Detection of Bilirubin using Raman Spectroscopy in a Neonatal Skull

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Introduction
Bilirubin is a naturally occurring chemical in the human body, whose main function is to be hydrogenated by intestinal bacteria. In high enough concentrations, though, especially in areas around the brain, it can lead to jaundice, neurological defects, seizures and even death. This accumulation, known as hyperbilirubinemia, is mainly a symptom of newborn infants, who lack the intestinal bacteria that facilitate the breakdown of bilirubin into a soluble substance. Thus, it is of extreme importance for doctors to be able to effectively measure bilirubin concentrations in the neonatal skull.

Currently, blood samples drawn from an infant’s heel or visual indicators from skin coloration are used to determine bilirubin levels. When found in excess, phototherapy is used to help reduce levels. Bilirubin is extremely sensitive to light, and isotermeters is an excretionable substance (lumirubin) when exposed to blue light.

The aim of this project was to develop a non-invasive method of detecting bilirubin concentrations that takes advantage of this property of photosensitization. Raman spectroscopy was used to obtain optical spectra of bilirubin at different stages of isomerization. When near-infrared (NIR) excitation is used, Raman spectroscopy has the potential ability to penetrate through the skull of a neonate to provide useful information concerning intracranial fluids. To investigate this possibility, a phantom skull was created to mimic the optical behavior of human bone. Our findings show the feasibility of measuring the photosensitization of bilirubin non-invasively and initial results on modelling the optical properties of a human skull.

Methods and Materials
Two types of bilirubin samples were prepared with a 20%g/L concentration, the lethal threshold for neonates:

- Mixed with 1M NaOH, 5mL H2O, and 75 mL HCl
- Mixed with 2% Intral Lipid solution (to simulate in vivo scattering properties)

The samples were placed in a closed box (fig. 3) and exposed to blue light emitted from 8 LED’s at 800 µW/cm² to simulate clinical lighting equipment for 4 hours.

The Raman spectra of the samples were measured every 30 minutes using HAMMER (Hadamard Aperature Mask Multi-Exraction Raman) system (fig. 2).

The changes in bilirubin concentration were measured and fluctuations in the Raman spectrum peak height were analyzed using a partial least squares regression.

Results
Bilirubin has several major peaks in the Raman spectrum that can be used as probes (fig. 4). For this experiment, peaks at wavelengths shown in Table 1 were chosen. They can be categorized into 3 different groups:

- Group A: C=C double bonds stretching in five member ring
- Group B: Stretching in CH and umbrella motion in CH bend
- Group C: C=C stretching between ring and CH=CH2

Over 2 hours of exposure, the bilirubin concentration in our sample decreased from 24.4 mg/dL to 17.5 mg/L. Changes in the Raman spectrum can be seen in Figure 5. A partial least square regression vector in fig. 6 shows the correlation between spectral changes and bilirubin concentration. Note the decrease in intensity for group B peaks and increase in group A and C peaks, as expected with a change from bilirubin into lumirubin. These changes in peak intensity highlight the possibility of quantitative determination of bilirubin concentration.

Conclusions
Our hypothesis was supported: Raman spectroscopy can non-invasively measure the decrease in bilirubin concentration during phototherapy.

- Changes in key peaks of bilirubin’s spectrum due to photosensitization can lead to quantitative evaluation of bilirubin concentration.
- Our results demonstrate the possibility of non-invasive detection through a neonatal skull is possible with the creation of a phantom skull that mimics the optical properties of human bone, as it research into other intracranial phenomena.
- Future research: Non-invasive detection through phantom skull, creation and use of substance to mimic properties of intracranial fluids, quantitative analysis of Raman peak changes to determine bilirubin concentration.

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References
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Figure 1: Diagram of the transformation of bilirubin (left) into lumirubin (right) as a result of photoisomerization.

Figure 2: HAMMER Raman spectrograph apparatus

Figure 3: Image of phantom skull created on Solidworks

Figure 4: Spectral fingerprint of 99% bilirubin powder taken from literature.

Figure 5: Raman spectra of 20 mg/dL bilirubin sample at different stages of photoisomerization. Exposures taken every 30 min. for 4 hours.

Figure 6: Partial least square regression vector of figure 5.

Figure 7: Image of phantom skull created on Solidworks machine, scaled down -50% from original size to mimic optical properties of a neonatal skull.

Figure 8: Plot of experimental measurements of transmitted light intensity vs. plastic thickness, with expected ideal fit (solid line) drawn in.

Figure 9: Phantom infant skull with an average thickness of approximately 3.41 mm is the best optical model for this project.

Table 1: Raman spectrum peaks and assignments for bilirubin and lumirubin.

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<thead>
<tr>
<th>Frequency (cm⁻¹)</th>
<th>Group Description</th>
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<tbody>
<tr>
<td>491.5</td>
<td>C -OH and C -CH₂ bending</td>
</tr>
<tr>
<td>1339.4</td>
<td>CCH bending in CH₃ and C-C stretching between ring and CH=CH₂</td>
</tr>
<tr>
<td>1611.4</td>
<td>A C=C stretching in five membered ring</td>
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