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Mapping/Targeting Telomerase Activity

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Microwave hyperthermia holds promise in the treatment of malignant breast tumors, with fewer side effects using non-ionizing radiation. However technical limitations continue to limit its use. This project seeks to overcome two such limitations. The first area of research is developing biocompatible vectors with high microwave absorbing and scattering materials, enhancing in-vivo localization of target cells, where the activity of specific markers is present. The second area of research seeks to optimize the microwave energy delivery system, studying the efficiency of pulsed versus continuous energy deposition for frequency from 30 MHz to 3 GHz, where the hyperthermia has shown best therapeutic results and detection systems are designed.

First, we identify and measure biocompatible materials that would enhance the absorption and/or the scattering of microwave photons in water based inhomogeneous medium. We first expanded our understanding of dipolar polarization and conduction mechanisms, determining the efficiency of microwave heating. A measurement system was then established and a series of measurements was taken on water-soluble conductive polymers. The first measurements were promising. Future work will focus on the absorption mechanism as well as including these materials in liposomes, optimizing the external delivery system.

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## Introduction

Microwave hyperthermia has shown promise in the treatment of malignant breast tumors with minimal side effects due to the use of non-ionizing radiation. However the full potential has yet to be realized due to technological limitations. This project seeks to move this technology toward its full potential in two areas.

The first area of research is developing biocompatible vectors with high microwave absorbing and scattering materials. These would enhance in-vivo localization of target cells, where the activity of specific markers such as Telomerase or Hepatocyte Growth Factor is present. The second area of research seeks to optimize the microwave energy delivery system, studying the efficiency of pulsed versus continuous energy deposition for frequency from 30 MHz to 3 GHz, where the hyperthermia has shown best therapeutic results therapy and detection systems are designed.

Due to a delay in the delivery of the measurement system for microwave material characterization and a 6-month leave of absence of the PI, a one-year no-cost extension was approved. Thus the following technical report is based on the results obtained in the period May – July 2000.

As first step, we are identifying and measuring biocompatible materials that would enhance the absorption and/or the scattering of microwave photons in water based inhomogeneous medium. The first month was dedicated to deepening our understanding of dipolar polarization and conduction mechanisms, which determine the efficiency of microwave heating. A measurement system was then established and a series of measurements was taken on water-soluble conductive polymers.

A common misunderstanding that molecular motion, and thus heating, is caused by microwave absorption into rotational energy levels. However, it is gaseous water that has quantized rotational energy levels in the microwave region, and is thus responsible for heating.

In the liquid state, for all practical purposes, the quantization of rotational levels is non-existent. The easiest way to visualize the true mechanism is to picture microwaves for what they are - high frequency oscillating electric and magnetic fields. In this field, anything electrically or magnetically polarized at the oscillation frequency will be affected. Two phenomena occur normally in the heating process: dipolar polarization, and conduction mechanisms. A third mechanism – interfacial polarization – also occurs. This mechanism is of relevance in the case of small particles, such as those under investigation in this study.

Since heat is the form of energy transfer associated with two bodies at different temperatures, the first effect of microwave absorption in a particle cluster is the increase of its internal energy. This happens because as a particle follows the field, it instead finds the incoherent inertia of the particles in the cluster, due to their random initial polarization states. Then, if cluster entropy does not change significantly, its temperature will rise causing the system to transfer energy (as heat) to the surrounding environment at a lower temperature ( $k$  is the Boltzman's constant):

$$W_{\text{In}} \rightarrow U \nearrow \rightarrow T = \frac{\partial U}{k \partial \sigma} \nearrow \rightarrow Q_{\text{Out}}$$

## The math of microwave properties

To understand quantitatively how materials react to microwave radiation, it is important to consider the concept of complex dielectric constant,  $\epsilon^*$ , which describes the dielectric properties of homogeneous materials and is expressed as the sum of real and complex dielectric constants:  $\epsilon^* = \epsilon' + i\epsilon''$

The real part of  $\epsilon^*$ ,  $\epsilon'$ , represents the ability of a material to be polarized by an external electric field. At very high and very low frequencies, and with static fields,  $\epsilon'$  will equal the total dielectric constant of the material. Where electromagnetic energy is converted to heat by the material,  $\epsilon''$  is non-zero, and quantifies the efficiency with which the electromagnetic energy is converted to heat.

A further quantity, the loss angle  $\delta$ , is also commonly used in the literature, and is more usually given in the form of its tangent. It is related to the complex dielectric constant by  $\text{Tan}\delta = \frac{\epsilon''}{\epsilon'}$ . The angle  $\delta$  is the phase difference between the electric field and the polarization of the material.

Magnetic polarization may also contribute to the heating effect observed in materials where magnetic properties exist, and similar expressions for the complex permeability of such materials may be formulated. Although such cases are relatively uncommon, a familiar example of its importance is in the microwave heating of  $\text{Fe}_3\text{O}_4$ . Here, we will be focusing our attention on dielectric materials.

## Dipolar Polarization<sup>1</sup>

Dipolar polarization is the phenomenon responsible for the majority of microwave heating effects observed in solvent systems. The different electronegativities of individual atoms result in the existence of a permanent electric dipole on the molecule. For a molecule in a polar liquid such as water (methanol, ethanol, THF, etc), there are intermolecular forces, which give any motion of the molecule some inertia.

The dipole is sensitive to external electric fields, and will attempt to align with them by rotation, the energy for this rotation being provided by the field. This realignment is rapid for a free molecule, but in liquids instantaneous alignment is prohibited by the presence of other molecules (intermolecular inertia). A limit is therefore placed on the ability of the dipole to respond to a field, which affects the behavior of the molecule with different frequencies of electric field.

Under very low frequency irradiation, the dipole may react by aligning itself in phase with the electric field uniformly and no significant random motion results. The molecule gains some energy, and some is also lost in collisions but the overall heating effect is small.

Under the influence of a very high frequency electric field, on the other hand, the dipoles will attempt to follow the field, but they do not have sufficient time to respond to the field, and so do not rotate. As no motion is induced in the molecules, no energy transfer takes place, and therefore, no heating.

Between these two extremes, at frequencies, which are approximately those of the response times of the dipoles, is the microwave region. The microwave frequency is low enough that the dipoles have time to respond to the alternating field, and therefore to rotate, but high enough that the rotation does not precisely follow the field. As the dipole

reorients to align itself with the field, the field has itself already changed, creating a phase difference between the orientation of the field and that of the dipole. This phase difference is randomly distributed among the particles causing energy to be lost from the dipole in disordered collisions, and to give rise to dielectric heating.

For any material, both the real and complex dielectric constants will vary with frequency. The variation of  $\epsilon'$  and  $\epsilon''$  with frequency for de-ionized (DI) water is shown in Figure 1.<sup>2</sup>

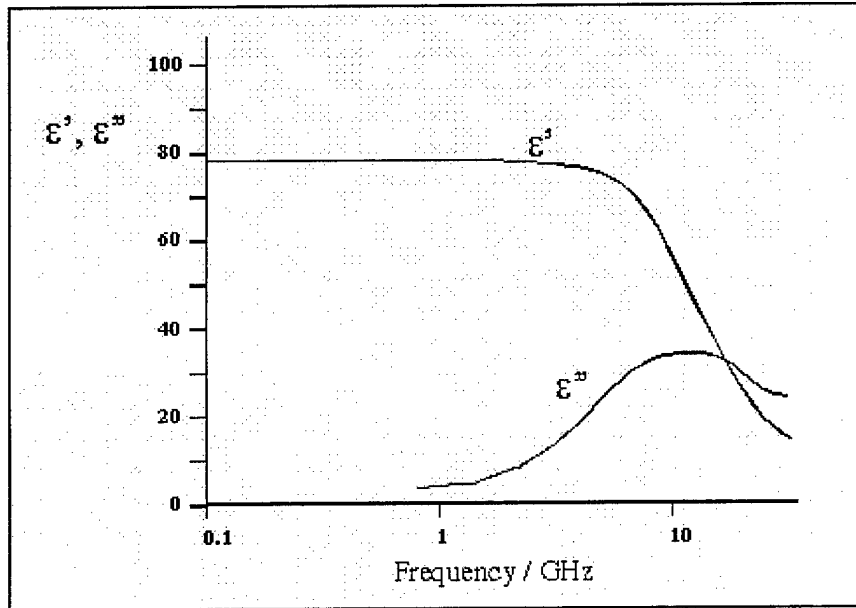


Figure 1: Complex dielectric constant for DI water in the microwave frequency range.

The difference is clear with quantum spectroscopic absorption: in the gas state the absorption linewidths are typically of the order of nanometers, while in the liquid state, the range of frequencies over which the dielectric loss is non-zero is relatively large.

As we reach the maximum in the dielectric loss  $\epsilon''$ , the dielectric constant  $\epsilon'$  goes through a point of inflexion as it decreases with increasing frequency.

Our current understanding of dielectrics is strongly based on the theoretical work of Debye<sup>3,4</sup> who expressed mathematically the frequency and temperature dependence of  $\epsilon'$  and  $\epsilon''$ :

$$\epsilon^* = \epsilon_\infty - \frac{\epsilon_\infty - \epsilon_s}{1 + i\omega\tau} \rightarrow \begin{cases} \epsilon' = \epsilon_\infty - \frac{\epsilon_\infty - \epsilon_s}{1 + \omega^2\tau^2} \\ \epsilon'' = \frac{\epsilon_\infty - \epsilon_s}{1 + \omega^2\tau^2} \omega\tau \end{cases}, \text{ with } \tau = \frac{4\pi\eta r^3}{kT}.$$

In the previous expressions,  $\epsilon_\infty$  and  $\epsilon_s$  are the dielectric constants at very high and very low frequencies (static). Infrared and higher frequency resonances are not taken into account. Debye derived the relaxation time,  $\tau$ , from Stoke's theorem using the molecular radius  $r$ , the viscosity  $\eta$ , the Boltzmann's constant  $k$  and the absolute temperature  $T$ .

The importance of Debye's work is clear when plotting his equations normalized to the product frequency-relaxation time (as in Figure 2) and comparing them with the experimental values for de-ionized water. Most of the dielectric solvents show similar behavior.

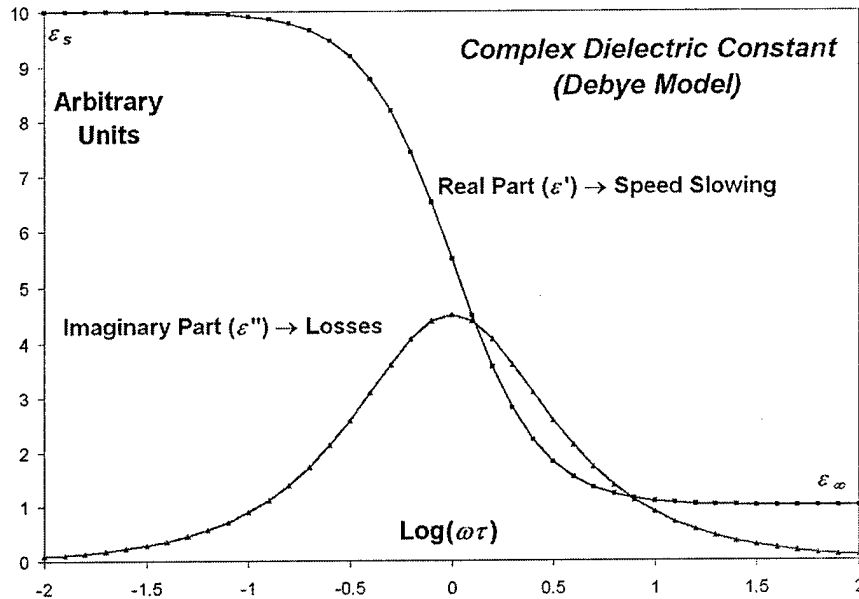


Figure 2: Debye's expressions for  $\epsilon'$  and  $\epsilon''$  calculated as a function of  $\omega\tau$  ( $\epsilon_s = 10$  and  $\epsilon_\infty = 1$ ).

Usually in medical and consumer applications the frequency of the electric field is chosen far from the maximum absorbency of water at 22 GHz, to have efficient heating, while avoiding absorption of energy not on the surface of the body or food.

Thus, we are interested in materials that present a very high absorption at a lower frequency than water, so that the heating process can be enhanced in the regions where the material is located within a water-based body. Thus, a larger relaxation constant is needed, while trying to keep the absorption? as high as possible. Increasing the average dipole radius and the viscosity would be beneficial in that sense.

## Conduction

Where the irradiated sample is an electrical conductor, the charge carriers (electrons, ions, etc) are moved through the material under the influence of the electric field,  $E$ , resulting in a polarization,  $P$ . These induced currents will cause heating in the sample due to any electrical resistance. This happens in the majority of solids, but it is also present in ionic solutions.

For a very good conductor, complete polarization may be achieved in approximately  $10^{-18}$  seconds, indicating that under the influence of few GHz radiations, the conducting electrons move precisely in phase with the field. Thus, if the sample is too conducting (small resistance to charge movements), such as a metal, most of the microwave energy is reflected. This property can be used for imaging systems, which base their accuracy on the power reflected back from the target.

The complex dielectric constant may be expressed to take account of these losses by including a separate conduction term:

$$\varepsilon'' = \frac{\varepsilon_\infty - \varepsilon_s}{1 + \omega^2 \tau^2} \omega \tau - \frac{\sigma}{\omega \varepsilon_s}$$

For example, if one takes pure water and heats it in a microwave oven, where the polarization mechanism dominates, we find that the heating rate is significantly less than if one takes the same volume of water and adds salt. In the latter case, both mechanisms occur, increasing the heating effect.

## Interfacial Polarization

This mechanism is important for systems comprised of conducting inclusions in a second, non-conducting material. An example would be a dispersion of metal particles in sulphur. Sulphur is microwave transparent, while metals reflect microwaves; however, the combination forms an extremely good microwave absorbing material (so good, in fact, that interfacial polarization effects are the basis of 'Stealth' radar absorbent materials)<sup>5</sup>.

Where a dielectric material is not homogeneous, but consists of inclusions of one dielectric in another, it is still possible theoretically to treat the material. If the dielectric properties and geometry of the inclusions are known, it is possible to arrive at expressions for the dielectric behavior of the bulk sample. However, determining the dielectric properties of the components from that of the system is generally insolvable except in the simplest of cases.

For a (non-superconducting) metal, there will always be a very thin surface layer in which some of the incident microwaves are attenuated, and in which induced currents will give rise to heating. For a bulk metal, this heating effect is so small as to be irrelevant, but in powders this surface layer makes up a large proportion of the material. However, the polarization induced in the metal is also subject to the properties of the surrounding medium, decreasing its effectiveness. Under these circumstances, the polarization of the metallic particles does not take place instantaneously, but lags behind the induced field, because of the polar molecule in the dipolar polarization mechanism. Hence, the frequency dependence of the sample's heating properties is similar to that of the dipolar polarization mechanism, despite being in actuality a conduction mechanism. Thus, interfacial polarization is most easily viewed as a combination of the conduction and dipolar polarization effects.

To derive a model for this phenomenon, the most basic geometrical situation was considered by Maxwell<sup>6</sup>. This consisted of a plate capacitor of  $n$  dielectric sheets of dielectric properties and conductivities  $\varepsilon_1 \sigma_1, \varepsilon_2 \sigma_2, \dots \varepsilon_N \sigma_N$ . Maxwell showed his model to be capable of explaining the observed data for dielectric relaxation in such systems.

Wagner<sup>7</sup> and Sillars<sup>8</sup> developed further Maxwell analysis for spherical enclosures. Their models led to expressions similar to the Debye's equations. The original values of  $\tau, \varepsilon_\infty$  and  $\varepsilon_s$  are modified to an effective value dependent on particle relative size and the volume fraction.

In reality little can be deduced about the dielectric properties of a heterogeneous material unless the shapes of the inclusions are well known. Agreement of the theoretical models with real systems has been demonstrated by the inclusion of 3% copper phthalocyanine in paraffin wax<sup>9</sup>. At higher concentrations, interparticle electrostatic

interactions must be taken into account. Attempts to do this have shown reasonable agreement with up to 30% water droplets in woolwax .13 and with 27.5% nitrobenzene in polystyrene<sup>10</sup> (Figure 3).

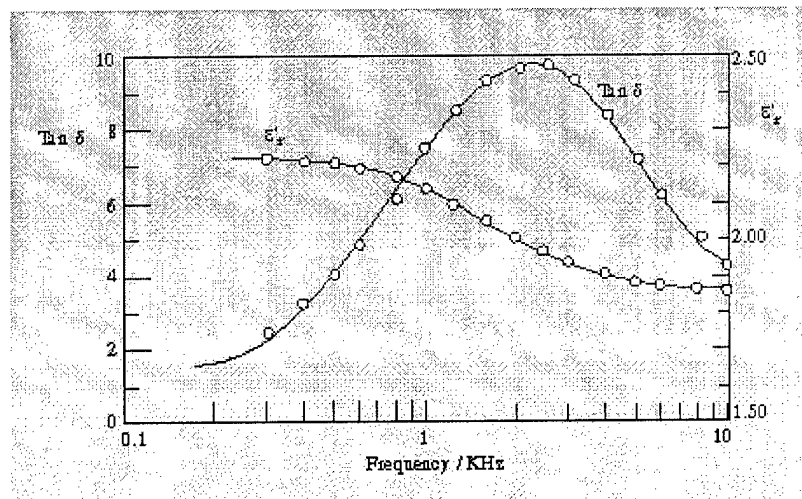


Figure 3: Dielectric properties of 27.5% volume nitrobenzene in polystyrene (Upper curve of each pair are experimental measurements, lower curve is the theoretical values)

Thus, when studying small conductive particles immersed in a dielectric, this phenomenon may lead to interesting properties. For this reason, we are focusing our attention on conductive polymers immersed in water and other biocompatible solvents. Together with the heating, these surface currents may lead a small polymeric enclosure (such as liposome) to break and release its content under low power microwave radiation. If the polymer does not absorb the microwave energy efficiently, it may still be so conductive that it scatters the energy, thus enhancing microwave detection systems. Conductive polymers seem to be a promising choice in this direction.

## Conductive Polymers

Alongside ceramic processing, polymer chemistry forms probably the largest single discipline in microwave chemistry. The dielectric properties of polar starting materials and very often products are an excellent indicator of reaction progress. The ability to control syntheses with high accuracy and with direct heating of the reactants has the advantage of large potential savings in energy. Economic analyses suggest that the costs of curing polymers may be reduced of a factor of 10 by switching to the use of microwaves<sup>11</sup>.

Investigations have indicated that the rate of curing depends less upon the total power than on the method through which microwave pulses are applied to a sample. This is most probably related to the relaxation mechanism involved in the polymeric reaction.

For example, with the epoxy resin DDS (4,4' diaminophenolsulphone), short high power pulses with a low time-averaged power ( $2 \times 10^{-3}$  sec, 700 Watts) were found to give comparable results to those of longer pulses, with high time-averaged power ( $2 \times 10^{-2}$  sec, 1500 Watts)<sup>12,13</sup>. Moreover, the pulse causes selectivity in bond formation: self-polymerization is preferred under short pulse irradiation. For each reaction (such as a

cross-link) it seems to exist an optimum pulse frequency based on the polar linking agent<sup>14,15,16</sup>.

These studies together with others<sup>17,18</sup>, seem to suggest that energy transfer is more efficient with the use of pulsed microwaves than by continuous power at least when polymers are in a heterogeneous medium. Inherently<sup>19</sup> conducting polymers such as polyaniline p-toluene sulphonate or polypyrrole p-toluene sulphonate are excellent microwave absorbers and are commonly used in welding of plastics.

Recent studies<sup>20</sup> attempt to explain the observed phenomena with relaxation processes occurring at different sites of the molecules. The imaginary part of the complex dielectric constant can be still expressed as the sum of polarization and conduction. We are in the progress of deepening our understanding of these models in order to interpret correctly the results presented below.

## Measurement Setup and First Results

We are interested in measuring the complex dielectric constant of liquid materials in the frequency range 20 MHz – 2 GHz using two methods: the reflection and the transmission techniques. In the reflection technique, the microwave power is sent to one side of the sample through a probe and the scattered power is recollectd from the same side. In the transmission technique, the power is sent from both sides using a transmission line with the sample inside. This method provides multiple means by which to calculate the dielectric properties, all of which can be combined to increase accuracy.

Due to the high price (15k\$) of commercial transmission-based systems, we are in the process of constructing our own transmission lines to confirm the results that we are collecting with the HP 85070 dielectric probe, which is based on the reflection method.

Both techniques make use of a network analyzer that sends and recollects microwave power and calculates the ratios of reflected and transmitted to incident powers.

The HP 85070 kit allows measurements of the complex permittivity for a wide range of semi-solid, pliable-solid, and liquid materials in the range 0.02-20 GHz. It performs all of the necessary network analyzer control, calculation, and data presentation functions. The software controls the network analyzer to measure the complex reflection coefficient of the material under test (MUT). Then it converts the reflection coefficient into the complex permittivity of the MUT. Finally it displays the measurement results in a variety of graphical and tabular formats.

The dielectric probe provides a convenient and repeatable method for measuring various dielectric materials. The convenience is a result of needing only to press the probe against (or immerse it in) the MUT to make a measurement. The probe is used with a vector network analyzer, in our case the HP8722D to take advantage of the analyzer's measurement flexibility, speed, and accuracy. Use of the vector network analyzer allows the software to calibrate against a variety of measurement errors and thus enhance accuracy.

An additional adjustable stand is used to adapt the probe to the samples dimensions (Figure 4).

This system needs to be calibrated as the external surroundings, especially metal parts, influence the measurements. Assuming that the environment does not change significantly, we can correct the measurements based on environmental influences. This

calibration process needs to be performed also when the sample size changes, as container walls and the metal stand would affect the results.

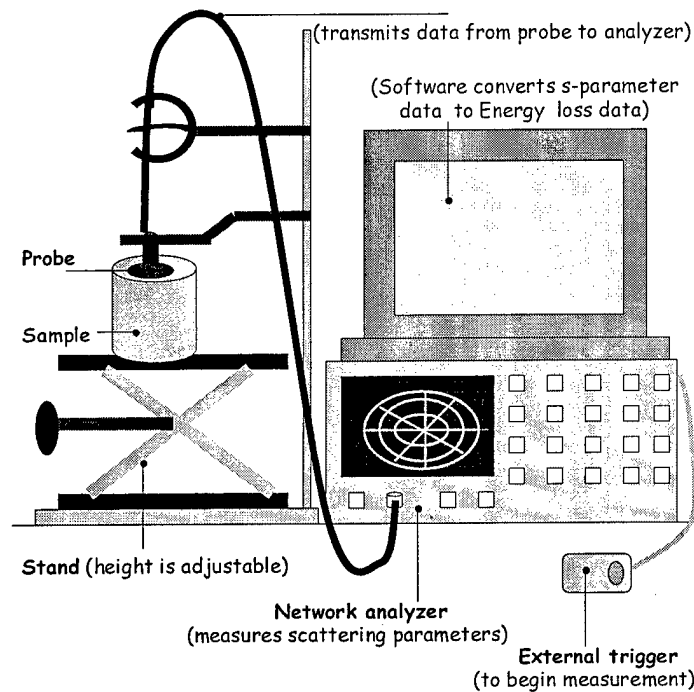


Figure 4: Reflection-based measurement setup.

First of all, due to the price of the material under investigation\* only a limited amount was available (1-2 mL). Thus initially it was necessary to prove that container size could be reduced to 0.3 mL with the proper calibration without affecting the quality of the results. We performed these measurements with DI (define, please) water. As shown in Figure 5, all the samples gave identical results after proper calibration.

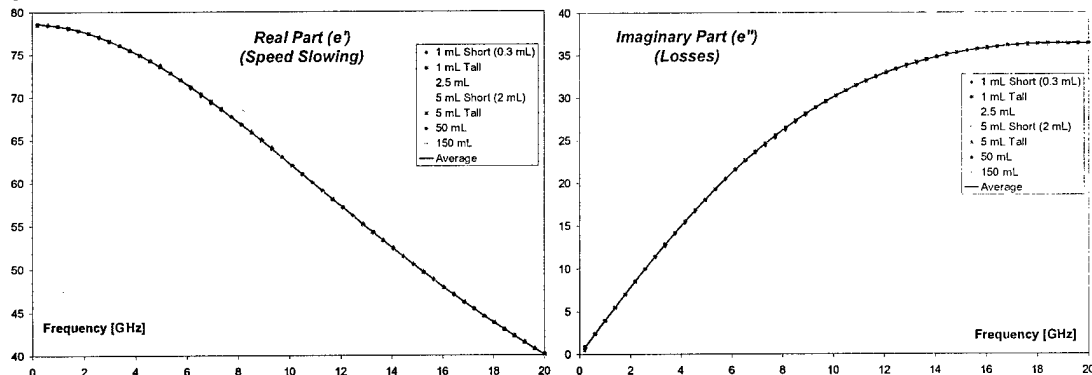
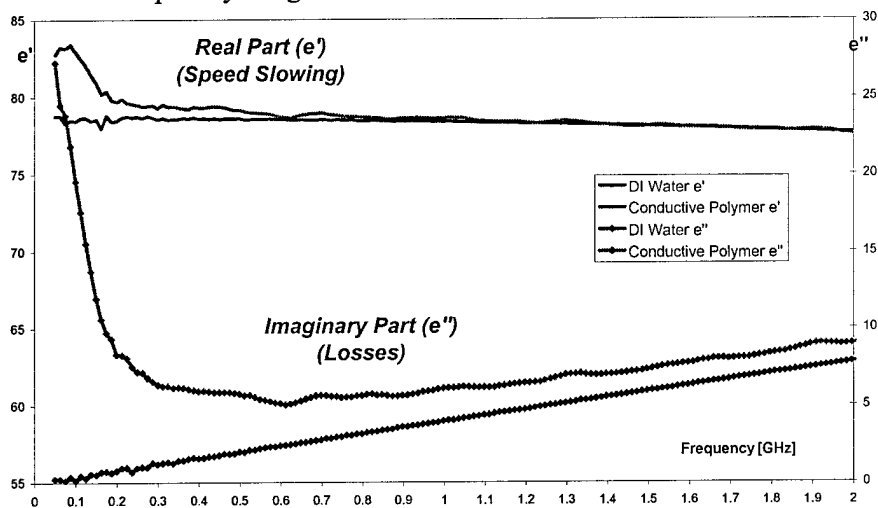


Figure 5: Imaginary and real part of the complex permittivity of DI water for several sample sizes.

Once the dimension of the sample was no longer a concern we performed our first measurement of a mixture of water-soluble conducting polymers. Since the material is

\* The material is a mixture of water-soluble conducting polymers, which is still in its experimental stage, and cannot be disclosed in detail at this juncture in its development.

relatively new, to our knowledge, these measurements are the first to be performed at the 20 MHz to 2 GHz frequency range.



These results clearly suggest higher energy absorption than normal DI water in the whole range, with a drastic increase of loss at lower frequency where current microwave phased array thermal therapy is applied (100-200 MHz)<sup>21</sup>.

As expected these results are difficult to fully interpret, due to the combination of dielectric polarization and conduction effects. They need to be confirmed and compared to the ionized water (with salt concentration of a typical human body). Our future work will include further measurements that will help improve the understanding of their relaxation process.

## Conclusions and Future Work

In this report we presented our progress in understanding the mechanisms of dielectric absorption and scattering as well as the first measurements of the material we are planning to use to enhance the hyperthermia treatment and the tumor detection.

Future work will provide additional information regarding the correctness of the choice of material based on a more complete understanding of the relaxation phenomena involved in a solution of conductive polymers. Additionally, the encapsulation of the selected material in a vector such as a liposome will be the next step followed by the analysis of the leakage, the heating efficiency and scattering properties of such systems. The final goal is to optimize the microwave power delivery system based on the frequency and type (pulsed versus continuum energy) that make best use of the material properties.

## Key Research Accomplishments

- Since the material is relatively new, to our knowledge, these measurements are the first to be performed at the 20 MHz to 2 GHz frequency range.
- These results clearly suggest higher energy absorption than normal DI water in the whole range, with a drastic increase of loss at lower

frequency where current microwave phased array thermal therapy is applied (100-200 MHz)<sup>22</sup>.

## Reportable Outcomes

N/A; pending completion of measurements, experimentation and analysis.

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