

Noninvasive Detection of Bilirubin in Discrete Vessels

Mark McEwen, Karen J. Reynolds

Abstract—Bilirubin, at the levels encountered in hyperbilirubinaemia, makes a similar contribution to blue light absorption as reduced haemoglobin makes to red light absorption of blood. Whilst this supports the possibility of extending pulse oximetry into the blue part of the spectrum for the noninvasive monitoring of bilirubin, the modulating effects of discrete blood vessels tend to disrupt the relationship between blood absorption coefficients and pulsations in light transmission through tissue at these wavelengths.

The relationship between the pulsatile attenuation of blue and green light and serum bilirubin concentration was investigated with a Beer-Lambert pulse oximetry model, a discrete blood vessel model, and in vivo. Whilst the Beer-Lambert model indicated a positive relationship between the blue/green light pulsatile attenuation ratio and serum bilirubin, both in vivo testing and the discrete blood vessel model demonstrated negative correlations. The variability in results from in vivo tests indicated that these correlations could not be used for reliable bilirubin estimation.

Index Terms—bilirubin, noninvasive, photoplethysmography

I. INTRODUCTION

In a Beer-Lambert model of pulse oximetry, the pulsatile attenuation of red and infrared light in vascularised tissue, ΔA , can be determined using (1) from the maximum intensity of transmitted light (I_{\max}) at diastole, the minimum intensity of transmitted light (I_{\min}) at systole, the extinction coefficient ε and concentration c of the blood, and the pulsatile change in light path length through blood, Δd [1]:

$$\Delta A = \text{Log} \left(\frac{I_{\max}}{I_{\min}} \right) = \varepsilon c \Delta d \quad (1)$$

Comparison of the pulsatile absorbancies at different wavelengths allows estimation of the relative concentrations of oxygen-saturated haemoglobin (HbO) and desaturated haemoglobin (Hb), and hence allows a noninvasive estimation of oxygen saturation of the blood.

This theory for pulse oximetry assumes that there are only

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two major absorbers of red and near infrared light in blood, HbO and Hb. This assumption is not quite correct, as dyshaemoglobins such as carboxyhaemoglobin may be present [2] (although not usually in high concentrations).

In addition to haemoglobins, there is another major visible light absorber in blood – bilirubin (Fig. 1). Bilirubin is a by-product of haemoglobin breakdown, and has a distinct yellow colour. If we include bilirubin into our Beer-Lambert model, then (1) becomes:

$$\Delta A_{\lambda_n} = (\varepsilon_{\text{Hb}} c_{\text{Hb}} + \varepsilon_{\text{HbO}} c_{\text{HbO}} + \varepsilon_{\text{Bili}} c_{\text{Bili}})_{\lambda_n} \Delta d \quad (2)$$

Approximately 60% of newborn babies experience hyperbilirubinaemia, which is classified as a serum bilirubin concentration above 86 μM (5 mg/dL) [3]; in some cases this can exceed 300 μM . Serum bilirubin concentration, in babies, 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.

An indirect method for monitoring serum bilirubin noninvasively is available; transcutaneous bilirubinometry involves measuring the skin reflectance of various wavelengths of light to assess the “yellowness” of the skin [4]. This is then correlated to the serum bilirubin concentration. The accuracy of transcutaneous bilirubinometry is however debated. In some cases it been reported to be an accurate screening method for hyperbilirubinaemia [5], while other papers identify significant errors between transcutaneous bilirubinometry readings and laboratory serum bilirubin tests [4]. The accuracy of transcutaneous bilirubinometry is reported to be lower in dark skinned babies than in light skinned babies, and the accuracy reportedly drops when phototherapy and blood transfusions are performed [6].

Because bilirubin exists in blood and is a strong absorber of light (Fig. 1) 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, and would provide real-time results to clinicians.

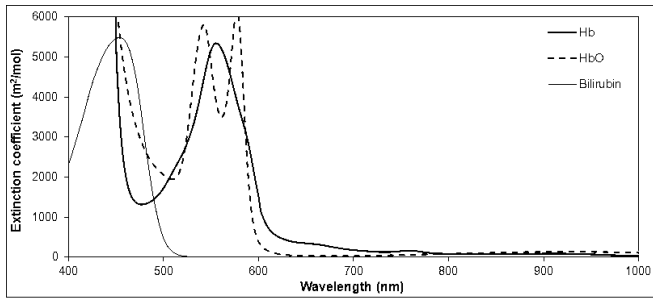


Fig. 1. Extinction coefficients for oxygenated (HbO) and reduced (Hb) haemoglobin (400 nm – 450 nm) [7], (450 nm – 1000 nm) [8], (850 nm – 1000 nm) [9], and bilirubin [10].

Haemoglobins strongly absorb light at wavelengths where bilirubin absorbs light. While this substantially masks bilirubin absorption, the minima in the haemoglobin absorption spectra around 500 nm enable bilirubin to be detected theoretically [11]. When hyperbilirubinaemia is present, bilirubin accounts for approximately 4% of 480 nm light absorption by blood; this is comparable to the contribution made by reduced haemoglobin to the 660 nm light absorption (approximately 16%) [11]. This supports the notion of expanding pulse oximetry to the monitoring of bilirubin. However the feasibility of noninvasively estimating serum bilirubin in a similar fashion to pulse oximetry depends on the relationship between the pulsatile attenuation of light in tissue and the absorption coefficients of blood. Scattering affects this relationship [12], [13]. Discrete blood vessels also modulate it [12], [14] and this modulation is greatest for wavelengths of light that are strongly absorbed by blood, such as 470 nm – 500 nm, which are absorbed by bilirubin (Fig. 1) [14].

This work investigates the influence of discrete blood vessels on the feasibility of noninvasively monitoring bilirubin using pulse oximetry techniques.

II. METHODS

A. In Vivo Pulsatile Ratios

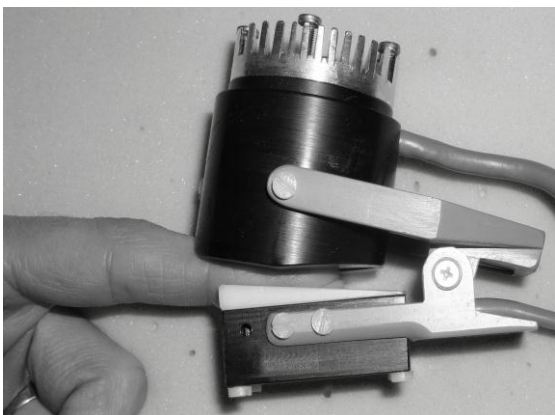


Fig. 2. Custom photoplethysmography instrument (described previously [14], [15]) used to measure the pulsatile transmission of light through a subject's finger.

Ethics committee approval was obtained for performing tests on patients, who were selected on the basis that they were having blood extracted for haemoglobin and bilirubin

analysis, and were willing to undergo non-invasive testing. Patients (23 males, 30 females, mean age 58 ± 18 years) rested in a sitting or supine position during testing. A custom photoplethysmography instrument (Fig. 2), described elsewhere [14], [15], was used to measure the transmission of light from a series of light emitting diodes (LEDs) through the fingers of patients. The LEDs generated 465 nm, 500 nm and 526 nm light as well as other wavelengths, including those normally used in pulse oximetry. Index fingers were used unless they had injuries, in which case middle or ring fingers were used. Three sets of measurements were recorded from each patient, each lasting 10-20 seconds. The mean values of total haemoglobin concentration (tHb) and serum bilirubin in these patients were 130 g/L and 57 μ M respectively.

Measurements were recorded at 90 samples/sec. Data from some tests (approximately 15%) were excluded due to patient voluntary movement during measurements. The pulsatile maxima and minima for each LED were extracted from recorded signals. Pulsatile attenuation was calculated for each max/min pair using (2), and averaged over all max/min pairs for each test on each patient. These average pulsatile attenuations, for in vivo tests, were determined for 465 nm, 500 nm and 526 nm light. The 465 nm / 526 nm and 500 nm / 526 nm pulsatile attenuation ratios were calculated and graphed against the serum bilirubin concentration (as determined from blood samples).

B. Theoretical (Beer-Lambert) Pulsatile Ratios

The pulsatile absorption (2) was calculated for 465, 500 and 526 nm light, using published extinction coefficients for haemoglobin and bilirubin (Fig. 1) and a pulsatile path length of 0.05 mm [11]. These calculations were performed for blood with a tHb of 130 g/L, arterial haemoglobin oxygenation (SaO₂) of 97%, and serum bilirubin concentrations ranging from 0 μ M to 400 μ M.

The whole-blood bilirubin concentration was calculated according to (3) [16]:

$$[\text{Serum Bilirubin}](1 - \text{Hct}); \quad \text{Hct} = \frac{\text{tHb}}{330\text{g/L}} \quad (3)$$

Ratios of pulsatile attenuations for 465 nm / 526 nm and 500 nm / 526 nm light were calculated and graphed against serum bilirubin concentration.

C. Modelling Pulsatile Ratios With Discrete Vessels

The presence of discrete blood vessels results in a range of light path lengths through blood. To simulate this, the light transmission through fingertip models at diastole and systole was used to determine the pulsatile absorption. A 12 mm diameter [17], [18] fingertip model cross section was defined [14], containing three pairs of arteries that pulsed from 1.00 to 1.05 mm diameter (diastole to systole) [19], [20]. Blood in these modelled arteries had 130 g/L tHb, 97% SaO₂, and bilirubin concentrations ranging from 0 μ M to 400 μ M. The transmission of light, T , along multiple parallel paths (1 μ m spacing) throughout the fingertip model cross sections was determined in accordance with the Beer-Lambert Law (1), rewritten here in terms of transmission:

$$T = 10^{-\epsilon c d} \quad (4)$$

The overall light transmission, at diastole (I_{\max}) and systole (I_{\min}), was calculated as the sum of all the individual transmissions, and used to determine the pulsatile attenuation of light in accordance with (1). Ratios of pulsatile attenuations for 465 nm / 526 nm and 500 nm / 526 nm light were graphed against serum bilirubin concentration.

III. RESULTS

Theoretical (Beer-Lambert) Pulsatile Ratios

Positive relationships between the theoretical (Beer-Lambert model) 465 nm / 526 nm and 500 nm / 526 nm pulsatile absorption ratios and blood bilirubin concentration were encountered (Fig. 3). The relationship involving the 465 nm / 526 nm ratio was stronger than that involving the 500 nm / 526 nm ratio as indicated by the higher gradient in Fig. 3.

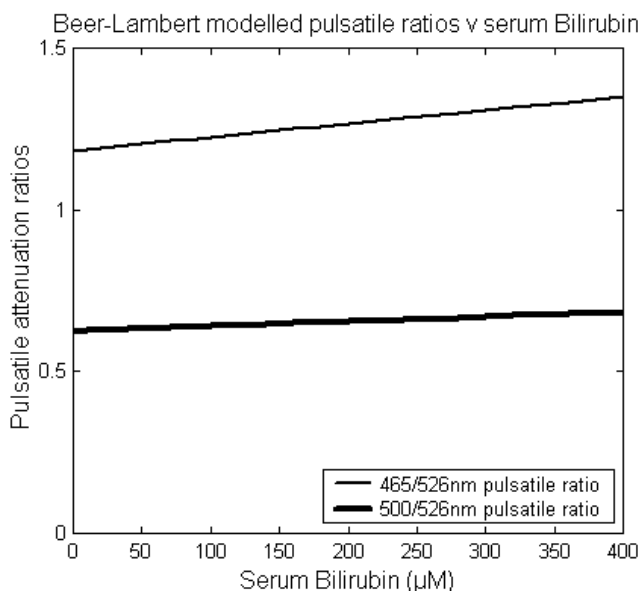


Fig. 3. Relationships between the theoretical (Beer-Lambert model) 465 nm / 526 nm and 500 nm / 526 nm pulsatile absorption ratios and blood bilirubin concentration.

Pulsatile Ratios Calculated Using Discrete Vessel Finger Models

Discrete blood vessel models generated weak negative relationships between 465 nm / 526 nm and 500 nm / 526 nm pulsatile absorption ratios and serum bilirubin (Fig. 4).

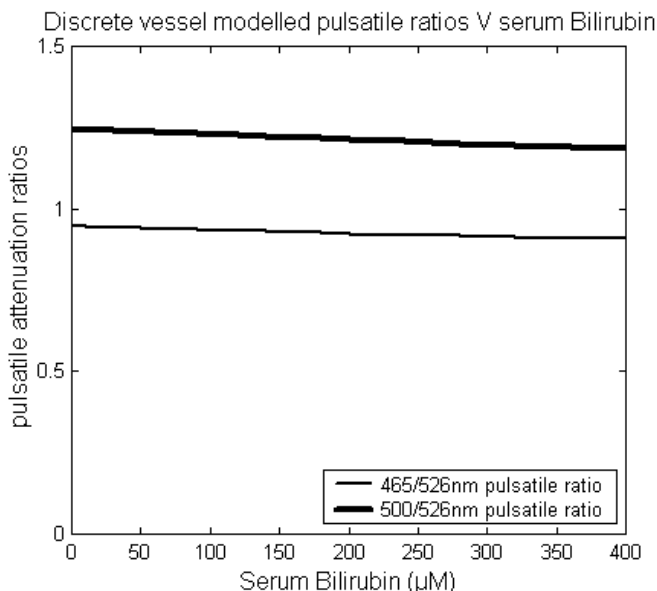


Fig. 4. Relationships between 465 nm / 526 nm and 500 nm / 526 nm discrete vessel model pulsatile absorption ratios and serum bilirubin.

In Vivo Pulsatile Ratios

In vivo results from 61 patients showed variations in pulsatile attenuation ratios that were not strongly associated with serum bilirubin concentration (Fig. 5). The least squares lines of best fit indicate that weak negative correlations between in vivo 465 nm / 526 nm and 500 nm / 526 nm pulsatile attenuation ratios and serum bilirubin were encountered. The correlation involving the 500 nm / 526 nm ratio (Fig. 5b; correlation coefficient -0.557, $p < 0.0001$) was stronger than that involving the 465 nm / 526 nm ratio (Fig. 5a; correlation coefficient -0.268, $p = 0.001$).

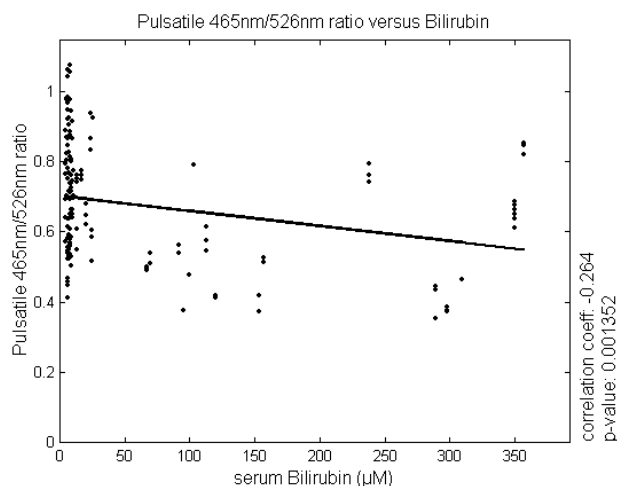


Fig. 5a. Pulsatile attenuation ratio (465 nm / 526 nm) versus bilirubin for 61 patients

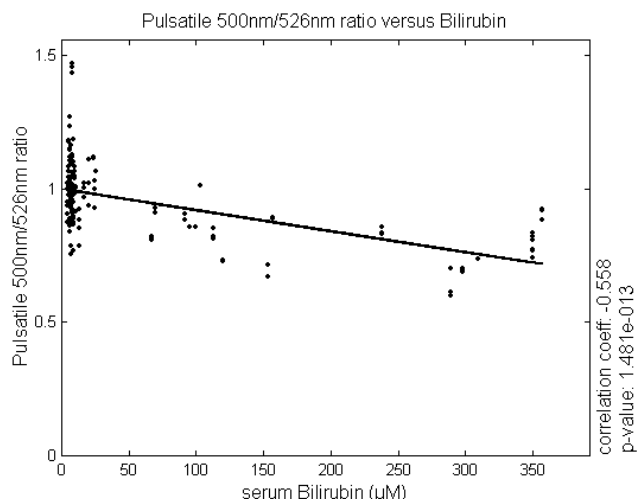


Fig. 5b. Pulsatile attenuation ratio (500 nm / 526 nm) versus bilirubin for 61 patients

Comparison of models

Table I gives a comparison of slopes between Beer-Lambert and discrete vessel models and in vivo tests, from Figs. 3, 4 and 5 respectively. The Beer-Lambert model generated ratios which increased with bilirubin concentration, whereas the discrete vessel model and in vivo testing generated negative relationships.

TABLE I
COMPARISON OF SLOPES BETWEEN BEER-LAMBERT AND DISCRETE VESSEL MODELS AND IN VIVO TESTS

Ratio (nm/nm)	Model	Slope (μM^{-1})	Correlation coefficient	p value
465/526	Beer-Lambert	+0.000 416	-	-
	Discrete vessel	-0.000 095	-	-
	In vivo	-0.000 429	-0.27	0.001
500/526	Beer-Lambert	+0.000 151	-	-
	Discrete vessel	-0.000 152	-	-
	In vivo	-0.000 791	-0.56	<0.001

IV. DISCUSSION

In previous simulations of photoplethysmography with finger models [14], we found the results from Monte Carlo simulations were similar to the results of calculating the transmission of light, according to the Beer-Lambert Law, along multiple paths throughout finger model cross sections - as used in this study.

The negative correlations, in Fig. 4 (discrete vessel models) and Fig. 5 (in vivo results) between the 465 nm / 526 nm and 500 nm / 526 nm pulsatile attenuation ratios and serum bilirubin concentration (tSB) indicate that, as the bilirubin concentration increased, the pulsatile attenuation of 526 nm light was increasing more than that of 465 nm and 500 nm light. This is opposite to the mechanism of light

absorption inside a spectrophotometer; if the concentration of bilirubin in a cuvette was increased, the absorption of 526 nm light would increase but the attenuation of 465 nm and 500 nm light would increase by a greater amount, in accordance with the absorption spectrum of bilirubin (Fig. 1). The modulating effects of discrete blood vessels [14], [22] may account, at least in part, for the negative correlations seen in Fig. 4 and Fig. 5.

Although Fig. 5 shows statistically significant correlations between tSB and the 500 nm / 526 nm and 465 nm / 526 nm pulsatile attenuation ratios, by themselves they could not be used to noninvasively estimate tSB, because the spread of results was too great. For instance, the variation in pulsatile ratios for patients with normal tSB ($< 20 \mu\text{M}$, as identified in the 'Admissions Transfers and Separations' (ATS) system at Flinders Medical Centre) was approximately 3 times the mean change in the pulsatile ratio when tSB increased from 0 to $350 \mu\text{M}$. The large spread of results may be at least partly due to variations in blood vessel size and tHb between individuals, which have been shown to affect pulsatile attenuation ratios [14], [23], [24]. It may also be attributed to light scattering - which is widely acknowledged in pulse oximetry [12], [13].

Modelling relationships between serum bilirubin and pulsatile attenuation ratios demonstrated that substantially different results could be obtained from the Beer-Lambert pulse oximetry model and discrete blood vessel modelling. This may be due to the modulating effects of discrete blood vessels on the proportionality between the pulsatile attenuation of light and the absorption coefficients of blood [14].

Table I shows that the slopes of the least squares lines of best fit from in vivo tests (Fig. 5) were closer to the results obtained from discrete blood vessel finger modelling (Fig. 4) than the results obtained from Beer-Lambert based law modelling (Fig. 3). However, the large difference between in vivo test results and models indicates that neither model fully represented in vivo situations.

Previously we showed that the light absorption of bilirubin was sufficiently different to that of haemoglobin to, in theory, allow direct non-invasive serum bilirubin monitoring using light absorption around 470 nm, in a similar fashion to pulse oximetry [11]. This work indicates that using a simple pulse oximetry-like algorithm to estimate tSB could generate unreliable results.

V. CONCLUSION

The noninvasive estimation of serum bilirubin, using simple pulsatile attenuation ratios (as per pulse oximetry), involving blue and green light, was not successful.

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