3O_4/MCM-41 and Fe_3O_4/MCM-41/SO_3H were investigated by TGA. The obtained thermograms are shown in Figure 4.12 and 4.13. The weight loss in the range of 40-100 ºC corresponding to the loss of moisture was found in both thermograms. However, thermogram of Fe_3O_4/MCM-41/SO_3H also shows the weight loss about 18% of total weight at 250-550 ºC which indicates a difference in composition of these adsorbents. The weight loss at this range of temperature is probably the result of the loss of organic compounds which would probably be propyl sulfonic acid groups and residual mercaptopropyl groups.

From the obtained information, it could be concluded that the functionalization was successfully achieved.

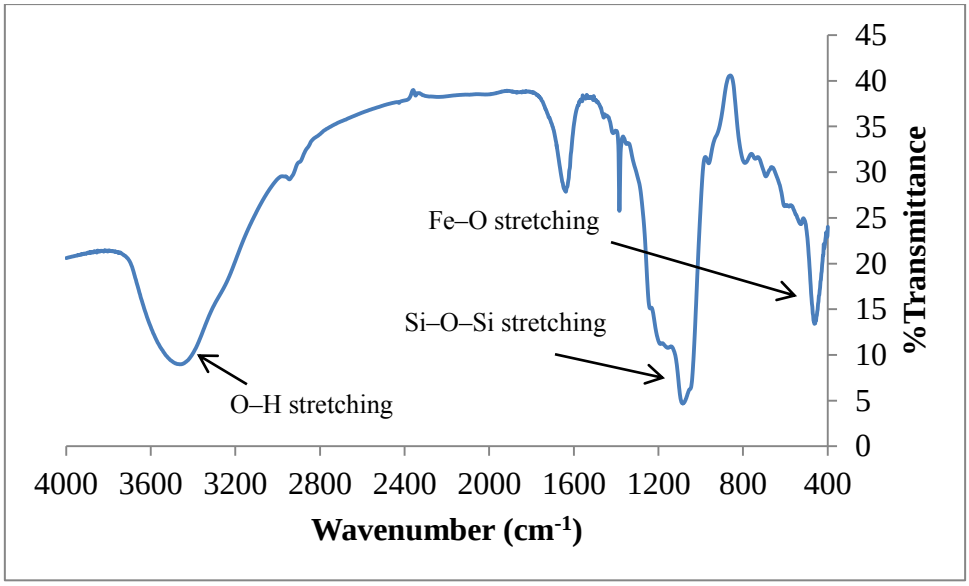


Figure 4.11 IR spectra of the prepared Fe_3O_4/MCM-41/SO_3H.

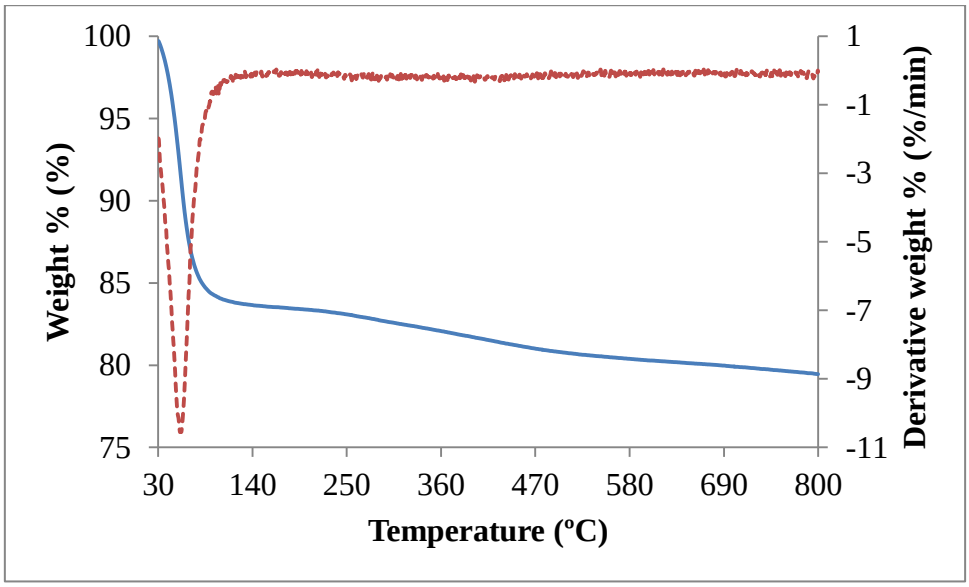


Figure 4.12 Thermogram of the prepared Fe_3O_4/MCM-41.

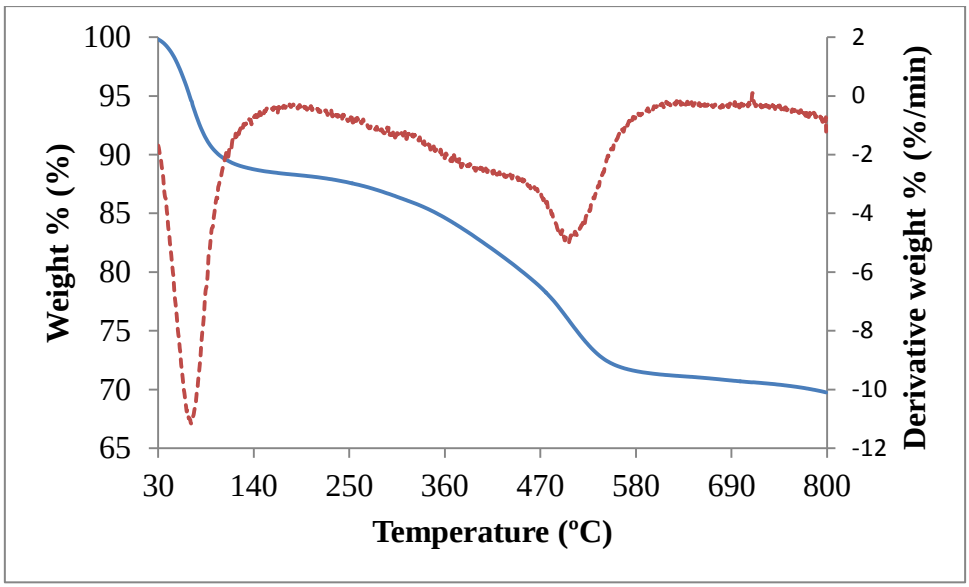


Figure 4.13 Thermogram of the prepared Fe_3O_4/MCM-41/SO_3H.

After functionalization process, the properties of the synthesized adsorbent i.e. surface area, total pore volume and average pore diameter were investigated by surface area analyzer. The results are shown in Table 4.1.

Table 4.1 Surface area, total pore volume and average pore diameter of the synthesized Fe_3O_4/MCM-41/SO_3H

Sample name	Surface area (Multipoint BET, m^2/g)	Total pore volume (mL/g)	Average pore diameter (Å)
Fe_3O_4/MCM-41/SO_3H	179.49	0.1139	25.38

4.2 Influence of extraction parameters

Influence of extraction parameters on the extraction and elution efficiency were studied under various experimental conditions such as different amount of adsorbent, sample pH, sample volume, eluent concentration, extraction time and elution time to find a suitable condition of the extraction process. Furthermore, leaching of ferric ions from the adsorbent into solutions at various sample pH was also investigated.

In the extraction procedure, urine samples were 50 times diluted before extraction process. Therefore, creatinine standard solution at concentration of 60 mg/L which is the highest creatinine concentration possibly found in diluted urine sample (creatinine concentration in human urine is in the range of 400-3000 mg/L), was chosen as creatinine sample in this study.

The creatinine extraction and elution efficiency are presented in the term of percent extraction (%extraction) and percent elution (%elution), respectively. These values can be calculated by using equation 4.2 and 4.3;

$$\%\text{Extraction} = \frac{M_i - M_f}{M_i} \times 100 \qquad (4.2)$$

$$\%\text{Elution} = \frac{M_{Eluted}}{M_i - M_f} \times 100 \qquad (4.3)$$

where M_i = initial amount of creatinine in the solution before extraction (mg)

M_f = final amount of creatinine in the solution after extraction (mg)

M_{Eluted} = amount of creatinine in eluted solution (mg)

When the proposed method was employed for the determination of creatinine in samples, the percent recovery of creatinine can be calculated by using equation 4.4;

$$\%\text{Recovery} = \frac{M_{found}}{M_i} \times 100 \qquad (4.4)$$

where M_{found} = amount of creatinine found in eluted solution (mg)

M_i = initial amount of creatinine sample solution (mg)

Furthermore, the leaching of Fe from the adsorbent to the sample during extraction process was determined and presented in the term of percent ferric ions leaching (%Fe leaching) which can be calculated by using equation 4.5;

$$\%\text{Fe leaching} = \frac{M_{Fe}}{m_{adsorbent}} \times 100 \qquad (4.5)$$

where M_{Fe} = amount of leached Fe in the extracted solutions plus amount of leached Fe in the eluted solutions (mg)

$m_{adsorbent}$ = amount of the adsorbent used in the extraction process (mg)

4.2.1 Sample pH

From preliminary study, the leaching of Fe into extracted and eluted solution were observed when the extraction of creatinine was performed using Fe_3O_4/MCM-41/SO_3H in solution of pH 1-2 (%Fe leaching at pH 1 and 2 were 0.17 % and 0.12 %, respectively). This event may be caused by incomplete coverage of Fe_3O_4 by silica network. Therefore, the effect of sample pH on the extraction efficiency was studied over the range of 3-5. The obtained results (Table 4.2) show that higher extraction efficiency could be achieved by lowering solution pH. It could be explained by an increase in the number of protonated creatinine (pK_{a1}=4.83, pK_{a2}=9.20, [41]) when the acidity of solution increases. The highest percent extraction was achieved at pH 3 with a low value of percent Fe leaching. Therefore, it was selected as optimum pH.

Table 4.2 Effect of sample pH on extraction efficiency and percent Fe leaching from the adsorbent at pH over the range of 3-5 (n=3)*

pH	%Extraction	%Fe leaching
3	92.4±0.2	0.05±0.01
4	86.7±1.0	0.05±0.01
5	83.5±1.1	0.03±0.01

* Extraction condition: 1 mL of 60 mg/L creatinine standard solution (the volume was subsequently adjusted to 5 mL with HNO_3 solution at different concentration), 50 mg of adsorbent and 1 hour of extraction time.

4.2.2 Amount of adsorbent

Adsorbent amount is an important parameter in extraction. If too little adsorbent is used for creatinine extraction, the error occurred during the process due to adsorbent loss become more serious. Moreover, the adsorbent amount should be large enough in order to achieve quantitative extraction. The effect of amount of adsorbent on the extraction efficiency was studied using 50 and 100 mg adsorbent. The obtained results (Figure 4.14) show that an increase of adsorbent amount from 50 to 100 mg was not beneficial. Therefore, 50 mg of adsorbent was selected for further study. In addition, these results also show that the extractions limit come from depletion of extractable protonated creatinine ions, not from using of too little adsorbent.

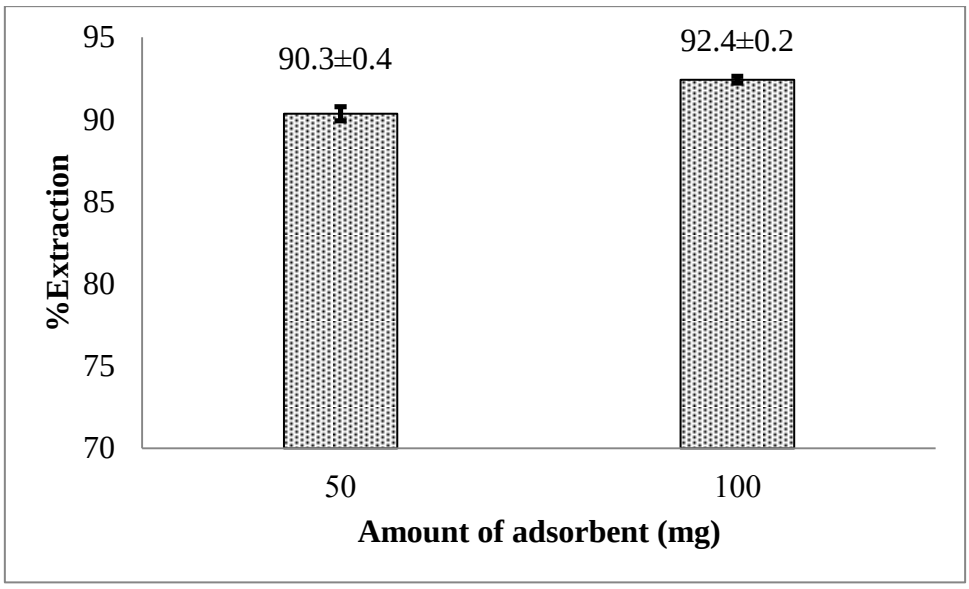


Figure 4.14 Effect of adsorbent amount on extraction efficiency (n=3); extraction condition: 1 mL of 60 mg/L creatinine standard solution (the volume was subsequently adjusted to 5 mL with 1 mM HNO_3 solution) and 1 hour of extraction time.

4.2.3 Sample volume

If too much sample volume is used for creatinine extraction, the poor extraction efficiency will be observed because excessive amount of creatinine cannot be extracted by the limited amount of adsorbent. Moreover, higher concentration of NaCl solution must be employed for creatinine elution. The former works have reported about the effect of alkali ions concentration in analyzed solution on sensitivity of Jaffé method [21]. Higher alkali ions concentration can cause lower sensitivity of the Jaffé method. Therefore, the effect of sample volume on the extraction efficiency was studied over the range of 0.50-1.00 mL. The percent extraction values observed when the studied range of sample volume was used (Figure 4.15) were not significantly different at 95% confidence interval. Thus, 1.0 mL of sample volume was selected to obtain high sensitivity of the method. Furthermore, these results also show that the extractions efficiency are confined by limited content of protonated creatinine ions, not from using of too much sample volume.

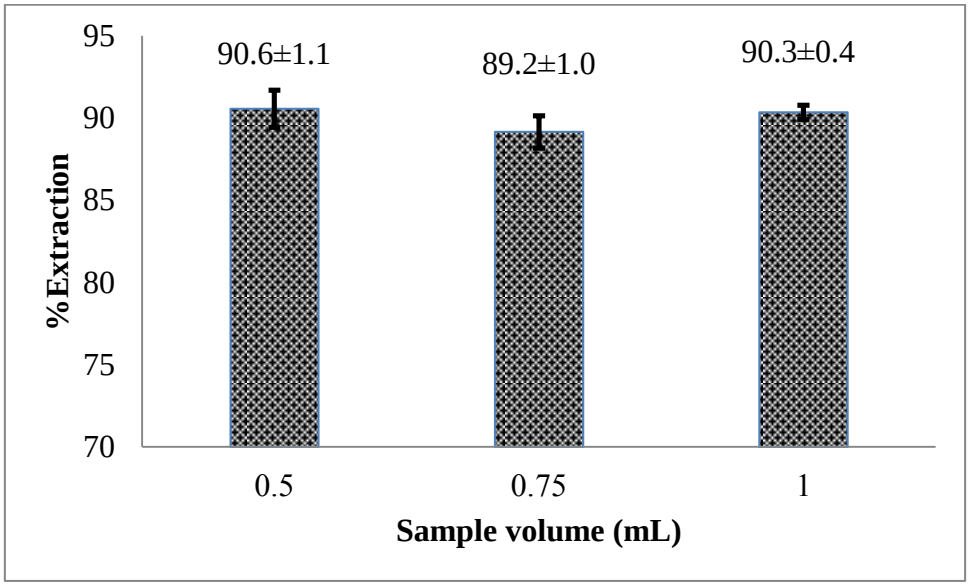


Figure 4.15 Effect of sample volume on extraction efficiency (n=3); extraction condition: 60 mg/L creatinine standard solution (the volume was subsequently adjusted to 5 mL with 1 mM HNO_3 solution), 50 mg of adsorbent and 1 hour of extraction time.

4.2.4 Eluent concentration

In this work, NaCl solution was used for elution of creatinine after extraction process. NaOH solution cannot be employed for this proposed because of the swelling of silica materials in a basic solution. However, if the concentration of sodium ions in analyzed solution is higher, the lower sensitivity of the Jaffé method will be observed. Therefore, the concentration of NaCl eluent should be as low as possible but enough for elution of creatinine with high elution efficiency.

The influence of sodium chloride concentration in the range of 0.5-1.5 mol/L on the elution efficiency was determined. The results indicate that elution efficiency increased when higher concentration of NaCl was used (Figure 4.16). Nevertheless, when NaCl concentration was increased from 1.0 to 1.5 mol/L, the percent elution values were not significantly different at 95% confidence interval. Therefore, 1.0 mol/L of NaCl was chosen as eluent.

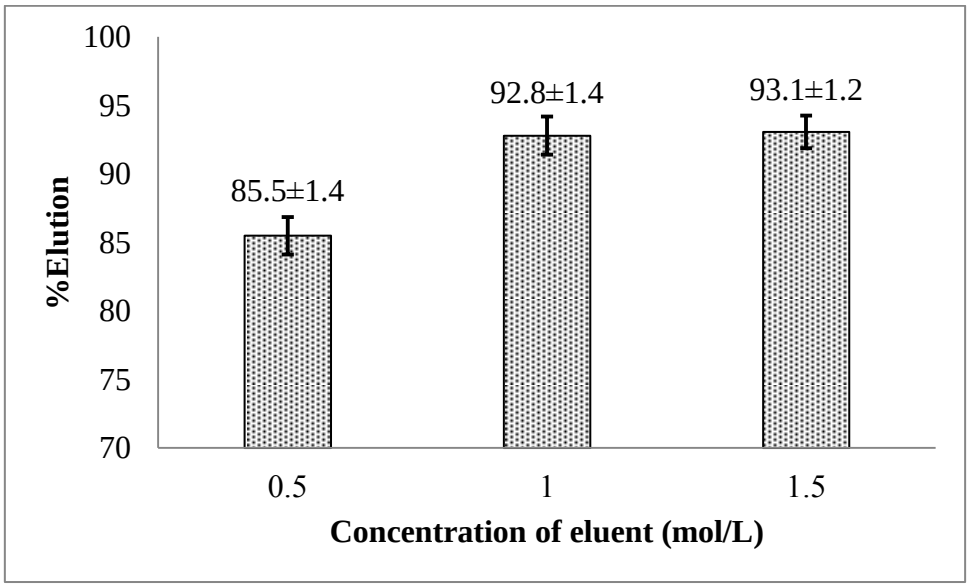


Figure 4.16 Effect of eluent concentration on elution efficiency (n=3); extraction condition: 1 mL of 60 mg/L creatinine standard solution (the volume was subsequently adjusted to 5 mL with 1 mM HNO_3 solution), 50 mg of adsorbent and 1 hour of extraction and elution time.

4.2.5 Extraction time

In this experiment, the effect of extraction time within the range of 10-60 minutes on the extraction efficiency was investigated to determine suitable time for creatinine extraction (Figure 4.17). The obtained results show that the extraction efficiency is almost constant over the extraction time studied. Furthermore, statistical hypothesis testing also indicated that the %extraction observed in the extraction time range of 10-60 minutes were not significantly different at 95% confidence interval. It could be concluded that adsorption of creatinine on the solid phase reached the equilibrium within 10 minutes. Thus, extraction time of 10 minutes was chosen for creatinine extraction.

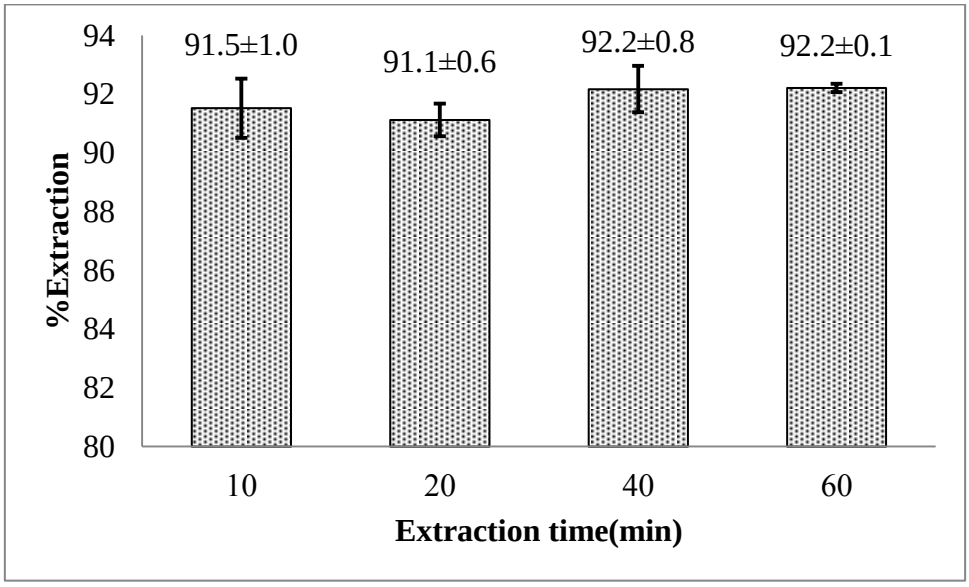


Figure 4.17 Effect of extraction time on extraction efficiency (n=3); extraction condition: 1 mL of 60 mg/L creatinine standard solution (the volume was subsequently adjusted to 5 mL with 1 mM HNO_3 solution) and 50 mg of adsorbent.

4.2.6 Elution time

The effect of elution time on the elution efficiency was studied in a range of 10 to 60 minutes (Figure 4.18). The obtained results show that the elution efficiency increased when increased the elution time from 10 to 20 minutes. However, the percent elution values were not significantly different at 95% confidence interval when the elution time was longer than 20 minutes. It indicates that equilibrium of creatinine elution was reached within 20 minutes. Therefore, twenty minutes of elution time was chosen for creatinine elution.

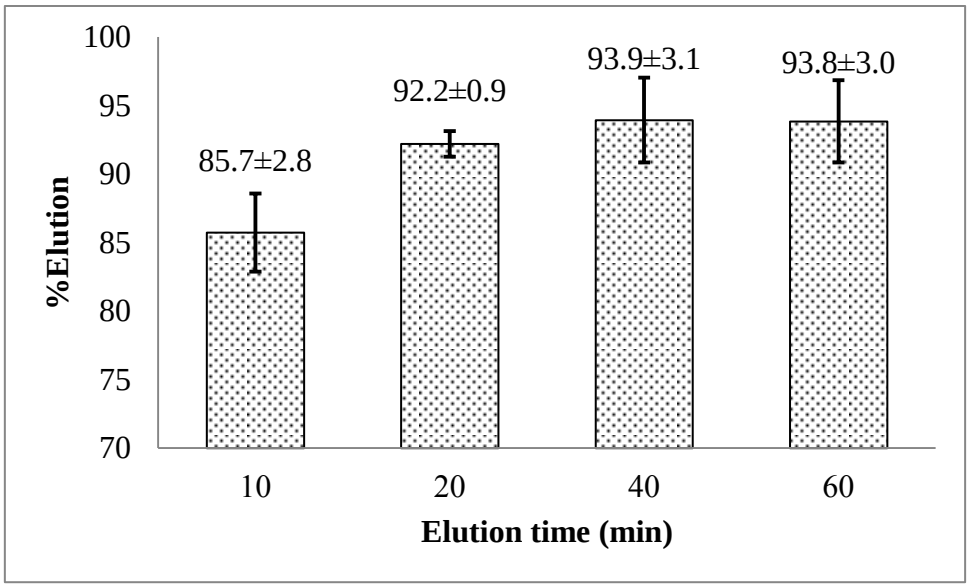


Figure 4.18 Effect of elution time on elution efficiency (n=3); extraction condition: 1 mL of 60 mg/L creatinine standard solution (the volume was subsequently adjusted to 5 mL with 1 mM HNO_3 solution), 50 mg of adsorbent and 10 minutes of extraction time.

4.3 Adsorbent reusability

In this section, the reusability of the prepared adsorbent was investigated by using the same adsorbent for repeated adsorption/elution cycles. The adsorbent was employed for creatinine extraction following the described method under chosen condition for 3 cycles. The obtained results (Figure 4.19) show that the adsorbent can be reused at least three times without losing efficiency.

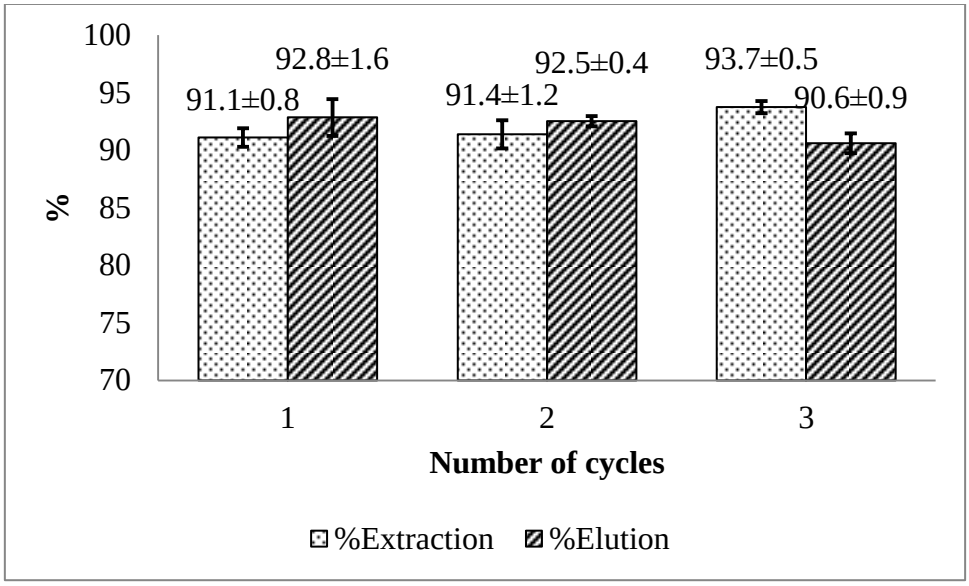


Figure 4.19 The reusability of the adsorbent (n=3); extraction condition: 1 mL of 60 mg/L creatinine standard solution (the volume was subsequently adjusted to 5 mL with 1 mM HNO_3 solution), 50 mg of adsorbent, 10 minutes of extraction time and 20 minutes of elution time.

4.4 Influence of interfering species

The results obtained from the analysis of 60 mg/L creatinine standard solution containing various types of interfering species at several concentrations by proposed method are shown in Figure 4.20. Percent recoveries of creatinine from pure standard solution and from standard solution containing glucose, ascorbic acid or urea at various concentrations were not significantly different at 95% confidence interval. This phenomenon can be explained by considering pK_a data of these species. In the case of glucose (pK_a=12.28), ascorbic acid (pK_{a1}=4.17, pK_{a2}=11.57) and urea (pK_b=13.82), most of these species were present in neutral forms at pH 3, so it could not be extracted by the adsorbent. From the above mentioned data, it can be concluded that the presence of glucose, ascorbic acid and urea in these concentrations in sample did not affect the accuracy of the results obtained by the proposed method

In the case of interference effect from bovine serum albumin (BSA), %recovery of creatinine from standard solution with and without BSA at concentration of 6 mg/L was not significantly different at 95% confidence interval. However, the %recovery observed in the determination of creatinine in standard solution containing 60 mg/L of BSA was significantly different from that obtained from the determination of pure creatinine standard solution. It indicates that under extremely high concentration of BSA in sample, BSA could also be extracted onto the adsorbent, eventually resulting in positively error in the determination of creatinine. This event can be explained by considering pI value of BSA (pI=4.7) and the nature of mesoporous materials. At pH 3, BSA has positive charge on the molecules and could be extracted by the cation exchanger sites. However, BSA could be only extracted on cation exchanger sites on the outside surface of mesoporous silica due to a bulky structure of BSA and small pore diameter of the adsorbent. Probability of BSA to reach these cation exchanger sites for adsorption increased when BSA of higher concentration co-existed in solution. This is a reason why BSA could be extracted at high concentration. From the above mentioned information, it can be concluded that the accuracy of the proposed method did not affected by the presence

of BSA when its concentration is not higher than its maximum concentration possibly found in urine.

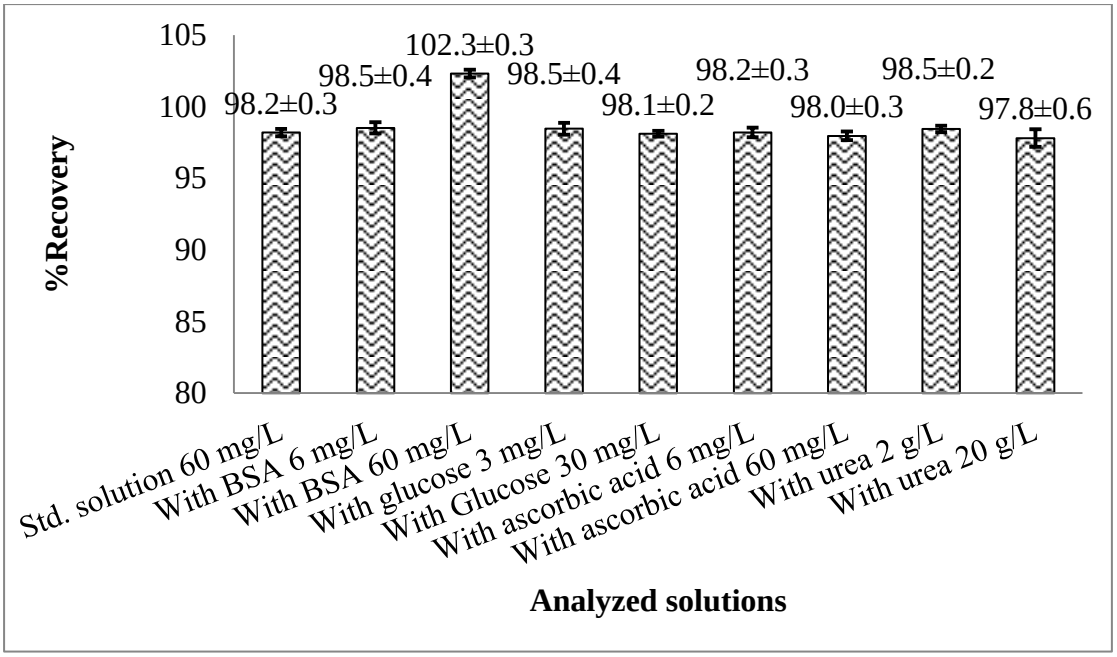
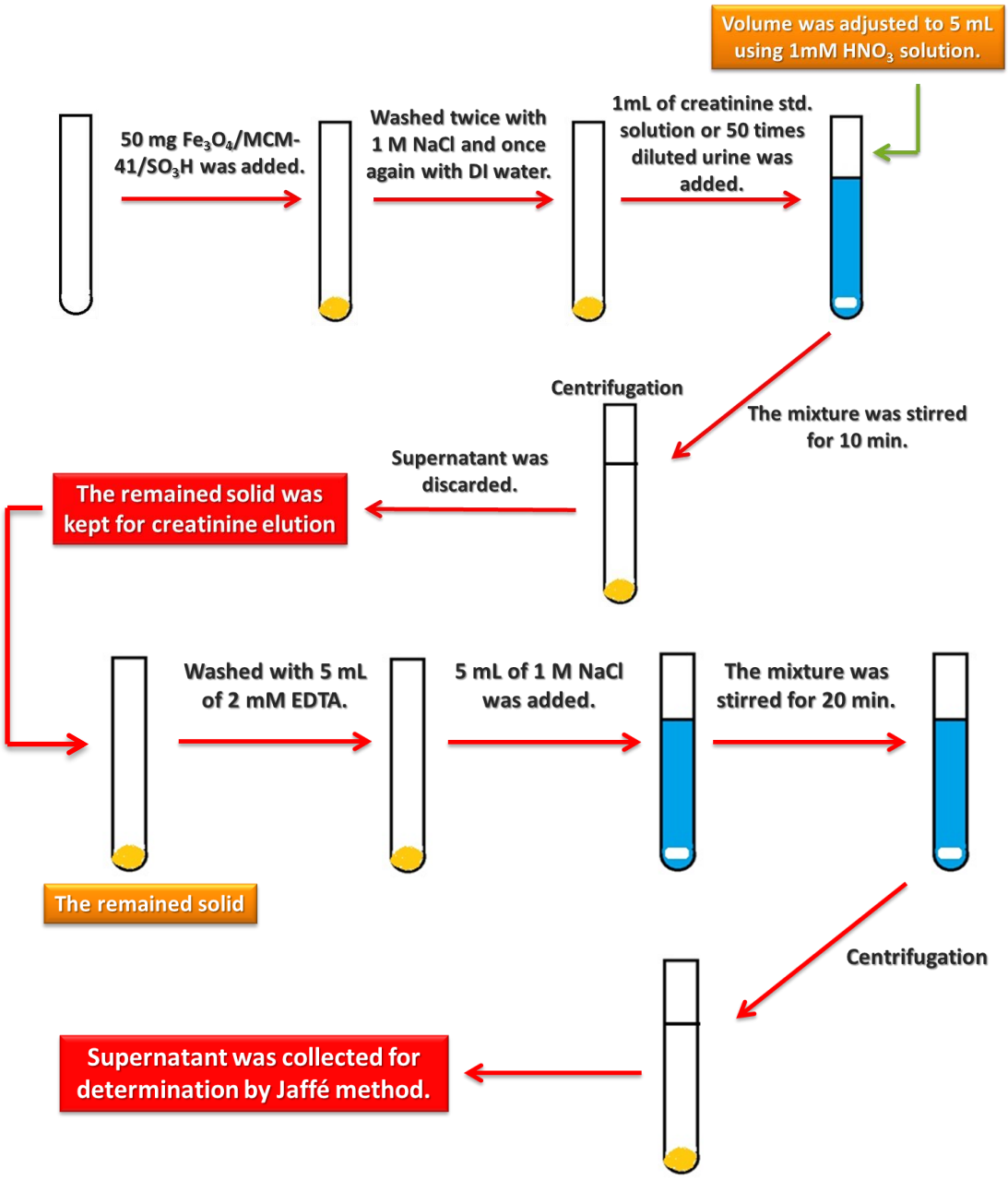


Figure 4.20 Influence of interfering species on the determination of creatinine by proposed method (n=3); extraction condition: 1 mL of 60 mg/L creatinine standard solution (the volume was subsequently adjusted to 5 mL with 1 mM HNO_3 solution), 50 mg of adsorbent, 10 minutes of extraction time and 20 minutes of elution time.

4.5 Method validation



Scheme 4.2 The proposed method for determination of creatinine.

In this section, the proposed method (Scheme 4.2) was validated under the selected extraction condition. The accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) of the method were determined and reported as follows. Furthermore, the linear range and repeatability of the method were also investigated and reported.

4.5.1 Accuracy and precision

Creatinine in standard solutions at the concentration of 10 and 50 mg/L were extracted by the proposed extraction method. The eluted solutions were analyzed by Jaffé method to determine accuracy and precision of the method that are reported in the term of percent recovery (%recovery) and percent relative standard deviation (%RSD), respectively (Table 4.3). The intra-day results were obtained from 6 replicate measurements within a day while the inter-day results were obtained from 12 experiments within 6 days. The results show that accuracy and precision of this method of creatinine analysis are acceptable, compared to the acceptable values in method validation [42].

Table 4.3 Accuracy and precision of the proposed method in creatinine determination.

Concentration of creatinine standard solution (mg/L)	%Recovery		%RSD	
	Intra-day[a]	Inter-day[b]	Intra-day[a]	Inter-day[b]
10	95.4 - 98.5	95.3 - 98.8	1.4	1.2 - 1.8
50	96.7 - 98.2	96.2 - 98.7	0.6	0.5 – 0.7

[a] Results from 6 replicate analysis within a day.

[b] Results from 12 experiments within 6 days.

4.5.2 Limit of detection (LOD) and limit of quantification (LOQ)

In this work, limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the signal obtained by equation 4.6 and 4.7, compared to standard calibration curve;

$$A_{LOD} = y_B + 3S_B \quad (4.6)$$

$$A_{LOQ} = y_B + 10S_B \quad (4.7)$$

where A_{LOD} = the absorbance at LOD concentration

A_{LOQ} = the absorbance at LOQ concentration

y_B = the mean of absorbances of the method blanks*

S_B = the standard deviation of absorbances of the method blanks*

* The method blanks were obtained from extraction of 1 mM HNO_3 by the proposed extraction method.

The LOD and LOQ were calculated using the information obtained by ten measurements of method blanks. The obtained results are shown in Table 4.4.

Table 4.4 Concentration at limit of detection (LOD) and limit of quantification (LOQ) of the proposed method

Variables of interest	Concentration (mg/L)
LOD	1.5
LOQ	3.0

Considering the results in Table 4.4, since the urine sample was diluted 50 times before the analysis, it could be concluded that the lowest concentration of creatinine in urine samples that could be detected and quantified were 74.6 and 150.0 mg/L, respectively.

4.5.3 Linearity and repeatability

Standard solutions containing creatinine in a concentration range of 8-60 mg/L were analyzed by the proposed method. The results from 6 replicate measurements within a day were used for calibration curve construction (Figure 4.21). The calibration plot shows clearly linear profile with R^2 = 0.9999 over the working range (8 - 60 mg/L). The repeatability of the method was also determined and reported in the term of standard deviation (SD) of intercept and slope of the calibration curves, as shown in Figure 4.21.

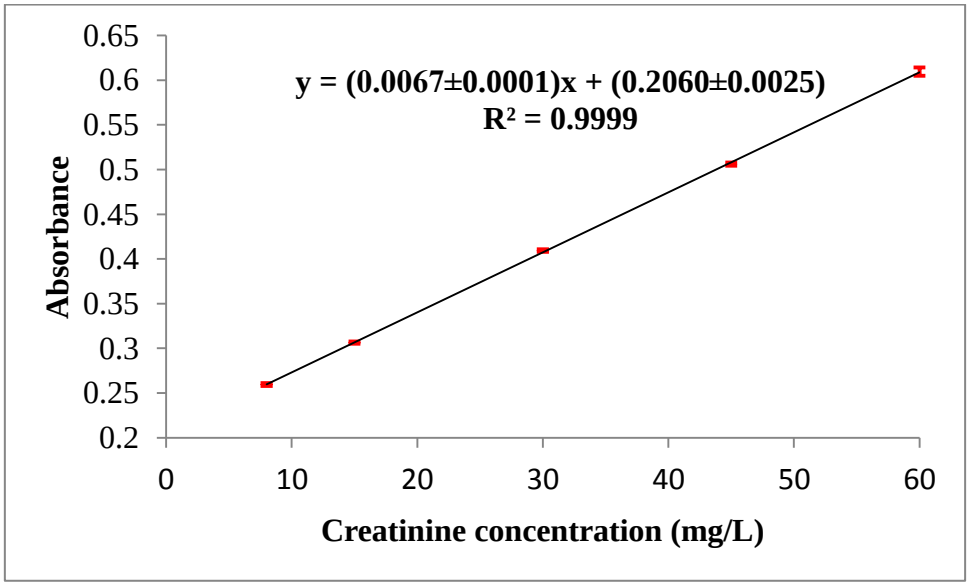


Figure 4.21 Calibration curve of creatinine constructed through extraction process (n=6).

4.6 Application to human urine samples

The method was employed to determine creatinine concentration in human urine samples (Table 4.5). The recoveries and relative standard deviation in the range of 96.7 - 99.5 % and 0.2 – 1.0, respectively, were observed from analysis of urine spiked samples, indicating an acceptable accuracy and precision of the method.

Table 4.5 Determination of the creatinine in human urine samples (n=3)

Sample origins	Creatinine concentration (mg/L)		%Recovery	%RSD
	added	found		
A healthy 24 years old male	0	2546	-	-
	500	3037	98.2	0.2
	1000	3519	97.3	0.5
A healthy 28 years old female	0	1584	-	-
	500	2068	96.7	1.0
	1000	2779	99.5	0.3
A healthy 54 years old female	0	1278	-	-
	500	1768	98.0	1.0
	1000	2265	98.6	0.2

CHAPTER V

CONCLUSION

5.1 Conclusion

Fe_3O_4 magnetic particles were synthesized by means of co-precipitation of ferric and ferrous ions in alkaline solution. The obtained magnetite particles were characterized by FT-IR and XRD and then they were coated by MCM-41 mesoporous silica. IR spectra and XRD diffractogram of the prepared Fe_3O_4/MCM-41 indicated the presence of Fe_3O_4 and MCM-41 while TEM images showed the successful coating of MCM-41 on Fe_3O_4 surface. The prepared Fe_3O_4/MCM-41 was further functionalized with 3-mercaptopropyltrimethoxysilane via grafting method. The functionalized thiol groups were subsequently oxidized by H_2O_2. IR spectra and TGA thermogram of Fe_3O_4/MCM-41 and Fe_3O_4/MCM-41/SO_3H indicated the successful synthesis of Fe_3O_4/MCM-41/SO_3H. The obtained Fe_3O_4/MCM-41/SO_3H had moderate surface area and showed response to external magnetic force.

The synthesized Fe_3O_4/MCM-41/SO_3H was applied as adsorbent for extraction and isolation of creatinine from matrix interferences in human urine samples prior to the determination by conventional Jaffé method. The adsorption of creatinine in acidic solution onto Fe_3O_4/MCM-41/SO_3H occurred mainly via ion exchange mechanism between creatinine ions and sodium ions attached on sulfonate groups on adsorbent. The extractions of creatinine in sample solutions were performed by batch method. Influence of various extraction parameters on the extraction and elution efficiency was studied to find suitable condition of the extraction process (Table 5.1).

Table 5.1 The condition of the creatinine extraction.

Parameters	Suitable condition
Sample pH	3
Amount of adsorbent	50 mg
Sample volume*	1 mL
Eluent (NaCl) concentration	1 mol/L
Extraction time	10 minutes
Elution time	20 minutes
Reusability	$\geq$ 3 times

* to make up to 5 mL with 1 mM HNO_3 solution.

Under the suitable condition, the synthesized adsorbent showed ability to extract creatinine with high recovery (83.6 - 85.9%) and it could be reused at least three times without loss of extraction or elution efficiency.

Moreover, the proposed extraction using the prepared adsorbent and determination method was validated. The analytical performance of the method is displayed in Table 5.2. The method showed acceptable accuracy and precision in creatinine determination.

Table 5.2 Analytical performance of the proposed method.

Variables of interest	Observed value
Intra-day accuracy (Recovery, n = 6) (%)	95.4 - 98.5[a], 96.7 - 98.2[b]
Inter-day accuracy (Recovery, n = 12) (%)	95.3 - 98.8[a], 96.2 - 98.7[b]
Intra-day precision (RSD, n = 6) (%)	1.4[a], 0.6[b]
Inter-day precision (RSD, n = 12) (%)	1.2 - 1.8[a], 0.5 - 0.7[b]
Limit of detection (3 SD, n = 10) (mg/L)	1.5
Limit of quantification (10 SD, n = 10) (mg/L)	3.0
Linear range (n=6) (mg/L)	8 - 60 mg/L
Repeatability (SD of calibration slope and intercept, n = 6)	0.0001 (slope), 0.0025 (intercept)

[a][creatinine] = 10 mg/L, [b][creatinine] = 50 mg/L

Finally, the method was applied to determine creatinine in human urine samples. The good recoveries and low relative standard deviation in the range of 96.7 - 99.5 and 0.2 - 1.0, respectively, were observed in the analysis of urine spiked samples. The results show that the proposed method was successfully applied for analysis of creatinine in human urine samples.

5.2 Suggestions for future work

- The extraction condition of the proposed method should be further optimized to obtain the better sensitivity method.

- The proposed method should be developed to use in extraction of creatinine in human plasma samples which encounter more serious interferences problem.

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