MAGNETIC MESOPOROUS SILICA NANOPARTICLES AS POTENTIAL MRI CONTRAST AGENTS

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By

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MAGNETIC MESOPOROUS SILICA NANOPARTICLES AS POTENTIAL MRI CONTRAST AGENTS

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ABSTRACT

Magnetic resonance imaging is a non-invasive diagnostic technique that utilizes native protons in biological samples to produce three-dimensional images. MR imaging relies on the native differences in relaxation time ($T_1$ and $T_2$) and tissue composition (amount of lipids, water, etc. present) in order to generate images. While different tissues have varying $T_1/T_2$, the low sensitivity and distinguishability of MRI, especially when comparing diseased and healthy tissue, has necessitated the use of contrast agents (CAs).

This unique class of compounds can be administered prior to taking an image to increase the minute signal intensity differences between tissues. Contrast agents are particularly useful when trying to differentiate between healthy and diseased tissue, allowing for the visualization of lesions and small tumors not normally seen without the use of such compounds. To date two main CAs are used: gadolinium chelates and iron oxide nanoparticles. Many issues still exist for these CAs including: metal leaching, agglomeration, and metal toxicity.

Previously, the use of metal-oxo clusters grafted into polymer nanobeads, utilizing the miniemulsion technique, has been explored. The encapsulation of these clusters proved to be highly advantageous as not only did they address the issues mentioned previously, as well as many more, the magnetic properties were also vastly improved with encapsulation.
Still, issues exist with this polymer nanobeads system. These nanobeads cannot be redispersed once the colloidal miniemulsion is disturbed. Furthermore the encapsulation of the metal-oxo cluster leads to several disadvantages. Although there is a homogenous metal distribution, only clusters closest to the surface are utilized leading to inefficient metal usage. The complete encapsulation also does not allow for direct metal-proton interaction, a crucial relaxation pathway, thus further limiting the relaxation efficiency of these materials.

The goal of this research is to produce new CAs using silica nanoparticles and metal-oxo clusters to address the issues associated with the polymer nanobeads. The use of mesoporous silica allows for cluster anchoring, preventing leaching, but also exposes the metal to the environment allowing for better metal-proton interactions. This introduction of a new relaxation pathway should improve upon the contrast abilities of the polymer nanobead system.
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Eternally Grateful,

Billy
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Chapter 1: Introduction and Background

1.1 Magnetic Resonance Imaging: History

MRI cannot be discussed without first mentioning nuclear magnetic resonance (NMR), which was first discovered in 1924, when Gerlach and Stern first demonstrated the quantum nature of the magnetic moments of silver atoms.\textsuperscript{1–4} Although the experiment failed, the first reported attempt at observing nuclear spin transitions in solids was reported by Cornelius J. Gorter in 1936. A second attempt by Gorter in 1942 also failed, but is credited as being the first time the term “nuclear magnetic resonance” appeared in the literature.\textsuperscript{2} Soon after the first experiment by Gorter, in 1938 while working in the physics department at Columbia University, Isidor Rabi began studying the magnetic properties of nuclei. Rabi showed that the nuclear spin states of lithium and chlorine can be flipped when exposed to an oscillating magnetic field while in a molecular beam.\textsuperscript{2–4} In doing so, he eventually was able to determine the nuclear magnetic moments, and in doing so, earned a Nobel Prize in Physics in 1944.\textsuperscript{4}

In 1946, Edward Purcell and Felix Bloch further expanded the field. At MIT a group led by Purcell worked with solid paraffin within a cavity tuned to resonate at
30 MHz. They then observed a 0.4% decrease in cavity output and this was associated with the nuclear spin relaxation of hydrogen atoms present.\(^2,5\) Meanwhile, at Stanford University, Bloch and coworkers utilized similar experiments that demonstrated the same phenomenon but with water rather than a solid. They discovered that when at the right resonant frequency one coil, the transmit coil, could excite the protons in water and detect a signal with another coil, the receive coil.\(^2,6\) In essence, both independently discovered, that upon exposure to a magnetic field; some nuclei absorb energy and emit that energy when returning to the ground state.\(^2-8\) This led to the use of NMR as the spectroscopic technique we know today as well as a Nobel Prize for both Bloch and Purcell in 1952.\(^2,4,7\)

After the Bloch and Purcell discovery, roughly the next three decades were spent fine-tuning the new phenomenon into the powerful analytic tool it is today. The next big discovery came when Henry C. Torrey and Erwin L. Hahn, once again as with the case of Bloch and Purcell, independently showed how feasible pulsed NMR was and observed free Larmor precession.\(^2\) Hahn is also further credited with the being the first to generate and observe spin echoes using pulsed NMR. He would later utilize pulsed NMR to observe spin-spin coupling and study molecular diffusion in liquids.\(^2-4,8\)
It wasn’t until the 1970s that the next major breakthrough in NMR would be made. In 1972, Raymond Damadian, while working at Downstate Medical Center in Brooklyn, N.Y., discovered that the $T_1/T_2$ relaxation times of excised rat tumors differed from that of healthy tissues with accuracies between 96%-98%. With this realization, despite initial skepticism, he would patent the use of NMR as a method for detecting cancer.4,9–12

Paul Lauterbur, his results published in Nature in 1973, was then the first person to use an NMR apparatus to generate an image. While at SUNY Stony Brook, his experimental set-up included placing two 1 mm capillaries filled with water submerged in heavy water. The images were then acquired while magnetic field gradients in various directions were applied to generate 1-D images which were then reconstructed into 2-D image (Figure 1.4).2–4,8,13 The next year saw, at the University of Nottingham, Peter Mansfield developed a technique to generate images by using selective radiofrequency irradiation.14,15 He introduced criteria paramount to image generation including slice selection and “snap shot” images where by a 2-D image could be generated in as little as ten milliseconds.2 Another crucial discovery by Mansfield, was the realization that there was a similarity between the MRI signal and the Fraunhofer diffraction pattern of scattering plane
waves.\textsuperscript{8} A diffraction pattern is subsequently generated and when the Fourier transform is taken, an image is produced. While the plane wave in this case is theoretical, this work was paramount in aiding the study and design of pulse sequences. These studies ultimately lead to the concept of reciprocal space wave vector, or k-space as it is referred to today, a crucial component of modern MR imaging.\textsuperscript{8}

\textbf{Figure 1.4: Lauterbur’s original experimental set up.} The water filled capillaries are aligned with the z-axis with their x-y plane projection. Magnetic field gradients are applied in various directions (red lines). Based on the positioning of the water (blue lines), one-dimensional projections are generated. These projections (blue and red lines) are then combined to form a two dimensional image. Inset: Lauterbur’s original NMR images.\textsuperscript{2}
The final, often forgotten major breakthrough, was made by Richard Ernst.\textsuperscript{2-4} A decade and a half earlier, Ernst introduced Fourier Transform NMR Spectroscopy. After attending a conference in which Paul Lauterbur gave a talk in 1974, Ernst realized that, rather than use Lauterbur’s back-projection technique to generate images, one could use switched magnetic field gradients instead.\textsuperscript{4} Then in 1975 at the Swiss Federal Institute of Technology at Zurich, he hypothesized that 2-D or 3-D images could be generated through by applying the same switched magnetic field gradients as the NMR signals were acquired and then applying Fourier transform.\textsuperscript{2} This discovery is, to this day, still the method most often utilized in generating modern MR images.

These studies jumpstarted the notion that NMR could be used as an imaging technique subsequently referred to as nuclear magnetic resonance imaging (NMRI).\textsuperscript{8,15} Given the general public’s concern with the word nuclear, thus the name was changed to what we know today as simply MRI. The last years of the 1970s saw images taken of a human finger, hand, thorax, head, wrist, and, in 1986 even a single cell was successfully imaged using MRI.\textsuperscript{2,4,15}

In 1980 a postdoctoral fellow at the University of Aberdeen, William Edelstein, generated the first clinically useful image of a human.\textsuperscript{2} This ultimately led the MRI renaissance, as prior to this point, MRI research was primarily restricted to the research laboratories in academia but Edelstein’s findings led to the beginning of extreme commercial interest in MRI with clinical trials soon following. In 1983,
companies such as Toshiba and Siemens were the first to bring commercialized MRI scanners to market. The magnets used were 0.15 T (Toshiba) and 0.35 T (Siemens). General Electric with the help of Edelstein himself, developed and began selling a 1.5 T whole body MRI scanner, two years later. To this day, General Electric remains a leading pioneer in MRI manufacturing and design.

Following the use of MRI to generate images for some time, in 1985 the FDA finally approved the technique for clinical diagnostic use. At this time, 70% of the 200 MRI instruments (Figure 1.5) operating worldwide were based in the United States. Over the last thirty years, these numbers have ballooned to an estimated 30,000 operational instruments worldwide with the number of exams at over 100 million. Today, MRI represents one of the most important imaging techniques both clinically and in research. This success is due to the ability to generate high-resolution three-dimensional images using non-invasive techniques. Furthermore being able to gather information on anatomy and other physiological parameters using a single exam is unmatched by other instrumentation.

Figure 1.5: Representative set up of an MR system. (Top) Location of coils. (Bottom) Typical system arrangement.
1.2 Magnetic Resonance Imaging: How it works

As with NMR, the fundamental property behind why MR imaging exists is due to many subatomic particles having spin.\textsuperscript{12} While individual components of atoms have spin, not all nuclei have an overall spin. The number of protons and neutrons determines the nuclear spin, an odd number of protons or neutrons lead to an overall nuclear spin. Naturally occurring elements, such as carbon and oxygen, do not have nuclear spin since both elements have an equal number of protons and neutrons. If, however, one were to look at isotopes of both elements (\textsuperscript{13}C and \textsuperscript{17}O), these versions would have an overall nuclear spin since the addition of one neutron leads to an odd number.\textsuperscript{12} Luckily, in the case of MRI, the overwhelming species used for imaging is hydrogen, which naturally has nuclear spin without relying on less abundant isotopes (Table 1.1).
Images are produced by utilizing an external magnetic field to manipulate the native protons, primarily water, in the body and detecting the emitted signal. While many parameters, such as proton spin density and temperature, influence image contrast the $T_1$ (longitudinal or spin-lattice) and $T_2$ (transverse or spin-spin) relaxation times impact image generation the most.\textsuperscript{16,18–23}

Other extrinsic parameters can further affect $T_1$ and $T_2$, primarily repetition time (TR) and echo time (TE).\textsuperscript{2,22} TR is defined as the time duration between each RF pulse for each tissue slice. The TE is the time span between applying the RF pulse and measuring of signal. The magnitude of the longitudinal relaxation in between pulses is dictated by TR, thus determining how much $T_1$ relaxation can occur. Likewise, the transverse magnetization allowed to occur is dictated by TE, which

---

**Table 1.1: Properties of various biologically relevant nuclei.\textsuperscript{12}**

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>Spin</th>
<th>Gyrometric ratio (MHz/T)</th>
<th>Natural Abundance</th>
<th>Conc. In Human Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen ($^1$H)</td>
<td>1/2</td>
<td>42.58</td>
<td>~100%</td>
<td>88 M</td>
</tr>
<tr>
<td>Sodium ($^{23}$Na)</td>
<td>3/2</td>
<td>11.27</td>
<td>~100%</td>
<td>80 mM</td>
</tr>
<tr>
<td>Phosphorus ($^{31}$P)</td>
<td>1/2</td>
<td>1.131</td>
<td>~100%</td>
<td>75 mM</td>
</tr>
<tr>
<td>Oxygen ($^{17}$O)</td>
<td>5/2</td>
<td>-5.77</td>
<td>0.04%</td>
<td>16 mM</td>
</tr>
<tr>
<td>Fluorine ($^{19}$F)</td>
<td>1/2</td>
<td>2.627</td>
<td>~100%</td>
<td>4 mM</td>
</tr>
</tbody>
</table>
ultimately controls the amount of $T_2$ relaxation. Because of the effects these parameters have on $T_1/T_2$, one can control which of $T_1$ or $T_2$ predominate. Because of favoring of one or the other, the proper selection of these parameters allows for "weighted" images to be generated. $T_1$-weighting of images occurs when the TR of the sequence is comparable to the longitudinal relaxation time, $T_1$. Areas characterized with short values of $T_1$ will recover more frequently than areas of longer values. Thus, because of the larger signals because of shorter $T_1$, these areas would have more intensity and appear white. Likewise a longer $T_1$ would lead to darker images. For example, healthy tissues containing more lipids than water, such as cerebral white matter, appear whiter when compared to cerebral grey matter because the $T_1$ values of fat are shorter than that of water. $T_2$-weighting occurs when the TE is comparable to the transverse relaxations time, $T_2$. The effect, however, is opposite compared to $T_1$-weighting. Areas characterized with short values of $T_2$ will be darker than areas of longer $T_2$ times. This is because when these two times, TE and $T_2$, are close enough, irreversible signal attenuation as dephasing of the transverse magnetization occurs between the initial RF excitation and the measuring of the signal. In this instance, areas containing more lipids appear darker in a $T_2$-weighted image than areas where water is the predominate proton source.

All tissues, to varying degrees, contain lipids, water, as well as other biological material that can influence $T_1$ or $T_2$ relaxation times. These slight environmental changes cause the protons of the imaged area to relax at various
rates leading to differences in signal strength between tissues (Table 1.2). It is these differences in signal strength and, by proxy $T_1$ and $T_2$ relaxation times, that lead to contrast, defined as an image having a mixture of high signal with areas of low signal.\textsuperscript{7,12,15,19–21,23} This causes an image to have white (high signal) areas mixed with darker (low signal) areas.

**Table 1.2: Relaxation properties of various tissues.\textsuperscript{8,12,22}**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$T_1$ (ms)</th>
<th>$T_2$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain (Gray)</td>
<td>920-950</td>
<td>100</td>
</tr>
<tr>
<td>Brain (White)</td>
<td>600-780</td>
<td>80-90</td>
</tr>
<tr>
<td>Muscle</td>
<td>900</td>
<td>50</td>
</tr>
<tr>
<td>Spinal Fluid</td>
<td>4500</td>
<td>2200</td>
</tr>
<tr>
<td>Fat</td>
<td>250-260</td>
<td>60-80</td>
</tr>
<tr>
<td>Blood</td>
<td>~1200-1400</td>
<td>100-260</td>
</tr>
<tr>
<td>Meningioma</td>
<td>400</td>
<td>80</td>
</tr>
<tr>
<td>Metastasis</td>
<td>1800</td>
<td>85</td>
</tr>
</tbody>
</table>

MR imaging relies on the native differences in relaxation time ($T_1$ and $T_2$) and tissue composition (amount of lipids, water, etc. present) in order to generate images. This is unique in the imaging world as other techniques, such as positron emission tomography (PET) or computed tomography (CT), rely on radioactive tracers to be the signal source.\textsuperscript{24–27} This non-invasive nature of MR imaging is one of the biggest advantages the technique has over other imaging modalities (Table 1.3). Furthermore, aside from metallic objects patients may use or have implanted (e.g., pacemakers), there are no known side effects to be exposed to magnetic fields.\textsuperscript{9,12} Some other advantages MRI has over other imaging modalities include contrast
resolution between differing tissues, multi-planar imaging, and the ability to manipulate an image accordingly.\textsuperscript{7,28}

**Table 1.3: Some differences in select imaging techniques.** PET (Positron Emission Spectroscopy), SPECT (Single Photon Emission Computed Tomography), CT (Computed Tomography), and Ultrasound.\textsuperscript{28}

<table>
<thead>
<tr>
<th>Imaging Technique</th>
<th>Spatial Resolution</th>
<th>Penetration Depth</th>
<th>Temporal Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>PET</td>
<td>1 – 2 mm</td>
<td>None</td>
<td>10s – min</td>
</tr>
<tr>
<td>SPECT</td>
<td>0.5 – 1 mm</td>
<td>None</td>
<td>min</td>
</tr>
<tr>
<td>MRI</td>
<td>25 – 100 μm</td>
<td>None</td>
<td>min – hr</td>
</tr>
<tr>
<td>CT</td>
<td>50 – 200 μm</td>
<td>None</td>
<td>min</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>50 – 500 μm</td>
<td>mm – cm</td>
<td>s – min</td>
</tr>
</tbody>
</table>

While different tissues have varying $T_1/T_2$, the low sensitivity and distinguishability of MRI, especially when comparing diseased and healthy tissue, has necessitated the use of contrast agents. This unique class of compounds can be administered prior to taking an image to increase the minute signal intensity differences between tissues. Contrast agents are particularly useful when trying to differentiate between healthy and diseased tissue, allowing for the visualization of lesions and small tumors not normally seen without the use of contrast agents.\textsuperscript{18–20}

The increase in MRI use has contributed to rapid development of contrast agents, molecules utilized to improve upon the contrast and resolution of images. These compounds have proved readily useful when trying to distinguish healthy tissue from diseased, as the natural contrast and resolution isn’t good enough alone to differentiate between them.
1.3 Contrast Agents

Lauterbur was once again pivotal in the development of MRI. Also in 1973, he was the first to demonstrate how the use of foreign substances, in this case Mn$^{+2}$, can alter relaxation times.$^{13,29,30}$ The highly paramagnetic ions shortened the T$_1$ relaxation time of water protons. This discovery was further implemented when a solution of Mn$^{+2}$ was injected into a dog with a blocked coronary artery.$^{29}$ It was soon realized that the T$_1$ relaxation rates were faster the higher the Mn$^{+2}$ concentration became. The Mn$^{+2}$ localized only in the healthy heart tissue thus the healthy tissue was brighter (since it had higher signal intensity) than the clogged artery. This was the first instance of, not only showing the medical diagnostic power of MRI, but also showing an ingenious way to get around the native limitations of the technique by utilizing foreign compounds. These compounds are now referred to as contrast agents.$^{16}$

As one might assume, the purpose of contrast agents is to help differentiate tissues which would otherwise be unseen by an MRI scan alone. Furthermore, with MR imaging being 10$^6$ times less sensitive than other imaging modalities, contrast agents help to improve this limited sensitivity.$^{8,16,18,25}$ This tends to be the case when the tissue in question has very similar properties to the background or when the malady is too small. Contrast agents work by altering the native tissue properties via changing the local magnetic field.$^{9,16,22,31}$ Therefore this magnetic field affects the T$_1$ and T$_2$ relaxation times of protons in close proximity. This amplifies the signal
differences between those tissues close enough to be affected by the contrast agent and the background that remains unchanged. It's this amplification that allows for greater distinguishability. Likewise the better a contrast agent is, the greater the amplifying affect and the more protons can be affected at once.

As previously mentioned, a contrast agent works by changing the local magnetic field. This local change is due to the presence of an internal magnetic field caused by the agent being paramagnetic, superparamagnetic, or ferromagnetic.\textsuperscript{22} This field is a defining characteristic of a contrast agent, without it there would be no change. This magnetic interaction can be achieved many different ways. These kinds of materials, via their unpaired electron spins, interact with the protons of the surrounding tissue. This interaction causes a change in the magnetization because the magnetic moment of an electron is roughly 700 times greater than that of a proton.\textsuperscript{22} While many materials possess these characteristics, most suffer from low biocompatibility due to toxicity.

Contrast agents can be categorized as either $T_1$ or $T_2$. This designation is based on what the agent predominately affects, either the longitudinal ($T_1$) or transverse ($T_2$) relaxation time.\textsuperscript{16,18} With an increase in signal intensity, $T_1$ contrast agents are also referred to as positive contrast agents. These materials generally contain Gd$^{3+}$, a paramagnetic metal ion with seven unpaired electrons.\textsuperscript{18} The unpaired electrons give rise to a very high magnetic moment, which is what affects the surrounding protons. Transition metal ions such as Mn$^{2+}$ and Fe$^{3+}$ are also
suitable metals like Gd$^{+3}$ due to the five unpaired electrons they possess (Table 1.4). By affecting the $T_2$ relaxation time, these agents decrease the amount of times relaxation occurs. This leads to a decrease in signal intensity and darkening of images. Thus these agents are also referred to as negative contrast agents. Many of these agents are comprised of superparamagnetic iron oxide ($\text{Fe}_3\text{O}_4$) nanoparticles.

**Table 1.4: Electron configuration and magnetic moment of select metal ions.**\(^{28,32}\)

<table>
<thead>
<tr>
<th>Metal Ion</th>
<th>Electron Configuration</th>
<th>Magnetic Moment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{24}\text{Cr}^{+3}$</td>
<td>$\uparrow\uparrow\uparrow\downarrow\downarrow$</td>
<td>3.88</td>
</tr>
<tr>
<td>$^{25}\text{Mn}^{+2}$</td>
<td>$\uparrow\uparrow\uparrow\uparrow\uparrow$</td>
<td>5.92</td>
</tr>
<tr>
<td>$^{26}\text{Fe}^{+3}$</td>
<td>$\uparrow\uparrow\uparrow\uparrow\uparrow$</td>
<td>5.92</td>
</tr>
<tr>
<td>$^{29}\text{Cu}^{+2}$</td>
<td>$\uparrow\downarrow\uparrow\downarrow\uparrow\downarrow\uparrow\downarrow$</td>
<td>1.73</td>
</tr>
<tr>
<td>$^{63}\text{Eu}^{+3}$</td>
<td>$\uparrow\downarrow\uparrow\uparrow\uparrow\uparrow\uparrow\uparrow$</td>
<td>3.4</td>
</tr>
<tr>
<td>$^{64}\text{Gd}^{+3}$</td>
<td>$\uparrow\uparrow\uparrow\uparrow\uparrow\uparrow\uparrow\uparrow$</td>
<td>7.94</td>
</tr>
<tr>
<td>$^{66}\text{Dy}^{+3}$</td>
<td>$\uparrow\downarrow\uparrow\downarrow\uparrow\downarrow\uparrow\downarrow\uparrow\downarrow\uparrow\uparrow\uparrow$</td>
<td>10.65</td>
</tr>
</tbody>
</table>

MRI efficiency is quantified based on relaxivity (r). This is defined as the change in $T_i$ (where $i = 1$ or 2) per concentration of contrast agent.\(^{18}\) By varying contrast agent concentration and measuring the subsequent relaxation rates, the relaxivity can be determined by the following equation:\(^{33}\)

$$\frac{1}{T_{i,\text{observed}}} = r_i[CA] + \frac{1}{T_{i,\text{tissue}}}$$

Perhaps the biggest piece of insight relaxivity can give is what type of contrast agent a particular prospective compound will have. The ratio of $r_2/r_1$ can
give a quantifiable value for comparison. As such, the greater the value of that ratio the more likely that particular compound is going to be a T<sub>2</sub> contrast agent or darken images. Likewise a lower number is indicative of that compound being a T<sub>1</sub> contrast agent or brighten images. 34

The benefits to using contrast agents are obvious, however there are a variety of unfortunate disadvantages that have to be addressed with most agents. For example, while the Mn<sup>2+</sup> used by Lauterbur is essential to humans, high concentrations lead to a neurodegenerative disorder similar to Parkinson’s disease referred to as manganism. 35 Ionic manganese also interferes with cell metabolism via the calcium fluxes. 18, 29 Similar issues arise when considering other contrast agents. The Gd<sup>3+</sup> of many T<sub>1</sub> contrast agents, since Gd<sup>3+</sup> has no natural metabolic pathway, is toxic when released into the body. Symptoms include splenic degeneration, enzyme inhibition, liver necrosis, interference with synapses, and calcium channel blockage. 36 Contrast agents containing Gd<sup>3+</sup> have also been linked to nephrogenic systemic fibrosis (NSF), a harmful side effect for patients with renal issues. 34, 36–40 Iron oxide nanoparticles suffer from a heterogeneous distribution of metal content leading to inefficient contrast. The particles also suffer from broad particle size distribution, are also easily oxidized to antiferromagnetic Fe<sub>2</sub>O<sub>3</sub>, and readily agglomerate in the presence of a magnetic field. These all diminish the usefulness of these kinds of contrast agents. 35, 41 An ideal contrast agent should have a number of important qualities, including but not limited to a high number of
unpaired electrons, low toxicity, solution stability, easily modifiable, and biocompatible.\textsuperscript{33}

1.3.1 \textit{T_1 Agents}

The mechanism in which \textit{T_1} contrast agents are best described is the Solomon-Bloembergen-Morgen (SBM) theory.\textsuperscript{18} Gadolinium chelates, since the emergence of gadolinium(III) diethylenetriaminepentaacetic acid as potential contrast agent in 1988, have been used extensively.\textsuperscript{9,16,29} Using Gd\textsuperscript{3+} as an example, when considering it as a metal center it provides a relaxation pathway for the water protons.\textsuperscript{18} This interaction, considered an electron-proton dipolar coupling, creates a fluctuating magnetic field leading to relaxation. This interaction fluctuates based on proton distance from the metal center, the number of coordinated water molecules and their exchange rate, and a slow tumbling rate of the contrast agent.\textsuperscript{18}

1.3.2 \textit{T_2 Agents}

\textit{T_2} contrast agents, like superparamagnetic iron oxide nanoparticles have a large magnetic moment, cannot be explained using SBM theory. The mechanism is better described the bulk susceptibility effect. The large magnetic moments produce strong magnetic fields emanating from the particles.\textsuperscript{42,43} These fields cause a rapid lose of phase coherence causing an increase in water relaxation.\textsuperscript{18}
1.4 Metal-oxo Clusters

Clusters are unique compounds in that their size is in between that of bulk solids and molecules. The simple composition of most clusters can be described as a number of metal ions covalently bonded to some combination of organic ligands or other metals via bridging atoms. Because of their unique intermediate size and unique structure, clusters have gained traction as promising materials for a variety of applications. In particular, interest in using clusters as contrast agents has grown since they combine the preferred properties of metal chelates and iron oxide nanoparticles. The numerous paramagnetic metal centers allow for higher relaxivity when compared to a compound with only one metal center. Furthermore, a cluster with a high magnetic moment is also of important as this would mean an effective dose needed for successful contrast would be lower than that of a cluster with a low magnetic moment.

Metal-oxo clusters provide a unique route for future contrast agent development. They provide an avenue for containing the magnetic metal ions, preventing any potential leaching. Furthermore many clusters allow for ligand exchange where by the outer ligands are exchanged for ligands of a more favorable moiety while still maintaining the core of the cluster. While many clusters exist, a few guiding principles should be considered. First, a prospective cluster should be easily synthesized. Complicated syntheses take away from the long-term feasibility. Likewise the metal composition should be of metals with as many lone electrons as
possible while still being biocompatible. Trivalent cations, like Gd$^{3+}$, have the maximum number of unpaired electrons but as previously mentioned are toxic. Many of its lanthanide counterparts suffer from the same issue. The first row transition metals offer an alternative. While the maximum number of unpaired electrons is lower (5 compared to 7), many including manganese and iron are biologically crucial for and possess a biological pathway.$^{32}$ This means that if any metal were to escape, the body has a natural defense mechanism to counter. Finally, as alluded to prior, the cluster in question should also be able to ligand exchange with other more desirable ligands. While seemingly trivial, this is arguably the most important criterion. The ability to ligand exchange allows for a more adaptable system including utilizing nanomaterials for further enhancement. As will be discussed later, all three of these rules have, in one way or another, shaped the outcome of this work.

Previous studies by Mertzman and Pablico have shown how promising clusters can be as contrast agents and how nanomaterials can be used to enhance their properties.$^{47-49}$ These studies, as well as others, prove how nanomaterials can be crucial in improving the native properties of a particular cluster by utilizing the ligand exchange technique.$^{19}$ As such much of this work focuses on exploring how nanomaterials can be utilized for further improvements.
1.5 **Nanomaterials**

Richard Feynman, Nobel laureate and renowned physicist, first described the concept of nanotechnology at an American Physical Society meeting in 1959 at Caltech.\(^\text{50}\) Three decades later, in September of 1989, physicist Donald M. Eigler at IBM precisely arranged xenon atoms to create “IBM” at the atomic scale using scanning tunnel microscope, a technique he in fact developed (Figure 1.6).\(^\text{50}\) Almost another thirty years later, nanotechnology has cemented itself as one of the most important and fastest growing fields in worldwide scientific research and economics.\(^\text{51,52}\)

Nanotechnology, as the name suggests, is the study, development, and use of materials in the nanometer size range.\(^\text{53}\) The properties of these sized materials often differ from their bulk counterparts. These differences arise from a relative increase in surface area and dominant quantum confinement effects; impacting such properties as mechanical, optical, magnetic, as well as catalytic.\(^\text{32,50,54}\)

Arguably one of the most important impacts these materials have had is in Figure 1.6: Eigler’s IBM spelt using xenon atoms. Each letter is 50 nm top to bottom. (Reprinted from Seminars in Radiation Oncology, 21/2, Jan Grimm, David A. Scheinberg Will Nanotechnology Influence Targeted Cancer Therapy?, 80-87, Copyright (2011), with permission from Elsevier)\(^\text{50}\)
medicine. Nanomedicine is the application of nanotechnology towards human health including diagnosis, treatment, and monitoring of diseases and other health related concerns. These materials are needed more than ever as anti-bacterial properties of certain nanomaterials are needed to combat the emergence of superbugs, such as certain strains of E. coli and salmonella, become increasingly more resistant to last resort anti-biotics.

Many nanoconstructs, composed of various materials, have found use in biological applications. As such the exploration of using magnetic nanoparticles for such applications was a natural progression. These materials have been used for both diagnostic and therapeutic purposes. Nanomaterials also allow for adequate cell uptake and delayed excretion otherwise foreign to native clusters due to their small size.

As will be discussed later in greater detail and implied previously, the size of many unadulterated clusters limit their viability for biological applications. As such, efforts have been taken to modify clusters in order to address this issue as much as possible. An obvious solution is the incorporation of these clusters into or onto nanomaterials. Easy exchange of native ligands, such as acetates, has been a characteristic of many metal-oxo clusters. The lability of these acetate ligands surrounding the cluster is what all for easy ligand exchange, as any ligand with a carboxylate group has the potential to be exchanged. The first studies to demonstrate the incorporation of a metal-oxo cluster, after a successful ligand
exchange, were using $\text{Mn}_{12}$ the prototypical single molecule magnet first synthesized by Lis in 1980.\textsuperscript{45,47,68,69} These studies paved the way for ligand exchange being utilized to incorporate metal-oxo clusters into a wide array of nanoconstructs.

The utilization of nanomaterials provides a unique avenue to improve upon many of the disadvantages associated with many unadulterated contrast agents. Examples include metal leaching, solubility, and stability. Herein two nanomaterials, polystyrene nanobeads and mesoporous silica nanoparticles, are discussed.

1.5.1 \textit{Polymer Nanomaterials}

Nanoparticles also have the distinct advantage of having a high surface to volume ratio, enhanced colloidal stability, and increased chemical resistance compared to other nanomaterials.\textsuperscript{32,50} The colloidal stability and chemical resistance are of particular importance as they allow for the incorporation of other functionalities. These functionalities, for example, include fluorescent dyes, targeting molecules, or drugs.\textsuperscript{70} The additional functionalities allow for a single nanoparticle to be multimodal, addressing multiple diagnostic and/or therapeutic techniques at once.

Hybrid nanomaterials, the combination of inorganic and organic components, have garnered a great deal of attention in the nanotechnological world. This attention arises from the unique physical properties, due to the presence of
both parent materials, and their subsequent interactions within the same material. As such the incorporation of inorganic components into polymers has been an ongoing area of interest. These types of materials have already been utilized in numerous applications including biosensors, medical diagnostics, and catalysis.\textsuperscript{69} For the purpose of metal-oxo cluster contrast agents, the polymer adds stability, prevents metal leaching, and allows for further particle modification beyond the simple ligand exchange.

While numerous approaches exist to make these hybrid materials, our group has utilized the miniemulsion technique in order to make our hybrid polymer nanoparticles.\textsuperscript{48,49,71} The direct miniemulsion allows for the formation of homogenous, stable, monodispersed polymer nanoparticles in a size range (50 - 500 nm) suitable for biological applications.

The miniemulsion technique involves the combination of two different phases, the continuous and dispersive. The combination of phases is homogenized via ultrasonication in the presence of a hydrophobe and surfactant. From this, stable nanodroplets are formed and, due to mass transport and restricted secondary nucleation, the droplet diameter corresponds to the polymerized bead diameter.\textsuperscript{71} As will be discussed in a later chapter, the continuous and dispersive phases can change depending on the desired characteristics and properties of the resulting polymer bead.
While miniemulsions have been used to encapsulate a wide array of inorganic materials and forming many structure types, for the purposes of this work, the focus will lie on the synthesis of magnetic polymer nanobeads. In many magnetic materials, including Fe\(_3\)O\(_4\), many times the purpose of the polymer coating is to preserve the magnetic properties of the inorganic component. This is due to the fact that many of these materials can readily react with their surroundings to form materials with inhibited magnetism or, as in the case with Fe\(_3\)O\(_4\), turning into something completely void of any magnetism.\(^{32,72}\) The polymer coating acts as a protective barrier, shielding the magnetic core from the potentially reactive and detrimental outside environment. Styrene, the monomer used by our group, is also biostable and the necessary conditions to synthesize monodispersed nanoparticles is well defined.

While the complete encapsulation prevents metal leaching and preserves the magnetic properties, the cluster exposure to water is also prevented. Since water exchange is one of the pathways for increased relaxivity, a balance between cluster entrapment and water exposure would be a theoretical improvement.

1.5.2 **Silica Nanoparticles**

Another promising avenue to explore for cluster containment is mesoporous silica nanoparticles. These kinds of nanoparticles are the next logical choice. The cluster would be entrenched in the pores, via covalent attachment, while still being
exposed at the surface of the particle. The porous nature of the silica itself would allow for water to flow freely within while also providing the structure to decrease the rotation correlation time similar to the polymer nanobeads discussed previously. The exposure to water should allow for an enhancement in relaxation compared to previous.

The first example of mesoporous silica nanoparticles was synthesized by the Mobil Corporation in the early 1990s.\textsuperscript{73,74} Since then, these materials, similar to polymer nanoparticles, have been utilized in a wide array of areas including catalysis, filtration, ion exchange, and even garnering attention in the forensic science community recently.\textsuperscript{61,67,75–81}

The particles are synthesized via the Stöber method. This method was first developed in the 1960s in order to synthesize monodispersed nonporous silica nanoparticles with a specified size range.\textsuperscript{82} The synthesis entails the hydrolysis and condensation of a silica source in a basic solution (Figure 1.7). This is done by adding a silica source, usually an organic silane, to an aqueous solution containing a surfactant that acts as a template for pore formation. The surfactant was later adopted in order to produce mesoporous versus nonporous materials.\textsuperscript{82} A base is used to catalyze the reaction and allow the silica source to hydrolyze around the surfactant micelles. After this process begins the silicate micelles self-assemble into cylinders at which point silica continues to condense forming the final set structure.\textsuperscript{74,83} Once allowed to age, the silica matrix is stable and the templating
surfactant can be removed via calcination, reflux in acidified ethanol and/or an ammonium nitrate solution, or reflux in a high boiling point organic solvent such as TOPO.\textsuperscript{83–86}

![Figure 1.7: Hydrolysis and condensation of a silica precursor, TEOS, under basic conditions. (Reproduced with permission from the Royal Society of Chemistry)\textsuperscript{86}](image)

The properties of these types of materials are what make them so versatile. The controllable size, mesopores, and high biocompatibility are key components, but one could make the argument that the ability to chemically modify the particle is the most crucial of these properties.\textsuperscript{73,75,79,81} The surface of these particles is coated with silanol (Si-OH) groups and it is these groups that allow for very rapid and complete surface modification. The presence of another silane with a more desirable functionality can be added, either post-synthesis (grafting) or a direct synthesis (co-condensation), via condensation reaction. The method chosen dictates in what area the modification occurs. Direct synthesis leads to deeper pore modification while
grafting leads to modification primarily at the pore entrance.\textsuperscript{83} This is what allows for cluster incorporation, as the silica nanoparticle surface can be modified in order to ligand exchange with native cluster. Unlike with the previous polymer nanoparticles, the nanomaterial is modified to accommodate the cluster rather than the cluster for the nanomaterial. Due to this key feature and the previously mentioned exposed cluster, it is hypothesized that these types of nanoparticles will be an improvement on the previously mentioned polymer nanoparticles.

1.6 Thesis Objectives

This thesis will look to devise new methodologies to improve upon current MR imaging technologies, both in general and specifically to our research group. Each chapter will take a more focused look at a component of the contrast agent equation our group has devised. The breakdown of this thesis is as follows:

- Chapter 2: Metal-Oxo Clusters
- Chapter 3: Mesoporous Silica Nanoparticle Contrast Agents
- Chapter 4: Future Directions/Closing Remarks
CHAPTER 2: METAL-OXO CLUSTERS

2.1 INTRODUCTION

The world of metal-oxo cluster chemistry has come a long way since the first single molecule magnet, Mn$_{12}$ [Mn$_{12}$O$_{12}$(O$_2$CCH$_3$)$_{16}$(H$_2$O)$_4$], was first synthesized by Lis more than 30 years ago.$^{68}$ It was not until thirteen years later, in 1993, that Sessoli and coworkers realized the magnetic potential of this cluster.$^{87}$ The cluster is composed of Mn$^{3+}$ and Mn$^{4+}$. Eight Mn$^{3+}$ are on the outside of the cluster while the Mn$^{4+}$ are internally located. Similar to bulk oxides, they are bridging oxide ions holding the core structure together with acetate ions on the periphery. Unlike the bulk materials, the inclusion of the acetates act as a barrier, preventing further growth and accounting for the finite sizes of the particles.$^{87}$ Furthermore, aside from the fascinating magnetic properties made possible because of the nanoscale dimensions, transition metal-oxo clusters have been used as biological models for many metalloenzymes and metalloproteins.$^{88}$

Today some clusters are big enough to be considered nanoparticles all on their own, having a large number of metals bonded together (Figure 2.1a).$^{89}$ Recently, as described by Zeng and co-workers, the largest bimetallic nanocluster at over 100 metals was synthesized (Figure 2.1b).$^{89}$ While smaller, other mixed valence manganese-oxo clusters have a ground spin almost double ($S = 17$) that of Mn$_{12}$.$^{90}$ Cluster synthesis has blossomed to include a wide variety of metals and ligands. Today there are clusters comprised of many metals, including but not
limited to: lanthanides such as gadolinium, lanthanum, uranium, and dysprosium; other transition metals such as zinc, gold, silver, and vanadium; even elements such as tin and antimony have garnered attention as clusters.  

\[89,91-100\]

Figure 2.1: Examples of modern day clusters. a) Palladium core of \(\text{Pd}_{145}(\text{CO})_{x}(\text{PEt}_3)_{30}\) and b) Bimetallic core of \(\text{Au}_{80}\text{Ag}_{30}\) first reported by Zeng and co-workers (Au: orange, Ag: green). ((a):Reprinted from Coordination Chemistry Review, 248/21-24, Paul J. Dyson, Catalysis by low oxidation state transition metal (carbonyl) clusters, 2443-2458, Copyright (2004), with permission from Elsevier) b: Reprinted (2016) with permission from (Zeng, J.-L.; Guan, Z.-J.; Du, Y.; Nan, Z.-A.; Lin, Y.-M.; Wang, Q.-M. J. Am. Chem. Soc. 2016, jacs. 6b04471..). Copyright (2016) American Chemical Society.)\(^{89,100}\)

While many clusters composed of a variety of metals exist today, the first cluster \(\text{Mn}_{12}\), was originally chosen by our group to explore its viability as a contrast agent. This cluster has a large spin state (\(S=10\)) and the magnetic anisotropy associated with the eight \(\text{Mn}^{+3}\) Jahn-Teller axes.\(^{47,87,101}\) For even more potential
contrasting power, the cluster also has four exchangeable water molecules in addition to the large number of unpaired electrons contained within each manganese. The measured relaxivity, in acetic acid solutions, determined by Mertzman et al. in 2009 result in this cluster determined to be a $T_2$ contrast agent ($r_1 = 3.0 \text{ mM}^{-1}\text{s}^{-1}$, $r_2 = 48 \text{ mM}^{-1}\text{s}^{-1}$). As one might presume, a major drawback to this cluster is having to use solutions of acetic acid in order to perform these experiments. Furthermore, Mn$_{12}$ readily decomposes in water without excess acetic acid severely limiting it’s viable use as a contrast agent without assistance.$^{47,101}$

The next generation cluster tested by the group, Mn$_8$Fe$_4$ [Mn$_8$Fe$_4$O$_{12}$(O$_2$CCH$_3$)$_{16}$($\text{H}_2\text{O})_4$] (Figure 2.2), was first synthesized by Schake and co-workers in 1994.$^{102}$ This iron analog of Mn$_{12}$ overcomes the solubility issues previously discussed. As reported by Pablico, this cluster also was determined to be a $T_2$ contrast agent ($r_1 = 2.38 \text{ mM}^{-1}\text{s}^{-1}$, $r_2 = 26.65 \text{ mM}^{-1}\text{s}^{-1}$).$^{49}$ Furthermore, ligand exchange reactions were successfully utilized in order to allow for incorporation of the Mn$_8$Fe$_4$ cluster into polystyrene nanoparticles (Figure 2.3). Bead incorporation greatly changed the relaxation properties of the native cluster, specifically, altering them such that the cluster encapsulated particles behaved more like a $T_1$ contrast agent, the more desired type of contrast agent.$^{48,49}$

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Mn$_8$Fe$_4$O$_{12}$O$_2$CCH$_3$$_{16}$H$_2$O$_4$ [Mn: aqua, Fe: yellow, O: red, C: gray. Co-crystallized solvents, water, and hydrogen atoms omitted for clarity.$^{104}$]}
\end{figure}
Pablico explored clusters aside from Mn_{12} and Mn_{8}Fe_{4}, identified as having promising contrasting abilities while still being comprised of biocompatible and inexpensive metals (Table 2.1). These clusters, aside from using manganese and iron, also have similar organic components—primarily the use of carboxylate ligands. This was done purposely, as the ligand exchange reactions reported for Mn_{12} and Mn_{8}Fe_{4} are key for bead incorporation.

**Table 2.1: Relaxivities of various previously explored metal-oxo clusters for potential use as contrast agents.**

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Relaxivity (mM^{-1} metal s^{-1})</th>
<th>( r_2/r_1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r_1 )</td>
<td>( r_2 )</td>
</tr>
<tr>
<td>Mn_{3}pyr</td>
<td>19.0 ± 1.5</td>
<td>155.8 ± 3.1</td>
</tr>
<tr>
<td>Mn_{3}bpy</td>
<td>12.4 ± 0.7</td>
<td>145.0 ± 2.2</td>
</tr>
<tr>
<td>Mn_{8}Fe_{4}</td>
<td>2.3 ± 0.1</td>
<td>24.2 ± 1.4</td>
</tr>
<tr>
<td>Mn_{8}Fe_{4}Cl</td>
<td>0.74 ± 0.01</td>
<td>7.2 ± 0.1</td>
</tr>
<tr>
<td>Mn_{12}</td>
<td>0.43 ± 0.02</td>
<td>2.9 ± 0.5</td>
</tr>
</tbody>
</table>

**Figure 2.3: Mn_{8}Fe_{4} after a successful ligand exchange (L).** A TEM image of polymer nanoparticles after the incorporation of substituted cluster (R, Scale bar = 100 nm). (Reprinted (2016) with permission from (Pablico, M. H.; Mertzman, J. E.; Japp, E. A.; Boncher, W. L.; Nishida, M.; Van Keuren, E.; Lofland, S. E.; Dollahon, N.; Rubinson, J. F.; Holman, K. T.; Stoll, S. L. *Langmuir* 2011, 27, 12575.). Copyright (2011) American Chemical Society.)

\(^{48}\)
While smaller trinuclear manganese-oxo clusters have been studied previously, as models for water oxidation, their use as contrast agents are yet unexplored. This chapter focuses on the potential for Mn$_3$(O$_2$CCH$_3$)$_6$(bpy)$_2$ [Mn$_3$ bpy] to be utilized in a similar fashion to Mn$_{12}$ and Mn$_8$Fe$_4$.

Mn$_3$ bpy is linear cluster first synthesized by Ménage et al. The cluster itself consists of three manganese atoms bound to acetate ligands with the outer metals also bound to bipyridine ligands, one on each end, essentially capping the metal-oxo chain and preventing further cluster growth similar to Mn$_{12}$ as mentioned previously. Each pair of metal atoms has two bidentate acetates acting as bridges while the last acetate is only monodentate (one oxygen atom (Figure 2.4)).

It was hypothesized that by ligand exchanging polymerizable carboxylate groups, specifically 4-vinylbenzoic acid and methacrylic acid (VBA and MA), the cluster could be cross polymerized as demonstrated for Mn$_8$Fe$_4$. This chapter will focus not only on Mn$_3$ bpy and the corresponding ligand exchange reactions but each new clusters potential inclusion into polystyrene nanobeads, as achieved by Pablo with Mn$_8$Fe$_4$, will also be explored.

Figure 2.4: Mn$_3$(O$_2$CCH$_3$)$_6$(bpy)$_2$ [Mn: aqua, O: red, N: blue, C: gray. Co-crystallized solvents, water, and hydrogen atoms omitted for clarity.]
2.2 Experimental

Manganese(II) acetate tetra hydrate, 2,2-bipyridine, ethanol (200 proof), 4-vinylbenzoic acid, methacrylic acid, hexanes, styrene, 2-hydroxyethyl methacrylate, acetonitrile, and 2,2'-azobis(2-methylpropionitrile) were all purchased from Sigma Aldrich. All chemicals, except for the monomers (styrene and 2-hydroxyethyl methacrylate) which were filtered through alumina (Al₂O₃) to remove inhibitors, were used as received.

Fourier-transform infrared spectroscopy (FTIR) measurements were recorded in the range 4,000-400 cm⁻¹, from pressed pellets in KBr on a Perkin Elmer Spectrum Two. The bulk polymerizations were analyzed using an ATR tip attachment in the 4,000 – 600 cm⁻¹ range. Elemental analysis was performed on a Perkin Elmer 2400 Elemental Analyzer, using aceta-nilide as standard. Thermogravimetric analysis was studied and the data was collected using a platinum pan with a heating range of 20-400°C at 10°C/min.

Single crystal X-ray diffraction data were collected at 100(2) K on a Siemens SMART three-circle X-ray diffractometer equipped with an APEX II CCD detector (Bruker-AXS) and an Oxford Cryosystems 700 Cryostream, using Mo-Kα radiation (λ= 0.71073 Å). The crystal structure was solved by direct methods using SHELXS, and all structural refinements were conducted using SHELX-2014-6.¹⁰⁵,¹⁰⁶ No decay
correction was applied to either structure. Figures were generated using CrystalMaker v9.2.7.107

**Mn<sub>3</sub>bpy** [Mn<sub>3</sub>(O<sub>2</sub>CCH<sub>3</sub>)<sub>6</sub>(bpy)<sub>2</sub>]. This metal-oxo cluster was prepared in accordance with the literature. In two Schlenk flasks, manganese(II) acetate (1 mmol) was dissolved in 10 ml ethanol. In the second flask was a 10 ml ethanolic solution (1 mmol) of 2,2'-bipyridine. The bipyridine solution was then cannula transferred to the first and the resulting pale yellow solution was stirred. The solvent was then partially evaporated under reduced pressure causing pale yellow microcrystals to appear. These microcrystals were isolated via vacuum filtration and washed with ethanol. IR (cm<sup>-1</sup>): 3425 (br), 3000 (w), 1603 (s), 1494 (w), 1477 (w), 1425 (s), 1019 (m), 771 (s), 649 (m), 511 (w), 415 (w). Found (Calculated): C, 46.11 (46.23); H, 4.00 (4.12); N, 6.63 (6.74).

**Mn<sub>3</sub>bpyVBA** [Mn<sub>3</sub>(O<sub>2</sub>CCH<sub>4</sub>H<sub>4</sub>CH=CH<sub>2</sub>)<sub>6</sub>(bpy)<sub>2</sub>]. This substituted metal-oxo cluster was prepared in a similar matter as other ligand exchange reactions in our group.48,49 In a Schlenk flask, Mn<sub>3</sub>bpy (0.125 mmol, ~0.1039 g) was dissolved in 20 ml of warm ethanol via a water bath and stirring. Once dissolved, 4-vinylbenzoic acid (1.5 mmol, ~0.2222g) was added to the clear yellowish solution. After approximately 20 minutes, the clear yellow solution precipitated a pale yellow powder. The Schlenk flask was then removed from the water bath. The powder was then isolated via vacuum filtration and subsequently washed with ethanol. For crystallization, the same protocol was used except the hot plate was turned off and
the flask was allowed to remain in the water bath to slowly cool. After ~48 hours, pale yellowish crystals formed. These crystals were then, as before, isolated via vacuum filtration and subsequently washed with ethanol. IR (cm⁻¹): 3063 (br), 1598 (s), 1551 (s), 1475 (w), 1441 (w), 1402 (s), 1179 (w), 1106 (m), 1017 (m), 912 (w), 867 (m), 795 (s), 762 (m), 650 (w), 615 (w), 451 (w). Found (Calculated): C, 64.52 (65.35); H, 4.31 (4.30); N, 3.91 (4.12).

**Mn₃bpyMA** [Mn₃(O₂C(CH₃)C=CH₂)₆(bpy)₂]. This substituted metal-oxo cluster was prepared in a similar matter as other ligand exchange reactions in our group.⁴⁸,⁴⁹ In a Schlenk flask, Mn₃bpy (0.125 mmol, ~0.1039 g) was dissolved in 20 ml of warm ethanol via a water bath and stirring. Once dissolved, methacrylic acid (1.5 mmol, ~0.1272 ml) was added to the clear yellowish solution. After approximately 4 hours, the clear yellow solution remained. This solution was then layered with hexanes (2:1 volume ratio) and allowed to sit undisturbed and in the dark for approximately 14 days. After this time the solvent had evaporated and yellowish microcrystals remained. The microcrystals were then isolated via vacuum filtration and subsequently washed with ethanol. IR (cm⁻¹): 3444 (br), 3106 (w), 2952 (w), 2922 (w), 1644 (m), 1567 (s), 1415 (s), 1099 (w), 1017 (m), 863 (w), 831 (m), 765 (s), 649 (w), 509 (w), 412 (m). Found (Calculated): C, 52.95 (53.51); H, 4.51 (4.69); N, 5.62 (5.67).

**Bulk Polymerization.** In a Schlenk flask, substituted cluster and monomer (200:1 monomer to cluster ratio) were combined and the mixture was allowed to stir. An
initiator, AIBN [2,2′-azobis(2-methylpropionitrile), ~45 mg] was then added to the cluster/monomer mixture. The flask was then placed in a hot water bath (~65°C) and allowed to stir under nitrogen until the polymerization was visibly complete.

*Mn₃bpyVBA + HEMA:*

IR (ATR, cm⁻¹): 2923 (m), 2853 (m), 1724 (s), 1451 (w), 1381 (w), 1256 (m), 1149 (s), 1075 (m), 1052 (m), 1028 (w), 896 (w), 816 (w).

*Mn₃bpyMA + HEMA*

IR (ATR, cm⁻¹): 3287 (br), 2996 (w), 2925 (m), 2853 (m), 2241 (w), 1720 (s), 1640 (w), 1481 (s), 1229 (m), 1174 (s), 1076 (m), 1026 (m), 817 (w).

*Mn₃bpyMA + Styrene*

IR (ATR, cm⁻¹): 3026 (w), 2998 (w), 2926 (s), 2856 (m), 2241 (w), 1744 (w), 1601 (w), 1461 (s), 1228 (m), 1177 (s), 1028 (w), 821 (w), 757 (m), 697 (s).
2.3 Results/Discussion

Similar to previous reports with Mn$_{12}$ and Mn$_8$Fe$_4$, ligand exchange was utilized on Mn$_3$bpy to synthesize new substituted analogues.$^{48,49}$ These reactions were carried out using an excess of ligand to ensure complete ligand exchange as shown in the following scheme:

$$\text{Mn}_3(O_2CCH_3)_6(bpy)_2 + 6\text{RCOOH} \rightleftharpoons \text{Mn}_3(O_2CR)_6(bpy)_2 + 6\text{CH}_3\text{COOH}$$

Where $R = -\text{C}_6\text{H}_4\text{CH}=$CH$_2$ (VBA) and -C(CH$_3$)=CH$_2$ (MA). The choice of ligand is crucial, as it has to have a carboxylate group in order to attach itself to the core of the cluster and an olefin for polymerization. This observation was the motivation behind the ligands chosen.

An excess of ligand was used, however, the complete substitution was achieved with only one round of ligand exchange rather than multiple rounds. Another time saving advantage, for the Mn$_3$bpyVBA synthesis, was that the substituted cluster crashed out of solution after approximately 20 minutes. This was markedly improved compared to the Mn$_8$Fe$_4$VBA ligand exchange which would take 4 hours for each ligand exchange, followed by the time needed for the layered solvents to evaporate in order for product precipitation to occur. Much like the ligand exchange reactions of Mn$_8$Fe$_4$, the color of the substituted Mn$_3$bpy cluster (VBA or MA) becoming paler.
2.3.1 *Fourier Transform Infrared Spectroscopy (FTIR)*

To evaluate any ligand substitution, FTIR was used due to its sensitivity towards different types of bonding. Replacing acetates with either VBA or MA, results in a complex with new bond (e.g. C=C). By comparing with the original compound (Figure 2.5), the fingerprint region (400 – 700 cm\(^{-1}\)) is dominated by the Mn-O stretches of the cluster. The presence of the CH out of plane bending vibrations of the bipyridines is exhibited at 771 cm\(^{-1}\) and 649 cm\(^{-1}\),\(^{108}\) At higher frequency, the stretches associated with the carboxylate ligands can be found. Most notably, the asymmetric and symmetric carboxylate stretches appear at 1603 cm\(^{-1}\) and 1477 cm\(^{-1}\) respectively, the characteristic stretches of the –O-C-O bidentate bridging motif,\(^{69,101}\) as well as the partially obscured stretches of the bipyridine rings (1494 cm\(^{-1}\) and 1477 cm\(^{-1}\)) located in the same region.\(^{108}\) Further down, the CH stretches can be found at approximately 3000 cm\(^{-1}\).
Arguably the biggest difference when comparing Mn₃bpy and Mn₃bpyVBA is the splitting of the asymmetric carboxylate stretch at 1603 cm⁻¹ into two noticeably different stretches at 1598 cm⁻¹ and 1551 cm⁻¹ (Figure 2.6). This observation coincides with the addition of a more olefins, the conjugated vinyl group and benzene ring of the VBA, thus splitting the peak. Due to this incorporation, the C=C stretch that was overshadowed by the asymmetric carboxylate stretch, is now clearly visible at 1598 cm⁻¹. Furthermore stretches at 912 and 867 cm⁻¹ appear due to C-H bending vibrations on the olefin based on the successful incorporation of VBA.
As described previously, methacrylic acid was also exchanged onto the cluster. There are noticeable differences when comparing the FTIR spectra (Figure 2.7). Similar to VBA the MA substituted cluster exhibits an asymmetric carboxylate splitting but not as drastic. A shoulder emerges at 1644 cm$^{-1}$ again differentiating the C=C stretch from the asymmetric carboxylate stretch. This stretch corresponds to the addition of a new, non-aromatic and unconjugated vinyl group. It’s this non-aromaticity that makes this peak appear only as a shoulder, at higher wavenumbers, rather than the splitting observed in the Mn$_3$bpyVBA spectrum. The vinyl peak can be differentiated from the other C=C in the bipyridine rings based on this lack of aromaticity. In addition, the number of C=C bonds in MA are less than VBA, reducing the split of the asymmetric carboxylate stretch. Once again, as before, two stretches, 863 cm$^{-1}$ and 831 cm$^{-1}$ appear. As in the case of VBA, this corresponds to the

**Figure 2.6: FTIR comparing Mn$_3$bpy (blue) and Mn$_3$bpyVBA (red).**
successful incorporation of the CH bending vibrations on the olefin due to the incorporation of the MA ligand.

![FTIR comparison](image)

**Figure 2.7: FTIR comparing Mn₃bpy (blue) and Mn₃bpyMA (orange).**

Based on the FTIR analysis, the ligand exchange reactions between Mn₃bpy and the olefin ligands, VBA and MA, were successful after a single reaction using excess of each ligand. Summarized below (Table 2.2) are the comparisons of Mn₃bpy and its substituted analogues Mn₃bpyVBA and Mn₃bpyMA.
Table 2.2: Select FTIR frequencies for substituted Mn₃bpy.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Asym. COO⁻</th>
<th>Sym. COO⁻</th>
<th>Olefin C=C</th>
<th>Olefin C-H Bending</th>
<th>Cluster Stretches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn₃bpy</td>
<td>1603</td>
<td>1477</td>
<td>Not Visible</td>
<td>N/A</td>
<td>649, 511, 415</td>
</tr>
<tr>
<td>Mn₃bpyVBA</td>
<td>1551</td>
<td>1403</td>
<td>1598</td>
<td>912, 867</td>
<td>650, 615, 451</td>
</tr>
<tr>
<td>Mn₃bpyMA</td>
<td>1567</td>
<td>1415</td>
<td>1644</td>
<td>863, 831</td>
<td>649, 509, 412</td>
</tr>
</tbody>
</table>

2.3.2 X-ray Diffraction

X-ray quality single crystals of both new clusters were obtained. Single crystals of Mn₃bpVBA (Figure 2.8) were obtained by slow cooling of a saturated ethanol solution. Single crystals of Mn₃bpMA (Figure 2.9) were obtained after two days by layering hexanes on top of an ethanol solution of Mn₃bpMA. The crystals were collected via vacuum filtration and washed with ethanol.
Mn₃bpyVBA crystallizes in the Triclinic space group P-1. As such the asymmetric unit contains only 1/2 of the molecule with the other half being symmetry generated, as such only Mn₁ and Mn₂ are labeled, giving a Z = 1. Each of the 4-vinylbenzoate ligands are disordered over two sites, hence the large ellipsoids representing the carboxylates surrounding the manganese atoms. The Mn-O, O-C, and C-C bond distances of the disordered groups are restrained to be similar. A co-crystallized disordered ethanol molecule, half of which was in the asymmetric unit, was also found. Despite significant efforts a good refinement model containing this ethanol could not be obtained. As such the solvent molecule was removed via the SQUEEZE subroutine of PLATON. The electron count from the “squeeze” model converged with good agreement with the number of ethanol molecules predicted by

**Figure 2.8:** Thermal ellipsoid plot of Mn₃bpyVBA displayed at the 50% probability level. [Mn: aqua, O: red, N: blue, C: black. Co-crystallized solvents, minor disorder components, and hydrogen atoms omitted for clarity.]
the first refinement model. H atoms were included as riding idealized contributors. H atom U's were assigned as 1.2 times carrier $U_{eq}$. Crystallographic details for Mn$_3$bpVBA can be found in Table 2.3.

\textit{Mn$_3$bpyMA}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Mn3bpyMA_thermal_ellipsoid.png}
\caption{Thermal ellipsoid plot of Mn$_3$bpyMA displayed at the 50\% probability level. [Mn: aqua, O: red, N: blue, C: black. Minor disordered components and hydrogen atoms omitted for clarity.]}\end{figure}

As with the previous cluster, Mn$_3$bpyMA crystallizes in the Triclinic space group P-1. Again the asymmetric unit contains only 1/2 of the molecule with the other half being symmetry generated, as such only Mn$_1$ and Mn$_2$ are labeled, giving a $Z = 1$. Each of the methacrylate ligands are disordered over two sites, hence the large ellipsoids representing the carboxylates surrounding the manganese atoms. The Mn-O, O-C, and C-C bond distances of the disordered groups are restrained to be similar. Methyl H atom positions, R-CH$_3$, were optimized by rotation about R-C
bonds with idealized C-H, R--H and H--H distances. Remaining H atoms were included as riding idealized contributors. Methyl H atom U’s were assigned as 1.5 times carrier U$_{eq}$; remaining H atom U’s were assigned as 1.2 times the carrier U$_{eq}$. Crystallographic details for Mn$_3$bpyMA can be found in Table 2.3.
Table 2.3: Crystal data and structure determination details for Mn$_3$bpyVBA and Mn$_3$bpyMA.

<table>
<thead>
<tr>
<th>Identification code</th>
<th>Mn$_3$bpyVBA</th>
<th>Mn$_3$bpyMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C$<em>{74}$H$</em>{58}$Mn$_3$N$<em>4$O$</em>{12}$</td>
<td>C$<em>{44}$H$</em>{46}$Mn$_3$N$<em>4$O$</em>{12}$</td>
</tr>
<tr>
<td>Formula weight</td>
<td>1360.06</td>
<td>987.67</td>
</tr>
<tr>
<td>Temperature</td>
<td>100(2) K</td>
<td>100(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P-1</td>
<td>P-1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Unit cell dimensions</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a = 11.3148(10) Å</td>
<td>α = 67.6660(10)°</td>
<td>a = 9.418(3) Å</td>
</tr>
<tr>
<td>b = 11.5755(10) Å</td>
<td>β = 76.1500(10)°</td>
<td>b = 10.979(3) Å</td>
</tr>
<tr>
<td>c = 14.1432(13) Å</td>
<td>γ = 77.1910(10)°</td>
<td>c = 12.048(4) Å</td>
</tr>
</tbody>
</table>

| Volume               | 1645.9(3) Å$^3$ | 1093.0(6) Å$^3$ |
| Z                    | 1               | 1               |
| Density (calculated) | 1.372 Mg/m$^3$  | 1.500 Mg/m$^3$  |
| Absorption coefficient | 0.633 mm$^{-1}$ | 0.921 mm$^{-1}$ |
| F(000)               | 701             | 509             |
| Crystal size         | 0.50 x 0.19 x 0.17 mm$^3$ | 0.66 x 0.20 x 0.17 mm$^3$ |
| Theta range for data collection | 1.580 to 24.995° | 1.871 to 24.998° |
| Index ranges         | -13<h<13, -13<k<13, -16<l<16 | -11<h<11, -13<k<13, -14<l<14 |
| Reflections collected | 15728          | 10563          |
| Independent reflections | 5785 [R(int) = 0.0293] | 3835 [R(int) = 0.0325] |
| Completeness to theta = 24.995° | 99.7 %        | 99.7 %        |
| Absorption correction | Integration    | Integration    |
| Max. and min. transmission | 0.7457 and 0.5767 | 0.7457 and 0.6637 |
| Refinement method    | Full-matrix least-squares on F$^2$ | |
| Data / restraints / parameters | 5785 / 1088 / 721 | 3835 / 548 / 457 |
| Goodness-of-fit on F$^2$ | 1.031          | 1.047          |
| Final R indices [I>2sigma(I)] | R1 = 0.0375, wR2 = 0.0928 | R1 = 0.0335, wR2 = 0.0781 |
| R indices (all data) | R1 = 0.0591, wR2 = 0.0997 | R1 = 0.0463, wR2 = 0.0831 |
| Largest diff. peak and hole | 0.409 and -0.258 eÅ$^{-3}$ | 0.334 and -0.278 eÅ$^{-3}$ |
To compare all three clusters (Mn$_3$bpyAC, Mn$_3$bpyVBA, and Mn$_3$bpyMA) select bond lengths are reported (Table 2.4). When comparing the average Mn-O bonds, the lengths differ depending on the manganese (either Mn$_1$ or Mn$_2$) in the asymmetric unit. In the new substituted clusters, the average Mn$_1$-O bond length is larger than the native cluster (Mn$_3$bpyVBA/MA vs. Mn$_3$bpyAC). This is likely due to increased steric hindrance with the new ligands versus the native acetates. It should be mentioned that one Mn-O interaction was much longer (~2.4 Å) than the previously observed bonds. This interaction was considered to be too long and was not considered a real bond. An interesting Mn$_1$-O bonding observation occurs in the Mn$_3$bpyMA structure. The disorder is due to the alternation of mono and bidentate bonding in the methacrylic acid (O5 an O6 in Figure 2.9). The Mn$_1$-N bond length of the unsubstituted Mn$_3$bpy and the Mn$_3$bpyMA cluster has similar lengths. However it is longer in the Mn$_3$bpyVBA cluster. This could be due to the increase in steric hindrance when comparing the bulkier VBA ligand with the relatively smaller acetate and MA ligands. This hindrance limits how the proximity of the VBA ligand to the metal center and is further emphasized when comparing the density of each. Finally the last group of bond lengths is focused on the C-O of the carboxylate ligands. These lengths were similar for all three clusters. This is interesting because one might expect the different ligands to have a varying degree of electron withdrawing groups or steric hindrance.
Table 2.4: Select bond lengths of Mn₃bpy(¹) and the VBA/MA analogues.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Bond Lengths (Å)</th>
<th>Carboxylate Groups (OCO)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mn₁-O</td>
<td>Mn₁-N</td>
</tr>
<tr>
<td>Mn₃bpy(AC)₆</td>
<td>2.067(4)</td>
<td>2.272(4)</td>
</tr>
<tr>
<td></td>
<td>2.101(4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.155(4)</td>
<td></td>
</tr>
<tr>
<td>AVG</td>
<td>2.108</td>
<td>2.254</td>
</tr>
<tr>
<td>Mn₃bpy(VBA)₆</td>
<td>2.257(2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>04A-C: 1.255(4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O4B-C: 1.261(9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>05A-C: 1.293(5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>05B-C: 1.294(6)</td>
</tr>
<tr>
<td>AVG</td>
<td>2.180</td>
<td>2.277</td>
</tr>
<tr>
<td>Mn₃bpy(MA)₆</td>
<td>2.240(2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>04B-C: 1.259(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>05B-C: 1.280(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>06B-C: 1.251(9)</td>
</tr>
<tr>
<td>AVG</td>
<td>2.173</td>
<td>2.259</td>
</tr>
</tbody>
</table>
Before the bulk polymerization experiments could take place, solubility tests for each substituted cluster in a variety of solvents and monomers was done (Table 2.5). Interestingly enough, the solubilities between the two substituted clusters were vastly different. Comparatively, the MA substituted cluster was more soluble in a wider range of solvents than its VBA counter part. The MA substituted cluster appears to have a wider solubility, particularly in polar solvents, compared to the VBA version. Finally this discrepancy in solubility also arises when comparing monomer solubility as MA substituted cluster is soluble in both HEMA and styrene, while VBA substituted cluster is only soluble in HEMA. Previously, Mertzman found that \( \text{Mn}_8\text{Fe}_4(\text{VBA})_{16} \) more cleanly polymerized with styrene compared with \( \text{Mn}_8\text{Fe}_4(\text{MA})_{16} \). Therefore, lack of solubility of \( \text{Mn}_3\text{bpyVBA} \) in styrene posed a potential issue.
Table 2.5: Cluster solubility in various solvents and monomers, HEMA (2-hydroxyethyl methacrylate) and styrene.

<table>
<thead>
<tr>
<th>Solvents (Polarity Index)</th>
<th>Clusters</th>
<th>Clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mn₃bpyVBA</td>
<td>Mn₃bpyMA</td>
</tr>
<tr>
<td>Hexane (0)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Dichloromethane (2.4)</td>
<td>Y</td>
<td>Slightly</td>
</tr>
<tr>
<td>Toluene (2.4)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Benzene (2.7)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Ether (2.8)</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>THF (4.0)</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>Methanol (5.1)</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Acetonitrile (5.8)</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>DI H₂O (9.0)</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>HEMA (N/A)</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Styrene (N/A)</td>
<td>N</td>
<td>Y</td>
</tr>
</tbody>
</table>

When comparing the IR spectrum of the bulk polymerization with that of pure poly(HEMA) and VBA substituted cluster (Figure 2.10), the bulk polymerization matches almost perfectly with that pure polymer. The broad stretch at ~3400 cm⁻¹ indicates the presence of an OH group. A defining feature of the VBA substituted cluster is the presence of vinyl group stretch at ~1600 cm⁻¹ as highlighted (Figure 2.10). When compared to the bulk polymerization, this defining stretch disappears, which indicates a successful polymerization. Based on these
results, the incorporation of VBA substituted cluster into bulk HEMA was conclusively achieved. Preliminary TGA results also indicate an incorporation of cluster (Figure 2.11) within the polymer, given at the same temperature, the weight percents were different indicating something, presumably a metal oxide, was present. It is worth noting, however, that when looking at the polymer product that the color is not uniform throughout. This indicates a heterogeneous mixing of cluster within the polymer.

Figure 2.10: Overlay of Mn₃bpyVBA + HEMA (blue), pure poly-HEMA (orange), and Mn₃bpyVBA cluster (yellow). The highlighted region shows the loss of the vinyl group stretches comparing Mn₃bpyVBA (yellow) and Mn₃bpyVBA + HEMA (blue). Inset: picture shows the Mn₃bpyVBA + HEMA after polymerization.
As with the VBA + HEMA, the co-polymerization of Mn₃bpyMA + HEMA and pure poly(HEMA) spectra look almost identical. The broad peak at ~3400 cm⁻¹ is associated with the hydroxyl group of the polymer. Because of the olefin, the MA substituted cluster also has the signature vinyl IR stretches as the VBA cluster does. As before the disappearance of the vinyl group stretch at ~1600 cm⁻¹ indicate that the olefin has indeed polymerized (Figure 2.12). Preliminary TGA results, also seem to indicate an incorporation of cluster (Figure 2.13) within the polymer, given at the same temperature, the weight percents were different indicating something, presumably a metal oxide, was present. Similarly, the polymer lacks color uniformity seemingly indicating the heterogeneous mixing of cluster within the polymer.

Figure 2.11: Thermogram comparing poly-HEMA (blue) and Mn₃bpyVBA + HEMA (yellow) suggesting cluster incorporation.
With the final bulk polymerization, Mn$_3$ bpyMA + styrene, as with the previous polymerizations the disappearance of the vinyl group stretches at $\sim$1600 cm$^{-1}$ and $\sim$1400 cm$^{-1}$ indicate the cluster has polymerized as expected (Figure 2.14).
Characteristic stretching assignments suggest the presence of a mono-substituted benzene, which would be the styrene itself. As there is no hydroxyl group in styrene, no stretch at ~3400 cm\(^{-1}\) is expected nor observed. Harder to see in Figure 2.14, the color of the polymer lacks uniformity, again suggesting a heterogeneous mixing of cluster incorporation within the polymer.

![Figure 2.14: Overlay of Mn\(_3\)bpyMA + styrene (blue), pure poly-styrene (orange), and Mn\(_3\)bpyMA cluster (yellow). The highlighted region shows the loss of the vinyl group stretches comparing Mn\(_3\)bpyMA (yellow) and Mn\(_3\)bpyMA + styrene (blue). Inset: picture shows the Mn\(_3\)bpyMA + styrene after polymerization.](image)

### 2.4 Conclusions

The ligand substitution of Mn\(_3\)bpy, to our knowledge, has not been reported yet and as such is first to be reported here using 4-vinylbenzoate (VBA) and methacrylate as the new ligands. As such the synthesis and subsequent
characterization are reported in this chapter. The ligand exchange reactions, once finely tuned, proved easier and more straightforward than the previously explored \( \text{Mn}_{8}\text{Fe}_{4}\text{VBA} \) and \( \text{Mn}_{8}\text{Fe}_{4}\text{MA} \) clusters. In addition to two new crystal structures via single crystal x-ray diffraction, FTIR and elemental analysis confirm the full substitution.

Further studies were done to explore the viability of polymer nanoparticle encapsulation of each \( \text{Mn}_3\text{bpy} \) substituted cluster. The solubilities of each cluster were wildly different as the VBA substituted cluster dissolved in very few solvents, with no apparent trend, and only one of the chosen monomers. Likewise, the MA substituted cluster was soluble in many more solvents, primarily polar in nature, and both the chosen monomers for potential polymer nanoparticles.

To further investigate nanoparticle viability, bulk polymerization experiments were attempted to see if either cluster would co-polymerize. These reactions were characterized using FTIR and TGA. Based on these results, the cluster/monomer combinations all seemed to successfully polymerize. One major drawback, however, was that all three showed a heterogeneous distribution of metal content. Any potential nanoparticles containing either compound should be homogenously distributed to maximize contrast agent ability.
CHAPTER 3: MESOPOROUS SILICA NANOPARTICLES

3.1 INTRODUCTION

Due to the interesting properties materials possess on the nanoscale, the use and study of nanotechnology has vastly increased the last thirty years since the time of Richard Feynman and Donald M. Eigler, two of the pioneering scientists of the field. Quite possibly the biggest impact nanotechnology has had is in medicine. Nanomedicine is the application of nanotechnology towards human health. This encompasses the diagnosis, treatment, and monitoring of diseases and other health related concerns. These materials are needed more than ever as anti-bacterial properties of certain nanomaterials are needed to combat the emergence of superbugs, such as certain strains of E. coli and salmonella, become increasingly more resistant to last resort anti-biotics.

While today nanomaterials are being used to fight pathogens, these materials have previously played a vital role in contrast agent development and overall improvement. As discussed previously the most prevalent metal used in modern $T_1$ contrast agents is Gd$^{3+}$. While the contrast ability of this metal is unquestioned, it has many undesirable side effects. The most troubling being that free Gd$^{3+}$ is toxic, having no metabolic pathway. Symptoms include splenic degeneration, enzyme inhibition, liver necrosis, interference with synapses, and calcium channel blockage. Contrast agents containing Gd$^{3+}$ have also been linked to nephrogenic
systemic fibrosis (NSF), a harmful side effect for patients with renal issues, associated with free Gd$^{+3}$ released from its chelate.$^{34,36-40}$ As such arguably the biggest detriment to contrast agents containing Gd$^{+3}$ is the potential for metal leaching and this leading to metal poisoning. This has caused an influx in the study of nanomaterials containing Gd$^{+3}$ rather than using the multi-dentate chelates to entrap the Gd$^{+3}$ ion(s) in current clinical use. Some examples include gadolinium oxide nanoparticles, silica nanoparticles where the Gd$^{+3}$ is covalently anchored to the pore of the silica particle, and fullerenes containing Gd$^{+3}$ ions (Figure 3.1).$^{109-111}$


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Another commonly investigated class of nanomaterials for contrast agent applications is iron oxide nanoparticles. These metal oxide nanoparticles are a standard T₂ contrast agent. Iron oxide nanoparticles have been observed in biological systems, as geomagnetic navigational aids and as iron storage proteins.\textsuperscript{112,113} While these materials do not suffer from the toxicity issues seen in Gd\textsuperscript{3+} contrast agents, they are not without disadvantages. Typically these materials suffer from a heterogeneous distribution of metal content leading to inefficient contrast. Additional problems include: 1) the particles suffer from broad particle size distribution, 2) are easily oxidized to antiferromagnetic Fe\textsubscript{2}O\textsubscript{3}, and 3) readily agglomerate in the presence of a magnetic field and in close proximity to each other. These all diminish the usefulness of these kinds of iron oxide nanoparticle contrast agents.\textsuperscript{35,41}

An ideal contrast agent should have a number of important qualities, including but not limited to a high number of unpaired electrons, low toxicity, solution stability, easily modifiable, and biocompatible.\textsuperscript{33} Arguably the most important characteristic for any contrast agent is the type of agent it is. A T\textsubscript{1} contrast agent is more valuable than a T\textsubscript{2} because of the brightening effect associated with T\textsubscript{1} agents. For the purposes of oncology research, a critical consideration for our contrast agent design methodology, the ability to synthesize agents with a homogenous metal content and tunable size range is also important.
Contrast agents are typically administered intravenously as this is the most efficient method to reach any particular target organ or tissue via the circulatory system.\textsuperscript{114,115} Because of this, the size of the contrast agent in question is crucial because it controls, in part, biodistribution. When using metal-oxo clusters, the relatively small size of the cluster becomes a crucial factor. This is due to rapid excretion, leading to low circulation time, and thus inefficient cellular uptake. This is also the reason why Gd\textsuperscript{3+} does not work for longer research applications.

Previous work by Pablico has shown how utilizing nanomaterials, specifically polystyrene nanobeads, can successfully crosslink the Mn\textsubscript{8}Fe\textsubscript{4} cluster.\textsuperscript{48,49} This was done by utilizing ligand exchange reactions to form olefin type clusters to be used in a miniemulsion polymerization reaction (Figure 3.2). The incorporation of the cluster into the nanobeads was paramount as many previous issues were solved. The complete homogenous encapsulation of the cluster severely limits the possibility of metal leaching. As mentioned previously, the increase in contrast agent size allows for longer circulation time as well as a method to control particle diameter using the miniemulsion synthesis. This limits how fast the body can excrete these particles thus increasing the efficiency of cellular uptake and contrast agent biocompatibility and viability. The miniemulsion technique also allows for possible surface functionalization, leading to further enhancements being made. The cluster encapsulation into the nanobeads had an unforeseen impact on the relaxivity. The polystyrene beads with encapsulated cluster exhibited a lower $r_2/r_1$ and thus became more of a $T_1$ contrast agent compared to free cluster (Figure 3.3).
Figure 3.2: Substituted \( \text{Mn}_8\text{Fe}_4 \) cluster, \( \text{Mn}_8\text{Fe}_4\text{VBA} \) (Right) and the TEM of the polystyrene nanobeads containing the substituted cluster (Reprinted (2016) with permission from (Pablico, M. H.; Mertzman, J. E.; Japp, E. A.; Boncher, W. L.; Nishida, M.; Van Keuren, E.; Lofland, S. E.; Dollahon, N.; Rubinson, J. F.; Holman, K. T.; Stoll, S. L. *Langmuir* 2011, 27, 12575.). Copyright (2011) American Chemical Society.)).

Figure 3.3: The change in contrast intensity comparing bare \( \text{Mn}_8\text{Fe}_4 \) (left) with \( \text{Mn}_8\text{Fe}_4\text{VBA}-\text{co-polystyrene nanobeads} \) (right). The \( T_1 \) increases while the \( T_2 \) decreases drastically (Reprinted (2016) with permission from (Pablico-Lansigan, M. H.; Hickling, W. J.; Japp, E. A.; Rodriguez, O. C.; Ghosh, A.; Albanese, C.; Nishida, M.; Van Keuren, E.; Fricke, S. T.; Dollahon, N.; Stoll, S. L. *ACS Nano* 2013, 7, 9040.). Copyright (2013) American Chemical Society.)).
While many problems were solved using the miniemulsion method to generated Mn₈Fe₄ encapsulated polystyrene nanobeads, this system still suffers from several disadvantages. Although homogenous, much of the metal within the bead is wasted as only the cluster closest to the surface is utilized in the relaxation process. This encapsulation also poses another serious drawback, as the metal-proton contact is completely limited. This rapid water exchange interaction is a critical relaxation pathway the polystyrene nanobead system cannot employ.

In this chapter, the utilization of mesoporous silica nanoparticles will be explored as a viable technique to entrap the metal-oxo cluster, Mn₈Fe₄. As the previous work by Pablico has shown, the contrast properties of the native cluster changed from a T₂ contrast agent to a T₁ contrast agent when encased in the polystyrene nanoparticles. The use of mesoporous silica should optimize the efficiency of the anchored Mn₈Fe₄. By anchoring Mn₈Fe₄ into the pore, leaching is still prevented. The exposure of the cluster to the environment coupled with the structural porosity allows for water to move freely. This exposure should allow for rapid water exchange, a key relaxation mechanism severely hindered for the polystyrene nanobead system, for increased relaxivity. As such the use of mesoporous silica nanoparticles will be examined to see if further improvements can be made by exposing the Mn₈Fe₄ cluster to the environment while still being firmly bond to the silica mesopore.
3.2 Experimental

Manganese(II) acetate tetrahydrate, potassium permanganate, acetone, ethanol (200 proof), sodium hydroxide, cetyltrimethylammonium bromide, tetraethyl orthosilicate, triethanolamine, (2-cyanoethyl)triethoxysilane, and concentrated hydrochloric acid were all purchased from Sigma Aldrich and used as received. Concentrated acetic acid and sulfuric acid were purchased from EMD and JT Baker respectively and also used as received.

Fourier-transform infrared spectroscopy (FTIR) measurements were recorded in the range 4,000-400 cm\(^{-1}\), from pressed pellets in KBr on a Perkin Elmer Spectrum Two. Diffraction patterns were obtained using a Rigaku Ultima IV X-ray powder diffractometer with CuK\(\alpha\) radiation at 40 kV and 44 mA with a scintillation counter detector, a fixed monochromator, a focusing method slit of 285mm, and no filter attachment. Nitrogen adsorption and desorption isotherms were obtained at 77K using a Quantachrome Autosorb-1 MP. Unmodified silica nanoparticle samples were outgassed at 573K for 19 hrs before measurements were performed. Modified silica nanoparticles containing Mn\(_8\)Fe\(_4\) were outgassed for 4 hrs at 328K. Specific surface area values were obtained using the Brunauer-Emmett-Teller (BET) equation.

Metal content of the cluster loaded MCM41/MSNs was determined by inductively coupled plasma mass spectrometry (ICP-MS, Aglient 7700x). A series of freshly made standards (1000ppm standards of Mn, Fe, Si purchased from Fluka) with
known concentrations (5 ppm, 3 ppm, 2 ppm, 1.5 ppm, 1 ppm, 0.75 ppm, 0.5 ppm, and 0.05 ppm) were made for both metals and silicon. These standards were used to construct a calibration curve (counts per second [CPS] vs. concentration). The silica nanoparticles containing Mn$_8$Fe$_4$ samples were prepared by making a 1mg/1mL solution in concentrated HCl. This solution was then diluted to 100 ppm in deionized water. The corresponding metal content was determined by calculating the concentration of metal using the equation generated from the calibration curve.

SEM images were taken with a Zeiss SUPRA 55-VP scanning electron microscope, at an acceleration voltage of 20 kV with an in-lens detector. High resolution TEM was performed with a JEOL JEM 2100F Field Emission Gun Transmission Electron Microscope, at 200 kV, at the University of Maryland Nanoscale Imaging Spectroscopy and Imaging Laboratory.

NMR relaxation data were obtained using a Bruker AM 300 MHz spectrometer interfaced with a TecMag DSpect acquisition system. The proton relaxivities of cluster loaded silica particles were measured in D$_2$O. Fresh solutions were prepared (ranging from 0.015 mM metal to 0.12 mM metal) immediately prior to use. This was done by dissolving, with sonication, the necessary dried Mn$_8$Fe$_4$ containing silica powder. T$_1$ measurements were recorded using the saturation recovery pulse sequence. The T$_2$ values were measured using a conventional spin echo sequence. The relaxivity values ($r_1$ or $r_2$) were determined by plotting the $1/T_i$ (where $i = 1$ or 2) versus metal concentration. This relationship is based of the following equation:
\[
\frac{1}{T_{i, \text{observed}}} = r_i [CA] + \frac{1}{T_{i, \text{tissue}}}
\]

Where \(1/T_{i, \text{observed}}\) is the relaxation rate with cluster present and \(1/T_{i, \text{tissue}}\) is the relaxation rate sans cluster.

**Mn\textsubscript{8}Fe\textsubscript{4}(Ac)\textsubscript{16}** \([\text{Mn}\textsubscript{8}\text{Fe}\textsubscript{4}\text{O}_{12}(\text{O}_2\text{CCH}_3)_{16}(\text{H}_2\text{O})_4]\). This metal-oxo cluster was prepared in accordance to the literature.\textsuperscript{48,49,102} Potassium permanganate (6.4 mmol) was added to a slurry of iron(II) acetate (16.3 mmol) in 40 mL 60\% (v/v) acetic acid/H\textsubscript{2}O and slowly heated to 60°C. The resulting reddish-brown solution was subsequently cooled to room temperature, layered with 40 mL acetone and allowed to stand undisturbed for several days. The resulting black crystals were collected via vacuum filtration, washed with acetone and dried. Found (Calculated): C, 21.56 (20.95); H, 3.43 (3.52). IR (cm\textsuperscript{-1}): 3441 (br), 1709 (m), 1548 (s), 1452 (s), 1349 (m), 707 (s), 644 (m), 616 (m), 565 (m), 538 (m).

**MCM41 nanoparticles.** The particles were prepared in accordance to the literature.\textsuperscript{117} Distilled water (240 mL) and a 2M solution of sodium hydroxide (1.75 mL) are combined with stirring. Cetyltrimethylammonium bromide (0.5 g, 1.4 mmol) was added with stirring and heated to 80°C. Tetraethyl orthosilicate (2.5 mL, 11.2 mmol) was added dropwise. The clear solution then becomes white. The solution was stirred for 2 hours, filtered and washed with 100 mL of distilled water.
The white product was allowed to dry on the bench top overnight. MCM-41 nanoparticles are calcined at 550°C for 8 hours to remove the organic template.

**Radially MS nanoparticles.** These particles were made in accordance with the literature with only slight modifications. Distilled water (16.02 mL) and absolute ethanol (1.84 mL) are combined with stirring. Cetyltrimethylammonium bromide (0.64 g, 1.77 mmol) was added with stirring and heated to 80°C. Triethanol amine (1.03 g, 6.9 mmol) was added and mixed until dissolved. Tetraethyl orthosilicate (1.454 mL, 6.5 mmol) was added dropwise. The clear solution then becomes white. The solution was stirred for 2 hours, filtered and washed with 100 mL of distilled water. The white product was allowed to dry on the bench top overnight. MCM-41 nanoparticles are calcined at 550°C for 8 hours to remove the organic template.

**Nitrile Modification:** The particles were prepared in accordance with the literature with only slight modifications. The mesoporous silica nanoparticles (either MCM41 or MS nanoparticles, 100 mg) was added to a Schlenk flask. The flask was then heated under vacuum with stirring overnight to remove any adsorbents on the silica surface. Then, while under a nitrogen atmosphere, anhydrous toluene (25 mL) was added to the flask. Once suspended, (2-cyanoethyl)triethoxysilane (3.3 mL, 14.75 mmol) was added drop wise and the suspension was allowed to stir continuously for 24 hours at room temperature. After which the suspension was collected and centrifuged. The pelleted particles were then redispersed in toluene, sonicated, and then collected again. This process was repeated for a total of 3 times.
after which the last toluene washed sample was washed once with ethanol (200 proof) and allowed to dry at room temperature.

**Carboxylate Modification:** Once dried the aforementioned nitrile modified silica particles (MCM41 or MS nanoparticles) are added to a solution of 50% sulfuric acid and allowed to reflux for approximately 3 hours. After, the suspension was collected and the particles were collected via centrifugation. The pelleted particles were then redispersed in deionized water and then collected. This process was repeated for a total of 3 times after which the last water washed sample was washed once with ethanol (200 proof) and allowed to dry at room temperature.

**Mn₈Fe₄(Ac)₁₆ Anchoring:** The particles were prepared in accordance with the literature with only slight modifications.¹⁰³ A solution of Mn₈Fe₄(Ac)₁₆ was made by dissolving cluster (20 mg) in acetonitrile (10 mL). Carboxylated modified silica particles (MCM41 or MS nanoparticles, 100 mg) were then added to the concentrated cluster solution. This was allowed to stir at room temperature for approximately 2 hours to ensure enough time for the ligand exchange reaction to take place. After which the suspension was collected via centrifugation with the dark acetonitrile solution decanted. The now brown powder was redispersed in fresh acetonitrile via sonication. This process was repeated for a total of 3 times after which the last acetonitrile washed sample was washed once with ethanol (200 proof) and allowed to dry at room temperature.
The first type of mesoporous silica nanoparticle synthesized was MCM41, first synthesized by the Mobil Corporation in the early 1990s. The MCM41 nanoparticles are synthesized via a modified Stöber process. The synthesis entails the hydrolysis and condensation of a silica source in a basic solution (Figure 3.4). This is done by adding a silica source, usually an organic silane TEOS, to an aqueous solution containing a surfactant that acts as a template for pore formation. The surfactant a modification of the Stöber process, which is used to prepare porous materials. A base is used to catalyze the reaction and allow the silica source to hydrolyze around the charged head group of the surfactant micelles. During this process the silicate micelles self-assemble into cylinders and the silica continues to condense forming the final structure. Over time, the silica matrix is stable and the templating surfactant can be removed via calcination, reflux in acidified ethanol and/or an ammonium nitrate solution, or reflux in a high boiling point organic solvent such as TOPO. For the purposes of this chapter, all silica nanoparticles were calcined in order to remove the surfactant molecules.
The porous nature of mesoporous silica nanoparticles is advantageous because it allows for a site for metal-oxo cluster anchoring, thus limiting leaching, while still allowing for water from the environment to interact with the metal center. This interaction should allow for an increase in relaxivity compared to the polystyrene nanobeads. A second type of mesoporous silica nanoparticle was synthesized. This type, herein referred simply as radial MSNs to differentiate from MCM41, improves on MCM41 by having many more pores per particle. The increase in pore number allows for more cluster per bead because the cluster plugs the pores. This increase in metal concentration as well as a more uniform coverage of the silica particle as a whole should lead to even higher relaxivities than both MCM41 and the polystyrene nanobeads.

As synthesized, the particle surface contains silanol (Si-OH) groups. In order to graft Mn₈Fe₄ into the pore openings, the surface of the pore needs to be

Figure 3.4: Hydrolysis and condensation of a silica precursor, TEOS, under basic conditions. (Reproduced with permission from the Royal Society of Chemistry)
chemically modified. The goal is to create a carboxylate surface for ligand exchange. Unfortunately, it is not possible to obtain a carboxylate silane directly since it would self condense. Therefore an appropriate modifiable silane, in this case a nitrile group, is chosen so that it can be converted to a carboxylate after the silane has reacted with the surface (Figure 3.5). It is this final step that allows for the anchoring of Mn₈Fe₄ onto the surface as simple ligand exchange, similar to before. The only difference being that the nanoparticle is modified to accommodate the cluster rather than the cluster for the nanoparticle. The attachment is a condensation reaction between the hydrogen of the silanol surface and the leaving group of the new silane. Depending on what is originally bound to the reacting silane, alcohols (ethanol or methanol), hydrogen chloride gas, etc. can be generated. Ultimately the silane used for this step, (2-cyanoethyl)triethoxysilane, was chosen because the leaving group generated (ethanol) was less potentially hazardous and the reactant was more readily available for purchase compared to other silanes. The final modification necessary prior to ligand exchange was oxidation of the nitrile group into carboxylates. This was done by taking the nitrile surface modified particles and refluxing them in 50% sulfuric acid. This aqueous acidic environment would then allow for successful modification to occur. Once modified carboxylate-modified particles could be used in ligand exchange reactions with Mn₈Fe₄ to generate the final desired product.
Figure 3.5: Reaction summary of Mn$_8$Fe$_4$ attachment to silica nanoparticles. **Top:** Conversion of Si-OH groups to a nitrile group using CTES ((2-cyanoethyl)triethoxysilane). **Middle:** Conversion of the nitrile group into a carboxylate group via 50% H$_2$SO$_4$ (aq) under reflux. **Bottom:** Ligand exchange between carboxylate groups on silica surface and Mn$_8$Fe$_4$ cluster in acetonitrile solution.
As with the substituted clusters of Chapter 2, FTIR was used in order to monitor successful silica particle modifications. The spectrum of unmodified silica nanoparticles is relatively simple (Figure 3.6). The silanol (Si-OH) stretch appears at \(~3400\ \text{cm}^{-1}\). The Si-O-Si stretch then appears at \(~1100\ \text{cm}^{-1}\). Then, at \(~400\ \text{cm}^{-1}\), the Si-O stretch appears. It should be noted that this spectrum was taken after calcination, at which point the surfactant has been removed. This is evident as if the surfactant had not been removed successfully, the presence of CH stretches at \(~2900\ \text{cm}^{-1}\) and a Si-O-C stretch of the Si-O-Si peak would appear at \(~1200\ \text{cm}^{-1}\). Since neither of these peaks is present, it can be concluded that surfactant has been successfully removed.

![Figure 3.6: FTIR spectrum of calcined and unmodified silica nanoparticles.](image)
Since the unmodified silica nanoparticles FTIR spectrum is simple, the addition of any new surface moieties and subsequent modifications are easily observed (Figure 3.7). The green spectrum represents the nitrile modification step. A new stretch at ~2250 cm\(^{-1}\) appears assigned as the carbon-nitrogen nitrile stretch.\(^{120-124}\) After that, the green spectrum shows the transformation from a nitrile terminated group to a carboxylate. The carbon-nitrogen nitrile stretch disappears and the asymmetric and symmetric carboxylate stretches appear. Finally the red spectrum shows successful ligand exchange. The same asymmetric and symmetric carboxylate stretches are now broader and more distorted, indicative of successful bonding between the carboxylated surface of the silica nanoparticle and the cluster as well as the acetates on the Mn\(_8\)Fe\(_4\) contribution. Finally an increase in intensity is observed in the CH stretches region at ~3000 cm\(^{-1}\) further suggesting the presence of the Mn\(_8\)Fe\(_4\) cluster on the silica surface.
While the silica is not crystalline, the repeating packing of tubes allows for characterization using low angle powder x-ray diffraction (PXRD) for both unmodified and modified samples of MCM41 (Figure 3.8). PXRD does not work for the radial MSNs since the distribution of the pores of that particle are random and not periodically repeated.

**Figure 3.7:** FTIR spectra of modified silica nanoparticles. The first shows the successful modification of silica surface with nitrile groups (orange, bottom). This is followed by the conversion of the nitrile groups into carboxylate groups (green, middle) and successful ligand exchange with Mn$_8$Fe$_4$ (red, top). Highlighted regions show new stretches that appear, disappear, or increase in intensity.

3.3.2 **Powder X-ray Diffraction (PXRD)**

While the silica is not crystalline, the repeating packing of tubes allows for characterization using low angle powder x-ray diffraction (PXRD) for both unmodified and modified samples of MCM41 (Figure 3.8). PXRD does not work for the radial MSNs since the distribution of the pores of that particle are random and not periodically repeated.

**Figure 3.8:** Graphical representation of the hexagonal mesoporous array that allows for PXRD characterization of MCM41 (Reprinted (2016) with permission from (Beck, J. S.; Schmitt, K. D.; Higgins, J. B.; Schlenkert, J. L. J. Am. Chem. Soc. 1992, 114, 10834. Copyright (1992) American Chemical Society.)$^{125}$)
This technique is used to confirm that the hexagonal mesoporous array is maintained. This is due to the periodic variation of electron density between the empty space of the pore and the pore wall. This is an important observation because this is the basis for using PXRD to further monitor the sequential modification of the MCM41 (Figure 3.9). As the pore becomes plugged the differences in electron density between the wall and the pore decreases. Therefore the intensities of the powder patterns should decrease with each modification in size order. This is exactly what is seen in Figure 9. Unmodified MCM41 (blue pattern) has the highest difference between electron density thus it has the most intense peak. The remaining patterns (orange, green, and red) have the electron density differences decrease and thus the overall peak intensity decreases with each modification reaching a minimum with the last modification, ligand exchange with Mn₈Fe₄.
Gas sorption studies were done in order to determine the pore diameter, pore volume, and surface area of porous materials. These experiments support the structure and bonding of Mn$_8$Fe$_4$ to the pore. Successful plugging of the pore would decrease the surface area, pore volume, and pore diameter. If there is no change in these parameters, it could mean the Mn$_8$Fe$_4$ cluster is attached to the surface rather than within the mesopore entrance. In these types of experiments typically nitrogen gas is used. This is because it is cheap, readily available, and since it has a permanent quadrupole, forms a well-defined monolayer on surfaces. In a typical experimental setup, a small amount of solid sample (~0.20 g) is placed in a sample tube and outgassed in order to remove any adsorbed water or other atmospheric gases. The tube is then evacuated under vacuum and placed in a liquid nitrogen
reservoir. Nitrogen gas is then introduced incrementally while the volume of gas added and equilibrium pressure is recorded. The resulting isotherm is plotted as volume of gas added (cm$^3$/g) versus the partial pressure ($P/P_0$). The mesoporosity of the MCM41 and MCM41-Mn$_8$Fe$_4$ nanoparticles was then determined from the adsorption-desorption isotherms generated (Figure 3.10).

![Figure 3.10: Nitrogen isotherm of MCM41 (blue) and MCM41-Mn$_8$Fe$_4$ (red).](image)

The hysteresis loop exhibited in Figure 3.10 is characteristic of type IV isotherms, typical of mesoporous materials.$^{126,127}$ The loop indicates the presence of open ended cylindrical pores, as expected for MCM41. With the addition of Mn$_8$Fe$_4$ the original pore diameter, pore volume, and surface area all decrease (Table 3.1).
Table 3.1: Data determined from nitrogen isotherms comparing MCM41 and MCM41-Mn₈Fe₄.

<table>
<thead>
<tr>
<th></th>
<th>MCM41</th>
<th>MCM41-Mn₈Fe₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET Surface Area (m²/g)</td>
<td>1397.5</td>
<td>772.5</td>
</tr>
<tr>
<td>Pore Volume (cc/g)</td>
<td>2.939</td>
<td>1.084</td>
</tr>
<tr>
<td>Pore Diameter (nm)</td>
<td>2.9775</td>
<td>1.734</td>
</tr>
</tbody>
</table>

The decreases in surface area, pore volume, and pore diameter when comparing unmodified MCM41 with MCM41-Mn₈Fe₄ suggests the successful plugging of the mesopores with Mn₈Fe₄ cluster. These observations coupled with the PXRD and FTIR data are consistent with the successful modification and attachment of Mn₈Fe₄.

3.3.4 Relaxivity

As discussed, contrast agents efficacy is measured by their relaxivities, r₁ and r₂. Relaxivity is determined through a linear relationship between the inverse T₁ or T₂ versus concentration. The slope of this relationship is rᵢ, where i = 1 or 2 depending on which of T₁ or T₂ is being measured. These values measure how much contrast agent concentration affects the relaxation rate; hence the units are mM⁻¹ s⁻¹. The higher the value the better the contrast agent. The r₂/r₁ ratio is used as a standard to determine whether a potential contrast agent will behave more like a T₂ contrast agent or a T₁. The higher this ratio the more likely that contrast agent will be a T₂ agent, likewise the lower the value the more likely it is a T₁. These types of
experiments were done on a number of MCM41-Mn\textsubscript{8}Fe\textsubscript{4} samples. As expected the final results yielded \( r_1 \) and \( r_2 \) values much higher than Mn\textsubscript{8}Fe\textsubscript{4} alone (Figure 3.11).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.11}
\caption{Average relaxivity values for MCM41-Mn\textsubscript{8}Fe\textsubscript{4}.}
\end{figure}

Upon further inspection, and clearly indicated by the error bars, the values were wildly inconsistent, even though the metal content was determined for each sample and each subsequent stock solution was made with the same starting concentration, 0.12 mM metal (Table 3.2). The corresponding metal per bead also indicated no reason for the large range in values, as all were roughly what would be expected.
Table 3.2: Individual relaxivity values for MCM41-Mn8Fe4 samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>mg metal/mg silica</th>
<th>Relaxivity (mM⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r₁</td>
</tr>
<tr>
<td>1</td>
<td>0.0208</td>
<td>30.2 ±1.8</td>
</tr>
<tr>
<td>2</td>
<td>0.0170</td>
<td>24.0 ±1.2</td>
</tr>
<tr>
<td>3</td>
<td>0.0174</td>
<td>21.9 ±1.0</td>
</tr>
</tbody>
</table>

To ensure the discrepancies were not originating from the instrumentation, relaxivity values of Gd-DTPA were determined and compared (Figure 3.12 and Table 3.3). The same stock solution was used for three separate experiments (Gd-DTPA_1) along with two independent samples (Gd-DTPA_2 and Gd-DTPA_3). The
values of each sample set or sample fit relatively well within the range of what is reported in the literature.

Table 3.3: Experimentally determined relaxivity values of Gd-DTPA using the same sequences as were used for the MCM41-Mn$_8$Fe$_4$ above. Literature range is presented in the last row.\textsuperscript{33,109–111,128,129}

<table>
<thead>
<tr>
<th>Sample</th>
<th>Trial</th>
<th>Relaxivity</th>
<th>$r_2/r_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$r_1$</td>
<td>$r_2$</td>
</tr>
<tr>
<td>Gd-DTPA_1</td>
<td>1</td>
<td>5.17±0.09</td>
<td>3.00±0.15</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.08±0.07</td>
<td>2.76±0.21</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.78±0.13</td>
<td>2.85±0.06</td>
</tr>
<tr>
<td>Gd-DTPA_2</td>
<td>-</td>
<td>5.46±0.44</td>
<td>2.51±0.08</td>
</tr>
<tr>
<td>Gd-DTPA_3</td>
<td>-</td>
<td>4.95±0.12</td>
<td>2.45±0.06</td>
</tr>
<tr>
<td>Literature Range</td>
<td>-</td>
<td>3.1–4.7</td>
<td>4.2–6.1</td>
</tr>
</tbody>
</table>

An experiment similar to Gd-DTPA_1 was done whereby the same batch of MCM41, synthesized in bulk then divided into 3 samples for the final step: was used as it was surmised that even though separately synthesized batches could vary, one bulk synthesis divided with only the cluster attachment remaining, should provide some consistency in the relaxivity measurements. These experiments were done without measuring the metal content prior to the NMR experiments. This was done purposely as the entire point of the experiment was to determine if the bulk
substituted samples had, for the same mass and volume of D₂O, the same relaxivities. Having the same relaxivities would have conclusively proven that the discrepancy of the original relaxivity values was due to particle variance from sample to sample of the MCM41. This, however, was not the case as the calculated relaxivities still varied (Table 3.4) even though the same mass and volume were used for all measurements. Although sample 1 was repeated a second time, freshly made, since it was the most egregious of the three bulk samples and the values (r₁ = 13.03 ± 0.34 mM⁻¹ s⁻¹ and r₂ = 190.21 ± 0.34 mM⁻¹ s⁻¹) compared favorable, relatively speaking, to the original relaxivities of that sample.

Table 3.4: Bulk synthesis values for MCM41-Mn₈Fe₄ samples.

<table>
<thead>
<tr>
<th>Bulk MCM41-Mn₈Fe₄</th>
<th>Relaxivity</th>
<th>r₂/r₁</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r₁</td>
<td>r₂</td>
</tr>
<tr>
<td>Sample 1</td>
<td>10.76 ± 0.26</td>
<td>151.77 ± 13.35</td>
</tr>
<tr>
<td>Sample 1_1</td>
<td>13.03 ± 0.34</td>
<td>190.21 ± 0.34</td>
</tr>
<tr>
<td>Sample 2</td>
<td>3.08 ± 0.18</td>
<td>33.99 ± 13.62</td>
</tr>
<tr>
<td>Sample 3</td>
<td>2.13 ± 0.14</td>
<td>10.19 ± 6.66</td>
</tr>
<tr>
<td>Avg.</td>
<td>7.25</td>
<td>96.54</td>
</tr>
</tbody>
</table>
Given the ever-present inconsistencies in the relaxivity measurements, even with bulk synthesis and sampling, experiments were devised to try and determine the root cause of these variations. Two hypotheses were developed. First the metal content, even given the bulk modifications, could still vary sample to sample when divided for $\text{Mn}_8\text{Fe}_4$ attachment due to the uncontrollable nature of such reactions. This would mean that the metal content for each sample could actually be different and thus the value discrepancies are because of varying metal content. The second hypothesis was also contingent on there being variances in the bulk modification despite vigorously trying to maintain consistency. This MCM41 synthesis had previously been shown to produce nanoparticles ranging in size, from 45 to 85 nm. Different sized nanoparticles would cause a variance in relaxivity because changes in density and rotational correlation time would change with nanoparticle size.

The samples selected for further analysis were sample 1/1_1 and sample 3 with the goal of trying to deduce what about sample 1 makes the relaxivity so much higher than sample 3. ICPMS was performed on each sample to determine if there was an abnormal amount of metal in each iteration of sample 1 versus sample 3 (Table 3.5). Based on these results it is evident that the metal content of each sample is not the reasoning behind the variance in relaxivity as the metal per bead is essentially equal for each.
Table 3.5: Metal concentration of select bulk MCM41-Mn₈Fe₄ nanoparticles.

<table>
<thead>
<tr>
<th>Sample</th>
<th>mg metal/mg silica</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.347434705</td>
</tr>
<tr>
<td>1_1</td>
<td>0.347087923</td>
</tr>
<tr>
<td>3</td>
<td>0.346703192</td>
</tr>
</tbody>
</table>

The TEM images of sample 1, 1_1, and 3 are shown in Figure 3.13. Based on these images there are no real clues if the size of the nanoparticles is the reason behind the variation in relaxivity values simply because there are not enough nanoparticles in each image to offer any real significant statistical evidence. One clue, however, could be that in each TEM image, there seems to be interparticle connections. Given the harsh environment during the calcination process, this is not a surprising observation. The uncontrollable nature of such conditions leading to these connections could be the reasoning behind the values as any agglomeration of particles would in essence skew the relaxivity towards the agglomerate rather than any individual, unconnected nanoparticles present in solution. And if this distribution of agglomerated particles was not evenly distributed throughout all of the bulk samples, which in is near impossible to accomplish, then it makes sense that the relaxivities range is as wide spread as has been observed.
Figure 3.13: TEM images of select bulk MCM41-
Mn$_8$Fe$_4$ samples. Top: Sample 1. Middle: Sample 1_1. Bottom: Sample 3.
In order to test the interparticle agglomeration theory, monodispersed mesoporous silica nanoparticles must be synthesized in order to compare these particles to MCM41. As mentioned earlier, the structure of MCM41 is that of a hexagonal close packing of tubes within a sphere. This means that the pore openings are only located on opposite ends of the nanoparticles. This is another factor that can lead to discrepancies in relaxivity measurements as this could, like that of iron oxide nanoparticles, lead to heterogeneous metal distribution.

Due to these newly realized requirements, the radial MSNs were synthesized and their viability as a replacement for MCM41 was investigated. These particles already had promise as they were used already by Davis and co-workers as potential contrast agents since they were used to anchor Gd$^{3+}$ chelates. The radial distribution of pores allows for a more homogenous distribution of metal. And as such the same modification reactions used previously were implored for these particles. The interparticle connections seen in MCM41 were not nearly as rampant in the radial MSNs (Figure 3.14 and Figure 3.15) even when calcined.
Figure 3.14: SEM image of calcined radial MSNs. Inset: TEM image.

Figure 3.15: SEM image of calcined radial MSNs-Mn$_8$Fe$_4$. Inset: TEM image.
It is clear that the morphology of the radial MSNs is vastly improved when compared to the MCM41 originally used. Even calcined, these particles have limited interparticle agglomeration and are much more monodispersed than MCM41. This morphology is maintained throughout the entire modification process. The first set of relaxivity data, while promising, still needs to be repeated in order to verify the problems that plagued the MCM41-Mn$_8$Fe$_4$ silica nanoparticles are not an issue when using these particles (Figure 3.16). The calculated metal per bead for this preliminary sample was 0.0350 mg metal/mg silica.

![Figure 3.16: Preliminary relaxivity of radial MSNs-Mn$_8$Fe$_4$.](image)

3.4 Conclusions

The use of nanomaterials to overcome native limitations of potential contrast agents has become a wide spread practice. These materials have many advantages
such as metal leaching prevention or the ability to enhance circulation time thus allowing for better cellular uptake and adequate biodistribution. Recently, Pablico showed the use of polystyrene nanobeads to encapsulate the metal-oxo cluster $\text{Mn}_8\text{Fe}_4$. In doing so the relaxivity of the contrast agent changed from a $T_2$ contrast agent to a $T_1$ contrast agent when encapsulated.

This observation was taken into consideration when considering the mesoporous silica family of nanoparticles. These nanomaterials should allow for adequate anchoring of the $\text{Mn}_8\text{Fe}_4$ while allowing for environmental water so come into contact with the metals of the cluster, an important relaxation pathway not utilized in the polystyrene nanobeads scheme. The inclusion of this relation pathway should allow for an increase in relaxivity relative to the polystyrene nanobeads, thus improving the established system.

This chapter was dedicated to exploring these mesoporous silica nanoparticles metal-oxo cluster hybrid materials as potential contrast agents. The first example of silica nanoparticle, MCM41, was successfully synthesized, characterized, and modified in order to accommodate the anchoring of $\text{Mn}_8\text{Fe}_4$ into the pore of the material. The modification process involved reacting a more desirable silane, in this case a silane with a nitrile group. This was done ultimately to further modify this moiety into a carboxylate group. Once carboxylated, the surface of these materials would have an adequate anchoring site for successful ligand
exchange reactions involving Mn₈Fe₄. PXRD and gas adsorption experiments proved conclusively the Mn₈Fe₄ cluster successfully plugged the pore of the MCM41.

Determining the relaxivities of these materials proved quite challenging as all samples analyzed had a wide range of relaxivities casting doubt on the materials viability as a contrast agent. Many factors were explored to try and conclusively determine what caused the variances. As of yet, no conclusive explanation as to why the MCM41-Mn₈Fe₄ relaxivities vary has been determined. Although, an interesting observation observed through TEM imaging was the clear presence of interparticle agglomeration. This could very well be the reasoning behind the varied range of relaxivities observed.

In light of these interparticle connections and agglomeration a new mesoporous silica nanoparticle, dubbed radial MSNs, was utilized. This material vastly improved upon the interparticle agglomeration seen in MCM41 and thus the same modification steps used on MCM41 were carried out on these nanoparticles. Preliminary relaxivity experiments on these materials are promising but further studies are needed to cement these hybrid materials potential as a contrast agent.
CHAPTER 4: FUTURE DIRECTIONS/CLOSING REMARKS

While the accomplishments presented in previous chapters are important, work still remains for the further improvement of these materials as potential contrast agents. This chapter is dedicated to the advances made not readily obvious or quantifiable from results not previously presented. Much work on this project has been done that has not been as successful but still paramount in regards to the learning process. This chapter stands to address that work. Furthermore, insight into possible solutions to previously mentioned problems that were not yet attempted or have only preliminary results will be discussed. Finally, each contrast agent component will be broken down and discussed in order to suggest possible future directions to take the project forward.

4.1 METAL-OXO CLUSTERS

As of this time, three metal-oxo clusters (Mn_{12}, Mn_{8}Fe_{4}, and Mn_{3}bpy) have been explored for their viability as potential contrast agents beyond the preliminary round of experiments. A central characteristic these clusters share, aside from the obvious manganese and acetate ligands, is the ability to be easily synthesized. For any usage outside of a laboratory setting, the synthesis of a cluster should be relatively easy to accomplish with high yields and high purity. While other clusters exist with more metals per cluster, the synthetic steps necessary to make these compounds could potentially inhibit their contrast agent potential. Furthermore,
one should not equate more metal as automatically the best cluster. This is no better emphasized then when comparing the clusters so far explored as it is the smallest cluster, Mn\textsubscript{3}bpy, that has the highest relaxivity.\textsuperscript{104} Although recent reports show that clusters can be used as building blocks in making nanomaterials themselves, this is not necessarily advantageous.\textsuperscript{130} This synthetic route, while increasing the metal per cluster by orders of magnitude, would also severally limit any potential post-synthetic modifications. Any new cluster under consideration for use as a contrast agent should ideally be relatively small. This is to ensure that the cluster does not limit any subsequent nanomaterial incorporation, whether encapsulated in a polymer nanoparticle, entrenched in a mesopore, or incorporated with any other potential nanomaterial vehicle.

While the metal and number of unpaired electrons are important criteria in cluster consideration, the structure, specifically the cluster core, is arguably just as an important consideration. For both Mn\textsubscript{12} and Mn\textsubscript{8}Fe\textsubscript{4}, there is a central [Mn\textsuperscript{+4}O\textsubscript{4}]\textsuperscript{+8} cubane structure essentially acting as a lock, holding the rest of the cluster together. When going through a ligand exchange reaction, this core acts as a foundation, holding everything together while the native acetates are exchanged. This is a major draw back for Mn\textsubscript{3}bpy, and subsequently Mn\textsubscript{3}bpyVBA and Mn\textsubscript{3}bpyMA. This could account for the increased disorder associated with the crystal structure compared to their Mn\textsubscript{8}Fe\textsubscript{4} counterparts. Any cluster without any bridging O\textsuperscript{2-} ions holding the metals in place could potentially be subject to this. The maintained core structure of Mn\textsubscript{8}Fe\textsubscript{4}, as well as Mn\textsubscript{12}, allows for ligand exchange while still maintaining the
magnetic properties that made it a promising contrast agent in the first place. This is important when considering cluster selection. The same cannot be said with substituted Mn$_3$bp (while actual magnetic experiments may prove otherwise), it’s hypothesized that because it lacks a well maintained core structure, the same magnetic properties that made Mn$_3$bp promising, are not guaranteed to remain the same from ligand exchange to ligand exchange.

Cluster shape is also important as seen when comparing linear and spherical clusters. The spherical clusters have a longer rotational correlation time and, as such, higher relaxivity than their linear counterparts. Furthermore the spherical nature of the clusters lends to more homogenous distribution of metal. This could be considered another advantage of spherical versus linear clusters. Chemical modification is a crucial component of the cluster selection process, not only for nanomaterial incorporation, but also for possible coupling to various molecules. A cluster that is easily modifiable can then be adapted to whole host of other applications, such as for use in drug delivery or as optical probes. The multimodal nature allows for an ideal scenario where by the contrast agent can be used for both diagnostic and therapeutic treatment.

Ultimately, the potential for these compounds to be used as contrast agents is limitless but certain guidelines should be adhered to when choosing any new cluster for these applications. Any new cluster should be easy to synthesize, chemically
modifiable, relatively spherical and small in size, as well as have some structure element ensuring a stable core.

4.2 Nanomaterials

4.2.1 Polymer Nanoparticles

The ability for the cluster to ligand exchange is also a very important criterion as the surrounding ligand can drastically change native properties. An example of this is readily apparent in the ligand exchange reactions previously observed for both Mn$_8$Fe$_4$ and Mn$_3$bpy. Both native clusters, with acetates, are soluble in water. But when exchanged with VBA, the cluster solubility changes. Mn$_8$Fe$_4$VBA mimics that of its new ligand and becomes soluble in non-polar solvents like hexanes while Mn$_3$bpyVBA becomes highly insoluble in many solvents. Likewise, in using the direct miniemulsion technique, Pablico further showed that the native relaxivity of Mn$_8$Fe$_4$ changed drastically when encapsulated in polystyrene nanobeads.$^{48,49}$ As such the polymerization aspect of these materials should be taken into account when trying to design further contrast agent improvements.

During a typical direct miniemulsion synthesis, two phases are made. The first, a continuous phase, is composed of water and a surfactant, sodium dodecyl sulfate (SDS). The other dispersive phase is the organic material including the
monomer styrene, a cross-linker divinyl benzene (DVB), a hydrophobe (hexadecane) to prevent Ostwald ripening, and the initiator (AIBN) that starts the polymerization process. When including substituted cluster, the cluster is also in the dispersive phase. The two phases are combined and pre-emulsified prior to ultrasonication. Finally, the entire mixture is heated to the appropriate temperature (60°C for the case of AIBN) to generate free radicals and allowed to polymerize for 6 hours.

Much time was spent trying to improve upon the direct miniemulsion synthesis developed by Pablico by varying select aspects of the synthetic process. This was done in hopes of gaining further insight into how to optimize the synthesis further in order to produce size selective nanobeads. It was hypothesized that, based on factors such as rotational correlation time, Mn8Fe4 infused polystyrene nanobeads should have size dependent affects on relaxivity assuming equal metal content. Unfortunately this hypothesis was never verified but attempts were made to elucidate the correct necessary conditions in order to achieve polystyrene nanobeads of varying sizes and what select factors impacted this size selectivity the most (Table 4.1). This is made even more interesting when considering the varying sizes throughout the circulatory system. As such, a contrast agent with a varying nanoparticle size range would be ideal as this range could be used advantageously as the size variation could lead to target specificity.
Table 4.1: The varying of three parameters (Output power for sonication, Surfactant:monomer ratio, and initiator) yielded particles of varying sizes. The particle sizes were determined using dynamic light scattering (DLS) with the polydispersity index (PDI) acting as a measure of the monodispersity of the synthesized beads.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Avg. Diameter (nm)</th>
<th>Avg. PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Output Power (W)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>74.59</td>
<td>0.339</td>
</tr>
<tr>
<td>20</td>
<td>52.49</td>
<td>0.175</td>
</tr>
<tr>
<td><strong>Mole Percent (SDS:Styrene)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.192</td>
<td>115.52</td>
<td>0.476</td>
</tr>
<tr>
<td>0.530</td>
<td>84.22</td>
<td>0.383</td>
</tr>
<tr>
<td>6.14</td>
<td>54.16</td>
<td>0.184</td>
</tr>
<tr>
<td><strong>Initiator</strong></td>
<td>KPS</td>
<td>0.193</td>
</tr>
</tbody>
</table>

The colloidal suspension synthesized from the direct miniemulsion process is incredibly stable. Although, once disturbed, the particles agglomerate irreversibly and the resuspension of these particles into solution is impossible. Thus, a potential improvement to be made would be to synthesize redispersible polystyrene nanobeads. This was attempted by utilizing an inverse miniemulsion technique in using a hydrophilic monomer, 2-hydroxyethyl methacrylate (HEMA).\textsuperscript{131} This technique is called an inverse miniemulsion because the aforementioned reagents for the direct miniemulsion are inverted meaning that the continuous phase is now organic, the dispersive phase the aqueous, etc. After many attempts a successful
procedure and synthesis was achieved. Unfortunately, the same issue resulted as collected polyHEMA nanobeads would not resuspend in solution. The reasons for such an observation were soon realized. First, for a polymer particle to have a more hydrophilic surface, the hydrophilic component has to be on the outside of the particle. It was not readily obvious until after the fact that using the inverse miniemulsion technique would always result in the hydrophilic end pointed toward the center of each particle, as the continuous phase was extremely non-polar in order to form the necessary nanodroplets. Therefore the alcohol-terminated end of the HEMA, the part needed for increased hydrophilicity, would never be on the outside of the particle, as it was needed. The second reason for the lack of polyHEMA nanobeads resuspension was that it was later realized polyHEMA absorbs multiple times its own weight of water. With this increase in both size and density the likelihood of being able to resuspend such particles is extremely low, even the aforementioned hydrophilic end not exposed to the outside. Luckily a potential solution would be to copolymerize both HEMA and styrene using the original direct miniemulsion technique. Copolymerization would allow for the incorporation of hydrophilic HEMA units into the hydrophobic polystyrene nanobeads. And because the continuous phase would once again be water, the hydrophilic end would correctly be situated on the surface of the nanobeads. This would then provide a potential route in synthesizing redispersible nanobeads.
Adapting the silica nanoparticles represents the broadest of possibilities as both the cluster used and the silica nanoparticle can be altered further to produce a viable contrast agent.

Other clusters could have potential for attachment aside from Mn$_8$Fe$_4$. Mn$_3$bpy, the unsubstituted cluster mentioned in Chapter 2, could represent a future candidate. While the single crystal structures of the VBA and MA analogues were disordered, they did also show that the general structure was maintained. This observation could indicate that the central cubane structure mentioned above is not necessarily as crucial as originally perceived to be. If that is the case, then Mn$_3$bpy represents an obvious candidate for future silica attachment, as the relaxivity of this cluster, compared to unmodified Mn$_8$Fe$_4$, was actually better. Another potential innovative avenue to explore would be by attaching either Mn$_3$bpyVBA, Mn$_3$bpyMA, or VBA/MA substituted Mn$_8$Fe$_4$ to the silica through an olefin metathesis reaction. This similarly relies on the modification of the silica surface to something that contains a vinyl group (e.g. 3-(trimethoxysilyl)propyl methacrylate). With such a modification, the substituted Mn$_3$bpy or Mn$_8$Fe$_4$ could be covalently attached the silica mesopores while still being exposed to the environment for rapid water exchange. One would have to take into account a few synthetic aspects such as how much substituted cluster: silica to use to prevent cluster bridging silica nanoparticles, whether fully substituted cluster would be best, and what if any
solubility limitations these systems might have. If such issues were overcome and these clusters were successfully attached this would represent a new synthetic route to explore that, as of the writing of this thesis, has not been explored in the literature.

As with the cluster component, many aspects of the mesoporous silica nanoparticle synthesis could be changed to better the material for potential contrast agent applications. Arguably the most important quality any silica nanoparticle should have is a highly monodispersed distribution of particle size. As described in Chapter 3, the varying MCM1 bead size was likely one of the root causes for the wildly ranging relaxivity values. By elucidating a synthetic procedure that makes nanoparticles of roughly the same size repeatedly is of the utmost importance. Furthermore the use of calcination to remove surfactant, more so in the case of MCM41, showed interparticle agglomeration and further complicated the size selectivity of the MCM41 synthesis. A possible alternative to avoid this is by doing away with the calcination technique altogether. Two routes exist for doing so, the use of acidified alcohol or ammonium nitrate solutions and liquid calcination.\textsuperscript{74,83–85,132–137} Although preliminary relaxivity data comparing the same batch of radial MSNs that were either calcined or used acidified ethanol for surfactant removal show promise (Table 4.2), further studies (e.g. SEM/TEM, multiple trials, etc.) would have to be done in order to conclusively verify the validity of these values. Liquid calcination, the method in which high boiling organic solvents, such as TOP or TOPO, was attempted but was unable to produce the same results as reported.
Table 4.2: Preliminary relaxivity values of radial MSNs using two different surfactant removal techniques.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surfactant Removal</th>
<th>$r_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acidified EtOH</td>
<td>0.96 ± 0.056</td>
</tr>
<tr>
<td>2</td>
<td>Calcination</td>
<td>0.89 ± 0.108</td>
</tr>
</tbody>
</table>

Other attempts to ensure that monodisperse silica nanoparticles were synthesized and gathered was by increasing the overall volume of the reaction and using syringe filters of select pore lengths to filter each batch. Although cumbersome and more time consuming, these methods proved to be viable options based on the preliminary DLS data (Table 4.3).

Table 4.3: Preliminary DLS data of three independent radial MSNs samples that were syringe filtered. The synthesis also included the use of a higher water volume (50 mL) and the surfactant was removed using acidified ethanol.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diameter (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58.84</td>
<td>0.153</td>
</tr>
<tr>
<td>2</td>
<td>58.17</td>
<td>0.112</td>
</tr>
<tr>
<td>3</td>
<td>52.04</td>
<td>0.119</td>
</tr>
</tbody>
</table>
As with the polymer nanobeads, the ability to selectively control the synthesized sizes of the radial MSNs is also of potential interest as it is surmised that the relaxivity of these particles would similarly vary based on particle size. This can be done by changing any of the following three parameters: silica source, base concentration, and the additional alcohol solvent used (Figure 4.1). A final characteristic of these particles to be further exploited is the ease in which the surface can be modified. As shown in Chapter 3, the route in which carboxylation of the silica surface occurs is relative simple. This aspect can be further utilized by modifying the surface of these particles with other useful moieties. Examples include utilizing PEGylation to further help and ensure colloidal stability of these particles, the addition of molecules such as folic acid to act as targeting agents, and adding a fluorophore such as indocyanine green (ICG) to make the particles dual modal. ICG is a fluorophore of the cyanine family that absorbs and fluoresces in the near infrared (NIR) part of the electromagnetic spectrum. This is crucial as biological matter is transparent in this region. ICG is the only NIR dye approved by the Federal Drug Administration.

![Figure 4.1: Schematic representation of how changing reaction parameters affects particle size](Reprinted (2016) with permission from Valtchev, V.; Tosheva, L. Chem. Mater. 2013, 113, 6734. Copyright (2013) American Chemical Society)
(FDA) for use in clinical applications and is readily available for purchasing. The combination of these diagnostic techniques should provide for better CAs in the present while allowing for new insights into agent development in the future.\textsuperscript{140–147}
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