Chapter 5

SYNTHESIS OF IRON OXIDE (Fe₂O₃) INCORPORATED MAGNETIC MESOPOROUS SBA–15 SILICA

5.1. Introduction

Magnetic materials have a range of applications in the field of biomedicine that include their use as contrast agents in magnetic resonance imaging (MRI), as drug delivery systems that can be targeted to desired locations by an applied magnetic field, as a controlled delivery system where the release can be triggered by an external magnetic field, in magnetic separations, as a hyperthermic agent for annihilating cancer cells and in biosensors [1–7]. The suitability of a magnetic material for a specific biomedical application depends upon a number of factors. These include the dimensions of the particle, its shape and magnetization values that will enable the magnetic field to guide the material to the desired location. However, once the magnetic field is removed, the particles should not exhibit a tendency to form agglomerates as it may lead to formation of blocks in the blood vessels, which may turn fatal. A low remanence and a low coercivity value for the material will prevent agglomeration after removal of the applied magnetic field [8]. Additional requirements for these magnetic particles to be used in vivo are biocompatibility, surface functionalities that can be easily modified to impart specific properties such as target specificity, desired residence time, enhanced cell permeability and optical visibility [9–12]. In addition, the material should be inert and should neither involve in undesirable reactions with the biological components nor elicit an adverse biological response against it [13].
Thus far, different types of magnetic micro– and nanoparticles have been developed and efforts are on to improve existing materials for biological compliance. In particular, applications of porous magnetic materials have attracted great attention due to their potential to become multi–functional [14]. Not only can these materials be surface modified with suitable ligands to confer specificity, non–immunogenicity, enhance cell permeation and optical visibility, the pores can serve to accommodate suitable guest molecules [15] that can be used for therapeutic purposes. The introduction of magnetic property to these porous materials can confer magnetic visibility to these structures thereby enabling manipulation of its location, kinetics, visibility and release properties by the magnetic field. Such types of multi–functional materials belong to a class, popularly known as ‘theranostic materials’, which is a hybrid material capable of both diagnosis and therapy. Mesoporous silica possesses tunable pores, functionality and morphology and are likely candidates that can fulfill the requirements of a theranostic agent and hence has become a hot topic for research in the present day.

Incorporation of magnetic properties in porous materials has been through introduction of magnetic nanoparticles into the materials. Research for the development of porous magnetic materials has primarily been centered on two approaches:

(i) *Post–synthesis or grafting technique* which involves the introduction of magnetic nanoparticles in a pre–formed porous matrix

(ii) *Pre–synthesis or co–precipitation technique* which involves synthesis of the porous template around the magnetic nanoparticles

A few attempts have also been developed to include a magnetic precursor into the porous template either by post–synthesis or pre–synthesis method followed by the conversion of
Synthesis of Iron Oxide (Fe$_2$O$_3$) Incorporated Magnetic Mesoporous SBA–15 Silica

the incorporated magnetic precursor into the magnetic nanoparticle through chemical reactions. The most commonly employed magnetic nanoparticle for modification has been iron and its oxides though cobalt doped mesoporous silica materials have also been reported widely in literature [16, 17]. But most biomedical applications that are being explored currently prefer the iron–based porous materials probably due to their ease of synthesis and controllable properties and relatively lesser toxicity. Many groups have investigated the magnetic functionalization of mesoporous materials such as SBA–15, MCM–41, and MCM–48 by the grafting technique [18–21]. These ordered mesoporous silica materials have gained considerable attention for the following reasons:

(i) Mesoporous materials possess a network of channels and voids of well–defined size in nanoscale range (2–50 nm). This highly regular pore architecture makes them suitable candidates for hosting a variety of molecules whose dimensions can be tuned by modifying the pore dimensions

(ii) They can easily be functionalized because of their high specific surface area and abundant –Si–OH groups on the pore walls

(iii) Silica is generally considered non–toxic and bioinert and hence may be explored for applications in biomedical field [22–23]

Hierarchical structures of metal oxides in the mesoporous silica framework have been developed using various methods. The co–precipitation techniques have been used to develop composite frameworks consisting of metal–oxygen–silicon links [24]. Some groups have attempted to transform iron metal into iron oxide through chemical reactions after entrapping them into the mesopores [25]. Garcia et al. reported a method based on the sol–gel technique for fabricating ordered mesoporous aluminosilicate materials with superparamagnetic $\gamma$–Fe$_2$O$_3$ particles embedded in the walls [26]. In this method, a sol
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prepared by hydrolysis of (3–glycidoxypropyl) trimethoxysilane and aluminum secondary butoxide (9:1 molar ratio) was added in a solution containing iron(III) ethoxide and a structure–directing agent of a block copolymer. After stirring for a few hours followed by evaporation of solvent and calcination, mesoporous aluminosilicate materials with superparamagnetic γ–Fe₂O₃ particles embedded in the walls were obtained. This throws a new light on a facile synthesis route for the preparation of nanocomposites with nanocrystals in the silica walls.

Wang et al. [27] have suggested that the –OH groups on the matrix surface generated by hydrolysis of a silica source, e.g., TEOS, were favorable for nucleation of iron oxide particles. Yiu et al. have reported the synthesis of iron oxide–silica nanoparticles (γ–Fe₂O₃–maghaemite and α–Fe₂O₃–haematite) with the iron oxide inside the mesochannels of silica. Internalization of these particles inside mesenchymal stem cells and human osteoblasts has also been reported [28]. Further, iron metal–silica and magnetic silica (Fe₃O₄–SBA–15) have been synthesized by temperature programmed reduction [29]. The iron–to–silica ratio is a critical factor in determining the magnetization property of the nanoparticles. Alam et al. have used mesoporous silica as template to synthesize Fe₂O₃ nanoparticles with uniform and tunable size by a ‘nanosieve’ approach [30]. Yiu et al. have demonstrated that the iron oxide SBA–15 nanoparticles coated with cationic polyethylene imine could be used for magnetofection of DNA into cells [31]. Vinu et al. have synthesized iron oxide nanoparticles that were confined in the mesoporous silica framework of KIT–6, a type of mesoporous silica with three–dimensional pores and large pore volumes [32]. Wang et al. have reported the synthesis of a core–shell iron oxide silica mesoporous structure by post–synthesis for protein
Synthesis of Iron Oxide (Fe₂O₃) Incorporated Magnetic Mesoporous SBA–15 Silica separation [33]. A novel approach to incorporate iron oxide inside the mesopores of a silica framework was reported by Hess et al. Here, an iron containing protein ferritin was incorporated into a mesoporous silica framework followed by calcinations during which the organic template and the protein were eliminated leaving behind the iron as iron oxide in the mesopores [34].

An interesting facet that emerges from these studies is that the magnetic property as well as the morphology and pore architectures are highly dependent on the reaction precursors and reaction conditions which in turn will have a major influence not only on the magnetic properties of the nanoparticles confined in the mesopores but also on the biological relevance of these materials. An important factor that also influences the magnetic properties of the mesoporous samples is the metal to silica ratio. Li et al. had investigated the influence of different ratios of iron to silica ranging between 0.1 and 0.3 on the textural properties of the mesoporous silica membrane developed using a porous alumina template [35]. However, effects of high loads of iron on mesoporous architecture have not been explored thoroughly yet. Another major concern in designing strategies for incorporating magnetic properties to the mesoporous silica is in preserving the highly ordered framework. Though numerous reports are available on the synthetic strategies for structural and textural control of the mesoporous silica framework and incorporation, not much attention has been focused on the compatibility of these materials in vivo. This work aims to systematically investigate the effects of pH on the structural and textural properties of magnetic mesoporous silica synthesized using the pre–synthesis route and employing different ratios of iron to silica. The pH of the reaction was also varied to understand the conditions that would promote high levels of iron oxide incorporation into the mesopores without compromising on the oriented pore
5.2. Materials & Methods

5.2.1. Materials

Tetraethylorthosilicate (TEOS), sulphorhodamine–B and pluronics P–123 were purchased from Sigma Chemicals, USA. Hydrochloric acid, methylene blue, methyl orange and iron(III) chloride were purchased from Merck, India. All chemicals were of GR grade and were used as such without any further purification. Iron oxide nanoparticles of dimensions 6–8 nm used in the post–synthesis method were a kind gift from Prof. M. S. Ramachandra Rao, Indian Institute of Technology (Madras), Chennai.

5.2.2. Synthesis of iron oxide incorporated SW through grafting technique

In this method, mesoporous SBA–15 silica and iron oxide synthesized separately were used to obtain the final product. Initially, the mesoporous SBA–15 silica (SW) synthesized as discussed in earlier chapters was dispersed in deionized water followed by addition of nanostructured iron oxide particles with constant stirring. The final product was filtered, dried and subjected to analysis. The various stages used for the synthesis are presented as a flow sheet in Figure 5.1.
5.2.3. *Synthesis of iron oxide incorporated SW through co–precipitation technique*

Five grams of P–123 was added to 60 g of water and stirred at room temperature until a clear solution was obtained. The pH in different trials was maintained below 1, 3 and 5 by addition of HCl. Calculated amount of iron(III) chloride dissolved in 5 mL of deionized water and 9 g of TEOS were added as precursors. The silica to iron ratio was varied (1:1, 1:0.5, 1:0.25 and 1:0.125). The reaction mixture was stirred at room temperature for 24 h and then transferred to a round bottom flask fitted with a condenser and refluxed at 80°C for 24 h in an oil bath. The precipitate obtained was cooled, filtered, washed several times with deionized water, dried and calcined in a muffle furnace for 6 h at 550°C. Figure 5.2 depicts the various stages involved in the synthesis process.
5.2.4. Characterization of mesoporous samples

Fourier transform infrared spectroscopy (FT–IR) (Spectrum 100, Perkin Elmer, USA) was used to confirm the complete removable of surfactant in the synthesized silica samples. FT–IR analysis was performed between 4000 and 450 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\) averaging 30 scans for all samples. The moisture–free samples were pelletized with KBr (IR grade, Merck, Germany) and subjected to analysis.

X–ray diffraction (D8 Focus, Bruker, Germany) was used to characterize the mesoporous silica. The samples were finely ground using an agate mortar and pestle and the fine powder was mounted on a polymer slide. The analysis was performed from an angle of 0.5° to 10° (2\(\theta\)) in steps of 0.001° and the wide angle was done from 10° to 60° in steps.
Synthesis of Iron Oxide (Fe$_2$O$_3$) Incorporated Magnetic Mesoporous SBA–15 Silica of 0.001° at the scan rate of 1 second per step. Cu$_{K\alpha}$ radiations were used to irradiate the samples for analysis.

The morphology of the synthesized mesoporous silica was studied using a cold field emission scanning electron microscope (FE-SEM) (JSM 6701F, JEOL, Japan). The samples were placed on a conducting alloy stub using carbon paste, sputter coated with a thin layer of platinum and were imaged using SEM. The transmission electron microscope (FE-TEM) images were obtained using a high–resolution transmission electron microscope (JEM 2100F, JEOL, Japan). The samples were prepared by dispersing in ethanol and deposition on a copper grid before imaging. Energy dispersive X–ray analysis (Oxford Instruments, UK) was carried out to confirm the presence of iron in the IO–SW. The characteristic peaks were collected for 220 counts at an applied voltage of 20 kV and a working distance of 15 mm. X–ray photoelectron spectroscopy (Multilab 2000, Thermo Scientific, UK) was carried out to confirm the oxidation state of iron in the iron oxide doped SBA–15. Surface area analysis of mesoporous silica samples was carried out by BET method (Quantachrome, USA). The outgas temperature was 300°C and the outgas time was 3 hours and the samples were degassed for 14 hours prior to analysis.

The magnetic measurements for the IO–SW samples were carried out between the temperature range 1.8 K–300 K using a superconducting quantum interference device vibrating sample magnetometer (SVSM, Quantum Design, USA).
5.2.5. Animal model and implantation

The tissue response of the biological system against the iron oxide incorporated mesoporous SBA–15 silica (IO–SW) was carried out using rat models. For implantation, a pellet of 10 mm x 10 mm x 2 mm was made from the iron oxide incorporated mesoporous silica. The pellet was sterilized by autoclaving at a temperature of 120°C for 15 minutes and similar pellets were made from mesoporous SBA–15 silica (SW) and starch for comparison.

Twenty seven Wistar rats, *Rattus Norvegicus*, each weighing about 200–250 g were used for animal experiments. The animals were randomly divided into three groups of nine rats each. All rats were kept in an individual cage and were housed in a temperature–controlled facility. All the surgical procedures were approved by the Animal Ethics Committee of SASTRA University (61/SASTRA/IAEC/RPP 02/07/2009).

Every group (9 rats) was assigned randomly to three different time points (2, 4 & 8 weeks) with three rats for each time point. An intra–peritoneal injection of ketamine (30 mg/kg body weight) and xylazine (13 mg/kg body weight) were administered to anesthetize the animals. The dorsal area of the animals were shaved and sterilized with 70% ethanol solution (Figure 5.3A). Using a sterile surgical blade No. 22 (Magna Marketing, India), an incision of about 12 mm was made on the dorsum of animal (Figure 5.3B). A subcutaneous pouch was created on both sides of the incision and an implant was inserted into each pocket (Figure 5.3C). Upon implantation of the material into the pouch, the cut was sutured using a non–absorbable surgical black braided silk thread (Relyonpac, India) (Figure 5.3 D–F). Animals in Groups I, II and III
were implanted with starch, SW and IO–SW respectively. The sutures were removed seven days post surgery.

Figure 5.3: Subcutaneous implantation of SW, IO–SW & starch in rat model

5.2.6. Histology studies

At the end of each time point (2, 4 & 8 weeks), rats were euthanized using an overdose of pentobarbital (75 mg/kg) followed by carbon dioxide asphyxiation. The implant and the
tissues surrounding the implant were excised. The tissues surrounding the implant were fixed in 10% formalin solution for seven days. Before embedding in paraffin wax, the tissue samples were dehydrated in an Automated Tissue Processor (Yorco YSI103LT, Yorco Scientific, India) by transferring through a series of graded concentrations of alcohol. The tissue samples were embedded in paraffin using an embedding machine (EG1150 H&C, Leica Microsystems, Germany) and were sectioned with a microtome (Rotary Microtome Leica RL2125RT, Leica Microsystems, Germany) stained with hematoxylin and eosin. The stained samples were viewed under a light microscope to observe the inflammatory responses on the region of the implant.

The inflammatory response to the implanted materials was determined based on the averaged number of cell types (neutrophils, lymphocytes, macrophages, and giant cells) present in the tissue surrounding implant. The average number of inflammatory cells was determined by counting in a 40X magnification from at least ten different fields by an independent pathologist. Tissue responses were evaluated as minimal, mild or moderate using an evaluation system suggested in literature and shown in Table 5.1 [36]

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
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<tbody>
<tr>
<td>Minimal</td>
<td>0 – 10 cells (lymphocytes / plasma cells / neutrophils / eosinophils / giant cells) per field at 40X magnification</td>
</tr>
<tr>
<td>Mild</td>
<td>10 – 20 cells (lymphocytes / plasma cells / neutrophils / eosinophils / giant cells) per field at 40X magnification</td>
</tr>
<tr>
<td>Moderate</td>
<td>More than 20 cells (lymphocytes / plasma cells / neutrophils / eosinophils / giant cells) per field at 40X magnification</td>
</tr>
</tbody>
</table>
5.3. Results & Discussion

5.3.1. Post-synthesis or Grafting technique

The scanning electron micrographs of iron oxide grafted mesoporous silica (IOG–SW) are shown in Figure 5.3. These images show that the surface of the mesoporous silica is decorated with the iron oxide nanoparticles. When the grafting process was carried under sonication instead of stirring, an decrease in the iron oxide nanostructures on the surface were observed (Figures 5.4 A & B) when compared to those obtained by sonication (Figures 5.4 C & D).

Figure 5.4: FE–SEM images of iron oxide doped mesoporous SW silica (IOG–SW) through grafting technique
Figure 5.5 shows the x–ray diffraction patterns obtained for the various samples. The iron oxide nanoparticles show intense signals at 2θ values 27° and 36° indicating the presence of Fe₃O₄ form of iron oxide [JCPDS 85–1436]. The same peaks are observed in the mesoporous samples IOG–SW prepared by stirring and sonication thereby confirming the presence of iron oxide in them.

The post–synthesis or grafting technique primarily promotes the interaction between the surfaces of mesoporous silica and the iron oxide nanoparticle. Therefore, in the resulting mesoporous product, the iron oxide nanoparticles mainly occupy the surface of the silica
Synthesis of Iron Oxide (Fe$_2$O$_3$) Incorporated Magnetic Mesoporous SBA–15 Silica framework and a few get localized in the mesopore channels. As the iron oxide is retained in the mesoporous silica through physical interactions it can be easily separated during processing or application of the magnetic field (Figure 5.6). Hence, an alternate method needs to be designed to overcome these limitations. The co–precipitation technique could provide a solution to this issue by enabling the entrapment and retention of the iron oxide nanoparticles in the mesopores.

![Figure 5.6: Schematic representation of the removal of surface bound iron oxide due to weak physical interactions on application of magnetic field](image)

5.3.2. Pre–synthesis or Co–precipitation technique

Figure 5.7, presents the scanning electron micrographs, showing the surface morphology of iron oxide incorporated mesoporous silica (IO–SW) samples synthesized with different silica to iron ratios (1:1, 1:0.5, 1:0.25 & 1:0.125) and pH (<1, 3, 5 & 7). An interesting observation from the scanning electron micrographs is that the characteristic rod–like morphology of SBA–15 gets heavily distorted as the pH is increased from acidic to neutral (Figure 5.7). This may be attributed to the fact that a decrease in acidity would lead to retardation in the reaction rates thereby promoting formation of structures with greater curvature leading to deviations from the characteristic morphology of SBA–15. The isoelectric point of silica lies around pH 2. Hence, pH values below its isoelectric point promote formation of the cationic silicate species that tend to interact effectively
Synthesis of Iron Oxide (Fe₂O₃) Incorporated Magnetic Mesoporous SBA–15 Silica with the surfactant. However, even at pH below 1, the typical highly elongated rod–like morphology of SBA–15 is retarded and shorter rods are observed at pH <1 as the silica to iron ratio changes from 1:0.125 to 1:1. This distortion in morphology can be attributed to the presence of the iron chloride salt that tends to interfere with the micelle aggregation. It is seen that as the percentage of iron decreases in the IO–SW, the deviation from the typical SBA–15 morphology is reduced. At higher pH, both low acidity and presence of iron chloride tend to destruct the morphology of the mesoporous silica.

<table>
<thead>
<tr>
<th>pH</th>
<th>Si/Fe ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>1:0.5</td>
</tr>
<tr>
<td>&lt;1</td>
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</tr>
<tr>
<td>3</td>
<td><img src="image5" alt="Image" /></td>
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<tr>
<td>7</td>
<td><img src="image13" alt="Image" /></td>
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</table>

Figure 5.7: Scanning electron micrographs of IO–SW synthesized under various pH and silica to iron ratios
The high–resolution transmission electron microscopic images (HR–TEM) for the IO–SW synthesized under various conditions are shown in Figure 5.8.

Figure 5.8: TEM images of IO–SW synthesized with 1:1 silica to iron ratio at pH <1 (A), 3 pH (B), 5 pH (C) & 7 pH (D)

The transmission electron micrographs for the samples synthesized with silicon to iron ratio of 1:1 at various pH shows a marked change in the pore morphology as one moves from acidic to neutral pH. While the ordered porous framework is observed in the sample synthesized at pH lesser than 1, the other samples show the absence of oriented pores. The presence of iron oxide is observed inside the pores of mesoporous silica for IO–SW synthesized with 1:1 silica to iron ratio at pH <1 while in the other samples, the
Synthesis of Iron Oxide (Fe₂O₃) Incorporated Magnetic Mesoporous SBA–15 Silica

Iron oxide seems to be randomly localized. Thus it is evident that the synthesis strategy at pH < 1 with silica to iron ratio of 1:1 offers high amounts of iron oxide incorporation without compromising on the textural properties and oriented mesoporous framework.

The FT–IR spectra of the IO–SW with silica to iron ratio of 1:1 synthesized at various pH as–synthesized are shown in Figure 5.9.

![FT–IR spectra of IO–SW synthesized at various pH](image)

The absorption bands between 1090 and 800 cm⁻¹ show the presence of –Si–O–Si– and –Si–O– of –Si–OH groups in all the samples confirming the formation of silica. The band at 590 cm⁻¹ appears only in the iron oxide incorporated samples, which may be attributed to the Fe–O bond. This band is absent in pristine mesoporous silica indicating the absence of iron oxide in the samples. Figure 5.9 shows the FT–IR spectra of IO–SW
Synthesis of Iron Oxide (Fe$_2$O$_3$) Incorporated Magnetic Mesoporous SBA–15 Silica synthesized at pH < 1 with various silica to iron ratios of 1:1, 1:0.5, 1:0.25 and 1:0.125. The characteristic bands for –Si–O–Si– and –Si–OH appear at 1100 cm$^{-1}$ and 950 cm$^{-1}$ in all the samples. The Fe–O band appears at 550 cm$^{-1}$ in all ratios indicating the presence of iron oxide in the mesoporous silica.

The x–ray diffraction patterns for the pristine mesoporous silica (SW) and IO–SW synthesized at a pH below 1 with different silica to iron ratios 1:1, 1:0.5, 1:0.25 and 1:0.125 are shown in Figure 5.10.

Figure 5.10: X–ray diffraction patterns of SW (a) and IO–SW synthesized at pH below 1 with different silica/iron ratios. b) 1:1, c) 1:0.5, d) 1.0.25 & e) 1:0.125
The peaks at 27° and 36° observed in all the IO–SW samples confirm the presence of iron oxide in the samples. The low intensity peaks are due to the presence of nanostructured iron oxide in the mesoporous samples. The pristine mesoporous SBA–15 silica does not show any peaks in this region indicating the absence of iron oxide. Figure 5.11 shows the x–ray photoelectron spectra (XPS) of the IO–SW synthesized at pH <1 using silica to iron ratio of 1:1. The binding energy of 711 eV shows that the iron exists in the trivalent state in the sample thus confirming the conclusion arrived at from the x–ray diffraction patterns that the mesoporous silica contains iron in the form of Fe₂O₃.

Figure 5.11: X–ray photoelectron spectra of IO–SW synthesized at pH <1 with 1:1 silica to iron ratio
Table 5.2 summarizes the surface properties derived from the nitrogen adsorption–desorption isotherms of the various IO–SW samples synthesized at different pH and silica to iron ratios.

<table>
<thead>
<tr>
<th>pH</th>
<th>Ratio</th>
<th>Name of the Material</th>
<th>BET Surface area (m²/g)</th>
<th>Micropore area (m²/g)</th>
<th>Pore Volume (cm³/g)</th>
<th>Pore diameter (nm)</th>
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<td>1:1</td>
<td>&lt;1</td>
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<td>118</td>
<td>0.7657</td>
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<td>349</td>
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</tr>
</tbody>
</table>

It is interesting to note that the surface area does not vary much with different percentages of iron loading for the iron oxide incorporated mesoporous samples synthesized at pH <1. However, in all the other pH conditions (3, 5 and 7), the surface area was found to decrease dramatically as the content of iron in the sample increased. Also, the surface area changes were significantly reduced when compared to conventional mesoporous SBA–15 silica. This may be attributed to the disruptions in the mesoporous architecture brought about by a combination of the iron precursor as well as the low acidity. The surface area range of 598–604 m²/g and pore diameter range of
Synthesis of Iron Oxide (Fe$_2$O$_3$) Incorporated Magnetic Mesoporous SBA–15 Silica

5.1–7.3 nm obtained for the samples synthesized at pH < 1 is closely related to the reported values for conventional SBA–15. This observation is in agreement with the conclusions drawn from the transmission electron micrographs where non-interruption of mesoporous framework in the samples synthesized below pH 1 was observed.

5.3.2.1. Magnetic properties of IO–SW

The vibrating sample magnetometer (VSM) data obtained at room temperature (300 K) and low temperature (1.3 K) of IO–SW synthesized at pH <1 with 1:1 ratio of Si/Fe are shown in figure 5.12.

![Figure 5.12: VSM of IO–SW synthesized with 1:1 silica to iron ratio at pH <1](image-url)
The magnetization curve exhibits zero magnetic memory and zero coercivity indicating a superparamagnetic behavior for the IO–SW sample. This may be attributed to the small nanodimensional iron oxide particles distributed in the mesoporous channels. The saturation magnetization for the IO–SW sample is 5.5 emu g\(^{-1}\) at 300 K and 5.2 emu g\(^{-1}\) at 1.8 K indicating its potential therapeutic and diagnostic relevance.

### 5.4. Tissue compatibility studies

The histopathology slides of the tissue surrounding the implant from different groups at various time points are shown in Figure 5.13.

![Histopathology slides](figure5_13.png)

**Figure 5.13:** Light microscopy of subcutaneous nodules of SW (A, B & C) & IO–SW (D, E & F) at 2 weeks (A & D), 4 weeks (B & E) and 8 weeks (C & F), (L – Lymphocytes, F – Fibroblasts, P – Plasma cells, IM – Iron laden macrophages, GC – Giant cells). All images are 40× the original magnification.
Careful observation of the tissue surrounding the implants shows a thin fibrous encapsulation around the mesoporous silica (SW) and IO–SW implants. While the structural integrity of SW was maintained even after 8 weeks, the IO–SW had disintegrated inside the fibrous capsule. The starch control showed mild inflammatory response after 2 weeks, which then became minimal after 4 and 8 weeks. During the entire duration of study the tissue surrounding the starch implant had very few lymphocytes and fibroblasts. In the case of the mesoporous SBA–15 silica (SW), the tissue in close contact with the implants was characterized by an abundance of lymphocytes, plasma cells and very few fibroblasts and neutrophils indicating a moderate tissue response. At the end of even four weeks, the tissue still exhibited a moderate response which reduced to a mild response after 8 weeks as characterized by a reduction in the number of lymphocytes. In the case of IO–SW, the tissue in contact with the implant was characterized by lymphocytes, a few macrophages and fibroblasts eliciting a moderate inflammatory response. This reduced to a mild response after 4 weeks and remained mild at the end of 8 weeks post implantation. At the end of 8 weeks, however, a few iron–laden macrophages were visible in the tissue in the immediate vicinity of the implant. Table 5.3 summarizes the tissue responses of the three materials over the period of study.

Table 5.3: Tissue response to SW and IO–SW implants at different time points

<table>
<thead>
<tr>
<th>Material</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>Mild</td>
<td>Minimal</td>
<td>Minimal</td>
</tr>
<tr>
<td>SW</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Mild</td>
</tr>
<tr>
<td>IO–SW</td>
<td>Moderate</td>
<td>Mild</td>
<td>Mild</td>
</tr>
</tbody>
</table>
Based on the inflammatory responses, a material can be classified as a level 1 material if it elicits a mild inflammatory response in the period of study. A level 2 material elicits mild to moderate inflammatory response that reduces with time while a level 3 material causes moderate to severe inflammatory response. Finally a level 4 material is one that causes severe inflammatory response that does not diminish with time [62]. The in vivo tissue compatibility studies that were carried out for the SW and IO–SW show that both these materials can be categorized as level 2 materials and hence can be used for in vivo applications.

5.5. Conclusions

This work has successfully incorporated iron oxide in the mesoporous framework without much compromising the textural properties of the mesoporous silica using a co–precipitation technique employing ferric chloride as the iron precursor. The silica to iron ratio of 1:1 was found to have suitable magnetic property that can be explored for biomedical applications. The in vivo tissue compatibility of these magnetic materials reported here for the first time show that these materials are well tolerated by the biological system and the moderate inflammatory response elicited in the first couple of weeks diminishes with time thus making it a level 2 biomaterial that can be safely used for short term applications. However, the appearance of iron laden macrophages after eight weeks indicates potential hemosiderosis–like conditions that need to be further investigated to understand the long term safety of these materials.
Synthesis of Iron Oxide (Fe₂O₃) Incorporated Magnetic Mesoporous SBA–15 Silica

5.6. References


Synthesis of Iron Oxide (Fe₂O₃) Incorporated Magnetic Mesoporous SBA–15 Silica


Synthesis of Iron Oxide (Fe₂O₃) Incorporated Magnetic Mesoporous SBA–15 Silica


