NONINVASIVE DETECTION OF BILIRUBIN USING PULSATILE ABSORPTION

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ABSTRACT

Bilirubin, the yellow substance usually responsible for neonatal jaundice, is currently monitored invasively or by observing/measuring skin colour. This paper investigates the feasibility of monitoring serum bilirubin concentration using light absorbance in a similar fashion to pulse oximetry. The light absorbance of bilirubin is shown to be sufficiently different to haemoglobin to in theory allow direct noninvasive serum bilirubin monitoring using light absorbance around 480nm.

KEY WORDS

Bilirubin, noninvasive, absorption, pulse oximetry, haemoglobin, Beer Lambert law.

1. Introduction

Oxygenated and reduced haemoglobin are major absorbers of visible and near infrared light in blood. This strong light absorbance is exploited in pulse oximetry where the ratio of oxygenated to total haemoglobin is determined, based on the ratio of light absorbance at 2 different wavelengths.

Theoretically, the Beer Lambert law states:

$$\frac{I_o}{I_i} = e^{-\varepsilon c d} \quad \text{equation 1}$$

$$\text{Absorbance} = \ln\left(\frac{I_i}{I_o}\right) = \varepsilon c d \quad \text{equation 2}$$

$$\therefore \text{Concentration} = \frac{\text{Absorbance}}{\varepsilon d} \quad \text{equation 3}$$

Where:

I_o = Light intensity leaving a non scattering light absorbing solution
I_i = Light intensity entering a non scattering light absorbing solution
ε = Absorption coefficient of light by the absorbing substance (L.mol^{-1}cm^{-1})
c = concentration of absorbing substance in the solution (mol.L^{-1})
d = Light pathlength through the solution (cm)

If base-10 logarithms are used, equation 2 becomes:

$$\log_{10}\left(\frac{I_i}{I_o}\right) = \frac{\varepsilon c d}{\ln(10)} \quad \text{equation 4}$$

Pulse oximetry is carried out by measuring the absorbance of light either shining through tissue (eg fingertip or earlobe) or reflected from/within tissue. With these configurations light passes through several layers of tissue and blood, and encounters several light absorbing and scattering substances.

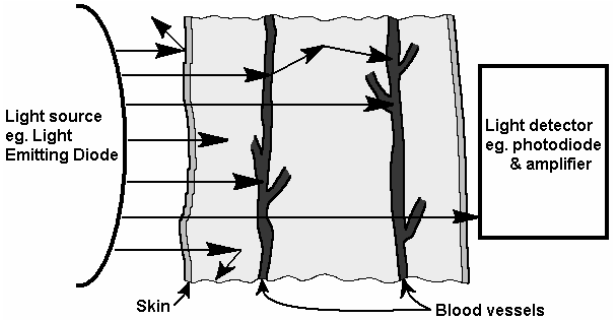

Figure 1: Simple model of light scattered, absorbed and transmitted through tissue - as occurs in pulse oximetry

However, as can be seen in figure 1, the conditions of the Beer-Lambert law are not satisfied in pulse oximetry: Tissue is not a non-scattering pure light absorbing solution. The mean distance between light scattering events encountered by a photon travelling through tissue has been estimated at between 50 and 200μm [1]. Due to this multiple scattering, light travelling through tissue becomes effectively isotropic within a short distance of entering tissue [2].
The pathlength of light through blood (within tissue) and the intensities of light entering and leaving blood (within tissue) are unknown.

These problems are overcome in pulse oximetry by analysing only the pulsatile component of light absorbance (figure 2), quoting results as a percentage – allowing simplification of equation 3, and by empirical calibration.

The volume of arterial blood between light source and detector pulsates with the cardiac cycle; therefore the light pathlength through blood will pulsate, and the amount of light absorbing substance (mostly haemoglobin) will also pulsate.

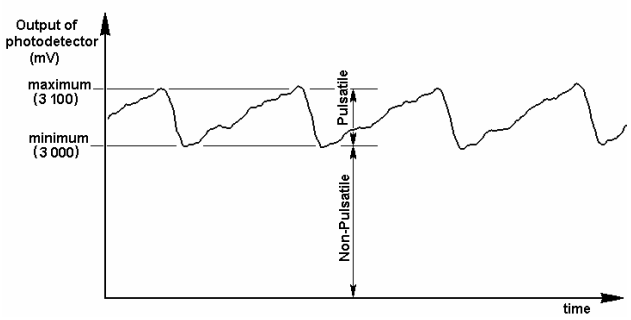

Figure 2: Photodetector output when 940nm light was shone through an adult finger onto the photodetector, showing the pulsatile and non pulsatile components

Providing light propagation changes due to in vivo rouleaux formation are negligible throughout the cardiac cycle, the maximum and minimum light intensities striking the detector correspond to the intensities of light entering and leaving the pulsatile volume of haemoglobin solution respectively (I_i and I_o in equations 1 & 2)

By measuring the ratio of absorbance of light at wavelengths where oxyhaemoglobin and reduced haemoglobin have different absorption coefficients, the ratio of oxygenated to total haemoglobin can be determined.

Pulse oximetry assumes that there are only 2 major absorbers of red and near infrared light in blood. This assumption is not quite correct, as haemoglobin can exist as carboxyhaemoglobin or methaemoglobin, as well as oxyhaemoglobin and reduced haemoglobin [1], although carboxyhaemoglobin and methaemoglobin are not usually present in high concentrations.

Apart from haemoglobins, there is another major light absorber that is sometimes present in blood – bilirubin. Bilirubin is a non-polar molecule [3, 4] with a distinct yellow colour, which is a by-product of haemoglobin breakdown.

Most newborn babies (approximately 60%) experience hyperbilirubinemia, which is classified as a serum bilirubin concentration above 86µM (5mg/dL) [4]. Serum bilirubin concentration has traditionally been determined from blood samples obtained via a "heel-stick" procedure, which involves pain and infection risk as well as delays associated with laboratory tests remote to the patient.

A method for monitoring serum bilirubin noninvasively is available; Transcutaneous Bilirubinometry (TcB) involves measuring the skin reflectance of various colours of light (wavelengths) to assess the "yellowness" of the skin [5], which is correlated to the serum bilirubin concentration. TcB has sometimes been reported to be an accurate screening method for hyperbilirubinemia [6] although some papers identify significant errors between TcB readings and laboratory serum bilirubin tests [5]. The accuracy of TcB is reported to be lower in dark skinned babies than in light skinned babies, and the accuracy reportedly drops during phototherapy and blood transfusions [7].

Because bilirubin exists in blood and is a strong absorber of light (figure 3) there is the possibility that it could be monitored in a similar fashion to pulse oximetry. Such a method would be independent of skin melanin concentration, phototherapy and blood transfusions.

2. Method

Published absorption spectra were collected for haemoglobin (Hb), oxyhaemoglobin (HbO), carboxyhaemoglobin (cHb), methaemoglobin (metHb) and bilirubin (figures 3 & 4).

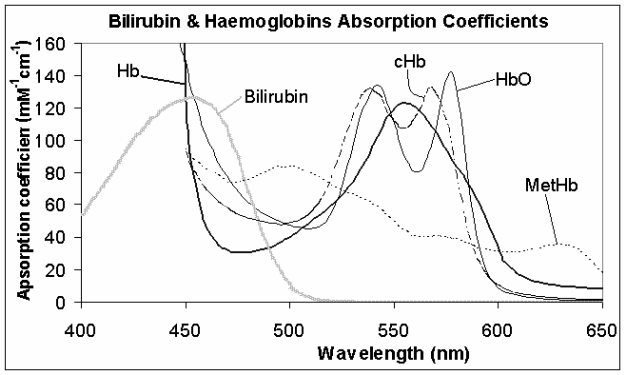

Figure 3: Absorption spectra for Haemoglobins, & Bilirubin in the 400-650nm range [2, 8-13]

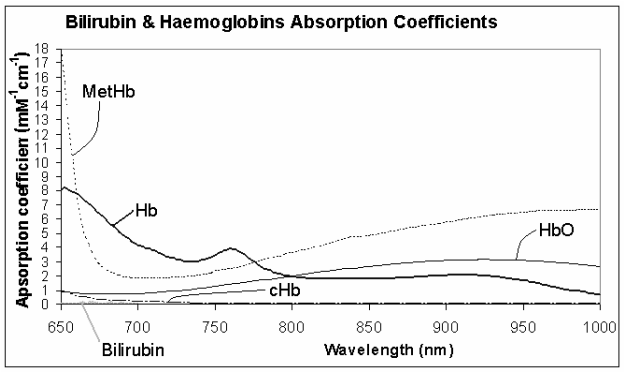

Figure 4: Absorption spectra for Haemoglobins, & Bilirubin in the 650-1000nm range [2, 8-13]

Figures 3 & 4 represent absorption coefficients for use with natural logarithms (equation 2), not base-10 logarithms (equation 4).

Concentrations were assigned to these analytes as follows:

Total haemoglobin: 15g/dL

Oxyhaemoglobin: 97.5% of total haemoglobin = 14.625g/dL ≈ 2.285mM

Reduced haemoglobin: 2% of total haemoglobin = 0.3g/dL ≈ 0.047mM

Bilirubin: 5mg/dL (the lower limit for hyperbilirubinemia) ≈ 0.086mM

Methaemoglobin: 0.25% of total haemoglobin = 37.5mg/dL ≈ 0.006mM

Carboxyhaemoglobin: 0.25% of total haemoglobin = 37.5mg/dL ≈ 0.006mM

A nominal pulsatile distance (ie the change in light pathlength through blood between diastole and systole – d in equations 1-4) was calculated using the measured photodetector outputs (figure 2) and the graphed absorption coefficients (figure 4).

$\varepsilon_{HbO,940} = 3.11 mM^{-1}cm^{-1}$ (figure4)

$\varepsilon_{Hb,940} = 1.85 mM^{-1}cm^{-1}$ (figure4)

$\varepsilon_{cHb,940} = 0.09 mM^{-1}cm^{-1}$ (figure4)

$\varepsilon_{metHb,940} = 6.36 mM^{-1}cm^{-1}$ (figure4)

$\varepsilon_{Bili,940} = 0 M^{-1}cm^{-1}$ (figure4)

$$A_{940} = \sum \varepsilon c d = d \sum \varepsilon c$$

$$\therefore d = \frac{A_{940}}{\sum \varepsilon c} = \frac{\ln\left(\frac{I_i}{I_o}\right)}{\sum \varepsilon c}$$

$$= \frac{\ln\left(\frac{3100}{3000}\right)}{\varepsilon_{HbO} c_{HbO} + \varepsilon_{Hb} c_{Hb} + \varepsilon_{cHb} c_{cHb} + \varepsilon_{metHb} c_{metHb} + \varepsilon_{Bili} c_{Bili}}$$

$$= \frac{\ln\left(\frac{3100}{3000}\right)}{(3.11)(2.285) + (1.85)(0.047) + (0.09)(0.006) + (6.36)(0.006) + 0}$$

$d = 0.0045$cm

$\therefore$ use $d \approx 0.005$cm

The absorbance of light by each species listed above, was calculated using the Beer-Lambert law (equation 1), eg at 660nm:

$\varepsilon_{HbO,660} = 0.77 mM^{-1}cm^{-1}$

$c_{HbO} = 2.285 mM$

$d = 0.005$cm

$\mathrm{A_{HbO}} = \varepsilon c \mathrm{d} = (0.77 mM^{-1}cm^{-1})(2.285 mM)(0.005 cm)$

$\mathrm{A_{HbO}} = 0.0088$ absorbance units

$\varepsilon_{Hb,660} = 7.78 mM^{-1}cm^{-1}$

$c_{Hb} = 0.047 mM$

$d = 0.005$cm

$\mathrm{A_{Hb}} = \varepsilon c \mathrm{d} = (7.78 mM^{-1}cm^{-1})(0.047 mM)(0.005 cm)$

$\mathrm{A_{Hb}} = 0.00183$ absorbance units

$\varepsilon_{cHb,660} = 0.553 mM^{-1}cm^{-1}$

$c_{cHb} = 0.006 mM$

$d = 0.005$cm

$\mathrm{A_{cHb}} = \varepsilon c \mathrm{d} = (0.55 mM^{-1}cm^{-1})(0.006 mM)(0.005 cm)$

$\mathrm{A_{cHb}} = 0.000017$ absorbance units

$\varepsilon_{metHb,660} = 7.46 mM^{-1}cm^{-1}$

$c_{metHb} = 0.006 mM$

$d = 0.005$cm

$\mathrm{A_{metHb}} = \varepsilon c \mathrm{d} = (7.46 mM^{-1}cm^{-1})(0.006 mM)(0.005 cm)$

$\mathrm{A_{metHb}} = 0.000224$ absorbance units

$\varepsilon_{bili,660} = 0 mM^{-1}cm^{-1}$

$c_{bili} = 0.086 mM$

$d = 0.005$cm

$\mathrm{A_{Bili}} = \varepsilon c \mathrm{d} = (0 mM^{-1}cm^{-1})(0.086 mM)(0.01 cm)$

$\mathrm{A_{Bili}} = 0$ absorbance units

Therefore the total absorbance at 660nm was
8.8+1.83+0.017+0.224
= 10.871milli absorbance units

The percentage absorbances due to the 5 chemical species were:

$$\mathrm{HbO}: \frac{8.8}{10.871} \times 100\% = 80.9\%$$

$$\mathrm{Hb}: \frac{1.83}{10.871} \times 100\% = 16.8\%$$

$$\mathrm{cHb}: \frac{0.017}{10.871} \times 100\% = 0.2\%$$

$$\mathrm{metHb}: \frac{0.224}{10.871} \times 100\% = 2.1\%$$

$$\mathrm{Bilirubin}: 0\%$$

The absorbance due to each species was calculated throughout the range 400-1000nm, and area graphs were generated, showing the absorbance of each species versus wavelength of light.

Pulse oximeters generally use Light Emitting Diodes (LEDs) as sources. LEDs generate light which spans a range of wavelengths, as shown by the LED spectrum in figure 5, the bandwidth for this LED at 50% maximum intensity being approximately 40nm.

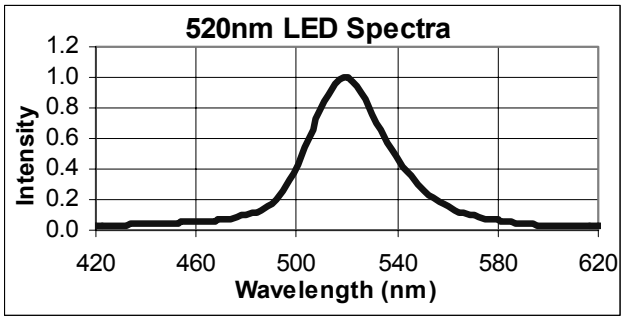

Figure 5: Normalised spectrum recorded from a LED with centre wavelength of 520nm

An instrument that monitors serum bilirubin concentration, using a similar method to pulse oximetry, may use LEDs as light sources. To see the effect of using LEDs as light sources, two sets of absorption graphs were produced – one showing the absorption of light with a bandwidth of 1nm in wavelength, the other showing the absorbance of light from a 40nm wide band of wavelengths. The latter graphs were produced by averaging the absorption coefficients for the chemical species shown in figures 3 & 4, over 40nm.

3. Results

The contributions of bilirubin and the 4 haemoglobin species to the absorption of light, with 1nm bandwidth, are shown in figures 6 & 7.

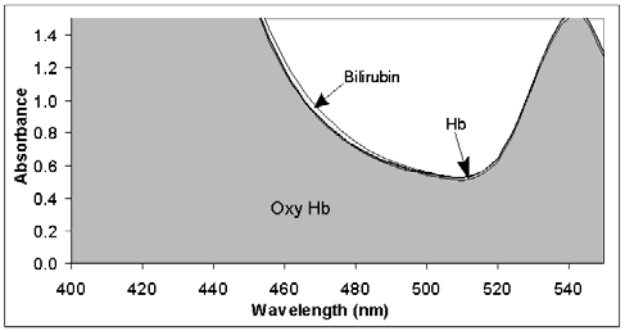

Figure 6: Absorbance in the 400-550nm range due to Bilirubin & haemoglobin species, with light bandwidth = 1nm

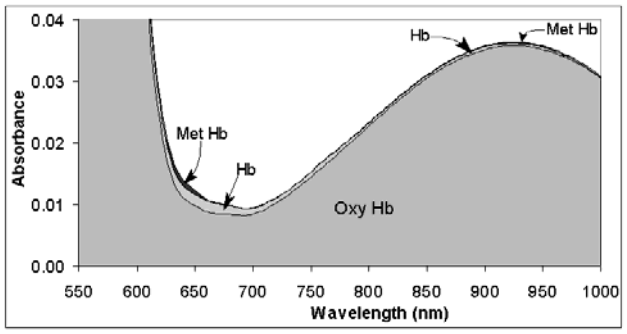

Figure 7: Absorbance in the 550-1000nm range due to Bilirubin & haemoglobin species, with light bandwidth = 1nm

The contribution of bilirubin and the 4 haemoglobin species to the absorption of light, with 40nm bandwidth, is shown in figures 8 & 9.

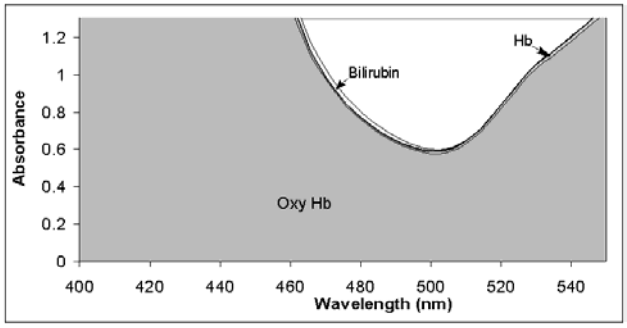

Figure 8: Absorbance in the 400-550nm range due to Bilirubin & haemoglobin species, with light bandwidth = 40nm

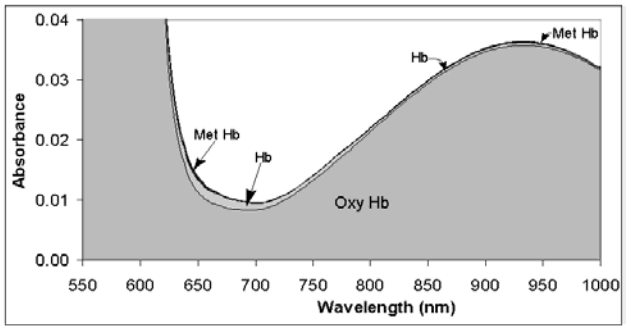

Figure 9: Absorbance in the 550-1000nm range due to Bilirubin & haemoglobin species, with light bandwidth = 40nm

With 97.5% oxygen saturation of haemoglobin and 5mg/dL serum bilirubin concentration:

At 660nm, reduced haemoglobin accounts for 16.8% of absorbance of 1nm bandwidth light, and 15.8% of 40nm bandwidth light.

At 804nm, reduced haemoglobin accounts for 1.9% of absorbance of 1nm bandwidth light, and 2.1% of 40nm bandwidth light.

At 940nm, reduced haemoglobin accounts for 1.2% of absorbance of both 1nm and 40nm bandwidth light.

At 480nm, bilirubin accounts for 3.9% of absorbance of 1nm bandwidth light and 3.7% of 40nm bandwidth light.

4. Discussion

Comparing the contributions made by bilirubin and reduced haemoglobin, to the absorption signals enables the difficulty in measuring bilirubin concentration to be compared to the difficulty in performing pulse oximetry. 660nm is an important wavelength in pulse oximetry; approximately 15.8% of light absorbance at this wavelength is due to reduced haemoglobin, while only 3.7% of light absorbance at 480nm is due to bilirubin. Therefore bilirubin's contribution to the overall absorbance signal at 480nm is only about one quarter of the contribution of reduced haemoglobin to the absorbance signal at 660nm. This means that the task of noninvasively measuring bilirubin using light absorbance could be around 4 times more difficult than pulse oximetry, but given the advances in technology since pulse oximetry was first introduced it should still be possible.

The absorbance area graphs show:

There is much higher overall absorbance around 480nm (where bilirubin may more easily be detected) than in the 660 to 940 nm region (where pulse oximetry is performed); so much higher power light sources would be required for bilirubin detection than for pulse oximetry.

As the bandwidth of light from the source becomes narrow, the percentage absorbance due to bilirubin at 480nm increases only slightly, indicating that there is no real advantage in using narrow bandwidth light sources in this instance. This suggests that LEDs are suitable light sources to be used in noninvasive serum bilirubin monitoring.

There are areas attributable to methaemoglobin absorbance, in figures 6-9 around 640nm and 900nm – indicating that the presence of methaemoglobin can render pulse oximetry and the proposed bilirubin detection method inaccurate if it is not compensated for.

The absorbance due to carboxyhaemoglobin (at 0.3mg/dL) was not large enough to be easily seen in figures 6-9.

Quantifying results from noninvasive light absorbance measurement is perhaps the most difficult obstacle to overcome – due to the difficulties in determining an effective pathlength through the pulsatile component of blood. Pulse oximetry avoids this problem by quoting results as a percentage, allowing pathlength to be cancelled from calculations. It may be possible to perform a similar calculation – bilirubin concentration could be calculated as a percentage of total haemoglobin concentration. Otherwise there is the possibility of calibrating an instrument on each patient against blood sample laboratory tests, then subsequent tests can all be done noninvasively.

The effect of other light absorbing substances in blood (apart from the 4 haemoglobin species and bilirubin) has not been taken into account in this analysis.

5. Conclusion

This analysis shows that serum bilirubin in theory should be able to be monitored directly and noninvasively in a similar fashion to pulse oximetry, providing that high power light sources are used, and all potential confounding light absorbing substances, eg methaemoglobin, are also measured and included in calculations. Ideally, an instrument measuring bilirubin concentration using this means would be similar to a pulse oximeter but with additional capability. Results could be quoted as a percentage of haemoglobin concentration, alternatively absolute concentrations could be determined if an instrument is calibrated on each patient against an initial laboratory serum bilirubin test.

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