Smart Oxygen Cuvette for Optical Monitoring of Dissolved Oxygen in Biological Blood Samples

A smart Oxygen Cuvette is developed by coating the inner surface of a cuvette with oxygen sensitive thin film material. The coating is glass like sol-gel based sensor that has an embedded ruthenium compound in the glass film. The fluorescence of the ruthenium is quenched depending on the oxygen level. Ocean Optics phase fluorometer, NeoFox is used to measure this rate of fluorescence quenching and computes it for the amount of oxygen present. Multimode optical fibers are used for transportation of light from an LED source to cuvette and from cuvette to phase fluorometer. This new oxygen sensing system yields an inexpensive solution for monitoring the dissolved oxygen in samples for biological and medical applications. In addition to desktop fluorometers, smart oxygen cuvettes can be used with the Ocean Optics handheld Fluorometers, NeoFox Sport. The Smart Oxygen Cuvettes provide a resolution of 4PPB units, an accuracy of less than 5% of the reading, and 90% response in less than 10 seconds.

1 INTRODUCTION

1.1 Microorganisms in blood

Microorganisms are one celled organisms such as viruses, fungi and bacteria. Presence of microorganisms is harmful and cause diseases. The presence of microorganisms in blood cultures plays an important role in the diagnosis of different diseases. Different methods have been in existence to detect the presence of microorganisms in blood cultures. Early detection of such organisms is of primary importance to the selection of appropriate therapies and doses to be adopted on patients . Blood culturing systems are bioreactor system which involves the process of selectively growing microorganisms under optimized conditions. Blood culturing systems are closed culture systems where blood along with the growth media is operated under constant temperature along with continuous mixing. The numbers of microorganism increase due to respiration process and establish reactions with blood components changing the forms of hemoglobin. In the absence of microorganisms irrespective of the growth media present, the blood components do undergo changes due to aging of the red blood cells. As the microorganism’s density increases in the blood culture, partial pressure of oxygen is reduced and partial pressure of carbon dioxide is increased as a part of respiration process.

Automated systems are being developed to continuously monitor the different metabolic changes happening in the blood contents along with the changes in the partial pressure of oxygen/carbon dioxide consumed/generated respectively. The instruments primarily constitute the detection system to capture the data points at different intervals to form mathematical models to study the behavior of microorganisms and their growth patterns. The information collected using such systems helps us to understand the time period when the microorganisms have grow and aid in the selection of system parameters optimum to detect the different microorganisms. Some of the changes such as conversion of oxy to deoxyghemoglobin within the red blood cells have been detected using spectroscopy methods which provide growth behavior of organisms . As oxygen is necessary for cell respiration and is consumed during the growth phase of a cell processes for aerobic microorganisms. The cells reproduce and their cell density increases during the growth phase leading to increased oxygen consumption by the cells. The cells consume the dissolved oxygen from the liquid media (blood culture).

This paper presents the application of smart cuvette coated with oxygen sensitive sol gel coating which acts as a detection system to measure the dissolved partial pressure of oxygen in blood culture systems and the trend in oxygen consumption in response to the increasing density of microorganisms

2 SYNTHESIS OF NANO POROUS SOL GEL MATRIX AS A MOLECULAR PROBE FOR DISSOLVED OXYGEN

A ruthenium compound was immobilized in an organically modified silicate (ORMOSIL) using sol gel process. Methyltrimethoxysilane (MTMS) was used as the sol gel precursor. Appropriate amount of water and alcohol is added to the precursor to induce hydrolysis and condensation polymerization. Sub ppb levels of DO were able to be detected using the sol gel coating. Organically modified silicate (ormosil) sol-gel thin films have many advantages over their inorganic sol-gel and polymeric counterparts for sensing applications .

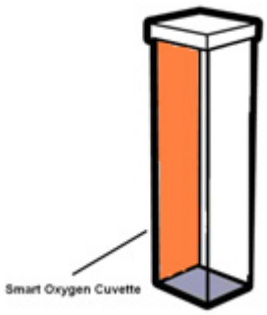
3 MATERIALS AND METHODS

3.1 Cell culture system design (Bioreactor)

An optical system is integrated to monitor the oxygen levels in a bioprocess system in a continuous fashion. The system built is a small scale version of the bioreactor. The integration involves the optical oxygen sensing system with the bioprocess system built to grow cells at constant temperature. Each of the components is described in detail in the following section:

3.2 Smart Oxygen Cuvette

Smart oxygen is a revolutionary oxygen sensing product designed for monitoring the dissolved oxygen in samples for biological and medical applications. Smart oxygen cuvette consists of a sensor coating formulation trapped in a sol gel matrix, immobilized and protected from the package contents. .The cuvette (Glass flourometer cell, Rectangular, Starna Cells Inc, CA) is the cell growth container of the bioprocess system. The Smart Oxygen cuvette has oxygen sensor coating formulation integrated with the cuvette on the inner lining of one of the side as shown in Figure 1



3.3 Qpod

The qpod is a complete sample compartment for fiber optic spectroscopy, including a peltier-controlled cuvette holder with magnetic stirring, and fused silica lens systems with SMA fiber optic connectors. The collimating /imaging/mirror optics enables the collection of rays and focus on the collection fiber. The qpod is equipped with Quantum Northwest TC125 Temperature Controller for temperature control and magnetic stirring to enable the cells in the

cuvette to be in continuous stirring mode. As the cells have to be in a continuous stirring mode in a bioreactor, so magnetic stirring feature enables a good control on the stirring aspect integrated into the system .

3.4 NeoFox

The NeoFox Phase Fluorometer is an instrument platform for measurement of fluorescence lifetime and phase. This frequency domain electronics uses a blue LED excitation and a photodiode for detection. A fluorescence method is used to measure the partial pressure of dissolved or gaseous oxygen. A bifurcated optical fiber carries excitation light produced by the blue LED to the thin-film coating of the Smart Cuvette. Fluorescence generated at the surface of the patch is collected by the probe and carried by the optical fiber to the detector of PF. The phase shift between the blue LED excitation and emission signal of fluorescence is used to calculate the lifetime. The Figure 2 below is a representation of the phase measurement. A new compact phase flourometer, NeoFox developed by Ocean Optics is used in this system design.

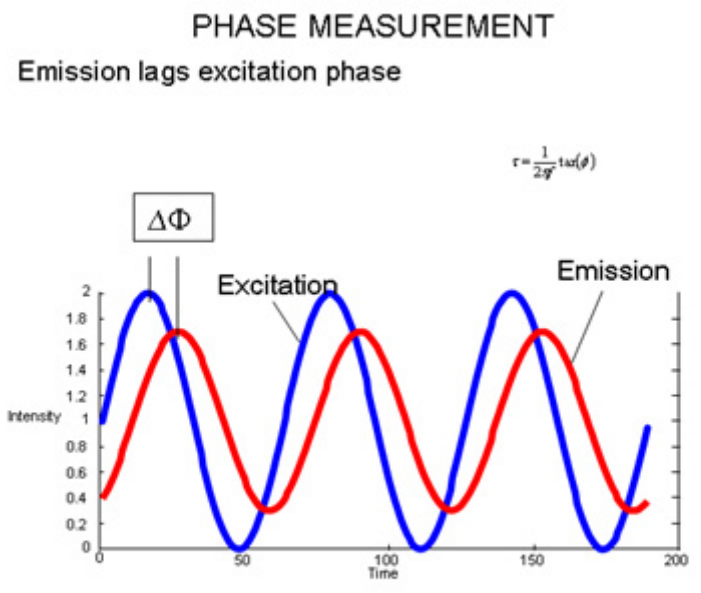


Figure 2 shows the principle of phase fluorometry, the phase difference between the excitation and emission and the relation between phase differences to the lifetime of fluorescence quenching

Oxygen as a triplet molecule is able to quench efficiently the fluorescence and phosphorescence of certain luminophores. This effect (first described by Kautsky in 1939) is called “dynamic fluorescence quenching.” Collision of an oxygen molecule with a fluorophore in its excited state leads to a non-radiative transfer of energy. The degree of fluorescence quenching relates to the frequency of collisions, and therefore to the concentration, pressure and temperature of the oxygen-containing media. When oxygen in the gas or liquid sample diffuses into the thin-film coating, it quenches the fluorescence. The degree of quenching correlates to the level of oxygen pressure.

4 SENSOR CALIBRATION

In order to make accurate oxygen measurements inside the cuvette, the calibration of the Smart Oxygen Cuvette was performed using the Linear (Stern-Volmer) algorithm. Since this experiments were performed at room temperature (~25C), temperature compensation during

the calibration was not required.
Temperature does not affect the fluorescence decay time, fluorescence intensity, collision frequency of the oxygen molecules with the fluorophore, and the diffusion coefficient of oxygen as long as the temperature is maintained between ± 1 °C of the calibrated temperature.

Linear (Stern-Volmer) Algorithm: The Linear (Stern-Volmer) algorithm requires at least two standards of known oxygen concentration. The first standard must have 0% oxygen concentration and the last standard must have a concentration in the high end of the concentration range. The Smart Oxygen Cuvette patch was calibrated at 0% and 20.9% oxygen. The calibration curves were generated from these standards and the linear algorithm was used to calculate oxygen concentration values for unknown samples.

The fluorescence lifetime (?) can be expressed in terms of the Stern-Volmer equation where the fluorescence is related quantitatively to the partial pressure of oxygen:

$$\frac{\tau_0}{\tau} = 1 + k\, p_{O_2}$$

Where t0 is the lifetime of fluorescence at zero pressure of oxygen, ? is the lifetime of fluorescence at a pressure p of oxygen, and k is the Stern-Volmer constant.

For a given media, and at a constant total pressure and temperature, the partial pressure of oxygen is proportional to oxygen mole fraction. The Stern-Volmer constant (k) is primarily dependent on the chemical composition of the sensor formulation. The Stern-Volmer constant (k) is temperature dependent. All measurements should be made at the same temperature (± 1 °C) from the calibration experiments. If temperature compensation is needed, then the relationship between the Stern-Volmer values and temperature is defined as:

$$\tau_0 = a_0 + b_0 * T + c_0 * T^2$$

$$k = a + b * T + c * T^2$$

The lifetime of fluorescence at zero pressure of oxygen depends on details of the optical setup: the power of the LED, the optical fibers, loss of light at the probe due to fiber coupling, and backscattering from the sample. It is important to measure the lifetime of fluorescence at zero pressure of oxygen (I0) for each experimental setup .

5 OPTICAL SENSING SYSTEM INTEGRATION WITH CELL CULTURE SYSTEM

The Smart Oxygen cuvette is placed in a qpod and the side of the cuvette which has the oxygen sensor coated material is interfaced with the bifurcated reflectance probe. The bifurcated reflectance probe is connected to the NeoFox system. The LED source on the NeoFox provides the excitation light and is coupled to one of the legs of the bifurcated optical probe. The reflected florescence light is coupled back into the other leg of the bifurcated probe and terminated at the detector surface on NeoFox. The NeoFox interfaces with the NeoFox Viewer Software (Ocean Optics Inc.) which measures the oxygen levels. The complete system used to measure oxygen levels is shown in Figure 3

5.1 Experimental Setup

The oxygen sensing experiment was carried out in a Smart Oxygen Cuvette. To build a two point calibration, the nitrogen gas is diffused into the cuvette for 0% oxygen and then air is diffused into the cuvette for 20.9% oxygen. The two points are captured and a calibration curve is built to quantify the oxygen levels in the range of 0 – 21% from the life time measurements. We start our experiment by placing Whole goat blood and water (1:1.5) in the cuvette, magnetic stirrer is placed in the cuvette and the stirring speed is set to a maximum using the qpod temperature and magnetic controller interface. The temperature is set at room temperature. The NeoFox viewer software starts logging the data from the instant diluted blood is placed in the cuvette. The oxygen concentration in blood starts at a low concentration of oxygen and increases until almost air saturation. Once the oxygen level increases and is stable, yeast cells are added to the blood in the cuvette. The oxygen quenching is observed over a period of time. After each run all of the dissolved oxygen sensor data is logged. The cuvette is washed and dried and placed back into the qpod for the next run. The experiment is conducted 3 times.

To replicate the bioprocess conditions, nutrients were added to the blood to study the rate of dissolved oxygen in the cell culture media. The experiments were repeated with the yeast cells of 200mgrams. The sensor data was logged for a period of 30 minutes and after each run, the cuvette was rinsed and dried with vacuum for the next run. The experiment was repeated three times.
Another set of experiments was run to study the time it takes to quench the dissolved oxygen in a closed cuvette. Different amounts of yeast were added to the diluted blood and the time it takes to quench the dissolved oxygen is recorded.

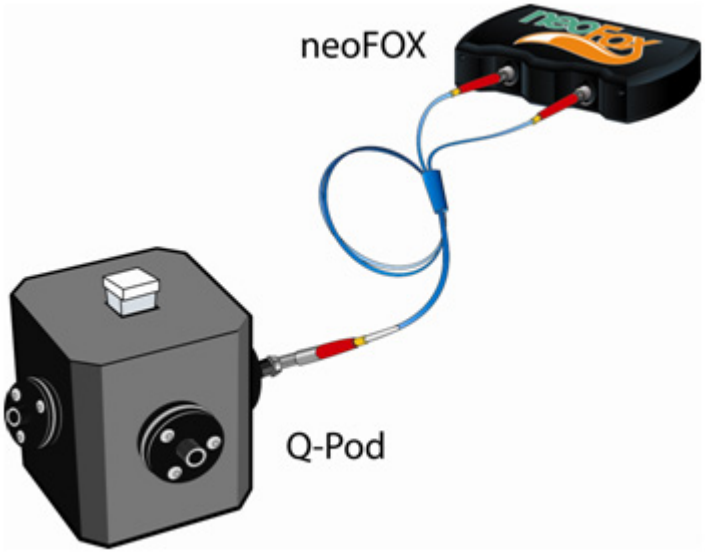


Figure 3 shows the bioreactor setup with oxygen sensor patch interfaced with bifurcated fiber optic probe. The legs of the fiber optic probe are connected to the LED excitation source and detector on the NeoFox. The USB interface on the NeoFox transfers the data to the NeoFox Viewer Software for the data logging process

6 RESULTS AND DISCUSSION

The Smart Oxygen cuvette is a small-scale system used to study the effects on the oxygen partial pressure of the blood sample in the presence of microorganisms in the blood. During the experiment while the blood is diluted with water and added to the cuvette very low concentration of dissolved oxygen is present. Due to stirring the blood in a closed system cuvette the oxygen level in the dissolved blood eventually rises up to air saturation. Once the dissolved oxygen level is stabilized at air saturation, the yeast cells are added to observe the consumption of the oxygen

The yeast cells when dissolved in blood started consuming the oxygen through the liquid cell membrane interface by the diffusion process. The system is calibrated and the dissolved oxygen levels are monitored when the yeast cells are added and the measurements have been carried out for a time period of approximately 30 minutes. The experimental results (n = 3) in Figure 4 show the performance of Smart Oxygen Cuvette in measuring the oxygen levels continuously as the bioprocess happens in the cell culture system. As the cells are consuming the oxygen in the liquid media through diffusion, the oxygen depleted in the liquid media is what the Smart Oxygen Cuvette is really sensing. The same experiment can be extended to a single cell, in a micro fluidic well culture system. The one side of the cuvette has the oxygen sensing coating which measures the oxygen level depleted in the liquid media surrounding the cell. Using the diffusion parameters of the cell, one can calculate the oxygen consumed by each cell. It is observed that adding 200 milligrams of yeast to about 2.5mL of diluted blood can quench the oxygen to approximately 1 % within 20 minutes. The three runs show very similar results as shown in Figure 4.

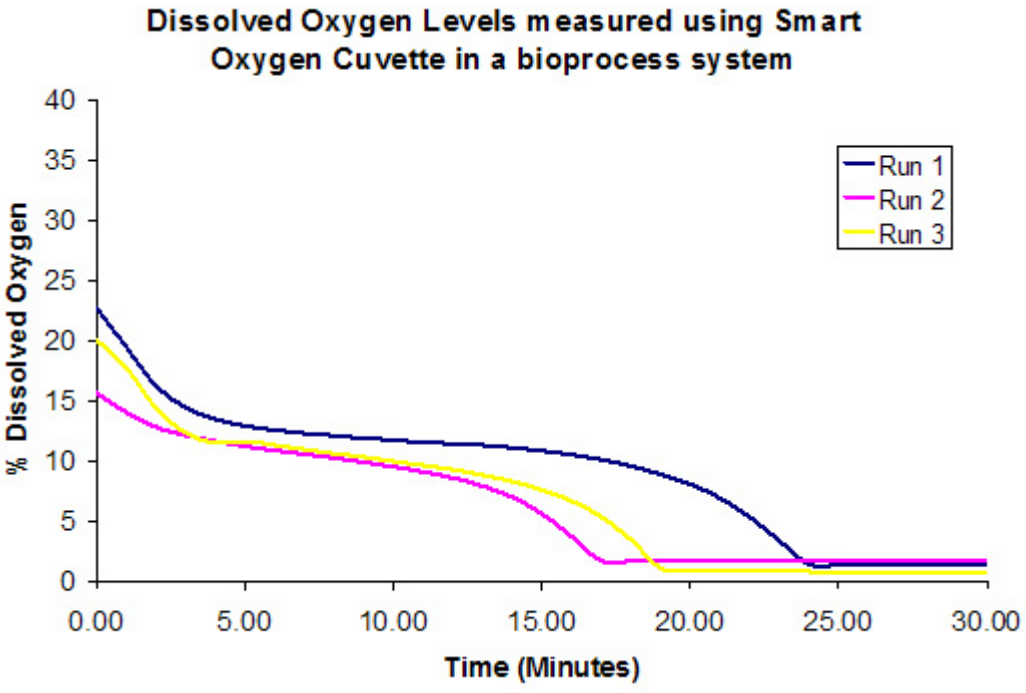


Figure 4 shows the dissolved oxygen levels in a bioreactor system measured using a Smart Oxygen Cuvette

The small scale culture applications have the advantage of the studying the effect of multiple nutrients/environmental conditions on the oxygen levels consumed and also on the process throughput. With an objective to study the performance of the performance of Smart Cuvette

in sensing oxygen levels in the cell culture, we have performed another set of experiment varying the amount of yeast dissolved in blood. The oxygen is consumed by the cells faster if the amount of cells is more. Figure 5 shows the performance of Smart Oxygen Cuvette in measuring the dissolved oxygen levels in cell culture environment with different yeast amount added to diluted blood.

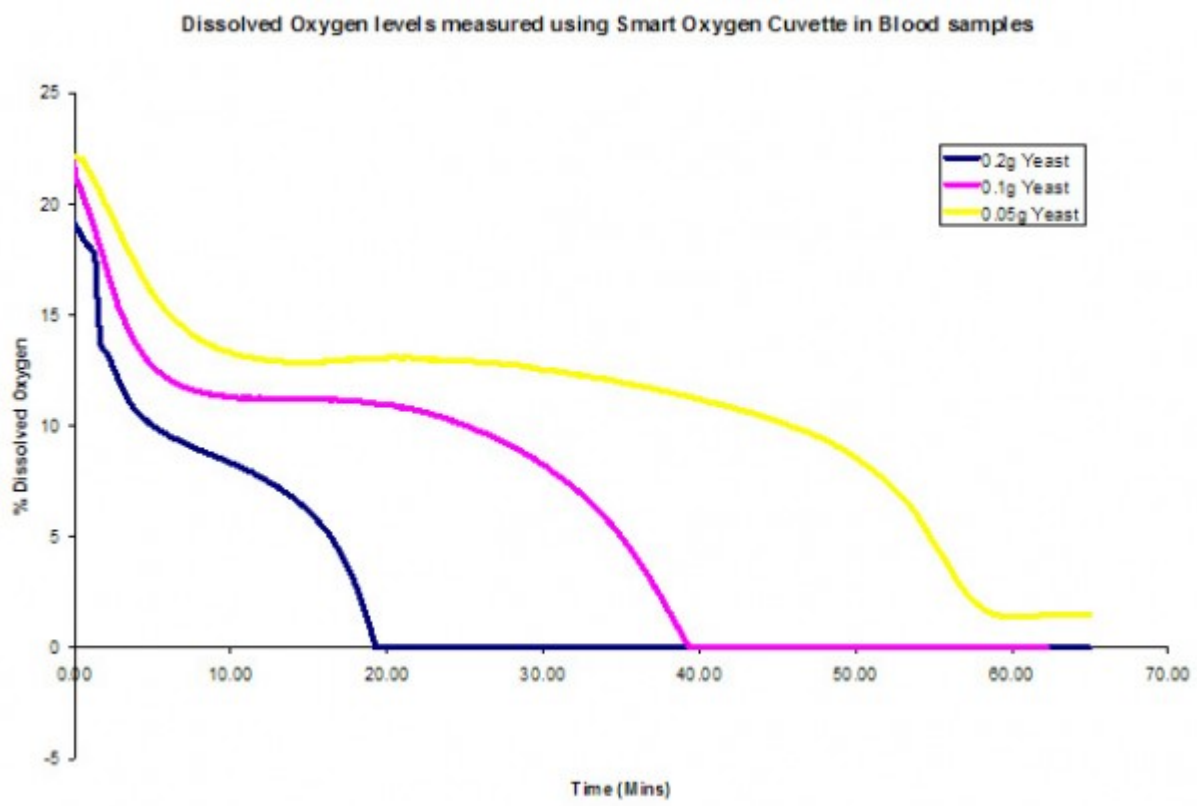


Figure 5 shows the oxygen consumed by cells when different amounts of yeast is added to diluted blood as measured by Smart Oxygen Cuvette

7 CONCLUSION

A Smart Oxygen cuvette is reported to provide superior measurements of dissolved oxygen in important biological experiments such as in blood culture/bioreactor systems. The integration of Smart oxygen cuvette when combined with advanced phase fluorometry can be used to develop portable systems to measure presence of bacteria in different blood cultures. The fluorescent technology based on oxygen quenching has already proven it success in the mycobacterial growth indicator(TB test) and is used to accurately identify mycobacteria .Development of a cost effective system integrated with multiplexing capabilities would open a new approach to study the presence of microorganisms in blood culture system. As healthcare costs are rising and especially with the increasing incidence of TB cases, the proposed system can be used in the preventive healthcare to diagnose the presence of bacteria at an early stage from blood sample. Systems of this nature would accelerate the intervention procedures and facilitate the reduction of healthcare costs.