engineering proceedings

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Proceeding Paper

A System-on-Chip Assay for Bilirubin Levels Measurement in Whole Blood †

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† Presented at the 8th International Electronic Conference on Sensors and Applications, 1–15 November 2021; Available online: https://ecsa-8.sciforum.net.

Abstract: Bilirubin (BR) is clinically confirmed as a biomarker for liver health and is used to assess the prognosis of cirrhosis. Optical and chemical methods have been utilized for blood BR biosensing. While optical methods offer real-time monitoring and are handy and immune to infection, measurements may not be practical due to the instrument complexity and space requirements. This study investigated the dual-wavelength (DWL) technique for BR estimation using a system-on-chip (SoC). The SoC includes an optical module with blue (455 nm) and green (530 nm) LEDs which were used for DWL measurement. Porcine blood was used as a surrogate of human blood and BR levels were kept within the pathophysiological ranges projected from healthy individuals (<1.2 mg/dL) to cirrhotic patients (up to 50 mg/dL). Our findings show a high BR sensitivity in blood. This lays the groundwork for point-of-care testing for BR levels, primarily for hyperbilirubinemia infants and cirrhotic adults out in homes or in-community settings.

Keywords: liver cirrhosis; biosensing; bilirubin; biomarker; blood; point-of-care testing

Citation: Ndabakuranye, J.P.; Prawer, S.; Ahnood, A. A System-on-Chip Assay for Bilirubin Levels Measurement in Whole Blood. *Eng. Proc.* **2021**, *10*, 74. https://doi.org/10.3390/ecsa-8-11295

Academic Editor: Stefano Mariani

Published: 1 November 2021

1. Introduction

Bilirubin is a by-product of heme catabolism [1], where less than 1.2 mg/dL is always present in the blood of healthy individuals [2]. It exists as conjugated and unconjugated bilirubin (UCB) in the bloodstream. Unconjugated bilirubin (UCB) is toxic and water-insoluble; hence, it should be excreted. The excretion starts with UCB binding with albumin and is transported to the liver. At this point, UCB is enzymatically converted into conjugated bilirubin (CB). The latter is less toxic and water-soluble, allowing for renal or intestinal excretion [3]. However, in the case of pathophysiological events, bilirubin levels are elevated, and the liver fails to live up to its conjugation ability, leading to irreversible neurological damage or death [4]. High bilirubin levels have been correlated with hepatic and hemolytic disorders [5,6].

Several studies on BR levels estimation have been reported [7,8], but these techniques suffer from the instrument complexity and cost. This mini-paper investigated the dual-wavelength (DWL) technique for BR estimation using a system-on-chip (SoC).

2. Materials and Methods

Materials: Bilirubin (PN: 14370), Dimethyl Sulfoxide (DMSO) and sodium citrate (NaCHO) commercial standards were supplied from Sigma-Aldrich, Australia.

Blood sample preparation: Porcine blood was procured from the abattoir immediately after sacrifice and was preferred as a prominent replacement for human blood due to its biochemical resemblance [9] and low cost. Solutions were prepared by mixing BR at varied concentrations and blood with anticoagulant (4% w/v)-to-blood ratio of 1:9 (v/v) [10].

BR-blood samples were stored at ~5 °C in the dark to prevent photodegradation. For extended preparation steps, refer to the study performed by Ndabakuranye et al. [11].

Optical measurement: A SoC platform was used for BR measurement. The setup consisted of a MAX86916 optical module, a MAX32630 host and an optical stage designed to operate in transflection mode. Transflection mode implies that a rear optical reflector (E39-R42, Omron) was used on top of the sample. Therefore, the spectral response contained both light intensity backscattered from the sample and light transmitted through the sample then reflected by the reflector to be retransmitted back through the sample, thereby doubling the path length (Figure 1). Major features of the system-on-chip are summarized in Table 1.

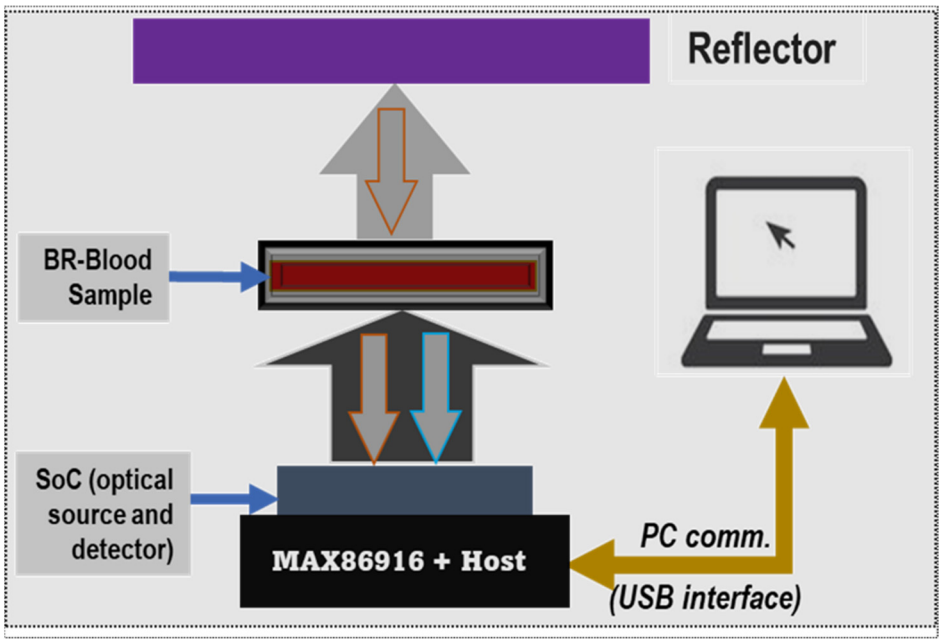

Figure 1. Illustration of the DWL measurement in transflection mode.

Table 1. Major features of MAX86916 optical module.

Feature	Value
ADC resolution	19-bit
Size	3.5 × 7.0 × 1.5 mm
Spectral sensitivity	400–1100 nm
Data communication protocol	I2C
Radiant sensitive area	1.51 mm^2

Feature selection: Several features were optimally selected using theoretical investigations and simulations to ensure the accuracy, reliability, and safety of bilirubin measurements. These features are summarized in Table 2.

Table 2. Optimal features for DWL measurement.

Method Parameter	Optimized Parameter
Operating wavelengths	Blue and green
Path length	200 µm
Solvent	DMSO
Bilirubin concentration range	1.2–50 mg/dL
Anticoagulant	Sodium citrate

3. Results and Discussions

The analytical (470 nm) and reference (525 nm) wavelengths were obtained by analyzing the distinct optical signatures of blood and BR. It does not significantly absorb green light but strongly absorbs blue light (ε_{525} = 214, ε_{470} = 46188 [cm^{-1} M^{-1}]) [12]. However, although the LEDs on the SoC emit the maximum power at the wavelengths of 455 nm and 530 nm, the LEDs' emission bands are sufficiently wide to also cover 470 nm and 525 nm, since the LEDs are not ideal monochromatic sources.

The DWL measurement was performed using a revised 2-dimensional Beer's law as shown in Equation (1) which suggests that BR concentration can be correlated with its absorbance ratio at 470 and 525 nm (t: path length [cm], ε: extinction coefficient, C: concentration).

$$\begin{pmatrix} A_{470\text{nm}} \\ A_{525\text{nm}} \end{pmatrix} = \begin{bmatrix} \varepsilon_{BR,\,470} & \varepsilon_{Hb,\,470} \\ \varepsilon_{BR,\,525} & \varepsilon_{Hb,\,525} \end{bmatrix} \begin{pmatrix} C_{BR} \\ C_{Hb} \end{pmatrix} \times t \quad (1)$$

To investigate *BR*'s sensitivity in blood, *R*-parameters were deduced by collecting the MAX86916 ADC counts data and calculated using Equation (2). Figure 1 shows the SoC platform used to measure the ADC data used to compute *R*-parameters ($\widetilde{R}$). The plot of R-parameters as a function of *BR* concentrations is shown in Figure 2, and results showed a strong linear relationship with the coefficient of determination greater than 0.991.

$$\widetilde{R} = \frac{\log[ADC\ count_{Blue}]}{\log[ADC\ count_{Green}]} \quad (2)$$

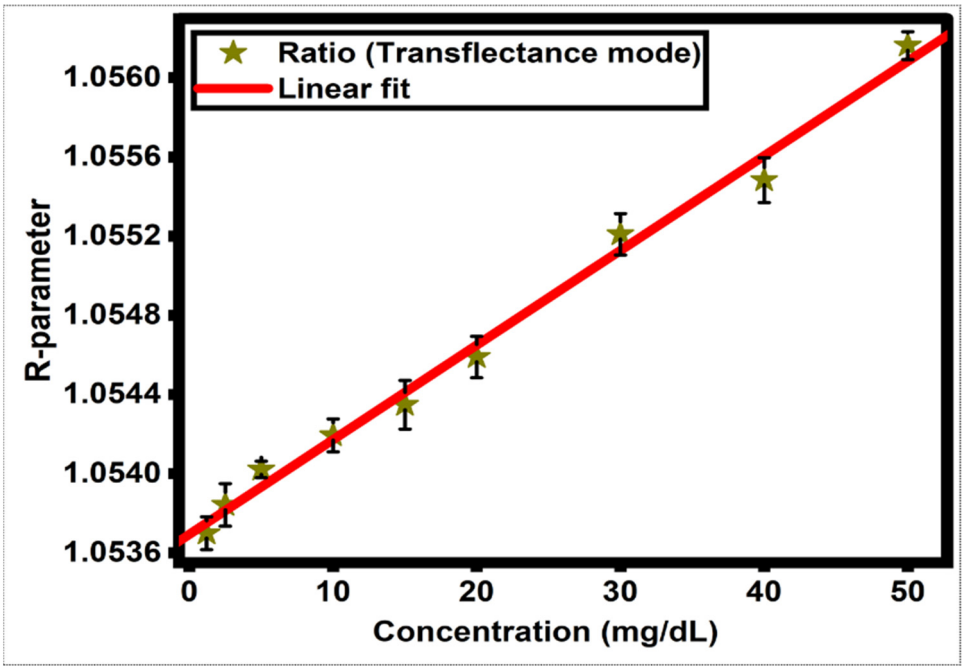

Figure 2. The graph of R-parameters as a function of bilirubin concentrations as obtained from DWL measurements on whole blood using the SoC platform.

Although this technique provides a robust and simple way of measuring BR, it may be susceptible to errors due to LEDs' spectral and spatial distribution inadequacies, residual bilirubin, other hemoglobin forms (COHb or MetHb), S_aO_2 and Hb levels variability.

4. Conclusions

The feasibility of BR monitoring by the DWL method was investigated, and BR's sensitivity in blood was explored at pathophysiological ranges (1.2–50 mg/dL) using an Soc. The SoC includes a miniature MAX86916 optical module with integrated signal conditioning and processing capabilities. Results showed a strong correlation between R-parameters and BR concentration. Our findings lay the groundwork for point-of-care testing for BR levels, primarily for hyperbilirubinemia infants and cirrhotic adults out of clinical settings.

Author Contributions: Methodology, J.P.N.; Formal Analysis, J.P.N.; Investigation, J.P.N.; Data Curation, J.P.N.; Original Draft Preparation and Writing, J.P.N.; Conceptualisation, J.P.N., A.A. and S.P.; Writing—review and editing, J.P.N., A.A. and S.P.; Supervision, S.P. and A.A.; Project Administration, S.P. and A.A.; Funding Acquisition, S.P. and A.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Australian Research Council, grant number LP160101052.

Institutional Review Board Statement: Experiments were carried out on samples from the abattoir, and thus, neither ethics application nor written consent was required.

Acknowledgments: Jean Pierre gratefully acknowledges the Melbourne Research Scholarship (the University of Melbourne), The School of Physics (The University of Melbourne) and the STEM college (RMIT University).

Conflicts of Interest: Authors declare that they have no conflicting financial or non-financial interests.

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