

SILICA-COATED MAGNETITE NANOPARTICLES PREPARED BY CO-PRECIIPITATION METHOD FOR PROTEIN DETECTION

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Abstract

Fe₃O₄ magnetite nanoparticles (MNPs) were prepared using the co-precipitation method at room temperature. The MNPs were modified by coating with SiO₂ and APTES to enhance their efficiency for protein detection applications. The MNPs were characterized by Transmission Electron Microscopy (TEM), Scanning Electron Microscopy with Energy Dispersive X-ray spectroscopy (SEM/EDXS), Fourier Transform Infrared (FT-IR), Thermogravimetry Analysis (TGA), X-ray diffraction (XRD), and Vibrating Sample Magnetometer (VSM). The results indicated differences in the size of particles, as around 10-40 nm and there was observed agglomeration among the sample, which, according to SEM image. Moreover, FT-IR analysis confirmed the presence of silica and NH₂ coating on the magnetic nanoparticle surface. The immobilization of amine molecules (-NH₂) onto the SiO₂ surface is evident in TGA. The crystalline structure of the MNPs was identified as the cubic inverse spinel structure of pure-phase polycrystalline magnetite through XRD. Results from the vibrating sample magnetometer (VSM) revealed that the prepared MNPs exhibited a high magnetization saturation (Ms) of 61.31 emu/g and a coercive field strength (Hc) of 30.94 Oe (near ferromagnetism). After being surface modification with SiO₂, Ms reduced to 43.24 emu/g, which further decreased to 40.17 emu/g following modification with APTES. Additionally, the Bradford assay was used to measure the percentage binding for BSA, assessing the efficiency of protein detection application.

Keywords: Co-precipitation; Fe₃O₄ nanoparticles; magnetite; protein detection

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Introduction

Magnetite nanoparticles are highly valued for their magnetic properties, making them particularly useful in various biomedical applications such as magnetic resonance imaging (MRI) for contrast enhancement, drug delivery, and hyperthermia treatment. Adding a silica (SiO_2) coating enhances their biocompatibility and stability in biological environments, providing a versatile surface for functionalization with specific biomolecules and expanding their application in biosensing detection. Furthermore, magnetite nanoparticles (Fe_3O_4 NPs) have undergone significant development for various applications, including biomedicine (Hao *et al.*, 2010; Cardoso *et al.*, 2018), agriculture (Zhu *et al.*, 2018; Ju *et al.*, 2019), and environmental catalysis (Zhang *et al.*, 2019). These nanoparticles exhibit distinct characteristics and functionalities suitable for specific areas, owing to their formation from different metal elements and coating with oxides exhibiting magnetic characteristics (Kefeni *et al.*, 2017). Particularly noteworthy is the superparamagnetic magnetite (Fe_3O_4 NPs) type, known for its high biocompatibility and low toxicity, making it widely utilized in contrast agents for MRI (Assa *et al.*, 2016; Farjadian *et al.*, 2017). Recently, Fe_3O_4 NPs have garnered attention for their applicability in diverse areas, especially in biological (Lu *et al.*, 2017) and biomedical applications (Mohammed *et al.*, 2017). Their surface chemistry can be controlled by modifying their physicochemical properties, enabling their use in hyperthermia, MRI, immunoassays, drug delivery, and cell separation (Weissleder *et al.*, 1995; Kumar and Mohammad, 2011), including the immobilization of proteins on the surface for magnetic hyperthermia application (Rajkumar *et al.*, 2010; Marin *et al.*, 2016; Gawali *et al.*, 2020; Kim *et al.*, 2007). Hence, there is a need to study the mechanical characterization of superparamagnetic behavior using co-precipitation preparation methods or hydrothermal synthesis (Li *et al.*, 1998). The co-precipitation method (Liu *et al.*, 2008; Marques *et al.*, 2008; Faiyas *et al.*, 2010; Wu *et al.*, 2011) is a popular technique for synthesizing and characterizing superparamagnetic Fe_3O_4 nanoparticles with various biological applications (Bansal *et al.*, 2017).

This article aims to investigate the synthesis of Fe_3O_4 nanoparticles using the co-precipitation method and employs various characterization techniques. Transmission electron microscopy (TEM) and scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDXS) were used to image and determine the particle size of various elemental materials. Fourier transform

infrared spectroscopy (FT-IR) and thermogravimetric analysis (TGA) was employed to confirm the silica coating on the magnetic nanoparticle surface. X-ray diffraction (XRD) patterns indicated that the magnetic nanoparticles consist of Fe_3O_4 with a specific phase structure. Vibrating sample magnetometer (VSM) instruments were used to study the magnetic properties or behaviour of magnetite Fe_3O_4 nanoparticles. The coated magnetite nanoparticles have also been utilized for the pre-concentration of biomarkers in complex media. Moreover, the silica-coated magnetite nanoparticles synthesized through the co-precipitation method show promise for protein detection and biomedical applications.

Instrumental Measurements

The surface morphology was investigated using transmission electron microscopy (TEM, JEOL model JEM-2100), operated at 200 kV, and scanning electron microscope FEI Quanta 450 with energy dispersive X-ray spectroscopy (SEM/EDXS model QUANTA 450) FT-IR measurements were recorded on an FT-IR instrument (Bruker, model INVENIO S) within the wavenumber range of 4,000-400 cm^{-1} . The thermogravimetric analysis (TGA) was performed using NETZSCH model STA 449 F5 Jupiter, thermal analyser, ranging from 25°C up to 900°C with a rate of 2.5°C/min under N_2 atmosphere. X-ray diffraction (XRD) patterns were recorded with a RIGAKU Smart Lab, where the X-ray beam was nickel-filtered to reduce $\text{Cu-K}\beta$ ($\lambda = 1.392 \text{ \AA}$), and only $\text{Cu-K}\alpha$ ($\lambda = 1.5405 \text{ \AA}$) was introduced to the samples. X-ray radiation operated at 40 kV and 30 mA, with the scanning range varied from $2\theta = 20^\circ$ to 80° and a step size of 0.03° . The magnetization behavior was measured using a hand-made vibrating sample magnetometer (VSM). This instrument measures the sample's magnetization using an applied magnetic field (H). In the case of the M-H curves, we can determine the saturation magnetization (M_s), remanence magnetization (M_r), and coercive force (H_c) at room temperature under applied magnetic fields (H) ranging from -17.5 kOe to +17.5 kOe. These results yield characteristic M-H curve, classifying the types of magnetic exhibited in the samples.

Experimental Details

Fe_3O_4 nanoparticles were synthesized using a simple

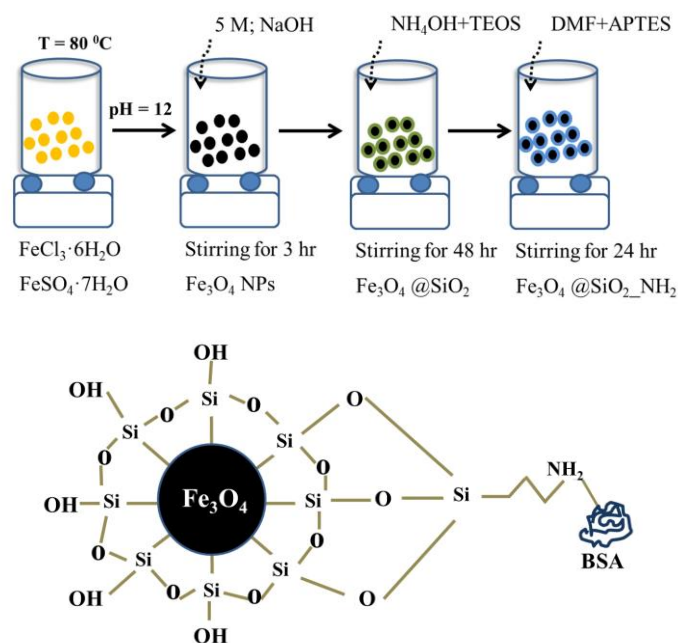


Figure 1. Illustration steps for the synthesis of Fe_3O_4 core NPs silica coating on MNPs, and the surface functionalization with amine ($-\text{NH}_2$) before albumin immobilization

chemical co-precipitation method. Typically, 0.01 mol of Iron (II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and 0.07 mol of Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) were dissolved in 400 mL of deionized water and stirred at room temperature, resulting in an orange solution. Sodium hydroxide (NaOH , 5 mol) was then slowly added dropwise (3 mL/min) to the solution to instantaneously adjust pH to 12-13. After 3 h of NaOH addition, the obtained black precipitate was separated using an external magnet. The Fe_3O_4 nanoparticles were filtered, washed several times with ethanol and deionized water, followed by the addition of 2 mL aqueous ammonia hydroxide (NH_4OH), and 0.5 mL of tetraethyl orthosilicate (TEOS) was slowly added to the reaction solution under mechanical shaking. The resulting dispersion was mechanically stirred for 48 h at room temperature, followed by carefully washing with ethanol and deionized water three times, and finally dried at 50°C for 6 h. For functionalization, the nanoparticles were treated with 3-aminopropyltriethoxysilane (APTES). Initially, the $\text{Fe}_3\text{O}_4 @ \text{SiO}_2$ NPs were dispersed in a 25 mL dimethylformamide (DMF) solution. Then, 1.5 mL of APTES was added, and the mixture was stirred for 24 h at room temperature. Afterwards, the particles were washed twice with ethanol, followed by washing with deionized water, and finally dried at room temperature for characterisation. However, nanoparticles intended for protein binding were stored in deionized water. This process resulted in

the immobilization of APTES ($\text{Fe}_3\text{O}_4 @ \text{SiO}_2 - \text{NH}_2$) on the surface of the nanoparticles. The interaction between amine groups ($-\text{NH}_2$) and bovine serum albumin (BSA) is of significant interest in the fields of biochemistry and molecular biology. Further, this interest is due to the implications it has in drug delivery, protein stabilization, and biomaterial science. Meanwhile, BSA is a serum albumin protein derived from cows and is well-studied. So, it is known for its high stability and solubility in water, which makes it an excellent model protein for studying protein-ligand interactions, as illustrated in Figure 1.

Results and Discussion

Transmission Electron Microscopy (TEM)

The particle size and morphology of MNPs were investigated using TEM at a magnification of $200,000\times$ as shown in Figure 2. The majority of particles appeared to be spherical in shape, with agglomeration observed in the range of 8-15 nm for the iron core nanoparticles in Figure 2(a). Fe_3O_4 NPs were coated with silica (Figure 2(b)) and amine groups (Figure 2(c)). The TEM image showed that the size of Fe_3O_4 increased from a mean particle size of 12 nm to 40 nm after coating. The estimated average particle size and particle distribution, along with a standard deviation, were fitted using a long-normal distribution function, as shown in

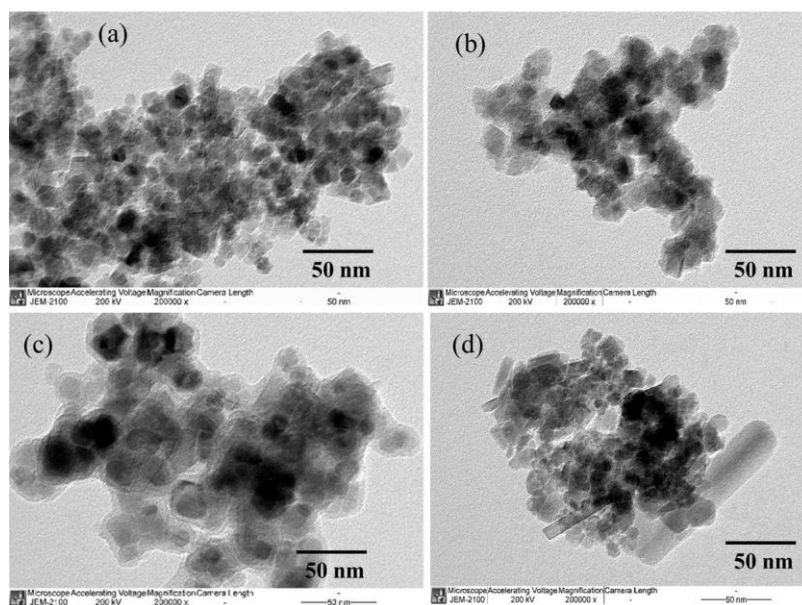


Figure 2. Transmission electron microscopy (TEM) image for (a) Fe_3O_4 NPs (b) $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (c) $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ and (d) $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$, respectively

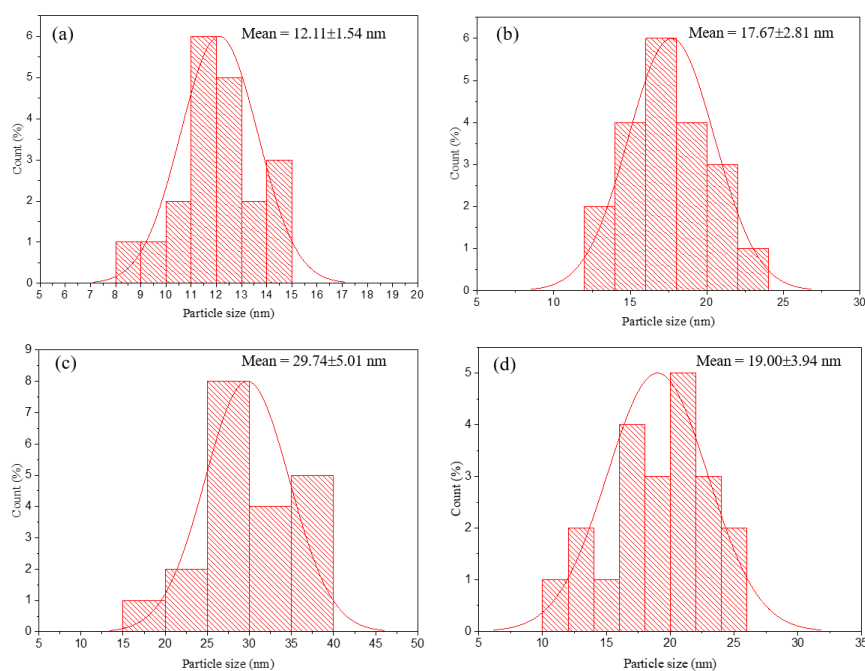


Figure 3. Histograms of the particle size distribution for (a) Fe_3O_4 NPs (b) $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (c) $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ and (d) $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$, respectively

histograms of Figure 3. After coating with SiO_2 and NH_2 , TEM images revealed that the core-shell structure, evident from the lighter contrast shadow surrounding the MNPs. These results confirm that Fe_3O_4 NPs exhibit a core-shell after coating with

SiO_2 and -NH_2 , with the mean diameters and standard deviations of the nanoparticles gradually increasing. Additionally, it appears that in Figure 2(d) the presence of rod-shaped particles is indeed observed. This occurrence may arise from either the

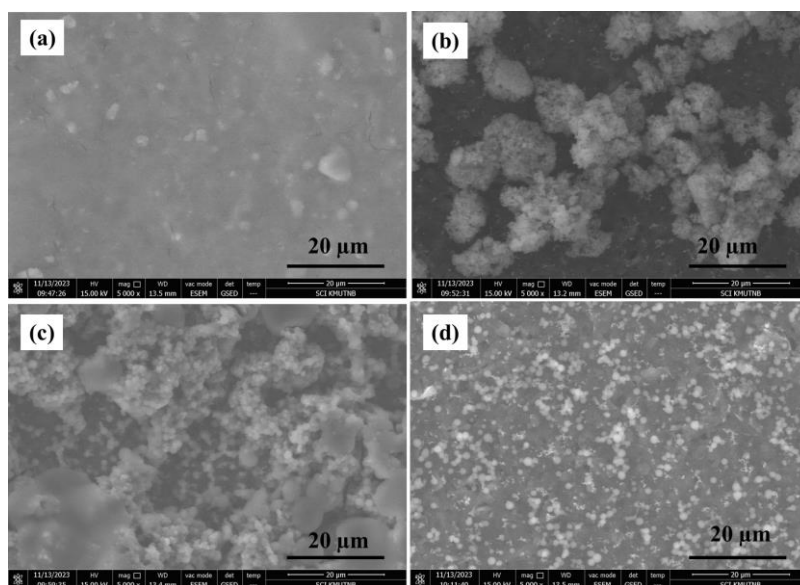


Figure 4. SEM image of (a) Fe_3O_4 NPs (b) $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (c) $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ and (d) $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$, respectively

aggregation of particles during the immobilization process or specific interactions between the surface-modifying, such as silica or -NH_2 , and the nanoparticles leading to preferential growth along certain crystallographic directions.

Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDXS)

Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDXS) is utilized for surface analytical techniques owing to its ability to provide high-resolution images of surface topography and an excellent depth of field. In this study, SEM images of Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{SiO}_2$, $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$, and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$ have provided crucial information about the surface morphology of all materials. In the first case, the surface of Fe_3O_4 NPs indicates aggregated and semi-spherical particles as shown in Figure 4(a). The atomic percentages of Fe and O elements presented on the surface were 18.3% and 75.0%, respectively. Second, $\text{Fe}_3\text{O}_4@\text{SiO}_2$ is more dispersed due to the formation of a silica (SiO_2) layer on magnetic nanoparticles, which distributed the nanoparticles (NPs) apart from each other and reduced their magnetic interactions (Aljarrah *et al.*, 2018), as shown in Figure 4(b). Moreover, the atomic percentages of Fe, O, and Si elements presented on the surface were 4.1%, 34.6%, and 4.6%, respectively. Finally, the nanoparticles demonstrated similar characteristics after attaching the amino silane agent (NH_2) onto the magnetite/silica core-shell, as indicated with

$\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ and BSA (Figure 3(c) and 3(d)). Additionally, the amine group (NH_2) grafting onto $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticle can lead to changes in the atomic presence of the Si element by increasing from 4.6% to 10.2%, and 10.3% of Si for $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$. However, Fe and O elements were presented in all samples, confirming the successful coating process of magnetite nanoparticles, as listed in Table 1.

Fourier Transform Infrared (FT-IR) Spectroscopy

Figure 5 presents the FT-IR spectra of Fe_3O_4 NPs, $\text{Fe}_3\text{O}_4@\text{SiO}_2$, and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ samples. In the spectrum of the Fe_3O_4 NPs, a peak at 542.26 cm^{-1} corresponds to the Fe-O vibration of tetrahedral and octahedral sites (Abkenar *et al.*, 2015).

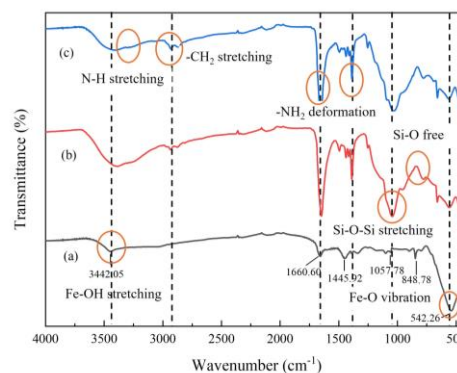
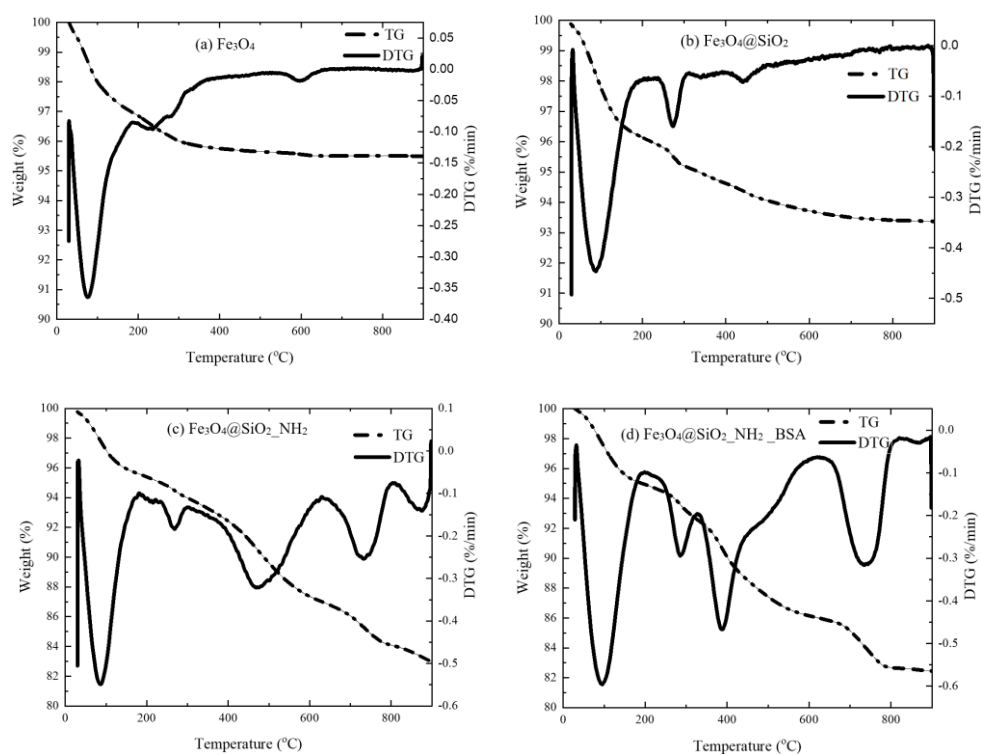


Figure 5. FT-IR spectra of (a) Fe_3O_4 NPs (b) $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and (c) $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$

Table 1. EDXS composition analysis of (a) Fe_3O_4 NPs (b) $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (c) $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$

Sample	O	Fe	Cl	C	Si
Fe_3O_4	75.0	18.3	6.3	-	-
$\text{Fe}_3\text{O}_4@\text{SiO}_2$	34.6	4.1	4.3	52.3	4.6
$\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$	33.0	2.1	4.9	49.9	10.2
$\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$	47.1	0.6	42.0	-	10.3

**Figure 6. TGA curves of (a) Fe_3O_4 NPs (b) $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (c) $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$, and (d) $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$ weight loss with temperature, respectively**

Meanwhile, a strong band of $-\text{OH}$ at $3,442.05\text{ cm}^{-1}$ is attributed to the stretching vibrations of Fe-OH groups at the surface and physically adsorbed water (Zhang *et al.*, 2013). The spectrum of Fe_3O_4 exhibits three characteristic peaks at $3,442.05\text{ cm}^{-1}$, $1,057.78\text{ cm}^{-1}$, and 848.78 cm^{-1} . These correspond to the stretching vibration of the silanol group (Si-OH) due to hydrogen bonding with physisorbed water molecules on the surface, the siloxane bond in the spin (Si-O-Si), and the free silanol group, respectively (Araghi and Entezari, 2015; Ghasemzadeh *et al.*, 2015). These results confirm the effective coating of silica on Fe_3O_4 NPs. Moreover, the spectrum of amine immobilization ($\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$) displays the same characteristic bands as the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ spectrum. Two new weak peaks around $2,938\text{ cm}^{-1}$ and $2,870\text{ cm}^{-1}$ are observed, corresponding to the asymmetric and

symmetric stretching modes of the $-\text{CH}_2$ of propyl groups. The corresponding N-H stretching vibration at $3,324\text{ cm}^{-1}$ is more difficult to observe because of its weak dipole moment (Pasternack *et al.*, 2008). Additionally, the $-\text{NH}_2$ deformation mode was observed at $1,653.73\text{ cm}^{-1}$ and $1,388.81\text{ cm}^{-1}$, respectively. Moreover, the terminal amino group of the $-\text{NH}_2$ molecule has loaded onto the SiO_2 surface forming a monolayer, leading to some hidden characteristic bands. These results confirm the amine functionalization of $\text{Fe}_3\text{O}_4@\text{SiO}_2$, which is important for preparing the surface before protein detection.

Thermogravimetric Analysis (TGA)

An investigation was conducted to study the thermal stability of Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{SiO}_2$, $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$, and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$

samples using the TGA technique with a heating rate of 2.5°C/min, as shown in Figure 6(a-d). Figure 6(a) shows that pure Fe_3O_4 has a weight loss of 4.5%. This weight loss can be attributed to the loss stemming from the evaporation of physically adsorbed water or/and structure water on the surface nanoparticles. In Figure 6(b), the TGA curve of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ shows weight loss values of 6.63%, consisting of two major transitions. The first weight loss below 88.2°C (0.45%/min) is attributed to the vaporization of the physically adsorbed water. The second minor weight loss is about (0.16%/min) between 150°C and 450°C due to the desorption of physisorbed molecules (Ulu *et al.*, 2018). For the $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$, the immobilization of amine molecules (-NH₂) onto the SiO_2 surface is evident in Figure 6(c). The weight loss is about 16.99% can be identified in three steps. The first step of the weight loss occurs at 86.6°C (0.55%/min), and 268.6°C (0.18%/min), the second step at 468°C (0.32%/min), and the last step at 731.7°C (0.25%/min), respectively. A major step occurring in the range of 300°C to 600°C may be reasonable for the pyrolysis of the amino groups on the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (Aljarrah *et al.*, 2018), as shown in Figure 6(c). Interestingly, in the case of detection of BSA in the amine group, a weight loss occurs below 95.3°C (0.60%/min), and at a high temperature of 898.1°C, residual mass remains at 82.45%. These results in the temperature range of 200-500°C are attributed to the decomposition of linked amine molecules, which confirms the strong binding of the amine group and BSA (Araghi and Entezari, 2015). Furthermore, the fourth peak in the range of 600-800°C is attributed to the phase transition from magnetite (Fe_3O_4) to hematite (Fe_2O_3) structure (Mazo-Zuluaga *et al.*, 2003; Mahdavi *et al.*, 2013). Hematite is thermodynamically stable above 570°C in the phase diagram of the hematite system, followed by a decrease due to the deoxidation of hematite (Zhao *et al.*, 2006).

X-ray Diffraction (XRD)

The structure patterns of Fe_3O_4 NPs, $\text{Fe}_3\text{O}_4@\text{SiO}_2$, $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$, and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$ were analyzed using XRD spectroscopy, as shown in Figure 7. The XRD pattern of Fe_3O_4 powder clearly demonstrates the spinel structure with a face-centered cubic (fcc) configuration, referring to the JCPDS database. Additionally, the Fe_3O_4 structure exhibits characteristic peaks at $2\theta = 21.14^\circ$, 30.12° , 35.52° , 43.22° , 53.38° , 57.08° , and 62.76° , corresponding to the (111), (220), (311), (400), (422), (511), and (440) phases of magnetite material, respectively (Liu *et al.*, 2004; Faiyas *et al.*, 2010). The average particle size was found to be 17.23 nm for

magnetite. On the other hand, a broad peak in the range from 20° to 29° indicates the presence of amorphous silica particle-coated Fe_3O_4 nanoparticles, which was slightly lower than that of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$. This difference might be due to OH ions generated by the hydrolysis of NH_4OH (Kim *et al.*, 2007; Rajkumar *et al.*, 2010). Therefore, it can be concluded that magnetite nanoparticles were coated by silica and no peaks were detected that could be assigned to the stability of the crystalline phase also with step of functionality amine (-NH₂) and BSA. However, nanoparticles can be crystalline, amorphous, or quasi-crystalline, therefore XRD particle size calculations are not quite accurate (Wu *et al.*, 2011). The impurities correspond to the EDXS analysis in Table 1.

Vibrating Sample Magnetometer (VSM)

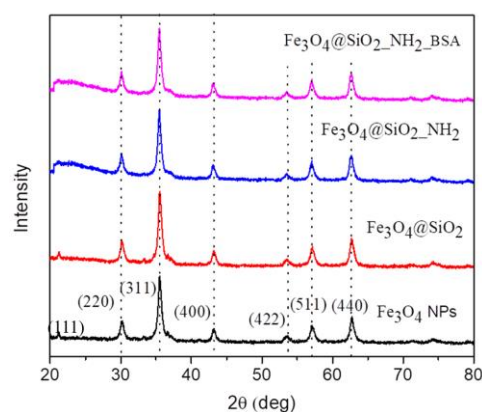


Figure 7. XRD spectra of Fe_3O_4 NPs, $\text{Fe}_3\text{O}_4@\text{SiO}_2$, $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$

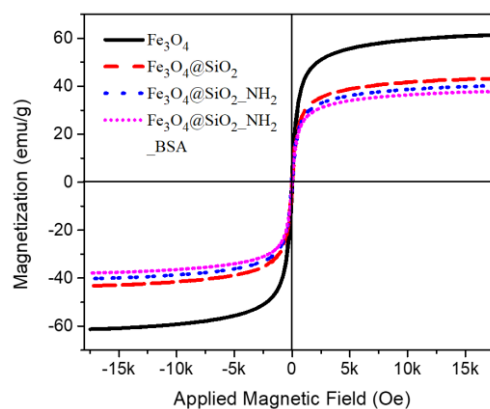


Figure 8. The magnetization (M-H) curves of Fe_3O_4 NPs, $\text{Fe}_3\text{O}_4@\text{SiO}_2$, $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$

Figure 8 presents plots of magnetization hysteresis loops for the four conditions obtained through the co-precipitation method, with measurements conducted at applied temperatures atmosphere. The M-H curves of the prepared samples reveal their magnetic features under a magnetic field ranging from -17.5 to +17.5 kOe. The results for the obtained samples indicate superparamagnetic behaviour, as evidenced by the demonstration of a hysteresis loop, with coercivity and remanence values approaching zero (Lin and Ho, 2014). On the other hand, the M-H curves demonstrate a significant decrease in the values of saturation magnetization (M_s), with 61.31 emu/g, 43.24 emu/g, 40.17 emu/g and 37.74 emu/g for Fe_3O_4 NPs, $\text{Fe}_3\text{O}_4@\text{SiO}_2$, $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2\text{-BSA}$, respectively. This decrease in M_s values can be attributed to the isolating effect of the coated SiO_2 on the nanoparticle surface (Meng *et al.*, 2018). Additionally, a newly established interface for $\text{Fe}_3\text{O}_4@\text{SiO}_2$ indicates that the SiO_2 layer and amino groups (NH_2) exhibit a consistent trend of decreasing coercivity. However, the functionalized coating of silica and the immobilized amine possesses sufficient magnetization, providing the benefit of effective separation from the solutions using an external magnetic field (Mutneja *et al.*, 2016).

The Percentage Binding Efficiency

This experiment utilized $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ nanoparticles with a solid content of 2% (w/w), equivalent to 50 mg of powder nanoparticles dissolved in a 2.5 mL solution. A BSA standard curve was created using 6 different concentrations ranging from 0.000 to 0.500 mg/ml, with optical density (O.D.) as $y = 1.3627x + 0.0433$ and

$R^2 = 0.9865$, as shown in Figure 9(a). Results from 50 $\mu\text{g/mL}$, 250 $\mu\text{g/mL}$, and 500 $\mu\text{g/mL}$ samples are presented in Figure 9(b), which displays the percentage binding efficiency. The $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ nanoparticles demonstrated exceptional binding efficiency of BSA immobilization, determined as 90.60% from 50 $\mu\text{g/mL}$ (with bound BSA at 45.18 $\mu\text{g/mL}$), 93.50% from 250 $\mu\text{g/mL}$ (with bound BSA at 245.22 $\mu\text{g/mL}$), and 95.21% from 500 $\mu\text{g/mL}$ (with bound BSA at 490.24 $\mu\text{g/mL}$). The results indicate that the amount of BSA immobilized on the APTES-treated $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles increase with BSA concentration. This is due to the high concentration of APTES, whose active amino groups ($-\text{NH}_2$) react with aldehydes, which in turn conjugate with BSA molecules. This reaction facilitates the covalent bonding of BSA to the nanoparticle surface, leading to enhanced immobilization with increasing BSA concentration (Liu *et al.*, 2004).

Conclusions

The magnetic properties of magnetite (Fe_3O_4) nanoparticles can be influenced by the co-precipitation synthesis method. Fe_3O_4 nanoparticles, synthesized at 80°C for 2 h, showed a high magnetic saturation (M_s) of 61.31 emu/g at room temperature. After coating the nanoparticles with silica, the M_s decreased to 43.24 emu/g. Further surface modification with NH_2 resulted in M_s of 40.17 emu/g, indicating superparamagnetic behaviour. FT-IR spectroscopy confirmed the successful SiO_2 coating on Fe_3O_4 nanoparticles and immobilizing amine (NH_2) on the $\text{Fe}_3\text{O}_4@\text{SiO}_2$ surface. The surface composition was found to be

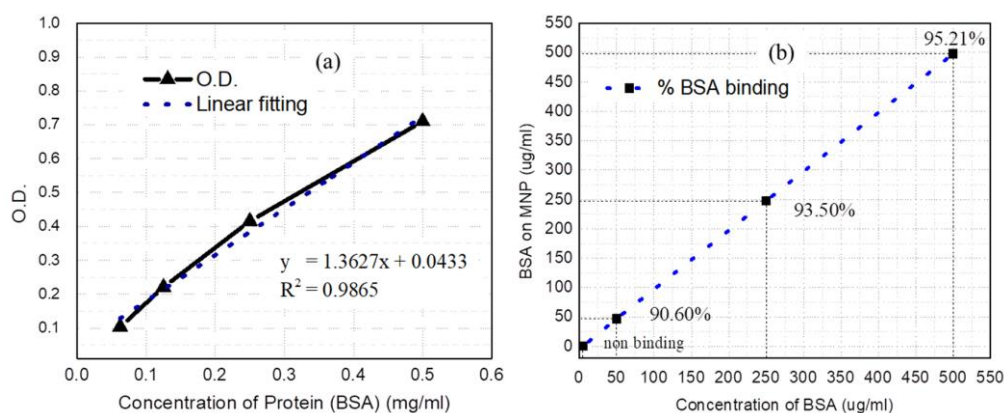


Figure 9. (a) BSA standard curve, (b) Concentrations of BSA and percent binding efficiency of binding BSA at concentrations as 50, 250, and 500 $\mu\text{g/mL}$

4.1% Fe, 34.6% O, and 4.6% Si. SEM imaging and EDXS confirmed the characteristics after attaching the amino agent. Moreover, BSA antibody binding to the nanoparticles exhibited a high percentage binding efficiency of 95.21%. This process has the potential to develop low-cost sensors for biomolecules and disease markers. Finally, the process of attaching amine groups to BSA involves a complex interplay of various interactions, including electrostatic interactions, hydrogen bonding, and, to a lesser extent, hydrophobic interactions. Understanding these interactions is crucial for designing applications of BSA in drug delivery, biomaterials, and analytical chemistry. This highlights the significance of studying protein-ligand binding for the advancement of biomedical research and development.

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