URSI GASS 2021, Rome, Italy, 28 August - 4 September 2021

Dielectric Spectroscopy for Lactic Acid Detection and Design of Liquid and Gel Tumor Phantoms

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Abstract

Lactic Acid levels are considered metabolic biomarkers of several pathologies. The aim of this work is to evaluate the feasibility of a real time detection of lactate in a tissue under test by means its dielectric characterization. Design of phantoms mimicking liquid tissues and solid tumors is proposed for microwave biomedical applications

1 Introduction

Microwave sensing is a constantly developing technology that is proposed for industrial and medical applications. In particular, biosensors and biomarkers-based techniques for detection of early cancer and metabolic abnormalities implicated in several pathologies, have received broad interest [1]. This detection is not trivial due to the biomarkers low *in vivo* concentrations under normal conditions.

In this framework, Lactic Acid (LA) levels are considered a metabolic biomarker of different pathologies. LA has been proposed as an early marker of brain pathologies [2], as a predictive value in infants with perinatal asphyxia in the early postnatal period [3], for detecting spinal cord ischemia [4], as diagnostic tool for diagnosing early-stage cancers, or the aggressiveness of cancer [5]. A low power microwave sensor for analysis of lactic acid in cerebrospinal fluid has been proposed in [6] by measuring the amplitude of the reflection coefficient when samples were inserted in a cylindrical cavity working in the 800 MHz – 2000 MHz frequency range.

For sensors design, broadband dielectric spectroscopy is mandatory both for an accurate characterization of the biological matter and to define the most appropriate frequency ranges suitable for cells and/or biomolecules discrimination. In this work we present the preliminary results of a broad-band (60 MHz-4.5 GHz) electromagnetic characterization of LA solutions at different concentrations (range from low, physiological up to pathological levels). Then, phantoms mimicking liquid tissue (blood) and spheroids models were realized in order to test the feasibility to detect or distinguish healthy and tumor tissues on the base of LA concentration. In section 2 we describe the prepared phantoms and the measurement technique. Section 3 is devoted to show some significant examples of the obtained results. In section 4 we draw the concluding remarks.

2 Materials and Methods

Solutions (10 ml) of L(+)-lactic acid BIOXTRA (Sigma – Aldrich) in both distilled water and blood phantom were prepared to cover a concentration range from low, physiological up to supra-physiological levels (0 mM, 0.5 mM, 2 mM, 4 mM, 8 mM, 16 mM, 32 mM, 64 mM). Similar concentrations were investigated in [6]. Blood phantoms were realized with Fetal Bovine Serum (FBS, Sigma – Aldrich) a fluid whose biological components are similar to those of blood. This was confirmed by comparing the blood dielectric spectra [7] with that of FBS. A good agreement was found in the 2.45 GHz- 4.5 GHz while in the low frequency range (60 MHz-2.45 GHz) an aliquot of distilled water was added in order to obtain a mixture that better reproduced blood conductivity. The Looyeng mixture rule [8] was applied to optimize the volumes of these constituents. Permittivity measurements were carried out with the truncated coaxial cable technique. This is a very common technique for electromagnetic characterization of biological tissues. It is based on the measurement of the reflection coefficient (amplitude and phase) at the tip of a truncated coaxial cable immersed in the sample and connected to a Vector Network Analyzer once the calibration with a short circuit, an open circuit and a known liquid is carried out.

Permittivity values are then calculated following the approach described in [9]. A good agreement (less than 1%) between measurements of a reference load (NaCl 0.1 M) and literature data assures the reliability of the technique. Uncertainty budget of the measurements was evaluated on the basis of criteria established in [10], estimating 2% and 3% uncertainties for the real part of permittivity and the conductivity respectively. Permittivity measurements were carried out with a strict temperature control. In addition to liquid phantom spheroidal phantoms were employed. They were made of Cryogel® FG/3, which has chemical and physical properties similar to collagen extracted from beef, and had a diameter of 3-4 mm. Spheroids were realized with and without LA. To this end, cryogel was soaked in lactate (64 mM) to recreate tumor cells (labeled as LACTATE Sheroids), other spheroids were only made of gelatin and were meant to mimic healthy tissues (labeled as EMPTY spheroids).

3 Results

The comparison between these preliminary data obtained in the different conditions indicate that it is possible to distinguish a difference between samples with low LA levels (0mM - 8 mM, mimicking healthy tissues) respect to that with high LA concentration (16 mM - 64 mM, mimicking pathologic tissues). As an example, the Cole-Cole plot of LA in blood phantoms with different concentrations and in the 60 MHz-2.45 GHz is shown in Fig.1. It appears that differing concentrations of lactate can be determined based on the changing dielectric properties. Concerning gel phantoms, the real part and conductivity of the measured permittivity of two mixtures composed by 8mL of H_2O and 2g of spheroids, one with EMPTY spheroids and the other one with LACTATE spheroids are presented in Figure 2. The theoretical permittivity of H_2O was used as reference, mean values and standard deviations of 7 independent measurements were considered.

4 Conclusions

The determination of lactate has relevance in cancer biology and other diseases. A broad band dielectric spectroscopy of LA solutions has been presented.

Liquid and spheroidal phantoms with different lactate concentrations were employed to mimic healthy and tumor tissues. Mixtures composed by water and spheroids were analyzed in order to get information about their electromagnetic properties. Results have shown that it is feasible to distinguish healthy cells from tumor ones, since they present differences in permittivity and conductivity.

More specifically, it was observed that mixtures containing lactate spheroids are more conductive and show higher real permittivity compared to mixtures which mimic healthy tissues.

The original spheroidal phantom features could be exploited not only for developing biosensors but also as tools in other radiofrequency and microwave medical applications (i.e. microwave imaging, hyperthermia).

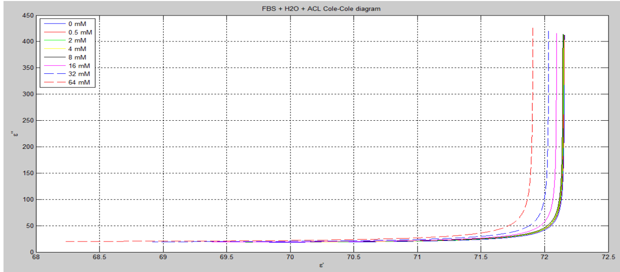
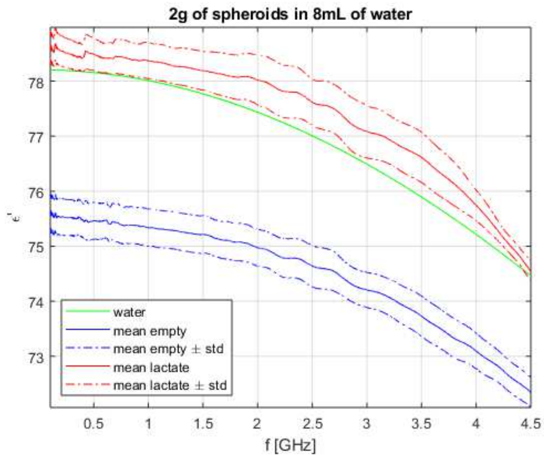
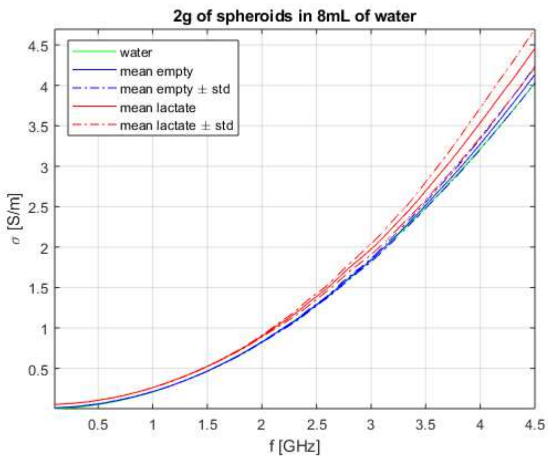


Figure 1. Cole-Cole diagram of lactic acid in blood phantom (60 MHz-2.45 GHz frequency range, 0 mM-64mM LA concentration).



a)



b)

Figure 2. Real part of the permittivity (a) and conductivity (b) of mixtures containing 2g of EMPTY and LACTATE spheroids in 8ml of H_2O compared with theoretical water (200 MHz-4.5 GHz frequency range).

5 References

1. Mehrotra, P.; Chatterjee, B.; Sen, S. “EM-Wave Biosensors: A Review of RF, Microwave, mm-Wave and Optical Sensing”. *Sensors,* **19,** 5, February 2019,1013

2. KLH Carpenter, I. Jalloh, PJ Hutchinson “Glycolysis and the significance of lactate in traumatic brain injury”. *Front. Neurosci*. 9, April 2015, 112

3. M. K. Zarifi, L. G. Astrakas, T. Y. Poussaint, A. du Plessis, D. Zurakowski, A. A. Tzika “Prediction of adverse outcome with cerebral lactate level and apparent diffusion coefficient in infants with perinatal asphyxia”, *Radiology*; 225, December 2002, pp 859–870

4.N. Khaladj et al., “The role of cerebrospinal fluid S100 and lactate to predict clinically evident spinal cord ischaemia in thoraco-abdominal aortic surgery”, *Eur J Vasc Endovasc Surg* 36, March 2008, pp 11-19

5. F.Hirschhaeuser, U.G.A. Sattler, W. Mueller-Klieser “Lactate: A Metabolic Key Player in Cancer”, *Cancer Res*; 71(22); 6921–5.

6. J. H. Goh, A. Mason, A. I. Al-Shamma’a M. Field, M. Shackcloth, P. Browning “Non Invasive Microwave Sensor for the Detection of Lactic Acid in Cerebrospinal Fluid (CSF)”, J. Phys.: Conf. Ser.307 012017

7. D.Andreuccetti, R.Fossi, C.Petrucci: An Internet resource for the calculation of the dielectric properties of body tissues in the frequency range 10 Hz - 100 GHz.. Based *on data published by C.Gabriel et al. in 1996.* IFAC-CNR, Florence (Italy), 1997 *[Online]. Available:* http://niremf.ifac.cnr.it/tissprop

8. H. Looyenga “Dielectric constants of heterogeneous mixtures”, Physica, 31, 3, March 1965, pp. 401-406

9. R.Massa, M. D. Migliore, G. Panariello, D. Pinchera, F. Schettino, E. Caprio, R. Griffo, “Wide Band Permittivity Measurements of Palm (*Phoenix Canariensis*) and *Rhynchophorus ferrugineus* (Coleoptera Curculionidae) for RF Pest Control, *J. of Microwave Power and Electromagnetic Energy*, **48**, 3, 2014, pp. 158-169

10. C. Gabriel and A. Peyman, “Dielectric measurement: error analysis and assessment of uncertainty”, *Phys. Med. Biol,* **51**, 23, December 2006, pp. 6033-6046