Nanomaterials of Iron Oxide, Boron, and Boron Nitride: on the Path to Magnetic Guided Bioconjugates for Medical Applications

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ABSTRACT

NANOMATERIALS OF IRON OXIDE, BORON AND BORON NITRIDE: ON THE PATH TO MAGNETIC-GUIDED BIOCONJUGATES FOR MEDICAL APPLICATIONS

John R. Wozny, M.S.
Department of Chemistry and Biochemistry
Northern Illinois University, 2019
Narayan S. Hosmane, Director

The functionalization of boron-based bioconjugates has long been studied for their potential use as a drug candidate for boron neutron capture therapy (BNCT). Also, magnetic nanoparticles have received greater attention due to their interesting magnetic properties and behaviors for selectively guiding them with an external magnetic field through the blood. A combination of the two ideas gives the framework for nanotechnology that has the potential to revolutionize drug delivery systems. This thesis focuses systematically on individual elements required for the many moving pieces required for magnetic drug targeting.

The research discussed in this thesis is divided into three parts. The first part investigates the synthesis and the encapsulation of iron oxide magnetite core nanoparticles and their characterization with a variety of spectroscopic techniques including, but not limited to, transmission electron microscopy (TEM), scanning transmission microscopy (SEM), electron energy-loss spectroscopy (EELS), energy-dispersive x-ray spectroscopy (EDX), and Mössbauer spectroscopy (MS). The second part focuses on the synthesis, encapsulation, and characterization of boron nanoparticle cores by pyrolysis of nido-decaborane (B_{10}H_{14}). The third part is an exploratory investigation on the synthesis of boron nitride nanotubes (BNNT) with an emphasis on the purification of the product to functionalize them for future boron-based bioconjugates for medical applications.
NORTHERN ILLINOIS UNIVERSITY
DEKALB, ILLINOIS

DECEMBER 2019

NANOMATERIALS OF IRON OXIDE, BORON, AND BORON NITRIDE: ON THE PATH TO MAGNETIC-GUIDED BIOCONJUGATES FOR MEDICAL APPLICATIONS

BY

JOHN RYAN WOZNY
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A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

Thesis director:
Narayan S. Hosmane
DEDICATIONS

Dedicated to my beloved father and guardian angel,
who worked two jobs my entire life to provide for my five brothers and me
and still found the time to be actively present at my sporting events.

Dedicated to my mother,
who showered me with unconditional love and also watched my children,
abling me to pursue my dreams and educational goals.

For my loving wife, JoHanna,
and my three daughters (Hailey, Natalya, and Sabrina),
whom I love with all my being and soul.

To my teachers, who fostered professional and personal growth, and
to the people I love and the people who love me.
ACKNOWLEDGMENTS

I wish to express my most profound appreciation and admiration to my mentor, Dr. Narayan S. Hosmane, who offered the once-in-a-lifetime opportunity to conduct research in his lab and utilize chemical strategies to fight cancer. Over the years, he has repeatedly reminded me to stay positive, to remain focused, work hard, and to submerge myself into research. Anyone who has spent any credible amount of time with him will recognize the mantra “F.A.I.L.” stands for your “first attempt in learning.” have learned many lessons related to chemistry, life, and writing through his vivid stories and funny jokes. He has an uncanny ability to elegantly explain complex concepts in a very simplistic manner, then associate them with real-world examples. His unwavering support and positive attitude have motivated me to improve continuously and to push personal boundaries while learning from my mistakes. It is for this reason I am forever indebted to Dr. Hosmane for the time, wisdom, and knowledge he has shared with me through the years.

Dr. Timothy J. Hagen and Dr. Chong Zhang are significantly acknowledged for being an integral part of my education and thesis review committee. They have provided suggestions and guidance to further my understanding of chemistry and research in the laboratory. Both committee members have advised me on a myriad of subjects ranging from the drying of solvents to the regeneration and maintenance of the glove box.
I am emphatically thankful to Dr. Yasuo Ito, Dr. Dennis Brown, and Mason Hayward from the Physics Department for their collaboration with Dr. Hosmane and myself on this research project. They have assisted and spent countless hours obtaining and refining the TEM EELS and Mössbauer data for the characterization of the iron oxide core nanoparticles. Their hard work is reflected in many of the data and pictures present throughout this thesis.

I want to thank Dr. Hiren Patel, Dr. Prabhuodeyara Gurubasavaraj, Dr. Fatima Ghaida, Dr. Vijayaraghavan Kalavakunda, and Dr. Eyrusalem Bedasso for teaching various aspects of research techniques and their methodologies; Lori Brass and Paige Bothwell from Biological Sciences for the TEM analysis; Dr. Tao Li and Eric Sarnello for the preparation of SAXS samples from the iron oxide nanoparticles and the collection of SAXS data; Dr. Tao Xu and Jue Gong for SEM analysis on the boron nanoparticles; Dr. Scott Grayburn, Dr. Barrie Bode, and Nipin Shreshtha for the ultrahigh centrifuge training and experiments related to the purification of boron nitride nanotube and boron nanoparticles. I want to thank the following student researchers: Joon Park, Nitya Nagarajan, Blake Mirman, Jake Kimball, Richard Miller, Nathan Scanlon, and all previous group members, whom I had the honor of working with during my time in the lab.

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I am greatly appreciative of Dr. Kyu Taek Cho from the Mechanical Engineering Department and our collaborators: Dr. Lu Zhang, Dr. Yuyue Zhao, and Dr. Jingjing Zhang, from the Division of Chemical Sciences and Engineering, which funded an independent research project.

Finally, I wish to thank my parents and family, who have made many sacrifices and have been my foundation throughout my educational journey.
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<th>Description</th>
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<tr>
<td>0D</td>
<td>Zero-dimensional</td>
</tr>
<tr>
<td>1D</td>
<td>One-dimensional</td>
</tr>
<tr>
<td>2D</td>
<td>Two-dimensional</td>
</tr>
<tr>
<td>APTES</td>
<td>3-aminopropyl)triethoxysilane (C₉H₂₃NO₃Si)</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated total reflection</td>
</tr>
<tr>
<td>BNCT</td>
<td>Boron neutron capture therapy</td>
</tr>
<tr>
<td>BNNT</td>
<td>Boron nitride nanotubes</td>
</tr>
<tr>
<td>BPA</td>
<td>Boronphenylalanine</td>
</tr>
<tr>
<td>BSH</td>
<td>Mercaptoundecahydrododecaborate</td>
</tr>
<tr>
<td>DI</td>
<td>Deionized</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy-dispersive X-ray spectroscopy</td>
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<td>EELS</td>
<td>Electron energy-loss spectroscopy</td>
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<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>FTIC</td>
<td>Fluorescein isothiocyanate (C₂₁H₁₁NO)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>HAADF</td>
<td>High-angle annular dark field</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HiPco</td>
<td>High-pressure carbon monoxide process</td>
</tr>
<tr>
<td>HRTEM</td>
<td>High-resolution transmission electron microscopy</td>
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<tr>
<td>IOP</td>
<td>Iron oxide nanoparticles</td>
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<tr>
<td>MNP</td>
<td>Magnetite nanoparticle</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MS</td>
<td>Mössbauer spectroscopy</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PEI</td>
<td>Polyethylenimine</td>
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<td>Small-angle X-ray Scattering</td>
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<td>Scanning electron microscopy</td>
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<tr>
<td>SPION</td>
<td>Superparamagnetic iron oxide nanoparticle</td>
</tr>
<tr>
<td>STEM</td>
<td>Scanning transmission electron microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TEOS</td>
<td>Tetraethyl orthosilicate</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid (C₂HF₃O₂)</td>
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<td>UV-Vis</td>
<td>Ultraviolet-visible spectroscopy</td>
</tr>
<tr>
<td>Abbreviation</td>
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<tr>
<td>XEDS</td>
<td>X-ray energy-dispersive spectroscopy</td>
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<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
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<td>XRD</td>
<td>X-ray diffraction</td>
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CHAPTER 1: NANOMATERIAL INTRODUCTION

1.1 Nanotechnology

“Nano-“ is the prefix used in front of a unit of measurement to change its value by orders of magnitude based on the metric system.¹ As illustrated in Table 1.1, a nanometer is one billionth of a meter.¹,² Nanotechnology is the process of producing a chemical substance or material whose size is on the nanometer scale in one or more dimensions between 1nm to 100nm.³ This scale can be challenging to imagine, but for gauging the relative size of a nanometer, if the diameter of the Earth represented one meter, then one nanometer would represent the approximate size of a marble.
Table 1.1 The Metric Units for Measurements.1
The table summarizes the prefixes, symbols, multipliers, exponentials, and notation of the metric system.

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<th>Symbol</th>
<th>Multiplier</th>
<th>Exponential</th>
<th>Scientific Notation</th>
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<td>1.0x10^{21}</td>
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<td>1.0x10^{9}</td>
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<td>10^{6}</td>
<td>1.0x10^{6}</td>
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Richard Feynman is the father of nanotechnology for a delivered presentation titled “There Is Plenty of Room at the Bottom,” at CalTech on December 29th, 1959.4 In his presentation, he described the trends of miniaturization and the future ideological framework that scientists would manipulate and control materials on the atomic and molecular scales.4 Fifteen years later, the phrase “nanotechnology” would be coined by Professor Norio Taniguchi.

Taniguchi advocated that nanotechnology consisted of the processing, separation, consolidation, and the deformation of materials by one atom or molecule at a time.5 The development of the scanning tunnel microscope by Gerd Binnig and Heinrich Rohrer at IBM allowed scientists to study materials for the first time at the nanoscale.6,7,8 Eric Drexler then popularized the term

There would be two additional scientific discoveries that would propel nanotechnology into the 21st century and cement it as a newly emerging field. The first discovery was of a new allotrope of carbon called buckminsterfullerene (C\textsubscript{60}) by Harold Kroto, Robert Curl, and Richard Smalley in 1985, for which they were awarded the Nobel Prize in 1996.\textsuperscript{10,11} The second discovery was the controlled growth of the first carbon nanotubes by Sumio Iijima in 1991; the carbon nanotubes tensile strength was approximately one hundred times greater than that of steel of the same diameter but with more excellent elasticity.\textsuperscript{14,15} The unique properties of carbon nanotubes elevated nanomaterials to the forefront of material science and various forms of carbon nanomaterials have been discovered as illustrated in Figure 1.1.

![Figure 1.1 Various forms of carbon nanomaterials.\textsuperscript{12}](image)

In 1993, Richard Smalley redirected his research group’s focus onto carbon nanotubes, with the firm belief system that nanotechnology offered solutions to the world’s biggest
problems.\textsuperscript{16} He would later become one of the world-renowned experts in carbon nanotubes with advanced synthesis techniques of laser ablation and the high-pressure carbon monoxide process (HiPco).\textsuperscript{17,18} In the HiPco process, iron carbon monoxide (Fe(CO)\textsubscript{5}) is utilized as a catalyst to create high-purity single-wall carbon nanotubes.\textsuperscript{18} In 1999, Smalley testified in front of the U.S. House of Representatives in support of the National Nanotechnology Initiative. Smalley made his testimony while suffering from leukemia, and his testimony was remembered as riveting as he spoke about how nanotechnology could revolutionize multiple industries.\textsuperscript{19}

The United States government would be pivotal in the research development efforts of nanotechnology funding, starting in 1991. It would fully commit to a national nanotechnology initiative with the inception of the National Nanotechnology Initiative (NNI) in 2000. Since 2000, over sixty countries have developed nanotechnology initiatives, which has resulted in a global proliferation of nanotechnology activities that continue to grow today.\textsuperscript{20} Nanotechnology research and applications can now be found all over the world and is truly a global market, which is expected to grow to $125 billion by 2024.\textsuperscript{21}
1.2 Examples of Premodern Nanotechnology

Examples of premodern nanotechnology have been dated back as early as the classical antiquity period of the 4th century with the Lycurgus Cup, a cage cup that is an example of color-changing dichroic glass with the presence of colloidal metal alloy nanoparticles in the glass. The cup’s unusual optical properties are attributed to the presence of 15-100 nm silver-gold alloy nanoparticles, which initially appear as an opaque green color when reflecting light and turn to a translucent red when transmitting light, as shown in Figure 1.2.²²,²³

![Figure 1.2](image)

**Figure 1.2** The Lycurgus Cup color change with silver-gold nanoparticles.²²-²⁴

a) An example of the Lycurgus Cup color change when the light is transmitted through the interior of the cup to the reflection of light from the cup’s exterior, b) the TEM image of a silver-gold alloy nanoparticle within the glass of the Lycurgus Cup, c-d) different crystallinity patterns with a strong Bragg spot verified by electron microdiffraction of the silver-gold nanoparticles.

Similar to the Lycurgus Cup, traces of metals nanoparticles and their oxide derivatives were found in decorated glazes and glasses between the 5th and 16th centuries. In the 9th century, the Abbasid Dynasty in Bashra (Iraq) was a significant producer of tin oxide ceramics with decorated metallic glazes, giving the effect of iridescent.²⁵ The thin surface (from a few
hundreds of a nanometer up to several microns) of metallic silver/copper nanoparticles became known as silver stain or luster decorations. The metal particles of the glazes ranged from 2 to 50 nm, and the process involved an ion exchange between Ag$^+$ and Cu$^+$ ions, followed by firing to remove Na$^+$ and K$^+$ ions. The final stage was the nucleation and growth of copper/silver nanoparticles.$^{26,27}$ As shown in Figure 1.3, the pottery made with this technique is known as lusterware with the polychrome lusters of red copper, white silvery, and black silver lusters, which change color based on the reflection or transmission of light.$^{28-30}$

![Figure 1.3 An example of the polychrome lusterware]

The change in the appearance of the polychrome bowl under different lighting settings. The British Museum Collection Online provided these images.

The incorporation of metal and metal oxides into a decorated glass would continue and spread to Syria (11th-14th centuries), and later to the stained-glass windows of the Renaissance cathedrals from the 15th-18th centuries. In the stain glass preparations, the artisans' techniques
altered the shape and size of gold and silver nanoparticles to get the desired color effect from the glass displayed in Figure 1.4.\textsuperscript{32,33}

\begin{center}
\textbf{Figure 1.4} The Notre-Dame Cathedral North Rose Window.\textsuperscript{34-36} The appearance of the stain glass window under different lighting in the Notre-Dame Cathedral located in Paris, France. The British Museum Collection Online provided these images.

The metal-based glass composites were not the only examples of premodern nanomaterials found. As shown in Figure 1.5, the presence of multiwalled carbon nanotubes and cementite nanowires found were in weapons within the Damascus Sabre. Historians believe the steel cakes of “wootz” were produced and obtained from ancient India and may predate to other swords as early as the 6\textsuperscript{th} century B.C.\textsuperscript{38}
Figure 1.5 The Damascus Sabre steel is strengthened with the presence of nanomaterials.\textsuperscript{37,38}

a) A picture of the Damascus Sabre; b) the HR-TEM image of a straight multiwalled carbon nanotube after dissolution of sample in HCl; c) the HR-TEM image of a bent multiwalled carbon nanotube after dissolution of sample in HCl; and d) the HR-TEM image of cementite nanowires encapsulated by the carbon nanotubes, which prevented the nanowires dissolution in HCl.

1.3 Nanomaterial Classification

Nanomaterials can be classified based on their dimensionality or by the axis of growth, as illustrated in Figure 1.6. The nomenclature to describe these structures based on dimensionality is 0D, 1D, 2D, and 3D. Zero-dimensional (0D) nanostructures are classified when all external dimensions are between 1 and 100 nm. Zero-dimensional nanostructures include various types of nanoparticles, nanocrystals, nanoclusters, highly symmetrical dendrimers, branched macromolecules, fullerenes (an allotrope of carbon), star-shaped crystal structures (vanadium oxide) and flower/tree-like structures (silicon carbide, magnesium oxide, and molybdenum disulfide). For 0D nanomaterials, the material can exhibit quantum effects due to the electrons entrapped in a dimensionless space making electron delocalization impossible. Some applications for 0D material have included nanovectors for drug delivery, cell markers, emulsifiers, quantum dots, and reinforcement for solid-state matrixes.\textsuperscript{39} One-dimensional (1D)
nanomaterials have two external dimensions at the nanoscale, while the third dimension is at the microscale. Examples of 1D nanomaterials include nanorods, nanotubes, nanowires, and nanofibers.\textsuperscript{39} Two-dimensional (2D) nanomaterials have one external dimension at the nanoscale and the other two dimensions at the microscale. The 2D nanomaterials comprise thin nanofilms, nanocoatings, and nanoplates. The thin nanofilms are utilized in physics and electronics for their insulating or conductive properties. They are composed of a few atomic layers of metal or ceramic. Nanoplates have a thickness of a few nanometers with varying widths and lengths, based on the material.\textsuperscript{39} Three-dimensional nanomaterials display internal nanoscale features but no external dimensions at the nanoscale. Nanocomposites are multiphase solid materials with at least one external nanoscale.\textsuperscript{39} The dimensionality is not the only way to classify nanomaterials, but it is the most common type of classification found in the literature.

![Nanomaterials classification based on dimensionality.](image)

\textbf{Figure 1.6} Nanomaterials classification based on dimensionality.\textsuperscript{39}

\section*{1.4 The Effects of Nanomaterial Scaling}

Materials synthesized at the nanoscale level have unique properties when compared to the bulk material of the same material. The traditional scientist's view of the physical properties,
such as melting point, fluorescence, electric conductivity, magnetic permeability, and chemical reactivity, is based on large macroscopic systems in which the material reacts under certain conditions. These measurements of the physical properties of the bulk material assumed that the number of atoms or molecules is more substantial than a mole (>10^{23} atoms). The scaling of nanomaterials is different from the bulk material. The scaling causes a shift in the dominant forces and models to explain the physical phenomenon observed at the nanoscale when compared to the bulk material. In the nanoscale, there are four essential ways that the relative scaling changes the properties of the material. The first way is that electromagnetic forces dominate, rather than gravitational forces. The gravitational force is a function of mass and distance, which effectively becomes infinitely small on the nanoscale and makes the effect of the gravitational force negligible relative to the electromagnetic force. The electromagnetic force is a function of charge and distance, which remains independent from the mass of the material and better describes the properties of the material when the mass is minimal. The second way scaling affects nanomaterials is by quantum effects to describe motion and energy levels of nanomaterials rather than classical mechanics. Newtonian mechanics and laws begin to break down with objects that are very small or at very high velocities. The reason is that the bulk material forces are the average of all quantum forces asserted on all the atoms of the material. Understandably, as the scale of the material becomes smaller and smaller, averaging becomes less practical to describe the specific behavior of materials made up of a few atoms or molecules. This breakdown is due to electron confinement and the discontinuous behavior from the completion of shells in systems with delocalized electrons.

The third way that scaling alters the properties of nanomaterials is the effect on the surface to volume ratio. As the material is divided into smaller and smaller pieces, it results
in an increase of surface area to volume ratios, as depicted in Figure 1.7. This shows the relationship between the scaling of the surface area of a cube relative to the volume of the cube. The surface area scales with the length of the edge (u) squared, as the volume of a cube scales with the length of the edge cubed. The surface area to volume ratio increases as the size is reduced and becomes critical as larger surface areas allow for additional contact with the surrounding material. The increase in surface area for chemical reactions allows for higher reactivity and is utilized for catalytic effects. Additionally, the cohesive energy, which is the bond energy per atom, will linearly decrease due to a lower mean coordination number as a function of the inverse radius. The lower coordination results as a consequence of a lower stabilization of atoms or molecules at the surface of the material. This lowers the melting point at the surface of the material.

![Figure 1.7 The increase in surface area to volume ratio.](image)

The final way scaling affects nanomaterials differently than the bulk material is that Brownian motion can be observed in the nanomaterials. The Brownian motion is the random
movement of particles suspended in a fluid (a liquid or a gas). The bulk material is largely aggregated, so when an applied force or stress is on the material, the atoms vibrate in place. The structure of bulk solids is rigid and cannot easily change shape as the force is dissipated through an extensive bond and lattice network. However, when the size of the material is reduced to the nanoscale with the same force applied, the material of the solid lacks the extensive atomic network, making the movement more observable and dramatic in the nanomaterial. 42

Ultimately, the four different ways that scaling causes the nanomaterial to deviate from the bulk material properties have unique properties based on the materials' size, shape, and morphology. The physical phenomenon can cause a noticeable reduction in melting point or a shift in the color appearance. This makes the study of nanomaterial systems essential for tuning, optimization, and application for various fields of science and engineering because of quantum confinement’s effects on materials with delocalized electron states.
1.5 Fabrication Strategies of Nanomaterials

Engineered nanomaterials have two primary routes of synthesis: “top-down” and “bottom-up” approaches, as illustrated in Figure 1.8. The “top-down” approach takes the bulk material and reduces the size to the nanoscale by mechanical, chemical, or physical methods.\textsuperscript{44,45} This process typically produces polydisperse particles with the particle sizes larger than 800nm containing a more significant number of structural deformation and defects.\textsuperscript{44} An analogy to this process is to take a granite statue and to cut the statue until ground to specks of silica-alumina dust/powder. Examples of the “top-down” approach are lithographic cutting techniques, anodizing, grinding, and ball-milling.\textsuperscript{45}

![Figure 1.8 Top-down and bottom-up synthesis routes.\textsuperscript{44}](image)

The “bottom-up” approach is the most common route of synthesis, which utilizes atomic or molecular components that self-assemble through chemical reactions or physical processes to produce the nanomaterial.\textsuperscript{45,46} An analogy is to take individual atoms of silicon, oxygen, and aluminum and build molecules, and then join the molecules together to form silica-alumina powder. Examples of the “bottom-top” approach are sol-gel, electrodeposition, pyrolysis,
chemical vapor deposition, atomic layer deposition, and molecular beam epitaxy, with additional examples presented in Figure 1.9.\(^{45}\)

**Figure 1.9** Top-down and bottom-up synthetic methods tree chart.\(^{46}\)

1.6 Biomedical Applications for Iron Oxide and Boron Nanomaterial Composites

Magnetic iron oxide nanoparticles have long been explored for biomedical applications as an MRI contrasting agent, target-specific drug therapy, carrier for gene therapy, therapeutic agent, magnetic sensing probes for in vivo diagnostics, and nano-adjuvants for vaccines.\(^{47}\) Furthermore, magnetic iron oxide nanoparticles are considered as a noninvasive cancer treatment through magnetic induction heating.\(^{48}\) Alternatively, the surface of the iron oxide nanoparticles can be altered with an encapsulating agent, shell, and appended boron bioconjugate for the second type of cancer treatment called boron neutron capture therapy (BNCT).\(^{49}\) G.L. Locher, in
1936, was the first to propose the therapeutic potential of neutron capture therapy in the treatment of cancer.\textsuperscript{50}

Cancer is the leading cause of death globally and ranked second in the United States of America. In 2012, a staggering 32.6 million people were living with some diagnosed form of cancer, resulting in 8.2 million cancer-related deaths for the year. On average, cancer kills 22,450 people a day, with an age-standardized cancer incidence rate approximately 25\% higher in men than women.\textsuperscript{51} New cancer cases in the U.S. are projected to increase by 45\% between 2010-2030.\textsuperscript{52} Cancer research has become more prominent in addressing this growing epidemic.

Glioblastoma multiforme (GBM) is a malignant form of brain cancer that is by far the most common and aggressive type.\textsuperscript{53} Approximately 700,000 Americans have been diagnosed with this form of malignant brain tumor, and an estimated 78,980 people will receive a primary brain tumor diagnosis by the end of 2018.\textsuperscript{54} The current median survival rate for adults diagnosed with glioblastoma is 11 to 15 months. There are currently only three types of treatment for GBM: radiation, chemotherapy, and surgery. Unfortunately, these types of treatment options are inefficient due to the adverse and troublesome side effects and the high incidence rate of reoccurrence. Chemotherapy can cause cell damage to nearby healthy tissue, and surgery may require multiple surgeries to remove the tumor. Many times the brain tumor is inoperable due to its location in the brain, which excludes surgery as an option for treatment.

There is an alternative radiation method for inoperable brain tumors using boron neutron therapy portrayed in Figure 1.10. There has been a resurgence into BNCT research with the death of John McCain from GBM on August 25\textsuperscript{th}, 2018.\textsuperscript{55} Currently, BNNT is one of the most
promising treatments for GBM. This therapy uses preferentially enriched $^{10}\text{B}$ isotope conjugates that when irradiated with thermal neutrons create a very unstable $^{11}\text{B}$ isotope. This $^{11}\text{B}$ isotope decomposes instantaneously with alpha decay, releasing a high-kinetic helium ion ($\alpha$-particle), lithium-ion, and gamma radiation.$^{56}$ Due to the alpha particle’s restricted path length of 8 to 10 micrometers, the alpha particle damage is localized to the cancer cell. The in situ generation of lithium is removed through the renal system, leaving no trace of the original $^{10}\text{B}$ isotope conjugate.

![Figure 1.10 The process of BNCT on a cancer cell.$^{57}$](image)

The limitations for BNCT are of drugs to target the cancer cell, only two have gained FDA approval, boronophenylalanine (BPA) and borocaptate sodium (BSH). The concentration of $^{10}\text{B}$ atoms required to kill the cancer cell with BNCT effectively is $10^9$ $^{10}\text{B}$ atoms per cell or approximately 35 $\mu$g of $^{10}\text{B}$ per gram of tumor tissue.$^{56,57}$ Biodistribution and toxicity of enriched $^{10}\text{B}$ conjugates are the two most important factors for drug development and clinical trials. Iron oxide nanoparticles appear a natural fit for this application, and their unique magnetic properties
would guide and deliver the drug towards the site of the tumor, which in theory would allow for a higher cellular uptake of $^{10}$B isotope into the cancer cells.$^{57}$
1.7 References


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35. Ken and Nyetta. “North Rose Window in Notre Dame Paris.” *Flickr*, 16 June 2014, 3:39, [https://www.flickr.com/photos/71279764@N00/11553619656](https://www.flickr.com/photos/71279764@N00/11553619656). License: [https://creativecommons.org/licenses/by/2.0/deed.en](https://creativecommons.org/licenses/by/2.0/deed.en). The picture was adapted, changes included crop, and resized.


CHAPTER 2: INSTRUMENTATION

2.1 Introduction

A chemist has a variety of instruments available for characterizing the properties of matter that are useful for qualitative and quantitative analysis. The instruments for chemical analysis convert information about the physical and chemical characteristics of the matter and then translates and transforms the information with a computer so humans can quickly interpret the data.\textsuperscript{1} Generally, instruments for chemical analysis will have an energy source, a system under study, and a response that converts information from the detector to data.\textsuperscript{1} In this thesis, the system under study was nanomaterials, which requires instrumentation with high sensitivity and magnification. This chapter will discuss the appropriate instrumentations utilized for studying and characterizing the nanomaterials. The following is a list of instrumentation covered in this chapter: transmission electron microscopy (TEM), scanning transmission electron microscopy (STEM), electron energy-loss spectroscopy (EELS), Fourier transform infrared spectroscopy (FTIR), Mössbauer spectroscopy (MS), nuclear magnetic resonance (NMR), and small-angle X-ray scattering (SAXS).

2.2 Transmission Electron Microscopy / Scanning Transmission Electron Microscopy\textsuperscript{2-6}

The working principle is the same for the transmission electron microscopy (TEM) and scanning tunneling electron microscopy (STEM), as both techniques utilize electrons to obtain
the images of the specimen. The main difference is how the microscopes create the image, in TEM, the electron beams are focused perpendicular to the plane of the specimen. The TEM can generate the image of the specimen by three types of signals: backscattered electrons, secondary electrons, and X-ray emission. The interactions of the electron beam with the specimen can be divided into elastic/inelastic interactions resulting in either transmitted or scattered electrons as they interact with the specimen. Table 2.1 compares the difference between SEM and TEM based on the type of electrons, scattered or transmitted. If the interaction with the electron beam and the sample is elastic, the interaction will only affect the trajectory of the electrons, conserving the electron kinetic energy, and does not change the energy level of the sample. When the electron beam interacts with the sample in an inelastic manner, part of the electron’s energy is transferred to the sample. This excites the specimen’s atoms, and the excited atoms must emit energy in the form of secondary electrons, Auger electrons, X-rays, or longer wavelength photons to return to its ground state.

In the TEM, as shown in Figure 2.1, the specimen is placed within the column, and the electrons are transmitted through the sample to a phosphor screen, CCD, or film and cast a 2D image. Both microscope types have similar components as they require an electron source, a series of electromagnetic and electrostatic lenses to control the shape and trajectory of electrons, and electron apertures. Also, they both require high vacuums to prevent air particles causing interference by deflecting electrons.

The STEM electron beam is finely focused with a convergence angle of about 7-8 mrad for 200 keV electrons without aberration correction. The beam is rastered over the sample and transmits the data as a function of the location of the beam. There are multiple detectors for
STEM imaging (Figure 2.2), including bright-field (small angles, 0-10 mrad), annular dark-field (larger angles, 10-50 mrad), and high-angle annular dark-field (angles 50-100 mrad). In HAADF, the high angle prevents the elastically scattered electrons from reaching the detector, so the image is generated from only inelastically scattered electrons. This improves the contrast for lower atomic numbers when compared to TEM.

Table 2.1 The Comparison of SEM and TEM

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<td>Transmitted electrons</td>
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<td>2D projection of an image of the inner surface</td>
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<td>Max Magnification</td>
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<td>More than 50 million times</td>
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<tr>
<td>Optimal spatial resolution</td>
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</tr>
<tr>
<td>Spectra formation</td>
<td>Electrons captured by the detector translated to image on the PC screen</td>
<td>Direct imaging on a fluorescent screen or PC screen with CCD</td>
</tr>
<tr>
<td>Sample Preparation</td>
<td>Little or no sample preparation</td>
<td>Laborious sample preparation</td>
</tr>
</tbody>
</table>
Figure 2.1 Schematic diagram of components of a TEM column construction.7
Figure 2.2 The position of detectors in a TEM system.\textsuperscript{8,9}
BF=bright-field (TEM), DF=dark-field (TEM), HAADF (STEM)=high-angle annular dark-field, EDS=energy-dispersive X-ray spectroscopy, EELS=electron energy-loss spectroscopy
2.2.1 Electron Energy-Loss Spectroscopy\textsuperscript{3,10}

Electron energy-loss spectroscopy (EELS) is an analytical technique that gives a wealth of structural and chemical information of a solid substance about the spatial resolution at the atomic scale. EELS detects the changes in energy from the primary electron beam that is predominant forward scattering.\textsuperscript{10} The energy distribution of the electrons can be used to determine local properties of the specimen, such as the type and quantity of atoms, the bonding and chemical state of the atoms, and the collective interaction of atoms with their surrounding neighbors. EELS is typically incorporated with modern scanning transmission electron microscopes using high-energy electrons between 60-300 keV. The electrons that pass through a specimen can interact with the atoms present and can either act with elastic (no net energy exchange) or inelastic (energy loss) scattering.\textsuperscript{10} The inelastic scattering with the loss of energy leaves the atoms in the sample in an excited state in which the material can relax back to the ground state by the release of energy in the form of photons, x-rays, or Auger electrons. The intensity of the scattering vs. the energy loss can be plotted to obtain a spectrum, which is divided into three regions: electrons with no change in energy (zero-loss peak), electrons that have interacted with the weakly bound electrons in the specimen (low-loss distribution), and the electrons that have interacted with the tightly bound core electrons of the atoms (the ionization edges or core-loss region).\textsuperscript{10} The nomenclature of the core-loss edges is illustrated in Figure 2.3, with the electrons shells from closest to farthest from the nucleus as; K, L, M, N, and O, with the increase of energy as the electrons moves away from the nucleus.\textsuperscript{11}
2.3 Energy-Dispersive X-ray Spectroscopy (EDX)\textsuperscript{12,13}

Energy-dispersive X-ray spectroscopy is an atomic spectrometric technique based on a two-step process that begins with an X-ray beam exciting an inner shell electron from an atom and ejecting it from the atom.\textsuperscript{12,13} This ejected inner shell electron leaves a vacancy in the orbital, which is filled by an outer shell electron at a higher energy state. As the outer shell electron transitions to fill the vacancy in the lower energy orbital, it releases X-ray radiation. The difference between the two electron energy levels is characteristic of the element, and the element can be determined by measuring the emitted photon energy released.\textsuperscript{12,13} The intensity of the emitted photons can determine the concentration of each element in the sample, as shown in Figure 2.4.\textsuperscript{12,13}
Figure 2.4 The process of EDX.\textsuperscript{13}

a) An incident X-ray beam begins by exciting an inner shell electron (K-shell) of a boron atom, resulting in the ejection of the electron from the atom and leaving a vacancy in the inner shell.

b) The inner shell vacancy is filled by an outer shell electron (L-shell), which during the transition from a higher electron energy level to a lower energy level emits X-ray radiation.

The characterization technique has some limitations as low atomic number atoms are undetectable, there can be overlaps between similar energy peaks, and the presence of carbon peaks is often an artefact from overcoating with a thin carbon film to make the sample conductive.\textsuperscript{12,14}

2.4 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a form of molecular absorption spectroscopy that records measurements in the mid-IR region of the electromagnetic spectrum. FTIR spectroscopy determines the chemical species of a sample both qualitatively and quantitatively.\textsuperscript{1} At the center of the FTIR instrument is a Michelson interferometer, shown in Figure 2.5.\textsuperscript{15} The FTIR spectrometer first collects a signal of a background interferometer over a range of frequencies and compares it to the sample interferogram.\textsuperscript{15} The computer will then
perform a Fourier transformation to generate a spectrum as the computer processes the data from the detector (Figure 2.6).  

The Michelson interferometer works by taking a monochromatic infrared source and uses a beam splitter to separate the beam into two beams with different path lengths. One beam (A) travels through a fixed mirror path length, and a second delayed beam (B) travels to a moveable mirror, which varies its pathlength. When beams A and B are recombined, it produces an interference. A detector then measures the intensity variations of the recombined emergent beam as a function to the path lengths in real time. Constructive interference is observed when beams A and B interferences are entirely in phase and a maximum beam intensity reaches the detector. Destructive interference is observed when beams A and B interferences are entirely out of phase and a minimum beam intensity reaches the detector. The differences between the maximum and minimum interferences cause the changes in the transmission as a function to the frequency and produce an interference pattern. From this data, the fringe pattern is calculated by Equation 1:

\[
d = \frac{(10)(n)}{2(\nu_1 - \nu_2)}
\]  

where \(d\) = thickness (in mm), \(\nu_1\) = frequency at the first maximum or minimum occurrence (in \(\text{cm}^{-1}\)), \(\nu_2\) = frequency at the last maximum or minimum occurrence (in \(\text{cm}^{-1}\)), and \(n\) = number of complete peak-to-peak fringes between the two maxima or minima at \(\nu_1\) and \(\nu_2\). The data is analyzed and deciphered for function groups, bond types, and qualitative information such as concentration.
The recombinant beam of the delayed split beam varies in intensity as a function to the path length of the moving mirror, causing the incident beam ($I_0$) to pass through the sample, in which molecules in the sample can absorb the infrared light at a specific frequency inherent to the molecule’s vibrational energy.\textsuperscript{15} The molecule’s inherent vibrational properties are inherent by the mass of atoms, the bond strength, and the environment around the molecules. The frequency of vibration can be mathematically calculated using Equation 2:\textsuperscript{18}

$$\nu = \frac{1}{2\pi c} \sqrt{\frac{k}{\mu}}$$

(2)

where $\nu$ = the frequency of vibration, $c$= speed of light, $k$ = force constant, and $\mu$ = reduced mass.
If a molecule changes in the dipole moment as it vibrates, the molecules will absorb a portion of the infrared light at a specific frequency. If there is no change in the dipole moment, the infrared light will pass through the sample without the absorption occurrence. After the incident light passes through the sample, the emergent beam recombined detector collects the radiant beam (I), and a computer can calculate the transmission by $I/I_0$ or the absorbance by Equation 3; and the concentration of the analyte is linearly related to absorbance by Equation 4 (Beer-Lambert law).

$$T = \log \frac{I_0}{I} \quad (3)$$

$$A = \varepsilon bc = -\log(T) = 2 - \log(\%T) \quad (4)$$

where $I_0$ = intensity of incident radiation, $I$ = intensity of transmitted radiation, $A$ = absorbance, $T$ = transmittance, $b$ = sample path length of the inner cell in cm, $c$ = concentration of the absorber (grams/L), and $\varepsilon$ = molar absorptivity.

Figure 2.6 A diagram of a basic FTIR spectrometer$^{16}$
2.5 Mössbauer Spectroscopy$^{10-20}$

Mössbauer spectroscopy is a spectroscopic technique based on the Mössbauer effect. The Mössbauer effect is a nuclear phenomenon of efficient emission of absorbance of γ-ray, where a nucleus at the ground state will become excited at discrete energy thresholds and transition to an excited nuclear energy state. This transition requires a substantial change in energy; for example, $^{57}$Fe first excited energy state is at 14.41 keV above its ground state. For the excited nucleus to relax back to its ground state, it needs to emit a γ-ray. The emission of the γ-ray from an excited nucleus is subsequently followed by the absorption of a γ-ray by a second nucleus. For the second nucleus to absorb the emitted γ-ray efficiently, both nuclei must be embedded in the solid’s lattice. The γ-radiation release is also known as the detection of the hyperfine interaction and can be separated into three interactions: isomer shift, quadrupole splitting, and magnetic hyperfine splitting; these interactions are illustrated in Figure 2.7.$^{19}$

![Figure 2.7 Hyperfining interactions of Mössbauer spectroscopy.](image)
In the presence of the magnetic field, the nuclear spin moment experiences a dipolar interaction with the magnetic field called the Zeeman effect, this effect is observed for $^{57}$Fe in Figure 2.8. The total effective magnetic field at the nucleus is given by Equation 5:

$$B_{\text{eff}} = (B_{\text{contact}} + B_{\text{orbital}} + B_{\text{dipolar}}) + B_{\text{applied}}$$

(5)

where $B_{\text{contact}}$ = spin of the electrons polarizing the spin density of the nucleus, $B_{\text{orbital}}$ = is the orbital movement on those electrons, and $B_{\text{dipolar}}$ = is the dipole field due to the spin of those electrons.

![Diagram of magnetic splitting of $^{57}$Fe nuclear energy levels.](image)

**Figure 2.8** Magnetic splitting of $^{57}$Fe nuclear energy levels.

The line position of the Mössbauer spectra relates to the splitting of energy levels, as the intensity of spectra relates to the angle between the γ-ray and nuclear spin moment.
2.6 Nuclear Magnetic Resonance

In 1924, Wolfgang Pauli proposed that specific atomic nuclei will have properties of spin and magnetic moments that can split the nuclei energy levels when exposed to an external magnetic field.\(^1\) Two decades later (1946), Felix Bloch and Edward Purcell would validate and demonstrate the energy splitting of nuclei under an applied magnetic field.\(^1\) This discovery led to the development of nuclear magnetic resonance (NMR) spectrometry, which is now a standard and versatile analytical technique for elucidating the structure of a chemical substance regardless of phase.\(^1\)

In Figure 2.9, the upper left picture shows a sample with nuclei with either an integral or fractional spin having an electric field applied to the sample.\(^2\) In the sample, the nuclei opposite of the applied magnetic field will spin opposite \((-\frac{1}{2})\) of the applied magnetic field, represented by the blue circle with a downward arrow. In contrast to the nuclei parallel to the applied magnetic field, the nuclei will spin in the same direction \((+\frac{1}{2})\) of the applied magnetic field, represented by the red circle and upward arrow. Once the nuclear spin splits the nuclei energy levels, the nuclei can be excited with pulses of radio frequency (10-15μs); if the nuclei are in resonance with the pulse, the nuclear spin will flip. The nuclei will eventually relax back to equilibrium, which is detectable by voltage oscillations in the NMR probe. The pulses of radio frequency are repeated many times more, and the summation of data is recorded as a free induction decay (FID) signal. A computer processes the FID signal into an NMR spectrum with a Fourier transform (FT). In Figure 2.10, a generic NMR spectrometer and its components show how the NMR detects changes in the oscillating voltage to create a spectrum with a Fourier
transform computer. Figure 2.11 shows the cross-section of an NMR spectrometer and how the samples are positioned to apply a magnetic field in the split energy levels.

Figure 2.9 The splitting of energy levels in NMR active nuclei in a magnetic field.²¹

Figure 2.10 Block diagram of a generic pulse NMR spectrometer.²²
Figure 2.11 Diagram of a generic NMR spectrometer and components.\textsuperscript{23,24}  
\(\text{a)}\) depicts the cross-section of an NMR spectrometer  
\(\text{b)}\) shows how the NMR sample tube is positioned between the north and south magnetic poles to induce a magnetic field

2.7 Small-Angle X-ray Scattering\textsuperscript{25,26}

Small-angle X-ray scattering (SAXS) is an analytical technique that measures the intensities of X-rays scattered by a sample of material as a function of the scattering angles, which provides localized structural information on the microstructure, phase behavior, and the interaction of multicomponents in the magnetic iron oxide nanomaterial. Measurements are typically in the range of 0.1 to 5 degrees and produces constructive or destructive waves as shown in Figure 2.12.\textsuperscript{25} The framework for SAXS analysis was observed by Lawrence Bragg and William Henry Bragg in 1913, in which they found that crystals, at specific wavelengths and incident angles, will produce intense peaks of reflecting radiation. From their research, they inferred Bragg’s Law from Figure 2.13 (Equation 6).\textsuperscript{26}

\[2d \sin \theta = n \lambda\]  
\text{(6)}
where $d$ represents the interplane distance of atoms, ions, or molecules; $\theta$ represents the scattering angle; $n$ represents the integer of an order of diffraction; and $\lambda$ represents the wavelength of the X-ray.

**Figure 2.12** Schematic of X-ray interacting with atoms and wave propagation.$^{25}$

**Figure 2.13** Schematic of inferred Bragg’s law.$^{26}$
SAXS data collection and analysis were performed on the bare magnetic iron oxide product to check the dispersion, the shape, and to validate the preliminary TEM and Mössbauer data analyses. However, SAXS may be utilized in the future to develop better coprecipitation methods for the creation of monodisperse nanoparticles of specific size and spherical shape. The small microscopic changes in nanoparticles can affect macroscopic function and aid in optimizing the cellular uptake of boron-bioconjugate systems once the magnetic nanoparticle is encapsulated and functionalized.
2.8 References


CHAPTER 3: SYNTHESIS AND ENCAPSULATION OF IRON OXIDE NANOPARTICLES

3.1 Synthesis

Iron oxide nanoparticles were synthesized in a controlled coprecipitation method of iron chlorides in a 1:2 molar ratio of FeCl\textsubscript{2}/FeCl\textsubscript{3} with ammonium hydroxide as the precipitating agent. Iron oxide nanomaterials considered for medical applications require superparamagnetic properties, which are associated with magnetite nanoparticles (MNP) less than 20nm in size.\textsuperscript{1} The magnetic properties of the MNP are highly dependent on the size and shape of the nanoparticles, which is mainly determined by the route of the synthesis.\textsuperscript{2,3} All coprecipitation methods are relatively sophisticated and require strict control of the precipitation conditions, including temperature, stirring rate, initial pH, stabilizers, reactants, the concentration of reactants and stabilizers, and finally, the oxidation of the nanoparticles.\textsuperscript{2,3} The size of the nanoparticles is not the only factor that influences the magnetization properties of the nanoparticles; the disorder of the crystallinity, irregularities of morphologies, spin disorder layer, and agglomeration from the magnetostatic interactions between particles can influence its effects.\textsuperscript{3} This project evaluated the properties of bare iron oxide nanoparticles without the aid of the stabilizer to establish a baseline as a comparison for the encapsulated and functionalized material.
3.2 Iron and Magnetite

Iron is the 26th element in the Periodic Table, positioned in the fourth period and the eighth group with an atomic number of five. Iron has an intense magnetic dipole moment from four unpaired electrons in the d orbital with the electron configuration of [Ar] 3d⁶4s². The d orbitals must contain unpaired electrons, or there would be no net magnetic dipole and the material will no longer be magnetic. Figure 3.1 depicts the Fe³⁺ iron site having five unpaired electrons in the d orbital, and Fe²⁺ iron site having four unpaired electrons in the d orbital.

![Figure 3.1](image)

Figure 3.1 The spin configuration of the octahedral (Fe²⁺,³⁺) and tetrahedral (Fe³⁺) iron sites in Fe₃O₄.

The most common iron oxides in nature and studied for biomedical applications are magnetite (Fe₃O₄) and maghemite (γ-Fe₂O₃), with magnetite being the primary focus of the synthesis for the first part of this thesis (Figure 3.2). Magnetite is a black crystalline powder that has the most potent magnetism of the naturally occurring minerals. Magnetite is alternatively named iron (II, III) oxide and contains both ferrous oxide (FeO) and ferric oxide (Fe₂O₃) within its crystal lattice. The molecular formula of FeO • Fe₂O₃ can also be written as Fe₃O₄.
Figure 3.2 The representative structure of magnetite and maghemite.\(^6\)

(a) Face-centered cubic spinel structure of magnetite, and (b) the magnification of one tetrahedron and adjacent octahedron with adjoining oxygen atoms. The lattice parameter side represents the (001) plane, and \(c\) represents the plane perpendicular to the (001) plane.

The magnetite crystal lattice was one of the first mineral structures that Bragg and Nishikawa applied X-ray diffraction to in 1915.\(^4\) Magnetite structure has an inverse spinel with a face-centered cubic unit cell base of 32 \(\text{O}^{2-}\) ions packed along with the [111] plane. The unit cell length is \(a = 0.839\) nm with eight formula units per unit cell.\(^4\) Magnetite differs from other iron oxides as it contains both divalent and trivalent cations of iron with the general formula \(Y[XY]O_4\), where \(X = \text{Fe (II)}, Y = \text{Fe (III)}\), and brackets denote the octahedral site stacked along the [111] plane. Maghemite has the same crystal lattice, except it does not contain the vacancies in the octahedral sites that usually are occupied by the divalent iron.\(^4\) In Figure 3.3, the O-O distance and layer spacing relationship for various iron oxides are observed relative to the unit cell hexagonal close-packing and cubic-packing.
Magnetite is ferrimagnetic at room temperature with a Curie temperature of 850K. The presence of two different cation sites, tetrahedral (Fe$^{3+}$) and octahedral (Fe$^{2+}$), forms two interpenetrating magnetic sublattices.\(^4\) The spins on the tetrahedral and octahedral are antiparallel below the Curie point with two unequal magnitudes of spin, which causes ferrimagnetism portrayed in the lower-left corner of Figure 3.4. Also, magnetite nanoparticles under 20nm show superparamagnetic properties. Particles in this size range are a single domain, and all the individual atoms with magnetic moments from nanoparticles act as one giant magnetic moment.

**Figure 3.3** The relationship between the O-O distance in hexagonal close-packed and cubic-packed for various iron oxides\(^4\)
Magnetite has an unequal magnitude dipole moment with the divalent and trivalent iron. This makes it ferrimagnetic at room temperature. This type of magnetic behavior has been suggested for magnetically targeted drug delivery and nanovectors since the earliest magnetic polymer carriers of the 1970s. The main reason is that MNPs are smaller than the target cells, can be encapsulated with a biomolecule forcing interaction or binding to the target, and can be magnetically guided with an external magnetic field. Much of the current research for magnetic guided drugs focuses on the optimization of the magnetic nanoparticles as vectors by improving magnetic properties and functionalizing them to increase cellular uptake and targeting active sites of cancer/tumor cells. This is the suggested mode of delivery, as illustrated in Figure 3.5, for boron-based bioconjugates based on carboranes and boron nitride nanotubes for BNCT.
3.4 EPR and Iron Oxide Magnetic Induction

One of the hallmarks of cancer is the ability of cancer cells to induce angiogenesis with vascular endothelial growth factor (VEGF). The creation of these new blood vessels with VEGF causes the vessels to become more porous and leakier when compared to healthy blood vessels, resulting in enhanced permeability and retention (EPR) localized to the tumor tissue. Pathologists observed that cancer cells grew preferentially around new blood vessels to supply oxygen and the nutrients needed for rapid cell growth. The creation of new blood vessels is required for the tumor to grow larger than 1mm in size. The tumor cells located more than 0.2 mm away from blood vessels were found to be nongrowing, while tumor cells farther away were
seen dying. This threshold of 0.2 mm represents the distance that oxygen can effectively diffuse through living tissue. Tumor cells found farther than the 0.2 mm threshold distance from the blood vessel consequently suffer from moderate to severe hypoxia and low pH. Tissue suffering from hypoxia can become necrotic and trigger apoptotic cell death.\textsuperscript{12}

Magnetic induction heating takes advantage of the enhanced permeability and retention, allowing delivery of biocompatible magnetic iron oxide nanoparticles to achieve concentrations valid for hyperthermia. When applying alternating magnetic fields to opposite sides of iron oxide nanoparticles, the microscopic domains interact with the magnetic field.\textsuperscript{13} The microdomains become aligned to the applied magnetic field; by alternating the magnetic field, it causes the iron oxide particles to realign, creating a constant thermal motion. The thermal motion over time starts to heat the iron oxide and eventually reaches a temperature threshold allowing for the ablation of the cancer cell.\textsuperscript{13} The concentration of the iron is biodistributed in squamous cell carcinoma mouse models with a tumor to non-tumor ratio > 16. Iron oxide tumor concentrations of 1.9 mg Fe/g tumor reached temperatures of greater than 60°C within 2 minutes with an applied field of 38kA/m at 980 kHz. This localized the ablating of the cancer cells while leaving the healthy tissue intact.\textsuperscript{13} This cancer treatment is also highly dependent on biodistribution and toxicity of the magnetic iron oxide delivery to the cancer cell.

3.5 Materials and Methods

3.5.1 Materials

Equipment and chemicals: spatula, weigh paper, 100mm x 100mm Fischer Scientific weigh paper, 250 mL Büchner flask, Mettler BB244 precision balance, vacuum pump, Z366 Hermle
high-performance centrifuge, Fischer Scientific Q125 sonicator, FEI Talos (S)TEM with Super-
X 4SDD XEDS, Hitachi H-600 TEM, and Bruker 300mHz NMR.

3.5.2 Methods

3.5.2.1 The Synthesis of Iron Oxide Nanoparticles

Reactions:

\[
\begin{align*}
\text{Fe}^{3+} + 3\text{OH}^- & = \text{Fe(OH)}_3 \, (s) \quad (1) \\
\text{Fe(OH)}_3 \, (s) & = \text{FeOOH} \, (s) + \text{H}_2\text{O} \quad (2) \\
\text{Fe}^{2+} + 2\text{OH}^- & = \text{Fe(OH)}_2 \, (s) \quad (3) \\
2\text{FeOOH} \, (s) + \text{Fe(OH)}_2 \, (s) & = \text{Fe}_3\text{O}_4 \, (s) + 2\text{H}_2\text{O} \quad (4)
\end{align*}
\]

Giving an overall reaction:

\[
2\text{Fe}^{3+} + \text{Fe}^{2+} + 8\text{OH}^- = 2\text{Fe(OH)}_3\text{Fe(OH)}_2 \, (s) \rightarrow \text{Fe}_3\text{O}_4 \, (s) + 4\text{H}_2\text{O} \quad (5)
\]

\[2\text{Fe (III)} + \text{Fe (II)} + 8\text{OH}^- \rightarrow \text{Fe}_3\text{O}_4 \quad \text{Nanoparticles} + 4\text{H}_2\text{O}\]

**Figure 3.6** A schematic for the synthesis of iron oxide magnetite nanoparticles.

**Table 3.1** The Reactant Quantities for SPIONs Reaction

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Quantity</th>
<th>Moles</th>
<th>Molar Mass</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl₂•4H₂O</td>
<td>0.800g</td>
<td>4.02E-3 mol</td>
<td>198.862 g/mol</td>
<td>1.93 g/cm³</td>
</tr>
<tr>
<td>FeCl₃•6H₂O</td>
<td>2.250g</td>
<td>8.033E-3 mol</td>
<td>270.295 g/mol</td>
<td>1.82 g/cm³</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>75.00mL</td>
<td>4.154 mol</td>
<td>18.01528 g/mol</td>
<td>0.99777 g/cm³</td>
</tr>
<tr>
<td>Ammonium Hydroxide</td>
<td>61.00mL</td>
<td>1.532 mol</td>
<td>35.04 g/mol</td>
<td>0.880 g/cm³</td>
</tr>
</tbody>
</table>
The synthesis of iron oxide nanoparticles: Ferric chloride hexahydrate (FeCl₃•6H₂O, 2.250g) and iron (II) chloride tetrahydrate (FeCl₂•4H₂O, 0.800g) were dissolved in 75.00 mL of deionized water in a fresh 250 mL Büchner flask. The solution of aqueous iron and chloride ion was sonicated for 30 minutes. The flask was transferred into an ice bath placed on a magnetic stir plate and allowed cool to ~9.4°C while stirring with a PTFE magnetic stir bar. The addition of 61.00 mL of ammonium hydroxide (NH₄OH) was added dropwise 1.00 mL per minute via a graduated burette. Upon the delivery of the ammonium hydroxide into the solution, the amber-colored solution formed a black precipitate. The solution and product were magnetically stirred overnight to allow for the completion of the reaction. The product was separated from the solution by transferring the solution into a fresh glass tube and centrifuging for 10 to 30 minutes at 3,000 RPM, or until a pellet formed at the bottom of the glass tube. The supernatant liquid was decanted into a waste beaker and discarded into the inorganic waste container. The separation process was repeated a second time to ensure the full transfer of solution and product from the flask. The product was then washed five times with deionized water, and the wastewater was decanted. The flask was transferred to a Schlenk line and the residual water dried under vacuum with a liquid nitrogen cold trap. After drying, the product was collected, weighed, and transferred to a vial. The product was stored under argon, and the vial was labeled with the date, initials, and the product ID. The product was then exposed to a strong neodymium magnet to test the magnetism of the product before committing to additional characterization through TEM, STEM, EELS, XRD, FTIR, NMR, EDX, and MS.
3.6 Encapsulation of Iron Oxide Core Nanoparticles

3.6.1 Reaction with TEOS

![Schematic representation of the synthesis of TEOS-Fe₃O₄ nanocomposites.](image)

**Figure 3.7** A schematic representation of the synthesis of TEOS-Fe₃O₄ nanocomposites.¹⁴

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Quantity</th>
<th>Moles</th>
<th>Molar Mass</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe₃O₄ NP 7-20-18</td>
<td>0.050g</td>
<td>2.14E-4 mol</td>
<td>233.55 g/mol</td>
<td>5.18 g/cm³</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>1.00mL</td>
<td>5.54E-2 mol</td>
<td>18.01528 g/mol</td>
<td>0.99777 g/cm³</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>10.00mL</td>
<td>1.715E-1 mol</td>
<td>46.07 g/mol</td>
<td>0.80281 g/cm³</td>
</tr>
<tr>
<td>Ammonium Hydroxide</td>
<td>5.20mL</td>
<td>1.306E-1 mol</td>
<td>35.04 g/mol</td>
<td>0.880 g/cm³</td>
</tr>
<tr>
<td>TEOS</td>
<td>20μL</td>
<td>8.96E-5 mol</td>
<td>208.33 g/mol</td>
<td>0.933 g/cm³</td>
</tr>
</tbody>
</table>

**Table 3.2** The Reactant Quantities for SPIONs Encapsulation with TEOS

Iron oxide tetraethyl orthosilicate encapsulation procedure:

A silicon oil bath was preheated to 40°C while being magnetically stirred with an aluminum paper clip. The magnetite nanoparticle product was pulverized on a folded piece of 100mm x 100mm weight paper using a pestle. The deionized water, 95% ethanol, and ammonium hydroxide were measured with a 10 mL graduated cylinder and transferred into a 25
mL Erlenmeyer flask. The magnetite nanoparticles and tetraethyl orthosilicate were added to the solution in a 25 mL Erlenmeyer flask. The mixture was sonicated at 20% amplitude for 15 minutes, followed by two sonications at 40% amplitude for 15 minutes each. After the third sonication treatment, a magnetic stir bar was placed in the flask. The flask was then lowered into the preheated silicon oil bath and magnetically stirred for four hours. The flask was removed out of the silicon oil bath and the solution mixture transferred to a centrifuge tube. The mixture was centrifuged, and the supernatant was decanted into a waste beaker. The product was then washed four times using a 1:1 ratio of 95% ethanol and deionized water solution. A volume of 100 mL of wash solution was prepared by measuring 50.0 mL of 95% ethanol with 50.0 mL of deionized water in a 100mL graduated cylinder. Each wash was performed during 30 minutes of centrifugation at 3,000 rotations per minute. The liquid after each centrifuge was decanted, and the solid was resuspended through shaking. After the 100mL of wash solution was used and decanted, the tube was filled with 95% ethanol and transferred to a 50 mL side-arm flask. The flask was attached to a Schlenk line under vacuum with a liquid nitrogen cold trap to dry the product. The product dried on the line for eight hours, with sporadic refilling of the liquid nitrogen trap. After four hours of drying, the product was scraped and collected from the sides of the container to ensure a uniform dried product.
3.6.2 Reaction with PEG

![Figure 3.8](image.png)

Figur e 3.8 A schematic representation of the synthesis of PEG-Fe₃O₄ nanocomposites.¹⁵

Table 3.3 The Reactant Quantities for SPIONs Encapsulation with PEG

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Quantity</th>
<th>Moles</th>
<th>Molar Mass</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe₃O₄ NP 7-20-18</td>
<td>0.050g</td>
<td>2.14E-4 mol</td>
<td>233.55 g/mol</td>
<td>5.18 g/cm³</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>1.00mL</td>
<td>5.54E-2 mol</td>
<td>18.01528 g/mol</td>
<td>0.99777 g/cm³</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>10.00mL</td>
<td>1.715E-1 mol</td>
<td>46.07 g/mol</td>
<td>0.80281 g/cm³</td>
</tr>
<tr>
<td>Ammonium Hydroxide</td>
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<td>1.306E-1 mol</td>
<td>35.04 g/mol</td>
<td>0.880 g/cm³</td>
</tr>
<tr>
<td>Polyethylene glycol</td>
<td>20μL</td>
<td>8.96E-5 mol</td>
<td>208.33 g/mol</td>
<td>0.933 g/cm³</td>
</tr>
</tbody>
</table>

3.7 Results and Discussion

The first test performed on the product was a magnetic test to verify that the product had magnetic properties before proceeding onto other characterization methods. As observed in Figure 3.9, the product was dark black and showed an excellent magnetic response. The next step was to check the scale of the product on the nanoscale, and this was performed with TEM.
TEM grid was prepared by suspending the product in 95% ethanol with a sonication treatment.

![Figure 3.9](image)

**Figure 3.9** Images of the MNP product in the absence and presence of a magnet.

The TEM analysis confirmed the particle size distribution between 8-20nm in size with the highest particle distribution between 10-12nm. The particles did show varying degrees of clustering and aggregation as the sonication treatment did not fully overcome the strong magnetostatic interactions between nanoparticles in the absence of a stabilizer. Larger agglomerates were also present in many of the encapsulated nanoparticle products but likely due to the frequency of sonication rather than the duration, as sonication treatments above two hours showed no discernable changes in TEM images.

The (S)TEM imaging and its related spectroscopic data (EELS\EDX) acquisitions were performed at the Center of Nanoscale Materials (CNM) at Argonne National Laboratory by collaborators Dr. Yasuo Ito and Mason Hayward. Bright-field TEM images of the iron oxide nanoparticles showed with FTT (**Figure 3.11** and **Figure 3.12**), and inverse FTT filters (**Figure 3.13**) showed crystallinity and consistency between nanoparticles. This implies that iron oxide nanoparticles are nanocrystallines. Additional images with the HAADF detector (**Figure 3.14**) were taken and offered its atomic number dependencies into the iron oxide analysis.
Figure 3.10 TEM images of the iron oxide nanoparticles [Hitachi H-600 TEM].

Figure 3.11 Bright-field TEM of the iron oxide nanoparticles [FEI Talos].
Figure 3.12 Bright-field TEM image of the iron oxide nanoparticles with FFT [FEI Talos].

Figure 3.13 The inverse FFT images of the iron oxide nanoparticles [FEI Talos].
In Figure 3.15, the EDX analysis of the IOPs showed that the sample was composed of 70.2% iron (Fe) and 24.8% oxygen (O) by weight. The presence of palladium (Pd) is due to the sputtering of the EDX, leaving only trace impurities of silicon. The silicon contamination is probably a result of the transfer of glass impurities from the glass flask, either from silicon residue from previous encapsulation reactions or traces of glass impurities transferred when scraping the sides of the glass flask with a metal spatula. The atomic percentage of iron to oxygen ratio is appropriate for magnetite but is not conclusive as a stand-alone test. The tall iron peak at 6.398 keV correlates to the Kα peak, and the small iron peak to the right of that peak is Kβ peak. The Fe peak close to 0.705 keV corresponds to the Lα peak at 0.705 keV. The O peak at 0.525 keV aligns with the oxygen Kα peak.
Figure 3.15 EDX data and spectrum for iron-oxide nanoparticles  [JEOL JSM-5610LV].

The EELS analysis (Figure 3.16) of the F-L edges and L$_3$/L$_2$ intensity ratio was in strong agreement with literature values for magnetite and within the margin of the standard error of measurement. The relative L$_3$ and L$_2$ edges across the EELS spectrum showed relative consistency throughout the iron oxide nanoparticles sample. Additionally, when looking at the oxygen K-edge (Figure 3.17), intense peaks at 530 and 540 energy loss is observed, with strong similarities to peaks found in the literature for magnetite. The signal for the K-edge of the iron is high, and an improved signal-to-noise ratio is required to determine the valence structure from the K-edge of the oxygen. However, with all the supporting characterization data, it is strongly supported that the product is magnetite.
**Figure 3.16** EELS spectrum of Fe L-edges and $L_3/L_2$ intensity ratio of iron oxide nanoparticles.\(^{16}\)

<table>
<thead>
<tr>
<th>Iron L-edge Spectra</th>
<th>$L_3/L_2$ Intensity Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.7</td>
</tr>
<tr>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>5.3</td>
</tr>
<tr>
<td>5</td>
<td>4.8</td>
</tr>
<tr>
<td>6</td>
<td>5.1</td>
</tr>
<tr>
<td>7</td>
<td>5.4</td>
</tr>
<tr>
<td>8</td>
<td>5.0</td>
</tr>
<tr>
<td>9</td>
<td>4.9</td>
</tr>
<tr>
<td>Average</td>
<td>5.1 $\pm$ 0.2</td>
</tr>
<tr>
<td>Literature</td>
<td>5.00 $\pm$ 0.09</td>
</tr>
</tbody>
</table>

**Figure 3.17** EELS spectrum of O K-edge of iron oxide nanoparticles.\(^{16}\)
Figure 3.18 The TEM images of IOPs encapsulated with TEOS.
Figure 3.19 The TEM images of IOPs encapsulated with PEG.
The TEM images of the encapsulated IOPs with TEOS (Figure 3.18) and PEG (Figure 3.19) displayed a contrast in particle size and aggregation relative to the bare IOPs in Figure 3.10. Unfortunately, it is difficult to resolve the encapsulation layer and iron oxide core in the absence of HR-TEM images. The TEOS-Fe$_3$O$_4$ particle size distribution range was between 50-110nm, and the PEG-Fe$_3$O$_4$ particles were too aggregated to resolve an accurate particle size distribution, but several isolated particles measured between the size of 12-19nm. An FTIR analysis was taken on the encapsulated products to check for the presence of the encapsulating additive functional groups. As seen in Figure 3.20 and Figure 3.21, both FTIR spectra for PEG-Fe$_3$O$_4$ and TEOS-Fe$_3$O$_4$ shell cores showed additional peaks not present in the Fe$_3$O$_4$ core FTIR spectra. The TEOS-Fe$_3$O$_4$ particles displayed much stronger peaks potentially as a function relative to the thickness of the encapsulation layer. Bond stretching was assigned based on the location of the peaks and compared to the literature values found in the Handbook of Chemistry and Physics (91st edition) and Silicon Compounds: Silanes and Silicones.$^{17,18}$ The FTIR peaks of both TEOS-Fe$_3$O$_4$ and PEG-Fe$_3$O$_4$ are in agreement with literature and supports the encapsulation of the IOPs with their respective encapsulating agent. The FTIR spectrum cannot conclusively prove that the encapsulating agent is chemically bonded to the IOPs. However, in collaboration with the TEM images, it does suggest that the IOPs were encapsulated and that the reactions were successful.
Figure 3.20 The FTIR spectra of IOPs before and after encapsulation with PEG.
Figure 3.21 The FTIR spectra of IOPs before and after encapsulation with TEOS.
The SAXS data analysis showed no sharp, intense peaks that would be typical of monodispersed spheres or single-core iron oxide nanoparticles, as shown in Figure 3.22. All analysis to this point has supported that the nanoparticles were uniform and spherical, but clustered into multicore particles. The reason for the lack of definitive peaks is likely due to aggregation and agglomeration from the strong magnetostatic interactions between nanoparticles that were observed in the original TEM images with the presence of clusters (Figure 3.14). The low quality of the SAXS data makes it unsuitable for estimating the particle size or analyzing
with pair distance distribution function. However, there is still some pertinent information that can be acquired with a couple of plots. The blue line is the actual sample of bare IOPs in solution, and the red line is the solvent background (Figure 3.23); the green line is the data determined after subtraction of the background (Figure 3.24). The first plot is the Guinier plot, which graphs the log I(s) vs. $s^2$, and the exponential decay curve is suggestive of one or more of the following: aggregation, polydispersity, and improper background subtraction. Figure 3.25 is called a Kranky plot; the curve is distinctive to a compact sample, folding, or globular structures in that region. In the case of nanoparticles, the interruption would suggest a high structure of crystallinity, which is supported by TEM with FFT. Unfortunately, this is the limit of the SAXS analysis from this data set, but the possibility of stabilization/encapsulation may improve the quality of data for a more comprehensive analysis of the structure of the nanoparticles.

Figure 3.23 The SAXS data for iron oxide core nanoparticles.
Figure 3.24 The Guinier plot for iron oxide core nanoparticles.

Figure 3.25 The Kranky plot for iron oxide core nanoparticles.
The NMR analysis of the encapsulation of MNPs with TEOS and PEG did not demand further analysis; no peaks were observed except for the D$_2$O solvent peak ($\delta$=4.80ppm), which is observed in the $^1$H-NMR spectrum in Figure 3.26. This suggests either the encapsulating agent did not solubilize in the D$_2$O or the concentration of the TEOS, and PEG was below the limits of detection for the sensitivity of the instrument.

Figure 3.26 The $^1$H-NMR and $^{13}$C-NMR data for iron oxide nanoparticles.
The Mössbauer data collection was performed at Northern Illinois University by collaborators Dr. Dennis Brown and Mason Hayward. For data collection, the Mössbauer spectrometer was calibrated using an iron foil, shown in Figure 3.27, to perform the analysis on the iron oxide nanoparticles. The radioactive form of $^{57}\text{Co}$ isotope was utilized as the source to excite the $^{57}\text{Fe}$ atoms for the data collection. The cobalt isotope was placed on the drive of the instrument, operating at 11mm/s to generate the Doppler effect needed for resonant absorption. The data collected was accumulated over seven days, with a count rate of 1,387 counts per second.

Iron oxide nanoparticles sample preparation for Mössbauer spectroscopy analysis:
19.3mg of the iron oxide nanoparticles were added to 53.8mg of ground carbon and mixed to make a uniform blend. The Mössbauer analysis used a three-site fit, with the MS spectra (Figure 3.28) representing iron at different sites and valence states. The red line correlated to the fitting of the Fe$^{3+}$ oxidation site with a magnetic field strength of 46.9T and an isomer shift of 0.34. The green line was fitted to the Fe$^{3+}$ in the paramagnetic state (nonmagnetic) with an isomer shift of 0.32. The blue correlated with Fe$^{3+/2}$ oxidation sites with a magnetic strength of 39.7T and an isomer shift of 0.37. The fitting of the blue line validated the presence of both divalent and trivalent iron sites, and the unequal magnitude of magnetic strength between the Fe sites is supportive that the sample is ferrimagnetic and with structural features comparable to magnetite.
Figure 3.27 The Mössbauer $^{57}$Fe calibration data from iron foil.\textsuperscript{16}

The calibration data points are shown in the spectra as the white circles, and the calibration fit is displayed as a red line.

Figure 3.28 The Mössbauer spectra of iron oxide nanoparticles.\textsuperscript{16}
Additionally, the broadening of the signal peaks shows superparamagnetic relaxation effects that are typical of nanoparticle sizes of 20nm or less.\textsuperscript{16} A second sample of iron oxide nanoparticles encapsulated in TEOS was tested for a comparison study (\textbf{Figure 3.29}). The Mössbauer spectrum collapsed to a quadrupole doublet, which has two possible interpretations. The first interpretation is that the magnetic relaxation of particles reached sizes below 5nm, which destroyed the magnetic long-range signal from the magnetic relaxation between particles. The second possible interpretation is the magnetic field between particles fell off as $1/r$, meaning the interaction of the nanoparticles became weaker as the MNPs became further separated from their neighboring nanoparticle. This would be indicative that the shell separated the nanoparticles away from each other to such a degree that the signal reduced to a quadrupole doublet.

\textbf{Figure 3.29} EDX data and spectrum for iron oxide nanoparticles encapsulated in TEOS.\textsuperscript{16}
3.8 Conclusions

Superparamagnetic magnetite nanoparticles were synthesized with a particle size distribution of 8-20nm. A variety of analytical techniques were used to characterize the IOPs, TEOS-Fe$_3$O$_4$, and PEG-Fe$_3$O$_4$. The IOPs showed properties of ferrimagnetism and superparamagnetism based on the Mössbauer analysis. The characterization with MS, EELS, and XRD is highly suggestive that the iron oxide is magnetite (Fe$_3$O$_4$) with both divalent and trivalent cation iron sites present in the nanomaterial. The only drawback is the arrangement of clusters and aggregates, which can influence the overall magnetic saturation properties of the SPIONs. Further characterization and testing of the encapsulated material in a systematic way can bring new insight into the interaction of the encapsulation additive with the iron oxide core.

Further functionalization of IOPs with a biomolecule or boron-based bioconjugate is required, and characterization of the nanocomposite will require additional characterization. This is a step to realizing the goal of magnetic-guided drugs, but there is still a long road to travel after connecting the bioconjugate to the MNPs; toxicological assays, biodistribution studies, and other tests are required to continue making progress on the path to magnetic-guided bioconjugates becoming a realization for medical applications.
3.9 References


CHAPTER 4: SYNTHESIS AND ENCAPSULATION OF BORON NANOPARTICLES

4.1 Synthesis Methods

Boron nanoparticles (BNPs) have been synthesized with a variety of techniques: pyrolysis, CVD, thermal plasma, arc discharge, ball milling, and liquid phase growth. In this research, the physical vapor deposition of nido-decaborane by pyrolysis was utilized to synthesize the BNPs. The encapsulation of BNPs utilized amorphous boron cores with PEG, TEOS, or PEI as shells. This was followed by a subsequent reaction with FTIC-APTES, which will be used to connect the biomolecule to the BNPs in the future.

4.2 Boron

Boron is the 5th element in the Periodic Table, positioned in the second period and thirteenth group with an atomic number of five. It has three valence electrons and an electron configuration of [He] 2s^22p^1. In its amorphous state, boron is dark brown, and in its crystalline form is silvery to black. Boron gives a green flare in flame tests. The element boron was discovered days apart by independent research groups. On June 21st, 1808, Louis Joseph, Gay-Lussac, and Louis Jacques Thenard announced the discovery of a new element they called “bore.” The French chemist group obtained boron by reducing boric acid with potassium. On June 30th, 1808, Sir Humphrey Davis submitted an article to the Royal Society of London on the discovery of the new element he called boracium. Davy prepared boron by electrolysis, but both groups’ synthesis compounds were no more than 50% pure. In 1909, Ezekiel Weintraub
reduced boron halides with hydrogen and noted that his product was 99%, i.e., pure boron. However, it is debated if his product was pure, as pure boron polymorphs started to appear in literature in the late 1950s.\textsuperscript{9,10}

Boron and gadolinium are unique elements in that they are capable of capturing a neutron, due to their large cross-sections, and are the only two elements suitable for neutron capture therapy.\textsuperscript{11} Boron is further unique due to its electron-deficient bonding, making it sensitive to small concentrations of impurities and reactive with elements with varying properties. Boron is the only element where all the known polymorphs are superhard and have distinct crystal structures.\textsuperscript{10} Its crystalline form is the second hardest on the Mohs scale with a melting point of 2075°C.\textsuperscript{10} Boron has two naturally occurring isotope, \textsuperscript{10}B and \textsuperscript{11}B, with \textsuperscript{10}B making up 19.9% and \textsuperscript{11}B making up 80.1% of all boron. Thirteen radioisotopes of boron exist from mass numbers of 7 to 21, but the radioisotopes of boron are extremely unstable with a half-life of decay usually in the order of milliseconds or below.\textsuperscript{12}

4.3 Applications\textsuperscript{13}

There are many applications for boron nanoparticles outside of biomedical applications, as previously discussed in Chapter 1. Some potential applications for BNPs are heat shields, biocides, ignition aids, corrosion inhibition, photoelectric chemistry, electromagnetic radiation shielding, and regulating nuclear reactors' energy levels.\textsuperscript{13} The ability to mass synthesize boron nanoparticles systematically has many potential applications outside of the medical field.
4.4 Materials and Methods

4.4.1 Materials

Equipment and chemicals: spatula, weigh paper, pestle, mortar, 150mL sublimation apparatus, two-neck round-bottom flask, a quartz tube, a compressed tank of argon gas, Innovative Technology Inc. LM-100 glove box, Mettler BB244 precision balance, vacuum pump, Lindberg 550X tube furnace, OTF-1200X MTI furnace, Z366 Hermle high-performance centrifuge, Fischer Scientific Q125 sonicator, Tescan Vega II SBH SEM, JEOL JSM-5610LV, Hitachi H-600 TEM, and Bruker 300mHz NMR.

4.4.2 Methods

Purification of Nido-Decaborane (B\textsubscript{10}H\textsubscript{14}) Procedure:

Sublimation of nido-decaborane was performed for four hours to purify decaborane for boron nanoparticle synthesis. A vial was weighed with the cap on a Mettler Toledo AG104 analytical scale, and the scale was then tared. The weight of the vial was recorded as 11.2805 grams; unpurified decaborane was transferred to the vial with a clean metal spatula. The cover of the vial was secured and reweighed on the scale showing 5.1468 grams of decaborane was added to the vial. The decaborane was transferred to the fume hood to a clean unassembled sublimation vessel. The vessel was assembled with silicone grease to seal the vessel. Dry ice and a 2-propanol bath were prepared for cooling the top of the sublimation apparatus. The sublimation vessel was connected to a vacuum line and the pump turned on, as displayed in the left image of Figure 4.1. The sublimation vessel with the decaborane was then heated with a mineral oil bath at 70°C to sublime the decaborane. After four hours, the vessel was disassembled; and the purified decaborane was collected from the finger and placed into a vial; as shown in the right image of Figure 4.1. The fume hood, vessel, glassware, and spatula were all cleaned with 2-
isopropanol. The total weight of purified product after removal of tare weight was 4.6142 grams, which is an 89.6518% yield of purified product. A $^1$H-NMR analysis was used to verify the purity of the sublimed nido-decaborane and was comparable to the literature results of Pérez et al.\textsuperscript{14}

![Figure 4.1 Purification of nido-decaborane (B$_{10}$H$_{14}$).](image)

**Boron Nanoparticle Core Synthesis Procedure:**

**Reaction:** B$_{10}$H$_{14}$(s) $\rightarrow$ 10B(s) + 7H$_2$(s)

A gas-phase pyrolysis reaction was performed on the sublimed nido-decaborane using a Lindberg L550X furnace under a fume hood (**Figure 4.2**). The pyrolysis apparatus was assembled and the connection points of the glass joints lined with silicon grease, with the apparatus ending in a water trap. The 0.7g sample of sublimed decaborane (5.73mmol) was transferred to a two-neck round-bottom flask with one neck of the flask fitted with a glass joint.
with a PTFE stopcock. This stopcock joint connected the flask to the argon carrier gas line. The other neck of the flask connected to a 90° elbow joint, which connected the flask to the quartz tube placed inside a horizontal tube furnace insulated with high-temperature glass wool. At the other end of the quartz tube was the collection tube that connected to a gas bubbler and water trap. The function of the water trap was to capture any stray nanoparticles carried past the collection tube with the carrier gas. The round-bottom flask and the quartz tube were purged with dry argon gas for 15 minutes, and the furnace was brought up to 700°C, with an argon flow of 0.22 L/min. The mineral oil bath holding the round-bottom flask containing the sample of sublimed decaborane was brought up gradually to 110°C and the reaction allowed time to progress. The decaborane was decomposed over six hours while passing through the quartz tube and the product deposited at the cooler end of the collection tube. The shutdown procedure involved closing the stopcock, shutting off the argon gas flow, powering down the furnace, and allowing the furnace and glassware to cool to room temperature. The deposited dark brown boron powder was collected in a vial, and 160-300 mg of boron nanoparticles (23.3-43.5% yield) was collected per reaction. The product was attached to a vacuum line overnight to remove any residual moisture during the collection of the product and exposure to air.
Figure 4.2 Experimental setup for the pyrolysis of nido-decaborane (B_{10}H_{14}) to synthesis BNPs.

4.5 Encapsulation of the Boron Core

Procedure: In a 250mL three-neck round-bottom flask, anhydrous ethanol, ammonium hydroxide, and amorphous boron powder were combined into a mixture and sonicated for 30 minutes at 40% amplitude. A magnetic stir bar was added to the flask and a glass joint with a Teflon stopcock attached to the flask. The two other neck openings were sealed with a glass stopper and a rubber septum. The tetraethyl orthosilicate (TEOS/PEG/PEI) was added by removing the glass joint. The flask was resealed and attached to the Schlenk line. The gas inside the flask was evacuated with a vacuum pump and the stopcock closed. The one-pot synthesis was
then magnetically stirred for 18 hours under vacuum. Fifty microliters of a (3-
aminopropyl)triethoxysilane and fluorescein isothiocyanate (APTES-FTIC) solution was
pipetted into the one-pot solution and magnetically stirred for an additional 12 hours. Samples of
the solutions were taken before and after the addition of APTES-FTIC.

4.5.1 Reaction with TEOS

![Reaction scheme of BNP with TEOS](image)

**Figure 4.3** Reaction scheme of BNP with TEOS.¹⁵

<table>
<thead>
<tr>
<th>Table 4.1 The Reactant Quantities for BNPs Encapsulation with TEOS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reactant</strong></td>
</tr>
<tr>
<td>Ammonium Hydroxide</td>
</tr>
<tr>
<td>Anhydrous ethanol</td>
</tr>
<tr>
<td>BNPs</td>
</tr>
<tr>
<td>TEOS</td>
</tr>
</tbody>
</table>
4.5.2 Reaction with PEG

![Reaction scheme of BNP with PEG](image)

**Figure 4.4** Reaction scheme of BNP with PEG.\textsuperscript{16}

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Grams</th>
<th>Moles</th>
<th>Molar Mass</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Hydroxide</td>
<td>1.00mL</td>
<td>2.56E-2 mol</td>
<td>35.046 g/mol</td>
<td>0.9 g/cm\textsuperscript{3}</td>
</tr>
<tr>
<td>Anhydrous ethanol</td>
<td>62.5mL</td>
<td>1.07 mol</td>
<td>46.07 g/cm\textsuperscript{3}</td>
<td>0.789 g/cm\textsuperscript{3}</td>
</tr>
<tr>
<td>BNPs</td>
<td>0.05g</td>
<td>4.71E-3 mol</td>
<td>10.81 g/cm\textsuperscript{3}</td>
<td>2.34 g/cm\textsuperscript{3}</td>
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<tr>
<td>PEG</td>
<td>0.058g</td>
<td>-</td>
<td>-</td>
<td>1.2 g/cm\textsuperscript{3}</td>
</tr>
</tbody>
</table>

**Table 4.2** The Reactant Quantities for BNPs Encapsulation with PEG
4.5.3 Reaction with PEI

Table 4.3 The Reactant Quantities for BNPs Encapsulation with PEI

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Grams</th>
<th>Moles</th>
<th>Molar Mass</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Hydroxide</td>
<td>1.00mL</td>
<td>2.56E-2</td>
<td>35.046 g/mol</td>
<td>0.9 g/cm³</td>
</tr>
<tr>
<td>Anhydrous ethanol</td>
<td>62.5mL</td>
<td>1.07 mol</td>
<td>46.07 g/cm³</td>
<td>0.789 g/cm³</td>
</tr>
<tr>
<td>BNPs</td>
<td>0.051g</td>
<td>4.71E-3</td>
<td>10.81 g/cm³</td>
<td>2.34 g/cm³</td>
</tr>
<tr>
<td>PEI</td>
<td>0.10g</td>
<td>2.3E-3</td>
<td>43.069 g/mol</td>
<td>0.8321 g/cm³</td>
</tr>
</tbody>
</table>

4.5.4 The FTIC-APTES Solution Preparation

This FTIC-APTES solution was prepared in a glove box under argon gas with the reactant quantities 1.36 grams of APTES and 0.105 grams of FTIC dissolved in 20.0mL of anhydrous ethanol in a fresh 50 mL round-bottom flask and secured over a magnetic hot plate to stir for 12 hours magnetically.
4.6 Results and Discussion

The characterization of BNP through TEM showed a particle size distribution between 17-48nm with aggregation and branch-like agglomerates. Sonication did not help to overcome the interparticle forces holding the BNPs together. The boron was a light brown when the furnace was hot in the collection tube but was dark brown when cooled to room temperature. This is suggestive that the boron is amorphous, as crystalline boron is silvery black in color.

The SEM analysis (Figure 4.7) of the BNPs product was problematic as the nanoparticles were too small to get a clear resolution with the electron microscope. It does show some character of morphology and the surface appears porous, which is indicative of amorphous boron, rather than a highly crystallized and orderly structure. The EDX analysis of the BNPs showed that the sample was composed of 58.77% boron (B), 18.66% carbon (C), and 22.58% oxygen (O) by weight. The quantification of the carbon and oxygen is highly suspect as EDX is notorious for the abysmal accuracy of carbon and oxygen due to the carbon film and water present in the film. In the EDX data, the carbon peak counts on the surface of the material. The only way to verify the presence of carbon and oxygen in the sample would be with X-ray photoelectron spectroscopy (XPS). All the carbon counts were found in one location, localizing it to one specific measurement point on the surface of the boron sample. Boron and oxygen were found in all four locations. Regarding that nido-decaborane has no carbons and was sublimed before pyrolysis and characterized with no carbon in $^{13}$C-NMR, it appears less likely for the source of the carbon contamination to originate from anywhere but the EDX preparation. The intense boron peak at 0.183 keV correlates to the $K_a$ peak, and the small carbon peak to the right at 0.277 keV corresponds to the $K_a$ peak. The O peak at 0.525 keV aligns with the oxygen $K_a$ peak. The gold peak (Au) is a trace contaminate from the sputtered atoms of the EDS.
Figure 4.6 The TEM images of boron nanoparticles core.
Figure 4.7 The SEM images of BNPs [Tescan Vega II SBH].
Figure 4.8 EDX data and spectrum for boron nanoparticles [JEOL JSM-5610LV].

were much lower than the oxygen, with the overall counts obtained from four locations

Next, an NMR analysis of the boron nanoparticles was performed to check for
decaborane contamination from the incomplete pyrolysis of the reactant. As observed in Figure 4.9, the $^1$H-NMR spectra, there were two hydrogen peaks, a sharp solvent peak of CDCl$_3$ at 7.24 ppm and a weak signal at 1.53 ppm, which potential is a water contaminate from the CDCl$_3$ solvent. Also, $^{11}$B-NMR spectra showed no boron peaks, which are generally observed for BNPs.

Only the BNP-PEI core/shell nanocomposite of the three reactions maintained the ideal particle size for further functionalization. The BNP-PEI core/shell was highly aggregated but did
not have the microscale globular appearance as the TEOS-BNP and PEG-BNP core/shell in the TEM images. The FTIC-APTES addition after encapsulation did not aid in the dispersion or stabilization of the core/shell.

Figure 4.9 The NMR spectra for BNPs in CDCl$_3$. The $^1$H-NMR showed a solvent peak of $\delta=7.24$ ppm and a possible water peak at $\delta=1.53$ ppm. The $^{11}$B-NMR showed no boron peaks, which is typical for BNPs.
Figure 4.10 The TEM images of BNP-TEOS before and after the Addition of APTES-FTIC. The TEM images of the BNPs encapsulated in TEOS were over 500nm and were too large to be considered nanoparticles.
Figure 4.11 The TEM images of BNP-PEG before and after the addition of APTES-FTIC. The TEM images of the BNPs encapsulated in PEG were over 500nm and were too large to be considered nanoparticles.
The TEM images of the BNPs encapsulated in PEI were under 100nm in size but highly aggregated with poor image resolution.

**Figure 4.12** The TEM images of BNP-PEI before and after the addition of APTES-FTIC. The TEM images of the BNPs encapsulated in PEI were under 100nm in size but highly aggregated with poor image resolution.
4.7 Conclusion

Boron nanoparticles were synthesized with particle sizes ranging between 17-48nm. The encapsulation with PEG and TEOS was not on the nanoscale, and the reactions need additional improvements to narrow the size of the encapsulated nanomaterial below 100nm. The BNP-PEI was the only encapsulation that had the correct particle size but was more aggregated than encapsulated MNPs. The $^1$H-NMR showed a solvent contaminant at 1.53ppm for the BNPs, which is likely water from either the NMR solvent or the BNPs. The EDX analysis, in addition to boron, carbon, and oxygen elements, remains inconclusive without XPS to verify if the BNP is a mixture or if the carbon and oxygen is from the carbon film. The SEM images supported that the BNPs were amorphous, which was also indicative of the dark brown color of the product.
4.8 References


CHAPTER 5: SYNTHESIS OF BORON NITRIDE NANOTUBES

5.1 Synthesis Methods

BNNTs can be synthesized using different methods, such as arc pyrolysis, discharge, substitution reactions, chemical vapor depositions, ball milling, laser ablation, and low-temperature synthesis methods.\textsuperscript{1-7} In this project, the BNNTs were synthesized using furnace pyrolysis in a one-pot reactor with sodium borohydride (NaBH\textsubscript{4}), ammonium tetrafluoroborate (NH\textsubscript{4}BF\textsubscript{4}), zinc powder (Zn), and sodium azide (NaN\textsubscript{3}) at 700°C. Most synthesis methods for the BNNTs range between temperature profiles of 1100-1700°C,\textsuperscript{1-7} regardless of the methodology, boron precursor, catalyst, mode of heat, or the duration of the reaction.

5.2 Toxicity

BNNTs have been proposed for biomedical applications due to superior mechanic, chemical, and electrical properties compared to their carbon analogs.\textsuperscript{8-10} Unfortunately, the initial cell interaction, higher chemical stability, and poor dispersion limited the concentration of BNNTs to below 20 μg/mL, although the BNNTs were stabilized with highly positive charge groups like poly-L-lysine and polyethyleneimine to improve dispersion in blood.\textsuperscript{11} The problem stemmed from utilizing tetrazolium salts that are generally used in standard cytotoxicity tests to evaluate cell proliferation and metabolism in toxicological tests. BNNT was able to interact with the (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide), which created a false negative result.\textsuperscript{12} It was observed that by utilizing a different metabolic salt (WST-1, based on 2-
(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) and a glycol-chitosan polymer that concentrations of BNNTs can reach past 100μg/mL with no significant difference from the control group, proving that BNNT has low cytotoxicity and high biocompatibility when functionalized and tested with a metabolic assay that does not interact with the BNNTs.13

5.3 Drug Delivery

BNNTs have been explored as possible drug vectors and carriers for boron atoms for neutron capture therapy, which remains a hopeful treatment to target aggressive and inoperable cancer types such as cerebral glioblastoma multiform. There have been at least three research projects investigating the drug delivery potential of BNNTs that suggest BNNTs are as suitable for drug targeting. The first study by Ciofani et al. synthesized a multifunctional BNNT-based nanocarrier with an iron catalyst and coated with PEI and quantum dots.14 The use of an iron catalyst resulted in impurities in the BNNT, revealing magnetic properties that are considered ideal for drug delivery systems.14 In the second study by the same research group (Ciofani et al.), a core/shell structure of BNNTs with sodium gadolinium fluoride was doped with europium. The BNNT-NaGdF₄ composite maintained both magnetic and fluorescent properties.15 The group further tested the composite in the human prostate cancer line LNCaP with the absence and presence of an external magnetic field. The conclusion is that the uptake of the drug was increased in the presence of the magnetic field, which has the potential to increase the chemotherapy efficiency of magnetically guiding drugs.15

The last study, by the Li et al. research group, utilized a negative surface charge BNNTs functionalized with mesoporous silica (BNNT-MS).16 Also, the group prepared a positively
charged surface-area BNNT by appending an amine group to the BNNT-MS composite to create a new BNNT-MS-NH₃ composite material. The structure was then loaded with doxorubicin (dox), which loading was independent of the surface charge. The biodistribution studies showed that the BNNT-MS-NH₃ had a higher cellular uptake in the LNCaP prostate cancer cell line due to the preference of the positive surface charge, resulting in the conclusion that the ability to functionalize the surface charge of BNNTs will be ideal for targeting specific cancer cell lines and has high potential for cancer therapy. All of the studies show strong support that BNNTs can be ideal for nanovectors for drug delivery and, if further catalyzed or incorporated with a trace metal impurity, can be further magnetically guided in the presence of an external magnetic field.

5.4 Materials and Methods

5.4.1 Materials

5.4.1.1 Chemicals

All chemicals used in this reaction were obtained from Sigma-Aldrich and used without further purification: sodium borohydride (NaBH₄), ammonium tetrafluoroborate (NH₄BF₄), zinc powder (Zn), and sodium azide (NaN₃).

5.4.1.2 Equipment

Equipment and chemicals: spatula, 100mm x 100mm Fischer Scientific weigh paper, pestle, mortar, a compressed tank of argon/nitrogen gas, Innovative Technology Inc. LM-100 glove box, Mettler BB244 precision balance, stainless-steel reaction vessel, screws, nuts, wrench, OTF-1200X MTI furnace, and Z366 Hermle high-performance centrifuge.
5.5 Synthesis of Boron Nitride Nanotubes

Reaction:

$$2\text{Li(BH}_4\text{s}) + 2\text{NH}_4\text{BF}_4(s) + 8\text{NaN}_3(s) \rightarrow 2[(\text{BN})_x\text{-Li-(BN)}_x\text{]}(s) + x\text{N}_2(g) + x\text{H}_2(g) + 8\text{NaF(s)}$$

Table 5.1 The Reactant Quantities for BNNT Synthesis

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Quantity</th>
<th>Moles</th>
<th>Molar Mass</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaBH$_4$</td>
<td>0.284 g</td>
<td>7.507E-3 mol</td>
<td>37.83 g/mol</td>
<td>1.07 g/cm$^3$</td>
</tr>
<tr>
<td>NH$_4$BF$_4$</td>
<td>0.787 g</td>
<td>7.506E-3 mol</td>
<td>104.85 g/mol</td>
<td>1.871 g/cm$^3$</td>
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<tr>
<td>Zn</td>
<td>0.504 g</td>
<td>7.708E-3 mol</td>
<td>65.38 g/mol</td>
<td>7.133 g/cm$^3$</td>
</tr>
<tr>
<td>Sodium Azide (NaN$_3$)</td>
<td>1.972 g</td>
<td>3.033E-2 mol</td>
<td>65.01 g/mol</td>
<td>1.85 g/cm$^3$</td>
</tr>
</tbody>
</table>

All reactants and equipment were transferred into the glove box. In the glove box, under a nitrogen atmosphere, the reactant ingredients were weighed using a Mettler BB244 precision balance. The reactants were then moved to a mortar and finely ground with a pestle. The pulverized mixture of reactants was placed into a stainless-steel reactor with a graphite gasket. The reactor vessel was sealed and tightened with screws, nuts, and two wrenches while in the glove box. Then the vessel was transferred outside of the glove box to finish tightening the screws and nuts with two wrenches.

The vessel was first carefully placed within the apparatus, as shown in Figure 5.1, and the line was sealed using the middle bolt to ensure no leaks could occur. The vessel was then tightly secured with the use of two red screws. Once the vessel was secured, the vacuum pump was turned on, and the vacuum pulled for five minutes. The valves to the Schlenk line were opened, and the fittings connecting the apparatus to the Schlenk line were drawn open one valve
at a time from left to right to pull a vacuum through the apparatus. It is important to note that each valve was opened and the vacuum allowed to stabilize before opening the next valve. Once all valves were open and the reactor vessel was under vacuum, all the nitrogen gas was evacuated from the reactor vessel for five minutes. Once the vessel was under vacuum, the valves were closed from right to left (6,5,1), and the middle bolt loosened; and the vessel was disconnected from the apparatus. The reactor vessel was then placed into the OTF-1200X MTI furnace and run under a preset program, as shown in Table 5.2.

Figure 5.1 Procedure for evacuating the nitrogen gas from the vessel.
After the reaction, the furnace was turned off and allowed to cool to room temperature. The reaction vessel was collected and transferred back into the glove box after loosening the screws. The ash-colored BNNT crude product was collected from the reactor vessel. The crude product was weighed and transferred into a glass jar with the lid secured in the glove box. The weight was recorded and the jar labeled with the date of synthesis and ID number. A vial filled with ethanol was transferred to the glove box, and a small portion of the crude was placed into the vial of ethanol for TEM analysis and the lid secured. The vial was transferred outside of the glove box and sonicated for 30 minutes; a few drops of the sonicated solution were transferred onto a TEM grid. The purpose of the TEM sample was to check the crude for boron nitride nanotubes before investing in further processing, purification, and functionalization of the BNNT crude.
5.6 Purification of the Boron Nitride Nanotubes

Three hundred milligrams of ground BNNT crude product was weighed and transferred to a fresh 50 mL pear-shaped flask. The product was washed three times with a 1:3 solution of trifluoroacetic acid (C₂HF₃O₂) to deionized water. The product was agitated in solution by vigorously shaking the flask for a minute and allowing the precipitation of the solid to the bottom of the flask. The remaining whitish supernatant was decanted and collected in a fresh pear-shaped flask. This process was repeated three times on the crude BNNT product flask to extract the insoluble boron nitride nanomaterial contained within the whitish supernatant. The extracted product was then washed with a 1:1 solution of ethanol and deionized water. The extracted product was agitated and allowed to precipitate to the bottom and supernatant discarded. This process was repeated for 3 to 5 washes or until the supernatant was clear in appearance. Substituting the solution for anhydrous ethanol in the final wash, the purified product dried on a Schlenk line with a liquid nitrogen cooling the cold trap. The resulting product was white, as observed in the right image of Figure 5.2.

![Extraction with TFA](image1)

![Final Wash](image2)

**Figure 5.2** Images of the processing of the crude BNNT with the TFA acid wash (left) and a photo taken during the final wash (right).
5.7 The Separation of BNNT-Purified Crude with Centrifugation and Sucrose Gradient

Four 50 mL sucrose gradients were prepared with the following concentrations: 10%, 20%, 30%, and 40%. The sucrose gradients were prepared by diluting a 40% sucrose stock solution with deionized water. The volumes of sucrose stock solution and deionized water were calculated by using the dilution equation \( c_1v_1 = c_2v_2 \). After the preparation of the sucrose gradients, the gradients were utilized with a centrifuge at 5000 and 16000 RPMs to see if differences in density could separate the purified product. Low-speed centrifuge experiments were performed on each sucrose gradient in the biology building in collaboration with Dr. Scott Grayburn. Each sample utilized 100 microliters of purified solution placed on top of 1mL of the sucrose gradient with a pipette. After the centrifugation, the upper supernatant layer was collected with a pipette for each of the sixteen samples and transferred to a 2mL disposable plastic vial, as shown in **Figure 5.3**.

![Figure 5.3 Images of the 2mL vials after centrifugation.](image-url)
5.8 Results and Discussion

The TEM images that are shown in Figures 5.4 and 5.5 validated the presence of boron nitride nanotubes and boron nitride nanocages within the BNNT crude. Unfortunately, the TEM images also showed the presence of unreacted material and side products, e.g., boron nitride nanoparticles requiring further purification before encapsulating or functionalization of the BNNT. A dilute TFA wash was used to remove the water-soluble reactants and metal from the BNNT crude. Unfortunately, this process did not remove similar chemically natured compounds like boron nitride nanoparticles. In an attempt to separate the BNNT from other products, a series of experiments with sucrose gradients and centrifugation were performed, and the samples are awaiting further characterization by TEM. This methodology is not based on any previous research or methodology and is the first attempt in purifying boron nanomaterials in this way. Previous attempts to purify the BNNT crude with sonication and more concentrated acid wash caused structural damage to the BNNT, which was verified with TEM images (not shown).

5.9 Conclusions

BNNTs were synthesized using the furnace pyrolysis in a one-pot reactor with sodium borohydride (NaBH₄), ammonium tetrafluoroborate (NH₄BF₄), zinc powder (Zn), and sodium azide (NaN₃) at 700°C. The shape, size, and order of the BNNTs can vary highly, even within the same sample. The addition of boron nitride nanocages and nanoparticles complicates the purification process as an acid wash can remove the water-soluble reactants and metal, but not the boron nitride side products. Purification is required before additional characterization methods can be utilized effectively to find the properties of these nanotubes.
Figure 5.4 The TEM images of the synthesized boron nitride nanotubes.
Figure 5.5 A TEM image of a boron nitride nanocage.
5.10 References


CHAPTER 6: CONCLUSION AND FUTURE WORK

6.1 Summary and Future Work

The synthesized bare iron oxide nanoparticles were evaluated with TEM, STEM, and SAXS, which confirmed the particle size distribution between 8-20nm. The EELS, XRD, and Mössbauer analyses confirmed the presence of both trivalent (Fe$^{+3}$) and divalent (Fe$^{+2}$) cations of iron, ferrimagnetic/superparamagnetic properties, and atomic spacing appropriate for magnetite. The characterization of BNP through TEM shows particle size range between 17 to 48 nanometers with aggregation and branch-like structuring. The source of the 1.53 ppm peak in the $^1$H-NMR boron nanoparticles is inconclusive but is probably a residual trace of water contaminant (1.56ppm) from either the NMR solvent or exposure of the BNPs to air during product collection. The information from the EDX, NMR, SEM, and the color of the product suggests the physical form is amorphous BNPs, which would be more reactive than crystalline boron. The experiment apparatus will need a glass stopcock joint to prevent the exposure of air and water to the boron nanoparticles when disconnecting the collection tube from the furnace.

The encapsulating of iron oxide nanoparticles with PEG and TEOS has been verified, and now further experiments by adjusting the thickness of the encapsulation can give insight into the effects of the encapsulation on the magnetic properties, ideally for optimization of the magnetic
properties of the coating for its application for magnetic-guided drug systems. A more thorough characterization approach with HR-TEM is needed to verify the thickness of the encapsulation layer on the IOPs before the addition of the biomolecules to create a magnetic bioconjugate. Additionally, the BNP encapsulation reactions need to be optimized to achieve desired particle size and dispersion of the nanoparticles before the project can move ahead with additional characterization with HR-TEM. The next step after encapsulation is to attach the carborane biomolecules to the magnetic nanoparticles to make it an authentic bioconjugate product. The bioconjugate will need biodistribution and toxicity studies performed on cancer cell lines to evaluate its effectiveness as a drug.

Furthermore, the methodology of BNNT’s purification needs additional work, either by continuing to work with centrifuge with the sucrose gradient or by another means of purification, such as solution purification with chlorosulfonic acid. The research in this thesis is a reflection of steps on the path of making magnetically guided bioconjugates for medical applications; it is not an easy path but worth the effort once applications are fully realized.
APPENDIX: CARBORANE CHEMISTRY

This appendix is ongoing research for the last step of making boron-based bioconjugates by the functionalization of the nanomaterial with the carborane cage.

Figure A.1 Generic carborane chemistry reaction scheme with R=Me, Ph, and -COOH.
**Boron-Based Bioconjugates Reactions**

Reaction with 1-methyl-o-carborane, n-butyl lithium and chloroacetic acid – rxn 1 product:

Dry dichloromethane (100.0mL) was used to dissolve 0.762 grams of 1-methyl-o-carborane in a 250mL 2-neck round-bottom flask. The solution was cooled with an ice bath and flask purged with argon gas, allowing the argon gas to flow out of the Teflon stopcock glass joint. The other neck had a rubber seal placed in it to be able to inject the n-butyl lithium with a syringe. The syringe was purged with argon gas, and then 2.30mL of 1.8M n-butyllithium in hexane was added slowly by syringe under argon gas flow to control the rate of the reaction. After the addition of n-butyl lithium, 0.5345g of chloroacetic acid was dissolved into the solution and stirred magnetically, removed from the ice bath, argon gas flow turned off, stopcock closed, and the solution could stir overnight. The total amount of product from this reaction was 1.3652 grams.

Rxn 1 Product with Fluorescein Free Acid – 1:1.1 Molar Ratio:

In a 50mL single-neck round-bottom flask, 0.2523mg of RXN 1 product was dissolved in 10 mL of dried methanol. Then 0.34612g of fluorescein free acid and sulfuric acid were added by pipette until the pH was approximately 2. The reaction stirred overnight, and then the acid was neutralized with bicarbonate and filtered (by Jimmy). The resulting product had silica added to it and the solvent removed on a rotovap. The product was then purified via silica column, initially starting with washes of hexane, then a blend of hexane and ethyl ether, and finally with blends of methanol and ethyl ether. Neutralization of the acid destroyed the carborane cage, as NMR analysis showed the carborane cage was not intact.
Rxn 1 Product with Fluorescein Free Acid – 2:1 Molar Ratio:

This was the same procedure as above, except the amount of fluorescein free acid added was modified to 0.17306 g.

N, N’-Dicyclohexylcarbodiimide Reaction:
Chemicals: 0.109g of phenyl carborane -COOH derivative, 20 mL of DCM, 0.407g of fluorescein free acid, and 0.103g of N, N’-dicyclohexylcarbodiimide.

A two-neck 100mL round-bottom flask with phenyl-carborane derivative, fluorescence-free acid, and dry dichloromethane cooled to -20°C with dry ice and 2-propanol bath. The round-bottom flask was flushed with argon gas for five minutes, and the N, N’-dicyclohexylcarbodiimide was added to the solution. The solution was magnetically stirred and allowed to stir overnight while being brought up to room temperature. No carborane cage or any boron peaks were observed in the $^{10}$B-NMR of the isolated, purified product.

Thionyl Chloride Reactions with Carborane Derivatives ($\text{B}_{10}\text{C}_{4}\text{O}_{4}\text{H}_{12}$, $\text{B}_{10}\text{C}_{5}\text{O}_{2}\text{H}_{15}$, and $\text{C}_{10}\text{B}_{10}\text{O}_{2}\text{H}_{17}$):
Chemicals: ~ 200 mg of carborane derivative, 20.0 mL of dry dichloromethane, and 2.00mL of thionyl chloride.

Quantity of the Reactants for the Carborane Derivatives:

$\text{B}_{10}\text{C}_{4}\text{O}_{4}\text{H}_{12}$: 0.2349 grams, $\text{B}_{10}\text{C}_{5}\text{O}_{2}\text{H}_{15}$: 0.2176 grams, and $\text{C}_{10}\text{B}_{10}\text{O}_{2}\text{H}_{17}$: 0.1468 grams.

A fresh three-neck 50 mL round-bottom flask, 20 mL of dry dichloromethane, one of the carborane derivative products listed above, and 2mL of thionyl chloride were added to a one-pot synthesis and allowed to stir for 12 hours with a magnetic stir bar.
Results and Discussion

The $^1$H-NMR and $^{10}$B-NMR analyses support that many of the attempted carborane reactions either failed to attach the fluorescein free acid or destroyed the carborane cage in the process. It is currently unknown if the carborane cage is destroyed during the reaction or when neutralizing the acid. Reactions have been moved to click reactions in attempts to maintain the integrity of the carborane cages while maintaining the fluorescence of the molecule. An alternative path can also attach the fluorescence to the biomolecule first and then react it with the carborane cage. I am in the process of conducting an extensive literature review before moving forward with the carborane project.