J Infrared Milli Terahz Waves (2013) 34:316–323
DOI 10.1007/s10762-013-9972-8

Determination of Leaf Water Content from Terahertz Time-Domain Spectroscopic Data

**R. Gente · N. Born · N. Voß · W. Sannemann ·
J. Léon · M. Koch · E. Castro-Camus**

Received: 31 January 2013 / Accepted: 1 February 2013 /
Published online: 20 March 2013
© Springer Science+Business Media New York 2013

Abstract We present a numerical method to determine the volumetric water content of leaves from transmission terahertz time-domain spectroscopy data. The method is based on iterative optimization of parameters of an effective medium model for vegetative tissue. We found a very good agreement between measurements performed with this method and direct fresh/dry leaf weight-based water measurements.

Keywords Terahertz · THz · Plant · Water · Drought · Stress · Barley

1 Introduction

The water content in vegetative tissues is a parameter of high importance to plant scientists. Currently this parameter is measured by destructive methods such as comparison of the fresh and dry weight of leaves. None of these methods allow for the instantaneous or continuous monitoring of the water content in live tissues. For this purpose a non-destructive technique that preferably requires very weak interaction

---

R. Gente · N. Born · N. Voß · M. Koch
Department of Physics and Materials Sciences Center, Philipps-Universität Marburg, Renthof 5,
35032 Marburg, Germany

W. Sannemann · J. Léon
Institute of Crop Science and Resource Conservation (INRES), University of Bonn,
Katzenburgweg 5, 53115 Bonn, Germany

E. Castro-Camus (✉)
Centro de Investigaciones en Óptica A.C., Loma del Bosque 115, Lomas del Campestre,
León, Guanajuato 37150 México
e-mail: enrique@cio.mx

with the plant in order to avoid altering its dynamics is desirable. The strong attenuation of terahertz (THz) radiation by water [1] makes radiation in this frequency band a very sensitive non-contact probe of hydration with enormous potential to perform non-destructive in-vivo detection of the water content. Furthermore, the recent advances in terahertz technology have improved the reliability, cost and ease of use of terahertz spectroscopy systems, which are now commercially available, making possible their implementation in biological research laboratories.

Terahertz time-domain spectroscopy (TDS) is a technique that has matured over the last three decades allowing the observation of physical [2, 3], chemical [4, 5] and biological [6–9] phenomena in the far-infrared band (300 GHz to 3 THz; 100 μm to 1 mm). This technique has the additional advantage of operating in the sub-microwatt power regime maintaining excellent signal–to–noise performance, which makes its effect on the live sample negligible.

Most of the previous publications reporting water status monitoring in plants using terahertz spectroscopy have reported the transmittance or absorption coefficients as qualitative indicators of water content [10–13]. However the actual volumetric water concentration was not quantified from the measurements.

In this article, we present a method to extract the volumetric fraction of water in leaf tissue from THz-TDS data. This method is based in the implementation of a numerical algorithm that calculates the parameters using an effective medium theory model of the dielectric function of vegetative tissue.

2 Theoretical Model

A leaf is a complex and heterogeneous structure made up of water, air, and “solid” tissue mostly composed of proteins, sugars and other compounds as illustrated in Fig. 1. The water content of the mixture is associated to different environmental and physiological conditions. In a previous publication [14] Jördens et al. demonstrated that effective medium approximation is an appropriate model to obtain the terahertz dielectric function of hydrated tissue. In particular they used an extended Landau–Lifshitz–Looyenga model [15] that relates the dielectric function of a heterogeneous

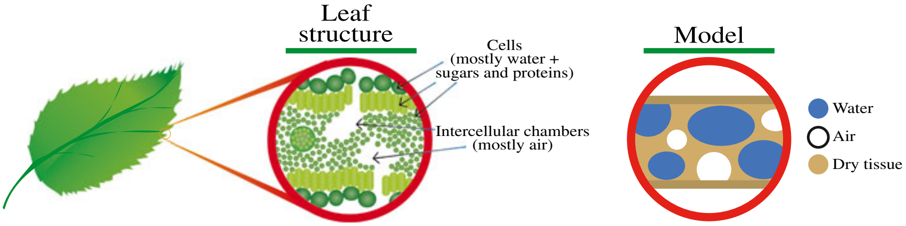

Fig. 1 Cross section of leaf structure. The leaf is composed mainly of air cavities, water and other compounds like sugars and proteins. Using effective medium theory it is possible to obtain the effective dielectric function of this complex-structured mixture as a combination of the dielectric functions of air, water and dry tissue

mixture to the dielectric functions of its components. Within this model the dielectric function of the leaf is given by

$$\sqrt[3]{\varepsilon_L(f)} = a_W \sqrt[3]{\varepsilon_W(f)} + a_S \sqrt[3]{\varepsilon_S(f)} + a_A \sqrt[3]{\varepsilon_A(f)}, \tag{1}$$

where ε_i are the dielectric functions and a_i are the relative volumetric concentrations of the different compounds, the indices refer to leaf (L), water (W), solid/dry tissue (S) and air (A) respectively. The water dielectric function was obtained from a dual Debye model [16]. In order to determine the dielectric function of solid/dry tissue, a compact pellet can be prepared by compressing dried leaves using a hydraulic press, subsequently a THz-TDS transmission measurement should be performed.

According to Ref. [14], in order to obtain the correct transmission coefficient for the leaf, it is critical to take into account the scattering produced by the rugose surface of the leaf. The complex transmission coefficient is given by

$$T(f) = \frac{E_{\text{sam}}(f)}{E_{\text{ref}}(f)}, \tag{2}$$

where $E_{\text{sam}}(\omega)$ and $E_{\text{ref}}(\omega)$ are the Fourier transformed electric fields measured by THz-TDS. The surface scattering can be included in the model by using a Rayleigh roughness factor [17], which means that a loss equivalent to an increased absorption coefficient $\alpha = \alpha_{\text{abs}} + \alpha_{\text{scat}}$ has to be considered, where

$$\alpha_{\text{scatt}} = \left(\Delta\varepsilon(f) \frac{4\pi\tau \cos(\theta)}{\lambda} \right)^2 \frac{1}{d}, \tag{3}$$

with

$$\Delta\varepsilon(f) = \sqrt{\epsilon_L(f)} - 1. \tag{4}$$

In such model the roughness τ is the standard deviation of the height profile of the leaf, d is the thickness of the leaf and θ is the angle of incidence.

The algorithm described in the previous section is assumed to be valid for leaves of any species provided that the correct dielectric functions, volumetric fractions, roughness and thickness are used. However, if the dielectric function of the leaf and its components is known, it is not trivial to determine the relative volumetric proportions of such components. In the following paragraphs we present a method to calculate the relative volumetric fractions of each component of the leaf given the dielectric functions of the hydrated leaf and its components.

The terahertz based hydration measurement is performed by experimentally determining the transmission coefficient $T_L^{\text{Exp}}(f)$ of the leaf using THz-TDS. Subsequently, an initial set of values a_i and d are assumed, with them, Eq. 1 is used to calculate a theoretical value of the dielectric function, with this dielectric function in combination with Eq. 3 the real and imaginary parts of the refractive index are computed and finally from them a theoretical transmission coefficient $T_L^{\text{Theo}}(f)$ is calculated. The values of a_i and d are then changed using an iterative sequential quadratic programming algorithm [18] in order to minimize the difference between the experimental transmission coefficient (Eq. 2) of the leaf and the theoretical one.

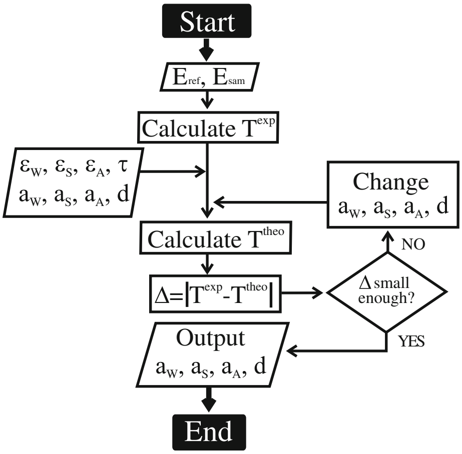

Fig. 2 Flow chart of iterative algorithm used to determine the volumetric fraction of water in a leaf. The algorithm calculates the experimental transmission function and compares it to a theoretical transmission coefficient calculated from the combination of the dielectric functions of its components according to the model presented

In other words, this optimization adjusts the parameters a_W, a_S and a_A as well as the leaf thickness d in order to fit the experimental transmission coefficient. The algorithm is schematically represented in the flow chart in Fig. 2. The conversion of volumetric $V_\% = a_W \times 100$ to weight water percentage $W_\%$ can be carried out using the parameters resulting from the iterative algorithm

$$W_\% = \frac{a_W \rho_W}{a_W \rho_W + a_S \rho_S} \times 100. \tag{5}$$

where $\rho_W = 1000\,\mathrm{kg/m^3}$ is the density of water and ρ_S is the density of the solid/dry tissue.

3 Experiment

In order to test the validity of the algorithm described in the previous section, which is in principle valid for any plant, we performed a series of measurements on Barley leaves (*Hordeum vulgare* ssp. *vulgare*) as a exemplary model system. The measurements were carried out using a THz-TDS system based on an Er:Fiber laser providing ∼65 fs pulses with a central wavelength of 1550 nm at a repetition rate of ∼80 MHz. A fraction of the pulses was sent through a polarization maintaining piezo-driven fiber-stretcher producing a delay of ∼15 ps at 10 Hz. These pulses were used to excite an LT-InGaAs stripline photoconductive emitter. The remaining fraction of the pulses was used to gate an LT-InGaAs dipole photoconductive detector. After emission, the THz radiation was collected and refocused by a pair of polyethylene lenses producing a ∼3 mm (FWHM) focus where the sample leaf was placed so that the radiation transmitted through the center of each leaf. Two additional lenses were used to collimate and refocus the radiation transmitted through the sample onto the photoconductive detector.

Barley plants where grown under optimal watering and illumination conditions. Ten fully expanded and healthy leaves were cut from the plants (at $t = 0$) and both

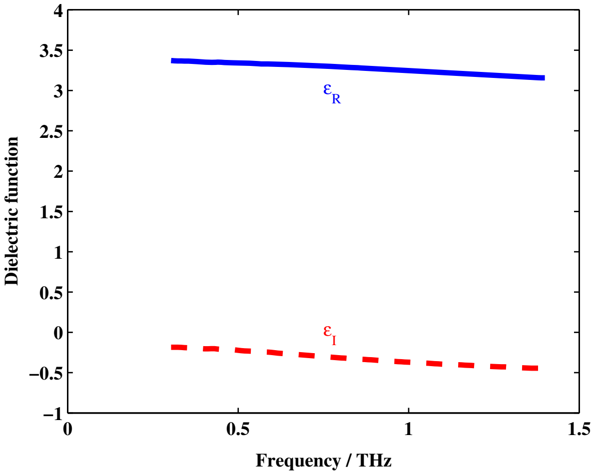

Fig. 3 Real (*continuous*) and imaginary (*dashed*) parts of the dielectric function of dry compact tissue measured by THz-TDS

the terahertz and gravimetric measurements started immediately as described in the rest of this section. The following steps were repeated 12 times:

(i) Each leaf was weighted.
(ii) A THz transmission spectrum was taken for each leaf.
(iii) The leaves were dried in an oven at 60 °C for 10 min.

Subsequently, the leaves were left in the oven until they were completely dehydrated in order to obtain their dry weight W_{dry}.

The weight percentage of water at each time step is

$$W_{\%}^{(i)} = \left(\frac{W_i - W_{\mathrm{dry}}}{W_i} \right) \times 100, \tag{6}$$

where W_i is the weight measured at the ith measurement cycle. In plant science this is known as gravimetric determination of water content. The gravimetric water content for the ten leaves was averaged at each time step.

It is worth mentioning that the surface scattering parameters are fixed by the user and not by the iterative algorithm. The radiation was incident at $\theta = 0$ and a value of $\tau = 1$ μm was taken, which is a reasonable estimation for barley leaves. As stated in the previous section, the dielectric function of the dry/solid tissue was determined by THz-TDS spectroscopy on a pellet made from compressed dried barley leaves. This dielectric function is shown on Fig 3.

4 Results and Discussion

The terahertz spectra obtained in each measurement cycle were processed with the algorithm presented in Section 2. The averaged weight percentage obtained from the algorithm for the ten leaves as function of drying time is shown in Fig. 4 (circles). The gravimetric data (triangles) is also presented in the plot for comparison purposes. As shown in Fig. 4a this measurement yielded a water content around 80 % at the time the leaves were cut from the plant ($t = 0$). They presented a relatively fast decrease of the water content at an average rate of ~0.16 % min^{-1}. The THz-based measurements correlate very well with the gravimetric ones. This demonstrated that the iterative algorithm converges to the correct water, dry tissue and air proportions for the leaf.

The algorithm was tested a number of times using different randomly chosen initial values for a_W, a_S, a_A and d in order to test the convengence of the algorithm obtaining the same results shown in Fig. 4. It is important to mention that the terahertz data acquisition was performed over a period of 2.5 seconds for each leaf (25 averages at 10 Hz). Longer data acquisition would result in lower noise in the spectra and consequently in the THz-based water quantification. The validity of this method was therefore proven for barley plants, yet, the structure and composition of most leaves is similar and therefore it is reasonable to assume that using the appropriate parameters, this model can be applied to leafs of any vegetative species.

In terms of computational performance, the algorithm was tested on 16 GB ram, 2.5 GHz dual core Intel core i5 processor computer. The program was coded in Matlab using under 600 MB of memory while running on a single core. The optimization process for each THz measurement took ~50 ms.

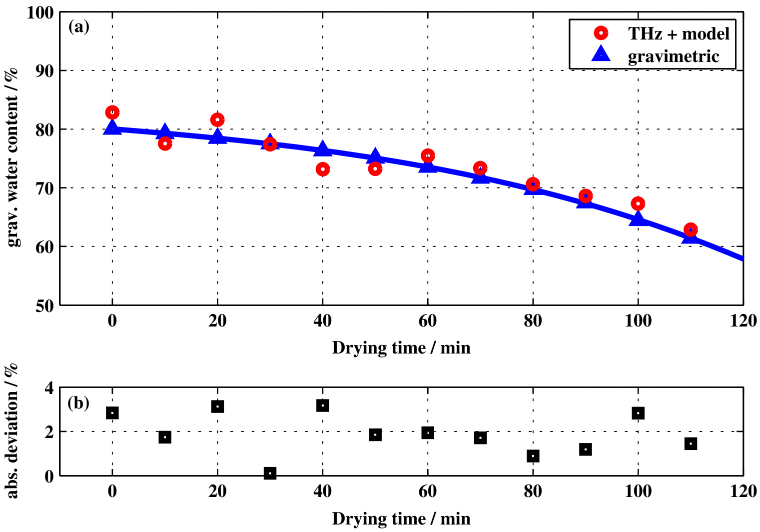

Fig. 4 Terahertz-based (*circles* calculated with Eq. 5) and gravimetric (*triangles* calculated with Eq. 6) quantification of weight-percentage of water contents in leaves. At $t = 0$ the leaves were separated from a live plant and they were maintained in an oven at 60 °C between measurements in order to accelerate their dehydration. The *continuous line* is supplied as a "guide to the eye" and does not represent any actual measurement. The *lower panel* shows the absolute deviation between the terahertz and the gravimetric measurements

5 Conclusions

We have presented a series of measurements of water quantification in plant leaves. The method presented uses THz-TDS transmission data which is subsequently processed with an iterative algorithm that calculates the relative volumetric fraction of water present in the tissue. These measurements correlate very well with direct determination of the water content by the well established gravimetric method. This experiment was carried out in a completely automated fiber-coupled THz-TDS system. In the future it is possible to incorporate the water calculation iterative algorithm to the data acquisition software. This would produce a turn-key system which requires no previous experience in THz-TDS to be operated or for the data analysis. Given that terahertz spectroscopy presents the considerable advantages of being a non-destructive and non-contact technique over traditional methods for water content determination, we expect that it could become a standard tool for hydration monitoring in plants in the near future.

Acknowledgments This work was partly funded by the German Federal Ministry of Education and Research BMBF, competence network "CROP.SENSe.net", subprojects S4 and GS2. We would also like to thank CONACyT (Mexico) [Grant no. 131931] for financial support.

References

1. J Xu, K W Plaxco, and S J Allen. Absorption spectra of liquid water and aqueous buffers between 0.3 and 3.72 THz. *J. Chem. Phys.*, 124:036101, 2006.
2. R Huber, F Tauser, A Brodschelm, M Bichler, G Abstreiter, and A Leitenstorfer. How many-particle interactions develop after ultrafast excitation of an electron-hole plasma. *Nature*, 414:286–289, 2001.
3. C Richter and C A Schmuttenmaer. Exciton-like trap states limit electron mobility in TiO2 nanotubes. *Nat. Nanotechnol.*, 5:769–772, 2010.
4. A G Davies, A D Burnett, W H Fan, E H Linfield, and J E Cunningham. Terahertz spectroscopy of explosives and drugs. *Mater. Today*, 11:18–26, 2008.
5. M B Johnston, L M Herz, A L T Khan, A. Köhler, A G Davies, and E H Linfield. Low-energy vibrational modes in phenylene oligomers studied by THz time-domain spectroscopy. *Chem. Phys. Lett.,* 377:256–262, 2003.
6. S Ebbinghaus, S J Kim, M Heyden, X Yu, U Heugen, M Gruebele, D M Leitner, and M Havenith. An extended dynamical hydration shell around proteins. *Proc. Natl. Acad. Sci. U. S. A.,* 104:20749–20752, 2007.
7. E Castro-Camus and M B Johnston. Conformational changes of photoactive yellow protein monitored by terahertz spectroscopy. *Chem. Phys. Lett.*, 455:289–292, 2008.
8. S W Smye, J M Chamberlain, A J Fitzgerald, and E Berry. The interaction between terahertz radiation and biological tissue. *Physics in Medicine and Biology*, 46(9):R101, 2001.
9. R J Falconer and A G Markelz. Terahertz spectroscopic analysis of peptides and proteins. *Journal of Infrared, Millimeter and Terahertz Waves*, pages 1–16, 2012.
10. S Hadjiloucas, L S Karatzas, and J W Bowen. Measurements of leaf water content using terahertz radiation. *IEEE Transactions on Microwave Theory and Techniques,* 47(2):142–149, 1999.
11. B Breitenstein, M Scheller, M K Shakfa, T Kinder, T Müller-Wirts, M Koch, and D Selmar. Introducing terahertz technology into plant biology: A novel method to monitor changes in leaf water status *Journal of Applied Botany and Food Quality*, 84(2):158, 2011.
12. W L Chan, J Deibel, and D M Mittleman. Imaging with terahertz radiation. *Rep. Prog. Phys.*, 70:1325–1379, 2007.
13. B B Hu and M C Nuss. Imaging with terahertz waves. *Optics Letters*, 20(16):1716–1718, 1995.

14. C Jördens, M Scheller, B Breitenstein, D Selmar, and M Koch. Evaluation of leaf water status by means of permittivity at terahertz frequencies. *J. Biol. Phys.*, 35:255–264, 2009.
15. H Looyenga. Dielectric constants of heterogeneous mixtures. *Physica*, 31(3):401–406, 1965.
16. H J Liebe, G A Hufford, and T Manabe. A model for the complex permittivity of water at frequencies below 1 THz. *International Journal of Infrared and Millimeter Waves*, 12:659–675, 1991.
17. P Beckmann and A Spizzichino. *The scattering of electromagnetic waves from rough surfaces*. Norwood, MA, Artech House, Inc., 1987.
18. P T Boggs and J W Tolle. Sequential quadratic programming. *Acta numerica*, 4(1):1–51, 1995.