DEVELOPMENT AND APPLICATION OF MAGNETIC NANOPARTICLES FOR DETERMINATION OF TETRACYCLINES

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BY

PAKANAN LAOLERTWORAKUL

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This work presents the use of Fe_3O_4 magnetic nanoparticles as an adsorbent for tetracycline detection. Fe_3O_4 magnetic nanoparticles were synthesized by the co-precipitation method and characterized functional groups and pH at point of zero charge (Pzc) using Fourier transform infrared spectroscopy (FTIR) and the pH drift method, respectively. The effective parameters for tetracycline adsorption and desorption conditions, including pH, amount of magnetic nanoparticles, contact time, desorption solution, concentration and volume of desorption solution, and desorption time were investigated. Under optimal conditions, the adsorption isotherm was well described by the Langmuir model showing a maximum adsorption capacity of 32.57 mg/g. For the study of reusability, Fe_3O_4 magnetic nanoparticles showed a high adsorption efficiency with good precision after the ninth cycle. The proposed method was successfully applied to adsorb tetracycline in real pharmaceutical matrix. The content of tetracycline using the developed method was 95.53% compared to the content on the drug label. This proposed method is simple, low-cost, environmentally friendly, and easy phase separation.

Keyword : Fe_3O_4 magnetic nanoparticles, Tetracycline, Isotherm, Adsorption, Desorption

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CHAPTER 1
INTRODUCTION

Background

Antibiotics are a type of antimicrobial drug used to treat and against bacterial infection in human beings and animals. They work by destroying or inhibiting the growth of bacteria. Antibiotic drugs are commonly divided into two types based on their mechanism of drug actions, namely bactericidal antibiotics, e.g., Rifamycin, Quinolone, and Sulfonamide, and bacteriostatic antibiotics, e.g., Lincosamide, Macrolides, and Tetracyclines (Singh, 2015).

Tetracyclines (TCs) are a group of broad-spectrum antibiotics active against Gram-positive and Gram-negative bacteria. They are widely used for the treatment of human and animal disease from bacterial infection and used as a growth stimulant. They have many favorable properties such as being effective against various pathogens, low cost, low side effects, and they can be administered orally (Chopra & Roberts, 2001). However, the use of a high dose over a long period of time leads to an excessive residual of TCs in animals and their product, such as meat, milk and may induce an allergic reaction in humans. Moreover, long term treatment of TCs can contribute to antibiotic resistance in the human body (Y. Wang et al., 2016). Antibiotic resistance leads to higher medical costs, prolonged hospital stays, and increased mortality (Organization, 2020). In addition, tetracycline residues from human and animal consumption can be released into the environment source. Therefore, the efficient method for determination of tetracycline is considerably important.

Several methods have been reported for tetracyclines determination in various samples (i.e., pharmaceutical formulations, food, and biological samples), including flow injection analysis (FIA) (Rodriguez, Espinosa-Ramírez, Aguilar, Ibarra, & Miranda, 2010); (Rodríguez, Pezza, & Pezza, 2016), high-performance liquid chromatography (HPLC) (Wu, Wang, Yang, Liu, & Wang, 2016); (Yang et al., 2019), and fluorescence spectrophotometry (An, Zhuo, Zhang, & Zhu, 2015); (Qu, Sun, Liu, Zhao, & You, 2016). However, these detection methods still need some disadvantages, such as sample

preparation step before analysis. A number of sample preparation techniques have been performed including liquid-liquid extraction and solid-phase extraction. Nevertheless, these methods have some drawbacks of high solvent consumption, time-consuming, expensive, and environmental toxicity.

Currently, magnetic nanoparticles (MNPs) have been used widely for a variety of applications due to many unique and novel characteristics compared to a bulk magnet. MNPs are usually synthesized from a salt of metal ions and have a size between 1 - 100 nanometers (S. Liu, Yu, Wang, Shen, & Cong, 2020). Fe_3O_4 magnetic nanoparticles (Fe_3O_4 - MNPs) are often developed and intended to use as an adsorbent for preconcentration and extraction of many substances and removal various contaminants in environment sample (El-Dib, Mohamed, El-Shamy, & Mishrif, 2020). These materials offer excellent properties, such as high surface to volume ratio, ease of synthesis, easy phase-separation, chemical stability, and low toxicity and provide the promising material for a wide range of applications. In particular, the use of Fe_3O_4 magnetic nanoparticles as adsorbents have more attractive than other non-magnetic adsorbents because of the possibility to separate them from the solution using magnetic field (Pourhakkak, Taghizadeh, Taghizadeh, & Ghaedi, 2021).

In this work, the Fe_3O_4 magnetic nanoparticles were synthesized and used as an adsorbent for tetracycline detection. The adsorption and desorption properties of tetracycline onto Fe_3O_4 magnetic nanoparticles in aqueous solution were investigated. The applicability of tetracycline adsorption in real sample matrix was also studied.

Objectives of the research

The purpose of this research consisting of two goals are listed below

1. To develop a method for adsorption of tetracycline using Fe_3O_4 magnetic nanoparticles as an adsorbent.

2. To apply the developed method for detection of tetracycline in pharmaceutical sample.

Scope of the research

1. The synthesis and characterization of Fe_3O_4 magnetic nanoparticles using the co-precipitation method

2. The study of tetracycline adsorption and desorption conditions by optimization of various parameters, including pH, the amount of magnetic nanoparticles, contact time, types and concentration of desorption solution, volume of desorption solution, and desorption time.

3. The study of analytical performance of Fe_3O_4 magnetic nanoparticles in terms of adsorption isotherm and reusability.

4. The application of the developed method for the detection of tetracycline in pharmaceutical sample.

Expected Outputs

1. A rapid, simple, and effective adsorption method was obtained for the adsorption of tetracycline using synthesized Fe_3O_4 magnetic nanoparticles.

2. The developed method was successfully applied to detect tetracycline in real pharmaceutical sample.

CHAPTER 2

Literature review

In this work, the related concept and literature reviews of analytical methodologies have been presented by the following topics:

1. Tetracyclines
2. Magnetic nanoparticles
3. Adsorption and desorption process
4. Literature reviews

1. Tetracyclines

Tetracyclines are antibiotics with a broad range that were identified from *Streptomyces* bacteria. It has a bacteriostatic effect by reducing bacterial proliferation. Tetracyclines are used to treat a variety of pathogens, including gram-negative and gram-positive bacteria, as well as certain mycoplasma, virus, and rickettsia (Calixto, Cervini, & Cavalheiro, 2012). Tetracyclines are known to affect bacterial protein synthesis by blocking the 30s component of a bacterial ribosome, as shown in Figure 1. They are a vast family of antibiotics with more than 20 variants that have just been identified. Tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), and doxycycline (DT) are the most prevalent types utilized in veterinary medicine (Ibarra, Rodriguez, Miranda, Vega, & Barrado, 2011). Tetracyclines have a fundamental chemical structure of linear-fused rings with several functional groups such as alkyl, hydroxyl, and amine on the upper and lower sides of the molecule, as illustrated in Figure 2. Chemical functional groups attached at the upper and lower regions produce variably antibiotic and non-antibiotic characteristics.

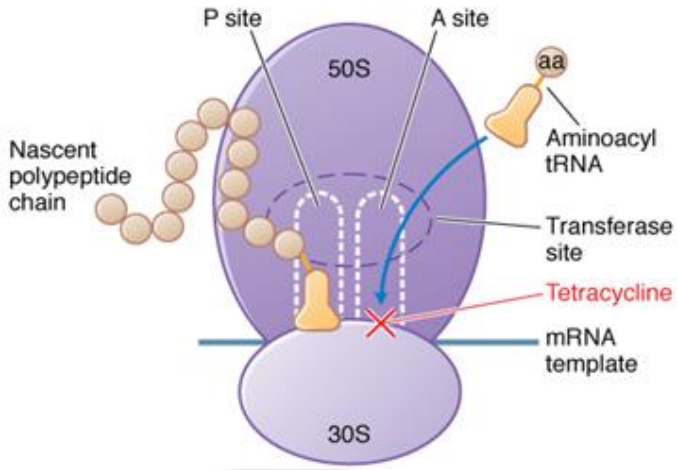
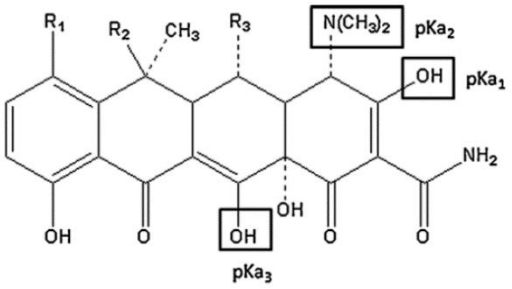


Figure 1. The mechanism action of tetracyclines.

Source: Antibiotics, T. N. I. P. o. (2016). Antibiotic drugs. Retrieved from: http://www.antibiotics-info.org/tetracycline.html



Common denomination	Symbol	R Groups			pKa		
		R1	R2	R3	pKa_1	pKa_2	pKa_3
Tetracycline (TC)	TC	-H	-OH	-H	3.2	7.5	8.9
Oxytetracycline (OT)	OT	-H	-OH	-OH	3.3	7.8	9.6
Doxycycline (DT)	DT	-H	-H	-OH	3.0	8.0	9.2
Chlortetracycline (CT)	CT	-Cl	-OH	-H	3.3	7.6	9.3

Figure 2. Chemical structures of tetracyclines.

Source: Ibarra, I. S., Rodriguez, J. A., Miranda, J. M., Vega, M., & Barrado, E. (2011). Magnetic solid phase extraction based on phenyl silica adsorbent for the determination of tetracyclines in milk samples by capillary electrophoresis. Journal of Chromatography A, 1218(16), 2196-2202.

Tetracyclines are widely utilized in human treatment, human prophylactics, and veterinary medicine due to their good antibacterial characteristics and lack of severe negative effects. They are also low-cost antibiotics that have been widely employed as growth enhancers in animal feed. As a result, tetracyclines are commonly accumulated in animals, and their derivatives are associated with increased antibiotic resistance (Chopra & Roberts, 2001). According to the World Health Organization (WHO), antimicrobial resistance is a global concern, and tetracyclines are one of the quickly increasing antibiotic resistance issues. Antibiotic resistance issues result in high antibiotic dosages, high medical expenses, and higher mortality (Organization, 2020). As a result, the Codex Alimentarius Commission determined that a safe daily consumption of tetracyclines is 0 - 30 g/kg. The maximum residual limit (MRL) for tetracycline, oxytetracycline, and chlortetracycline in milk is 100 g/kg (Commission, 2018), as stated in Table 1. According to the food and drug administration (FDA), the total maximum residual of tetracyclines in milk is 300 g/kg (Ibarra et al., 2011).

Table 1. Maximum residue limit of tetracyclines in foods

CHLORTETRACYCLINE/OXYTETRACYCLINE/TETRACYCLINE (antimicrobial agent)				
JECFA Evaluation		45 (1995); 47 (1996); 50 (1998); 58 (2002)		
Acceptable Daily Intake		Group ADI for chlortetracycline, oxytetracycline and tetracycline: 0-30 µg/kg bw (JECFA50). Group ADI for chlortetracycline, oxytetracycline and tetracycline.		
Residue Definition		Parent drugs, singly or in combination		
Species	Tissue	MRL (µg/kg)	CAC	Notes
Cattle	Muscle	200	26 (2003)	
Cattle	Liver	600	26 (2003)	
Cattle	Kidney	1200	26 (2003)	
Cattle	Milk (µg/l)	100	26 (2003)	
Fish	Muscle	200	26 (2003)	Applies only to oxytetracycline
Giant prawn (*Paeneus monodon*)	Muscle	200	26 (2003)	Applies only to oxytetracycline
Pig	Muscle	200	26 (2003)	
Pig	Liver	600	26 (2003)	
Pig	Kidney	1200	26 (2003)	

Table 1. (continued)

Species	Tissue	MRL (µg/kg)	CAC	Notes
Poultry	Muscle	200	26 (2003)	
Poultry	Liver	600	26 (2003)	
Poultry	Kidney	1200	26 (2003)	
Poultry	Eggs	400	26 (2003)	
Sheep	Muscle	200	26 (2003)	
Sheep	Liver	600	26 (2003)	
Sheep	Kidney	1200	26 (2003)	
Sheep	Milk (µg/l)	100	26 (2003)	

Source: Commission, T. C. A. (2018). Maximum Residue Limits (MRLs) and Risk Management Recommendations (RMRs) for residues of veterinary drugs in foods. Retrieved from: http://www.fao.org/fao-who-codexalimentarius/codex-texts/maximum-residue-limits/en/

2. Magnetic nanoparticles

Magnetic materials in particles and nanoparticles can currently display magnetic properties that are connected to the composition of the material and the size of the products (Wallyn, Anton, & Vandamme, 2019). As a result, materials of various sizes, such as nanometers and bulk materials, react differently in a magnetic field. Magnetic nanoparticles with diameters ranging from 1 to 100 nanometers respond to magnetic fields as a superparamagnetic material (S. Liu et al., 2020). Each molecule's size and magnetic property are affected by atom rearrangement (Siri Sineenat & Sutthiluck, 2017). A magnetic state is classified into six states based on the magnetic dipole moment, which describes the magnetic strength and direction of an item that generates a magnetic field (Siri Sineenat & Sutthiluck, 2017). The primary relevant forms of magnetism and their features are shown in Figure 3.

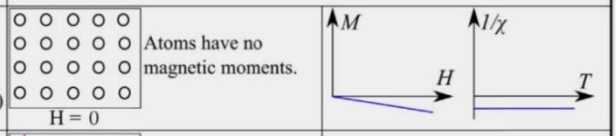
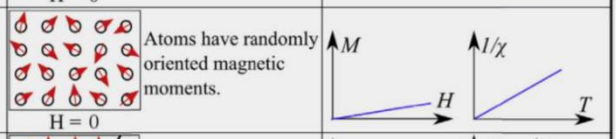
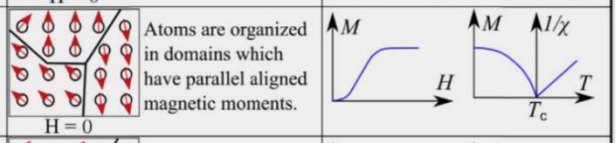
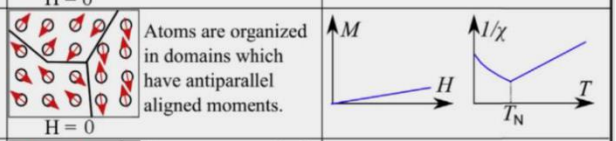
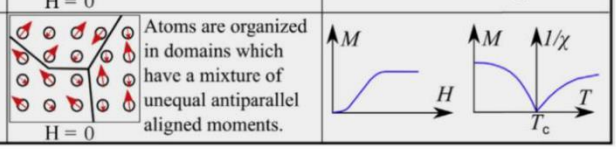
Magnetism	Examples	Magnetic behaviour
Diamagnetism	Bi, Si, Cu, inert gases Susceptibility small and negative (-10^{-6} to -10^{-5})	
Paramagnetism	Al, O_2, MnBi Susceptibilty small and positive (10^{-5} to 10^{-3})	
Ferromagnetism	Fe, Ni, Co, Gd Susceptibility large (generally > 100)	
Antiferromagnetism	Cr, MnO, FeO Susceptibilty small and positive (10^{-5} to 10^{-3})	
Ferrimagnetism	Fe_3O_4, $MnFe_2O_4$, $NiFe_2O_4$ Susceptibility large (generally > 100)	

Figure 3. Summary of the primary relevant forms of magnetism

Source: Nisticò, R., Cesano, F., & Garello, F. (2020). Magnetic Materials and Systems: Domain Structure Visualization and Other Characterization Techniques for the Application in the Materials Science and Biomedicine. Inorganics, 8, 6.

Diamagnetism appears in a material with no permanent magnetic moments in the atomic structure. When a material is placed in a magnetic field, electrons circling the nucleus lose their balance, forming magnetic poles within the atom. When magnetic poles oppose a magnetic field, magnetic susceptibility (X_m) is slightly negative. Quartz, bismuth, graphite, marble, and rock salt are examples of diamagnetic materials.

Paramagnetism appears in a material that the electrons in an atom have a random arrangement in the absence of a magnetic field, and the total magnetic moment is zero. When a material is placed in a magnetic field, electrons circling the nucleus of a material arrange in the same direction as the magnetic field, and the value of magnetic susceptibility is positive, in the range of 10^{-6} to 10^{-2}. Aluminum, oxygen, and titanium are examples of elements that behave in this manner.

Unlike paramagnet, a ferromagnetic material becomes a magnet upon induction by an external magnetic field and retains the magnetic property even after the external field is removed. The material has a permanent magnetic moment in the structure. It consists of magnetic domains, and each domain is divided by a partition called a domain wall. In each domain, dipole moments align in the same direction. Their magnetic susceptibility is positive and usually greater than 100. Certain types of metals, including iron, cobalt, nickel, gadolinium, and dysprosium, demonstrate ferromagnetism.

Antiferromagnetism is a phenomenon that, although the material has a permanent magnetic moment in the structure, the arrangements of magnetic moments in each domain are in the opposite direction in the same magnitude. Therefore, the magnetic moments offset each other, and the magnetic susceptibility is zero. An example of antiferromagnetic is hematite.

Ferrimagnetism is found in a material that has a permanent magnetic moment. This occurs from domains of magnetic moments of unequal magnitude arranged in opposite directions. The residual magnetization allows the material to have a large positive magnetic susceptibility. The materials with this type of property are ferrites, such as magnetite (Fe_3O_4).

Superparamagnetism occurs in a nanoscale ferromagnet or ferrimagnet. When the material size is small, domain walls become unstable, and each nanoparticle acts virtually as a single magnetic domain. Furthermore, when the size becomes smaller, reaching the specific critical value, ferromagnetic magnetic particles become superparamagnetic. Superparamagnetic materials only show their magnetic properties when exposed to external magnetic, and their properties vary by temperature. The different relationships between external magnetic field strength, H, and the magnetization (dipole moment per volume), M, in substances with different types of magnetism (Nisticò, Cesano, & Garello, 2020) are shown in Figure 4.

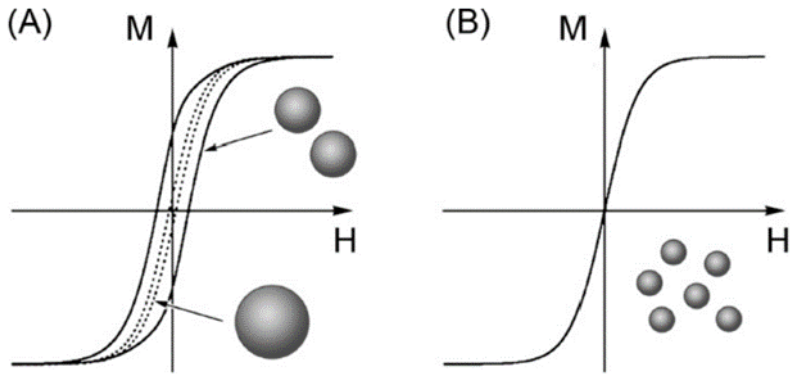


Figure 4. The relationship between the external magnetic field and the magnetization value of ferromagnetic (A) and superparamagnetic (B).

Source: Wallyn, J., Anton, N., & Vandamme, T. F. (2019). Synthesis, Principles, and Properties of Magnetite Nanoparticles for In Vivo Imaging Applications-A Review. *Pharmaceutics, 11*(11), 601.

2.1 Magnetite nanoparticles

Iron oxide nanoparticles are one of the nanoparticles widely used in theranostics, drug delivery, and imaging (Akbarzadeh, Samiei, & Davaran, 2012). They are based on three main types of iron oxide, including hematite (α-Fe_2O_3), maghemite (γ-Fe_2O_3), and magnetite (Fe_3O_4 or $FeO.Fe_2O_3$) (Wallyn et al., 2019), of which the properties are shown in Table 2. Hematite is the first recovered iron oxide that has kinetic and thermodynamic stability. Maghemite only has kinetic stability and slowly changes to hematite, which drops the magnetization. Magnetite composed of Fe^{2+} and Fe^{3+} is a black iron oxide with the most robust magnetic behavior (Wallyn et al., 2019).

Table 2. The chemical formula and color of significant species of iron oxides.

Iron Oxide	Chemical Formula (Current Name)	Color
Ferrous oxide (iron(II) oxides)	FeO (Wüstite)	Black
Mixed-oxide (iron(II, III) oxides)	Fe_3O_4 or $FeO.Fe_2O_3$ (Magnetite)	Black
Ferric oxides (iron(III) oxides)	α-Fe_2O_3 (Hematite) β-Fe_2O_3 γ-Fe_2O_3 (Maghemite) ε-Fe_2O_3	Grey, brown, red

Source: Wallyn, J., Anton, N., & Vandamme, T. F. (2019). Synthesis, Principles, and Properties of Magnetite Nanoparticles for In Vivo Imaging Applications-A Review. Pharmaceutics, 11(11), 601.

Magnetite crystallizes in two structures: spinel and inverse spinel. The spinel structure, AB_2O_4, contains a cubic-closed pack oxygen atom with A metal atoms in tetrahedral sites and B metal atoms in octahedral sites. In contrast, the inverse spinel structure differs in that all atoms of A and half of atoms of B are located in octahedral sites, while another half of atoms of B are in tetrahedral sites (Thitirat, 2014). For example, the inverse spinel of Fe_3O_4, as shown in Figure 5(a), consists of all Fe^{2+} and half of Fe^{3+} in the octahedral holes of oxygen while the remaining Fe^{3+} are in the tetrahedral holes.

Magnetite is one of the materials that has ferrimagnetic properties. Figure 5(b) shows the difference in the spin direction of ions in the tetrahedral and octahedral sites, which leads to a non-zero total magnetic moment. In addition, magnetite has a superparamagnetic property when magnetite in nanosize reaches a single magnetic domain's specific critical size (Thitirat, 2014).

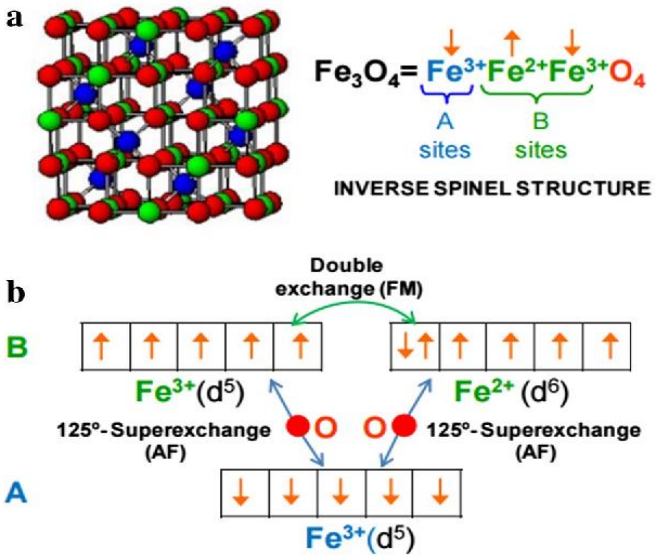


Figure 5 (a). The inverse spinel structure of magnetite with tetrahedral (A) and octahedral (B) site. 4(b) spin direction of magnetite.

Source: Fodjo, E. K., Gabriel, K. M., Serge, B. Y., Li, D., Kong, C., & Trokourey, A. (2017). Selective synthesis of Fe_3O_4AuxAgy nanomaterials and their potential applications in catalysis and nanomedicine. Chemistry Central Journal, 11.

Fe_3O_4 magnetic nanoparticles also have a high surface-to-volume ratio and surface interaction, leading to an adsorbent for substance separation and preconcentration (El-Dib et al., 2020). For example, Shariati-Rad et al. (Shariati-Rad, Irandoust, Amri, Feyzi, & Ja'fari, 2014) proposed a preconcentration step of methyl orange in water samples using SiO_2 coated Fe_3O_4 nanoparticles. Abd Ali & Omar. (Abd Ali & Omar, 2018) proposed a preconcentration step of Cd^{2+} ions in drinking water using bared Fe_3O_4 nanoparticles. In addition, Saad et al. (Saad et al., 2020) have developed magnetic-nanoparticle-assisted dispersive liquid-liquid microextraction to use in preconcentration and extraction of chloramphenicol residue in water.

Furthermore, Fe_3O_4 magnetic nanoparticles have been demonstrated as an effective catalyst to generate reactive oxygen species (ROS), which is similar in activity to a natural peroxidase (L. Gao et al., 2007).

2.2. Synthesis of Iron Oxide Magnetic Nanoparticles

In general, there are two approaches to nanoparticle synthesis: top-down and bottom-up. The production of nanoparticles from bigger compounds is known as top-down synthesis. Bottom-up synthesis is the chemical or biological aggregation of atoms or molecules to generate nanoscale compounds (Isaacoff & Brown, 2017).

Bottom-up chemical methods are gaining popularity because they provide quick synthesis, high yield, and homogeneous magnetic nanoparticle size (Siri Sineenat & Sutthiluck, 2017). Figure 6 depicts the synthesis approaches for forming iron oxide magnetic nanoparticles in an aqueous solution. The quickest nucleation occurs when the precursor concentration is raised to supersaturation, resulting in tiny crystalline nuclei. Following that, nuclei are formed by diffusing a solution onto the grain surface. Surfactants and stabilizers are used to regulate the increasing grain surface. Clusters, aggregates, or large nanoparticles are generated in the presence of uncontrolled optimal conditions, with the potential of Ostwald ripening (Wallyn et al., 2019).

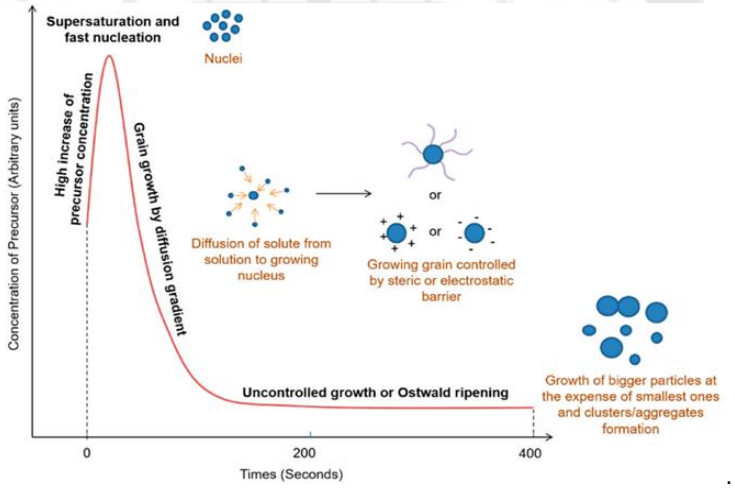


Figure 6. The Illustration of the formation of nanoparticles.

Source: Wallyn, J., Anton, N., & Vandamme, T. F. (2019). Synthesis, Principles, and Properties of Magnetite Nanoparticles for In Vivo Imaging Applications-A Review. Pharmaceutics, 11(11), 601.

The synthetic methods for iron oxide magnetic nanoparticles, e.g., solution precipitation, sol-gel, polyol, thermal decomposition, hydrothermal, and co-precipitation methods, are described below.

- Solution precipitation method is the traditional method for preparing iron oxide nanoparticles from homogeneous solutions. During the precipitation, precipitant is added to generate insoluble products with uniform size and high yield. However, this result still depends on the prevention of nucleation during the growth step of particles (Gul, Khan, Rehman, Khan, & Khan, 2019).

- Sol-gel is the process of synthesis of nanoparticles containing hydroxylation and condensation. When the precursor is hydrolysis with water, the polymerization of the precursor occurs in the condensation steps. After these steps, the product can be obtained by drying or heating (Siri Sineenat & Sutthiluck, 2017).

- Polyol method is the heating process of the precursor dissolved in polyol, such as polyethylene glycol. When the temperature increases gradually, the reduction of metal ions proceeds. The metal nuclei are generated, and nucleation occurs. At the end of the reaction, magnetic nanoparticles are in submicrometer size (Kim, Jeong, & Moon, 2006).

- Thermal decomposition method generates magnetic nanoparticles from the degradation of organometallic or metal acetylacetonate compounds (examples of metals; Fe^{3+}, Mn^{3+}, Co^{2+}, Ni^{2+}, and Zn^{2+}) in an organic solvent using surfactants, e.g., oleic acid and hexane, as a capping agent. A temperature around 200-340 °C is used to decompose precursors depending on the nature of the organometallic compound. Thermal decomposition achieves control of the size distribution, shape, and dispersion behavior of magnetic nanoparticles (Thitirat, 2014). For example, the iron oxide nanoparticles range of 6-20 nm is generated by the polymer-catalyzed decomposition of the reaction of $Fe(CO)_5$ (Gul et al., 2019). However, the risk of using high temperature and pressure of organic liquids during the reaction is still needed to consider (Gul et al., 2019).

- Hydrothermal method is one of the most successful methods for synthesizing magnetic nanoparticles using metal salts as a precursor. This method uses high temperature (125-250 °C) and high pressure (0.3-4 MPa). The size and size distribution of magnetic nanoparticles are controlled by precursor concentration. However, this method still has slow kinetics, which means that it needs much reaction time to produce nanoparticles. Meanwhile, the reaction time significantly affects the average size of particles (Gul et al., 2019).

- Co-precipitation method is easy and suitable to synthesize iron oxide nanoparticles at room temperature (Gul et al., 2019). Iron oxide nanoparticles are generated from salts of Fe^{3+} and Fe^{2+} in a mole ratio of 2:1 using base as a precipitant, as shown in equation 1.

$$Fe^{2+} + 2Fe^{3+} + 8OH^{-} \rightarrow Fe_3O_4 + 4H_2O \quad \text{--------(1)}$$

On the other hand, magnetite nanoparticles can easily be oxidized to maghemite nanoparticles in the presence of oxygen (shown in equation 2).

$$Fe_3O_4 + 2H^{+} \rightarrow Fe_2O_3 + Fe^{2+} + H_2O \quad \text{--------(2)}$$

The main advantages of this method are rapid and easy scale-up magnetic nanoparticles. However, the size and shape depend on the pH of a solution, mole ratio between Fe^{3+}/Fe^{2+}, and nature of salts (chloride, nitrate, and sulfate) (Thitirat, 2014).

3. Adsorption and desorption process

3.1. Adsorption

Adsorption is a phenomenon that happens on the adsorbent's surface. Adsorbents may draw molecules or substances, known as adsorbate, to trap on their surface. As seen in Figure 7, adsorption processes are classified into two types: physical adsorption and chemical adsorption. They have also happened in several phases of matter, including gas and liquid, gas and solid, liquid and liquid, and liquid and solid (J. Wang & Guo, 2020).

3.1.1. Types of the adsorption process

3.1.1.1. Physical adsorption

By weak van der Waals contact, the adsorbate accumulates and forms a multilayer on the adsorbent surface. Physical adsorption has a low enthalpy. Physical adsorption is a method that is advantageous at low temperatures. Furthermore, depending on the interaction between adsorbate and adsorbent, the process is reversible. As a result, when the adsorption situation changes, such as temperature or pressure, the adsorption capacity drops and desorption occurs. Adsorbents can be reused thanks to desorption.

3.1.1.2. Chemical adsorption

Chemical adsorption is more specific than physical adsorption since it is based on chemical bonding between the adsorbate and the adsorbent. On the adsorbent surface, the adsorbate forms a monolayer. Chemical adsorption has a high enthalpy. As the temperature rises, so does the adsorption. Furthermore, because it involves the production of a compound, the process is typically irreversible (Cuong, 2020).

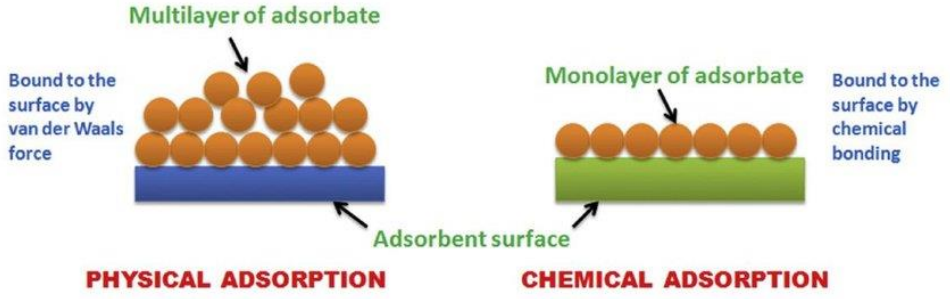


Figure 7. The difference between physical and chemical adsorption

Source : Cuong, D. (2020). Eco-friendly biochar-based materials in removal of contaminants from aqueous solutions: Fabrication, characterization and applications.

3.1.2. Adsorption equilibrium

The adsorption equilibrium is caused by the relationship between adsorbate concentration in the liquid phase and the solid phase. As the adsorption

process continues, the desorption process will occur in parallel. The adsorption equilibrium happens when the adsorption and desorption processes are equal. The adsorption process, the distribution of adsorbate in 2 different phases, and the amount of adsorbate on the adsorbent surface can be described by the adsorption isotherm.

Adsorption isotherms are classified into 6 types by IUPAC (Alothman, 2012) as shown in Figure 8. The different adsorption isotherms depend on the type of adsorbent, type of adsorbate, and adsorbent-adsorbate interaction (Kumar et al., 2019).

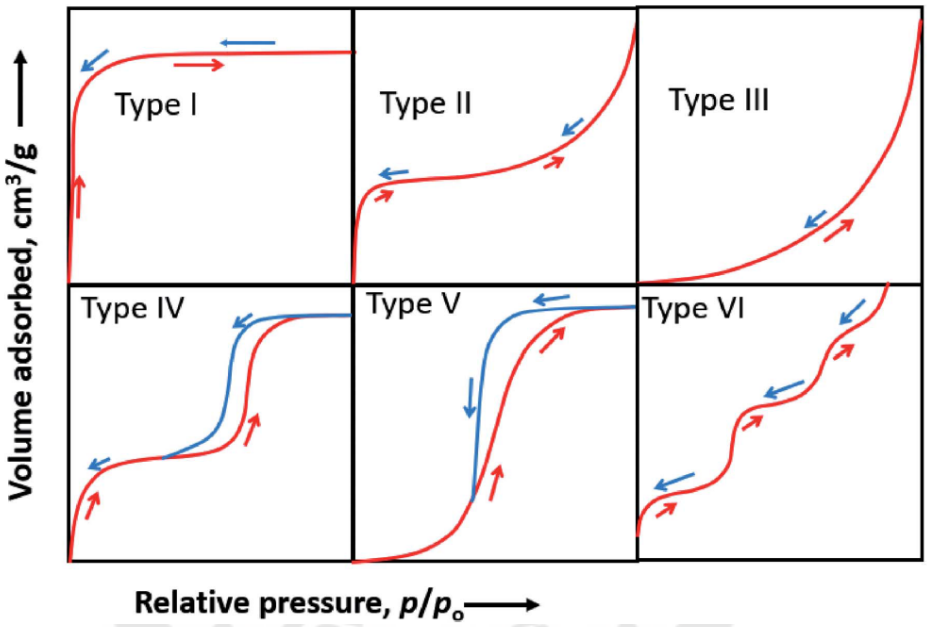


Figure 8. The different types of adsorption isotherms

Source : Kumar, K. V., Gadipelli, S., Wood, B., Ramisetty, K. A., Stewart, A. A., Howard, C. A., Brett, D J. L., Rodriguez-Reinoso, F. (2019). Characterization of the adsorption site energies and heterogeneous surfaces of porous materials. Journal of Materials Chemistry A, 7(17), 10104-10137.

The different types of adsorption isotherms can be explained as follows.

- Type I isotherm is an adsorption isotherm for the microporous adsorbent. The adsorption process mainly occurs in small porous, and the rearrangement of molecules in porous is a monolayer. Sometimes this type of isotherm is called Langmuir or L-shape isotherm

- Type II isotherm is an adsorption isotherm with nonporous or mesoporous adsorbent. In the adsorption process, the monolayer is completely formed before the multilayer. They are also called this isotherm as Sigmoid or S-shape isotherm.

- Type III isotherm is an adsorption isotherm with predominantly adsorbate-adsorbate interaction and less adsorbate-adsorbent interaction.

- Type IV isotherm is an adsorption isotherm for the adsorbent that has a porous bigger than the diameter of the adsorbate. The early adsorption process of this isotherm is similar to type II isotherm. Then the level of isotherm changes due to condensation in pores.

- Type V isotherm is an adsorption isotherm with predominantly adsorbate-adsorbate interaction with barely adsorbate-adsorbent interaction, which is similar to type III. Moreover, the adsorbent has pores in the same range as in type IV.

- Type VI isotherm has multiple layers of adsorption on the adsorbent surface, and each layer of adsorption is available to layer by layer.

3.1.3. Adsorption isotherm

3.1.3.1 Langmuir isotherm

Langmuir isotherm is used to explain the equilibrium between adsorbent and adsorbate. All of the adsorption sites on the adsorbent surface are identical. The adsorption is a monolayer, and the adsorbed molecules have no interaction with other molecules on the adsorbent surface (Kecili & Hussain, 2018). The following equation can demonstrate the Langmuir isotherm model:

$$q_e = q_{max}K_LC_e / 1+KC_e \quad \text{--------(3)}$$

It can also be arranged into a linear equation as follows:

$$C_e/q_e = 1/K_Lq_{max} + C_e/q_{max} \quad \text{--------(4)}$$

Where; C_e = the concentration of adsorbate at equilibrium (mg/L)

K_L = the Langmuir constant (L/mg)

q_e = the amount of adsorbate on the adsorbent surface (mg/g)

q_{max} = the maximum adsorption capacity (mg/g)

3.1.3.2 Freundlich isotherm

Freundlich isotherm model is another empirical equation used for the adsorption occurring on heterogeneous surfaces (Sun et al., 2021), and there are multilayers of adsorption (Kecili & Hussain, 2018). The following equation can demonstrate the Freundlich isotherm model:

$$\text{Log } q_e = 1/n \log C_e + \log K_F \quad \text{--------(5)}$$

Where; C_e = the concentration of adsorbate at equilibrium (mg/L)

K_F = the Freundlich constant (L/mg)

n = the constant related to the heterogeneity of adsorbent surface.

q_e = the amount of adsorbate on the adsorbent surface (mg/g)

4. Literature reviews

Application of the adsorbent used for analysis tetracycline

In 2010, Kang Liu et al. (Kang, Liu, Zheng, Qu, & Chen, 2010) was investigated the adsorption of tetracycline and copper onto chitosan. The adsorption of tetracycline and copper occurred rapidly in a few hours before 90% completely adsorbed at 11-12 and 6 hours, respectively. The adsorption equilibrium of tetracycline and copper occurred 24 hours. The adsorption of tetracycline was increased with increasing pH and copper presence. In the other hand, the adsorption of copper was decreased with the presence of tetracycline. The adsorption isotherm of both were fitted by the Langmuir isotherm. The maximum adsorption capacity of tetracycline was 53.82 to 93.04 mmol/kg as the copper concentration range from 0 to 0.5 mmol/L. The maximum adsorption capacity of copper was 1856.06 to 1486.20 mmol/kg as the absence and presence of tetracycline.

In 2013, Lin et al. (Lin, Xu, & Jia, 2013) synthesized graphene oxide functionalized magnetic particles (GO-MPs) as an adsorbent for the removal of four tetracyclines from aqueous solution, including tetracycline, oxytetracycline, chlortetracycline, and doxycycline. The pH solution and ionic strength had essentially no influence on tetracycline adsorption in the factor experiments. Tetracycline has a

maximum adsorption capacity of 39.1 mg/g according to the Langmuir isotherm. The GO-MPs have the capacity to remove a low level of tetracyclines from huge volumes of water samples from the environment.

In 2016, Guo et al. (Guo et al., 2016) used organic acid-functionalized magnetic nanoparticles to create a novel adsorbent for tetracycline absorption. Magnetic nanoparticles were created by co-precipitating $FeCl_3$ and $FeCl_2$ using nitrogen gas to eliminate the oxygen gas in solution and ammonia as a precipitant. Organic acids such as oleic acid, undecenoic acid, caprylic acid, and hexanoic acid were employed to modify the surfaces of magnetic nanoparticles. The capacity of four different types of modified magnetic nanoparticles to remove tetracycline from aqueous solution was tested. The magnetic nanoparticles treated with undecenoic acid had the maximum adsorption efficiency. The adsorption capacity was 222 mg/g at 318 K according to the Langmuir isotherm, and it was linearly related to temperature.

In 2017, Liu et al. (M.-k. Liu et al., 2017) developed a novel adsorbent for enhancing the adsorption efficiency of tetracycline hydrochloride. In this investigation, carbon nanomaterials such as graphene oxide (GO), activated carbon (AC), and carbon nanotubes (CNT) were employed. Before detaching from the fluoride substrate, the hybrid carbon membranes were combined in a mass ratio of 2:1 and vacuum filtered over a porous polyvinylidene fluoride sheet. The GO/AC had the highest adsorption efficiency, with a capacity of 449 mg/g.

In 2017, Ma, Sun, & Yu. (Ma, Sun, & Yu, 2017) developed the new adsorbent for adsorption of tetracycline using activated graphene. The activated graphenes were synthesized by the KOH-activated method. The KOH-activated graphene exhibited excellent tetracycline adsorption capacity (532.59 mg/g) compared with bare graphene (272.70 mg/g). However, metals such as Cu^{2+} and CrO_4^{2-} in aqueous solution could significantly interfere the adsorption of tetracycline.

In 2017, Hu et al. (Hu et al., 2017) developed a new adsorbent to remove tetracycline from wastewater using the assembly of Fe_3O_4 magnetic nanoparticles on the surface of graphene oxide. Graphene oxide was synthesized using the reaction of

preoxidized graphite in sulfuric acid with $NaNO_3$ and $KMnO_4$. Magnetic nanoparticles used as a modifier were synthesized by the co-precipitation between $FeCl_3$ and $FeSO_4$ using NaOH as a precipitant. The modified graphene oxide exhibited excellent adsorption efficiency and good reusability. From the Langmuir isotherm, the adsorption capacity was 1272.45 mg/g at 318 K. The modified graphene oxide adsorption capacity was proportional to temperature but inversely proportional to pH value.

In 2019, Huízar-Félix et al. (Huízar-Félix et al., 2019) prepared nanocomposites of reduced graphene oxide (RGO) with ferromagnetic α-Fe_2O_3 nanoparticles for tetracycline adsorption by a thermal treatment. The modified nanocomposite exhibited ferromagnetic behavior at room temperature under a magnetic field that enabled to separate tetracycline from the aqueous phase. Tetracycline adsorption was well described by the pseudo-second-order kinetic and Langmuir isotherm equations. The tetracycline adsorption of the modified nanocomposite was substantially pH dependent. The α-Fe_2O_3 nanoparticles hindered the adsorption sites of RGO surface, reduced the adsorption of tetracycline and affected its adsorption capacity. The maximum adsorption capacity of RGO was 44.23, 39.94, and 15.82 mg/g at pH 7, 10, and 4, respectively. The maximum adsorption capacity of modified nanocomposite was decreased to 9.69, 10.25, and 18.47 mg/g at pH 7, 10, and 4, respectively.

In 2019, Jannat Abadi et al. (Jannat Abadi, Nouri, Zhiani, Heydarzadeh, & Motavalizadehkakhky, 2019) modified a zeolite that is used as an adsorbent for tetracycline adsorption. Fe(III) from $FeCl_3$ was used to modify the produced zeolite. The modified zeolite was utilized in a batch setup to study tetracycline adsorption. The results revealed that tetracycline adsorption on modified zeolite was substantially pH dependent, with pH 6 providing the greatest adsorption capacity. The maximal capacity for adsorption was 200 mg/g.

In 2020, Zhao et al. (Zhao et al., 2020) used activated carbon as an adsorbent for tetracycline adsorption. Waterwork sludge and *Phragmites australis* were used to modify activated carbon. The BET surface area of the improved activated

carbon was developed to 949.90 m^2/g. Tetracycline adsorption was well described by the pseudo-second-order kinetic and Freundlich isotherm equations. The highest adsorption capacity measured was 153.4 mg/g.

In 2020, Vu et al. (Vu et al., 2020) used modified laterite to study tetracycline adsorption. Polystyrene sulfonate was used to modify laterite (PSS). PSS-modified laterite was employed in a batch system and removed tetracycline with a high efficiency of 88 percent in pH 4, 180 minutes. Tetracycline adsorption was well described by the pseudo-second-order kinetic and Langmuir isotherm equations. Tetracycline removal effectiveness in wastewater was around 94%, increasing to 66% after five reuse cycles.

In 2021, Hamoudi et al. (Hamoudi, Hamdi, & Brendlé, 2021) developed a novel geomaterial-based adsorbent. Maghnia clay, activated carbon, cement, and PVA polymer were among the geomaterials used. Tetracycline adsorption was well described by the pseudo-second-order kinetic and Langmuir isotherm equations. Furthermore, the kinetics of tetracycline adsorption on the adsorbent were rapid, with equilibrium attained in 30 minutes. The adsorbent's adsorption capability was affected by the pH value. The adsorption capacity was 12.58 mg/g at pH 2 and declined to 10 mg/g at pH 8.

CHAPTER 3
RESEARCH METHODOLOGY

In this research, the methodology is described by the following topics.

1. Instrumentation
2. Materials and chemicals
3. Synthesis and characterization of Fe_3O_4 magnetic nanoparticles
4. Study optimum conditions for adsorption and desorption of tetracycline using Fe_3O_4 magnetic nanoparticles
5. The study of analytical performances of adsorbent material
6. Application of the developed method for detection of tetracycline in real sample

1. Instrumentation

- UV-VIS spectrophotometer (model V-750, JASCO, Japan)
- Fourier-transform infrared spectrometer (FTIR) (model Tensor27, Bruker, Germany)
- Analytical microbalance (model PA224C, OHAUS, China)
- Hotplate and stirrer (model HTS-1003, LMS, Japan)
- Hot air oven (model SM 400, Memmert, Germany)
- pH meter (model 827 pH Lab, Metrohm Autolab, Switzerland)
- Ultrasonic bath (model GT SONIC-D13, GT SONIC, China)
- Vortex mixer Genie 2 (model G560E, Scientific Industries, USA)
- Water purification system (model Direct-Q® 3 UV, MERCK MILLIPORE, USA)

2. Materials and chemicals

- Tetracycline Hydrochloride ($C_{22}H_{24}N_2O_8$.HCl) from Fisher Scientific (USA)
- Ammonia (NH_3, 30%) from PanReac (Spain)
- Di-Sodium hydrogen orthophosphate dihydrate ($Na_2HPO_4.2H_2O$) from Ajax Finechem (Australia)

- Ethanol absolute anhydrous (C_2H_5OH) from Carlo Erba (Italy)
- Ferric chloride hexahydrate ($FeCl_3.6H_2O$) from Qrec (New Zealand)
- Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) from AnalaR (U.K.)
- Glacial acetic acid (CH_3COOH) from RCI Labscan (Thailand)
- Hydrochloric acid (HCl, 36%) from Ajax Finechem (Australia)
- Sodium acetate trihydrate ($CH_3COONa.3H_2O$) from Ajax Finechem (Australia)
- Sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$) from Ajax Finechem (Australia)
- Sodium hydroxide anhydrous (NaOH) from Ajax Finechem (Australia)
- External magnet
- Mortar and pestle

3. Experimental method

3.1. Synthesis and characterization of Fe_3O_4 magnetic nanoparticles

Magnetic nanoparticles were synthesized with the modification from the previous report (Limchoowong et al., 2017). First, ferric chloride hexahydrate ($FeCl_3.6H_2O$) was mixed with ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) in a mole ratio of 2:1 in 50 mL of deionized water, and then the solution was stirred for 5 minutes. Next, the solution was precipitated using 15 mL of 30% (w/v) ammonia solution. The color of the solution turned to black, which is the color of formed Fe_3O_4 magnetic nanoparticles. The magnetic nanoparticles were collected and washed several times with deionized water until a neutral pH. After that, magnetic nanoparticles were washed with ethanol and dried in a hot air oven at 60 °C for 3 hours. The nanoparticles were then kept in a desiccator before use.

The functional groups of synthesized Fe_3O_4 magnetic nanoparticles were characterized by Fourier-transform infrared spectrometer (FTIR). The FTIR spectrum was recorded between 500 and 4000 cm^{-1}. The pH at point zero charges of Fe_3O_4 magnetic nanoparticles was studied using the pH drift method (Li, Han, Xie, Wang, & Li, 2018). The pH of 0.05 M sodium chloride was adjusted to pH 1-14 by adding either 0.1 M hydrochloric acid or 0.1 M sodium hydroxide solutions. Magnetic nanoparticles were

then added to the solution, and the mixture was sonicated for 30 min before measuring the final pH. A graph between the initial pH and final pH was plotted to determine the point zero charge at the intersection of the curves.

3.2. Study optimum conditions for adsorption and desorption of tetracycline using Fe_3O_4 magnetic nanoparticles

3.2.1. The effect of adsorption parameters

3.2.1.1. Effect of pH of the solution

The standard tetracycline solution was prepared in 0.1 M buffer solution in pH ranging from 3 – 10. Magnetic nanoparticles (5 mg) and tetracycline solution (5 mL, 25 mg/L) were mixed in a vial and stirred vigorously using vortex for 1 minute. Magnetic particles were separated to the vial side by an external magnet. The residual tetracycline concentration in the supernatant was measured using a UV-Vis spectrophotometer at a wavelength of 356 nm. The amount of tetracycline adsorbed was deducted to calculate the adsorption capacity of magnetic nanoparticles (q_e, mg/g). according to the following:

$$q_e = (C_o - C_e) \times V/ m$$

Where; C_o = the initial concentration of tetracycline (mg/L)

C_e = the equilibrium concentration of tetracycline after adsorption (mg/L)

V = the volume of solution (L)

m = amount of magnetic nanoparticles adsorbents (g)

3.2.1.2. Effect of amount of magnetic nanoparticles

The amount of magnetic nanoparticles in the range of 1-10 mg was studied. After adding magnetic nanoparticles and tetracycline in 0.1 M acetate buffer (pH 5.0) solution (5 mL, 25 mg/L), the solution was stirred vigorously for 1 minute. The adsorbent was separated by an external magnetic field. The concentration of tetracycline was measured using a UV-Vis spectrophotometer at a wavelength 356 nm. The adsorption amount of tetracycline was expressed as the adsorption capacity of Fe_3O_4 magnetic nanoparticles and %adsorption.

3.2.1.3. Effect of contact time

The contact time in the range of 1-10 minutes was studied. Magnetic nanoparticles (1 mg) and tetracycline in 0.1 M acetate buffer (pH 5.0) solution (5 mL, 25 mg/L) were added to the vial and stirred vigorously. The adsorbent was separated by an external magnetic field. The tetracycline residue in solution was measured using a UV-Vis spectrophotometer at wavelength 356 nm. The adsorption amount of tetracycline was expressed as the adsorption capacity.

3.2.2. The effect of desorption parameters

3.2.2.1. Effect of desorption solution

The various desorption solution, namely sodium hydroxide, potassium hydroxide, and sodium carbonate, were studied at constant concentration (0.1M). Magnetic nanoparticles (1 mg) and tetracycline in 0.1 M acetate buffer (pH 5.0) solution (5 mL, 25 mg/L) were added to the vial and stirred vigorously for 5 minutes. The adsorbents were separated to the vial side by an external magnet. The solution residue was poured out of the vial. After that, the adsorbent loaded with tetracycline was treated with the desorption solution (3 mL, 0.1 M) and stirred vigorously for 5 minutes. The adsorbent was separated by an external magnetic field. The tetracycline concentration in desorption solution was measured using a UV-Vis spectrophotometer at a wavelength 356 nm. The desorption amount of tetracycline was expressed as %desorption.

3.2.2.2. Effect of the concentration of desorption solution

The concentration of sodium hydroxide in the range of 0.01-0.5 M was studied for desorption efficiency. After the adsorption of 25 mg/L of tetracycline in 0.1 M acetate buffer (5 mL, pH 5.0) with 1 mg of magnetic nanoparticles for 5 minutes, the adsorbent was collected to the vial side by an external magnetic field. The adsorbent was then resuspended in 3 mL of solution and stirred vigorously for 5 minutes. The desorbed tetracycline concentration in solution was measured using a UV-Vis spectrophotometer at wavelength 356 nm. The desorption amount of tetracycline was expressed as %desorption.

3.2.2.3. Effect of volume of desorption solution

The volume of 0.25 M sodium hydroxide in the range of 2-5 mL was studied for evaluation of desorption efficiency and enrichment factor. The experiment was carried out by mixing magnetic nanoparticles (1 mg) with 25 mg/L of tetracycline in 0.1 M acetate buffer solution (5 mL, pH 5.0), followed by stirring vigorously for 5 minutes. The adsorbent was magnetically collected from the solution and resuspended in 0.25 M sodium hydroxide. The solution was stirred for 5 minutes. The adsorbent was separated by an external magnetic field. The solution was measured using a UV-Vis spectrophotometer at a wavelength 356 nm for measurement of tetracycline concentration. The desorption amount of tetracycline was expressed as %desorption and enrichment factor. The enrichment factor (EF) was calculated using following the equation:

$$EF = C_{ext} / C_{ini}$$

Where; C_{ext} = the final concentration of tetracycline after desorption (mg/L)

C_{ini} = the initial concentration of tetracycline (mg/L)

3.2.2.4. Effect of desorption time

The effect of desorption time in the range of 1-5 minutes was studied. Magnetic nanoparticles (1 mg) and 25 mg/L of tetracycline in 0.1 M acetate buffer solution (5 mL, pH 5.0) were added to the vial and stirred vigorously for 5 minutes. The adsorbent was isolated from solution by an external magnetic field. After that, the adsorbent was suspended in 0.25 M sodium hydroxide (2 mL) and stirred vigorously in various times. The adsorbent was then separated by magnet. The desorption solution was measured using a UV-Vis spectrophotometer at a wavelength 356 nm for determination of desorbed tetracycline. The desorption amount of tetracycline was expressed as %desorption.

3.3. The study of analytical performances of adsorbent material

3.3.1. Adsorption isotherm

The adsorption isotherm experiments were performed with various initial tetracycline concentration ranging from 1 to 75 mg/L for investigation of mechanism of adsorption process. Tetracycline solutions (5 mL, pH 5.0) at different concentration were mixed with 1 mg of Fe_3O_4 magnetic nanoparticles and stirred vigorously for 5 minutes. The adsorbent was magnetically separated from the solution. The equilibrium concentration of tetracycline solutions was measured using a UV-Vis spectrophotometer. The adsorption capacity (q_e) was calculated and plotted with the equilibrium tetracycline concentration (C_e) using the Langmuir and Freundlich isotherm model equations as follows

$$C_e/q_e = 1/K_L q_{max} + C_e/q_{max}$$

$$\text{Log } q_e = 1/n \log C_e + \log K_F$$

Where q_{max} is maximum adsorption capacity (mg/g), K_L is the Langmuir constant (L/mg), K_F is the Freundlich constant (L/mg) and n is constant related to the heterogeneity of adsorbent surface.

The adsorption process was investigated using the Langmuir and Freundlich isotherm models fitting. All experiments were carried out in batch mode with triplicate measurement.

3.3.2. Reusability of Fe_3O_4 magnetic nanoparticles

The reusability of Fe_3O_4 magnetic nanoparticles was studied in batch experiments and expressed in terms of %efficiency and adsorption capacity (mg/g). The adsorption-desorption cycle was repeated ten consecutive cycles with the same adsorbent. The optimal and desorption condition and desorption conditions were used in each cycle. After that, the adsorbent was magnetically isolated from the solution and washed several times with deionized water before using in the next cycle.

3.4. Application of the developed method for detection of tetracycline in real sample

The developed method was applied to detect tetracycline in pharmaceutical sample. Tetracycline capsules 250 mg were purchased from local drugstore. Five capsules were weighed, and well mixed. The average amount of one capsule was accurately weighed and dissolved in deionized water. The sample solution was then diluted to an appropriate concentration using 0.1M acetate buffer (pH 5.0) before analysis using the developed method. Sample was analyzed in triplicate and compared tetracycline content obtained from this method with label value.

CHAPTER 4
RESULTS

The results and discussion of this research are described under the following topics:

1. Synthesis and characterization of Fe_3O_4 magnetic nanoparticles

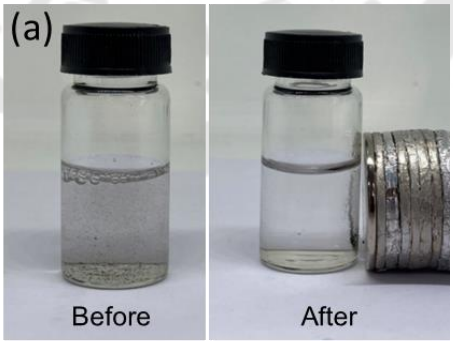
2. Study optimum conditions for adsorption and desorption of tetracycline using Fe_3O_4 magnetic nanoparticles

3. The study of analytical performances of adsorbent material

4. Application of the developed method for detection of tetracycline in real sample

1. Synthesis and characterization of Fe_3O_4 magnetic nanoparticles

The black Fe_3O_4 magnetic nanoparticles were successfully synthesized with magnetic property as shown in Figure 9a. The functional groups of the Fe_3O_4 magnetic nanoparticles were confirmed using FTIR spectroscopy as shown in Figure 9b, the FTIR spectrum shows strong characteristic peak at 580 cm^{-1}. The adsorption bands at 570-585 cm^{-1} corresponded to the Fe-O stretching vibration in Fe_3O_4 (Sun et al., 2021).



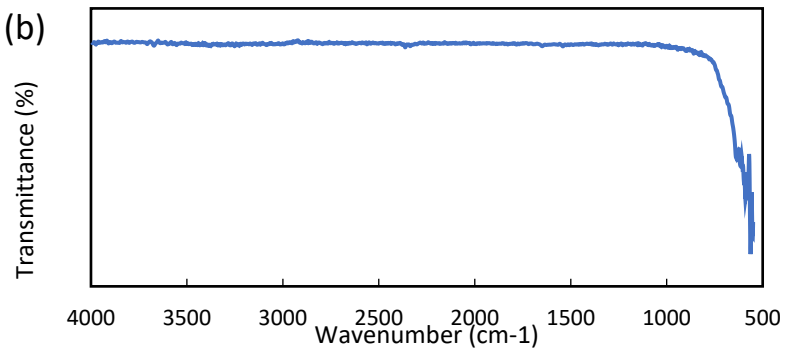


Figure 9. (a) photograph of the Fe_3O_4 magnetic nanoparticles before and after magnetic separation (b) FTIR spectrum of Fe_3O_4 magnetic nanoparticles

The pH at the point of zero charges (Pzc) is one of important parameter for characterization of Fe_3O_4 magnetic nanoparticles. The point of zero charges or isoelectric point is the pH value at which the total charge of Fe_3O_4 magnetic nanoparticles surface is equal to zero. The results showed that the pH at point zero charge of material was 5.41, as shown in Figure 10. Therefore, the anion species can be adsorbed on the surface of material at pH lower than 5.41 because the surface of the magnetic nanoparticles is positively charged. On the other hand, at pH higher than 5.41 the cation species can be adsorbed onto the negatively charged surface of Fe_3O_4 magnetic nanoparticles (Zhang et al., 2020).

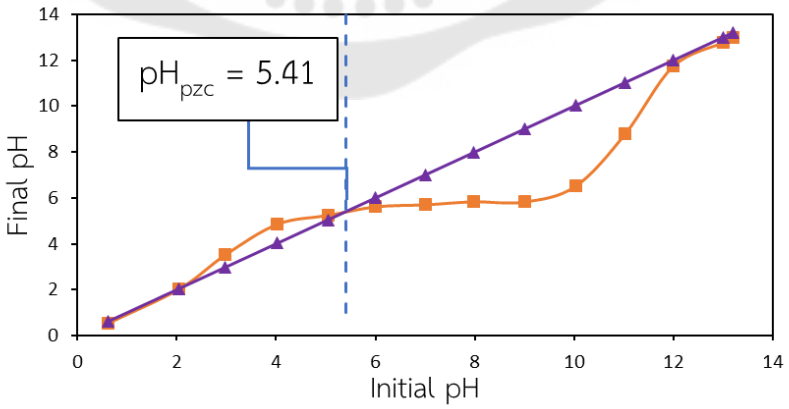


Figure 10. The point zero charges of Fe_3O_4 magnetic nanoparticles

2. Study optimum conditions for adsorption and desorption of tetracycline using Fe_3O_4 magnetic nanoparticles

2.1. The effect of adsorption parameters

2.1.1. Effect of pH of the solution

The adsorption capacity (q_e) of Fe_3O_4 magnetic nanoparticles was investigated for the tetracycline adsorption at pH ranging from 3-10. It can be seen that the adsorption capacity for tetracycline was dependent on pH. The phenomenon occurs because pH-dependent charges of both tetracycline molecules and surface of magnetic adsorbents. Tetracycline exists as cation species at pH < 3.3, zwitterion species at pH 3.3-7.7, or anion species at > 7.7. As shown in Figure 11, the adsorption amount of tetracycline on Fe_3O_4 magnetic nanoparticles gradually increased with increasing pH from 3 to 5. The highest adsorption capacity appeared at pH 5. At this pH range, the mainly species of tetracycline was zwitterion which can be adsorbed onto the surface of Fe_3O_4 magnetic adsorbents. On the other hand, there is almost no tetracycline adsorption on magnetic materials for pH>5. While at higher pH (pH > Pzc of Fe_3O_4), the electrostatic repulsion between the anionic tetracycline and negative charge on the surface of Fe_3O_4 adsorbent, resulting in a decrease of tetracycline adsorption. Therefore, the optimum pH for adsorption of tetracycline was 5.0.

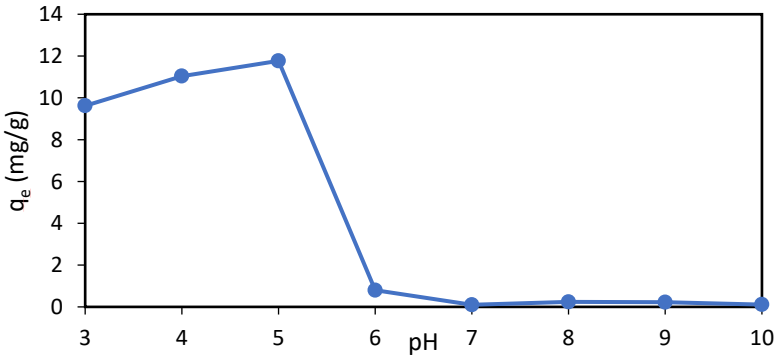


Figure 11. The adsorption capacity of Fe_3O_4 magnetic nanoparticles for tetracycline at different pH values

2.1.2. Effect of amount of magnetic nanoparticles

The amount of magnetic nanoparticles is one of significant factors that impact on adsorption efficiency. The effect of magnetic adsorbent amount on the adsorption of tetracycline was studied in range from 1 to 10 mg. Figure 12 demonstrated that the higher amount of magnetic nanoparticles increased %adsorption but the adsorption capacity decreased, possibly due to the ineffective dispersion of magnetic nanoparticles. The highest adsorption capacity of magnetic nanoparticles for tetracycline was obtained at 1 mg. The adsorption capacity of tetracycline was 14.10 mg/g. Consequently, 1 mg of synthesized magnetic nanoparticles was chosen as the optimum amount for further tetracycline adsorption studies.

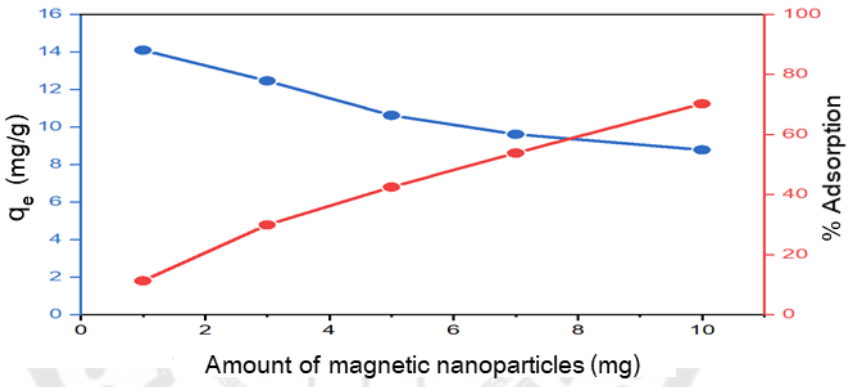


Figure 12. Effect of adsorbent amount on the adsorption capacity of Fe_3O_4 magnetic nanoparticles for tetracycline

2.1.3. Effect of contact time

Contact time is one of effective factors on adsorption efficiency in batch adsorption process. In this work, the magnetic nanoparticles and tetracycline solution was stirred vigorously by varying time from 1-10 min. The effect of contact time on the adsorption of tetracycline showed in Figure 13. As illustrated in Figure 13, the adsorption capacity increased with increasing contact time from 1 to 5 minutes. There was no significant change in the adsorption capacity after 5 minutes. The highest adsorption capacity of tetracycline was 20.48 mg/g at 5 minutes. Therefore, the contact time at 5 minutes was chosen for adsorption of magnetic nanoparticles with tetracycline.

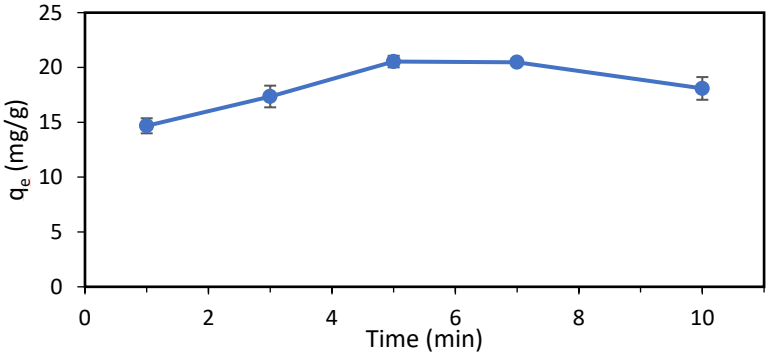


Figure 13. Effect of contact time on the adsorption capacity of Fe_3O_4 magnetic nanoparticles for tetracycline

To investigate the interaction of tetracycline and Fe_3O_4 magnetic nanoparticles, the infrared experiments of magnetic materials before and after tetracycline adsorption were performed. The experiments were done under the optimum adsorption condition by mixing Fe_3O_4 magnetic nanoparticles (1 mg) with tetracycline solution at pH 5.0 for 5.0 minutes before IR measurement. As shown in Figure 14, the characteristic peaks of Fe_3O_4 magnetic nanoparticles and tetracycline were observed at 580 cm^{-1} and 1200-1700 cm^{-1}, respectively. The IR spectrum of tetracycline shows the peaks at 1675 cm^{-1} for C=O stretching, at 1600-1650 cm^{-1} for C=C stretching of an aromatic ring, at 1310-1460 cm^{-1} for O-H bending, and at 1200-1250 cm^{-1} for C-C stretching and bending, N-H bending, and C-N stretching (Hashemikia, Hemmatinejad, Ahmadi, & Montazer, 2015). The results demonstrated that adsorbent spectrum after tetracycline adsorption was different from pure adsorbent spectrum. After tetracycline adsorption, the characteristic peaks at 1329, 1408, and 1597 cm^{-1} of tetracycline had appeared in FTIR spectrum of adsorbent. It can be confirmed the adsorption of tetracycline on Fe_3O_4 magnetic nanoparticle surface.

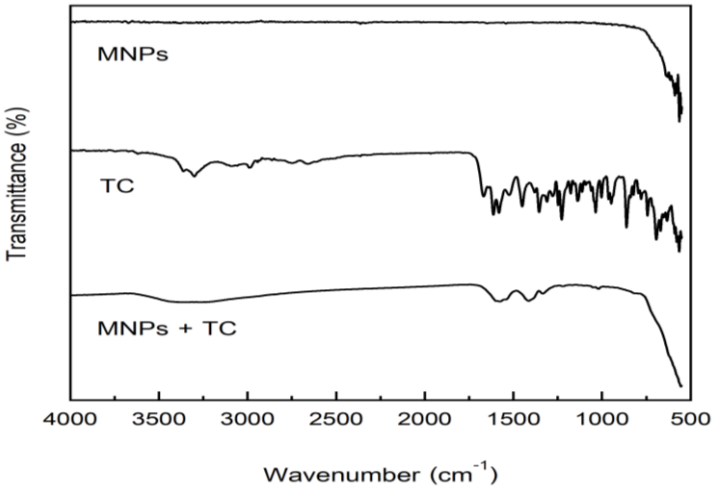


Figure 14. Infrared spectra of Fe_3O_4 magnetic nanoparticles before and after tetracycline adsorption

2.2. The effect of desorption parameters

2.2.1. Effect of desorption solution

From the adsorption experiments, it can be seen that the adsorption of tetracycline was enhanced in acidic condition. On the other hand, tetracycline adsorption was hindered in neutral and alkaline conditions. In this work, the desorption of tetracycline was therefore studied under alkaline conditions. The effect of various base solutions, including sodium hydroxide, potassium hydroxide, and sodium carbonate was studied for desorption of tetracycline from the surface of the magnetic nanoparticles. The tetracycline from Fe_3O_4 adsorbent was eluted with 3.0 mL of desorption solution for 5.0 min at room temperature. The desorption amount of tetracycline was calculated and expressed as %desorption, as shown in Figure 15. The results show that desorbed tetracycline was significantly achieved under alkaline conditions due to the electrostatic repulsion between negatively charged of both tetracycline molecule and surface of the magnetic nanoparticles. It can be observed that sodium hydroxide exhibited the highest desorption efficiency with good precision results. Consequently, sodium hydroxide was chosen as a desorption solution in this work.

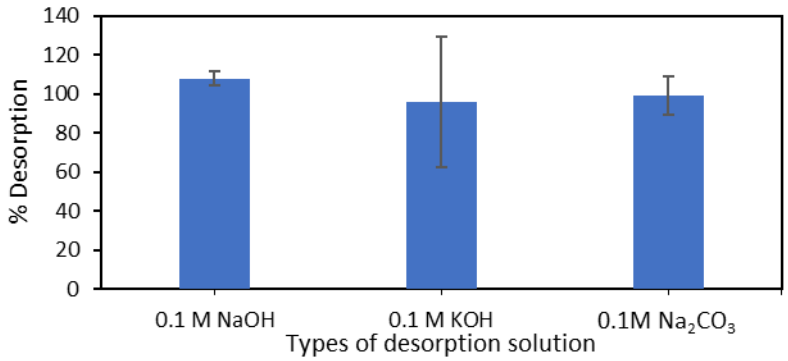


Figure 15. The desorption of tetracycline in different types of desorption solution

2.2.2. Effect of concentration of desorption solution

The concentration of desorption solution in the range of 0.01 - 0.5 M was studied for desorption of tetracycline. Desorption experiment was carried out using 3.0 mL of sodium hydroxide solution for 5.0 min at room temperature. The desorption amount of tetracycline was calculated and expressed as %desorption as depicted in Figure 16. The results showed that the recovery of desorbed tetracycline increased significantly with an increase in the concentration of sodium hydroxide from 0.01 to 0.1 M. The recovery results were approximately 100% at 0.1 M and no significant difference values with further increasing of sodium hydroxide concentrations. In this work, 0.25 M sodium hydroxide was chosen as the optimized desorption solution with good repeatability and cost-effectiveness.

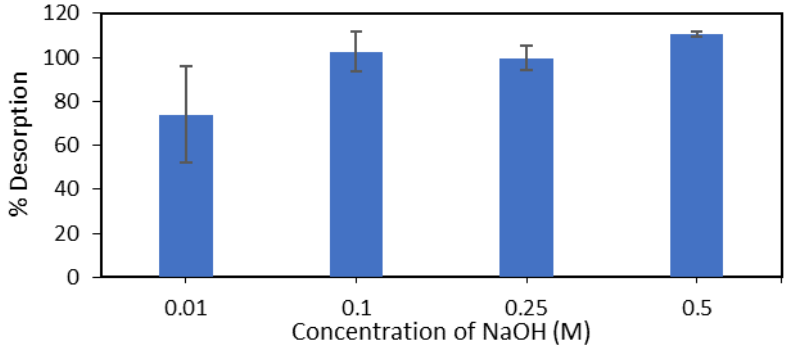


Figure 16. The desorption of tetracycline in different concentrations of sodium hydroxide solution

2.2.3. Effect of volume of desorption solution and the enrichment factor

The effect of the volume of 0.25 M sodium hydroxide was studied for the desorption of tetracycline from the magnetic nanoparticles. The volume of desorption solution in the range of 2.0 - 5.0 mL were examined using 0.25 M sodium hydroxide as desorption solution for 5.0 min at room temperature. Figure 17 shows the desorption amount of tetracycline expressed as %desorption and enrichment factor. It can be seen that the increasing of desorption volume had no obvious effect on the desorption results. However, the enrichment factor was significantly decreased with increasing the desorption volume. Thus, 2 mL of 0.25 M sodium hydroxide was chosen in this work.

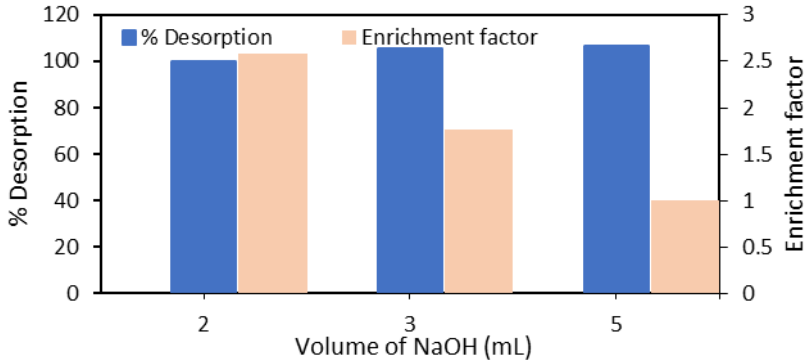


Figure 17. The desorption of tetracycline and the enrichment factor using different volumes of sodium hydroxide as desorption solvent

2.2.4. Effect of desorption time

The effect of desorption time in the range of 1-5 minutes was studied for desorption of tetracycline. Figure 18 illustrates the effect of desorption time on the desorption of tetracycline from the surface of magnetic adsorbent using 2.0 mL of 0.25 M NaOH as desorption solution. As shown in the results, tetracycline was completely desorbed within 1 minute. It indicates that the desorption process easily occurs, and the desorption equilibrium can be obtained rapidly. Therefore, the desorption time of 1.0 min was selected as the optimal desorption condition.

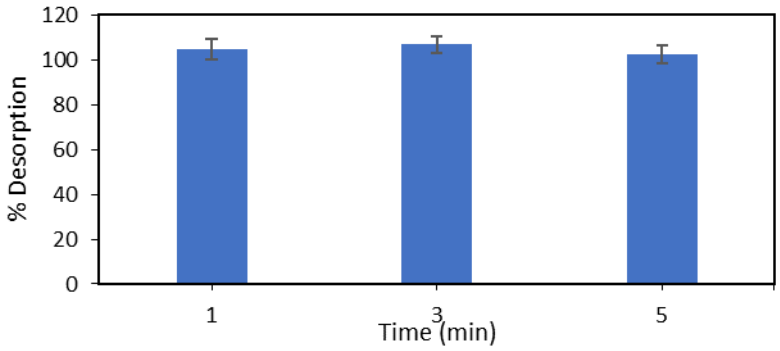
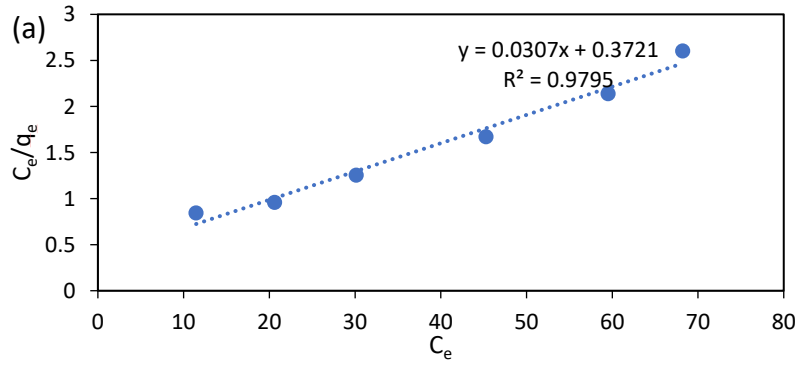


Figure 18. The desorption of tetracycline in sodium hydroxide in different desorption time

3. The study of analytical performances of adsorbent material

3.1. Adsorption isotherm

The adsorption isotherm of Fe_3O_4 magnetic nanoparticles for tetracycline adsorption at room temperature was investigated at different tetracycline concentrations. The Langmuir and Freundlich isotherm models were used to elaborate the adsorption isotherm results. Figure 19 shows the adsorption isotherm fitted according to the linear Langmuir and Freundlich model equations. The related isotherm parameters of each isotherm model are listed in Table 3. As can be seen in Table 3, correlation coefficient (R^2) value for the Langmuir isotherm model is higher than that for the Freundlich model. The result indicates that the isotherm experimental data were better fitted by the Langmuir adsorption model. Thus, the Fe_3O_4 magnetic adsorption of tetracycline on the adsorbent surface was monolayer adsorption.



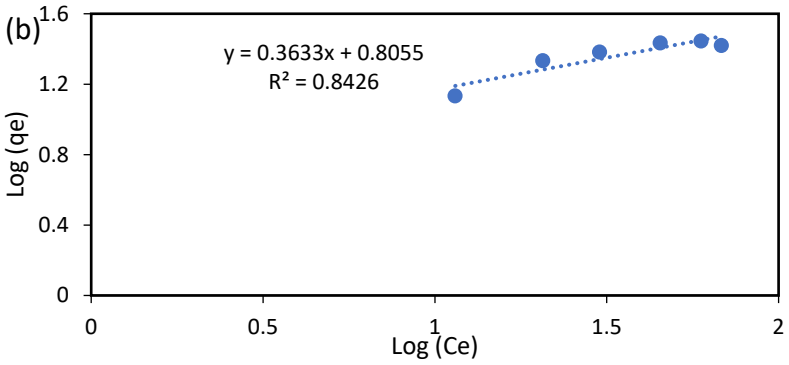


Figure 19. Adsorption isotherm of (a) Langmuir model and (b) Freundlich model for tetracycline adsorption on Fe_3O_4 magnetic nanoparticles

Table 3. Adsorption isotherm parameters and correlation coefficients of tetracycline adsorption on magnetic adsorbent at room temperature

Model	Langmuir isotherm			Freundlich isotherm		
Parameters	q_{max} (mg/g)	K_L (L/mg)	R^2	K_F (L/mg)	n	R^2
	32.57	0.0825	0.9785	2.3223	2.7525	0.8426

It can also be seen in Table 3, the calculated maximum adsorption capacity of the adsorbent from Langmuir isotherm was 32.57 mg/g. In addition, Table 4 shows the comparison of the maximum adsorption capacity of Fe_3O_4 magnetic nanoparticles for tetracycline adsorption in this work with previously reported adsorbents. The results showed that Fe_3O_4 magnetic nanoparticles had an adsorption capacity higher than some of adsorbents due to a result of nanostructure and surface area properties. Moreover, the adsorbent in this work also took less adsorption time than the others. It can therefore be said that Fe_3O_4 magnetic nanoparticles is a good adsorbent with high potential for adsorption of tetracycline.

Table 4. The comparison of adsorption capacity and adsorption time for tetracycline adsorption using various adsorbents.

Adsorbent	q_{max} (mg/g)	Adsorption time	References
Chitosan	23.92	24 hours	(Kang et al., 2010)
Silica oxide	12.21	250 minutes	(Brigante & Schulz, 2011)
Graphene oxide	313	90 minutes	(Y. Gao et al., 2012)
GO functionalized magnetic particle	39.1	10 minutes	(Lin et al., 2013)
Reduced graphene oxide with α-Fe_2O_3 nanoparticles	18.47	90 minutes	(Huízar-Félix et al., 2019)
Fe modified zeolite	200	400 minutes	(Jannat Abadi et al., 2019)
Fe_3O_4 magnetic nanoparticles	32.57	5 minutes	This work

3.2. The reusability

Reusability is one of important factors for evaluating adsorbents performance. If, the adsorbent has ability to regenerate for reuse in multiple cycles, the economic feasibility of the adsorption process should be enhanced. In order to evaluated the reusability of Fe_3O_4 magnetic nanoparticles, 10 consecutive adsorption-desorption experiments were repeated using the same adsorbent. The results from Figure 20 showed that the high efficiency of the adsorbent was obtained without a significant loss of efficiency at the end of the 10^{th} cycle. The adsorption capacity was slightly declined from 18.85 mg/g to 17.62 mg/g, as presented in Figure 20. However, compared with the first cycle, it still maintained a high adsorption performance. Unfortunately, the precision of result drastically decreased at the 10^{th} cycle. Therefore, these results indicate that Fe_3O_4 magnetic nanoparticles is a reusable adsorbent for tetracycline treatment without any loss in the adsorption efficiency and precision up to 9^{th} cycle.

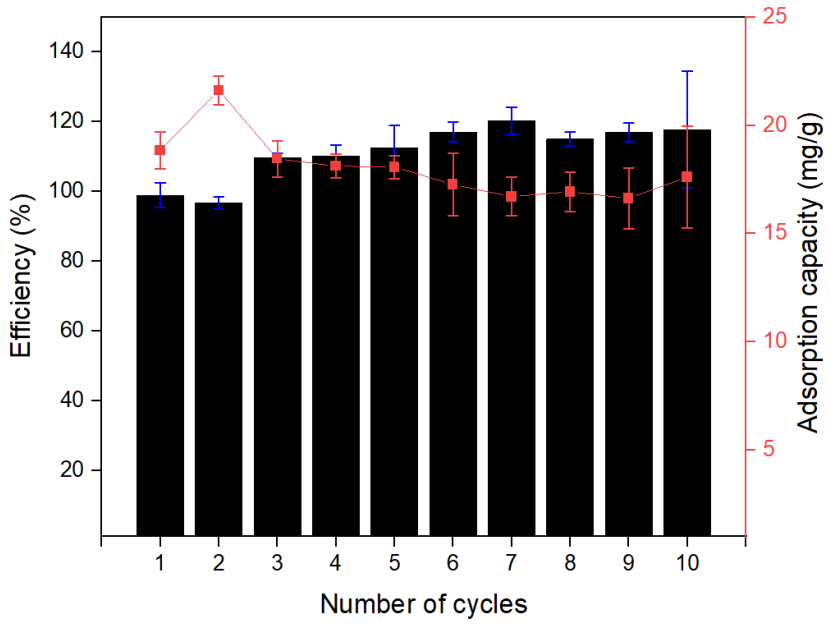


Figure 20. The adsorption-desorption cycles of magnetic nanoparticles

4. Application of the developed method for detection of tetracycline in real sample

To evaluate the adsorbent's performance in the real application, Fe_3O_4 magnetic nanoparticles was applied to adsorb and detect tetracycline in pharmaceutical sample. The experiment was performed using the optimize adsorption and desorption conditions. The result obtained from the developed method were compared with label value. The result showed, the content of tetracycline in pharmaceutical sample is 238.84 ± 5.83 mg/capsule. The tetracycline content obtained from this method and label value (250 mg/capsule) was in good agreement. The satisfactory result proved that the sample matrix had no influence on the adsorption of tetracycline by magnetic material. Therefore, Fe_3O_4 magnetic nanoparticles has a potential to be a great adsorbent for adsorb and remove tetracycline from real sample.

CHAPTER 5
SUMMARY DICUSSION AND SUGGESTION

Herein, Fe_3O_4 magnetic nanoparticles synthesized by a simple co-precipitation synthesis method were used as an adsorbent for the detection of tetracycline in pharmaceutical sample. The tetracycline adsorption and desorption performances using Fe_3O_4 magnetic nanoparticles was investigated by optimizing various variables. In addition, the adsorption capacity and reusability of the adsorbent for tetracycline adsorption were also evaluated.

Fe_3O_4 magnetic nanoparticles were synthesized from ferric chloride hexahydrate and ferrous sulfate heptahydrate in a mole ratio of 2:1 using co-precipitation method. The Fe_3O_4 nanoparticles were obtained as a black powder with magnetic properties. The characterization result from FTIR indicated that the synthesized Fe_3O_4 magnetic nanoparticles had a characteristic peak of Fe_3O_4 magnetic nanoparticles at 586 cm^{-1}, which Fe-O stretching peak. The synthesized magnetic nanoparticles were studied the pH at the point of zero charge (Pzc) using pH drift method. The result showed that the Pzc of Fe_3O_4 magnetic nanoparticles was found to be at 5.41.

In the adsorption and desorption experiment of tetracycline using Fe_3O_4 magnetic nanoparticles as adsorbent, the several parameters were investigated. The results showed that the highest adsorption capacity was achieved at pH 5, which is mild acidic condition. Therefore, pH 5 was chosen for subsequent experiments. In addition, the use of 1 mg of Fe_3O_4 magnetic nanoparticles with 5 minutes of contact between adsorbent and tetracycline provided the highest adsorption capacity of 21.5 mg/g. From the desorption experiments, 0.25 M sodium hydroxide at 2 mL provided a high desorption efficiency close to 100% and a highest enrichment factor of 2.59. For these reasons, 0.25 M NaOH at 2 mL was selected as desorption solvent in subsequent desorption experiments. It can also be seen that tetracycline rapidly and completely desorbed from Fe_3O_4 magnetic nanoparticles within 1 minute with desorption efficiency more than 99%. The experimental data demonstrated that the cycle of adsorption and

desorption can be completed with 10 minutes. Under optimum conditions, the adsorption isotherm of tetracycline on adsorbent surface was well described by the Langmuir model. The maximum adsorption capacity of adsorbent was 32.57 mg/g at room temperature. The reusability of adsorbent results shows a slight decrease of adsorption capacity after each reuse cycle. However, the adsorption capacity still maintained a high efficiency with good precision at the 9^{th} cycle. This confirms that the Fe_3O_4 magnetic nanoparticles adsorbent can be used repeatedly up to 9 times, which increase cost effective application value and reduces the waste generation. Finally, the proposed method was successfully applied to adsorb tetracycline in real pharmaceutical sample matrix. The content of tetracycline using the developed adsorbent was 238.84 mg/capsule, representing a 95.53% compared to the content on the drug label.

The proposed method was simple, low-cost, environmentally friendly, easy phase-separation and rapid in both of synthesis process and adsorption-desorption experiment. Furthermore, this proposed method provides a promising potential for tetracycline adsorption in real matrix sample and may offers a potential of being an alternative choice of adsorbent for the other sample applications in future.

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