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Photopolymerization Synthesis of Magnetic Nanoparticle Embedded Nanogels for Targeted Biotherapeutic Delivery

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Photopolymerization Synthesis of Magnetic Nanoparticle Embedded Nanogels for Targeted Biotherapeutic Delivery

by

Daniel J. Denmark

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy Department of Physics College of Arts and Sciences University of South Florida

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Keywords: stimuli-responsive polymer, linear response theory, drug delivery, magnetic nanoparticle, composite device, alternating magnetic field

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DEDICATION

For Dad, Mom, and Rebekah who always pushed me to excel. For Paige who made this possible through her love, support, and encouragement. For Piper and Gavin who inspire me to better myself so that they may have advantages in life.
I would like to express my sincere gratitude to my advisors Dr. Sarath Witanachchi and Dr. Pritish Mukherjee, for the opportunities they have made available to me, the academic and professional advice they have freely given, and their unwavering guidance and support through this doctoral program.

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<tr>
<td>AMF</td>
<td>alternating magnetic field</td>
</tr>
<tr>
<td>ATR</td>
<td>attenuated total reflection</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>CCD</td>
<td>charged coupled device</td>
</tr>
<tr>
<td>cm(^{-1})</td>
<td>wavenumber</td>
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<td>CMC</td>
<td>critical micelle concentration</td>
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<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
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<td>DIW</td>
<td>deionized water</td>
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<td>EM</td>
<td>electron microscopy</td>
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<tr>
<td>FTIR</td>
<td>fourier transform infrared</td>
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<tr>
<td>ICCD</td>
<td>integrated charged coupled device</td>
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<tr>
<td>IOMNP</td>
<td>iron oxide magnetic nanoparticles</td>
</tr>
<tr>
<td>ITO</td>
<td>indium tin oxide</td>
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<td>IR</td>
<td>infrared</td>
</tr>
<tr>
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<td>potassium hydroxide</td>
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<td>lower critical solution temperature</td>
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<td>LRT</td>
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<td>mg</td>
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<tr>
<td>ms</td>
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<td>MNP</td>
<td>magnetic nanoparticle</td>
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<tr>
<td>MPixel</td>
<td>mega pixel</td>
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<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
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<tr>
<td>μm</td>
<td>micrometer</td>
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<td>NDF</td>
<td>neutral density filter</td>
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<td>NIPAM</td>
<td>N-isopropylacrylamide</td>
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<td>NIR</td>
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<td>particle size distribution</td>
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<td>PVP</td>
<td>polyvinylpyrrolidone</td>
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<td>optical density</td>
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<td>OMA</td>
<td>optical multichannel analyzer</td>
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<td>stimuli-responsive polymer</td>
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<td>targeted biotherapeutic delivery</td>
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ABSTRACT

Conventional therapeutic techniques treat the patient by delivering a biotherapeutic to the entire body rather than the target tissue. In the case of chemotherapy, the biotherapeutic is a drug that kills healthy and diseased cells indiscriminately which can lead to undesirable side effects. With targeted delivery, biotherapeutics can be delivered directly to the diseased tissue significantly reducing exposure to otherwise healthy tissue. Typical composite delivery devices are minimally composed of a stimuli responsive polymer, such as poly(N-isopropylacrylamide), allowing for triggered release when heated beyond approximately 32 °C, and magnetic nanoparticles which enable targeting as well as provide a mechanism for stimulus upon alternating magnetic field heating. Although more traditional methods, such as emulsion polymerization, have been used to realize these composite devices, the synthesis is problematic. Poisonous surfactants that are necessary to prevent agglomeration must be removed from the finished polymer, increasing the time and cost of the process. This study seeks to further explore non-toxic, biocompatible, non-residual, photochemical methods of creating stimuli responsive nanogels to advance the targeted biotherapeutic delivery field. Ultraviolet photopolymerization promises to be more efficient, while ensuring safety by using only biocompatible substances. The reactants selected for nanogel fabrication were N-isopropylacrylamide as monomer, methylene bisacrylamide as cross-linker, and Irgacure 2959 as ultraviolet photo-initiator. The superparamagnetic nanoparticles for encapsulation were approximately 10 nm in diameter and composed of magnetite to enable remote delivery and enhanced triggered release properties. Early investigations into the interactions of the polymer and nanoparticles employ a pioneering experimental setup, which allows for coincident turbidimetry and alternating magnetic field heating of an aqueous solution containing both materials. Herein, a low-cost, scalable, and rapid, custom ultraviolet photo-reactor with in-situ, spectroscopic monitoring system is used to observe the synthesis as the sample undergoes photopolymerization. This method also allows in-situ encapsulation of the magnetic nanoparticles simplifying the process. Size characterization of the resulting nanogels was performed by Transmission
Electron Microscopy revealing size-tunable nanogel spheres between 50 and 800 nm by varying the ratio and concentration of the reactants. Nano-Tracking Analysis indicates that the nanogels exhibit minimal agglomeration as well as provides a temperature-dependent particle size distribution. Optical characterization utilized Fourier Transform Infrared and Ultraviolet Spectroscopy to confirm successful polymerization. When samples of the nanogels encapsulating magnetic nanoparticles were subjected to an alternating magnetic field a temperature increase was observed indicating that triggered release is possible. Furthermore, a model, based on linear response theory that innovatively utilizes size distribution data, is presented to explain alternating magnetic field heating results. The results presented here will advance targeted biotherapeutic delivery and have a wide range of applications in medical sciences like oncology, gene delivery, cardiology and endocrinology.
CHAPTER 1:
INTRODUCTION

Almost 25% of all deaths in the United States can be attributed to heart disease making it the number one cause of death nationally [1]. Granted, there has been significant success in treating and preventing (e.g. by educating patients to manage diet and exercise) this top killer of Americans. However, conventional treatments continue to utilize drugs that are administered to the patient’s entire body. Some of these drugs are known to be toxic in excessive quantities. Furthermore, when the drug does not damage cells that were not the target of treatment the drug is necessarily wasted. There is new interest in employing targeted drug delivery (TBD) to treat heart disease patients more effectively [2, 3]. In this chapter, an introduction to the field of TBD is provided with an emphasis to the active targeting devices that are of interest in the work described in this dissertation. The first section will broadly describe the TBD field by illustrating how devices are designed to treat specific tissues and outline the hierarchy of various types of these devices. Then, each of the main components of the TBD device that is the goal of this work will be treated in separate sections. The second section introduces the stimuli-responsive polymer (SRP) whose function is to encapsulate a biotherapeutic only to release it when prompted by an environmental cue. The third section deals with the magnetic nanoparticles (MNPs) which serve to carry the entire device to a target tissue in response to a static magnetic field and then initiate a cue to the SRP by way of alternating magnetic field (AMF) heating. After that, a fourth section discusses several methods for synthesizing these TBD devices. These concepts are summarized in a fifth section. Finally, an outline of the dissertation is presented to serve as road map for the remaining chapters.

1.1. Targeted Biotherapeutic Delivery (TBD)

There are a variety of medical treatments whose administration to the patient is justifiably characterized as imprecise. For example, chemotherapy, a relatively standard technique for treating
cancer, intravenously dispenses a cellular toxin to all of the patient’s tissues indiscriminately. The result is that otherwise healthy cells are attacked along with the target of the treatment, cancer cells, leading to the infamous side effects of chemotherapy [4, 5]. Now consider the relatively broad field of gene delivery in which genetic diseases (e.g. sickle-cell disease, Down syndrome, cystic fibrosis, etc.), cancer, and HIV/AIDS are being combated in novel ways. In general, gene therapy works by either swapping healthy genes for mutated ones, deactivating mutated genes, or delivering a new gene to cells for combating some disease. Gene therapy, a controversial technique, usually involves the introduction of genetic information, which did not originate in the patient, to both afflicted and healthy patient cells [5]. One final instance of nonspecific medical interventions involves hemostasis using artificial platelets. This relatively new technology has been shown to significantly decrease exsanguination time in a rat model through intravenous administration [6, 7]. Such a tool would prove indispensable in preventing traumatic deaths which were responsible for approximately 47 % of deaths in the US in 2009 [8]. The idea is that artificial platelets work by being administered shortly after the trauma is sustained, giving the patient enough time to reach an operating room. Although artificial platelets can effectively slow bleed rate, they are free to circulate throughout the patient increasing the risk of embolism. All these treatments would benefit from a more controlled administration that would allow for their beneficial aspects to affect only the cells or tissues of interest, while preventing their detriment to otherwise healthy parts of the patient.

The field of TBD, more commonly known as targeted drug delivery, promises to transport some medically beneficial payload to a specific tissue. In this dissertation, ‘biotherapeutic’ is more precise than ‘drug’ since it refers not only to compounds like chemotherapy, but also to viruses carrying certain desirable genetic information and potentially even as a carrier for artificial platelets. Through this technique, only diseased or damaged tissues are treated and otherwise healthy ones are not. Put another way, the purpose of TBD is to convey medical aid to the core of the afflicted tissue for intentional treatment. TBD literature describes a hierarchy of micro- and nano-sized devices at various stages of development. The two major groups of devices are categorized by whether or not they target their intended tissue passively or actively. Devices that employ passive targeting are designed to naturally accumulate in the vicinity of a tumor due to its unusual surface as compared to otherwise healthy tissue. More recent efforts have seen the development of devices capable of active targeting of a particular tissue. Active targeting devices can be
further subcategorized into those that use biological targeting and those that target remotely, from outside of the patient. Biological targeting, not the focus of this work, involves attaching chemical receptors on the surface of the device enabling it to bind to certain tissues. This type of tissue targeting has met with limited research success [9-12]. Remotely directing a biotherapeutic device to a target tissue typically involves employing magnetic nanoparticles since they are attracted to a static magnetic field maintained in the vicinity of the diseased cells [13, 14].

The human body maintains a relatively constant temperature and any deviation in that homeostasis can serve as a trigger for biotherapeutic release. For this reason, stimuli responsive polymers in conjunction with magnetic nanoparticles, for localized heating capability, are ideal for TBD applications [15]. The work presented in this dissertation aimed to synthesize TBD devices composed of magnetic nanoparticles, to enable remote control tissue targeting and provide thermal stimulus triggering biotherapeutic release, and a stimuli responsive polymer, to serve as biotherapeutic carrier and enable release upon temperature increase. The TBD device is schematized in Fig. 1.1 (a) as a stimuli responsive nanogel that encapsulates MNPs and the biotherapeutic (e.g. artificial platelet). The device will release the biotherapeutic, as in Fig. 1.1 (b), to the surrounding tissue once an AMF has induced temperature increase. Such devices can be intravenously administered to a patient as shown in Fig. 1.1 (c).

In terms of drug delivery, the benefits of tissue specific targeting are two-fold: less drug necessary to treat the target tissue and elimination of drug exposure to otherwise healthy tissue. Regarding gene delivery, the benefits are three-fold: less genetic material administered to the patient, less incubation time necessary to achieve modified cells, and elimination of likelihood to treat otherwise healthy cells. These devices are easily degraded and eliminated from the patient’s body due to their small size and composition of biocompatible components [11, 16, 17]. This can be a benefit in terms of not polluting the body, but can be a disadvantage regarding residence times in the body that are potentially too short for effective treatment [18, 19]. One disadvantage of using MNPs in this application is that their relative small size means that many of them would be necessary to achieve the remote guidance capability since on a discrete level they would only exert a small magnetic force on the rest of the TBD device in response to a magnetic field [20-22]. One advantage is that their small size ensures superparamagnetic (SPM) behavior. More details regarding SPM are given in Sect. 1.3.
This section serves as introduction to TBD. Motivation for the field was presented in terms of providing more precise treatment for conditions such as cancer, genetic disease, and trauma. The field was described as being organized by a hierarchy that branches it into the two main methods of targeting a certain tissue: active and passive. Active targeting projects are then further subcategorized into biological and remote control targeting. The TBD device under consideration in this project is described as a

Figure 1.1 Schematics for illustrating the aspects of TBD corresponding to (a) the MNP and biotherapeutic embedded nanogel prior to payload delivery, (b) the same nanogel in the process of releasing payload to target tissue after receiving an appropriate stimulus, and (c) the intravenous administration of TBD device to a patient.
A polymer that responds to some environmental prompt by undergoing a change of physical state is known as a stimuli responsive polymer (SRP) [23-25]. Several stimuli are known to bring about these phase transitions including temperature, pH, ions, glucose, stress/strain, electromagnetic fields, ultrasound, and many others [19, 26]. The stimulus of interest in this dissertation is temperature. It is possible to crosslink several SRPs together to form a mesh network similar to that shown in Fig. 1.2 (a). When this SRP network encounters its stimulus it will either contract or relax [25, 27]. The entire SRP mesh network constitutes a three dimensional, stimuli responsive nanogel. A tremendous change in size can be invoked by a minute change in any of the stimuli listed above, which causes SRPs to be excellent candidates for

**Figure 1.2** SRPs undergo a VPT upon experiencing a particular environmental prompt (e.g. heating or cooling past a LCST), (a) which causes its polymeric mesh to collapse or expand and (b) manifests as a discontinuous change in volume.
Table 1.1 List of several stimuli-responsive polymers and their corresponding transition temperatures.

<table>
<thead>
<tr>
<th>Material</th>
<th>Transition Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(N-isopropylacrylamide)</td>
<td>30 - 34</td>
</tr>
<tr>
<td>Poly(N-vinylcaprolactam)</td>
<td>25 - 50</td>
</tr>
<tr>
<td>Poly(vinyl methyl ether)</td>
<td>~ 37</td>
</tr>
<tr>
<td>Poly(N,N-diethylacrylamide)</td>
<td>25 - 34</td>
</tr>
<tr>
<td>Poly(methacrylic acid)</td>
<td>~ 75</td>
</tr>
<tr>
<td>Poly(vinyl methyl oxazolidone)</td>
<td>~ 65</td>
</tr>
<tr>
<td>Poly(siloxylene glycol)</td>
<td>10 - 60</td>
</tr>
<tr>
<td>Poly(vinyl pyrrolidone)</td>
<td>~160</td>
</tr>
<tr>
<td>Gelatin / collagen</td>
<td>~ 40</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>30 - 50</td>
</tr>
<tr>
<td>Methylcellulose</td>
<td>~ 80</td>
</tr>
<tr>
<td>Hydroxypropylcellulose</td>
<td>~ 55</td>
</tr>
<tr>
<td>Polynaphosphazene derivatives</td>
<td>33 - 100</td>
</tr>
<tr>
<td>Poly(silamine)</td>
<td>~ 37</td>
</tr>
<tr>
<td>Poly(vinyl alcohol)</td>
<td>~ 125</td>
</tr>
<tr>
<td>P188</td>
<td>27 - 53</td>
</tr>
</tbody>
</table>

applications calling for materials that behave as switches. A discontinuous change in volume as brought on by a change in temperature elucidating a VPT is simulated in Fig. 1.2 (b) [28].

Soft materials that undergo phase transition in response to a stimulus are categorized into the four groups: homopolymers, modified copolymers, triblock copolymers, and natural polymers. Homopolymers, like poly (N-isopropylacrylamide) (PNIPAM), are polymer chains of only one monomer type [29]. Modified copolymers, like PNIPAM-PEG, are polymer chains composed of one monomer type at one end of the chain and a different monomer type at the other end of the chain [30]. Triblock copolymers, like P188, have three different monomers constituting the polymer chain [31]. Finally, natural polymers, like gelatin, are not synthesized in the laboratory [32]. Table 1.1 lists a few SRPs along with their transition temperatures.

SRPs are particularly well suited to TBD applications. They are known to undergo discontinuous VPTs which means that the polymeric mesh network that they are made up of shrink in a sudden fashion potentially squeezing out any contents (i.e. a biotherapeutic) they may contain. Many SRPs already undergo this phase change near physiological temperatures, or they can be modified to do so. What follows
next is a closer look at the polymer specifically chose for the work presented in this application. That subsection will be followed by a brief review of some applications for SRPs.

1.2.1. Poly(N-isopropylacrylamide) (PNIPAM)

The stimuli-responsive polymer chosen for the TBD application discussed in this dissertation was poly(N-isopropylacrylamide) (PNIPAM). Its molecular structure is given in Fig. 1.3 (a). The hydrophobic acryl section, shown in red, of the monomer NIPAM forms the backbone of the polymer chain. As a monomer that section has a double bond between the two carbons. That double bond becomes a single bond when monomer units are chemically linked, which can be assessed spectroscopically to confirm polymerization (see Sect. 2.3.3) [33-35]. The hydrophilic amide section, shown in blue, is where hydrogen bonding takes place. Finally, the isopropyl section, shown in green, is hydrophobic in nature. One of the things that makes PNIPAM so attractive for biomedical applications is that it is biocompatible [36, 37]. However, PNIPAM is not biodegradable [23].

PNIPAM belongs to a unique group of polymers that exhibit reverse solubility when its temperature is increased. Below the lower critical solution temperature (LCST) it readily bonds with water, remaining in solution (hydrophilic state), and when it is above its LCST it precipitates out of solution (hydrophobic state) [38-40]. A short PNIPAM chain (three monomers polymerized together) is schematized in Fig. 1.3 (b) demonstrating the conformational transition of its phase transformation [41]. At temperatures less than the LCST the monomer units expand so that the amide section is exposed to surrounding water molecules allowing hydrogen bonding to occur. After the temperature has exceeded the LCST the molecule collapses on itself hiding the hydrophilic amide within the hydrophobic acryl and isopropyl sections. The photographs in Fig. 1.3 (c) demonstrate the increase in turbidity, upon heating past the LCST, corresponding to the polymer precipitating out of solution. The LCST of PNIPAM is approximately 32 °C, but this can vary with certain properties of the solution as discussed later. This switch-like behavior of PNIPAM is the inspiration for many of the applied uses of PNIPAM, including targeted biotherapeutic delivery systems [37, 40].

The mechanism by which PNIPAM undergoes its phase transition is best described by the Gibbs free energy, $G$, of the system, where $H$ is the enthalpy, $T$ is the temperature, and $S$ is the entropy of the system.
The organization of PNIPAM, the solute, in solution with water, the solvent, is a consequence of the hydrogen bonding of the polymer with the already slightly ordered water. This is important since water

\[ \Delta G = \Delta H - T\Delta S \]  

(1.1)

The organization of PNIPAM, the solute, in solution with water, the solvent, is a consequence of the hydrogen bonding of the polymer with the already slightly ordered water. This is important since water
necessarily rearranges itself in the vicinity of nonpolar parts of PNIPAM because they cannot hydrogen bond. The consequence of this hydrophobic effect is a reduced entropy when solute and solvent come together. As the solution heats, the entropy term becomes more prevalent when compared to the enthalpy term associated with the formation of hydrogen bonds between solvent and solute which initiates dissolution. Phase separation occurs at the LCST which marks the transition to positive free energy ($\Delta G > 0$) of the solution. The result is a transition from a sample with water and PNIPAM joined through hydrogen bonds to a sample in which the PNIPAM has precipitated out of solution. The concentration dependence of the LCST in PNIPAM can be understood in terms of a transition from the enthalpy to entropy dominance of the free energy. When the fraction of PNIPAM chains to solvent is increased, the multiplicity of states is necessarily increased, which means that the entropy increases as well. Therefore, the LCST is reached sooner upon heating for larger concentrations of PNIPAM. [36, 40]

PNIPAM is one of the most heavily studied SRPs for targeted drug delivery applications [42]. Literature searches like that shown in Fig. 1.3 (d) reveal the dramatic growth of interest in the polymer since the first citation in the mid-1950s. Interestingly, the monomer NIPAM was originally noteworthy as a rodent repellant [43]. PNIPAM can be made to assume many different forms such as fibers, coatings, membranes, thin films, latexes, nanogels, macroscopic gels, and single chains. The methods for synthesizing such structures include free radical initiation, redox initiation, ionic polymerizations, crosslinking methods, and photopolymerization (see Sect. 1.4) [44, 45].

In this subsection, the polymer chosen for the work presented in this dissertation, PNIPAM, was introduced. Specifically, the biocompatible chemical structure of PNIPAM was presented with special attention being paid to its hydrophobic acryl and isopropyl sections and to the hydrophilic amide section. Then, the unusual collapse behavior of PNIPAM with increasing temperature was discussed. This phase transition of PNIPAM was explained in terms of its thermodynamics. Finally, a brief description of a PNIPAM literature search was presented.

1.2.2. Applications

Here, several of the many diverse applications of SRPs will be discussed. Besides targeted biotherapeutic delivery, some of the applications for which SRPs are known to be useful include tissue
engineering, gene delivery, waste water recovery, gel actuators, sensors, membranes, and thin films [23, 46]. Since this dissertation is focused on targeted biotherapeutic delivery this section will forgo that topic. For further information on that topic see Sect. 1.1. Here, a sampling of work in fields significantly different from targeted biotherapeutic delivery that employ SRPs will be presented.

The field of waste water recovery benefits from membrane technology in efforts to remove pollutants from clean water. This is due to the fact that well designed membranes are compact, consume less energy, and yield cleaner water as compared to other methods [47, 48]. The inevitable fouling of a membrane with pollutants presents the highest hurdle to overcome since it necessitates frequent cleaning, or replacement, thereby increasing cost [49-51]. Ngang et al. managed to synthesize a PVDF matrix membrane composited with SiO$_2$/PNIPAM particles dispersed throughout [52]. The SiO$_2$ component served to increase hydrophilicity and discouraged pollutant adsorption to the PVDF matrix [53, 54]. Their composite membranes effectively trapped oil at temperatures above LCST and released the oil when heated above the LCST. In other words, the thermo-responsive action of the dispersed particles resulted in pollutant entrapment and subsequent release from the PVDF membrane. Obviously, this is a convenient method for water purification.

Another interesting application of SRPs is for the detection of various compounds to advance public security and biomedicine efforts. Consider a plasmonic nanostructure which has the capacity to change refractive index when bound to a particular substance of interest in a certain application. These devices are well known for their importance as sensors, but suffer from limited change in optical properties that usually require the use of enhanced detection efforts [55]. What is desired is a sensor that can indicate a target substance with naked eye observations alone. One way to make the plasmonic nanostructure more practical is to composite it with a SRP having switch-like behavior to amplify the smaller change in the original material [56, 57]. Recently, T. Wang et al. successfully inserted a PNIPAM brush layer into the cavity of their Ag nanovolcano array [58]. Their composite device was used to make an anti-counterfeit photonic paper capable of naked eye detection. The optical properties changed quickly (~ 1 second) and consistently. The authors predict they will be able to detect proteins, genetic material, and other compounds in the future.
Yet another exciting application of SRPs is the transfer of genetic material to target cells for medical therapy, i.e. targeted gene delivery. As an example, for nearly three decades one form of cancer treatment has involved physical delivery of donor genes to a patient’s cells such as micro-injection, electroporation, or gene gun [59, 60]. These methods are now considered to be impractical due to manipulation challenges and subsequent use of viruses to achieve gene delivery came to prominence as a result. Unfortunately, patient deaths during clinical trials resulted in the prohibition of the virus vector method [61, 62]. SRP nanogels are excellent substitutes for viruses because of their capacity for tailorable structure and size and low immunogenicity [63, 64]. Positively charged Polyethylenimine (PEI) is considered particularly well suited to carry negatively charged DNA, but this positive charge can also lead to cell death [65]. It turns out PNIPAM can effectively encapsulate PEI thereby protecting cells that are not the target of therapy [66]. Furthermore, PNIPAM has been shown to preferentially target cancer cells when heated above its LCST [67, 68]. In a new publication, Zhang et al. managed to create a composite SRP nanogel consisting of a PEI core for holding the DNA and a PNIPAM shell for release of DNA at the target cells [69]. Their narrow size distribution particles effectively encapsulated the cytotoxic PEI and only released the payload in a switch-like manner upon temperature stimulus. The composite particle showed reduced cancer growth when compared to PEI particle alone.

Finally, tissue engineering has benefited from the unique thermo-responsive behavior of PNIPAM for about twenty years. Traditionally, the synthesis of fibronectin and collagen sheets is commonly achieved via physical adsorption to some substrate. The merging of SRPs with this technique allows for simple cell sheet detachment. This is because above the LCST of PNIPAM, when the polymer is shrunken and hydrophobic, cells tend to adhere and below the LCST, when the polymer is swollen and hydrophobic, cells detach from the polymer thin film [70-72]. Healy et al. compositied PNIPAM with another polymer, to serve as anchor between the thin film and a plastic substrate, in order to promote this technique for superior cell sheet separation and investigate the role temperature at the time of initial polymer thin film synthesis plays in cell sheet separation [73]. The authors were able to successfully culture, and detach via cooling below the LCST, a sheet of human pulmonary microvascular endothelial cells. Because the cell sheets detached in relatively shorter times, it was determined that the polymer thin films synthesized beneath the LCST are optimal, as compared to those thin films synthesized above the LCST.
Again the applications for SRPs include the fields of targeted biotherapeutic delivery, gene delivery, thin films, sensors, waste water recovery, tissue engineering, gel actuators, and membranes to name a few. This section specifically highlighted some recent work in the areas of tissue engineering, sensors, gene delivery, and waste water recovery. A careful reading of the section will reveal that often times some of these fields overlap. Certainly, all these fields benefit from the switch-like thermo-responsive behavior of PNIPAM.

1.3. Magnetic Nanoparticles

In this section, the all-important other half of the TBD device, the MNP will be presented. Recall that the purpose of the MNP is not only to enable remote control guidance of the TBD device to a target tissue, but also to stimulate the VPT of the SRP so that the biotherapeutic is released. This section is broken up into three parts. First, a basic review of magnetism will be presented. Then, the desirable quality of SPM will be discussed. Finally, several applications for MNPs will be reviewed.

1.3.1. Basic Review of Magnetism

As the title implies, this part of the dissertation will present a brief review of some basic concepts from magnetism. Some important terms will be defined and equations will be presented that relate those terms. Then, different types of magnetic materials will be discussed with an emphasis on materials that relate to the targeted biotherapeutic delivery application presented in Sect. 1.1. In that section, MNPs were presented as a necessary component of the objective TBD device since they will not only guide the device to the target tissue, via static magnetic field, but will also enable temperature increase of the device, via alternating magnetic field.

Being composed of atoms having electron orbits, magnetic materials are made up of magnetic dipoles. An externally applied magnetic field (represented by \( \mathbf{H} \) and having units of amperes per meter) can influence magnetic dipoles to change their orientation. The applied magnetic field produces an internal field, known as magnetic flux density (represented by \( \mathbf{B} \) and having units of teslas, T), as a consequence of the reorientation of the magnetic dipoles. The degree to which all the individual magnetic dipoles are commonly oriented in the material can be characterized by its magnetization (represented by \( \mathbf{M} \) and with units of...
amperes per meter); the material has a net volumetric magnetic moment. Note that all three of these quantities are vectors; they have magnitude as well as direction. These three parameters are related through a fourth parameter known as the magnetic permeability (represented by μ and with units of webers per ampere-meter) that describes material through which the applied magnetic field propagates and in which the magnetic flux density measurement takes place. The magnetic flux density is directly proportional to the sum of the applied magnetic field and the magnetization through the magnetic permeability of the material, as expressed in Eqn. 1.2.

\[ \mathbf{B} = \mu (\mathbf{H} + \mathbf{M}) \]  

(1.2)

The magnetization is directly proportional to the applied magnetic field, as expressed in Eqn. 1.3, through the magnetic susceptibility (represented by \( \chi \) and having no units).

\[ \mathbf{M} = \chi \mathbf{H} \]  

(1.3)

The magnetic susceptibility will be germane to the topics found in the next section and Ch. 5. More details on these concepts can be found in assorted textbooks. [74-76]

Now, the various types of magnetic materials are categorized by how their constituent magnetic dipoles respond overall to an applied magnetic field, and this is often illustrated with plots of magnetization as a function of applied magnetic field, commonly referred to as M-H curves. At the start, it is worthwhile to consider the magnetic response of all materials involved in the targeted biotherapeutic delivery application such as the patient’s various tissues and PNIPAM, in addition to the MNPs. First, consider a sample of matter whose magnetic dipoles align themselves in a direction that opposes the applied magnetic field, as represented by the M-H curve of Figure 1.4 (a). These materials are referred to as diamagnetic and include molecules composed exclusively of nitrogen, carbon, oxygen, and hydrogen such as proteins native in the patient’s circulatory system and also PNIPAM. Next, Fig 1.4 (b) shows the magnetic response of a material whose magnetic dipoles align preferentially with applied magnetic field, but do not retain lasting magnetism after removal of the field. Materials of this sort are classified as paramagnetic. The protein hemoglobin is paramagnetic since it contains iron. Both paramagnetic and diamagnetic materials have relatively weak responses to applied fields and will be ignored from here on regarding their magnetic response. [13, 74, 77]
What remains left to explain is the magnetic response of the MNPs for guidance and triggering of the biotherapeutic delivery composite. It’s most instructive to begin by pondering upon a comparatively larger piece of the material from which the MNPs are made. Note that the IOMNPs characterized in Ch. 3 were advertised by the manufacturer to be comprised of magnetite. Fig. 1.4 (c) schematizes a microscopic view of a piece of magnetite with an eye toward its magnetic features. Here, the magnetic dipoles, represented by small yellow dots, individually contribute to the overall magnetization, represented by the
large yellow arrows, of a given magnetic domain. The black irregular lines separate magnetic domains which are characterized by regions in which all the magnetic dipoles align themselves similarly. In other words, for a large enough piece of magnetite, adjacent domains may exhibit dipoles that orient themselves in slightly different directions if a change in applied magnetic field has only just occurred. The reason for this is that energy from the magnetic field is required to force the domain walls to move in the vicinity of grain boundaries or impurities. The crystal lattice of magnetite and its magnetic anisotropy can also be attributed to the phenomenon. This manifests itself in the M-H curve by way of a sigmoidal shaped response exhibiting hysteresis in the magnetization when the direction of the applied magnetic field is reversed, as illustrated in Fig. 1.4 (d). The remanence, \( M_r \), of such a material is the degree to which it remains magnetized when the applied field is returned to zero. The coercivity, \( H_c \), of the material is the amount of applied magnetic field, directed opposite to the original field, which must be applied to return the magnetization to zero. At large enough magnitudes of the applied magnetic field magnetization saturation, \( M_s \), is reached at which point all magnetic dipole moments are aligned in the same orientation. [78-80]

A basic introduction to magnetism as it relates to the MNPs used in the realization of a PNIPAM/MNP composite was presented here. The magnetic parameters of flux density, permeability, applied field, magnetization, and susceptibility were all defined. Furthermore, equations relating these parameters were presented. Then, the different types of magnetic materials applicable to the targeted biotherapeutic delivery application were illustrated by way of their corresponding M-H curves. In the application coercivity, and hysteresis in general, is undesirable. The elimination of coercivity is the motivation for using superparamagnetic MNPs and will be discussed in Sect. 1.3.2. All these concepts are fundamental to the topics presented in Chs. 3 and 5.

1.3.2. Superparamagnetism

In the last section, magnetite was reported to be a ferromagnetic material. This is important since the MNPs used in this work were composed of magnetite. Recall from Sect. 1.3.1 that when MNPs of this type are subject to a changing applied magnetic field they exhibit hysteresis like that illustrated in Fig. 1.4 (d); these MNPs do not trace out the same path in magnetization when the applied field changes direction. This is undesirable since the MNPs would require additional energy from the magnetic field in order to
return the magnetization to zero. Superparamagnetic materials do not exhibit any such hysteresis and so are optimal for the targeted biotherapeutic delivery application discussed in Sect. 1.1. This section will clearly describe the magnetic response of superparamagnetic materials, justify their favored use over ferromagnetic particles in this application, and set the ground work for explaining how they generate heat.

Below a particular size threshold, the magnetic dipole moment of a MNP can flip randomly as a consequence of thermal energy. A representation of M-H curves typical of superparamagnetic materials is illustrated in Fig. 1.5 (a). Note, here, that as the applied magnetic field changes direction the magnetization of the MNP traces out the exact same path. In other words, these MNPs exhibit no hysteresis. Furthermore, the particles exhibit no left over magnetization when the applied magnetic field is removed; they have no remanence. Also, no applied magnetic field is required to return the magnetization of the MNP to zero; the particles display no coercivity. Just as for ferromagnetic materials, superparamagnetic materials will reach saturation magnetization at large enough applied magnetic field amplitudes. [13, 79]

It is generally well established that relatively large multi-domain MNPs have low coercivity. The problem with this is that such particles are potentially too large for the application at hand. As the size of a multi-domain MNP is decreased its coercivity approaches a maximum that corresponds to threshold diameter transitioning into the realm of single-domain MNPs. So while these MNPs may now be appropriate for compositing with a PNIPAM nanogel their relatively high coercivity makes them undesirable. As the size of the particles are further decreased the coercivity decreases again to the point of another critical diameter that marks the transition into superparamagnetic MNPs. Now the particles at hand are both small in size, making them appropriate for encapsulating into PNIPAM, as well as possessing minimal coercivity, making them ideal in terms of their magnetic response. These trends in coercivity as a function of MNP size are illustrated in Fig. 1.5 (b). As a reference point for the discussion of nanoparticle characterization in Ch. 3 and the model for MNP heating presented in Ch. 5, the literature reports a critical diameter of 30 nm marking the regime switch between multi-domain and single domain for IOMNPs. [81-84]

Now a brief introduction to the mechanism behind the heating capability of MNPs will be presented. As was established earlier, because the entire magnetic dipole moment of the MNP is uninhibited in its reaction to the environmental thermal energy, it is considered superparamagnetic. These reactions to the environment come in the form of particle rotations or relaxations. There are two kinds of relaxations that
contribute to an overall MNP relaxation. First, the magnetic dipole moment itself can rotate within the otherwise stationary particle and this is known as a Néel rotation. A Brownian rotation is defined as the entire MNP rotating in the solvent in which it is dispersed carrying the magnetic dipole moment along with it. These are both schematized in Fig. 1.5 (c). Assuming the anisotropy (K) is uniaxial the time ($\tau_N$) in which a MNP undergoes a Néel rotation is determined through

\[ M_r \approx 0 \]

\[ H_c \approx 0 \]

Figure 1.5 In SPM particles, (a) their magnetic response exhibits no hysteresis, (b) their small size means they require no additional applied magnetic field to return their magnetization to zero, and (c) their heating mechanism, in response to applied AMF, is explained in terms of their Néel and Brownian relaxations.
The volume of the MNP is expressed through the parameter $V$, the thermal energy is given by $k_B T$, and $\tau_0$ is a material dependent constant called the attempt time. The amount of time ($\tau_B$) corresponding to a Brownian rotation is given by

$$\tau_B = \frac{3 \eta V_h}{k_B T}. \quad (1.5)$$

The viscosity of the solvent is given by $\eta$ and the hydrodynamic volume is represented by $V_h$. Note that Néel relaxation time compares the size of the MNP to the thermal energy of the environment. This highlights the correspondence of small particles to superparamagnetism since the two energy types are similar near the thermal energy of physiological temperatures. On the other hand, Brownian relaxation time is more dependent on the properties of the solvent [85, 86]. With the two types of relaxation time in hand, an effective relaxation time ($\tau$) can be determined through

$$\frac{1}{\tau} = \frac{1}{\tau_N} + \frac{1}{\tau_B}. \quad (1.6)$$

Another requirement for the observation of superparamagnetic behavior has to do with the measurement time ($\tau_m$) of the equipment used in a given trial. When $\tau_m >> \tau$ the MNP can react faster than it is measured so that no lag in magnetization is observed. Contrary to this is the situation where $\tau_m << \tau$, because now the relaxations are slow compared to the measurement, which means that lag in magnetization changes will be apparent. For the heat generating AMF experiments of Ch. 3 and 4 and for the model predicting heat generation of Ch. 5 an AMF frequency of 307 kHz to 308 kHz was the standard. The effective relaxation time calculated later on was two orders of magnitude less than the measurement time ensuring superparamagnetic behavior [87, 88].

In closing, superparamagnetic MNPs exhibit no hysteresis, remanence, or coercivity. Similarly, they tend to have much lower magnetic susceptibility than ferromagnetic materials do. Since there is only one domain in these superparamagnetic particles it is not possible for other domains to interact in the material. It follows, then, that superparamagnetic materials have no remaining magnetization after the applied magnetic field has been removed. While larger multi-domain MNPs do have reduced coercivity they are likely too large for the targeted biotherapeutic delivery application making the case for superparamagnetism even stronger. Néel and Brownian relaxation times of the MNPs combine to given its effective relaxation time. The distinction of superparamagnetism on a material depends on how its anisotropic energy compares
to the thermal energy of the environment and on how the experimental measurement time compares to the effective relaxation time. The IOMNPs used in this work will be characterized in Ch. 3 with plenty of evidence to suggest they are in fact superparamagnetic. The introductory review of superparamagnetism presented in this section will be more thoroughly explored in terms of linear response theory (LRT) in Ch. 5.

1.3.3. Applications

Many diverse fields of research have employed the use of MNPs composed specifically of magnetite for approximately three decades (see Fig. 1.6). Applications include developing new and improved sensors, memory devices, environmental remediation, and catalysis as well as biomedical efforts such as the magnetic separation of labelled cells, contrast enhancement for magnetic resonance imaging (MRI), AMF tumor ablation via hyperthermia, and targeted biotherapeutic delivery. Recall that one of the attractive aspect of IOMNPs for biomedicine is their biocompatible nature; they are excreted by the liver. Since the first two parts of this section have already covered the basic physics of IOMNPs, this section aims to present a brief review of fields that benefit from their use. So a short collection of recent works has been assembled here detailing their motivations, methods, and accomplishments. The topic of targeted biotherapeutic delivery was already covered in Sect. 1.1 and will not be discussed further in this part.

![Figure 1.6 Literature search, conducted in May 2017, for MNPs composed of magnetite in regard to the number of citations as a function of the year in which they were published.](image)
In magnetic hyperthermia, MNPs are administered into or near a tumor with assistance from an applied static magnetic field and/or employment of surface modification to enhance tumor targeting. Once the particles are in the location of treatment an AMF is applied to induce them to elevate the temperature surrounding cells. Since cancer cells are more susceptible to elevations in temperature than healthy cells cytotoxicity is specific to the cancer and the tumor is ablated [16, 89]. In clinical trials, typical AMFs used have frequencies ranging between 80 kHz and 100 kHz as well as field strengths ranging between 1.5 kA/m and 18.0 kA/m (19 – 226 Oe) [90]. For patient safety and comfort it is critical that the product of the applied field and its frequency not exceed $4.85 \times 10^8$ A m$^{-1}$ s$^{-1}$ [91]. In one recent effort to optimize the magnetic heating of MNPs, while minimizing the requisite applied AMF, Nemati et al. synthesized magnetite MNPs with unique nano-octopod shape [92]. They demonstrated that their particles exhibited superior heating efficiency as compared to the spherical analog.

In general, cell labelling and separation can be thought of as the identification of cells of interest and the subsequent removal of those cells from their native environment. One of the primary aims of oncology is management of what’s known as circulating tumor cells which can leave a primary tumor and colonize a new tumor elsewhere in the patient [93, 94]. Researchers have learned much in their efforts to develop magnetic nanoparticles functionalized with ligands that adsorb to circulating tumor cells. Once adsorption has occurred a static magnetic field can allow for separation of these dangerous cells from the patient’s blood [95, 96]. The technique does face the challenge of effective separation of small numbers of tagged cells in a typical volume of blood containing various other molecules and healthy cell types. In particular, it is well known that some separation devices developed so far have suffered from unintentional adsorption to proteins native in the patient’s blood reducing their efficacy [97, 98]. The abandonment of micro sized particles in favor of IOMNPs and functionalizing with polyethylene glycol (PEG) based copolymer was recently reported by Lin et al. in an attempt to advance cell separation [99]. The authors reported that their device showed improved efficacy in separating out metastatic cells from patient blood than the industry standard device.

The operation of MRI takes advantage of the vast numbers of protons in a patient’s bodies. First a strong magnetic field, ranging between 0.5 T and 3.0 T, aligns the magnetic moments of the protons then a second alternating magnetic field established resonance in those moments. When the AMF is switched
off the relaxation time of the magnetic moments is enhanced and measured. Different relaxation times can indicate different tissue types in a patient's body [100, 101]. SPM particles can be absorbed by different human tissues based on their sizes (e.g. ~ 30 nm absorbed preferentially by spleen and liver while ~ 10 nm preferred by bone marrow and lymph nodes) [102, 103]. Some researchers have targeted the circulatory system, tumors, and the central nervous system [104-106]. In a new article, Nakamura et al. have reported on a device they synthesized that enabled multimodal diagnostic imaging [108]. Their device composited a MNP with a fluorescent NP to enable MRI contrast functionality as well as enable cell tracking capability.

The capacity for iron as a potent reducing agent makes its use in environmental remediation an obvious choice; various pollutants can be degraded by iron in water sources leaving behind more benign compounds. The high surface to volume ratio of IOMNPs make them ideal for metal removal since they exhibit high adsorption efficacy. In one study, Wanna et al. demonstrated removal of lead, mercury, copper, and cobalt ions from water by applying an AMF [108]. Electrostatic attraction was demonstrated to be responsible for cadmium, chromium, copper, and nickel ion adsorption to IOMNPs and the effect was heavily influenced by particle surface area, and environmental conditions such as pH and temperature [109].

To say the uses for IOMNPs are many is an understatement. This is true for the biomedical field (e.g. targeted biotherapeutic delivery, hyperthermia ablation of tumors, MRI contrast, cell labelling etc.) and otherwise (e.g. memory devices, environmental remediation, catalysis, sensors, etc.). It’s no wonder they have enjoyed the intense scrutiny evident in literature searches like the one plotted in Fig. 1.6.

1.4. Synthesis Techniques of Composite Devices

This section will present a brief review of methods to synthesize biotherapeutic delivery systems. The main component of such systems is usually a hydrogel which is composed of a 3D network of polymer chains that dissolve in water and can swell/shrink when dispersed depending on the environmental conditions [110]. Hydrogels are attractive as platforms for TBD systems due to their large capacity to retain water in the polymeric mesh, 3D structure variability, biocompatibility, and mechanical properties [111-113]. Many researchers have already successfully composited nanomaterials with hydrogels such as carbon nanotubes, magnetic nanoparticles, inorganic clays, and quantum dots [114-116]. In general, a hydrogel’s
polymeric mesh can be crosslinked either physically or chemically. Chemical crosslinking is more pertinent to this dissertation. In particular, free radical polymerization is a reaction in which a polymer chains grow by binding to unpaired monomers with their unpaired electron chain end [117] and has been studied thoroughly in the preparation of PNIPAM hydrogels [118-120]. The benefits of using nanogels include sizes that can be dialed-in ranging from nanometers to several micrometers, a polymeric mesh for retaining bio-related compounds, and large surface areas where bioconjugation can be made to happen. There have already been a large number of works that successfully demonstrated retention and release of proteins, drugs, genetic material, and carbohydrates within nanogels. Modern efforts in this field are focused on developing devices that can be tested in vivo by overcoming challenges such as nanogel biodegradability to ensure eventual expulsion from patient, designing devices smaller than 200 nm to promote their uptake by cells while impeding their uptake by the immune system’s mononuclear phagocytes, developing new modified surfaces for the targeting of target tissues, and ensuring the devices remain in the circulatory long enough for effective treatment [121-123]. The synthesis methods presented here include photolithography/micromolding, microfluidics, spray drying, and emulsion polymerization.

In photolithography, a precursor solution containing unreacted monomers and photoinitiators is constrained to fill replica molds and then irradiated with UV light in order to cure the nanogels, as schematized in Fig. 1.7 (a). This technique has been able to produce submicron-sized nanogels having diverse chemical composition, particle shape, and particle size. A mold was composed of perfluoropolyether that allowed for photo-irradiation curing. The molds were constructed via electron beam lithography. In addition to the photoinitiator, the precursor solution contained dimethacrylate-functionalized oligomers that permeated facile release of the cured nanogels. The product is resistant to solvents, durable, and exhibits chemical robustness. These devices can be made to have sizes ranging between 200 nm to several microns. They can be made to possess trapezoidal, arrow, bar, and conical shapes. The smaller of these nanogels were made to encapsulate genetic material, small molecules, and proteins. [124-126]

Similar to photolithography, micromolding technique incorporates the added benefits of eliminating the use of expensive cleanroom facilities and lithographic equipment. Yeh et al. fabricated nanogel prisms, disks, or stings that contained animal cells through a micromolding process. Namely, they prepared aqueous solutions of either poly(ethylene glycol diacrylate) or methacrylated hyaluronic acid dispersed with
a photoinitiator. After depositing onto poly(dimethylsiloxane) patterns the ensemble was irradiated with UV light to enable crosslinking [127].

The experimental equipment for the microfluidic synthesis of TBD devices is central to understanding the technique. Typically, microfluidic equipment consists of tapered microinlets, as seen in Fig. 1.7 (b), where two immiscible liquids are mixed forming an emulsion. The equipment is assembled

**Figure 1.7** TBD devices can be synthesized using a multitude of techniques including (a) photolithography, (b) microfluidics, (c) spray drying, and (d) emulsion polymerization.
through soft lithography and tends to be composed of either polyurethane elastomers or poly(dimethylsiloxane): elastomers. Once the emulsion has been achieved the synthesis terminates with either poly-condensation or photopolymerization of the TBD devices. By controlling the rate of reaction, droplet proximity to neighbors, and microfluidic flow rate the TBD devices can be made to have an assortment of monodisperse morphologies and shapes [128]. In one noteworthy project, ionic crosslinking was employed to achieve external gelation of monomer droplets stably dispersed by way of a surfactant. Zhang et al. successfully produced stable monodisperse spherical alginate microgels whose diameters could be made to range between 50 to 70 microns depending on the microfluidic flow rate [129]. The morphology of their product could be changed from gradient to capsular to uniform depending on both the crosslinker concentration and crosslinker diffusion time.

The synthesis of TBD devices has also been investigated utilizing atomizers and drying chambers in a technique commonly referred to as spray drying. Here, precursor solutions containing monomers, crosslinkers, nanoparticles, drugs, reaction accelerants, etc. can all be sprayed into fine droplets at which point polymerization takes place in flight prior to product capture at the bottom of the drying chamber. The spray dry technique is schematized in Fig. 1.7 (c). The diameter of the TBD device is determined by crosslinking density, atomizer nozzle size, rate of atomization, spray flow rate, and droplet evaporation rate [130-133]. Researchers have produced devices ranging in size from hundreds of microns to as small as one micron [134-137]. One notable effort manufactured nanogels composed of silica/poly(L-lysine)/alginate by way of spray drying [138]. The resulting nanogels contained magnetic cobalt silicate nanoparticles for potential guidance and heating capability. The authors demonstrated TBD potential as evidenced by endocytosis of their product into animal cells and even deterioration of the nanogel by immune system cells. The fact that immune system cells naturally remove the device from a patient reflects its promise in regard to safety.

In emulsion polymerization a water in oil dispersion of fine water soluble monomers within a continuous phase of oil solvent is made stable by way of a surfactant. After the emulsion has been established initiators are introduced to polymerize the TBD devices, as diagrammed in Fig. 1.7 (d). There are three basic types of emulsion polymerization including inverse emulsion, reverse micelle, and membrane emulsification. As described above, inverse emulsion achieves an emulsion of fine water soluble
monomers droplets within oil solvent by employing vigorous agitation of the dispersion with either a high-speed mechanical stirrer or a homogenizer. Microgels synthesized using inverse emulsion can range in size from a few microns to several hundred microns [110, 139-141]. The reverse micellar emulsion polymerization is distinct in that significantly larger amounts of surfactant are used so that smaller devices can be produced. Nanogels synthesized via the reverse micellar technique have diameters as small as tens of nanometers to as large as hundreds of nanometers [142-144]. Finally, in membrane emulsification the water in oil dispersion is achieved by permeation of aqueous phase through a membrane, due to pressure differential, into the oil phase. Membrane pores studied so far range in size from as small as 100 nm to as large as 18 microns yielding TBD devices of diverse monodisperse diameters [145-147]. Wang et al. used membrane emulsion polymerization to achieve insulin loaded monodisperse microgels that could be made as small as 4 microns to as large as 15 microns [148]. They found that the solidification of their product, which took about two hours, could affect various parameters of the application including insulin release in vitro, device morphology, drug activity, and insulin encapsulation.

In closing, there exists impressive diversity in regard to the methods for producing TBD devices. Unfortunately, only a few of those techniques could be explored here in terms of their methodologies and some examples using them. The techniques that were discussed here included TBD devices synthesized via photolithography/micromolding, microfluidics, spray drying, and emulsification polymerization. The synthesis technique used for the work presented in this dissertation was inspired heavily by the spray dry method, but is most similar to emulsion polymerization and incorporates UV energy to drive the reaction.

1.5. Summary

In Sect. 1.1 an introduction to TBD was presented. The field was established as being necessary since it can provide more exact treatment for conditions like cancer, genetic disorders, and traumatic injury. A hierarchy exists in the field that branches it into the two main methods of targeting a particular tissue: active and passive targeting. Active targeting efforts are further subcategorized into those that target certain tissues biologically and those the employ remote control targeting. In this project, the TBD devices under consideration was described as composite particles that consist, at least, of a SRP, for transport and squeeze release of the biotherapeutic, and MNPs, for remote guidance to a target tissue and heating
mechanism for triggering release. The section concluded by identifying the advantages of such devices and areas in which the devices need to be improved.

In Sect. 1.2, SRPs were presented as particularly well suited to TBD applications. They undergo discontinuous VPTs; the polymeric mesh network they are composed of shrinks in a sudden fashion squeezing out any contents (i.e. a biotherapeutic) they contain. Several of them naturally undergo this phase change near physiological temperatures, or can be made to. Then, came a closer look at the polymer specifically chose for the work presented in this application. After that came a brief review of some applications for SRPs. The polymer chosen for the work presented in this dissertation, PNIPAM, was introduced. In particular, the biocompatible chemical structure of PNIPAM was presented with special attention being paid to its hydrophobic acryl and isopropyl sections as well as the hydrophilic amide section. Next, the unusual collapse behavior of PNIPAM with increasing temperature was discussed. This phase transition of PNIPAM was explained in terms of its thermodynamics. This was followed by a brief description of a PNIPAM literature search. The applications for SRPs include the fields of TBD, gene delivery, thin films, sensors, waste water recovery, tissue engineering, gel actuators, and membranes to name a few. Specifically, some recent work in the areas of tissue engineering, sensors, gene delivery, and waste water recovery was reviewed. All these fields benefit from the switch-like thermo-responsive behavior of PNIPAM.

Section 1.3 began with a basic introduction to magnetism as it relates to the MNPs used in the realization of a PNIPAM/MNP composite. The magnetic parameters of flux density, permeability, applied field, magnetization, and susceptibility were all defined. Furthermore, equations relating these parameters were presented. Then, the different types of magnetic materials applicable to the targeted biotherapeutic delivery application were illustrated by way of their corresponding M-H curves. In TBD, coercivity and hysteresis are undesirable. The elimination of coercivity is the motivation for using superparamagnetic MNPs and will be discussed in Sect. 1.3.2. All these concepts are fundamental to the topics presented in Chs. 3 and 5.

In the next part of Sect. 1.3, it was established that superparamagnetic MNPs exhibit no hysteresis, remanence, or coercivity. Similarly, they tend to have much lower magnetic susceptibility than ferromagnetic materials do. Since there is only one domain in these superparamagnetic particles it is not possible for other domains to interact in the material. It follows, then, that superparamagnetic materials
have no remaining magnetization after the applied magnetic field has been removed. While larger multi-domain MNPs do have reduced coercivity they are likely too large for the targeted biotherapeutic delivery application making the case for superparamagnetism even stronger. Néel and Brownian relaxation times of the MNPs combine to given its effective relaxation time. The distinction of superparamagnetism on a material depends on how its anisotropic energy compares to the thermal energy of the environment and on how the experimental measurement time compares to the effective relaxation time. The IOMNPs used in this work will be characterized in Ch. 3 with plenty of evidence to suggest they are in fact superparamagnetic. The introductory review of SPM presented will be more thoroughly explored in terms of linear response theory (LRT) in Ch. 5.

In the last part of Sect. 1.3, several diverse uses for IOMNPs were presented including for the biomedical field (e.g. targeted biotherapeutic delivery, hyperthermia ablation of tumors, MRI contrast, cell labelling etc.) and otherwise (e.g. memory devices, environmental remediation, catalysis, sensors, etc.). It's no wonder they have enjoyed the intense scrutiny evident in literature searches like the one plotted in Fig. 1.6.

Finally, in Sect. 1.4, an impressive range of methods for producing TBD devices was presented. Unfortunately, only a few of those techniques could be explored here in terms of their methodologies and some examples using them. The techniques that were discussed included TBD devices synthesized via photolithography/micromolding, microfluidics, spray drying, and emulsification polymerization. The synthesis technique used for the work presented in this dissertation was inspired heavily by the spray dry method, but is most similar to emulsion polymerization and incorporates UV energy to drive the reaction.

1.6. Outline of Dissertation

The synthesis and assessment of an IOMNP embedded PNIPAM nanogel through UV photopolymerization of aqueous precursor solutions was studied by way of the following investigations. Fabrication of the PNIPAM nanogels is monitored in situ using a novel spectroscopic tracking technique that observes UV source attenuation through the sample. The resulting nanogels are characterized and control of their diameters is demonstrated (Chapter 2). Next, the IOMNPs are characterized in terms of their morphology, magnetization, aqueous transmittance versus wavelength, and propensity toward settling out
of solution. In addition, evaluation of the heating capability of the IOMNPs dispersed in water in response to an AMF is presented (Chapter 3). Then, the IOMNPs heating capability, upon AMF exposure, is determined when dispersed in aqueous PNIPAM, but not encapsulated. Encapsulation of the IOMNPs within the PNIPAM is described. The resulting TBD devices are evaluated for heating response upon AMF exposure (Chapter 4). After that, a model to predict the AMF heating of IOMNPs is developed to explain the experimental results (Chapter 5). Finally, a thorough summary of all results as well as a brief discussion of future directions is presented (Chapter 6).
CHAPTER 2:
IN-SITU MONITORING OF AQUEOUS PHOTOPOLYMERIZATION OF PNIPAM

In this chapter, the results from all experiments related to the synthesis of a stimuli responsive nanogel will be presented. As discussed in Ch. 1, PNIPAM was the stimuli responsive polymer used in this work. The chapter will begin by documenting two nanogel synthesis techniques, emulsion polymerization and spray, that were ultimately abandoned in favor of a faster method. Next, the chapter will describe the successful nanogel synthesis method: UV photopolymerization. After that, the characterization of a PNIPAM nanogel is presented. Finally, the chapter will conclude by demonstrating control of the size property of the nanogels.

2.1. Early Synthesis Attempts

The purpose of this section is to document all attempts that were made to synthesize stimuli-responsive nanogels in this work. Early in the project emulsion polymerization was employed to prepare PNIPAM micelles. These were subjected to TEM and DLS to determine morphology and demonstrate stimuli-responsive behavior. Ultimately, this method proved problematic when MNP encapsulation was attempted and was abandoned to pursue spray processes. Both aerosol and nebulous sprays were investigated for the production of nanogels. A number of experimental apparatus were assembled to prepare samples for SEM in order to establish whether or not nanogels could be formed in this way. A brief review of those setups is presented here along with the results obtained.

2.1.1. Emulsion Polymerization

Chapter 1 introduced the concept of using a stimuli responsive polymer such as PNIPAM to serve as the carrier of the biotherapeutic intended for delivery to a target tissue. Once the carrier arrives at the target tissue it can be stimulated, via temperature increase past its LCST, to undergo phase transition
causing the biotherapeutic to be released to the surrounding environment. Emulsion polymerization was
introduced as one of many chemical methods to synthesize stimuli responsive nanogels. Early in the project
emulsion polymerization was employed, by Dr. Kirpal S. Bisht, to prepare PNIPAM micelles. This section
documents the analysis of stimuli-responsive micelles synthesized using emulsion polymerization. These
were subjected to TEM and DLS characterization in order to determine their size and morphology, and to
demonstrate their stimuli responsive behavior.

The microscope used to analyze the micelles was an FEI Morgani TEM. It is capable of a 3 kX –
824 kX magnification range. All images were captured using an electron voltage of 60 kV. Within the
microscope, the sample was subject to a working pressure of 5 x 10⁻³ Pa (5 x 10⁻⁵ Torr) and an ultimate
pressure < 1 x 10⁻³ Pa (< 10⁻⁵ Torr). For imaging, a 1.4 MPixel sidemount CCD camera and 16.7 MPixel
bottom mount CCD camera are used. Samples were typically dropped with a pipette onto a carbon coated
copper grid and left to dry for several minutes. This could be achieved either under ambient conditions or
under vacuum at approximately 45 °C if a faster preparation time was desired. Alternatively, drying could
be accelerated by removing some excess sample with wet filter paper. One final method for quickly drying
a TEM grid was to remove some excess sample with the same pipette from which the sample was dropped.
In some of the samples imaged, uranyl acetate (UA) was used to achieve negative staining of the micelles;
the background was stained while the specimen was left unstained. To achieve the negative stain a small
drop of UA was placed on the already prepared copper grid and allowed to sit. After approximately 30
seconds the excess stain was removed with wet filter paper and the grid was allowed to dry thoroughly
before imaging. It was important to remove UA after only 30 seconds of contact with the sample to prevent
overstaining [149]. Initial TEM imaging produced poor quality representations of the micelles that are not
shown here. Ultimately, centrifuging was employed to improve the quality of the imaging represented in this
section.

The DLS instrument used for in-situ characterization of PNIPAM micelles was a Malvern
Instruments Zetasizer Nano S Model ZEN1600. The system utilized a laser with wavelength of 632.8 nm,
beam diameter of 0.63 mm, and 4 mW maximum power output. The micelles were dispersed in filtered DIW
within a polystyrene optical cuvette. The sample was subject to three trials of DLS analysis at several
temperatures ranging from 10 – 50 °C. The DLS system was programmed to wait two minutes after
achieving a certain temperature to ensure enough time for sample equilibration before the measurements would commence.

The high quality PNIPAM micelles, synthesized via emulsion polymerization, are shown in Fig. 2.1 (a). The round shape in this micrograph suggests the micelles are approximately spherical. Any apparent physical connections in the micrograph are an artifact of proximity of individual micelles. In fact, when the same sample was imaged under TEM at lower concentrations the physical connections are either less
prevalent or absent altogether. Upon close inspection of the micelles, some texture is apparent on their surface that is suggestive of the crosslinked PNIPAM. By fitting the counts of PNIPAM micelles versus their size with a Gaussian function the average size was determined. Figure 2.1 (b) shows these desiccated micelles have an average size of 119 ± 4 nm. The error in average size is equivalent to one standard deviation from the size corresponding to peak counts. Apparently, these PNIPAM micelles are easily characterized as monodisperse since the error is much less than a tenth of the average size.

When the micelles are still in solution, DLS is more appropriate for sizing. This is because TEM preparation and measurement subjects the micelles to desiccation and pressures very different from those of the application described in Ch. 1. Furthermore, TEM cannot perform temperature dependent studies of the micelles. Figure 2.1 (c), a temperature study of PSDs from DLS trials, shows the effect temperature has on PNIPAM micelles. Here, it is apparent that changing the temperature can have a significant effect on micelle size. For example, when heated to 50 °C the micelles are about 100 nm and when they are cooled to 20 °C they expand to nearly 300 nm. Again, fitting each particle size distribution in Fig. 2.1 (c) with a Gaussian, allows the plotting of PNIPAM micelle size versus temperature, shown in Fig. 2.1 (d). As before, error was determined by evaluating one standard deviation from the peak of the Gaussian. Here, it is obvious that these micelles undergo a discontinuous, volume phase transition at 35.9 ± 1.1 °C. This was determined by taking the derivative of the data in Fig. 2.1 (d) to identify an inflection point corresponding to the LCST. The resulting derivative data was fitted with a Gaussian to determine error as before.

Here, the results of TEM and DLS characterization of the PNIPAM micelles, synthesized via emulsion polymerization, are presented. TEM revealed the PNIPAM micelles were spherically monodisperse at almost 120 nm in diameter. Keep in mind, however, that this technique exposes the micelle to conditions significantly different from what should be considered in-situ. A better technique for ascertaining PSDs while the micelles are in solution is DLS. This method also allows for temperature dependent size measurements. With this technique the stimuli responsive nature of the micelles was demonstrated. In fact, the micelles discontinuously change their size when being heated or cooled past about 36 °C. Ultimately, emulsion polymerization proved problematic when IOMNP encapsulation was attempted, and subsequently abandoned to pursue spray processes. In Ch. 3, the reason for this difficulty will be attributed to the agglomeration of IOMNPs.
2.1.1.1. PNIPAM micelle centrifugation. Early in this work samples of PNIPAM micelles were acquired for characterization to determine their usefulness for the targeted biotherapeutic delivery application defined in Sect. 1.1. TEM was the initial tool of choice since it had the potential to allow for descriptions of micelle morphology and size. Unfortunately, initial imaging yielded micrographs that at first seemed out of focus, but in hindsight were better described as hazy. It was as if one was seeing the micelles through a fog. This short section documents centrifugation efforts to improve the TEM imaging. It’s well known that centrifugation can serve to separate a sample based the densities of its constituent materials [150-154]. After obtaining the low quality micrographs it was decided to employ centrifugation in an attempt to separate the micelles from any unreacted chemicals left in the solution.

As explained at the beginning of Sect. 2.1.1, the PNIPAM micelles used here were synthesized via emulsion polymerization. A Sorvall Instruments, MicroSpin centrifuge, rated at 100 W, 1.1 A, and 115 V, was used to carry out the separation. Aliquots of the sample were placed in 1.5 mL centrifuge tubes after being ultrasonically agitated for approximately 30 minutes. The tubes were then centrifuged at 10,000 rpm for about 15 minutes. After this the supernatant was carefully removed without disturbing the precipitate at the bottom of the tube. The tube was filled back up to its original volume with filtered DIW. This entire process was repeated four times. In this way, the precipitate would have been cleansed of unreacted materials a total of five times. After the last precipitate was reconstituted with filtered DIW and sonicated a drop of it was allowed to dry on a copper coated TEM grid. The electron beam was set to a constant 60 keV for all imaging.

The micrograph shown in Fig. 2.2 (a) is typical of those obtained during the first TEM session. While the micrographs serve to definitively say micelles are in the sample, there was no detailed information that could be taken from them. Imaged here are, seemingly, unfocused artifacts with no definite shape or edge. In other words, it was impossible to comment on morphology or to obtain a particle size distribution. This uncentrifuged sample was at a concentration of 0.5 wt. % before desiccation on the TEM grid.

Once exhaustive centrifuging was accomplished, TEM imaging was much improved. Figure 2.2 (b) reveals the PNIPAM micelles once the unreacted chemicals had been removed from the solution. Now the micelles are obviously round suggesting a spherical morphology. Their definite edges make their size
measurement possible as was reported in the last section. There is even surface morphology apparent in these micrographs that is indicative of PNIPAM crosslinking.

After centrifuging it was impossible to report on sample concentration. Removing the supernatant and replacing with clean water necessarily confuses that parameter. In later experiments lyophilization was used on different samples. That technique would have made it possible to reacquire the concentration after weighing out the dehydrated micelles before adding to a known volume of water. It’s important to note the high quality micrographs portray the micelles as agglomerating together. It will be shown in Sect. 2.3 that TEM sample preparation necessarily pulls artifacts together upon drying. Only characterization involving samples still in solution remove this tendency to agglomerate. Ultimately, centrifugation served to improve TEM micrographs of PNIPAM micelles. Presumably, these micelles were obtained with unreacted chemicals in the sample that obscured the electron beam during imaging.

2.1.2. Spray

Recall from Ch. 1 that the stimuli responsive nanogel is the component of the biotherapeutic delivery device responsible for carrying the biotherapeutic within the patient and also responsible for release
of the biotherapeutic upon temperature increase. Here, efforts to synthesize a stimuli responsive nanogel using spray technique, arguably significantly different from the emulsion polymerization technique detailed in Sects. 1.4.1 and 2.1.1, are documented. There are various applications, including biotherapeutic delivery, that utilize spray generation [130-133], but this work investigated a piezoelectric gold crystal in one effort and a repurposed vibrating mesh from a medical inhaler in another effort. Specifically, the goal of these investigations was proof of principle in nature; the experiments had the aim of demonstrating the spray concept of nanogel synthesis had practical potential. Of particular interest was developing a nanogel synthesis method that was relatively lower cost, faster, and/or simpler in design when compared to techniques used by other researchers (e.g. emulsion polymerization, other spray techniques, etc.). Ultimately, successful synthesis would rely on the chemistry introduced in Sect. 1.4.

The first spray technique investigated was that whose spray was generated from the precursor solution via piezoelectric gold crystal. The unit, shown in Fig. 2.3 (a), was a Sonaer model 241 (see Appendix A) piezoelectric ultrasonic nebulizer operating at a frequency (f) of 2.4 MHz. The nebulizer was mounted to the bottom of a vessel designed for the mount and to hold the precursor solution, as shown in Fig. 2.3 (b). In these experiments the precursor solution was initially just DIW, but was later changed to NaCl, gellan gum, or agarose dispersed in DIW. These solutes were purchased from Alfa Aesar and were used as received without further purification. Originally, the temperature of the precursor solution was monitored with a thermocouple, but when it was realized that the high frequency nebulizer was interfering with the measured temperature that data was abandoned. The mist forming at the surface of the precursor solution could only exit the vessel vertically through a copper tube designed to direct the nebulous mist out. Brief, low flow rate puffs of N₂ were directed into the vessel through a separate copper tube in order to facilitate the transformation of the nebulous mist into a spray. The spray was directed through copper tubing to an ITO glass substrate for sample collection. That sample was allowed to dry thoroughly, then taken for coating via sputtering of AuPd under vacuum to improve contrast during SEM imaging. The SEM used was a JEOL JSM-6390LV having a tungsten filament. It’s imaging parameters were set to 4 keV accelerating voltage, 10 mm working distance, and a spot size of 30 for all sample characterization described in this section.
One of the earliest findings with this piezoelectric technique was that the nebulizer could not operate unless the viscosity of the precursor solution was very close to that of water. Therefore, it would not operate with significant concentrations of gellan gum or agarose. Nebulizing salt water was less challenging and served the purposes of forcing the droplets to carry some solute. The formation of the nebulous mist during a trial can be seen in Fig. 2.3 (b). Also, apparent in the picture is the prevalent

Figure 2.3 In early attempts to synthesize a TBD device (a) a Sonaer model 241 vibrating ultrasonic nebulizer (b) was mounted to a chamber that directed mist out to a collection substrate where (c) microdroplets were collected for SEM imaging.
condensation of the nebulous mist onto the walls of the quartz glass vessel. In fact, the mist would also condense within the copper tube on its way out toward sample collection. More often than not a sample substrate would be ruined when a large drop would come out of the copper tube and land on the ITO glass substrate. Condensation was a common occurrence that manifested in any attempt to redirect the nebulous mist. Figure 2.3 (c) is a representative SEM micrograph demonstrating one of the few successful spray droplet captures. This artifact is best described as the left over remnant, or stain, left behind after the droplet from the spray landed on the ITO coated glass and completely dried out. The stain is the left over solute on the ITO revealing the size of the droplet as it spread out on the substrate. For the purposes of determining the original droplet size, it was assumed that such remnants were approximately cylindrical having diameters \(d_{cyl}\) that could be measured using software on the SEM computer. Using a profilometer the height \(h\) of several stains were measured. The volume of the cylinder \(V_{cyl}\) was determined through

\[
V_{cyl} = h \frac{\pi d_{cyl}^2}{4}. \tag{2.1}
\]

It was assumed that this volume was approximately equivalent to the volume of the spherical droplet \(V_{sph}\) which landed on the substrate. In that case, the size of the original droplet \(d_{sph}\) could be determined through

\[
V_{sph} = \frac{\pi d_{sph}^3}{6}. \tag{2.2}
\]

The calculated volumes for several similar cylindrical artifacts are recorded in Table 2.1, which reports an average volume of approximately 790 ± 590 cubic microns. Here, the error is the standard deviation of the trial volumes. For a spherical droplet of similar volume the droplet must be about 11 ± 10 microns across, as determined using Eqn. 2.2. Note that the specifications for Sonaer’s nebulizer state that it should produce

\[
V_{cyl} = h \frac{\pi d_{cyl}^2}{4},
\]

\[
V_{sph} = \frac{\pi d_{sph}^3}{6}.
\]
droplets around 1.7 microns in diameter. Sonaer makes this claim by taking the surface tension (T) of water as 0.0729 N/m and the density (ρ) of water as 1000 kg/m$^3$ and evaluating for the droplet size using

$$d_{\text{sph}} = 0.73 \sqrt[3]{\frac{T}{\rho f^2}}.$$

The results presented here seem to indicate that the droplets were colliding midflight producing larger droplets. This conclusion is also indicated in the error reported here; the droplets are not monodisperse but likely range from as small as 1 micron to as large as 21 microns in diameter. This is likely a consequence of constraining the nebulous mist to flow within the narrow and bending copper tube. Of course, the sample size is rather small and contributes to the error.

Discouraged by the performance and design restrictions of the piezoelectric nebulizer a different technique for generation of spray was investigated. Next, a medical inhaler that utilizes a vibrating metal mesh in contact with the medicine (or in this case the precursor solution) was evaluated for feasibility in this work. The particular unit was a ClinicalGuard Ultrasonic Portable Nebulizer HL100 (see Appendix B) operating at a frequency of 120 kHz. During characterization of the nebulizer, the metal mesh was removed from the rest of the unit for imaging under SEM. One of the resulting micrographs is shown in Fig. 2.4 (a) at a low enough magnification to observe most of the holes on the entire mesh. The insert to this figure is a high magnification micrograph showing one of the holes in great detail. A survey of several of these holes confirms the manufacturer’s assertion that the holes are approximately 4 microns across. The mesh was mounted to the bottom of a Teflon housing with the appropriate electronics for activation and seals to prevent precursor solution leakage. The precursor solution was stored in the Teflon housing during a trial. Various concentrations of NIPAM (monomer), MBA (crosslinker), and Irg 2959 (photoinitiator) were dispersed in DIW to constitute the precursor solution. These solutes were purchased from Alfa Aesar and used as received without further purification. Figure 2.4 (b) depicts the experimental setup in these investigations including the Teflon housing and cylindrical drying chamber, both of which were custom built and designed in house. It can be seen from the experimental setup that the mist forming at the nebulizer mesh falls into the drying chamber. That chamber is wrapped in heat tape, which is controlled by a Variac for the purposes of encouraging and controlling the amount evaporation of any given droplet in the mist. The temperature of the precursor solution and within the drying chamber was monitored via thermocouple. A sample of the mist was collected on an ITO coated glass piece affixed to a variable height plate.
Figure 2.4 In another attempt to synthesize a TBD device (a) a vibrating mesh from a medical inhaler, imaged here by SEM, was powered in (b) a spray dry experimental setup that produced (c) sample collection substrates documenting microdroplet remnants, (d) which exhibited minimal size at a collection distance of approximately 10 cm from the vibrating mesh.

Preparation of the substrate was similar to that described for the piezoelectric investigations above. Briefly, after the sample dried thoroughly it was coated with AuPd to enhance contrast under SEM imaging. SEM parameters (i.e. spot size, accelerating voltage, and working distance) were identical to those described above.
First, it’s important to note that this nebulizer also would not operate unless the viscosity of the sample was very similar to that of water. So, as before, the concentration of solutes (monomer, crosslinker, and photoinitiator) in DIW had to be impractically low to produce a mist. Once the nebulizer was operating, trials were conducted in which stains of the droplets comprising the mist were characterized. Figure 2.4 (c) shows a representation of an SEM micrograph of these stains and the PSD is inset in the figure. When surveying the sizes of the stains care was taken not to consider asymmetric remnants since these were likely the result of droplets that had coalesced from smaller droplets. Average sizes were determined by fitting the PSD with a Gaussian function whose standard deviation from the peak was attributed to the reported error. The first study conducted was aimed at optimizing the distance between the nebulizer mesh and plate upon which the sample was collected. The results of this constant trial time study are given in Fig. 2.4 (d). At a sample collection distance of nearly 4 cm the remnants were approximately as large as 250 microns. This relatively large remnant size can be attributed to the fact that there is so little space between the mesh and substrate that the mist had no time to spread out before being collected and droplets landed in ever increasing pools on the substrate. On the other end of the range for tested collection distances, another relatively large, albeit smaller than collection at 4 cm, remnant size was measured. At this collection distance of 15 cm an average remnant size of almost 100 microns was determined. This large remnant size can likely be attributed to the fact that the collection plate is now so far away from the mesh that falling droplets have time to coalesce in flight before striking the substrate. The relatively large error in this measurement can be taken as further evidence of the unsuitable nature of this collection distance since it indicates a range of droplets. The two trials performed at collection distances between 7 and 12 cm yielded the smallest remnant size and corresponding error. Based off this information, it was decided to set a constant collection distance of 10 cm for the next study since it lies in the optimal range and is easy to remember.

Because the droplets had very low concentrations of solute it was necessary to consider controlled evaporation of the solvent from the droplets. Therefore, the heat tape described above was used to control the temperature of the environment in which the droplets fell to induce evaporation. A study of the effect of drying chamber temperature on the remnant size was conducted for the purposes of determining a recipe for synthesis of nanogels. The results of that study are provided in Fig. 2.5 (a). Overall remnant size was
found to decrease with chamber temperature at an approximate rate of -0.24 μm/°C demonstrating that the final concentration and size of droplets could be controlled. However, when none of the trials produced nanogels upon UV photo-irradiation the technique came into doubt. This was especially the case after the Teflon Housing was allowed to run dry and solute was found within. This raised the question of the

Figure 2.5 (a) Average droplet remnant size as a function of drying chamber temperature, indicating to what extent evaporation can be controlled while the droplet is falling. (b) Artifacts synthesized after exposing the falling spray from EXAIR’s stainless steel atomizer to a UV source. (c) Proposed experimental setup to synthesize stimuli-responsive microgels.
possibility of any solute making it through the vibrating mesh in the first place. Not long after, the technique was abandoned.

Finally, a pneumatic atomizer (see Appendix C) was used in a final attempt to create stimuli responsive nanogels. The atomizer was manufactured by EXAIR which asserted that the droplets in the spray were between 39 and 57 microns in size. Now obviously this is in violation of the application described in Ch. 1 since the resulting microgel would be larger than the upper size limit of a human capillary of about 10 microns. However, it was hoped that successfully synthesizing a too large microgel would inform on synthesizing the smaller variety. Precursor solutions were prepared as before to contain the monomer, crosslinker, and photoinitiator dispersed in DIW at substantial concentrations. A UV lamp (described more fully in Sect. 2.2.2) was made to irradiate the vertical space between the atomizer nozzle and the ITO coated glass substrate in order to initiate polymerization. SEM imaging and preparation of the sample was similar to that described above.

A typical SEM micrograph of the artifacts created during these atomizer trials is given in Fig. 2.5 (b). The larger image shows a wide field view of the approximately 100 micron artifacts while the inset shows the surface topography of one of these specimens. Again, the relatively large size of these artifacts makes them ineligible for biotherapeutic delivery to the various tissues of the human body. The artifacts certainly have a plastic-like quality to them giving the impression that polymerization has taken place. However, the artifacts are quite flat whereas a spherical shape was expected. While a reduction of the concentration would allow for too large droplets that are later evaporated down to a reasonable size and concentration, as described above, the incorporation of this atomizer nozzle into the proposed experimental design turned out to be impractical.

At the candidacy evaluation stage of this work the proposed experimental design for synthesis of stimuli responsive nanogels was presented and generally accepted as likely to succeed. That design is presented in Fig. 2.5 (c). Briefly, a precursor solution could be sprayed into a heated evaporation chamber. The optimized for size and concentration droplets would then fall into a chamber where photo-irradiation would commence forming nanogels. A cross-section of the irradiation zone reveals a UV source at its center surrounded by a quartz tube so that N₂ could keep the bulb of the UV source clean of mist while preventing O₂ from absorbing the polymerizing energy. Concentric with the first quartz tube is another quartz tube of
larger diameter to confine the mist in a ring where the photo-polymerization takes place. An optional third layer concentric with the first two might be composed of a PVC pipe for the purposes of a water jacket which could regulate the temperature of the entire reactor. After passing through the reactor the product finally falls out of the system for collection and analysis. As indicated above when it finally came down to assembling this system there was the problem of directing the spray into the second-most ring. The force of the atomized spray was of such magnitude that it was not possible to prevent condensation on the inner or outer walls of the quartz tubes rendering the spray little more than a too heavy stream and completely eliminating all droplets.

Any attempt to control a flow of nebulous mist by directing it into a spray necessarily resulted in some degree of condensation. It turns out that condensation was perhaps the most challenging problem in any attempt to succeed in synthesis of nanogels using the spray technique. The next most challenging problem was controlling the concentration since all nebulizers required the precursor solution be similar to water in viscosity. This required the complication of consideration of droplet evaporation before polymerization could take place. Unfortunately, in the interest of time and consideration of resources the proposed setup had to be abandoned along with the spray technique for completion of this dissertation. In the next section, a new approach will be described that still utilized UV photopolymerization, but eliminates the complexity of the spray technique. The next technique avoids condensation altogether by eliminating spray in the synthesis. Ultimately, it will be shown that the early attempts to control final droplet concentration and size through evaporation can be accomplished much more directly. In effect, the techniques presented here are relatively more complicated than those presented next.

2.2. Description of Photopolymerization Experimental Setup

In the last section, attempts at synthesis of stimuli responsive nanogels using emulsion polymerization and the spray technique were documented. It turned out that micelles synthesized via emulsion polymerization had a peculiar morphology when encapsulation of IOMNPs was attempted and so that technique was abandoned. Also, all attempts at nanogel synthesis using a spray technique ultimately failed mostly due to the complexities of synthesis engineering, like condensation of the spray, as well as the restrictions, placed by optimal nebulizer operation, on spray concentrations. The remaining sections of
this chapter focus on presenting successful synthesis of stimuli responsive nanogels. This section documents the system, designed and assembled in house, for monitoring the synthesis of the biotherapeutic carriers.

Figure 2.6, document the system that was capable of not only the synthesis of nanogels, but also monitoring their synthesis while it is occurring; the assembly of the nanogels is observed in situ. Both the schematic, shown in Fig. 2.6 (a), and the photograph, shown in Fig. 2.6 (b), relate how this was possible. A hand held UV lamp was used as the source of UV energy for photopolymerization. The lamp was affixed to the reaction chamber which had a volume of approximately $3.6 \times 10^{-3} \text{ m}^3$. The atmosphere of the reaction chamber was purged with $\text{N}_2$ gas at a rate of nearly 1 LPM for at least 10 minutes prior to the start of any trial. This was enough time to rid the chamber of $\text{O}_2$ about three times over. The purpose for removing $\text{O}_2$ was to eliminate the possibility of it absorbing the UV energy in the generation of ozone ($\text{O}_3$). Any light from the UV source that made its way through the sample containing, quartz cuvette was detected by the ICCD camera (Appendix D) after being directed through the spectrometer (Appendix E) and fiber optic cables (Appendix F) [155].

Figure 2.6 The experimental setup for in-situ monitoring of photopolymerization synthesis of stimuli-responsive microgels as illustrated by (a) schematic and (b) photograph.
Each of the plastic (polystyrene), glass, and quartz cuvette types was analyzed for absorbance versus wavelength in order to determine the most suitable one for synthesis trials. The results of the investigation of the cuvettes available for the synthesis, described in this chapter, are provided in Fig. 2.7 (a). Here we see that the plastic cuvette (black line data) is the worst one for UV transmittance. The plastic cuvette displayed saturated absorbance from a little less than 200 nm to a little less than 300 nm. It will be shown in Sect. 2.2.2 and 2.3 that much of the critical photochemistry is occurring in this part of the spectrum which the plastic cuvette would have prevented by totally absorbing the energy. The glass cuvette (red dashes data) is only a little bit better in that it permits some energy into this critical part of the spectrum but the absorbance is still significantly high here. The quartz cuvette (green dots data) exhibits the overall lowest absorbance at any given wavelength compared to the other two cuvette types. Its absorbance only deviates from zero at wavelengths just under 200 nm.

Further investigation of the interaction of environmental parameters (i.e. atmosphere, cuvette, and solvent) with incident light are reported in Fig. 2.7 (b). Here, the absorbance versus wavelength of the air in the chamber, empty quartz cuvette, and solvent (DIW for all trials discussed in this chapter) is given. Now we see that the solvent (gray short dashes and short dots data) has a higher absorbance than the cuvette
(green dots data) does at wavelengths less than about 270 nm. This information will be revisited in Sect. 2.3. Above 270 nm the cuvette exhibits only a little higher absorbance. The data corresponding to the air in the chamber (long dashes and long dots data) exhibits no apparent absorbance at any given wavelength on this scale.

In Sect. 2.2.1 a more detailed description of the spectrometer and ICCD camera will be provided. First, an accounting of response of the OMA system to wavelength is given. In other words, the full spectrum this system is capable of measuring will be defined. Then, the calibration of the OMA system is documented. Finally, the neutral density filters (NDFs) used in this work to protect the ICCD chip from saturation are discussed. Later, in Sect. 2.2.2 the UV source is described in detail. Its spectrum, as measured by the OMA system, will be presented first. Next, a study of the intensity of the UV source as a function of distance to the bulb is given. Last, analysis of the timing of the UV source’s output is discussed.

2.2.1. Optical Multi-Channel Analyzer (OMA)

2.2.1.1. Characterization. Here a detailed description of the distinctive measured intensities of the OMA system in response to particular wavelengths is given. This was necessary early in the work in order to determine what parts of the spectrum could be monitored during later trials. It also served the purpose of determining how the sensitivity of the ICCD detector changes across the measurable spectrum. In fact, the manufacturer provides the quantum efficiency versus wavelength data that is plotted in Fig. 2.8 (a). This demonstrates that the detector is generally more sensitive in the visible and NIR parts of the spectrum than it is in the UV. The section seeks to confirm this data and that the OMA system has not lost sensitivity after prolonged inactivity over the years.

To achieve these goals, the source of another spectrometer was used to assess the performance of the OMA system. The sources were a deuterium lamp for the UV range and a tungsten lamp for the visible to NIR range. These sources were part of a Perkin Elmer High-Performance Lambda 950 spectrometer. Figure 2.8 (b) shows how the source from the Perkin Elmer spectrometer was directed into the fiber optic cable of the OMA system. In this way, the source of the Perkin Elmer spectrometer was used as a monochromator and the OMA system was able to measure and record the resulting spectra. It should
be noted that neutral density filters (NDFs) were used to prevent the source from saturating the ICCD detector. In each case the data was corrected by using the following

\[ \frac{1}{I_o} = 10^{\text{OD}}. \tag{2.4} \]
In Eqn. 2.4, the measured intensity when using a NDF is given by $I$, while the original, unfiltered intensity is given by $I_o$. The optical density of any given NDF is given by OD and was provided by the manufacturer of the filter. Section 2.2.1.3 documents attempts to experimentally determine the OD. The data reported here in the form of intensity versus wavelength plots have been corrected for the NDF used in that particular measurement using Eqn. 2.4.

As an example, a sample spectrum of the Perkin Elmer source is given in Fig. 2.8 (c). Here, the Perkin Elmer system was used to direct light from its deuterium source at 220 nm into OMA’s fiber optic cable. The peak was fit with a Gaussian to determine its center, and one quarter of its width yielded the error reported. Obviously, OMA is capable of detecting light in the UV part of the spectrum as indicated by the presence of this peak. Similar spectra were obtained for several wavelengths throughout the operational domain of the OMA system. The maximum intensities, after correction using Eqn. 2.4, were plotted as a function of corresponding wavelength and the full response of the OMA system is shown in Fig. 2.8 (d). The vertical line at 320 nm indicates the transition from deuterium to tungsten source within the Perkin Elmer spectrometer. Apparently, the OMA system has a relatively weak sensitivity in the UV region compared to the visible and NIR regions. While the intensities in the UV regime appear relatively low compared to other data points it is important to note that these intensities were significantly higher than zero. Again, the response of the OMA system is significant from ultraviolet up to near infrared. Keep in mind that data in other sections of this work predominantly focus on the UV.

This section has established that the OMA system is, in fact, operational in the UV, visible, and NIR regions of the spectrum. It has also been verified that the sensitivity of the ICCD detector is not constant in these regions. Note, that the quantum efficiency of the detector, according to its manufacturer, is similar in shape to the response versus wavelength plotted in Fig. 2.8 (d). Since, no characterization of the Perkin Elmer source was performed the data in Fig. 2.8 (d) is not suitable for corrections of future spectra. Therefore, all spectra of future sections recorded with the OMA system will have their intensities corrected using the data plotted in Fig. 2.8 (a). It is interesting to note the minor discrepancy between the wavelength measured in Fig. 2.8 (c) and the wavelength programmed into the Perkin Elmer spectrometer. This suggests some calibration was necessary to bring these two wavelengths into agreement which is the topic of the next subsection.
2.2.1.2. Calibration. It was shown in the preceding subsection that OMA is capable of recording spectra covering the UV, visible, and NIR regions of the spectrum. However, it is imperative that the spectrum, recorded by OMA, of some standard sample compare reasonable close to the already documented spectrum of said sample. Here, an accounting of the calibration of OMA software, known as WinSpec, as well as an assessment of that calibration is provided. First, a description of the process the software uses to achieve calibration is presented. Then, a test of that calibration is described using the Perkin Elmer spectrometer discussed in Sect. 2.2.1.1. Finally, the calibration will be further evaluated against a Hg standard calibration bulb.

The initial calibration of the OMA system used for the in-situ, study of PNIPAM’s photopolymerization was conducted using the WinSpec operating software for the spectrometer and camera. The standard calibration bulb used for this process was a He bulb. The software organizes this process into three parts that consist of an offset, adjustment, and dispersion. The purpose of the offset step is to recognize that when the user commands the software to tell the spectrometer to move its mounting to a particular wavelength that mechanical tolerances prevent this from being achieved precisely. In the adjustment step of software calibration a linear correction to measured wavelengths is applied in order to match a standard. In this work, the standard calibration bulb used to determine this linear correction was He. Finally, the WinSpec software applies a dispersion correction by measuring a particular standard peak both on the left side of the spectral window and the right side. The discrepancy between these two values is accounted for in the dispersion step. Again, this process was followed for several of the strongest peaks corresponding to He in order to achieve calibration. What follows is two efforts to demonstrate successful calibration.

It has already been stated that a standard He bulb was used for the WinSpec software calibration of OMA. For the first round of evaluation of that calibration the Perkin Elmer source was used as described in the previous subsection. Essentially, a wavelength was dialed in to the Perkin Elmer software to command its source to output at that particular wavelength. Then the OMA system recorded a very narrow peak like that discussed in Sect. 2.2.1.1. The center of the peak was found by fitting it with a Gaussian function and its standard deviation corresponded to one-fourth of its width. Note in Fig. 2.8 (c) the discrepancy between the Perkin Elmer spectrometer output wavelength and the OMA measured wavelength. For this example, even though the Perkin Elmer spectrometer was instructed to output 220.0
± 0.3 nm, the OMA system measured the peak at 218.6 ± 1.6 nm. Apparently, despite software calibration, some minor correction is necessary to bring the Perkin Elmer spectrometer and OMA into agreement.

So measured OMA wavelengths versus Perkin Elmer spectrometer output wavelengths were plotted. This linear correction is shown in Fig. 2.9 (a). If it was desirable to calibrate OMA data to the Perkin Elmer spectrometer, solving the linear equation in Fig. 2.9 (a) for \( \lambda_{PE} \), Perkin Elmer wavelength, and applying that to the recorded data would suffice. However, close inspection of the data and consideration of the excellent R-squared value suggest the correction is unnecessary and the WinSpec software calibration was sufficient.

However, the practice of using the deuterium and tungsten sources in the Perkin Elmer spectrometer as calibration standards quickly came into question. Therefore, it was decided to produce a similar calibration correction using a standard calibration Hg bulb. The reason a Hg bulb was selected was that it was known to possess strong peaks in the UV region which is so important in this dissertation. In this round of calibration assessment the fiber optic cable of the OMA system was mounted on an optical bench and directed to gather light from the Hg calibration bulb. The measured spectra was matched and compared to National Institutes of Standards and Technology (NIST) data [156]. The wavelengths of the characteristic

---

**Figure 2.9 (a)** By comparing the Perkin Elmer spectrometer dialed-in wavelength to the measured ICCD detector wavelength a calibration curve was generated, which is similar to the (b) calibration curve generated by comparing ICCD detector measured wavelengths of a Hg calibration lamp to the corresponding wavelengths reported by NIST.

![](image)

OMA vs Perkin Elmer
\[ \lambda_{OMA} = 0.9949 \lambda_{PE} + 0.7559 \]
\[ R^2 = 0.9999 \]

OMA vs NIST
\[ \lambda_{OMA} = 0.9980 \lambda_{NIST} + 0.3179 \]
\[ R^2 = 0.9999 \]
Hg peaks as measured by OMA were plotted against the corresponding NIST wavelengths in Fig. 2.9 (b). As before, the data is well described by the linear fit which is indicated by the excellent R-squared value. Therefore, software calibration was sufficient and no further correction of OMA data is necessary.

Here, the WinSpec software algorithm for calibration of the OMA system was presented. This was achieved by considering the offset, adjustment, and dispersion of raw data necessary when reconciling it against how standard peaks should measure. Also, discussed were two assessments of the WinSpec calibration in order to demonstrate the validity of all OMA data presented from here on. The comparison of OMA measurements to the Perkin Elmer source prescribed negligible corrections to bring the two into agreement. Likewise, the comparison of OMA measurements of a Hg standard calibration bulb to those reported by NIST archives suggested only minimal adjustment to match the two. This second assessment is particularly pertinent to the work since the source of UV energy discussed in Sect. 2.2.2 was, in fact, a Hg-gas filled bulb. In the next subsection (Sect. 2.2.1.3), the NDFs used to protect the OMA system from saturation are described.

2.2.1.3. Neutral Density Filters. In order to prevent the saturation of the CCD chip in the OMA system camera, it was necessary to utilize neutral density filters (NDFs). The NDF allows for the reduction of light independent of wavelength. In photographic applications, for example, this can cause an overall dimming but no change in color [157]. The ratio of measured intensity to the original, unfiltered intensity of light is equivalent to the transmittance of light as expressed in Eqn. 2.5.

\[
\text{Transmittance} = \frac{I}{I_o} = 10^{-OD}
\]  

Here OD refers to the optical density of the filter; it is the degree to which incident light is attenuated through the NDF [158]. While the manufacturer of commercially purchased NDFs provide specifications including the OD, one can also solve Eq. 1 for OD, as shown in Eq. 2, and perform experiments that should corroborate the specifications.

\[
OD = - \log \left( \frac{I}{I_o} \right)
\]  

Using the OMA system, all NDFs used in this work were subjected to a series of experiments to determine an exact value for its OD. Commercially purchased NDFs were advertised to have optical densities of 0.1, 0.2, 0.6, 0.7, and 0.8. Three custom built holders were fashioned for each of the five NDFs making a total of fifteen filters that were characterized. These filters are shown in the photograph of Fig.
2.10 (a). The fiber optic cable, which directs light into the spectrometer, was mounted into a holder secured to the optical bench. The NDF being characterized during any given trial was affixed in front of the fiber optic cable, as shown in Fig. 2.10 (b). A spectrum was obtained of a calibration Hg bulb with the NDF in place to obtain the filtered intensity, \( I \). Then the filter was removed and the exact same spectrum was recorded to serve as the original, unfiltered intensity, \( I_o \). Of course, care was taken to ensure that, even unfiltered, the intensity would not saturate the detector. These quantities were then used in Eqn. 2.6 to determine the experimental OD. Every spectrum described in this experiment was the accumulation of at least 100 scans and centered at 270, 400, 560, 720, and 880 nm, so that the entire wavelength domain – 190 nm to 930 nm – of the spectrometer could be tested with each filter.

The results of the NDF characterization experiments are summarized in Table 2.2. Here each OD is the average of four peaks, as determined using Eqn. 2.5, found in the spectrum observed in a particular experiment. In the first round of experiments the spectrograph was centered at 270 nm and included the wavelengths from 190 nm to 350 nm. Here the filters do appear to increase in optical density with the advertised OD, with the exception of the advertised 0.80 NDF. Note, however, that most of the experimental values are not similar to the advertised OD values. Also the standard deviations suggest that data is poor in this UV exclusive part of the spectrum. In the second round of experiments, those centered at 400 nm,
some negative values for OD were obtained which indicate that the filtered light was more intense than the unfiltered light. This seemingly unphysical result occurs in later rounds of the experiments but only for the lowest OD values of NDF: 0.10 and 0.20. Beginning with the second round and for the remaining experiments (centered at 400 nm, 560 nm, 720 nm, and 880 nm) the largest valued experimental ODs appear to not only trend with the advertised values but have smaller relative error. However, there is still a significant difference between the advertised and experimental ODs.

When the first negative ODs were calculated during analysis it was immediately clear from the data that filtered intensities were greater than the unfiltered intensities. The NDFs were not attenuating the incident light, but, rather they were augmenting it. That is to say the filters were behaving as lenses. Immediately, the NDFs were visually inspected and observed to be warped by the tape holding them against the black plastic seen in Fig. 2.10 (b). The physical size of the NDFs used here make straightening difficult. In fact, between experiments it can be expected that NDF curvature and therefore OD will change as a result of the tape settling or shifting during storage. It was also learned that these filters had previously been used in experiments that may have caused heat damage which leads necessarily to warping and curving of the NDF.

Since all these concerns make the preparation and preservation of well characterized NDFs difficult it was decided to only characterize those filters that are used in a particular experiment described in other sections of this dissertation. Concerning those sections, when a sample’s spectrum was obtained it may or
not may not have originally emitted light that was saturating the camera. When this was the case one or more NDFs were selected to ensure all strong peaks were not damaging the camera. The spectrum was recorded using those NDFs and immediately the spectrograph was re-centered to exclude strong peaks, but contain weaker peaks that do not saturate the camera when filters are excluded. Another spectrum was quickly obtained without the filters and common peaks between this and the first spectrum were compared to determine the experimental OD. It was important to obtain the second spectrum quickly in order to guarantee that the light source was not behaving significantly different.

2.2.2. Ultraviolet Source Parameters

2.2.2.1. Wavelength. The source of UV irradiation in this work was achieved with Spectroline E-series handheld lamps. There were a number of replacement tubes available for purchase with the lamps. Three types of tubes were purchased for this work including those advertised to be centered at 254 nm, 312 nm, and 365 nm. In addition, one lamp was shipped containing a tube having markings which indicated it was also centered at 365 nm and that the tube was integrally filtered. As discussed in Sect. 2.2.1.2, the ionizing gas within all four of the lamps was Hg.

One of the lamps to be characterized was fixed to a chamber that was sealed to keep out ambient light from the room. Despite this precaution all trials were conducted with the room light off to reduce background radiation. In addition, N₂ was flowed into the chamber to prevent O₂ from absorbing the radiation of interest. The fiber optic cable of the OMA system was fixed to the opposite side of the chamber and directed toward the lamp. Exposure times for each spectrum was 200 ms and 100 of those spectra were summed to enhance the signal and reduce noise. NDFs were used to protect the OMA camera from saturation due to particularly strong peaks (see Sect. 2.2.1.3). Intensity data has been corrected in terms of sensitivity of the OMA system as a function of wavelength (see Sect. 2.2.1.1).

Initially, the OMA system was used to observe and record the UV, visible, and NIR parts of a particular bulb’s spectrum. Those spectra are shown in Fig. 2.11. Specifically, the 254 nm bulb’s spectrum is given in Fig. 2.11 (a), the 312 nm bulb in Fig. 2.11 (b), the 365 nm bulb in Fig. 2.11 (c), and the filtered 365 nm bulb in Fig. 2.11 (d). For the data shown here the grating used had a spacing of 150 grooves per millimeter, which was the coarsest resolution available. Note that many of the peaks are shared in all 4
spectra (e.g. approximately 400 nm and 430 nm). This can be attributed to the fact that all bulbs contain Hg, and in fact compare favorably to NIST data [156]. It was interesting that the bulbs emit strongly in the visible and NIR parts of the spectrum. Equally surprising, Fig. 2.11 shows considerably weaker peaks in the UV part of the spectrum. These characteristics were not expected since the bulbs were advertised and

Figure 2.11 The OMA system measured these UV/Vis/NIR spectra of the bulbs identified by the manufacturer as (a) 254 nm, (b) 312 nm, (c) 365 nm, and (d) 365 nm integrally filtered.
purchased for their ability to emit UV radiation. In order to better resolve the UV portion of the bulb’s spectrum the grating was then changed to 1200 grooves per millimeter, which was the highest resolution available. In Fig. 2.12, the highly resolved UV peaks that warrant the advertised wavelength, are displayed. Again, Fig. 2.12 (a) is the 254 nm peak, Fig. 2.12 (b) is the 312 nm peak, Fig. 2.12 (c) is the 365 nm peak, and Fig. 2.12 (d) is the integrally filtered 365 nm peak.
Overall, the spectra of all 4 bulbs exhibit strong peaks in the visible and near infrared portions of the spectrum. Upon close inspection of Fig. 2.11 there are some apparently, relatively weaker UV peaks. Only when the grating was changed from the 150 grooves per mm to the 1200 grooves per mm, and the spectrograph centered on the region of interest, did the advertised UV peaks become more prominent. Overall, the spectra do match the strong peaks of Hg as reported by NIST. Ultimately, the bulb labelled 254 nm was chosen for the experiments described later in this chapter. This decision was reached since the 254 nm peak provides approximately the correct energy needed to break the chemical bonds to initiate polymerization. More details on this photochemistry will be discussed in Sect. 2.3.

2.2.2.2. Distance. The purpose of this section is to demonstrate the relationship of intensity as a function of separation between the UV light source and the sample contained in the quartz cuvette that is to undergo photopolymerization for the synthesis of targeted biotherapeutic delivery devices. General physics students learn that the intensity of a light source varies inversely with the square of the separation from the source [159-161]. More specifically, for a point source, the relationship between intensity (I) and separation (r) can be expressed with

\[ I = \frac{P}{4 \pi r^2}. \]

In other words, the intensity of light at a certain point away from the light source is the quotient of the power (P) of the light source to the surface area of a sphere that contains the point on its surface and centered on the source. Note that this relation is only true when the light can be approximated as a point source; Eqn. 2.7 is valid when the source is relatively small and/or far from the location of interest.

In order to experimentally determine whether or not Eqn. 2.7 would apply to the investigations described in Sect. 2.3 and Sect. 2.4 a study of intensity versus sample separation from the 254 nm light source was conducted. Using the OMA system spectra were collected from the bulb at separations that ranged between 5 cm and 50 cm. The spectrometer was centered on the strongest peak of the UV light source, 545 nm, in order to record the maximum intensity for a given sample/source separation. The exposure time of the ICCD camera was set to 1.0 seconds and 100 accumulations were acquired for any one data point graphed below. The fiber optic cable was directed toward the UV light source of which a photograph is inset in Fig. 2.13 (a). The schematic comprising Fig. 2.13 (a) shows an aerial view of the experimental setup.
The intensity versus sample/source separation results are plotted in Fig. 2.13 (b). Interestingly, the data is not well described by the expected inverse square law. However, the data is well described by a line as indicated in the figure. To be precise, the intensity decreases by nearly 66,000 counts per centimeter and the intensity at the location of the source (i.e. the origin of light) would be approximately 4.6 million counts. Note that the $R^2$ value for this linear fit is approximately 0.993. The linear fit revealed that the ratio of uncertainty in slope to the slope was approximately 3.9 %, while the ratio of uncertainty in the y-intercept to the y-intercept was approximately 1.8 %.

According to one source, [162] an in-door gardening industry rule of thumb is that the $1/r^2$ relation only holds when the separation from the source is more than 5 times the largest dimension of the source. Since the largest dimension of the UV source is almost 25 cm (see Fig. 2.13) the inverse square law shouldn’t apply for separations less than 125 cm from this source. So the inverse square law cannot apply to the experimental apparatus described in this chapter. To further complicate the issue special consideration of all the UV source’s geometric parameters are of interest. As can be seen in Fig. 2.13 (a), the bulb has a cylindrical shape meaning that photons are emitted from the ionized mercury gas contained within at various point sources. Furthermore, the source contains a concave reflector behind the bulb that...
redirects light toward the sample. For these reasons it is not surprising that the intensity decreases linearly with sample/source separation and not the inverse of the separation.

2.2.2.3. Timing. Humans have specialized cells in their retinas for collecting light called cones and rods. Cones being the cells that are sensitive to wavelength (i.e. color perception), have better time resolution than rods with a detection threshold just beneath 60 Hz. The cells that are sensitive to light intensity (i.e. variation in light), known as rods, exhibit reduced time resolution with a detection threshold of approximately 15 Hz [163, 164]. It is well known that common indoor light sources, including the UV lamps used in this work, operate at frequencies fast enough so that they appear as if they are always on. However, knowing that the UV light source would vary in this way raised questions in regard to how spectra should be acquired. Of particular interest was how long an OMA exposure should be before error associated with a single pulse could be neglected. In other words, if a spectra was acquired by exposing the camera for a period of time comparable to the period of a pulse from the UV source then the spectral signal would be suspect. So it was decided that the camera should be exposed for intervals that were significantly longer than the period of one pulse. That made it necessary to know the exact frequency of the light source. Additionally, the consistency of the UV source was of concern. That is, this section aims to demonstrate to what degree the maximum intensity of the source varied with every pulse.

To determine the answer to these queries a series of simple measurements were taken of the UV source in terms of its intensity as a function of time. The UV source was manufactured by Spectronics Corporation and advertised to be centered at 254 nm, as was discussed in Sect. 2.2.2.1. The lamp was specified to use a 6 W, 120 V, 60 Hz, and 0.20 A power supply. The light from the source was directed onto a SiC photodiode from Electro Optical Components, Inc. The photodiode was secured inside a chamber with the UV source that was sealed from outside light sources and the room lights were turned off for the acquisition of data. Also, the chamber was purged with N₂ to prevent O₂ from absorbing the UV radiation of interest. When light from the UV source, powered by the lamp, was incident on the photodiode a plot of measured voltage as a function of time could be displayed on a Tektronix TDS 2002B Oscilloscope. While data acquired by the oscilloscope was in terms of voltage as a function of time, it was plotted in terms of light intensity as a function of time. This practice assumes that the intensity of UV light is proportional to the measured voltage across the diode.
The voltage of AC sources are well known to vary in a sinusoidal manner with time [159, 165]. While the lamp powering the UV bulbs is an AC electronic device, the bulbs do not emit in a sinusoidal manner as measured by the photodiode and oscilloscope and shown in Fig. 2.14 (a). Here it is obvious that whether voltage be positive or negative the Hg in the UV bulb ionizes in such a way as to produce the same response as a function of time. So even though the power source has a standard 60 Hz operating frequency, the UV is emitted from the bulb at nearly double that, namely $f = 119.968 \pm 0.029$ Hz. This value for the UV bulb emission frequency was determined by calculating the average (mean) of 128 distinct signals acquired with the oscilloscope. The error reported here is the standard deviation of the 128 distinct signals. Figure 2.14 (b) demonstrates how consistent the intensity of any given emitted UV pulse of light is. Here, only the apex of ten data sets is plotted in terms of intensity as a function of time in order to demonstrate any variation. Apparently, the maximum intensity of a pulse varies by no more than approximately 2 % from some arbitrary average maximum intensity.

By taking the inverse of the frequency the period of any given pulse is found to be approximately 8.34 ms. Because of this, it was decided that any given exposure of the OMA camera in this work should be kept to 200 ms. This would allow the acquisition time of a spectrum to be nearly 24 times longer than
any given pulse. Also, since several pulses are acquired with slightly different maximum intensities it is possible to ignore their differences. In this way, the variability of the light source can be neglected. Recall that this is a consequence of assuming that the intensity of light is proportional to the voltage measured across the photodiode. In fact, time evolution data of UV source spectra will be shown in Sect. 2.3 indicating that the maximum intensities have larger variation than only 2 %. This implies the original assumption was incorrect and that the light intensity measured by the photodiode is not proportional to its measured voltage.

**2.2.2.4. Long-term operation study.** The UV through NIR spectrum of the bulb, advertised by the manufacturer, having emission around 254 nm was presented in Sect. 2.2.2.1. Then, in Sect. 2.2.2.3 the emission rate at which the UV source, used for synthesizing PNIPAM nanogels via photopolymerization of the precursor solution, was presented. Instead of a short-term study of the bulb’s emission, this section reveals a long-term study of the bulb’s performance. The motivation of the experiment described here was to gauge bulb stability so as to achieve repeatable nanogel synthesis (see Sects. 2.3 and 2.4).

Two fiber optic cables were securely aimed at the UV source to direct its light toward the OMA spectrometer. One fiber optic cable was instructed, via the WinSpec software, to observe the 546 nm peak. This was the peak observed in the concentration study for nanogel synthesis discussed in Sect. 2.4. The other fiber optic cable was instructed to observe the 254 nm peak, which was shown to be responsible for initiating polymerization in Sect. 2.3.4. In each case, the spectrometer’s grating was set to its highest resolution of 1200 grooves per millimeter. Both cables and the UV source were enclosed in a box to prevent ambient light from reaching the spectrometer. In addition, the laboratory’s room lights were switched off during any data acquisition. The peaks were observed at random intervals over the course of about 35 days. During that time the experimental apparatus, including the UV source, was left operational and undisturbed. For any given data point the detector was exposed for approximately 0.1 seconds a total of 100 times in order to improve the signal to noise ratio. Note that this exposure time is nearly 12 times longer than the emission pulse of the bulb, as discussed in Sect. 2.2.2.3. The environmental conditions of the laboratory were monitored to document stability. Specifically, the relative humidity was 57 ± 3 % and the temperature was 22.2 ± 0.2 °C.

The normalized intensities of the 546 nm (green open circles) and the 254 nm (violet open squares) as a function of the UV source’s time of operation are plotted in Fig. 2.15. Apparently, the 546 nm peak
varies by as much as about 20 % over the course of the observed time interval. This contradicts the findings of Sect. 2.2.2.3 in which the intensity of the bulb was found to vary by only about 2 %. However, as discussed in that section, that result was likely attributable to a relatively constant photodiode voltage measured by the oscilloscope. Otherwise, there are no obvious deviations from this relatively constant output of the bulb at this visible region wavelength. On the other hand, the intensity of the 254 nm bulb exhibits a sharp decrease in output within the first several hours of operation. To be precise, Fig. 2.15 (b) suggests that the UV peak is relatively stable between 2 and 6 hours of operation. After that time the source only varies by about 10 % at that UV region wavelength.

The variation in intensity of the UV source’s 546 nm peak, of approximately 20 %, will be documented again in Sect. 2.4. Obviously, the nearly 2 % error reported in Sect. 2.2.2.3 cannot be assigned to variation in the UV source but, rather, to the variation in measured voltage of the photodiode. Since the bulb was found to become stable only after about 2 hours of operation time all synthesis experiments discussed later employed a shutter between the UV source and the sample so that the bulb could reach stability without initiating reaction of the precursor solution. Initially, it was thought that this study would reveal the amount of time in which the UV source was no longer useful. However, in the nearly 35 days of

Figure 2.15 Plotted here are the results of the long-term UV source study displaying (a) all recorded data and (b) only the first six hours.
uninterrupted operation that this UV source was observed there was no indication the bulb was reaching the end of its usefulness. Instead, the experiment revealed an initial warm-up time for the bulb.

2.3. Nanogel Characterization

The last section was concerned with describing the experimental parameters (i.e. OMA system and UV source) associated with the synthesis of PNIPAM nanogels. This section serves to present on all the tools for characterization that the PNIPAM nanogels were subject to. First, the nanogels’ morphology and desiccated PSDs will be described via microscopy (namely TEM). Then, the in-situ PSDs, as measured by NTA, will be provided. After that, ATR-FTIR will be used to demonstrate successful polymerization of NIPAM into PNIPAM. Finally, ultraviolet absorbance of the precursor solution and synthesized product will be discussed in an effort to demonstrate the photochemistry involved in the nanogel synthesis. All characterization presented in this section was performed on one sample. The precursor solution had 3 parts NIPAM (monomer) to 1 part MBA (crosslinker) to 1 part Irg 2959 (photoinitiator), by weight, dispersed into filtered DIW. The solution was agitated both mechanically and ultrasonically to ensure homogenous distribution of the solutes. The sample was exposed to UV for approximately 35 minutes. Further details regarding the synthesis are provided in Sect. 2.4 where the time-evolved spectrum of the UV source is provided to monitor the reaction in-situ. The sample that was removed from the chamber was the product upon which all characterization described in this section was performed.

2.3.1. Electron Microscopy

Monodisperse and stimuli-responsive PNIPAM micelles synthesized via emulsion polymerization were characterized in Sect. 2.1.1. That method of synthesis was abandoned for creating stimuli responsive nanogels for biotherapeutic delivery when the micelles were unsuccessful at encapsulating IOMNPs. In this subsection, microscopic characterization of the PNIPAM nanogels synthesized via exposure to UV is presented. Confocal microscopy, SEM, and TEM will be used to describe the morphology of the nanogels and to report on their size. Microscopy is uniquely suited to comment on PNIPAM nanogel morphology since it provides a visual representation of the artifacts. On the other hand, the electron microscopy used here to report on size requires the desiccation of the sample meaning that the reported sizes are likely
smaller than a hydrated sample would be during the intended application. Furthermore, electron microscopy lacked the capability of measuring sizes at varying temperatures. These shortcomings will be resolved later.

The details regarding the mixing of the precursor solution and subsequent exposure of the precursor solution to the UV source were recorded at the beginning of this section. After synthesis, the product was agitated briefly mechanically and ultrasonically just prior to removal of a drop for microscopy. One drop was placed onto a microscope slide for confocal microscopy, another was dropped onto ITO coated glass for SEM, and a third was pipetted onto a copper coated grid for TEM. Confocal microscopy was conducted by Nawal Khadka in Dr. Jianjun Pan laboratory using the provided wet sample. The sample for SEM analysis was coated with AuPd to improve contrast and the microscope was operated at 6 keV. As with SEM, the TEM sample was allowed to dry at room temperature just prior to inserting it into the specimen chamber where it was exposed to vacuum further drying out the sample. Then TEM was operated at 60 keV.

A sample micrograph, recorded by Nawal Khadka, is presented in Fig. 2.16 (a). The image shows several of the PNIPAM nanogels, apparently, agglomerating together in the DIW solvent. Note the multiple focal planes in the micrograph causing some smaller artifacts to appear out of focus. It should be noted that confocal microscopy was the first test of the sample after synthesis. Therefore, it wasn’t until later that confirmation of discrete nanogels was obtained. One of the first indications that the sample contained smaller nanogels was provided by SEM. The SEM micrograph in Fig. 2.16 (b) reveals individual nanogels some of which are isolated, but are for the most part in proximity to each other. They appear approximately round and are all relatively close in size to each other. Finally, the micrograph in Fig. 2.16 (c) provides high resolution representations of the nanogels. Again, they are round in appearance with some indication of a bumpy texture on their surfaces. The histogram in Fig. 2.16 (d) reveals the PSD of this sample of PNIPAM nanogels as measured using the TEM software. They have an average size of 203.3 ± 28.4 nm. The histogram was fit with a Gaussian whose peak corresponded to the average size and whose standard deviation from the center corresponded to the error in average size. The nanogels are almost monodisperse in that the error is a little more than one-tenth of the average size.

Like SEM, most TEM micrographs also show the nanogels in proximity to each other. However, in SEM and TEM this can be attributed to sample preparation in that the drop is necessarily desiccated. As
the solvent evaporates, the nanogels are forced together giving the false appearance of agglomerates. Note, in Fig. 2.16 (b) most nanogels are in proximity to each other, but a few nanogels are, in fact, isolated. In the case of confocal microscopy, the comparatively lower magnification capability tends to bias the observations toward larger artifacts. Note, in Fig. 2.16 (a) that smaller artifacts are left out of focus while the largest structure is made clear. While these nanogels may not be as monodisperse as the PNIPAM
micelles synthesized via emulsion polymerization (see Sect. 2.1.1), their size distribution could easily by cleaned up using techniques such as centrifugation, filtration, and refinement of the final product harvesting after synthesis. The next subsection will demonstrate that these nanogels do not agglomerate when in solution.

2.3.2. Nanogel Tracking Analysis

The last section utilized TEM sizing data of PNIPAM nanogels to report an average size of almost 200 nm. However, those micrographs revealed most nanogels in close proximity to each other with only a few in isolation. In this section, NTA data will be used to establish the fact that the nanogels, synthesized as described at the beginning of this section, are not agglomerating in solution, but exist as discrete entities. The same data will be used to describe the in-situ PSD of PNIPAM nanogels. These NTA results will be more pertinent to the application of biotherapeutic delivery since the sample was in solution and not desiccated as in TEM. Recall the PNIPAM micelles of Sect. 2.1.1 were revealed, by DLS, to be temperature responsive. Similarly, NTA data will be used in this section to gauge the temperature response of the PNIPAM nanogels.

Detailed specifications regarding the NTA equipment are provided in Malvern's brochure [166]. The schematic in Fig. 2.17 (a) diagrams how the sample is probed for the size and concentration data that are output by the software. Data sets of this sort allow for the determination of the prevalence, based on concentration, of distinct nanogel size populations. In short, a He-Ne laser propagates through the sample in such a way that the scattered light is captured by a microscope and directed to a digital camera. A video of the nanogels’ scattered light is analyzed for their Brownian motion and the Stokes-Einstein equation can be used to determine their size. The data presented below represents the average of five videos lasting three minutes each. This practice demonstrates repeatability and generates error. The raw data sets range in size from 10 nm to 2000 nm. Samples were allowed to equilibrate for about 15 minutes anytime the temperature was changed in order to ensure a thermally stable sample.

The sample was initially subjected to NTA’s measurements at a temperature of approximately 20.0 °C. The data corresponding to those measurements is plotted in Fig. 2.17 (b) in terms of concentration versus nanogel size. Note that this data has been cropped to exclude data that appeared relatively flat.
compared to these peaks. Immediately apparent are five distinct nanogel populations based on size. Each peak was separately fitted with a Gaussian whose peak identified the average size and whose width specified the error after dividing by four. The result of this analysis is collected in Table 2.3. Also in Table 2.3 is an accounting of the prevalence of a particular size population based off their peak concentration measurements. For example, the most prevalent size population measures about 217.7 ± 6.6 nm and it constitutes nearly 25.4 % of the sample. Obviously, this is a narrower PSD than that reported in the previous

Figure 2.17 The NTA equipment, as schematized in (a), allowed for (b) the demonstration of various monodisperse PNIPAM nanogel size populations, (c) whose temperature-dependent concentration versus size study reveal (d) increasing size with elevated temperatures.
section, but it would necessarily grow wider when all five size populations are considered together. The advantage of seeing the size populations resolved in this manner demonstrates that the nanogels are distinct and not agglomerating.

Next, the sample was analyzed by the NTA system again keeping all parameters constant except the temperature. Data sets comparable to that collected at 20.0 °C were also collected at 30.0 °C and 40.0 °C. For the purposes of this temperature study the distinct peaks were ignored and the entire data set was fit with a Gaussian. As before, the peak of the Gaussian identified the average size of all nanogels at that particular temperature and one-fourth of its width gave the error. The Gaussian fits for the sample at each of the three temperatures are plotted in Fig. 2.17 (c). Apparently, the average nanogel increases in size as its temperature is raised. Also, the concentration of a typical size population decreases at increased temperature. In other words, there is a wider distribution of larger sizes at elevated temperature.

Finally, the average sizes and corresponding error were plotted against temperature in Fig. 2.17 (d). As before, there is a clear trend for these nanogels to grow as the temperature is increased. Unfortunately, the error is rather large in that it overlaps. Recall that this error was the result of considering every size population in the sample. It would be possible separate out the distinct size populations using centrifugation or dialysis. The data was fit with a line to determine that the size of the nanogels is growing with temperature at an approximate rate of 5.8 nm/°C.

In closing, this section served to demonstrate a temperature-dependent, in-situ PSD for the PNIPAM nanogels. Also, the data reveals that the nanogels are not agglomerating as suggested by microscopy data of the previous section. Recall that the PNIPAM micelles synthesized via emulsion
polymerization (see Sect. 2.1.1) were discontinuously shrinking upon temperature increase passed the LCST. The reason the nanogels of this section behaved differently may be attributable to the fact that they were synthesized with a relatively high concentration of MBA (crosslinker) to NIPAM (monomer). This may have caused them to be so dense with crosslinker that they effectively lost the more familiar stimuli-responsive behavior of PNIPAM. This may not disqualify them, however, for biotherapeutic delivery. It is conceivable that future researchers may learn that these nanogels do release a biotherapeutic compound upon temperature increase perhaps due to the polymeric mesh opening up and enabling exit. That being said, the familiar behavior of PNIPAM can likely be restored by changing the synthesis recipe to contain less MBA. Later, in Sect. 3.1.2, NTA will again be used to characterize the IOMNPs used in this dissertation.

### 2.3.3. Fourier Transform Infrared Bond Identification

In Sects. 1.2 and 1.4 the molecular structure and polymerization of precursor solutions containing a monomer, crosslinker, and initiator (e.g. NIPAM, MBA, and Irgacure 2959 respectively) were discussed in detail. Some of those earlier concepts from Ch. 1 will be important here. In this section, FTIR spectroscopy of solutions, having varying concentrations, containing only NIPAM, MBA, or Irgacure 2959 is presented to establish Lambert-Beer linear fits. The purpose of this data is to enable the potential to determine the concentration of some unknown solution from its FTIR spectrum. This section also presents FTIR spectra of precursor solutions, those containing all three solutes dispersed in filtered DIW, both before and after UV exposure. This second set of data demonstrates how FTIR spectroscopy can be used to confirm successful photo-polymerization. Throughout this section the various atomic bonds of PNIPAM will be identified in the course of accomplishing the above two tasks.

The basic concepts regarding the significance of a FTIR spectrum will be helpful before proceeding to the presentation of the results. Briefly, the interaction of some molecule with light can be understood in terms of relating the molecule to a simple spring system. Molecules have natural frequencies at which they vibrate due to their own unique properties as expressed in

\[
\nu = \frac{1}{2 \pi c} \sqrt{\frac{k}{\mu}}.
\]

The speed of light is given by \(c\), and its purpose is to force the frequency given by \(\nu\) to have units of wavenumbers (cm\(^{-1}\)). The “spring constant”, \(k\), of a particular molecule and its reduced mass, \(\mu\), are both
unique to that molecule. When a molecule is subject to incident light of a particular frequency then resonant absorption is achieved resulting in a FTIR peak. So, in order to observe one of these characteristic absorption peaks the incident light’s frequency must be the same as the unique vibration of the molecule. Another requirement for the absorption of light is that the unique frequency must force the dipole moment of the molecule to change. [33]

In order to obtain infrared spectroscopy a Bruker Optics, Vertex 70 FTIR spectrometer was used. The attenuated total reflectance (ATR) module was used in order to avoid using a sample holder which would have reduced the 3400 cm$^{-1}$ to 1300 cm$^{-1}$ range observed in these experiments. A total of 1000 scans were collected for any given sample to improve the quality of the data. These scans were performed at a resolution of 4 cm$^{-1}$ and carried out at approximately 22 °C. The resulting data was baseline corrected. The NIPAM solutions, MBA solutions, and Irgacure 2959 solutions were prepared by weighing out the desired amount of solute and dispersing it in enough filtered DIW to achieve the prescribed concentration. Homogeneous dispersions were achieved using both mechanical and ultrasonic agitation. Similarly, the precursor solution was prepared by weighing out the monomer, crosslinker, and photo-initiator separately and then adding them all to one vessel containing the requisite amount of filtered DIW to yield the desired concentration. The post UV sample was prepared by exposing the precursor solution to the UV source for approximately 35 minutes. The precursor solution discussed in this section had a monomer to crosslinker to photo-initiator ratio of one to one to one.

Figure 2.18 provides the results of the concentration study of each of the solutes (NIPAM, MBA, and Irgacure 2959) dispersed in DIW separately. The FTIR spectra of four NIPAM concentrations of 1.2 wt. %, 1.0 wt. %, 0.80 wt. %, and 0.40 wt. % were obtained and are plotted in Fig. 2.18 (a). The most prominent peak in the entire measured range, around 1560 cm$^{-1}$ corresponding to the stretching of NH, in the spectrum is shown here for each of the concentrations. As expected from the Lambert-Beer law, the NH stretching is more readily observed at higher concentrations of NIPAM. For example, at a concentration of only 0.40 wt. % the peak exhibits an absorbance of about 1.0 x 10$^{-2}$ arbitrary units. On the other hand at a NIPAM concentration of 1.2 wt. % the absorbance increased to nearly 2.2 x 10$^{-2}$ arbitrary units. For MBA, three concentrations of 0.030 wt. %, 0.0030 wt. %, and 0.00030 wt. % were tested. Similarly, the absorbance of
the crosslinker nearly doubles when the concentration is increased by a factor of 100, as seen in Fig. 2.18 (b). For Irgacure 2959, three concentrations of 0.068 wt. %, 0.020 wt. %, and 0.010 wt. % were examined. Again, the absorbance of the photo-initiator also increases, by a factor of almost five, when the concentration is made about seven times greater, as seen in Fig. 2.18 (c). Note the higher absorbance of NIPAM, in general, compared to that of MBA and Irgacure 2959 as indicated by the y-axis labels. The
tendency toward higher absorbance of NIPAM peaks is more obvious in Fig. 2.18 (d). Here Lambert-Beer law linear fits are plotted providing a method for determining the concentration of some unknown sample given the FTIR absorbance. While NIPAM may have higher absorbance, in general, its rate of absorbance increase with concentration increase is less than that of both MBA and Irgacure 2959 at only 0.013 arbitrary units per wt. % of the monomer. On the other hand, the crosslinker and photo-initiator exhibit increases of absorbance with concentration of 0.11 and 0.071 arbitrary units per wt. %, respectively.

Next, FTIR experiments were performed to provide evidence of the conversion of monomer to polymer upon exposure of the precursor solution to UV light (i.e. to confirm successful photopolymerization). As indicated above, FTIR can be used for bond identification by exciting certain atomic bonds with infrared light producing characteristic spectroscopic peaks. Figure 2.19 (a) gives the FTIR spectroscopic fingerprint of a 20 wt. % sample of NIPAM. Many of its atomic bonds are identified by the labelled peaks [33, 34, 35]. Now, a FTIR spectrum for a precursor solution, whose concentration and preparation were described above, was obtained. Then the precursor solution was exposed to the UV source described in Sect. 2.2 for about 35 minutes. After that exposure the sample was taken for FTIR spectroscopy. One peak around 1600 cm\(^{-1}\) exhibited more than 50 % decrease in absorbance after the UV exposure, as shown in Fig. 2.19 (b). This peak is well known to correspond to the C = C bond. In order to understand how this conspicuous change in peak absorbance indicates successful polymerization it is necessary to understand the significance of the C = C bond in the polymerization process. Figure 2.19 (c) shows the molecular structure of a single NIPAM monomer. Note that the C = C bond is broken during polymerization so that the backbone of the polymer forms. During polymerization that covalent bond breaks so that other links (monomers) in the chain (polymer) can be added, as seen in Fig. 2.19 (d). In this polymer, C = C is critical to the formation polymeric chain. So, the reduction in absorbance observed in Fig. 2.19 (b) is indicative of the formation of PNIPAM’s polymeric backbone.

The FTIR spectrum of PNIPAM is in essence a fingerprint of the molecule describing, in detail, its constituent atomic bonds. However, in this section FTIR spectroscopy was used to accomplish two other tasks. The first of these was to establish Lambert-Beer lines that allow for the determination of the concentration of one of the three solutes, NIPAM, MBA, or Irgacure 2959, if the FTIR absorbance is known. These experiments were accomplished by obtaining FTIR spectra of different concentrations of solutions
containing the three solutes separately and plotting the maximum absorbance of any peak against the corresponding concentration. The second task was to use FTIR spectroscopy to provide evidence of successful photo-polymerization by monitoring peak absorbance changes after a sample was exposed to UV. This evidence of polymerization is complimentary to that presented in the next subsection.

Figure 2.19 (a) Most of the observable vibrational energies using ATR-FTIR spectroscopy of an aqueous NIPAM solution remain unchanged after exposure to UV irradiation, (b) except the C=C bond at approximately 1600 cm⁻¹, which is indicative of the transformation of the (c) monomer to (d) polymer.

containing the three solutes separately and plotting the maximum absorbance of any peak against the corresponding concentration. The second task was to use FTIR spectroscopy to provide evidence of successful photo-polymerization by monitoring peak absorbance changes after a sample was exposed to UV. This evidence of polymerization is complimentary to that presented in the next subsection.
2.3.4. Ultraviolet Absorbance

As in the last section, the chemistry of polymerization discussed in Sects. 1.2 and 1.4 will be useful for the understanding of the results presented here. In this section, the ultraviolet characterization of the materials used to synthesize PNIPAM nanogels will be presented, as well as that for the product after synthesis is complete. First, detailed concentration studies of absorbance versus wavelength for each of the solutes NIPAM, MBA, and Irgacure 2959 will be presented. The results of those studies allow for the application of the Lambert-Beer law to each of the solutes for the purpose of concentration determination of potential unknown samples later. In addition, these UV absorbance spectra allow for chemical bond identification. Second, an experiment in which a precursor solution containing the monomer, crosslinker, and photoinitiator was exposed to UV is discussed. The precursor solution, finished product, and DIW are all evaluated for absorbance versus wavelength and presented here. The characterization of the UV source, as presented in Sect. 2.2.2.1, will be invoked as pertinent to the mechanism of photochemistry presented here.

Again, a spectroscopic characterization study of the three solutes needed to synthesize a stimuli-responsive nanogel via UV photopolymerization is presented. The monomer, NIPAM, crosslinker, MBA, and photoinitiator, Irgacure 2959, were all purchased commercially and used without further purification. Solutions were prepared by mixing the powders in DIW. Mechanical and ultrasonic agitation were used to ensure homogenous samples. UV absorption spectroscopy was conducted in order to exhibit bond identification, the role of solute concentration on the chemical fingerprint, and to determine the optimal wavelength of light for activating the photoinitiator. The equipment used was a Perkin Elmer High-Performance Lambda 950 spectrometer. The resolution of the data presented is 1 nm per data point. Note that for all UV spectra shown here data was obtained from as low as 185 nm and up to 700 nm. However, since the data exhibited no peaks in the visible part of the spectrum that data is not shown. In a UV-grade quartz cuvette, samples were scanned 10 times at any given concentration to investigate repeatability and error. To eliminate cross-contamination and maintain measurement consistency, a single cuvette was cleaned before and after any one sample was scanned, involving soaking of the cuvette overnight in the solvent, DIW, lightly scrubbing the cuvette with a cotton swab after the soak, and rinsing the cuvette with DIW prior to using with the next sample. Background spectra of the air in the spectrometer, quartz cuvette,
and DIW in the cuvette were analyzed to ensure there was no absorption in the regions discussed below. However, these background spectra were already presented and discussed in Sect. 2.2. The conclusion drawn from that data is that the cuvettes do not interfere with the data presented here. After the UV characterization of the individual solutes was carried out, a custom built photoreaction chamber was assembled and designed in house. The chamber was purged with N\textsubscript{2} to prevent the absorption of UV wavelengths by molecular oxygen. The temperature of the reaction chamber was monitored with a thermocouple.

The unit monomer NIPAM functions as a link in a chain made up of several other NIPAM links which, when attached to each other, form the polymer PNIPAM. It has already been shown that PNIPAM absorbs light at approximately 220 nm. This was attributed to the absorption of light causing an electron transition between π and η orbitals on the C=O bond of the acrylamide [167]. The absorbance as a function of wavelength for several concentrations of NIPAM is shown in Fig. 2.20 (a). As in the other study, a shoulder peak is observed at approximately 220 nm which, again, is indicative of the C=O bond. However, there is also a peak at 200 nm in Fig. 2.20 (a). Presumably, this peak can be attributed to the C=C bond, within the acryl part of the monomer, since the difference between this data and the previous study was that this data represents the unreacted monomer. In other words, polymerization has not yet taken place and so the peak at 200 nm is observed. It can be seen in Fig. 2.20 (a) that when higher concentrations of NIPAM are dispersed in DIW more light is absorbed at 200 and 220 nm. For example, at a NIPAM concentration of 1.8 x 10\textsuperscript{-3} wt. % the absorbance of the 200 nm peak occurs at approximately 1.9 arbitrary units, while at 1.4 x 10\textsuperscript{-3} wt. % the absorbance has dropped to about 1.5 arbitrary units. Similar decreases in absorbance were observed as the concentration was reduced. The absorbance of DIW is included in Fig. 2.20 (a) to demonstrate how the solute compares with the solvent.

The crosslinker MBA is the molecule that will allow chains of PNIPAM to interconnect forming a mesh network capable of carrying the biotherapeutic. A careful inspection of the molecular diagrams presented in Ch. 1 suggest that the UV spectra for NIPAM and MBA should be similar. Indeed both molecules have the acrylamide sections in common. The concentrations study of UV absorbance for MBA is presented in Fig. 2.20 (b) and, as expected, it is similar to Fig. 2.20 (a), the UV absorbance of NIPAM. There is a peak at approximately 220 nm that has been attributed to the C=O bond and a peak at
approximately 200 nm thought to be due to the C=C bond. Interestingly, for a given concentration, the absorbance at 200 nm is higher in the MBA spectra. This is attributable to the fact that there are two of the C=C bonds in MBA as compared to only one such bond in NIPAM. Again, increasing the concentration of aqueous MBA solutions caused the absorbance at 200 and 220 nm to increase as shown in Fig 2.20 (b).

Note that at a MBA concentration of 1.4 x 10^{-3} wt. % the 200 nm peak has an absorbance of around 2.4
Irgacure 2959 is a water soluble molecule that, when exposed to UV light, will cleave and form radicals that can react with the monomer and crosslinker to initiate polymerization. The UV absorbance for several concentrations of the photoinitiator were obtained and are plotted in Fig. 2.20 (c). The markedly different spectra shown here can be attributed to the significantly different chemical structure of the photoinitiator as compared to NIPAM and MBA. More precisely, the peaks at approximately 221 nm and 279 nm inform upon the wavelengths of light this molecule effectively absorbs. Presumably, when the molecule absorbs radiation of this energy, it then cleaves forming the radicals necessary to begin the polymerization process. As before, when the amount of Irgacure 2959 is varied in solution a proportional amount of variation in absorbance is observed. Observe how when the concentration of Irgacure 2959 is around $5.0 \times 10^{-3}$ wt. % the 279 nm peak exhibits absorbance of almost 3 arbitrary units, while at a reduced concentration of about $2.0 \times 10^{-3}$ wt. % the absorbance has decreased to approximately 1.3 arbitrary units.

As discussed in the last section, the phenomenon of proportional variation between absorbance and solute concentration is consistent with the Lambert-Beer law \[168\]. Figure 2.20 (d) plots the maximum absorbance for the 200 nm peak as a function of NIPAM concentration. The same 200 nm peak was used for the plotting of MBA maximum absorbance versus concentration data. Also, the maximum absorbance versus Irgacure 2959 concentration corresponding to its 279 nm peak is plotted in Fig. 2.20 (d). All data is shown to be well described to a line having error bars smaller than the data point markers. The equations shown in Fig. 2.20 (d) allow for the determination of the concentration of some unknown solution containing these solutes.

The custom-built experimental apparatus used to expose a precursor solution, one containing all three solutes dispersed in filtered DIW, is shown in Fig. 2.21 (a). Visible in the image is the sample cuvette on a pedestal that keeps the sample at a height roughly equal to the center of the UV source. It was thought that the ends of the bulbs were less effective than the center at emitting light. The image also shows that two UV lamps were attached to the chamber, the N$_2$ inlet hose, and thermocouple for monitoring the temperature inside the chamber. Three different samples of the constant concentration solution were subjected to varying durations (90, 45, and 22 minutes) of UV exposure using the source advertised by its
manufacturer to be centered at 254 nm and characterized in Sect. 2.2.2.1. Ninety minutes was chosen as an upper limit on exposure time since literature suggests this time would be sufficient to fully polymerize a bulk sample of PNIPAM [169]. However, the results from the three experiments are indistinguishable. Therefore, only the results corresponding to the UV exposure lasting 45 minutes are presented here.

Figure 2.21 In a typical UV irradiation of a precursor solution, photographed in (a), (b) the reaction chamber’s temperature increased by about 13 °C and (c) normalized absorbance of each solvent is changed by the UV source to that shown in (d).
Figure 2.21 (b) indicates that the temperature of the reaction chamber did increase during the reaction. To be precise, the initial temperature of the chamber was approximately 22 °C and the final temperature was around 35 °C yielding a relative temperature increase of around 13 °C. These elevated temperatures are not surprising considering the significant infrared peaks of the UV source reported on back in Sect. 2.2.2.1. This temperature increase occurred despite the fact that the chamber was constantly purged with N\textsubscript{2} which must have served to carry some excess heat out of the chamber. Recall, from Ch. 1, that one benefit of UV photopolymerization is that it doesn’t require the elevated temperatures commonly needed in reactions like emulsion polymerization. The act of increasing the temperature of those reactions necessarily increases their cost. Fortunately, this observed temperature increase doesn’t approach the temperatures utilized in those other techniques keeping this method of synthesis significantly unique in that regard. In other words, the reaction here is not thermally initiated, but triggered by UV irradiation.

Now, in an effort to describe the mechanism for the photopolymerization occurring here, a plot of the normalized absorbance of the three different solutes has been reconstructed to include the normalized intensity of the 254 nm source used in this experiment. Note that the concentration of the three different solutes was a constant $1.0 \times 10^{-3}$ wt. %. These are all shown in Fig. 2.21 (c). Although the 254 nm intensity peak is not precisely coincident with any of the solute absorbance peaks, it does overlap the 279 nm absorbance peak associated with Irgacure 2959. This seems to suggest that the photoinitiator is sensitive to the UV source.

Finally, the results of the 45 minute UV exposure on the precursor solution are shown in Fig. 2.21 (d). Note that in the before UV exposure data there is a barely detectable peak at 200 nm corresponding to the C=C bond in NIPAM and MBA. The peak at 220 nm indicating the C=O bond, common to both NIPAM, MBA, and Irgacure 2959, is easier to observe. There is also a prominent peak at 279 nm that was seen in Fig. 2.21 (c) and indicative of Irgacure 2959 in the solution. Regardless of UV exposure duration all absorption peaks were eliminated as a result of UV exposure. Upon close inspection, however, there was an apparent increasing difference between the DIW curve and the post UV exposure curve as the duration was reduced. This may indicate that relatively short times are necessary to photopolymerize NIPAM. Apparently, all of the photoinitiator was consumed in the reaction as indicated by the absence of a peak at 279 nm in the data set corresponding to the sample after UV exposure. The peak at around 220 nm is also
missing. There is the slightest indication in the after UV exposure data that a shoulder peak exists at around 200 nm. This could be attributed to the C=O bond after conjugation of that bond with C=C has been eliminated through successful polymerization [170].

The preceding serves as UV absorbance versus wavelength characterization of monomer, crosslinker, and photoinitiator as they pertain to photopolymerization in synthesis of PNIPAM nanogels. Increases in any solute with respect to the solvent, DIW, leads to increases in absorbance at characteristic wavelengths. This absorbance dependence upon concentration is linear in nature and consistent with Lambert-Beer’s law. It has been shown that NIPAM and MBA have peaks at approximately 200 nm and 220 nm. If polymerization is successful the peak at 200 nm is expected to diminish indicating the double C=C bond becomes a single bond. When a solution containing all solutes was photo irradiated all peaks were diminished. However, this could be a shift of the C=O peak to shorter wavelengths as conjugation is eliminated. The spectra of Irgacure 2959 provided here was ultimately used in deciding the optimal UV irradiation source. In other words, Irgacure 2959 absorbs at 221 nm and 279 nm and so should be most readily reacted by a UV source that emits controllably at those wavelengths.

2.4. Concentration Study of Nanogel Synthesis

In Sect. 2.2 the experimental setup for the UV photopolymerization synthesis of PNIPAM nanogels was presented. Part of that setup included the OMA system that allows for the in-situ monitoring of the synthesis which can track the attenuation of the UV source through an increasingly turbid sample while undergoing photopolymerization. Here, OMA data will be presented to demonstrate how the attenuation of the UV source was quantified and what role the initial concentration of the precursor solution had on that attenuation. Characterization of the synthesized nanogels, including their desiccated size measurement via TEM, was discussed in Sect. 2.3. This section also reports on systematic changes in the precursor solution concentration leading to changes in synthesized nanogel size. Thus, this section demonstrates the ability to control the ultimate size of synthesized nanogels via choice of the precursor solution concentration.

As discussed in Sect. 2.2, the UV source chosen for this work was known to possess a relatively strong peak at around 254 nm thought to be responsible for the photochemistry involved in this synthesis. Unfortunately, in preliminary experiments, that peak was lost amongst the noise of the spectrum as the
synthesis progressed in time. So it was not a useful peak to track as the sample became increasingly turbid. However, the peak at 546 nm did persist in remaining significantly distinct from the spectral noise throughout all trials, and so it served as acceptable for monitoring the UV source. Therefore, the OMA spectrometer was centered at 546 nm, set at the highest resolution grating of 1200 grooves per millimeter, and made to collect a spectrum every 200 ms. Three different samples, with regard to NIPAM to MBA to Irgacure 2959 ratios, were prepared. Samples R1, R2, and R3 had monomer to crosslinker to photoinitiator ratios of 3:1:1, 10:1:1, and 1:1:1 respectively. Further changes to the precursor solution concentration were achieved by diluting a sample by either one-half or one-tenth. Table 2.4 provides the nomenclature for all samples studied in this experiment. For example, the 0.5R1 sample was made by weighing out three parts NIPAM, one part MBA, and one part photoinitiator. These solutes were homogenously dispersed in filtered DIW using both mechanical and ultrasonic agitation. Then, approximately 1.25 mL of the precursor solution was put in the quartz cuvette with another 1.25 mL of filtered DIW to reduce its concentration by half.

One of the earliest indications that polymerization was occurring, upon exposure of a precursor solution to the UV source, was an apparent increase in turbidity of the sample upon visual inspection. Figure 2.22 (a) documents the appearance of the precursor solution both before and after exposure to UV lasting approximately 35 minutes. The leftmost picture indicates that prior to UV exposure the sample was apparently transparent and seemed indistinguishable from water. The rightmost picture relates how a whitish material formed at the front window of the cuvette. This material could be scraped off the cuvette wall for sample removal and ultimate analysis using TEM. It should be noted that the back wall of the cuvette did not possess this whitish material. This suggests that the formation of the whitish material on the front of the cuvette prevents unreacted solutes behind it from polymerizing.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>NIPAM</th>
<th>MBA</th>
<th>Irgacure 2959</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0R1</td>
<td>1.0</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.5R1</td>
<td>0.5</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.1R1</td>
<td>0.1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1.0R2</td>
<td>1.0</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.5R2</td>
<td>0.5</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1.0R3</td>
<td>1.0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Recall from the description of the experimental setup in Sect. 2.2 that the fiber optic cable for direction of light to the spectrometer was located behind the cuvette. Therefore, when the product began to form on the front window of the cuvette the light from the UV source was increasingly prevented from reaching the detector in the OMA system. The progress of the attenuation of the 546 nm peak is shown in Fig. 2.22 (b). This data corresponds to the UV exposure of the undiluted precursor solution, 1.0R1. This

Figure 2.22 The precursor solution attenuates the UV source as documented by (a) photographs as well as (b) the OMA system and (c) the attenuation was found to depend on the degree to which the solution was diluted.

Recall from the description of the experimental setup in Sect. 2.2 that the fiber optic cable for direction of light to the spectrometer was located behind the cuvette. Therefore, when the product began to form on the front window of the cuvette the light from the UV source was increasingly prevented from reaching the detector in the OMA system. The progress of the attenuation of the 546 nm peak is shown in Fig. 2.22 (b). This data corresponds to the UV exposure of the undiluted precursor solution, 1.0R1. This
figure is a three dimensional plot of intensity on the y-axis, wavelength on the x-axis, and time on the z-axis. To make this plot, eleven spectra from the entire 35 minute trial were selected at equally spaced time intervals. Note that the maximum intensity of the 546 nm peak is reduced as synthesis progresses. So the manifestation of the whitish material on the front window of the cuvette is the cause of the attenuation of the 546 nm peak as the nanogels are formed.

The maximum intensity of the UV source’s 546 nm peak versus synthesis duration, for all three potencies of R1, are plotted in Fig. 2.22 (c). Recall that in Sect. 2.2 the variation in the intensity of the source was determined to be no greater than about 2 % based on analysis with a photodiode and oscilloscope. Any of the four data sets shown here reveals that intensity can vary by as much as about 20 % and no less than about 10 %. Apparently, the error in intensity discussed in Sect. 2.2 was actually error in the voltage measurement of the photodiode. Note that all the data sets here exhibit an initial sharp increase in intensity that can be attributed to the warm-up time of the source. In later trials this was eliminated from the data by introducing a shutter into the experimental setup, between the source and the sample cuvette, which would allow for the activation of the source while preventing its light from reaching the sample until warm-up was achieved. Filtered DIW was also subjected to UV exposure to serve as a control and is indicated by the red open square data points. Observe that the DIW data indicates that the source tends to stabilize within about 20 minutes to a constant intensity. Obviously, the undiluted precursor solution (i.e. sample 1.0R1) shows the most dramatic attenuation of the 546 nm peak by almost 80 % of its maximum intensity. The undiluted data set suggests that the photoreaction is complete after only about 20 minutes. Meanwhile, when R1 is diluted by half (i.e. sample 0.5R1) the source is attenuated by about 50 % by the end of the synthesis trial. Apparently, diluting R1 to only one-tenth of its original concentration (i.e. sample 0.1R1) makes the change in attenuation of the source not much different from that of the control, DIW sample. This data set was the first indication that the various concentrations of precursor solution were producing PNIPAM nanogels of different sizes. Other researchers have already reported upon the fact that turbidity of a solution specifies the size of the particles dispersed therein [171, 172].

The whitish material, or product, synthesized in the experiments described above were taken for sizing measurements using TEM, as described in Sect. 2.3. Synthesis experiments as well as TEM analysis was also carried out on two other ratios, R1 and R2, in order to produce a wider range of PNIPAM nanogel
sizes. Figure 2.23 (a) demonstrates that nanogels of particular sizes can be tuned-in by carefully selecting the concentration of the precursor solution. This data is presented in terms of the average size of the nanogel as a function of the dilution of the original precursor solution, and it is organized by the solute ratio. For example, R1 is represented by closed black square data points and indicates that at this ratio the resulting nanogels will range in size from as small as about 50 nm to as large as around 200 nm. Note that all error was determined from the standard deviation of the PSD as described in Sect. 2.2 and may be hidden by the data point in some cases. For the precursor solution ratio R2 (open red square data points) the nanogel sizes were always less than 100 nm. Chapter 3 will reveal that this nanogel size is too small for the biotherapeutic delivery application and so a one-tenth dilution was not prepared for R2. So, a third ratio, R3, was prepared to establish the possibility of relatively large nanogels. The closed green circle data point here indicates that at this concentration the nanogels are almost 750 nm in diameter. This final, undiluted, concentration was that used for the purposes of the encapsulation experiment discussed in Ch. 4. In general, dilutions were only necessary for the R1 concentration since it always leads to smaller nanogels and the application calls for a nanogel large enough to at least encapsulate the IOMNPs. When comparing the different ratios it is obvious that increasing the monomer content leads to less variation in nanogel size; R2 exhibits less nanogel size range than R1.
Figure 2.23 (b) is an alternative arrangement of the data in Fig. 2.23 (a) that makes other trends more obvious. Here, the data is organized by both solute ratio and dilution factor in a plot of average nanogel size versus solute ratio. Obviously, increasing the monomer content produces nanogels that are smaller. The alternative interpretation is to observe that increasing the crosslinker content leads to nanogels that are relatively larger at any given dilution factor. This general result is common knowledge to polymerization researchers [173, 174]. As before, the data reveals that dilutions only serve to reduce the size of the synthesized nanogels. Note the wide range in possible nanogel size when the precursor solutions are not diluted as indicated by the points connected by the unbroken blue line.

In closing, this section has served to describe how the OMA data was used to measure the attenuation of the UV source as the precursor solution underwent polymerization. Photographs of the sample cuvette after it was exposed to UV show that the product only formed on its front window. This suggests that future apparatus should be designed in such a way so as to expose more of the sample to the source and the precursor solution should flow through the system in order to prevent any change in its concentration. The formation of the whitish material, or product, corresponds to the attenuation of the source as measured by OMA. An initial spike in the intensity of the source through a sample necessitated the use of a shutter in the experimental apparatus to ensure a more stable UV exposure. Results indicate that more concentrated precursor solutions will attenuate the UV source to a greater degree as the synthesis nears completion. It would be possible for researchers continuing this work to use the attenuation of light through their samples to predict a size of the nanogels without the need for electron microscopy. The attenuation of the source’s intensity as the exposure progressed suggest that the synthesis was complete after only about 20 minutes which is significantly faster that more traditional chemical synthesis techniques like emulsion polymerization (see Ch. 1). The section also demonstrated that the size of the synthesized nanogels could be controlled via choice of precursor solution concentration. It was confirmed that crosslinker prevalence in the precursor solution relates to the ultimate size of the synthesized nanogels. It will be shown in Ch. 3 that the IOMNPs used in this work tended to agglomerate while in solution necessitating large enough PNIPAM nanogels to ensure successful encapsulation.
2.5. Summary

This chapter began with a description of the PNIPAM micelles synthesized via emulsion polymerization that were to serve as the stimuli responsive carrier. The micelles, synthesized by emulsion polymerization, were characterized by TEM and DLS in Sect. 2.1.1. The spherically, monodisperse micelles were revealed by TEM to have an approximate diameter of 120 nm. However, electron microscopy necessitates the micelle be observed under conditions significantly different from what should be considered in-situ. DLS was shown to be superior to electron microscopy, in this regard, for evaluating PSDs while the micelles are in solution. Another benefit of DLS is that it allows for temperature dependent size measurements. This allowed for the demonstration of the stimuli responsive nature of the micelles. DLS results revealed that these micelles undergo a VPT at approximately 36 °C. In the end, this method of synthesis resulted in irregularly shaped PNIPAM nanogels when IOMNP encapsulation was attempted which will be attributed to IOMNP agglomeration in Ch. 3. Emulsion polymerization was set aside to pursue spray fabrication of the stimuli responsive carrier.

Next, Sect. 2.1.2 gave a brief accounting of all attempts to design and assemble experimental apparatus for spray synthesis of stimuli responsive carriers. It was shown here that all attempts to direct a nebulous mist into a spray resulted in condensation. Ultimately, condensation was a particularly challenging problem in developing a spray technique for synthesis of nanogels. After that, controlling the precursor solutions’ concentration was the next most challenging problem since all nebulizers required the sample’s viscosity be similar to that of water. This necessitated the consideration of droplet evaporation prior to polymerization so that the concentration would increase prior to polymerization, after being decreased prior to nebulization. In order to complete this dissertation in a timely manner the spray process was abandoned so as to avoid the complications of spray condensation and droplet concentration.

After that, an approach was pursued using UV photopolymerization for the synthesis of PNIPAM nanogels to serve as the stimuli responsive carrier. The ultimate technique, used in this work, avoids condensation altogether by never employing spray in the fabrication. It was shown that the attempts to control final droplet concentration and size through evaporation of falling droplets can be realized in a more straightforward manner. The techniques presented early in Ch. 2 are relatively more difficult to achieve.
than that presented in later sections. At the start of Sect. 2.2, a concise description of the experimental apparatus for nanogel synthesis and the monitoring of that synthesis was presented.

The demonstration of the UV photopolymerization technique began with characterization of the OMA spectrometer in Sect. 2.2.1.1; all wavelengths that were observable using this system were documented. Here it was shown that the OMA system can observe in the UV, visible, and NIR regions of the electromagnetic spectrum. Also, the sensitivity of the ICCD detector across the observable spectrum was shown to not be constant. For example, the quantum efficiency of the detector, as per the manufacturer, is similar to the response versus wavelength plotted in Fig. 2.8 (d). That data was not used to correct any spectra contained in this work since no characterization of the Perkin Elmer source was performed. Because of that, all spectra recorded with the OMA system had their intensities corrected using the data plotted in Fig. 2.8 (a). A minor discrepancy between the wavelength measured in Fig. 2.8 (c) and the wavelength programmed into the Perkin Elmer spectrometer was observed. This warranted calibration to bring the two wavelengths into agreement, which was discussed next.

The software controlling the OMA system, WinSpec, was used to carry out calibration of the spectrometer. Section 2.2.1.2 details how WinSpec accomplishes this by correcting for offset, adjustment, and dispersion of raw data upon comparison of a measured peak to its accepted standard. After that initial calibration, the spectrometer was evaluated in two different ways so as to demonstrate successful calibration. The first assessment revealed that the output of the Perkin Elmer spectrometer source, as measured by the calibrated OMA system, warranted negligible corrections to bring those two systems into agreement. Similarly, when the calibrated OMA system was used to measure the spectrum of a standard Hg calibration bulb the discrepancy between the data and NIST archives was minimal. Agreement between OMA measurement and NIST’s Hg data is important since all spectra measured by OMA in this work comes from a UV source that is a Hg gas discharge tube. After calibration, Ch. 2 discussed an attempt to characterize the NDFs used to prevent saturation of the ICCD detector.

In Sect. 2.2.1.3, the attempt to confirm the measured optical density of the NDFs was similar to that claimed by their manufacturer was unsuccessful since they had previously been exposed to heat and were warped as a result. Therefore, it was decided that if a set of NDFs used in any given experiment required characterization, then that would be accomplished at the end of said experiment. A peak that does not
saturate the detector, when unfiltered, is required for characterization. The peak must also be observable when the NDF(s) requiring characterization are in place. A comparison of the peak’s intensity when filtered to the intensity when it is unfiltered leads to experimental determination of the OD. The filtered and unfiltered intensities were collected in quick succession in order to ensure the UV source was operating under similar conditions during both measurements.

Now, with the description of the monitoring apparatus (Sect. 2.2.1) out of the way Ch. 2 moved into a more thorough evaluation of the UV source (Sect. 2.2.2) in terms of its spectrum, intensity as a function of distance from the source, the rate at which it emits, and a long-term study of its performance. It was initially surprising when it was discovered that the bulbs acquired for this work all exhibited strong emission in the visible and NIR regions of the electromagnetic spectrum, as revealed in Sect. 2.2.2.1. Significant time and resources were dedicated toward acquiring a strong source of UV radiation for the photopolymerization of stimuli responsive nanogels. The spectra presented in the characterization of photopolymerization source section do reveal relatively weak UV peaks upon close inspection. When high resolution spectra, centered on the wavelength referred to by the manufacturer, were obtained the peaks of interest were made apparent. It’s important to note that the entire measureable spectra of all four bulb types do match, in general, the spectrum of Hg, as per NIST. The bulb labelled 254 nm by the manufacturer was selected for this work since its UV peak was proximate to the UV absorbance peaks of the precursor solution discussed later in the chapter.

Once its spectrum was presented, a study of the effect of distance from the UV source on its measured intensity was discussed in Sect. 2.2.2.2. In-door gardening literature suggests that the $1/r^2$ rule for light intensity drop off only applies when the separation from the source exceeds five times the greatest length dimension of the source. The largest dimension of the UV source used in this work suggests the inverse square law only holds when the sample and source are more than 1.25 meters apart. Because the samples discussed here are always much closer to the source than this, the intensity does not drop off as many undergraduate physics students would expect. The unique geometry of the UV source further distinguishes it from expected intensity drop off. It was shown that the source has a cylindrical shape which can only mean that light reaching the sample originated from a multitude of point sources. Furthermore, the manufacturer included a concave reflector behind the bulb which only augments its intensity on the sample.
For these reasons a linear decrease in intensity with increasing distance between source and sample was observed instead of the more familiar $1/r^2$ drop off.

Then, Sect. 2.2.2.3 addressed the issue of the timing of the UV source by detailing just how quickly it emitted its spectrum. The frequency of the source’s emitted pulses, as measured by a photodiode connected to an oscilloscope, was inverted to yield a period of approximately 8.34 ms. With this knowledge in hand it was decided that a typical exposure, using the OMA system, should be kept to not much less than 200 ms. In this way, the length of time of detector exposure would be almost 24 times longer than the UV bulb’s pulse duration. Furthermore, by acquiring almost 24 pulses the discrepancies in their individual maximum intensities can be neglected. The relatively small variation in intensity of the individual pulses reported on here was a result of assuming that the measured voltage was proportional to the intensity of light. Later in Ch. 2, time dependent data of the UV source’s intensity gave a more realistic measure of its error, which turned out to be larger than the 2 % variation reported in this section. Of course, this implies it was inappropriate to assume the measured voltage across the photodiode was proportional to the intensity of the bulb.

Following a study of short-term timing of the UV source, a long-term study of its performance was presented in Sect. 2.2.2.4. Here, for the first time, evidence of variation in the source’s intensity of the 546 nm peak of nearly 20 % was encountered. This directly contradicts, and corrects, the reported error in intensity reported on in Sect. 2.2.2.3. The long-term study revealed that the source only became stable after about 2 hours of operation, which necessitated the use of a shutter in later synthesis experiments to protect the sample from the UV light until the bulb became stable. While the study revealed a useful lifetime of the bulb that exceeds 35 days, it also indicates a warm-up time of approximately 2 hours. With that, the description of the custom designed system, that was built in-house, for the synthesis and in-situ monitoring of a targeted biotherapeutic delivery device was complete.

Section 2.3 addressed the characterization of the stimuli responsive nanogels by presenting subsections that dealt with their electron microscopy, NTA, bond identification through FTIR spectroscopy, and ultraviolet absorbance. First, Sect. 2.3.1 presented micrographs that showed the nanogels typically in close proximity to each other. This tendency toward apparent agglomeration was attributed to the way in which samples were prepared for imaging. This involved allowing a drop of the sample to desiccate so that
all the nanogels contained therein were necessarily brought together as the solvent evaporated. The micrograph of Fig. 2.16 (b) relates how most nanogels are in proximity to each other, while only a few nanogels are observed isolated under these conditions. Confocal microscopy was only useful in quickly determining that a sample was worth further investigation because its limited magnification capability naturally biased the observer toward larger artifacts. Future researchers continuing this work could further narrow the already monodisperse PSDs, reported on here, by employing techniques like centrifugation and/or filtration of the final product after synthesis. After electron microscopy, the chapter turned to NTA to demonstrate that the nanogels do not agglomerate while in solution.

Section 2.3.2 presented NTA data which effectively allows for determination of a temperature dependent, in-situ nanogel PSD. Even more important, the section established that the apparent agglomeration observed under electron microscopy is no longer the case according to NTA. The nanogels of this section did not exhibit a discontinuous VPT like the PNIPAM micelles of Sect. 2.1.1. A high density polymeric mesh due to high concentrations of MBA (crosslinker) during synthesis may explain the loss of stimuli responsive behavior. It could be, however, that temperature increase of these nanogels would allow for the slow release of a biotherapeutic as these nanogels gradually increase in size and their polymeric mesh opens up. On the other hand, future researchers could restore the VPT by using less MBA than reported here.

Section 2.3.3 resumed the nanogel characterization by detailing chemical bond identification using FTIR spectroscopy. Besides confirming the molecular fingerprint of PNIPAM this section accomplishes two other tasks. First, Lambert-Beer lines were presented that allow for concentration determination of the three components of the precursor solution. An absorbance versus concentration study of the three solutes NIPAM, MBA, and Irgacure 2959 enabled this. Second, and more critical to this work, FTIR provided evidence of successful photopolymerization following exposure of the precursor solution to UV. Similarly, the subsequent section used UV absorbance to provide further evidence of successful photopolymerization.

UV absorbance spectroscopy was complimentary to FTIR spectroscopy in that it not only served to provide a potential method to determine the concentration of some unknown solution and identify chemical bonds, but it also provided evidence of successful polymerization. Section 2.3.4 served as UV absorbance characterization of NIPAM (monomer), MBA (crosslinker), and Irgacure 2959 (photoinitiator)
as they pertain to photopolymerization in the fabrication of PNIPAM nanogels. Preliminary investigations of the precursor solutes confirmed that increases in concentration lead to increases in absorbance at characteristic wavelengths. As with FTIR, this is consistent with Lambert-Beer’s law. Regarding bond identification, NIPAM and MBA were shown to have peaks at approximately 200 nm and 220 nm, which correspond to C=C and C=O bonds respectively. A diminishing of the peak at 200 nm was attributed to the elimination of a C=C bond upon successful polymerization. After a precursor solutions was exposed to UV radiation all peaks were diminished. It is possible that the C=O peak shifted to shorter wavelengths when conjugation, sharing of electron density between nearby multiple bonds, was eliminated. UV absorbance of Irgacure 2959 informed upon an appropriate choice of UV source out of the four that were originally purchased and characterized back in Sect. 2.2.2.1. That is to say that the Irgacure 2959 absorbance peaks at approximately 221 nm and 279 nm would respond optimally to the UV bulb demonstrated to have an emission peak around 254 nm. Thus ended Sect. 2.3, the characterization of the stimuli responsive nanogels, composed of PNIPAM, for the application of targeted biotherapeutic delivery device.

Finally, Sect. 2.4 reported on how OMA data demonstrates an attenuation of the UV source while the precursor solution underwent polymerization. A whitish polymeric material manifested on the front window of the cuvette after UV irradiation of the precursor solution, as was documented in photographs. Future researchers continuing this work will want to redesign the apparatus so that more of the precursor solution is exposed to UV radiation with methods such as stirring the solution during exposure, flowing the sample through a region of UV exposure, or strategically placing the UV source in relation to the solution. Some of these steps can also prevent a change in concentration that would lead to nanogels of varying size. Certainly, the manifestation of the whitish material correlates to the attenuation of the UV source, as measured by OMA. Preliminary experiments revealed a brief, sharp increase in intensity of the source as it passed through the precursor solution. This was attributed to the warm-up time of the UV bulb, which necessitated the use of a shutter in the experimental apparatus to ensure relatively unvarying UV exposure. Higher concentrated precursor solutions exhibited a greater degree of attenuation as synthesis neared completion. Future researchers can take advantage of the measured attenuation as an indication of synthesized nanogel diameter, thus eliminating the need, and cost, for electron microscopy characterization. A synthesis time of approximately 20 minutes is suggested by monitoring the attenuation
of the UV source while the nanogels are being fabricated. This is relatively faster than the synthesis of PNIPAM micelles using techniques such as emulsion polymerization discussed in Ch. 1. Section 2.4 also demonstrated control over ultimate nanogel size by careful selection of the precursor solution's concentration. Apparently, the amount of MBA in the precursor solution played a prominent role in determining the final size of the PNIPAM nanogels. The ability to control the nanogel size will prove to be of paramount importance in Ch. 4 when encapsulation of IOMNPS is presented. This is because the IOMNPs agglomerate in solution, as discussed next in Ch. 3.
CHAPTER 3:
MAGNETIC NANOPARTICLE CHARACTERIZATION AND EFFICACY

Recall, from Ch. 1, that the IOMNP serves two purposes in the application previously discussed. First, it will provide a TBD device with remote guidance capability utilizing a static magnetic field to target some diseased tissue. It will also respond to an AMF by heating the surrounding stimuli responsive polymer, prompting it to undergo its phase transition which will release the biotherapeutic cargo. In this chapter, the second basic component of the biotherapeutic delivery device, the IOMNP, will be characterized and evaluated for heating response to an AMF. Following their characterization, a detailed reporting of the heating of IOMNP dispersions responding to an AMF is given. All the work discussed in this dissertation pertains to a 5 g sample of IOMNP powder from US Research Nanomaterials, Inc. The nanopowder was advertised by the manufacturer to be made of magnetite and containing nanoparticles ranging in size from 15 – 20 nm.

3.1. Magnetic Nanoparticle Characterization

A PSD for discrete IOMNPs and the agglomerates they form in solution will be presented. Then a solution of IOMNPs dispersed in DIW, at a concentration typical of samples discussed later in the dissertation, will be evaluated for transmittance of light as a function of wavelength. This will inform as to the ability of the photochemical energy source, discussed in Ch. 2, to penetrate the solution during synthesis of the IOMNP embedded PNIPAM nanogels detailed in Ch. 4. Further characterization, in terms of the magnetization as a function of applied magnetic field, will be provided in order to establish the superparamagnetic nature of the nanoparticles. Characterization will conclude with results from the investigation of IOMNP sedimentation, while dispersed in DIW.
3.1.1. Transmission Electron Microscopy (TEM)

In this subsection, TEM results will be presented to establish the size and morphology of the discrete IOMNPs purchased from US Research Nanomaterials, Inc. TEM was also used to determine the thickness of the PVP coating added to the surface of the IOMNPs. The manufacturer included PVP to facilitate dispersal in water. It should be stated that two varieties of magnetite nanopowder were acquired: one claiming to be 15-20 nm in diameter and another claiming to be 20-30 nm. Both were subjected to rigorous size measurements and the results from each were indistinguishable in terms of average size. Ultimately, the advertised 15 – 20 nm sample was used throughout this entire work since its size distribution was more monodisperse. While TEM will be shown to be effective at characterizing an individual IOMNP, the results can be misleading. The preparation of a sample and the conditions of its imaging causes this confusion.

TEM experiments were carried out in the Electron Microscope Core Lab in the Department of Integrative Biology at the University of South Florida. That lab is equipped with a FEI Morgani TEM that was used for this work. The microscope is capable of a 3 kX – 824 kX magnification range. All images were captured using an electron voltage of 60 keV. Within the microscope the sample was subject to a working pressure of $5 \times 10^{-3}$ Pa ($5 \times 10^{-5}$ Torr) and an ultimate pressure of $< 1 \times 10^{-3}$ Pa ($< 10^{-5}$ Torr). For imaging, a 1.4 MPixel sidemount CCD camera and 16.7 MPixel bottom mount CCD camera are used. Obviously, exposure of the IOMNPs to this low pressure environment is unlike the environment described by the targeted biotherapeutic delivery application discussed in Ch. 1.

Sample preparation for TEM imaging, described briefly here, is a process that removes IOMNPs from solution. Typically, a small droplet of IOMNPs, diluted to low concentration in DIW, was placed on a copper coated TEM grid to dry for about 15 minutes. The grid was suspended in a petri dish from tweezers and the grid was covered to prevent dust from landing on the grid during the time it took to dry the droplet completely. On some occasions an oven under low pressure was used to dry the sample more quickly. Uranyl acetate was tried on one occasion to improve the contrast during imaging but showed no pronounced benefit as compared to when the stain was not used. This is likely due to the already high electron density of magnetite. Again, these desiccated conditions are dissimilar from what the IOMNP would encounter in a targeted biotherapeutic delivery application in which a solvent is present.
A good representation of the TEM images obtained during this experiment is shown in Fig. 3.1 (a). This image has been magnified 824 kX. Immediately apparent is the seeming nonconformity in mostly round shape which can be characterized as irregularly spherical. Some of the nanoparticles appear darker than others which can be attributed to the stacking of neighboring particles on one another, upon close inspection. For stacked nanoparticles, the spacing provided by the PVP is not observed and is presumed to be in a plane parallel to the grid. Otherwise, there is a consistent spacing between neighboring particles.
which is attributed to the PVP coating. Note that there are no IOMNPs imaged here that are isolated by more than about 2 nm in space from any neighbor. That is to say, there are no obviously discrete IOMNPs evident in TEM imaging.

Many TEM images were taken and many of the nanoparticles in those images were measured for largest diameter through the center using software on the microscope computer. A histogram of counts of IOMNPs as a function of size population comprises the PSD plotted in Fig. 3.1 (b). That data was fit with a Gaussian function whose peak corresponded to an average IOMNP size of approximately 10 ± 1 nm. The error in this measurement represents one standard deviation from the Gaussian center. Therefore, the IOMNPs are relatively monodisperse. The histogram reveals some bias toward measuring larger IOMNPs as indicated by the higher number of counts on the large particle size side of the Gaussian. This is a natural consequence of the fact that larger particles are easier to measure and present less technical challenge for the microscopist. It is interesting to note that the average size lies outside the size range advertised by the manufacturer (15 – 20 nm). This peculiarity is further compounded by the fact that the alternative purchased sample advertised a 20 – 30 nm size range, and its PSD was similar to that of Fig. 3.1 (b), albeit broader.

In order to determine the thickness of the PVP coating around the IOMNP exhaustive measurements were recorded of the nearest spacing between the particles. These spacing measurements were recorded and analyzed in much the same way as was the particle sizes corresponding to Fig. 3.1 (b). The spacing size distribution is given in Fig. 3.1 (c). Once an average spacing between IOMNPs of about 2 nm was determined it was assumed that this was due to two PVP shells around two distinct IOMNPs coming into contact with each other. Therefore, one shell of PVP must be about 1.0 ± 0.2 nm thick. The error here comes from one standard deviation in Fig. 3.1 (c) that was then divided by two to coincide with the fact that two shells are measured in one spacing.

Here, the purchased IOMNPs were reported to be irregularly spherical, about 10 nm in diameter, and coated with an almost 1 nm thick PVP shell. The low quality of the IOMNPs, indicated by their size discrepancy to that of the advertised size and inconsistent morphology, are likely attributable to their low cost. Discrete IOMNPs were not observed, but rather they were always found and measured in larger agglomerations. In the section following this one, a report of a NTA investigation of IOMNP interactions in solution will be presented. There will be shown that TEM imaging of IOMNPs gives a false sense of discrete
particles that do not interact. The reason for this is that TEM measurements occur under desiccated conditions, while NTA occurs under aqueous conditions like those of the intended use. Therefore, it is likely that the large agglomerates typically imaged under TEM correspond to the clusters measured by NTA. However, because NTA will observe agglomerates it will be unable to report on the hydrodynamic size of discrete IOMNPs. At least TEM was able to inform that a minimum hydrodynamic size of approximately 12 nm will be appropriate in Ch. 5 when determining the Brownian relaxation.

3.1.2. Nanoparticle Tracking Analysis

The purpose of this section is to detail the characterization of the purchased IOMNPs using Malvern’s NTA [166]. The goal with this experiment was to acquire a PSD of the IOMNPs under conditions similar to those of the application this dissertation is concerned with, discussed in Ch. 1. Recall from the discussion of NTA data in Ch. 2 that this technique provides sizing data while the sample is in solution. In other words, the IOMNPs are free to interact with each other, and the solvent, while data is acquired just as they would in their targeted biotherapeutic delivery role. In this regard, NTA data is superior to the TEM results presented in Sect. 3.1.1 since there the sample is desiccated and constrained to lie flat on a copper grid. This means that NTA will inform upon whether the IOMNPs are relatively isolated in solution, rarely interacting with their neighbors, or if they are agglomerating while dispersed in DIW. Recall that these IOMNPs were coated by the manufacturer in PVP to facilitate dispersal in water. It is possible that, during synthesis, several IOMNPs were trapped together in a shell of PVP as has been the case in other works [175]. It has also been shown that dipole-dipole interactions have been associated with linear configurations of MNPs [176]. The possibility of formation of ensembles will have consequences on conclusions drawn based on the assumption that IOMNP solutions are composed of uniform, discrete particles, especially in regard to AMF heating discussed later.

The fundamental design and operation of Malvern’s NTA was introduced in Sect. 2.3.2., but will be reviewed briefly here. By passing a He-Ne laser through a sample of IOMNPs dispersed in DIW light is scattered off the particles, as shown in Fig. 3.2 (a). A digital camera can be made to record the Brownian motion of the particles. Malvern’s software is then utilized to deduce particle size from the rate of motion using the Stoke-Einstein equation. Each particle in the video’s frame is analyzed providing visual
confirmation of any agglomeration. NTA is capable of observing particles ranging in size from 10 nm to 2000 nm and assumes a spherical shape for analysis.

For the data presented here, the sample was subjected to rigorous ultrasonic agitation in order to ensure large, stable agglomerates were removed from the dispersion. Additionally, differential centrifugation was attempted, but with only limited success. Therefore, it was decided the extra effort and
time associated with centrifugation would not be employed in the eventual encapsulation experiments. The results of the centrifugation efforts are presented next in Sect. 3.1.2.1. However, some other important parameters of the NTA experiment include the duration of video and number of videos captured. The power of this technique is achieved only when particles are observed for long time periods. Therefore, it was decided to observe a sample for as long as three minutes and to capture five such videos. The results of those five trials were then averaged to demonstrate repeatability and generate error.

Before proceeding to the results it will be informative to provide a description of converting back and forth between the concentrations in particles per milliliter of this section to concentrations in milligrams per milliliter encountered in most of the remainder of this dissertation. A convenient place to begin is to take the size of one IOMNP from TEM analysis given in Sect. 3.1.1, approximately \( d = 10 \text{ nm} \), for use in the volume of a sphere formula

\[
V = \frac{4}{3} \pi \left( \frac{d}{2} \right)^3. \tag{3.1}
\]

This yields a volume of almost \( 5.2 \times 10^{-25} \text{ m}^3 \) which when multiplied by the density of magnetite, \( \rho = 5,200 \text{ kg/m}^3 \) (see the appendix on magnetite parameters), gives a mass of nearly \( 2.7 \times 10^{-15} \text{ mg} \) per ideal IOMNP. This, then, is the conversion factor allowing for the generation of Table 3.1. Note that the first four entries in Table 3.1 inform that the \( 10^6 - 10^9 \) particles per milliliter NTA concentration range correspond to \( 10^{-9} - 10^{-6} \text{ mg/mL} \).
10^6 milligrams per milliliter concentration range. On the other hand, the concentration range under consideration in Sect. 3.2. and Ch. 5 of 1 – 5 milligrams per milliliter correspond to a 10^{14} – 10^{15} particles per milliliter range that the NTA would be incapable of testing, as seen in the last five entries of Table 3.1.

As implied above, NTA did capture video of light scattering off rather large agglomerates, compared to the discrete 10 nm size reported by TEM, of which an example is shown in Fig. 3.2 (b). Since this picture is of light scattered off particles at different depths of field, a scale bar is not possible here. Nonetheless, as the video plays the largest seeming particle in the frame rotates in such a way that its irregular shape can be observed over the course of about 30 seconds. Fortunately, NTA provides raw data in the form of an array for size and a corresponding array for concentration from analysis of the video.

Figure 3.2 (c) is a plot of concentration in particles per milliliter as a function of IOMNP size in nanometers for each of the five, three minute videos recorded. The uniqueness of each trial is discernible, but each particle size distribution has the same basic shape. This demonstrates the repeatability of the results. Studying any single trial informs on certain size populations present in the sample. For example the fourth trial indicates there are distinct size populations at approximately 150 nm, 220 nm, and 300 nm.

All five trials were averaged together to give the plot shown in Fig. 3.2 (d) with error bars representing the standard error of the five trials at any given particle size. Apparently, there are no discrete IOMNPs or agglomerates observed that are less than about 100 nm in size. This is not to say that those smaller size populations do not exist in the sample, only that the larger agglomerates are so much more prevalent that detection of smaller agglomerates is obscured. Both 1(c) and (d) indicate there are more than one size population ranging between approximately 100 and 600 nm. The average of the five trials was fit with a Gaussian function to report the average IOMNP agglomerate size of 268.4 ± 23.4 nm.

By employing NTA, a PSD has been obtained for dispersions of IOMNPs in DIW that were sonicated and centrifuged in order to ensure the largest stable agglomerates were eliminated. An average IOMNP agglomerate size of nearly 270 nm was detected, with none below about 100 nm and none above approximately 600 nm. Apparently, the nearly 10 nm discrete IOMNPs, as detected by TEM reported on in Sect. 3.1.1, are either agglomerating together in larger ensembles or forming linear assemblies when dispersed in DIW. This 270 nm average size for IOMNP agglomerates places a size limitation on the encapsulating PNIPAM nanogel discussed in Ch. 2. Obviously, a nanogel synthesis recipe will have to be
employed that yields a size large enough to envelope these IOMNP agglomerates. Now, the size of these agglomerates should present no challenge to the overall size restriction imposed by the application. Recall that capillaries tend to be about 5 – 10 microns in diameter and these agglomerates would easily pass through those vessels. Ultimately, the effective size of the IOMNPs will play a large role in AMF heating as will be seen in Ch. 5. At that point, NTA data will prove to be necessary in predicting the heat generated by IOMNP agglomerates.

3.1.2.1. IOMNP centrifugation. In Sect. 3.1.2, NTA experiments were described, which revealed that the IOMNPs used in this work tend to agglomerate in clusters averaging almost 270 nm in diameter. In an effort to obtain smaller agglomerates, if not discrete IOMNPs, differential centrifugation was employed. Differential centrifugation is used by cell biologists, for example, to separate out the constituent organelles of the cell for further study. This technique allows for separation of a diverse solution, in terms of particle size and density, into its various particle types [150-154]. In this section, the efforts to separate out a homogenous dispersion of IOMNPs are presented. First, a detailed description of the general technique and steps followed in this work are presented. Next, a model is presented whose purpose was to predict the centrifugation spin rates necessary to pull a particular IOMNP agglomerate size out of solution. Finally, results from a systematic separation attempt, using centrifugation, are presented.

The centrifuge used in this experiment was a Sorvall Instruments MicroSpin centrifuge, rated at 110 W, 115 V, and 1.1 A. At the start of the experiment the sample was dispersed in several 1.5 mL centrifuge tubes. They were ultrasonically agitated for approximately 5 minutes and mechanically agitated briefly just prior to being placed in the centrifuge. At that point the samples were considered homogenous like the leftmost tube shown in the schematic of Fig. 3.3 (a). The only difference is that there were several different particle sizes in the real sample not just the two shown in the schematic. It is important to keep in mind that when large particles are precipitated out of solution they sometimes carry smaller ones with them. Therefore, a multistep centrifugation process that graduates up to faster and faster spin rates is optimal for fine separation of IOMNPs. The tubes were then centrifuged at 1,000 rpm for approximately 15 minutes. Next, the supernatant was carefully transferred to new centrifuge tubes and enough filtered DIW was added to bring the volume back up to 1.5 mL. That entire process was repeated with the new tubes except that they were centrifuged at 4,000 rpm to obtain the next supernatant. Once that supernatant was brought back
up to a 1.5 mL volume the process was repeated again centrifuging at 10,000 rpm. Each cycle of centrifuging served to precipitate out a different size population of IOMNPs. At each stage a small aliquot was removed for NTA analysis. All these samples would have required tremendous investment of time to replicate the robustness of the previous section. Instead, the NTA data from this experiment represents
It was hoped that a mathematical description of differential centrifugation would allow for size (D) prediction of precipitated IOMNP agglomerates corresponding to a given centrifugation spin rate (ω). Modification of Stokes’ law describes sedimentation of IOMNPs according to

$$D = \sqrt{\frac{18 \eta \ln(R_f/R_i)}{(\rho_p - \rho_f) \omega^2 t}}.$$  \hspace{1cm} (3.2)

Here, it was assumed the viscosity (η) and density (ρ) of the solvent was equivalent to that of water, 8.90 x 10^{-4} Pa s and 1,000 kg/m^3 respectively. All samples were centrifuged for at least 15 minutes making time (t) a constant in Eqn. 3.2. The particle density (ρ_p) was taken to be equivalent to the density of magnetite, 5,150 kg/m^3. The final radius of rotation (R_f) and initial radius of rotation (R_i) were allowed to range over the horizontal component of the centrifuge’s longest dimension. Finally, the centrifuge spin rate (ω) was made to range from zero to 11,000 rpm: the centrifuge used in this experiment was restricted to these rates.

Simulations of Eqn. 3.2 using the parameters as described above are plotted in Fig. 3.3 (b). As expected, for faster centrifuge spin rates smaller and smaller IOMNP agglomerates can be expected to fall out of the sample’s water column. Note that samples spun at 10,000 rpm can be expected to yield IOMNP agglomerates around 50 nm in diameter. This would be the smallest theoretical agglomerate obtainable using the centrifuge available for this experiment.

The IOMNP agglomerate size distributions, as measured by NTA, for the sample centrifuged according to the steps given above, are plotted in Fig. 3.3 (c). While the nature of the single NTA video capture makes the data noisy, it is possible to observe trends indicating some separation, due to centrifugation, did occur. This is confirmed by tracking the rightmost prominent peak for each data set. In each data set, except the supernatant after spinning at 10,000 rpm, there is a tendency to obtain smaller and smaller agglomerates. In fact, this process has allowed the most prevalent agglomerates in a sample that were originally as large as 250 nm to be reduced down to almost 90 nm in diameter. This, however, is significantly different from the 50 nm predicted by the model. Note that each data set was normalized to itself to make this analysis simpler.

Future researchers wishing to continue with the work presented in this dissertation may find it desirable to obtain more narrow IOMNP agglomerate size distributions than those presented in Sect. 2.3.2.
and this technique does enable that. However, the model described above is inadequate to give accurate predictions of precipitated IOMNP agglomerate size. Certainly, the assumptions associated with Eqn. 3.2 can lead to some shortcomings (e.g. neglecting ramp up and ramp down times of centrifuging). However, if agglomerated IOMNPs are trapped within their surfactant, as described in the previous section, then no amount of centrifuging would yield smaller agglomerates.

### 3.1.3. Transmittance versus Wavelength

The fraction of light passing through a typical dispersion of IOMNPs in DIW as a function of wavelength is presented here. The biotherapeutic delivery devices described in Ch. 1 are synthesized utilizing UV photopolymerization as detailed in Ch. 2. Later in Ch. 4, the encapsulation of IOMNPs into the delivery devices will be presented. Because of that, the interaction of IOMNPs with the UV source was a logical investigation to perform prior to any encapsulation attempt. It should be noted that a priority for this investigation was the interaction of UV light with a typical dispersion of IOMNPs and not necessarily the IOMNPs themselves. That is to say, IOMNP dispersion parameters most similar to those encountered during the encapsulation investigations of Ch. 4 were of the utmost importance. Therefore, it is not the goal to report on optical energy band gaps, Urbach energy, Fermi energy, etc., especially since this has already been reported elsewhere [177].

Spectroscopy was carried out using a Perkin Elmer High-Performance Lambda 950 spectrometer which is capable of absorption or reflectance spectroscopy in the UV, visible, and NIR regions. Specifically, this spectrometer can observe from 175 nm to 3300 nm. The spectra presented here were recorded at a resolution of 5 nm. The sample was contained in a quartz cuvette that was already characterized in Sect. 2.2. The purchased IOMNPs were weighed out and dispersed in enough filtered DIW to achieve a 0.50 mg/mL concentration. This concentration is comparable to that used in the IOMNP encapsulation by PNIPAM nanogels described later in Sect. 4.2. Note that this concentration is not optimized for providing detailed information regarding the structure of an individual IOMNP. The spectral range investigated here was from 190 nm to 930 nm which corresponds to the spectral range investigated during characterization of the UV source of Sect. 2.2.
The transmittance as a function of the entire spectral range investigated here is presented in Fig. 3.4 (a). The error bars indicate the standard deviation of ten trials. The relatively large error is a consequence of the high IOMNP concentration. Recall from the discussion of Sect. 2.3 that samples subject to this type of spectroscopy must have relatively low concentrations to provide structural information. So this data can only characterize the global IOMNP dispersion. While the error is larger than desirable, some general conclusions can be drawn from apparent trends. For example, it is clear from the data presented...
in Fig. 3.4 (a) that the sample does not in general allow much light to pass through it regardless of the wavelength. To be precise, light is virtually blocked by the sample in the UV region and only a little more light is permitted through in the visible and NIR regions.

The same data is presented in Fig. 3.4 (b) except that only the UV part of the spectrum (190 nm to 400 nm) is shown in order to take advantage of the spectral resolution achieved by the data. Here, care should be taken not to interpret too much from the features observed at around 300 nm since the error in transmittance is relatively high. However, there certainly is a slight increasing trend in transmittance as wavelength increases. At around 200 nm only about 0.4 % of the light is passing through the sample while at around 400 nm approximately 0.7 % of the light is transmitted. Recall that the photopolymerization energy source has a peak near 254 nm believed to be responsible for the synthesis of Ch. 2. This IOMNP dispersion only allows less than 0.5 % of the light through at that crucial wavelength. Apparently, the energy source for the photo reaction is being absorbed by the IOMNPs in solution. Apparently, the IOMNP dispersion is practically opaque to the photopolymerization energy source.

Again, Fig. 3.4 (c) is the same data plotted in (a) only with the visible region data (400 nm to 700 nm) plotted exclusively. Here, the transmittance of the sample is more abruptly increasing with wavelength, as compared to the UV data. The transmittance increases from nearly 0.7 % at 400 nm, almost linearly, to about 10 % at 700 nm. It was previously reported that the color of such dispersions is reddish, brown [178].

Finally, the transmittance versus wavelength data corresponding to the NIR region (700 nm to 930 nm) is plotted in Fig. 3.4 (d). As before, features like that around 850 nm should not be focused on since the error in transmittance is relatively high. On the other hand, there is obviously an overall trend toward higher transmittance as wavelength increases: at 700 nm the transmittance is about 10 % and at 900 nm the transmittance has increased to nearly 14 %. Despite the relatively higher fraction of light passing through the sample, as compared to the UV region, there is still significant infrared light absorbed by the sample. It was shown in Sect. 2.2 that the photopolymerization energy source does have some relatively strong NIR peaks. If the IOMNP dispersion absorbs NIR energy it must necessarily increase the temperature of the photo reaction presented later in Ch. 4.

Overall, the IOMNP dispersion has been shown to exhibit rather strong opaqueness to UV, visible, and NIR light. The sample is most opaque to UV which may present a challenge to the photopolymerization
goals of this work (i.e. the photochemistry, presented in Sects. 2.2 and 2.3, requires an UV energy source). Visible light only transmits through the dispersion a little more readily than UV does. Finally, NIR light transmits most readily, but still not at a very high percentage of the original light. This presents a different challenge in the form of unintentional heating of the sample undergoing photopolymerization discussed in Ch. 4. Recall from the discussion of Ch. 1, one of the goals of this work was to avoid temperature induced chemical synthesis of the delivery devices.

3.1.4. Magnetic Response to Applied Magnetic Field

At this point, the magnetic response of the purchased IOMNPs as a function of the applied magnetic field will be presented. Data of this type can be used to demonstrate a superparamagnetic quality of MNPs, which is desirable since those nanoparticles exhibit minimal coercivity in response to an applied field; there would only be a negligible applied field necessary to return the magnetic flux density to zero. Similarly, superparamagnetic nanoparticles exhibit very little residual magnetic flux density, known as remanence, when the applied field is removed. Data of this sort will also be used to report on the size of the IOMNPs used in this work [179]. Finally, some implications of the data to the model of Ch. 5 will be made apparent here.

The raw magnetization (emu/g) versus applied magnetic field (Oe) data was provided by Dr. Javier Alonso working in Dr. Manh-Huong Phan’s and Dr. Hariharan Srikant’s lab group. The measurements were carried out at room temperature on a sample of the purchased IOMNPs used in this work. That raw data was presented previously [178]. However, it will be necessary in Ch. 5 to consider, instead of magnetization (M), the magnetic polarization (J) of the IOMNPs. Therefore, the vertical axis has been scaled by a factor of the permeability of free space \( \mu_0 = 4\pi \times 10^{-7} \text{ N/A}^2 \) and a factor of the density of magnetite \( \rho = 5.2 \text{ g/cm}^3 \) according to the expression

\[
J = \mu_0 \rho M.
\]

This equation gives the polarization in units of Teslas (T). In this form, the data will be more readily useful for the modeling of AMF heating presented in Ch. 5 without losing the characteristic shape necessary to ascertain a superparamagnetic quality of the IOMNPs. Also, the raw data from the experiment gave the magnetization in Oersted (Oe) so that the array had to be multiplied by a factor of \( 10^3/4\pi \) to give a new
array in Amperes per meter (A/m), which was more applicable to the rest of the work presented in this dissertation.

The modified results of the experiment, magnetic polarization as a function of applied magnetic field, are presented in Fig. 3.5 (a). The response of the IOMNPs can be characterized as sigmoidal in shape, exhibiting no apparent magnetic hysteresis. That is to say, there is no discernible coercivity or remanence which indicates that the IOMNPs are apparently superparamagnetic in nature. It was already reported that a Langevin expression was fit to the expression in order to further confirm superparamagnetism. The result of that fit was to report on the magnetic size of the IOMNP of 7.8 nm. The discrepancy between this magnetic size and that measured by TEM was attributed to surface spin disorder. Also reported, was the saturation magnetization of the sample of around 60 emu/g, which will be used in Ch. 5 to determine the equilibrium susceptibility, $\chi_0$ of the IOMNPs. The difference between this saturation magnetization and that of bulk magnetite (~90 emu/g) was also attributed to surface spin disorder [178]. These results are in good agreement with similar data made freely available by the manufacturer of the IOMNPs.

Figure 3.5 Polarization, being converted from magnetization, as a function of applied magnetic field across (a) all measurable field strengths and (b) the field strengths applicable to the AMF heating experiments described in Sect. 3.2.
Later on in Ch. 5 the AMF heating of IOMNP dispersions will be simulated using LRT to predict the heating rates presented in Sect. 3.2. One of the critical assumptions of LRT is that the magnetization of the IOMNPs must respond linearly to the applied magnetic field. The amplitude of the applied field of Sect. 3.2 does not exceed 59 kA/m. In Fig. 3.5 (a) the response of the IOMNPs may be linear but this is unclear. Figure 3.5 (b) plots the same data only in the range of applied fields of interest corresponding only to the AMF heating discussed later in this dissertation. Here, the experimental data is shown not to coincide, in general, with a linear fit. This is more apparent by noting the $R^2$ value of the linear fit is only about 0.9833, not particularly good.

The magnetic response of the IOMNPs presented here demonstrates that they can be characterized as superparamagnetic; they respond relatively strongly to an applied magnetic field. The data suggests that the IOMNP magnetic size, about 8 nm, is slightly smaller than that measured by TEM, about 10 nm, but that is expected when surface spin disorder is taken into consideration. These particles may not be responding exactly linearly with the applied magnetic field, a central assumption of LRT, which will be considered later when accounting for deviations between the experimental results of Sect. 3.2 and the simulations predicting AMF heating rates of Ch. 5.

### 3.1.5. Nanoparticle Sedimentation

The purpose of this section is to describe the tendency of the IOMNPs to settle out of solution when mixed with DIW. It is well established in the magnetite nanoparticle literature that sedimentation is a reality which researchers must address [180, 181]. The IOMNPs used in this work were coated by the manufacturer with polyvinylpyrrolidone (PVP) in order to facilitate dispersal in water. Despite this measure, early experiments with dispersions of the nanopowder revealed settling occurred within minutes of vigorous ultrasonic and mechanical agitation.

To determine the rate at which sedimentation occurred an experimental setup similar to that described in section 4.1 was used to probe the turbidity of the sample as a function of time. Briefly, a He-Ne laser was made to pass through the sample and collected with a photoresistor. The photoresistor was monitored with a Keithley multimeter. The resistance was recorded at the beginning of a trial and every minute for six minutes. At least seven trials were collected in order to demonstrate reproducibility and
generate error. Photoresistance was converted to relative transmittance according to Appendix G. Four samples with different concentrations were prepared by measuring out the desired amount of nanopowder and adding to that the requisite volume of DIW to achieve the target concentration. Next, the samples were sonicated for at least one hour followed by vigorous mechanical agitation. Finally, the samples were immediately pipetted from the original vessel and transferred to a polystyrene cuvette. That cuvette was capped and always agitated just prior to a trial to ensure the IOMNPs were well dispersed.

The results for the sedimentation of IOMNPs experiment are given in Fig. 3.6. As expected, the lower the concentration the more readily the laser penetrates the sample as indicated by the higher relative transmittances for lower concentration solutions. So the 1 mg/mL sample became more transparent to the laser by about 27 % at the end of 6 minutes, whereas the 4 mg/mL sample only became about 8 % more transparent. It is clear from Fig. 3.6 (a) that the IOMNPs in all solutions settled out over time. Figure 3.6 (b) show the same data as in (a) except that now the error bars, as calculated from standard deviation of at least seven trials, is shown. In general, the error is more than 10 % of the measured relative transmittance causing most of the error bars to overlap between samples.
Much of the data discussed in sections 3.2 and 5.2.2 assume a dispersion of IOMNPs with an unchanging concentration. The data presented in Table 3.2 could be of use in correcting any disagreement between experimentally determined heating rates (section 3.2) and those determined theoretically (section 5.2.2.). Of particular importance is the realization that concentrations are not constant during a heating trial. Specifically, during a 5 minute trial, a 1 mg/mL sample decreases to about 0.7 mg/mL, a 2 mg/mL sample decreases to about 1.7 mg/mL, a 3 mg/mL sample decreases to about 2.8 mg/mL, and a 4 mg/mL sample decreases to about 3.8 mg/mL. Such information could be used to adjust experimental data to match results obtained using Linear Response Theory (see Ch. 5). Table 3.2 also show the average sedimentation rate of a sample after 5 minutes of settling has been allowed to occur. This was determined according to the equation,

\[
\frac{\Delta C}{\Delta t} = \frac{C_f - C_i}{t_f - t_i}
\]  

(3.4)

where \(C_f\) and \(C_i\) are the final and initial concentrations and \(t_f\) and \(t_i\) are the final and initial times. Interestingly, average sedimentation rates do not show an easily discernible trend as indicated in Table 3.2. This may be related to the error overlap shown in Fig. 3.6 (b).

In closing, data presented here shows that IOMNPs are obviously settling out of solution over time even though they have been coated with PVP. While error analysis suggests the data is not precise, there are obvious trends demonstrating that the samples become more transparent to the laser over time. The results here will be applied to Ch. 5 to resolve discrepancies between theory of IOMNP heating with applied AMF and experiment.

<table>
<thead>
<tr>
<th>Initial Concentration (mg/mL)</th>
<th>Final Concentration (mg/mL)</th>
<th>Sedimentation Rate (mg/mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.7</td>
<td>0.05</td>
</tr>
<tr>
<td>2.0</td>
<td>1.7</td>
<td>0.07</td>
</tr>
<tr>
<td>3.0</td>
<td>2.8</td>
<td>0.04</td>
</tr>
<tr>
<td>4.0</td>
<td>3.8</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 3.2 Final concentrations and average sedimentation rates of IOMNP dispersions in DIW measured for five minutes.
3.2. Alternating Magnetic Field (AMF) Heating of IOMNPs Dispersed in DIW

The preceding section presented characterization results on IOMNP dispersions including discrete particle and agglomerate size distributions, magnetization in response to applied field, and transmittance versus wavelength. Now, this section presents the results of exposing IOMNP dispersions to an alternating magnetic field and the temperature increase culminating from that. Recall that in the ideal TBD device described previously, the IOMNPs are embedded within a SRP that also contains the biotherapeutic. Chapter 1 described how the IOMNPs serve two functions in the TBD device of which they are a part: guidance to a target tissue and heat generation for remote triggering of biotherapeutic release [13, 14, 78]. In effect, it was the purpose of the experiments described here to ascertain the capability of the IOMNPs to heat their local environment, DIW only in this section, remotely. What was of particular interest, however, was the determination of an optimum IOMNP concentration(s) for the use in the AMF heating of samples that also contain the stimuli responsive polymer PNIPAM, which is the topic of section 4.1.

The induction heating equipment used to heat the IOMNP dispersions was an Ambrell EasyHeat LI heating system fitted with an 8 loop (N), 1.25 cm radius (a) sample heating coil. For all experiments described in this section a current (I) of 184.8 A was selected which equates to a nearly 59 kA/m magnetic field amplitude (H) as calculated from

$$H = \frac{N I a^2}{2 (z^2 + a^2)^{3/2}}.$$  \hspace{1cm} (3.5)

In Eqn. 3.5, z is the distance from the center of the coil on the z axis. This field alternated at a frequency (f) of about 307 kHz. Note that the product of field strength and frequency is approximately $1.8 \times 10^{10}$ Am$^{-1}$Hz which is in excess of the patient pain threshold set forth by Atkinson and Brezovich over 30 years ago [91, 183]. Chapter 5 will provide evidence that this assumed ideal field of 59 kA/m is not the likely effective field strength after all, but a significantly lower value. Furthermore, it was only the goal of these experiments to establish the heating effect as a consequence of applied AMF to IOMNPs and not intended for clinical trials.

IOMNP nanopowder was measured out and added to enough filtered DIW to provide five IOMNP dispersions ranging in concentration from 1 – 5 mg/mL. These dispersions were vigorously agitated and then sonicated to ensure minimization of agglomeration, but was never eliminated as was described in Sect. 3.1. Each of the five samples was subjected to ten trials in order to generate error and demonstrate repeatability. Each trial lasted approximately five minutes. A fiber optic temperature sensor by Photon
Control, and its associated software, was used to measure the change in temperature of a dispersion during the course of a trial. The time resolution of the data sets are all one second, and the sensor is capable of ±0.05 °C accuracy. Any given trial started at approximately the same temperature as any other trial (i.e. $T_i = 23.5 ^\circ C$). Then, the temperature change was determined by subtracting the temperature ($T$) at any given time from the initial temperature ($\Delta T = T - T_i$).

The average heating curves resulting from the IOMNP concentration study trials described above are plotted in Fig. 3.7 (a). As the concentration of the dispersions range from 1 through 5 mg/mL the final changes in temperature are approximately 7 °C, 12 °C, 16 °C, 19 °C, and 25 °C respectively. Obviously, the more IOMNP nanopowder there is in a solution the more potential heating that sample is capable of at a particular field strength and frequency. Keep in mind that this data only informs upon the entire, global sample and does not provide information on how effective the IOMNP agglomerates are at the local level when heating their surroundings. Note how the heating curves are not linear, but tend toward eventual saturation. This is due to heat energy losses to the environment of the experiment. However, the initial 20 – 30 s of data was assumed to be linear in nature. Such initial, linear heating rates ($\Delta T/\Delta t$) can be used to calculate the specific absorption rate (SAR) which does allow for comment on the local heating environment of an IOMNP agglomerate through

Figure 3.7 AMF heating trials of DIW dispersed IOMNPs reveal (a) increasing temperature change with increasing concentration as well as (b) decreasing SAR with increasing concentration. – Reproduced by permission of The Royal Society of Chemistry
In Eqn. 3.6 the specific heat of the solution is given by $c_{sol}$, the mass of the solution is given by $m_{sol}$, and the mass of the nanopowder is given by $m_{IOMNP}$. It can be seen from the expression that SAR is effectively normalized by the mass of the IOMNPs allowing for the comparison of heating efficiencies without regard for nanopowder mass.

From the initial, linear heating rates of each of the five concentrations the SAR was determined using Eqn. 3.6, and the data is plotted in Fig. 3.7 (b). The figure also provides an important juxtaposition of $\Delta T/\Delta t$ and SAR both as a function of IOMNP concentration. First, note, that the $\Delta T/\Delta t$ data points (red circles) again show that there is an unsurprising, increasing trend upward with increasing IOMNP concentration; the more nanopowder there is in solution, the more effective the AMF heating of the entire, global sample. However, the SAR data points (black squares) seem to behave in much the opposite way; SAR is relatively high at low IOMNP concentrations and relatively low at higher concentrations. Since SAR is a measure of how effective the local heating is in the sample this implies that IOMNP agglomerates are negatively impacting each other at higher concentrations when confined to smaller spaces between neighboring agglomerates.

Now the juxtaposition of $\Delta T/\Delta t$ and SAR also allows for an informed choice of optimum IOMNP concentration to be used in the experiments described later in Sect. 4.1. On the one hand, a choice of high IOMNP concentration would mean the dispersion has effective global heating but the agglomerates are not very effective at heating their local environment. On the other hand, a low concentration would mean effective local heating, but that the whole dispersion would heat relatively slowly. Figure 3.7 (b) clearly shows the choice of either 2 or 3 mg/mL dispersion would provide both effective local and effective global heating of the sample. The need for two IOMNP concentrations was to allow for the comparison of the effect of different heating rates on PNIPAM’s LCST detailed further in Sect. 4.1.

Also plotted in Fig. 3.7 (a) are two data sets corresponding to 2 and 3 mg/mL IOMNP that both have 0.8 wt. % PNIPAM concentration dispersed therein. These two data sets were included since the question was raised as to whether PNIPAM plays some role in the temperature change of a sample exposed to an AMF. The data clearly shows that at 2 mg/mL the presence of PNIPAM serves to generate slightly higher temperature increase at any given time as compared to when the stimuli responsive polymer is not
present. More striking is the effect when the 3 mg/mL IOMNP sample is considered. It was previously reported that TEM of IOMNPs shows markedly smaller agglomerates when PNIPAM is present as compared to when it is not [178]. Apparently, the presence of PNIPAM serves to reduce agglomeration, which in turn makes nanoparticle heating more effective overall [81, 184].

Here, evidence has been presented establishing that the IOMNPs are, indeed, capable of heating their local environment when exposed to an AMF. Specifically, \( \Delta T/\Delta t \) has been shown to increase with IOMNP concentration, while SAR behaves very differently in that it decreases with IOMNP concentration. Heating rate behavior is easily explained through an understanding that more agglomerates, heat sources, are available at high concentrations. SAR behavior is best understood in terms of larger and more frequent agglomerates at higher IOMNP concentrations interfering with an individual agglomerate’s ability to effectively heat its local environment due to the necessarily increased proximity. These two ideas allowed for the determination of an optimum IOMNP concentration(s) that provided for both effective local and global heating of samples analyzed later in Sect. 4.1. By including data from two samples having PNIPAM, in addition to the IOMNPs and DIW, amongst the heating curves of Fig. 3.7 (a) there is evidence that the polymer discourages agglomeration of the nanoparticles making their global heating more effective. This result will be of interest to any researcher studying similar biotherapeutic delivery devices. Consider in Ch. 5 the LRT model developed for this work predicts that the heating rate is relatively diminished when PNIPAM is present due to the effect of increased solution mass and specific heat. This is contradicted by the experimental results presented here. Apparently, the physical effect PNIPAM has on IOMNP agglomeration is stronger than the mathematical accounting of its presence in solution.

3.3. Summary

The purpose of this chapter was to introduce, characterize, and evaluate the AMF heating of the IOMNPs used in this work. These nanoparticles were purchased from US Research Nanomaterials, Inc. They were advertised by the manufacturer as composed of magnetite and ranging in size from 15 – 20 nm. First, the discrete and agglomerated nanoparticle size distributions were investigated using TEM and NTA, respectively. Second, spectroscopy was conducted to investigate the transmittance as a function of wavelength for a typical dispersion of the IOMNPs in DIW. Third, the magnetization as a function of applied
magnetic field of the nanoparticles was presented. Fourth, an appraisal of the sedimentation of the IOMNP agglomerates dispersed in DIW was conducted. Finally, samples of IOMNPs dispersed in DIW were subjected to an AMF to establish their potential to heat the surrounding water in response to the field, and to optimize an IOMNP concentration for use in AMF trials that would also include the stimuli responsive polymer, PNIPAM.

Through TEM investigation the purchased IOMNPs were revealed to be irregularly spherical, about 10 nm in diameter, and coated with an almost 1 nm thick PVP shell. These low cost IOMNPs were shown to be significantly smaller than advertised and to exhibit a not quite spherical morphology, which gave the general impression of low quality. All TEM micrographs recorded reveal IOMNPs in larger ensembles or agglomerates of nanoparticles and never discrete or isolated. Recall that TEM measurements required a sample be dried out, while NTA measurements were performed on wet samples like those of the biotherapeutic delivery application described in Ch. 1. Most likely, the large ensembles imaged with TEM correspond to the agglomerates reported by NTA.

Using NTA, a PSD was presented that corresponded to dispersions of IOMNPs in DIW that were sonicated and centrifuged to remove the largest, stable agglomerates. IOMNP agglomerates ranged in size from 100 nm to 600 nm with a strong peak centered around 270 nm. This evidence suggests the discrete, 10 nm IOMNPs, revealed by TEM, are associating with each other in either larger irregular ensembles or forming linear assemblies when dispersed in DIW. A size limitation on the encapsulating PNIPAM nanogel, discussed in Ch. 2, is imposed by this 270 nm average size of IOMNP agglomerates. In other words, a nanogel synthesis recipe will have to be utilized to ensure a size large enough to envelope the IOMNP agglomerates. Fortunately, the agglomerates’ size should present no problem to the upper size restriction imposed by the application. From basic human anatomy, capillaries tend to be about 5 – 10 microns wide so that these agglomerates easily pass through the vessels. Note, since NTA observes only the larger ensembles it cannot report on the hydrodynamic size of discrete IOMNPs. NTA is capable of measuring the discrete IOMNPs, but that would require the agglomerates be prevented from forming.

When spectroscopy was used to investigate the transmittance versus wavelength of the IOMNP dispersion it revealed strong opaqueness to UV, visible, and NIR light. The sample was most opaque to UV which presents a problem in realizing the photopolymerization goals of this work (i.e. Ch. 2 describes UV
light as providing the energy to realize the photochemistry). Visible light transmitted through the sample a little more easily than UV did. Finally, NIR light passed most easily, yet still was still generally opaque. The absorption of NIR from the radiation source for device synthesis presents a problem in terms of unintentional sample heating while undergoing photopolymerization. Recall from the discussion of Ch. 1, one of the goals of this work was to avoid temperature induced chemical synthesis of the delivery devices.

Superparamagnetic characteristics of the IOMNPs was demonstrated by magnetization versus applied magnetic field data; the nanoparticles exhibit no apparent remanence or coercivity. The data reveals the IOMNP 8 nm magnetic size is slightly smaller than the 10 nm TEM measurement, which is accounted for when surface spin disorder is taken into consideration. It is possible these particles are not responding quite linearly with the applied magnetic field, a central assumption of LRT, which may explain deviations between the experimental results of Sect. 3.2 and the simulations predicting AMF heating rates of Ch. 5.

Through turbidimetry analysis of IOMNP solutions the samples were exposed for having a strong tendency to exhibit significant settling out of solution over time even though they have been coated with PVP and sonicated to promote homogenous dispersal. Despite the fact that error analysis indicates this data is not precise, it is obvious the samples become more transparent to the laser over the course of only a few minutes.

Finally, in Sect. 3.2, evidence was presented establishing the IOMNPs are capable of heating their local environment when exposed to an AMF. There, ΔT/Δt was shown to increase with IOMNP concentration, while SAR behaves quite the opposite since it decreases with IOMNP concentration. High concentrations of IOMNP agglomerates translate into a higher number of heat sources available for heat transfer, which explains the ΔT/Δt behavior. On the other hand, when IOMNP concentration is high the agglomerates tend to crowd each other out negatively impacting an individual’s ability to heat its surrounding leading to a decreasing SAR trend with increasing IOMNP concentration. Consideration of these concepts requires an optimum IOMNP concentration allow for effective global heating of the entire sample and effective heating of the local environment for experiments investigating AMF heating that include PNIPAM in the solution (see Sect. 4.1). The two heating curves corresponding to samples having 0.8 wt. % concentration of PNIPAM included with IOMNPs and DIW in the sample suggest the polymer reduces agglomeration since heating was more effective as compared to when the polymer was not
present. Any researcher studying the AMF heating of IOMNPs incorporated into a SRP (e.g. TBD devices) will find that result interesting.

Minimally, TEM informed that a lower limit hydrodynamic size of approximately 12 nm is appropriate in Ch. 5 when determining the Brownian relaxation. It will be seen in Ch. 5 that the effective size of the IOMNPs is critically important when considering AMF heating. There it will be shown that NTA is vital to predictions of heat generated by IOMNP agglomerates. The concerns of UV and NIR absorption of a sample undergoing photopolymerization to synthesize IOMNP embedded PNIPAM nanogels will be revisited in Ch. 4. Non-linear magnetization versus applied magnetic field, applicable to this work, of the IOMNPs will be considered in explaining certain shortcomings in predictions of AMF heating based on LRT presented in Ch. 5. Sedimentation of IOMNP results will be applied in Ch. 5 in an attempt to resolve differences between the model to predict AMF heating and the actual experiment presented in Ch. 4. Interestingly, the LRT model developed for this work predicts that the heating rate is relatively diminished when PNIPAM is present due to the effect of increased solution mass and specific heat. This is contradicted by the experimental results presented in Sect. 3.2. Apparently, the physical effect PNIPAM has on IOMNP agglomeration is stronger than the mathematical accounting of its presence in solution. All of the data presented in this chapter will form the basis of the model to predict AMF heating of IOMNPs presented in Ch. 5.
CHAPTER 4:
MAGNETIC NANOPARTICLE ENCAPSULATION

This chapter reports on investigations pertaining to the incorporation of IOMNPs with the stimuli responsive polymer PNIPAM in order to realize a targeted biotherapeutic delivery device. First, an investigation into the AMF heating of non-encapsulated IOMNPs dispersed in aqueous solutions of PNIPAM is presented. Next, the efforts to successfully encapsulate IOMNPs within a PNIPAM nanogel are documented along with evidence to demonstrate that. Finally, experiments demonstrating effective AMF heating of the IOMNP embedded PNIPAM nanogels will be detailed.

4.1. AMF Heating of Aqueous PNIPAM and Non-encapsulated IOMNP Solutions

In Sect. 1.2.3 a review of PNIPAM, the SRP selected to serve as the nanogel biotherapeutic carrier, was presented. Later, in Sect. 1.3, a review of MNPs was given, whose purpose it is to direct the biotherapeutic delivery device to the target tissue and to provide the stimulus to release the payload. Here, a report is given of the behavior of PNIPAM when it is in aqueous solution with the non-encapsulated IOMNPs and subjected to an AMF. Documenting the behavior of PNIPAM when in the vicinity of IOMNPs, while not enveloping them, was thought to be a crucial step prior to encapsulation efforts. A careful explanation of the experimental setup that allowed for the determination of PNIPAM’s LCST, while AMF heating is occurring, will be provided. Also, some factors that do and do not affect the LCST of PNIPAM will be discussed. These results were reported on previously [178, 185].

The experimental setup used to probe the phase transition of aqueous PNIPAM and non-encapsulated IOMNP solutions is portrayed in Fig. 4.1 (a). The induction heating equipment was an Ambrell EasyHeat LI heating system and was already described in Sect. 3.2. The current control, seen in the schematic, was set to deliver a current corresponding to a field strength of approximately 59 kA/m at a frequency of about 308 kHz at the location of the coils. A given sample was secured in the coils using
Styrofoam and a He-Ne laser was carefully aligned to pass through the coil and sample to be detected by a cadmium-sulfide detector. That detector was monitored by a Keithley 2100 Multimeter as controlled by custom LabView software to record changes in turbidity of the sample. The nature of this first data set was photoresistance as a function of time. It was converted to transmittance percent as a function of time.
according to the procedure detailed in Appendix G. At the same time, the temperature of the sample was monitored by a fiber optic temperature sensor, made by Photon Control, whose accuracy was 0.05 °C. The nature of this second data set was temperature as a function of time. A data point was collected for each second of the trial which lasted about five minutes. During analysis, the two data sets were brought together to provide a third data set that described the turbidity of the sample as a function of time. All trials were repeated 10 times in order to demonstrate repeatability and to investigate error. Note, in Fig. 4.1 (a) a fan was used to remove excess heat from the space between the coils. The reason for this was to prevent the sample from heating due to phenomena other than from the IOMNP response to the AMF. In later experiments adjusted KOH pellets were dissolved in the solution to introduce ions for the determination of ionic influence on LCST.

The results of a PNIPAM concentration study, at a constant 2 mg/mL IOMNP concentration, are given in Fig. 4.1 (b). Here the LCST of PNIPAM at a particular concentration is defined by the inflection point of the corresponding data set. Immediately apparent is a concentration dependence on LCST. In general, low PNIPAM concentrations yield gradual transitions from clear to opaque as probed by the He-Ne laser, while higher PNIPAM concentrations yield a more step-like transition. For example, the 1.0 wt. % PNIPAM solution appears to undergo its phase change from about 30.5 °C to 33 °C. On the other hand the 0.3 wt. % solution begins its transition at approximately 31.0 °C and nears completion at about 37.0 °C. Obviously, solutions having higher concentrations of PNIPAM in them are associated with a reduced LCST.

In order to rigorously identify the LCST, the derivative was taken of data sets like those shown in Fig. 4.1 (b). The modulus of the derivative data was fit with a Gaussian function. The temperature corresponding to the peak of the Gaussian represented the LCST at a particular PNIPAM concentration. One standard deviation of the Gaussian represented the error in the corresponding LCST. Two examples of this are shown in Fig. 4.1 (c). At 1.0 wt. % PNIPAM concentration the LCST is 31.7 ± 0.1 °C, while at a concentration of 0.3 wt. % the LCST is 33.9 ± 0.3 °C. Again, higher PNIPAM concentrations yield a well-defined phase transition over a relatively small temperature range, while lower concentrations yield a transition that occurs over a wider temperature range. In the literature, PNIPAM has a critical micelle concentration (CMC) above which the LCST is constant and below which the LCST can be made to vary with PNIPAM concentration [40, 186]. In the data presented here, the LCST varies since the experimental
apparatus required all PNIPAM concentrations be below the CMC. In other words, at high enough PNIPAM concentrations the sample became opaque to the He-Ne laser regardless of its temperature making turbidimetry impossible.

In addition to the PNIPAM concentration study at constant 2 mg/mL IOMNP concentration a separate study was conducted at 3 mg/mL IOMNP concentration as well as a complimentary study involving no IOMNPs. This last study merely involved heating the sample using a hot bath to bring about PNIPAM phase transition. In all these experiments turbidimetry was still employed to determine the LCST. Those LCSTs are plotted as a function of PNIPAM concentration in Fig. 4.2 (a). Note, here, that data points lie within the margin of error of those from other experiment types, but with the same PNIPAM concentration.

In general, regardless of IOMNP concentration or method of heating there is no change apparent in the resulting LCST. Recall, from Sect. 3.2, higher IOMNP concentrations result in higher heating rates (ΔT/Δt) for a given AMF. These results indicate that the LCST of PNIPAM is constant whether heated fast or slow and using either AMF or heat bath to stimulate the phase transition. As before, higher PNIPAM concentrations can be seen to exhibit reduced LCSTs.

Figure 4.2 (a) Study of the effect of heating mechanism/rate on the LCST of PNIPAM as a function of polymer concentration. (b) Study of the effect of heating mechanism, presence of IOMNPs, and presence of KOH on the LCST of PNIPAM as a function of polymer concentration. *Reproduced by permission of The Royal Society of Chemistry*
Finally, experiments were conducted to determine what role ions (to simulate the conditions of blood) and IOMNPs had on PNIPAM's LCST. The results of those investigations are plotted in Fig. 4.2 (b). For the sake of clarity, data corresponding to hot bath heating is shown in green and data corresponding to AMF heating is shown in black. First, observe when KOH is present in a solution having no IOMNPs, and necessarily heated by hot bath, that the LCST is effectively decreased at a particular PNIPAM concentration as compared to the case when no KOH was present. Next, notice when IOMNPs are added to a solution containing no KOH the LCST is again reduced as compared to the case where there is only PNIPAM present in solution. Now, note, when both KOH and IOMNPs are present the LCST is reduced even further than in the two previous cases. Previously, this was explained in terms of the introduction of negative charges that serve to structure the DIW molecules. This can shrink hydration shells around the acryl and isopropyl sections of NIPAM forcing them to associate with neighboring NIPAM molecules earlier in thermal agitation leading to reduced LCST. Alternatively, ordered DIW molecules translate to polymers characterized by higher entropy meaning the Gibbs free energy transitions sooner than would be the case without the structured DIW. Once again, increasing PNIPAM concentrations, while keeping them beneath the CMC, serves to reduce the LCST.

This section has served to document the behavior of PNIPAM when in the presence of non-encapsulated IOMNPs as an important step toward understanding the interaction of IOMNPs and PNIPAM. If the devices, whose synthesis is described in Sect. 4.2, have a PNIPAM concentration greater than the CMC, then their LCST should be constant. Fortunately, the rate at which the devices are heated (or method of heating) does not change PNIPAM's LCST. Negatively charged ions in solution and the PVP coated IOMNPs, with negatively charged surfaces, act to reduce the LCST of PNIPAM. In Sect. 4.3, the targeted biotherapeutic delivery devices will be subjected to AMF to investigate how effectively PNIPAM encapsulated IOMNPs heat their local environment. Later, in Ch. 5, a model to predict the AMF heating of IOMNPs will be presented.

4.2. Encapsulation of Clusters with Confirmation

Recall from Ch. 1, the goal of this work was to realize a IOMNP embedded PNIPAM nanogel for the purposes of targeted biotherapeutic delivery. The purpose of this section is two-fold: it details the
monitoring of the encapsulation of IOMNP clusters by PNIPAM nanogels via photopolymerization and it describes how evaluation of that synthesis was achieved. The tracking of the encapsulation process is achieved via the OMA system as was described in Ch. 2; the 545 nm peak of the UV source is observed for an amount of time that ensures the reaction is complete. Evaluation of the synthesis of PNIPAM nanogels containing clusters of IOMNPs is carried out with transmission electron microscopy. The expectation was that the morphologically spherical PNIPAM nanogels would be made to reveal the IOMNP agglomerates contained within. This section will conclude with another turbidimetry experiment performed on the product of the encapsulation synthesis for the purpose of establishing that IOMNPs have been incorporated into the PNIPAM nanogel.

In order to decide upon the concentrations of monomer, crosslinker, and photoinitiator (NIPAM, MBA, and Irgacure 2959 respectively) the concentration study of Sect. 2.4 was consulted. Recall that in Sect. 3.1 the IOMNPs were shown to agglomerate in clusters about 270 nm in size. That means encapsulation would only be possible if the nanogel were larger than 270 nm. Therefore, the largest nanogel achieved in this work was selected, calling for a 1:1:1 ratio of monomer, crosslinker, and photoinitiator. Specifically, a precursor solution was prepared containing 0.6 wt. % NIPAM, 0.6 wt. % MBA, and 0.6 wt. % Irgacure 2959. The next step was to choose an IOMNP concentration that would yield significant temperature increase upon AMF exposure while still allowing for substantial light propagation through the sample. Eventually, it was discovered that 0.2 mg/mL IOMNP dispersed in the precursor solution described above did allow the 545 nm peak to be observed at the beginning of a trial, albeit notably attenuated compared to the trials discussed in Sect. 2.4.

With the precursor solution’s parameters decided upon, approximately 50 mL was prepared so that after about 6 mL was removed for a photoreaction there would be sufficient amount left for potential future trials and characterization. The initial 6 mL was divided equally into two quartz cuvettes and placed within the photoreaction chamber described in Sect. 2.2. The chamber was sealed and nitrogen was made to flow inside to purge the environment for approximately ten minutes which was enough to cleanse the chamber three times over. It was desirable to eliminate oxygen from the environment to prevent the absorption of UV energy during the conversion of O₂ to O₃. The characterization of the bulb addressed in Ch. 2 revealed that the UV source has a warm-up time. Therefore, a shutter was employed between the source and sample
to guarantee the bulb was stable throughout the synthesis. As explained earlier, the bulb’s 254 nm peak provides the energy responsible for the photo-chemistry in this synthesis. However, that peak is relatively weak in intensity and particularly difficult to observe when IOMNPs are present in solution. Therefore, the strongest peak of this bulb, 545 nm, was monitored throughout the course of synthesis since it continues to be significantly stronger than background in the spectrum regardless of its attenuation. The OMA system was made to collect a spectrum from 537 nm to 555 nm every two seconds. The grating on the spectrometer with highest resolution was chosen: 1200 grooves per millimeter. The synthesis was carried out over the course of about 35 minutes and data was collected throughout that time. Finally, in order to determine transmittance as a function of time a sample of filtered DIW was subjected to the same UV exposure and OMA data collection just described. The data corresponding to the precursor solution was divided by the data corresponding to the water yielding the transmittance.

After the sample had been photo-irradiated and stored overnight, under refrigeration, it was observed to have separated. That is, a material appeared to have precipitated out of solution onto the bottom of the vessel, while the rest of the liquid retained a slight reddish/brown quality. Gently, an aliquot of the upper part of the product was removed and is, hereafter, referred to as “suspended”. Then, the remaining sample was vigorously agitated with a mechanical mixer so that the precipitate was made to go back into the water column of the vessel. Another small aliquot was removed from this and will be called the “vortexed” sample from here on. Each of these aliquots was separately diluted by a tenth and a hundredth of what was removed from the original. Small drops of each of the six samples were pipetted onto a carbon coated copper grid for drying at room temperature or under vacuum at approximately 45 °C. These grids were used for TEM imaging. The parameters of the TEM were as follows: 80 keV accelerating voltage and magnification ranging between 47.5 kX and 824 kX. See, for example, Sects. 2.3 or 3.1 for more details regarding TEM sample preparation and imaging.

Just prior to securing the samples within the photoreaction chamber photographs were captured to document their appearance prior to synthesis. The corresponding photo is labelled “Before” in Fig. 4.3 (a). The precursor solution had a reddish/brown color through which only a little light passed according to the naked eye; the sample was translucent. After the reaction was complete the sample was removed from the
chamber and another photograph was taken to document its appearance. This photograph is labelled “After” in Fig. 4.3 (a). Here, can be seen the material, first reported on in Sect. 2.4, collected predominantly at the front window of the cuvette closest to the UV source. Notice, now, that the previously whitish material has retained some of the reddish/brown quality associated with the iron oxide powder.

Figure 4.3 (a) Photographs were taken of the precursor solution, containing monomer, crosslinker, and photoinitiator, before and after UV irradiation, to document the change in appearance corresponding to (b) the attenuation of the UV source recorded by OMA, and TEM micrographs of the product reveal IOMNPs apparently incorporated into the PNIPAM matrix, as revealed in (c) and (d).
Transmittance as a function of time data, presented in Fig. 4.3 (b), quantitatively corroborate the qualitative description of precursor solution evolution upon photo-irradiation provided by Fig. 4.3 (a). Again, recall that each data point represents the intensity of the 545 nm peak at a given time during the UV photoreaction (see Ch. 2). The attenuation of the light source is similar to that presented earlier in this dissertation; the 545 nm peak is initially, relatively strong and quickly drops off to a significantly less intensity and does not change for the remainder of the trial. Specifically, only about 15 % of the light is transmitted through the precursor solution, as compared to the solvent, at the start of the synthesis. The fraction of transmitted light reaches a constant level of between 1 % and 6 % after only about 10 minutes. Apparently, the material that collected at the front window of the cuvette, seen in Fig. 4.3 (a), is responsible for the attenuation of light and quantitatively documented in Fig. 4.3 (b).

After synthesis, TEM was conducted to characterize the IOMNP embedded PNIPAM nanogels. The micrographs in Figs. 4.3 (c) and 4.3 (d) are typical of the artifacts documented during TEM analysis. The 122 kX magnification micrograph of Fig. 4.3 (c) reveals PNIPAM nanogels with irregular morphology; they are not easily discernible as spherical. Some of the irregularity can be attributed to the tendency of nanogels to draw together upon sample drying during TEM preparation (see Sect. 2.3). However, recall that IOMNPs agglomerate while dispersed in DIW as per the characterization presented in Sect. 3.1. While these nanogels were undergoing synthesis they were in the presence of IOMNP agglomerates and not discrete IOMNPs. At least two of these IOMNP agglomerates can be seen in Fig. 4.3 (c). One of those agglomerates is magnified to 824 kX in Fig. 4.3 (d). Here the IOMNPs appear either to be only semi-encapsulated by the PNIPAM nanogel or possibly incorporated into the surface of the nanogel’s polymeric matrix. Observe how IOMNPs are only obvious in relatively small and isolated nanogels. For example the large, dark nanogel in the lower right corner of Fig. 4.3 (c) could very well contain IOMNPs, but the electron beam cannot detect them due to the high electron density of the surrounding nanogel. Also in Fig. 4.3 (d), note the non-encapsulated IOMNPs appear in focus, while those apparently contained within the PNIPAM seem out of focus. This demonstrates how seemingly dark spots in the nanogel could, in fact, indicate hidden IOMNP agglomerates.

Now in order to further demonstrate that the IOMNPs were successfully fused into the polymer matrix, turbidimetry was conducted on the product of the encapsulation synthesis described in this section.
The same He-Ne laser used to evaluate the LCST of PNIPAM when heated by IOMNPs exposed to AMF was used in this experiment. The laser had a power rating of 25 mW, 633 nm wavelength, and was manufactured by CW Radiation. The fraction of transmitted light was determined as described in 4.1 and also in Appendix G. Three samples were evaluated for this experiment and are identified as DIW, Control, and Magnet. The DIW sample was probed with the laser to establish an original intensity for the purposes of determining transmittance percent through the ratio of measured intensity to original intensity. The Control sample was measured 16 hours after a homogeneous aliquot of the encapsulation synthesis product was dispersed in the cuvette. The Magnet sample, initially prepared in the same way as the Control, had a strong magnet placed flush with the side window of the cuvette during the same 16 hours after being placed in the cuvette.

The overriding assumption of this experiment was that the encapsulation synthesized product contained several different types of particles: naked IOMNPs, PNIPAM nanogels encapsulating IOMNPs, and empty PNIPAM nanogels. Based on the IOMNP sedimentation characterization presented in Sect. 3.1.5 it was thought that gravity would have a particularly strong influence to remove naked IOMNPs from the path of the He-Ne laser in the Control sample. This would leave empty PNIPAM nanogels and those that successfully encapsulated the IOMNPs still suspended in the water column. That buoyancy could be attributed to the relatively low density of PNIPAM as compared to magnetite (see Table 5.1). Now, the

**Figure 4.4** IOMNPs dispersed in filtered DIW are attracted to a magnet. Over the course of several minutes an initially translucent sample eventually appears transparent after the nanopowder has collected on the cuvette wall nearest the magnet.
Magnet sample introduces a strong magnetic force which effectively attracts IOMNPs encapsulated within PNIPAM nanogels. This leaves only empty PNIPAM nanogels in the path of the laser. As an illustrative example, consider how a strong magnet easily separates an aqueous dispersion of IOMNPs shown in Fig. 4.4.

The final transmittances of the three samples are collected in Table 4.1. Naturally the transmittance through the DIW was approximately 100% since the intensity of light through it did not change over the course of 16 hours. The transmittance of the Magnet sample was determined to be 72.6 ± 0.5%. The transmittance of the Control sample was determined to be 59.9 ± 0.5%. Error was determined by considering variation in calculated transmittances after only about 30 minutes into the experiment. Obviously, the Magnet sample was more transparent to the laser than the Control sample after about 16 hours. This indicates that the Magnet sample contained significantly less material suspended in the water column. This result along with the considerations laid out above suggest that some PNIPAM nanogels are fused with some IOMNP agglomerates.

Now that a case has been made for some degree of IOMNP impregnation into PNIPAM nanogels, additional characterization of the nanogels is given. Not all TEM micrographs of the final product showed irregular nanogels like those of Fig. 4.3 (c) and (d). Those micrographs, in particular, were exhibited to demonstrate the association of IOMNPs with PNIPAM. Many of the micrographs obtained from the same experiment reveal approximately spherical PNIPAM nanogels. Such micrographs were not discussed earlier in this section because IOMNPs cannot be observed within PNIPAM. As was discussed earlier, a likely reason for this is that the TEM simply could not resolve IOMNPs within the encapsulating PNIPAM. In other words, only when the encapsulation was imperfect, like in Fig. 4.3 (c) and (d), could both materials

<table>
<thead>
<tr>
<th>Sample</th>
<th>Transmittance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIW</td>
<td>100</td>
</tr>
<tr>
<td>Magnet</td>
<td>72.6 ± 0.5</td>
</tr>
<tr>
<td>Control</td>
<td>59.9 ± 0.5</td>
</tr>
</tbody>
</table>
Figure 4.5 (a) Low magnification TEM, (b) high magnification TEM, and (c) SEM of presumed IOMNP embedded PNIPAM nanogels reveal (d) a nearly monodisperse average size of approximately 1 micron, as measured by TEM.

be observed. Figure 4.5 reports on the size and morphology of those IOMNP embedded nanogels that could not be peered into.

A typical wide-field TEM micrograph of the IOMNP embedded nanogels is given in Fig. 4.5 (a). Note, the dark appearance of the nanogels in comparison to those shown in Fig. 2.16 (c). It could be that
the increase in electron density as a result of embedding IOMNPs within the PNIPAM nanogel accounts for the inability to observe the interior of the nanogel as was possible in Ch. 2. As was the case in Ch. 2, the artifacts in Fig. 4.5 (a) do tend to agglomerate due to sample preparation for TEM imaging; the nanogels are drawn together as the solvent evaporates on the TEM grid. This agglomeration is likely artificial and not the case in situ as would be demonstrated by NTA. Observe, the irregular material at the periphery of the cluster of nanogels. This material was not observed in Ch. 2 and may indicate incomplete photopolymerization. As was demonstrated in Fig. 3.4, the IOMNPs drastically inhibit the transmittance of UV light possibly inhibiting the polymerization process. Thus, much of the material in the sample may remain unreacted which would benefit from some stirring action.

Further evidence of the fact that these IOMNP embedded PNIPAM nanogels are not agglomerating in every scenario is provided in Fig. 4.5 (b). Here, one of several TEM micrographs is provided demonstrating the observation of the artifacts in isolation from its neighbors. Again, the electron density of isolated nanogels exceeds what the TEM can peer through so IOMNPs are not evident here but assumed to be impregnated within the nanogel.

Additionally, SEM was used to make observations of the sample containing monomer, crosslinker, photoinitiator, and IOMNPs all dispersed in DIW and exposed to UV irradiation. The finished product was diluted by one-tenth of its original concentration and drop cast onto a TEM copper grid. Note, that in this case a TEM grid was used, instead of the usual ITO coated glass substrate, since that particular grid yielded good images under TEM imaging and seemed promising for SEM imaging. Figure 4.5 (c) shows a wide-field view of a small section of the copper grid where IOMNP embedded PNIPAM nanogels are observed. Similar to Fig. 4.5 (a) an irregular material is observed around the periphery of the nanogel suggesting, perhaps, incomplete reaction that may benefit from stirring action while UV photopolymerization is taking place. Researchers wising to continue this work in the future may see improved product by incorporating such a mechanism into a new synthesis design. A magnified IOMNP embedded nanogel is shown in the lower left inset of Fig. 4.5 (c) to document the artifacts in greater detail according to SEM. In general, SEM is well suited to observing the surface of these artifacts; SEM tends to have insufficient electron voltage to observe the interior of the nanogels. Also, the IOMNPs used in this work are on average 2 orders of
magnitude smaller than these nanogels. For these two reasons, observing discrete IOMNPs embedded within the PNIPAM nanogels, using SEM, is particularly challenging.

A PSD of IOMNP embedded nanogels is provided in Fig. 4.5 (d). These diameters were measured from the TEM micrographs since artifacts were easier to find and more highly resolved with that method. An average size of 1.01 ± 0.19 μm was determined by fitting the PSD with a Gaussian function. The Gaussian’s peak corresponds to the average diameter and one-fourth of its width represents one standard deviation. These nanogels are nearly monodisperse. One explanation for the wide PSD is the fact that the IOMNPs are not discrete but tend to agglomerate. This may cause the PNIPAM nanogel to have some shape other than spherical. Furthermore, recall from that when precursor recipe R3 is used the resulting nanogels have a wide distribution to begin with. It is also interesting to note that the IOMNP embedded nanogels imaged here are, on average, nearly 260 nm larger than the empty nanogels reported in Fig. 2.23. This is consistent with an average IOMNP agglomerate size of almost 270 ± 20 nm as measured by NTA and reported in Fig. 3.2 (d).

Photographs of the precursor solution document a macroscopic change took place when exposed to a 254 nm UV light source for approximately 35 minutes. In-situ monitoring of the light source provide a quantitative record of its attenuation revealing the synthesis was complete after only about ten minutes. This is significantly faster than the synthesis of PNIPAM micelles using emulsion polymerization discussed in Ch. 1. Future researchers could broaden the photographic documentation of sample appearance, shown in Fig. 4.3 (a), by recording what the sample looks like not only before and after photopolymerization but at various times throughout synthesis as well. These time-dependent photographs would enhance the transmittance versus synthesis time data of Fig. 4.3 (b). TEM characterization of the product reveal irregular nanogels fused with the IOMNP agglomerates. While sample preparation for TEM could account for difficulty in observing IOMNPs encapsulated within PNIPAM nanogels, it could also be that the nanogel synthesis was inhibited by the irregularity of the IOMNP agglomerates. Furthermore, UV transmittance as a function of wavelength of the IOMNPs presented in Sect. 3.1 indicate the nanoparticles absorb the energy necessary for nanogel formation. Consequently, the nanogels imaged here were synthesized with less energy available for polymerization. Regardless of their appearance in micrographs, there do appear to be IOMNPs fused with the PNIPAM. Additionally, the turbidimetry experiment described here suggests that
some IOMNP agglomerates have been incorporated into some PNIPAM nanogels. EM micrographs of presumed IOMNP embedded PNIPAM nanogels did not demonstrate conclusively the encapsulation of NPs within the SRP. This was attributed to increased electron density when the two materials were fused in the case of both TEM and SEM and significantly smaller size of the NPs in the case of SEM. However, an average diameters of the synthesized product was reported to be a little over one micron which means that it will suffice for the TBD application since human capillaries tend to range between five and ten microns. It is convincing to note the increase in diameter of the PNIPAM nanogels corresponds to the sum of sizes of nanogels synthesized in isolation and the IOMNP agglomerates. These findings warranted AMF heating investigations of the samples in order to evaluate their capacity to exhibit temperature increase upon exposure to RF, which is the topic of the next section.

4.3. AMF Heating of IOMNP Embedded PNIPAM Nanogels

It was shown in section 4.2 that clusters of IOMNPs were successfully encapsulated within the stimuli responsive polymer, PNIPAM. As described in Chs. 1, 3, and 5, MNPs attempt to align with an AMF causing them to generate heat via their Néel and Brownian relaxations. The resultant heat induces a temperature change in the surrounding PNIPAM nanogel. Chapter 1 describes how this temperature responsive polymer undergoes a discontinuous VPT as it is heated past its LCST. Should the IOMNP embedded PNIPAM nanogel be loaded with a biotherapeutic then that will be squeezed out as a result of environmental heating past the LCST. In this section, the heating response of the IOMNPs encapsulated by PNIPAM to an AMF is reported upon. First, the heating response of filtered DIW, containing no IOMNPs, was investigated at various AMF amplitudes to serve as a control experiment. Then, the suspended and vortexed samples described in section 4.2 were separately subjected to various AMF amplitudes. Finally, special consideration was given to the SAR of these samples as a function of the square of the AMF amplitude in order to confirm a linear relationship as predicted by LRT.

The AMF and temperature recording equipment were both described in Ch. 3. Briefly, a 1 mL sample was suspended in the coils and insulated to reduce unintentional heating. Despite this insulation there is still some temperature increase of the sample as reported further down. Therefore, the DIW heating curves were subtracted from those of the samples to yield the data presented here. This is a common
technique to correct for unintentional heating in the field of hyperthermia [187]. Each data set represents the average of three trials demonstrating repeatability and generating error. After any given trial the sample was placed in a water bath to return the temperature to a common starting point (~ 22.4 °C). Since the starting temperature was not always exactly identical, every data set was made to begin at the origin (t = 0 s, ΔT = 0 °C). Therefore, temperature change (usually increase) is reported and not absolute temperature of a sample. In order to determine the SAR value, the initial positive slope of the heating curve was plugged into Eqn. 3.6. As discussed previously (see Ch. 3), this allows for consideration of the heat generated by the IOMNPs independent of their mass. The LINEST function in Excel was used to linearly model the SAR versus the square of the field data for each of the suspended and vortexed samples. The y-variance in the data points from the line of best fit is represented by error bars.

The results of the control experiment are plotted in Fig. 4.6 (a). Here an approximately 1 mL sample of filtered DIW was subjected to the four applied magnetic fields of 16.1, 32.3, 47.7, and 59.1 kA/m (203, 405, 600, and 743 Oe respectively). Since no IOMNPs are present in these samples then all the heat generated in the sample leading to temperature increase can only come from the unintentional, conventional heating of the AMF heating coils which are in proximity to the sample. Note, that this heating occurs even though the coils are cooled with circulating water and the sample has been insulated from the coils. Unsurprisingly, the higher the applied field (corresponding to higher current flowing through the AMF coil) the higher the temperature increase of the sample. Obviously, when more current flows through the coils they will get hotter. For example, note that when the sample was subjected to a relatively small 16.1 kA/m field it experienced less than 0.5 °C rise in temperature. On the other hand, when the sample was subjected to a 59.1 kA/m field, then it increased in temperature by almost 7.5 °C. These heating curves were subtracted from the corresponding data sets of samples containing IOMNPs in order to remove the heating effect associated with being in proximity to the AMF heating coils. It should be noted that when conducting the “suspended” and “vortexed” experiments discussed next, that the applied field of 16.1 kA/m produced no significant heating of those samples so that data is not presented.

Recall from section 4.2 the precursor solution that was UV photo reacted had an IOMNP concentration of 0.20 mg/mL. This relatively low nanoparticle concentration was decided upon in order to minimize the initial attenuation of the 545 nm light from the UV source used to monitor the synthesis
process. Consequently, comparatively low temperature increase would be expected for low IOMNP concentration samples after the discussion of section 3.2. This is confirmed by the data plotted in Figs. 4.6 (b) and 1(c). In Fig. 4.6 (b), the sample was gently pipetted from the mid to upper parts of the water column after the product was allowed to sit undisturbed overnight. In this way heavier, high density matter in the solution would be allowed to settle out while lighter, low density matter was suspended. The logic here is

Figure 4.6 AMF heating trials of (a) the solvent, DIW, as well as presumed IOMNPs embedded in PNIPAM nanogels both (b) suspended naturally and (c) re-suspended by agitating the final UV irradiated sample yield heating curves that, when compiled, (d) reveal a linear response of the SAR versus the square of the applied magnetic field.

"Figure 4.6 AMF heating trials of (a) the solvent, DIW, as well as presumed IOMNPs embedded in PNIPAM nanogels both (b) suspended naturally and (c) re-suspended by agitating the final UV irradiated sample yield heating curves that, when compiled, (d) reveal a linear response of the SAR versus the square of the applied magnetic field."
that in the “suspended” sample, clusters of IOMNPs encapsulated by PNIPAM nanogels are represented. Of course, empty PNIPAM nanogels are also possible in this sample but they will not contribute to AMF heating and are ignored. No attempt was made to further separate the IOMNP loaded nanogels from the empty nanogels and this point will be worth revisiting for future researchers. Again, the heating curves presented here are the differences of the heating curves of the filtered DIW subtracted from the heating curves of the sample at the indicated field (the remaining discussion assumes this). This fact may explain the lack of smoothness in Figs. 4.6 (b) and (c). At an applied field of 32.3 kA/m the sample increased by only about 0.1 °C after nearly 5 minutes. When the field was increased to 47.7 kA/m the same sample increased by about 0.4 °C in the same time. Finally, at an applied field of 59.1 kA/m the sample increased by a little more than 1.0 °C at the end of the trial.

Now, by agitating the synthesized product (i.e. the precursor solution described in sect. 4.2 after being UV photo-reacted) with a Vortex Genie and removing a 1 mL sample the matter that settled overnight is captured. In this way the “vortexed” sample was prepared. Here, AMF heating necessarily includes the contribution from naked IOMNP clusters. In this case, higher relative temperature increase is expected since many of the nanoparticles are not encapsulated and have no surrounding nanogel to heat before the solution’s temperature can begin to climb. The heating curves from that sample are plotted in Fig. 4.6 (c). A quick comparison between Figs. 4.6 (b) and (c) confirm that, indeed, the vortexed sample exhibits relatively higher temperature increase at all applied magnetic fields. To be precise, at 32.3 kA/m the sample increases by almost 0.6 °C, at 47.7 kA/m it increases by a little more than 1.2 °C, and at 59.1 °C it also increases by almost 1.2 °C.

LRT describes the rate of heat generation of MNPs subject to an AMF as being proportional to the SAR which is in turn proportional to the square of the applied field (see Ch. 5). In an effort to validate the results presented in this section with the predictions of LRT described by Rosensweig [188], the SAR as a function of the square of applied magnetic field was plotted in Fig. 4.6 (d). As expected, the data is very well described by a line as suggested by the excellent R-squared values shown in the plot. Additionally, linear regression of the data reveals that the error in the slope is approximately 6 % of the “suspended” slope and approximately 8 % in the “vortexed” slope. Observe that when the results are analyzed in this way there is little difference between the “suspended” and “vortexed” samples. This can be attributed to the
fact that SAR (or rate of heat generation) is predominantly a property of the MNP. That is, any effect the PNIPAM has on the heating rate is negligible.

Perhaps initially surprising, it was demonstrated here that filtered DIW does heat during AMF trials. However, this is a consequence of convective heating that occurs with any matter in proximity to the heating coils despite their being cooled with internal circulating water. Data obtained from this control experiment was used to correct for the same unintentional heating in later AMF heating of samples containing IOMNPs. In this way, the heating curves presented in Figs. 4.6 (b) and (c) represent only the heat that is generated as a result of Néel and Brownian relaxations of the nanoparticles. While temperature increases were not large compared to those presented in Ch. 3 (due to the low IOMNP concentration in the precursor solution), it was shown that IOMNP clusters encapsulated in PNIPAM nanogels do heat the “suspended” sample upon exposure to an AMF. The heating in Fig. 4.6 (b) is important evidence that encapsulation of IOMNP clusters was achieved, since the sample was taken from a part of the vessel that likely had very few if any naked IOMNP clusters. The “vortexed” sample exhibited slightly elevated temperature increases but this can be attributed to the likelihood that this sample contained naked (or non-encapsulated) IOMNP clusters. Surely, the act of encapsulating a MNP would retard its ability to generate heat since the energy would require more time to exit the nanogel and translate into a temperature change of the whole sample. It was also confirmed that the samples tested here have a SAR that is linearly proportional to the square of the applied magnetic field in agreement with LRT. The results presented here demonstrate that the IOMNP embedded PNIPAM nanogel can be subjected to an AMF in order to induce temperature change which could be used to bring about the phase transition of the PNIPAM nanogel triggering the release of the biotherapeutic.

4.4. Summary

First the chapter documented the behavior of PNIPAM in proximity to non-encapsulated IOMNPs. This was a critical first step toward understanding the IOMNP embedded PNIPAM nanogel discussed in the remainder of the chapter. These nanogels only have a constant LCST when the PNIPAM concentration
is above the CMC. It was shown that varying the AMF heating rate does not affect PNIPAM’s LCST. Evidence was presented demonstrating that negative charges (i.e. ions from salts or IOMNP PVP coating) in solution effectively reduce the LCST of PNIPAM. In the next chapter, a model to predict the AMF heating of IOMNPs will be presented.

The appearance of the precursor solution was dramatically changed upon exposure to a 254 nm UV light source for about 35 minutes. The OMA system allowed for a quantitative demonstration of the attenuation of the UV source, while synthesis was occurring, suggesting the reaction was complete after only ten minutes. Contrast this with a synthesis technique such as emulsion polymerization (see Ch. 1) that takes significantly longer. This work would benefit from a broadening of photographic documentation of sample appearance throughout the synthesis process: not only at the start and end of synthesis. Such an effort will enhance the transmittance versus synthesis time data presented here. The IOMNP embedded PNIPAM nanogel was revealed by TEM as agglomerates of IOMNPs fused with the surfaces of irregularly shaped nanogels. Due to the limitations of the TEM, the possibility of IOMNP agglomerates hidden within dark appearing PNIPAM nanogels has not been ruled out. It’s likely that the non-uniform IOMNP agglomerates inhibited PNIPAM nanogel synthesis causing them to lose their round shape. Additionally, evidence was presented in Ch. 3 demonstrating that the IOMNPs absorb UV energy that would otherwise go toward initiating PNIPAM nanogel synthesis. Therefore, the devices imaged in this chapter were synthesized with less available energy as compared to those presented in Ch. 2. Nevertheless, the micrographs presented suggest the IOMNP agglomerates are, at least, fused with the surface of the nanogel. Complimentary turbidimetry was presented to further demonstrate the apparent successful incorporation of PNIPAM nanogels and IOMNP agglomerates. Further EM was exhibited documenting the presumed IOMNP embedded PNIPAM nanogels that were more regular in appearance. While IOMNPs were not be observed in the nanogel, there was an increase in nanogel diameter, compared to that reported in Ch. 2, accounting for the added diameter of the encapsulated IOMNP agglomerate. Encouraged by those findings, AMF heating experiments were conducted to determine temperature increase.

Interestingly, filtered DIW was shown to exhibit temperature increase during AMF heating trials even when there are no IOMNPs dispersed therein. This can be explained by the fact that the DIW was in proximity to the heating coils causing them to heat via convection. This control experiment allowed for
correcting later data sets by subtracting the temperature increase attributed to convection. Therefore, heating curves presented in this chapter represent temperature increases attributable to the Néel and Brownian relaxations of the IOMNPs. Even though temperature increase was not particularly impressive compared to the results of Ch. 3, there was detectable heating of IOMNP agglomerate embedded PNIPAM nanogels. Care was taken to demonstrate significant heating corresponding to the part of the synthesized sample that was unlikely to contain non-encapsulated IOMNP agglomerates. On the other hand, the sample likely to contain non-encapsulated IOMNP agglomerates exhibited relatively higher temperature increase upon exposure to AMF. Certainly, an encapsulated MNP would necessarily experience inhibited AMF heating since extra time would be required for its generated heat to escape the surrounding nanogel. Furthermore, the IOMNP embedded PNIPAM nanogels exhibited SARs that were linearly proportional to the square of the applied magnetic field in agreement with LRT. The chapter demonstrates AMF induced temperature change of the IOMNP embedded PNIPAM nanogels for bringing about PNIPAM’s LCST, which would trigger biotherapeutic release.
CHAPTER 5:
MODELING MAGNETIC NANOPARTICLE HEATING

The purpose of this chapter is to predict the change in temperature of a sample of IOMNP embedded PNIPAM nanogels when exposed to an AMF. As was discussed in Ch. 1, the IOMNPs respond to the AMF by heating the surrounding PNIPAM nanogel which in turn squeezes out the biotherapeutic in response to increasing temperature. Here a comparison of eddy currents and linear response theory (LRT) is provided as potential mechanisms for the generation of heat. For each, the phenomenon will be theoretically detailed. Also, eddy currents and LRT will both be applied to this work by using the parameters of the IOMNPs to determine the heating response as a function of time. Finally, the chapter will be summarized with special attention being paid to the model that best represents the experimental results from the previous chapter and deliberation of reasons the other model did not perform as well.

5.1. Eddy Currents

Many common technologies utilize the capability of an AMF to generate elevated temperatures in a conductor such as melting metals, welding, cooking, and sealing food containers [189-192]. These technologies and the temperatures they generate are explained via eddy currents: circulating currents in bulk pieces of conductors induced by changing magnetic fields.

5.1.1. Theoretical Development

As stated earlier, eddy currents are circulating currents in bulk pieces of conductor that are induced either because of relative motion through a magnetic field or as a result of exposure to a changing magnetic field. A common demonstration in physics lectures involves fixing a metal plate to the end of a swinging rod that is allowed to pass between the poles of an electromagnet. When the electromagnet is activated the swinging metal plate dramatically comes to a stop between the magnetic poles. The reason for this is easily
explained with Lenz’s law. As the metal plate experiences changing magnetic field lines perpendicular to its surface an electric current must flow to create a magnetic field that will counter the change induced from the electromagnet. This will in turn generate a magnetic force pointing in the opposite direction as the plate’s velocity bringing it to a stop [76, 159].

While in several applications eddy currents are undesirable (e.g. motors and transformer cores), since they lead to the generation of internal energy from mechanical energy, in this work they are potentially responsible for the heat that triggers the release of the biotherapeutic from PNIPAM nanogels. The rate at which heat energy is generated by eddy currents, $P_{EC}$, is found through Maxwell’s equations as they pertain to the material in question, magnetite in this case. To arrive at the result expressed by Eqn. 5.1, it is assumed that the material has no magnetic domains, which is appropriate due to the significantly small size of the IOMNPs [193].

$$P_{EC} = \frac{\pi^2}{6} \frac{\sigma d^2 J_P^2 f}{\rho}$$

(5.1)

Here, $\sigma$ is the conductivity of the magnetite, $J_P$ is the peak polarization, $f$ is the frequency of the AMF, and $\rho$ is the density of magnetite. The size of the conducting piece of magnetite was allowed to vary from as small as discrete 10 nm IOMNPs to 270 nm size clusters of the IOMNPs to a single piece of magnetite with the same mass as the IOMNP powder dispersed in the solvent, as schematized in Fig. 5.1.
The discrete, 10 nm IOMNP simulation was conducted to address the smallest reasonable size since this is the average size of the individual IOMNPs and is diagrammed in Fig. 5.1 (a). Here, each IOMNP has its own unit cell of DIW. As was discussed in Ch. 3, NTA revealed the IOMNPs do not disperse effectively in DIW despite being coated in PVP. That data revealed that most of the particles were agglomerating in clusters that were approximately 270 nm in diameter. This explains the choice of 270 nm as an agglomerate size to test in the eddy current model, as shown in Fig. 5.1 (b). In this diagram, each cluster has its own unit cell of DIW. Finally, to treat the other end of reasonable sizes for use in the eddy current model it was decided to assume all IOMNP powder dispersed in DIW somehow managed to agglomerate together forming one chunk of magnetite. For example, suppose a sample contains 1 mL of
DIW. If the sample is to have an IOMNP concentration of 2 mg/mL then it must have 2 mg of IOMNP powder in it. Dividing by the density of magnetite reveals a magnetite chunk volume of almost $4 \times 10^{-10} \text{ m}^3$. Knowing the volume of this sphere, one can solve for its size using

$$d = \sqrt[3]{\frac{6V}{\pi}}$$

This suggests the largest possible size to evaluate eddy current heating should be about $9.1 \times 10^{-4} \text{ m}$ and is illustrated in Fig. 5.1 (c). Now, all of the IOMNPs heat a single piece of DIW.

The dependent variable in this model was taken to be $P_{EC}$ and has dimensions of power per unit mass (i.e. W/kg). The amount of time the IOMNPs were exposed to the AMF was the independent variable (units of seconds) and allowed for the determination of heat per unit mass, $q_{EC}$, using the definition of power (energy per unit time) according to:

$$q_{EC} = P_{EC} \Delta t$$

The constants $\sigma$ and $\rho$ in Eqn. 5.1 represent the conductivity and density of magnetite respectively. Table 5.1 provides some commonly used values for these parameters. The frequency, $f$, of the AMF ranged between 307 and 308 kHz for all experiments discussed in this dissertation. The peak polarization, $J_p$, of the IOMNPs was determined from the magnetization data of the IOMNPs shown in Fig. 5.2 (a). From this it can be seen that for the experimental AMF amplitude of approximately 59,000 A/m, or about 740 Oe, (a

---

**Figure 5.2 (a)** The peak polarization of nearly 0.2 T, as obtained from the magnetization versus applied magnetic field presented in Ch. 3, was required to predict the heating rates of IOMNPs interacting under the idealized scenarios illustrated by Fig. 5.1.
constant field amplitude used in all AMF heating experiments discussed in this dissertation) a peak polarization of almost 0.19 T was measured.

With the heat per unit mass, as provided in Eqn. 5.3, one must multiply by the mass of one IOMNP to find the heat generated by one nanoparticle, Q_{MNP}. This mass was determined by inserting the average 10 nm size of one IOMNP into Eqn. 5.2 and solving for the volume. That volume was multiplied by the density of magnetite revealing a mass of almost $2.7 \times 10^{-21}$ kg. Now to find the total heat generated by all the magnetite in solution, $Q_{tot}$, the heat generated by one nanoparticle was multiplied by the number of particles in solution (see Table 3.1). This quantity is then divided by the mass and specific heat of DIW, $m_{DIW}$ and $c_{DIW}$ respectively, to find the increase in temperature as in Eqn. 5.4 [208, 209].

$$\Delta T_{tot} = \frac{Q_{tot}}{m_{DIW} c_{DIW}}$$

(5.4)

Since this is a temperature increase per second, consistent with Eqn. 5.1, this quantity could simply be multiplied by the independent variable to find how much the temperature increases at any given time.

### 5.1.2. Application

Both the eddy current and LRT models are in many ways different from each other fundamentally. One way in which they are similar is that they both yield a value for the heat energy provided by MNPs that are subject to an AMF. That heat necessarily determines a temperature increase for the surrounding environment. Suppose, for example, an ensemble of MNPs is homogenously dispersed in solution and subjected to an AMF. In that case, one of these MNPs would lie in, and heat, a unit cell of the surrounding environment whose size and mass would be determined by the concentration of MNPs and density of the solvent. With the quantity of heat provided by the models discussed in this chapter, and some basic thermodynamics, the temperature increase of the unit cell can be determined. Then the temperature increase of the entire sample can be found by adding up all the contributions from each unit cell, or each MNP, in the sample.

As mentioned earlier and schematized in Fig. 5.1, three possible MNP dispersions were considered here. To account for the ideal situation one of these possibilities assumed the 10 nm MNPs do not agglomerate when mixed in DIW. So to determine the unit cell mass, $m_{DIW}$, Table 3.1 was used to determine that a 2 mg/mL sample contains about $8 \times 10^{14}$ MNPs per milliliter. Taking the inverse means that a 1 mL
sample is comprised of DIW unit cells that weigh about $1 \times 10^{15}$ g (it is assumed that for DIW, $1$ g $\approx 1$ mL). Another simulation data set was produced assuming the MNPs clustered in agglomerates similar in size to those reported upon by NTA analysis in Ch. 3. For this case, the determination of $m_{\text{DIW}}$ was similar to the discrete case detailed above except that the concentration in particles per mL was divided by about 3400 since this was the approximate number of IOMNPs in a 270 nm agglomerate. Finally, the last possibility was to assume the worst case scenario in terms of agglomeration; it was assumed that every single MNP clustered with its neighbors eventually forming a single chunk of magnetite with the same mass as the powder dispersed in the solvent. For the final case, the unit cell was equal to the mass of all DIW in the sample, or 1 g. In this way it was thought that every possible scenario of agglomeration was addressed. All three of these possibilities are simulated in the temperature increase versus time graph of Fig 5.2 (b).

Now to discuss the results of the simulations, first, consider the PVP coating around each MNP is effective at preventing particles from agglomerating and that the dispersion is homogenous. This simulation is represented by the black line in Fig. 5.2 (b). In this case, the temperature of the entire sample will increase at a rate of $1.41 \times 10^{-7}$ °C/s. So by the end of the 5 minute trial the entire solution should increase in temperature by about $4.2 \times 10^{-5}$ °C overall. Note that this is significantly less than the temperature increase reported in Ch. 3. In that case, the temperature increase of a 2 mg/mL sample of IOMNPs dispersed in DIW was nearly 12 °C after only 4.5 minutes. Consider that the conductivity, $\sigma$, used here in the discrete, 10 nm IOMNP case and in the 270 nm agglomerate case to follow was appropriate for their small size as specified in Table 5.1.

Next, if MNPs are coming together in such a way so as to form agglomerates of approximately 270 nm size the heating rate decreases. The red line in Fig. 5.2 (b) represents this scenario. Now, the whole sample increases in temperature at a rate of $9.38 \times 10^{-9}$ °C/s. Therefore, when the AMF trial has concluded the solution’s temperature has increased by less than $5 \times 10^{-6}$ °C. Clearly, this result is also suspicious in light of the results of Ch. 3.

Finally, if all the MNPs agglomerated together upon dispersal into solution then one single piece of magnetite would form. As stated earlier, since 2 mg of IO powder was dispersed in DIW the resulting chunk would be nearly 0.91 mm in size based off the density of magnetite and use of Eqn. 5.2. This simulation is represented by the green line in Fig. 5.2 (b). In this case, the entire sample is heating at $1.44 \times 10^{-7}$ °C/s. If
that were the case, at the end of the AMF heating trial the sample’s temperature would be $4.3 \times 10^5$ °C. It should be noted that in this scenario the conductivity, $\sigma$, was taken to be that of bulk magnetite and specified in Table 5.1. Immediately, one can conclude that the IO powder is not forming a single piece of magnetite if eddy currents are responsible for the heating.

5.2. Linear Response Theory

Contrary to the concepts of eddy currents, cutting edge technologies like hyperthermia treatment of cancer and TBD begin with linear response theory (LRT), which considers the rotational relaxation of a nanoparticle, to describe the heating mechanism of MNPs [13, 79, 210].

5.2.1. Theoretical Development

Literature suggests the field of targeted drug delivery benefits from considering IOMNP heating while subject to an AMF as arising from relaxation losses from the rotation of the nanoparticle. This rotation necessarily has two components since the magnetic moment of a nanoparticle can rotate in two different ways as illustrated in Fig. 5.3 (a). First, the magnetic moment can spontaneously flip, within the nanoparticle, in response to the changing magnetic field in a process called Néel rotation [211]. The magnetic moment can also be carried around with the thermal motions of the nanoparticle in a process called Brownian rotation [188]. Both of these two possibilities contribute to the overall rotational relaxation of a nanoparticle which heats the surrounding environment as described below. This treatment has been coined linear response theory (LRT). At the core of LRT is the assumption that the magnetization of the nanoparticle responds linearly with the applied field. That is to say, the magnetic susceptibility is considered constant in the range of applicable applied magnetic field. Magnetization data from Ch. 3 reveals that the IOMNPs are approximately linear in the range of applied fields used in this work consistent with LRT. There are also the assumptions that magnetization can only undergo coherent rotation, all MNPs are identical, and they also do not interact with each other. In Ch. 3 TEM was used to show that the MNPs are not identical, while NTA provided evidence that the particles are agglomerating and therefore interacting. So already there are some violations of LRT. [79]

The amount of time necessary for a nanoparticle to undergo Néel relaxation is given by
where $\Gamma$ is the ratio of anisotropic energy to thermal energy determined through the following:

$$
\Gamma = \frac{K V}{k_B T}
$$

The constants in these expressions are the attempt time $\tau_o$, anisotropy $K$, and Boltzmann's constant $k_B$.

Table 5.1 provides acceptable values used for these constants. The temperature is known to vary during

$$
\tau_N = \tau_o \sqrt{\frac{\pi}{2}} \frac{1}{\sqrt{\Gamma}} \exp(\Gamma)
$$

Figure 5.3 The Néel and Brownian relaxations of IOMNPs, as schematized in (a), are combined to reveal an (b) effective relaxation time, which can be used to determine the behavior of (c) SAR as a function of hydrodynamic size and to predict (d) the heating rates of IOMNPs interacting under the idealized scenarios illustrated by Fig. 5.1.
the experiment making this a difficult parameter work with. Simulations of the Néel relaxation time were plotted in Fig. 5.3 (b) with three temperatures that are representative of those a sample experiences during a typical AMF trial. The simulations show that temperatures typical in this work do not have a drastic effect on the outcome.

Brownian relaxation time is expressed as

$$
\tau_B = \frac{3 \eta V_h}{k_B T}
$$

(5.7)

Of course, the viscosity of DIW, $\eta$, is temperature dependent and allowed to vary with $T$ in this model. The hydrodynamic volume, $V_h$, includes the nanoparticle itself, its surrounding PVP (for facilitation of dispersal in solution), and the surrounding hydration shell. To determine a minimum hydrodynamic volume the TEM data presented in Ch. 3 was used to measure the space between MNPs, assuming this space was the PVP keeping the particles from touching, and dividing by two to determine the PVP shell thickness. Again, simulations in Fig. 5.3 (b) indicate that allowing $T$ to vary does not significantly change the Brownian relaxation time. Therefore, it was assumed that for both relaxations room temperature, $T = 295$ K, was to be used. Figure 5.3 (b) also shows the Brownian relaxation has a relatively gradual increase with MNP size. On the other hand, Néel relaxation time increases exponentially with MNP size.

The combination of Néel and Brownian relaxation times, or effective relaxation time of the MNP, $\tau$, is achieved using the following equation.

$$
\frac{1}{\tau} = \frac{1}{\tau_N} + \frac{1}{\tau_B}
$$

(5.8)

Just like electronic parallel resistors, the lesser of the two components in Eqn. 5.8 dominates their amalgamation. In other words, relatively slow Néel relaxation times, which only occurs for the smallest MNPs, and the effective relaxation time of the IOMNP are approximately equal. On the other hand, for larger MNPs, Brownian relaxation and the effective relaxation are nearly the same. This is true for larger IOMNPs since the Brownian relaxation time is shorter than the Néel relaxation time. These observations are simulated in Fig. 5.3 (b).

Now, rotational relaxation of the MNP determines the imaginary part of the magnetic susceptibility, $\chi''$ (unitless), through

$$
\chi'' = \chi_o \frac{2 \pi f \tau}{1 + (2 \pi f \tau)^2}
$$

(5.9)

Here, the saturation magnetization, $M_s$, determines the equilibrium susceptibility, $\chi_o$ (unitless), through
\[ \chi_0 = \frac{\mu_0 M_s^2 V}{k_B T} \tag{5.10} \]

The above expression contains the factor \( \mu_0 \) known as the permeability of free space. The volumetric power, \( P \), of the MNP is determined by the susceptibility according to

\[ P = \mu_0 \pi \chi'' f H^2 \tag{5.11} \]

Here, \( H \) is the applied magnetic field. From the volumetric power (having units \( \text{W/m}^3 \)) one can find the specific absorption rate (SAR) using Eqn. 5.12.

\[ \text{SAR} = \frac{P}{\rho} \tag{5.12} \]

SAR has units of Watts per kilogram. At this point, the volumetric heat per MNP, \( q \) (with units of Joules per cubic meter), can be found for a given time interval, \( \Delta t \).

\[ q = \rho \left( \text{SAR} \right) \Delta t \tag{5.13} \]

The heat per MNP, \( Q \), is calculated by multiplying the above result by the volume of one MNP.

\[ Q = q V \tag{5.14} \]

Finally, that result can be divided by the mass of the solvent and its specific heat, as in Eqn. 5.4. However, that only gives the temperature increase, \( \Delta T \), of one MNP. In order to obtain the total temperature increase one can assume each MNP contributes equally to the whole and multiply by the number of MNPs, \( N \), in the sample according to.

\[ \Delta T_{\text{tot}} = N \Delta T \tag{5.15} \]

At this point, it is instructive to further consider the role of MNP size specifically as it pertains to SAR. Simulations of SAR versus MNP hydrodynamic size were carried out on particles that were 10 nm, 15 nm, and 20 nm. The results are plotted in Fig. 5.3 (c). For 10 nm MNPs the SAR response has a peak value near that of the same hydrodynamic size. MNPs that are about 15 nm exhibit SAR response that also peaks near the hydrodynamic size of 10 nm. Finally, if the MNP is about 20 nm in average size then the SAR response has no peak, but a saturation value instead that is reached at about 10 nm hydrodynamic size. These simulations are reminiscent of recent literature suggesting that when SAR response exhibits a peak then the MNPs can be characterized as superparamagnetic. On the other hand, when the SAR response has a saturation value, rather than a peak, the MNP losses are characterized by hysteresis.
5.2.2. Application

As was explained in Sect. 5.1 the simulations that follow also suppose a unit cell (see Fig. 5.1) for a given MNP to derive the corresponding heat. That heat can then be used to determine a temperature increase of the unit cell (see Eqn. 5.4) and then of the entire sample by adding up the contribution of every MNP (see Eqn. 5.14) in the sample. These simulations suppose a total sample volume of 1 mL whose solvent is DIW. Unless otherwise specified the particles are assumed to be homogeneously dispersed in the solvent and are not otherwise interacting with each other. The model also assumes that the AMF has an amplitude of 59,000 A/m, a frequency of 307 kHz, and that the heating trial lasts for 300 seconds (or 5 minutes). These last three parameters were typical of the experiments discussed in Chs. 3 and 4. Finally, in all simulations discussed below, and plotted in Fig. 5.3 (d), the concentration is assumed to be 2 mg of MNP in 1 mL of DIW. Recall that the heating of non-encapsulated IOMNPs in the presence of PNIPAM discussed in Ch. 4 was achieved with 2 and 3 mg/mL concentrations.

Again, three possible dispersions of the MNPs were considered here. The rise in temperature as a function of time simulations of the discrete, 10 nm IOMNP ensemble, the 270 nm agglomerate ensemble, and single 0.91 mm piece of magnetite are shown in Fig. 5.3 (d). These simulations are remarkably different from those shown in Fig. 5.2 (b) as can be immediately noted by observing the difference in the y-axis scales.

While there are slightly different heating rates when comparing the three different simulations, they are obviously out of the realm of realistic sample heating. When applying LRT to each scenario, the heating rate is nearly $1.8 \times 10^{14} \degree C/s$. This heating rate slightly increases as one considers agglomeration more and more likely to occur. Overall, each of the three simulations predicts that the sample will increase in temperature by about $5.3 \times 10^{16} \degree C$. For some perspective, the surface of the sun is about $5.5 \times 10^{3} \degree C$, the center of the sun is about $1.5 \times 10^{7} \degree C$, and the early universe was about $1 \times 10^{10} \degree C$ shortly after the Big Bang. Up to this point, LRT seems implausible for explaining the heating of a sample containing IOMNPs and its execution, as well as its assumptions, must come into question.

One possible reason that LRT is deficient to predict the results presented in Chs. 3 and 4 is that the IOMNP clusters are larger than the superparamagnetic size regime [87, 92, 194]. It has been shown elsewhere that heat losses can only be attributed to Néel relaxations when the IOMNPs are smaller than
20 nm. Specifically, previous researchers have satisfactorily demonstrated that there is a relatively small peak in heat losses for IOMNPs around 10 nm in size. As progressively larger sizes are considered there is at first relatively little heat loss predicted followed by another relatively larger predicted heat loss that cannot be attributed to Néel relaxation. Additionally, there is a restriction that the applied field be less than 15 kA/m. If these two criteria are violated, then Néel relaxations must give way to hysteresis losses. Recall that the IOMNPs measured by NTA (see Ch. 3) revealed agglomerates approximately 270 nm in size. Furthermore, the applied field in this work was 59 kA/m.

Consider now the ideal versus actual IOMNP whose heating behavior under the influence of an AMF, LRT attempts to explain. First, LRT assumes the IOMNP exhibits uniform magnetization, as depicted in Fig. 5.4 (a). In reality, the IOMNPs used in this work will suffer from an effect known as surface spin disorder in which magnetic moments along the surface are not aligned with the magnetization of the uniform core [212, 213]. Also, these IOMNPs were coated upon manufacture with a layer of PVP in order to facilitate dispersal in DIW. This layer of PVP most certainly would retard the heat transfer from the IOMNP to the surrounding environment. Both of these are depicted in Fig. 5.4 (b).

If the IOMNP was actually described by Fig. 5.4 (a) (i.e. uniform magnetization and no PVP coating) then LRT predicts an increase in temperature of only one IOMNP by as much as 69 °C for a sample concentration of 2 mg/mL exposed to 59 kA/m AMF for about 5 minutes. On the other hand, it was shown in Ch. 3 that under the same experimental conditions the entire sample only increased in temperature by about 12 °C. This yields a difference in temperature increase of about 57 °C between the LRT prediction and experiment. Perhaps this difference can be attributed to the surface spin disorder and PVP coating of the IOMNP. Upon comparison of the experimental temperature increase, ΔT_E, to theoretical temperature increase, ΔT_T, which is a ratio represented by η, it is discovered that these two realities of the nanoparticle lead to a reduction in theoretical temperature by almost 17 % as demonstrated below:

\[
\eta = \frac{\Delta T_E}{\Delta T_T} = \frac{12 \ ^\circ C}{69 \ ^\circ C} \approx \frac{1}{6}
\]

Assuming the correction is constant, or at the very least representative of general corrections to be made to theoretical calculations of temperature increase, then one can modify Eqn. 5.14 as follows:

\[
\Delta T_{tot} = N (\Delta T - 57 \ ^\circ C)
\]
Here the temperature increase of one IOMNP, \( \Delta T \), must be corrected by subtracting from it the difference between theoretical and experimental heating before it is multiplied by the total number of IOMNPs in solution. However, note that \( \Delta T_E \) corresponds to an ensemble of MNPs, while \( \Delta T_T \) corresponds to a single MNP. This means that it may not be appropriate to compare these two quantities.

Certainly, the step of multiplying by the number of IOMNPs, \( N \), in solution prescribed by Eqns. 5.15 and 5.17 is what leads to the non-physical temperature increase shown in Fig. 5.3 (d). Another way to reduce this contribution is to consider how many nanoparticles make up one cluster. If the agglomerate is

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**Figure 5.4** To account for difficulties in predicting heating rates of IOMNPs consider that (a) the particle is idealized as being composed of a unidirectional magnetic moment when in reality (b) it has surface spin disorder and is encapsulated by a layer of PVP, in addition to the fact that its (c) idealized heat transfer is (d) significantly irregular.
about 270 nm in size (see Ch. 3) then its volume is about $1.4 \times 10^7 \text{nm}^3$ according to Eqn. 5.2. Similarly, the volume of a single 10 nm IOMNP is about $4.2 \times 10^3 \text{nm}^3$. The ratio of these two volumes yields the approximate number of IOMNPs per cluster as $3.4 \times 10^3$. This, then, can be divided into the total number of IOMNPs for a 2 mg/mL solution, provided by Table 3.1, to give the total number of clusters in solution. This effectively reduces the number of heat sources, and temperature increase, by a factor of $10^3$. While such a correction does move the temperature increase down in the correct direction, it is still too high by several orders of magnitude. Of course, agglomerates of this size necessarily precludes the applicability of LRT to this work anyway.

Continuing with the comparison of ideal versus actual IOMNP dispersions in DIW, consider now the interaction of particles. Again, LRT assumes there is no interaction of particles and so agglomeration cannot occur. LRT can be applied to a particle like the one depicted in Fig. 5.4 (c) in which heat transfers equally away from the particle in all directions. However, NTA already demonstrated that the IOMNPs used here (see Ch. 3) were predominantly clustered around 270 nm in size. Heat transferring away from, and within, an agglomerate like this is depicted in Fig. 5.4 (d). Thus IOMNPs that are larger than the superparamagnetic size regime, have non-uniform magnetization, are coated to facilitate dispersal in DIW, and interact (or agglomerate) will heat their local environment, in response to applied AMF, at a rate that is not described well by LRT.

Later on, a different treatment of LRT and its equations yield far more reasonable results which are plotted in Fig. 5.5. For the most part the calculations did not change up to the point of determining the SAR. In other words, Eqns. 5.5 – 5.11 were utilized to find SAR. Once the SAR was found it was used to determine the heating rate according to

$$\frac{\Delta T}{\Delta t} = \frac{m_{\text{MNP}}}{m_{\text{SOL}} c_{\text{SOL}}} \text{SAR} \tag{5.18}$$

Here, $m_{\text{MNP}}$ is the mass of all of the MNPs, $c$ is the specific heat, and the subscript SOL stands for solution. This method allows for avoiding the step of multiplying by the number of MNPs in solution, $N$. It is also superior since it yields the heating rate more directly than the previous method.

The black squares correspond to the LRT simulation parameters described earlier. For comparison purposes the corresponding experimental results from Ch. 3 (red circles) have also been plotted in Fig. 5.5 (a). On the one hand, LRT is behaving much more reasonably by yielding a heating rate per IOMNP
concentration of 0.052 °C/s/mg/mL. On the other hand, the experimental data from Ch. 3 exhibited a 0.029 °C/s/mg/mL heating rate per IOMNP concentration. These values are nearly 57 % different. It is possible the difference could be due to settling of the MNPs explored in Ch. 3. Therefore, the IOMNP settling data was used to correct the ideal data. These corrections to the ideal data (green triangles) is also shown in Fig. 5.5 (a). While the slope did not change significantly, the theoretical values are now, overall, closer to

Figure 5.5 By avoiding the step of scaling up the heating rates of one IOMNP by a factor of all particles present in solution (a) the model comes much closer to the experimental heating rates presented in Ch. 3, as well as can be used to yield ideal heating curves for (b) various aqueous IOMNP concentrations with no PNIPAM present, (c) constant IOMNP concentration with varying concentrations of PNIPAM, and (d) various IOMNP concentrations with constant PNIPAM concentration.
the experimental values. Other differences between ideal and theoretical values can be attributed to violations of LRT (i.e. MNP interactions) discussed above.

Now that LRT is yielding more reasonable results it can be used to predict heating rates of samples. First, the IOMNPs dispersed in DIW only had LRT applied to them and those heating rates are shown in Fig. 5.5 (b). As was expected, the higher the IOMNP concentration the greater the heating rate of the sample and a decreased concentration corresponds to a slow heating rate. As an example, a 2 mg/mL sample has an approximate heating rate of 10 °C/s meaning it will increase in temperature by about 31 °C at the end of a 5 minute trial.

Next, samples containing various concentrations of PNIPAM and constant IOMNP concentration of 2 mg/mL were considered with LRT. These simulations are plotted in Fig. 5.5 (c). Take for instance a sample containing 20 wt. % PNIPAM. In that case the sample will increase in temperature by nearly 30 °C after 5 minutes. It is immediately apparent that increasing the PNIPAM content only serves to decrease the heating rate ever so slightly. This happens since the denominator in Eqn. 5.17 increases with increasing PNIPAM content which serves to decrease the heating rate. Note that in Ch. 4 it was discovered that the presence of PNIPAM serves to better disperse the IOMNPs which only helps to increase heating rates. So, ultimately, any variation in heating rates shown in Fig. 5.5 (c) are unimportant since they will be overshadowed.

Finally, LRT was applied to scenarios in which the PNIPAM content was held constant at 20 wt. % and the IOMNP concentration was allowed to vary just as in Fig. 5.5 (b) when no PNIPAM was present. These results are shown in Fig. 5.5 (d). Specifically, the 2 mg/mL IOMNP concentration sample will heat by almost 29 °C. Again, here we see that the calculations dictate there should be a slight decrease in heating rate that will be dominated by the effect of better dispersal of MNPs. Clearly, the avoidance of multiplying by the number of IOMNPs, N, has resulted in better predictions of LRT.

5.3. LRT Applied to NTA Data

Here, an approach to LRT that is more representative of the purchased IOMNPs characterized in Sect. 3.1 is presented. Throughout the development of applying LRT to the IOMNPs described in chapter 3 it became clear that idealizing them as uniformly 10 nm in size was not appropriate (see Sect. 5.2.2). As
discussed earlier, that average size came from measuring individual IOMNPs, contained in larger clusters of said IOMNPs, under TEM. On the other hand, NTA revealed that dispersions of the nanopowder was comprised of clusters of various sizes with most of the clusters averaging between 100 nm and 400 nm in size. It was decided that a more appropriate application of LRT should predict the heating rate of a sample that contained the same IOMNP cluster size populations and in the same proportions as an actual sample. In other words, the heat generated by the very few individual IOMNPs present in solution would be different from that generated by the more prevalent 200 nm clusters of IOMNPs. Fortunately, NTA provides data sets that are nanoparticle concentration as a function of size. So, here, LRT will be used to predict the heating rate of a sample by inputting NTA data representing every possible IOMNP cluster size, according to proportion of prevalence, rather than an assumed discrete particle size. Furthermore, if this technique succeeds in predicting the heating rate then it provides further evidence that the IOMNPs are in fact clustered together and not contributing heat as a discrete nanoparticle, but rather as an ensemble (i.e. an agglomerate).

Again, NTA yields data that is concentration in particles per milliliter as a function of particle size in nanometers. For this exercise it was decided to use a data set corresponding to a sample of purchased IOMNPs at an initial concentration of 1 mg/mL that was initially sonicated vigorously and centrifuged at 1,000 rpm for 15 minutes. The supernatant was removed, added to more water, and centrifuged at 4,000 rpm for 15 minutes. Then that supernatant was removed, added to water, and centrifuged at 10,000 rpm for 15 minutes. Finally, that supernatant was removed and added to enough water to return the sample to its original volume yielding a sample with unknown concentration, but it would have the largest stable clusters removed ideally. A part of this sample was subjected to NTA for five trials lasting three minutes each. The data presented in Fig. 5.6 (a) represents the average of those trials.

The NTA data was input into LRT in a manner already described in Sect. 5.2, but briefly described here. First, the size array from the NTA data set was converted to a volume array using Eqn. 5.2. This volume array was then used to determine the ratio of anisotropic energy to thermal energy, known as $\Gamma$, using Eqn. 5.6. From there Néel relaxation time was found using Eqn. 5.5. Now, in order to determine the Brownian relaxation time it was assumed that the discrete IOMNP had a 1 nm shell of PVP (added by the manufacturer to facilitate dispersal in water) around it contributing to the hydrodynamic volume when
evaluating Eqn. 5.7. Of course, this does not account for a hydration shell around the IOMNP which was assumed to be negligible in size compared to the PVP shell, since it could not be measured by NTA. The Néel and Brownian relaxation determined the effective relaxation through Eqn. 5.8, which allowed for the evaluation of imaginary magnetic susceptibility as described by Eqn. 5.9. This, then, made possible the

Figure 5.6 (a) NTA data, presented in Ch. 3, can be input into LRT as one array of IOMNP agglomerate sizes and the corresponding array of concentration (or prevalence at a certain size) to report upon (b) the heating rate of a particular concentration in the sample (or size population), which compiled together contribute to a heating rate of the whole sample for given (c) large and (d) small magnetic field strengths.
determination of volumetric power via Eqn. 5.11, and, consequently, the SAR with Eqn. 5.12. With the SAR in hand the heating rate was found according to Eqn. 5.18. So from the various size populations identified by NTA it was possible to determine various heating rates associated with them. All the constant IOMNP parameters used by LRT were kept the same as reported in Sect. 5.2.

Next, the NTA concentrations, in particles per milliliter, were converted to milligrams per millilitre in the same way that Table 3.1 was developed. The resulting heating rate as a function of IOMNP concentration is plotted in Fig. 5.6 (b). Apparently, smaller clusters, and discrete IOMNPs, contribute minimal heating rates to the NTA sample. As cluster sizes increase toward 250 nm the heating rate contribution peaks out to more than $1.5 \times 10^{-5} ^\circ C$. This is a natural consequence of the fact that there are more IOMNP clusters around that size; clusters larger than 250 nm are less prevalent, as seen in Fig. 5.6 (a). Populations of the largest clusters contribute less to the overall heating rate of the entire sample, as indicated by the inset data in Fig. 5.6 (b). This inset displays the middle part of entire data set showing that at ever increasing cluster size the contribution to sample heating rate decreases.

The final step in this approach involves scaling up the aforementioned results from NTA applied to LRT to simulate a total heating rate and total concentration that corresponds to one of the five data points from the AMF heating experiment of IOMNPs described in Sect. 3.2. First, the concentrations converted from particles per milliliter (raw NTA data) to milligrams per milliliter are summed to determine the total concentration of the NTA sample. The inverse of this total NTA sample concentration was multiplied by an integer between one and five, which was then taken as a multiplicative factor for scaling up every agglomerate size population in the original array to a new array. When this new array was summed it equaled 1, 2, 3, 4, or 5 mg/mL depending on what integer scale was selected. Now, each cell in the new concentration array represented the mass of the IOMNPs for that particular agglomerate size population. So that quantity could be used in Eqn. 5.18 with the corresponding SAR quantity calculated from the agglomerate size quantity (raw NTA data) described above to determine the heating rate for that agglomerate size population. When this new, scaled up, heating rate array is summed it represents total heat rate of IOMNPs at a concentration of 1, 2, 3, 4, or 5 mg/mL. Therefore, NTA data used as an input into LRT generated simulations of the IOMNP heating via AMF reported on in Sect. 3.2.
The simulations of heating rates versus IOMNP concentration are provided in Figs. 5.6 (c) and 5.6 (d). Once the more basic IOMNP parameters (i.e. anisotropy, attempt time, magnetite density, etc.) were decided upon as reported on in Sect. 5.2, the main parameter that determined the ultimate heating rate turned out to be the applied magnetic field amplitude, H. It was shown in Sect. 3.2 that for an AMF heating coil of constant geometry, the field is determined by Eqn. 3.5. There it was reported that the applied field used was approximately 59 kA/m as calculated from setting the current to 184.8 A (the frequency during those experiments, reported on in Ch. 3, was approximately 307 kHz). Therefore a natural applied field to input into this treatment provided an upper bound of possible heating rates. It can be seen from the solid black square data points in Fig. 5.6 (c) that such a field yields a heating rate of approximately 1.44 °C/s/mg/mL. This is significantly different from the 0.029 °C/s/mg/mL which was the actual heating rate versus IOMNP concentration determined through AMF heating of purchased IOMNPs reported on in Sect. 3.2. Since this was obviously not a close comparison, it was decided to input into this model an applied field that corresponded to the outermost parts of the sample exposed to AMF. The field at the corners of the sample cuvette would be about 39 kA/m. The resulting simulation is represented by the empty black square data points in Fig. 5.6 (c) characterized by a slope of almost 0.63 °C/s/mg/mL. While this is less drastically different from the actual experiment, it is still many factors higher than reality. Recall that the application of LRT assuming a uniform size of almost 10 nm IOMNPs yielded a closer to reality heating rate versus IOMNP concentration of approximately 0.052 °C/s/mg/mL as demonstrated by the open, red circular data points plotted in Fig. 5.6 (d). Up to this point it would seem as if the application of NTA data to LRT yields poor predictions to actual AMF heating of IOMNPs dispersed in DIW.

However, consideration of the effect of applying current to a conductor provides a solution to the discrepancy. It is well known that applying a significant current to a conductor, as happens to the heating coil during an AMF trial, causes the conductor to increase in temperature. This change in temperature leads to a change in resistivity of the copper heating coil according to

\[ \rho = \rho_0 (1 + \alpha \Delta T) \]  \hspace{1cm} (5.19)

Of course, this then would mean the resistance of the coil would change since

\[ R = \rho \frac{1}{A} \]  \hspace{1cm} (5.20)
Assuming a constant potential difference, Ohm’s law would necessitate a corresponding change in the current, which would ultimately result in a new effective applied magnetic field according to Eqn. 3.5 [74, 159]. So by relating the effect of a temperature change on current via Ohm’s law to the dependence of applied magnetic field on current it can be shown that a change in temperature gives rise to a change in applied magnetic field according to

$$H_1 = H_2 \left(1 + \alpha \Delta T \right)$$

(5.21)

Apparently, the AMF heating of IOMNPs is subject to applied magnetic fields that are much weaker than those predicted by Eqn. 3.5.

With these realizations in mind it was decided to determine what applied field would be achieved if the copper in the heating coil were to nearly melt. In this way, the melting temperature of copper (1085 °C) provides a lower limit to the possible applied magnetic field of approximately 6.6 kA/m. The open, green triangular data points of Fig. 5.6 (d) represent a minimum to the possible heating rate as a function of IOMNP concentration when NTA data is fed into the LRT model. The slope of this data set is 0.018 °C/s/mg/mL which is still 46 % different from the experimental data set, albeit much improved from the higher applied fields discussed in this section. At this point, it seemed interesting to ascertain what applied field would produce a slope most similar to that of the experimental data set indicated by closed red circles. Therefore, a variety of applied magnetic fields were input for this parameter until a data set most like the experimental data was produced. It turns out that when an applied field of 9.4 kA/m is used as a parameter in the model, then the 0.037 °C/s/mg/mL slope of heating rate versus IOMNP concentration turns out to be about 24 % different from that of the experimental data set. Furthermore, it can be seen in Fig. 5.6 (d) that this simulation, and its closed, green triangular data points, have relatively good crossover with the data points and error bars of the experimental data set. Assuming the ideal applied magnetic field is that calculated near the four corners of the sample cuvette (~39 kA/m) and the effective applied magnetic field is 9.4 kA/m serves to predict a change in temperature of the heating coil of around 810 °C. While this increase in coil temperature is surprising it is well below the melting temperature of copper and therefore certainly possible. Note that a comparison with a 59 kA/m field was not evaluated since this is the field strength only at the center of the sample and under ideal conditions, so it is not physical.
By utilizing NTA data, LRT was more successfully used to predict the response of IOMNPs by calculating the heating rate of each cluster size population as a contribution to the whole sample. This approach to LRT turned out to be superior to assuming the sample was composed of discrete nanoparticles. Unexpectedly, this approach seems to suggest that significant temperature change is occurring within the AMF heating coil, despite the fact that the coil is internally cooled with circulating water. Fortunately, the predicted increase in the coil’s temperature does not exceed the melting temperature of copper. It should be stated that many other factors could lead to reductions in effective applied field. For example, excess space in the design of AMF heating coil leads has been shown to contribute to power losses [214].

5.4. Summary

Eddy current losses can be thought of as a classical description of the heating of a solution containing IOMNPs that is exposed to an AMF. When magnetite is chosen as the material making up the IOMNPs then many of the parameters leading to the heating power generated by eddy currents are constants (e.g. peak polarization, conductivity, frequency, and density). Now, the heating power is determined by the square of the diameter of the IOMNP. With the heating power in hand, the heat energy generated in a sample can be found, which leads to the temperature increase using standard calorimetry calculations. Figure 5.2 (b) demonstrates if discrete, 10 nm IOMNPs are the case then a heating rate that is significantly low compared to the results presented in Ch. 3 is predicted. On the other hand, assuming all the magnetite powder agglomerates into a single 0.91 mm chunk yields a heating rate that is also too small. By assuming that the IOMNPs cluster in 270 nm agglomerates, consistent with NTA data presented in Ch. 3, simulations yields a heating rate that is once again too small. These results suggest, unsurprisingly, that eddy currents are not appropriate for explaining data presented in Chs. 3 and 4.

LRT can be considered a more modern approach to explaining the heating of a sample containing IOMNPs that is exposed to an AMF. It supposes that the relaxation of an IOMNP, due to Néel and Brownian rotation, gives rise to its heating ability. The relaxation time determines the imaginary part of the magnetic susceptibility, which determines the power loss of the particles. With that power, the specific absorption rate can be found which is related to the heating rate of the sample. Surprisingly, Fig. 5.3 (b) demonstrates
that the predicted heating rates of a typical sample is unphysical implying that LRT is not appropriate for this work.

This motivated a closer look at the assumptions of LRT. As was discussed previously, LRT supposes, first and foremost, that the magnetization of the particles is linear with applied magnetic field. In Ch. 3 this data was given and it did appear that for the range of applied field (0 – 59,000 A/m) used in this work that the magnetization was linear. However, upon linear fit of the data the $R^2$ value was determined to be 0.98 which is not very encouraging. Furthermore, the ratio of error in the y-intercept to the y-intercept was nearly 72%. This evidence suggests the magnetization is not linear with applied field. The LRT also assumes that particles do not interact. Again, in Ch. 3 NTA data provided evidence that the particles are clustered and therefore necessarily interacting. This indicates that heat transfer will be inhibited among and away from IOMNPs that are agglomerated. If their PVP coating is holding several IOMNPs together, then when one responds to an applied field it must exert a force on the rest of the cluster. Also, the PVP coating must be retarding some of the heat transfer from IOMNP to local environment. For these reasons one must conclude that LRT is a less than ideal description of the heating observed in this work but much superior to the eddy current description.

By avoiding the use of Eqn. 5.14 LRT can be made to yield reasonable heating rates that are close to those determined experimentally. The fact that the theoretical and experimental heating rates are more than 10% different can be attributed to violations of LRT (i.e. non-linear magnetization versus applied field, interacting IOMNPs, etc.). Predictions of minor decreases in heating rates when PNIPAM is present in a sample should be negated by the fact that in a real sample the polymer acts to better disperse the IOMNPs allowing them to heat more effectively.

Finally, LRT was made to produce acceptable predictions of AMF heating experiments by embracing the fact that these IOMNPs do cluster together in DIW solution and fully abandoning the assumption that the purchased IOMNPs used in this work were discrete, as was advertised, and approximately 10 nm in diameter. This was achieved by repurposing the size distribution data from NTA as an input for the LRT. In this way, each size population of IOMNP clusters could be used to determine their contribution to the heating rate to the entire sample. This is important since clusters of a particular size contribute a different amount of heat than clusters of a different size. Then the heating rate, at a particular
cluster size, could be scaled up based on the concentration of that particular cluster size. Ultimately, it was discovered that the experimental results reported on in Sect. 3.2 were well simulated by assuming the effective applied magnetic field was 9.4 kA/m. This discovery suggests that the AMF heating coil may be heating to as much as 810 °C. Alternative explanations for the apparent reduction in effective applied field include apparent flaws in the design and setup of the experimental apparatus such as the placement of coil leads.
Chapter 6: Conclusions

This aim of this project was to make progress toward a TBD device capable of actively delivering a biotherapeutic to a target tissue by way of MNPs encapsulated within a SRP for the more precise treatment of conditions such as heart disease, cancer, genetic disorders, and traumatic injury. Specifically, the device that was sought after during this work is capable of what is commonly referred to in the TBD field as active targeting since it would employ a static magnetic field in the vicinity of the target tissue to reach its destination prior to releasing the biotherapeutic. The gold-standard in SRP research, biocompatible PNIPAM, was selected for this work since it undergoes a discontinuous VPT near physiological temperature and the thermodynamics of its phase transition are well understood. Biocompatible IOMNPs were selected to achieve remote control targeting and enable optimal device heating upon AMF exposure due to their propensity toward no residual magnetization in the absence of an applied magnetic field (i.e. they are SPM). For some perspective, several synthesis methods of TBD devices were discussed with emphasis on techniques and specific applications for the fabricated devices.

Early attempts, in this work, to synthesize PNIPAM micelles via emulsion polymerization resulted in monodisperse structures that were apparently incapable of maintaining their spherical shape while encapsulating IOMNPs, and spray synthesis attempts that never produced stable nanogels of suitably small size. Ultimately, aqueous UV photo-irradiation synthesis produced stable PNIPAM nanogels, whose progress was monitored in-situ by observing the spectroscopic attenuation of the UV source. OMA, the system for innovative, in-situ monitoring, was characterized and calibrated in terms of the range of wavelengths it could observe. Likewise, the source of energy to power the polymerization of NIPAM was characterized in terms of its wavelength, distance from the sample, timing of emission, and its long-term use. The PNIPAM nanogels were characterized using electron microscopy to describe their size and morphology, NTA to determine in-situ average diameter and demonstrate their discrete, non-agglomerated
nature, as well as FTIR and UV absorbance to demonstrate successful polymerization. Furthermore, control of PNIPAM nanogel diameter was demonstrated by varying the initial solute concentrations of the precursor solution.

The IOMNPs were systematically characterized to demonstrate their SPM nature and inform upon the challenges in successfully compositing them with PNIPAM. Electron microscopy revealed the discrete IOMNPs were approximately 10 nm in diameter, safely beneath the generally accepted threshold for single domain size. Unfortunately, the particles were never imaged in isolation suggesting a tendency to agglomerate. In order to determine how the IOMNPs interacted with each other in-situ they were subject to NTA where it was found they do agglomerate in clusters predominantly around 270 nm in diameter. Magnetic response to applied magnetic field revealed no hysteresis indicating the IOMNPs are SPM. Sedimentation experiments revealed that despite being coated in PVP, the particles fall out of solution in relatively fast times making encapsulation within PNIPAM problematic. Furthermore, transmittance as a function of wavelength of aqueous IOMNP dispersions reveal significant inability of UV light to penetrate a solution which may impede photopolymerization.

To report on the efficacy of IOMNPs dispersed in water to heat a sample they were subjected to AMF and their heating rates were recorded. Unsurprisingly, increasing concentrations of IOMNPs dispersed in DIW corresponded to increasing heating rates of the sample. However, what was interesting was a trend in SAR to decrease with increasing IOMNP concentration, which the literature attributes to a growing tendency of the particles to interact with their neighbors as they become crowded.

AMF heating experiments were also conducted on samples of aqueous PNIPAM samples in which IOMNPs were dispersed, but not yet embedded. Using a novel, custom-assembled experimental setup, the phase transition of PNIPAM could be monitored while the AMF heating was occurring. This was made possible by carefully aligning a He-Ne laser through the induction coils and sample so that turbidimetry could be carried out and matched to heating data. These trials revealed that the IOMNPs were reducing the LCST of PNIPAM.

The embedding of IOMNP agglomerates into PNIPAM nanogels was achieved via UV photopolymerization of precursor solutions and monitored, in situ, with the OMA system. The presumably IOMNP embedded nanogels were characterized using TEM to exhibit diameters consistent with the sum of
the empty nanogel from Ch. 2 plus the IOMNP agglomerate from Ch. 3. IOMNP agglomerates could only be observed, using TEM, in nanogels that were malformed, which was explained by way of high electron density in well-formed nanogels appearing opaque in the micrographs. Furthermore, a turbidimetry experiment on a photo-irradiated sample suggests that a static magnetic field was effective at removing IOMNP embedded PNIPAM nanogels from the water column. Ultimately, AMF heating trials were conducted to determine the efficacy of the IOMNP embedded PNIPAM nanogel to heat in response to applied fields.

Finally, a model was presented to assist in understanding the results of the AMF heating of IOMNPs dispersed in DIW. First, eddy current heating was considered in order to establish a limit/boundary on acceptable heating rates. Next, the more appropriate LRT was considered since these IOMNPs are, after all, SPM nanoparticles. Initial application of LRT yielded wholly unphysical heating rates due to the practice of considering the heat generated by each IOMNP or IOMNP cluster. By calculating sample heating rates directly from the corresponding SAR values more realistic heating rates were found. However, these were still significantly inconsistent with the experimental results of Ch. 3 since they assumed a discrete 10 nm IOMNP diameter. Ultimately, the model was only successful after a pioneering application of NTA data to LRT; consideration of various size populations, and their relative prevalence, was necessary to predict heating rates.

The results presented here advance the field of TBD by introducing novel methods for carrying out TBD device synthesis while monitoring its progress in situ, conducting turbidimetry coincident with AMF heating, and developing LRT models to predict AMF heating based on size distributions (e.g. NTA) of MNPs. These findings will be of interest to researchers wishing to develop their own biotherapeutic efficient, delivery platform.
REFERENCES


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Appendix A: Piezoelectric Nebulizer

Manufacturer: Sonaer Ultrasonics Inc.
Model: 241
Input Power: 0 – 24 ± 1 VDC
Frequency: 2.40 – 0.05 MHz
Generator Power: 12 – 15 W
Processing Rate: 200 – 250 mL/hr (@ 66 °C), 70 – 100 mL/hr (@ 21 °C), 0 mL/hr (@ 1 °C)
Mating Connector: MOL705-43-0003
Element: Gold Plated
Seals: Viton
Particle Size: 1.7 μm (assuming droplets are water)

Figure A.1: Schematic for mounting the nebulizer unit to a vessel, which was utilized in this work. This mount accommodates one nebulizer by means of securing the unit with screws through the 0.23 cm TAP holes. The larger 1.7 cm hole allows the gold plated part of the nebulizer to make contact with the liquid in the containing vessel.
Appendix B: Medical Inhaler

Distributor: Assist Medical Supplies
Model: CE-331
Power Supply: 1.5 V alkaline battery x 2, or
Input: 100-240 VAC, 50/60 Hz, 0.4 A
Output: 3 VDC, 1A
Ultrasonic Freq.: 120 kHz
Power Consumption: ~ 2.0 W
Nebulization Rate: 0.2 mL/min (@ 10 °C – 40 °C and 30 % – 85 % relative humidity)
Particle Size: mass median aerodynamic diameter 5 μm (assuming droplets are water)
Fill Volume: 0.5 mL – 8.0 mL
Battery Life: 1.5 hours (continuous use), 4 days (20 min operation per day)
Storage Conditions: -20 °C – 70 °C and relative humidity < 85 %
Weight: 98 g
Dimensions: 5.56 cm x 4.20 cm x 10.92 cm
**Appendix C: Pneumatic Atomizer**

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Appendix D: ICCD Camera System Specifications

PI Acton PI-MAX:512 UNIGEN Digital ICCD Camera System:

Manufacturer: Princeton Instruments Acton
Model: 7361-0017
Serial Number: 2911060001
Thompson TH7895M scientific grade 1, front-illuminated CCD, 512 x 512 pixels
1.27:1 fiber-optic bonded 18 mm grade 1, Gen III extended blue image intensifier ideal for 350 nm
to 900 nm range.
Fiber-optic input, proprietary UV coating
19 x 19 µm pixels (12.4 x 12.4 mm image area)
< 5 ns gating capable

ICCD USB2 Controller:
with 16-bit 100 kHz and 16-bit 1 Mhz digitizers
ST-133 B/U-PTG-Dual Model 7513-0001
Serial Number: 2911060002

Programmable Timing Generator (PTG):
Delay Range: ~0 ns to 20 ms
Timing Resolution: 40 ps
Timing Jitter: 40 ps rms
Repetition Rate: 50 kHz sustained; 500 kHz burst
Gate Width: 5 ns to 10 ms; 40 ps resolution
Fast Gate: 2 ns; 5 kHz repetition rate
Bracket Pulse: 5 kHz repetition rate; 500 ns lead time
Insertion Delay: 15 ns
Appendix E: Spectrometer Specifications

Manufacturer: Acton Research Corporation
Model: 1237 SpectraPro-500
Serial Number: 500173S
Controller Serial Number: 100-240V 2A
Focal Length: 500 mm

Optical System: Czerny-Turner type with in-line (180°) optical path, and optical multi-port configuration featuring 90° and 180° optical path.

Wavelength Scanning System: Direct Digital Scanning with exclusive Autotrack™ electronics.
Scan Linearity: Scans linear with respect to wavelength.
Triple Indexable Gratings: 150, 600, and 1200 grooves/mm.
Resolution: 0.05 nm with standard 1200 g/mm grating, 10 μm wide x 4 mm high slits, measured at 435.8 nm.
Reciprocal Linear Dispersion: 1.67 nm/mm with 1200 g/mm grating (nominal).
Aperture Ratio: f/6.9
Wavelength Operating Range: Up to the far infrared with available gratings. 185 nm to 1.4 μm with 1200 g/mm grating.
Wavelength Accuracy: ± 0.2 nm/500 nm with a 1200 g/mm grating.
Wavelength Reproducibility: ± 0.5 nm with 1200 g/mm.
Focal Plane Detector Compatibility: 25 mm focal plane extends 0.750 inch (19 mm) beyond housing for easy positioning of focal plane detectors. Provides nominal 280 nm coverage with 150 g/mm grating, 140 nm with 300 g/mm grating, 70 nm with 600 g/mm grating, and 35 nm with 1200 g/mm grating.
Slits: Standard slits are bilaterally adjustable from 10 μm to 3.0 mm, via external micrometer. Standard slit height is 4 mm. Other slit heights up to 20 mm can be supplied.
Computer Compatibility: RS-232 port – 9600 baud, no parity, 8 data bits, 1 start bit, 1 stop bit; or optional IEEE 48 port.

Figure E.1: Spectrograph optical layout.
Appendix F: Fiber Optic Bundles

Standard bundles come with 200 µm diameter fibers in silica for UV-VIS. Fibers are arranged as a slit pattern (in a 10 mm diameter ferrule) on the spectrograph end, with a round configuration (in SMA 905 connectors) on the source end.

Two-Leg Fiber Bundle
Model BFB-455-7: a 1 m long UV_VIS fiberoptic bundle for 190 to 1100 nm. It contains two groups of 200 µm diameter fibers (~245 micron diameter with cladding), with seven fibers per group and ~1 mm spacing between groups.

Four-Leg Fiber Bundle
Model QFB-455-3: a 1 m long UV_VIS fiberoptic bundle for 190 to 1100 nm. It contains four groups of 200 µm diameter fibers (~245 micron diameter with cladding), with three fibers per group and ~1 mm spacing between groups.

![Figure F.1. 10 mm diameter ferrule on spectrograph end for (a) two-leg fiber bundle and (b) four-leg fiber bundle.](image)
Figure F.2. The three fiber configuration on the source end of the four-leg fiber bundle. An approximate 528 µm diameter fiber bundle zone (including cladding), an approximate 483 µm diameter light collection zone (not including cladding at outer edge).
Appendix G: Determination of Relative Transmittance from Photoresistance

In the turbidimetry experiments described in Sect. 4.1, a He-Ne laser was passed between the induction heating coils and through the sample so that it could be monitored by a photoresistor in series with a multimeter. Photoresistance was recorded as a function of time using LabView software, written in-house, for the purposes of this experiment. The software stored time in one array, as shown in column A of Fig. A4. The photoresistance at a particular time was stored in a second array. The average photoresistance of ten trials, and the corresponding standard deviation, is shown in columns B and C respectively. The maximum photoresistance is calculated in column D. Next, the difference between maximum photoresistance and that at any given time is calculated in column E. Then the maximum of all those differences is determined in column F. Finally the relative transmittance of the sample at any given time is found by multiplying the ratio of column E to column F by 100.

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*Figure G.1: This sample spreadsheet, from the 0.4 wt. % aqueous PNIPAM dispersed with 2 mg/mL IOMNPs data set, serves to illustrate how photoresistance was converted to relative transmittance.*
Appendix H: NTA Data Applied to LRT

The figures in this appendix serve to document how NTA data was used as an input to LRT for predicting the heating rates of IOMNPs in response to an AMF. In Figs. H.1 through H.4, the first three rows are a collection of physical constants and other environmental parameters that are necessary for LRT calculations. For more information on how to use these parameters, or make any of the calculations described here, see the discussion of Ch. 5. In some cases the parameters are flexible like the temperature, which surely will fluctuate during any given AMF trial. In other cases, the parameter was taken from the literature and not allowed to vary, like the magnetic anisotropy. Still other parameters were determined by the experimental conditions (e.g. frequency) or are particular to the IOMNPs used (e.g. saturation magnetization). In that case, the calculation is based on characterization data presented in Ch. 3. What follows is a discussion meant to inform upon how MNP size distribution data, as supplied by NTA or DLS, can be applied to LRT to yield practical heating rates.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anistrp (J/m^3)</td>
<td>Sat. Mag. (A/m)</td>
<td>Temp. (K)</td>
<td>Atmpt Time (s)</td>
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<tr>
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<td>23000</td>
<td>3.08E+05</td>
<td>296.65</td>
<td>1.00E-09</td>
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<tr>
<td>2</td>
<td></td>
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</table>

Figure H.1 Shown here are some physical constants and parameters pertinent to LRT in the upper rows of this spreadsheet with the conversion of NTA size populations to LRT quantities in the lower rows.
Columns A and B of Figure H.1 represent size populations as established by the NTA software. That size is converted to a magnetic and hydrodynamic volume of the MNP in columns C and D respectively by assuming the particles are ideal geometrical spheres and that the hydrodynamic volume minimally includes the 1 nm thick PVP layer in addition to the MNP agglomerate. With the magnetic volume the ratio of magnetic anisotropic to thermal energy, known as gamma, is determined in column E. For particularly large size populations, gamma became too large for the software to effectively proceed with the subsequent steps and had to be fixed, in column F, at sizes larger than about 63 nm. That is to say, beyond this MNP agglomerate size, gamma was set at a value of approximately 700. The discussion surrounding Fig. 5.3 (b) explains why this is inconsequential. It suffices for the purposes of this appendix to say that at higher values of gamma the results are indistinguishable from what is shown here. From gamma, the Néel relaxation time was calculated in column G and the Brownian relaxation time was calculated from the hydrodynamic volume in column H, as seen in Fig. H.3. These then, were used to determine the effective relaxation time in column I. The equilibrium susceptibility, in column J, was found using the magnetic volume in column C as well as the parameters thermal energy (product of cells O3 and C3), saturation magnetization (cell B3), and permeability of free space (cell N3). With the equilibrium susceptibility (column J), the effective relaxation

<table>
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<tr>
<th>Ang Freq (rad/s)</th>
<th>Apld Fld (A/m)</th>
<th>Fe₃O₄ ρ (kg/m³)</th>
<th>Fe₃O₄ ρ (mg/m³)</th>
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</thead>
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<tr>
<td>1928937.889</td>
<td>9400</td>
<td>5100</td>
<td>5100000000</td>
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<table>
<thead>
<tr>
<th>Gamma Fix (unitless)</th>
<th>Neel Relax (s)</th>
<th>Brun Relax (s)</th>
<th>Combo Relax (s)</th>
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</thead>
<tbody>
<tr>
<td>0.000367544</td>
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<td>5.52E-09</td>
<td>5.09E-09</td>
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<tr>
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<tr>
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<td>9.71E-08</td>
<td>3.07E-09</td>
</tr>
</tbody>
</table>

**Figure H.2** Shown here are some physical constants and parameters pertinent to LRT in the upper rows of the spreadsheet with LRT quantities in the lower rows.
time (column I), and the parameter angular frequency in hand (cell F3), the imaginary susceptibility was calculated in column K, as seen in Fig. H.3. This allowed for the determination of volumetric power dissipated by the MNPs in column L, which allowed for the evaluation of SAR, found in columns M and N. From there, the heating rate was calculated in column O, seen in Fig. H.4, so that a heating rate for each size population in the sample was found.

Now NTA also outputs an array of concentrations corresponding to each of the size populations, which was placed in column P. It was converted in the next several columns from particles per mL to milligrams per milliliter in column Q. Note that the concentration of the entire sample could be found by summing every entry in this array. At this point the inverse of the sum of column Q was found so that it could be multiplied by each entry in column Q. In this way, column S, seen in Fig. H.5, represents the prevalence (or concentration) corresponding to a particular heating rate. In effect, this practice yields an array of concentrations pertaining to specific size populations for a sample at an idealized total concentration and no longer the concentration of the sample taken to NTA. Column Q represents a 1 mg per mL sample. The prevalence arrays for 2, 3, 4, and 5 mg per mL are found in columns U, W, Y, and AA.
respectively, see Fig. H.5. Finally, the heating rate of one of these ideal solution concentrations was determined, in columns T, V, X, Z, and AB, from the SAR in column N. Therefore, this algorithm yields predicted AMF heating rates based on actual size distribution data coming from experiments such as NTA or DLS.

Figure H.4 Shown here are some physical constants and other parameters pertinent to utilizing LRT in the upper rows of the spreadsheet as well as LRT specific quantities in the lower rows and concentration arrays output from NTA.
**Figure H.5** Shown here are size population specific prevalence (or concentrations) and heating rates within samples idealized at a particular IOMNP concentration.

**Figure H.6** Shown here are size population specific prevalence (or concentrations) and heating rates within samples idealized at a particular IOMNP concentration.
Appendix I: Publications and Presentations

Refereed Publications


Refereed/Contributed Conference Presentations


• **D. J. Denmark, D. Mukherjee, G. Marcus, S. Witanachchi, and P. Mukherjee,** “A fundamental understanding of the competing Néel and Brownian relaxation mechanisms in the remote RF heating of thermoresponsive polymers using Fe$_3$O$_4$ magnetic nanoparticles.” *2015 Materials Research Society Spring Meeting & Exhibit* (oral presentation)

• **J. Bradley, D. J. Denmark, S. Witanachchi,** “Systematic studies of phase transitions in thermo-responsive polymers in targeted drug delivery.” *2015 American Physical Society Spring Conference* (Distinguished Undergraduate Poster Presentation)

• **D. J. Denmark, N. Bernal, S. Shakespeare, M. Mamone, K. S. Bisht, S. Witanachchi, P. Mukherjee,** “Nebulized and aerosol synthesis of optimized targeted drug delivery composites.” *2015 Biomedical Engineering Society Annual Meeting* (oral presentation)

• **N. Bernal, D. J. Denmark, and S. Witanachchi,** “Vertical spray-dry synthesis of nebulized smart polymer carriers of nano-therapeutics.” *2015 Biomedical Engineering Society Annual Meeting* (poster presentation)

• **D. J. Denmark, D. Mukherjee, G. Marcus, S. Witanachchi, and P. Mukherjee,** “Resolving susceptibility loss into the competing Néel and Brownian relaxations in the remote RF heating of thermo-responsive polymers using Fe$_3$O$_4$ nanoparticles for targeted drug delivery”, *American Vacuum Society 2015 Florida Chapter Symposium* (oral presentation)

• **D. J. Denmark, D. Mukherjee, S. Witanachchi, and P. Mukherjee,** “Remote triggering of thermoresponsive polymers using radio frequency heating of Fe$_3$O$_4$ magnetic nanoparticles for targeted drug delivery applications”, *NanoFlorida 2014 The 7th Annual NanoScience Technology Symposium* (oral presentation)
APPENDIX J: Copyright Permissions

Fig. 1.3 (b) was adapted with permission from Z. Ahmed, E. Gooding, K. Pimenov, L. Wang, and S. Asher, “UV Resonance Raman Determination of Molecular Mechanism of poly(N-isopropylacrylamide) volume phase transition,” *J. Phys. Chem. B*, Vol. 113, pp. 4248-4256 (2009). Copyright 2009 American Chemical Society.

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Figs. 3.7, 4.1, and 4.2 Reproduced by permission of The Royal Society of Chemistry.
Daniel Jonwal Denmark received his Bachelor of Science in Astronomy and Bachelor of Arts in Physics degrees from the University of Florida in 2004. Before enrolling in the doctoral physics program at the University of South Florida, he was employed as a physics and astronomy instructor by the School District of Hillsborough County from 2005 until 2012. Besides physics and astronomy, he taught integrated science, biology, and earth science at Armwood and Brandon High Schools. In that role, he also served as a district astronomy curriculum writer. He enrolled in the University of South Florida in 2012 and achieved doctoral candidacy status in 2015. In addition to his academic and research pursuits, he served as Research Experience for Undergraduates coordinator and mentor during the summers of his graduate work. He was awarded the Frank E. Duckwall Graduate Fellowship and Fred L. & Helen M. Tharp Endowment Scholarship during the last two summers of his graduate work. The investigations he and his undergraduate assistants conducted led to two refereed publications and ten conference presentations, for which he has received a distinction for oral presentation organization.