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Determination of Wavelength Candidates for Non-Invasive Hemoglobin Measurement Devices and Energy Spectrum Analysis

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Abstract. Hemoglobin (Hb) is an important parameter in human medical treatment. At present, hemoglobin level is measured by invasive method. This method require blood sample, less accurate and the result can be delayed. So that, human hemoglobin spectrophotometry characterization research to obtain a spectrophotometric spectrum in the development of non-invasive hemoglobin measuring devices is needed. The advantages of non-invasive method are do not require blood samples, high accuracy, painless, and allow for quick. Hemoglobin level measurement in non-invasive method can be done by measuring light absorption at different wavelengths. This non-invasive device needs appropriate LED wavelength to detect the level of hemoglobin. Hemoglobin is the dominant absorbent at 800-1600 nm. This experiment used near infrared (NIR) spectrum analysis in 1000-2500 nm. The result of this experiment is the candidates for LED wavelength that can be used in non-invasive hemoglobin measurement devices are 1200 nm and 1300 nm. This determination is based on the lowest standard deviation value at 1293 nm, the highest correlation value at a wavelength of 1266 nm, and is in the near infrared region vibration of C-H functional group at the 3rd energy level, 1252 nm in harmonic vibration. At a wavelength of 1200 nm and 1300 nm it shows low infrared absorption. It shows that non-invasive hemoglobin measuring devices that use an infrared reflectance sensor can be used.

Keywords: hemoglobin, infrared, non-invasive

INTRODUCTION

Hemoglobin is one of important parameters in human medical treatment and used in laboratory tests in medical research [1]. Recently, hemoglobin level measurement done by invasive method by taking blood samples using finger-prick or stab on the fingers with a needle. This method is less accurate and the result can be delayed [2,3]. Several attempts have been made to find appropriate alternatives for hemoglobin non-invasive measurement. Hemoglobin non-invasive measurement allows information for quick, real time and accurate for efficient medical action [4,5]. In addition, non-invasive measurement do not require blood sampling taken by finger-prick that cause pain [2,6].

Hemoglobin level measurement in non-invasive method can be done by measuring light absorption at different wavelengths [7,8]. Synthesis materials or natural materials absorb infrared radiation in certain regions of spectrum. Hemoglobin is dominant absorbent at 800-1600 nm [5]. This allows to use infrared spectroscopy with wavelength in range 400-4000 nm. This experiment used near infrared (NIR) spectrum analysis in 1000-2500 nm. This wavelengths is needed to determine appropriate LED that can be used in non-invasive hemoglobin measurement device.

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METHOD

This research used an experimental clinical test method with a code of ethics 076 / IT3.KEPMSM-IPB /SK/ 2018. This was conducted to obtain human blood samples. Blood samples were taken from subjects 16-65 years old. Respondents who will not be included (exclusion) are pregnant or breastfeeding and fear of needles or could not stand pain. Blood samples taken from 11 random respondents by doctor in December 2018, from a vein located in the fold of the arm (median cubic vein). About 3 mL blood samples were used in hemoglobin level measurement with invasive method in laboratory. About 6 mL blood samples were used in absorbance measurement using Buchi NIRFLEX 500 spectroscopy. This measurement in wavelength variations from 1000-2500 nm, with 3 times repetitions for each sample.

This research used 3 parameters to determine appropriate LED wavelength can be used in non-invasive device. Those the lowest value of standard deviation, the highest value of Pearson correlation, and infrared spectrum of the blood samples. Standard deviation analysis used to determine the distribution and absorbance value with the closest value to the average of each wavelength variation in determining data stability. Pearson correlation analysis used to find the relationship between two variables, the result from hemoglobin measurement with invasive and non-invasive method. Near infrared (NIR) analysis aimed to see the functional group of blood samples absorption region. The NIR result was a graph of absorbance (%). The peaks in the graph show the molecular bonds identified in hemoglobin. This data are used to plot a graph to determine appropriate LED wavelength can be used in non-invasive device. Spectrum energy analysis used to get more information about the dissociation energy and level energy of the hemoglobin functional group.

RESULT

Hemoglobin spectrum analysis shows the presence of functional groups C=O, O-H, and C-H at the ends of the chemical bonds in the heme. Those functional group vibrate due to absorbing the infrared. The C=O functional group at wavelength 1923 nm. The O-H functional group at wavelength 1449 nm and 2160 nm. The C-H function group at wavelength of 1159-1802 nm. The lowest standard deviation value at wavelength 1293 nm, the highest correlation value at wavelength 1266 nm. C-H vibration functional group is at the 3rd energy level with 1252 nm in harmonic vibration. Wavelengths 1200 nm and 1300 nm show low infrared absorbance.

DISCUSSION

NIR spectrum hemoglobin analysis

Non-invasive hemoglobin level measurement can be done by measuring light absorption at different wavelengths [7,8]. The infrared spectrum used to analyze the functional groups of a molecule or sample. Molecular bonds samples characteristic are easy to know if they have similar absorption region with the literature that provide similarity information of functional groups and vibration modes [9]. The figure 1 shows functional groups C-H, O-H, and C=O from the blood samples absorption region. Those functional groups at the ends of heme, this vibrations occurred because infrared absorption. Table 1 shows the functional groups absorption region from NIR experiment.

TABLE I. Average wavelength of hemoglobin functional groups bond.

Functional Groups	Wavelength (nm)	
	Experiment	Literature[14]
C=O	1923	1800-2000
O-H	1449	1300-1500
O-H	2160	2000-2200
C-H	1159	1100-1300
C-H	1802	1600-1800

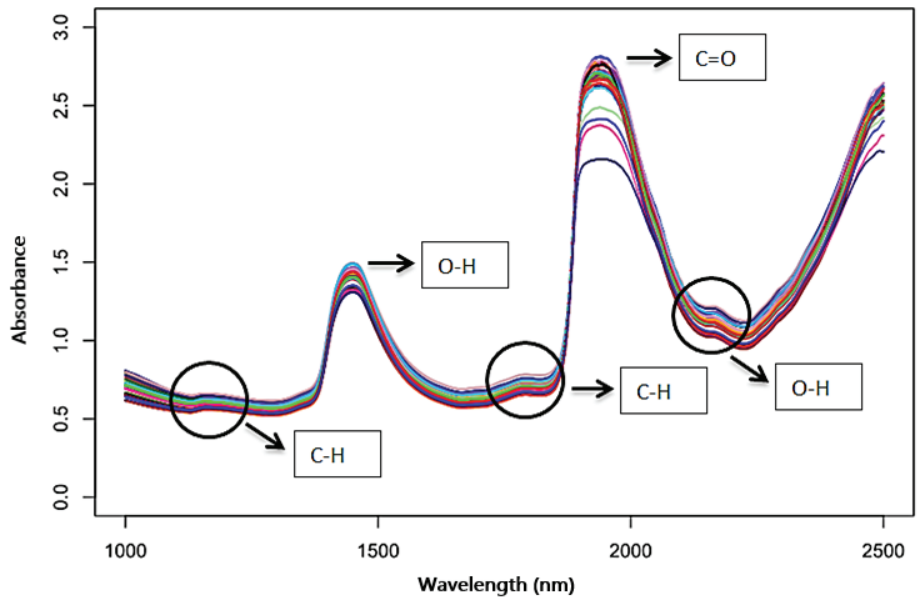

FIGURE 1. NIR spectrum of hemoglobin.

Hemoglobin spectrum analysis shows the presence of functional groups C=O, O-H, and C-H at the ends of the chemical bonds in heme. Those functional groups vibrate due to absorbing the infrared. The C=O functional group at a wavelength 1923 nm. The O-H functional group at wavelength 1449 nm and 2160 nm. The C-H functional group at wavelength 1159-1802 nm. This shows appropriate LED can be used at a wavelength 1200 nm and 1300 nm to detect O-H and C-H functional groups at the end of heme.

Standard Deviation Analysis

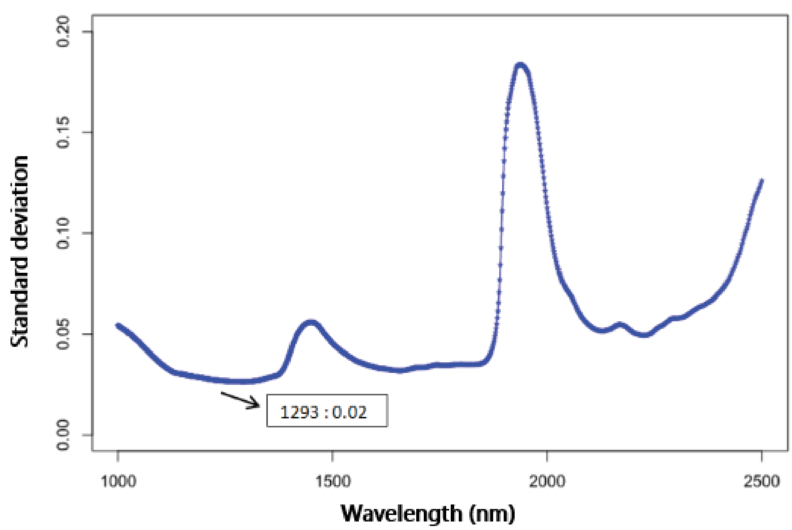

FIGURE 2. Graph of standard deviation value at wavelength of hemoglobin spectrum.

Standard deviation analysis used to determine the distribution and absorbance value with the closest value to the average of each wavelength variation in determining data stability [10]. In this research, the lowest standard deviation shows small difference value at wavelength absorbance of each sample. The figure 2 shows the standard deviation value in the variation of wavelengths with range 1000-2500 nm from entire samples. Low standard deviation value in the range 1155-1372 nm with a standard deviation value 0.0299-0.0298. The lowest standard deviation value is 1293 nm with an absorbance value of 0.0263. The lowest standard deviation value shows the accuracy of measurements [10]. So that, hemoglobin level can be detect at those wavelength because it has the highest accuracy.

Pearson Correlation Analysis

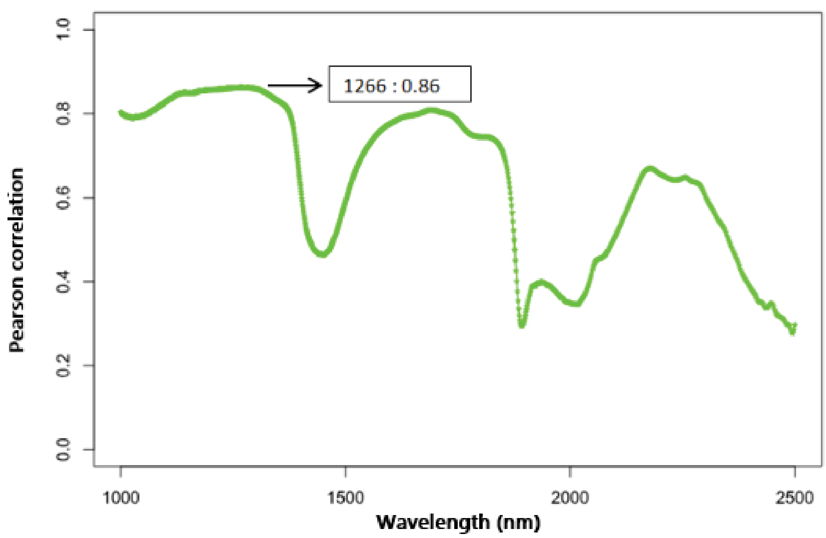

FIGURE 3 Graph of Pearson correlation value at wavelength of hemoglobin spectrum.

Pearson correlation analysis shows a correlation of two quantitative data [11]. In this research we calculate the correlation of the wavelength variation absorbance values each sample and hemoglobin level invasive measurement by laboratory. Correlation value of those data is 0.5-1.0. It mean that they have strong correlation [20]. The Figure 3 shows the correlation value in wavelength variation ranges in 1000-2500 nm from entire samples. High correlation in the range 1139-1326 nm has a value of 0.85-0.84. The highest correlation value is 0.86 at wavelength 1266 nm. Both of these correlation values have a strong correlation because they are in the range of 0.5-1.0. This shows that the wavelength range can be used for hemoglobin detection devices and proves that the measurement of blood hemoglobin can be done by non-invasive methods.

Determination the wavelength candidates in non-invasive measurement devices

The graph shows the relation of standard deviation, Pearson correlation and absorbance in ratio of 1 with available LED wavelengths. This LED used to detect hemoglobin level in non-invasive device. Appropriate LED determinate based on lowest standard deviation value, highest Pearson correlation value and low absorbance value, Those at wavelength range 1139-1327 nm. The vertical lines on the graph intersect with three curves with horizontal lines. Intersection between the highest peak of green curve and the lowest peak of blue curve are vertical line (d) representing LED with wavelength 1200 nm and vertical line (e) representing LED with wavelength 1300 nm. This shows that those LED wavelengths can be used in non-invasive blood hemoglobin measurement devices. At these wavelengths, hemoglobin is more reflected than transmitted (red curve), indicated by a low absorbance value that allows the use of a measuring instrument with the reflectance working principle.

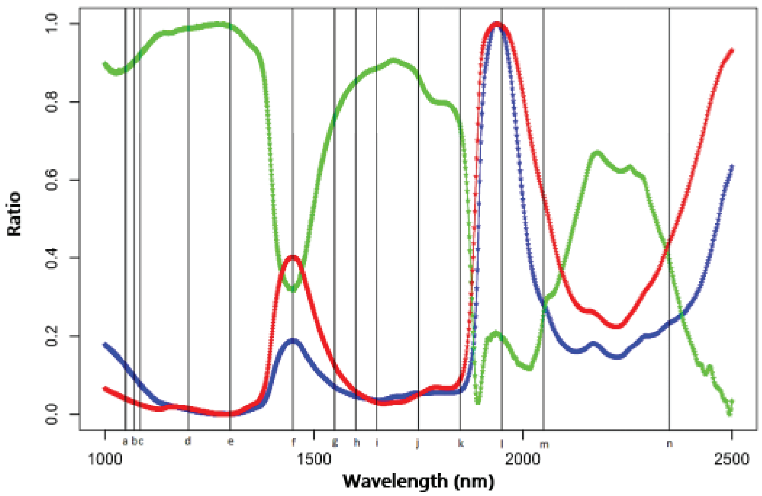

FIGURE 4. Graph of the ratio of standard deviation (blue curve), Pearson correlation (green curve), and absorbance (red curve) and vertical lines representing LED wavelengths (a) 1050 nm, (b) 1070 nm, (c) 1085 nm, (d) 1200 nm, (e) 1300, (f) 1450 nm, (g) 1550 nm, (h) 1600 nm, (i) 1650 nm, (j) 1750 nm, (k) 1850 nm, (l) 1950 nm, (m) 2050 nm, (n) 2350 nm.

Energy spectrum analysis

Dissociation energy analysis can be used to determine the energy needed to break molecular compound bonds [13]. Table 2 shows the experimental dissociation and calculations energy on O-H and C-H functional groups. Dissociation energy of O-H and C-H functional groups according the calculation are 4.49 eV and 3.62 eV. The difference of experimental dissociation energy and the calculation energy of O-H and CH functional groups are 0.08 eV and 0.11 eV. The difference is the energy value of the potential energy in the ground state or level 0.

TABLE II. Comparison of calculation dissociation energy and experimental dissociation energy in the O-H and C-H functional groups.

Functional Groups	Do[15] (eV)	De (eV)	Difference (eV)
O-H	4.41	4.49	0.08
C-H	3.51	3.62	0.11

Potential energy at level 0 used by O-H and C-H function groups to vibrate at equilibrium. Increasing energy levels to the next level of the ground state causes H atoms in O-H and C-H functional groups to vibrate away from the balanced point and require greater energy. Determination of candidate wavelength can be seen from the energy value of the O-H and C-H functional groups. In O-H functional group energy in the near infrared range at a wavelength of 1252 nm and at the 3rd energy level of harmonic vibration.

TABLE III. Energy value in the O-H functional group.

Level Energy	Energy Value	
	Ev	nm
0	0.08	15490
1	0.25	4959
2	0.42	2952
3	0.99	1252
4	1.27	976

LED wavelength candidates for non-invasive hemoglobin measurement devices can be determined from the standard deviation, correlation, absorbance, and vibration energy values. The lowest standard deviation shows high accuracy and close distance with absorbance value of a sample and average absorbance value of entire sample. The highest correlation value indicates a strong correlation between the two data, hemoglobin level invasive measurement value from laboratory and blood sample absorbance value. Energy levels indicate the presence of vibrations in a functional group when absorbing infrared. Based on these parameters, the candidate LED wavelength that can be used are 1200 nm and 1300 nm because it approaches the lowest standard deviation value at 1293 nm wavelength, the highest correlation value at wavelength 1266 nm, and in the near infrared region C-H vibration function groups at the 3rd energy level in 1252 nm harmonic vibrations. At wavelengths 1200 nm and 1300 nm shows low infrared absorbance. So that, infrared reflectance sensor can be used in non-invasive hemoglobin measurement devices.

CONCLUSIONS

LED wavelength candidates for non-invasive hemoglobin measurement devices can be determined from the lowest standard deviation value, highest correlation value, absorbance, and vibration energy values. Based on these parameters, the candidate LED wavelength that can be used are 1200 nm and 1300 nm.

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