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Fe_3O_4/SiO_2 Core/Shell Nanocubes: Novel Coating Approach with Tunable Silica Thickness and Enhancement in Stability and Biocompatibility

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Abstract

Magnetic nanoparticles are frequently coated with SiO_2 to improve their stability, biocompatibility and functionality for they become promising for many biomedical applications, such as MR imaging contrast agents, magnetically-targeted drug delivery vehicles, agents for hyperthermia, etc. In our study, we demonstrated a novel and time reducing modified sol-gel approach for obtaining a uniform Fe_3O_4/SiO_2 core/shell nanocubes. Furthermore, the thickness of the silica shell is easily controlled in the range of 5-16 nm by adjusting the reaction parameters. The core/shell nanocubes samples were characterized by X-ray diffractometry (XRD), Transmission Electron Microscopy (TEM), Energy Dispersive Spectrometer (EDS), fourier transform infrared spectroscopy (FTIR), and vibrating sample magnetometer (VSM). The as-prepared Fe_3O_4/SiO_2 core/shell nanocubes showed good stability in air for at least 4 month as well as against annealing condition of up to 300°C in presence of H_2 gas as a strong reducing agent. Furthermore, high magnetization value of 50.7 emu/g was obtained for the sample with thin silica thickness (5 nm) as a consequence of shell thickness controlled. Moreover, the biocompatibility of the core/shell nanocube was enhanced in comparison to that of pristine Fe_3O_4 nanocubes. In addition, the Fe_3O_4/SiO_2 nanocubes were functionalized by Aminopropy-ltriethoxysilane, and then conjugated with streptavidin-Cy3 successfully as indicated by fluorescence microscopy.

Keywords: Fe_3O_4/SiO_2 nanocubes; Core/shell; Bio-compatibility; Stability; Magnetic properties

Introduction

In the last few years, considerable efforts have been devoted to surface functionalization of magnetic nanoparticles as such a coating could lead to obtain significant properties thereafter. Even though there are many kinds of materials available for coating of the magnetic nanoparticles, like metal oxide, noble metals and polymer material, the silica is still considered to be the best candidate for surface functionalization because it is highly stable against degradation. Furthermore, silica act to improve the biocompatibility, hydrophilicity as well as the surface functionality due to the availability of abundant silanol groups (-SiOH) on the surface [1], which makes it as a promising materials for different kinds of bio-applications.

For the time being, the Stober method as well as microemulsion method are the prevailing choices for surface coating of magnetic nanoparticles NPs with silica [2,3]. Following these well-known methods, many groups have been succeeded with some modification to coat silica on magnetic nanoparticles. However, these methods are complicated and require long time for the coating of silica. For example, Hui et al. have used the Stober method for coating silica to magnetite nanosphere within 24 h [4]. Roca et al. have used the sol-gel method to coat silica on maghemite nanosphere in the time range of 6-48 h [1]. Palani et al., Caruana et al., Zhang et al. and Ding et al. used microemulsion and reverse microemulsion methods for coating iron oxide nanosphere with silica in the time range of 16-48 h [5-8]. And, Shiva et al. have used the so-called general method for coating of silica to the iron oxide nanoparticles within 16 h [9]. Even though these methods provide great contribution and benefits in the controlling of silica shell thickness as well as nanoparticles dispersion, but they needed much amount of surfactant, long synthesis time and consequently involves high costs which of course inhibits their usage in the large scale of industrial applications.

On the other hand, shape of the nanoparticles becomes an important aspect for consideration while evaluating their use in bio-applications. Furthermore, the surface to volume ratio (S/V) of nanomaterial plays a significant role on the biomolecules immobilization for various bio-applications. Particularly, the geometries other than spherical are, however, preferred for applications involving magnetic-based bio-sensing devices, where a larger contact area can lead to more-robust binding to a sensor platform [10,11]. Thus, nanocubes could be the good candidate comparing with other shapes for the bio-application because of its high surface to volume ratio which can increase the density of biomolecules on the surface [12]. Therefore, integration of the cubic structure of magnetic NPs in the form of magnetite and thereafter functionalization with silica as a shell would obviously enhance its performance and thus becomes a promising material for these applications. The uniform coating of silica on magnetite nanocubes with controlled thickness being a challenging task for researchers because of the high tendency of nanocubes towards the aggregation and problem with retention of cubic shape after silica coating [11]. However, recently Kolhatkar et al. have reported 55 nm silica coated FeCo nanocubes using Stober method with long synthesis times [11]. Even though they succeeded in the coating process of silica onto nanocube shape,

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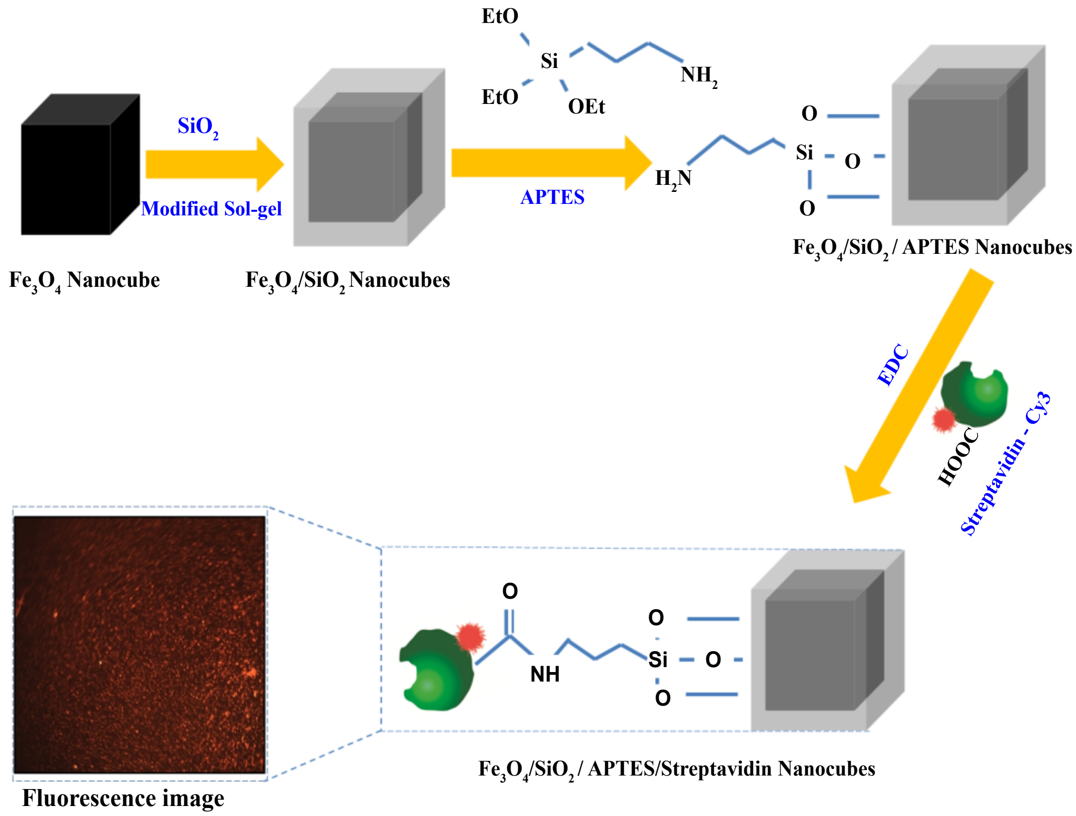

Figure 1: Schematic representation for the functionalization of the Fe_3O_4 nanocubes with SiO_2 and Streptavidin-Cy3.

their data do not provide adequate information about the silica shell thickness and also on the mechanism of controlling the shell thickness. Therefore, it is felt necessary to develop a time reducing-facile approach for synthesis and controlled surface coating onto the cube shaped iron oxide nanoparticles.

Herein, we first developed a novel and facile approach for the coating process of silica to the magnetic nanocubes shape within only four hours' reaction time and without the need of any much amount of surfactant. Furthermore, we could easily control the thickness of silica shell by just changing the ratio of Tetraethyl orthosilicate (TEOS) to Fe_3O_4 nanocube. The prepared core/shell nanocubes were characterized and analyzed by different techniques like XRD, TEM, EDS, FTIR, and the study of the magnetic properties versus silica thickness was done using VSM measurements. Afterwards, we mainly investigated the importance of silica coating in terms of stability (either in normal air or severe condition), biocompatibility and the affinity for biomolecule conjugation by attaching $-NH_2$ groups and then modified with streptavidin (SA).

Experimental Section

Materials

Iron (II) sulphatehepta hydrate ($FeSO_4.7H_2O$), Tetraethyl orthosilicate (TEOS), *Polyvinyl pyrolidone (PVP* M.W. 40.000*)*,(3-Aminopropyl) triethoxysilane (APTES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), Hydroxysuccinimide (NHS), Streptavidin-Cy3from streptomyces avid in protein, Sodium hydroxide (NaOH), Ammonia, and Ethyl alcohol (C_2H_5OH) (99%) were purchased from Sigma-Aldrich, Ltd. All chemicals were of analytical reagent grade and used as received without any further purification and the synthesis process was carried out under an ambient temperature.

Synthesis of hydrophilic magnetite (Fe_3O_4) nanocubes

The synthesis of magnetite(Fe_3O_4) nanocube was done here based on our previous reported work of one-pot sonochemical method with small modification [12,13]. In briefly, 8.5 mM of $FeSO_4.7H_2O$ was dissolved in 100 mL distilled water, and then the solution was transferred to ultrasonication reactor chamber. NaOH was injected in the reaction after 15 min of starting ultrasonication. The reaction continued up to 75 min and then turns off the ultrasonics. The obtained magnetite nanocube was washed for five times in water and ethanol while collecting the precipitate using a magnet, and then dried in a vacuum evaporator.

Synthesis of core/shell Fe_3O_4/SiO_2 nanocubes

The surface coating of the magnetite nanocube with silica was done using the modified sol- gel method. For a typical synthesis, two different bottles namely (a) and (b) were used; in the first bottle (a) we mixed 5 gm of PVP (M.W. 40.000) with 55 mL distilled water for 5 min using magnetic stirrer, and in the second bottle (b) we mixed 100 mg of a hydrophilic Fe_3O_4 nanocubes with 15 mL distilled water for 5 min. After that, we mixed the contents of both the bottles (a and b) using magnetic stirrer for two hour, then separated using a permanent magnet. The resulted PVP adsorbed magnetite nanocubes were added to a solution containing 100 mL ethanol and 5 mL ammonia, after that we added 1 mL of TEOS dissolved in 2 mL ethanol to the above solution while stirring the mixture for 2 hours. Then, the solution was washed several times using ethanol and water and collected the precipitate

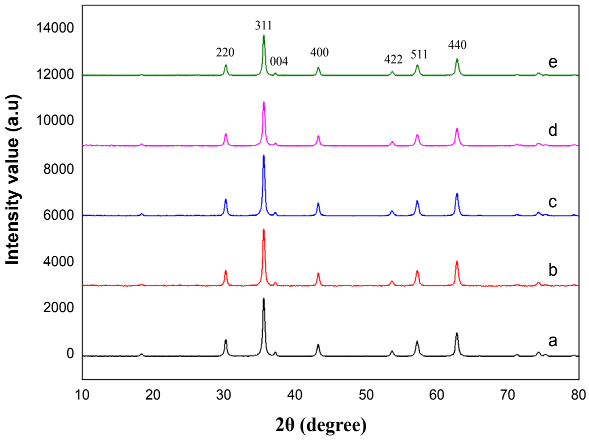

Figure 2: XRD pattern of (a) As-prepared Fe_3O_4 nanocubes and (b) Fe_3O_4/SiO_2 with 1 mL TEOS, (c) Fe_3O_4/SiO_2 with 1.5 mL TEOS, (d) Fe_3O_4/SiO_2 with 2 mL TEOS and (e) Fe_3O_4/SiO_2 with 2.5 mL TEOS.

using a magnet. It was subsequently dried in a vacuum oven to obtain ultrafine core/shell Fe_3O_4/SiO_2 nanocubes. By adjusting the amount of TEOS to Fe_3O_4, core/shell of Fe_3O_4/SiO_2 nanocubes with different shell thickness ranging from 5 to 16 nm can be achieved.

Streptavidin-Cy3 loading on APTES modified Fe_3O_4/SiO_2 nanocube

A solution of APTES in ethanol was prepared at a concentration of 200 µg/mL. 1 mL of as prepared APTES solution was added to Fe_3O_4/SiO_2 nanocubes (150 µg). To this solution, 10 µl of 29% ammonium hydroxide was added as a catalyst. The reaction mixture was sonicated for 3h. The particles were harvested by applying an external magnetic field and removed supernatant solution. APTES modified Fe_3O_4/SiO_2 nanocubes were washed with deionized (DI) water and dispersed in 1 mL PBS buffer, pH 7.4. To this, a 3 mg/mL streptavidin-Cy3 stock solution in PBS buffer (pH 7.4), NHS and EDC were added and kept for 12 hours at 4ºC. After this, Fe_3O_4/SiO_2 nanocubes were isolated by an external magnetic field followed by three washes with distilled water. Streptavidin modified Fe_3O_4/SiO_2 nanocubes were dispersed again in PBS buffer, pH 7.4 and stored at 4ºC. Figure 1 represents the scheme of this work which outlines the procedure we used to obtain the final structure of Fe_3O_4/SiO_2/APTES/Streptavidin-Cy3 (inset figure shows the fluorescence image of Streptavidin-Cy3-Fe_3O_4/SiO_2 nanocubes).

Characterization

The crystal structures of the synthesized core/shell nanocubes were analyzed by XRD (Regaku D/max-2500) at a voltage of 40 kV, a current of 300 mA and a scanning rate of 2°/min with step size 0.01º. The size and morphology of the nanoparticles were characterized using TEM (Tecnai G2 F20 operated at 200 kV). The chemical compositions of nanocubes were analyzed by EDS which is coupled withthe TEM. The FT-IR Spectroscopic data was taken in the spectral range from 4000-400 cm^{-1} to interpret the traces of surface coating of silica on the nanoparticles by FT-IR spectroscopy (Brucher Optic GmbH, Germany). Fluorescence microscopy was used for confirmation of immobilization of streptavidin on the core/shell nanocubes surface. The magnetic properties of the synthesized core/shell nanocubes were measured by VSM (Lake Share 7400) with an external magnetic field ranging from −15 kOe to +15kOe. An ultrasonic processor (VibraCell-VCF1500, Sonics and Materials) with a maximum power of 1500W was used for preparation of the nanocubes. The sonoreactor was equipped with a titanium horn having 5 cm^2 of irradiating surface area, and piezoelectric transducer supplied by a 20 kHz generator was immersed below the surface of the sonicated liquid.

Cell viability test

HeLa cells cultivated in Dulbecco's Modified Eagle Medium (DMEM) including the 10% fetal bovine serum and 1% penicillin-streptomycin. The cultured cells having 80~90% confluency were collected after treating them with 0.25% trypsin-EDTA and centrifuged at 1000 rpm for 1 min. Approximately, 1.0×10^4 HeLa cells were kept in the suspension load on cell culture plates and incubated for 12 hr. Then, nanoparticles with different concentration (0.01, 0.1 and 1 mg/ml) are loaded on HeLa cells and incubated for different times and up to 72 hours. The LIVE/DEAD Cell Viability assay kit was used to examine the cytotoxicity of nanoparticles. For this purpose, the HeLa cells were treated with 2 µM calcein AM and 4 µM ethidium homodimer-1 for 30 min. The stained cells were monitored by a fluorescence microscope (TE-2000, Nikon, Japan) equipped with a high resolution CCD camera (Coolsnap, Roper Science, USA). The software Image Pro (Mediacybernetics, MD, USA) was used for the fluorescence analysis of each obtained image.

Results and Discussion

The X-ray diffraction patterns of as-prepared magnetite nanocubes and silica coated samples are shown in Figure 2. The Figure 2 (line a) represents the peaks of the as-prepared Fe_3O_4 nanocubes with the crystal planes of (220),(311),(222),(400),(422),(511),(440), respectively. The strong and sharp peaks of Fe_3O_4 nanocubes indicate the formation of magnetite with a cubic inverse spinel structure. Figure 2 (lines b-e) represents the patterns of core/shell Fe_3O_4/SiO_2 nanocubes with silica shell thickness ranging from 5-16 nm. It is seen that the core/shell Fe_3O_4/SiO_2 reveals similar diffraction patterns to the Fe_3O_4 nanocubes which indicates that after the coating of silica the core nanocubes perseveres its crystallinity.

The structural morphology of Fe_3O_4 and Fe_3O_4/SiO_2 core/shell nanocubes was observed through TEM. Figure 3 shows the TEM images of as-prepared nanocubes (Figure 3a) and core/shell nanocubes (Figures 3b-3e) with different shell thickness ranging from 5-16 nm with respect to the amount of TEOS used for the coating of silica on Fe_3O_4 nanocubes. Figure 3a shows the as-synthesized magnetite sample, where the cubic shape with well crystallinity is clearly displayed, and it's consistent with the sharp peaks observed in XRD. The size of the nanocubes before coating is nearly 40 nm with narrow and uniform size distribution. Figures 3b-3e represents the TEM images of the coated silica samples which reveal that, the silica is successfully coated on Fe_3O_4 nanocubes to form core/shell nanostructures which can be clearly identified by the two different contrasts observed for Fe_3O_4 and silica in images. The TEM images indicate that by increasing the amount of TEOS (From 1 mL to 2.5 mL), the silica shell thickness also increases sharply in the range from 5-16 nm. In ordered to observe the detail structure of the Fe_3O_4/SiO_2 core/shell nanocubes, we used high resolution TEM for the sample 2 mL TEOS used for coating process (Figure 3f). Generally, the magnetic nanoparticles with high surface free energy tend to aggregate for achieving a stabilized state [14]. Furthermore, in the absence of an efficient surface coating, the formation of agglomeration and aggregates results from the strong attraction force between the magnetic nanoparticles [15]. However, even after coating with silica, still there is some aggregation between nanoparticles, which can be seen from TEM images.

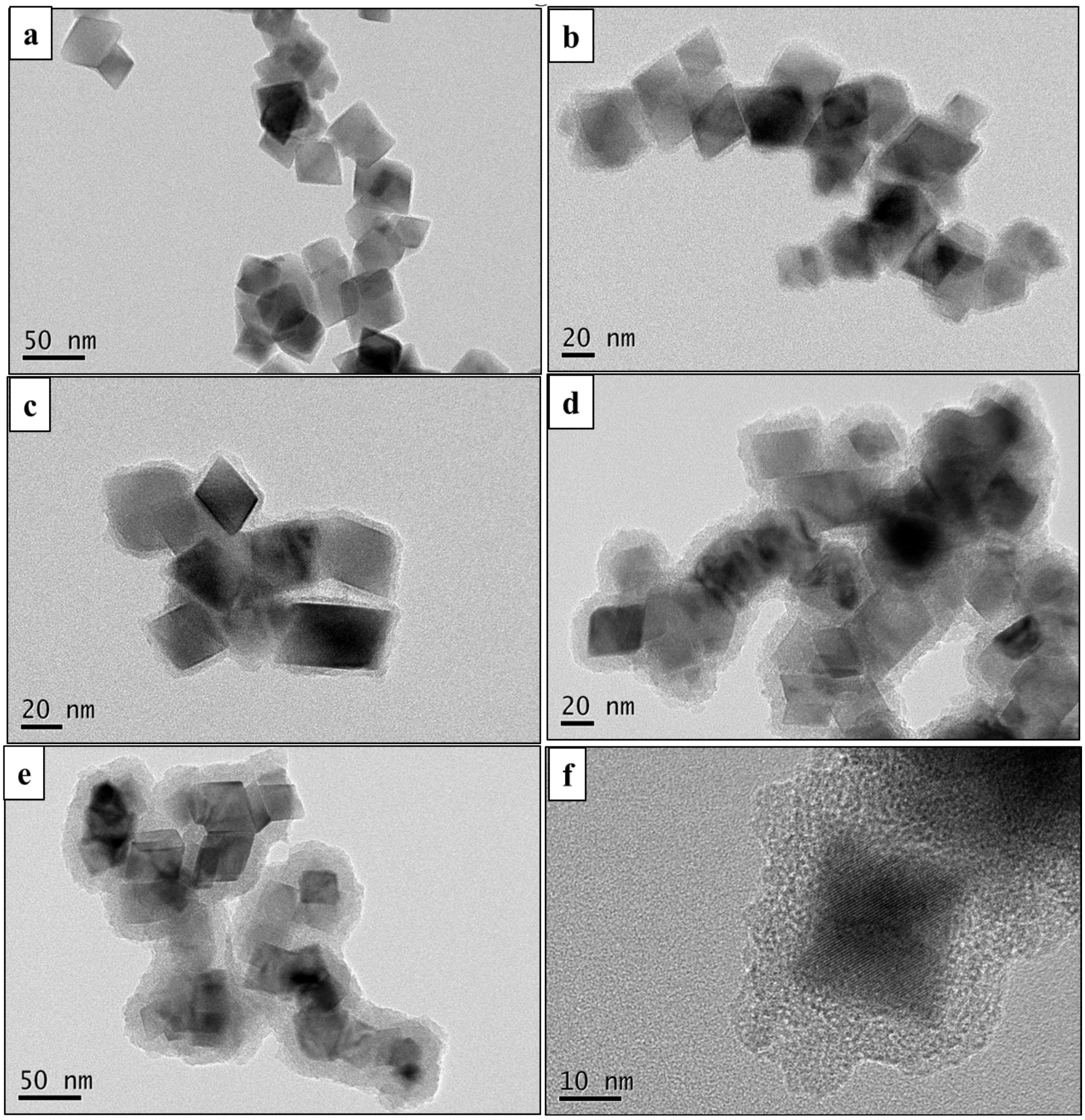

Figure 3: TEM images of (a) As-prepared Fe_3O_4 nanocubes, (b) Fe_3O_4/SiO_2 with 1 mL TEOS, (c) Fe_3O_4/SiO_2 with 1.5 mL TEOS , (d) Fe_3O_4/SiO_2 with 2 mL TEOS and (e) Fe_3O_4/SiO_2 with 2.5 mL TEOS, and (f) HRTEM of Fe_3O_4/SiO_2 with 2 mL TEOS.

The further confirmation of the core/shell nanostructures was observed by EDS analysis. The nominal elemental composition of Fe_3O_4 nanocubes and Fe_3O_4/SiO_2core/shell nanocubes was shown in Figure 4. From the Figure 4, it is clear that the EDS spectrum for Fe_3O_4 contains only iron and oxygen elements whereas the presence of iron, oxygen and silicon in core/shell spectrum confirms that the synthesized core/shell nanostructure is composed of Fe_3O_4/SiO_2. There are no any other impurity elements present in Fe_3O_4 nanocubes and Fe_3O_4/SiO_2 core/shell nanocubes which indicate that the physicochemical properties shown by nanocubes are completely an outcome of its own elements.

The further characterization of the synthesized Fe_3O_4/SiO_2 core/shell nanocubes with different TEOS content is obtained through the FTIR spectrum which is shown in Figure 5. The absorption peak observed at around 542 cm^{-1} represents the vibration band of Fe-O functional group which is a characteristic peak of Fe_3O_4 was observed for both Fe_3O_4 nanocubes and Fe_3O_4/SiO_2 core/shell nanocubes. Furthermore there is no longer any peak around 632 cm^{-1} is observed which the representative peak for Fe_2O_3 indicating that the samples are purely magnetite Fe_3O_4 [16]. The broad absorption band appeared around 1130 cm^{-1} in Fe_3O_4/SiO_2 core/shell nanocubes is related to the asymmetric vibration of Si-O-Si bond. In addition, the absorption peak at around 900 cm^{-1} represents Si-OH bond. As expected the intensity of these peaks increases with added volume of TEOS for various samples due to the increase in silica shell thickness. The broad peak at around 3500-3000 cm^{-1} attributed to the stretching bend of –OH functional group of chemisorbed water which is significantly increased with increasing the TEOS content due to the presence of silica. Consequently suggests another confirmation of the presence of silica on the magnetite nanocubes.

The coating approach we used in our study is based on the well –established sol-gel method of Graf et al. [17]. However in their study, they coated the noble metal with silica and not magnetic materials. Hence the coating process involves mainly two reaction of nucleation (hydrolysis) and growth (condensation), and the silica

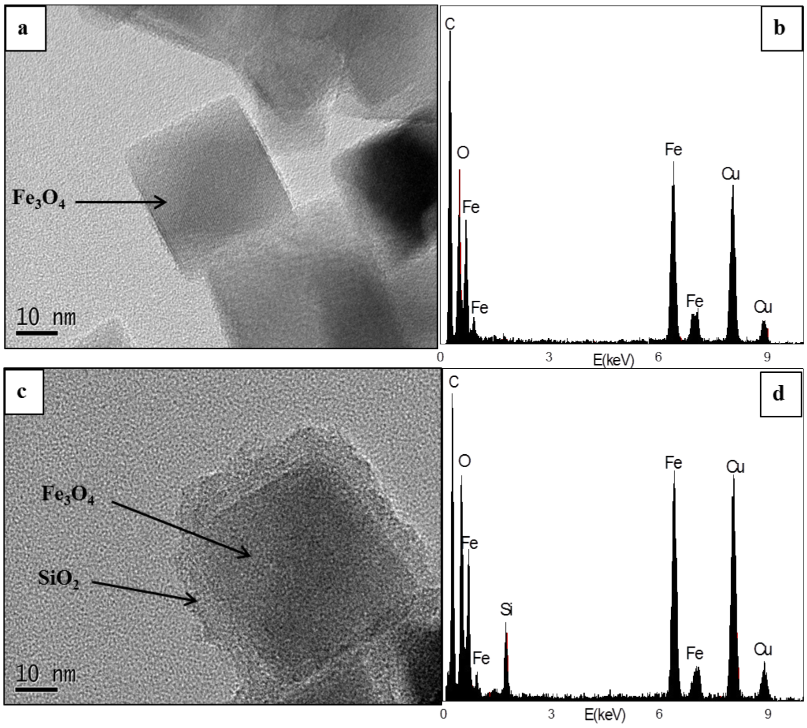

Figure 4: (a) TEM image of Fe_3O_4 nanocube which is to be used for EDS measurement, (b) EDS spectrum of Fe_3O_4 nanocube, (C) TEM image of Fe_3O_4/SiO_2 nanocube and (d) EDS spectrum of Fe_3O_4/SiO_2 nanocube.

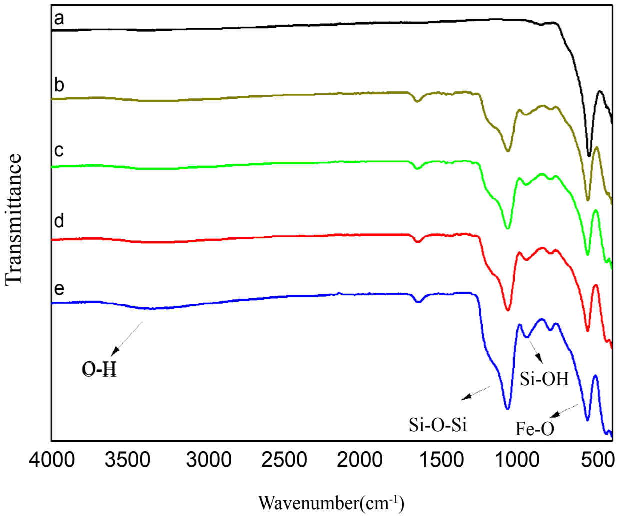

Figure 5: FTIR analysis data for (a) As-prepared Fe_3O_4 nanocubes and (b) Fe_3O_4/SiO_2 with 1 mL TEOS, (c) Fe_3O_4/SiO_2 with 1.5 mL TEOS , (d) Fe_3O_4/SiO_2 with 2 mL TEOS and (e) Fe_3O_4/SiO_2 with 2.5 mL TEOS.

tends to predominantly coat onto the magnetic nanoparticles as the growth rate is much higher than nucleation rate [4], one must be very careful of reaction parameters optimization. Based on the reported method, it was found that lower temperature, lower pH value, lower TEOS concentration, and less H_2O are predominant in facilitating the condensation process and consequently the coating process [18-20]. However, in our study, we found that, synthesis of 40 nm hydrophilic Fe_3O_4 nanocubes and functionalization with PVP are important step for easy coating with silica. Since the PVP is an amphophilic polymer that is soluble in water and many non-aqueous solvents [21,22], and this behavior arises from the presence of a highly polar amide group within the pyrolidone ring and polar methylene and methine groups in the ring and along its backbone [23]. Furthermore, and to be exact, we found that mixing of 4 g PVP in 55 mL dis water to 100 mg of a hydrophilic Fe_3O_4 nanocube in 15 mL dis water to 100 mL ethanol in 5 mL ammonia is resulting in a better performance. Interestingly, the time of the total coating process is only four hours with respecting to the reported work which takes more than 20 hours as clearly indicated from the comparison with some other groups work in Table 1. The thickness of silica coated on magnetite nanocubes can be tuned in the range from 5 to 16 nm as evident from the TEM images in Figures 3b-3f. A part from Figure 6 shows the relation between the TEOS amount used versus the silica shell thickness obtained at constant experimental parameters with four hours of reaction time. On the other hand, increasing the time of reaction for more than two hours resulted in formation of separated silica nanospheres, as clearly appeared from the TEM images in Figures7a and7b. Further, the intensity of the silicon (Si) peak was increased sharply as observed from the EDS analysis (Figure 7c). We also studied the influence of Fe_3O_4 nanocubes concentration on the coating process and found that the concentration of the Fe_3O_4 does not affect the thickness of the silica shell, and this result was in agreement with the earlier report [4].

The magnetic hysteresis loops of the prepared Fe_3O_4/SiO_2 core/shell nanocubes samples were measured at room temperature using VSM in an external magnetic field ranging from −15 kOe to + 15kOe, and compared with the uncoated magnetite nanocube as shown in Figure 8. The saturation magnetization value, extracted from the corresponding

Coating method	Time used	References
Stober method	24 h	[4]
Reverse Microemulsion method	> 16 h	[8]
General method	> 16 h	[9]
Reverse Microemulsion method	48 h	[7]
Sol-gel method	6-48 h	[1]
Microemulsion method	24 h	[6]
Microemulsion method	20 h	[5]
Modified Sol-gel method (Our method)	4 h	-

Table 1: Total coating process.

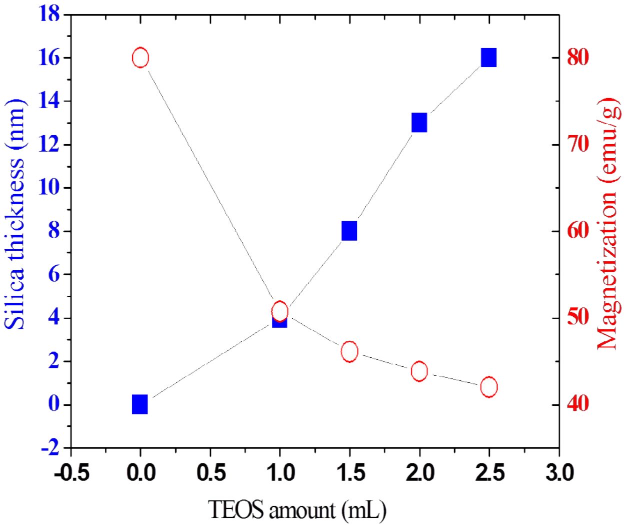

Figure 6: The relation between magnetization values (Ms), TEOS amount and silica thickness.

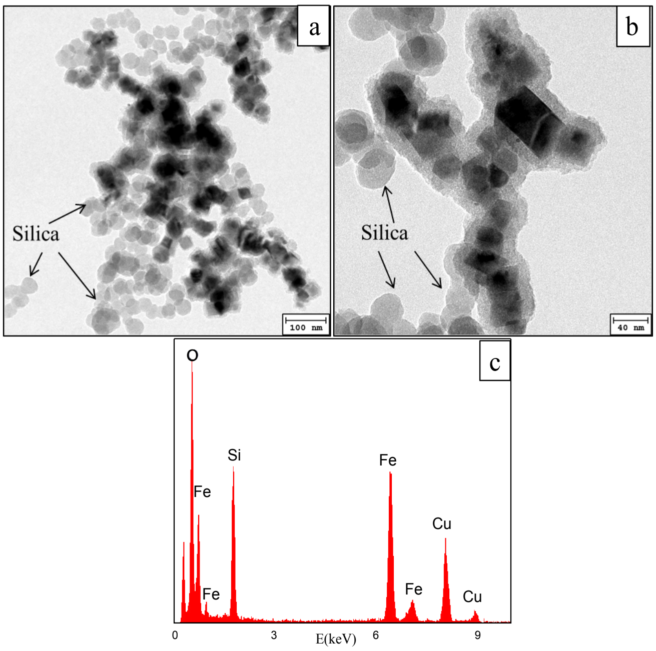

Figure 7: TEM images (a and b), and EDS analysis (c) of Fe_3O_4/SiO_2 nanocubes in case of increasing the coating time more than two hours.

hysteresis loop, for the uncoated magnetite sample at 300 K is 80 emu/g, and it decreased for the coated samples as expected to be 50.7, 46.1, 43.8, and 42 emu/g, respectively with increasing the thickness of silica shell. The decrement in magnetization value after coating with silica in all the samples may be attributed to the incorporation of non-magnetic silica shell around the core magnetite nanocubes. Another possible mechanism that could be proposed here for the reduction in the magnetization value after coating is the presence of chemical bonds such as (Fe-O-Si), as a consequence of surface functionalization with SiO_2. Because of the silica coating, the Fe ions particularly at the surface tend to bonding with silica as Fe-O-Si, and as a result the magnetic moment of the Fe ions would get disappeared [24]. Similar results of decrement in magnetic moment value after functionalization with silica have been reported [25,26]. Figure 6 partly shows the relation between the saturation magnetization values verses different silica shell thickness based on the amount of TEOS used in the coating process. Noteworthy, it may be noted that the cubic shape of the nanoparticles seem to have affected the value of magnetization compared to that of the spherical shape. Since, in most of the reported paper concerned with the silica coated magnetic nanospheres, there was a remarkable reduction in the magnetic moment after coating with silica which may be ascribed to their corresponding geometries. Interestingly, the relatively high magnetization valueof 50.7 emu/g (for the sample with 4 nm silica thickness) which reported in our work as a consequence of controlling in the silica shell sickness is favorable for many applications, especially the targeted drug delivery application which need magnetic nanoparticles with high magnetic moment for easy manipulation. On the other hand, and as it's expected based on the reported studies [26,27], the coercivity (Hc) values are little decreased in the range from 203 Oe for the bare magnetite nanocube to 190 Oe with increasing in the thickness of the coated silica samples. The reason for such decreasing of coercivity value after coating with silica may be because lower specific absorption rate (SAR) for the silica coated samples compared with the pure magnetite nanoparticles [28] and this decrease in SAR ascribed to surface spin effects [26]. Moreover, the silica shell encapsulating the magnetite particles screens and decreases the magnetic dipole coupling interactions between neighboring magnetic nanoparticles, and thereby reducing the coercivity value from hysteresis loops measurements [29,30].

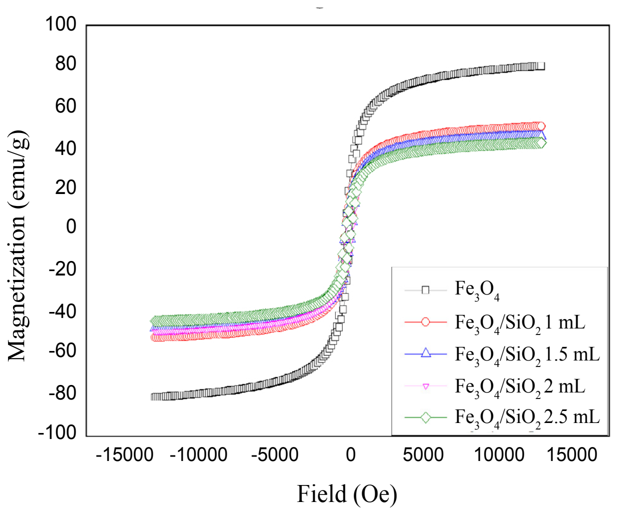

Figure 8: The magnetization curves for both Fe_3O_4 nanocube and Fe_3O_4/SiO_2 nanocube with different TEOS amount (1, 1.5, 2, and 2.5 mL) measured by VSM at 300 K.

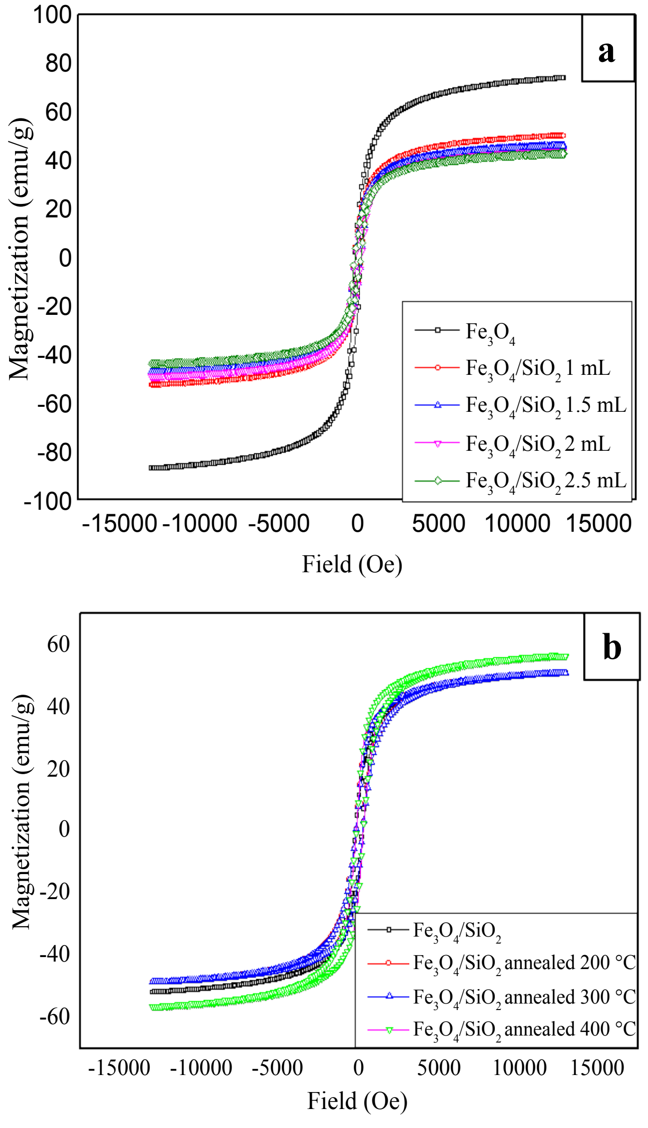

Figure 9: The magnetization curves for both Fe_3O_4 nanocube and Fe_3O_4/SiO_2 nanocube with different TEOS amount (1, 1.5, 2, and 2.5 mL) measured by VSM at 300 K in case of (a) after 4 month of exposure to air and (b) after annealed the sample at different temperatures in presence of H_2 gas.

Since the stability of the core/shell nanostructure is considered to be very important issue for practical applications. Thus, the stability of the prepared Fe_3O_4/SiO_2 core/shell nanocube was investigated and analyzed the changes in terms of their magnetic measurements. Figure 9a shows the magnetization curves for the as-prepared Fe_3O_4 and the Fe_3O_4/SiO_2 core/shell nanocubes after exposure of both the samples to normal air for 4 month. From the figure, it is clearly apparent that there is a marginal change in the magnetization value of the Fe_3O_4 by recording a decrease of its value from 80 emu/g to 72 emu/g; however, the silica coated sample does not experience any change in its magnetization value even after such an exposure. Further, the confirmation about the stability of the core/shell nanocubes against severe condition was tested by annealing the coated samples in the presence of hydrogen gas as a strong reducing agent at different temperature. Figure 9b shows the hysteresis curves of the coated nanocubes samples at 200, 300, and 400°C. From the figure, it is clearly shown that the sample is completely stable at 200 and 300°C, where as a further increase in the annealing temperature up to 400°C resulted in little increment in the magnetization value from that of 50 emu/g to 56 emu/g, which could be ascribed to increased structural order, increase in particle size and also to a minor degree of cracks in the silica layer.

Hence, the streptavidin (SA) is one of the most common globular protein used in imaging, sensing, drug delivery, and surface modification of nanoparticles which used in combination of nanoparticles very often [31], therefore, in our study we used streptavidin for the surface functionalization of core/shell nanocubes to confirm the capability of our nanocubes for binding the biomolecules. After preparing the Fe_3O_4/SiO_2 core/shell nanocubes, we functionalize the surface with APTES as illustrated in the schematic diagram (Figure 1) for the conjugation of the streptavidin. Figures 10a and 10b shows the bright field and fluorescence images of the $Fe_3O_4/SiO_2/$ APTES/streptavidin complex, respectively. The $Fe_3O_4/SiO_2/$ APTES/streptavidin complex shows good fluorescence intensity. From the fluorescence image it is clear that the Fe_3O_4/SiO_2 core/shell nanocubes are successfully functionalized with streptavidin-Cy3 protein. The mechanism of binding of streptavidin-Cy3 to the nanocubes could be explained by following way, the oxygen from SiO_2 layer is easily bonded to APTES to form terminal amine groups on the nanocubes surfaces and this amine group is conjugated by the carboxylic group presented in the streptavidin-Cy3 protein via covalent amide bond.

On the other hand, since the bio-compatibility is one of the

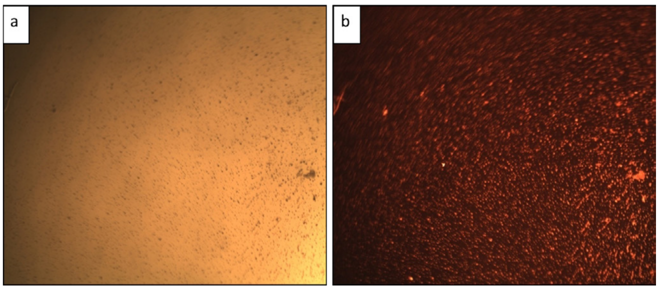

Figure 10: The electron microscopy images of the Fe_3O_4/SiO_2/APTES/SA samples with (a) Bright field image and (b) Fluorescence image.

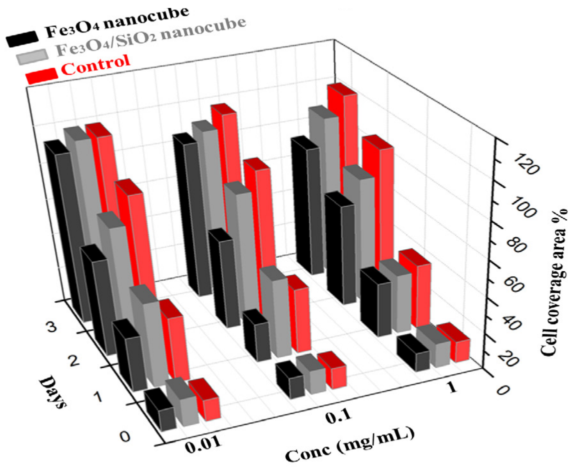

Figure 11: The biocompatibility test for the Fe_3O_4 and Fe_3O_4/SiO_2 nanocubes and the control.

important issues from the view point of practical bio-application, our study also focused on studying the effect of concentration of the prepared Fe_3O_4/SiO_2nanocubes on the growth of HeLa cells. In order to study the effect of core/shell nanocubes on the viability of HeLa cells, the cells were incubated with different concentrations (0.01, 0.1 and 1 mg/mL) of the Fe_3O_4 as well as Fe_3O_4/SiO_2 core/shell nanocubes samples at different times of 1-3 days and compared with the control (Figure 11). The cell survival rate increased with the culture days increased and concentration. Thus, we consider that our core/shell nanocubes exhibited good biocompatibility to the HeLa cells up to the dose of 1mg/mL concentration. The origin images from which we estimated the results in Figure 11 are provided as supplementary information (Figure S1).

Conclusion

In summary, we demonstrated a facile and novel approach for the synthesis of Fe_3O_4/SiO_2 core/shell nanocubes in which magnetite nanocubes can be functionalized with uniform silica shell. Furthermore, we could easily control the silica thickness in the range from 5 to 16 nm with just changing the amount of the TEOS from 1 to 2.5 mL. The synthesized core/shell nanocubes showed excellent magnetization value, bio-compatibility as well as the bio-molecule binding affinity. We expect that the high magnetization value of 50.7 emu/g and good stability, biocompatibility as well as affinity for binding biomolecule of our core-shell nanocubes could make them as promising materials for various bio-sensing applications.

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