CHAPTER 3

METHYLENE BLUE LOADED SILICA ENCAPSULATED MAGNETITE NANOPARTICLES: A POTENTIAL DRUG DELIVERY VECTOR FOR PHOTODYNAMIC THERAPY

3.1 INTRODUCTION

One of the concerns of public about the modern day medicine is ‘side effects’. Therapies targeting only the diseased cells are the solution for this\(^1\,\text{,}^2\). The targeted delivery of drugs is one of the main challenges that all cancer therapies are dealing with\(^3\,\text{-}^5\). Beside very few exceptions, almost all the therapies for cancer are only extending the life of patients by few months instead of really targeting the cancer cells and cure patients\(^6\).

Photodynamic therapy (PDT) is a noninvasive medicinal modality for the treatment of cancers\(^7\). The therapy can be applicable to both neoplastic and non-neoplastic disease\(^7\,\text{,}^8\). PDT is based on the concept that certain therapeutic molecules called photosensitizers (PS) can be preferentially localized in malignant tissues, and when these PSs are activated with appropriate wavelength of light, they pass on their excess energy to surrounding molecular oxygen. This results into the generation of reactive oxygen species (ROS), such as free radicals and singlet oxygen (\(^1\text{O}_2\)), which are toxic to cells and tissues\(^9\,\text{,}^{10}\). This leads to a number of biological effects including damages to proteins, nucleic acids, lipids, and other cellular components, and often resulting in cell death and possible activation of the immune system to attack the tumor\(^11\,\text{-}^{13}\). Its advantage lies in the inherent dual selectivity. First, selectivity is achieved by a preferential localization of PS in the target tissue, and second, the photo-irradiation and subsequent photodynamic action can be limited to a specific area of interest. Because the PS is nontoxic without light exposure, only the irradiated areas will be affected, even if the PS does infiltrate normal tissues.
The concept of PDT began with studies by Oscar Raab in 1900 and, since then considerable work has been done to understand the process and to maximize the efficacy using animal models. These pre-clinical and clinical studies recently resulted in the approval of first PS drug (Photofrin\textsuperscript{®}) for the treatment of selected tumors. Equal biodistribution of PS in the body after intravenous injection significantly lowers the performance of PS in PDT as the PS dose, time window for treatment (after injection of PS) and energy density depend on the balance between concentration of drug in tumor tissues and in normal tissues at the time of light irradiation. If PS is loaded in a drug delivery vehicle (DDV), which can be effectively targeted towards a tumor site, and have preferential binding ability with tumor tissues then it will cause absolute damage to tumor cells when exposed to light. Hence, the required dose of PS and time of treatment would decrease significantly.

Magnetic nanostructures are one of the most popular materials, which can be use as a DDV. Pure magnetic nanoparticles (MNPs) may not be useful in practical applications because of the following reasons: (i) they are prone to form large aggregates, (ii) their original structure may be altered if they are not stabilized, resulting in the change of magnetic properties, and (iii) they can be very toxic when exposed directly to the biological systems.

In order to overcome these limitations, MNPs are usually used in two kinds of structures: (1) core-shell structures that consist of cores of superparamagnetic particles and shells and (2) composite structures that consist of superparamagnetic particles dispersed in other matrices. These shells or matrices usually possess biocompatibilities and surface activities, and can be conjugated or trap biomolecules and therapeutic drugs. Out of various coatings, silica is a promising and important matrix. It has rich and well-documented biocompatible chemistry, which is good for practical implementation of MNPs in magnetically guided drug delivery and tumor targeting.

In the present research, magnetite nanoparticles are encapsulated in silica matrix. Synthesis protocols have been established to tune the morphology of core-shell structures in the form of sphere and capsules. Photosensitizer (PS) drug “Methylene Blue” (MB) is loaded in the spherical nanostructures. Smaller size,
biocompatibility, tunable loading of PS and capabilities of magnetic guidance can make this DDV, a potential candidate for the treatment of malignant tumors by PDT.

3.2 SYNTHESIS OF MAGNETIC DRUG DELIVERY VECTOR

In general, there are four methods use for the preparation of magnetic-silica core-shell nanostructures. The first method is based on in-situ formation of MNPs inside the pores of pre-synthesized silica using metal compounds\textsuperscript{24}. The second one involves aerosol pyrolysis. In this method precursor mixture of silicon alkoxides and metal compounds are agitated in alcohol medium followed by the decomposition of metal compounds in a flame environment\textsuperscript{25,26}. Microemulsion is the third technique, in which non-ionic surfactants are used to form water/oil inverse microemulsion for the preparation of MNPs and silica layers are formed around the magnetic particles by hydrolysis and condensation of TEOS\textsuperscript{27-29}. Fourth method is the sol-gel technique. In this method, silica layers are formed on MNPs using silicon alkoxides in a basic alcohol mixture\textsuperscript{29}.

Sol-gel technique has received considerable attention because of numerous advantages like mild reaction conditions, low cost and surfactant free synthesis\textsuperscript{30-33}. Recently, Zhang et al.\textsuperscript{34}, Deng et al.\textsuperscript{35} and Lu et al.\textsuperscript{36} have carried out systematic investigation on preparation of silica encapsulated magnetic nanoparticles (SEMNPs) using this technique. Despite of ultrathin coating, their systems suffer from low saturation magnetization, typically 0.1 – 15 emu/g at room temperature\textsuperscript{33-35}. Apart from this, there are no reports on the synthesis of anisotropic (tubular) core-shell nanostructures of SEMNPs.

In this section, synthesis protocols for a novel drug delivery vector (DDV) for photodynamic therapy (PDT) is described. Magnetite nanostructures are produce by well-established chemical co-precipitation technique\textsuperscript{37} and encapsulated in silica shell by modified process of hydrolysis and condensation of tetraethyl orthosilicate (TEOS)\textsuperscript{38}. MB is grafted into the pores of spherical silica shell by demethylation reaction. Kinematical conditions have been established for tunable loading of PS in DDV. These core-shell structures possess higher saturation magnetization (i.e. 39\% for nanospheres and 61\% for nanocapsules) then reported by other groups\textsuperscript{33-35}.
3.2.1 SYNTHESIS OF MAGNETITE NANOPARTICLES

Magnetite nanoparticles were synthesized by coprecipitation technique. Stoichiometric aqueous mixture of ferric (5 mM) and ferrous (2.5 mM) ions were prepared using ferric and ferrous salts. To this 20 mM aqueous solution of ammonium hydroxide was added drop-wise under continuous stirring. Black precipitates were formed immediately. The pH of the slurry was maintained at 10.5. After continuous stirring for 20 min at room temperature (300 K), the black precipitates were magnetically decanted and washed several times with warm HPLC grade water. Finally, the water-wet slurry of Fe₃O₄ nanoparticles was equally divided in four parts. One part was preserved in ethanol as control. Each of the other three parts was ultrasonically dispersed in mixtures of ethanol-water.

3.2.2 SYNTHESIS OF MAGNETITE - SILICA NANOSPHERES

Ethanol-water mixture (300 mL) was prepared by diluting 240 mL absolute ethanol with 60 mL HPLC grade water. Presynthesized magnetite nanoparticles were ultrasonically dispersed in 50 mL ethanol-water. An RBF (cap. 500 mL), equipped with a condenser and thermometer was filled with 250 mL ethanol-water and under continuous vigorous stirring, 2 mL TEOS was added drop-wise to it. Upon addition, the hydrolysis and condensation of TEOS begins immediately, which nucleates silica nanoparticles into the reaction mixture. After 20 min of commencement of hydrolysis and condensation, solution of ethanol-water containing magnetite nanoparticles was added drop-wise into the RBF.

Presence of presynthesized silica nanoparticles suppresses magnetic dipolar interaction and prevents the agglomeration of magnetite nanoparticles. The solution was stirred at room temperature (300 K) for 10 h. During this time, a fine layer of silica is formed over magnetite nanoparticles. Now the mixture was heated at 323 K for another 8 h to further hydrolyze the TEOS. During the entire reaction period, pH of the reaction was maintained at 11 ± 0.1. High temperature (323 K) increases the rate of reaction and produces larger size silica nanospheres. During their growth, these secondary silica particles, encapsulate finely coated magnetite nanoparticles and produced silica encapsulated magnetite nanospheres.
The mixture was cooled to room temperature. Silica encapsulated magnetite nanospheres were isolated by magnetic decantation. Unreacted TEOS (if any), and surface impurities were removed from the nanospheres by washing them with absolute ethanol. Part of these nanospheres was vacuum dried at 300 K, while the others were preserved at room temperature (300 K) in ethanol.

3.2.3 Preparation of Magnetite–Silica Nanocapsules

Presynthesized magnetite nanoparticles were ultrasonically dispersed in ethanol-water mixture by following the methodology explained in section 3.2.2. TEOS (0.2 mL) was added drop-wise into 250 mL of ethanol-water under continuous vigorous stirring. Hydrolysis and condensation of TEOS nucleates silica nanoparticles. When the solution containing ultrasonically dispersed magnetite in ethanol-water was added to the reaction mixture, these presynthesized silica particles prevent the agglomeration of magnetite nanoparticles. The mixture was stirred at room temperature (300 K) for 10 h. During this period, fine coating of silica was achieved on magnetite nanoparticles.

In the second step, 0.8 mL of fresh TEOS was added to the mixture and the reaction was allowed to proceed at room temperature (300 K) for another 24 h. Fresh addition of TEOS leads to the anisotropic growth of silica. During this preferential growth in the form of nanocapsules, finely coated nanoparticles were encapsulated into these anisotropically grown silica nanostructures. The entire reaction was carried out at pH 9 ± 0.1. These core-shell structures were purified and dried at room temperature (300 K) by following the methodology explained in section 3.2.2.

3.2.4 Synthesis of MB Loaded Silica Encapsulated Magnetic Nanospheres

Presynthesized Fe₃O₄ nanoparticles was dispersed in a mixture, containing 30 mL methanol and 15 µL Tetra methyl ammonium hydroxide (TMAH) by ultra-sonication. At the same time, an ethanol-water mixture (300 mL) was prepared by diluting 240 mL absolute ethanol with 60 mL HPLC grade water. TEOS (0.05 mL) was added under vigorous stirring and pH was adjusted to 11 with ammonium hydroxide. After 20 minutes of sonication, both suspensions were mixed in a 500 mL RBF. 10 mL aqueous suspension of MB (2.67 mM) was added and pH was again adjusted to 12 with the help of NH₄OH. The demethylation reaction was carried out for 22 h at 300 K. MB loaded Fe₃O₄-SiO₂ core-shell nanostructures have been isolated from the...
solution by magnetic decantation. This DDV were was hed with absolute ethanol until traces of MB were observed in Uv-visible spectrum of the supernatant. Same experiment was repeated by varying the pH of the demethylation reaction from 11 – 13 to understand its effect on the loading of MB in silica encapsulated magnetite nanoparticles.

3.3 CHEMICAL KINETICS OF HYDROLYSIS AND CONDENSATION OF TEOS

Magnetite-silica core-shell nanospheres have been produced by adopting a two-step process. In the first step, presynthesized magnetite nanoparticles were delicately coated with a thin layer of silica. The addition of TEOS in the mixture of ethanol-water begins the hydrolysis and condensation of TEOS. First, TEOS hydrolyzed into silicic acid\(^{39}\), i.e.

\[
≡\text{Si-OR} + \text{H}_2\text{O} \rightarrow ≡\text{Si-OH} + \text{ROH}
\]

Hydrolysis reaction

Here R is an alkyl group.

Hydrolysis of alkoxysilanes occurred by nucleophilic mechanism. Under the basic condition, water dissociates to produce nucleophilic hydroxyl anions (OH\(^-\)), and then the hydroxyl anion attacked the silicon atom. When an alkoxide group (OR) is replaced by a hydroxyl group (OH), the electron density of silicon is reduced, accelerating the hydrolysis rate of other alkoxides. Once an alkoxide group is hydrolyzed, the others would hydrolyze rapidly\(^{39}\).

Next, the silicic acid condenses into primary silica particles either by water or alcohol condensation mechanism\(^{39}\).

\[
≡\text{Si-OR} + \text{HO-Si} ≡ \rightarrow ≡\text{Si-O-Si}≡ + \text{ROH}
\]

Alcohol Condensation

\[
≡\text{Si-OH} + \text{HO-Si} ≡ \rightarrow ≡\text{Si-O-Si}≡ + \text{H}_2\text{O}
\]

Water Condensation

Alkoxide groups produced by the hydrolysis are polymerized into SiO\(_2\) by the formation of siloxane bonds through water or alcohol condensation. The condensation of Si(OH)\(_4\) will nucleate embryos of silica. These embryos are colloidally unstable and readily aggregate into larger particles with surface charge large enough to prevent the irreversible Brownian aggregation. These silica
nanoparticles will chemically adsorbed on the surface of magnetite nanoparticles by the formation of covalent bond between Fe of magnetite and OH\(^-\) ions on the surface of silica\(^{27,31}\).

In ideal condition, the condensation reaction should commence only after the completion of hydrolysis process. However, in practice, it is not possible to suppress both the reactions completely. Therefore, at the end of first step, each magnetite nanoparticles are coated with a fine layer of silica.

In the second step, by keeping the growth conditions intact, the reaction mixture was heated to 323 K and refluxed at this temperature for another 8 h. Increase in temperature enhances the hydrolysis and condensation rate of TEOS. The hydrolyzed silica embryos diffuse rapidly and produce cages of silica with a well-defined morphology in which, lightly coated magnetite nanoparticles were entrapped. The high temperature and strong pH environment leads to the isotropic growth\(^{40}\), which, results into the formation of monodisperse, nanospheres of silicon encapsulated magnetic nanoparticles (SEMNPs).

Two-step process is necessary to preserve the monodomain nature of magnetic nanoparticles. Instead of two-step process, if entire reaction was carried out (either at room temperature or at high temperature (323 K)) in single step, then cluster of magnetite nanoparticles will be encapsulated into thick silica cages. Apart from this, it will also produce lot of free silica cages that cannot be separated from the encapsulated nanostructures and hence, will degrade the magnetic properties of the final product.

Tubular structures may provide an opportunity to work with more efficient structures than the isotropic ones as they have high drug loading capacities and good cell penetration qualities. These nanocapsules have larger inner volume to be used as the drug container, large aspect ratios for numerous functionalization, and the ability to be readily taken up by the cell\(^{41}\). Drug encapsulation has shown to enhance water dispersibility, better bioavailability, and reduced toxicity. Encapsulation of molecules also provides a material storage application as well as protection and controlled release of loaded molecules\(^{42}\).
In our knowledge, no efforts have been made to prepare anisotropic nanostructures of silica, which is one of the most important elements of inorganic coating on magnetic drug delivery systems. We have developed a two-step process to produce silica encapsulated magnetite nanocapsules. Kinematical conditions have been established to tailor the morphology of the nanostructures in the form of nanocapsules. The first step is almost identical to that followed for the synthesis of nanospheres with the following two marked differences. (i) The concentration of TEOS has been considerably reduced (from 2 mL to 0.2 mL) and (ii) The pH of the reaction was lowered to 9 from 11. Mild reaction conditions and lower concentration of reactant will decrease the rate of growth than nucleation. Hence, more number of monomers will be available in the mixture. Slow growth rate allows the polymerization along a preferred direction, which leads to anisotropic coating of silica on presynthesized magnetite nanoparticles. At the end of first step, reaction mixture contains silica coated magnetite nanocapsules, which will act as seeds during the second step of the reaction.

In the second step, reaction rate needs to be kept low in order to preserve the morphology of seeds. It is known that high temperature increases the reaction rate and may lead to the isotropic growth. To avoid that, entire reaction in the second step was carried out at room temperature (300 K). Multiple injection of reactant (TEOS) is also very important to control the morphology of the nanostructures. We have found that injection of TEOS in two steps (0.2 mL + 0.8 mL) is necessary to produce nanocapsules of SEMNPs. If entire 1 mL TEOS was added at once, isotropic core-shell nanostructures were appeared.

3.4 Investigation of Nanostructures

Structural investigation of magnetite nanoparticles and magnetite-silica nanospheres and nanocapsules has been carried out by powder X-ray diffraction (XRD). Figure 1 shows the X-ray diffractograms of magnetite nanoparticles and magnetite-silica nanospheres and nanocapsules.
Figure 1. XRD spectrum of (A) as-synthesized magnetite nanoparticles, (B) magnetite-silica nanospheres and (C) magnetite-silica nanocapsules. Reflections are indexed with inverse spinel structure

Six peaks are observed in the diffractogram of magnetite nanoparticles. These peaks are centered at 30.24°, 35.49°, 43.37°, 53.71°, 57.15° and 62.64° and corresponds to the face centered cubic (fcc) inverse spinel structure. The space group of the system is $Fd\overline{3}m$ and the calculated value of lattice parameter obtain from the highest intense reflection (311) is 8.37 Å. These results are consistent with those reported in the powder diffraction data base (PDF card No #751610). The line broadening of X-ray diffraction peaks is primarily due to the small size of particles. Assuming the first order diffraction, the crystallite size calculated from the highest intense reflection (311) (Figure 1A) using classical Scherrer formula is found to be 8.36 nm. X-ray diffraction patterns of magnetite-silica nanospheres (Figure 1B) and nanocapsules (Figure 1C) are almost identical to magnetite nanoparticles with a marked difference at 23°. A broad hump is observed at 23° in the XRD patterns of both nanospheres and nanocapsules. This broad hump is the characteristic signature of the presence of amorphous silica in nanostructures. It is not possible to determine the size of nanospheres and nanocapsules from the line broadening as silica is in the amorphous state.
FTIR spectroscopy is an appropriate technique to understand adsorption of polymers on nanoparticles. The characteristic absorption bands at 440, 580 and 620 cm$^{-1}$ in the FTIR spectra of magnetite (Figure 2A) are attributed to asymmetric stretching vibrations of Fe-O. Bands at 580 and 620 cm$^{-1}$ are due to the splitting of the absorption band observed at 570 cm$^{-1}$ in the spectrum of bulk magnetite. Similarly, the band at 440 cm$^{-1}$ originates from another absorption band of Fe-O of bulk magnetite located at 375 cm$^{-1}$. The results are consistent with those observed by Zhang et al.\textsuperscript{46,47}. The band due to the stretching vibration of Fe-O is blue shifted to 469 cm$^{-1}$ and 598 cm$^{-1}$ for magnetite-silica nanospheres (Figure 2B) and nanocapsules (Figure 2C), respectively. A prominent absorption band centered at 1081 cm$^{-1}$ (Figure 2B) and 1088 cm$^{-1}$ (Figure 2C) is assigned to the asymmetric stretching vibration of Si-O-Si\textsuperscript{31}. The band at 615 cm$^{-1}$ (Figure 2B) and 597 cm$^{-1}$ (Figure 2C) is attributed to the asymmetric stretching vibration of Fe-O-Si. This band is blue shifted from its original position of 580 cm$^{-1}$, which is an indicative of chemical adsorption of silica on magnetite nanoparticles\textsuperscript{31,48}. The characteristic absorption peaks observed at 615 & 597 cm$^{-1}$ (Fe-O-Si) and 1081 & 1088 cm$^{-1}$ (Si-O-Si) confirms the binding of silica with the magnetite and the formation of the silica shell.

Figure 2. FTIR spectra of (A) magnetite nanoparticles, (B) magnetite-silica nanospheres and (C) magnetite-silica nanocapsules.
Optical microscopic image of magnetite-silica nanospheres is shown in Figure 3A. Particles are perfectly spherical in shape with nearly monodisperse in size. The observed white patches in the image are due to the evaporation of the ethanol. The size distribution histogram of hundred particles is shown in Figure 3B. It follows the lognormal size distribution. The average size of the nanospheres is 540 nm ± 51 nm. Polydispersity of the system is 9%. Enlarge version of magnetite-silica nanosphere was obtained by transmission electron microscopy. A typical image is shown in Figure 3C. It shows that mono-domain magnetite nanoparticles are dispersed in the silica matrix, indicating encapsulation of individual magnetite nanoparticles into the silica shell. SAED pattern of the nanosphere is shown in Figure 3D. Diffraction rings are due to magnetite core and they are relatively broad because of the smaller size of the particle and its polycrystalline nature. They are indexed well with the inverse spinel structure and agree well with XRD results.

![Figure 3](image)

**Figure 3.** (A) Optical microscopic image (B) size distribution histogram (C) TEM image and (D) SAED pattern of magnetite-silica nanospheres.

TEM image of magnetite-silica nanocapsules is shown in Figure 4A along with its size distribution histogram (Figure 4B). Average size of nanocapsules is 149 ± 19 nm having moderate polydispersity of 11%. Nanostructures possess perfect tubular structure. High resolution version of a nanocapsule is shown in Figure 4C. As can be seen from the micrograph, individually coated magnetite nanoparticles are
encapsulated in a nanocapsule of silica. TEM image of MB loaded silica encapsulated magnetite nanospheres is shown in Figure 5. Image shows that the magnetite-silica nanospheres have nearly spherical morphology. Size distribution histogram obtained from DLS measurement is also shown as an inset in Figure 5. The average size and polydispersity index obtained from DLS are 49.8 nm and 16 %, respectively.

Figure 4. (A) TEM image (B) size distribution histogram and (C) high resolution TEM image of magnetite-silica nanocapsules

Figure 5. TEM image of MB loaded magnetite-silica nanospheres. Inset shows the size distribution histogram of the same system obtained from DLS measurements

Dynamic light scattering is an appropriate technique to determine the physical size of nanostructures. Size distribution histogram of magnetite nanoparticles and
magnetite-silica nanospheres and nanocapsules are shown in Figure 6. The average size and polydispersity index obtained from DLS are presented in Table 1. As can be seen from the table, the results are in good agreement with those observed from microscopic analysis.

![Figure 6](image)

**Figure 6.** Size distribution histogram of (A) magnetite nanoparticles (B) magnetite-silica nanospheres (C) magnetite-silica nanocapsules

**Table 1.** Size and size distribution of magnetite nanoparticles, magnetite-silica nanospheres & nanocapsules and MB loaded magnetite-silica nanospheres obtained from DLS and microscopic techniques.

<table>
<thead>
<tr>
<th>System</th>
<th>DLS</th>
<th>Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size (nm)</td>
<td>Polydispersity (%)</td>
</tr>
<tr>
<td>Magnetite</td>
<td>11</td>
<td>29</td>
</tr>
<tr>
<td>Magnetite-silica nanospheres</td>
<td>550</td>
<td>9.4</td>
</tr>
<tr>
<td>Magnetite-silica nanocapsules</td>
<td>151</td>
<td>15</td>
</tr>
<tr>
<td>MB loaded Magnetite-silica nanospheres</td>
<td>50</td>
<td>16</td>
</tr>
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</table>
Encapsulation of MB in magnetite-silica nanosphere is confirmed by UV-visible spectroscopy. **Figure 7(A)** shows the UV-visible spectrum of MB loaded DDV. A broad hump centered at 657 nm is observed, which corresponds to the characteristic absorption of MB molecule in water. The effect of pH on the drug loading is shown in **Figure 7(B)**. Drug loading capacity was determined by the method explained by Zhang et al.\(^{47}\) As can be seen from the figure, loading of MB in DDV increases as the pH increases, attains a maxima at pH 12 and then again falls. This can be attributed to the highest rate of demethylation at pH 12.

**Figure 7.** (A) UV-visible spectrum of MB loaded magnetite-silica nanosphere (B) Tuning of MB loading in DDV by pH

Magnetization of magnetite nanoparticles and magnetite-silica nanospheres, nanocapsules and MB loaded magnetite-silica nanostructures as a function of applied magnetic field is shown in **Figure 8**. The saturation magnetization (\(M_s\)) of magnetite nanoparticles is 64 emu/g, which is 70% of its bulk value (92 emu/g). Reduction in \(M_s\) might be due to the decrease in particle size accompanied by an increase in surface area, and is consistent with the results observed by Mohapatra et al.\(^{49}\). The \(M_s\) value of magnetite-silica (nanospheres and nanocapsules) and MB loaded magnetite-silica nanospheres are 39 emu/g, 54 emu/g and 35 emu/g respectively. The observed 29% (for nanospheres), 16% (for nanocapsules) and 45% (for MB loaded magnetite-silica nanospheres) decrease in the \(M_s\) value is due to the presence of diamagnetic shell of silica on magnetite nanoparticles and in the case of PS loaded nanospheres reduction of magnetization is partially due to the encapsulated MB. No remanence or coercivity has been observed, which indicates that magnetite nanoparticles are
superparamagnetic and no clustering has been occurred during the formation of core-shell structures. The observed magnetization for magnetite-silica nanostructures and PS loaded nanostructures is considerably higher (38% for nanospheres and 61% for nanocapsules) than reported by other groups. This might be due to the prevention of creation of clusters of magnetite and exclusion of formation of structures of free silica during hydrolysis and condensation.

Figure 8. Magnetization curve of (A) as-synthesized magnetite nanoparticles (B) magnetite-silica nanocapsules (C) magnetite-silica nanospheres and (D) MB loaded magnetite-silica spheres

3.5 Drug Content and Drug Loading Efficiency

To determine drug content and drug loading efficiency of DDV, optical density of collected supernatant was measured at 657 nm using UV-vis spectrophotometer. The concentration of MB that remained unincorporated into the magnetite-silica nanospheres was determined by Beer-Lambert law, $A = \epsilon c l$, where $A$ is the optical density at sample concentration $c$, $l$ is the path length of sample cell and $\epsilon$ (73230 cm$^{-1}$/M at 656 nm) is molar absorptivity. The drug content and loading efficiency were determined using the following equations,
Drug content = \frac{\text{Weight of Drug in Nanoparticles}}{\text{Total weight of Nanoparticles}} \times 100 \quad (1)

Drug loading efficiency = \frac{(W1 - W2)}{W1} \times 100 \quad (2)

Where \( W1 \) is the total weight of drug and \( W2 \) is the weight of free drug, which did not incorporate into the nanoparticles. Drug content and drug loading efficiency of MB loaded magnetite-silica nanospheres are determined by using equation (1) and (2) respectively and tabulated in Table 2 as a function of the reaction pH.

**Table 2.** Drug content and drug loading efficiency as a function of reaction temperature

<table>
<thead>
<tr>
<th>pH of reaction</th>
<th>Drug content (%)</th>
<th>Drug loading efficiency (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Theory</td>
<td>Experiment</td>
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<tr>
<td>11.0</td>
<td>5.0</td>
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<td>12.5</td>
<td>8.5</td>
<td>58</td>
</tr>
<tr>
<td>13.0</td>
<td>7.5</td>
<td>49</td>
</tr>
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**3.6 KINETICS OF DRUG RELEASE**

A method similar to drug loading capacity was established to study kinetics and response of drug release from the drug-conjugated nanoparticles. The *in-vitro* release of drug from the nanoparticles was studied under the sink condition, using double diffusion chambers separated by a membrane (Durapore, Millipore) with a porosity of 10 nm. A suspension of drug-loaded nanoparticles in PBS buffer (pH ~ 7.4) was placed in the donor chamber and pure PBS in the receiver chamber. The chambers were placed in an orbital shaker (NOVA). The shaker was maintained at 27 °C with a shaking speed of 100 RPM. At different time intervals, the entire volume of the receiver chamber was removed and replaced with fresh PBS. The amount of drug release was determined by measuring the optical density of sample extracted from the receiver chamber. Identical experiments were performed in the presence of static magnetic field (477 kAm\(^{-1}\)). A permanent slab magnet was used to produce static magnetic field.
Cumulative drug release from drug delivery vectors (DDV) synthesized at different pH as a function of time is shown in Figure 9. As evidenced from the figure, the nature of the release is independent of the amount of drug content. For each system initial burst release (for first 3 days) followed by a sustained release is observed over a period of 7 days. The release mechanism of drug from the nanospheres is largely governed by the diffusion of MB from the pores of silica in PBS medium. The results are the average of three experimental runs and statistically significant.

**Figure 9.** Drug release profile of MB loaded magnetite-silica nanospheres synthesized at different pH (A) 11 pH (B) 11.5 pH (C) 12 pH (D) 12.5 pH (E) 13 pH.

### 3.7 CONCLUSIONS

Kinematical conditions have been established to tailor magnetite-silica nanostructures in the form of nanospheres and nanocapsules. The process produces stable, non-aggregated superparamagnetic magnetite-silica nanostructures having isotropic as well as anisotropic morphologies. These nanostructures possess excellent magnetization, which is considerably higher than reported by any other group. Magnetite-silica nanocapsules can be a prominent candidate for carrier system for magnetic drug targeting as it provides numerous advantages over isotropic nanostructures.
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