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Comparison of Multiple Moisture-Measurement Techniques Applied on Biological Tissue

Vergleich mehrerer Feuchtigkeitsmessmethoden für biologisches Gewebe

DOI 10.1515/teme-2021-0065

Abstract: This paper presents a measurement setup which combines three different methods indicating the moisture fraction of a sample. This is managed by measuring the weight, the absolute electrical impedance with a harmonic excitation between 10 Hz to 1000 Hz and the surface reflectance of the sample with a spectroscope between 160 nm to 1100 nm. The measurements are carried out over 50 h every 5 min using a piece of porcine meat. The results show clear evidences that the dehydration occurs on the surface until the water inside is diffused to the surface completely. This can be seen on significant wavelengths of absorption bands in the reflected spectrum like 540 nm, 575 nm and 970 nm (oxyhaemoglobin and water) and in the impedance measurement that are both set into ratio with the weight loss of the sample.

Keywords: Moisture, bio-tissue, spectroscopy, impedance.

Zusammenfassung: Dieser Artikel präsentiert einen Messaufbau, welcher drei Arten der Feuchtigkeitsbestimmung einer Probe kombiniert. Dafür wird das Gewicht, die absolute elektrische Impedanz, mit einer harmonischen Anregung zwischen 10 Hz bis 1000 Hz, und die Oberflächenreflektion mit einem Spektroskop im Bereich von 160 nm bis 1100 nm gemessen. Die Messung wird über 50 h alle 5 min auf einem Stück Schweinefleisch durchgeführt. Die Ergebnisse zeigen klar auf, dass die Austrocknung von der Oberfläche startend in das Innere der Probe vordringt. Dies kann an den signifikanten Wellenlängen der Absorptionsbändern, wie zum Beispiel 540 nm, 575 nm und 970 nm (Oxyhämoglobin und Wasser) im reflektierten Spektrum sowie bei den Impedanzmessungen gesehen werden. Diese Messungen werden dabei in Abhängigkeit zum Gewichtsverlust der Probe gesetzt.

Schlüsselwörter: Feuchtigkeit, biologisches Gewebe, Spektroskopie, Impedanz.

1 Introduction

The properties of almost all materials depend on environmental impacts e.g. temperature, humidity and age. Regarding extinct biological materials like e.g. soft tissue these influences change the measured values in a tremendous way. Furthermore the ageing and dehydration of the sample is an immense factor which is hardly possible to prevent and also to indicate whilst ongoing measurements.

While measuring the soft tissue of the vocal folds the authors were confronted with this effects [3, 7], to bring light to this sparsely illuminated field of their research, a measurement device combining different moisture measurement techniques is developed and applied on different samples. By doing so, not only the processes of dehydration could be studied but also the correlation between different techniques and measurement locations, which does not necessarily measure the same sort of moisture. The weight measurement represents a mean value of moisture of the whole sample, where local dehydration is not distinguished.

The absolute impedance indicates the moisture inside the sample, depending on the penetration depth of the electrodes.

And to complete the study a spectroscopy analysis detects the dehydration on the sample's surface.

The aim of this study is to gain knowledge about the processes during dehydration and furthermore to establish the base to set a link between the spectral reflectance of a sample to its stage of dehydration. After this introduction the measurements will be described, followed by an explanation of the measurement setup combining those methods, further the results are presented and discussed later on.

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2 Methods

Determination of indicators representing the samples water fraction is done in three different ways in this study. The theoretical background and measurement principles will be described in this section.

2.1 Weight measurements

The fraction of Water ν_{water} in the sample is simply determined through

$$\nu_{water} = \frac{m_{fresh} - m_{dry}}{m_{fresh}} \cdot 100, \quad (1)$$

where the masses, of the fresh and dry sample are set to ratio [4].

2.2 Absolute electrical impedance spectroscopy

2.2.1 Application

In order to determinate the samples absolute impedance a sinusoid sweep signal in the range of 10 Hz to 1000 Hz with an amplitude of 1 V is produced by a function generator (MFG-2120MA, GW-Instek, Taiwan) and brought into the sample with silver coated copper electrodes. Thereby not only the resistant behaviour over the frequency range can be determined but also electro-chemical reactions inside the tissue are avoided, which can occur while obtaining a constant voltage. To calculate the samples absolute impedance $|Z_{sample}|$, information about the flowing current is required. This is done by measuring the voltage $\hat{U}_{ref}$ over a known reference resistor R_{ref} , so the voltage between both electrodes is calculated with the voltage divider rule. Thus only the maximum values of the resistance over the sinusoid sweep signal are of interest, the peaks in the measured signal are detected and then referred to the current frequency f . These maximum peak-to-peak values are marked with the (^) sign, and the absolute impedance is calculated then by,

$$|Z_{sample}(f)| = \frac{[\hat{U}_{all}(f) - \hat{U}_{ref}(f)] \cdot R_{ref}}{\hat{U}_{ref}(f)}. \quad (2)$$

In order to observe a possible electrode movement affecting the resistance, the cross section impedance is calculated, defined as $Z_{cross} = Z_{sample}/d$ with d representing the distance between the electrodes. Therefore the markers are placed on the electrodes to detect their positions.

2.2.2 Warburg model

The contact between metallic electrodes and an electrolyte results in a charge distribution, which creates a half-cell potential and furthermore an impedance [6]. To describe this effect various models have been developed, one of the most common is the Warburg-model, which we will apply on the measured data. The Warburg-model characterizes each electrode as a capacitor and resistor in row, which brings with in a frequency depended behaviour to the measurement. This electrode-electrolyte interface may be calculated for low-current densities as

$$|Z_{war}| = \frac{0.005 \text{ cm}^2}{S} \sqrt{(Af^{-\alpha})^2 + (Bf^{-\beta})^2} \quad (3)$$

with the electrode Surface S , and material parameters A in Ω/Hz , α representing the resistance and B in Ω/Hz , β the capacity [6]. The material parameters values are mentioned in the result section.

2.3 Optical Spectroscopy

To complete the study, the diffuse reflection spectrum S in the visible and near infrared range 160 nm to 1100 nm is taken. The analysis of the spectrum changes is a common method to detect the dehydration [5]. The sample is illuminated with a halogen lamp, with a color temperature of 2700 K, to ensure a coverage over the whole spectrum range. The intensity curve of the reflected light can be seen in Figure 1. Due to the fact that the intensity in the lower frequency range is quite low, the results in this range are affected to high noise. Thus wavelength below 400 nm are not used for further processing. The regarded wavelengths are towards the upper limit of the spectrum, so it does not effect the measurement massively.

The measurement setup is placed inside a light-tight thermobox, to take potential background light into account a dark-spectrum S_{dark} is recorded in advance, which is subtracted from each spectrum.

In order to observe the change of the samples reflection, every measurements spectrum S_i is divided by the spectrum of the first measurement S_1 , which acts as a reference. The so called relative absorption I_{Absorb} can be written as

$$I_{Absorb}(i) = \frac{S_i - S_{dark}}{S_1 - S_{dark}}. \quad (4)$$

In the recorded spectrum are absorption bands of different molecules present. These are picked out, for the use as an indicator, compare Table 1.

Oxyhaemoglobin is an indicator for the oxygen saturation in the cell, and correlates linear between the spec-

tral reflectance and the saturation [9]. The wavelength of 970 nm represents the water absorption band and indicates the fraction of water in the near surface tissue.

Fig. 1: The relative spectrum of the reflected light is plotted against the wavelength. Underneath 400 nm the reflectance is less than 10% of the maximum and thence not used for further processing.

Table 1: The chosen indicator wavelengths [1, 2, 10].

#	Wavelength in nm	
1	540	Oxyhaemoglobin absorption band
2	575	Oxyhaemoglobin absorption band
3	700	Significant changes in spectrum
3	970	Water absorption band

2.4 Measurement setup

The measurement setup combining weight-, impedance- and spectroscopy-measurements can be seen in Figure 2. The sample, placed on the load-cell (TAL221, HT-Sensors, China), is assembled with two electrodes for the impedance measurement. The surface moisture is detected via optical spectroscopy using the AvaSpec AS7010 spectroscope and the factory software (Avantes, Netherlands), which enables an analysis in the range of 160-1100 Hz. The position of the measurement can be changed by rotating the servo-arm on which the spectroscope is mounted on. The sample is illuminated during measurements with a halogen lamp which provides a wide spectrum of light. The environmental conditions are tracked with an temperature and humidity sensor (DHT 11). In order to keep those parameters constant, the lamp and a fan are controlled by the micro-controller (Arduino Uno, Arduino, Italy) which receives commands and sends measurement data via serial-port from and to the computer. On the computer

the control and data processing is managed by Matlab 2018b (Mathworks, USA), which controls the function generator (MFG-2120MA, GW-Instek, Taiwan) and also reads out the samples of the impedance measurements that are recorded with a NI-Card (NI-9324, National Instruments, USA).

The measured raw data, saved on the hard drive, are subsequently further processed and visualized with Matlab.

Fig. 2: The setup combining weight-, impedance- and spectroscopy-measurements is placed inside a thermobox to isolate the measurement from environmental distortions. Different parts and devices are described in the pictures, where the arrows are pointing on the related positions.

2.5 Measurement procedure

In this study different samples of porcine meat, provided by the local butcher, are measured. The meat was cut in to a slice of about 9 g. One sample is picked out to demonstrate the technique and measurement methods. The investigation is performed by placing the sample on the load-cell after it got prepared by cutting it into a slice and attaching the electrodes with a distance of approx. 10 mm. The electrodes are cleaned with acetone between before every measurement, which is a common procedure in impedance measurements [6]. During the experiment the distance will be traced with the camera taking pictures with every measurement cycle and detecting the marker positions on the electrodes. Therefore the viewing angle has to be set correctly in advance, if this is granted, the thermobox can be closed and dark

Fig. 3: The reflectance spectrum for each measurement is plotted over the wavelengths. The coloring of each plot indicates the Weight Loss in percent.

reference spectra are recorded once. The dehydration is measured over 51 h 54 min in time intervals of 5 min, where the lamp is turned on in order to illuminate the sample for the picture and the spectroscopy. Next the Impedance is measured, and finally the van aerates the measurement chamber to prevent a overheating due to the illumination and to keep the environment conditions constant.

Fig. 4: Pictures of the sample at the beginning of the measurement and total dry-state 51 h 54 min later. Further the marker for the electrode position detection can be seen. The distance between the electrodes decreases from 11.64 mm at the begging to 9.60 mm in the dry state.

3 Results

First of all the visual change of the tissue can bee seen clearly in the images presented in Figure 4, the color, the structure and also the size of the sample changes drastically.

3.1 Weight Loss

The observed water-loss, can bee seen in Figure 5 it clearly shows an exponential decrease and could be determined by an exponential fit to $7\% \text{ h}^{-1}$, which matches to drying

models in literature [8].

Fig. 5: Weight loss over the elapsed time is plotted. The Water loss follows a exponential decrease of $7\% \text{ h}^{-1}$, determined by fitting the data.

3.2 Absolute impedance

The measured absolute impedance of the sample depends on the applied frequency and the degree of dehydration represented by the weight-loss. The electrical properties are shown as a contour plot in Figure 6. The impedance is illustrated as different colors, according to the colorbar on the side, as a function of the water loss and the frequency on the x and y-axis. The resistance shows, after a first drop below 100 Hz, an almost constant value over the remaining frequency range but a clear rise with dehydration until it rises high above a water-loss of 40 %. Between 5 % to 15 % water-loss a small plateau can be seen, which can be traced back to chemical reactions between electrodes and sample [6].

Fig. 6: The absolute impedance $|\hat{Z}_{sample}(f)|$ indicated in Ω are illustrated in different colors as a function of the applied frequency 50 Hz to 1000 Hz (y-axis) and the weight-loss in % (x-axis).

3.2.1 Warburg-model

In the visualization of frequency dependent behaviour of the impedance measurement an interaction between electrode and tissue is not considered. Therefore it will be regarded closer by applying the Warburg-model, described in section 2.2.2, exemplary to one impedance measurement. The coefficients for silver electrodes determined by Ragheb et al. are $A = 104$, $B = 307$, $\alpha = 0.674$ and $\beta = 0.741$ [6]. For the Warburg-compensation a constant resistance R_{const} equal to the measured resistance at 1000 Hz is assumed. The Warburg-compensation is then calculated through:

$$|Z_{comp}(f)| = 2Z(f) + R_{const} - 2Z(1000); \quad (5)$$

with $Z(f)$ calculated with the values above and Equation 3 with a electrode surface area of about 0.00028 cm^2 . As one can see in Figure 7, the model describes the frequency trend adequate.

3.3 Reflectance spectrum

The reflectance spectrum changes drastically while dehydration, as one can see in Figure 3. The shown spectrum is set in to ratio to the first measurement, to illustrate the variation depending on the water loss. During dehydration some wavelength reflect less some more due to the fact of the growing absence of specific molecules. Certain absorption bands of oxyhaemoglobin 540/575 nm and water 970 nm are chosen as moisture indicators [1, 2, 10]. Furthermore the red color with wavelength of 700 nm is chosen as an indicator, because of its almost linear change. The reflection changes for those wavelengths are plotted

Fig. 7: Shown are the measured Impedance data and in comparison the compensated Impedance $|Z_{comp}|$ with a constant resistance of 809.26Ω and a electrode surface area of 0.00028 cm^2 are assumed.

in Figure 8. It can be seen quite clear, that the red color decreases with time, where the other wavelengths are increasing. It seems like oxyhaemoglobin is decreasing linearly with the elapsed time, where water shows an exponential rise, directly correlating to the water loss of the tissue.

Fig. 8: The change of certain chosen wavelengths, explained in Table 1 are plotted against the elapsed time since measurement start.

4 Discussion

It was possible to measure three different moisture indicators in the same measurement setup. The spectral reflectance and the absolute impedance measurements are set into ratio to the weight-loss. Thus certain wavelengths could be chosen as indicators for the surface moisture of the sample. Furthermore, the moisture inside the sample is determined by the resistance for various excitation fre-

quencies. Comparing the moisture indicators of surface and inside the sample, it could be shown, that the sample starts dehydrating on the surface until the dehydration reaches the inner locations, where the electrodes are located. This can be seen in the sink in the beginning due to the water resigning to the surface and the connecting constant rise of the water-absorption band wavelength, see Figure 8 In contrast to that the resistance shows a slow increase until it suddenly rises drastically at about 65 % weight-loss, compare Figure 6. The frequency trend of the absolute impedance can be modelled adequate with the Warburg-model, the assumed electrode surface-area is higher than the one we measured on the used electrodes, furthermore is the current density in our application presumable higher than granted in the model [6]. Nevertheless it is evidential to show that the measured data does not represent the frequency trend of the tissue, which can be assumed as roughly constant. Future measurements with a number of various samples are planed to build the basis to develop a model to describe the degree of dehydration. The overall aim would be to be able to trace back to the degree of dehydration with two non-invasive spectrometer measurements, one at the time of interest and on reference measurement at the dry state of the tissue. Regarding different wavelengths which are set in to ratio, this could be possible. Which hopefully further investigations will bring up.

Acknowledgment: This research was funded by the Austrian Science Fund Grant No. I 3806-B28 and by the German Research Foundation (DFG) No. DO1247/9-1.

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