CHAPTER-4

SURFACE COATING /FUNCTIONALIZATION OF IRON OXIDE NANOPARTICLES

4.1 INTRODUCTION

Magnetic iron oxide nanoparticles have a wide range of biological applications such as protein adsorption and purification; cell sorting, nucleic acid, enzyme immobilization, detachment; hyperthermia and drug release [148-152]. A magnetic iron oxide nanoparticle requires set of necessary particle properties for biological applications such as uniform size distribution, morphology and stability under physiological conditions. It is known that surface coating of magnetic iron oxide nanoparticles by polymers or proteins can satisfy the above conditions. In the absence of any surface coating/passivation, iron oxide nanoparticles are easier to agglomerate through hydrophobic interaction and form large clusters, resulting in increased particle size [153]. In addition, surface coating provides the ligand to bind special molecules such as proteins, antibodies, nucleic acids etc., on the surface of iron oxide nanoparticles [154-159].

In the recent years, researchers are concentrating on heating properties of iron oxide nanoparticles for magnetic hyperthermia applications. The heating properties of magnetic nanoparticles can be influenced by alternative magnetic field (AMF), its frequency, magnetic particle size, particle size distribution, surface treatment, concentration of nanoparticles and viscosity of the medium [160]. Surface treatment is essential to increase heating rate, stabilizing the nanoparticles within the carrier liquid and to increase the cellular uptakes [161, 162]. Recently [163], in vitro studies have been performed to
explore the mechanism and differential uptakes of biocompatible BSA coated iron oxide nanoparticles in different cancerous and isogenic cell types. All of these studies showed that BSA coated nanoparticles can provide a platform for targeted tumor thermal therapy. Some of the in vivo experiments have been performed for hyperthermic effect of dextran, aminosilane, liposom coated iron oxide nanoparticles and found that they are suitable for the efficient hyperthermic treatment due to their excellent biocompatibility, high accumulation in target tissue and water solubility [164-167].

In the present work, BSA has been chosen as modeling protein for coating the Fe₃O₄ nanoparticles and heating characteristics were investigated under different AMF of field strength and different frequency. In addition, researchers are interested on study of interaction between BSA and nanoparticles [168-170]. Wang et al have investigated the adsorption characteristic of BSA onto the magnetic chitosan [171]. The electrostatic attractions have been observed between positively charged magnetic chitosan and negatively charged BSA. In the recent years, the syntheses of metallic and iron oxide nanocomposites have attracted much attention due to their biomedical applications [172-174]. The metallic and iron oxide core/shell nanocomposites not only provided the stability to the nanoparticles in solution but also help in binding drugs, proteins, enzymes, antibodies and nucleotides on their surface for targeted delivery to specific organ [175-177].

In the recent years, considerable attention has been made on synthesis of multifunctional silica nanocomposite with unique magnetic or/and luminescent properties. These nanocomposite offer high potential applications such as MRI contrast agent, drug delivery carrier, cell sorting, labeling, diagnostic analysis, enzyme immobilization and bioseparation based on their unique magnetic, luminescence properties and low cytotoxicity and good biocompatibility [178-187]. Among optical materials investigated for such composites, organic
fluorescent compounds and quantum dots (QDs) are the most widely used as fluorescent labels although both of them have inherent limitations. For example, organic fluorescent compounds typically undergo rapid photobleaching [188-190], whereas semiconducting QDs contains heavy metals like Cd$^{2+}$, which raised serious health and environmental concerns [191]. Therefore, we explored heavy-metal-free fluorescence nanoparticles such as carbon nanodots. A fluorescence carbon nanodot is new class of fluorescent materials with less toxicity and good biocompatible material [192]. Recently, several methods have been developed by using QDs and magnetic nanoparticles [193, 194]. To synthesis magnetic-luminescent materials with high selectivity, good sensitivity and manipulation capability, we introduced superparamagnetic Fe$_3$O$_4$ nanoparticles to couple with the carbon nanodots using silica sphere as template. The method developed here is simple and straightforward and could be described as the assembly of carbon nanodots and Fe$_3$O$_4$ nanoparticles on silica sphere through electrostatic interaction.

4.2 ENHANCED HEATING CHARACTERISTICS OF BOVINE SERUM ALBUMIN COATED Fe$_3$O$_4$ NANOPARTICLES FOR MAGNETIC FLUID HYPERThERMIA APPLICATION

4.2.1 Preparation of BSA Coated Superparamagnetic Fe$_3$O$_4$ Magnetic Fluid

All reagents were of analytical grade and used without further purification. In a typical experiment, FeCl$_3$.6H$_2$O and FeSO$_4$.7H$_2$O were dissolved in deionized water with a molar ratio of 2:1 under vigorous mixing at 80 °C. Then, 120 mg of BSA was added into the mixture as a capping agent. Finally, NaOH was slowly added into the mixture until pH reached up to 11.
On addition of NaOH, the precipitate turned black, indicating the formation of Fe₃O₄ nanoparticles. Further, the mixture was stirred vigorously for 15 minutes and aged for 30 min at 70 °C and then the reaction mixture was cooled down to room temperature. Fe₃O₄ nanoparticles were separated by magnetic separation and washed by deionized water repeatedly to remove the impurities. Finally, the as synthesized BSA coated Fe₃O₄ nanoparticles were dispersed in deionized water by ultrasonication for 30 min to prepare the magnetic fluid.

4.2.2 Characterization of BSA Coated Fe₃O₄ Nanoparticles

The heating measurements were made with AMF generator incorporating a solenoidal with ferrite core [195]. The schematic of representation AMF setup is shown in Fig. 4.1.

![Schematic of AMF setup](image)

**Figure 4.1** Schematic representation of alternating magnetic field setup.

The measurements were performed at magnetic field amplitude of 2.9-3.5 kA m⁻¹ and frequency of 130-300 kHz. Approximately 0.7 ml of magnetic fluid with concentration of about 15 mg ml⁻¹ was placed in a sample
tube. Temperature was monitored using optical thermometer (FX-9020, Anritsu Meter CO.,). SAR was calculated from the relation,

\[
SAR = \frac{C_{\text{water}}}{c} \left( \frac{\Delta T}{\Delta t} \right)
\]

where, \( C_{\text{water}} \) is the specific heat capacity of water (4.18 J g\(^{-1}\) K\(^{-1}\)), \( c \) is concentration of sample (15 mg ml\(^{-1}\)) and \( \Delta T/\Delta t \) (°C s\(^{-1}\)) is initial slope of the temperature with respect to time.

### 4.2.3 Results and Discussion

Fig. 4.2 shows the XRD pattern of BSA coated Fe\(_3\)O\(_4\) nanoparticles, which exhibits the diffraction peaks for inverse spinel ferrite structure and consistent with JCPDS card (No: 19-0629). The average particle size of 9 nm was obtained using the Scherrer’s equation from full width at half maximum of the strongest peak (311). No impurity peaks were detected, indicating the high purity of the synthesized product. Fig. 4.3 (a) shows the TEM image of the BSA coated Fe\(_3\)O\(_4\) nanoparticles from which the size of the particles is estimated as 10 nm. The histogram shows the uniform distribution of BSA coated Fe\(_3\)O\(_4\) nanoparticles [Fig. 4.3 (c)]. This is consistent with the size obtained from the XRD.

The monodispersity of nanoparticles was achieved by reducing the interaction between superparamagnetic nanoparticles during synthesis through coating of BSA on the surface of the Fe\(_3\)O\(_4\) nanoparticles. Fig. 4.3 (b) shows HRTEM image of magnetic fluid, which implies that high crystalline quality Fe\(_3\)O\(_4\) nanoparticles were surrounded by BSA shell (indicated by the arrow). The interlayer spacing was calculated to be 0.25 nm, which corresponds to the (311) lattice planes of Fe\(_3\)O\(_4\).
Figure 4.2  X-ray diffraction pattern of BSA coated Fe$_3$O$_4$ nanoparticles.

Inset of Fig. 4.3 (a) is the corresponding SAED pattern, revealing that the nanoparticles are polycrystalline crystalline nature and can be indexed to inverse spinel structure of Fe$_3$O$_4$. Fig. 4.3 (d) shows the schematic representation of the formation of BSA coated Fe$_3$O$_4$ nanoparticles. The hydrodynamic diameter of nanoparticles is obtained from DLS measurement.

The hydrodynamic diameter of nanoparticles was found to be 27 nm (Fig. 4.4). The large hydrodynamic diameter as compared to the result obtained using TEM and XRD is due to the minor particle aggregation. BSA is composed of several amino acids. The $\zeta$ potential of BSA coated Fe$_3$O$_4$ nanoparticles are measured as -24 mV. This negative charge arises from the
unequally charged amino acids constituting the BSA molecules and providing the negative charge at pH = 7.4. This confirms the presence of BSA coating on Fe₃O₄.

Fig. 4.3 (a) TEM, (b) HRTEM micrograph, (c) Particles size distribution and (d) schematic representation for reaction of BSA coated Fe₃O₄ nanoparticles.

\[
\text{FeCl}_2 + 2\text{FeCl}_3 \xrightarrow{8\text{NH}_3, \text{H}_2\text{O}} 8\text{NH}_4\text{Cl} + 4\text{H}_2\text{O}
\]

Fig. 4.5 shows FTIR spectrum of pure BSA and BSA coated Fe₃O₄ nanoparticles. The pure BSA bands at 3446, 3066, 1653, and 1534 cm⁻¹ are assigned to the stretching vibration of OH, amide A (mainly N-H stretching vibrations), amide I (mainly C=O stretching vibrations) and amide II (the coupling of the bending vibration of N-H and the stretching vibration of C-N) bands, respectively [196]. All of the characteristic bands of BSA are observed from BSA coated Fe₃O₄ nanoparticles with characteristic band of Fe-O of iron oxide at 627 cm⁻¹. From the above results, it is concluded that existence of
interaction between the Fe$_3$O$_4$ nanoparticles and the N-H and COOH groups of BSA.

![Graph showing hydrodynamic diameter distribution](image)

**Figure 4.4** Hydrodynamic diameter distribution of BSA coated Fe$_3$O$_4$ magnetic fluid.

The conformational change of BSA molecules with nanoparticles can be specifically identified by the change in the intrinsic fluorescence of tryptophan (Trp) residues. BSA has two Trp residues located at internal part of domain II (Trp-213) and another one is buried on the surface of the molecules domain I (Trp-134) [197]. Fig. 4.6 (a, b) shows the PL spectra of BSA and BSA coated Fe$_3$O$_4$ nanoparticles. The both BSA and BSA coated Fe$_3$O$_4$ nanofluid show Trp emission peak at 342 nm. This confirms the presence of BSA in the BSA coated Fe$_3$O$_4$ nanoparticles. However, the intensity of Trp emission peak from BSA coated Fe$_3$O$_4$ is drastically reduced. This quenching of Trp emission of BSA in the presence of Fe$_3$O$_4$ nanoparticles implies the conformational change
of BSA due to the electrostatic and coordinative interaction with Fe$_3$O$_4$ nanoparticles [198].

Fig. 4.7 shows the thermogravimetric analysis of BSA coated Fe$_3$O$_4$ nanoparticles under nitrogen atmosphere at a heating rate of 5 °C min$^{-1}$ from 30 to 800 °C. From the TG curve of the BSA coated nanoparticles three stage of weight losses are observed. In the first stage, from 30 to 170 °C, because of removal of water take place from surface of the nanoparticles. The second stage, from 170 °C to 560 °C is probably due to the escape of the several kinds of small organic molecules, resulting from the intermolecular disintegration of the BSA molecules. Third stage, from 560 °C to 780 °C is due to the combustion of the residues [199]. The total weight loss was 49 % from 30 to 800 °C.

Figure 4.5 FTIR spectra of (a) BSA and (b) BSA coated Fe$_3$O$_4$ nanoparticles.
Figure 4.6  PL spectrum of (a) PL spectrum of BSA and (b) BSA coated Fe$_3$O$_4$ magnetic nanoparticles.

Figure 4.7  TGA curve of BSA coated Fe$_3$O$_4$ nanoparticles.
Fig. 4.8 shows magnetization curve for BSA coated Fe₃O₄ nanoparticles, which represent the no hysteresis loop and are completely reversible at room temperature; this indicates the superparamagnetic nature of nanoparticles.

The $M_s$ value of the BSA coated Fe₃O₄ nanoparticles was determined to be 35.5 emu g⁻¹. The magnetic particles diameter ($D$) was calculated using by Langevin equation.

$$D = \left( \frac{18k_BT}{\pi M_s} \sqrt{\frac{\chi_i}{3m_s H_0}} \right)^{\frac{1}{2}}$$

where $k_B$ is the Boltzmann constant and $T$ is the absolute temperature, $M_s$ and $m_s$ are saturation magnetization of bulk and nanosized Fe₃O₄ respectively, $\chi_i$ is the initial susceptibility calculated in the lower-field region where the $M$-$H$ relation is linear. $(1/H_0)$ is obtained by extrapolating $M$ to 0 at high field where the relationship between $(M)$ and $(1/H)$ is linear [200]. The calculated size of the nanoparticles is 8 nm, which is slightly smaller than particles size calculated from TEM measurement. This discrepancy can be explained by interparticles interactions and broader size distribution.

Magnetite nanoparticles were studied by ZFC and FC procedures. The magnetization as a function of temperature was performed between 5 K and 300 K under a field of 100 Oe and is shown in Fig. 4.8 (b). The FC and ZFC magnetization curves are splitted below blocking temperature ($T_b$) ($T_b$, the transformation temperature from ferromagnetism to superparamagnetism) and overlapped with each other above $T_b$ as the remanence and coercivity have vanished. The $T_b$ of as synthesized BSA coated Fe₃O₄ nanoparticles is 87.4 K.

The heating experiment of magnetite nanoparticles was performed at different frequency and magnetic field amplitude to suit hyperthermia application. The maximum field amplitude of 6.5 A m⁻¹ along with frequency of about 300 kHz can possibly be applied for the treatment of tumors [201].
However, magnetic field amplitude and frequency tolerance for normal tumor patients are not clearly known yet. In the present work, the magnetic field (3.5-290 kA m\(^{-1}\)) and frequency (130-300 kHz) have been taken for the heating measurement.

![Magnetization curve](image1.png)

*Figure 4.8*  (a) Magnetization curve (b) ZFC and FC magnetization curves at a magnetic field $H = 100$ Oe of BSA coated Fe\(_3\)O\(_4\) nanoparticles.
Table 4.1  SAR and slopes of the correlation curves with respect to field amplitude (at $f_{\text{const}} = 300$ kHz).

<table>
<thead>
<tr>
<th>Magnetic Field Amplitude (kA m$^{-1}$)</th>
<th>Initial Heating slope ($^\circ$C s$^{-1}$)</th>
<th>Specific Absorption Rate (W g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>0.346 ± 0.004</td>
<td>96.55</td>
</tr>
<tr>
<td>3.3</td>
<td>0.238 ± 0.003</td>
<td>64.18</td>
</tr>
<tr>
<td>3.1</td>
<td>0.157 ± 0.004</td>
<td>43.81</td>
</tr>
<tr>
<td>2.9</td>
<td>0.140 ± 0.002</td>
<td>39.06</td>
</tr>
</tbody>
</table>

Table 4.2  SAR and slopes of the correlation curves with respect to AC frequency (at $H_{\text{const}} = 3.5$ kA m$^{-1}$).

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>Initial Heating slope ($^\circ$C s$^{-1}$)</th>
<th>Specific Absorption Rate (W g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>0.346 ± 0.004</td>
<td>96.55</td>
</tr>
<tr>
<td>250</td>
<td>0.206 ± 0.002</td>
<td>57.48</td>
</tr>
<tr>
<td>200</td>
<td>0.155 ± 0.003</td>
<td>42.94</td>
</tr>
<tr>
<td>130</td>
<td>0.06 ± 0.002</td>
<td>16.74</td>
</tr>
</tbody>
</table>
Figure 4.9 Heating rate of magnetic fluid with respect to time for (a) different magnetic field amplitude (b) different frequency.
The heating rate of magnetite suspension was increased with increasing applied magnetic field amplitude and frequency for the time interval 0-250 s. Table 4.1 and 4.2 shows the SAR and slopes of the correlation curves with respect to AC frequency and field amplitude respectively. The maximum SAR of 96.5 W g\(^{-1}\) was observed at 300 kHz and field amplitude of 3.5 kA m\(^{-1}\). The SAR of BSA coated Fe\(_3\)O\(_4\) magnetic fluid obtained in the present study is relatively high compared to the uncoated and Dexran-coated Fe\(_3\)O\(_4\) nanoparticles [164, 201]. This is attributed to the narrow size distribution of the BSA coated Fe\(_3\)O\(_4\) nanoparticles and surface structure of the material. We have also found that the experimentally obtained SAR of BSA coated Fe\(_3\)O\(_4\) nanoparticles depends on both the frequency and field amplitude.

The specific absorption rate of superparamagnetic magnetite nanoparticles can be influenced by two possible relaxation mechanisms, namely Néel and Brown relaxation. Néel relaxation is the reorientation of magnetic moments within nanoparticles by a magnetic field, in which an anisotropy energy barrier \(E_a = kV\) is exceeded and thereby the heat is produced. The Néel relaxation time is given by following equation

\[
\tau_N = \tau_0 \exp \left( \frac{kV}{k_B T} \right) \text{..........................(4.3)}
\]

Brown relaxation is the rotation of magnetic nanoparticles within the carrier liquid and heat is produced by frictional force between nanoparticles [202] and carrier liquid and the Brown relaxation time is given by

\[
\tau_B = \frac{3\eta V_H}{k_B T} \text{..........................(4.4)}
\]

where \(\tau_0 = 10^{-9}\) s, \(K\) is the anisotropy constant 8 k J m\(^{-3}\), \(V\) is the volume of particle core [m\(^3\)], \(k_B\) is the Boltzmann constant [J k\(^{-1}\)], \(T\) is the absolute
temperature [K], $\eta$ is the viscosity of carrier liquid [k gm$^{-1}$ s$^{-1}$] and $V_H$ is hydrodynamic diameter of nanoparticles [m].

In order to distinguish the contribution of the Néel and the Brown relaxation times, the values were calculated using equation (4.3) and (4.4) respectively. Calculated Néel relaxation and Brown relaxation time were found to be $1.003 \times 10^{-9}$ s and $6.38 \times 10^{-3}$ s respectively. The Neel relaxation time is six order of magnitude shorter than the Brown relaxation time. Therefore, Neel relaxation is able to follow the faster change of applied magnetic field at low frequency. So the Néel relaxation is the only relaxation mechanism to dominate the SAR [203]. The critical size for a dominating Néel relaxation to SAR is about 16 nm. It may be noted that the Néel relaxation time is increases exponentially with diameter, whereas the Brown relaxation time increases linearly with respect to diameter which is seen from the eq. (4.3) and (4.4) mentioned earlier [4]. However, Brown relaxation may be considered ineffective in biological tissue [201].

4.3 SYNTHESIS AND CHARACTERIZATION OF POLYETHYLENIMINE CAPPED Fe$_3$O$_4$ NANOPARTICLES AND STUDY OF INTERACTION WITH BOVINE SERUM ALBUMIN

4.3.1 Synthesis of Polyethylenimine Capped Fe$_3$O$_4$ Nanoparticles

All reagents used were of analytical grade and used without further purification. In a typical experiment, $\text{FeCl}_3.6\text{H}_2\text{O}$ and $\text{FeSO}_4.7\text{H}_2\text{O}$ were dissolved in deionized water according to the molar ratio of 2:1 under vigorous mixing at 80 °C. Then 5 mmol of Polyethylenimine (PEI) was added into the solution as a capping agent. Finally, NaOH was slowly added into the mixture
until the pH increased to 11; while mixing of NaOH into the mixture black color precipitation was obtained. This indicates the precipitation of Fe$_3$O$_4$ nanoparticles. The mixture was stirred vigorously for 15 min and aged for 30 min at 70 °C. Then the reaction mixture was cooled down to room temperature. The Fe$_3$O$_4$ nanoparticles were separated by magnetic separation under magnetic field and washed by three times using deionized water to remove the impurities and reduce the pH values.

4.3.2 Sample Preparation For $\zeta$ Potential Measurements

The specific amount (10, 7, 5 and 2 mg) of protein was pre-dissolved in 15 ml of deionized water. The protein was pre-dissolved to favor homogeneous mixing of the added protein with the Fe$_3$O$_4$ colloidal suspension. In each case, the addition of the 15 ml of water from the dissolved protein solution reduced the volume fraction of the stock solution to exactly 3 %. A protein-free reference sample was prepared by simply adding 12 ml of water to a Fe$_3$O$_4$ colloidal suspension. For test samples, the pre-dissolved protein solution was added to the Fe$_3$O$_4$ colloidal suspension under stirring conditions for 1 h.

4.3.3 Results and Discussion

The crystalline nature and phase purity of the PEI capped iron oxide nanoparticles were determined by XRD as shown in Fig. 4.10. All XRD peaks can be indexed as inverse spinel structure of Fe$_3$O$_4$ phase, which is well consistent with standard value of bulk Fe$_3$O$_4$ (JCPDS Card No.75-0033). The crystalline size is calculated as 5 nm using Sherrer’s formula from the full width half maximum of strongest peak. The size of the synthesized nanoparticles is 5 nm and shown in Fig 4.11 (a) and consistent with particle size calculated from XRD. The HRTEM image shows that the particles are single crystalline
The interplanar spacing were calculated, which corresponds to the (311) plane of Fe$_3$O$_4$.

**Figure 4.10** XRD pattern of PEI capped Fe$_3$O$_4$ nanoparticles.

**Figure 4.11** (a) TEM and (b) HRTEM of PEI capped Fe$_3$O$_4$ nanoparticles.
Fig. 4.12 (a) shows the relative magnetization curve as a function of magnetic field at room temperature. The superparamagnetic property was observed with saturation magnetization ($M_s$) of 34 emu g$^{-1}$, which is lower than that of bulk (92 emu g$^{-1}$). This is attributed to surface spin canting effect for smaller particles. The magnetic particles diameter ($D$) can be calculated using by Langevin equation,

$$D = \left( \frac{18k_BT}{\pi M_s \sqrt{3m_s H_0}} \right)^{1/3} \text{....................(4.5)}$$

where $k_B$ is the Boltzmann constant and $T$ is the absolute temperature. $M_s$ and $m_s$ are saturation magnetization of bulk and nanosized $\text{Fe}_3\text{O}_4$, respectively. $\chi_i$ is the initial susceptibility calculated in the lower-field region where the $M$-$H$ relation is linear. $(1/H_0)$ is obtained by extrapolating $M$ to 0 at high field where the relationship between $M$ and $(1/H)$ is linear [200].

The calculated magnetic particles size is 3 nm, which is slightly smaller than that calculated from TEM and XRD measurements. This discrepancy is due to existence of nonmagnetic PEI on surface of the $\text{Fe}_3\text{O}_4$ nanoparticles. Temperature dependent magnetic properties of the nanoparticles measured by ZFC and FC measurements. Fig. 4.12 (b) shows ZFC and FC curve of PEI capped $\text{Fe}_3\text{O}_4$ nanoparticles from 300 K to 0 K under magnetic field of 100 Oe. The FC and ZFC magnetization curves are deviates below blocking temperature ($T_b$) and overlap with each other above $T_b$ as the remanence and coercivity have vanished [204]. The $T_b$ of as synthesized PEI capped $\text{Fe}_3\text{O}_4$ nanoparticles is 35 K.
Figure 4.12 (a) Hysteresis (b) ZFC and FC properties of PEI capped Fe$_3$O$_4$ nanoparticles.
In the present work, the interaction between BSA and PEI capped Fe₃O₄ nanoparticles were studied as a function of pH. The ζ potential of BSA and PEI capped Fe₃O₄ nanoparticles under various pH is shown in Fig. 4.13. The ζ potential of PEI capped nanoparticles were positive within pH range of 3-8.3 meanwhile ζ potential of BSA is negative within pH range of 4.9-11 and hence the electrostatic attractions are stronger. The adsorption of BSA was investigated under different pH for various concentrations. The moving of isoelectic point (IEP) for Fe₃O₄ nanoparticles is more for more adsorbed BSA molecules, which is obviously shown in Fig 4.13. The IEP of 10 mg of BSA with Fe₃O₄ nanoparticles colloidal suspension was very close to IEP of BSA because of one layer of BSA masked on surface of Fe₃O₄ nanoparticles.

![Figure 4.13 ζ potentials of Fe₃O₄ colloidal suspensions (3 vol %) with different amount of BSA under pH (3-11.5).](image-url)
4.4 SILVER COATED Fe₃O₄ NANOPARTICLES AND ITS PROPERTIES

4.4.1 Synthesis of Ag Coated Fe₃O₄ (Fe₃O₄@Ag) Nanocomposites

Fe₃O₄@Ag nanocomposites were prepared by two step process using microemulsion of cetyl trimethyl ammonium bromide (CTAB), 1-butanol as a co-surfactant and cyclohexane as an oil phase. In the first step, Fe₃O₄ nanoparticles were synthesized by co-precipitation of FeSO₄·7H₂O in water-in-oil microemulsion system. To form microemulsion (A), the oil solution was prepared by adding 0.8 mol of CTAB and 0.25 mol of butanol into 0.11 mol of cyclohexane (oil). The 0.07 mol of FeSO₄·7H₂O containing 10 ml of aqueous solution was added into oil solution and then NaOH solution was mixed under vigorous magnetic stirring to maintain the pH = 11. In the second step, the Ag shell was formed on Fe₃O₄ (core) by reducing AgNO₃. microemulsion (B), similar to microemulsion (A) having 10 ml aqueous solution containing 0.4 mol of AgNO₃ instead of FeSO₄·7H₂O. The microemulsion (A) and microemulsion (B) were mixed under magnetic stirring at room temperature and kept for 3 h. Then, 10 ml aqueous solution containing 0.8 mol of trisodium citrate dihydrate was added to the mixture in order to reduce the AgNO₃ as Ag. This mixture was kept in magnetic stirring overnight. Finally, reddish-brown precipitate was separated using a permanent magnet and subsequently centrifuged with methanol and water.

4.4.2 Results and Discussion

Fig. 4.14 show XRD patterns of (a) pure magnetite with cubic spinel structure (space group Fd3m), (b) silver nanoparticle with cubic close packed structure (space group Fm3m) and (c) Fe₃O₄@Ag with cubic close packed
structure (space group Fm3m). The XRD patterns of the samples were recorded from 20° to 90° (2θ). The sharp peaks of Fe₃O₄@Ag nanocomposites at 2θ = 38.719°, 44.65°, 64.82° and 81.867° correspond to the (111), (200), (220) and (222) Bragg’s reflection of Ag.

![XRD patterns](image)

**Figure 4.14** XRD patterns of (a) pure Fe₃O₄ nanoparticles, (b) pure Ag nanoparticles and (c) Fe₃O₄@Ag nanocomposites.

However, no other peaks are observed for Fe₃O₄ because thickness of the Ag shell is too high to penetrate the X-ray into Fe₃O₄ core [205]. This is a strong evidence for a complete coverage of the Fe₃O₄ nanoparticles by Ag. The TEM images of Fe₃O₄ nanoparticles and Ag coated Fe₃O₄ nanocomposites are shown in Fig 4.15. The Fe₃O₄ nanoparticles are spherical with average diameter
7 ± 2 nm [Fig. 4.15 (a)]. Ag coated Fe₃O₄ nanocomposites are also spherical with average diameter of 10 ± 15 nm [Fig. 4.15 (b)]. The coating of Ag on Fe₃O₄ nanoparticles were confirmed by the increase in the particles size compared to pure Fe₃O₄ nanoparticles. In addition, the Ag coated Fe₃O₄ nanoparticles are assembled as a branched like nanostructures. The formation of branched like nanostructures are discussed as follows:

Coupled coating of Ag on Fe₃O₄ nanoparticles and self-assembly of Fe₃O₄@Ag nanocomposites could be formed by microemulsion techniques. The coating of Ag on Fe₃O₄ nanoparticles achieved by percolation of AgNO₃ and Fe₃O₄ micelles in microemulsion system followed by reduction of AgNO₃ through trisodium citrate dihydrate. The following possible mechanism is proposed for the formation of Fe₃O₄@Ag nanoparticle assembly. The micelles in microemulsion can be stabilized by the tendency of the non polar tails to avoid contact with water and the repulsion among the polar or charged heads of CTAB. The aggregation of Fe₃O₄@Ag micelles takes place due to the following reasons: the hydrocarbon tails of CTAB avoid contacts with the water molecules pointing toward the aggregate interior of Fe₃O₄@Ag micelles, which may cause the lack of water. Instead, the repulsion among the positively charged heads of CTAB on the surface of the micelles is attenuated by the presence of oppositely charged ions (counter-ions) of CTAB such as Br⁻. Thereby, Fe₃O₄@Ag nanocomposites are attracted and self assemble.
Figure 4.15 TEM images of (a) Fe$_3$O$_4$ nanoparticles and (b) Fe$_3$O$_4$@Ag nanocomposites.

Fig. 4.16 shows hydrodynamic size distribution of pure Fe$_3$O$_4$ nanoparticles and Fe$_3$O$_4$@Ag nanocomposites respectively. Light scattering
technique such as DLS was used to measure the hydrodynamic diameter of the spherical nanoparticle. The hydrodynamic diameter of pure Fe₃O₄ nanoparticle about 28 nm was related to the translation diffusion of Fe₃O₄ nanoparticle [206]. No aggregation was found for pure Fe₃O₄ nanoparticles but in the case of Fe₃O₄@Ag nanocomposites the aggregation of nanoparticles was observed. Two distinguished peaks for Fe₃O₄@Ag nanocomposites at 986 nm and 5017 nm indicates the small and large aggregation of nanoparticles respectively.

Figure 4.16 Hydrodynamic diameters of (a) Fe₃O₄ and (b) Fe₃O₄@Ag nanocomposites at pH 7.4.

UV-Visible spectra of pure Fe₃O₄ nanoparticles, Ag nanoparticles and Fe₃O₄@Ag nanocomposites are presented in Fig. 4.17. The broad hump in the
absorption spectrum of pure Ag nanoparticles at 403 nm is due to resonant excitation of surface plasmons (SPR) in pure Ag nanoparticles [62]. Fe₃O₄@Ag nanocomposites show a clear SPR peak at 422 nm, this is due to the SPR of the Ag surface. The peak showed red shift, broadens and decay in intensity in comparison to pure Ag nanoparticles that were synthesized by chemical reduction method. The red shift was due to Fe₃O₄, which increase the effective local dielectric constant of Ag shell [207]. This effect is explained by the classical Mie theory. In contrast, the pure Fe₃O₄ nanoparticles do not show any absorption band.

![UV-Visible absorption spectrum of (a) Fe₃O₄, (b) Ag and (c) Fe₃O₄@Ag nanocomposites.](image)

Figure 4.17 UV-Visible absorption spectrum of (a) Fe₃O₄, (b) Ag and (c) Fe₃O₄@Ag nanocomposites.
The magnetization versus applied magnetic field \((M-H)\) curves obtained at room temperature for pure \(\text{Fe}_3\text{O}_4\) and \(\text{Fe}_3\text{O}_4@\text{Ag}\) nanocomposites are shown in Fig. 4.18 (a) and 4.18 (b). It can be observed the pure \(\text{Fe}_3\text{O}_4\) nanoparticles exhibit superparamagnetic behavior whereas \(\text{Fe}_3\text{O}_4@\text{Ag}\) nanoparticles show ferromagnetic property. The magnitude of the magnetization for \(\text{Fe}_3\text{O}_4@\text{Ag}\) (40 emu g\(^{-1}\)) is much smaller than the reported for bulk \(\text{Fe}_3\text{O}_4\) (92 emu g\(^{-1}\)) and \(\text{Fe}_3\text{O}_4\) nanoparticle (60 emu g\(^{-1}\)). The decrease in saturated magnetization values could be due to the contribution of overall mass from the nonmagnetic Ag shells [208].

Figure 4.18 Magnetization curves of (a) pure \(\text{Fe}_3\text{O}_4\) and (b) self assembled \(\text{Fe}_3\text{O}_4@\text{Ag}\) nanocomposites at room temperature.
4.5 NOVEL MULTIFUNCTIONALIZED NANOCOMPOSITES: MAGNETIC, LUMINESCENT AND SILICA SPHERES

4.5.1 Synthesis of amino-functionalized SiO₂ sphere

Silica nanospheres were synthesized at room temperature by a Stöber method. In a typical synthesis, 2.5 g of tetraethoxysilane (TEOS) was added into 4 ml of 5 mol ammonia solution in 80 ml of ethanol and 20 ml of water under stirring to hydrolyze TEOS. After 24 h of stirring, the solution was centrifuged and the precipitate was washed twice with ethanol and dried under vacuum resulting the formation of silica nanospheres. To synthesis the amino-functionalized silica sphere, silica spheres were dispersed into 80 ml of ethanol containing 0.1 ml of aminopropyltriethoxysilane (APS) under magnetic stirring. Finally, the amino-functionalized particles were collected by centrifugation and obtained amino-functionalized spheres were redispersed in 10 ml of water.

4.5.2 Synthesis of Citric acid Stabilized Fe₃O₄ Nanoparticles

In typical synthesis, FeCl₃·6H₂O (0.010 mol) and FeSO₄·7H₂O (0.005 mol) were dissolved in 50 ml of deionized water into three neck flask. Then NaOH solution (20 ml, 2 mol) was rapidly added into the flask under vigorous stirring at 70 °C in a nitrogen atmosphere. After being stirred for 30 minutes, the black precipitates were obtained and separated from the solution by a permanent magnet, washed with water three times to remove the unreacted impurity. The magnetic nanoparticles were dried under vacuum overnight. Citric acid stabilized Fe₃O₄ nanoparticles synthesized through procedures as reported by Steitz et al [209]. Final nanoparticles were then
subjected to a centrifugation to remove the excess citrate. The resulting product was redispersed in 30 ml of deionized water by ultra sonication.

### 4.5.3 Synthesis of Fluorescent Carbon Nanodots

Carbon nanodots (CDs) were synthesized by procedures described previously with little modification [210]. A 2 g of L-Ascorbic acid was dissolved in 30 ml of deionized water into glass beaker and then 30 ml of ethanol was added into the solution to form a colorless clear solution under magnetic stirring. 10 ml of ZnCl₂.6H₂O (7 mmol) solution was added into the mixture. Then, 30 ml of the mixture was transferred into a 50 ml Teflon-lined stainless steel autoclave and heated at 180 °C for 4 h and then cooled to room temperature naturally. A dark brown solution was obtained, which implied the formation of the CDs. After extraction with chloroform, the water-phase solution was dialyzed using cellulose ester dialysis membranes (Molecular Weight: 8000–14000 Dalton unit) for two days to remove all impurities. Finally, a yellow aqueous solution containing CDs was obtained.

### 4.5.4 Synthesis of Fe₃O₄@CDs@SiO₂ nanocomposites

A dispersion of 8 mg of citric acid stabilized Fe₃O₄ in 30 ml water and 20 ml aqueous solution of CDs were subsequently added with 10 mg of amino-functionalized SiO₂ in 30 ml of deionized water under sonication for half an hour at room temperature. The resultant product was isolated by magnetic separation. Finally, the synthesized products washed by water for several time to remove the impurities.
4.5.5 Results and Discussion

Fig. 4.19 shows the XRD patterns of the Fe$_3$O$_4$@CDs@SiO$_2$ nanocomposites, Fe$_3$O$_4$ nanoparticles and bulk Fe$_3$O$_4$. Fe$_3$O$_4$@CDs@SiO$_2$ nanocomposites corresponds to the face-centered cubic structure (Fd3m space group) of Fe$_3$O$_4$ nanoparticles and broad peak around 2θ = 22° attributed to amorphous phase of silica.

Fig. 4.20 (a) shows the general self-assembly strategy of the CDs and Fe$_3$O$_4$ nanoparticles on silica sphere. In a typical process, SiO$_2$ spheres were prepared by Stöber method [211]. Surface charge of the SiO$_2$ spheres were modified through functionalization by APS.

Figure 4.19 XRD of (a) bulk Fe$_3$O$_4$ (b) citrate stabilized Fe$_3$O$_4$ nanoparticles and (c) Fe$_3$O@CDs@SiO$_2$ nanospheres.
Figure 4.20 (a) Schematic representation to self assembly of magnetic, luminescent carbon nanodots on silica sphere, (b) TEM image of carbon nanodots, (c) TEM image of citrate stabilized Fe$_3$O$_4$ nanoparticles, (d) FESEM image of silica sphere, (e) FESEM image of Fe$_3$O@CDs@SiO$_2$ nanospheres and (f) TEM image of Fe$_3$O@CDs@SiO$_2$ nanosphere.

The $\zeta$ potential of CDs and citric acid stabilized Fe$_3$O$_4$ nanoparticles are measured by $\zeta$ analyser at pH = 7. The $\zeta$ potential of CDs and citric acid stabilized Fe$_3$O$_4$ nanoparticles are -30 mV and -25 mV respectively. The negative charge of CDs originated from the ionization of the carboxylic acid and hydroxyl groups that were located on the surface [212]. The amino-functionalized SiO$_2$ spheres shows the negative charge due to the alkylamines exist predominantly as positively charged R–NH$_3^+$ groups at pH = 10 [213]. The negatively charged CDs and citrate stabilized Fe$_3$O$_4$ nanoparticles were
assembled on the surface of the positively charged amino functionalized SiO$_2$ sphere through electrostatic interaction.

Fig. 4.20 (b) shows the representative TEM image of CDs nanoparticles. The synthesized CDs were well dispersed with a particle diameter of 2.0-5 nm. The size of Fe$_3$O$_4$ nanoparticles is 7 nm [Fig. 4.20 (c)]. The field emission scanning electron microscopy images of amino-functionalized SiO$_2$ spheres and Fe$_3$O@CDs@SiO$_2$ spheres are shown in Fig 4.20 (d) and 4.20 (e). Fe$_3$O$_4$ nanoparticles and CDs are well assembled on 100 nm amino-functionalized SiO$_2$ spheres and consistance with TEM image [Fig. 4.20 (f)].

Figure 4.21 FTIR spectra of (a), carbon nanodots, (b) citrate stabilized Fe$_3$O$_4$ nanoparticles and (c) Fe$_3$O@CDs@SiO$_2$ nanospheres.
The FTIR spectra were recorded to characterize the conjugation of Fe$_3$O$_4$ and CDs on silica sphere. Fig 4.21 shows the FTIR spectra of CDs, citrate stabilized Fe$_3$O$_4$ and Fe$_3$O$_4$@CDs@SiO$_2$. The band at 1650 cm$^{-1}$ is attributed to C=O vibrations due to the aromatization of L-ascorbic acid during the hydrothermal treatment [Fig. 4.21 (a)]. The bands in the range 800-900 cm$^{-1}$ assigned to C-H vibrations mode. The broad band from 3600 to 3100 cm$^{-1}$ is assigned to -OH stretching vibration arising from hydroxyl groups on nanoparticles. The band at 624 cm$^{-1}$ can be assigned to C-Cl vibration. From this band, it is confirmed that the CDs were synthesized in ZnCl$_2$ atmosphere. Fig. 4.21 (b) shows the strong IR band at 567 cm$^{-1}$ and this is due to Fe-O vibration [214, 215]. The existence of both CDs and Fe$_3$O$_4$ nanoparticles on the silica sphere was confirmed from Fig 4.20 (c).

![Figure 4.22](image_url)

**Figure 4.22** Absorption spectra of colloidal carbon nanodots (blue line) and the as synthesized Fe$_3$O$_4$@CDs@SiO$_2$ nanospheres in water (black line).
UV-Vis spectroscopy and PL spectrum were used to monitor the optical properties of CDs and Fe₃O₄@CDs@SiO₂ nanocomposites. Fig. 4.22 shows the absorption spectra of the CDs and Fe₃O₄@CDs@SiO₂ nanocomposites. The CDs shows absorbance at 413 nm (Fig. 4.22), whereas absorption of Fe₃O₄@CDs@SiO₂ nanocomposites is 437 nm which is shifted to higher wavelength side (Fig. 4.22). This may be attributed to the increase in scattering efficiency from larger particles or the absorbance from iron oxide nanoparticles [81]. Both CDs and Fe₃O₄@CDs@SiO₂ nanocomposites show the emission peak at 395 nm. However, emission intensity of the nanocomposite is drastically reduced compared to CDs (Fig. 4.23). This is due to the energy transfer between Fe₃O₄ nanoparticles and CDs on the silica sphere [216].

![Figure 4.23](image.png)

**Figure 4.23** PL spectra of colloidal carbon nanodots (blue line) and the as synthesized Fe₃O₄@CDs@SiO₂ nanospheres in water (black line).
Figure 4.24 Magnetic measurements of Fe₃O₄@CDs@SiO₂ showing (a) magnetization-applied magnetic field (M-H) and (b) ZFC and FC magnetization curves. The inset in (a) is a magnified view of the magnetization curve at low applied fields.
Magnetic properties of the Fe$_3$O$_4$@CDs@SiO$_2$ nanocomposite were characterized by VSM measurement. Fig. 4.24 (a) shows the hysteresis loop of nanocomposite at 300 K and 5 K. The nanocomposites are superparamagnetic at room temperature and ferromagnetic at 5 K. The magnified view of the magnetization curves at 300 K shows no hysteresis [inset of Figure 4. 24 (a)], which represent that the nanocomposite exhibit superparamagnetic behavior at room temperature. The saturation magnetization of the nanocomposite at room temperature is 2.5 emu g$^{-1}$ [217].

Fig 4.24 (b) shows the ZFC and FC curves of the nanocomposite under an applied field of 100 Oe. ZFC and FC curves coincide at high temperature ($T_b = 138$ K) and begin to separate as the temperature is decreased to 50 K. Such a behavior is characteristic of superparamagnetism due to the progressive deblocking of particles as the temperature increases [218]. This superparamagnetic property will allow the nanocomposite to be used in biomedical applications such as targeting and cell separation, due to the strong magnetic attraction between nanocomposite and the external field [219].

4.6 CONCLUSION

In conclusion, superparamagnetic BSA coated Fe$_3$O$_4$ magnetic fluid was synthesized by co-precipitation technique. The magnetic nanoparticles were spherical in morphology with a uniform size distribution. The mean diameter of the particles was 10 nm. The saturated magnetization of the nanoparticles reached 33.7 emu g$^{-1}$ and the nanoparticles showed superparamagnetism. The heating effect of ferrofluid was investigated for different AC magnetic field amplitude and frequency. The highest SAR of 96.5 W g$^{-1}$ was obtained under maximum field of 3.5 kA m$^{-1}$ and frequency of 300 kHz. The adsorption characteristics of BSA onto the PEI capped Fe$_3$O$_4$ nanoparticles have been investigated in this work. The PEI capped Fe$_3$O$_4$ nanoparticles were prepared
by the co-precipitation Fe$_3$O$_4$ particles within PEI. The morphology of PEI capped Fe$_3$O$_4$ nanoparticles were observed from TEM microgram. It was found that the diameter of PEI capped Fe$_3$O$_4$ nanoparticles were from 5 nm and the nanoparticles suspending in the aqueous solution could easily attached by a external magnet, which suggested that the nanoparticles had good magnetic characteristics. By measuring the zeta potential of BSA solution and the magnetic nanoparticles, it was found that the surface of BSA took the negative charge, but the magnetic nanoparticles took the positive charge.

The Fe$_3$O$_4$@Ag nanocomposites were synthesized by microemulsion method. The nanocomposites were self-assembled as a branched like nanostructure, which was visualized by TEM image. The sizes of nanocomposites were of 10-15 nm. The hydrodynamic diameter of nanoparticles was evaluated by DLS studies. The optical properties of Fe$_3$O$_4$ nanoparticles have modified by surface plasmon resonance of Ag shell. Vibrating sample magnetometer studies of nanoparticles were reported the superparamagnetic properties with saturation magnetization 25.5 emu g$^{-1}$. The resulting nanoparticles are superparamagnetic coupled with plasmonic properties gives them potential in diagnostic and therapeutic applications. The simple approach developed for synthesis of multifunctionalized nanocomposites based on a assembly of superparamagnetic Fe$_3$O$_4$ nanoparticles and fluorescence carbon nanodots on silica sphere through electrostatic interaction. The average particle size of the obtained composite nanoparticles is around 90 nm. TEM and FESEM micrograms confirm the encapsulation of Fe$_3$O$_4$ nanoparticles and CDs on silica spheres. The as-synthesized nanocomposites possess dual functionalities: superparamagnetism (saturation magnetization: 2.5 emu g$^{-1}$) and photoluminescence (maximum emission: 395 nm). This type of nanocomposites can be potentially used in simultaneous biolabeling, imaging and targeting applications.