Bioelectromagnetics 33:346–355 (2012)

Complex Permittivity of Representative Biological Solutions in the 2–67 GHz Range

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The main purpose of this study is to provide experimental data on the complex permittivity of some biological solutions in the 2–67 GHz range at room and human body temperatures. The permittivity measurements are performed using an open-ended coaxial probe. Permittivity spectra of several representative monomolecular solutions of proteins, amino acids, nucleic acids, and carbohydrates are analyzed and compared. Furthermore, measurements have also been performed for complex biomolecular solutions, including bovine serum albumin (BSA)–DNA–glucose mixture, culture medium, and yeast extract solution. The results demonstrate that for concentrations below 1%, the permittivity spectra of the solutions do not substantially differ from that of distilled water. Measurements carried out for 4% and 20% BSA solutions show that the presence of proteins results in a decrease in permittivity. For highly concentrated RNA solutions (3%), a slight increase in the imaginary part of the permittivity is observed below 10 GHz. Experimental data show that free water permittivity can be used for modeling of the culture medium above 10 GHz. However, at lower frequencies a substantial increase in the imaginary part of the permittivity due to ionic conductivity should be carefully taken into account. A similar increase has also been observed for the yeast extract solution in the lower frequency region of the considered spectrum. Above 10 GHz, the high concentration of proteins and other low-permittivity components of the yeast extract solution results in a decrease in the complex permittivity compared to that of water. Obtained data are of utmost importance for millimeter-wave dosimetry studies. Bioelectromagnetics 33:346–355, 2012.

Key words: millimeter waves; coaxial probe; dielectric properties; permittivity measurements; culture medium; proteins; DNA; RNA; glucose; yeast extract

INTRODUCTION

Millimeter waves in the 30–100 GHz range have been used for numerous applications. In particular, the 57–64 GHz frequency range has been recently identified as extremely promising for wireless applications essentially due to the high data transmission rates (up to 10 Gb/s) and low interference with existing systems [Wells, 2009]. In 2009, the Wireless Gigabit Alliance (WiGig) was created to develop a new standard for wireless high-definition multimedia content transmissions at 60 GHz [WiGig, 2011]. Furthermore, the recent progress in millimeter-wave technologies and miniaturization of radiating structures has triggered research activities aimed at developing future millimeter-wave body area networks (BAN). Today, several research groups focus on the characterization of the body channel and development of millimeter-wave body-worn antennas [Zhadobov et al., 2011].

The biocompatibility issues of these radiations have been investigated for more than 40 years. The first results, published by Grundler et al. [1977] and Fröhlich [1978], demonstrated that low-power millimeter waves can impact living systems including

Grant sponsors: Agence Nationale de la Recherche (ANR) (Bio-CEM project); Grant number: ANR-09-RPDOC-003-01; Centre National de la Recherche Scientifique (CNRS).

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Received for review 10 November 2010; Accepted 19 September 2011

DOI 10.1002/bem.20713
Published online 19 October 2011 in Wiley Online Library (wileyonlinelibrary.com).

biological cells. Recently, several in vivo and in vitro studies have reported their potential biological and health effects [Pakhomov et al., 1998; Nicolas Nicolaz et al., 2009; Zhadobov et al., 2009; Alekseev et al., 2010]. Most of these studies demonstrated that: (1) direct health effects are not expected for millimeter waves at exposure levels below the current exposure restrictions for the general public (10 W/m^2 under far-field conditions); and (2) because of the very local power absorption, medium- and high-power exposures can induce thermal effects that can potentially result in a biological response. The ongoing studies mainly focus on possible synergistic and combined electromagnetic/thermal biological effects, determination of power thresholds of induced effects, and identification of potential biomarkers of over-exposure.

For all these studies, the accurate dosimetric characterization of exposure levels is important for the reliable interpretation of the experimentally obtained biological/biophysical results. In particular, one of the major challenges for numerical millimeter-wave dosimetry is an accurate determination of the permittivity and conductivity of biological tissues and solutions, since most of well-established broadband experimental data for these characteristics are provided for frequencies below 20 GHz [Gabriel et al., 1996], with little data at millimeter waves. A precise knowledge of these dielectric properties is crucial and directly impacts the accuracy of any numerical dosimetry study.

In the millimeter-wave band, skin permittivity values for in vivo studies have been obtained experimentally [Alabaster, 2003; Alekseev and Ziskin, 2007; Alekseev et al., 2008] or by extrapolation of experimental results obtained from microwaves [Gandhi and Riazi, 1986; Gabriel et al., 1996; Alabaster, 2003]. However, in contrast to the lower part of the microwave spectrum [Kaatze and Feldman, 2006], few experimental data on the permittivity of biological solutions used in in vitro studies are available for frequencies around 60 GHz [Pottel et al., 1984; Alison and Sheppard, 1993; Fuchs and Kaatze, 2002]. To model the broadband dispersive behavior of biological samples, several analytical techniques have been introduced and used for the approximate evaluation of the permittivity [Takashima, 1989; Zhadobov et al., 2008]. However, for some biological solutions these theoretical models have never been validated experimentally for the upper part of the microwave spectrum, mainly because commercially available techniques for the dielectric characterization of lossy liquids are limited to 50 GHz.

The main purpose of this study is to experimentally determine and analyze the complex permittivity and dispersive behavior of several representative monomolecular and multimolecular biological solutions, including culture medium and yeast extract solution, for frequencies up to 67 GHz at room (24 °C) and human body (37 °C) temperatures.

MATERIALS AND METHODS

Biological Samples

Living organisms are mainly composed of water, organic molecules, and ions [Alberts et al., 2002]. Water constitutes 70–85% of the total body mass. It represents 59–72% of the skin and 75–80% of the cornea endothelium—the primary targets for millimeter waves [Duck, 1990]. The rest of the cellular mass is related to carbon-containing molecules, which can be divided into four classes: proteins, nucleic acids, carbohydrates (sugars), and lipids. In this study, we consider only proteins, nucleic acids, and sugar water solutions (Table 1). The lipids were eliminated because they are poorly soluble in water, which makes obtaining homogeneous solutions practically impossible. As several solutions considered in this study have nonzero static conductivity that partly determines their permittivity in the lower part of the considered spectrum, static conductivity of all solutions was measured using a HANNA HI 8733 conductometer (Woonsocket, RI) at 24 and 37 °C (Table 1).

Monomolecular Solutions

Depending on the type of cells, proteins represent 10–25% of the cell mass, the largest contribution after water. In blood serum, the most abundant protein is albumin, and its normal concentration is around 35–45 g/L (roughly 4%). Therefore, we selected a 4% bovine serum albumin (BSA) solution as a representative biological model. Furthermore, measurements have also been carried out for a 20% solution, which represents the maximum protein concentration in biological cells.

Proteins are polymers of amino acids. The concentration of free amino acids in cells is less than 1% of the cellular weight. To determine whether the permittivity values could vary as a function of the amino acid structure, we compared two amino acids with different properties: (1) tryptophan containing a side chain with a hydrophobic aromatic ring; and (2) lysine, a positively charged amino acid having basic and hydrophilic side chains.

DNA and RNA are polymers of nucleotides; they consist of purine and pyrimidine bases linked to

TABLE 1. Biological Solutions Used for the Permittivity Measurements

Molecules	Concentration (g/L)	Static conductivity at 24 °C (S/m)	Static conductivity at 37 °C (S/m)
Proteins and amino acids			
Bovin serum albumin (BSA)	40 (4%)	4.55×10^{-2}	5.65×10^{-2}
	200 (20%)	11.5×10^{-2}	15.1×10^{-2}
Tryptophan	10 (1%)	3.2×10^{-3}	4×10^{-3}
Lysine	10 (1%)	0.47	0.59
Nucleic acids			
DNA	10 (1%)	0.22	0.24
RNA	10 (1%)	0.22	0.25
	30 (3%)	0.47	0.55
Carbohydrates			
Glucose	1 (0.1%)	0.8×10^{-3}	1.5×10^{-3}
	10 (1%)	10^{-3}	1.4×10^{-3}
Mixture of three different molecules			
BSA	100 (10%)	7.6×10^{-2}	10.1×10^{-2}
Glucose	10 (1%)		
DNA	10 (1%)		

phosphorylated sugars. Depending on cell type, DNA, and RNA represent 0.3–1% and 0.8–6% of the total cell mass, respectively. We used a 1% DNA solution from the salmon sperm and a 1% RNA solution from the torula yeast (*Candida utilis*). To analyze in depth the dispersive behavior of these solutions at various concentrations, we also performed measurements for a 3% RNA solution, which corresponds to the maximal soluble concentration. A further increase in the RNA concentration results in sedimentation.

Glucose is the archetype of the carbohydrate class of molecules. In blood, the glucose level varies with food intake, but the normal range for most people is 0.8–1.1 g/L (roughly 0.1%). However, the level of sugars and their metabolites may represent 1% of the total cellular mass in bacteria or even more in eukaryotic cells. Therefore, we selected two concentrations of the glucose solutions, namely 0.1% and 1%.

Complex Biomolecular Solutions

Because biological cells are composed of various molecules, we also measured the permittivity of complex biomolecular solutions. First, a mixture containing three representative molecular types from each class, namely BSA, Glucose, and DNA, has been used (Table 1). Second, a culture medium (containing amino acids, nucleotides, sugars, ions, growth factors and hormones, etc.) commonly employed in in vitro bioelectromagnetic experiments has been selected for permittivity measurements. We used Dulbecco's modified Eagle medium (Gibco/Life Technologies, Paisley, UK), supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco/Life Technologies). Measured static conductivity of the culture medium equals 1.5 S/m (at 24 °C) and 2 S/m (at 37 °C). Finally, we used yeast extract solution as an experimental model, whose composition is much closer to the cytoplasmic medium compared to the above-described solutions. A 10% solution was prepared using the yeast extract (Amresco, Solon, OH) and was made by extracting and lyophilizing soluble cytoplasmic contents of yeast cells (peptides, amino acids, vitamins, sugars, and other soluble components). The yeast extract is rich in amino acids and protein fragments, which represent up to 55% of the powder. The measured static conductivity of this solution equals 1.1 S/m (at 24 °C) and 1.5 S/m (at 37 °C).

MEASUREMENT PROCEDURE

Experimental Set-Up

The permittivity measurement system implemented here consists of: (1) an Agilent 8510C vector network analyzer (VNA; 45 MHz–110 GHz) operated by a PC with Agilent 85070 software through the Agilent 82357A GPIB interface (Agilent Technologies, Santa Clara, CA); (2) an Anritsu 3670V50 semi-rigid coaxial cable (DC-70 GHz; Anritsu Emea, Luton, UK); and (3) a coaxial slim open-ended probe. This probe has been recently designed by Agilent Technologies for permittivity measurements of lossy liquids and semi-solids up to 67 GHz. The set-up, sample and probe dimensions are represented in Figure 1.

During the measurements, the coaxial probe is immersed in the sample located in a cylindrical

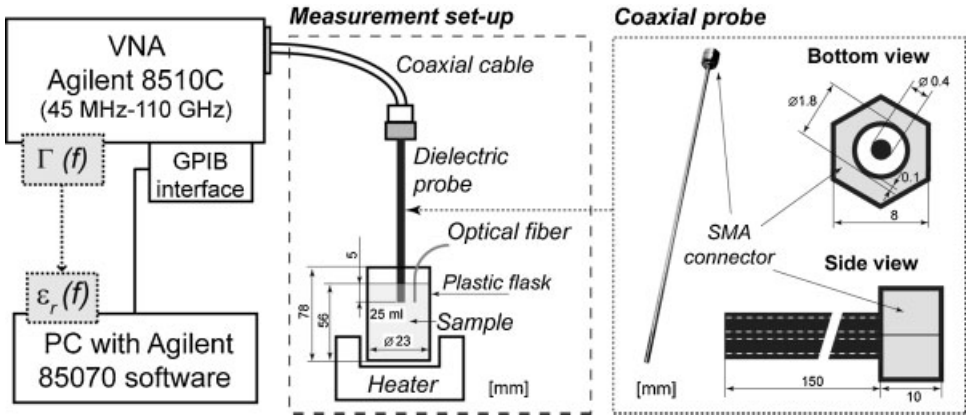

Fig. 1. Schematic representation of the measurement set-up, sample and coaxial probe dimensions (proportions are not to scale).

plastic flask, and the complex reflection coefficient (Γ) is measured using the VNA. The genetic algorithm implemented in the Agilent software is used to retrieve the complex permittivity (ε_r) of the sample under test from the measured values of Γ (for a detailed review on open-ended coaxial probes, refer to Blackham and Pollard [1997], Chen et al. [2004] and Kaatze [2010]). It is worthwhile to underline that the measured imaginary part of the permittivity accounts for both electrical and ionic losses [Zhadobov et al., 2008].

In the coaxial probe method, it is assumed that the material under test is nonmagnetic, isotropic, and homogeneous. This is the case for all the solutions measured here. Furthermore, the assumption of a semi-infinite test material should be satisfied [Chen et al., 2004]. Measurements performed for the samples of different sizes confirmed that this condition is also fulfilled because the considered samples are highly lossy and the sample dimensions are much larger than the probe aperture (at least 10 times).

To confirm the results obtained using the 67 GHz probe, we also used a commercially available 50 GHz Agilent 85070E slim probe. Comparative measurements demonstrated almost identical results up to 50 GHz for the distilled water and test solutions (Table 1). For the sake of brevity, we do not present the results obtained using the 50 GHz probe.

Calibration Procedure

The probe calibration was performed before each measurement. A three-term calibration was used to compensate for the directivity, tracking, and source match errors that are present in any reflection measurement. It was performed by measuring the reflection coefficient of known loads, namely air, a short circuit, and distilled water. The latter was chosen as a calibration standard as the biological solutions to be measured have 80–99% water content, and therefore it guaranteed the best measurement accuracy among available reference liquids (e.g., methanol, ethanol, and ethanediol). Furthermore, utilization of these liquids as references is appropriate only for frequencies below 10 GHz because millimeter wave permittivity is too low to obtain reliable results using the measurement technique considered in this study [Kaatze, 2007]. The difference between the predicted and actual values is used to remove the systematic errors from measurements. In order to avoid any artifacts during the measurements, the probe, and cable are fixed in a given position.

When performing the calibration with the open load at room temperature, the probe tip was suspended in the air inside a $5 \times 5 \times 5$ cm^3 box made of microwave absorbers. This avoids any calibration distortion due to reflections from surrounding objects. The deionized water used for calibration has a static conductivity lower than 10^{-3} S/m and a concentration of dissolved residual solids less than 10 mg/L. Particular care was taken to ensure a perfect contact between the probe tip and the liquid to avoid air bubbles that could strongly affect the accuracy of results. A reflex fiber optic thermometer (Neoptix, Québec, Canada) with a precision of ± 0.05 °C and an accuracy of ± 0.5 °C was used to locally monitor the temperature of the samples in proximity to the tip of the probe (Fig. 1). A heater (DB28510, Thermolyne, Duduque, IA) was used to heat the samples when needed and maintain their temperature within the measurement accuracy of ± 0.5 °C.

Although the measurement principles of the coaxial probe technique remain the same as at room temperature, at higher temperatures the measuring precision and accuracy decrease due to some technical difficulties essentially related to the presence of the differential thermal expansion and temperature gradients [Chen et al., 2004]. In addition, thermal elongation of the probe could result in a phase-shift error [Arai et al., 1995]. As there is no universal procedure to overcome these issues, several experimental calibration protocols have been implemented and compared for measurements at 37 °C. Hereafter, we describe the protocol corresponding to the best measurement accuracy. First, the probe calibration is carried out at a temperature slightly higher (39 °C) than the one of the sample under test (37 °C) using deionized water. After cooling of the reference liquid and probe tip to the target temperature, the calibration is refreshed. Then, the water is replaced by a test sample at 37 °C to perform the measurement. This protocol allows us to achieve reproducibility above 90% when measuring the complex permittivity in the 55–65 GHz range for liquids whose permittivity is close to that of distilled water. For the solutions with permittivity spectra substantially different from those of water (11% ethanol solution, 20% BSA, BSA–DNA–glucose mixture, and yeast extract solution), the experimental accuracy at 37 °C is not acceptable, particularly at millimeter waves, and therefore the results are presented exclusively at 24 °C for these solutions.

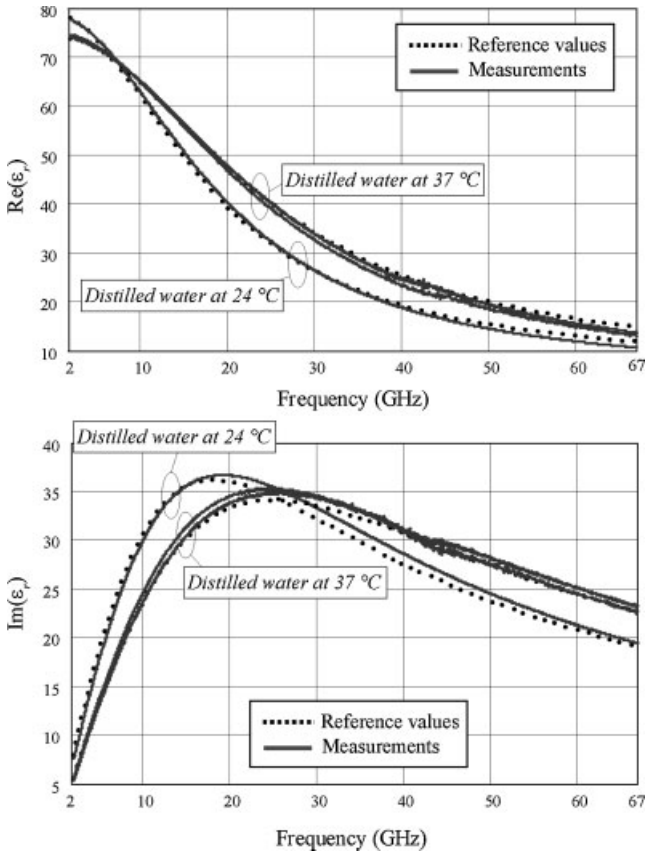

Fig. 2. Measured real and imaginary parts of the distilled water permittivity compared to the reference data. Results are for three independent measurements at each temperature.

RESULTS

Reproducibility and Accuracy

The reproducibility is assessed experimentally by measuring the complex permittivity of distilled water several times at 24 and 37 °C with independent calibrations. The measured data are compared in Figure 2 with reference values for pure water extracted from Ellison's database [Ellison, 2007].

These results demonstrate an excellent reproducibility when retrieving the real Re(ε_r) and imaginary Im(ε_r) parts of the complex relative permittivity at both temperatures even at millimeter-wave frequencies. For the sake of brevity, the term ''relative'' will henceforth be omitted. Maximal deviations compared to the reference values equal 8% and 4% within 55–65 GHz range for Re(ε_r) and Im(ε_r), respectively. The reproducibility of the results for the highly concentrated solutions (20% BSA, mixture, and yeast extract solution) is shown in the figures corresponding to the measured spectra of these solutions (Figs. 4, 9, and 11). The reproducibility of measurements for these solutions is similar to those obtained for water.

Figure 2 demonstrates that the permittivity spectrum also depends strongly on the temperature. For instance, the relaxation frequency is shifted from 19 to 25 GHz when the temperature is increased from 24 to 37 °C. This dependence should be carefully taken into account when defining the permittivity data for dosimetry studies. It is worthwhile to note that the reproducibility is better at room temperature than at 37 °C. This can be explained by the fact that the calibration procedure is straightforward and more accurate at room temperature.

To assess the accuracy of the method, several measurements with independent calibrations have been carried out for the 11% ethanol solution. Its dielectric properties differ from those of water, but are close to those of some complex biomolecular solutions considered in this study. The results have been compared with the reference data presented in the literature [Sato and Buchner, 2004] (Fig. 3). The

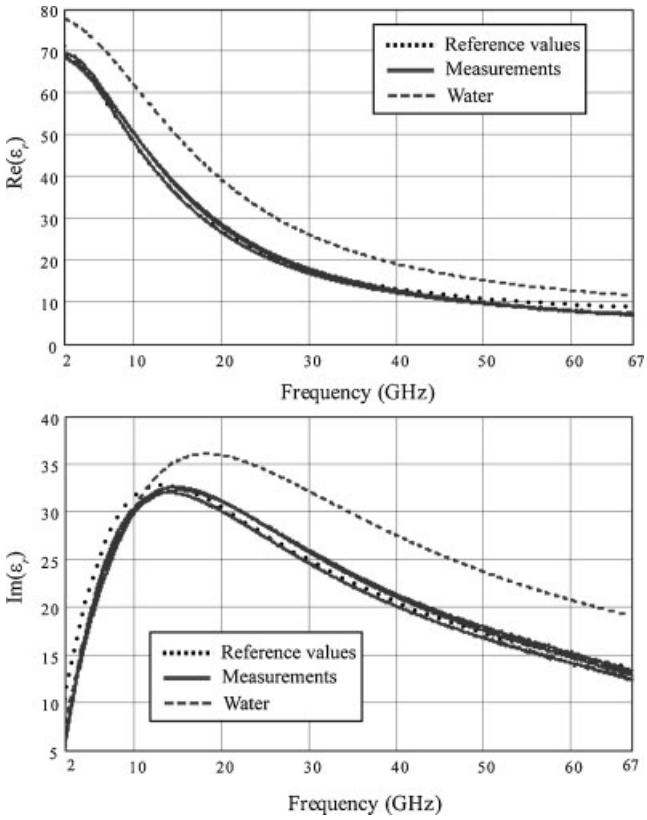

Fig. 3. Measured real and imaginary parts of 11% ethanol solution permittivity compared to the reference data [Sato and Buchner, 2004]. Results are given for three independent measurements at 24 °C.

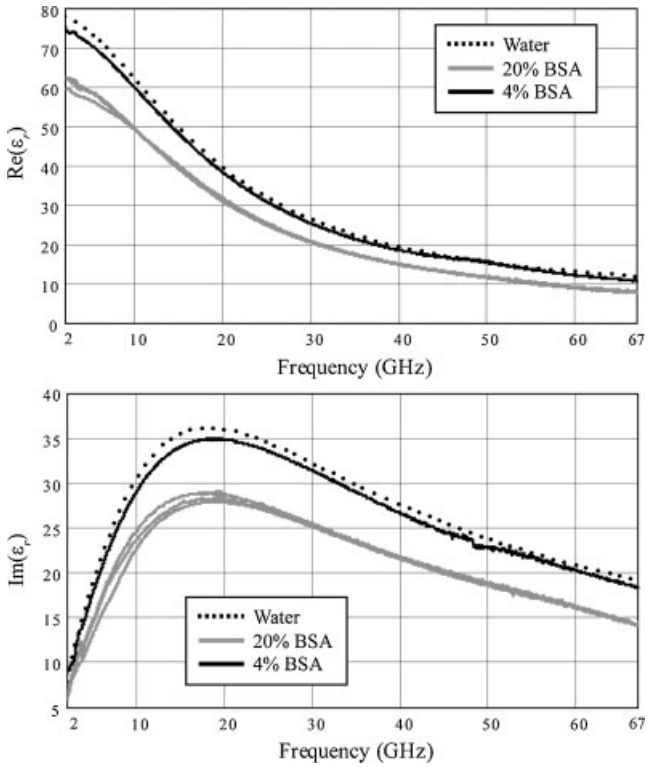

Fig. 4. Measured complex permittivity of 4% and 20% BSA solutions at 24 °C. For the 20% BSA solution, the results are presented for three independent measurements.

accuracy is better than 10% within 55–65 GHz range for both $\mathrm{Re}(\varepsilon_r)$ and $\mathrm{Im}(\varepsilon_r)$.

Permittivity of Biological Solutions

Monomolecular solutions. First, we measured the complex permittivity for 4% and 20% BSA solutions (Fig. 4). Each measurement was repeated at least three times to confirm the reproducibility. Whereas the difference between the permittivities of water and the 4% BSA solution is negligible, the results for the 20% BSA solution demonstrate a substantial decrease in both the real and imaginary parts. However, the relaxation frequency remains the same as for distilled water. Above 50 GHz, the major observed phenomenon is a strong decrease in the imaginary part of the permittivity compared to distilled water.

Figure 5 shows the permittivity of 1% solutions of lysine and tryptophan—two essential amino acids of human cells. The results show that the behavior of amino acids at 24 and 37 °C demonstrates the same dynamics as those of water at respective temperatures, even at a higher than normal concentration.

Figures 6 and 7 provide dielectric characteristics of 1% DNA and RNA solutions, respectively, at 24 and 37 °C. Again, the results are close to those of distilled water within the measuring accuracy. To study the behavior at higher concentrations, we increased the RNA ratio to 3%. Whereas the results are still similar to the dielectric characteristics of water at respective temperatures, an increase in the imaginary part of permittivity below 10 GHz is observed for both temperatures (Fig. 7). This increase is stronger compared to the one observed for the measured permittivity of a NaCl solution with equivalent static conductivity. These observations confirm the data previously reported for DNA solutions [Swicord and Davis, 1982; Takashima et al., 1984].

Figure 8 represents the results for 0.1% and 1% glucose solutions at 24 and 37 °C compared to the free water permittivity. Glucose is highly soluble in water, and our experimental results demonstrate that the complex permittivity of the solution is almost identical (within the measuring accuracy) to that of water at both temperatures. However, a shift in the relaxation frequency of 1.5 and 0.5 GHz toward the

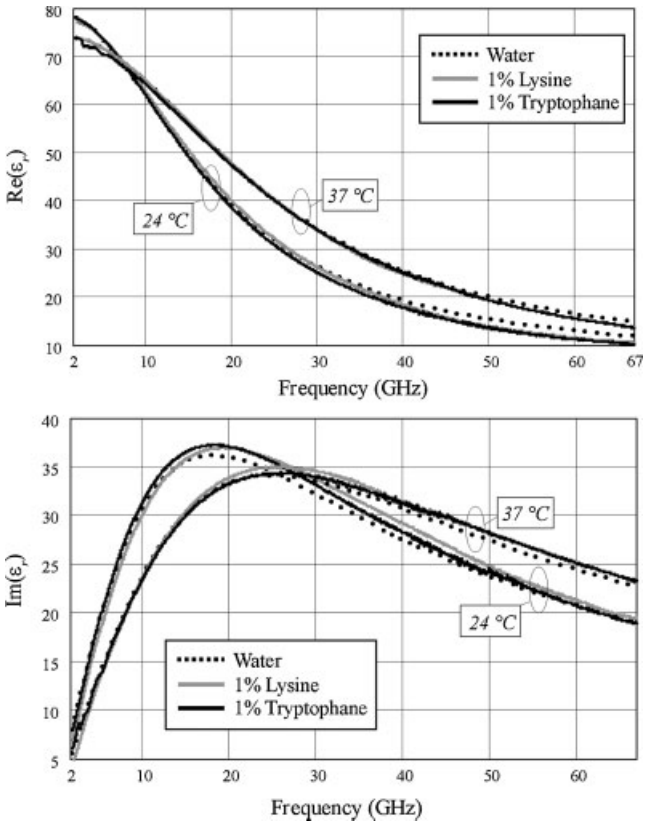

Fig. 5. Measured complex permittivity of 1% lysine and tryptophane solutions at 24 and 37 °C.

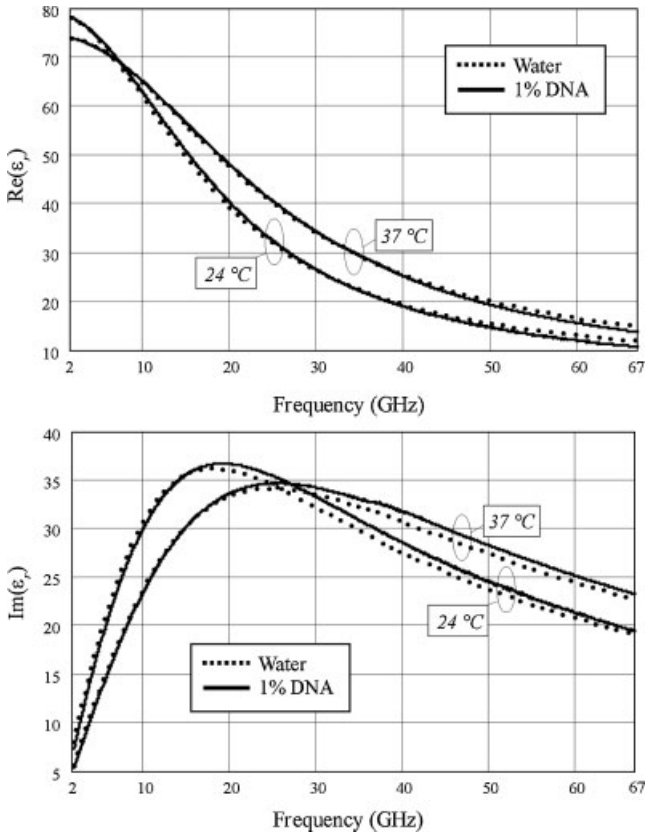

Fig. 6. Measured complex permittivity of 1% DNA solution at 24 and 37 °C.

lower frequencies is observed at 24 and 37 °C, respectively, when the concentration is increased from 0.1% to 1%.

Complex biomolecular solutions. The permittivity results obtained for a mixture of 10% BSA, 1% DNA, and 1% glucose are consistent with the results for the monomolecular solutions (Fig. 9). A decrease in the complex permittivity compared to the distilled water is related to the BSA, whereas small concentrations of other components have a weak impact on the resulting permittivity.

The complex permittivity spectra for the culture medium at 24 and 37 °C are plotted in Figure 10. Below 10 GHz, a substantial difference in the culture medium permittivity compared to free water is related to the presence of 1% salt in the solution (NaCl, $NaHCO_3$, etc.) [Peyman et al., 2007]. This is confirmed by comparison with the permittivity spectrum of the NaCl solution, which has an equivalent static conductivity (Fig. 10). In particular, at 24 °C the imaginary part of the culture medium permittivity is 2.5 and 1.2 times higher compared to distilled water at 2 and 5 GHz, respectively. It is interesting to note that this difference increases as a function of temperature as the ionic conductivity increases with temperature. For instance, at 37 °C the difference increases 4 and 1.4 times at 2 and 5 GHz, respectively. However, at millimeter waves the difference between the permittivities of the culture medium, NaCl solution, and water becomes negligible (it is lower than the measurement accuracy).

The permittivity spectrum of the yeast extract solution is represented in Figure 11. It reveals the contribution of different cell components. Comparison with the permittivity of the NaCl solution at the same static conductivity demonstrates that below 10 GHz, the increase in the imaginary part is partially due to the dominating ionic conductivity. It is interesting to note that this increase is more pronounced for the yeast extract solution compared to the NaCl solution. Above 10 GHz, the major contribution to the permittivity value is related to the presence of proteins (4%) and other low-loss dielectric cytoplasmic components, resulting in a decrease in both the real and imaginary parts of the permittivity.

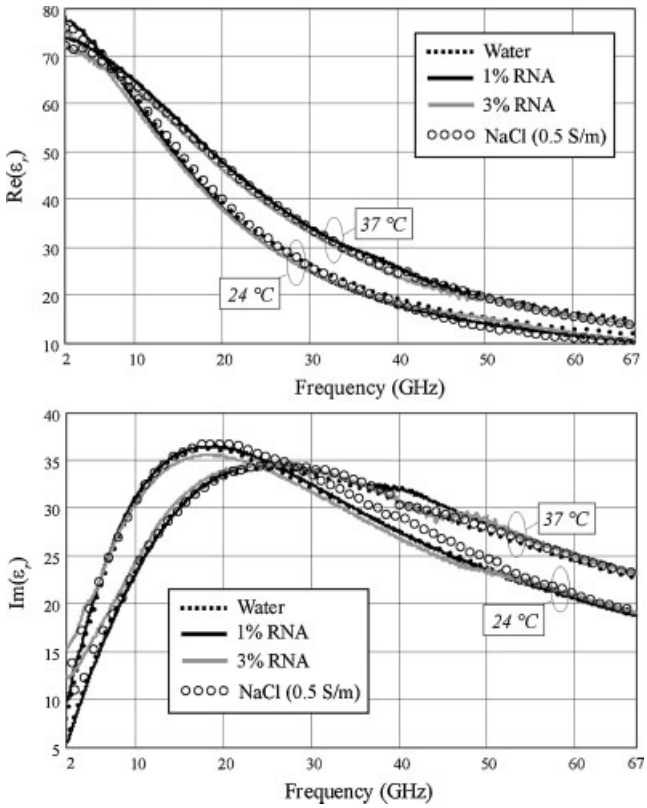

Fig. 7. Measured complex permittivity of 1% and 3% RNA solutions at 24 and 37 °C.

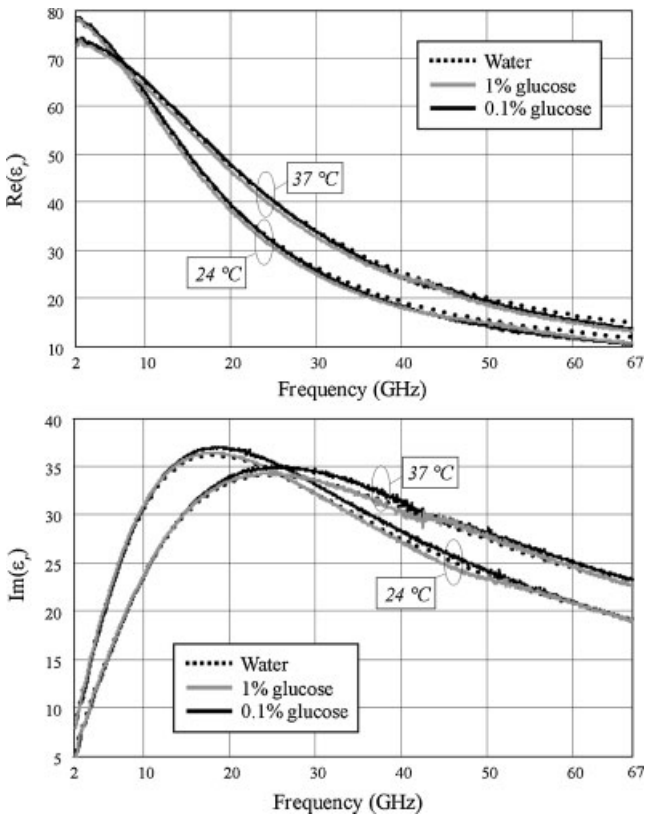

Fig. 8. Measured complex permittivity of 0.1% and 1% glucose solutions at 24 and 37 °C.

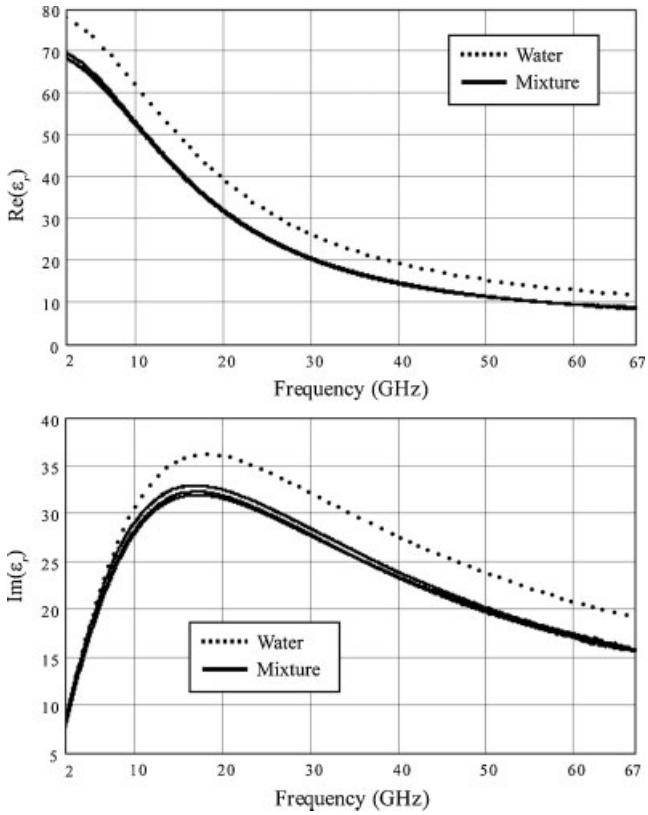

Fig. 9. Measured complex permittivity of water mixture containing 10% BSA, 1% DNA, and 1% glucose. Results are of three independent measurements at 24 °C.

DISCUSSION AND CONCLUSION

In this article, we reported and analyzed original experimental data on the complex permittivity of various biological solutions including major organic molecule solutions, culture medium commonly used in in vitro experiments, and a cellular cytoplasm model represented by the yeast extract solution. For the first time the permittivity of these solutions is measured up to 67 GHz. Furthermore, a specific calibration procedure has been implemented to perform reproducible and accurate measurements at the human body temperature (37 °C) in order to carefully account for the strong dependence of the permittivity on the temperature of high water content solutions. For the solutions demonstrating an increase in the imaginary part of the permittivity at frequencies below 10 GHz, the results are compared to NaCl solutions having equivalent static conductivity.

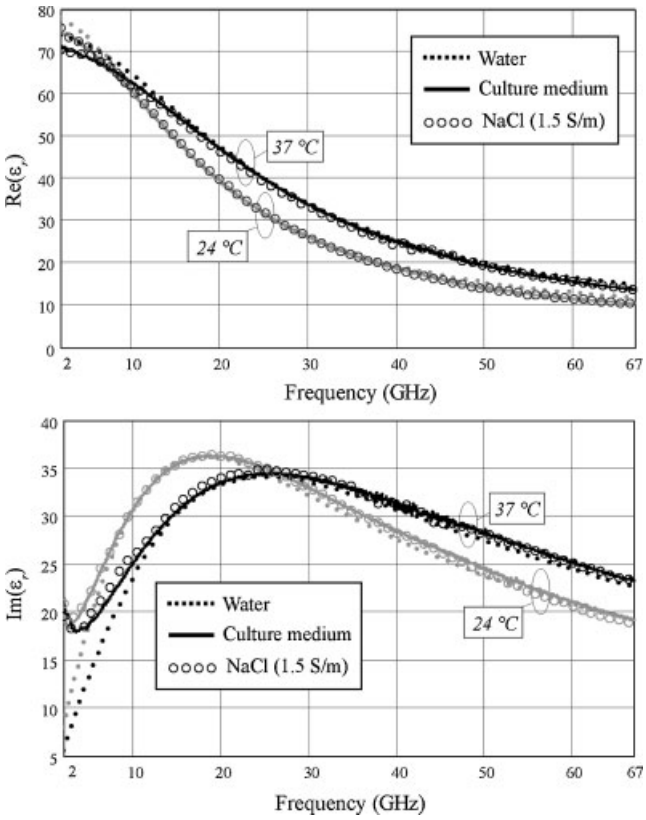

Fig. 10. Measured complex permittivity of the culture medium at 24 and 37 °C.

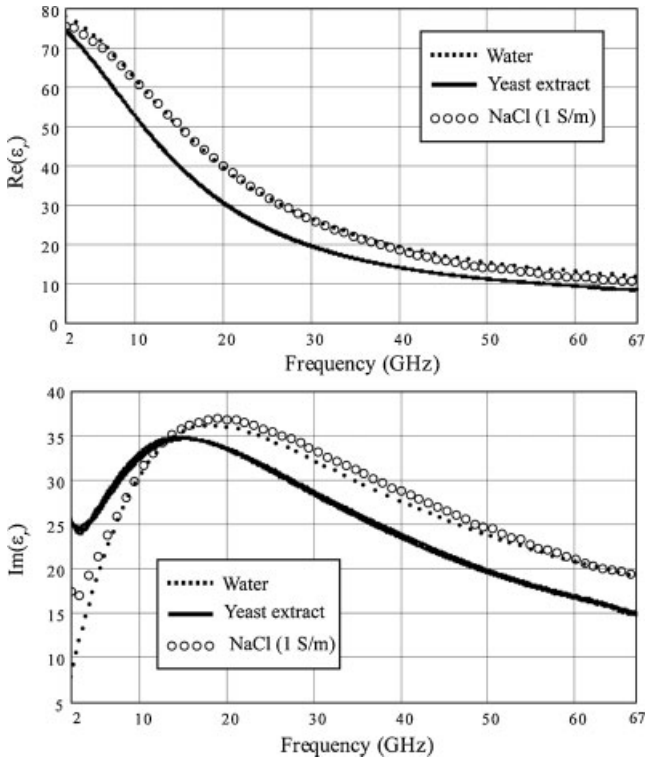

Fig. 11. Measured complex permittivity of the 10% yeast extract solution. Results are of three independent measurements at 24 °C.

Our results suggest that the dispersive behavior of the solutions at concentrations below 1% does not substantially differ from the free water permittivity at both room temperature and 37 °C. An exception is a shift in relaxation frequency on the order of 1 GHz toward the lower frequencies for the 1% glucose solution. Measurements performed for 4% and 20% BSA solutions demonstrate that the presence of proteins results in a decrease in both the real and imaginary parts of the permittivity. For the highly concentrated RNA solution (3%), a slight increase in the imaginary part of the permittivity has been observed for frequencies below 10 GHz, thereby confirming previously reported results [Swicord and Davis, 1982; Takashima et al., 1984].

It was experimentally demonstrated that the permittivity values of free water can be used for the numerical modeling of the culture medium for frequencies above 10 GHz. However, at lower frequencies a substantial increase in losses related to the ionic conductivity should be carefully taken into account. A similar increase has also been observed for the yeast extract solution in the lower part of the considered spectrum. In addition, above 10 GHz the high concentration of proteins and other low-permittivity cytoplasmic components in the yeast extract solution resulted in a decrease in the complex permittivity compared to that of water. It is interesting to note that these two phenomena result in a 4 GHz shift of the maximum of the imaginary part of the yeast solution permittivity toward the lower frequencies. However, as cells synthesize thousands of different molecules, the exact identification of all molecular factors contributing to the total permittivity is very challenging.

The results provided in this article are of importance for electromagnetic dosimetry studies, as well as for other applications requiring accurate permittivity data of biological solutions at microwaves and millimeter waves. Future cross-comparison of these results using other time- or frequency-domain dielectric measurement techniques [Kaatze, 2008] is of interest. The experimental protocol has also been extended to human skin measurements [Chahat et al., 2011], which are of increasing importance, particularly in the context of the emerging millimeter-wave body-centric wireless communications.

ACKNOWLEDGMENTS

The authors acknowledge Laurent Le Coq, IETR, University of Rennes 1, Rennes, France, and Shelley Begley, technical specialist, Agilent Technologies, Santa Clara, CA, USA.

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