HYDRATION LAYERS OF ALCOHOL AND PROTEINS ANALYZED BY THZ BIOMEMS

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ABSTRACT

A Terahertz (THz) dedicated microsystem is presented. Design, microfabrication and subTHz (0.5-0.11THz) measurements are shown in this article. Ethanol/Water mixtures measurements analysis show that THz spectroscopy is able to characterize hydration shells of a solute in water, in term of absorption, volume or hydration number. Moreover, first measurements with proteins in the microsystem show that lysozym concentration detection is possible.

KEYWORDS: terahertz spectroscopy, silicon-glass microchannels, hydration shell dynamics, hydrogen bond.

INTRODUCTION

The high absorption of biochemical weak bonds (like hydrogen ones) in THz frequency band (100GHz - 10THz) has opened a way for real-time marker-free non-destructive probing of biological samples [1]. However, huge THz-absorption of water makes it difficult *in-vivo* biological solutions investigation. This is why most of the THz measurements concerns dry samples today [2]. In other hand, this strong absorption can be used to characterize water around biomolecules. Indeed, proteins hydration-layer study is today a strong challenge because of its influence on the protein expression [3].

In order to perform THz spectroscopy with liquid samples despite the huge water absorption, we proposed to reduce analyzes samples volumes. Such a way has already been investigated by using micelles [4] or thin liquid films for spectroscopy [5]. Here is proposed another experimental way to control accurately, with a strong reproducibility of samples volume. A biology-dedicated microsystem (BioMEMS), integrating microfluidical and electromagnetical functions has been designed. First measurements have been performed with ethanol/water solutions and protein/water solutions in the subTHz band of frequencies (0.05-0.11THz). They show that hydration layers of molecules in aqueous solutions can be probed and characterized by THz spectroscopy.

THEORY

From radiofrequencies to infrared field, electromagnetic-waves/water interaction enhanced several water physical phenomena and structures. Water dipolar moment collective relaxation is shown by microwave excitement in dielectric spectroscopy [6]. Two major relaxation times are about 10ps and 1ps and respectively correspond to collective rotation and translation relaxations of water, constrained by hydrogen bonds in water clusters. Higher frequencies correspond to subpicosecond times are probed by THz spectroscopy. They deal with hydrogen-bond free water molecules relaxation (about 0.1ps) and water hydrogen-bond intermolecular-network bending or stretch (about 30fs) [7]. Infrared (IR) and Raman spectroscopy are in relationship with 10fs-0.1fs time constants ($400cm^{-1}/20000cm^{-1}$), and probe intramolecular individual librations (lower frequencies) and stretch (higher frequencies) [8]. Note that the continuous shape of water absorption and the phenomena overlap increasing frequency makes the Microwave/THz/IR frontiers difficult to discern clearly. Very simply, one can sum up saying that dielectric spectroscopy deals with collective relaxation, THz one with hydrogen bond network and IR/Raman with individual motions (fig 1).
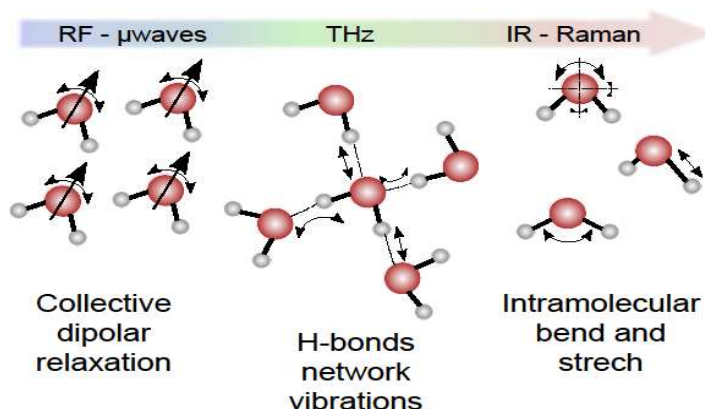

Figure 1: Water/Electromagnetic-wave interaction

When a solute (alcohol, sugar, protein for instance) is inserted in an aqueous solution, the water hydrogen bond network is hugely modified at the solute vicinity. Indeed, water molecules next to solute one form an "hydration shell" which behavior is different from "bulk water" [9]. This is the result of hydrogen bond forming between "hydration water" and solute. To characterize hydration shell is today a chemically and biological challenge. For instance, it is studied to

understand biopreservation phenomena with trehalose, protein dynamics and functionality or even solvent influence in protein expression [10].

This increase of hydrogen bonds can be indirectly probed by dielectric spectroscopy or IR/Raman spectroscopy because it influences collective motion and individual motions by decreasing water molecules mobility. However, THz spectroscopy seems to probe directly water hydrogen bond network, and might help hydration shell characterization.

EXPERIMENTAL

To perform a THz spectroscopy, we manufactured a two-function microsystem (fig 2). The first function is a microfluidical one with microchannels design to flow the samples towards THz-analysis micro-volume area. The microchannels are 150µm-deep and the measurement channel is 50µm-width. Silicon has been processed to realize them because of its ability to be accurately deep-etched (DRIE) and its compatibility for possible future integration in electronical systems. The second function is the electromagnetical one, composed of a THz planar waveguide, perpendicular to the measurement channel. The wave is propagated on a 5µm-large single-wire transmission-line called "Goubau line", which enables micronic-resolution probing [11]. This line is realized thanks to gold deposition on a glass substrate. Gold is used because of its low resistivity whereas glass substrate enables optical control thanks to its transparency. Silicon and glass substrates are then bound under a 2-bar pressure and a 130°C temperature during an hour.
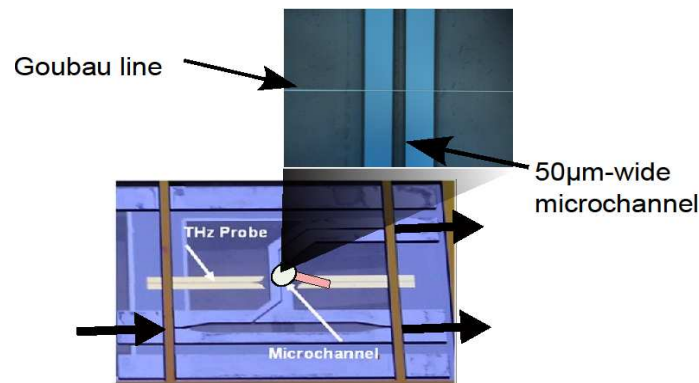

Figure 2: THz BioMEMS

Here are presented subTHz measurements of solutions absorption from 0.05 to 0.11 THz. They have been carried out by using a Vectorial Network Analyzer (VNA) which measures complex Scattering [S] parameters (transmission/reflection) of the propagated wave along the Goubau line. As VNA excites the device with coplanar wave, a coplanar/Goubau transition has been designed [12]. Real-time enzymatic reaction probing has already been performed with this device [13].

Once the microsystem has been fabricated, liquids are injected in the microchannels to measure the device [S] parameters as a function of the liquid injected. In a first time, ethanol/water mixtures were injected. Solutions with ethanol/water volumic ratio have been prepared: 0 – 0.0125 – 0.05 and each 0.05 up to 1. They were injected in a randomly chronological order to avoid any measurements bias. For each mixture, [S] parameters are measured with a 30-average factor. Between each measurement, water is re-injected in the microchannels and measured to check the measurements reproducibility.

RESULTS AND DISCUSSION

Figure 3 (left) shows the 95GHz absorption of the ethanol/water mixtures as a function of water concentration. As ethanol 95GHz-absorption is weaker than water one, increasing ethanol concentration decreases the solution absorption. Indeed, by water volumic replacement, the measured absorption decrease could be explained. However, such a decrease (volumic replacement) should be linear as the Lambert-Beer law prediction. Here, the measurements curved shape disagrees with a linear law. Moreover, Molecular Dynamics (MD) simulations of ethanol/water solutions confirm this shape, which cannot be only explained by water replacement.

MD simulations show that water at the vicinity of an ethanol molecule can form hydrogen bonds with ethanol. As a consequence, dynamics of this so-called "bond water" (hydration shell) should be modified and its absorption should decrease. Thus, solution absorption is the mean of (bulk)water absorption α_{bulk}, "bound water" absorption α_{shell} and ethanol absorption α_{eth}, which can be represented by a three-component model:

$$\alpha = \alpha_{eth} \frac{V_{eth}}{V} + \alpha_{shell} \frac{V_{shell}}{V} + \alpha_{bulk} \frac{1 - V_{eth} - V_{shell}}{V} \qquad (1)$$

where V_{eth}/V and V_{shell}/V are respectively ethanol and hydration shell volumic ratios.
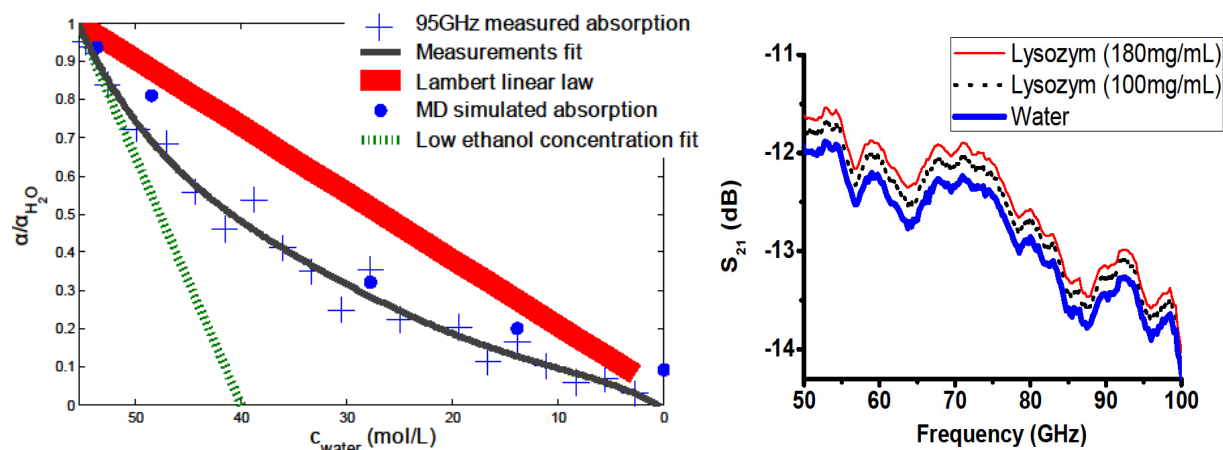
Figure 3 Left:Ethanol/water mixture 95GHz- absorption as a function of water concentration
Right: Lysozym solution transmission (dB) as a function of lysozym concentration and water

Considering the low-ethanol-concentration region, we assume that hydration shell volume is proportional to ethanol volume: there is no overlap between hydration shells of several ethanol molecules. Moreover, α_{shell} is supposed to be constant. By fitting this model to the measurements in this region, one can characterize the hydration shell. $\alpha_{shell} / \alpha_{H_2O}$ has been found equal to 0.72 ± 0.1, which confirm that hydration shell water should absorb less than bulk water, and V_{shell} / V_{eth} has been found equal to 13 ± 2.5. From this value, the ethanol hydration number, which is the number of water molecules in the hydration shell, has been found equal next to 30, which corresponds to the value extracted from MD simulations [14].

Same measurements have to be done with proteins. Lysozym has already been investigated, as shown in fig 3 (right). It shows that lysozym detection concentration is possible with the BioMEMS. Improvements in term of reproducibility and sensitivity in this case are in progress.

CONCLUSION

The microfluidic-system experimental-way is able to perform subTHz spectroscopy and to characterize hydration shell of ethanol in term of absorption and volume. For proteins, increasing sensitivity and accuracy is needed. Increasing frequency, designing electromagnetical functions (interferometers, resonators) or microfluidical functions (mixers, filters) are ways we are going to follow. Future works will so benefit from the microsystem-approach for THz spectroscopy.

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