DIGITAL HEALTH

Measurements of glucose concentration in aqueous solutions using reflected THz radiation for applications to a novel sub-THz radiation non-invasive blood sugar measurement method

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Abstract

The terahertz (THz) frequency range corresponds to molecular vibrations or relaxation modes such as those for the hydrogen bond. Most biomolecules are activated only in aqueous solutions, thus, to understand the function and structure of biomolecules, it is necessary to investigate the characteristics of electromagnetic waves in hydrated samples. THz radiation causes little damage to the human body, thus it is expected that it can be applied for noninvasive examinations. However, spectrometry of the transmitted light is difficult, since the absorption of THz radiation in water is extremely high. In this study, we used sub-THz radiation (frequencies near to 0.1 THz), where the absorption is lower than for THz radiation, to measure the reflectance of a glucose water solution. We found that the reflectance decreases in proportion with the glucose concentration. These results suggest that sub-THz radiation can be used in the noninvasive measurement of blood glucose levels.

Keywords

1. Introduction

1.1. Terahertz (THz) wave

Electromagnetic waves are classified into light waves and radio waves depending on the wavelength. Short wavelength electromagnetic waves such as gamma-rays, X-rays, ultraviolet rays, visible light, and infrared rays are generally referred to as light waves. On the other hand, electromagnetic waves with wavelengths longer than about 0.1 mm are referred to as radio waves. The frequency of THz radiation ranges from $0.1 \sim 10$ THz (1 THz $= 10^{12}$ Hz) and the corresponding wavelengths are from 30 μm to 3 mm.

THz radiation has both the characteristics of light and radio waves, in which the optical properties of light and the permeability of radio waves are realized. These characteristics enable us to construct an optical system and transmit THz radiation into non-polar materials such as plastics and rubber. Furthermore, the frequency of THz radiation corresponds to the vibration frequencies of the inter-molecular and absorption bands of water in the far infrared light region, thus it is effective for analyzing the inter-molecular vibration properties of a wide variety of chemical compounds.

Some crucial applications of THz radiation are high-density wireless telecommunications, very sensitive in the detection of water, discrimination between cancer cells and normal cells in biological tissue,[1] detection of drugs in paper envelopes[2] and so on. The energy of THz radiation is quite low compared with that of X-rays and gamma rays. The quantum photon energy at 6 THz simply corresponds to the thermal energy at

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room temperature. Therefore, THz radiation is considered to be harmless to human tissue, and it is expected it can be safely used for noninvasive biological testing. According to the "Federal Communications Commission (FCC) maximum permissible exposure," the limit for the general population for sub-THz radiation (under $100\,GHz$) is $1.0\,mW/cm^2$.

1.2. Diagnosis of blood glucose level

Glucose is the main calorimetric energy source for the human body, and it performs critical work in cellular metabolism. However, a high concentration of glucose leads to glycation of the cells, affecting the epithelial tissue or blood vessels in the retina, causing arteriosclerosis or diabetic retinopathy (DR). In the human body, insulin is continually secreted by the pancreas, which reduces the blood glucose level. In a healthy person, the fasting blood glucose level is under $100\,mg/dl$ ($0.10\,wt\%$), and after eating a meal, it becomes higher. However, the level drops below $140\,mg/dl$ ($0.14\,wt\%$) within a couple of hours after taking a meal, if the secretion rate of insulin is normal. On the other hand, in the case of a person with diabetes, the postprandial blood glucose level is greater than $200\,mg/dl$ ($0.20\,wt\%$) and the fasting blood glucose level is also high. Thus diabetes can be diagnosed by measuring the blood glucose level. The present method used to measure glucose levels is to take a sample of blood from the fingertip by puncturing it with a needle, transferring it to a chip and then measuring the glucose concentration using an oxygen electrode method or by colorimetric determination.[3,4]

An advanced method for determining blood sugar levels has also been developed. This method uses a wristwatch device called a "glucowatch," which determines the blood glucose level from electrical resistance measurements of the interstitial fluid using an electrode in contact with the skin. However, both methods involve some pain for the user. A noninvasive method without pain is required. Some researchers have proposed a new system for noninvasive measurements.[5,6] However this system has not been established for clinical applications. We propose a new approach using THz radiation that is painless. The specific vibration modes of many biomolecules are in the THz frequency range,[7] thus by using THz radiation, we can estimate and sometimes determine biological molecular bonding states. There has been some literature published on measuring the glucose concentration in the frequency above ca. 0.1 THz using Fourier transform infrared (FT-IR),[8] and THz time-domain spectroscopy (THz-TDS).[9] This study characterizes the variation of glucose concentration in a model solution at a frequency of 60 GHz. It is noted that this method, which is sensitive to the presence of proteins, would require an independent measurement of the total protein concentration to determine the absolute glucose concentration of the solution.

1.3. Hydration

The hydration state of the aqueous solution is an important factor in considering the function of biological molecules. The hydration of glucide or protein molecules is considered on the basis of a hydration model in which the surrounding water molecules are spherically linked.[10] Whereas the relaxation time of bulk water is of the order of picoseconds (10^{-12} seconds (s)), that of the proximal layer that is combined with the dissolved molecules is extremely fast (10^{-17} s) because of the effect of the hydrogen bonding.[11] The surrounding water is weakly bound by the dipole of the proximal layer, thus the relaxation time is of the order of $10^{-9} \sim 10^{-10}$ s.[11] At around 100 GHz, there are absorption peaks that correspond to the intermolecular vibration of water. Over 1 THz there are many absorption peaks in water,[12,13] thus the hydration of water molecules is closely related to THz spectroscopy, and we can determine the characteristics of solutions using THz radiation.

2. Experiment

2.1. Measured solution samples

We used deionized water, D-(+)-glucose and bovine serum albumin for the sample solutions. D-(+)-glucose is a major saccharide in the human body. In this study we call it glucose. Albumin is a plasma protein that is a major constituent in human blood. Albumin controls the osmotic pressure and in transporting nutritional material. Thus, to simulate human blood, we need to consider the effects of albumin. In this study, we used aqueous solutions comprising dissolved glucose or albumin in deionized water. The concentrations of glucose are from 0.05 to $0.20\,wt\%$. The concentrations of albumin in water are from 1.0 to $5.0\,wt\%$. The standard level of albumin in human blood ranges from 4.0 to $5.0\,wt\%$ and the normal level of postprandial blood glucose is under $0.14\,wt\%$. The fineness of D-(+)-glucose is over 98%.

2.2. Sub-THz oscillator

In this study, we used a TUNNETT diode and a GUNN diode for the oscillator (TUNNETT diodes are made in our laboratory). The output power of the TUNNETT diode is about 0.18 mW, and the line-width
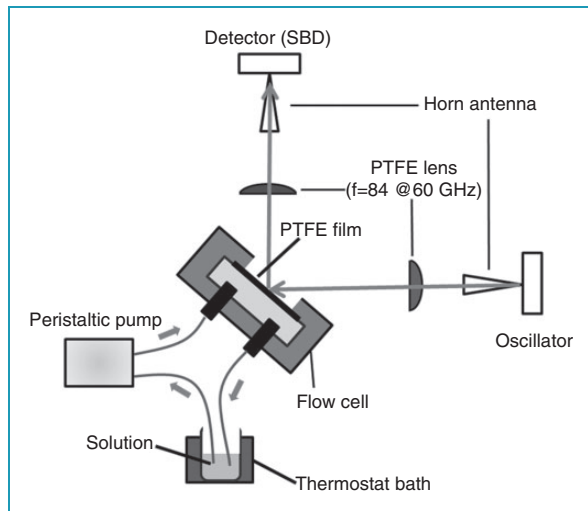
Figure 1. Schematic drawing showing the optical configuration of the sub-THz reflection measurement system.

is about 0.25 GHz. The power of the GUNN diode is about 60 mW.

2.3. Measurement methods

Figure 1 is a schematic drawing that shows the optical configuration of the sub-THz reflection measurement system. THz radiation, emitted from a semiconductor oscillator device, is focused by a polytetrafluoroethylene (PTFE) lens on to a flow cell in which the sample is being circulated by a peristaltic pump. After reflection from the flow cell, the THz radiation is focused by another PTFE lens, and then detected by a Schottky barrier diode (SBD). The signal-to-noise (S/N) ratio of the experimental system is about 140 dB. The angle of incidence is 45 degrees, and the reflectance is calculated from the following expression.

$$\text{Reflectance} = \frac{V_{sol} - V_{noise}}{V_{back} - V_{noise}} \times 100[\%]$$

(where V_{sol} is the voltage measured when the THz radiation is reflected from the liquid sample in the flow cell, V_{noise} is the voltage measured without the sub-THz light source, and V_{back} is the voltage measured when a metal plate is put in place of the flow cell for reference.) V_{back} is the voltage for the reference reflectance in this study. When the flow cell contains no liquid, the THz radiation passes through it, since it is made of polyethylene, which is highly transparent at these frequencies. Thus, we used a metal plate to measure the background reflectance.

We used a flow cell and a peristaltic pump to keep the concentration of the solution uniform. The flow cell is made of polyethylene, and the central region, where the THz radiation impinges, is 0.08-mm thick PTFE

(ASF-110FR, Chukoh Chemical Industries, Ltd). It is so thin that we can measure the reflectance of the sub-THz radiation from the solution. The area irradiated is 40 mm × 56 mm and the thickness of the cell is 1 mm. We use a Variable-Flow Peristaltic Pump (Fisher Scientific) to circulate the liquid sample without pressure perturbation. For this study, we performed two different kinds of experiments, one in which measurements were calculated as a function of sample concentration, the other in which measurements were calculated as a function of temperature. For the first kind, the measurements were calculated at 37°C, which is close to the temperature of the human body. We measured the dependence of the reflectance of 60 GHz radiation on the concentration of glucose at concentrations around that in human blood (from 0.05 to 0.20 wt%). Then, we measured the dependence on the concentration of albumin from 1.0 to 5.0 wt%. This range includes the range of values in human blood (from 4.0 to 5.0 wt%). Furthermore, we also calculated measurements on glucose solutions with albumin (albumin 4.32 wt%, glucose 0.10 ~ 0.20 wt%). Following these measurements, we measured the temperature dependencies of the reflectance for glucose solutions with concentrations of 0 and 0.10 wt% at temperatures from 25 to 45°C. In these studies we used a 60 GHz oscillator because there is an absorption peak due to the vibration of water molecules at around 100 GHz. The reflectance measurements were made with s-polarized light, and the reflectance was calculated from the output voltage from the SBD detector. These experiments were carried out at atmospheric pressure.

3. Results and discussion

First, we measured the dependence of the reflectance on the concentration of glucose. Figure 2 shows the concentration dependence of the reflectance for glucose concentrations from 0.05 to 0.20 wt%, and Figure 3 shows the concentration dependence of the reflectance for albumin concentrations from 1.0 to 5.0 wt%. The oscillator used was a TUNNETT diode (60 GHz). The error bars show the standard deviations calculated from 2000 measurements. The blood can be affected by many other substances other than glucose itself. In this study, we estimated the effect of the major plasma protein, albumin. As shown in Figures 2 and 3, the reflectance monotonically decreases with increasing concentration of the solutes. This is related to the effect of rotational relaxation. Bonding between the water and solute molecules inhibits rotational relaxation of the water molecules. Thus the absorption due to rotational relaxation decreases, leading to a reduction in reflectance. These factors are related to the change in refractive index. We can calculate the
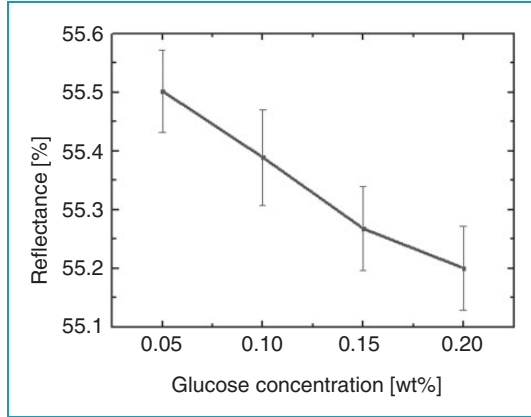
Figure 2. Concentration dependence of 60 GHz reflectance for glucose concentrations from 0.05 wt% to 0.20 wt% at 37°C.
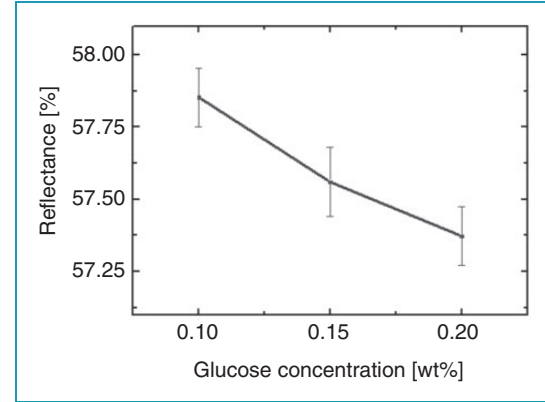

Figure 4. Concentration dependence of 60 GHz reflectance for glucose concentrations from 0.10 wt% to 0.20 wt% with a constant level (4.32 wt%) of albumin at 37°C.
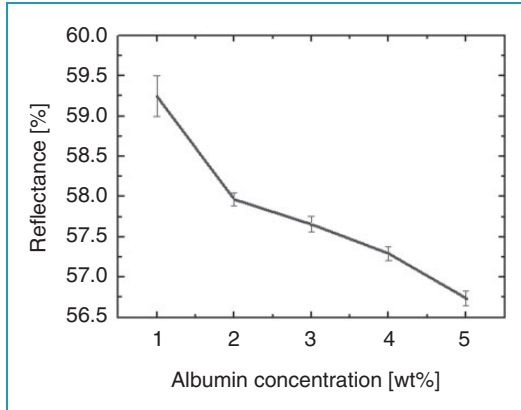

Figure 3. Concentration dependence of 60 GHz reflectance for albumin concentrations from 1.0 wt% to 5.0 wt% at 37°C.

fluctuation of the reflectance in the measured blood concentration. As a consequence, the effect of albumin on the variation in reflectance is larger than that due to glucose for unit solution concentrations. This is because of the difference in refractive index, or the difference in size of the molecules of the solutes. Thus, we can estimate the concentration of glucose in a short measurement time, because the range of albumin in normal human blood is usually very small.[14] Next, we measured the dependence of the reflectance on the concentration of glucose in a solution with albumin. Figure 4 shows the results for a glucose solution with a constant level (4.32 wt%) of albumin, where a TUNNETT diode (60 GHz) was used for an oscillator. The results demonstrate that we can measure differences in glucose concentration of 0.05 wt% for human blood with typical concentrations (0.1 ~ 0.2 wt%) even with the presence of albumin. Comparing Figures 2 and 4, the change in reflectance for the mixed solution with albumin and glucose is greater than that for the glucose-only solution. This difference is related to the effect of the matrix formed between glucose, albumin and water. Therefore, we have demonstrated that THz reflectance measurements can be used in applications to measure the blood glucose level. However, it is necessary to consider the effect of other solutes in the mixed solution. Other blood proteins are present in blood, thus, to reduce the effect of these on glucose measurements, the method of using the interstitial fluid has to be considered. In the interstitial fluid, there are fewer blood proteins, including albumin, than in blood vessels because of their large size,[15,16] so the effect of blood proteins in interstitial fluids is less than in blood vessels. The concentration of blood proteins is about 7 wt% in blood vessels and about 2 wt% in the interstitial fluid. The main protein in the interstitial fluid is insulin. Interstitial fluid is within a few hundred micrometers of the surface of the skin, thus it is possible to measure the concentration of glucose on the basis of the penetration depth of THz radiation.

Whereas glucose is active in aqueous solutions, biological molecules such as enzymes are most active at around body temperature. The bonding states between the molecules depend on temperature, thus it is necessary to clarify the temperature dependences of the measured results. Figure 5 shows the temperature dependences of the reflectance around human body temperature. This shows that the reflectance increases monotonically with increasing temperature, where a GUNN diode (60 GHz) was used for an oscillator. The slopes of the graphs are almost the same. It shows that when the temperature of the solution increases by 1°C, the reflectance increases by about 0.16%. The reflectance change per °C corresponds to a difference in glucose concentration of 0.05 wt%, thus the temperature of the solution is a significant factor for this measurement.
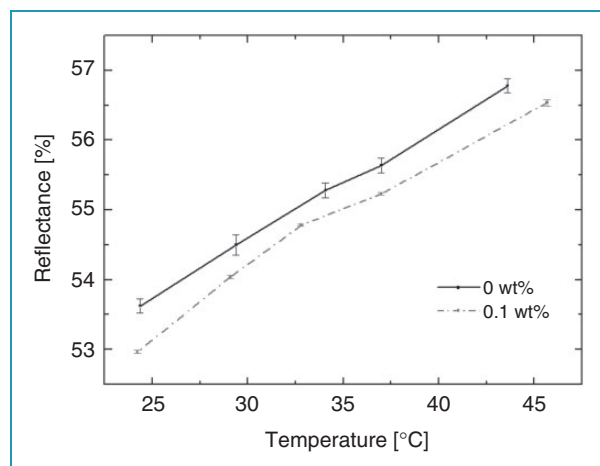
Figure 5. Temperature dependences of 60 GHz reflectance for glucose solutions with concentrations of 0 wt% and 0.10 wt% at temperature from 25 to 45°C.

4. Conclusion

We measured the dependence of the reflectance of sub-THz radiation on the concentration of glucose and albumin in aqueous solutions. We also investigated the effect of albumin on measurements of the glucose concentration by calculating measurements of a mixed solution. As a result, it was shown that the reflectance decreases in proportion to the glucose concentration. We can resolve differences in glucose concentration of 0.05 wt%. This level of sensitivity would enable us to discriminate between the blood glucose levels of diabetic and healthy individuals.

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