

Computer Modeling of Induced Hyperthermia Using Superparamagnetic Iron Oxide Nanoparticles and their Application

Chris Conklin*, Saroj Biswas, Feroze B. Mohamed⁺, Scott H. Faro⁺, Linda Knight⁺, Marvin Ziskin⁺, Daniel Strongin[°], Joan Z. Delalic

College of Engineering, Temple University

1947 N. 12th Street

Philadelphia, PA 19122

Tel: (215) 204-3382 Fax: (215) 204-5960

[°]College of Chemistry, Temple University

⁺School of Medicine, Temple University

*Corresponding author email: chris.conklin@temple.edu

Abstract

The goal of this research is to develop the mathematical realization of RF-induced hyperthermia models for the effective ablation of brain tumors. This process requires a precise evaluation of the magnetic field interactions with the SuperParamagnetic Iron Oxide (SPIO) nanoparticles. The nanoparticles are suspended in a gelatin that can emulate several properties of brain tissue. The physical system, Hot Shot RF Unit, developed by Ameritherm Inc. that produces an AC magnetic field through induction, is used for empirical data acquisition. The magnetic fluid is subjected to an alternating field whereby the magnetic moments of these single domain particles are constantly oscillating and resulting in frictional heat losses. With the development of a targeting ligand for efficient biodistribution, these particles would adhere to the neoplasm and destroy the tumor through controlled RF exposure. The first step in realizing the above-mentioned effects is to develop a model that can estimate the potential effects of changing certain parameters such as coil diameter, time of exposure, and field strength for future optimization.

Keywords: superparamagnetic, hyperthermia, iron oxide nanoparticles

Introduction

The aim of this research involves the realization of a comprehensive model for estimating the effects of AC magnetic field induced hyperthermia. This emerging therapeutic procedure when coupled with nanoparticles has the potential for specific targeting and ablation of carcinomas located within the body. Numerous researches have been conducted showing the feasibility of such a procedure. However, the heating characteristics of biocompatible nanoparticles, such as Feridex and Cross Linked Iron Oxides (CLIO), need to be gathered to aid in the modeling of this physical and biological system. These characteristics will be used to develop a model for empirical data validation, which will provide sufficient evaluation to be utilized in the comprehensive computer model. The following sections will discuss cancer treatments, and heating results enunciating the importance of pursuing a theoretical model for future optimization.

1. Current Cancer Treatment Modalities

Currently, the most common treatments for cancer are chemotherapy and radiation therapy. Chemotherapy is a treatment method that relies on the use of anti-neoplastic drugs, or drugs that inhibit and fight the development of tumors. This is accomplished by stopping or slowing mitosis of the quickly dividing cancerous cells. However, due to the potency of these drugs, they can adversely affect healthy cells, causing side effects such as nausea, vomiting, fatigue, hair loss, and a decrease in blood cell counts. Despite the myriad of health related problems associated with chemotherapeutic treatments, they are mitigated once treatment has stopped or the patient has become acclimated to the drugs. By contrast, radiation therapy involves the application of ionizing radiation, either internally or externally, to destroy neoplastic cells. This is accomplished by interrupting cell division through the destruction or alteration of the cell's genetic material.

External irradiation of radioactive isotopes is applied through the use of X-rays, gamma rays, particle beams, or proton beams. Internal irradiation is done by implanting the radiation source inside or near the tumor site. Side effects are closely correlated with the treatment area but typically disappear a couple months after the radiation therapy is complete [2].

2. Previous Work at Temple University

The basis for this research stems from the work initiated at Temple University by Asha Seshadri who showed the feasibility of heating superparamagnetic iron oxide nanoparticles for the clinical application of thermal ablation of tumors [3]. Numerous experiments were done by Seshadri and Conklin in 2007 to measure the heating capabilities of these particles based on particle size and concentration under a variety of field strengths. Since the system used produces a magnetic field through induction the temperature rise was measured against current. Particle sizes of 10 nm and 100 nm were suspended in 2ml of glycerol at concentrations of 5.25mg/ml and 11.25mg/ml creating a homogeneous ferrofluid that was exposed to fields induced by currents of 100, 150, and 200 A. Only the results from the 10 nm particles will be shown since single domain particles are the focus of this paper.

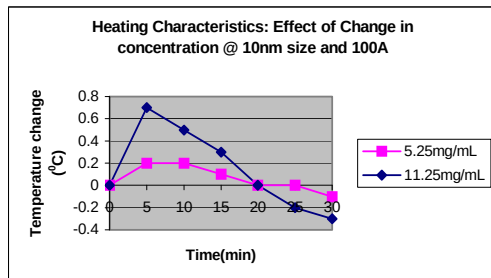


Figure 1: 10nm nanoparticles heated by 100A.

Figure 1 illustrates the heating effects on 10nm nanoparticles with 100A. It can be seen that the change in temperature is roughly 0.2°C after 5 minutes of exposure for a concentration of 5.25mg/mL while a rise of 0.7°C was observed over the same time period at double the concentration. Increasing the applied current to 150A had a much more significant effect, especially with the 11.25mg/mL solution as a Δt of 13.0°C was observed. This is in stark contrast to the 5.25mg/mL solution in which a 1.0°C rise was recorded. Figure 2 depicts this.

The final test was done using 10nm particles exposed to 200A. Figure 3 shows the tremendous increase in temperature after only 5 minutes as the solution raised almost 65°C from baseline at 11.25mg/mL. The halved concentration achieved a modest 25°C.

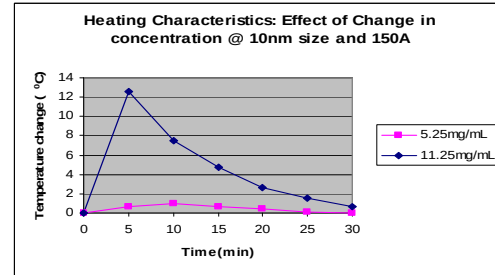


Figure 2: 10nm nanoparticles heated by 150A.

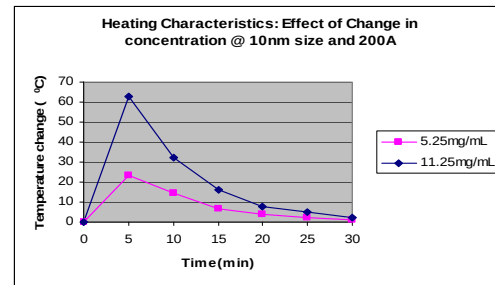


Figure 3: 10nm nanoparticles heated by 200A.

The results from this study clearly show the feasibility of using superparamagnetic iron oxide nanoparticles for hyperthermic purposes and provide the basis for the forthcoming discussion.

3. Introduction to Hyperthermia

The concept of hyperthermia dates as far back as 400 BC with the ancient Greeks. While first documented by Hippocrates, the Greek physician Parmenides gave his now famous quote “If I could create fever, I could cure all illness.”

The Romans, and more recently the Native Americans, used heat baths and sweat houses for medicinal ‘cleansing’ purposes [1]. These facts demonstrate that hyperthermia is an old idea that until the evolution of technology has been unrealizable for clinical practice.

Fever is the body’s natural response to disease and illness. Typically bacteria and viruses proliferate at the body’s core temperature and heat is one of the defense mechanism used to destroy any foreign agents. This heat increase can be attributed to both the working of the immune system in fighting off the pathogens as well as the body’s innate response to disease. Therefore, almost every human has experienced the effects of hyperthermia. For the context of this paper, however, hyperthermia will refer to the specific, local heating of tissue for clinical therapy.

Extensive work has been done to test hyperthermia as a potential treatment method for cancer, stemming from the pioneering work of R.K. Gilchrist in 1957. In his work [4], Gilchrist proposed the necessity for a new method of destruction of metastases present in lymph nodes. This need was precipitated by his evaluation of colorectal carcinomas extracted from patients who expired in the postoperative period. His solution involved injecting metallic particles into the lymph nodes surrounding the operative bed and subjecting them to an inductive based form of heating. While his heating mechanism was primarily based on dielectric loss due to alternating electric fields, his notion of controlling the necrotic effects through field strength and time of exposure were revolutionary.

More recent approaches to this problem involve the site specific heating of magnetic nanoparticles [5-18]. This method exploits the magnetic properties of the compounds as heat is generated through the magnetic losses experienced when the particles are placed in an alternating magnetic field. According to the National Cancer Institute (NCI), hyperthermia kills neoplastic cells, with minimal necrosis to the surrounding healthy tissue, if the temperature is elevated to the therapeutic range of 42°C – 45°C [2]. However, hyperthermia generally needs to be combined with traditional cancer therapies like irradiation and chemotherapy for the best, most reliable destruction of cancerous cells [5,7,12].

4. Applications of Magnetic Nanoparticles

Magnetic nanoparticles, specifically iron-oxide nanoparticles, have been researched extensively for clinical treatments due to their ease of synthesis and biocompatibility. Biocompatibility is arguably the most critical property of these compounds since irradiating tissue for hyperthermic purposes is stressful enough on the body. By ensuring that the magnetic agents are readily cleansed from the bloodstream, without adverse effects, by the reticulo-endothelial system (RES) additional sources of potential patient trauma can be eliminated. The RES is a component of the immune system responsible for identifying and organizing an immune response against all foreign antigens present in the body through the mobilization of monocytes and macrophages (tissue phase monocytes), collectively referred to as phagocytes. They typically aggregate in the lymph nodes and spleen and are essential for provoking an appropriate reaction against pathogens and choreograph much of the immunologic response to pathogens and neoplastic cells. However, despite the physiological marvels performed by the RES in maintaining proper synchronization of the body's

defenses, they pose a problem for many magnetic nanoparticles when active targeting (i.e. when conjugated with specific antibodies for site specific delivery) as they efficiently cleanse exogenous particles and the therapeutic agents (nanoparticles) from the bloodstream before reaching the neoplasm. There are several solutions to this problem such as coating the particles with a carbohydrate shell to 'mask' them from the phagocytes for a longer period of time thereby increasing their blood-half life as described in [20]. This topic will be investigated in more detail below.

Now that it has been established that iron-oxide nanoparticles create no adverse effects when injected into the bloodstream, it is natural to study their applications as they apply to medicine. Q.A. Pankhurst and others outlined the potential for using such agents and demonstrated their versatility among a wide variety of tasks [16-18]. These include the aforementioned hyperthermia as well as therapeutic gene and drug targeting systems, magnetic resonance imaging (MRI) contrast agents, and magnetic separation techniques between biomolecules and labeled cells. Magnetic separation is an interesting concept that could offer an effective mechanism for filtering out desirable and undesirable moieties within the human body. By 'tagging' these entities with magnetic labels through the use of antibody (or equivalent) coated iron-oxide nanoparticles and exposing them to a magnetic field gradient, the unwanted materials can be washed away while the magnetically tagged particles are typically filtered through steel wool for collection [17]. It is important to note that the field gradient must be sufficiently strong to overcome the hydrodynamic drag force experienced by the particle.

While the prospect of magnetic separation is interesting, hyperthermia and drug delivery are the most relevant topics for achieving our goal. In terms of hyperthermia, this article indicates that a tumor be heated above 42 C for 30 minutes for complete tumor ablation. This heating of superparamagnetic particles comes from the relaxation of magnetic moments and can be delivered to targeted areas through the use of biocompatible compounds, like dextran, with functional molecules or ligands for effective attachment of antibodies. This method of delivery is ideal in that it minimizes the amount of drug distribution to healthy areas while also reducing the required dosage. Unfortunately, for our purposes these compounds must be able to pass through the blood-brain barrier which has proven difficult. Another significant issue revolves around ensuring that the target site receives the required concentration

of magnetic particles. Despite the fact that the compound will be given intravenously, there is no guarantee that all of the SPM particles will reach its destination. This presents another challenge in that a lower concentration will require significantly more field strength or specific absorption rate (SAR) to generate the appropriate amount of heat, which may not be clinically acceptable for human patients. Lower field strength coupled with a lower frequency may render this technique useless [17].

Another variant to the previously mentioned methods of inducing hyperthermia within cancerous cells is magnetic fluid hyperthermia (MFH). MFH is a colloidal dispersion of magnetic nanoparticles to be used in the intravenous circulation of induction based heating agents. The size and magnetic properties of the particles greatly influence the amount of heating. Heating due to multi-domain ferromagnets is a consequence of hysteresis loss. In the presence of an alternating magnetic field, the domain magnetization of these particles is aligned with the field creating domain wall displacements. This is in stark contrast to superparamagnetic heating where domain walls do not exist. In this situation, the AC magnetic field supplies the necessary energy for these particles to overcome the energy barrier associated with magnetic moment reversal, also called Neel relaxation. The magnetic field also exerts torque on the moments subsequently creating frictional heat losses as the particles rotate in the suspending medium (Brownian) [16]. These concepts are explored in detail later.

In terms of its usefulness as a contrast agent, superparamagnetic nanoparticles exhibit higher relaxation than typical Gadolinium ions. This is due to the sub-domain characteristics of these single moments particles. While SPIO nanoparticles lose their magnetization when not in the presence of a magnetic field, the magnetic moment is significantly higher than paramagnetic materials. This creates a negative contrast, or signal reduction, on T2 weighted images enabling an understanding of the biodistribution of the particle [16, 21].

All of these applications exploit the same properties specific to iron-oxide nanoparticles, which are central for their potential use as clinical agents.

- Iron-oxide nanoparticles are easy to synthesize and have controllable size ranges spanning the nanometer scale. This implies that the particles are small enough to interact at a cellular level since their size enables them to pass

through membranes and other lipid based barriers while also being able to adhere to antigens through targeted delivery methods.

- Since the nanoparticles are magnetic by nature, they can be manipulated through typical electromagnetic field theory. In the presence of an alternating magnetic field these sub-domain, or superparamagnetic, particles are easily excited by the irradiating field thereby providing the particles with enough energy to overcome the barrier associated with magnetic moment reversal. Heat is generated by the energy dissipated upon the particle's return to the equilibrium state. An understanding of the physics behind this process enunciates the importance of choosing particles that are paramagnetic by nature.

5. Experiments

5.1. Experimental Heating Agents

In terms of targeted delivery of the SPIO nanoparticle, a molecule must exist that exhibits certain characteristics, namely biocompatibility, stability, long blood half life, and magnetic under the presence of an induced RF field. While Feridex, which is already an FDA approved agent, and monocrySTALLine iron oxide nanoparticle (MION) are viable candidates for such a task, the particle of primary interest is a crosslinked iron oxide (CLIO). Feridex is a 1st generation magnetic nanoparticle that contains a thin coating of dextran. Dextran is a polysaccharide comprised of glucose chains of variable lengths. This compound essentially increases molecular stability while also adding another level of biocompatibility. However, Feridex has a tendency to form polycrystalline clusters, which are quickly removed from the blood by macrophages and phagocytes in the reticulo-endothelial system (RES) [21]. In a pharmacokinetics study on SPIO particles, as outlined in [22], the effects on distribution, metabolism, bioavailability, and toxicity due to these particles are examined. It was found that the AMI-25 particle, the first SPIO approved for clinical trials, amassed relatively quickly in the liver and spleen. This seems to suggest that its blood half life is short due to the rapid response of the reticulo-endothelial system in clearing it from the blood. Similar work done by Weissleder showed that the blood half life of AMI-25 was 8 min; far below the average for most pioneer ultrasmall superparamagnetic iron oxide (USPIO) particles at 81

minutes [20]. Equally important was the observation that the iron contained within the molecule's core was broken down and utilized by new red blood cells in the form of hemoglobin. In addition, no adverse effects (ie. death or tissue damage) were noted at high levels of injection in both rats and beagles implying that toxicity is not an issue [22]. These facts characterized the bioavailability and potential use for SPIO particles as contrast agents for MR imaging and other applications due to the biocompatibility of iron-oxide particles.

While these advances were made almost 20 years ago, researchers have nonetheless continued to persist and the 2nd generation of magnetic nanoparticles was developed. Such particles consist of MION and CLIO. The primary difference between MION and Feridex is that MION have a more robust dextran, or polymer, coating and its molecular mass remains constant in solution. When coupled with roughly a 24 hour blood half life in humans, the ramifications are deeper tissue penetration due to the particle's ability to camouflage itself from phagocytes [21]. This is clearly advantageous, especially when dealing with brain tumors where the blood brain barrier (BBB) and skull provide the organ with additional layers of protection. The chemical structure of MION is a superparamagnetic iron oxide core typically 3-5 nm in diameter coupled with the dextran coating of variable size. Due to the restriction presented by the blood-brain barrier, a particle that minimizes the thickness of the dextran coating is optimal. Work has been done to increase the stability of such magnetic nanoparticles as outlined in [23]. In this work, MION was reacted with ammonia and epichlorohydrin to form a dextran cage about the iron oxide core creating a substance known as CLIO. As previously mentioned CLIO is chemically stable, however an additional benefit to crosslinking is that the compound can now be conjugated with ligands such as antibodies and radio tracers for targeted delivery of the superparamagnetic particles. This is of the utmost importance for the application being presented throughout this paper and for determination of the appropriate experimental hyperthermia agent.

5.2. Synthesized Heating Agents

For the purposes of this experimental procedure, a 2nd generation superparamagnetic nanoparticle, MION, was synthesized for a comprehensive characterization of its heating properties following the U.S. Patent by Palmacci, et al. which details the synthesis of polysaccharide covered superparamagnetic oxide colloids [24]. Below is a description of the steps to achieve this goal in the context of this preparation:

- 1) ~100mL of a 0.2 micron filtered aqueous solution of 45g of dextran and 3.155g FeCl_3 (ferric) was cooled to 2-4°C.
- 2) To this mixture, a freshly prepared solution of 4mL H_2O and 1.266g FeCl_2 (ferrous) was added and filtered through a 0.2 micron screen.
- 3) While the above solution is being vigorously stirred, 4.5mL of 29.7% $\text{NH}_4\text{OH}[\text{H}_2\text{O}]$ (cooled to 2-4°C) is added dropwise to said solution.
- 4) The solution was then heated to 81°C over a 1-hr heating interval and was maintained between 75°C<T<86°C for an additional 75min while being stirred.
- 5) Dialysis was run 4 times of the final solution to remove any impurities, such as excess $\text{NH}_4\text{OH}[\text{H}_2\text{O}]$, dextran, and NH_4Cl .

5.3. Iron Assay

In an effort to determine the iron concentration, an important factor for both heating and FDA approval, an iron assay was performed. This was accomplished by preparing a series of fresh iron standards at predefined concentrations. These standards were the result of reacting 0.2M $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ with 1N HCl. 10 μL of each standard, as well as several samples of known and unknown concentrations, were then added to a 6N HCl solution containing 0.3% H_2O_2 . The solutions were left to rest for an hour to ensure proper reaction and their absorbance was read at 410nm using an Ultrospec III UV scanning spectrophotometer. The absorbance of each standard was recorded and plotted against its concentration. For those preparations with unknown concentrations, the iron content was calculated by 'fitting the curve' against known standards. The plot of results is shown in Figure 4.

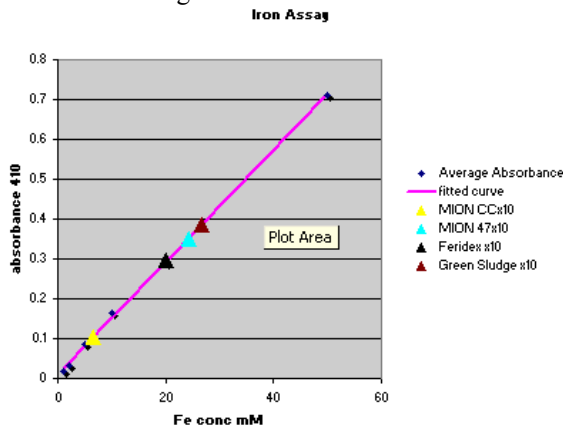


Figure 4: Iron Assay of synthesized MION.

Analysis of the plot yields concentration values for 4 iron-based colloids detailed in Table 1.

Table1: Summary of iron concentrations.

	Concentration (mg/mL)
MION 47	13.6 (11.2 advertised)
Feridex	11.3 (11.2 advertised)
MION CC (synthesized particles of interest)	3.6
Green Sludge (particles w/o dextran)	14.9

In Table 1, it is important to note that MION CC was not concentrated. Doing so would increase concentration 3 times.

5.4. Mathematical Cooling Model

While the overall goal of this project is to develop a comprehensive model for estimating temperature rise with variable parameters, the current data set being worked with does not promote such a system. However, given the extensive cooling data collected by Seshadri and Conklin an accurate cooling model can be implemented using the standard heat equation. Despite the fact that this PDE is only concerned with changes in one direction (x), it is the logical place to start.

$$\rho c_p \frac{\partial u}{\partial t} = k \frac{\partial^2 u}{\partial x^2} \quad 0 \leq x \leq l$$

To best replicate the conditions of the physical system currently in place in our labs the initial conditions and boundary values are set to the following:

$$\begin{aligned} \frac{\partial u}{\partial x}(0, t) &= 0 \\ \frac{\partial u}{\partial x}(l, t) &= -A(u(l, t) - T_0) \\ u(x, 0) &= f(x) \end{aligned}$$

where the $x=0$ condition represents the insulated end of the test tube within the induction coil and $x=l$ is the open, or irradiating end. $f(x)$ the function responsible for representing the temperature distribution function, which in this case will be assumed to be homogenously distributed with the temperature at the insulated end being 5°C hotter than the surface temperature.

For ease of mathematical manipulation all of the above equations were normalized for x , distance along the tube, values between 0 and 1 using the following substitution:

$$\hat{x} = \frac{x}{l} \quad 0 \leq \hat{x} \leq 1$$

In addition, the temperature is also represented as the difference between the actual temperature and the ambient temperature, which again is denoted as u for notational simplicity. This yields the equations in the normalized form as:

$$\frac{\partial u}{\partial t} = \hat{k} \frac{\partial^2 u}{\partial \hat{x}^2} \quad 0 \leq \hat{x} \leq 1 \quad \hat{k} = \frac{k}{\rho c_p l^2}$$

$$\frac{\partial u}{\partial \hat{x}}(0, t) = 0$$

$$\frac{\partial u}{\partial \hat{x}}(l, t) = -\hat{A}u(l, t) \quad \hat{A} = Al$$

$$u(x, 0) = f(x)$$

The above partial differential equation represents the cooling process of the fluid in the test tube with two unknown parameters, $\hat{k}$ and $\hat{A}$. In the rest of this section, an attempt will be made to estimate these two unknown parameters using experimental measurement data.

Using standard methods of solving partial differential equations, it can be shown that the solution of the above model can be expressed as

$$u(x, t) = \sum_{n=1}^{\infty} c_n e^{-\alpha_n^2 \hat{k} t} \cos(\alpha_n x)$$

$$u(x, 0) = f(x) = \sum_{n=1}^{\infty} c_n \cos(\alpha_n x)$$

$$c_n = \frac{\int_0^1 f(x) \cos(\alpha_n x) dx}{\int_0^1 \cos^2(\alpha_n x) dx}$$

where α_n satisfies the equation

$$X'' + \alpha_n^2 X = 0$$

$$X'(0) = 0$$

$$X'(1) + \hat{A}x(1) = 0$$

$$\hat{A} = \alpha \tan(\alpha)$$

$$X_n(x) = c_n \cos(\alpha_n x)$$

Assuming that there is only one term in the series (i.e. no second order harmonics)

$$u(x, t) = c_1 e^{-\alpha_1^2 \hat{k} t} \cos(\alpha_1 x)$$

$$u(1, t) = c_1 \cos(\alpha_1) e^{-\alpha_1^2 \hat{k} t}$$

$$u(0, t) = c_1 e^{-\alpha_1^2 \hat{k} t}$$

$$u(1, 0) = c_1 \cos \alpha_1 = T_1$$

$$u(0, 0) = c_1 = T_2$$

With the assumption that $T_2 = T_1 + 5$, then

$$c_1 = T_2$$

$$\cos \alpha_1 = \frac{T_1}{T_2} \therefore \alpha_1 = \cos^{-1}\left(\frac{T_1}{T_2}\right)$$

Finding α_1 enables for the determination of the thermal constant $\hat{k}$ by calculating the slope of the temperature $u(l, t)$ on a logarithmic scale. Using all of the above derivations yield a theoretical temperature cooling curve, as shown in Figure 5.

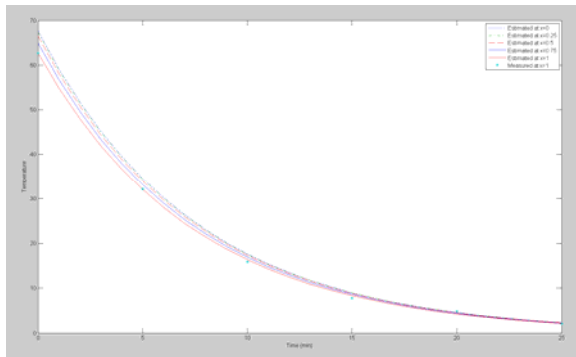


Figure 5: Theoretical cooling curve

As can be seen, the estimated temperature at $x = 1$ (surface of tube) closely mirrors the measured empirical data. The unknown coefficients for two of the legitimate data sets are shown in Table 2.

	Alpha	Log(temp) Slope	Thermal Constant (slope*1 ² / α ²)	Transfer Coefficient (α *tan(α)/1)
10 nm, 11.25 mg/ml, 200A	0.386 7	-0.1344	0.0022	3.1499
100 nm, 11.25 mg/ml, 200A	0.369 0	-0.1225	0.0023	2.8532

Table 2: Estimated coefficients for 2 accurate data sets

6. Conclusion:

Given all of the advances in the area of clinical hyperthermia over the past 20 years, it is not unrealistic to expect that sometime in the near future the use of superparamagnetic nanoparticles can be used to both characterize a tumor (through its use as a contrast agent) in terms of size and location while subsequently doubling as a therapeutic agent to immediately ablate the tumor through controlled RF exposure. The results shown for the model in development is the first step in realizing this goal of potentially providing a non-invasive modality of cancer treatment for the effective elimination of brain tumors.

Then given the accuracy of the results shown in Figure 5, the next steps involve the modification of the current physical system. This includes the implementation of a continuous data acquisition system that monitors both the surface temperature through the use of an IR thermometer as well as a thermocouple strategically placed near the bottom of the tube. By gathering data at regular intervals over the course of a 5-10 minute heating session and an additional 20-25 minute cooling period, a more comprehensive model can be developed that accurately estimates the heating effects of changing field strength, time of exposure, and frequency. All of this information will be critical in terms of getting FDA approval for future clinical use in patients with brain tumors.

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