

# Magnetic Nanoparticles: An Optimization Design Approach for a Targeted Drug Release Using an AC Magnetic Field

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## ABSTRACT

*Magnetic Nanoparticles while very versatile, show a promising and innovative method in drug targeting to help increase efficacy. The optimization of magnetic nanoparticles through the application of an AC magnetic field will enhance the release of cytotoxic drugs at the target area whilst preventing effects on healthy tissues. This article presents a study that describes the development of a Manganese Iron Oxide ( $MnFe_2O_4$ ) magnetic nanoparticle showing a therapeutic effect on T47D cells using Doxorubicin in a DC magnetic field. It also introduces and dissects the core of magnetic nanoparticles, its applications as well as the breakdown of a design approach that can be employed to enhance drug delivery to localized sites. In addition, it shows a comprehensive technique through mathematical modeling and discusses the AC susceptibility of these unique drug vehicles; magnetic nanoparticles.*

## Keywords

Nanobiotechnology, Magnetic Nanoparticles, Magnetic Hyperthermia, Ferromagnetic Nanoparticles, Superparamagnetic Nanoparticles, Drug Targeted Delivery, AC susceptibility, Neel Relaxation, Brownian Relaxation, Alternating Magnetic Field, Relaxation Loss, Optimization Design, AC Magnetic field, DC Magnetic field, Toxicity, Therapy, Heat Dissipation, Frequency, Magnetic Anisotropy, Boltzmann Constant, Surface Absorption Rate, Cytotoxic drugs, T47D cells, Doxorubicin.

## Introduction

Nanobiotechnology has many proven prospects- from biosensors, drug delivery, and microfluidics to tissue engineering. One of the many advantages is that these nanostructures usually sized from 1-100nm can directly interact with tissues on a molecular level based on their engineered designs. This allows these nanoparticles to exhibit their electrical, mechanical, chemical, structural, biological and magnetic properties. Pertaining to the use of drug delivery, they are able to stay in the blood circulatory system for a long time, enabling a slow release of drug per dose, and thus use their nanostructures to target diseased cells for reduced drug side effects [1].

Magnetic Nanoparticles (MNPs) are another revolutionary echelon of nanostructures that are making great strides in drug delivery therapies and diagnostics such as detection and treatment of cardiovascular disease, neurological diseases and cancer. There are various chemical compositions of MNPs that have been explored in biomedical application to enhance the magnetic moments and superparamagnetism which overall optimizes the response from its nanoscale magnetic phenomena. The structure of the MNP can be dissected into an inorganic nanoparticle core and a biocompatible coating surface (poly-ethylene glycol, starch, block co- polymers); the suitable surface coating provides stabilization and improved surface functionality [2-4]. The magnetic properties of materials can be characterized by their magnetic susceptibility ( $\chi$ ) which is the ratio of the induced magnetization ( $M$ ) to the applied magnetic field ( $H$ ). The magnetic susceptibilities of diamagnetic materials are small and negative because the magnetic moment is antiparallel to  $H$  and hence will not retain their magnetic ability when the external magnetic field is removed. On the other hand, paramagnetic materials have magnetic moments aligned parallel to the applied magnetic field and exhibit magnetic susceptibilities from  $10^{-6}$  to  $10^{-1}$ . Additionally, ferri and ferromagnetic materials

have magnetic moments aligned parallel to the applied magnetic field. Furthermore, ferromagnetic MNPs in very small sizes (in order of tens of nanometer) tend to become a single magnetic domain with one magnetic moment. In spite of this, at high temperatures (hyperthermia), the thermal energy induces a free rotation of the particle instituting a loss of net magnetization in the absence of magnetic field. This property ensures that the particles have colloidal stability and prevent aggregation in applications. Exploiting the superparamagnetic property from a single magnetic domain improves the magnetic susceptibility and performance of the MNP better than paramagnetic particles.

While superparamagnetism is desirable, however, the decrease in particle size generates its fair share of drawbacks such as non-collinear spins, spin canting and spin-glass-like behavior- affecting its magnetic property. Lee et al showed that MnFe<sub>2</sub>O<sub>4</sub> was non-toxic in-vitro with great magnetic susceptibilities. Coupling a cytotoxic drug such as doxorubicin (DOX) or Camptothecin to a biocompatible MNP and attracting them with an external magnetic field for a targeted release reduces the side effects of the chemotherapeutic drug on healthy tissues [3]. The thermo-responsive triggering mechanism is a way the drug is released from the magnetic particle. During exposure to an alternating magnetic field (AMF) or AC, the MNP generates heat from hyperthermia effect; increasing the temperature of the carrier particle. This rise surpasses the phase transition temperature of the thermosensitive polymer, instigating the release of loaded drugs after the structure swells and softens. Magnetic Nanoparticles under alternating magnetic fields create heat from Brownian and Neel relaxation together with the hysteresis loss. This effect is utilized to heat up diseased cells to at least 40 degrees Celsius for a stimulated drug release [5].

Electromagnetic radiation, made of magnetic and electric energy propagates at the speed of light-interacting with living tissues during exposure and causing heating of the absorbed area. This can be quantified physically as the Specific Absorption Rate (SAR), measured in units of W.kg<sup>-1</sup>. However, in magnetic hyperthermia, this magnitude of power would be partially absorbed by the magnetic nanoparticles, not by the cells/tissues in its entirety at dominating frequencies (i.e. for which only the electrical interaction is high) [6].

Since most in vitro and in vivo experiments are expensive and have some limitations, there is the need to develop an optimization design and explore mathematical modeling methods that explain the parameters that result in the targeted release of drugs from the magnetic nanoparticle. This paper presents an efficient method for a targeted delivery in an alternating magnetic field.

### Classes of Magnetic Nanoparticles

Magnetic Nanoparticles can be classified into 4 major areas;

- Metal Nanoparticles (Fe, Co)
- Alloys (Au/Fe, Fe/Co)
- Oxides ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>, Fe<sub>3</sub>O<sub>4</sub>, NiO)
- Ferrites (CoFe<sub>2</sub>O<sub>4</sub>, NiFe<sub>2</sub>O<sub>4</sub>, MnFe<sub>2</sub>O<sub>4</sub>, ZnFe<sub>2</sub>O<sub>4</sub>)

Cobalt nanoparticles, FeCo alloys and ferrites all show great magnetic properties that allow one to obtain magnetic resonance spectroscopy data. In addition, they add to the heating properties in magnetic fields for great drug delivery applications. Though nickel and cobalt are susceptible to oxidation which make their compounds toxic, they have very diminished usage for biomedical purposes. Their toxicities have been reduced by using polymer coatings such as polyvinyl alcohol, polyvinylpyrrolidone and polyethylene glycol). They have been found to show insignificant cytotoxicity at concentrations up to 150µg/mL.

Moreover, the toxicity of iron and its oxides have shown lower tendencies which ensue a limited use. A polymer coating, biopolymer nano-skin has been suggested as a solution to eliminate the oxidation process with external media.

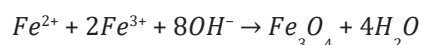
The most commonly used magnetic nanoparticles are Superparamagnetic Iron Oxide Nanoparticles (SPIONs)-magnetite (Fe<sub>3</sub>O<sub>4</sub>) or maghemite ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>). These are primarily used due to low toxicity, low-cost, superparamagnetism and biocompatibility [7].

### SPIONs Synthesis

There are various methods used in the synthesis of superparamagnetic iron nanoparticles (Figure 1). These are discussed below;

#### Chemical Methods

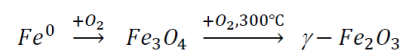
This is one of the most commonly used method for the synthesis of SPIONs nanoparticles. It involves the slow addition of a precipitating agent (aqueous solution of hydroxide, ammonia) to an aqueous solution of ferric and ferrous salts in a molar ratio of 2:1.



*Coprecipitation method of SPIONs synthesis.*

Oxidation of iron nanoparticles can also produce magnetite nanoparticles. It is obtained from the oxidation of iron and magnetite nanoparticles with atmospheric oxygen.

This reaction is an oxidation of magnetite to maghemite with atmospheric oxygen at 300°C.



*Oxidation Method of SPIONs synthesis.*

Thermal decomposition method produces maghemite nanoparticles with a small size. It involves a continuous heating of a solution made of a solvent, surfactants with the precursor compound subjected to a predefined temperature from which the nanoparticles begin to aggregate and grow. This is described as the “heating-up approach”. Alternatively, the “hot- injection” approach seeks to instigate a rapid and uniform nucleation; by the introduction of reagents into a hot solution of surfactant and finished with a controlled-growth phase [7].

## Physical Methods

There are two main ways that are being used in producing magnetite nanoparticles; the bottom-up and the top-down methods (Figure 1).

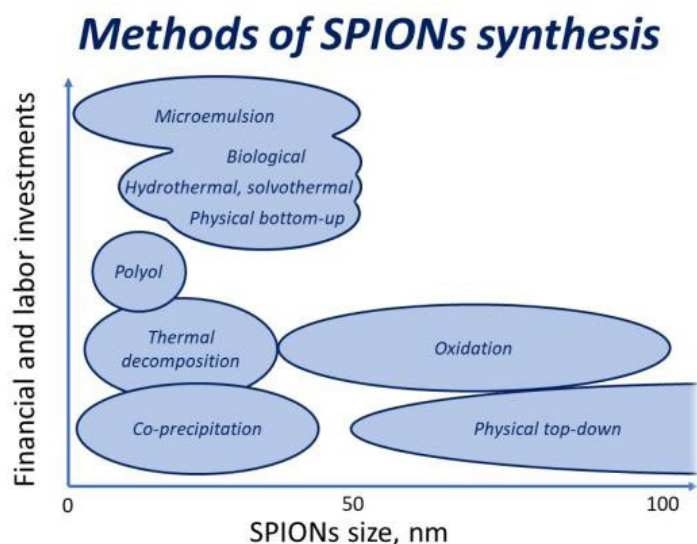
The top-down procedure requires the milling of the magnetic material down to a nanometer range. The bottom-up approach involves the laser evaporation of micron sized metal oxide powders to obtain the iron nanoparticles. This results in a sudden sharp temperature difference outside the evaporation zone while condensation and nucleation occur from the gas phase.

Particles of sizes 20 to 50nm are formed from this process. The major draw-back of these methods is the difficulty in size regulation and shapes of the nanoparticles [7].

## Biological Methods

Biomineralization is a biological process where a magnetotactic bacteria moves along geomagnetic fields with Magnetosomes contain nanometer-membrane protein-coated crystals which are formed by the accumulation of iron and deposition of a mineral particle of a specific size and orientation. Uniform particles sizes with core diameter of 20 to 45 nm can be formed under conditions of anaerobic synthesis in the laboratory similar to magnetotactic bacteria in normal living conditions. Even though magnetotactic bacteria exhibits great magnetic properties, however, they are yet to be used in medicine [7].

*other methods being used in creating superparamagnetic nanoparticles include thermal decomposition, microemulsion and polyol (Figure 1).*



**Figure 1:** Schematic describing SPIONs size and their financial/labor intensity.

## Toxicity of SPIONs

Over the years, SPIONs have demonstrated a great prospective efficacy in biomedical applications due to their low toxicity

rates in the human body. It has been found that CuO, TiO, ZnO, CuZnFe<sub>2</sub>O<sub>4</sub>, Fe<sub>3</sub>O<sub>4</sub> and Fe<sub>2</sub>O<sub>3</sub> metals show very little to no toxicity at concentrations of 20 to 100 ug/mL. Studies from *in vitro* experiments report that the toxicity levels depend on the cell type being tested and the availability of polymer shell coating on the iron oxide.

It should be noted that, during the synthesis of superparamagnetic nanoparticles of iron oxide, they would possess a possible outcome of being toxic. These nanoparticles have large surface areas that contain metal ions of variable valence that lead to formation of reactive oxygen species (hydrogen peroxide H<sub>2</sub>O<sub>2</sub>, superoxide anion of radical O<sub>2</sub>, hydroxyl radical OH). In turn, when reactive oxygen species are absorbed by cells, they activate anti-inflammatory mediators that could ultimately cause oxidative stress. Alternatively, the reactive oxygen species can damage healthy cells through lipid peroxidation, impaired signal transduction, protein changes, lipid peroxidation and modulation of gene transcription from macromolecule reaction which leads to cell death or apoptosis. To add to this, even though the dose of administered iron oxide nanoparticles for magnetic targeting is usually 1.25 -5% of the total iron supply in the body, this leads to the retention in target organs. As a result, the overload causes high levels of iron ion concentration and consequently to cytotoxicity, oxidative stress and inflammatory processes leading to DNA damage. However, SPIONs have low toxicity in-vivo which depend on their area of localization in various tissues. Moreover, the introduction of SPIONs into the human body does not only result in toxic effects, but also provide great health benefits based on levels of concentration (not excessive). They can serve as sources of iron for the body and aid in the formation of hemoglobin, lymphocytes, enzymes, thyroid hormones and other regulatory processes (toxins neutralization and conduction of nerve impulses) [7].

## Applications of Magnetic Nanoparticles

There are diverse applications of magnetic nanoparticles for biomedical purposes that have shown great prospects. They span from the therapeutic domain to diagnostics. In therapy, they include hyperthermia, target drug delivery and tissue engineering. Some diagnostic applications include magnetic resonance spectroscopy, immunoanalysis and bioseparation and purification (magnetic separation of proteins, cells and nucleic acids). In view of this, due to the objectives of this paper, magnetic hyperthermia and targeted drug delivery are areas to throw light on [7].

## Magnetic Hyperthermia

The inefficiency of causing cell death in cancer cells locally with minimal temperature of 40-43°C through hyperthermia can be curbed by the intravenous administration of magnetic nanoparticles. Subsequently, this aims at the tumor site after accumulation and proceeded by the application of an alternating magnetic field. This enhanced method is able to increase cell death at tumor sites without causing damage to healthy surrounding tissues. Magnetite or maghemite nanoparticles are known to be mostly used for magnetic hyperthermia.

Magnetic nanoparticles can be delivered to the tumor through intratumoral, intra-arterial, intravenous and intracavitary means. However, the most versatile form of delivery has been intravenous administration. Oral administration is not feasible since most of the nanoparticles will be excreted. Intratumoral, intra-arterial and intracavity are different administration techniques suited for specific cases.

Moreover, after delivery, the accumulation of magnetic nanoparticles of iron oxide in malignant areas are dependent on the effect of increased permeability and retention. In addition, this effect shows the susceptibility of the nanoparticles to primarily accumulate in target sites due to their permeability of vasculature and reduced lymphatic drainage. To enhance this effect, target ligands receptors on the surface of tumor cells can aid the absorption of the nanoparticles in target areas. This leads to the accumulation in the tumor for an efficient targeted heating under a magnetic field to preserve near-by healthy tissues [7].

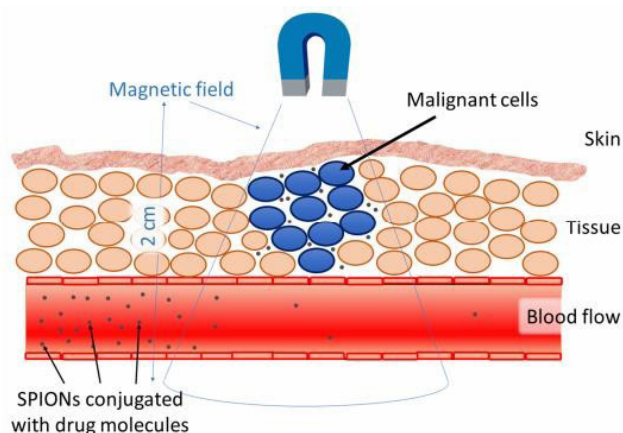
### Targeted Drug Delivery

Targeted drug deliveries have been explored for their promising prospects and results. There are two kinds of targeted drug deliveries; passive targeting and active targeting. Passive targeting consists of medicinal substances and carriers which are provided by the increase in permeability of capillaries in a lesion. Whereas active targeting delivery systems involve vector molecules like enzymes, hormones, antibodies, glycolipids, peptides and viruses. The most used active drug delivery carriers include carbon nanotubes, micelles, polymers, erythrocytes and others. The drawbacks of using organic nanoparticles such as liposomes and polymer nanoparticles are reduced mechanical and chemical swelling, susceptibility to microbiological attacks, low control rates in release of drugs and high cost. For an effective drug delivery modality, the magnetic nanoparticle carrier must be biocompatible and also non-toxic. The size of the magnetic nanoparticle is a vital physical element that not only affects the magnetic properties, charges and the chemistry but also hugely influences the bioavailability of particles in the body as a system. Larger sizes in range of 200nm or more are excreted by the spleen and liver whilst smaller particles ranged from 10nm or less are discarded by the renal discharge [7].

The optimal operating size for superparamagnetic iron oxide nanoparticles is 10 -100nm. There are two methods that are currently used in loading drugs onto the magnetic nanoparticle.

These include; physical and chemical types of loading. The physical method comprises of compounding a drug substance with a carrier through physical interactions, that is, hydrophilic or hydrophobicity and affinity. On the other hand, the chemical binding method is the conjugation of a drug substance with a carrier using chemical bonds such as covalent bonds formed due to carboxyl, amino and thiol groups on the surface of the magnetic nanoparticle and the therapeutic drug. The polymer coating from polyethylene glycol, dextran polyetherimide or chitosan allows the bonding of the functional groups and also enhances the biocompatibility of the drug carrier system [7].

One of the growing concerns of using targeted drug delivery through magnetic nanoparticles is the restriction of blood flow in the targeted area after accumulation of the drug nano-systems. Due to this, very high, strong magnetic fields are needed to retain these particles in larger arteries (Figure 2).

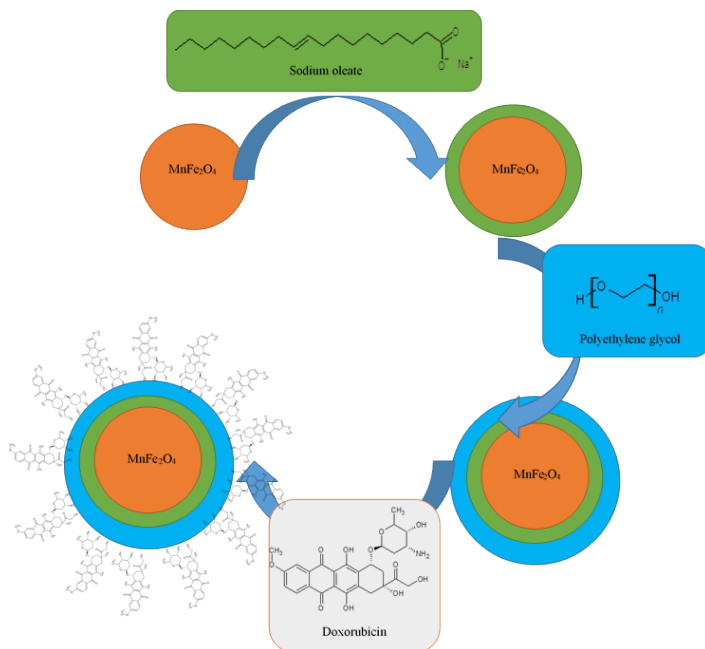


**Figure 2:** A Schematic for Targeted drug delivery using a magnetic field.

### Experimentation

#### Preparation and Analysis of $MnFe_2O_4$ -MNP

The  $MnFe_2O_4$  had a diameter of 60nm and density of 5.368g/cm<sup>3</sup>. To create the novel delivery system, a polymer coating was applied to the MNPs to couple Doxorubicin (DOX). This polymer coating consisted of an inner coating of Sodium Oleate (NaO) and an outer coating of Polyethylene glycol (PEG). The PEG coating provided an -OH residue that facilitated coupling to the amine group of the DOX molecule as shown in Figure 3.



**Figure 3:** DOX-MNP Pegylation and dox-coupling schematic. Graphic representation of the DOX-MNP production process. Chemical structures of sodium oleate, polyethylene glycol and doxorubicin are illustrated in the order in which they are applied to the DOX-MNP.

### Treatment of TD47 cells

To evaluate the effectiveness of the MNP delivery system, T47D human ductal epithelial breast cancer cells were treated with several combinations of MNPs, DOX and a DC magnetic field exposure. T47D cells were cultured in 48 well plates at a density of  $1.6 \times 10^6$  cells/well in 500  $\mu$ L RPMI-1640 supplemented with FBS, sodium pyruvate and streptomycin-penicillin until 50% confluent. The 160  $\mu$ g solutions of MNP-Dox, PEG-MNPs and nMNPs were resuspended in 160  $\mu$ L of supplement-free RPMI-1640 media. A stock solution of doxorubicin was prepared in supplement-free RPMI-1640 to match the final doxorubicin concentration coupled to the Dox- MNPs. Once 50% confluency had been reached, 486  $\mu$ L of fresh supplemented RPMI-1640 medium was added to each well. Each well was then augmented with 4  $\mu$ L of each treatment group (Dox, Dox-MNPs, PEG-MNP, nMNP) in quadruplicate so that MNP and DOX concentrations were equal where applicable. In addition, 10  $\mu$ L of 1X magfectin in non- supplemented medium was applied to each of the MNP treatments. Culture plates were then placed upon a super magnetic plate and incubated under standard conditions for 0-90 minutes. MTT assay was then performed to quantify the effect of each treatment on cell viability.

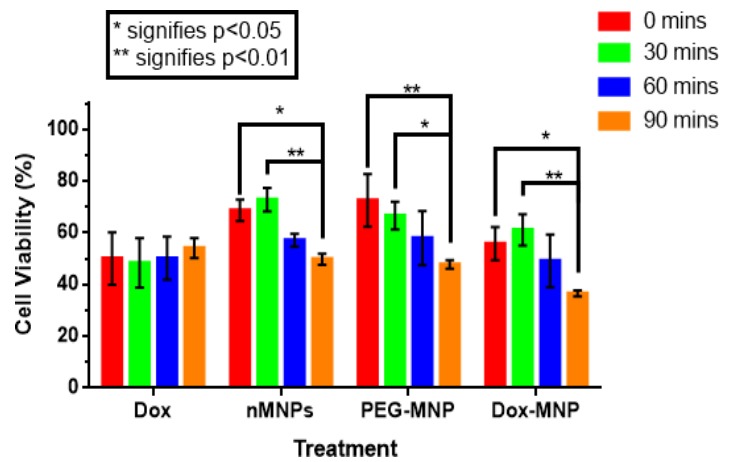
### Statistical Analysis

The mean  $\pm$  standard deviation for cell viability of each treatment was determined from each set of triplicates/quadruplicates. A one-way ANOVA was utilized to determine any significance between the mean MNP sizes after each stage of the polymer coating process. A fixed effect two-way ANOVA will be executed and Tukey's post-hoc test will be utilized to determine significance between each factor of the treatment groups. The factors utilized for the two-way ANOVA consist of magnetic field exposure time and actual treatment applied. Statistical tests were performed on Graphpad Prism software.

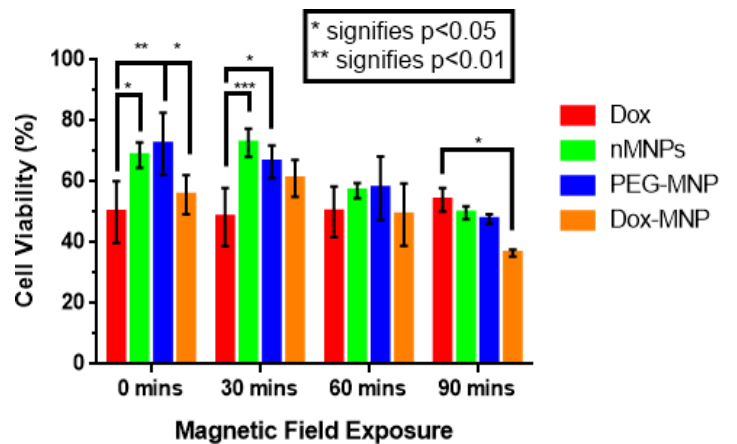
### Results

T47D breast cancer cells were treated with DOX alone, nMNPs, PEG-MNPs and DOX-MNPs along with exposure to a DC magnetic field for 0, 30, 60 or 90 mins (Figure 4). The cytotoxicity of each treatment was determined using the MTT assay. Cells were treated with DOX alone at a concentration of 1.8  $\mu$ M. The DOX-MNP treatment also had a total concentration of 1.8  $\mu$ M coupled to  $6.25 \times 10^9$  DOX-MNPs. PEG-MNPs and nMNP treatments were performed at the same density as the DOX-MNPs. Following magnetic field exposure, the cells were incubated for 72 hrs.

The most effective treatment was DOX-MNPs after 90 min magnetic field exposure, where a mean cell viability of 36.52% was observed (Figure 4). PEG-MNPs and nMNPs were not found to be significantly different at each of the magnetic field exposure times. DOX alone and DOX- MNPs were not significantly different until the 90 min magnetic field exposure time. These results suggest that a vehicle made using  $MnFe_2O_4$ -MNPs coated with PEG and coupled with DOX increases the efficacy of the drug after 90 min magnetic field exposure. PEG-MNPs and nMNPs also appear to be an effective treatment at the 90 min magnetic field exposure time, as they were slightly, but not significantly, more effective than DOX alone.



**Figure 4a:** Viability of T47D cells after treatment and magnetic field exposure grouped by treatment type T47D cells were treated with several combinations of DOX and MNP treatments along with various magnetic field exposure times (n=3).



**Figure 4b:** Viability of T47-D cells after treatment and magnetic field exposure grouped by magnetic field exposure time T47D cells were treated with several combinations of DOX and MNP treatments along with various magnetic field exposure times (n=3).

### Optimization Design

This optimization design will consist of two parts. The first will be centered on developing mathematical models for magnetic nanoparticle concentration behavior in a 2- dimensional space in a magnetic field. The second part will predict the heat dissipated by magnetic nanoparticle response in an AC magnetic field.

#### Part One: Dynamical Model

The single magnetic particle's equation is the combination of the effect of diffusion and advection with all the forces acting on the particles; magnetic force, hydrodynamic drag force, hydrodynamic lift force, gravitational force, buoyant force, a random Brownian force and the particle interaction forces shown in (equation 1) below;

$$m_p \frac{dv}{dt} = F_m + F_D + F_L + F_g + F_b + R + d \quad \text{equation (1)}$$

where  $m_p$  is the particle mass,  $v$  is the particle's velocity,  $F_m$  is the magnetic force,  $F_D$  is the hydrodynamic drag force,  $F_L$  the

hydrodynamic lift force,  $F_g$  the gravitational force,  $F_b$  the buoyant force,  $R$  is a random Brownian force and finally  $d$ , represents the particle interaction forces [8].

The magnetic charge model showing the magnetic force on a particle will be  $F_m = (m \cdot \nabla) B$  is used.

Very small nanoparticles of ranging from nanometers to hundreds of nanometers have a magnetic behavior of a single domain and is treated as magnetic dipoles. Therefore  $m = V M$ , where  $V$  is the volume of particle core and  $M$  is the magnetization.

Targeted drug delivery applications consist of large numbers of particles spread throughout fluids. The mass transport of particles is described in the continuity equation below;

$$\frac{dc}{dt} + \nabla \cdot j = S$$

Where:  $c$  will be the particle concentration (amount of particle per unit volume)

$j$  is the total flux of particles

$S$  is the volumetric source for  $c$ , the concentration

$S = 0$  when no particles are added to the system during time interval

The flux contains a diffusive component given by Fick's law  $jD = -D\nabla c$  ( $D$  is the diffusion coefficient) [8].

Advection of particles in the fluid where  $jA = v c$

The Final advection-diffusion equation will be

$$\frac{\partial c}{\partial t} = \nabla \cdot (D\nabla c) - \nabla \cdot (v c) \quad \text{equation (2)}$$

To find the spatial concentration distribution of particles, the PDE (equation 2) was solved below with predicted boundary values and initial values.

In a petri-dish (with a ferrofluid);

The no-flux boundary condition will be  $n \cdot (jD + jA) = 0$

$N$  is the normal outwards to the domain at the edge  $\partial\Omega$  which means that no particle can cross the boundary, the total particle mass in the domain  $\Omega$  is constant.

Leading to  $n \cdot (D\nabla c - v c) = 0$

The approximated sample space for a 2-dimensional space with length  $\Delta x$ , each with a certain concentration;

$$C_{i,j} = C(x_i, y_j) = c(x_0 + i\Delta x, y_0 + j\Delta x)$$

Then apply

$$\frac{\partial C_{i,j}}{\partial x} = \frac{C_{i+1,j} - C_{i-1,j}}{2\Delta x} \quad \text{and} \quad \frac{\partial^2 C_{i,j}}{\partial x^2} = \frac{C_{i+1,j} - 2C_{i,j} + C_{i-1,j}}{\Delta x^2}$$

To obtain

$$C' = A \cdot c$$

Where  $c$  will be the vector of concentrations at each of the four sides,

$A$ , will be a matrix containing magnetic and fluid dynamics [8].

There will be four magnetic coils governing each side of the 2D geometric space.

### Part Two: AC design Model for Magnetic Nanoparticles

This prediction procedure will revolve around magnetic hysteresis loops of superparamagnetism and steady orientation of axis in directions parallel, perpendicular or oblique to the AC magnetic field.

During microwave heating – food can be penetrated deeply due to the electromagnetic wave strengths and hence this can be applied to help penetrate deeper tissues and get to hidden malignant tissues inside the body.

All magnetic nanoparticles used in hyperthermia will have only one magnetic moment,

$\mu = M_s V$ , where  $M_s$  is the spontaneous magnetization and  $V$  is the volume of the core of the magnetic nanoparticle,  $V = \pi/6 \cdot d^3$

which makes such magnetic nanoparticles to be classified under ferromagnetic or superparamagnetic based on the direction of their magnetic moments (whether it thermally fluctuates or not) [9].

### Case One: Ferromagnetic Nanoparticles

When a ferromagnetic nanoparticle with uniaxial magnetic anisotropy, and constant  $K$  (anisotropy),  $V$  is considered large enough to make its magnetic anisotropy barrier with height  $KV$  which blocks the thermal fluctuations. A magnetic field,  $H$  applied in the antiparallel direction to  $\mu$ , becomes metastable (seen in Figure 5). When the barrier disappears at the anisotropy field  $H_K = 2K/(\mu_0 M_s)$ , then  $\mu$  reverses. This makes the Zeeman energy fall from  $\mu_0 \mu H_K$  to  $-\mu_0 \mu H_K$  and the remaining energy dissipates ( $\mu_0$  is the permeability of the vacuum) [9].

The work done in one cycle of the AC magnetic field,  $H_{ac} \sin(2\pi f t)$  is 0 for  $H_{ac} < H_K$  and  $4\mu_0 \mu H_K$  for  $H_{ac} > H_K$ . The loss of heat is called the hysteresis loss shown in Figure 5 below.

### Case Two – Superparamagnetic Nanoparticles

For the smaller superparamagnetic nanoparticles, with thermally fluctuating reversal of  $\mu$ , the reversal probability in a zero magnetic field will be expressed as

$$\tau_N^{-1} = f_0 \cdot \exp\left(\frac{-KV}{k_B T}\right)$$

Where  $\tau_N$  is the Neel relaxation Time,  $f_0$  is the attempt frequency of  $10^9 \text{ s}^{-1}$ ,  $k_B$  is the Boltzmann constant and  $T$ , the temperature.

Brownian Rotation if nanoparticles are displaced in a liquid phase in a zero magnetic field:

$$\text{Is now } \tau_B = \frac{3\eta V H}{(k_B T)}, \quad \eta \text{ is the viscosity of the liquid phase and } V H,$$

the hydrodynamic volume of nanoparticles. If parallel rotation occurs, the relaxation,  $\tau$  will be  $\tau^{-1} = \tau_N^{-1} + \tau_B^{-1}$  (Figure 6).

The  $\tau$  for very small superparamagnetic nanoparticles will be only determined by  $\tau_N$  since  $\tau_N^{-1}$  exponentially increases with decreasing  $V$  and the increase of  $\tau_B^{-1}$  is inversely proportional to  $V_H$  [9].

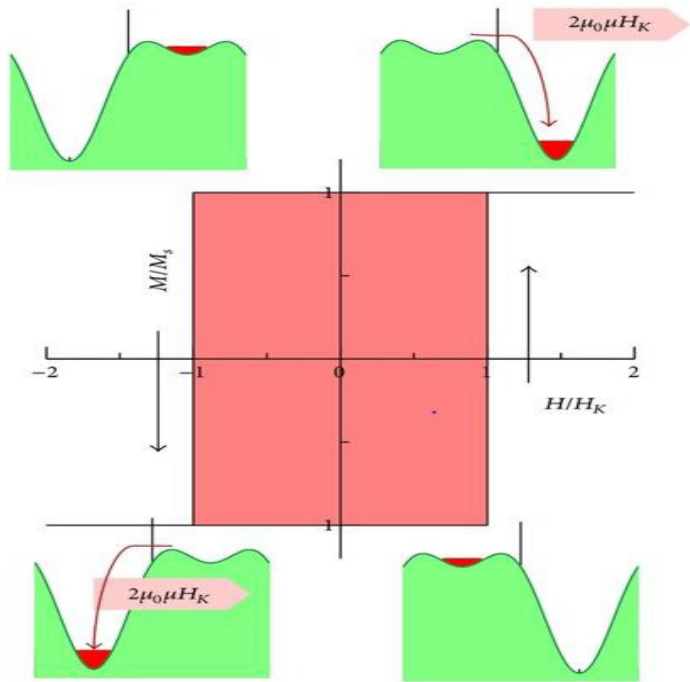


Figure 5: Schematic describing the hysteresis loss in an M-H loop.

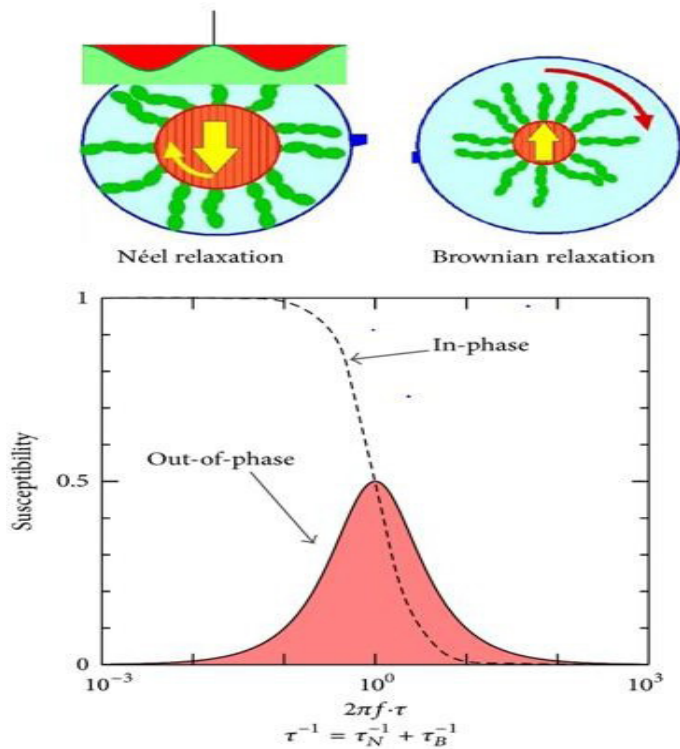


Figure 6: The relaxation loss from Neel and Brownian relaxation.

In an equilibrium state for a linear response, nanoparticles are assumed to have small  $H_{ac}$  which is the average-out of phase component of AC susceptibility,  $X''$  will be given by;

$$X'' = \frac{\mu_0 \mu^2}{(3kBT)} \cdot \frac{2\pi f \cdot \tau}{[1+(2\pi f \cdot \tau)^2]}$$

Subsequently, the relaxation loss and its heat dissipation  $P_H$  is given as;

$$P_H = \pi \mu_0 X'' \cdot H_{ac}^2 \cdot f \cdot \Omega^{-1}$$

$$= \frac{1}{2} \frac{[\mu_0^2 M_s^2 V / (3kBT\tau\rho)] H_{ac}^2 \cdot (2\pi f \cdot \tau)^2}{[1+(2\pi f \cdot \tau)^2]} \quad \text{equation (3)}$$

To maximize  $P_H$  for a superparamagnetic nanoparticle,  $f$  should be adjusted to  $\tau^{-1}$  and  $H_{ac}$  is maximized [9].

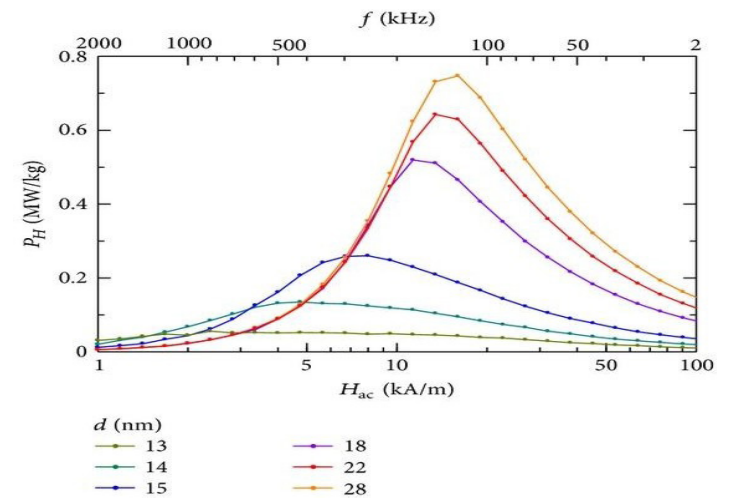


Figure 7a: Calculated Heat dissipation by core particles that are rotatable.

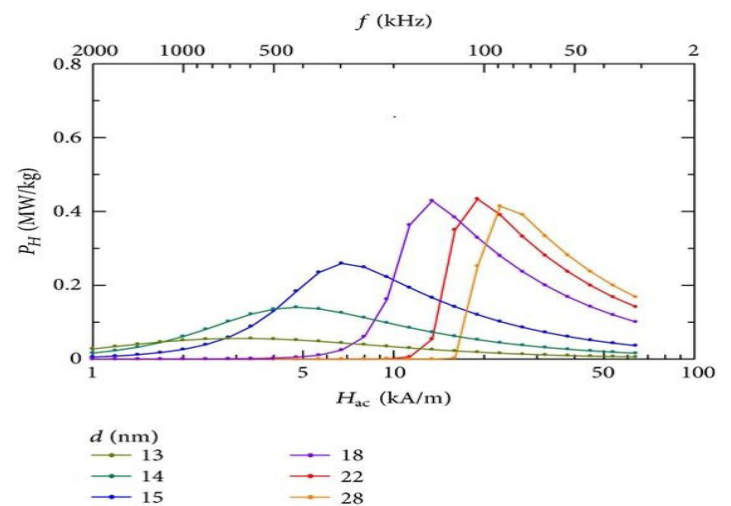


Figure 7b: Calculated Heat dissipation of core particles that are non-rotatable.

## Discussion

T47D breast cancer cells were treated with several combinations of MNPs, DOX and DC magnetic field exposure times. As the

DC magnetic field exposure times increased for each of the MNP treatments, a decrease in cell viability was observed. These observations suggest that lengthier magnetic field exposure times lead to a more efficient reduction in cell viability. nMNPs and PEG-MNPs were never found to be significantly different from each other, which demonstrates that the PEG coating does not reduce the magnetic responsiveness of the MNP. The DOX-MNPs produced in this study demonstrated no significant decrease in cell viability compared to DOX alone after 0, 30 and 60 min DC magnetic field exposure. Conversely, after 90 minutes of DC magnetic field exposure, cell viability dropped to 36.52%, which was found to be significantly more effective than DOX treatments alone (Figure 4).

For an optimization approach using an AC magnetic field, there is the need to synthesize ferromagnetic nanoparticles, with anisotropy field,  $H_k = 2K/(\mu_0 M_s)$  to match the amplitude  $H_{ac}$  of the AC magnetic field. However, when the relaxation loss is increased, it results in the synthesis of superparamagnetic nanoparticles that have  $\tau_N$  matching  $f$  of the AC magnetic field. In view of this, large number of reports have emphasized on controlling the shape, size, structure to optimize  $H_k$  and  $\tau_N$  [9].

In one study by Mamiya, a superparamagnetism nanoparticle (cobalt and manganese ferrites) was developed with a uniform diameter of 15nm to avoid aggregation with an oscillator to generate AC magnetic field of 500kHz ( $f$ ). With a measured magnetic anisotropy constant  $K$  ( $1.7 \times 10^4$  J/m<sup>3</sup>) and amplitude  $H_{ac} = 37.3$  kA/m, the heat dissipation  $P_H$  per unit weight reached 3MW/kg (3 kW/g) which was higher than using cobalt ferrite of 0.4 MW/kg or manganese ferrite of 0.2 MW/kg. The particle sizes of cobalt ferrite used alone and manganese ferrite alone were 12nm and 15nm respectively. The required heat dissipation for killing metastatic cancer cells is estimated to be within 0.3 and 1 MW/kg which corresponds to tumors of approximately 2kg/m<sup>3</sup> [9].

In contrast, when the nanoparticles with PH of 3MW/kg described above was obtained from irradiation in an AC field of  $H_{ac} = 37.3$  kA/m and  $f$  of 500kHz on a simple model body with electrical conductivity of  $\sigma = 0.2$  Sm<sup>-1</sup> and radius of  $r = 0.1$  m, the maximum voltage generated on the outer circumference  $V = \pi^2 2\pi f (\mu_0 H_{ac}) = 4,600$  V per revolution: the eddy current loss  $P_e = 1/2 \pi^2 \mu_0^2 \sigma r^2 f^2 H_{ac}^2 = 5$  MW/m<sup>3</sup>, this is the energy needed to raise the temperature in thermally insulated tissues by 10K. From this information, the eddy current loss on normal tissues cannot be neglected. With a nanoparticle of diameter = 15nm, it is seen in Figure 7 that if the diameter is slightly increased, the  $P_H$  at higher  $H_{ac}$  corresponds to a lower frequency and it is expected that adequate heat is generated inside hidden tumors with diameter 0.01m with no fatal effects on normal tissues from Pe constraints [9].

## Conclusions

In summary, this study describes the development of a novel MNP vehicle produced using MnFe<sub>2</sub>O<sub>4</sub>-MNPs and an optimization approach that could be utilized to efficiently release drugs for a

targeted delivery in therapy. Although using nanoparticle size of diameter 15nm and  $f$  (500kHz) and  $H_{ac} = 37.3$  kA/m may probably not lead to the maximum optimized design because there are other parameters that complicate the optimal combinations that need to be reviewed to facilitate an efficient hyperthermia.

## Future Works

Further studies are required to elucidate additional physical characteristics of the PEG-MNP vehicle produced. Scanning electron microscopy (SEM) could be utilized to further examine the surface properties of the MNPs at each step of the pegylation process and may provide clearer images of the rough exterior produced by the adsorption of PEG. X-ray diffraction may also be utilized to determine the relative size of the MnFe<sub>2</sub>O<sub>4</sub> core of the MNPs to ensure they are not aggregating prior to the addition of the polymer shell. Cellular uptake of the MNPs could also be determined through inductively coupled plasma mass spectroscopy (ICP-MS), which would measure the levels of MnFe<sub>2</sub>O<sub>4</sub> present within the cell culture medium following treatment.

Additional pegylation protocols should also be attempted using varying solutions of each of the polymers and incubation times to further minimize the diameter of the PEG-MNP vehicle. Other chemotherapeutic agents containing an amine functional group can be coupled to the PEG- MNPs to determine whether they are similarly effective.

Finally, the necessary experimentation should be conducted with varying nanoparticle diameter sizes with an oscillator that measures the magnetic field gradients to find the optimization frequency for a maximum therapeutic effect.

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