Monitoring Protein Denaturation of Egg White Using Passive Microwave Radiometry (MWR)

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Research Article

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Abstract

Passive microwave radiometry (MWR) is a measurement technique based on the detection of passive radiation in the microwave spectrum from different objects. This radiation in equilibrium is known to be proportional to the thermodynamic temperature of an emitting body. We hypothesize that living systems feature other mechanisms of emission that are based on protein unfolding and water rotational transitions. To understand the nature of these emissions, microwave radiometry has been used in several in vitro experiments.

In our study, we performed pilot measurements of microwave emissions from egg whites during denaturation induced by ethanol. Egg whites are 10% proteins such as albumins, mucoproteins, and globulins. We found a novel phenomenon that microwave emissions changed without a corresponding change of the water thermodynamic temperature. increase 100 times faster than thermodynamic temperature. We have also found striking differences between microwave emission and thermodynamic temperature kinetics. Therefore, we hypothesize that these two processes are unrelated, contrary to what was thought before. It is known that some pathologies like stroke or brain trauma feature increased microwave emissions. We hypothesize that this phenomenon originates from protein denaturation and is not related to the thermodynamic temperature. So, our finding could explain first time the reason for microwave emissions increase after trauma and postmortem. It could be used for the development of novel diagnostics methods.

The MWR method is inexpensive, and it does not require fluorescent or radioactive labels. It can be used in different areas of basic and applied pharmaceutical research, including kinetics studies in biomedicine.

Introduction:

Microwave radiometry is currently used in medical studies to measure natural microwave radiation from human tissues[1,2,3]. Commercially available microwave radiometers have been applied in a variety of clinical applications, such as breast cancer[4], cerebrovascular diseases[5,6], carotid artery pathology[7] and others.

Human tissues, like any other heated body, emit electromagnetic radiation across a wide frequency range. If a matched probe is applied to a human body, the power of the electric signal at the probe output will be proportional to the brightness or internal temperature (Eq. 1)

$P = kT_{br}$I″$f(1)$ Eq. 1

where P – electric noise power at the output of probe; T_{br} – brightness temperature; k – Boltzmann constant; Δf – receiver bandwidth.

In thermodynamics equilibrium the brightness temperature (T_{br}) is the thermodynamic temperature of a matched black body producing the same power of radiation that a measured body does. T_{br} is known to

be dependent on probe characteristics and human tissue dielectric properties. Therefore, the brightness temperature is roughly the average temperature in volume under the probe (Fig. 1). It is possible to noninvasively obtain information about the averaged thermodynamic temperature by measuring the power of the natural radiation. The area in which the probe receives electromagnetic radiation depends on the wavelength of the received signal. At a wavelength of 30 cm, the depth of measurement is 4–7 cm, depending on the moisture content of the tissues. At a wavelength of 10 cm, the depth of measurement is 2–5 cm^3. At an ambient temperature of 36°C and a bandwidth 0.8 GHz, the power of the received signals is about $3x10^{-12}$ W. This value is commensurate with the level of intrinsic noise of the receiving device and requires special methods to receive and process microwave signals.

Since the previous century, thermal imagers or infrared thermographs are being used in medicine[8]. These devices are measuring temperature basing on the radiation of tissues in the infrared (IR) wavelength range. The important difference is that with MWR it is possible to average temperatures up to 70 mm, allowing the detection of deeper processes, while IR sensors can only measure temperature of a surface.

Previous experiments featured passive-mode measurements of MWR of cytochrome P450CYP102A1(BM3) solution during hydroxylation reactions[9]. During another experiment, MWR measurements of a peroxidase reaction in solution with and without excitation of the solution were performed[10]. Recently[11], MWR was applied to measure the kinetic rate of bovine serum albumin (BSA) denaturation.

Human serum albumin is the most abundant protein in blood plasma and is very well studied. HSA is involved in important biological processes like osmotic blood pressure, transport, and metabolism of small molecules and drugs. Also, HSA is being used as a biomarker for diagnostics and treatment of many diseases like hypoalbuminemia[12]

The kinetics of albumin denaturation was studied using a variety of methods, including calorimetric studies, absorption spectroscopy, CD spectroscopy, fluorescence spectroscopy using thermal exposure, as well as chemical exposure by changing pH[13,14,15]. It was found that temperature exposure causes both denaturation and protein aggregation. Regarding the effects of alcohols, it was studied[16] by resonance light scattering (RLS), fluorescence spectroscopy, ultraviolet spectrophotometry (UV), circular dichroism (CD), and transmission electron microscopy (TEM). It was found that an ethanol concentration of more than 30% changes the conformation of the protein, making it more hydrophobic, i.e. causing his denaturation.

Traditional biochemical methods are based on electrochemistry, spectroscopy or calorimetry while widely used but have some disadvantages. Electrochemical methods are characterized by insufficient stability of the results associated with the state of the electrodes. Circular dichroism requires expensive spectral equipment. Fluorescent methods (which often use fluorescence of aromatic protein groups or labels) can provide additional information about details of the denaturation process, however, in this case, more [illegible]ric systems usually quite expensive due to the use of both an

optical radiation excitation scheme and a real-time recording circuit in one device. The traditional calorimetry process is a time-consuming technique. Since almost all biological processes are accompanied by thermal processes, it is difficult to relate them to a specific process or phenomena.

The presented experiment is a continuation of our albumin denaturation experiment[11] and easy to reproduce. We use the RTMM radiometer (www.mmwr.co.uk) with a round probe to measure microwave radiation from egg white during alcohol-induced denaturation (Fig. 2). For more detailed description of the technology see our paper[35].

The RTMM device is a precise Dicke null-balancing radiometer 3.8+/-0.4 GHz with 0.1 seconds integration time and 4 seconds post-processing averaging time. The device was calibrated with a pair of graduated waterfilled thermostats. Special software was developed for capturing time series.

A contact infrared thermometer (IR Thermometer, Thermlog 20) has been used to measure thermodynamic temperature aside from brightness. Fresh chicken eggs are required to perform this experiment.

Materias And Methods:

All equipment, water, ethanol and fresh eggs should be kept at room temperature for at least 6 hours, so their temperature is uniform and equal to room temperature. The RTMM device should be turned on at least for 20 min before carrying out measurements. The thermometer and the probe should put inside the cup (volume 70ml, the height 40mm). The probe should be covered with a latex finger cap, and inside the cup with egg white. The probe is oriented vertically in the cup and positioned within ½ of cup depth. The thermometer was put inside the cup nearby but not touching the microwave probe. The microwave emission temperature were monitored using specialized software which could record time series. When it stop changing within 2–3 min with fluctuations < 0.3°C, move to the next step add 25 mL of 96% ethanol (www.Kelsia.net, Spain) to the cup. The sensor and probe are noise protected, but there could be a strong external microwave signal (i.e., 5G) which increase the measurements error. If there are some short peaks, we recommend remove or turn off mobile phones. Alternatively, use another location in the room. After 5 min, stir the melting masses in the cup with a plastic spoon. Minimize any movement of the probe, and continue the recording session for another 5 min. All experiments were performed in triplicate. Using the obtained data we have calculated slope and determine the kinetics of denaturation both microwave and thermodynamic using Eq. 1. For control we have used 25 mL of tap water instead of ethanol (Fig. 3).

Results:

We observed that the addition of ethanol leads to a sharp increase in the microwave emission (brightness temperature) of the solution as shown in Fig. 4, while thermodynamic temperature rises almost 1000 times slowly as shown in Fig. 5. In contrary, adding water as a control does not produce any microwave (brightness) or thermodynamic temperature effect. Adding ethanol to water increases MWR and IR

temperatures but much slower rates in comparison with eggs (Fig. 6, 7). All rate constants are presented in Table 1

Table 1
Rate constants

Experiment	Linear slope (MWR), K/s	Linear slope (IR), K/s
Egg and alcohol	0,127 ± 0,007	$3{,}5\times10^{-4}\pm0{,}7\times10^{-4}$
Egg and water	$-7{,}5\text{x}10^{-3}\pm1{,}5\text{x}10^{-3}$	$2{,}5\times10^{-3}\pm0{,}2\times10^{-3}$
Water and alcohol	0,073 ± 0,01	0,015 ± 0,005

Discussion

It was shown earlier by us that the microwave emission occurs during egg white temperature denaturation, and addition of ethanol in this study leads to a sharp increase in the microwave radiation of the solution, while the infrared temperature of the solution rises very slowly.

It could be explained by following. Under the action of a chemical reagent (ethanol), the hydration shell of the protein is destroyed, which leads to the removal of steam - water molecules from the protein shell. In this case, 1) water-alcohol structures are formed, 2) the protein surface increases, which leads to an increase in the exposure of the hydrophobic groups to the solution. This lead, on the one hand, to the formation of protein aggregates. It is known that albumin aggregation is accompanied by protein aggregation, which, in turn, leads to an increase in the surface for vapor sorption — isomers of water molecules, which form a new hydrated shell of albumin with a larger surface than the native globular protein. As water is a nonequilibrium system[13], the degree of nonequilibrium is determined by the ratio of ortho- and para-isomers, the resulting shift in equilibrium between the ortho - and para-isomers of water towards the ortho - isomer of water in the solution leads to an even greater non-equilibrium spin of water. Since the system tends to equilibrium, as this system moves (water, protein, alcohol) to an equilibrium state, processes occur that remove the overpopulation of rotational water levels, including due to microwave radiation at ortho transitions water. Since this radiation has a quantum mechanical nature, it proceeds fairly quickly (2°C in ~ 10–20 sec), and its flow time is determined by Einstein's constant for spontaneous emission, as well as protein denaturation kinetics, leading to a change in the ratio of ortho-para- isomers of water.

The infrared temperature changed rather slowly (0.5°C for ~ 5 min), since it is determined by slower processes of heating the solution. This temperature is determined by Brownian motion. Note that earlier, it was shown[33] that not only chemical, but also mechanical excitation can lead to a change in the structure of water and the relationship between ortho- para- isomers of water. We have demonstrated that mechanical excitation of a fluid near singular points can lead to microwave radiation from water[34]. The kinetics of microwave radiation differs from the thermal relaxation temperature variation of solutions

upon mechanical excitation: thus, at a temperature near 4°C, a sharp increase in microwave radiation was observed, while the infrared temperature of the solution increased very slowly.

This work demonstrates that MWR allows direct monitoring of alcohol-induced denaturation of proteins, without the use of any labels. The process of microwave emission during denaturation is different from emission in the infrared spectrum measured by a thermometer. The data obtained in our work are consistent with the published data[16].

Analysis of the functional status of cells also is usually determined using traditional molecular biology methods[17]. These include standard invasive techniques, such as histological ones[18], the fluorescent method for determining damage to the plasma membrane of a cell[19] and electrophysiological[20,21],which are not very convenient and require either various dyes or electrical equipment where the readings are usually not stable due to the influence of buffer salts. For studying biological processes, one can distinguish methods based on Raman spectroscopy[28, 29]. These methods have a high potential for studying the structure of the active site of enzymes, the concentrations of cellular components during the cell cycle by analyzing vibrational modes, etc. The oscillatory modes in this method of analysis are in the range of hundreds to thousands of cm^{-1}.

Recently, methods of coherent four-photon scattering (FPS) spectroscopy have been emerged. These methods allow investigation of the properties of biological objects based on the registration of optical transitions in the range of ώ1-ώ2 ~ 0.1–100 cm^{-1}, which makes it possible to record the low-frequency region of the spectrum owing to phasing[30]. Phasing is realized in the macroscopic volume of molecular motions using two laser waves with frequencies ώ1 and ώ2, the difference between which (ώ1 -ώ2) is scanned in a wide spectrum from near infrared to microwave range. The frequency range from 0.1 to 1 cm^{-1} (corresponding to rotational transitions of water and being in the microwave range) was investigated to study the microenvironment of proteins and DNA in aqueous solutions[30, 31].

It was

Biological and objects can also be studied using microwave radiometry, which is simpler, less expensive and convenient in analytical biochemistry.

MWR methods provide additional information on biochemical processes, including the denaturation process, by radiation in the radio range, which may be associated with quantum-mechanical transitions in the biological system.

The MWR method is low cost, easy to use and can produce measurements without using optical and radioactive labels. It does not need to immobilize molecules on the surface of microchips, and it can be used in real time. The target processes are not only the processes of denaturation but also the processes associated with radiation during flow enzymatic reactions[9,10] and cellular processes including those associated with changes in the status of cancer cells[22]

As previously mentioned, the measurements should take place in a location with no high microwave noise. The probe should be completely immersed in the egg white. The experiment was performed at room temperature, but the device can work at a wider range of temperatures (5–60°C). Smaller probes can be used, but as the device measuring emission of the volume, the power of the signal should be higher. So, low-noise microwave room or a good microwave noise insulation is required to perform experiments. The second limitation of the method could be the time interval of the kinetic parameters of denaturation.

Unfortunately, on current stage of development MWR cannot separate different process or indicate specific mode of a process.

The advantage of the MWR method is its low cost, ease of use of the equipment, the ability to measure without using optical and radioactive labels, without using the processes of immobilizing molecules on the surface of the chips, in real time, not only the processes of denaturation, but also the processes associated with radiation during the flow enzymatic reactions[9,10], cellular processes, including processes associated with changes in the status of cancer cells[32], etc., for medical monitoring of the condition of various biomedical objects.

So far, nobody could explain effect of postmortem microwave emission increase in liver[36]

The mechanism of microwave emission increase after brain trauma injury [37], ischemic [38], and hemorrhagic strokes [39]. Neurogenic fever often develops significantly worsening the prognosis and outcomes of the disease. We hypothesize that this phenomenon is caused by proteins denaturation, and protein deficiency in the blood. For example, as a emergency therapy albumin has been used for many years in patients with cirrhosis and ascites [40], and in acute ischemic stroke [41]. The results could be used for further development of MWR based diagnostics systems.

Declarations

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DISCLOSURE:

Sergey Vesnin is a Director of MMWR LTD, UK. Lev Ovchinnikov is working for MMWR LTD. Igor Goryanin is a MMWR LTD Consultant. Yoshiyasu Rinsei Kobayashi, Igor Panarin and Anton Kulbachevsky have

no conflict of interests.

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Figures

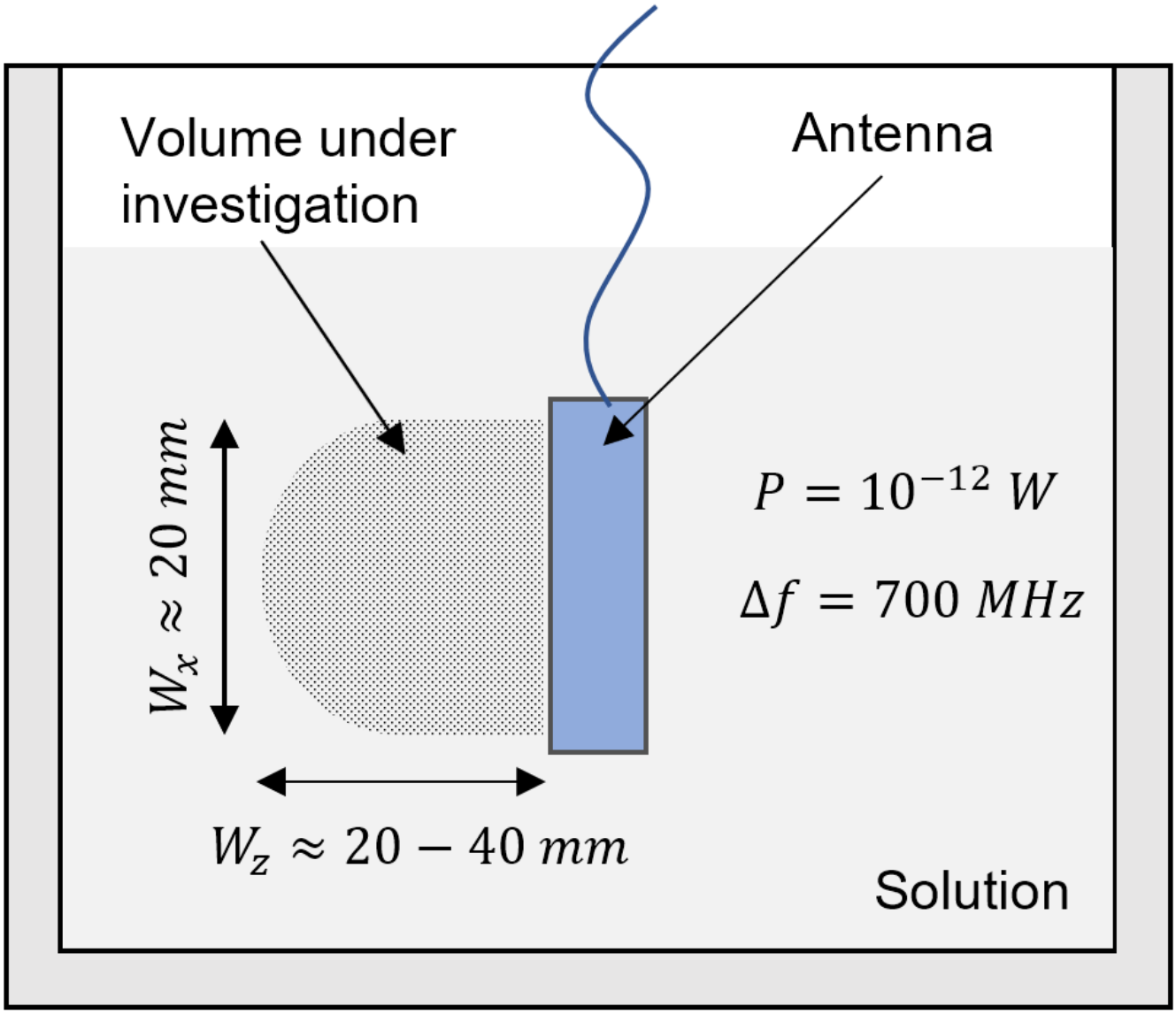


Figure 1

Microwave radiometry measurement principles. A probe is immersed into a liquid. The averaging volume is 5-30 mm in width and 20-50 mm in depth. Microwave emissions from a liquid is measured.

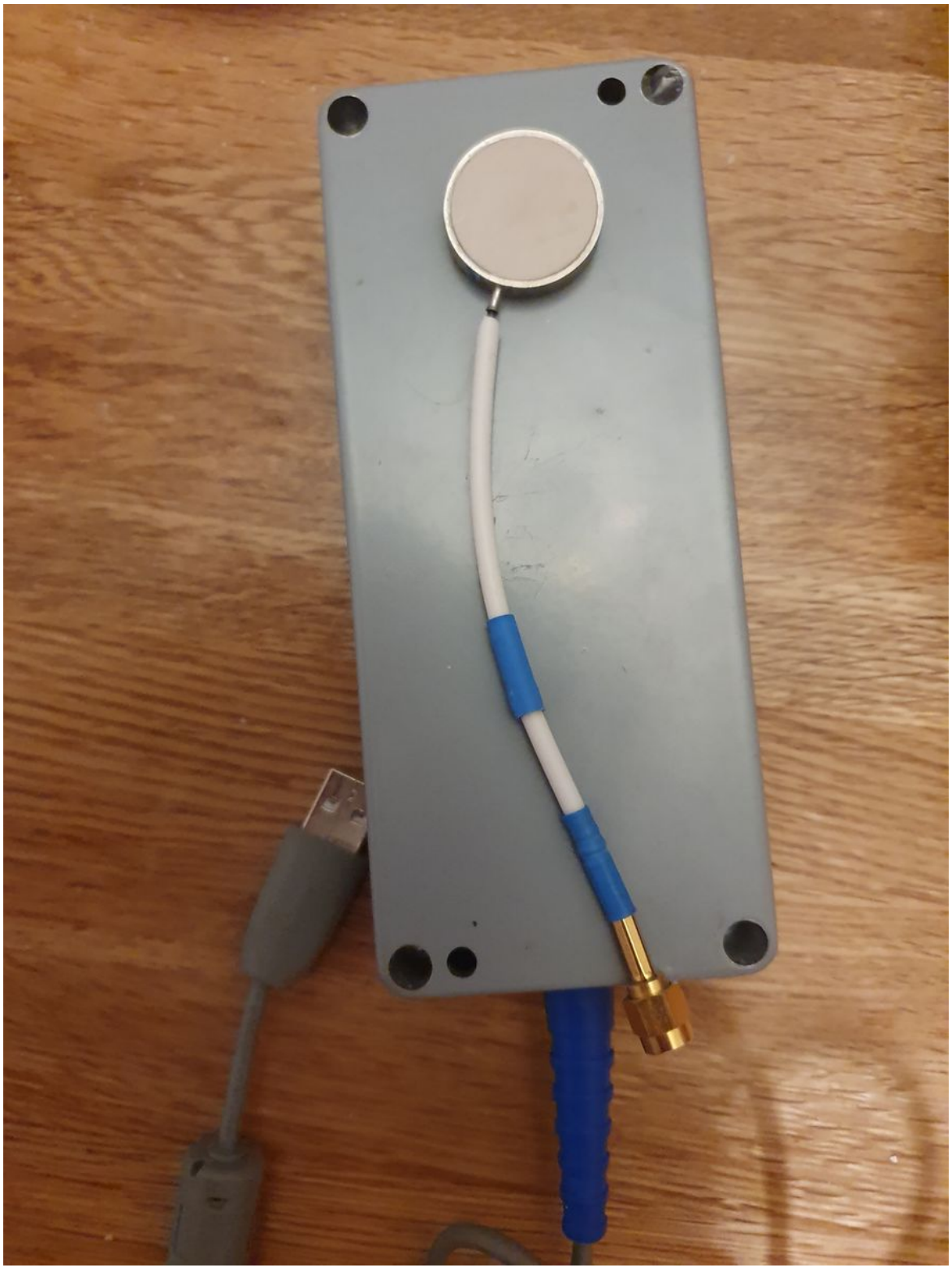


Figure 2

RTMM device with USB connector

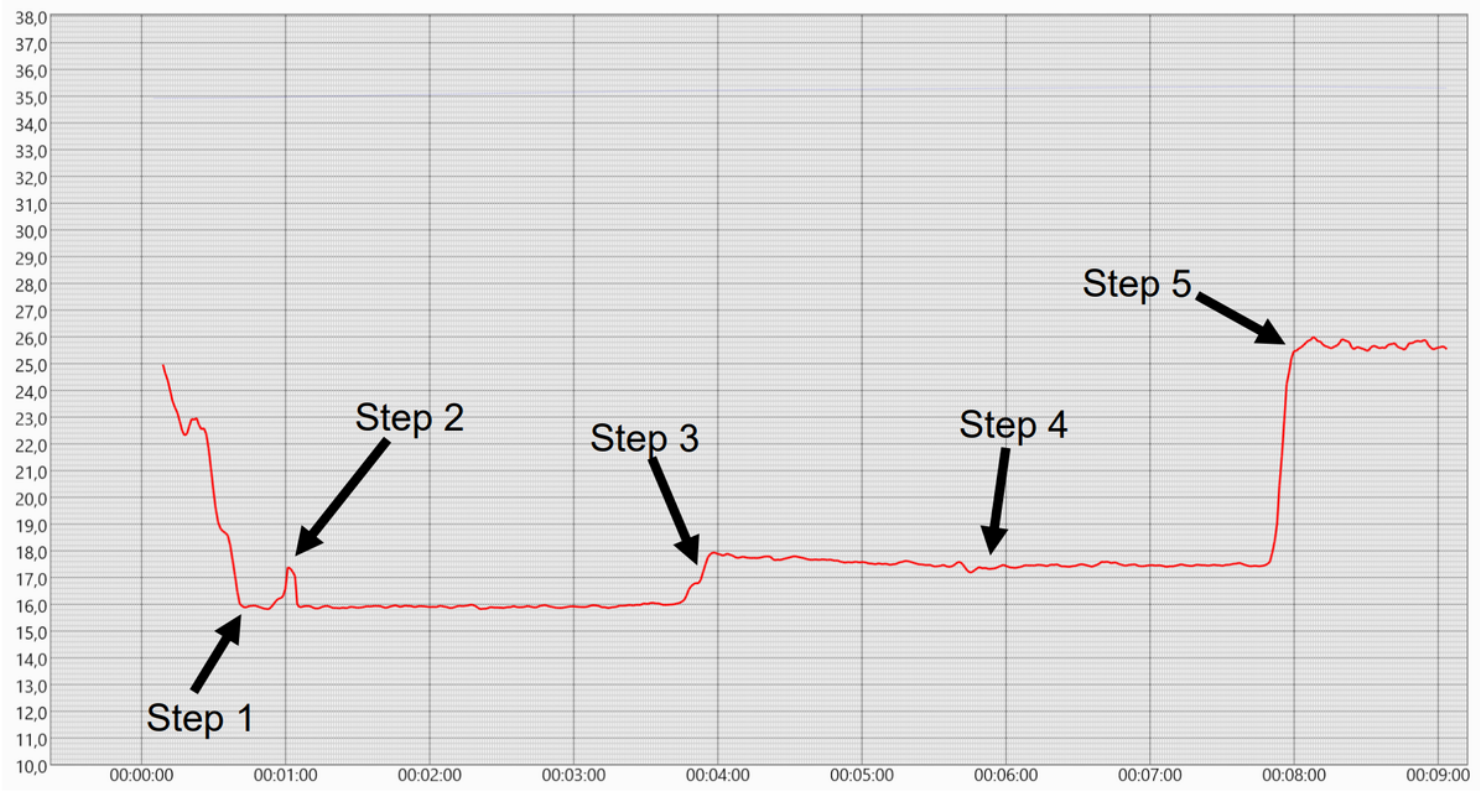


Figure 3

The Experiment. Time dependence. Step 1 Add egg whites. Step 2 Immerse probe and IR thermometer to the cup. Step 3 Add ethanol 96% Step 4. Stirring Step 5. Probe out.

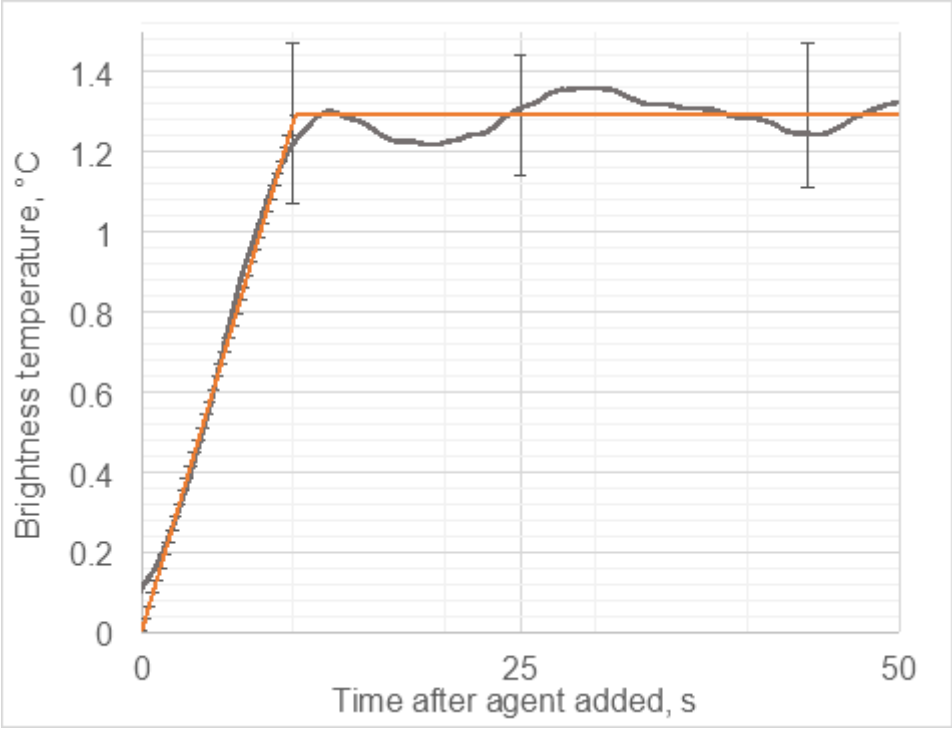


Figure 4

Microwave emissions (Brightness) temperature of the egg white during ethanol-induced denaturation. Black line denotes experimentally observed temperature; red line denotes approximation (slope). Different panel figures refer results from repeated experiment.

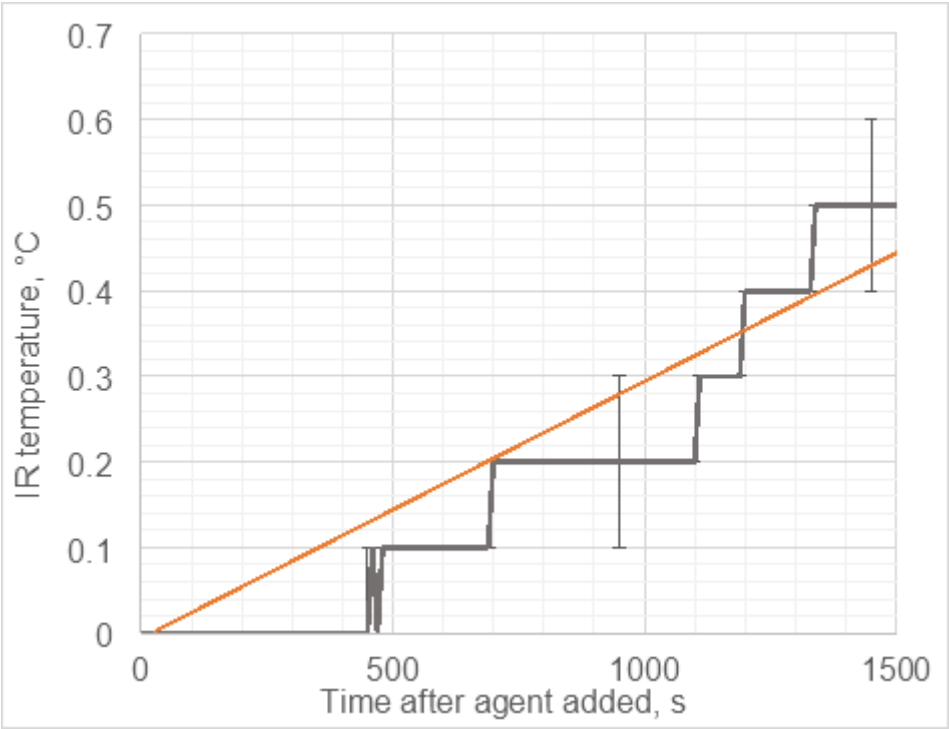
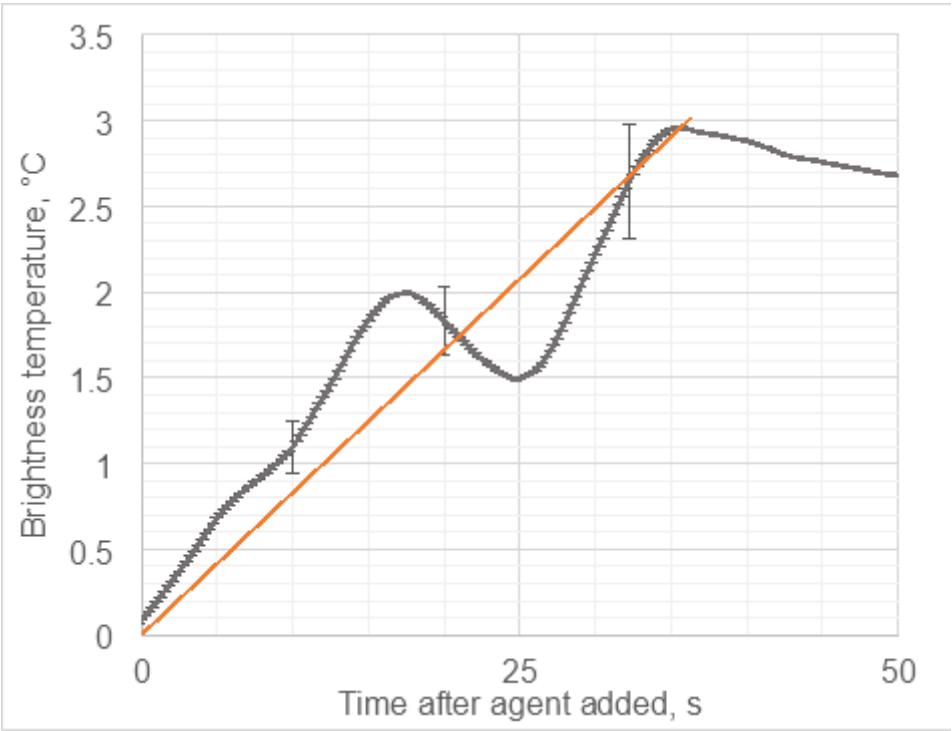


Figure 5

Thermodynamic (IR) temperatures during alcohol-induced denaturation. Solid green line denotes experimentally observed brightnesstemperature; blue line denotes approximation(slope).



Microwave emissions (Brightness temperature) of adding ethanol to tap water during control experiment. No egg white. Black solid line denotes experimentally observed. Orange line denotes approximation (slope)

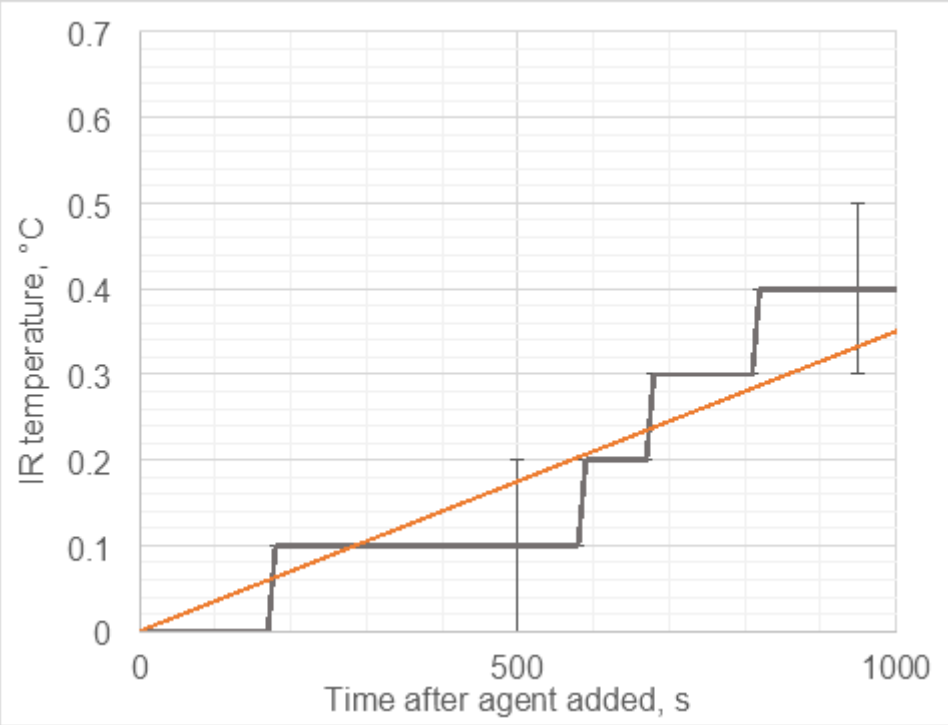


Figure 7

Microwave emissions (Brightness temperature) of adding ethanol to tap water during control experiment. No egg white. Black solid line denotes experimentally observed. Orange line denotes approximation (slope)