Surface functionalization of magnetic nanoparticles towards biomedical applications

Nawfel Abdullah

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Surface Functionalization of magnetic nanoparticles towards biomedical applications

By
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School of Mechanical, Materials, Mechatronic and Biomedical Engineering
Australian Institute for Innovative Material (AIIM)
Institute for Superconducting and Electronic Material (ISEM)

“This thesis is presented as part of the requirements for the award of the degree
Master of Philosophy
from the
University of Wollongong, Australia”

Supervisor: Dr. Md Shahriar A Hossain
Co-Supervisor: Prof Yusuke Yamauchi, Assoc. Prof Konstantin Konstantinov
and Dr. Vitor Sencadas Gomes da Silva Sencadas

March 2018
DECLARATION

I, Nawfel Abdullah, declare that this thesis, submitted in fulfilment of the requirements for awarding the degree of Master of Philosophy at the School of Mechanical, Materials, Mechatronic and Biomedical Engineering at the University of Wollongong, is entirely my own, unless otherwise stated, referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Nawfel Abdullah
March 2018
ACKNOWLEDGEMENT

Above all, I express my gratitude to Almighty for all the bounties granted to me and only with his guidance and help this achievement has become possible. It is my pleasure and proud privilege to express my heartiest regards and gratitude to my respected supervisors. I am much thankful to my principal supervisor Dr. Md Shahriar A Hossain for giving me the opportunity to join his research group, and for his guidance, support and encouragement throughout the MPhil program. I would like to express my deep respect and gratitude to Professor Yusuke Yamauchi for his expert supervision, constructive criticism, valuable advice, optimistic counseling, constant support, mentorship and encouragement throughout every phase of my study. I am deeply grateful to Associate Professor Konstantin Konstantinov for his support, expertise, guidance and mentorship. I am thankful to Dr. Vitor Sencadas Gomes da Silva Sencadas for his support as a co-supervisor with his extraordinary scientific knowledge on fundamentals of polymer.

I would like to extend my cordial gratitude to Alexander Morlando, Sheik Nazrul Islam, Mostafa Kamal Masud, Shunsuke Tanaka, Enad Abed Alsabaawi, Kyubin Shim, Junayet Hossain Khan for their constant inspiration and whole-hearted cooperation.

I owe special thanks to my fellow research group members, near and dear ones and specially Rabeya Sultana Meem who offered encouragement, information, inspiration and assistance during the period of constructing the research report.

Last but not the least, I would like to thank my family and friends for their care, love, understanding and encouragement during my research work.
ABSTRACT

Nowadays nanoparticles, especially mesoporous materials have drawn notable attention due to their wide variety of applications. Among them, mesoporous silica and iron oxide are novel types of such materials due to their unique physicochemical properties that can enable new horizons for their application in biosensing.

As preliminary research work, a detailed study of mesoporous silica was performed. As a fundamental study, it deals with the basic concepts of mesoporous material synthesis, the required characterization, and related scientific ideas to bring its practical application. It was then demonstrated as a first step that, by using a single structure directing agent for mesoporous silica, precise tuning of the pore wall thickness can improve the antireflection property of the resultant film. When the relative weight of the polymer with respect to the silica source is increased from 10 to 40 wt.%, the pore-to-pore distance is gradually reduced without a significant change in the mesopore size. Compared to glass substrate, the transmittance is increased by around 3.3 % in the wavelength range of 400 to 750 nm. Thus developed technological concept can also be applied to other plastic substrates such as triacetylcellulose (TAC) and poly methyl methacrylate (PMMA) films.

In the second step, a study was performed on mesoporous silica (MPS) as an inorganic filler material to improve the mechanical strength of silicone rubbers. Synthesis was performed to prepare ultraviolet curing type silicone rubbers containing mesoporous silica fillers. The main focus was to show the advantage of the pores of mesoporous silica (MPS) for improving the mechanical strength of silicon rubber as inorganic filler. MPS has abundant nanoscale pores and large pore volumes, and can be prepared through the spontaneous self-assembly of surfactants. The typical fabrication process of silicone rubber composites is laborious because of the many steps required to obtain the final product. So, we have investigated and proposed a newer single step method using ultra violet radiation.

In the third steps, soft-templated synthesis of mesoporous iron oxide was performed using asymmetric poly(styrene-b-acrylic acid-b-ethylene glycol) triblock copolymer. This polymer is known to form micelles consisting of a polystyrene (PS) core, a poly acrylic acid (PAA) shell, and
a poly ethylene glycol (PEG) corona in aqueous solutions. The calcination temperature was found to play a crucial role in determining the final morphology of the mesoporous iron oxide, as lower temperatures (<400°C) could not sufficiently remove the block copolymer, whereas very high temperatures (≥450 °C) led to the collapse of the mesoporous structure. The mesoporous iron oxide obtained at an optimized calcination temperature of 400 °C exhibited an average pore diameter of 39 nm, with a large specific surface area and pore volume of 86.9 m² g⁻¹ and 0.218 cm³ g⁻¹, respectively.

Finally, the synthesized mesoporous iron oxide products were then investigated for their potential peroxidase mimicking activities towards the catalytic oxidation of 3,3’,5,5’-tertamethylbenzidine (TMB) in the presence of hydrogen peroxide (H₂O₂). This mimetic feature was further exploited to develop a simple colorimetric (naked-eye) and electrochemical assay for the detection of glucose. Both our colorimetric (naked-eye and ultraviolet-visible (UV-vis)) and electrochemical assays estimated the glucose content in the linear range from 1.0 to 200 mM with the detection limit of 1.0 mM.
LIST OF PUBLICATION

1. Abdullah, Nawfel¹; Hossain, Md. Shahriar A¹; Fatehmulla, Amanullah²; Farooq, Wazirzada Aslam²; Islam, Md. Tofazzal³; Miyamoto, Nobuyoshi⁴; Bando, Yoshio⁵; Kamachi, Yuichiro⁵; Malgras, Victor⁵; Yamauchi, Yusuke¹; Suzuki, Norihiro⁵ (2018). **Preparation of Ultraviolet curing types silicone type silicone rubbers containing mesoporous silica fillers. Journal of Nanoscience and Nanotechnology.** Volume 18, Number 1, January 2018, pp. 86-89(4).

2. Abdullah, Nawfel¹; Hossain, Md. Shahriar A¹; Konstantinov, Konstantin¹; Tanabe, Hirofumi²; Matsuura, Mikiya²; Maekawa, Kazuhiko²; Fatehmulla, Amanullah³; Farooq, Wazirzada Aslam³; Islam, Md. Tofazzal⁴; Bando, Yoshio⁵; Kaneti, Yusuf Valentino⁵; Yamauchi, Yusuke¹ (2018). **Tuning wall thickness in mesoporous silica films for optimization of optical anti-reflective properties. Journal of Nanoscience and Nanotechnology.** Volume 18, Number 1, January 2018, pp. 100-103(4).

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<td>IUPAC</td>
<td>The international union of pure and applied chemists</td>
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<td>MPS</td>
<td>Mesoporous silica</td>
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<tr>
<td>nm</td>
<td>Nano meter</td>
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<td>MOF</td>
<td>Metal organic framework</td>
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<td>MCM</td>
<td>Mobil composition of matter</td>
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<td>m</td>
<td>Meter</td>
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<tr>
<td>CMC</td>
<td>Critical micelle concentration</td>
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<td>SDA</td>
<td>Surface-directing agent</td>
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<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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<td>PSiP</td>
<td>Porous silicon particle</td>
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<td>EISA</td>
<td>Evaporation-induced self-assembly</td>
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<td>AAO</td>
<td>Anodic aluminum oxide</td>
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<td>CTAB</td>
<td>Cetyltrimethylammonium bromide</td>
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<td>TEOS</td>
<td>Tetraethylorthosilicate</td>
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<td>MRI</td>
<td>Magnetic resonance spectroscopy</td>
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<td>RES</td>
<td>Reticuloendothelia system</td>
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<td>DDS</td>
<td>Drug delivery system</td>
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<td>AR</td>
<td>Anti-reflection</td>
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<td>x</td>
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<td>Wt%</td>
<td>Weight percent</td>
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<td>n₁</td>
<td>Surface refractive</td>
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<td>n₂</td>
<td>Refractive index</td>
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<td>Kilogram</td>
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<td>Centimeter</td>
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<td>N</td>
<td>Newton</td>
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<td>TAC</td>
<td>Thoria acetyl cellulose</td>
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<td>PMMA</td>
<td>Polymethyl methacrylate</td>
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<td>P</td>
<td>Porosity</td>
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<td>XRD</td>
<td>X-ray diffraction</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
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<td>CTE</td>
<td>Coefficient of thermal expansion</td>
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<td>LED</td>
<td>Light emitting diode</td>
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<td>PDMS</td>
<td>Polydimethylsiloxane</td>
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<td>NS</td>
<td>Non-porous silica</td>
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<td>BJH</td>
<td>Barrett-Joyner-Halenda</td>
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<td>2D</td>
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<td>V</td>
<td>Volume</td>
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<td>ρ</td>
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<td>Description</td>
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<td>DPR</td>
<td>Dead pore ratio</td>
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<td>TMA</td>
<td>Thermomechanical analysis</td>
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<tr>
<td>W</td>
<td>Weight</td>
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<tr>
<td>$\sigma$</td>
<td>Stress</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>Strain</td>
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<tr>
<td>E</td>
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<td>MPa</td>
<td>Megapascal</td>
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<td>P</td>
<td>Pressure</td>
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<tr>
<td>$L_0$</td>
<td>Original length of the specimen</td>
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<tr>
<td>$\Delta L$</td>
<td>Displacement of length</td>
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<td>TEM</td>
<td>Transmission electron microscopy</td>
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<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
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<td>TGA</td>
<td>Thermogravimetric analysis</td>
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<tr>
<td>$\text{Å}$</td>
<td>Angstrom (unit of length)</td>
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<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
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<tr>
<td>RAFT</td>
<td>Reversible addition-fragmentation</td>
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<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<td>GPC</td>
<td>Gel permeation chromatography</td>
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<td>DMSO-d$_6$</td>
<td>Dimethyl sulfoxide-d6</td>
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<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>mmol</td>
<td>millimole</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliter</td>
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<td>DLS</td>
<td>Dynamic light scattering</td>
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<td>BET</td>
<td>Brunauer-Emmett-Teller</td>
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<td>mV</td>
<td>Millivolt</td>
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<td>$R_h$</td>
<td>Hydrodynamic radius</td>
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<td>$^\circ$C</td>
<td>Degree Celsius</td>
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<td>k</td>
<td>scherrer constant</td>
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<tr>
<td>$\lambda$</td>
<td>Wavelength</td>
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<tr>
<td>$\beta$</td>
<td>Full width</td>
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<tr>
<td>$\vartheta$</td>
<td>Bragg angle</td>
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<td>SAED</td>
<td>Selected area electron diffraction</td>
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<td>TMB</td>
<td>3,3',5,5'-tertamethylbenzidine</td>
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<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
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<tr>
<td>GOx</td>
<td>glucose oxidase</td>
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<td>μM</td>
<td>Micromole</td>
</tr>
<tr>
<td>μL</td>
<td>Microliter</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>Maximum rate of conversion</td>
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<td>$[S]$</td>
<td>Substrate concentration</td>
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<td>$K_m$</td>
<td>Michaelis-Menten constant</td>
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<td>SPE-Au</td>
<td>Screen-Printed Gold Electrodes</td>
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<td>μA</td>
<td>Microampere</td>
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<td>μL</td>
<td>Microgram</td>
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1. Chapter 1: General Introduction

1.1 Background

Nanotechnology is an emerging field that is actively involved in the production and use of nanomaterials and devices. In the past, the porosity of material was a paramount issue, in choosing a material for industrial or commercial application. Porous materials are widely used in the fine chemical industry and biomedical sciences. ‘Porous materials’ refers to materials that have porous spaces, which may be filled by fluid either in the form of a liquid or a gas. The material are used for various purposes such as chromatography, molecular sieves, catalysts, and for ion exchanges.

Mesoporous material is one type of porous material which has pore diameters between 2 and 50 nm. Mesoporous nanoparticles can be used in the detection of glucose, for bio-mimicking the peroxidase enzyme, and also in the detection of micro-organisms. Such particles can also be used in diagnosing of cancer. These nanoparticles can be used to bind drugs and proteins and, they can penetrate into a tissue or an organ. They can also undergo a phase transition in a controllable way.

Nanomaterials have drawn attention in both the scientific fields and industry due to their capability of interacting with ions and molecules throughout the bulk of the material, having solely surface interactions. As the world population is growing very rapidly, the number and pathogenicity of various diseases such as cancer are also increasing in an equivalent manner.[1] Scientists all over the world have come up with different ideas and treatments to control cancer, and as well as increase the life span of cancer patients.[2] Several remedies, such as controlled drug release, site specific drug delivery, delivery of radionuclides, and gene therapy have been proposed as treatments for some cancer cases.[3] Among them, radio frequency is also a method used to catabolize tumors through hyperthermia.[4]

The use of nanoparticle based sensors has been increased in the past decade. These biosensors are used in the diagnosis of different chronic ailments. Some of the ailments require continuous
checking of some key factors for the patients. These tests are sometimes expensive and painful, and are thus considered stress intensive due to repetitive blood collection. In most cases, diseases such as cancer are not detected and treated in their earlier stages, so that, in many cases, death occurs. Early detection and effective treatment at the proper time are not only essential for cancer, but also very necessary for other pathogenic diseases. So, the advancement of diagnosis and monitoring of these disease profiles and prognosis is a burning issue.

Catalysts play a significant role in current science and technology as they can control reaction yield, promote exact enantioselectivity in an unequal synthesis and modify the temperature of chemical processes as required. Modern advances in the modification of morphology and surface functionalization of mesoporous nanoparticles has improved the biocompatibility of these materials. Current reports have shown that mesoporous silica particles loaded with a desired material can be internalized by plants cells and animal tissues. Functionalization of mesoporous silica nanoparticles with carbon-based nanostructures or moieties enables specific ion discharge, which provides molecular recognition capabilities to this mesoporous material, which can be used for target drugs, gene transfer, and detection applications.

Mesoporous silica and iron oxide nanoparticles materials with various types of measured ion discharge provide the features needed to utilize them in the construction of highly selective and specific sensory devices. Starting from the known concepts, and the ongoing research outcomes from the enormous application of magnetic materials in industry and biological fields, it is now clear that the prospect of working in this field is not only going with the recent trend in research, but also receiving and opportunity to contribute to the mankind by using its enormous field of application. Although various research outcomes have already been achieved, and research in industry and on biomedical application is ongoing, still more progress is needed to reveal and utilize their unique properties for the betterment of mankind.
1.2 Thesis Aims

Despite the superior analytical capabilities of current commercially available sensors, they often require complex and time-consuming amplification processes. Additionally, they are costly, involve laborious procedures, and their preparation and storage require a restricted environment. To aid material synthesis and functionalization for biomedical application (e.g. biosensors), the first part of this thesis work reviews the fundamentals of nanoparticles and the basic characterization techniques for mesoporous materials. After that based on the previous knowledge, the focus is on on the synthesis of magnetic nanoparticles for application in biosensing.

The first aim of the research described in this thesis was to show the advantages of the mesoporous silica over the nonporous towards improving the mechanical properties of silicone rubber as an inorganic filler.

The second aim of this research project was to use mesoporous silica as an antireflection coating material and show that a single structure directing agent can be used for precise tuning of the pore wall thickness to improve the antireflection property of the film.

The third aim of this project was to synthesize mesoporous magnetic material using the soft templating method and perform characterization, which include N₂ adsorption-desorption isotherms, X-ray diffraction, scanning electron microscopy, transmission electron microscopy, X-ray photoelectron spectroscopy, and thermogravimetric analysis.

The final aim of this research project was to investigate the synthesized mesoporous materials for their potential peroxidase mimicking activity. This intrinsic property of our synthesized mesoporous material can be used to develop a proof of concept for their use as a glucose sensor.
1.3 Literature Review on Porous and Mesoporous Nanomaterials

1.3.1 Introduction:

Nowadays porous materials have been receiving more attention although various research articles show that still more improvement is needed, such as the chemical modification or functionalization, which can add additional features for industrial or biomedical use. There are three categories of the porous materials based on the pore size diameter: microporous, <2nm, mesoporous, 2 nm to 50 nm, and microporous, > 50 nm. Within the material, the pores can be distributed in an asymmetrical or a symmetrical manner. The diameters of these pores tend to vary from one particle to the next. Currently, among all the nanomaterials, mesoporous silica has attracted great research attention due to its wide variety of applications in industry. The unique characteristics that promote the wide application of mesoporous silica are large pore volume, adjustable pore diameter, abundant nanoscale pores, greater surface area, and good thermal stability. Due to their unique qualities, mesoporous materials of different kinds are widely used in industry and the biomedical field. In the history of porous materials, the first nanoporous, crystalline material was zeolite. A Swedish mineralogist discovered these materials while performing an experiment. He observed that this type of material produced a great deal of water steam when rapidly heated. Alex F. Cronstedt concluded that the water molecules must have been absorbed in the micro pores. The adsorption, dehydration, and reversible cation exchange properties of zeolite have been established by various scientific works. The first hydrothermal synthesis of zeolite was reported by St. Claire Deville. The zeolite molecule contains micro pores which can accommodate a wide variety of positive ions. Zeolite currently has a wide variety of applications in industry due to its small pore size. The ability of the zeolite to capture positive ion has given it a unique property, the ability to sieve fission products from a nuclear reaction and permanently trap them. The preparation of these mesoporous materials is now conducted using the silica formation method. This process is closely followed by template removal by appropriate methods of solvent evaporation and calcination. Scientists have done various types of research work in drug design and delivery with the mesoporous nanomaterials especially with mesoporous silica. This is because they have unique characteristics, such as uniform pore size distribution, greater surface area, and void volume. In most of the cases, the researchers are always keen to
discover the impact of using the material from the environmental and ecological perspective. Researchers are still trying to investigate both the environmental and the biological impact of the microporous and mesoporous materials.

Several research studies have shown that, when the liquid quartz templating technique is involved, the material is dissolved with the surfactant. The particles need to be pre-hydrolyzed in the inorganic precursor. The process that is used is strongly influenced by the electrostatic interaction of one solvent molecule to another. Magnetic mesoporous silica nanoparticles are being researched for use as adsorbents for heavy metals from water or any other liquid. They can be used in water treatment application to allow efficient magnetic recovery of the contaminated particles. The microporous and mesoporous materials are used actively in drug transportations and human medical imaging. The main benefits of such processes are related to their low toxicity and good biocompatibility with minimum cost. More research work is still going on to make these processes more precise and better. These materials can also be used in the delivery of small molecule drugs. These include aspirin, ibuprofen, fluorouracil, and doxorubicin. The main parameters that are currently under research for the components of zeolites and mesoporous silica are the improvement of pore size and surface area to have better functionality. The researchers now have a very keen interest in using the silica material as a drug carrier because of its unique functionality. The drugs are placed in the silica pores. As mentioned earlier, silica material has a very uniform pore size distribution. The desired material(drug) is adsorbed within the pores of the mesoporous silica particle, and the loading is done with careful consideration so that the drug or particle which has been loaded can maintain its own chemical behavior. In some research studies, it is now established that silica nanotubes can also be used for efficient drug loading as well as delivery to the targeted sites. The drug-silica composite is delivered either in powder form in capsules or shells or in a compact forms such as small tablets. Each composite is produced according to the required concentration of the given drug in the solution. This procedure is known as the impregnation procedure.
1.3.2 Types of Porous and Mesoporous Material

There are generally three types of porous material, micro-porous, macro-porous, and mesoporous materials, as described above. The mesoporous materials are of key interest as they tend to have very uniform pore size distributions and large pore volumes. Some materials, such as zeolites, have especially large pore volumes. These can easily trap positive ions. This makes them highly useful in sieving or separation processes, and they can be used as catalysts. The pore walls tend to have an amorphous nature. There are many substances that can be used to produce porous materials. These substances are polymers, minerals, ceramics, silicates, and carbon. The mesoporous materials, in turn, exhibit poorer hydrothermal stability and weaker acidity as compared to non-porous materials. Much research has already been done and is still going on to improve the structure of the mesoporous material to achieve better diffusion rates. Some of the materials categorized as having mesoporous forms are niobium, zirconium, cerium, tin, tantalum,
titanium, and carbon-based materials. The amphiphilic surfactants tend to form micelle like structures. These micelles are then encapsulated by an inorganic material. The inorganic substance used in most research work is either a silicate or a silicate variant. A surfactant is used to control the micelle formation. There is a thermal processing technique that is used to eliminate the unused surfactant. [12]

![Classification of porous materials](image)

**Figure 2:** Categorization of porous materials based on the pore size.

All these classes are defined according to the pore diameter as reviewed by the IUPAC, as shown in the **table 1** below:

<table>
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<th>Type</th>
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<td>Microporous</td>
<td>&lt;2.0nm</td>
</tr>
<tr>
<td>Mesoporous</td>
<td>2.0nm-50nm</td>
</tr>
<tr>
<td>Macroporous</td>
<td>&gt;50nm</td>
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There are several types of the mesoporous materials which are used in the industry and biomedical sciences. These materials are categorized as three-dimensional (3D) nanostructures with surface areas of about 1000m$^2$ per gram. They tend to have superior quality as compared to the Zeolites and other types of 3D structured materials. These materials are formed following a self-assembly process. The original component are a sol-gel precursors and structure directing surfactants.

Two commonly used mesoporous material are:

i) Mesoporous silica materials

Among all the mesoporous materials, silica has some outstanding features. It is easy to synthesize and can be processed easily for commercial and biomedical applications. It has the unique ability to tolerate modification of its surfaces with functional groups. These functional groups are formed from several reactive silanol groups that are commonly found on amorphous silicates. Silica is a member of the class of mesoporous materials, while zeolite is a member of the class of microporous materials and hence features smaller pore sizes.

ii) Mesoporous carbon

Mesoporous carbon has ordered pores and is mesostructured, with a high surface area, and special chemical properties. It has the potential to be applied as a catalyst or separation medium in industrial applications. It can be formed with either soft-template or hard-template methods. This material has a large pore wall thickness and a rather small particle size with a considerable mesoporous nature.

1.3.3 General synthesis of mesoporous material

There are several chemical processes used in the synthesis of mesoporous material. These components are synthesized as silicas to make the inorganic kind of unique mesoporous material. The small size of pores in a mesoporous material holds the drug molecule. This characteristic involves the surface interaction characteristics of both the drug molecule and the pore walls. The Mobil Oil company first introduced the general synthesis of mesoporous silicas. Their process, uses the surfactant as a template for the synthesis and application of the silicas. Their MCM-41 mesoporous silica is notable because its, thermal stability is high, it has smaller pore size
distribution than other types, and it has a larger surface area. A template, for the synthesis of silicas, is a structure guiding agent that is a relatively simple molecule or ion. The framework of the final product is built around this template. Quaternary ammonium ions are usually obtained with long alkyl chains as templates. The ions belong to the hexadecyl group form the common patterns for the amalgamation of MCM-41.\textsuperscript{[13], [14]}

By disrupting the surface cohesive energy surfactants lower the surface tension. Their interaction with their favored environment depends on their arrangement. Micelles are defined as aggregates of surfactant molecules dispersed in a liquid colloid, where the hydrophobic portion of the surfactant molecule points inward within the cluster and the hydrophilic region is exposed towards the solvent. According to the concentration of the amphiphilic surfactants molecules, micelles are present in the bulk of the liquid. With more surfactant, more micelles will be formed by the molecules.\textsuperscript{[15]} The concentration at which the loaded surfactant surface tends to turn into micelles is defined as the Critical Micelle Concentration (CMC). Different surface-directing agent (SDAs) have separate values. Beyond the point of this CMC value, the micelle form 3D spherical shapes or two-dimensional (2D) rod like structured by self-assembly, and with increasing concentration this assembly helps in generation of the pores. These SDAs act as "placeholders" which produce nanoporous material by becoming void space. The shape and size of the pores depend on the design of the template.\textsuperscript{[16], [17]}
Research and investigation on the formation of mesoporous materials started around the year 1992. The "exo-template method" (nano-casting) and the endo-template method (soft templating) are the two surfactant assisted synthesis processes for mesoporous materials. The inorganic precursors orient themselves in the liquid in a regular order and form an inorganic-organic solid composite. This method consists of three steps; self-assembly of the surfactant, the orientation of the inorganic precursors over the surfactant arrangement to form an organic-inorganic composite, and removal of the template to obtain the mesoporous compound. Calcination of the inorganic-organic composite is performed to remove the template to obtain the porosity of the material (ex, mesoporous silica). It is notable that the removal of different template will require different temperature and the degree of the crystallinity of the mesoporous compound depends on the increase of the temperature. \cite{19}, \cite{20}
In the exo-template process, polystyrene beads, silica, or silica microspheres (colloidal crystals), carbon, etc. are used as the template instead of surfactant. That is why this process is called the hard-templating method. Here, as the template is situated outside of the inorganic material, the empty space of the exo-template structure becomes filled with the inorganic precursor, which is then cured in under suitable condition. In short, in this process the porous structure of the template acts as a negative image for the material. Then with the help of either hydrogen fluoride or sodium hydroxide solution at high temperature, the filled exo-template is removed to obtain the desired material. [22]

Lyotropic liquid crystal is used as a template to obtain uniform pore size in the range of 20-100Å. It can accommodate molecules that are large enough to fit within the pores of zeolite. This kind of arrangement allows a greater quantity of micelles to be created out of a given quantity of template. [23] If the concentration is increased further, this hexagonal liquid crystalline stage first transforms into a cubic liquid crystal-like phase. Due to this similarity between the liquid
crystalline phases and the Mobil Composition of Matter (MCM) arrangements, it is frequently presumed that the liquid glassy structures are the real and definite patterns of MCM-41 and MCM-48.

Again, a group of chemists, Yanagisawa and his colleagues, reported the generation and production of mesoporous silica materials. The material was obtained by intercalation of long chain ammonium cations known as alkyl trimethylammonium cations. The intercalation converts the silica into a layered silicate composite, which followed by calcination to remove the ammonium cations. \[24\] The final product has a three-dimensional structure that is condensed with nanoscale pores. MCM-41 has a notable attribute, in that the diameter of the pores in this material or product is controllable when different carbon chain lengths of the surfactant are utilized in the synthesis process. Researchers and experimenters observed that, with an increasing amount of template in water, different micelle orientations occurred. It could be seen that the micelles in spherical form gradually transformed into long tubes that looked like rods. \[25\] It was observed that one can hardly control the long-range order and the orientation of self-assembled structures. The long tube-like micelles are dipped into a hexagonal liquid crystalline structure that closely resembles the MCM-41 structure. \[26\] The transformation of the template to a finished product with the MCM-41, MCM-48 and MCM-50 structures is demonstrated below,
Figure 5: The MCM-41, MCM-48 and MCM-50 synthesis route demonstrated using a schematic structural diagram. [27]

There are some advantages to preparing three-dimensional structures using the above route. Soft amphiphilic templates are used in the structure formation. [4] This approach overcomes the barrier related to the pore size limitation observed in microporous materials such as the zeolites and allows for more facile diffusion of bulky molecules.
1.3.4 Application of mesoporous material

Catalysis:
Materials that have a higher surface area with nanoscale feature are now attractive to limit the use of energy and to control the generation of waste material or pollutants in industry. For example, in comparison to zeolite microporous solids as a catalyst, Aluminum MCM-41 exhibits better catalytic performance and enhanced conversion.

Adsorbents:
Various gases, toxins, and heavy metal can be adsorbed on the high surface area of a mesoporous material. The uptake of these substances varies based on the functionality. As an example, mesoporous silica is widely used to serve these purposes, as it has uniform pore size and high-density silanol groups. It can be used as membrane filter to separate out toxic or harmful gases. This is a very crucial process that aims at the extraction of CO$_2$ from bio gas processing and in industry. As mentioned, this process is known as adsorption. Adsorption and separation are still under research to ensure efficiency. At present, mesoporous material is very popular for the treatment of water. The mesoporous silica materials are very popular for the chromatographic process, as they have a high surface area with a very narrow pore size distribution. In size exclusion chromatography, gas chromatography, protein separation and in high performance liquid chromatography (HPLC), they are material of choice.

Bio-medical field:
The overexpression of drug efflux transporters is a key mechanism for multiple drug resistance in cancer cells. Using the mesoporous particles as a carrier for a cancer drug means that it can be delivered to its site of action to overcome this problem with sustained release. Based on their unique properties of it, mesoporous silica nanoparticles are used in the medical field, to make biomaterials, and for use in different medical equipment in hospitals and in the pharmaceutical field. [4] They offer uniform pore structures that can embed both large and small molecules. There are several key factors that make these silicas very attractive for use:

(i) They have high biological compatibility;
(ii) They are nontoxic;
(iii) These silicas can be easily modified by adjusting the pore size or changing the size of the functional surface as well as its main function;

(iv) There are several synthesis procedures that can be used to vary the composition, void size, and structure of the silicas. The nature of the template used in synthesis as well as the functionalization method can be adjusted to suit the design intended for the material.

Again, the field of medicine always faces many crisis due to new pathogens and diseases. Some of these diseases require preventive treatment and others are treated when a human being is infected. Among all of them, vaccinations are the crucial approach used to eliminate infections and non-communicable diseases. Several researchers have identified the recently developed porous silicon particles (PSiP) as a potential vaccine delivery tool. There are several advantages to the use of this porous silica variants in vaccination \[^4\]:

(i) Safety;

(ii) Compatibility and better performance;

(iii) It is biodegradable and as a result, it does not pose a risk to the human body;

(iv) It performs its functions depending on the application.

Tissue engineering, such as of bone, is a developing area focused on that the structure of the material to help the tissue to regrow and recover its functionality. Most of the drugs being used for different ailments today are developed using mesoporous particles. Due to their the chemical stability and characteristic of increased image contrast, mesoporous material is also an ideal candidate for use as a diagnostics material. The addition of functional moieties within the pores of the material endows the material with unique properties, for example as a sensing compound that can be to used as a glucose sensor. Due to their biocompatibility, low toxicity, and superior performance, they are also used to tract drug or imaging dyes inside the body.
1.4 Literature Review of Magnetic Nanomaterial

1.4.1 Introduction

Nanotechnology is a vast area where more development and research work are still required and it has been getting more attention worldwide due to its wide scope and variety of applications. As a branch of nanotechnology, the development and study of magnetic nanocomposites for potential use in biomedical, catalyst, diagnostics, industrial processing, telecommunications, electrical device, etc. applications, magnetic nanocomposites have grown in importance. Magnetic nanoparticles generally consist of a ferromagnetic material on the nanometer scale. In recent years, porous materials have attracted considerable attention due to their unique morphology and properties including a large surface area, large pore volume, narrow pore size distribution, and modifiable surface properties. Accordingly, porous magnetic materials are considered to have the advantages of not only for their magnetic property, but also their porous structures. Magnetism has been widely known from ancient times as a phenomenon by which some materials are attracted or repelled, depending on the materials used to make them e.g. loadstone, iron, and some steels. Attractive forces are due to ordered charged particles, which lead to the creation of a magnetic field.

Among all the magnetic nanoparticles, magnetite (Fe3O4) has a wide variety of applications for its super paramagnetic activity, although its application is also limited due to its high sensitivity to oxidation. Coating with another material such as a polymer not only provides a solution to this problem, but also adds some extra functionality to the composite. Carbon nanotubes, polymers, inorganic oxides, etc. are now widely used as coatings to synthesize diverse types of nanoparticle composites. Magnetic materials are now very promising for biomedical applications because magnetic materials can be controlled remotely from outside via the use of a non-invasive magnetic field. Specifically, applications of magnetic nanocomposites in the biomedical field includes drug delivery, hyperthermia based cancer treatment, magnetic resonance imaging, etc.

Additionally, the unique properties of the porous magnetic materials have attracted more attention due to their high pore volume, which can store drugs or any desired material, their large functional surface, which can easily be modified according to the reacting biomolecules, and their controllability by an external magnetic field. Even so, more research on magnetic
nanocomposites is required to diminish aggregation or decrease the energy associated with nano-magnetism. Also, as mentioned above, they are highly chemically sensitive, so that they require a coating to overcome of this limitation, although this modification or coating has some benefits as it adds additional functionality to the material, but at the same time, it may reduce the magnetic property. So, it is now a matter of study to overcome the challenge of how to precisely control the synthesis process of the magnetic composite to control the physical properties like size, surface area, pores etc. for a wide range of applications.

To date, many approaches have been tried to prepare magnetic materials such as hydrothermal, sol-gel, sonochemical, coprecipitation, etc. methods. As a simple, fast, and efficient method, the sol gel technique has no alternative for producing magnetic materials, where the concentration of the precursor is a critical parameter to optimize the final product [28], [29]. According to research, three factors affect particle size, including concentration of ligands, the heating temperature the dried gel, and the dilution coefficient of a solution. Recently, considerable efforts to develop various porous materials have been devoted to such methods as soft-templating, hard-templating, and hybridization.

Additionally, in magnetic resonance imaging, semiconductors, optical devices and similar applications, having a narrow particle size distribution is the main priority. It is now clear from the above that to have a successful application in any fields in industry and the biomedical fields, the precise control of the magnetism, pores, shape, size, structure, functional groups etc. has no alternative. This is also true for the extensive research work in this field.
1.4.2 General synthesis of magnetic material

Magnetic materials can be classified in diverse ways, depending on the strength and nature of their magnetic properties, and the materials used to enhance them. Some of the classifications used for magnet materials includes, ferromagnetic, diamagnetic, antiferromagnetic and paramagnetic. [30]

a) Diamagnetism: This is the weakest type of magnetism and is an essential property of all material. Diamagnetism is generally due to lack of cooperative behavior of orbital electrons under an applied force from external magnetic field. Diamagnetism is brought about because all the atoms have paired electrons hence there are no unpaired electrons in the shell. The result is that the net magnetic moment of an atom of a diamagnetic material is zero. It is should be noted, however, that, when a peripheral magnetic field is induced on these materials, the substances are magnetized in the opposite direction to the field, resulting in negative magnetization.

b) Para-magnetism: In para-magnetism, atoms and ions have partially unpaired electrons in their orbitals. This would mean that each atom present in a paramagnetic substance has at least a little magnetic moment. Nevertheless, there is no relationship between these atomic magnets. In the presence of a peripheral magnetic field, partial alignment of the atomic magnetic moment is experienced in the direction of the applied field resulting in net positive magnetization, unlike the case of diamagnetic materials, and positive susceptibility. When the field is zero, however, the magnetization also tends be zero.

c) Ferromagnetism

When we think of magnetic materials, the first things that come to our mind are nickel, iron, and magnetite. The above mentioned substances are referred to as ferro magnetic materials. The force between these substances is stronger than for any other magnetic materials. The strong forces that exist in between the atomic magnets are due to the parallel arrangement of electrons with respect to each other under the impact of exchange forces. It is estimated that the magnitude of the forces that exist in between these atomic magnets is as high as 1000 Tesla. This strength is $10^8$ times stronger than that of the Earth’s magnetic field.
On the other hand, the template method has been widely investigated by many scientific researcher’s due to its uncomplicated design. Templating methods are commonly utilized to fabricate nano-structured materials. In general, the template method requires three steps: The first step is the fabrication of the template followed by a common synthesis method. The last step is the removal of the template, resulting in a porous material. Template method is mainly classified into two categories: one for the soft-templating methods and the other for the hard-templating methods. Soft templates are relatively easier and simple to eliminate by heat treatment or dissolution in the proper solution. In contrast, the main advantage of hard-templating methods is their stable controllability to obtain the desired morphology. The selection of the template is the key to preparing porous materials with targeted structure.

**Soft-templating methods:** The soft-templating method is important for fabricating porous structured materials. In general, the materials for the soft template are surfactants, polymers and biopolymers which aggregate by means of intermolecular interaction forces such as van der Waals forces, hydrogen bonding, chemical bonding, and static electricity. Removal of the soft template is a relatively simple and facile process. A surfactant consists of molecules with hydrophilic and hydrophobic parts, and it forms versatile morphologies such as spherical, cylindrical, and lamellar micelles. The surfactant forms a liquid crystal phase at a high concentration in aqueous media, based on surfactant geometry. In the solution, the inorganic precursor interacts with the surfactant. In the preparation process, the choice of proper conditions plays a key role in determining the desired morphology, since the conditions directly affect the interaction between the organic and inorganic components at the interface between them. For example, the interaction between them is a weak hydrogen bond in strong acidic solution, whereas, a strong electrostatic force occurs in a strong alkaline solution. In most case, porous materials using surfactant template are obtained by aqueous solution synthesis or evaporation induced self-assembly (EISA) routes. Also, the removal of the template is an important process, so the proper process should be selected in accordance with the framework composition. There have been many reports on mesoporous materials using a surfactant as a soft template.
In recent years, various mesoporous transition metal oxides templated by surfactants were reported. The pore size of these materials is usually small, because the pores are obtained from low-molecular weight amphiphilic molecules, which have small hydrophilic heads and hydrophobic tails. Consequently, their short hydrophobic chains lead to small pore sizes. The diameter of the pore generally depends on the hydrophobic part. Polymer templates have been widely used to enrich the pore volume and surface area. Polymers, especially block copolymers, are widely employed in the synthesis of mesoporous materials due to their advantages such as large molecular weight, stability, and the fact that various molecular structures can be easily modified into the mesoporous structure. The structure and morphology are controllable by using different combinations of blocks and should be adapted to the application. It is clear that using the polymer as a template is beneficial to the enlarge pore size compared with surfactant. It is still a challenge, however, to fabricate well crystallized materials, because the wall composition tends to collapse at extremely high-temperature, where polymer faces difficulty in preserving its morphology. Biopolymers or biomolecules are another promising type of soft-template to fabricate porous materials owing to their natural availability, variety of structures, low toxicity, and facile process for the removal of the template. DNA, proteins, viruses, etc. are commonly used as biopolymer templates, and many reports have been published on porous materials templated by biopolymers. Recently, cellulose has been widely as a template because of its natural abundance and many sources. Huang et al. reported porous metal oxide templated by cellulose. Tobacco mosaic virus is very familiar as a biomolecule template for the synthesis of porous inorganic materials. In these achievements, it is obvious that biopolymers/molecules are a great templates to fabricate various kinds of structural materials, although it might be difficult to find an alternative template, that has same properties and/or morphology in nature. [31],[32]

**Hard-templating method**

The hard-templating method is an important strategy to fabricate porous metals, metal oxides, and carbon on the nanoscale since it directly gives a stable porous structure to the final products. A wide variety of hard template was previously reported such as polymeric microspheres, anodic aluminum oxide (AAO) and silica. Although polymer is well known as a soft-template, polymer beads are also widely used as hard templates because of their capability of maintaining a rigid
structure. The particle size is controllable by the rate of polymerization over a wide range. In a report by Xu et al., poly(methyl methacrylate) was successfully used as hierarchical ordered colloidal template to synthesize three-dimensionally ordered microporous material. In general, removal of the template is carried out by dissolution in appropriate solvents, calcination or pyrolysis. Silica template is often employed to fabricate porous metal oxides as a hard template because of its versatile morphology and adjustable size. Functionalization on the surface of silica is helpful to achieve the desired structure. Also, silica template has good stability, even under severe conditions such as vacuum, high temperature, and a strongly acidic environment, which leads to a better-quality framework. Three-dimensional ordered macroporous materials have been synthesized by colloidal silica template. In recent years, mesoporous silica has been arousing research interest as a template to fabricate mesoporous metal oxide frameworks. Bagheri et al. reported mesoporous MgFe$_2$O$_4$ templated by mesoporous silica.

**Classification of the functional magnetic nanocomposites given below:**

- Magnetic nanoparticles with multicomponent core shell type;
- Crystals colloids;
- Magnetic mesoscale nanocomposites;
- Magnetic functional polymers;

![Figure 6: Morphology of magnetic nanomaterials. Blue spheres represent magnetic nanoparticle.](image)

[31]
**Magnetic nanoparticles of the multicomponent core-shell type**

Hybrid particle consisting of two nanoscale compounds are receiving more attention due to their wide variety of application. In medical diagnosis, this type of hybrid composite has features making it suitable for dual imaging platforms, magnetic imaging processes with dual action features and multiple sensing capabilities. It has to be noted that the hybrid composite may be optimized independently of the properties of the individual particles. Additionally, it shows better performance as collective interaction is achieved between the combined particles. Several morphologies of this hybrid multi composite have been reported, such as core-shell and heterodimeric.

In this process, one type of nano particle of the hybrid composite is used for nucleation seeds and another one is distributed over it. By using this synthesis approach, a considerable number of heterostructures have been produced. Coating is a regular process for the stabilization of the magnetic core particles and functionalization of their surfaces for technical and biomedical purposes.

**Crystal colloids**

With a high translational order, self-assembly of the nanocomposite into a superlattice with two or three dimensions has attracted more attention these days. Most recently, applying a magnetic field by a drying medicated process from nanocubes of iron oxide, self-organized super crystals were developed. These types of super crystals reveal both translational and orientational order. By the interaction of different nanocrystals in self-assembly, these types of material provide scope for a large variety of composite materials with new cumulative properties.\(^{[33]}\)

**Magnetic mesoscale nanocomposites**

As explained above, materials with a pore diameter between 2 to 50nm are defined as mesoporous. The synthesis of magnetic mesoporous materials with desired functional groups and a narrow pore size distribution has received notable attention. Typically four steps required for the synthesis of the magnetic mesoporous nanoparticles.\(^{[34]}\)

- Super magnetic nanoparticle synthesis;
- Processing of nonporous dense SiO\(_2\) layer;
- Porous SiO\(_2\) growth using template;
• Removal of the template.

The synthesis process of SiO$_2$ superparamagnetic mesoporous spheres using cetyltrimethylammonium bromide (CTAB) template is given below:

**Figure 7**: Synthesis process for SiO$_2$ superparamagnetic mesoporous spheres. \[^{[31]}\]

**Magnetic functional polymers:**

To improve the stability, surface properties, and biocompatibility of the magnetic nanoparticles polymer coating has become very popular among researchers. An interesting aspect of this type of composite is that they can share their physical properties so that the magnetic nanoparticle and can transfer its magnetic field to the surface polymer coating which ultimately causes a change of size and movement of the nanocomposite. This type of composite has a wide variety of applications in industry and the biomedical field. Ferrogels represent one example of a functional magnetic polymer. \[^{[34],[35]}\]
1.4.3 Application of magnetic nanomaterials

Biomedical field:
Magnetic materials are useful in biomedical application in not only diagnosis, but also treatment in vivo and in vitro. Particle size, surface functionalization, surface area, and pore volume are important parameters, which must be adapted to applications. Although many approaches have been attempted for each application, it is still a challenge to develop magnetic materials for multiple applications. Here, recent reports on biomedical application of magnetic materials are summarized:

- Magnetic resonance imaging: In diagnosis, magnetic resonance spectroscopy (MRI) perform a very outstanding role. To obtain clear images, the contrast agent is very helpful. Currently, gadolinium chelate is used as the contrast agent, which makes images clearer in (longitudinal relaxation time)-weighted (T1) images but the agents make the image dark because of short intravascular half-lives and rapid renal excretion. Magnetic materials, in particular superparamagnetic materials, could be used to obtain clear images in T1, and (transverse relaxation)- weighted (T2) images as contrast agents. Nevertheless, it is still hard to deliver magnetic materials to the targeted site, since the magnetic materials are carried to the targeted site by the reticuloendothelial system (RES), which results in less effective detection. Magnetic materials can be carried to the targeted site by magnetic fields, and coating the magnetic materials with polymer, silica, gold, platinum could achieve the better detection and targeting as well as high biocompatibility.  

- Biosensor
In recent years, magnetic materials have been used as biosensor to detect glucose, DNA, viruses, bacteria, and cancer cells. It is required that the materials for the biosensor are superior in terms of sensitivity, size, power consumption, stability, response, resistance to aggressive media, price, and automatization level. Also, functionalization on the surface of materials is advantageous for the detection of molecular interactions, so nanostructured magnetic materials could meet these requirements because the large surface area allows materials to be functionalized more efficiently for interaction with targeting biomolecules. Immobilization of enzymes is of significant importance in the fabrication of a biosensor to retain bioactivity by utilization of magnetic
materials. Many efforts have been devoted to preparing materials for electrochemical biosensors by using magnetic materials with carbon and/or conductive polymers. [37]

**Drug delivery System**

The development of a drug delivery system (DDS) is crucial to reducing side effects and enhancing the effects of therapy in the treatment of disease. Considerable efforts have been devoted to developing DDSs by many approaches, such as stimuli-responsive polymeric nanoparticles, liposomes, metals/metal oxides, and exosomes, but there are still challenges to be overcome, including bio toxicity, targeting, difficulty of fabrication on a large scale, and economical availability. In those systems, drug is entrapped, attached, absorbed or encapsulated into or onto the carrier agent. Magnetic materials are beneficial to carry drugs to target a tumor via magnetic fields. A key parameter in DDS is the rate of adsorption and release, as well as the delivery of the drug to the targeted site. Nanostructured materials play important roles as drug vehicles, which are expected to store and release drugs efficiently. Nanostructured materials, especially porous magnetic materials have enormous potential for development of DDS systems to overcome these challenges. Cheng et al. demonstrated hollow Fe₃O₄ for anticancer drug delivery. The hollow Fe₃O₄ was functionalized to interact with cisplatin, which is a typical anticancer drug. The adsorption and release were conducted and their high magnetic property allowed efficient control to steer the particles to the tumor site. Also, magnetic nanoparticles coated by mesoporous silica for DDS were repoted. The iron oxide core plays a role in controllable steering, and the silica shell is used as the reaction site with biomolecules at the tumor site and provides storage for the drug. Furthermore, better controlled porous Fe₃O₄ was demonstrated by Mislav et al. Interestingly, both alternating and direct magnetic fields were shown to assist in efficient release of drugs. Surface functionalization for targeting, pore volume for storage of the drug and magnetic properties for better control, as well as magnetic field, are crucial factors in developing DDS systems using nanomaterials.

**Hyperthermia**

Not only DDS, but also magnetic hyperthermia is of foremost importance in cancer treatment. In this system, magnetic materials are made to heat up under alternating magnetic field, which is caused by hysteresis loss. The heating temperature for hyperthermia depends on the magnetic
property, the strength of magnetic field and the blood flow at the target tumor. Typically, cells exhibit signals of apoptosis when the temperature is elevated from 41 to 47 °C and signs of necrosis above 50°C. Nevertheless, there is also the difficulty that the normal cells/tissues are damaged while heating tumor the cell. Functionalization on the surface of magnetic materials is also important to target tumor site and minimize damage to healthy cells/tissues. Fe₃O₄ nanoparticles coated by oleic acid and polyethylene glycol were reported for magnetic hyperthermia, which resulted in increasing killing of breast cancer cells by 35% and 65%, respectively. In a further approach, Jiang et al. demonstrated the effectiveness of hyperthermia and MRI by using Fe₃O₄ nanoparticles functionalized by folic acid to react with tumor cells, which achieved great biocompatibility, resulting in a clear heating effect and good contrast imaging. The combination of diagnosis with hyperthermia is superior approach to treat cancer cells, since both diagnosis and treatment at the same time lead to less side effects and better efficacy. [38]

**Energy production and storage**

Metal oxides are useful in both lithium ion batteries and supercapacitors as anode and electrode materials, respectively. Although many approaches have been reported, the results are still far from the theoretical values. Therefore, further development of electrode materials is a key factor in optimizing the properties of those energy storage applications. The lithium ion battery is one of the most reliable types of energy storage in portable electronic devices. The development of better anode materials for the lithium ion battery leads to better performance, including higher power densities, enhanced safety, and longer cycle life. Carbon based materials, especially graphite, is currently used as the most common commercial anode materials, but there are limitations in terms of specific capacitance and safety. A novel approach to preparing anode materials is essential to overcome these problems. [39],[40]
1.5 Reference


2. Chapter 2: Tuning wall thickness in mesoporous silica films for optimizing of optical anti-reflective properties

2.1 Abstract
By changing the relative weight of the polymer to silica source, the pore-to-pore distance can be varied without a significant change of the mesopore size, thus allowing control of the refractive index and the porosity in the films. Compared to the glass substrate, the transmittance is increased by around 3.3 % in the wavelength range from 400 nm to 750 nm.

2.2 Introduction
Controlling the morphology of mesoporous silica materials is critical for optimizing their functional applications. Among various morphologies, continuous mesoporous silica films are quite useful for optical applications, because of their high transparency.[1] Mesoporous silica films have generally been prepared by solvent evaporation methods which enable coating of well-ordered mesoporous layers on various substrates, such as glass and plastic substrates.[2-4] Currently, mesoporous silica films have attracted great interest as a low dielectric constant and low refractive index materials, because of their high porosity and high thermal/mechanical stability.[5-6] To date, several types of mesoporous silica films with different pore structures have shown excellent low dielectric properties and an ultra-low refractive index. An anti-reflection (AR) coating is a type of optical coating which is applied to the surface of lenses and other optical devices in order to reduce reflectivity.[7-9] In order to enhance the antireflection properties an AR film having a low refractive index is desired. However, the conventional solvent evaporation method generally requires precise optimizations of the experimental conditions, such as controls of temperature and humidity, selection of templates, solvent compositions etc. Recently, there are a few reports on the use of mesoporous silica nanoparticles for antireflection films, but careful modifications of the internal and external surfaces of nanoparticles are critical, and also the selection of a proper matrix for the dispersion of nanoparticles is important.[10] Traditionally, antireflection films have been prepared by spraying, electrodeposition, chemical vapour deposition, physical vapour deposition, and so on. Wet-chemical methods are very convenient for practical industrial applications, but sometimes they are limited to specific
materials. In this work, we have developed a new technology for the formation of an ultra-low refractive index thin film by means of wet-chemical method. Specifically, we have successfully synthesized continuous mesoporous silica films with large spherical mesopores using new laboratory-made polystyrene-\textit{block}-polybutadiene-\textit{block}-polystyrene based triblock copolymers (HmSEBmS)[11], in which the polystyrene block is functionalized with hydrophilic sulfonic groups. By using a single structure directing agent, precise tuning of the wall thicknesses and optimization of the mesostructures for obtaining the desired antireflection films can be realized.

2.3 Experimental section

According to a previous report[11], the precursor solution was prepared by using the laboratory-made triblock polymer (HmSEBmS). The precursor solutions were spin-coated on silicon (Si) substrates at room temperature. The as-prepared films were calcined at 600 °C for 4 h in air. The weight ratios (x) were varied from 10 to 40 mass%. The calcined mesoporous silica samples are abbreviated as ‘meso\_x’, in which x indicates the weight ratio of sulfonated HmSEBmS to TEOS in the precursor solutions.

2.4 Results and Discussion

\textbf{Figure 1} shows low-angle XRD patterns and SEM images of mesoporous silica films prepared with various polymer amounts. From the SEM images of the top surfaces (\textbf{Figure 1b}), large-sized spherical mesopores are observed on the film surface for all the films, even when the x value is largely varied from 10 to 40 wt.%. Although the mesopores are closely packed and are mostly similar in size, their pore arrangement is not highly ordered, which is different to P123- and F127-templated mesoporous silica films.[2] Following a further increase in the polymer amount of up to 40 wt.%, the spherical mesopores still remain however the pore walls become thinner due to the decrease in the amount of silica available for construction of the walls.

To carefully investigate the structural details, such as pore-to-pore distances, pore sizes, and wall thicknesses, low-angle XRD measurement (\textbf{Figure 1a}) and N$_2$ adsorption-desorption isotherm were carried out for all the samples. The \textit{d}-spacing of the first diffraction peaks corresponds to the average pore-to-pore distance (\textbf{Figure 1a}), because the pore arrangement in the films are in
random. The average pore sizes can be measured by the BJH method. Hence, by subtracting these values from the \( d \)-spacing, the average pore wall thicknesses can be theoretically calculated. All the calculated values are summarized in Table 1. The most important point is that the peaks gradually shifted towards higher angles with the increase of the \( x \) values from 10 wt.% to 40 wt.%, indicating that the pore-to-pore distance is gradually decreased, although the size of the mesopores remains constant. At the same time, the wall thicknesses are gradually decreased.

Furthermore, Table 1 also show the measured refractive index and porosity for all the films. The data indicates that the porosity is increased with the increase in the amount of polymer, while the refractive index is decreased. Moreover, the results also support that the mesoporous silica thin film is stably formed when the quantity of the starting polymer is in the range of 10 wt.% to 40 wt.%.

Mesoporous silica film with low refractive index can be applied as an antireflection film. To design the antireflection film, the following equations should be considered:

Amplitude condition: \( n_2^2 = n_1 \cdot n_3 \) \hspace{1cm} (1)

Phase condition: \( 2n_2 \cdot d = \lambda / 2 \) \hspace{1cm} (2)

Where \( n_1 \) is a surface refractive (air layer) index (1.00), \( n_2 \) is the refractive index of the antireflection film, \( d \) is the film thickness, and \( n_3 \) is the refractive index of the substrate, respectively. As indicated by the above equations, the anti-reflection efficiency shows a strong dependence on the wavelength. Single layered antireflection film cannot provide 0 % reflectance ratio in all wavelengths. Here, we selected only the spectral sensitive wavelength of the human eye, which is around 550 nm. The required refractive index for realizing the minimum reflectance ratio in the range of around 550 nm can be estimated, by assuming that the refractive index \((n_1)\) of the substrate (non-alkali glass substrate) is 1.52. As a result, the required value is 1.23 in case that the film thickness is 100 nm. Thus, it can be concluded that the meso_20 film (prepared with 20 wt.% polymer) can provide the best antireflection layer.

Figure 2 shows a comparison of visible-light transmittance for the glass substrate and the antireflection film with mesoporous silica layer. The transmittance curves for the films exhibit maximum values in the wavelength region of 400-750 nm (i.e., The reflection curves are minimalized in this region). Compared to the glass substrate, the transmittance is increased by
around 3.3 % in the wavelength range of 400 to 750 nm, indicating a significant improvement in the antireflection performance. In addition, the antireflection films are often used on the outermost surface. Even after the abrasion test using a cheese cloth (1 kg cm\(^{-2}\), 50 times), the transmittance curve is not changed, showing a good mechanical stability. Furthermore, the fabricated mesoporous silica film also has an excellent acid resistance. Even after the films were immersed for 2 hours in a sulfuric acid aqueous solution (0.1 N), no peering was observed on the film surface. In addition, no change in transmittance was observed after the films were kept at high temperature and high humidity (85 °C, 85 %) for 100 hours. These results suggest that unlike CTAB-templated mesoporous silica films, the present mesoporous silica film is very stable due to the presence of thick pore walls.\(^{[2]}\)

This technology is applicable to other plastic substrates such as thoria acetyl cellulose (TAC) film and polymethyl methacrylate (PMMA) films. In this case, the polymer was removed by irradiation with vacuum ultraviolet light. It is confirmed that the mesoporous structure can be formed onto these plastic substrates, and the same mesostructures are formed. The technique based on the photochemical decomposition of organic molecules has been known as a photocalcination.\(^{[12-13]}\)

As shown in Figure 2b, the TAC film shows an average reflectance of 3.7 % in the visible light region. By forming a mesoporous silica layer, the average reflectance can be reduced to 0.4 %. In the case of PMMA film, after the coating of mesoporous silica layer, the average reflectance is decreased from 3.8 to 0.7 % in the visible light region. Importantly, it should be noted that the coating of mesoporous layers can reduce the reflectivity (i.e., increase the transmittance). The minimal reflectivities of these films (0.4-0.7 %) meet the requirement for antireflection coatings on substrates.
2.5 Conclusion
This work has demonstrated that using a single structure directing agent can achieve precise tuning the wall thicknesses, realizing the optimal mesostructure for desired antireflection films. When the relative weight of the polymer to silica source is increased from 10 to 40 wt.%, the pore-to-pore distance is gradually reduced without a significant change of the mesopore size. Compared to the glass substrate, the transmittance is increased by around 3.3 % in the wavelength range of 400 to 750 nm. The developed technological concept can also be applied to other plastic substrates such as TAC and PMMA films. It should be noted that the coating of mesoporous layers can largely reduce the reflectivity of up to 0.4-0.7 %. Such a minimal reflectivity meets the requirement for antireflection coatings on substrates.
Table 1 Summary on structural properties for mesoporous films prepared with various polymer amounts.

<table>
<thead>
<tr>
<th>Polymer amount (wt.%)</th>
<th>Film thickness (nm)</th>
<th>d-spacing (nm)</th>
<th>Pore size (nm)</th>
<th>Wall thickness (nm)</th>
<th>Surface area (m² g⁻¹)</th>
<th>Refractive Index*1</th>
<th>Porosity (vol.%)*2</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>96</td>
<td>33.3</td>
<td>21.2</td>
<td>12.1</td>
<td>101</td>
<td>1.302</td>
<td>31</td>
</tr>
<tr>
<td>20</td>
<td>117</td>
<td>28.7</td>
<td>22.3</td>
<td>6.4</td>
<td>211</td>
<td>1.210</td>
<td>51</td>
</tr>
<tr>
<td>30</td>
<td>82</td>
<td>26.8</td>
<td>22.1</td>
<td>4.7</td>
<td>358</td>
<td>1.171</td>
<td>60</td>
</tr>
<tr>
<td>40</td>
<td>117</td>
<td>26.8</td>
<td>22.7</td>
<td>4.1</td>
<td>473</td>
<td>1.135</td>
<td>68</td>
</tr>
</tbody>
</table>

*1 The refractive index was measured by spectroscopic ellipsometer. The refractive index is at a wavelength of 550 nm.

*2 The porosity (P) was calculated from the refractive index using the following Lorentz-Lorentz formula. The refractive index is at a wavelength of 550nm. P = 1 - (n₂⁻¹ - n₄⁻¹)/2 + (n₂⁻¹ - n₄⁻¹)^2, where nₐ is refractive index of the antireflection film, and n_s is the refractive index of silica (1.46).

Figure 1

Figure 1 (a) Low-angle XRD patterns and (b) SEM images of mesoporous silica films prepared with various amounts of polymer.
Figure 2 (a) Visible-light transmittance of non-modified glass substrate, and glass substrates with mesoporous layers before and after the abrasion tests. (b) Transmittance of TAC and PMMA films with and without mesoporous layers.
2.6 Reference

3. Chapter 3: Preparation of Ultraviolet Curing Type Silicone Rubbers Containing Mesoporous Silica Fillers

3.1 Abstract

Here we have been focusing on mesoporous silica (MPS) as inorganic filler material to improve the mechanical strength of silicone rubbers. The MPS particles are more effective in reducing the coefficient of thermal expansion (CTE) and hardening silicone rubber composites, compared to commercially available nonporous silica particles. In this study, we utilize ultraviolet curing type silicone rubbers and prepare MPS composites according to a simple single-step method. From an industrial viewpoint, simplifying the fabrication processes is critical. The thermal stability and mechanical strength are examined in detail in order to showcase the effectiveness of MPS particles as filler materials.

3.2 Introduction

Silicone rubber is a rubber-like material composed of silicone, carbon, hydrogen, and oxygen. Silicone rubbers have been widely used in industry because they are generally non-reactive, flexible, mechanically- and thermally-stable, and resistant to extreme conditions.\(^1\)-\(^3\) Due to these properties, besides its simple manufacturing and shaping process, silicone rubber can be found in a wide variety of products, such as LED cover, light guide films, and optical fibers. However, low mechanical strength, especially tensile strength, and high thermal expansion are critical drawbacks which hamper the practical use of silicone rubbers. Until now, many efforts aimed at reinforcing their mechanical strength, including the addition of inorganic fillers.\(^4\)-\(^9\)

We have been focusing on mesoporous silica (MPS) as inorganic filler material to improve the mechanical strength of polymers including silicone rubbers.\(^10\)-\(^16\) MPS have abundant nanoscale pores and large pore volumes, and can be prepared through the spontaneous self-assembly of surfactants.\(^17\)-\(^20\) Our previous papers have demonstrated that MPS particles are more effective in reducing the coefficient of thermal expansion (CTE) and hardening silicone rubber composites, compared to other inorganic fillers.\(^15\)-\(^16\) However, the typical fabrication process of silicone rubber composites is laborious because of the many steps required to obtain the final product. From an industrial viewpoint, simplifying the fabrication processes is critical. In this study, we
focus on ultraviolet curing type silicone rubbers and prepare MPS composites according to a simple single-step method. The thermal stability and mechanical strength are examined in detail in order to showcase the effectiveness of MPS particles as filler materials.

3.3 Experimental sections

MPS particles were prepared according to a previously reported method.\[21\] Dried MPS particles and silicone (liquid silicone rubber UV cure type (PDMS) provided from Shin-Etsu Chemical Co., Ltd.) were mixed with a THINKY vacuum mixer ARV-310 at room temperature until a bubble-free mixture was obtained. The obtained mixture was heated under reduced-pressure conditions and was further kept under UV light (365 nm) for 2-3 hours. We prepared a control sample consisting of a composite with nonporous silica (NS) particles (Admafine SO-C6; from Admatech Co., Ltd.) were used under the same conditions. The particle size is around 800 nm. Several silica/silicone composites were prepared with different concentration of MPS (or NS) particles. The total mass of the composite was fixed to 10 g. The details of each sample are listed in Table 1. Hereafter, MPS and NP composites are described as MPS_\(x\) and NS_\(x\), respectively, where \(x\) indicates the silica fillers-to-silicone weight percentage.

3.4 Result and discussion

Figure 1a shows the \(N_2\) adsorption/desorption isotherms of the starting MPS particles. A typical type-IV isotherm with a clear capillary condensation step can be observed. From the calculation of the pore size estimated by the Barrett-Joyner- Halenda (BJH) method, the average pore diameter is found to be 3.8 nm. The surface area and the pore volume are 883 m\(^2\) g\(^{-1}\) and 1.10 cm\(^3\) g\(^{-1}\), respectively. Figure 1b shows the low-angle XRD patterns for the MPS powders and a typical MPS_15 composite. In both samples, the two peaks assignable to the 10 and 11 diffractions of a 2D hexagonal mesostructure confirm that the long-range pore ordering is not affected by the process. Because the assessed value of \(d_{10}\) is 5.0 nm, the pore-to-pore distance is calculated to be ca. 5.8 nm. Figure 1c shows the photographs of the various obtained MPS/silicone composites. The composites show good flexibility. The fillers are uniformly
dispersed in the matrix and no voids or cracks were observed at the interface between the particles and the silicone rubber matrix.

The dead pore ratio (DPR) \( (i.e., \) the amount of mesopores filled with the silicone in the composites) can be theoretically calculated according to:

\[
V = \frac{1}{\rho_{\text{silicone}}} \left[ \frac{(100-x)}{100} \right] - \frac{1}{\rho_{\text{silica}}} \left[ \frac{x}{100} \right]
\]

DPR (\%) = \frac{V_0 - V}{V_0} \times 100 \tag{2}

Here, \( V_0 \) (cm\(^3\) g\(^{-1}\)) is the total pore volume, \( x \) (\%) is the additive filler weight ratio of the composites, and \( \rho_{\text{silicone}}, \rho_{\text{silica}}, \) and \( \rho \) (g cm\(^{-3}\)) are the densities of silicone (1.05 g cm\(^{-3}\)), silica (2.18 g cm\(^{-3}\)), and the composites. The densities were determined by the Archimedean method. The DPR for each sample is calculated to be above 70\%. Most of the mesopores are effectively loaded with the silicone.

The CTE values were determined from the gradient of the linear dependence between the mechanical expansion and temperature (see the thermomechanical analysis charts (TMA) in Figures 2a-b). The CTE values gradually decrease as the filler content in the composites is increased. For instance, the CTE value of MPS_35 is 216\times10^{-6} \degree C\(^{-1}\), which is 41.6 \% lower than that of silicone itself (370\times10^{-6} \degree C\(^{-1}\)). On the other hand, the CTE value of NS_35 is 245\times10^{-6} \degree C\(^{-1}\) which is higher than that of MPS_35. It is found that the MPS fillers are more effective at decreasing the CTE values, probably due to the physical confinement of the silicone inside the pores of the MPS. To make this point clear, the weight percentage of silicone present outside the MPS particles (\( W \)) was estimated according to the following formula (Table 1):

\[
W = \frac{V_0 \times \frac{x}{100} \times \frac{\text{DPR}}{100} \times \rho_{\text{silicone}}}{\left( \frac{100-x}{100} \right)} \times 100
\]

From Figure 2c, it can be observed that the CTE values decrease with \( W \) in a linear fashion. Thus, by being physically restricted inside the mesopores, the silicone shows a higher thermal stability. The CTE value at the y-intercept is around 50 \times10^{-6} \degree C\(^{-1}\), which is larger than that of silica itself. The silicone inside the mesopores, probably near the pore entrance, is slightly thermally expanded.
The values of hardness for each sample were obtained from the compression stress-strain ($\sigma$-$\varepsilon$) curves. As the filler content is increased, the stress required for bending the materials is also increase, bringing about improved mechanical hardness. The Young’s modulus ($E$) can be measured according to the following formula in the stress range within which the Hooke’s law holds:

$$E = \frac{\sigma}{\varepsilon} \quad (4)$$

**Figure 3a** shows the calculated Young’s modulus of the composites in function of $W$. For a specific filler concentration, the MPS appear harden the silicone rubber much more than the NS.

The enhancement of the mechanical strength in a typical MPS composite (MPS_15) is also monitored through tensile stress-strain measurement, as shown in **Figure 3b**. The typical curve of the silicone rubber sample exhibit a sharp increase in stress before fracture. The MPS composite shows a much higher fracture strength (8.5 MPa) in comparison with the original silicone rubber (4.2 MPa). It is important to notice that the fracture strain is mostly retained in the MPS composite (131 %), although the modulus significantly increased. In contrast, the hardness of the NS composite (NS_15) is increased, but its fracture strain is significantly decreased. This situation has been typically observed when adding inorganic fillers.

From the above results, it is found that the addition of mesoporous silica not only increases the modulus effectively but also retains the stretching property, leading to a significantly improved fracture toughness. Therefore, we should consider a different mechanism under stretch for better strain of the MPS-containing rubber system. It can be expected from silicone polymers to be released from the mesopores upon stretching. The usage of this stretching energy, typically responsible for breaking the covalent bond inside the polymer, can be redirected towards breaking the weak physical bonds between the silicone rubber and the mesopores wall, thus contributing to the larger fracture strain. To investigate the optimum fillers concentration, the tensile stress-strain curves for all the samples are measured. The relationship between the filler concentration and the elongation at break are plotted in **Figure 3c**. While the elongation break is approximately constant in the case of the NS composites, we can clearly see that the optimal concentration for the MPS composites ranges between 5 and 15 %.
3.5 Conclusion

To conclude, we have proposed a simple procedure to produce MPS/silicone composites. Our systematic study has demonstrated that mesoporous silica particles are a promising candidate as inorganic filler, unlike non-porous silica, with the potential to significantly improve the mechanical properties of silicone rubber. Our MPS/silicone composites exhibit lower coefficient of thermal expansion as well as larger tensile strength and Young’s modulus. These encouraging results will undoubtedly promote the use of MPS/silicone composites to practical applications benefiting from improved thermal and mechanical strength.
Table 1 Summary on density, DPR, silicone relative ratio inside/outside pores, and CTE for the obtained MPS- and NS-containing silicone composites.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount (g)</th>
<th>Density [g cm(^{-3})]</th>
<th>DPR [vol%]</th>
<th>Silicone relative ratio [wt%]</th>
<th>Outside silicone weight ratio [wt%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Silicones inside pores</td>
<td>Silicones outside pores</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silicone</td>
<td>0.00</td>
<td>100</td>
<td>1.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MPS_10</td>
<td>9.1</td>
<td>91.0</td>
<td>1.07</td>
<td>8.5</td>
<td>91.6</td>
</tr>
<tr>
<td>MPS_15</td>
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<td>86.7</td>
<td>1.08</td>
<td>12.7</td>
<td>87.3</td>
</tr>
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<td>25.9</td>
<td>74.0</td>
<td>1.14</td>
<td>32.9</td>
<td>67.1</td>
</tr>
<tr>
<td>NS_10</td>
<td>9.1</td>
<td>91.0</td>
<td>1.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NS_15</td>
<td>13.0</td>
<td>86.7</td>
<td>1.09</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NS_35</td>
<td>25.9</td>
<td>74.0</td>
<td>1.17</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 1. (a) N₂ adsorption-desorption isotherms of the MPS particles. Inset: Pore size distribution curve calculated from BJH method. (b) Small-angle XRD patterns for the MPS particles and MPS_15, and (c) photographs of the obtained MPS/silicone composites.
Figure 2. Length normalizing thermal mechanical analysis (TMA) charts for (a) MPS and (b) NS composites. The original length of the specimens and the displacement of length are $L_0$ and $\Delta L$, respectively. The CTEs are also displayed. (c) Relationship between the CTE and the weight ratio of the silicone outside the pores in the composites.
Figure 3  (a) Relationship between the Young’s modulus and the weight ratio of the silicone present outside the pores. (b) Tensile stress-strain measurement for MPS_15, NS_15, and bare silicone. (c) Relationship between the elongation at break and the filler content.
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4. Chapter 4: Mesoporous Iron Oxide Synthesized Using Poly(styrene-b-acrylic acid-b-ethylene glycol) Block Copolymer Micelles

4.1 Abstract
The synthesis of mesoporous metal oxides has gained extensive interests due to their high surface area, uniform and tunable pores, narrow pore size distribution, and unique properties and functionalities. Herein, we report the soft-templated fabrication of mesoporous iron oxide using the asymmetric triblock copolymer, poly(styrene-b-acrylic acid-b-ethylene glycol) (PS-b-PAA-b-PEG) block copolymer. To our best knowledge, this block copolymer has not yet been used for the fabrication of mesoporous iron oxide. This polymer is known to form a micelle consisting of a PS core, a PAA shell and a PEG corona in aqueous solutions. The calcination temperature was found to play a crucial role in determining the final morphology of the mesoporous iron oxide, as lower temperatures (<400 °C) could not sufficiently remove the block copolymer, whereas very high temperatures (≥450 °C) lead to the collapse of the mesoporous structure. The mesoporous iron oxide obtained at an optimized calcination temperature of 400 °C exhibited an average pore diameter of 39 nm, with a large specific surface area and pore volume of 86.9 m² g⁻¹ and 0.218 cm³ g⁻¹, respectively.
4.2. Introduction

Transition metal oxide nanomaterials are important classes of materials with broad potential applications in catalysis,\textsuperscript{1,2} sensors,\textsuperscript{3-5} energy storage and conversion,\textsuperscript{6-9} and biomedical fields.\textsuperscript{10-12} Among many transition metal oxides, iron(III) oxide has been considered as one of the most promising support materials for the loading of noble metal nanoparticles in catalytic applications due to their abundance, low cost, high chemical and thermal stabilities, as well as low toxicity and environmental friendliness.\textsuperscript{13} The functional performance of iron oxide materials, such as the catalytic activity and magnetic behaviour, can be greatly enhanced when their crystal sizes are confined to the nano-scale and the morphologies are appropriately controlled to have a high surface area.\textsuperscript{14} As such, many efforts have been carried out to fabricate diverse morphologies of iron oxide nanostructures with large surface areas, including nanorods,\textsuperscript{15} nanotubes,\textsuperscript{16} hollow spheres,\textsuperscript{17} and flower-like nanosheets.\textsuperscript{18} In recent years, the synthesis of mesoporous materials have attracted extensive research interests owing to their unique morphology, large surface area and pore volume, narrow pore size distribution, controllable wall composition and modifiable surface properties.\textsuperscript{19, 20} Mesoporous metal oxide materials, including mesoporous iron oxides have been fabricated mostly through two main approaches: hard and soft-templating methods.\textsuperscript{14, 21, 22} Hard-templating (nanocasting) method typically uses rigid materials (e.g., silica, polystyrene spheres, anodic aluminium oxide (AAO)) and the crystal growth is limited to the void space of the template, leading to subsequent replica production.\textsuperscript{14, 21, 23} Nanocasting is particularly useful for the fabrication of rigid metal oxide mesostructures with high crystallinity.\textsuperscript{24} However, it often involves complicated procedures due to the need for multiple steps, including the preparation of the hard template, then infiltration of the metal precursor into the pores, and lastly, the removal of the hard template and formation of the metal oxide phase.\textsuperscript{25, 26} Furthermore, in nanocasting, the removal of the hard template such as silica spheres usually requires the use of hydrofluoric acid, which is harmful and environmentally unfriendly.\textsuperscript{27, 28} On the other hand, soft-templating methods typically relies on the use of surfactants or block copolymers. Compared to nanocasting, these methods are easier to perform as they do not require the initial fabrication of the template, whilst also enabling control over the pore size,
depending on the choice of surfactants or polymers. In the case of mesoporous iron oxide obtained using surfactant-templated method (e.g., non-ionic surfactant P123, F127, and P108), the pores tend to be smaller and easier to collapse after crystallization. In comparison, the utilization of block copolymer as a soft template for the fabrication of mesoporous iron oxide is usually more advantageous as it enables the formation of larger pores, which may be advantageous for accommodating larger guest species or molecules. However, at present, there are still some challenges associated with the block copolymer-templated fabrication of mesoporous iron oxide, such as poor control over the reaction and crystal growth.

Herein, we report the soft-templated synthesis of mesoporous iron oxide using the poly(styrene-\textit{b}-acrylic acid-\textit{b}-ethylene glycol) (PS-\textit{b}-PAA-\textit{b}-PEG) block copolymer. In this reaction system, the PS-\textit{b}-PAA-\textit{b}-PEG block polymer forms tri-functional micelles in the aqueous solution: PS, PAA and PEG blocks which act as core, shell, and corona, respectively. The effect of calcination temperature was investigated to determine the optimized condition for the fabrication of well-defined mesoporous iron oxide. The composition, morphology, and textural characteristics of the mesoporous iron oxide have been characterized using various analytical techniques, such as X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) thermogravimetric analysis (TGA), and nitrogen (N\textsubscript{2}) adsorption-desorption isotherms.
4.3. Experimental

4.3.1. Chemicals

Poly(ethylene glycol) methyl ether (4-cyano-4-pentanoate dodecyl trithiocarbonate) was purchased from Sigma Aldrich (Japan). PEG-CTA (number-average molecular weight \( M_n \) = 2,400, degree of polymerization = 46) and 2,2'-azobis(2,4-dimethyl valeronitrile) (V-65, > 95.0%) were purchased from Wako Pure Chemicals (Japan) and were used without further purification. 2,2'-Azobis(isobutynitrile) (AIBN, > 98.0%) from Wako Pure Chemicals was recrystallized from methanol. Methanol was dried by molecular sieves (4 Å) and purified by distillation. 1,4-Dioxane and acrylic acid (AA, > 98.0%) were dried over 4 Å molecular sieves and distilled under reduced pressure. Styrene (> 99.0%) was purchased from Wako Pure Chemicals and washed with an aqueous alkaline solution and distilled from calcium hydride under reduced pressure. Tetrahydrofuran (THF), iron(III) nitrate nonahydrate (Fe(NO\(_3\))\(_3\)·9H\(_2\)O, 99.99%), sodium hydroxide (NaOH, 99.9%), gold(III) chloride trihydrate (HAuCl\(_4\)·3H\(_2\)O, 99.99%) and ethanol (99.5%) were purchased from Nacalai Tesque (Japan).

4.3.2. Preparation of PS-\(b\)-PAA-\(b\)-PEG triblock copolymer

PS-\(b\)-PAA-\(b\)-PEG triblock copolymer was prepared via reversible addition-fragmentation chain transfer (RAFT) radical polymerization (Scheme 1). First, poly(acrylic acid-\(b\)-ethylene glycol) (PAA-\(b\)-PEG) diblock copolymer was prepared as follows. PEG-CTA (1.50 g, 0.635 mmol), AA (6.74 g, 93.6 mmol), and AIBN (41.1 mg, 0.250 mmol,) were dissolved in 1,4-dioxane (93 mL). The polymerization process was carried out at 60 °C for 8 h under argon (Ar) gas. After the polymerization, the polymerization mixture was dialyzed against pH 10 aqueous solution for one day and against pure water for two days. The diblock copolymer (PAA-\(b\)-PEG) was recovered through a freeze-drying technique (5.42 g, 65.8 %). The degree of polymerization of the PAA block was estimated from \(^1\)H NMR spectrum in DMSO-\(d_6\) to be 113. The number-average molecular weight, \( M_n \)(NMR) for the block copolymer was estimated from \(^1\)H NMR to be \( 1.07 \times 10^4 \) (Figure S1a). The number-average molecular weight (\( M_n \)(GPC)) and molecular weight distribution (\( M_w/M_n \)) estimated from gel-permeation chromatography (GPC) were \( 1.32 \times 10^4 \) and 1.42, respectively (Figure S2).
Styrene (1.00 g, 9.56 mmol), V-65 (3.87 mg, 0.0156 mmol), and PAA-b-PEG (201 mg, 0.0190 mmol) were dissolved in methanol (4.7 mL). The polymerization was carried out at 50 °C for 48 h under Ar atmosphere. The polymerization mixture was dialyzed against methanol for two days and against pure water for three days. The triblock copolymer (PS-b-PAA-b-PEG) was recovered by a freeze-drying technique (0.653 g, 45.3 %). The degree of polymerization of the PS block was estimated to be 162 by 1H NMR in DMSO-d6 at 120 °C. The $M_n$(NMR) for PS-b-PAA-b-PEG was estimated by 1H NMR to be $2.74 \times 10^4$ (Figure S1b). The GPC measurements for PS-b-PAA-b-PEG was not performed, because the polymer could only be molecularly dissolved in DMSO at high temperatures.

4.3.3. Synthesis of mesoporous iron oxide

The formation mechanism of mesoporous iron oxide from the PS-b-PAA-b-PEG triblock copolymer micelles is shown in Scheme 2. In a typical procedure, 10 mg of PS-b-PAA-b-PEG block copolymer was dissolved into 2 mL of THF with sonication. After a complete dissolution, 20 µL of NaOH (0.1 M) was slowly added into the above solution under a constant stirring. Meanwhile, 42 mg of Fe(NO3)3·9H2O was dissolved in 80 µL of ethanol, before being added into the above polymer solution. Next, the mixture solution was stirred for 1 h and subsequently dried on the petri dish at room temperature overnight. After completely dried, the powder was calcined in air at 400 °C for 4 h, with a heating rate of 1 °C min⁻¹ to obtain the mesoporous iron oxide.

4.3.4 Characterization

1H NMR spectra were obtained with a Bruker DRX-500 spectrometer operating at 500 MHz. GPC measurements for PAA-b-PEG were performed using a refractive index detector equipped with a Shodex GF-7M HQ column working at 40 °C under a flow rate of 0.6 mL min⁻¹. A phosphate buffer (pH 8) containing 10 vol% acetonitrile was used as an eluent. $M_n$ and $M_w/M_n$ were calibrated with standard sodium poly(styrenesulfonate) samples. Dynamic light scattering (DLS) and zeta-potential measurements were performed using a Malvern Zetasizer Nano-ZS ZEN3600 equipped with a He-Ne laser light source (4 mW at 632.8 nm). The sample solutions were filtered using a syringe filter with a pore size of 0.45 µm. The data obtained were analyzed using Malvern
Zetasizer Software 7.11. The morphological observations of the polymeric micelles and mesoporous iron oxide samples were conducted using scanning electron microscope (Hitachi SU-8000) operated at an accelerating voltage of 10 kV and transmission electron microscope (JEOL JEM-2100F) operated at an accelerating voltage of 200 kV. The phase composition and crystal structure of the mesoporous iron oxide samples were identified using X-ray diffraction (Shimadzu XRD-7000) with Cu-Kα (1.54 Å). The surface composition of the samples was analyzed using X-ray photoelectron spectroscopy with a PHI Quantera SXM (ULVAC-PHI) 30 instrument using an Al-Kα X-ray source. Thermogravimetric analysis (TGA) of the samples was carried out from room temperature to 800 °C under an air atmosphere using a Hitachi HT-Seiko Instrument Exter 6300 TG. Nitrogen (N₂) adsorption-desorption measurements were performed using a Belsorp-mini II Sorption System at 77 K. The specific surface areas were calculated using the multipoint Brunauer-Emmett-Teller (BET) method at a relative pressure (P/P₀) range of 0.05 to 0.30, while the total pore volumes were calculated by the Barrett-Joyner-Halenda (BJH) method. Before the BET measurements, the samples were degassed under vacuum at 100 °C for overnight.
4.4. Results and discussion

The synthetic route to prepare poly(styrene-\textit{b}-acrylic acid-\textit{b}-ethylene glycol) (PS-\textit{b}-PAA-\textit{b}-PEG) triblock copolymer via RAFT controlled radical polymerization is shown in Scheme 1. To prepare the PS-\textit{b}-PAA-\textit{b}-PEG, we firstly prepared the diblock copolymer, poly(acrylic acid-\textit{b}-ethylene glycol) (PAA-\textit{b}-PEG) via RAFT using PEG-CTA. The GPC elution curve (Figure S2) for PAA-\textit{b}-PEG is unimodal with no indication of the presence of uncontrolled polymers. Furthermore, the $M_w/M_n$ value of PAA-\textit{b}-PEG is fairly narrow ($M_w/M_n = 1.42$), indicating that the polymerization is reasonably well-controlled. PS-\textit{b}-PAA-\textit{b}-PEG was then prepared via RAFT radical polymerization of styrene using PAA-\textit{b}-PEG as a macro chain transfer agent (CTA) combined with a polymerization-induced self-assembly (PISA) technique. Although the styrene monomer and PAA-\textit{b}-PEG can dissolve in methanol, the PS block in PS-\textit{b}-PAA-\textit{b}-PEG triblock copolymer cannot dissolve in methanol. Therefore, after the polymerization of styrene using PAA-\textit{b}-PEG macro CTA in methanol, the triblock copolymers become spontaneously aggregated in methanol.

Figure S1a shows the $^1$H NMR spectrum of PAA-\textit{b}-PEG in DMSO-$d_6$ at room temperature. The resonance peak observed at 3.5 ppm is attributed to the PEG block. The resonance bands observed at 1.2-1.9 ppm are attributed to the PAA block and CTA. The degree of polymerization and $M_n$(NMR) for the PAA block determined from the intensity ratio of these resonance bands are 113 and $1.07 \times 10^4$, respectively. Figure S1b shows the $^1$H NMR spectrum of PS-\textit{b}-PAA-\textit{b}-PEG in DMSO-$d_6$ at 120 °C. The resonance bands observed at 3.5 ppm and 6.3-7.2 ppm are attributed to the PEG block and the pendant phenyl groups in the PS block, respectively. The degree of polymerization of the PS block and $M_n$(NMR) for PS-\textit{b}-PAA-\textit{b}-PEG are 116 and $2.74 \times 10^4$, respectively, as estimated from the integral intensity ratio of resonance bands of the PEG methylene protons at 3.5 ppm and the pendant phenyl protons in the PS block at 6.3-7.2 ppm.

After the polymerization of styrene using PAA-\textit{b}-PEG macro CTA in methanol, the triblock copolymers become spontaneously aggregated, because the PS block cannot dissolve in methanol. The solution was purified using a dialysis method to remove the styrene monomer. The solution was subsequently changed from methanol to pure water by dialysis. After that, DLS measurements were performed for the aqueous solution of PS-\textit{b}-PAA-\textit{b}-PEG (Figure S3). The pH value of the aqueous solution was measured to be 4.86. The unimodal hydrodynamic radius ($R_h$)
distribution can be observed with $R_h = 54.3$ nm. This $R_h$ value suggests that the triblock copolymer formed a micelle structure composed of hydrophobic PS core, hydrophilic PAA shell and PEG corona in pure water. The zeta-potential for the polymer micelle is identified to be -48.0 mV in pure water at pH 4.86. This observation suggests that the pendant carboxyl groups in the PAA block are ionized.

**Scheme 2** illustrates the formation mechanism of the mesoporous iron oxide through a micelle assembly process using the asymmetric triblock copolymer PS-$b$-PAA-$b$-PEG. The use of THF helps to achieve a perfect dissolution of the PS-$b$-PAA-$b$-PEG block copolymer, as this polymer cannot be well-dissolved in water or ethanol alone. The introduction of NaOH into the reaction system induces the formation of spherical micelles, in which the PS block forms the core as the addition of a small amount of water makes the PS block insoluble in the solution due to its rigid nature.\(^{19}\) Furthermore, NaOH plays an important role in the polymer solution, not only in promoting the formation of the micelles but also for providing a negative charge at the PAA block.\(^{19, 30}\) The negatively-charged (anionic) PAA block then interacts with the positively-charged (cationic) metal ions (Fe$^{3+}$) in the solution and forms the shell. It is expected that the metal ions will bind to carboxylate anions of PAA block and the extended micelles will shrink with the addition of metal ions.\(^{31}\) The hydrophilic PEG block forms the corona of the micelles which prevents the formation of secondary aggregates through steric repulsion between the PEG chains.\(^{29}\)

The morphology of the PS-$b$-PAA-$b$-PEG block copolymer micelles was characterized using TEM. From **Figure S4**, it can be seen that the block copolymer forms spherical micelles with an average diameter of 39 nm. The size estimated from TEM is smaller than that estimated from DLS measurements, because the TEM sample was in a dried state. The effect of the calcination temperature on the morphology of the obtained mesoporous iron oxide was investigated using SEM. As evident in **Figure 1a-b**, at calcination temperatures of 300 °C and 350 °C, the obtained mesoporous iron oxide samples exhibit a rough surface and the block copolymer on the surface is not sufficiently removed. With the increase of calcination temperature to 400 °C, a well-organized mesoporous iron oxide product with an average pore size of 39 nm can be obtained, as shown in **Figure 1c**. However, the mesoporous structure collapses at higher calcination temperatures of 450 °C and 500 °C, mainly due to the very large crystal growth (**Figure 1d-e**).
From the above results, the mesoporous iron oxide obtained at an optimized calcination temperature of 400 °C was selected as the typical sample. The fact that the average pore size of the resultant mesoporous iron oxide is very similar to the average diameter of the spherical micelles provides a strong evidence that the spherical micelles serve as pore forming agents (Figure 1f).

Wide-angle XRD was employed to observe the crystal structure and phase composition of the mesoporous iron oxide samples obtained at various calcination temperatures from 300 °C to 500 °C. From Figure 2, it can be observed that the mesoporous iron oxide products obtained at 300 °C and 350 °C are mostly amorphous. However, the XRD pattern of the product obtained at a higher calcination temperature of 400 °C can be indexed to the (012), (104), (110), (113), (024), (116), (122), (214), and (300) peaks of α-Fe₂O₃ phase (JCPDS No. 33-0664). The diffraction peaks of the α-Fe₂O₃ phase become stronger and narrower with the further increase of calcination temperature. No diffraction peaks due to phases other than α-Fe₂O₃ are observed, thereby indicating the high purity of the obtained mesoporous iron oxide products. The average crystal size was calculated from the most intense peak ((104) peak) at 33° using the Scherrer’s formula:

\[ d = \frac{k\lambda}{\beta \cos \theta} \quad (4) \]

where \( d \) is the nanocrystal size, \( k \) is the Scherrer constant, \( \lambda \) is the wavelength of the X-rays (Cu-Kα), \( \beta \) is the full width at half maximum of the diffraction peak, and \( \theta \) is the Bragg angle. The average crystallite size values of the mesoporous iron oxide synthesized at 400 °C, 450 °C, 500 °C are 23.5 nm, 24.9 nm, 28.4 nm, respectively. This trend suggests that the increase in calcination temperature promoted an increase in the crystallite size, which eventually lead to the collapse of the mesoporous structure.

The mesoporous nature of the product obtained at 400 °C can also be confirmed through the TEM image (Figure 3a) and the selected area electron diffraction (SAED) pattern indicates the polycrystalline nature of the mesoporous iron oxide product (Figure 3b). The high-resolution TEM image of the mesoporous iron oxide product obtained at an optimum temperature of 400 °C displays well-defined lattice fringes with a \( d \)-spacing of 0.24 nm, which corresponds to the \( d \)-spacing of (111) plane of α-Fe₂O₃ (Figure 3c).
XPS analysis was performed in order to analyze the surface composition of the mesoporous iron oxide sample. The survey spectrum of the sample shown in Figure 4a confirms the presence of Fe and O elements. The deconvoluted O1s XPS peaks display two major peaks at binding energies of 529.4 eV and 531.4 eV, corresponding to the oxygen atoms in the Fe$_2$O$_3$ lattice and the adsorbed water present on the Fe$_2$O$_3$ surface, respectively (Figure 4b). The high resolution Fe2p XPS spectrum shows two distinct peaks at binding energies of 710.6 eV and 723.9 eV (Figure 4c), indexed to Fe2p$_{3/2}$ and Fe2p$_{1/2}$, which is characteristic of Fe$^{3+}$ in Fe$_2$O$_3$. This confirms the absence of Fe$^0$ and Fe$^{2+}$ valency states in the obtained mesoporous iron oxide sample. Thus, the combined observation of Fe2p and O1s XPS signals as discussed above confirm that the mesoporous iron oxide product is Fe$_2$O$_3$.

TGA was conducted to analyse the weight change of the pure PS-b-PAA-b-PEG triblock copolymer and the PS-b-PAA-b-PEG micelles (with Fe$^{3+}$) with increasing temperatures. As evident in Figure 5a, the block copolymer starts to undergo a sharp weight loss starting at around 150 °C and completely burns out at around 400 °C. In comparison, with respect to the PS-b-PAA-b-PEG micelles (with Fe$^{3+}$), an initial decline of the TG curve is observed at ~150 °C (~19%), which can be correlated to the loss of adsorbed water molecules, whereas the weight loss observed from 150 °C to 400 °C can be attributed to the decomposition of the PS-b-PAA-b-PEG template and/or the crystal growth of the iron precursor into α-Fe$_2$O$_3$ phase (Figure 5b). No further weight loss is observed after 400 °C, indicating the complete removal of the polymeric template. Figure 5c shows the N$_2$ adsorption-desorption isotherms and pore size distribution curves of the mesoporous iron oxide obtained at an optimized calcination temperature of 400 °C, respectively. The sample exhibits a type IV hysteresis loop, which is the characteristic of mesoporous materials. The specific surface area and pore volume of the mesoporous iron oxide sample are measured to be 86.9 m$^2$ g$^{-1}$ and 0.218 cm$^3$ g$^{-1}$, respectively. Such high surface area and pore volume may accommodate the incorporation of large guest species, whilst also providing good diffusion of reactant molecules during catalytic reactions.
4.5. Conclusions

In this work, we have successfully synthesized mesoporous iron oxide using the asymmetric PS-$b$-PAA-$b$-PEG triblock copolymer as a soft template. In this reaction system, the PS block forms the core of the micelles on the basis of its lower solubility in water. The anionic PAA block interacted with the cationic Fe$^{3+}$ ions in the solution to form the shell. The PEG block forms the corona of the micelles and stabilizes the micelles by preventing the formation of secondary aggregates through steric repulsion between the PEG chains.
4.6 References


Scheme 1 Preparation of poly(styrene-b-acrylic acid-b-ethylene glycol) (PS-b-PAA-b-PEG) triblock copolymer via reversible addition-fragmentation chain transfer (RAFT) radical polymerization.
Scheme 2 Schematic illustration showing the mechanism of the formation of mesoporous iron oxide from the PS-b-PAA-b-PEG triblock copolymer micelles.
Figure 1 SEM images of the mesoporous iron oxide calcined at (a) 300 °C, (b) 350 °C, (c) 400 °C, (d) 450 °C (e) and 500 °C, and (f) histogram of the pore diameter distribution of the mesoporous iron oxide calcined at 400 °C.

Figure 2 Wide-angle XRD patterns of the mesoporous iron oxide calcined at (i) 300 °C, (ii) 350 °C, (iii) 400 °C, (iv) 450 °C, and (v) 500 °C.
Figure 3  (a) A typical TEM image of the mesoporous iron oxide obtained at an optimized calcination temperature of 400 °C, (b) selected area electron diffraction (SAED) pattern, and (c) high resolution TEM (HRTEM) image.
Figure 4 (a) XPS survey spectrum, (b) high resolution O1s XPS spectrum, (c) high resolution Fe2p XPS spectrum of the mesoporous iron oxide calcined at 400 °C.
Figure 5 TG curves of (a) PS-\(b\)-PAA-\(b\)-PEG triblock copolymer and (b) PS-\(b\)-PAA-\(b\)-PEG micelles (with \(\text{Fe}^{3+}\)). (c) Nitrogen (\(\text{N}_2\)) adsorption-desorption isotherm of mesoporous iron oxide obtained at an optimized calcination temperature of 400 °C.
**Figure S1** $^1$H NMR spectra for poly(acrylic acid-\textit{b}-ethylene glycol) (PAA-\textit{b}-PEG) in DMSO-$d_6$ at room temperature and poly(styrene-\textit{b}-acrylic acid-\textit{b}-ethylene glycol) (PS-\textit{b}-PAA-\textit{b}-PEG) in DMSO-$d_6$ at 120 °C.

**Figure S2** Gel-permeation chromatography (GPC) curve for poly(acrylic acid-\textit{b}-ethylene glycol) (PAA-\textit{b}-PEG) using a phosphate buffer (pH 8) containing 10 vol% acetonitrile as the eluent at 40 °C. The elution curve at 17.2 min was solvent peak.
Figure S3. Hydrodynamic radius ($R_h$) distribution for poly(styrene-b-acrylic acid-b-ethylene glycol) (PS-b-PAA-b-PEG) in pure water at 25 °C.

Figure S4. A typical TEM image of the spherical micelles formed by the PS-b-PAA-b-PEG triblock copolymer (inset showing the size distribution histogram of the micelles).
5. Chapter 5: Synthesized Mesoporous Iron Oxide for Colorimetric and Electrochemical Detection of Glucose

5.1 Abstract
These highly porous materials have shown intrinsic peroxidase-like activities towards the catalytic oxidation of 3,3’,5,5’-tertamethylbenzidine (TMB) in the presence of hydrogen peroxide (H₂O₂). The apparent steady-state kinetic parameters of these mesoporous iron oxide materials for TMB oxidation reaction were also determined. This mimetic feature was further exploited to develop a simple colorimetric (naked-eye) and electrochemical assay for the detection of glucose. Both of our colorimetric (naked-eye and UV-vis) and electrochemical assays estimated the glucose in the linear range from 1.0 to 200 mM with the detection limit of 1.0 mM. We envisage that our integrated detection platform for H₂O₂ and glucose will find a wide range of applications in developing varieties of biosensors in the field of personalized medicine, food-safety detection, environmental-pollution control, and agro-biotechnology.
5.2 Introduction

Owing to their various intrinsic characteristics such as biofavorable network structure, good electronic conductivity, good biocompatibility and stability, low synthesis costs, and environmental friendliness, iron oxide-based nanomaterials have been widely used in a variety of biomedical applications, such as tissue engineering, magnetic resonance imaging, drug delivery and biosensing.\textsuperscript{1,2,3,6} In biosensing applications, the magnetic properties of these materials also provide an additional benefit of allowing magnetic mixing (intimate) and contactless sample preparation.\textsuperscript{7} Furthermore, the introduction of pores into the nanoparticles also resulted in the development of mesoporous iron oxide with a significantly higher surface area. These mesoporous nanostructures therefore, have an improved surface functionality (i.e., an increase in the uptake and release of target analyte) and superior catalytic activity (maximization of surface dependent mass transport compared to that of planar materials of similar mass). Moreover, mesoporous iron oxides have the capability of being highly selective in catalysis (via molecular sieving) while they remain stable against sintering.\textsuperscript{8} In recent years, these nanocomposites have been suggested as potential alternatives to natural enzymes for a wide range of applications. For example, they have been reported to imitate the structures and functions of horseradish peroxidase (HRP), serine proteases, cytochrome P450, dioxygenase, phosphodiesterase, etc.\textsuperscript{9} The replacement of these natural enzymes with a robust material, similar to the as-synthesized mesoporous iron oxide, can offer the following significant advantages in bioanalytical and translational research\textsuperscript{10}: (i) catalytic efficiency of natural enzymes are often vulnerable to the environmental changes due to the destabilization of the protein conformation, while mesoporous iron oxide exhibit better physical and chemical stabilities, (ii) unlike natural enzymes, nanoparticles are protected from protease digestion and, (iii) these mesoporous materials are relatively inexpensive, easy to synthesize, and they avoid the time-consuming preparation steps and specific storage requirements of natural enzymes. Following the synthesis and characterization of the mesoporous iron oxide products, we investigated the HRP-like activities of these materials towards the oxidation of TMB in the presence of H\textsubscript{2}O\textsubscript{2} followed by the evaluation of their steady-state kinetic parameters using the Michaelis-Menten model and Lineweaver-Burk model. Finally, the peroxidase-like feature was
used to develop a proof-of-concept glucose sensor, where enzymatic oxidation of glucose with glucose oxidase (GOx) was synergized with TMB/H$_2$O$_2$ catalytic reaction. The *in-situ* production of H$_2$O$_2$ from the former system was used in the latter for the oxidation of TMB in the presence of mesoporous iron oxides. This results in a blue-colored complex solution, facilitating the naked-eye evaluation of glucose. After quenching the reaction with acid, the blue-colored complex solution turned yellow, which is stable and electroactive, thus allowing the estimation of glucose *via* an electrochemical readout.

5.3. Experimental

5.3.1 Instrumentations

PBS tablet (0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4 at 25 °C), TMB, glucose and GOx were purchased from Sigma Life Science (Australia). Analytical grade H$_2$O$_2$, dimethyl sulfoxide (DMSO), hydrochloric acid (HCl) were purchased from Chem-Supply (Australia). Screen-printed gold electrodes (SPE-Au) (220BT) with three-electrode system printed on a ceramic substrate were acquired from Dropsens (Spain). UltrapureTM DNase/RNase-free distilled water (Invitrogen, Australia) was used throughout the experiments. All electrochemical measurements were carried out with a CHI650 electrochemical workstation (CH instrument, USA). All the chemicals were used without further purification.

5.3.2 Kinetics measurements and colorimetric and electrochemical detection of glucose

5.0 μg of mesoporous iron oxide was mixed in 80 μL of 0.2 M sodium acetate (NaAc, pH 3.5) buffer in the presence of freshly prepared 800 μM TMB (TMB dissolved in DMSO) and 700 mM H$_2$O$_2$ to study the peroxidase mimetics. The absorbance of the resultant blue-colored solution was measured at 652 nm using a spectrophotometer (SpectraMax). The steady-state kinetic parameters were selected by varying the concentration of H$_2$O$_2$ (0.01 to 1.0 M) at a fixed concentration of TMB (800 μM) and vice versa for the varying the concentration of TMB (0.01 to 1.0 mM) at 700 mM H$_2$O$_2$. The apparent kinetic parameters were calculated by considering a typical enzyme catalytic reaction;
where $E$, $S$, $ES$ and $P$ represent the enzyme, substrate, enzyme substrate adduct and product respectively. The Michaelis-Menten equation for this catalytic system is expressed as follows:

$$V_o = \frac{V_{\text{max}} [S]}{K_m + [S]}$$

where $V_0$ is the rate of substrate conversion, $V_{\text{max}}$ is the maximum rate of conversion, $[S]$ is the substrate concentration, and $K_m$ is the Michaelis-Menten constant. The absorbance data were converted to corresponding concentration terms by the Beer-Lambert Law using the value of $\varepsilon = 39,000 \text{ M}^{-1} \text{ cm}^{-1}$ (at 652 nm) for the oxidized product of TMB. The rearrangement of Michaelis-Menten equation gives the following Lineweaver–Burk equation, which was used to determine $K_m$ and $V_{\text{max}}$

$$\frac{1}{V} = \frac{K_m}{V_{\text{max}}} \frac{1}{[S]} + \frac{1}{V_{\text{max}}}$$

For chronoamperometric (CA) measurements, the reaction was stopped by adding 2.0 μL (2.0 M HCl) of stop solution. The resultant 80 μL of this yellow solution was pipetted onto a SPE-Au and $i-t$ was measured at 150 mV over 60 s.

For the glucose detection assay, 50 μL of glucose solution of different concentrations and 5.0 μL of GOx (20 mg/ml) were mixed in PBS solution (pH 7.0) and incubated at 40 °C for 30 min. Then, 200 μL of TMB (800 μM) solution and 5.0 μL of mesoporous iron oxide (1.0 μg/μL) were added to the mixture. The pH of the final solution was maintained by adding acetic acid solution and incubating for 15 min at 37 °C. The particles were separated by centrifugation (12000 rpm for 2 min) and the resultant blue-colored complex was used for the colorimetric detection. After quenching the reaction by acid, the yellow solution was transferred to a SPE-Au for a CA readout.
5.4. Result and discussion

It has been well studied that natural HRP/H₂O₂ could catalyze the oxidation of TMB which produced a blue-coloured complex product which turned yellow after the addition of acid to the reaction media. This yellow product is stable and electroactive. In this study, we first prepared mesoporous iron oxides at different calcination temperatures (400, 450 and 500 °C) via a novel soft-templated fabrication procedure using the PS-b-PAA-b-PEG block copolymer. Similar to natural peroxidases, the as-synthesized mesoporous iron oxide materials showed catalytic activity towards the oxidation of TMB substrate in the presence of H₂O₂ and produce a blue-colored product (with maximum absorbance at 652 nm). As seen in Figure 1a-c, all these three oxides showed significantly higher absorbance for the oxidation of TMB compared to those of the corresponding control experiments (in the absence of mesoporous iron oxide). It is also clearly evident that the mesoporous iron oxide obtained 400 °C exhibited the highest UV-visible response in naked-eye observation (clearly distinguishable from other two) and UV-visible detection (abs. 0.101 versus 0.085 and 0.083). After quenching the reaction by acid, an electrochemical readout was performed onto a SPE-Au, and as depicted in Figure 1a', b' and c', the amperometric response of the oxidized reaction product was in good agreement with the absorbance data, where the current response of the mesoporous iron oxide synthesized at 400 °C was almost 19-times higher than that of the negative control (50.76 versus 2.7 µA cm⁻²). Similar to the absorbance measurement, the electrochemical readout also indicates that this mesoporous iron oxide displayed a better response than those observed with mesoporous iron oxide samples prepared at 450 °C and 500 °C (Figure 1a' versus 1b' and 1c'; 50.76 versus 30.61 and 23.62 µA cm⁻²). All these experiments (i.e., naked eye observation, UV-vis and CA readouts) clearly confirm the peroxidase-like activity of our novel mesoporous iron oxide, where the mesoporous iron oxide obtained at calcination temperature of 400 °C performed better than the other samples. One possible explanation of this is the tendency of collapsing of the mesoporous structure of iron oxide obtained at 450 °C and 500 °C, while the mesoporous iron oxide obtained at 400 °C retained its structure. Thus, the iron oxide obtained at 400 °C with high surface area can bind an increased amount of positively charged TMB, which lead to both enhanced colorimetric and electrochemical responses. It is important to mention that, unlike most of the
reported mimetic nanoparticles, our mesoporous iron oxide nanoparticles exhibited such an enhanced activity at room temperature.\textsuperscript{9,12}

To further investigate the peroxidase-like activity of these mesoporous iron oxide products, their apparent steady-state kinetic parameters for TMB oxidation were determined by changing the concentration of H\textsubscript{2}O\textsubscript{2} and TMB following the initial rate method.\textsuperscript{41,43} As shown in Figure 2, a typical Michaelis-Menten-like curve was obtained in a designated concentration range of both H\textsubscript{2}O\textsubscript{2} (Figure 2a, b and c) and TMB (Figure 4a', b' and c'). The data were fitted using a non-linear least square fitting to calculate the catalytic parameters $K_m$ and $V_{\text{max}}$ (shown in Table 1). As depicted in the inset of Figure 2, these parameters were also determined from the Lineweaver-Burk double reciprocal plot ($1/ V$ versus $1/ [S]$).\textsuperscript{4} $K_m$ value is an indicator of the enzyme affinity towards its substrate and a lower $K_m$ value indicates the stronger affinity between enzyme and its substrate. The apparent $K_m$ value for the mesoporous iron oxide obtained at 400 °C with TMB was significantly lower than that of the reported $K_m$ value of HRP (0.153 versus 0.434 mM). This clearly suggests that the mesoporous iron oxide product has a higher affinity with TMB in comparison to that of HRP, thereby demonstrating its potential as a good alternative for HRP.

However, the apparent $K_m$ value of this mesoporous iron oxide with H\textsubscript{2}O\textsubscript{2} (as the substrate) was significantly higher than that of the reported value for HRP (86.43 versus 3.70 mM), which supports the fact that an increased amount of H\textsubscript{2}O\textsubscript{2} was required to obtain the highest mimetic activity of mesoporous iron oxide. As expected, the kinetic parameters of the mesoporous iron oxide obtained at 400 °C towards the oxidation of TMB were better than those achieved using the mesoporous iron oxide products obtained at calcination temperatures of 450 °C and 500 °C (Table 1). A similar level of the $K_m$ values for nanomaterials with TMB and H\textsubscript{2}O\textsubscript{2} have previously been reported.\textsuperscript{12,13} As previously outlined, this could be due to the mesoporous structures synthesized at 450 °C and 500 °C being relatively less stable. On the contrary, the mesoporous structure of iron oxide prepared at 400 °C is highly stable and the associated large pore volume can significantly enhance the reaction kinetics and peroxidase mimetic activity. We also assume that the transfer of lone-pairs electron density from the amino group of TMB towards the vacant d-orbital of Fe$^{3+}$ may contribute to the electron density and mobility of mesoporous iron oxide, thereby increasing its conductivity.\textsuperscript{14}
To demonstrate the potential application of the peroxidase-like activity of these newly synthesized mesoporous iron oxide products, we have developed a proof-of-concept colorimetric and electrochemical assay platform for the detection of glucose. Scheme 1 provides an overview of our assay which relies on the oxidation of TMB in the presence of the \textit{in-situ} produced-H\textsubscript{2}O\textsubscript{2} and mesoporous iron oxides. The reaction in the assay comprises two major steps: (i) catalytic oxidation of glucose by dissolved oxygen in the presence of glucose oxidase (GOx) at pH 7.0 which produced H\textsubscript{2}O\textsubscript{2} and (ii) the reaction of H\textsubscript{2}O\textsubscript{2} with the peroxidase substrate TMB in the presence of mesoporous iron oxides (at pH 3.5). This gives rise to a blue-colored diamine complex which can be evaluated by the naked-eye and UV-vis. After stopping the reaction with acid, the reaction mixture was further detected by CA measurements. The responses obtained from these readouts should have a clear correlation with the amount of H\textsubscript{2}O\textsubscript{2} produced \textit{in-situ}, which in turns provide the estimation of glucose concentration in the assay. Figure 3 illustrates typical absorbance (Figure 3a) and current density (Figure 3b) plots as a function of H\textsubscript{2}O\textsubscript{2} concentration, where the response was linearly correlated to the H\textsubscript{2}O\textsubscript{2} concentration. Figure 4 depicts the plots of responses in absorbance (Figure 4a) and current density (Figure 4b) as a function of glucose concentration. Clearly, both the UV-vis and electrochemical responses are correlated to the glucose concentration in the linear range of 1.0 mM to 200 mM (fitting curve in the inset of Figure 4) with a detection limit of 1.0 mM (signal to noise ratio = 3). This detection limit of the assay is comparable with previously reported glucose sensors, however, our assay requires only a very low amount of iron oxide to enable the detection via peroxidase mimetics, for example, Wei \textit{et al.}\textsuperscript{36,37} and Mitra \textit{et al.}\textsuperscript{15} sed 37 and 100 µg of iron oxide nanoparticles, respectively for glucose detection, whereas our assay requires only 5.0 µg of the mesoporous iron oxide.
5.5. Conclusions

The as-synthesized mesoporous iron oxide products were then investigated for their potential peroxidase mimicking activities. The typical Michaelis–Menten modelling of the reaction demonstrates the high affinity of mesoporous iron oxide obtained at an optimized calcination temperature of 400 °C towards the oxidation of TMB in the presence of \( \text{H}_2\text{O}_2 \). This intrinsic property of the mesoporous iron oxide was used to develop a proof-of-concept glucose sensor. Although, the applicability of the mesoporous iron oxide has successfully been shown only for the glucose detection, we believe that these materials will work as artificial nanoenzymes where nanoenzymes/\( \text{H}_2\text{O}_2 \) will mimic the conventional HRP/\( \text{H}_2\text{O}_2 \)-based sensing approaches used in a variety of bioassays for a wide range of applications in biotechnology, environmental sciences, energy, and agricultural sciences.
5.6 References


**Table 1** Kinetic parameter of mesoporous iron oxide

<table>
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<tr>
<th>Mesoporous iron oxide</th>
<th>Substrate</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ (Ms$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400_CT</td>
<td>H$_2$O$_2$</td>
<td>86.425</td>
<td>$3.05 \times 10^{-06}$</td>
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<tr>
<td></td>
<td>TMB</td>
<td>0.153</td>
<td>$2.94 \times 10^{-06}$</td>
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<tr>
<td>450_CT</td>
<td>H$_2$O$_2$</td>
<td>125.904</td>
<td>$2.89 \times 10^{-06}$</td>
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<tr>
<td></td>
<td>TMB</td>
<td>0.214</td>
<td>$2.74 \times 10^{-06}$</td>
</tr>
<tr>
<td>500_CT</td>
<td>H$_2$O$_2$</td>
<td>132.9175</td>
<td>$2.89 \times 10^{-06}$</td>
</tr>
<tr>
<td></td>
<td>TMB</td>
<td>0.236</td>
<td>$2.65 \times 10^{-06}$</td>
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**Abbreviation:** CT= calcination temperature

**Scheme 1** An overview of the developed assay for colorimetric and chronoamperometric detection of glucose using the peroxidase-like activity of mesoporous iron oxide.
Figure 1 Mean response of absorbance (UV-vis) (a, b, c, left panel) and chronoamperometric current signals (a', b', c') for negative and positive control samples. Inset images show the corresponding photos for naked eye evaluation and $i$-$t$ curves; (a, a' = 400_CT, b, b' = 450_CT, c, c' = 500_CT, CT denotes calcination temperatures). The error bar represents the standard deviation of three independent measurements.
Figure 2 Steady-state kinetic analysis using Michaelis-Menten model (main panel) and Lineweaver-Burk model (inset panel) for mesoporous iron oxide synthesized at various calcination temperatures (CT) by varying the concentration of (a, b, c) H$_2$O$_2$ and (a', b', c') TMB with fixed amounts of TMB (800 µM) and H$_2$O$_2$ (700 mM), respectively; (a, a' = 400_CT, b, b' = 450_CT, c, c' = 500_CT). The error bar represents the standard deviation of three independent measurements.
Figure 3 (a) UV-vis absorbance and (b) chronoamperometric responses for the designated concentration of \( \text{H}_2\text{O}_2 \) using 450_CT mesoporous iron oxide (insets show the corresponding fitting curves). The error bar represents the standard deviation of three independent measurements.

Figure 4 Response-concentration curves obtained with (a) UV-vis absorbance and (b) chronoamperometric measurements for the designated concentration of glucose using 450_CT mesoporous iron oxide. Insets show the corresponding linear dynamic ranges for detection of glucose. The error bar represents the standard deviation of three independent measurements.
6. Chapter 6: Conclusions and Recommendations

6.1 Conclusion

In this MPhil work, we have tried to investigate how the pores of mesoporous materials play a crucial role in different application in which they are superior to nonporous materials. Also, the soft template synthesis technique with triblock copolymer has been demonstrated. In addition, it has been found that the calcination temperature plays a key role in the morphology of the synthesized material. After that, the bio-sensing application of the synthesized mesoporous iron has been evaluated.

Firstly, we have used mesoporous silica as an inorganic filler material with a simple single-step method using ultraviolet radiation. We have shown that the composite prepared with the mesoporous silica is more mechanically stable than the composite prepared with non-porous silica. For instance, the CTE value of the mesoporous silica (MPS-35) containing composite is 41.6% lower than that of silicone itself. This is due to the physical confinement of the silicone inside the pore of the mesoporous silica.

Secondly, unique process has been developed for the formation of an ultra-low refractive index thin film composed of mesoporous silica by means of a the wet-chemical method. Compared to the glass substrate, after the film coating, the transmittance is increased by around 3.3% in the wavelength range of 400 to 750nm. This technology is also applicable to other plastic substrates such as thoria acetyl cellulose(TAC) and polymethyl methacrylate (PMMA) films. After coating with the mesoporous silica layer, the average reflectance of the PMMA film decreased from 3.8 to 0.7% in the visible light region.

Thirdly, mesoporous iron oxide was synthesized using an asymmetric triblock copolymer, poly(styrene-b-acrylic acid-bethylene glycol) block copolymer. Here, it was found that the calcination temperature plays a crucial role in the morphology of the material, as lower temperatures (<400°C) could not sufficiently remove the block copolymer, whereas very high temperatures (≥450 °C) lead to the collapse of the mesoporous structure. After investigation, it
was found that the iron oxide obtained after heating to 400°C exhibited an average pore diameter of 39nm with a large surface area and pore volume of 86.9 m² g⁻¹ and 0.218 cm³ g⁻¹, respectively.

Finally, as-synthesized mesoporous iron oxide was then investigated and showed its potential peroxidase mimicking activities towards the catalytic oxidation of 3,3´,5,5´-tertamethylbenzidine (TMB) in the presence of hydrogen peroxide (H₂O₂) to develop a simple colorimetric (naked-eye) and electrochemical assay for the detection of glucose in the linear range from 1.0 to 200mM with the detection limit of 1.0mM (signal to noise ratio = 3). It was also shown that in this detection the mesoporous iron oxide obtained at 400°C calcination temperature had better detection capability over the mesoporous iron oxide obtained at 450 °C and 500 °C. It was also shown that the synthesized iron oxide had a lower kₘ value towards TMB than HRP (0.153 versus 0.434 mM), which proves that mesoporous iron oxide has a higher affinity to TMB than HRP. It was also noted that this assay requires only a very low amount of iron oxide to enable detection via peroxidase mimetic compared to the previously reported one.
6.2 Recommendation

Nanoparticles have enormous potential to bring a revolution to the medical field. This synthesized unique mesoporous iron oxide can make a real contribution to this objective. Here, this study has demonstrated that modified mesoporous iron oxide can mimic horseradish peroxidase enzyme for the detection of glucose. Also, as it has a high surface area with a notable pore size, this unique material can be used for drug delivery by loading the desired medication into the pores of the mesoporous material. Additionally, these particles can also be used to deliver drugs to the site of action using an external magnetic field. To transfer this theoretical knowledge to practical application, however, more research is required. As is well known for medical application, various issues such as side effects, associated toxicities, patient compliance, etc., all need to be considered.