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Exploring Controlled Payload Release from Magnetoliposomes

Amanda Baxter

ABSTRACT

The use of chemotherapy drugs remains one of the dominant treatment methods for cancer patients even though these drugs are not specific to cancer cells. This causes damage to healthy cells and as a result unpleasant side effects for the patients undergoing these treatments. If these drugs were targeted to a tumor site and nearly instantaneously released, these side effects could be significantly reduced. Recent studies have been conducted to attempt to target these drugs to tumor sites; however, the problem remains that the drugs need to be released as quickly as possible in order to prevent the drugs from spreading to healthy cells throughout the body. This research explores a new possibility for nearly instantaneous liposomal drug release. Superparamagnetic nanoparticles are infused into liposomes and payload release is controlled by exposure to a pulsed magnetic field. The mechanical rotation of the superparamagnetic nanoparticles upon exposure to the pulsed magnetic field causes liposome membrane disruption, and ultimately a leakage of the payload. Impedance spectroscopy is used for analysis of the magnesium sulfate payload. Payload release is observed and is dependent on the presence of superparamagnetic nanoparticles

Keywords: liposomes, nanoparticles, impedance spectroscopy

INTRODUCTION

One of the greatest challenges that persists in current cancer treatment methods is targeting the drug to the cancer site and controlling the timing of the drug's release. Ideally the drugs would be targeted to the specific cancer site and released instantaneously. In the current model, cancer drugs are designed to target rapidly-dividing cells, a characteristic of cancer cells. Unfortunately this causes the drugs to attack healthy rapidly-dividing cells as well. Because of this, patients undergoing cancer treatments experience many negative side effects including hair loss, loss of appetite, decreased production of blood cells, and many more. If these drugs could be targeted to a specific tumor site, and instantaneously released, the damage to healthy cells could be minimized, thus minimizing these side effects. The goal of this research is to manipulate the magnetic properties of superparamagnetic nanoparticles that are infused into liposomes to control drug release.

A paramagnetic material is defined by its ability to align its atomic magnetic dipole with an external magnetic field. A superparamagnetic material has this property but at a much larger scale as the entire crystal aligns with the external field because of its single crystal nature (Thorek et. al 2006). Because the entire crystals mechanically move in the presence of an external field, they may be able to disrupt a liposome membrane and lead to payload leakage.

Liposomes were first described in 1964 by Alec Bangham (Bangham 1964) and have since been extensively studied as vesicles for drug delivery because of a unique surface chemical property in which specific ligands can attach and be used to target the liposome to a specific location in the body. Liposomes consist of an aqueous core surrounded by a phospholipid bilayer, and without an external stimulus they will hold their payload for an extended period of time. Previous research suggests that liposomes can encapsulate anticancer drugs in their core (Blanco, et. al 2011). If a liposome contains an anticancer drug and superparamagnetic nanoparticles in its core, it may be possible to magnetically control the release of the drug.

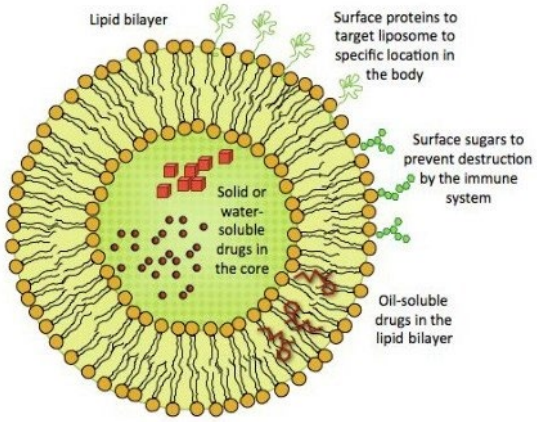

Figure 1. A model liposome nanoparticle for drug delivery. (Illustration by Shannon McArdel). Retrieved from Science in The News, Harvard Medical School. https://sitn.hms.harvard.edu/sitnflash_wp/2011/06/materials-for-drug-delivery/

So far, some liposome drug delivery systems have been developed (Moses et. al 2003 and Allen and Cullis 2004). The problem with these systems is that the payload is released too slowly which gives the liposomes substantial time to spread away from the

tumor site. The proposed solution is to use a strong pulsed magnetic field to cause the superparamagnetic nanoparticles to mechanically move, disrupting the liposome's membrane. Each pulse lasts only fifty microseconds, so the payload release is nearly instantaneous. The other advantage to using a pulsed magnetic field as opposed to an AC magnetic field is that the liposome payload release is induced by heat when an AC magnetic field is used (Bossmann 2009). This is disadvantageous because many drugs are relatively heat sensitive (Moses et. al 2003 and Allen and Cullis 2004).

MATERIALS AND METHODS

The pulsed magnetic field is an RLC (resistor-inductor-capacitor) circuit. First the capacitor bank is charged from a power supply. Next the charged capacitor bank is discharged through the inductive coils. When there are more turns in the coil, magnetic field strength is increased and increasing the diameter of the coil results in a greater area of magnetic field uniformity but decreases magnetic field strength. However inductance is increased when either the diameter or the number of turns is increased, so to maximize the rate of field switching, which is ultimately necessary for a shorter release time, a minimum diameter size and number of turns in the coil are used. A series of resistors then control the charging current. The pulsed magnet used for this research consists of a capacitor bank of (F=77.3 μF) Maxwell Laboratories. The power supply uses 100-240 V AC-50/60 Hz input and output of 10kV at 500 J/s in continuous operation and is from Lumina Power, Inc.

Liposome Preparation

247 microliters of 1,2-Dihexadecanoyl-sn-glycero-3-phosphocholine (DPPC) is mixed with 4 microliters of 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) and 12 microliters of cholesterol. Once these compounds have been added, the chloroform is evaporated off at approximately 55ºC. After evaporating the chloroform, apply a vacuum for at least one hour. The next step is hydration in which 125 microliters of Phosphate Buffered Saline (PBS), 838 microliters of double distilled water, and 37 microliters of 3M NaOH are added to the dried phospholipids. During this step 200 microliters of $FeFe_3O_4$ nanoparticles are added, in addition to 25 mg of solid $MgSO_4$. After everything has been added, vortex the mixture for a minimum of five minutes. This creates the multilamellar liposomes which are larger than the final desired product (unilamellar liposomes).

The next step is the freeze/thaw process. Place the mixture in dry ice for five minutes, and then place it in a 50ºC water bath for five minutes. Repeat this procedure ten times. End with the solution in the hot water bath. This process helps the heterogeneous multilamellar liposomes to more readily become the desired unilamellar end product (Sriwongsitanont and Ueno 2004). Next is the extrusion process where the multilamellar liposomes become the desired unilamellar liposomes, typically 50-250 nm in diameter. Keep the solution at 50ºC and push the solution back and forth through the filters eleven times, ending on the opposite side from where the liposomes began. The final step is gel filtration in which the unilamellar end product is isolated from anything else present in the solution. Make a slurry of sephadex and PBS, and fill the column with the slurry. Collect the first fraction.

After the liposomes have been synthesized, mix 500 microliters of the liposome solution with PBS to reach a total volume of 15 milliliters. Record the temperature of the solution and then measure the AC Impedance. Place the solution in the pulsed magnetic field for ten pulses. Place the solution in a room temperature water bath until the solution reaches the same temperature it was when the AC Impedance was measured the first time. Then measure the impedance again. Next place the liposomes in the Sonicator on the highest power for 20 minutes. This step will completely destroy all liposome membranes and leads to complete payload release. The liposome solution will be hot. Cool it until it reaches the temperature that it was when the other impedance measurements were taken. Measure the AC impedance a final time.

RESULTS

The results of this experiment are promising. Liposome payload release was observed in every experiment in which superparamagnetic nanoparticles were used. There were also control experiments that were implemented to ensure the superparamagnetic nanoparticles were in fact the cause of the liposome membrane disruption. In these experiments no payload release was observed. It can be concluded that the mechanical rotation of the superparamagnetic nanoparticles upon exposure to the pulsed magnetic field is responsible for liposome payload release. Calibration curves were constructed with concentrations of magnesium sulfate in phosphate buffered saline that ranged from 1×10^{-5} M to 0.01 M. Due to the extremely sensitive nature of impedance spectroscopy, minute changes in conditions lead to very significant changes in impedance (MacDonald 1992). Therefore, since different experiments were conducted on different days with different disposable screen-printed electrodes, the experiments had highly variable impedance values from one another. The same electrode, however, was used for the "before

magnetic field exposure", "after magnetic field exposure", and "after complete liposome destruction through sonication" measurements. This indicates it can be safely assumed that these values may be accurately compared to one another, but there is no reliable method to compare the data from trial to trial because the impedance values from one experiment are not necessarily comparable to the impedance values from the calibration curve or from the impedance values from other experiments. Since the calibration curve is nonlinear, extrapolation may not be an accurate method. The quantitation of the impedance data remains a challenge and as a result the amount of payload release is unknown, however, payload release can be qualitatively observed in every experiment in which superparamagnetic nanoparticles were used.

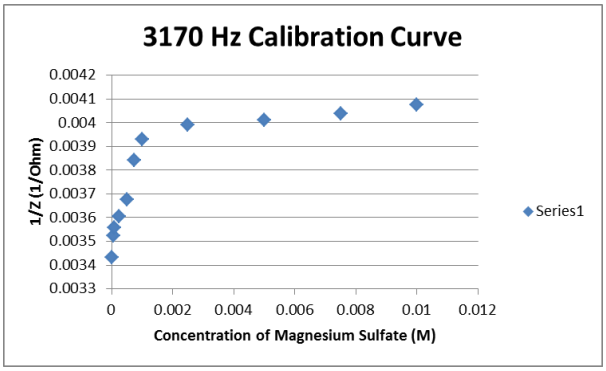

Figure 2. Calibration curve at a high frequency. Concentration of magnesium sulfate is plotted versus the inverse of impedance which is proportional to conductivity which increases with concentration.

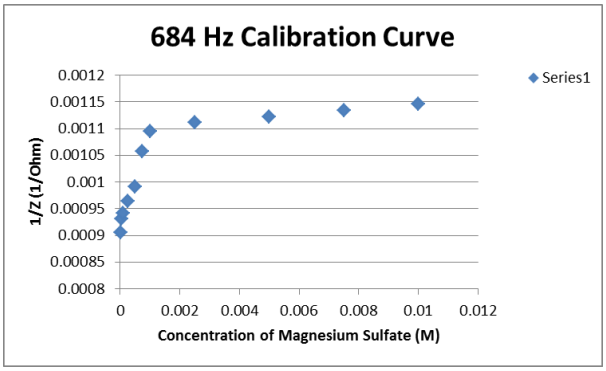

Figure 3. Calibration curve at an intermediate frequency.

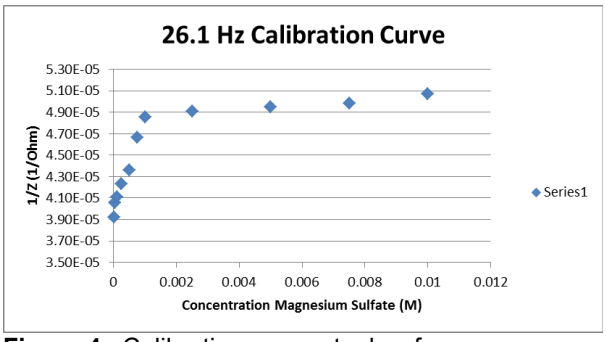

Figure 4. Calibration curve at a low frequency.

The calibration curves indicate that the same trend can be observed at many frequencies. For this reason frequencies may be arbitrarily chosen when comparing impedance data. Although the calibration curves are nonlinear, they may be broken into two linear domains.

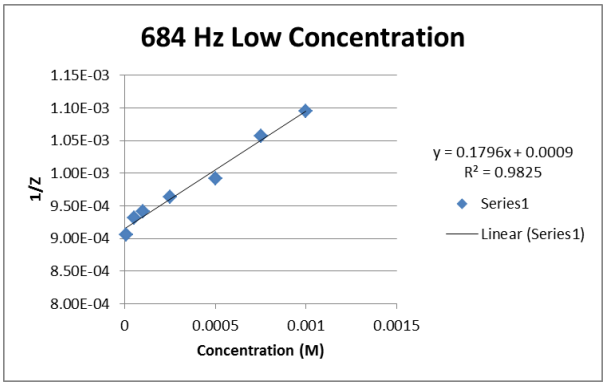

Figure 5. First linear domain consisting of lower concentrations at 684 Hz.

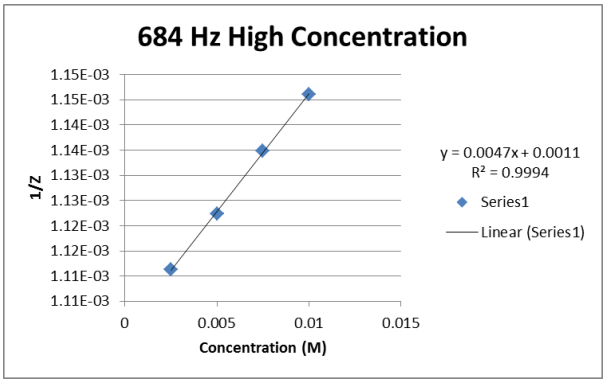

Figure 6. Second linear domain consisting of higher concentrations at 684 Hz.

This double linear domain trend was observed at all frequencies with only slight R^2 value differences.

There is clearly a relationship between impedance values and concentration. A decrease in impedance (or an increase in its inverse) signifies an increase in concentration. The predicament lies with the fact that

it is not a completely linear proportionality. Therefore the data cannot be effectively extrapolated, and since the impedance values from different experiments have such large ranges, extrapolation would be ideal. However it is clear that even at higher concentrations an increase in magnesium sulfate concentration leads to an increase in conductivity (1/Z).

Table 1. Impedance values before and after exposure to the pulsed magnetic field as well as after complete liposome destruction via sonication for trials with and without superparamagnetic nanoparticles. These values were obtained from at 684 Hz.

Nanoparticles	Before Magnet	After Magnet	After Sonication
With	998.8	958.7	884.5
With	1856	1543	1206
With	1570	1411	1250
Without	1149	1149	1073
Without	1372	1389	1179

Table 1 demonstrates how impedance decreases after pulsed magnetic field exposure then decreases again after complete liposome membrane disruption by sonication when superparamagnetic nanoparticles are used. This indicates magnesium sulfate concentration is increasing like expected and payload release is occurring after magnetic field exposure. Conversely when superparamagnetic nanoparticles are not used the impedance effectively remained constant. Interestingly however the impedance values in each column differ from one another by a rather large amount. In fact only the impedance values in the first row all fit in the 684 Hz calibration curve. Therefore the data cannot be quantified using the equations generated from the two domain calibration curves, except for the first trial. When the equations are used for the first trial, the concentration for before exposure is 0.000563 M, after is 0.000797 M, and 0.00651 M after complete liposome destruction. This indicates a 4% release. When other frequencies were used similar percent releases were calculated, with an average of about 5%. It is interesting that the impedance values are so different from trial to trial. Impedance spectroscopy is an incredibly sensitive technique in which tiny changes in condition lead to immense impedance changes. Different materials, electrodes and temperatures can lead to impedance changes up to ten orders of magnitude (MacDonald 1992). Temperature was controlled for in the experimental setup, as a result the discrepancies are most likely caused by the fact that different electrodes were used for different experiments. Disposable carbon screen-printed electrodes were used for these experiments. This explains why the impedance values are consistent throughout the same trial but not when comparing different trials.

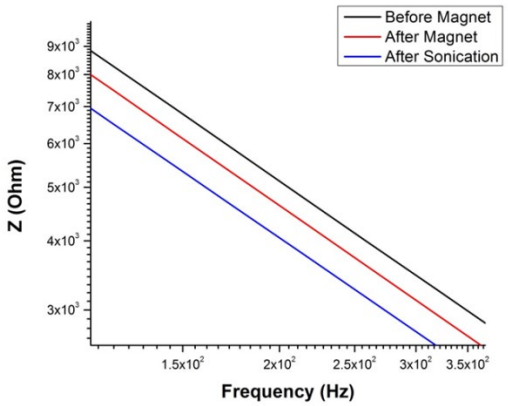

Figure 7. A Bode Plot presenting impedance versus frequency showing the data before exposure to the magnetic field, after exposure to the magnetic field, and after complete liposome destruction from sonication. A decrease in impedance is observed from before exposure to after exposure and again from after exposure to after destruction. This indicates an increase in magnesium sulfate concentration in solution because impedance is the inverse of conductivity which increases as ionic concentration increases. Liposome payload release is observed.

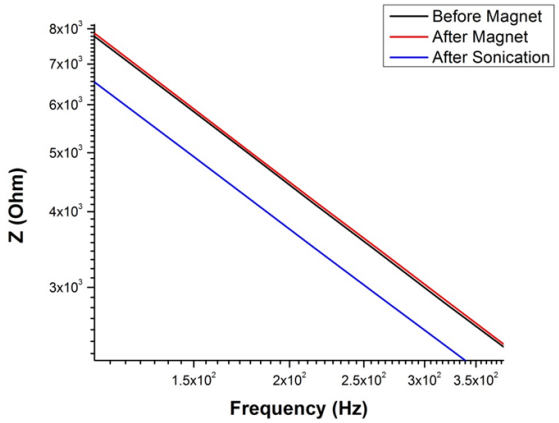

Figure 8. A Bode Plot presenting impedance versus frequency for a control experiment in which superparamagnetic nanoparticles were not present during liposome synthesis. The black and red lines are very close to one another indicating that magnesium sulfate concentration was relatively constant. No payload release is observed.

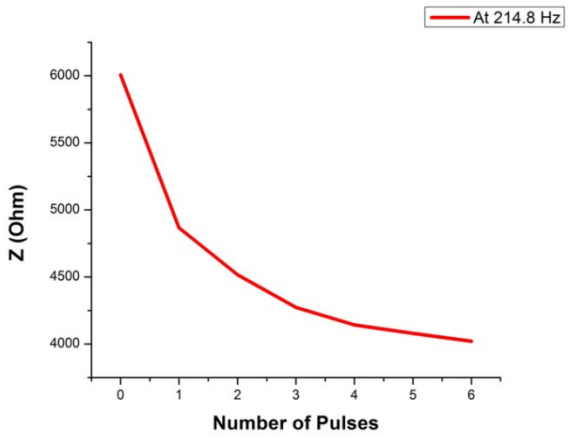

Figure 9. Another experiment was conducted to observe how many pulses should be used for optimum payload release. Impedance was measured after each pulse. There is significant release after only one pulse which is ideal because one pulse lasts only 50 microseconds. However, about six pulses cause the most release in the least amount of time.

DISCUSSION

Magnetoliposomes have been targeted as methods for drug targeting, and it is known that superparamagnetic nanoparticles that are encapsulated in a liposome respond to an external magnetic presence (Fortin-Ripoche et. al 2006). Also it is known that the size of polyethylene glycol stabilized liposomes (~200 nm) aids in the tumor targeting process by favoring tumor interstitium and interactions with tumor cells (Fortin-Ripoche et. al 2006). Because such studies have been conducted regarding targeting liposomes to tumor sites, the focus of this research was to optimize release time. Several strategies have been implemented for optimizing payload release by means of biological or physical triggers including: cell membrane fusion, pH sensitivity and heat sensitivity (Fortin-Ripoche et. al 2006). These methods have proven somewhat effective; however, the release time needs to be much further optimized so that the liposomes do not have sufficient time to spread throughout the body, damaging healthy rapidly-dividing cells. This research sought to fuse these two ideas: the use of manipulating the magnetic properties of superparamagnetic nanoparticles, and optimizing release time. A strong pulsed magnetic field was used to control payload release. The theory is that the mechanical rotation of the superparamagnetic nanoparticles in the presence of a pulsed magnetic field would lead to disruption of the liposome membrane and a leakage of the payload. The results are promising. Payload release was observed each time superparamagnetic nanoparticles were used. There were also trials in which superparamagnetic nanoparticles were not used, and payload release was not observed during these trials, indicating that the mechanical rotation of the superparamagnetic nanoparticles is in fact what is responsible for liposome membrane disruption. Due to complications involved with using disposable carbon screen-printed electrodes, the data remains challenging to quantify. Therefore, further studies should be conducted either using different analytical methods or improving upon these methods by using a glassy carbon electrode to obtain more reproducible impedance values.

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