

Synthesis and characterization of Fe₃O₄@SiO₂ core/shell nanoparticles for biological applications

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Abstract

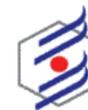
Magnetic nanoparticles have received extensive attention in biological applications such as enrichment, bioseparation, detection, and purification. In this report, nanoparticles of magnetite (Fe₃O₄) are prepared from the solution of ferrous/ferric mixed salt solution in an alkaline solution using the modified chemical coprecipitation process. Next, the synthesized magnetic nanoparticles were coated with silica shells by the Stober method. Then, the surface of Fe₃O₄@SiO₂ nanoparticles modified with organosilane for the investigation of biological applications. The core/shell nanoparticles were characterized by X-ray powder diffraction (XRD), Fourier transforms infrared spectrometer (FTIR), and scanning electron microscopy (SEM). The results of XRD indicated that the nanoparticles were composed of crystalline Fe₃O₄ core and SiO₂ shell. The SEM image of Fe₃O₄ nanoparticles showed the diameter could be tuned from 15 to 25 nm. The average diameter of the uniform spherical core/shell nanoparticles was approximately 170 ± 50 nm, and the average thickness of the silica shell was 30 nm. Finally, the protein adsorption studies on the nanoparticles revealed the ability of these nanoparticles for use in biological applications.

Keywords: Fe₃O₄, Fe₃O₄@Silica, nanoparticle, biological application, drug delivery.

Introduction

In the past decades, various types of applications were developed for nanoparticles within different fields such as separation processes, imaging, analytical chemistry, drug delivery, data handling, acoustic reproduction, energy production, and environmental transport, etc [1]. The most prevalent iron oxide found in nature includes magnetite (Fe₃O₄), maghemite (γ-Fe₂O₃), and hematite (α-Fe₂O₃) [2]. Since Fe₃O₄ magnetic nanoparticles do not have sufficient selectivity, high surface area adsorption capacity superparamagnetic properties, and also acid solution stability, some organic compounds have been functionalized. This ensures better chemical stability, cost-efficient, and environment-friendly [1].

Magnetic nanoparticles demonstrate the phenomenon of superparamagnetism and do not maintain magnetism after the operation of the magnetic field, giving the benefit of the risk of particle aggregation. The magnetic particles obtained from magnetic transition metals (iron, nickel, and cobalt) oxidized readily whereas iron oxide like magnetite is more stable against oxidation [3]. Fe₃O₄ nanoparticles have attracted much interest because they belong to a group of materials with non-toxicity and biological compatibility due to the presence of Fe



ions. Magnetic nanoparticles have been synthesized by various methods, which the most commons are thermal decomposition, microemulsion, coprecipitation, solvothermal, and sonochemical synthesis. The chemical coprecipitation has some advantages and desired product with excellent magnetic properties [4]. The coprecipitation is the most common, facile and convenient method. However, the magnetic nanoparticles under the influence of dipole-dipole interactions can be aggregated and formed the chains and cross-linked structures [5]. Magnetic iron oxide nanoparticles are commonly used for the core-shell nanostructures as it is non-toxic and simple in preparation [6].

Surface coating of magnetic nanoparticles is essential to provide multifunctionality, as well as improving their biocompatibility [7]. Silica due to properties such as biocompatibility, nontoxicity, chemical inertness, very high specific surface area with abundant Si-OH bonds on the surface, non-magnetic properties, and biostability has been widely used as the coating of magnetic nanoparticles [2, 7]. The Stöber synthesis method is one of the most common approaches for silica coating. Silica shell has some advantages, such as simple synthesis, improved water solubility, protecting the core from any oxidation and degradation, ease of further functionalization, and increase the resistance for the highly acidic solution, and easily furthered conjugation with various functional groups, thus enabling the coupling and labeling of bio targets with selectivity and specificity. The investigation of the bio-toxicity effects of nanomagnetic silica-coated ($\text{Fe}_3\text{O}_4@\text{SiO}_2$) demonstrates that this composite is biocompatible [8].

Table 1: A summary of different used $\text{Fe}_3\text{O}_4@\text{SiO}_2$ structure in biological applications

| Nanocomposite type | Application | Ref. |
|---|---|-------------|
| Lamivudine functionalized $\text{Fe}_3\text{O}_4@\text{SiO}_2$ | Drug delivery of Lamivudine | [9] |
| $\text{Fe}_3\text{O}_4@\text{SiO}_2$ @poly-l-alanine peptide brush-magnetic microsphere | Targeted drug delivery and protein enrichment | [10] |
| $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles | Immobilized enzyme Laccase | [11] |
| $\text{Fe}_3\text{O}_4@\text{SiO}_2$ magnetic core-shell nanoparticles | Purification of plasmid DNA from E. coli | [12] |
| pSBMA-Conjugated Magnetic Nanoparticles | Selective IgG Separation | [13] |
| magnetite cluster@silica@albumin double-shell | Drug delivery of curcumin | [14] |
| PAA-modified Fe_3O_4 @silica core/shell microspheres | adsorption and separation of lysozyme | [15] |

Table. 1 presents a summary of different used $\text{Fe}_3\text{O}_4@\text{SiO}_2$ structures in biological applications such as enrichment, separation, detection, and purification. Magnetic nanoparticles with functional ligands may have enhanced ability toward the targets with different functional groups. Herein, we prepare the amine-functionalized magnetic silica nanoparticles for biological applications. The surface modification of silica can be carried out with alkoxy silanes via a silylation reaction. Among the silanating agents, 3-aminopropyl-triethoxysilane (APTES) are of special interest owing to their biofunctional nature. The unique properties imparted by the amine group, along with its capability for reaction with other functionalities have been the source of its popularity as a silanating agent. Firstly, we synthesized the silica-coated magnetic nanoparticle with uniform and narrow size distribution through a co-precipitation method and hydrolysis of tetraethylorthosilicate (TEOS). Then, amine functionalization was performed by using the 3-aminopropyl-triethoxysilane. An ultraviolet and visible spectrophotometer measured the capacity of these particles for protein adsorption ability. The properties and surface morphologies of nanoparticles were investigated



by Scanning electron microscopy (SEM), Fourier transforms infrared spectroscopy (FT-IR), Powder X-ray diffraction (XRD), Dynamic light scattering (DLS).

Experimental

Materials

Ferrous chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), oleic acid (99 vol%), ammonia (25 vol%), ethanol, methanol, toluene (99.9 vol%), tetraethyl orthosilicate (TEOS, 99 vol%) were purchased from Merck. Bovine serum albumin (BSA) and 3-Aminopropyl-triethoxysilane (APTES) were obtained from Sigma-Aldrich.

Methods

Synthesis of $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-NH}_2$

Oleic acid-coated magnetic nanoparticles were prepared via the co-precipitation method as described previously [16]. Briefly, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ with a molar ratio of 2:1 were dissolved in deionized water under a nitrogen atmosphere with vigorous stirring. After the addition of the oleic acid, aqueous ammonia solution was added all at once. Oleic acid (0.1 ml) was added to the solution at every 5 min intervals with continued stirring at 80 °C for four times. The reaction was then allowed to proceed at 1000 rpm for 30 min. The resulting dark brown suspension was collected with a magnet and repeatedly washed with water. The synthesis of magnetite/silica core/shell nanocomposite (MS) was performed by the Stober method via the hydrolysis condensation of TEOS in the presence of Fe_3O_4 nanoparticles with slight modifications [4]. Briefly, the as-prepared magnetite nanoparticles were dispersed in toluene and ethanol, deionized water, and ammonia. Emulsion drops were added to the ethanol. The mixed solution was homogenized at room temperature and 400 rpm for 20 min. Then, 1 mL of TEOS was added to the solution by dropwise addition in 30 min under stirring at room temperature. The product was separated using a magnet and washed several times with ethanol and water. Finally, the $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ magnetic nanoparticle was functionalized with APTES according to a similar process presented in the literature [17]. Briefly, 1 g of dried $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ was dispersed in 100 mL of anhydrous toluene through ultrasonication, followed by the addition of APTES under vigorous stirring. The mixtures were sealed into autoclaves and heated at 90°C for 12 h. Then, the product was separated using a magnet and washed three times with toluene, methanol.

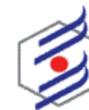
Performance in biological applications

Briefly, the protein solution of BSA with a determined concentration was prepared in PBS buffer. The magnetic nanoparticles produced at different concentrations of the solution were used, and the protein adsorption potential of it was determined using the Bradford assay.

Results and discussion

Structure characterization

The structure of synthesized particles was evaluated by X-ray diffraction characterization. Fig. 1 shows the spectrum of diffraction patterns taken from iron oxide nanoparticles and iron oxide nanoparticle /silica core/shell nanocomposite particles. The peaks shown in Fe_3O_4 spectrum are at 2θ equals to 21.6°, 35.3°, 41.6°, 50.6°, 63.2°, 67.5°, and 74.4° which are matched well to the characteristic peaks and their relative intensities of spinel structure of magnetite corresponding to the (111), (220), (311), (400), (422), (511), and (440) (hkl) planes (JCPDS 019-0629). The XRD pattern of $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ particles shows that the crystal structure of the magnetite nanoparticles core does not change after the formation of the silica



shell. The broad peak in the range $2\theta=20^{\circ}$ - 34° is due to the amorphous silica phase on the surface of magnetite nanoparticles [4, 7, 10].

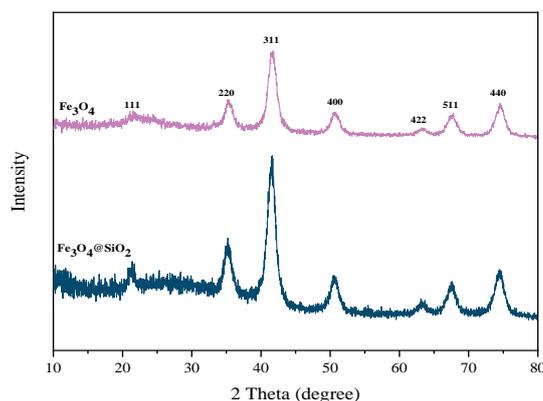


Figure 1. XRD spectrum of Fe_3O_4 and $\text{Fe}_3\text{O}_4@SiO_2$

Characterization of size and morphology

Fig. 2 presents the electron microscopy images of the synthesized particles. The Fe_3O_4 nanoparticles have a uniform diameter of 15 - 25 nm. The $\text{Fe}_3\text{O}_4@SiO_2$ particles, which were obtained via encapsulating of magnetite nanoparticles in a shell of silica, are near-spherical (170 ± 50 nm) and possess a core/ shell structure with a 110 ± 30 nm core and a 30 ± 10 nm silica shell.

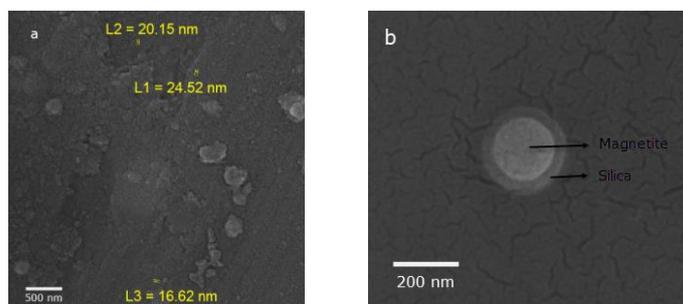


Figure 2. SEM of (a) Fe_3O_4 and (b) $\text{Fe}_3\text{O}_4@SiO_2$

FTIR analysis

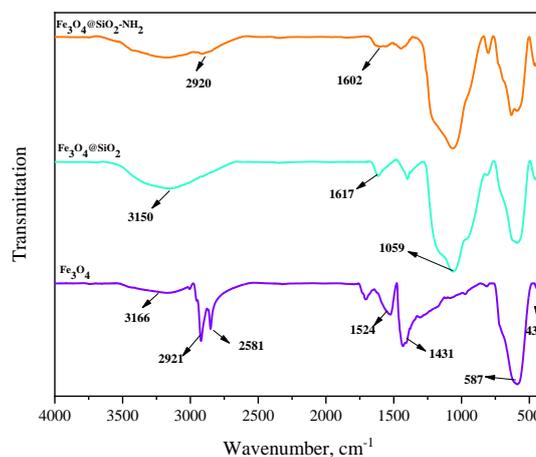


Figure 3. FTIR of Fe_3O_4 , $\text{Fe}_3\text{O}_4@SiO_2$, and $\text{Fe}_3\text{O}_4@SiO_2-NH_2$.

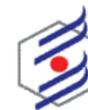


Fig. 3 shows the FTIR spectra of Fe_3O_4 and $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ particles. The peaks around 439 and 587 cm^{-1} in magnetite spectrum is the characteristic of Fe-O vibrations associated with the magnetite. The FTIR spectrum of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ particles indicates the peaks at 1059 cm^{-1} are assigned to Si-O-Si vibrations [4]. The presence of amino groups associated with the 3-aminopropyl of the alkoxy silanes was observed. The absorption bands 2920 cm^{-1} are ascribed to the C-N and C-H stretching vibrations, respectively. Broadband at 1602 cm^{-1} can be ascribed to the N-H stretching vibration, which is indicative of the free amino group [18].

Bioseparation by synthesized nanoparticles

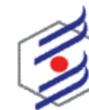
$\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-NH}_2$ was added to the BSA solution to PBS at pH 7. After one hour, magnetic separation was performed, and the remaining liquid analyzed with Bradford assay. Finally, a decrease in the concentration of BSA was observed that confirm with a decrease in the absorption of the solution in the UV spectrophotometer. Also, as indicated in the research method, the addition of nanoparticles to BSA solution to PBS was carried out with two concentration nanoparticle. The samples are placed inside the shaker for one hour at room temperature ($25\text{ }^\circ\text{C}$), and after this time using an external magnet, the nanoparticles isolated from the solution. Then, supernatant samples are diluted, and the absorption numbers were investigated in a UV spectrophotometer. Table. 2 shows the protein absorbance after 1 hour of exposure to a solution containing BSA with nanoparticles. Also, the DLS analysis performed to ensure the binding of amine nanoparticles with the protein. Before the protein adding, the nanoparticles had a size of 260 nm. After adding the proteins to the nanoparticles and the occurrence of the electrostatic interactions between the positive charge of amine and the negative charge of the carboxylic protein group, the protein binding occurred at the surface, and the particle size increase to 380 and 411 nm. The results of the adsorption protein showed the ability of the synthesized nanoparticle nanoparticles.

Table 2: Results of protein adsorption with synthesized nanoparticles

| sample | Concentration nanoparticle(mg/ml) | mg-protein/ g-NP | particle size (nm) |
|--------|-----------------------------------|------------------|--------------------|
| 1 | 2 | 20.68 | 380 |
| 2 | 4 | 19.55 | 411 |

Conclusions

We prepared a multilayered magnetic nano-bio hybrid structure with potential in the biological application. Capacity, high surface area, superparamagnetic properties, selectivity and also stability of the as-prepared nanoparticles makes them an appropriate candidate for target drug delivery, bioseparation, and immobilization enzymes. Magnetite nanoparticles cluster coated by silica shell as the inorganic phase of nanocomposite particles acts as the magnetic part with preserved magnetite nanoparticles from oxidation. By using the silanization reaction of amine molecules attached to the silica surface to provide more suitable conditions for increasing productivity in biological applications. Among the biological applications, bioseparation was selected to evaluate the performance of the synthesized nanoparticles. The results showed that the synthesized nanoparticles could be used as a matrix for the separation and delivery of biomolecules.



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