

# Synthesis of Novel Amino-Coated Fe<sub>3</sub>O<sub>4</sub> Magnetic Nanoparticle Functionalized with Ciprofloxacin (AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf) and Study of Its Effect on Cu(II) and Ni(II) Adsorption

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## ABSTRACT

Ciprofloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl) - 3-quinoline carboxylic acid, cpfH) is a member of synthetic antibacterial agent widely used in clinical practice for the treatment of various gram negative and positive microorganisms. In concurrent use of ciprofloxacin with cations, they may bind together and result to formation complex by chelating. Formation of chelates ultimately reduces drug adsorption from the gastrointestinal tract. So, it is recommended a minimum of 2-hour interval between usage of ciprofloxacin and compounds containing cations. The aim of this study was to develop a novel amino-coated magnetic nanoparticle Fe<sub>3</sub>O<sub>4</sub> functionalized by ciprofloxacin (AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf) to study the amount of cation which adsorbed on ciprofloxacin by Atomic Absorption Spectrophotometry (AAS). The synthesized AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf was characterized using FT-IR, VSM and TEM. Separation of the adsorbed elements from reactive environment was fascinated with the aid of a magnet. The amount of residual metal in the solution was measured by AAS. Moreover our study investigated the effects of various conditions, such as pH, amount of nanoparticle and contact time of drug and metal in complex formation. The optimal condition of Cu(II) and Ni(II) absorbing was obtained.

**Keywords:** Fe<sub>3</sub>O<sub>4</sub> Magnetic Nanoparticle, Ciprofloxacin, Atomic Absorption Spectrophotometry, AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf

Quinolones are synthetic antibacterial agents widely used in clinical practice. It was found that quinolones and some antibiotics such as Streptomycin, aspergillilic acid, usnic acid, and tetracycline react with several metal ions and the crystal structures of boron, cadmium, calcium, cerium, cobalt, copper, iron, magnesium, nickel, selenium, silver and zinc complexes with ciprofloxacin have been reported [1-2]. Ciprofloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid, cpfH) is a member of this large family (Fig 1) and is used for the treatment of certain diseases caused by various gram-negative and some gram-positive microorganisms [3-4].

In concurrent use of ciprofloxacin with cations, they may bind together and result to formation a complex by chelating agents. Formation of chelates ultimately reduces drug adsorption from the gastrointestinal tract. So, it is recommended a minimum of 2-hour interval between usage of ciprofloxacin and compounds containing cations [5-6]. In term of physico-chemical studies, it seems that the formation of this complexes leads to change in the concentration of body transition elements such as copper (Cu) and nickel (Ni) [7]. Chelating effects have been proposed by several drugs. Findings of previous study endorse the chelating effects of ethanbutol leading to a decrease in serum levels of

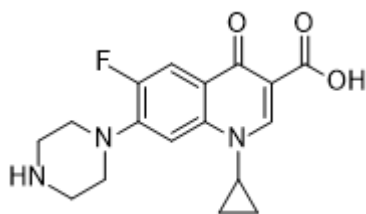


Fig 1. Molecular structure of ciprofloxacin

copper after 10 days [8]. Although the extent of the requirements to these transition elements in the body is low, but they play an important role in the vital body biochemical process [9].

Cu(II) is necessary for the development of connective tissue, nerve coverings, and bone and participates in both Fe and energy metabolism. Also, it acts as a reductant for the enzymes superoxide dismutase, cytochrome oxidase, lysyl oxidase, dopamine hydroxylase, and several other oxidases that reduce molecular oxygen. It is transported in the organism by the protein ceruloplasmin [10]. Good sources of dietary Cu(II) are liver and other organ meats, oysters, nuts, seeds, dark chocolate, and whole grains. Cu(II) deficiency in humans is rare, but when it occurs leads to normocytic, hypochromic anemia, leucopenia and neuropenia, and inclusive osteoporosis in children [11].

Also, Ni(II) is an essential constituent of methyl-CoM reductase, CO dehydrogenase and hydrogenase in some strains of bacteria. It is capable of activating or inhibiting a number of enzymes, altering cell membrane properties and influencing various redox processes [12-13].

Nanoparticles (NPs) with their basic properties such as extremely-small size and high surface-area-to-volume ratio and the ability to fabricate the materials and systems with the desired structures and functionalities provide better kinetics for the adsorption of metal ions from aqueous solutions [14]. To facilitate the recovery and manipulation of NPs, magnetisms incorporated with the NPs. Magnetic separation has been shown to be a very promising method for solid-liquid phase separation technique. This makes magnetic NPs excellent candidates for combining metal binding and selective adsorption properties with ease of phase separation [15]. Super paramagnetic particles are of interest especially for in vivo applications because they do not retain any magnetism after they are removed from the magnetic field. Iron oxides (Fe<sub>3</sub>O<sub>4</sub> and Fe<sub>2</sub>O<sub>3</sub>), in particular, have received considerable attention for their paramagnetism, biocompatibility and safety [16-17].

To date, many technologies, such as co-precipitation, microemulsion, thermal decomposition and hydrothermal synthesis, have been applied and reviewed for the production of these magnetic nanoparticles [18]. Among them, co-precipitation is a facile and convenient method that synthesizes iron

oxides from aqueous Fe(II)/ Fe(III) salt solutions in the presence of a base with a high yield and a relatively narrow size distribution. Also, for the dispersions, the particles should either be encapsulated by silica or stabilized with a surfactant. The surface modification reactions are not only important for engineering the surface energy and interfacial properties such as wetting, adhesion, and friction, but also for providing active surfaces to attach designated molecules that are immobilized on the surfaces of the magnetic nanoparticles. Several methods, including coating iron oxide with Au, polymers and silica have been developed to modify magnetic nanoparticles of the iron oxides. Among them, silica is the most prominent material for coating because it is chemically inert and therefore does not affect the redox reaction at the core surface [19-21].

The aim of this study was to define the adsorption quantity of trace Cu(II) and Ni(II) elements on ciprofloxacin with the aid of developing a novel adsorbing magnetic nanoparticle Fe<sub>3</sub>O<sub>4</sub> functionalized by ciprofloxacin (AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf) for measuring the amount of cations which adsorbed on AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf by atomic absorption spectrophotometry (AAS). The synthesized AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf was characterized using fourier transform-infrared (FT-IR), Vibration Sample Magnetometer (VSM) and transmission electron microscopy (TEM). Separation of the adsorbed elements from reactive environment was fascinated by using a magnet. Moreover our study investigated the effects of various conditions such as pH, amount of nanoparticle and contact time of AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf and metals.

## MATERIALS AND METHODS

### *Reagents and Characterization of AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf*

All chemicals were of analytical grade and were purchased from Merck (Tehran, IRAN). All the dilutions were prepared by deionized water. Ciprofloxacin was obtained in pure form from Pars Daru Pharmaceutical Company (Tehran, IRAN) and was used without further purification. Transmission electron microscopy (TEM) images were obtained from a JEM-2100 microscope (Philips CM 30, Tokyo, JAPAN) to measure the particle size and shape. An infrared spectrum was obtained using a Fourier Transform- Infrared Spectrometer (FT-IR, Perkin Elmer, spectrum 100, CT, USA) to identify the functional groups and chemical bonding of the coated materials. Magnetic property was estimated using a Vibrating Sample Magnetometer (VSM) (MDKFD, Iran).

The pH of the solutions was determined using a pH 540 GLP WTW (Wissenschaftlich- technische Werkstätten) pH-meter equipped with a WTW Sen Tix pH electrode. An ultrasonic bath was used for stirring the solution. The concentration of metal Cu(II) and Ni(II) was determined using a flame atomic absorption

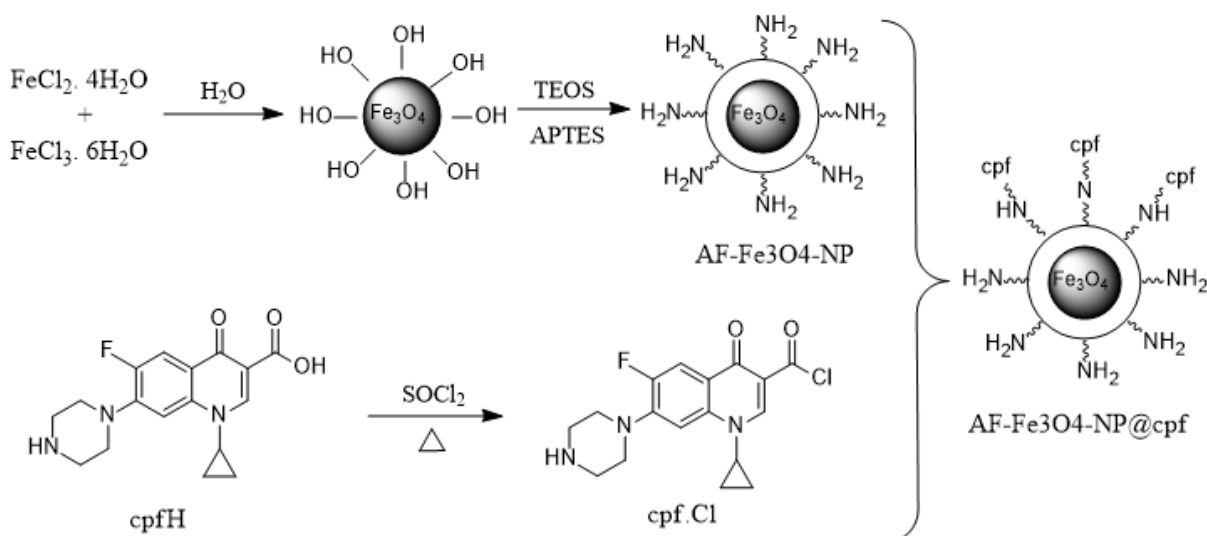


Fig 2. Schematic representation of the functionalizing silica, amine and ciprofloxacin on the magnetic nanoparticles,  $\text{Fe}_3\text{O}_4$

spectrometer (AAS, Thermo Elemental, Solaar UK). Calibration curves of metal ions Cu(II) and Ni(II) were created from 0, 0.5, 1, 1.5, 2, 5 and 10 ppm of each metal ion in deionized water ( $R^2$  for calibration curve of Ni(II) and Cu(II) is 0.9986 and 0.9963, respectively).

### Synthesis of magnetic nanoparticles with functional group

#### Preparation of $\text{Fe}_3\text{O}_4$ -NPs

To synthesize  $\text{Fe}_3\text{O}_4$  nanoparticles, the standard chemical co-precipitation method was followed [22]. According to this method,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (0.0862 mol) (Iron(III) chloride hexahydrate 97%, Sigma-Aldrich Chem. Co., USA) and  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  (0.0431 mol) (Iron(II) chloride tetrahydrate 99%, Sigma-Aldrich Chem. Co., USA) were added to deionized water (150 mL) and heated to 30 °C under Ar gas (99.999%, Seoul Special Gas, Inc., Seoul, Korea) for removal oxygen and created an inert condition in a round bottom flask, while magnetically stirring the mixture. Then, ammonia solution (8.57 mL), (25%, Sigma-Aldrich Chem. Co., USA) was injected into the mixture, and stirred for 30 min.  $\text{Fe}_3\text{O}_4$  nanoparticles were magnetically decanted and washed three times with deionized water and then dissolved in a mixture of ethanol: deionized water (2:1 v/v) solution.

#### Surface coating of $\text{Fe}_3\text{O}_4$ -NPs by Tetraethyl orthosilicate (TEOS) and (3-aminopropyl)-triethoxysilane (APTES)

Subsequently, 1.429 ml of Tetraethyl orthosilicate (TEOS) (Tetraethyl orthosilicate 99.999%, Sigma-Aldrich Chem. Co., USA) and 1.429 ml of 3-Aminopropyl triethoxysilane (APTES) (3-Aminopropyl

triethoxysilane 99%, Sigma-Aldrich Chem. Co., USA) were added, and the entire solution was stirred at 38 °C for 16 h. As a final step, amine functionalized  $\text{Fe}_3\text{O}_4$  nanoparticles (AF- $\text{Fe}_3\text{O}_4$ -NP) were magnetically decanted, washed three times with ethanol and dried leaving the dry nanoparticles in powder form (Fig 2).

#### Acylation of ciprofloxacin

Into the test tube were placed 0.08 g of ciprofloxacin and 4 mL of  $\text{SOCl}_2$  (thionyl chloride 98%, AR, Merck) and the solution was refluxed at 65 °C overnight. Unreacted thionyl chloride was removed under reduced pressure.

#### Synthesis of AF- $\text{Fe}_3\text{O}_4$ -NP@cpf

The mixture of AF- $\text{Fe}_3\text{O}_4$ -NP in DMF (N,N-Dimethylformamide, anhydrous 99.8%, Sigma-Aldrich Chem. Co., USA) was stirred at 0 °C for 0.5 h, followed by the addition of acylated ciprofloxacin and 4 drops of  $\text{C}_6\text{H}_{15}\text{N}$  (triethylamine, AR, Merck), and stirred at room temperature for 5 h. Black materials were separated via external magnet, and then washed with DMF.

#### Optimization of adsorption procedure

The adsorption procedure of Cu(II) and Ni(II) by means of AF- $\text{Fe}_3\text{O}_4$ -NP@cpf and AF- $\text{Fe}_3\text{O}_4$ -NP was optimized by evaluating several parameters including; pH, adsorption time (Ultrasonic Time) and the amount of absorbent. The pH of mixtures were adjusted 0 by using  $\text{CH}_3\text{COOH}$  and NaOH and stirred for 30 min in Ultrasonic bath. Thereafter, AF- $\text{Fe}_3\text{O}_4$ -NP@cpf and AF- $\text{Fe}_3\text{O}_4$ -NP with adsorbed heavy metal ion were

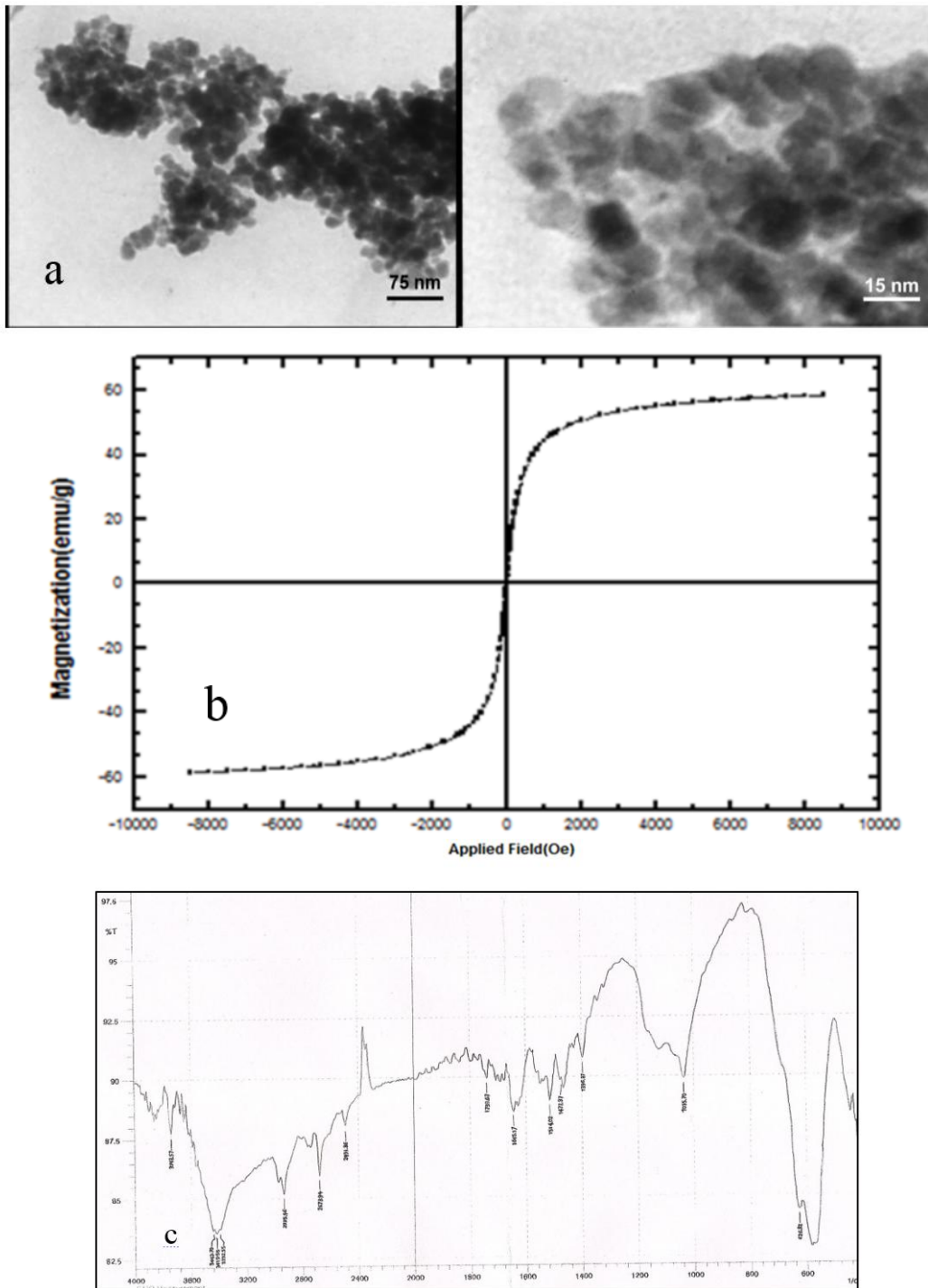


Fig 3. TEM image (a), VSM curve (b), FT-IR analysis (c)

separated from the mixture with a permanent hand-held magnet. The residual heavy metals in the solution were determined with AAS. Blank samples (containing only deionized water) were prepared and monitored for the duration of the experiment as a control.

#### Adsorption data analysis

The removal efficiency was determined by analyzing Cu(II) and Ni(II) concentration before and after the treatment and calculated by using Equation 1:

$$\text{Removal efficiency (R \%)} = \frac{C_0 - C_e}{C_0} \times 100\% \quad \text{Eq.(1)}$$

The adsorption capacity for each adsorbent,  $q_e$  (mg g<sup>-1</sup>), was determined by analyzing Cu(II) and Ni(II) concentration before and after the treatment and calculated by using Equation 2:

$$\text{Adsorption capacity (} q_e \text{)} = \frac{C_0 - C_e}{m} V \quad \text{Eq.(2)}$$

Where  $C_0$  and  $C_e$  are the initial and the equilibrium concentrations of the metal ions, respectively.  $V$  (L) is the volume of the solution whereas  $m$  (g) represents the weight of the adsorbent.

## RESULTS AND DISCUSSION

### Characterization of AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf

The TEM, VSM, and FT-IR of the CMNP were recorded. Typical results are summarized in Fig. 3(a-d). The morphology of AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf was investigated by transmission electron microscopy (TEM) and is presented in Fig. 3a. It is clear that Fe<sub>3</sub>O<sub>4</sub> microspheres are in good spherical shape, and it was obvious that these particles were fine with the average particle size around 15-75 nm. The nano structure could be roughly distinguished, where the resolution was limited by the magnetic interference.

The magnetic property of AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf was studied using a Vibrating Sample Magnetometer (VSM). Fig 3b shows the hysteresis loop at room temperature as the external magnetic field was changed from -9koe to 9koe. For super-paramagnetic particles, the magnetization became zero when the external field approached zero. The magnetization curve showed that the hysteresis curve passed through the zero point of magnetization and not observed coercivity ( $H_c$ ) and remanence ( $M_r$ ) and so it can be said that AF-Fe<sub>3</sub>O<sub>4</sub>-NPs@cpf are essentially super-paramagnetic and have a magnetization saturation value of 60 emu g<sup>-1</sup>. The outstanding magnetic property of the AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf is most likely due to the Fe<sub>3</sub>O<sub>4</sub>-containing particles which resulted in excellent magnetization [23]. These magnetization saturation values are high enough to separate AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf from aqueous solution because saturation magnetization of 16.3 emu g<sup>-1</sup> is sufficient for magnetic separation with a conventional permanent magnet [24].

In order to confirm the formation of Fe<sub>3</sub>O<sub>4</sub>, the prepared AF-Fe<sub>3</sub>O<sub>4</sub>-NPs@cpf was characterized by Fourier transform infrared spectroscopy (FT-IR) technique. Fig 3c shows the FT-IR spectrum of AF-

Fe<sub>3</sub>O<sub>4</sub>-NP@cpf. It shows the broad and strong peaks centered at 585 cm<sup>-1</sup> were ascribed to the stretching vibrations of Fe-O bond in the spectra. The wide absorption band at 3332-3442 cm<sup>-1</sup> is assigned to the N-H stretching vibrations and a band at 1635 cm<sup>-1</sup> N-H bending vibration. The IR absorption bands at 1620 and 1645 cm<sup>-1</sup> are the characteristic stretching frequencies of C=O in 4 position of quinoline ring and C=O connected to the N-H in ciprofloxacin, respectively.

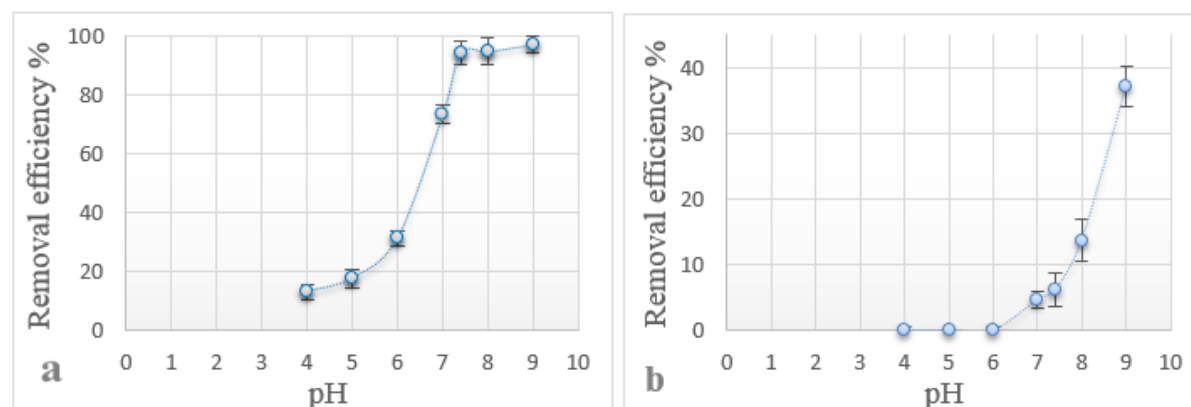
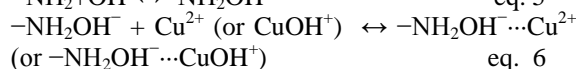
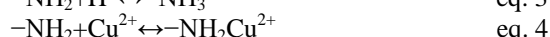
### Adsorption properties of AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf to Cu(II) and Ni(II) at different pH values

The pH value of the solution is an important controlling parameter in the adsorption process. The effect of pH value on the removal efficiency was due to its influence on the surface properties of the adsorbents as well as different species of the Cu(II) and Ni(II) in aqueous solution.

The adsorption of Cu(II) and Ni(II) were studied by mixing 10 mL of Cu(II) and Ni(II) solutions of initial concentrations at 1mg L<sup>-1</sup>(1 ppm) with 0.03 g AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf by varying pH ranging from 4 to 9 for 30 min.

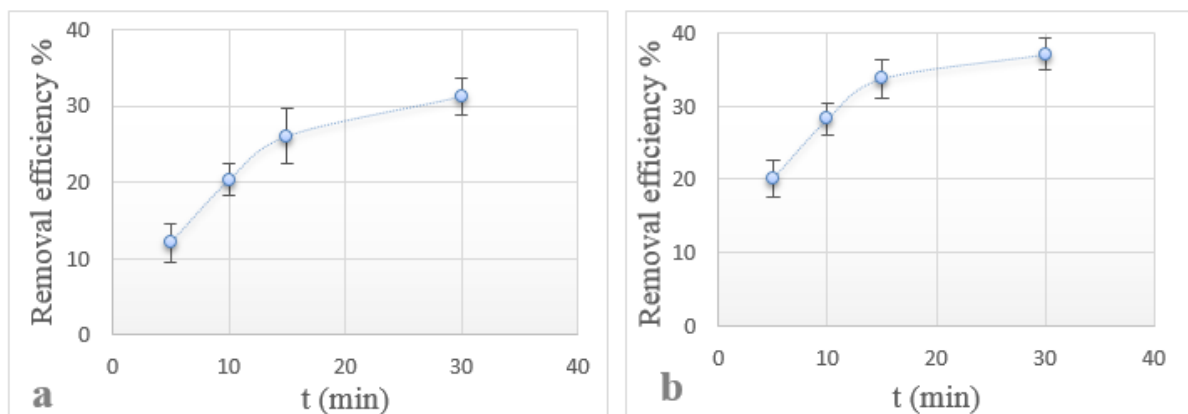
Fig. 4 shows the effect of pH on removal efficiency of Cu(II) and Ni(II) by AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf. It is clear that the adsorption of Cu(II) and Ni(II) on AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf was highly pH-dependent. Fig 4a shows the removal efficiency of Cu(II) on AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf increased with increasing pH which gets to maximum of 97% at high pH (pH=9).

Cu (II) forms several complexes in aqueous solution, such as Cu<sup>+2</sup>, Cu(OH)<sup>+</sup>, Cu(OH)<sub>2</sub>, Cu(OH)<sub>3</sub><sup>-</sup> and Cu(OH)<sub>4</sub><sup>2-</sup>. Also, Cu<sup>+2</sup> is the predominant species at pH values lower than 6.0 [25-26], while at pH values higher than 6, insoluble Cu(OH)<sub>2</sub> precipitated [27]. The interaction between the adsorbents amino group and the Cu(II) ions may be described by equation 3-6 [27-29].

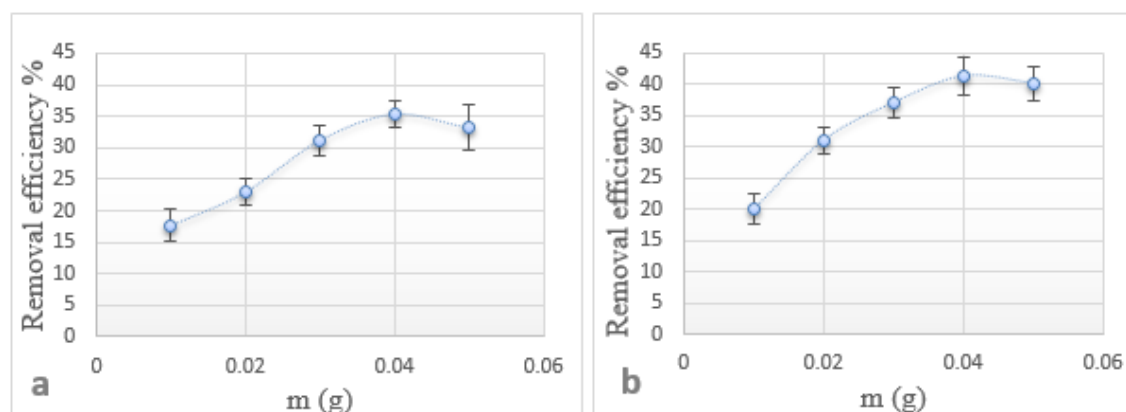


**Fig 4.** Removal efficiency %± SD as the effect of pH on the adsorption of Cu(II) (a) and Ni(II) (b) by AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf. Initial concentration of Cu(II) and Ni(II): 1 mg L<sup>-1</sup>(1 ppm); nanoparticles: 0.03 g; ultrasonic Time: 30 min and solution volume: 100 mL.

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**Fig 5.** Removal efficiency % ± SD as the effect of ultrasonic time on the adsorption of (a) Cu(II) and (b) Ni(II) by AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf. Initial concentration of Cu(II) and Ni(II): 1 mg/L; nanoparticles: 0.03 g; solution pH: 6.0 and 9.0 for Cu(II) and Ni(II) respectively; solution volume: 100 mL.



**Fig 6.** Removal efficiency % ± SD as the effect of adsorbent dose on the adsorption of (a) Cu(II) and (b) Ni(II) by AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf. Initial concentration of Cu(II) and Ni(II): 1 mg L<sup>-1</sup> (1ppm); ultrasonic time: 30 min; solution pH: 6.0 and 9.0 for Cu(II) and Ni(II) respectively; solution volume: 100 mL.

Eq. 3 indicates the protonation and deprotonation reactions of the amino groups of AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf. In solutions with lower pH values, the reaction in equation 3 favored the protonation of NH<sub>2</sub> to form NH<sub>3</sub><sup>+</sup>. The electrostatic repulsion between Cu<sup>2+</sup> and surface of the adsorbent increased. This effect resulted in the reduction of Cu(II) adsorption with decreasing solution pH.

With increasing solution pH, the reaction in equation 3 proceeded to the left, leading to an increased number NH<sub>2</sub> sites on the surface of the adsorbent and facilitating formation of surface complexes of Cu(II) through coordination interactions and thus increasing the adsorption capacity (eq. 4) [30].

However, at higher pH values, the reaction in eq. 5, which describes the adsorption of OH<sup>-</sup> from the solution through hydrogen bond formation, may proceed. This reaction on one hand can reduce the adsorption of Cu(II) through surface complex in eq. 4, but on the other hand may increase the adsorption of Cu(II) through electrostatic attraction as indicated in eq. 6. The abrupt increase of the removal efficiency (R %) at pH

higher than 6 may be due to the formation of insoluble Cu(OH)<sub>2</sub> precipitate [31]. So the optimum pH for Cu(II) ion adsorption is found in the pH range of 5–6 which is in accordance with previous reports [32–35]. As a result, the further experiments for Cu(II) adsorption were carried out at pH 6.0 which the percent removal is 31.23 ± 2.4.

Additionally, Fig 4b defines that the adsorption of Ni(II) was almost zero with pH between 4.0 and 6.0 and it reached to its maximum of 37.06 % ± 2.5 at pH 9. It is clear that nickel presents in the species of Ni<sup>2+</sup>, Ni(OH)<sup>+</sup>, Ni(OH)<sub>2</sub>, Ni(OH)<sub>3</sub> and Ni(OH)<sub>4</sub><sup>2-</sup> at different pH values. According to a research performed by Smith and Martell, they defined relative proportion of nickel species in various pH and reported Ni<sup>+2</sup> is the most specious of Ni(II) up to pH 9 [36]. Therefore, the low Ni(II) adsorption at low pH values is attributed partly to the competition between H<sup>+</sup> and Ni<sup>2+</sup> on the surface sites as well as electrostatic repulsion of Ni<sup>+2</sup> with protonated surface adsorbent (eq. 3) [35,37]. By increasing pH, the surface charge of the adsorbent becomes neutral due to the deprotonation process,

**Table 1.** Maximum removal efficiency of Cu(II) and Ni(II) at optimum condition

ADSORBENT	MAXIMUM REMOVAL EFFICIENCY %± SD	
	NI(II)	CU(II)
AF-Fe <sub>3</sub> O <sub>4</sub> -NP	25.5± 2.7	21.60± 2.2
AF-Fe <sub>3</sub> O <sub>4</sub> -NP@CPF	41.32± 3.1	35.04± 2.1

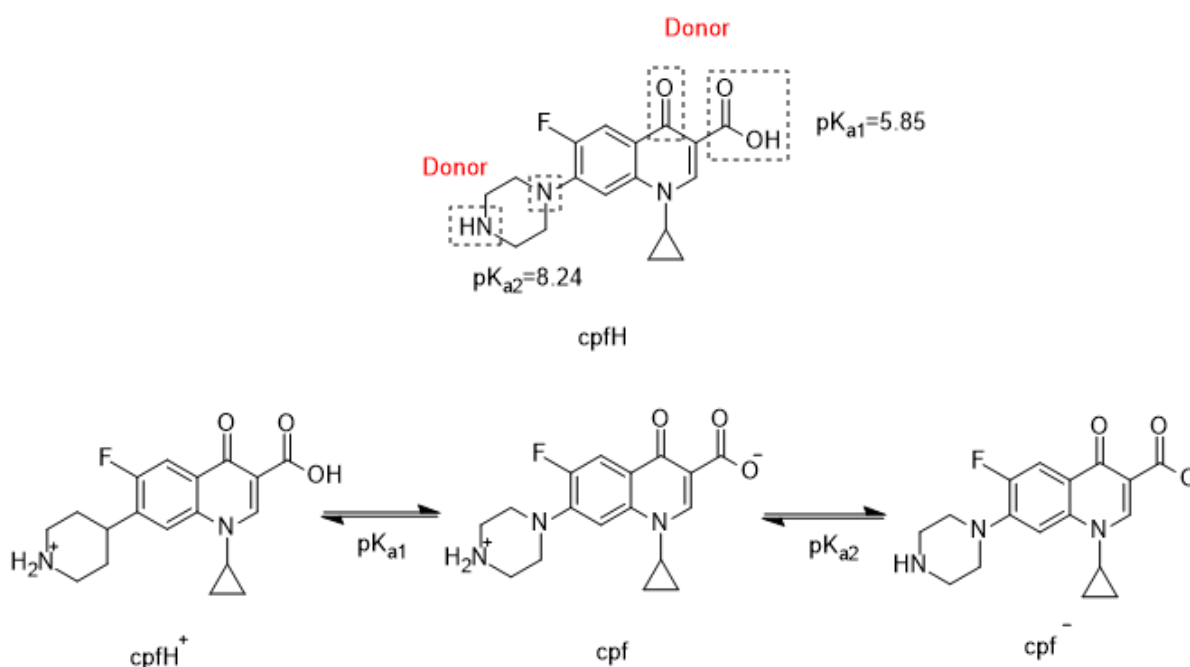
resulting in the electrostatic attraction of Ni<sup>2+</sup> to the deprotonated surface of AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf. Thus, the adsorption capacity increased sharply with pH increasing.

#### Effect of ultrasonic time on removal efficiency

The ultrasonic time is also a very important parameter in the adsorption procedure. The removal efficiency was studied by mixing of trace metal in initial concentrations of 1 mg L<sup>-1</sup> (1 ppm) with 0.03 g AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf and the mixture was adjusted to the optimum pH for each metal (pH= 6.0 and pH=9.0 for Cu(II) and Ni(II), respectively) in various ultrasonic times ranging from 5 to 30 min. Fig 5 shows the adsorption of Cu(II) and Ni(II) by AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf increased with the increasing of time. Fig 5a shows that the adsorption of Cu(II) proceed rapidly in the first 30 min. At time of 30 min, the percentage of Cu(II) uptake reached to its maximum of 31.23 % ± 2.4 and for Ni(II) reached to 37.06 % ± 2.5 (Fig 5b).

#### Effect of adsorbent concentration

It is necessary to get the optimum adsorbent in order to maximize the interactions between metal ions and adsorption sites of adsorbent in the solution. AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf with different concentrations were combined with a fixed amount of 1 mg L<sup>-1</sup> Cu(II) and Ni(II) and the mixture was adjusted to pH = 6.0 for Cu(II) and pH= 9.0 for Ni(II) by varying adsorbent dose ranging from 0.01 to 0.05 g for 30 min. As shown in Fig 6, the adsorption of heavy metal ions, Cu(II) and Ni(II), increased with the increase of adsorbent dose. At adsorbent dose of 0.04 g the percentage of Cu(II) uptake reached to its maximum (35.3%± 2.1) (Fig 6a) and for Ni(II) reached to 41.32%± 3.1 (Fig 6b). As a matter of fact, these observations show that an increase in the adsorbent dose would increase the number of available adsorption sites leading to the raise of the removal efficiency. However, when nearly all the heavy metals in the aqueous solutions were absorbed by the

**Fig 7.** Ionization state of ciprofloxacin

adsorbents, the number of occupied active adsorption sites grew, which would no longer contribute to the removal percentage, and thus result in the decrease of removal efficiency of the adsorbents. Moreover, high dosage may result in aggregation of the adsorbents and could further reduce the adsorption [38-40].

#### *Adsorption of Cu(II) and Ni(II) on AF-Fe<sub>3</sub>O<sub>4</sub>-NP and AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf in optimal conditions*

In order to determine the role of ciprofloxacin in adsorbing the quantity of Cu(II) and Ni(II), the removal efficiency of adsorbent before and after modifying with ciprofloxacin (AF-Fe<sub>3</sub>O<sub>4</sub>-NP and AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf) has been defined in the same and previous described optimal condition for two metal ions. Table 1 presents the results of maximum removal efficiency of Cu(II) and Ni(II) at optimum condition. Before functionalizing, Cu(II) absorbed by 16.6%± 2.2 while this amount increased to 35.04± 2.1% after modification (about 13.44% differences) and in the case of Ni(II), it observed higher differences in which the removal efficiency before and after is 25.5%± 2.7 and 41.32± 3.1, respectively (about 15.82% differences). These variations could be referred to the presence of ciprofloxacin as metal adsorbent. Moreover it can be driven that ciprofloxacin show much more removal adsorption efficacy for Ni(II) rather than that of Cu(II) which may be related to the lower ionic radius of Ni(II) than Cu(II) (70 and 77 pm, respectively)[41].

#### *Mechanism of Cu(II) and Ni(II) adsorption by AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf*

The aim this study is to propose an adsorption mechanism through formation of stable complexes between Cu(II) and Ni(II) with the chelating groups of AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf including: nitrogen atom of piperazine ring and 4-keto and 3-carboxylate oxygen of ciprofloxacin (Fig 7) that serve as the coordination and reaction sites.

At lower pH values, the adsorption capacity of the adsorbent was low due to the protonation of the amino groups (AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf and AF-Fe<sub>3</sub>O<sub>4</sub>-NP) and the oxygen end of two carbonyls in AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf that reduces the number of binding sites available for the ions adsorption. Moreover, the protonation of amino groups induces an electrostatic repulsion of Cu(II) and Ni(II) [42-43]. With an increase in pH value, first the protonated carbonyl and gradually NH<sup>+</sup> group will convert to neutral forms that provide favorable electron donating groups to adsorb Cu(II) and Ni(II). Additionally, the inhibitory effect of H<sup>+</sup> decreases by increasing pH value. Therefore, the probable mechanism of Cu(II) and Ni(II) at low pH is ion-exchanging with hydrogen ions on the binding sites.

### CONCLUSION

In the presented study, a novel magnetic nano-adsorbent has been developed by binding APTES and

ciprofloxacin on Fe<sub>3</sub>O<sub>4</sub>-NPs in order to identify the trace amount of Cu(II) and Ni(II). After the characterization of the adsorbent by FT-IR, TEM and VSM, its removal efficiency toward Cu(II) and Ni(II) was investigated. The obtained results can be concluded as follow:

- i. The results from FT-IR, TEM and VSM clearly indicated that preparation procedure was successfully performed.
- ii. Maximum adsorption yield was obtained at a solution pH of 6 and 9 for Cu(II) and Ni(II), respectively.
- iii. Maximum adsorption yield was obtained at ultrasonic time of 30 min and adsorbent amount of 0.04 g for both of the trace metal.
- iv. Compared to the AF-Fe<sub>3</sub>O<sub>4</sub>-NP, the AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf exhibited significant adsorption capability for Cu(II) and Ni(II) which as discussed previously it is related to the chelation mechanism and presenting more electron donating sites.
- v. The removal percentage of Ni(II) by AF-Fe<sub>3</sub>O<sub>4</sub>-NP@cpf is greater than Cu(II) as a result of lower ionic radius.

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