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ABSTRACT

Testing blood ex-vivo allows physicians to gain an understanding of what is happening within the human body. Once blood is extracted from the body, it begins to clot. This process can affect the outcome of blood tests, particularly for dielectric measurements. As a result, the dielectric measurements recorded are dependent on time after extraction. To solve the clotting problem, and to allow measurements at any time, anticoagulants are employed. However, a number of anticoagulants have been shown to greatly alter the dielectric properties of fresh blood. In this paper, we analyze the anticoagulant heparin, which works by a different mechanism to stop coagulation than other previously studied anticoagulants. Specifically, heparin acts as a cofactor, accelerating the natural anticoagulation process by several orders of magnitude rather than chelating with clotting factors. We compare intra-individually the dielectric measurements of pure and heparin blood samples at 37°C, taken from four healthy adult volunteers. We also compare the heparin results to previous data on other anticoagulants, specifically, Ethylene Diamene Tetra Acetic acid (EDTA) and sodium citrate. In this exploratory study, we found that the effects of heparin on blood are 2.66% or less for the relative permittivity and conductivity, and that heparin has the least effect on dielectric measurements when compared to the other commonly used anticoagulants. These results suggest heparin as the anticoagulant of choice for dielectric measurement.

Index Terms — **Anticoagulant, Blood, Dielectric Measurement, Microwave Frequency**

1 INTRODUCTION

BLOOD is an important biological material that can provide insight into what is happening inside the human body. Blood samples are commonly taken in clinical situations and analyzed in laboratories to aid in diagnosis and in monitoring of medical conditions. Dielectric measurement has been proposed as one possible test, and allows the water content and electrical properties of the material to be determined. Dielectric measurement has the potential to monitor conditions such as diabetes [1], anemia [2], and to aid in diagnosis of cancerous cells [3]. In these applications, the constituent components of blood, such as hemoglobin levels for anemia detection or monitoring, can be related to the dielectric measurements of blood [2]. This method has the potential to minimize laboratory resources by taking a small blood sample and inexpensively and immediately relating the dielectric properties to key constituent components of the blood [4]. Furthermore, the dielectric properties of blood are relevant to electromagnetic therapeutic technologies, such as hyperthermia and microwave ablation. These technologies use knowledge of dielectric measurements of biological tissues in order to optimize treatment efficacy [5], [6].

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Blood has been previously examined with dielectric measurements to determine the relative permittivity and conductivity of fresh healthy samples across frequency [7], across temperature [8], and to determine the difference between heathy blood and diabetic blood [8]. However, once fresh blood is withdrawn from the body, the electrical properties of blood begin to change with coagulation [9].

Coagulation is a natural process of forming a blood clot that happens within the body to help stop bleeding (hemostasis) [10]. The coagulation process also happens when blood is ex-vivo, after it has been extracted from the body. However, coagulating or coagulated blood may not be desirable for ex-vivo dielectric measurements as the blood properties change during the coagulation process until complete coagulation occurs [9]. In this case, anticoagulants that chemically stop coagulation are employed.

However, anticoagulants can affect the dielectric properties of blood. Common clinically-used anticoagulants, Ethylene Diamene Tetra Acetic acid (EDTA) and citrate (sodium or lithium bonded), have been shown to affect the dielectric properties of blood [11]. Both of these anticoagulants work by chelating with the clotting factor calcium, thereby stopping the coagulation cascade [12], [13].

Another anticoagulant that is less used ex-vivo is heparin. The protein antithrombin naturally inactivates thrombin, thus stopping the coagulation cascade [10], [14]. Unlike EDTA and citrate, heparin acts by binding with antithrombin and by acting as a cofactor, i.e. accelerating the natural anticoagulation process by several orders of magnitude [10], [12], [13]. In a 2014 comprehensive review [15], heparin was recommended for the study of electrolytes and ionized calcium. This result is important as the concentration of ions directly affects the electrical conductivity of a material sample.

Heparin has shown promise with dielectric measurements at lower frequencies [8], [16]–[18]. However, the impact of heparin on the dielectric properties of blood has not been examined across the microwave range. Therefore, in this exploratory study, we analyze the effect of heparin on fresh blood samples from 400 MHz to 20 GHz at body temperature. The aim is to examine the use of heparin as a means to preserve ex-vivo blood while minimizing the effect of an anticoagulant additive on the measured microwave dielectric properties. We also compare the overall intra-individual results of heparin to EDTA and citrate from a previous study of similar sample size. This is the first study to look at the effects of heparin on the dielectric properties of blood through the entire frequency range 400 MHz to 20 GHz and to use blood samples from both genders.

This paper is organized as follows: we present the methodology in Section 2; we discuss our findings in Section 3; and we conclude in Section 4.

2 METHODS

2.1 EXPERIMENTAL SETUP

Dielectric measurements of fresh pure blood and fresh blood with heparin additive were performed over the microwave frequency range of 400 MHz to 20 GHz at 37°C. This frequency range encapsulates the frequencies that most applications using microwaves, in particular, wideband microwave imaging [19], [20], ablation [21], hyperthermia [22] and is within the range of common off-the-shelf dielectric probes [23]. Body temperature is generally accepted as 37°C. Thus, we use this temperature in order to maintain the blood as close to in-vivo temperature as dielectric properties of tissues are temperature and frequency dependent [24]. The measurement equipment consisted of a commercially available slim-form probe (Ø 2.2 mm) from Keysight Technologies (Keysight Technologies, California, United States), connected directly to a Rohde & Schwarz ZVA50 vector network analyzer (VNA), as seen in Figure 1. In order to maintain the blood samples at body temperature, an apparatus consisting of a water bath, a specialized temperature-controlled beaker, and a water pump were connected together. The vacutainer tubes were inserted into a custom stand within the temperature-controlled beaker, while the circulating water from the water bath regulated the temperature. The full experimental setup is shown in Figure 1.

Figure 1. Experimental setup using a slim-form probe directly connected to the VNA. Blood samples were maintained at 37°C by pumping heated water from a large water bath to a temperature-controlled beaker. The beaker was then brought to the probe by a lifting platform, thereby minimizing uncertainties introduced by movement of the probe.

2.2 CALIBRATION AND VALIDATION

A 3-load calibration (open/short/load) was performed before commencing measurements. Validation was performed regularly throughout the experiment, and at both the beginning and end of each measurement set, using 0.1 M sodium chloride (NaCl). All calibration and validation measurements were performed at room-temperature (22.01 ± 0.83°C). The temperature was recorded before and after each measurement. Performing the calibration at a temperature different to the measurement temperature has been shown not to affect the results of the measurements [25]. Measurements were carried out over the frequency range of 400 MHz to 20 GHz with a linear spacing of 81 points. All experimental metadata was recorded in the format recommended by Porter *et al.* [26]. Deionized water and alcohol wipes were employed to clean the probe and the thermometer after each use.

2.3 MEASURMENT PROCEDURE

Fresh blood was collected from four healthy volunteers by a physician in accordance with ethical guidelines at the University of Malta. There were two female volunteers (denoted 'F1' and 'F2') and two male volunteers ('M1' and 'M2'). Four blood samples per volunteer were taken: two were placed in vacutainer tubes without anticoagulant and two were placed in vacutainer tubes with heparin (Vacuette® 4 ml lithium heparin tubes). Each sample contained 4 ml of blood. The collected samples were immediately placed into the temperature-controlled beaker to maintain the samples at approximately body temperature (36.98 ± 0.14°C).

Measurements were first performed on the fresh pure blood samples (in the tubes without anticoagulant) in order to reduce the effect of changes that occur with time from extraction, such as the degree of coagulation. Specifically, the fresh pure blood measurements were completed within 5 minutes of extraction to minimize the effects of coagulation. Measurements on the anti-coagulated blood (with heparin) were then performed.

For all samples, measurements were carried out at two different positions per sample and three measurements per position were taken. In this way, a total of 96 dielectric measurements of blood were obtained. A DTM 3000 thermometer (accuracy: ± 0.03°C) was used to measure the temperature of the blood samples. The sample temperatures were recorded before and after each measurement.

3 RESULTS AND DISCUSSION

3.1 UNCERTAINITY ANALYSIS

In order to evaluate the measurement uncertainty of the system, repeated measurements were taken on a standard liquid with known dielectric properties (0.1 M NaCl). The expanded standard uncertainty (ESU) was then calculated for the 0.1 M NaCl at 22°C, by comparing the measured data to the theoretical known properties. The theoretical 0.1 M NaCl model employed can be found in [27]. The resulting ESUs for relative permittivity (ε_r) and conductivity (σ) were 1.55% and 4.68%, respectively. These values demonstrate that the measurement system is capable of recording dielectric data with high accuracy and high repeatability.

3.2 FRESH PURE BLOOD VS. FRESH BLOOD WITH HEPARIN

The measured data was analyzed and compared intra-individual in order to prevent inter-individual confounders affecting the results e.g. gender and water content [28]–[30]. For each individual, 12 measurements were taken for each case, pure and with heparin additive (2 samples per case and person, 2 positions per sample, 3 measurements per position). The mean and standard deviation across all 12 measurements were calculated at each frequency.

Figures 2 and 3 show the variation per person between the pure and heparin blood samples for the two endpoint frequencies. In all cases, the range of properties for the heparin blood samples overlaps with the range of properties for the pure blood samples. Further, the mean of the heparin blood samples is within the error bars (3 standard deviations) of the pure blood samples in almost all cases.

The trend holds across the whole frequency range for both ε_r and σ, as shown in Figures 4 and 5. While some difference can be seen between ε_r curves of the pure and heparin blood samples, the difference is small. Similarly, the difference between σ curves of the pure and heparin blood samples is minor, with small changes at the upper end of the frequency range (> 10 GHz).

Intra-individual comparisons of the mean percentage differences between pure blood and blood with heparin are presented in Table 1. The mean percentage differences for ε_r and σ were 2.66% and 2.28%, respectively (across all frequencies and all individuals). The small mean percentage difference illustrates that the use of heparin as an anticoagulant has little effect on the dielectric measurements. In fact, this mean percentage difference would be within the experimental uncertainty in many studies (e.g. where uncertainty > 3%).

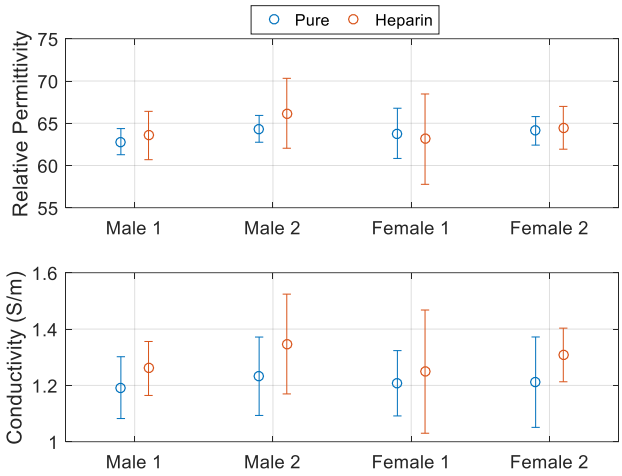

Figure 2. The mean recorded measurements of ε_r and σ per person at 400 MHz. The error bars represent 3 standard deviations, encapsulating 99.73% of the data for each person. For each case (specific person and pure/heparin), 12 measurements each were taken. For both ε_r and σ, all error bars overlap.

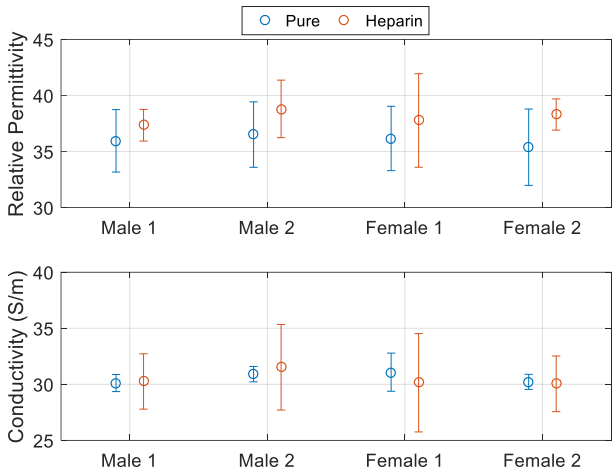

Figure 3. The mean recorded measurements of ε_r and σ per person at 20 GHz. The error bars represent 3 standard deviations, encapsulating 99.73% of the data for each person. For each case (specific person and pure/heparin), 12 measurements were taken. For both ε_r and σ, all error bars overlap, and the mean of the heparin data is within the range of data recorded for pure blood.

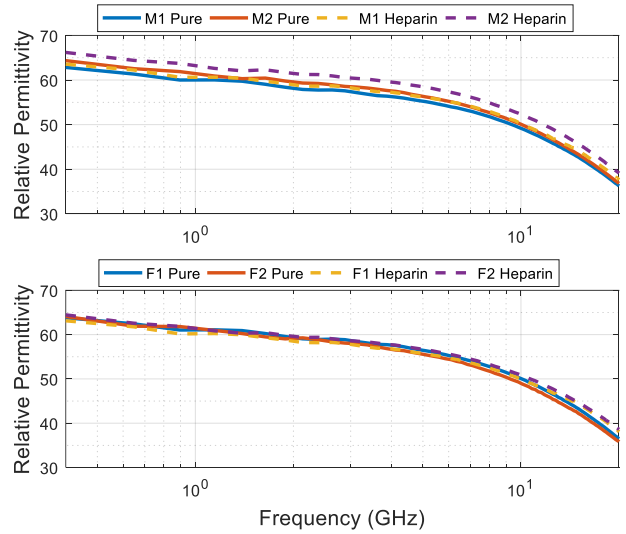

Figure 4. The measured ε_r for (top) male (M) and (bottom) female (F) samples from 400 MHz to 20 GHz. Very little difference can be seen between pure and heparin blood samples. The average standard deviation for each case (gender and pure/heparin) across the whole frequency range was less than 1.89, indicating very consistent measurements.

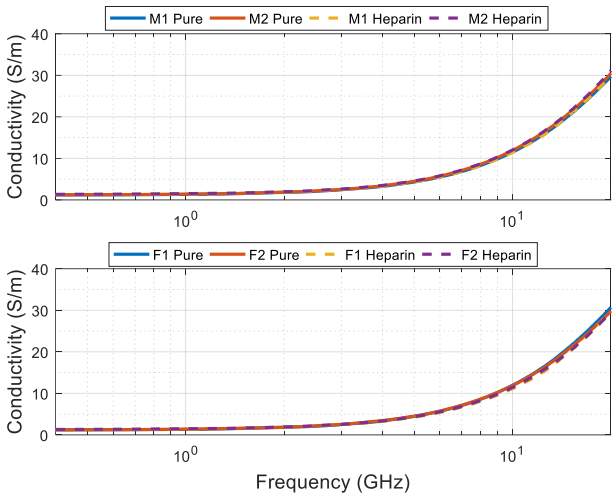

Figure 5. The measured conductivity for (top) male (M) and (bottom) female (F) samples from 400 MHz to 20 GHz. No noticeable difference can be seen below 10 GHz between the types of blood samples, and the difference above 10 GHz is minute. The average standard deviation for each case (gender and pure/heparin) across the whole frequency range was less than 0.57 S/m, indicating very consistent measurements.

Table 1. Mean percentage difference (PD) between pure and heparin blood for both ε_r and σ for each volunteer

Volunteer	ε_r Mean PD (%)	σ Mean PD (%)
Male 1	2.33	0.12
Male 2	4.35	1.43
Female 1	0.37	5.11
Female 2	3.58	2.46

3.3 HEPARIN VS. OTHER ANTICOAGULANTS

In the previous subsection, we observed very little difference between the dielectric properties of the pure blood and the fresh heparin blood. Citrate and EDTA are other common anticoagulants in clinically employed vacutainer tubes and have been previously used in dielectric studies of blood [7]. However, Salahuddin *et al.* [11] showed that these anticoagulants (sodium citrate and EDTA) can lead to dielectric measurements that vary from the true dielectric measurements of pure blood. Salahuddin *et al.* compared the intra-individual mean percentage difference for both ε_r and σ and for each of four volunteers. In Table 2, we present the overall percentage difference for each anticoagulant (sodium citrate, EDTA, and heparin). While the volunteers differed between the studies, in both cases the intra-individual comparison removes the effect of inter-individual confounders.

From Table 2, it is evident that blood with heparin has the lowest difference from pure blood for both ε_r and σ, followed by sodium citrate. For the effect on the σ, there is a factor of 2 in the difference between heparin and sodium citrate. The effect of heparin on ε_r is 57% less than the effect of sodium citrate. Unlike the other two anticoagulants, heparin has a lower difference in σ than ε_r. This low difference in conductivity aligns with [15], where heparin was recommended as the anticoagulant for use in electrolytes and ionized calcium studies. The addition of chemical compounds that alter the concentration of ions in a substance can affect the electrical conductivity. Therefore, the larger conductivity difference for EDTA and citrate could be due to chelation, in which a chelating agent forms two or more separate bonds to a single central metal ion [31], e.g., calcium within the blood sample. Thus, the redistribution of ions may be the cause of the conductivity change with EDTA and citrate.

Table 2. Mean percentage difference (PD) between pure and heparin blood for both ε_r and σ for each volunteer

Comparison	ε_r Mean PD (%)	σ Mean PD (%)
Pure vs. Heparin	2.66	2.28
Pure vs. EDTA	6.67	7.57
Pure vs. Sodium Citrate	4.63	4.73

4 CONCLUSIONS

In this paper, we performed an exploratory study of heparin as an anticoagulant for dielectric measurements. We found that heparin deviated from the pure blood measurement by 2.66% or less for both relative permittivity and conductivity. We also found that heparin had the minimum impact on dielectric measurements when compared to the commonly employed anticoagulants EDTA and sodium citrate. Future work in this area demands a larger study in order to determine the level of significance of the effect of heparin on the measured dielectric properties of blood, as well as to determine the effect of heparin with temperature beyond normal body temperature (37°C). Based on the current literature, with results derived from studies involving similar samples sizes, we currently recommend using heparin as the anticoagulant to preserve blood for dielectric measurements at microwave frequencies and normal body temperature. This study supports the possibility of storing and testing blood samples at later stages for dielectric spectroscopy. Furthermore, these results contribute to the field of dielectric data for biological tissues, which are key for electromagnetic medical technologies.

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