Glucose-water interactions at increasing concentrations and temperatures as revealed by Near-Infrared Spectroscopy

F S Rondonuwu$^{1,3,*}$, A Setiawan$^{2,3}$, J Muninggar$^3$ and F F Karwur$^4$

$^1$NIR Center, Faculty of Science and Mathematics, Universitas Kristen Satya Wacana, Diponegoro 52–60, Salatiga 50711, Indonesia
$^2$Study Center for Multidisciplinary Applied Research and Technology, Universitas Kristen Satya Wacana, Salatiga, Indonesia
$^3$Physics Department, Faculty of Science and Mathematics, Universitas Kristen Satya Wacana, Diponegoro 52–60, Salatiga 50711, Indonesia
$^4$Faculty of Health Sciences, Universitas Kristen Satya Wacana, Diponegoro 52–60, Salatiga 50711, Indonesia.

* E-mail: ferdy.rondonuwu@uksw.edu

Abstract. Water is an essential molecule and one of the most intensive research subjects, yet it is a peculiar molecule. The non-invasive measurement of blood glucose levels by near-infrared spectroscopy requires detailed information about the glucose-water interactions. The glucose in water induces strong hydrogen bonds and may influence the tetrahedral structure of water molecules. This knowledge is essential in constructing a non-invasive calibration of the blood glucose prediction model. How they are related to the absorption spectra of water and glucose is particularly valuable to comprehend. The evaluations were carried out using near-infrared spectroscopy, tracing changes in absorption intensity and shifts in corresponding peaks with glucose varying at an increasing concentration from a 0-0.9 molar fraction. The near-infrared spectra analysed were around 6900 cm$^{-1}$ and 4716 cm$^{-1}$ corresponding to the overtone and the combination band of water and glucose absorptions, respectively. The analysis suggests that glucose prefers to enter a water cluster, rather than bind to free molecules, and induce bond breaking at low glucose concentrations at which the molecular fraction is less than 0.05.

1. Introduction
Water is available in abundance on Earth and is essential to sustain life. The adult human body is made up of approximately 60% water, while a newborn baby is about 78% [1]. Whole blood consists of about 50% water, while plasma contains up to approximately 90% water. Water plays a critical function, including as nutrition for all living beings as it is the building material for cells, a medium for transporting carbohydrates and proteins to all parts of the body. Together with glucose, it functions as a cryoprotective agent [2]. Hence, in various phases, this molecule has been the subject of exciting research for decades by many researchers [3,4]. The conformations in both solid and liquid forms of water were studied through the atoms’ parameters of energy, bond length, structure, and orientation. How water molecules interact with other water molecules to form water clusters were also investigated [5,6]. Water clusters are influenced by additives such as glucose, where this additive can induce a structure breaker or a structure maker as well as limit the translational and rotational mobility of water [7,8]. Even then, water remains a mystery [9,10].

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd
Near-infrared is the electromagnetic absorption of water overtones and the combination band of symmetry and antisymmetric stretching and bending vibration modes [11]. Therefore, near-infrared spectroscopy is a great tool that has become a hallmark of rapidly advancing analytical techniques in the last few decades, including the nature of anharmonic molecular vibrations. Advances in NIR spectroscopy have also provided valuable information in quantum mechanical models and calculations regarding molecular vibrations [12].

This paper aims to report the near-infrared study of the interactions between water and glucose as an additive with the absorption in the spectral region of 5552–9000 cm$^{-1}$, corresponding to the absorption of overtone and combination band of the vibration mode. The dynamics of a glucose solution at various concentrations and temperatures and the effects of glucose on the water clusters are discussed. This information is particularly useful in designing a non-invasive measurement of glucose concentrations in blood using near-infrared spectroscopy.

2. Experimental method

2.1 Sample preparation

The D(+) -glucose monohydrate sample used in this study was purchased from Merch without further treatment. A total of 14 samples of glucose were dissolved in distilled water prepared with a mole fraction (c) of 2, 4, 6, 11, 17, 24, 32, 41, 51, 62, 76, and 91 x 10$^{-3}$. The D(+)-glucose in each solution sample was dissolved using a magnetic stirrer. Since the glucose solution can easily suspend, each sample was then stirred for a second time just before being loaded to the measurement cell ensuring even molecule distribution.

2.2 Data acquisition

The near-infrared absorption spectra were obtained by scanning samples loaded into a cuvette with a geometrical length of 1 mm using the BUCHI NIRFLEX N500 spectrometer (with liquid accessory). The targeted spectral regions were 5552–9000 cm$^{-1}$ with an interval of 4 cm$^{-1}$. Thus, the total data points in the specified spectral regions are 862. Each spectrum was averaged over 32 scans to achieve an acceptable s/n ratio. Sample temperatures were set at 25°C and 60°C using a built-in temperature controller (a repeatability of 0.5 °C and stability less than 0.2 °C).

2.3 Data analysis

Near-infrared spectra in almost all measurement modes often undergo baseline fluctuations. However, in this case, the baseline fluctuations were corrected by pushing each spectrum to zero at 9340 cm$^{-1}$. No additional corrections, including multiplicative scattering and smoothing, were applied. The absorption intensity in the spectral gap of 4820-5552 cm$^{-1}$ was intentionally set to zero due to the saturated water absorption around 5500 cm$^{-1}$. Since water absorption has approximately double the magnitude of absorption compared to glucose, the spectral evolution due to the increase of glucose mole fraction was not quite profound in the glucose-solution spectra. Therefore, the absorption spectrum of pure water was then subtracted from each spectrum measured at the same temperature. Hence, only the changes in the absorption spectrum due to the addition of glucose are shown. The same method was applied to groups of spectra measured at various temperatures with the same concentration. In this case, the background subtraction was carried out using a spectrum from the lowest concentration and temperature.

3. Results and discussion

Strong absorption from a combination band of symmetric overtone stretching and bending modes vibrations originated from water OH bond appears in the spectral region around 5200 cm$^{-1}$. The combination band of bending, overtone symmetric and antisymmetric stretching modes appears in an area of around 6900 cm$^{-1}$. On the other hand, the glucose absorption dissolved in water at various mole fractions appears in the spectral region 5700–6600 cm$^{-1}$, as shown in figure 1(a). There are two groups of spectra in this figure, those measured at 25 °C (black) and 60 °C (red). Absorption spectra
measured at 25 °C have a maximum absorption peak at 6880 cm⁻¹, while at 60 °C, the maximum absorption peak appears at 7000 cm⁻¹. There is a peak shift in absorption when the sample measured from lower to higher temperatures or vice versa. These results are consistent with previous reports observed in the range of 5400–4600 cm⁻¹ [13] and around 7000 cm⁻¹ [14]. When the number of glucose molecules increases, the water absorption intensity at 6880 cm⁻¹ and 7000 cm⁻¹ decreases. The decreasing absorption intensity is due to the reduced number of OH bond water molecules.

Along with increasing glucose concentration, the absorption band in the spectral region of 5700–6600 cm⁻¹ increases along with the increased glucose concentration. To carefully examine how the absorption spectra change with the addition of glucose, we subtracted the absorption spectra measured at 25 °C and 60 °C with the corresponding one at zero concentration for a baseline and depicted in figure 1(b). In this particular figure, the ordinate axis represents the change in absorption (ΔA), which has a negative value for a decrease in absorption intensity and positive value for increases in absorption intensity while glucose increases. Such spectral behavior implies absorption around 5552–6540 cm⁻¹ is originated from glucose molecules. Increasing glucose mole fraction, enabling decreasing absorption of water in the spectral region 6540–9000 cm⁻¹.

Figure 1. (a) Near-infrared absorption spectra (A) of a glucose solution in concentrations of 0, 2, 4, 6, 11, 17, 24, 32, 41, 51, 62, 76, and 91 x 10⁻³ mole fraction measured at 25 °C (black) and 60 °C (red). (b) Difference spectra (ΔA), absorption spectra minus the spectrum at 0 mole fraction for the set of spectra measured at 25 °C (black) and 60 °C (red).

To examine how the temperature modifies the spectrum of pure water and a glucose solution with a concentration of 0.091 mole fraction, a spectral acquisition of those samples was carried out at a temperature of 25–60 °C, as represented in Figure 2(a). The spectrum of pure water at various temperatures is represented with a black line, while the glucose solution at a molar fraction of 0.091 is represented with a red line. The heating effect of pure water and glucose solutions causes the water peak position to shift to the higher energy side. The spectra difference, i.e., the spectrum that has been subtracted from the spectrum of pure water at 25 °C, is shown in Figure 2(b). This shift eventually originated from the competition of the two water absorption peaks at 6880 cm⁻¹ (suppressing) and 7000 cm⁻¹ (growing) when the temperature increases. However, both peak positions remain invariant in the course of the heating process. Similar behavior is also valid for a glucose solution at a 0.091-mole fraction. These results indicate that, unlike water absorption, glucose absorption is insusceptible to temperature variations.
Figure 2. Near-infrared absorption spectra (A) measured at temperature from 25 °C to 60 °C (interval 5 °C) for (a) of pure water (black) and a glucose solution at concentrations of 91 x 10^{-3} mole fraction (red). (b) Difference spectra (ΔA), absorption spectra minus spectrum at 25 °C for a set of pure water spectra (black), and a glucose solution (red).

Figure 3. Plot of ΔA as a function of mole fractions (χ) measured at 25 °C (black) and 60 °C (red) for water peak positions of 6868 cm^{-1} (ρ) and 7084 cm^{-1} (o).

Figure 3 shows a plot of the difference absorbance (ΔA) for the increase of glucose mole fractions at 25 °C (black) and 60 °C (red) and two absorbance positions, namely 6868 cm^{-1} (ρ) and 7084 cm^{-1} (o). All peaks decrease when glucose is increasing. At 60 °C, the decrease in absorption intensity at 7084 cm^{-1} appears faster than that of the same peak at 25 °C. In contrast, the reduction rate of the absorption peak intensity at 6868 cm^{-1} was faster at 25 °C, which suggests that the water molecules at high temperatures more rapidly reduce the vibrational mode from antisymmetric to symmetric as the glucose mole fraction increases. Probably the distance between water molecules is elongated when glucose is added and is further enhanced as the temperature gets higher.
The decreases in absorbance intensities do not linearly diminish with the increasing glucose mole fraction. There are two decreasing phases; the first phase occurs below the mole fraction of 0.4 and the second phase is at or above the mole fraction of 0.04. The two decreasing phases appeared for the absorption peaks of 6868 cm\(^{-1}\) and 7084 cm\(^{-1}\) and at temperatures of 25 \(^{\circ}\)C and 60 \(^{\circ}\)C. The phase changes at a mole fraction of 0.4 can probably be related to water's nature, which can be in a cluster or as individual molecules (monomeric). Such a water molecules change their form continuously between monomeric and cluster forms in such a way that every time a water molecule changes from a monomeric to a part of the cluster, one of the cluster's water molecules changes back into a monomer and vice versa.

At low concentrations, glucose tends to enter the water cluster rather than bind to a free molecule in the monomeric form. In this way, the glucose molecule establishes a strong H-bonding resulting in a stable solution. However, the presence of glucose also induces the breaking of hydrogen bonds both peripherally and within clusters. Consequently, the cluster size was reduced, and so glucose functions as a structure breaker. Such a break mechanism is indicated by the increase in water absorption as the overall number of H-bonds increases. Meanwhile, free molecules tend to be close to each other (probably for sterically reasons) to increase their overall stability. As a result, water absorption will increase. When the glucose concentration increases, the water absorption resumes to its initial state. To a certain extent, the interactions between glucose will significantly dominant, so that water forms its clusters again. Thus, it functions as a structure maker. The threshold between the structure breaker and the structure maker for glucose is a mole fraction of 0.04. In addition, a shift in the absorption peak near 7000 cm\(^{-1}\) was observed. The shift became prominent when the water absorption spectra at the increasing concentration of glucose were represented by their 2\(^{nd}\) derivative spectra, as shown in figure 4. The position of the water absorption peaks shifts to the lower energy side when the glucose concentration increases, both at 25 \(^{\circ}\)C and 60 \(^{\circ}\)C. This shift occurs monotonically below the 0.04 mole fraction and then changes a bit beyond the 0.04 mole fraction, as depicted in figure 5. This threshold border at a 0.04 mole fraction is consistent with the above results. So, both the change in the intensity of water absorption and the analysis of the shift in the peak position near 7000 cm\(^{-1}\) support the earlier mentioned mechanism of glucose as a structure breaker and structure maker [13, 14] as well as the result of computational calculations using molecular dynamics simulations [15].

**Figure 4.** Calculated second derivative spectra of a glucose solution at 0, 2, 4, 6, 11, 17, 24, 32, 41, 51, 62, 76 and 91 x 10\(^{-3}\) mole fraction measured at 25 \(^{\circ}\)C (black) and 60 \(^{\circ}\)C (red). The inset in the upper-right corner shows a portion of the spectra for clarity, while the inset in the lower-right corner shows a similar calculation for pure water for comparison.
Figure 5. The shifts of water peak positions as a function of the molar fraction ($\chi$) measured at 25 °C (black) and 60 °C (red).

4. Conclusion
The near-infrared spectrum has been measured for increasing a glucose concentration of 0-0.09 mole fractions. In addition, the near-infrared spectra for a glucose solution at temperatures ranging from 25 °C to 60 °C have been measured. Compared to water absorption, glucose absorption is relatively low, which is about two magnitudes lower than that of water, but glucose absorption can also be observed from an apparent decrease in the water absorption intensity at 6868 nm$^{-1}$ and 7084 nm$^{-1}$. When glucose with a concentration of less than a 0.04-mole fraction is added to water, it tends to insert itself into the water cluster and form a stable hydrogen bond with water. At concentrations above a 0.04-mole fraction, a glucose solution tends to interact with other glucose. Meanwhile, water tends to revert to form clusters as before. Increasing the sample temperature does not affect the water absorption peak. However, increasing the sample temperature pushes the absorption intensity of the anti-symmetric stretching and symmetric stretching modes lower. In contrast to the temperature, increasing the glucose concentration induces a shift in the absorption peak of the anti-symmetric stretching vibration mode towards the lower energy side, supporting the statement that glucose functions as a cluster breaker at mole fraction less than a 0.04 and a cluster maker at mole fraction higher than 0.04.

Acknowledgments
The author is thankful to the Directorate of Research and Community Service, the Minister of Education and Culture of the Republic of Indonesia for funding this research in the 2020 funding year with the Higher Education Leading Basic Research scheme (PDUPT) through Satya Wacana Christian University with proposal ID: 05f61621-2266-407c-9a14-0751f1eccc41a. FSR would also like to thank Tafip Haryanto for helping prepare the NIR equipment and Sheila and Dina Samalukang for the sample preparations.

References
[1] Mitchell H H, Hamilton T S, Streggerda F R and Bean H W 1945 J. Biol. Chem. 158 625
[2] Elliott G D, Wang S and Fuller B J 2017 Cryobiology 76 74
[3] Philip B 2008 Chem. Rev. 108 74
[4] Tian X, Jiang L, Yuan Y, Wang M Q, Guo Y Z, Zeng X J, Li M L and Pu X M 2013 J. Mol. Model. 19 2525
[5] Ohmine I and Saito S 1999 Acc. Chem. Res. 32 74
[6] Dixit S, Crain J, Poon W C, Finney J L and Soper A K 2002 Nature 416 829
[7] S. Perticaroli, P. Sassi, A. Morresi and M. Paolantoni 2008 J. Raman Spectrosc. 39 227
[8] Moran G R and Jeffrey K R 1999 J. Chem. Phys. 110 3472
[9] Clarke C, Woods R J, Gluska J, Cooper A, Nutley M A and Boons G J 2001 J. Am. Chem. Soc. 123 12238
[10] Bellissent-Funel M C, Hassanali A, Havenith M, Henchman R, Pohl P, Sterpone F, Van Der Spoel D and Garcia A E 2016 Chem. Rev. 116 7673

[11] Tzenkova R, Kovacs Z and Kubota Y 2015 Aquaphotomics: near infrared spectroscopy and water states in biological systems Membrane Hydration: The Role of Water in the Structure and Function of Biological Membrane vol 71, ed E A Dislalvo (Springer) p 189

[12] Bec K B and Huck C W 2017 Front. Chem. 7 48

[13] Arfin A, Mafy N N, Rahman M M, Mollah M Y A and Susan Md A B H 2014 RSC Adv. 4 50906

[14] Cui X, Cai W and Shao X 2016 RSC Adv. 6 105729

[15] Chen C, Li W Z, Song Y C, Weng L D and Zhang N 2012 Comput. Theor. Chem. 984 85