applied sciences

MDPI

Article

Monitoring Theophylline Concentrations in Saline Using Terahertz ATR Spectroscopy

Kei Takeya [1,*], Keita Muto [1], Yasuhiro Ishihara [2] and Kodo Kawase [1]

[1] Department of Electrical and Electronic Engineering Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8603, Japan; muto.keita@f.mbox.nagoya-u.ac.jp (K.M.); kawase@nuee.nagoya-u.ac.jp (K.K.)

[2] Graduate School of Integrated Arts and Sciences, Hiroshima University, Higashi-Hiroshima 739-8521, Japan; ishiyasu@hiroshima-u.ac.jp

* Correspondence: takeya@nuee.nagoya-u.ac.jp; Tel.: +081-52-789-3169; Fax: +081-52-789-4440

Academic Editor: Christoph Peter Hauri
Received: 21 November 2015; Accepted: 24 February 2016; Published: 3 March 2016

Abstract: To assess the possibility of terahertz technology for the determination of drug concentration in blood, we endeavored to apply a terahertz (THz) attenuated total reflection (ATR) method to measure the levels of theophylline in saline. A change in reflected THz wave amplitude was observed in a theophylline concentration-dependent manner. This result was obtained with simple measurements of comparisons of the amplitude of the reflected wave, and suggests that it is possible to monitor concentration changes of drugs in liquid material using THz ATR measurements.

Keywords: terahertz; attenuated total reflection; therapeutic drug monitoring

1. Introduction

To assist in the appropriate usage of pharmaceutical agents, therapeutic drug monitoring (TDM) plays a fundamental role in clinical practice. TDM has been used for the determination of blood concentrations of drugs with narrow therapeutic ranges, drugs with marked pharmacokinetic variability, medications for which target concentrations are difficult to monitor, and drugs known to cause therapeutic and adverse effects. In this regard, TDM can allow for the optimization of individual dosage regimens [1]. However, because TDM is generally performed with immunoassays based on antigen-antibody reactions using serum, plasma, or whole blood, repeated blood collection can be stressful for patients. Additionally, it takes tens of minutes to determine the blood concentrations of agents, even though the measurement process is totally automated. Thus, the development of novel TDM methods for non-invasively and rapidly evaluating drug levels in blood is required.

Terahertz (THz) waves lie between the optical region and microwave region. The energy of a THz wave corresponds to the motion of a relatively large molecule; thus, THz waves offer the ability to observe absorption of a highly polymerized compound [2]. The energy is also related to the rotation of a hydrogen bond of water molecules and the rotation of free water molecules [3], so THz waves have the potential to observe water dynamics in a solution. Features of THz waves include transmission performance for an object, like microwaves, and spectral performance, like visible light. Although it has been difficult to use THz waves due to the lack of a good emitter to date, recent developments in the technology of THz emitters and detectors have allowed many applications of THz technology, such as non-destructive inspection, security checks, and communications [4,5]. Further, they also have potential for novel medical tools, because the photon energies of THz waves are relatively low and safe for the human body.

One feature of THz waves is that they are strongly absorbed by liquid water. This is mainly based on the modes of interaction with water mentioned above. This absorption becomes both an advantage and a disadvantage in some cases using THz waves to observe liquid materials. It is difficult to observe water or some solutions using THz transmission measurements, so the THz attenuated total reflection (ATR) method is used for measurements with water-related materials [3,6–8]. When light is reflected at an interface between a material of refractive index n_1 and one of n_2 (Figure 1), if the incident angle θ_c is greater than $\theta = \sin^{-1}(n_2/n_1)$, total light reflects at the interface. At this time, the light enters and propagates through a slight distance inside the second material. This is an evanescent wave. If the material is transparent for THz waves, then all evanescent waves pass through the second material with no absorption. However, if the second material, like water, has large absorption, the evanescent wave is absorbed and a decrease of light amplitude is observed even with a short passing distance of the light. Using the decrease, we can estimate some information. This is the THz ATR measurement, by which it is possible to measure a sample that has strong absorption in THz frequencies. Furthermore, because non-invasive characterization is possible with evanescent waves, they can be used for medical applications.

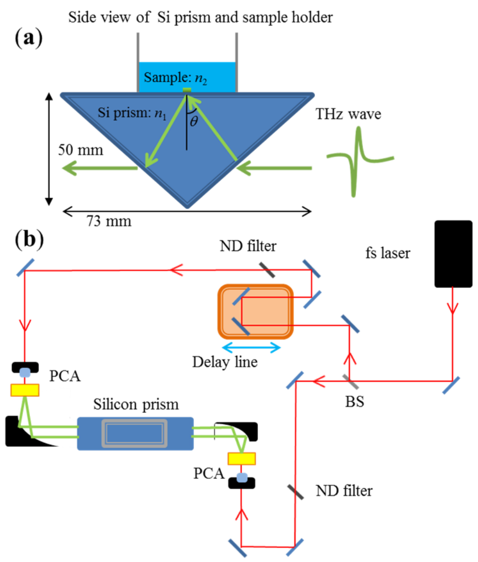

Figure 1. (**a**) Path of the propagating THz wave inside the Si prism. (**b**) Image of the measurement system using a typical THz-TDS system.

In the THz region, some studies for solutions have been reported using ATR methods [3,6–8]. For example, there are determinations of hydration numbers in solution and observations on the relaxation mode of water molecules [8]. These studies were primarily fundamental studies. In contrast, reports based on medical applications are rare. Moreover, there are very few THz studies on blood [9,10]. Because the energy level of THz waves corresponds to the dynamics of hydrogen bonds, they have the potential to observe the state of water molecules in a solute. Furthermore, a large absorption means a large interaction; thus, we can obtain meaningful information if we use suitable measurement methods to observe drug concentrations in solution. In this paper, we report a THz ATR study of saline, and a combination of saline and a drug, to discuss the potential of a THz-ATR method for medical applications, which, in this case, is that of determining the blood concentrations of drugs.

2. Experimental Section

We used techniques of a typical THz time-domain spectroscopy (THz-TDS) system for this experiment. Samples were measured with the THz-TDS system, equipped with dipole low-temperature-grown GaAs photoconductive antennas (Hamamatsu Photonics KK, Hamamatsu, Japan) as the emitter and detector, and a fiber laser (IMRA femtolite HFX-400, IMRA America, Inc. Ann Arbor, MI, USA, wavelength 780 nm, pulse width 48 fs, laser power max. 100 mW). The procedure to measure the sample, with the exception of the ATR method, is similar to that described in our previous paper [11].

It is difficult to measure aqueous samples with a transmission-type THz-TDS because of their strong absorption at terahertz frequencies. Thus, we chose an ATR THz-TDS using a silicon prism. The prism was 30 mm in width; the long axis of the triangle was 73 mm and it was 50 mm tall. The principle of the measurement using the prism is based on that of the Dove prism, although we used a triangular prism. The path of a THz wave propagating inside the Si prism is shown in Figure 1. The THz wave is guided by the two off-axis parabolic mirrors between the emitter and detector; thus, the shape of the THz wave entering the prism is a parallel beam (Figure 1). This shape is intended to use a wide area of the sample surface. The diameter of the parallel THz wave is about 20 mm, according to a knife-edge measurement.

A container with the sample solution was placed onto the surface of the Si prism. This was made of plastic walls (20 × 20 × 40 mm) and a resin adhesive. The sample solution in the container was connected directly to the Si prism. The entire propagating THz wave reflected at the border of the Si prism and the sample solution, and propagated onto the detector. Since we used the Si prism and plastic container, these materials had no influence on the measurement results. We used an aluminium mirror to measure the reference THz wave. We performed our analysis by comparing the reflected THz wave from the sample solution and the mirror.

The aim of this work was to assess the possibility of blood drug concentration measurements using THz techniques. We used saline as solvent for the drug. We prepared the saline by dissolving NaCl (Wako Pure Chemical Industries, Osaka, Japan) in pure water. Furthermore, we prepared a NaCl solution with various concentrations to measure the NaCl concentration dependence of the strength of reflected THz waves. We also used theophylline (Wako Pure Chemical Industries) as a test drug. Theophylline is an anti-asthmatic drug and has been used in clinical practice for over 30 years. The pharmacological effects of theophylline are closely related to its blood concentration, but high levels of theophylline in blood can result in various toxic effects, such as generalized convulsions and impaired consciousness [12]. Thus, TDM of theophylline was necessary for its safe administration.

3. Results and Discussion

The amplitude of THz wave reflectance of the samples was measured by comparing the sample spectrum and reference spectrum. Reference spectra were obtained using a mirror. Here, we discuss the evanescent field penetration depth, d_p, which is the distance between the interface and a point where the intensity of light becomes 1/e. Thus, d_p is described as

$$d_p = \lambda_2/2\pi\sqrt{(n_1{}^2/n_2{}^2)\sin^2\theta_1 - 1} \quad (1)$$

where λ is wavelength of the light, n_1 is the refractive index of ATR prism, n_2 is the refractive index of the sample, and θ_1 is the incident angle of the light. The frequency dependence of the penetration depth d_p in this study is shown in Figure 2. As the wavelength increases, the penetration depth, d, increases. This indicates that the evanescent wave at low frequency propagates inside objects, rather than a high frequency wave in the ATR method. Thus, we can expect that a terahertz wave at a lower frequency will have a larger interaction between light and a solution. Thus, we used data for a lower frequency THz wave for the analysis in this study.

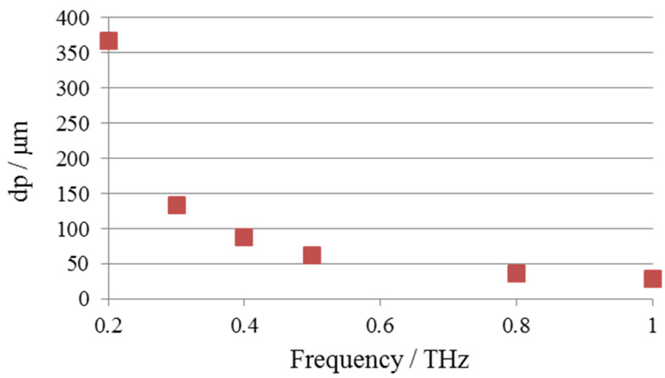

Figure 2. Frequency dependence of the penetration depth d_p.

In terms of NaCl solution, physiological saline is 0.9% NaCl solution. In the process of making saline solutions, we observed ATR spectra across different concentrations of NaCl solution using the ATR method. Reflection spectra were estimated by a division of the sample spectra by the reference spectra. As the concentration of NaCl increased, the amplitude of reflection spectra decreased (Figure 3). It is assumed that the decrease was caused by a change in the distance of the penetration depth due to the change of refractive index with the increase in NaCl concentration. The penetration depth is calculated with Equation (1). A change in refractive index causes a change in the value of d_p. Because water and solutions have strong absorptions for THz waves, a large change in ATR spectra occurs with a small change in the distance. Thus, it is possible to observe the concentration of solutions using THz ATR measurements.

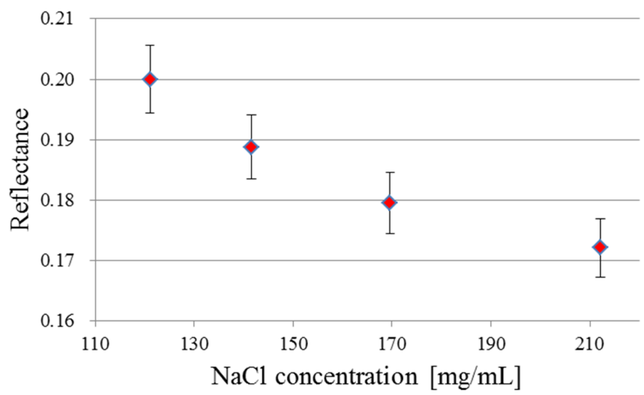

Figure 3. NaCl concentration dependence of amplitude of reflection spectra obtained at 0.86 THz.

Next, we measured the concentration dependence of theophylline in saline. The amplitude of the reflected spectra of the theophylline solution also changed with the change in theophylline concentration. The reflectance at 0.164 THz changed from 0.071 to 0.073 from the change of the concentration from 2.7 mg/mL to 5 mg/mL (Figure 4). The data shown in Figure 4 are from triplicate experiments. The concentration change was smaller than that of the NaCl solution. In the theophylline solution, the refractive index did not change with the concentration change, but amplitude changes in the ATR spectra were observed. These changes seemed to have occurred due to a change in the state of the solution. For water, strong absorptions exist below 1 THz. One of the absorptions is due to the Debye relaxation mode (τ_1), around 0.01 THz, and a second is due to the fast relaxation mode (τ_2) around 0.8 THz [3]. Theophylline in solution exists in molecular form and a hydrated state, because its dissociation constant is low. Thus, some water molecules make hydrogen bonds with theophylline, and they will change motions. The τ_1 mode is from the relaxation of the rotation of a water molecule, which connects with theophylline via a hydrogen bond, and the τ_2 mode is from the rotation of a free water molecule. Thus, the τ_2 mode does not shift, but part of the τ_1 mode shifts to a lower frequency region when more theophylline is present. The shift in the τ_1 mode causes decreased absorption of THz waves by water, so the amplitude of the reflected THz spectra becomes larger. This is consistent with the experimental results.

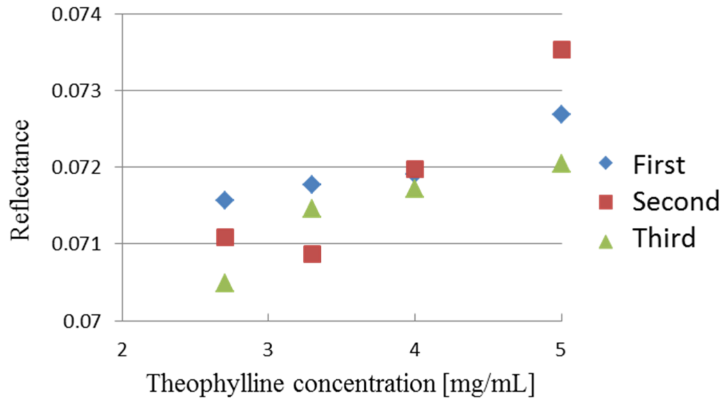

Figure 4. Theophylline concentration dependence of the reflectance of THz ATR spectra at 0.164 THz. With an increasing concentration, reflectance also increases. Errors were within ± 0.0003.

From the measurements of theophylline solutions, changes in the concentration of theophylline could be observed. This indicates the possibility of monitoring drug concentrations using THz techniques. The measurement is broadband spectroscopy because the method is based on THz TDS, but information on the concentration can be obtained by measurement of a single wavelength. Further, TDS usually requires both amplitude changes and phase shifts of the THz pulse, but the measurements in this study are from amplitude changes only. It is not necessary to perform a complex calculation to obtain data, and the method can be simplified. It is possible to make measurements by simply setting a sample onto the Si prism, with no complex procedure. Moreover, the measurement uses the evanescent wave; thus, it may be possible to make non-invasive measurements. The timing of THz ATR measurements is about a few minutes, clearly short enough for TDM. We expect further improvements in the measurement ability by improving THz spectroscopy in future.

4. Conclusions

We obtained a concentration dependence of reflected THz ATR spectra with NaCl solutions and theophylline solutions. In the case of NaCl solutions, a concentration dependence was observed with a change in the refractive index of the solution. For theophylline solutions, a concentration dependence was observed without a change in the refractive index of the solution. This change was likely to be due to a change in the hydrogen-bonding state of the water molecules. These results show that we can readily detect changes in drug concentrations in a solution using THz ATR measurements.

Acknowledgments: Kei Takeya is grateful to Yu Guan for helpful discussions.

Author Contributions: The manuscript was drafted by Kei Takeya; data was acquired by Keita Muto; data was analyzed and interpreted by Kei Takeya, Keita Muto, and Yasuhiro Ishihara; critical revision was done by Yasuhiro Ishihara; the research was planned and supervised by Kei Takeya, Kodo Kawase.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gross, A.S. Best practice in therapeutic drug monitoring. *Br. J. Clin. Pharmacol.* **1998**, *46*, 95–99. [CrossRef] [PubMed]
2. Tonouchi, M. Cutting-edge terahertz technology. *Nat. Photonics* **2007**, *1*, 97–105. [CrossRef]

3. Yada, H.; Nagai, M.; Tanaka, K. Origin of the fast relaxation component of water and heavy water revealed by terahertz time-domain attenuated total reflection spectroscopy. *Chem. Phys. Lett.* **2008**, *464*, 166–170. [CrossRef]
4. Kawase, K.; Ogawa, Y.; Watanabe, Y.; Inoue, H. Non-destructive terahertz imaging of illicit drugs using spectral fingerprints. *Opt. Exp.* **2003**, *11*, 2549–2554. [CrossRef]
5. Song, H.J.; Nagatsuama, T. Present and future of terahertz communications. *IEEE Trans. Terahertz Sci. Technol.* **2011**, *1*, 256–263. [CrossRef]
6. Nagai, M.; Yada, H.; Arikawa, T.; Tanaka, K. Terahertz time-domain attenuated total reflection spectroscopy in water and biological solution. *J. Infrared Milli. Terahz. Waves.* **2006**, *27*, 505–515. [CrossRef]
7. Arikawa, T.; Nagai, M.; Tanaka, K. Characterizing hydration state in solution using terahertz time-domain attenuated total reflection spectroscopy. *Chem. Phys. Lett.* **2008**, *457*, 12–17.
8. Arikawa, T.; Nagai, M.; Tanaka, K. Hydration structures of 2-butoxyethanol monomer and micelle in solution. *Chem. Phys. Lett.* **2009**, *477*, 95–101. [CrossRef]
9. Hirmer, M.; Danilov, S.N.; Giglberger, S.; Putzger, J.; Niklas, A.; Jäger, A.; Hiller, K.A.; Löffler, S.; Schmalz, G.; Redlich, B.; *et al*. Spectroscopic study of human teeth and blood from visible to terahertz frequencies for clinical diagnosis of dental pulp vitality. *J. Infrared Milli. Terahz. Waves* **2012**, *33*, 366–375. [CrossRef]
10. Reid, C.B.; Reese, G.; Gibson, A.P.; Wallace, V.P. Terahertz time-domain spectroscopy of human blood. *IEEE J. Biomed. Health Inform.* **2013**, *17*, 774–778. [CrossRef] [PubMed]
11. Takeya, K.; Fukui, T.; Takahashi, R.; Kawase, K. Dielectric constants of H_2O and D_2O ice in the terahertz frequency regime over a wide temperature range. *J. Opt.* **2014**. [CrossRef]
12. Hendeles, L.; Weinberger, M.M. Theophylline therapeutic use and serum concentration monitoring. *Individ. Drug Therapy Pract. Appl. Drug Monit.* **1981**, *1*, 31–66.