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THE OPTIMAL ABSORPTION OF BILIRUBIN USING AN OPTICAL FIBRE SENSOR

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ABSTRACT

This paper describes an optical fiber sensor for the monitoring of bilirubin concentration and commonly called jaundice An open path optical technique is used to analyze the absorption lines of bilirubin within the Ultra Violet/ Visible region. By using a wavelength corresponding to a bilirubin absorption peak, the Beer-Lambert Law can be used to relate the concentration of bilirubin surrounding the sensing portion to the amount of absorbed light. In the initial experiment, the absorption cross section for MAS bilirubin a product from Thermo Scientific was investigated and compare with theoretical data. Initially, an empty cuvette was used to measure incident intensity when the light passes through the empty cuvette. Then a cuvette was filled with bilirubin sample before measured the transmitted intensity. The theoretical absorbance of bilirubin shows maximum absorption in the range of 400 nm to 600 nm. The experimental result shows the absorption line for measured MAS bilirubin is in similar pattern but the maximum absorbance shows in range 600 nm to 700 nm. This is due to the type of sample used in the experiment and high attenuation of the optical fiber used at the lower wavelength of UV light. Future work would be carried out to study the cross sensitivity of bilirubin absorption spectrum with other human blood molecules like hemoglobin (Hb), oxygen (O_2) and carbon dioxide (CO_2) to yield the best wavelength for the absorption.

Keywords: fibre optic sensor, bilirubin, absorption.

INTRODUCTION

Jaundice or yellowish pigmentation of the skin is a common condition for most newborn. It is caused by increased levels of bilirubin in the blood and severe cases can lead to brain damage (Rai *et al.* 2002). Therefore, a close monitoring of bilirubin level is vital for the child future survival. Current technologies allow us to determine the value of bilirubin using several methods, both invasive and non-invasive. Non-invasive methods for bilirubin concentration measurement are attractive by their evident advantages such as real time monitoring, immunity to the infection, possibility to control the concentrations of bilirubin providing painless measurement as often as necessary (Yap *et al.* 2002). Therefore a new method of bilirubin detection using spectroscopic method is proposed.

For the purpose of fundamental studies, the research project of the jaundice monitoring is focused on the study of spectroscopy of the bilirubin molecules absorption. The absorption spectrum of the bilirubin must be thoroughly studied and the optimum wavelength for absorption must be determined and verified and compared with the theoretical data. Initial stage of experiments also includes the absorption cross sensitivity studies with other human blood molecules such as CO2, O2, hemoglobin, etc. This is to avoid any reading interference which can provide imprecision in the measurement of bilirubin level in the blood system.

Various methods have been developed for bilirubin analysis in clinical samples. The most commonly used methods for bilirubin analysis is the diazo reaction in which bilirubin reacts with diazotized sulfanilic acid(Watson, 1958). The diazo reaction of bilirubin is highly selective and accuracy in the determination of the bilirubin concentration but interferes with other heme containing proteins (e.g. haemoglobin) and pigments (Doumas *et al.* 1987). Other analytical methods such as voltammetry, polarography, and fluorometry have also been used for bilirubin analysis (Malloy and Evelyn, 1937). While providing higher sensitivity, these methods are less selective than the diazo reaction.

Electrochemical amperometric sensors and fiber optic sensors that make use of bilirubin oxidase have been recently fabricated and applied for bilirubin analysis in aqueous solutions and blood(Klemm *et al.* 2000). In these sensors, bilirubin oxidase is immobilized in a membrane that is attached to the surface of a carbon electrode or to the distal end of a fiber optic sensor. The measurement of the decreasing level of molecular oxygen when bilirubin is oxidized by the enzyme serves as an indirect indication of the bilirubin concentration. This analytical method suffers from interferences of electro active species and is characterized by a relatively long response time(Fevery, 2008).

Each molecule has its own unique absorption and reflection spectrum. For the bilirubin absorption detection, the Beer-Lambert Law was utilized. The Beer-Lambert law describes the relationship between absorbance and concentration of an absorbing species and its general form is shown in equation (1).

$$\frac{I}{I_0} = e^{(-\sigma Nl)} \tag{1}$$

Where I is the transmitted intensity, Io is the incident intensity, l (cm) is the distance that the light travels through sample, σ (cm2/Molecule) is the absorption cross

section and N (Molecules/cm3) is the concentration of the absorbing medium

In this paper, an initial experiment is carried out to study the absorption cross section of bilirubin molecules using UV-Visible application and to obtain the optimum wavelength that shows maximum absorbance for bilirubin molecules.

EXPERIMENTAL SETUP

There are three main parts need in experimental set up such as light source, cuvette as sample container and detector. The DH-2000-BAL Deuterium Tungsten Halogen Light Source with a wavelength range from 210nm to 1200nm is applied as light source for the initial experiment. Figure-1 shows the schematic of the experimental set up. The transmitted light interacts with the bilirubin sample in the cell prior to being coupled to another (receiving) optical fibre at the other end of the gas cell to the light detector. The light detector was an Ocean Optics MAYA 2000 Pro spectrometer. The spectrometer has a range from 200 nm to 1100 nm and it provides a spectral resolution to 0.65 nm. The spectrometer was interfaced with the computer using SpectraSuite software. SpectraSuite is a specifically designed program provided by Ocean Optics in order to acquire the data from the spectrometer in real time.
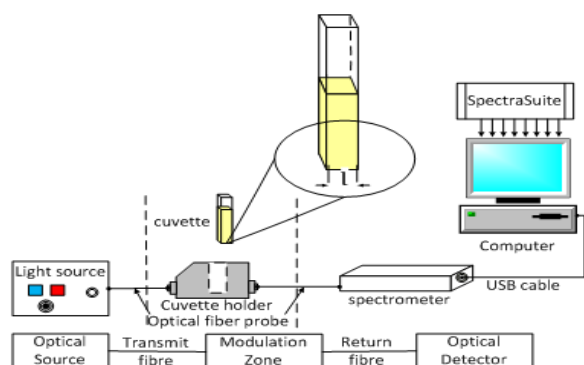

Figure-1. Experiment set up.

From the initial measurement, the absorbance of bilirubin sample can be calculated using below equation:

$$A(\lambda) = -log(T(\lambda))$$ (2)

Where

$$T(\lambda) = \frac{I(\lambda)}{Io(\lambda)}$$

The transmitted intensity, I and the incident intensity, Io were recorded and equation (2) is used to obtain the absorption cross section. These absorption values were plotted against the wavelength and compared with theory. Initially, an empty cuvette was used to measure incident intensity when the light passes through the empty cuvette. Then a cuvette was filled with bilirubin sample before measured the transmitted intensity. The

sample used in the experiment is MAS bilirubin a product from Thermo Scientific. The sample is prepared from a bovine serum base. Analyte levels are adjusted with bilirubin extracts and synthetic derivatives. Preservatives and stabilizers are added to maintain product integrity.

RESULTS AND DISCUSSION

In this work, the intensity of light was measured when the light pass through a cuvette contains with bilirubin sample and without bilirubin sample using a spectrometer. The spectrum was captured by SpectraSuite software for both conditions. Then the spectrum raw data from SpectraSuite was replotted using Kaleidagraph software. Figure-2 shows the spectrum for both conditions.

The absorption cross section is used to characterize the optical transmission properties of a medium whether in the solid, liquid or gaseous state. When a beam of light is passed through a medium, its propagation is affected in two important ways(Mohammed *et al.* 2012). In the first way, the intensity will always decrease to a greater or less extent as the light penetrates father into the medium. In second way, the velocity will be less in the medium compare to the velocity in free space. The loss of intensity is chiefly due to absorption (Mohammed *et al.* 2012).

The raw data from initial experiment can be used to calculate the absorption cross section using equation 2 and then plot a graph. Figure-3 shows absorption cross section of MAS bilirubin.
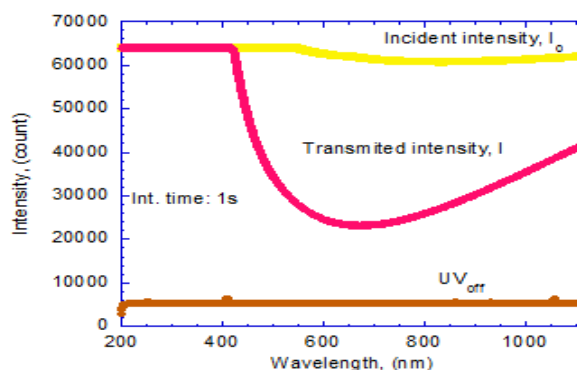

Figure-2. The intensity of MAS bilirubin (transmitted intensity) and without MAS bilirubin (incident intensity).
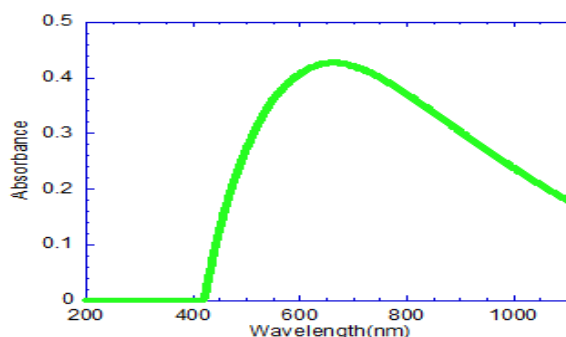

Figure-3. Absorbance of MAS bilirubin.

Figure-3 shows that the optimal absorbance for MAS bilirubin is obtained at 650 nm. The theoretical absorbance of bilirubin shows maximum absorption in the range of 400 nm to 600 nm. The experimental result shows the absorption line for measured MAS bilirubin is in similar pattern but the maximum absorbance shows in range 600 nm to 700 nm. All bilirubin in blood specimens from healthy neonates is practically unconjugated bilirubin (UBIL), and the absorptivity of UBIL in other protein matrices, particularly bovine serum, human serum albumin or bovine serum albumin are different from that in human serum(Lo and Doumas, 2011). As mentioned above, MAS bilirubin is prepared from a bovine serum base. This is one of the reason why the optimal absorbance of MAS bilirubin different from theoretical absorbance. This is also due to high attenuation of the optical fiber used at the lower wavelength of UV light transmitted, resulting in a low signal-to-noise ratio (Manap *et al.* 2009).

CONCLUSIONS

A novel optical fibre sensor for non-invasive jaundice monitoring is focused on the study of spectroscopy of the bilirubin molecules absorption. The absorption spectrum of the bilirubin must be thoroughly studied and the optimum wavelength for absorption must be determined and verified and compared with the theoretical data. Thus, it has been demonstrated that the UV-Visible range is well suited for the measurement of bilirubin. Future work will focus on cross sensitivity of bilirubin with other component of blood such as hemoglobin. This is to avoid any reading interference which can provide imprecision in the measurement of bilirubin level in the blood system.

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