FTIR spectroscopy of glucose

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Abstract

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Approximately 450 000 people have diabetes in Sweden today, and the number of diabetics only rises. Monitoring blood sugar several times a day is a fundamental part of managing the disease, and reducing the risks of complications. Today’s glucose monitoring devices are invasive and require small needle sticks for a measurement. Providing a painless method of monitoring the blood sugar level would relieve the lives of diabetics world-wide.

The objective of this project was to investigate the absorption spectra of aqueous glucose concentrations (100 to 5000 mg/dl) in the mid infrared region with Fourier Transform spectroscopy (FTIR), and finally implementing a hand-held monochromatic spectrometer to demonstrate a non-invasive concept. The method chosen for implementing the hand-held demo is due to the commercial availability of diodes and detectors at those wavelengths.

The results from the FTIR showed a trend among concentrations in all wavelengths, in between 1180 to 980 cm⁻¹, specifically at 1035 cm⁻¹, but also in the region 2920 to 2850 cm⁻¹. The hand-held spectrometer did not register any transmittance of the glucose samples. For future implementations, 1035 cm⁻¹ should be investigated more in-depth for a hand-held device.
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1 Introduction

Around 450,000 people have diabetes in Sweden in 2016\(^1\) and 422 million have diabetes across the world\(^2\). The diagnosis is chronic and has many complications, such as cardiovascular diseases, chronic kidney failure, blindness etc. The number of diabetics only increases each year, and so an easy, non-invasive method of monitoring the blood sugar level is vital for reducing the risks of further complications. Modern monitoring techniques are invasive and an easier, less painful method of monitoring one’s blood glucose should relieve difficulties for diabetic patients.

Fourier Transform Infra-red spectroscopy (FTIR) is a well-established method of identifying unknown substances and their composition. Mid infra-red (MIR) covers the electromagnetic region between 4000 to 900 cm\(^{-1}\) (alternatively, 2500 to 11000 nm or 120 to 27 THz). Different approaches for non-invasive glucose monitoring have been made before\(^3,4\), besides the MID-FTIR, specifically the near infra-red region (NIR) (14000 to 4000 cm\(^{-1}\)), alternatively, 800 to 2500 nm or 120 to 375 THz). Some of these approaches include analyzing the FTIR absorbance spectra of oral mucous\(^5\) and blood serum\(^6\).

The objective of this project was to investigate the absorption of varying glucose concentrations in the MID-FTIR spectra, and furthermore to look into the possibility of implementing a portable device for non-invasive measurements as a proof of concept.

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2 Theory

2.1 FTIR

Fig. 2.1. Michelson interferometer for FTIR

The optical instrumentation in an FTIR spectroscope requires an infra-red light source, a Michelson interferometer which consists of two mirrors, a beam splitter, and one detector. In the Michelson interferometer the infrared light strikes on the beam splitter, which reflects 50% of the incoming light on a stationary mirror and transmits the remaining 50% on a moving mirror. The two beams from the moving mirror and stationary mirror are then recombined and 50% of the combined beam travels to the detector through the sample, and the other 50% travels back to the light source. The interferogram, or interference pattern, is generated when the combined beam strikes through the beam splitter. The pattern varies relative to the position of the moving mirror. The interferogram is later converted to a spectrum through a Fourier Transform.

To further quantitative analyze the absorbance, integration of peaks can be calculated.

The absorbance at a wavelength is given by:

\[ A = \log_{10} \frac{1}{T} \]  \hspace{1cm} (1)

where T, the transmittance is given by

\[ T = \frac{I}{I_0} \]  \hspace{1cm} (2)

where I is the transmitted intensity and I₀ is the incoming intensity on the sample.
2.2 Glucose samples

![Chain structure of glucose](image)

Figure 2.2.1 Chain structure of glucose

The fundamental frequencies of glucose are found in the MID-IR region\(^7\). The fundamental frequencies have the strongest absorption of all the types of frequencies: fundamental, combination, first overtone, second overtone etc.

<table>
<thead>
<tr>
<th>Wavenumber [cm(^{-1})]</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3350</td>
<td>Stretch of OH</td>
</tr>
<tr>
<td>2920</td>
<td>Asymmetric vibration of CH</td>
</tr>
<tr>
<td>2850</td>
<td>Symmetric vibration of CH(^8)</td>
</tr>
<tr>
<td>1450</td>
<td>Bending vibration of CH</td>
</tr>
<tr>
<td>1035</td>
<td>Vibration of CO and vCC(^9)</td>
</tr>
</tbody>
</table>

Table 2.2.1 The fundamental frequencies of glucose and their corresponding assignments.

The blood sugar level of a healthy diabetic should be in between 90 and 130 mg/dl fasting, and less than 180 mg/dl after eating. A blood sugar level less than 50 mg/dl is a diagnostic for hypoglycemia, which might cause clumsiness, confusion, and seizures. A blood sugar level higher than 200 mg/dl is hyperglycemia, but may not be noticeable until higher levels of blood sugar are reached.


3 Experimental apparatus and procedure

3.1 FTIR

The spectrometer used in this project was a Bruker IFS 66 V/S. The light source used was a HeNe laser and the beam splitter KBr. The detector was a deuterated L-alanine doped triglycine sulfate (DLaTGS) detector. The measurements were done with 256 scans, and a resolution of 2 cm\(^{-1}\) between 4000 and 900 cm\(^{-1}\). In the following graphs, atmospheric compensation was made to remove H\(_2\)O and CO\(_2\) vapor bands in the spectra. These bands arise due to different H\(_2\)O vapor concentrations in the beam path. No baseline correction was made, since it was not considered to be necessary. Integration and processing the spectra were done with OPUS software\(^{10}\).

In the beginning of a new experiment the sample compartment was cleansed with ethanol to remove any traces of old samples and dirt. After each measurement the sample cell was purged three times with deionized water to remove any residue of alcohol. For a sample measurement, the sample compartment was filled with 30 µl of solution, slowly whisking around in the sample compartment to remove any bubbles. For the dry sample, 3 µl of 300 mg/ml was added on the sample surface and was set to dry.

For preparation of the samples, a glucose concentration of 300 mg/ml was diluted with deionized water according to table 9.1. The concentrations prepared for this experiment ranged between 100 to 5000 mg/dl, in steps of 100 mg/dl between 100 and 500 mg/dl and 1000 mg/dl between 1000 to 5000 mg/dl. Many attempts at verifying the concentrations with a glucometer were made, however it did not register any glucose.

3.2 Hand-held spectrometer

For selecting the external light source and detection, two regions of absorption were considered: The absorption peak at 1035 cm\(^{-1}\) (31,03 Tand the absorption band between 2920 and 2850 cm\(^{-1}\). The light sources and detectors 1035 cm\(^{-1}\) are expensive, and were considered to be too advanced for this project. The light sources in the absorption band between 2920 and 2850 cm\(^{-1}\) were within the limitations of this project. The light source selected was a LED34-PR\(^{11}\) and the photodiode chosen was a PD36-03-PR\(^{12}\). Both the LED and photodiode had parabolic reflectors for a narrower, more directed beam. A PCB-mdriver-P\(^{13}\) delivered a pulsed square wave for maximum peak output from the LED.

The aim for the demonstration was to manufacture a small, hand-held spectrometer for measuring the glucose samples. The design had a few criteria: It had to be small, not let any light in, and the led and photodiode should be stationary in the spectrometer since they were very sensitive.

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\(^{10}\) OPUS. (2016). Bruker.
\(^{11}\) Roithner LaserTechnik. Light emitting diode LED34-PR.
\(^{12}\) Roithner LaserTechnik. Photodiode PD36-03-PR.
\(^{13}\) Roithner LaserTechnik. PCB-mdriver-P.
The portable spectrometer was made of a small, black, plastic box with the measurements 5x4x3 cm. The box was filled with several layers of black, thin foam. In the middle of each foam layer, a hole for the sample container was cut out. In the middle layers, additional holes were cut out for the LED and photodiode. Two separate holes were drilled in the sides for the cables. The cables were isolated with tape to prevent any possible short-circuit. A lid was taped to the plastic box for blocking external light. The PerkinElmer spectroscopy UV/VIS cells was used for a sample container.

The driver delivered a 1% duty cycle 2 kHz 2 A square wave, for obtaining maximum peak power. The LED was connected to the driver. The output voltage was measured from the photodiode anode which was connected to the ground, and analyzed with an oscilloscope. For calculating the transmittance of the samples, the sample cell was filled with deionized water and the incoming intensity was measured. The transmittance was calculated relative the sample cell filled with DI water.

Figure 3.2.1. Schematic of the spectrometer

Figure 3.2.2. The hand-held spectrometer. To the left: The LED and photodiode setup. To the right: The open spectrometer with the sample cell
4 Results

4.1 FTIR

4.1.1 Absorption spectra and graphs

The following spectra have been slightly shifted to have as similar baseline as possible.

Figure 4.1.1. The entire absorbance spectra with all concentrations

Figure 4.1.2. Absorbance between 3020 and 2760 cm⁻¹. A trend can be noticed between 2000 and 5000 mg/dl.
Figure 4.1.3. Absorbance spectra between 1480 to 1200 cm\(^{-1}\), no trend between 100 and 500 mg/dl can be noticed.

Figure 4.1.4. Absorbance spectra between 1180 and 960 cm\(^{-1}\). A trend in absorption can be noticed specifically at 1035 cm\(^{-1}\), from 100 mg/dl to 5000 mg/dl.
The absorption peak at 1035 cm\(^{-1}\) were further analyzed by plotting absorbance relative to glucose concentration.

4.1.2 Integration graphs

For the following graphs, integration was calculated with baseline correction in OPUS software.
Figure 4.1.8 Integration from 1485 to 1395 cm\(^{-1}\) with linear trendline and R\(^2\) value

Figure 4.1.9 Integration from 1180 cm\(^{-1}\) to 996 cm\(^{-1}\) with linear trendline and R\(^2\) value.

Figure 4.1.10 Integration from 1066 to 1000 cm\(^{-1}\) with linear trendline and R\(^2\) value
4.2 Hand-held spectrometer
The hand-held spectrometer did not meet the design goals. However, it was small and portable, it was easy to change samples and it did not let in any external light. The output voltage was very low, and did not measure any incoming light from the LED using an oscilloscope. In Figure 4.2.1. the LED is being pointed directly toward the photodiode.

![Figure 4.2.1 Driver input (yellow curve), photodiode output (blue curve)](image-url)
5 Discussion

5.1 FTIR

From the absorbance of the dry sample in Figure 4.1.5, all of the absorption peaks of glucose can be viewed. It can be noticed that the region between 3000 and 2900 cm\(^{-1}\) and 1645 cm\(^{-1}\) has a very strong absorption, which does not register in the aqueous samples. This is because water has a very strong absorptivity in MID-IR region, which leads to a negative absorbance in the OH assignments.

Between 2000 and 5000 mg/dl, there was a trend in all of the absorption regions. Between 1180 and 993 cm\(^{-1}\) there was a visible correlation between absorbance and all the glucose samples. This indicates that the hand-held device may not register any transmittance of the lower concentrations, regardless if the spectrometer had worked or not. But it could perhaps work within the higher ranges of concentrations.

Since the results did not show an obvious trend in the lower concentrations in the 2920-2850 cm\(^{-1}\) region, the absorption region around 1430 cm\(^{-1}\) could be considered for future developments. It is possible to do so since it’s the partially same assignment but different vibrational mode, see table 2.2.1. The integration curve for 1430 cm\(^{-1}\) correlates better to the glucose concentrations.

As previously mentioned in results, there was a varying trend relative to the concentration in the aqueous solutions, both in absorption and integration. 1035 cm\(^{-1}\) had evidently the most linear correlation between glucose concentration and absorption, and integration value respectively. 1035 cm\(^{-1}\) is a characteristic peak of glucose, and has been investigated in previous research\(^{14}\)\(^{15}\). A potential source for 1035 cm\(^{-1}\) could be a Quantum Cascade Laser (QCL). A QCL was briefly considered for the hand-held spectrometer, but was out of budget.

When the samples were prepared, small amounts of glucose were diluted in large amounts of DI water. This could be a possible source of error for the low absorptivity in the lower concentrations. Unfortunately, the glucometer did not measure the solutions properly. Ideally, the concentrations should be checked. Another possible source of error is the location of the vacuum pump. In this experiment, the vacuum pump was located very close (ca 3 m) from the FTIR spectroscope. The optimal location for a vacuum pump is in another room, to lower vibrations.

5.2 Hand-held spectrometer

The cause of the photodiode not registering any input from the LED is unclear. It’s possible that the LED or photodiode was faulty from the beginning, or that some part was damaged during the handling (e.g. soldering) of the equipment. To pin-point what part of the apparatus


is faulty is difficult to say, since trouble-shooting was not successful within the time-frame of this project.
6 Conclusion

The higher concentrations (>2000 mg/dl) had a correlation in absorbance and concentration throughout the wavelengths, while the lower concentrations a correlation was observed between 1180 and 993 cm\textsuperscript{-1}. For future practical applications, the absorption peak at 1035 cm\textsuperscript{-1} should be further investigated. The results showed a positive trend both in the raw absorbance measurements and the qualitative analysis integrations as expected, which opens the perspective of future continuation of the work.

The hand-held spectrometer did not work, and trouble-shooting was not within the time-frame. Trouble-shooting should be carried out for future improvements.
7 Acknowledgements

I would like to thank Dragos Dancila for your patience, support, and for introducing this project to me.

I would like to thank Fredrik Nikolajeff, for your inspiring enthusiasm and willingness to help me.

I would like to thank Saroj Kumar, for helping me with the FTIR spectrometer, and for your positivity and encouraging words.

I am very grateful to all three of you for taking your time to help me with this project. It meant a lot to me!
8 References


13. Roithner LaserTechnik. PCB-mdriver-P.


## Appendix

<table>
<thead>
<tr>
<th>$c_2$ [mg/dl]</th>
<th>$v_1$ [µl] (glucose 300 mg/ml)</th>
<th>$v_2$ [µl] = $v_1$ + DI H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>40</td>
<td>12000=40+11960</td>
</tr>
<tr>
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<td>2000</td>
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</table>

Table 9.1. Dilution table.