Millifluidic Sensor Designed to Perform the Microwave Dielectric Spectroscopy of Biological Liquids

Yegor Kozhemyakin, Sophie Réhault-Godbert, David Dubuc, Katia Grenier

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Y. Kozhemyakin#1, S. Rehault-Godbert*4, D. Dubuc#2, K. Grenier#3
LAAS-CNRS, Toulouse, France
*INRAE, Université de Tours, BOA, Nouzilly, France
{1ykozhemyak, 2dubuc, 3grenier}@laas.fr, 4sophie.rehault-godbert@inrae.fr

***Abstract* — This paper presents a microwave sensor composed of one millifluidic channel that operates over a frequency range from 1 GHz to 4 GHz and is used for microwave spectroscopy of liquids with millilitre volumes. The accuracy of the device is first evaluated with BSA solutions of different concentrations and then with complex biological liquids such as hen egg yolk and albumen samples. Low standard deviations of the measured values of modulus and phase contrasts of the transmission coefficient S21 are obtained. These values correspond to 0.02 and 0,18 respectively for BSA solutions, as well as 0.05 and 0.03 for 10 samples of hen egg yolk and white respectively over the selected frequency range. Such accuracies enable the use of such a sensor for various applicative domains, including agronomy for which the investigation of the dielectric properties of eggs may be of interest for quality, freshness or status discrimination purpose.**

***Keywords* — microwave spectroscopy, millifluidic channel, coplanar waveguide, transmission coefficient, liquids.**

I. Introduction

Microwave dielectric spectroscopy is an analyzing technique able to characterize solids, liquids and gaz based on their dielectric properties. This analyzing method presents the main advantage of being non-invasive and quick. It is well used in different domains, including the control of processes [1], such as the evaluation of the quality of water, food, animal feed and others [2], [3], [4]. Not so long ago, microwave spectroscopy has broaden its application field with the study of intermolecular interactions [5], the properties of biological tissues [6], including the discrimination of malignant, benign and healthy tissues [7] and finally at the microscale the investigation of individual cells [8], [9]. Therefore, microwave dielectric spectroscopy is of great interest for the quantitative study of the composition of liquids, in various volumes conditions. Developments have already been performed at the macroscale with the use notably of large waveguide configurations, resonators, coaxial probes, as well as at the microscale with miniature microwave sensor configurations. There is however a lack of solution for the intermediate level, which involves millifluidic samples, whereas an increasing demand for the development of microwave spectroscopy devices for such a size exists at an affordable cost. Previously, a microwave sensor designed with two millifluidic channel has been developed [10], based on the Bianco and Parodi method for permittivity extraction. Even if this device is efficient in the characterization of millifluidic liquids, the presence of the two fluidic channel complicates the use of this sensor as the two channels require to be filled. It is also liquid-consuming. This paper therefore considers a simplified microwave device, which consists of a coplanar waveguide with only a single millifluidic channel.

Regarding its applicative context, which is related to precision farming, there is always a demand for devices that could facilitate a strict quality control of the products produced and delivered to the end consumer. Methods for checking the freshness of fruit, eggs and vegetables using microwave spectrometry have already been shown in [11], [12], [13]. In addition, methods for determining the moisture content of cereal grains and meat have also been described [14], [15]. These investigations demonstrate the high potential of microwave dielectric spectroscopy for studying the qualitative and even quantitative composition and condition of various materials ranging from chemistry to biology, including product quality controls.

In the present paper the construction of a millifluidic sensor with a single fluidic channel designed for microwave dielectric spectroscopy is first discussed. Several reasons of changing the microwave sensor with two millifluidic channels to the one with one millifluidic channel are given. In a next section, a description of the experiments performed from 1 GHz to 4 GHz for bovine serum albumin (BSA) solutions of different concentrations ranging from 5 ml/mg to 100 ml/mg are evaluated. Next section is dedicated to the investigation of more complex and sticky biological materials, which correspond to hen egg yolk and albumen, carried out with the developed microwave sensor.

II. Architecture and Fabrication of The Millifluidic Microwave Sensor Elaborated with a Single Fluidic Channel

In [10], a microwave sensor with two millifluidic channels is considered to characterize the permittivity of several liquids. In the present paper, the simplified microwave sensor is presented with the schematic shown in Fig. 1.

The operating frequency band of the sensor is from 1 GHz to 4 GHz. The microwave structure is fabricated on the Rogers RO2003 substrate with a thickness $h = 0.8$ mm, dielectric constant $\varepsilon_r = 4.3$ and dissipation factor $tan\delta = 0.003$.

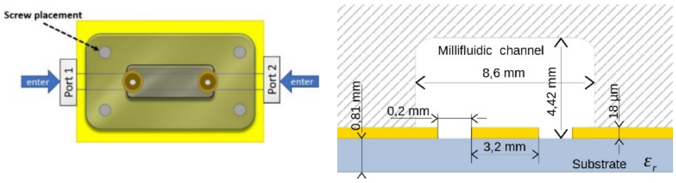

Fig. 1. Schematic of the simplified millifluidic and microwave sensor with two ports with dimentions. The coplanar waveguide is represente in yellow, whereas a plexiglas fluidic channel is placed on top.

The device consists of a coplanar line with a central conductor width of $w = 3.2$ mm and a slot of $s = 0.2$ mm. The line length L is equal to 56 mm. These dimensions have been defined to match with a 50Ω characteristic impedance in air. On top of the substrate, a drilled Plexiglas block is used to realize the millifluidic channel. It presents a length $l = 26$ mm long and has an approximate volume $V = 0.8$ mL. The Plexiglas block containing the millifluidic channel and two holes allowing the liquid injection on one side and the fluid output on the other side provides a precise positioning of the liquid under consideration on the coplanar line. In addition, the tight fit of the block to the metal substrate prevents any leakage of the liquid under test outside the channel. For this purpose, the Plexiglas block is fastened with four lateral plastic screws to a second Plexiglas substrate located below the Rogers one, thereby creating the necessary pressure on the metal substrate and ensuring a tight fit, as shown in Fig. 2.

In operation, the new microwave sensor, which integrates a single millilitre channel, has proven to be practical and easy to use. This fact is the consequence of three main advantages compared to the microwave sensor, which includes two milliliter channels as described in [10]:

- *The smaller required volume of the liquid to be tested*. As a result of the fact that the device consists of a single millilitre fluidic channel, the required minimum volume of the liquid to be tested is reduced by more than 35% compared to the device in [10],
- *Increased simplicity and ease of operation*. The microwave sensor with a single millilitre fluidic channel is a device that relies on proper installation, calibration of the device, as well as smooth and even filling of the channel with the liquid under test. These steps are simplified compared to the microwave sensor with two millifluidic channels,
- *Cleaning*. Insufficient cleaning of the millifluidic channel leads to additional measurement errors, which have a direct effect on the overall result of the experiments. Switching from microwave sensor with two millilitre fluidic channels to microwave sensor with one channel ensures that the probability of experimental error due to insufficient cleaning of working volume of the channel is lower.

Experimental analysis of the liquids under test is made with a measuring unit consisting of a measuring and a calculation block, a vector network analyser (VNA) Copper Mountain S5085 and the microwave millifluidic sensor (Fig.2).

In the next section the results of measurements on BSA solutions of different concentrations from 5 mg/ml up to 100 mg/ml as well as on egg yolks and albumens with the microwave millifluidic sensor will be presented.

III. Two Case Studies for Evaluating the Capabilities of The Microwave Millifluidic Sensor

A. *Microwave measurements of solutions of BSA with different concentrations*

The first sensitivity studying of microwave millifluidic sensor is carried out with 4 BSA solutions of different concentrations: 5, 10, 50 and 100 mg/ml. Each solution is measured 5 times, starting with the lowest concentration. De-ionized water is also measured between each BSA solutions as a reference.

In the BSA experiments, the modulus and phase of the S_{21} parameter are measured. Since the liquids present increased viscosity and adhesiveness, a special measurement protocol is developed for their experimental analysis with the microwave millifluidic sensor. Modulus and phase of the transmission coefficient are calculated using the formulae (1) and (2):

$$\Delta|S_{21}| = |S_{21}|_{liquid} - |S_{21}|_{water} \tag{1}$$

$$\Delta\varphi_{S_{21}} = \varphi_{S_{21}liquid} - \varphi_{S_{21}water} . \tag{2}$$

The obtained average values for the differences of modulus and phase contrasts for S_{21} are presented on fig. 3 and fig. 4 respectively. Fig. 5 shows these values depending on the concentration of the BSA solutions. The 2,7 GHz and 4 GHz frequencies are selected for the modulus and phase contrasts respectively. They have been chosen on the basis of a search for a frequency with a maximum modulus and phase contrasts values over the overall frequency range. The measurements enable to distinguish the

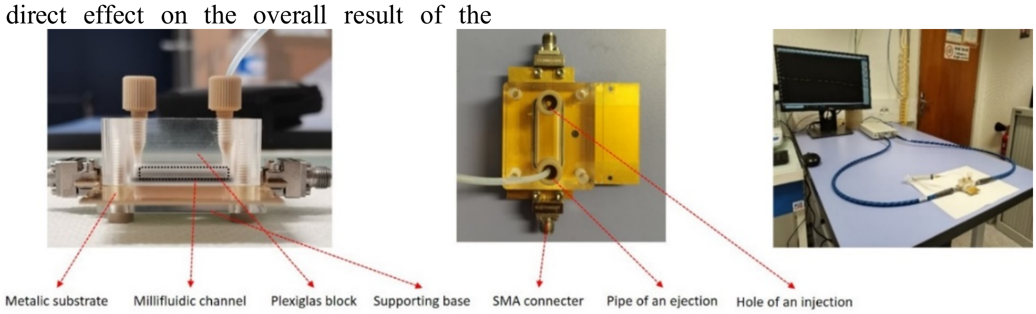

Fig. 2. Photographies of the millifluidic and microwave device, seen from the side (in the left) and from above (in the right), with the coplanar waveguide, the millifluidic channel, SMA connecters, Plexiglas block and the inlet and the outlet hole and pipe and an experimental test setup with the microwave millifluidic sensor

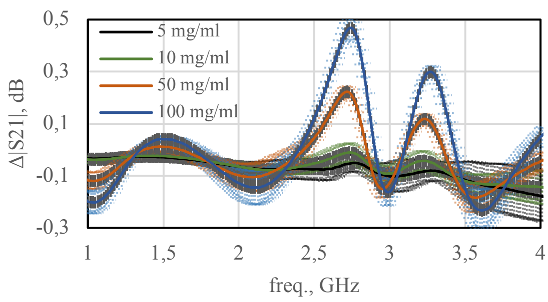

Fig. 3. The average values of differences of modulus contrasts of measured S_{21} for the BSA solutions for different concentrations, with marked standard deviations

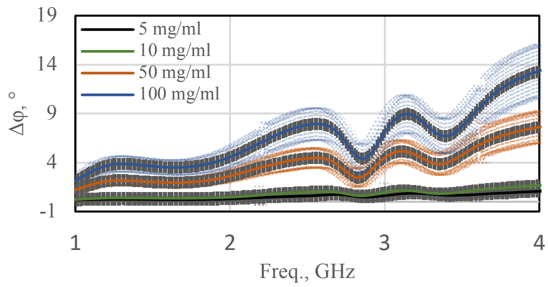

Fig. 4. The average values of differences of phase contrasts of measured S_{21} for the BSA solutions for different concentrations, with marked standard deviations

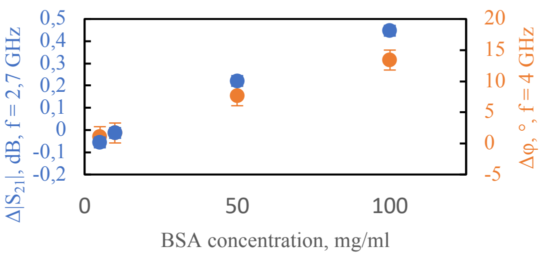

Fig. 5. Measured standard deviation and average modulus contrasts of S_{21} for each BSA solution versus concentration, at 2,7 GHz (in blue) and measured standard deviation and average phase of S_{21} for each BSA solution versus concentration, at 4 GHz (in orange)

solutions of BSA with different concentrations. Calculated average values and the standard deviations σ of measured modulus and phase contrasts of S_{21} for every BSA solution are shown in Table 1.

Table 1. Average values and dispersion of the module and phase contrasts of measured and referenced to water ΔS_{21} for the solutions of BSA with different concentrations at 2,7 GHz for the modulus contrasts and at 4 GHz for the phase contrasts calculated for 5 measurements for each solution

BSA Concentration	**Mean $\lvert\Delta S_{21}\rvert$, dB**	**σ $\lvert\Delta S_{21}\rvert$**	**Mean Δφ, °**	**σ Δφ**
5 mg/ml	-0,06	0,04	1,08	0,18
10 mg/ml	-0,01	0,04	1,67	0,37
50 mg/ml	0,22	0,02	7,66	1,6
100 mg/ml	0,45	0,04	13,4	2,7

It is possible to observe the σ - values for the modulus contrasts of S_{21} of the same order for all the BSA solutions. For phases, the standard deviation is increasing with respect to an increase of the BSA solution concentration, taking the maximum value as 100 mg/ml. This fact may be explained by a pollution of millifluidic channel because of higher viscosity of the liquid at high concentration. Despite a definite dispersion of values of σ for the modulus and the phase contrasts of S_{21} on examined frequencies, these standard deviations may be considered acceptable and applicable specially in the field of rapid, omnipresent and low-cost analyses.

B. *Microwave measurements of hen egg yolk and albumen*

This section presents the results of microwave measurements of the yolk and albumen of fertilized chicken eggs. Such a study can be applied in the agro-industrial sector in order to accumulate and process information on the quality and condition of the liquids that constitute the structure of a hen egg.

In order to quantify the degree of repeatability of measurements made with a microwave millifluidic sensor, yolk and albumen samples from two different eggs are examined and measured 10 times each in sequence. The measurements are then made for 9 yolks samples and 9 albumen samples of different hen eggs measured 1 time each to estimate the level of a reproducibility of experiments. This approach allows a qualitative evaluation of the microwave measurements of the tested liquids according to (1) and (2).

The choice of frequencies for the presentation of the modulus and the phases contrasts of the S_{21} coefficient is due to the maximum values of these quantities at the frequencies in question. 3 GHz and 4 GHz are chosen for calculations for the modules and phases respectively. Fig. 6 shows the dependencies of the measured modulus contrasts of the coefficient of transmission on its phase contrasts for 10 consecutive repetitive measurements of yolk and albumen of two fertilized hen eggs at 3 GHz.

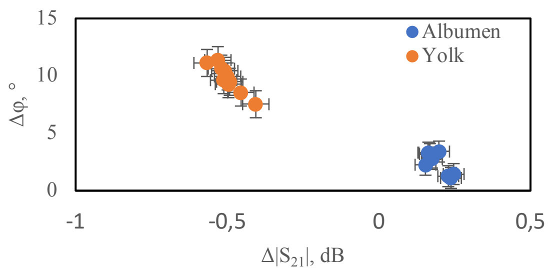

Fig. 6. Complex graph of S_{21} modulus on phase contrasts of 10 measurements for the yolk and the albumen of hen eggs with marked standard deviations at 3 GHz

Table 2 presents the measured and calculated quantities of the average values and the standard deviations of the modulus and the phase contrasts of S_{21} for the chosen frequencies.

Table 2. Average values and standard deviations of the modules contrasts at 3 GHz and phase contrasts at 4 GHz referenced to water $\Delta\lvert S_{21}\rvert$ for the yolk and the albumen measured 10 times in terms of the repeatability of experiments

Liquid	**Mean $\lvert\Delta S_{21}\rvert$, dB**	**σ $\lvert\Delta S_{21}\rvert$**	**Mean Δφ, °**	**σ Δφ**
Yolk	-0,5	0,05	-12,6	1,8
Albumen	0,2	0,03	-4,3	1,5

The standard deviations σ for the yolk and the albumen are 0,05 and 0,03 respectively. The difference with the measured values of S_{21} can be attributed to the contamination of the millifluidic channel during successive measurements. This created a negative effect on the accuracy of the experiment and the values obtained when measuring the same liquid. The egg constituents are indeed particularly sticky compare to

BSA samples. Nevertheless, achieved values of σ are of the same order than those obtained in [10].

The fig. 7 shows the results of measurements of S_{21} in terms of reproducibility of measurements at 3 GHz for 9 yolks and albumens samples from different fertilized hen eggs.

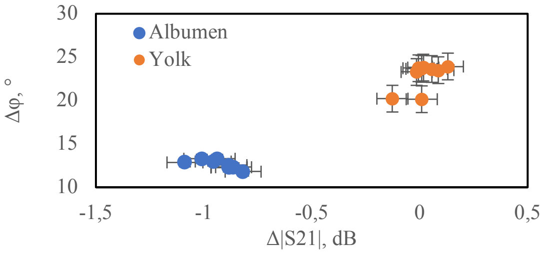

Fig. 7. Complex graph of S_{21} modulus on phase contrasts of 9 measurements for the yolks and the albumens extracted from 9 different fertilized hen eggs with marked standard deviations at 3 GHz

In Table 3 the obtained quantities of average values and standard deviations for the modulus and the phase contrasts of S_{21} at 3 GHz and at 4 GHz respectively are presented.

Table 3. Average values and standard deviations of the modules contrasts at 3 GHz and phase contrasts at 4 GHz referenced to water ΔS_{21} for 9 yolks and 9 albumens of different eggs measured 1 time in terms of the reproducibility of experiments

Liquid	**Mean $\|\Delta S_{21}\|$, dB**	**σ $\|\Delta S_{21}\|$**	**Mean Δφ, °**	**σ Δφ**
Yolk	0,02	0,07	41,7	2,7
Albumen	-0,9	0,08	21,7	0,8

The variation between the values of σ presented and those obtained in the previous case may be due, among other things, to differences in the chemical and biological composition of the yolks and albumens extracted from diverse hen eggs.

IV. CONCLUSIONS

A millifluidic microwave sensor operating in the frequency range from 1 GHz to 4 GHz is presented. The device features a single millifluidic channel to allow the small volume of liquid to be studied to interact with the electromagnetic waves over a wide frequency range.

BSA solutions of different concentrations were measured to determine the sensitivity of the microwave sensor. A small value for the standard deviation of 0.02 and 0.18 for the modulus and the phase contrasts of the transmission coefficient S_{21} respectively were obtained. The found results of the standard deviation for the modulus contrasts of S_{21} correspond to that expected in the modeling and development of the considered microwave sensor and present the same order as the values of a standard deviation obtained in [10].

Further work with the device was carried out with samples of yolks and albumens of fertilized hen eggs in the terms of the repetitiveness and the reproducibility of the measurements. The corresponding values of the calculated standard deviations for the modules of the coefficient of transmission S_{21} were for the yolks 0.05 and 0.07 and for the albumens 0.03 and 0.08 respectively. The results were used for the validation of the microwave sensor and methodology of studying complex liquids in millilitre volumes, which can be used in a variety of applications. This opens the door to the development of systems suitable for quality assessment and control of products in the agro-industrial sector.

ACKNOWLEDGMENTS

These experiments were performed under the framework of the PPILOW project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°816172.

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