Loading of anticancer drug anastrozole using $Fe_3O_4@SiO_2$

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Received: October 22, 2023 DOI: 10.14295/bjs.v3i2.497

Accepted: December 08, 2023 URL: https://doi.org/10.14295/bjs.v3i2.497

Abstract

Anastrozole is a prescription drug that is used to treat hormone-dependent breast cancer, mostly in women who have gone through menopause. Once a day, it is taken by mouth. Anastrozole stops the activity of an enzyme called aromatase, which changes androgens into oestrogens. But taking the drug often comes with side effects that depend on how much you take, such as tiredness, diarrhea, hot flashes, nausea, headaches, muscle and joint pain, and so on. Anastrozole has also been linked to other side effects and more bone loss. To overcome the side effects of anastrozole and for their efficient delivery anastrozole must be loaded on the surfaces which is biocompatible and stable towards human body. So, the co-precipitation method was used to make iron oxide nanoparticles, which were then covered with silica using the Stober method. The made $Fe_3O_4@SiO_2$ nanocomposite was taken out as a black powder and studied using FTIR, EDX, and SEM. The SEM picture showed that the Fe_3O_4 and $Fe_3O_4@SiO_2$ nanoparticles size ranges were between 30 and 45 nm and 55 to 70 nm respectively. We also looked at how contact time, pH, and the amount of nanocomposite affected the loading of the drug. The best adsorption (85.6%) happened when the reaction lasted 12 h, the pH was 4, and the adsorbent dose was 10 mg.

Keywords: Fe_3O_4, $Fe_3O_4@SiO_2$, anastrozole loading, adsorption.

Carregamento do medicamento anticâncer anastrozol usando $Fe_3O_4@SiO_2$

Resumo

Anastrozol é um medicamento prescrito usado para tratar o câncer de mama dependente de hormônios, principalmente em mulheres que já passaram pela menopausa. Uma vez ao dia, é tomado por via oral. O anastrozol interrompe a atividade de uma enzima chamada aromatase, que transforma andrógenos em estrogênios. Mas tomar o medicamento muitas vezes traz efeitos colaterais que dependem da quantidade que você toma, como cansaço, diarréia, ondas de calor, náuseas, dores de cabeça, dores musculares e articulares e assim por diante. O anastrozol também tem sido associado a outros efeitos colaterais e a mais perda óssea. Para superar os efeitos colaterais do anastrozol e para sua administração eficiente, o anastrozol deve ser carregado nas superfícies que sejam biocompatíveis e estáveis em relação ao corpo humano. Assim, o método de coprecipitação foi utilizado para produzir nanopartículas de óxido de ferro, que foram então recobertas com sílica pelo método de Stober. O nanocompósito $Fe_3O_4@SiO_2$ fabricado foi retirado como um pó preto e estudado usando FTIR, EDX e SEM. A imagem SEM mostrou que as faixas de tamanho das nanopartículas Fe_3O_4 e $Fe_3O_4@SiO_2$ estavam entre 30 e 45 nm e 55 a 70 nm, respectivamente. Também analisamos como o tempo de contato, o pH e a quantidade de nanocompósito afetaram a carga do medicamento. A melhor adsorção (85,6%) ocorreu quando a reação durou 12 h, o pH foi 4 e a dose do adsorvente foi de 10 mg.

Palavras-chave: Fe_3O_4, $Fe_3O_4@SiO_2$, carga de anastrozol, adsorção.

1. Introduction

Anastrozole is a popular anticancer medication that has been shown effective in treating aromatase-dependent cancers and reducing the chance of developing these cancers in postmenopausal women. The aromatase enzyme converts androgens into estrogens in a crucial stage in estrogen biosynthesis, and this compound is a highly

selective aromatase inhibitor.

Although anastrozole is a powerful anticancer medication, it does come with certain unwanted side effects, including accelerated bone loss and other issues connected to aromatase inhibition (AIBL). Conjugating anastrozole with a drug nanocarrier technology has the potential to increase its efficacy while decreasing its negative effects (Teixeira et al., 2019). A composite material is a synthetic structure comprising multiple components or elements that are created to accomplish certain features and characteristics. These parts include a suitable matrix binder comprising various substances like metal, carbon, resin, ceramic, etc. in addition to a specified strengthening agent or filler (Toto et al., 2022).

Drug loading and targeted delivery systems are only two of the numerous biological fields where magnetic iron oxide (Fe_3O_4) nanoparticles have shown promising potential in recent years (Li et al., 2016). Biocompatibility, chemical stability, and solubility in a wide range of liquid media at room temperature and different pH levels are all crucial for use as medicinal Fe_3O_4 NPs (Gul et al., 2019). In addition to protecting magnetic NPs from agglomeration and oxidation throughout a wide pH range, silica has been shown to be the most promising coating material.

Coating magnetic particles enhances their solubility in water and the delivery system's biocompatibility. In addition, silica's surface is often finished with a silanol group, which may react with different chemicals and silane coupling agents to form conjugates with different biomolecules and specific ligands. The compatibility and hydrophilicity of SiO_2 films increase their use in biological settings (Verma et al., 2013). In addition, it can be used as a platform for drug loading. Since the surface makeup of naked maghemite or magnetite magnetic particles is largely inert, they lack the capacity to create stronger interactions with functional molecules and are hence not advised for direct usage as drug carriers. Therefore, their silica coating improves their medication interaction (Kralj et al., 2011).

The new synthesis of $Fe_3O_4@SiO_2$ for regulated drug administration under ambient conditions forms the basis of the current investigation. Scanning electron microscopy, energy dispersive X-ray spectroscopy, and Fourier-transform infrared spectroscopy were all used to analyze the nanocomposites after they were created. The effects of varying the composite dosage, pH, and time on the anastrozole loading were studied.

2. Materials and Methods

2.1 Chemicals and reagents

Analytical grade chemicals and reagents were utilized in all experiments. China Wulian Chemical Co., Ltd. supplied the tetraethyl orthosilicate (TEOS) for this project. Hydrochloric acid was procured from sisco research laboratories Pvt Ltd while sodium hydroxide from Tianjin Qilun Chemical technology Ltd. The anticancer drug) anastrozole and the inorganic chemicals iron sulphate heptahydrate $FeSO_4.7H_2O$ and ferric chloride hexahydrate ($FeCl_3.6H_2O$), were purchased from Sigma-Aldrich.

2.2 Preparation of Fe_3O_4 nanoparticles

In this study Fe_3O_4 nano particles were synthesized using the co-precipitation technique. Initially, a homogeneous dark orange solution was formed by dissolving 2.50 g of $FeSO_4.7H_2O$ and 4.86 g of $FeCl_3.6H_2O$ in 100 mL of distilled water while stirring with a magnetic stirrer. Next, under vigorous stirring at room temperature for five hours, the aqueous solution of NaOH (5.1 g of NaOH diluted in 50 mL of distilled water) was added to the solution mentioned above. The product was filtered finally and purified by washing with anhydrous alcohol and distilled water and dried in oven for 100 ºC for two hours (Liu et al., 2020).

2.3 Preparation of $Fe_3O_4@SiO_2$ nanoparticles

The modified Stober sol-gel technique was used to create core-shell particles of $Fe_3O_4@SiO_2$. In a solution consisting of 160 mL ethanol, 40 mL water, and 10 mL concentrated ammonia (28 wt%), 1.6 g of as-prepared Fe_3O_4 submicrospheres were ultrasonically disseminated. The solution was sonicated while 0.4 mL TEOS was added drop by drop. After that, mechanical stirring was done for three hours at room temperature. After being magnetically separated, the particles were washed with ethanol and deionized water. After doing this numerous times, we dried the mixture at 60 ºC for two hours (Ghasemzadeh et al., 2015).

2.4 Characterization

FTIR, SEM, and EDX studies were used to evaluate synthesized Fe_3O_4 and $Fe_3O_4@SiO_2$. Different functional groups were identified using a Fourier-transform infrared spectrophotometer (Shimadzu-A60; Kyoto, Japan), and the atomic structure was identified using a scanning electron microscope (SEM; JEOL-Jsm-5910; Tokyo, Japan). The resulting $Fe_3O_4@SiO_2$ was examined for its elemental composition using an Oxford EDX (Inca-200).

2.5 Anastrozole loading for potential delivery

Twenty milliliters of the 20 ppm anastrozole solution was used in each adsorption measurement, and the samples were maintained in a shaker incubator at room temperature and 120 revolutions per minute $Fe_3O_4@SiO_2$ was used in varied concentrations, buffer solutions were adjusted for pH, and the adsorption processes were run for different amounts of time to get the required results. Using an external magnet, the adsorbent was extracted after adsorption, and the resulting reactive solutions were compared to the parent solution using a UV double beam spectrophotometer set to 206 nm. The following formula was used to determine the drug's percentage adsorption

$$\% \text{ loading} = Ci - Cf \times 100 / Ci$$

Where “Ci” denotes the drug's pre- and “Cf” post¬ concentration, respectively.

3. Results and Discussion

3.1 Images of Fe_3O_4 and $Fe_3O_4@SiO_2$

Co-precipitation was used to create iron oxide (Fe_3O_4) nanoparticles, which were subsequently coated with a silica layer using the Stober technique. Figure 1 (a) depicts black Fe_3O_4 nanoparticles, whereas Figure 1 (b) shows dark black $Fe_3O_4@SiO_2$ nanoparticles. After silica coating, the black colour and the magnetism of the Fe_3O_4 nanoparticles retains.

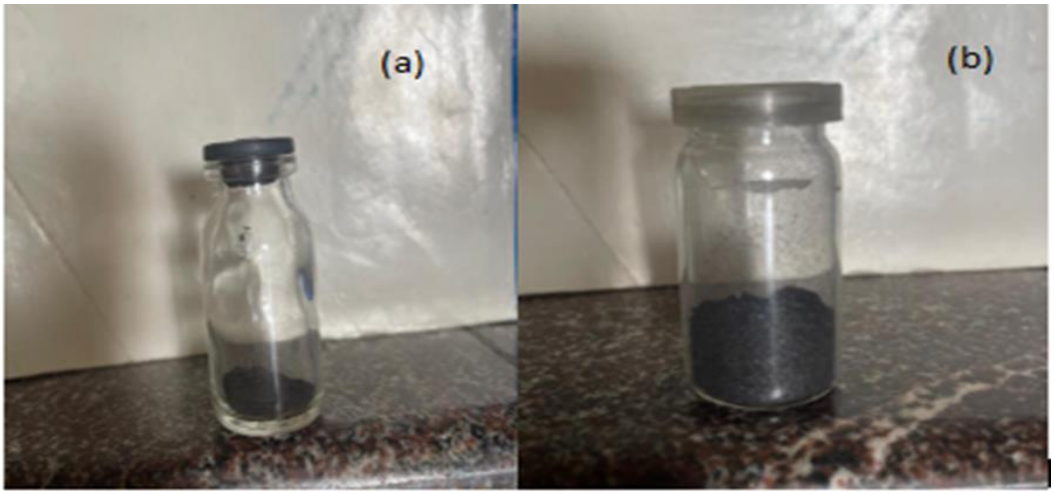

Figure 1. Images of (**a**) Fe_3O_4 and (**b**) $Fe_3O_4@SiO_2$. Source: Authors, 2023.

3.2 FTIR Spectrum of Fe_3O_4 and $Fe_3O_4@SiO_2$

Table 1 displays FTIR data of Fe_3O_4 and $Fe_3O_4@SiO_2$. The development of a distinctive peak at 538 cm^{-1} proved the presence of Fe-O bond. Vibrations of the Fe-OH were detected at 3346 cm^{-1} and 1571 cm^{-1} as the Fe_3O_4 nanoparticles' surface was shielded with OH group in an aqueous environment during their synthesis. The synthesis and FTIR spectral recording of Fe_3O_4 nanoparticles by Yang et al. is consistent with our findings. At 580, 1630, and 3405 cm^{-1}, they saw three distinct bands. The mobility of Fe-O linkages in the Fe_3O_4 the crystal structure was shown to be associated with absorption at 580 cm^{-1}.

The other peaks of the hydroxyl groups were found at 1630 cm^{-1} and 3405 cm^{-1} (Yang et al., 2010). For iron oxide (Fe_3O_4) nanoparticles, Ahangaran et al. used FTIR spectroscopy and found an absorption peak at 567 cm^{-1},

which they attributed to the Fe-O bending vibration (Ahangaran et al., 2013). Similar results were found by Hwang et al. when they examined iron oxide nanoparticles using FTIR spectroscopy; they found prominent peaks caused by an iron-oxygen connection around 577 and 631 cm^{-1}.

Additionally, a peak at 1631 cm^{-1} was found caused by the bending vibration of absorbed water, and another at 3431 cm^{-1} caused by the stretching mode of surface hydroxyl (Hwang et al., 2014). Similarly, the silica-coated iron oxide nanoparticles FTIR spectrum is shown in (Figure 2) Fe-O bond stretching was detected at 538 cm^{-1}, while the presence of a Si-O bond was indicated by an absorption peak at 1013 cm^{-1}. Coating of iron oxide nanoparticles with silica was verified by this absorption peak. Sodipo and Aziz coated iron oxide nanoparticles with silica.

The FTIR analysis revealed that an absorption peak associated with the Si-O bond occurred between 1110 and 1059 cm^{-1} (Sodipo; Aziz, 2015). The movement of oxygen in Si-O-Si antisymmetric stretching was also linked by Ahangaran et al. who observed a large, intense band at 1061 cm^{-1}. The existence of the Si-O-Fe bond was linked to the 570 cm^{-1} absorption band (Ahangaran et al., 2013).

Table 1. Functional groups of Fe_3O_4 and $Fe_3O_4@SiO_2$.

Wavenumber (cm^{-1})	**Functional group**
538	Fe-O
3346	OH
1571	Fe-OH
1013	Si-O
538	Fe-O

Source: Authors, 2023.

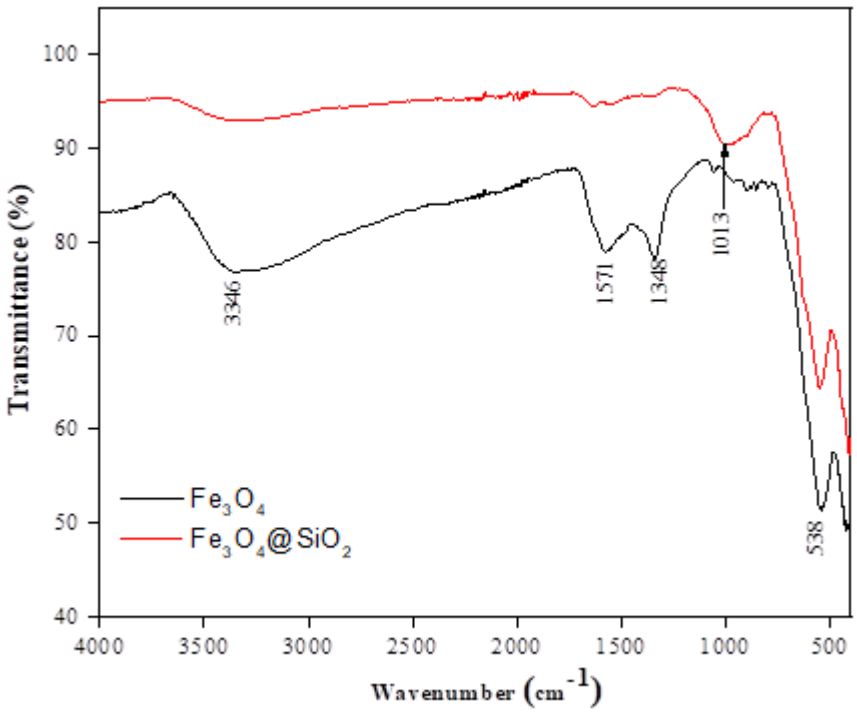

Figure 2. FTIR spectrum of Fe_3O_4 and $Fe_3O_4@SiO_2$. Source: Authors, 2023.

3.3 SEM images of Fe_3O_4 and Fe_3O_4@SiO_2

Morphology of nanoparticles of Fe_3O_4 and Fe_3O_4@SiO_2 was studied by SEM analysis, and results are shown in Figure 3 (a, A) and Figure 3 (b, B) respectively. Nanoparticles were round. In most cases, the nanoparticles were discovered clustered together because of the powerful magnetic contact between them. Due to their fundamental magnetic properties and the potent Van der Waals interaction between the particles that are present on their surface, pure Fe_3O_4 nanoparticles have a significant tendency to be agglomerated.

Image J was used to calculate the diameters of the nanoparticles. Sizes of the particles varied between 30 and 45 nm. Magnetite Fe_3O_4 nanoparticles were manufactured via a co-precipitation process, and similar results were reported by Homogen. Nanoparticles, on average measuring 20-40 nm in size, were found to be unevenly distributed throughout the sample due to agglomeration (Homogen, 2018). Hariani et al. manufactured Fe_3O_4 nanoparticles by co-precipitation using reagents such as ferrous chloride, ferric chloride, propylene glycol, NH_4OH, etc. and then used SEM to examine the morphology of the particles. They estimated that the size of Fe_3O_4 to be between 5 and 20 nm (Hariani et al., 2013).

Figure 3 (b, B) displays scanning electron micrographs of iron oxide coated with silica (Fe_3O_4@SiO_2) nanoparticles. Coating of SiO_2 on Fe_3O_4 nanoparticles was clearly visible in the photos, demonstrating a full phase-shifting structure. The particles did not distribute uniformly and had an odd shape. Particles typically measured between 55 and 70 nm in size.

The effective coating of silica on iron oxide nanoparticles was previously confirmed and reported by Javidi et al. For the purpose of selectively identifying clozapine from human serum, they developed core-shell magnetic molecularly imprinted polymers. They said that SEM scans show that the magnetic Fe_3O_4 particles were successfully coated, and that the particle size ranged from 16 to 24 nm (Javidi et al., 2015). Particle size distributions were found to be between 34.39 and 37.3 nm in the SEM picture of silica-coated magnetite (Asab et al., 2020).

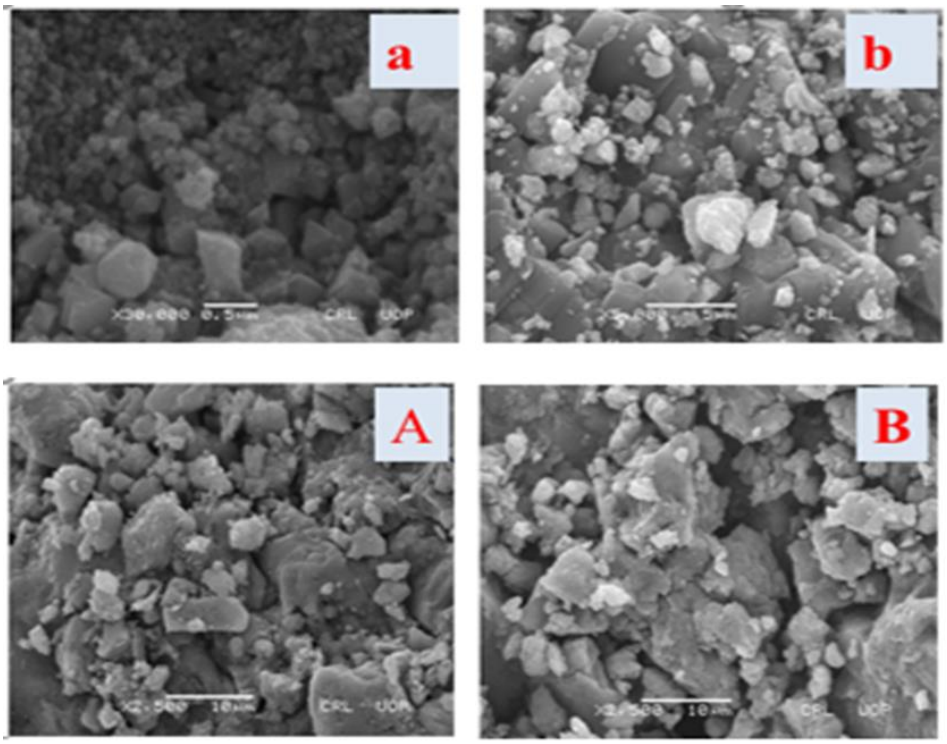

Figure 3. SEM images of (a, A) Fe_3O_4 and (b, B) Fe_3O_4@SiO_2.

3.4 EDX spectrum of Fe_3O_4@SiO_2 nanocomposite

EDX spectroscopy verified that the produced nanocomposite of Fe_3O_4@SiO_2 had the expected elemental distribution (Table 3). The nanocomposite EDX spectrum is shown in Figure 4. The EDX spectrum

demonstrated unequivocally that the silica shell encloses iron. The obtained findings suggest that this simple, fast, and cheap method may be used to effectively produce composites with several constituent parts.

Table 3. Elemental composition of Fe_3O_4@SiO_2 nanoparticles.

Element	Atomic Weight %
O	60.33
Si	24.74
Fe	14.93
Total	100

Source: Authors, 2023.

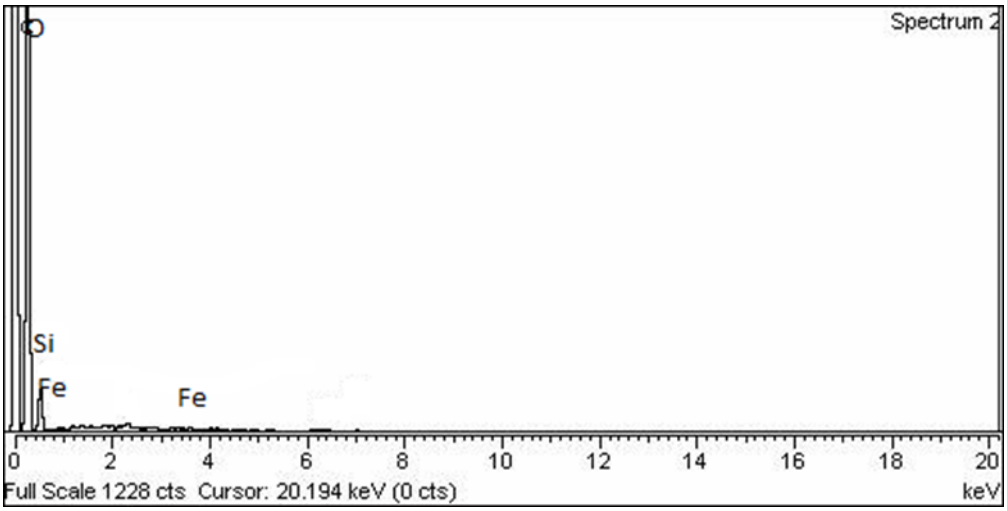

Figure 4. EDX spectrum of Fe_3O_4@SiO_2 nano composite.

3.5 Effect of pH on anastrozole adsorption

As can be shown in Figure 5, the adsorption capacity of the as-synthesized Fe_3O_4@SiO_2 for anastrozole was measured at pH values between 2 and 10. As predicted, the as-synthesized nanocomposites exhibited different degrees of anastrozole adsorption over the pH range. The medication's maximum adsorption of 85.6% was observed at a pH value of 4 for anastrozole. This is because at pH levels below 4, the as-synthesized adsorbent is positively charged; whereas the adsorbent nanoparticle surface is negatively charged at pH values above PZC. This is due to the basic structural form of anastrozole, the final structure of the Fe_3O_4@SiO_2 and the point of zero charge (PZC).

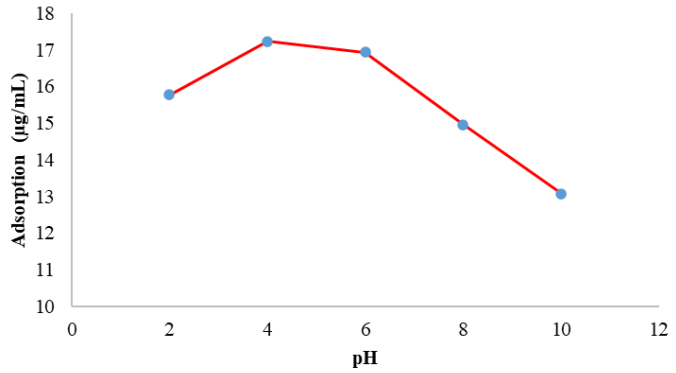

Figure 5. Effect of pH on anastrozole adsorption. Source: Authors, 2023.

3.6 Effect of adsorbent dose on anastrozole adsorption

Various adsorbent dosages were tested on the as-prepared $Fe_3O_4@SiO_2$ nanocomposite to determine its adsorption capacity. Adsorption of anastrozole increased from 5 to 10 mg when adsorbent was added to 20 mL of 20 µg/mL^{-1} anastrozole solution, as illustrated in (Figure 6). However, the further increase did not show any noticeable improvement in adsorption. Therefore, adsorbent weight of 10 mg was determined to be optimal.

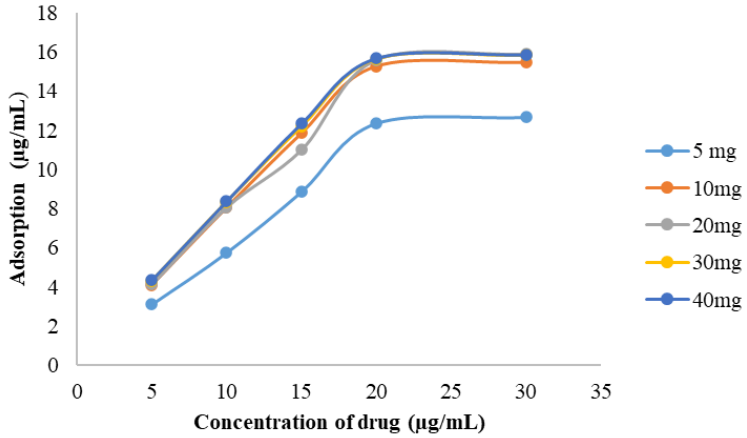

Figure 6. Effect of adsorbent dose on anastrozole adsorption. Source: Authors, 2023.

3.7 Effect of time on anastrozole adsorption

The appropriate adsorbent dosage was found to be 10 mg, and the pH value was found to be 4, using a 20 g/mL^{-1} solution of anastrozole. The investigation focused on how time affected the as-synthesized $Fe_3O_4@SiO_2$ adsorption capability. Results revealed that adsorption increased with time, with the maximum adsorption being achieved after 12 hours of adsorption i.e. 85.6% (Figure 7).

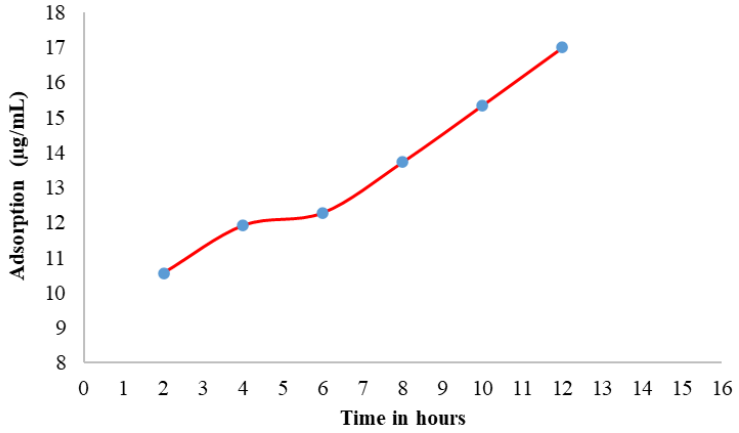

Figure 7. Effect of time on anastrozole adsorption. Source: Authors, 2023.

4. Conclusions

Iron oxide nanoparticles were prepared using precipitation method and successfully coated by silica through Stober method. The effective synthesis was demonstrated using a number of analytical instruments that includes SEM, EDX, and FTIR. The synthesized nanocomposites was evaluated for loading of anastrozole and found maximum loading. As such, these magnetic nanocomposites may be an efficient adsorbent for administering anastrozole and other medications in a targeted and regulated manner. In addition, the method may be utilized to create core-shell composites with shells made of a variety of different materials.

5. Acknowledgments

The author is greatly acknowledging the Department of Chemistry, Abdul Wali Khan University Mardan and University of Swat, Swat, Khyber Pakhtunkhwa, Pakistan for providing experimental facilities.

6. Authors' Contributions

Muhammad Ahsan: Conducted the experimental loading of anastrozole onto the $Fe_3O_4@SiO_2$ nanoparticles. Sobia Qasim: Analyzed the drug release kinetics and evaluated the efficiency of anastrozole loading on $Fe_3O_4@SiO_2$. *Ajmal Shah*: Contributed to the synthesis and characterization of $Fe_3O_4@SiO_2$ nanoparticles for drug loading in the research. *Nelofar*: Assisted in the preparation and optimization of the $Fe_3O_4@SiO_2$ carriers for effective drug loading. *Irum Nawaz*: Helped in the data interpretation and statistical analysis of the drug-loading process for anastrozole. *Muhammad Kashif*: Played a role in reviewing the literature and providing critical insights into the study's context. *Wisal Ahmad*: Contributed to the design and coordination of the research, ensuring the overall integrity of the project.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

9. References

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Funding

Not applicable.

Intitutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.