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Performance of brain tissue oxygen probes in vitro under varying physiological conditions

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Thesis

PERFORMANCE OF BRAIN TISSUE OXYGEN PROBES IN VITRO UNDER VARYING PHYSIOLOGICAL CONDITIONS

by

SAUMYA GUPTA

B.S., University of California San Diego, 2015

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Approved by

First Reader ______________________________
James Holsapple, M.D
Associate Professor of Neurosurgery and Pediatrics

Second Reader ______________________________
Zhaohui Aleck Wang, Ph.D.
Associate Scientist of Marine Chemistry and Geochemistry

DEDICATION

I would like to dedicate this work to my supportive parents and my dearest little sister.

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I would like to thank Dr. Holsapple for his guidance and mentorship this year. I am grateful for this research opportunity that Dr. Holspple has provided me to grow as a scientist and a future physician.

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PERFORMANCE OF BRAIN TISSUE OXYGEN PROBES IN VITRO UNDER VARYING PHYSIOLOGICAL CONDITIONS

SAUMYA GUPTA

ABSTRACT

Patients with Traumatic Brain Injuries (TBI) commonly experience periods of brain tissue hypoxia which can result in secondary brain injuries and worse outcomes during recovery. Currently, the Integra® Licox® brain tissue oxygen monitor is an FDA-approved clinical tool that is used in clinical practice to monitor brain tissue oxygenation ($PbtO_2$) to allow early intervention practices in the patients. The Licox probe is designed as a Clark electrode which measures the oxygen partial pressure (pO_2) in aqueous solutions by reducing oxygen gas into water. The current measured created by the reduction reaction is proportional to the oxygen partial pressure. There have been many studies that have tested the ability of the Licox probe to accurately measure the pO_2 value in vitro and there is a large debate about the probe's reability and stability under changing conditions in the injured brain. TBI patients undergo major metabolic changes during the course of recovery which alters the chemical environment of the cerebrospinal fluid (CSF) fluid. In order to confidently rely on the Licox system for clinical usage an understanding of its behavior under varying conditions must be tested.

Our study focused on the performance of the Licox probe under varying physiologic conditions of salinity, pH, temperature, and hydrostatic pressure. We

created a isolated experimental chamber where these conditions were manipulated in a controlled manner to observe the effects of these variables on in vitro Licox pO_2 values.

The results in our study show that the Licox is not sensitive to physiologic variations in pH, salinity, and hydrostatic pressure. In saline solutions with pO_2 values between 20-40mmHg, with other variables constant, as hydrostatic pressure was raised in the closed chamber from 0-70mmHg, there were no observable changes in the Licox pO_2 readout. In solutions with pO_2 values of 150mmHg with other variables constant, as salinity was increased there was no change in the Licox pO_2 readout and the value remained at 143.66mmHg, 95% CI [143.01, 144.32]. Lastly, in solutions with pO_2 value of 35mmHg, the pO_2 value remained at 34.33mmHg 95% CI [34.0, 35.3] across the different pH values tested while other variables were held constant.

The Licox probe was found to be sensitive to temperature for solutions with pO_2 values between 20-40mmHg. The Licox pO_2 rose by every degree with 95% CI [0.780, 0.977] ($p<0.001$). In the 20mmHg pO2 solution, the rise in Licox pO2 value was also found to be statistically significant. The Licox pO_2 also rose by every degree with 95% CI [0.864, 0.998] ($p=0.0003$).

The Licox probe measures pO_2 with acceptable accuracy under certain conditions, however there are significant observable changes in Licox pO_2 readouts under varying temperatures. These changes are especially of concern at the pO_2

value of 20mmHg as many clinical intervention decisions are made at this critical threshold value. There is a possibility these changes in pO_2 values may be explained by changes in dissolved oxygen concentrations which are simultaneously occurring in the solution due to fluctuations in temperature. In a clinical setting these results are important as they can help clinicans decide if the observed changes in pO_2 values can be attributed to actual changes in brain tissue oxygenation rather than fluctuations caused by Licox probe calibrations or changes in total dissolved oxygen content.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ILLUSTRATIONS

LIST OF ABBREVIATIONS

CSF	Cerebrospinal Fluid
GCS	Glasgow Coma Scale
ICP	Intracranial Pressure
O_2	Oxygen gas
$PbtO_2$	Brain Tissue Oxygen
PO_2	Partial Pressure of Oxygen
$SjVO_2$	Jugular Venous Oxygen
TBI	Traumatic Brain Injury

CHAPTER ONE

Traumatic Brain Injury Morbidity and Pathology

Traumatic Brain Injuries (TBI) are injuries that alter brain function. They are usually caused by a blow or jolt to the head or from penetrating injuries into the cerebral cavity (CDC, 2022). TBIs can be categorized in three of the following categories, mild, moderate, and severe as described by the Glasgow coma scale (GCS) (CDC, 2022). Data from 2020 recorded about 64,000 deaths that were related to traumatic brain injuries. Traumatic brain injuries can cause health problems that persist for days to weeks to a lifetime (CDC, 2022). Traumatic Brain Injury can be classified with its two phases of pathology (Stocchetti, et.al., 2017). The primary insult is created by the impact or collision of the skull by an outside force and can form skull fractures, hematomas, and destruction of brain tissue (Stocchetti, et.al., 2017). Secondary injuries can result from failing to meet the new increased anaerobic metabolism, and energy substrate depletion due to hypoxemia, hypercapnia, arterial hypotension, hyponatremia (Stocchetti, et.al., 2017). A majority of TBI research work is focused on detection and prevention of these secondary injuries that can occur while patients are in recovery.

Past Therapies

Even after many years of research work dedicated to TBIs no standard or accurate treatment has been established (Ngwenya, et.al., 2016). Most of the limitations occur as it is difficult to obtain information about the injured brain

(Ngwenya, et.al., 2016). One of the most common measurements made in TBI patients is intracranial pressure (ICP) (Ngwenya, et.al., 2016). The Monro-Kellie doctrine shows that intracranial pressure is a measure of brain tissue amount, Cerebrospinal Fluid (CSF), and blood found in the skull (Ngwenya, et.al., 2016). ICP can tell about the perfusion pressure in the skull and indirectly tells the amount of oxygen perfused to the brain (Ngwenya, et.al., 2016). However, ICP does not provide information about cerebral oxygenation, cerebral metabolism or cerebral blood flow (Ngwenya, et.al., 2016).

Many research studies have been dedicated to searching for other methods to detect, prevent, and treat the TBI and any injuries that may follow suit (Leach, et.al., 2021). Past research emphasized intracranial hypertension, a risk factor found in severe TBI (Leach, et.al., 2021). Intracranial hypertension creates hypoperfusion and induces brain tissue hypoxia that can cause further brain injury (Leach, et.al., 2021). The brain requires an appropriate amount of blood flow to meet metabolic demands as it does not have glucose or oxygen storage reserves (Leach, et.al., 2021). Techniques such as positron emission tomography, magnetic resonance spectroscopy, jugular venous oximetry, near-infrared spectroscopy, and cerebral microdialysis have shortcomings that make them not reliable to be used as therapy monitors for severe TBI (Leach, et.al., 2021).

New Oxygen Therapies

Ischemia and hypoxia are one of the more prevalent problems often found following TBIs and therefore increase deaths due to head injuries (Patchana, et.al.,2020). Longer periods of ischemia in the brain tissue is associated with worse outcomes for TBI patients (Karlis et.al.,2009). To bypass this, brain tissue oxygenation recording was brought up in the 1990s. Prior to this, jugular bulb oximetry was used to assess the cerebral hypoxia (Patchana, et.al.,2020). Jugular venous oxygen saturation (SjvO2) measures the venous blood oxygen that is exiting the brain to assess the brain's oxygen usage (Hirschi, et.al.,2019).

In 1993, a new brain tissue oxygenation system created by Integra® Licox® was placed into clinical settings (Integra® Lifesciences, Plainsboro Township, New Jersey) (Patchana, et.al.,2020). The Licox system integrates both temperature and oxygen measurement in one probe (Patchana, et.al.,2020). The new monitor also comes with an automated card calibration system so no manual calibration is required by the clinicians (Stewart et.al.,2008). The patients diagnosed with severe TBI are placed on the oxygen probes to monitor the brain tissue oxygen and guide treatment (Patchana, et.al.,2020).

Newer therapies developed for adult traumatic brain injury include monitoring brain tissue oxygen ($PbtO_2$) as it can be an important marker to direct the treatment (Zeiler, et.al, 2021). Oxygen probes measuring ($PbtO_2$) are inserted into the brain and recordings of extracellular oxygen are taken (Zeiler, et.al., 2021). Many studies suggest low $PbtO_2$ has a correlation with worse outcomes of adult TBI

(Zeiler, et.al., 2021). Normal established values $PbtO_2$ are 23 ± 7 mm Hg (Okonkwo, et.al.,2017). After a few days of an episode of TBI the $PbtO_2$ values can decrease to <20mmHg in over 70% of the patients (Okonkwo, et.al.,2017). It has been found that the fall of $PbtO_2$ values below this threshold have significant impact on the recovery of TBI patients (Okonkwo, et.al.,2017). It is hypothesized that strict management of $PbtO_2$ and appropriate intervention can improve the prognosis of TBI patients(Okonkwo, et.al.,2017). The critical question is how to detect the $PbtO_2$ values accurately and how to interpret the values to provide the optimal care for patients.

CHAPTER 2

Licox Clark Sensor

The Licox biosensor is a Clark Electrode that detects the partial pressure of oxygen (pO_2) via electrochemical reaction involving oxygen. The Clark Electrode contains a O_2 permeable Teflon membrane, auxiliary electrodes, and an intracellular electrolyte (Wei, et.al.,2019), also shown in Illustration 1(Cunningham, 2013). The selective oxygen permeable membrane allows avoidace of other factors that may disrupt the current signal (Niazi, 2016). This also aids the sensor's measurement function when placed in biological fluids (Niazi, 2016).

A voltage is applied to the electrodes and oxygen molecules are reduced as they pass through the selective Teflon membrane via a diffusion current which creates a measurable electrical current (Wei, et.al.,2019). The oxygen in the solution diffuses through the Teflon membrane and gets reduced at the cathode (Niazi, 2016). The flow of current between the anode and cathode is under diffusion limited current conditions (Niazi, 2016). The relationship between the current and oxygen concentration is linear. The lower the oxygen concentration the lower the current and vice versa. The linear relationship between oxygen tension (pO_2) and current is known to be variable with temperature (Steward et.al., 2008). Each of the Licox probes comes with its own current-temperature function which is formulated when the probe is manufactured and formula is then placed into the smart card for calibration (Steward et.al., 2008).

The design of the clark electrode system in an electrolyte solution is shown in Illustration 1 (Cunningham, 2013).

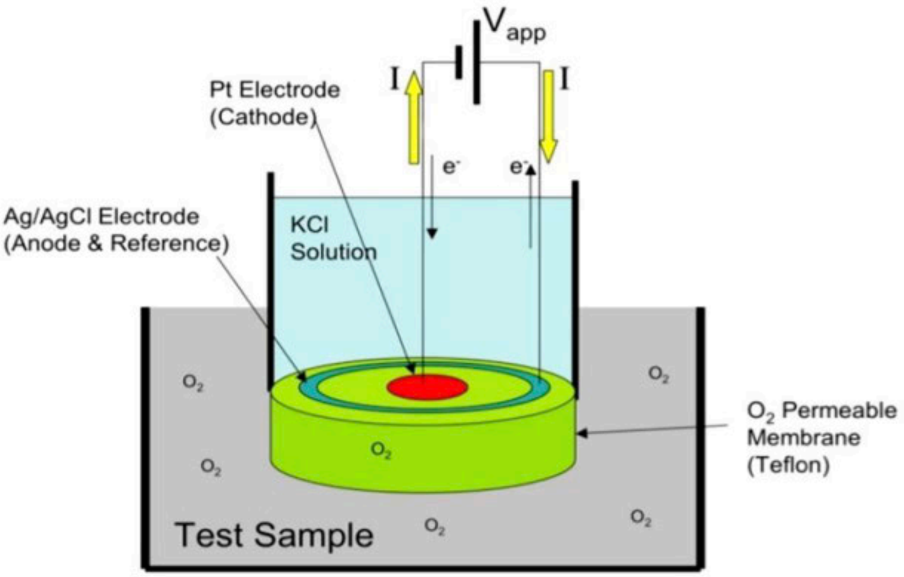

Illustration 1: Simplified Design of Clark Electrode.
This figure shows the mechanism behind the electrode. The illustration depicts the O2 permeable Teflon Membrane where oxygen gas molecules pass through via a diffusion gradient. The electrode has a Ag/Cl electrode on the Anode and a Pt electrode on the Cathode. The Anode and Cathode are placed in a KCl solution.
Illustration Sourced from: Cunningham, B., [Illinois] ECE 416 Electrochemical Sensors. 2013.

The reduction oxygen is demonstrated in the following equations below.

$$O_2 + 2H^+ + 2e^- \rightarrow H2O2 \quad (1)$$

$$H2O2 + 2H+ + 2e- \rightarrow 2H2O \quad (2)$$

$$O2 + 4H+ + 4e- \rightarrow 2H2O2 \quad (3)$$

Illustration 2: Reduction reaction equations of oxygen in the Clark Electrode.

Source: Niazi A. Real Time Measurement of Oxygen by Integrating a Clark Sensor with Low Cost Printed Circuit Board Technology and Solid Electrolyte Membrane. University of Brimingham; 2016. http://etheses.bham.ac.uk/id/eprint/6817

Oxygen is first converted to hydrogen peroxide and then further reduced to water as show in the first two equations, equations (1) and equation (2) (Niazi, 2016). Equation (3) shows the net reaction of the reduction of water from the first two equations.

Previous Licox Studies

There have been two other studies that have focused on the study of the accuracy of the Licox probes in a in vitro model. The first study by Stewart et. al focused on the accuracy of the Licox to measure the partial pressure of oxygen or oxygen tension (pO_2) and the accuracy of the Licox temperature probe. The Stewart et. al study used 2-chamber apparatus where oxygen gas of known partial pressure was passed through distilled water (Stewart et.al.,2008). The humidfied gas was then passed into a second chamber where the oxygen concentration was recorded by the Licox probe (Stewart et.al.,2008). Oxygen partial pressures of 2.5%, 9.7%, and 21% were tested (Stewart et.al.,2008). The study results indicated that Licox probes had good accuracy with the mean relative errors reported as -3.8+/- 3.5% for every oxygen partial pressure tested at the various temperatures, the difference had statistical significance ($p<0.001$) (Stewart et.al.,2008). With every increase in temperature, the Licox probe tended to under read the oxygen tension with a significance of $p=0.02$ for the difference between the 39^{o}C and 33^{o}C groups (Stewart et.al.,2008).

Since the Licox probe is designed as a Clark Polarographic Electrode, the detection of oxygen tension depends on the electrochemical reaction, with it's rate limiting step as the diffusion time of O_2 to travel to the electrode (Stewart et.al.,2008). So, it has been an important marker to study the response times of the Licox probes to changes in oxygen tension. The Stewart study found that the average response times for the Licox probes is about 113+/- 22 seconds (Stewart et.al.,2008). In two different solutions where the difference in oxygen gradient is larger, the Licox probe was slower to response since more time was needed to complete the oxygen diffusion (Stewart et.al.,2008). Another important issue the study addressed was the higher lipid content and viscosity of brain fluid versus the distilled water used in the study. The Licox probe response times were re-tested in a solution of oil and water (Stewart et.al.,2008). The results indicated that the response time of the Licox probe was slower in the solution with oil because of the reduce rate of oxygen diffusion (Stewart et.al.,2008). These results are clinically significant as clinicians need to follow the pO_2 changes over a longer period of time as the Licox probe does not reflect immediate changes in pO_2 that may be occurring in the TBI patients during recovery.

A second study by Karlis et. al also focused on the in vitro accuracy of the Licox probe against another brain tissue oxygen monitor, the Neurovent-PTO. The two oxygen probes were tested in a container filled with a buffer solution (Karlis et.al.,2009). The buffer solution was then equilibrated with different partial pressures of oxygen at $37^{o}C$ (Karlis et.al.,2009). The study found that the Licox pO_2 mean values were (0.1-0.42mmHg) from the calculated pO_2 value with a high standard deviations of (0.71-1.33) (Karlis et.al.,2009). The Karlis et. al study also studied the response times of the Licox probe in solutions with a low pO_2 value of 10mmHg and a high pO_2 value of 40mmHg. The study found that the response times for the Licox sensor from low to high oxygen partial pressure was 78.2 +/- 21s (Karlis et.al.,2009). The response time for the Licox sensor from high to low oxygen partial pressure was much longer with values reported as 215+/-63s (Karlis et.al.,2009). This study is in congruence with the Steward et. al study as it shows the significance of the long Licox response times.

Lastly, another recent clinical study focused on the effect of temperature on brain tissue oxygen tension in patients with traumatic brain injuries. The study attempted to correlate changes in brain tissue oxygenation ($PbtO_2$) with increases in temperature (Rass et.al.,2021). The study found that overall the ($PbtO_2$) increased ($\Delta P_{bt}O_2 = 0.9 \pm 6.1$ mmHg, $p = 0.022$) when TBI patients experienced episodes of temperature increases (Rass et.al.,2021). The figure below shows the mean $PbtO_2$ values were higher at higher temperatures ($p<0.001$) (Rass et.al.,2021). This trend is clinically important as at the critical threshold value of $PbtO_2$ at 20mmHg any increases in $PbtO_2$ may be falsely interpreted as increase in brain tissue oxygenation rather than just an effect of temperature. The decisions to actively intervene and restore brain tissue hypoxia are conducted at this critical value, so it is important to detect any variations in the Licox pO_2 levels at this value. Currently, it is unknown whether these changes in $PbtO_2$ are due to Licox probe calibrations with temperature or if these $PbtO_2$ are reflective of chemical changes that may be occurring in the injured brain.

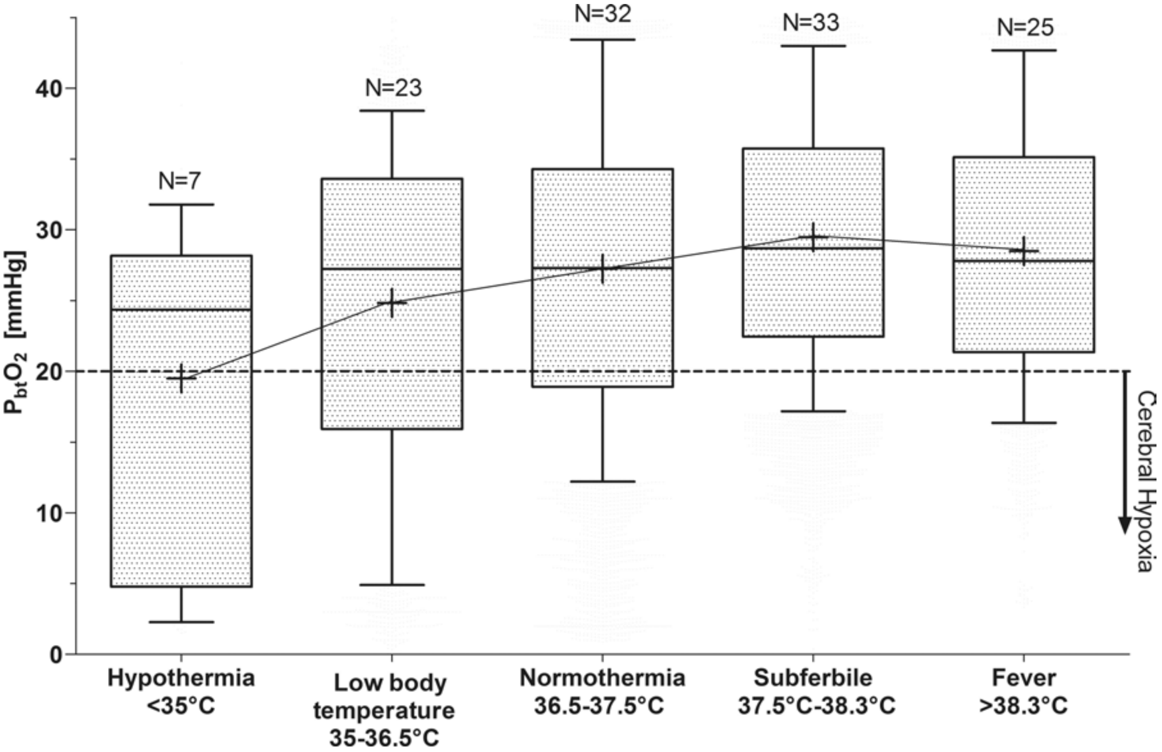

Figure 1: Mean PbtO$_2$ values at various temperatures

The figure shows the mean PbtO$_2$ values were higher at higher temperatures ($p<0.001$) (Rass et.al.,2021).

Source: Rass V, Huber L, Ianosi BA, et al. The Effect of Temperature Increases on Brain Tissue Oxygen Tension in Patients with Traumatic Brain Injury: A Collaborative European NeuroTrauma Effectiveness Research in Traumatic Brain Injury Substudy. *Therapeutic Hypothermia and Temperature Management*. 2021;11(2):122-131. doi:10.1089/ther.2020.0027

Dissolved Oxygen Concentration

While the Licox can indicate the partial pressure of oxygen pO_2 in (mmHg) in the aqueous solution, it does not entirely reflect the dissolved oxygen concentration (mg/L) or oxygen solubility within the solution.

Henry's law states that at a given temperature, the concentration of the dissolved oxygen in the solution is proportional to the partial pressure of the atmosphere above it (Alberty, et.al.,1996). The proposed relationship can be seen by the equation below.

$$P_i = y_i P = K_i x_i \text{ (Niazi, 2016)}$$

Source: Niazi A. Real Time Measurement of Oxygen by Integrating a Clark Sensor with Low Cost Printed Circuit Board Technology and Solid Electrolyte Membrane. University of Brimingham; 2016. http://etheses.bham.ac.uk/id/eprint/6817

The equation shows the the partial pressure of a gas P_i (Pa), is equal to the molar fraction of that specified gas y_i (%), multiplied by the gas pressure of all the gases P (Pa) (Niazi, 2016). The Pi (Pa) is equal to the amount of dissolved oxygen in a solution multiplied by the measured constant K_i (Pa) for the different gases and solutions (Niazi, 2016). The equation can be use to determine the concentration of dissolved oxygen in a solution at equilibrium (Niazi, 2016).

The Licox sensor focuses on the partial pressure of oxygen, pO_2. Other polarographic dissolved oxygen sensors such as the Thermo Fisher Orion Probe, input established dissolved oxygen trends within the sensor software to compensate for dissolved oxygen concentrations changes due changing parameters such as temperature, pressure, and salinity.

Real time measurement of dissolved oxygen concentration (mg/L) is not easily achievable (Wei, et.al.,2019). So, the dissolved oxygen probes are corrected and compensated for changing parameters of temperature, pressure, and salinity.

Traditional Clark Electrodes without these compensations detect only the partial pressure of oxygen in the gas phase within the solution. They do not measure the amount of oxygen that is in the liquid phase along with the solution. The new intelligent dissolved oxygen sensors do deal with the influencing parameters (Wei, et.al.,2019).

The dissolved oxygen concentration is affected the most due to changes in temperature of the solution (Wei, et.al.,2019). Many of the dissolved oxygen sensors come with temperature compensation technologies so that they can be used for real time monitoring of oxygen solubility (Wei, et.al.,2019). The dissolved oxygen sensors use an automatic preset temperature compensation and correction model in the microprocessor.

The solubility of oxygen in water is also affected by changes in salinity and must be compensated for as well (Wei, et.al.,2019). However, most of the dissolved oxygen sensors do not have automatic salinity compensation and rely on artificial input of the salinity values (Wei, et.al.,2019).

Dissolved oxygen sensors still need to be improved in order to detect real time oxygen solubility changes due to changing conditions involving pressure, temperature, and salinity (Wei, et.al.,2019).

Dissolved oxygen concentration provides the truest measurement of the brain tissue oxygen perfusion, so it is important find the relationship between dissolved oxygen concentration (mg/L) and the pO_2. These relationships bring up an important debate on whether both dissolved oxygen concentrations (mg/L) and pO_2 need to be monitored and if these measurements would provide valuable pieces of information to improve treatment protocols for TBI patients.

SPECIFIC AIMS

To determine if the Licox brain tissue oxygen ($PtbO_2$) monitoring can be a reliable method of therapy guidance for recovering traumatic brain injury patients the following aims will be investigated:

Aim 1. Determine the performance of the brain tissue oxygen monitoring system in reading partial pressure of oxygen (pO_2) in vitro under varying physiologic conditions of temperature, pH, salinity, and hydrostatic pressure.

Aim 2. Determine if the current brain tissue oxygen monitoring system can provide a reliable framework of the brain's oxygen usage.

Aim 3: To provide a more streamlined and standardized method of using brain tissue oxygenation levels ($PbtO_2$) to enhance recovery of TBI patients.

METHODS

To study the effects of Licox performance under varying conditions, a closed experimental chamber was created to accurately measure in vitro pO_2 values of aqueous solutions under altered physiological conditions of pressure, temperature, pH and salinity.

Creating Different pO_2 Concentrations

Three gas tanks, N_2, O_2, and CO_2 were individually attached to 3 mass flow meters. The flow meters allowed a calculated pO_2 value to be bubbled into a 1500ml saline and bicarbonate buffer solution in an erlenmeyer flask. The gasses were bubbled into the saline solution for 1 hour of equilibration time. Using a hot plate, the erlenmeyer flask was heated to a temperature of 37 degrees Celsius while the gasses were bubbled.

This step was repeated to every pO_2 value that was used in the study.

Closed Experimental Chamber

The bubbled saline buffer solution with the designated pO_2 value was then pumped from the erlenmeyer flask into the closed experimental chamber using a peristaltic pump. The solution was pumped through twice into the chamber to remove any room air from the empty chamber. The experimental chamber was placed into a 1000mL jacketed beaker attached to a water bath set to 37 degrees Celsius. After the solution was pumped, the chamber was sealed off from the atmosphere. The experimental chamber contained ports for Licox pO_2 Probe, the GE solar pressure catheter, Thermofisher dissolved oxygen probe, peristaltic pump tubing, and a pressure release port, as shown in Figure 3. The Licox probe was kept in the sealed experimental chamber for 15 minutes to allow for equilibration and stabilization, before beginning experiments.

Pressure Experiments

To test the licox performance against changes in hydrostatic pressure whilst mainting the temperature, pH and salinity of the solution, the peristaltic pump was used to precisely and incrementally create positive pressure into the closed chamber. For the first set of experiments, the pressure was raised incrementally by 1mmHg until 70mmHg of hydrostatic pressure was reached. For the second set of experiments the pressure was raised quickly from 0 to 70 mmhg within 10-15seconds. The pressure recordings were measured using the GE Solar Arterial pressure measuring system and the Integra® Camino® ICP measuring monitor.

Temperature Experiments

To test the licox performance against changes in temperature whilst mainting the pressure, pH and salinity of the solution, the solution was first prepared at 37 degrees Celsius and placed in the water bath at 37 degrees Celsius. The water bath was then set to 41 degrees Celsius to heat the system. Previousy boiled water was placed in the jacketed beaker to speed up the heating process. The system was heated from 37 degrees Celsius to 41 degrees Celsius in about 15 minutes.

The system was kept at 41 degrees Celsius for 15 minutes to allow equilibration time. The experimental chamber was then placed into a ice bath to cool the system back down.

A thermometer was placed in the water bath to monitor temperature. The Thermofisher DO probe was also used as a secondary device to monitor the temperature within the experimental chamber.

Salinity

The Licox system performance was tested against changes in salinity, as pH, temperature, and pressure were held constant. Three separate buffer solutions with salinities of 150meq/L, 500meq/L, and 1000meq/L were created at the same pO_2 value of 145mmHg. The Licox system was tested in each saline buffer environment separately, set at the same temperature, pressure and pH value.

pH

The Licox system performance was tested against changes in pH, as temperature, pressure, and salinity were held constant. Three separate solutions with pH of 7.25, 7.35, and 7.45meq/L were created at the same pO_2 value of 35 mmHg. Hydrochloric Acid (HCL) and Sodium Hydroxide (NaOH) was used to create the different solutions set at the various pHs. The Licox system was tested in each different pH saline buffer environment separately, set at the same temperature, pressure and salinity value.

Response Times

The Licox probes were tested for their response times to changes in pO_2 in the aqueous saline buffer solution. In a 12mL glass vial with a sealed rubber end cap, the licox probe was inserted. Two other ports, one for the peristaltic pump and one for fluid drainage was inserted into the rubber cap.

For the first set of experiments. A saline buffer solution with a pO_2 value of 10mmHg was pumped into the empty vial using the peristaltic pump set at 100ml/min. Food coloring was placed into the vial as well. The licox probe was left in the vial for 5 minutes to equilibrate and stabilize. Next, in the same vial, a clear saline buffer solution with a pO_2 value of 40 mmHg was pumped through using the peristaltic pump. The food coloring was used to watch the washout period between the two solutions. The transition period between the two solutions was about 18 seconds. As soon as the new solution began pumping through, a stopwatch was used to monitor the Licox probe to reach 90% of the new pO_2 value.

The same procedure was repeated in the opposite direction, where initially the vial began with a solution at pO_2 of 40mmHg and then a solution pO_2 of 10mmHg was pumped through the vial to monitor Licox response time.

Results

The results were recorded by all three systems, Licox® Integra®, Integra® Camino® ICP monitor, and the GE solar arterial pressure measuring system while the experiments were conducted. R studio was used for statistical analysis and data collection.

Methods: Set Up

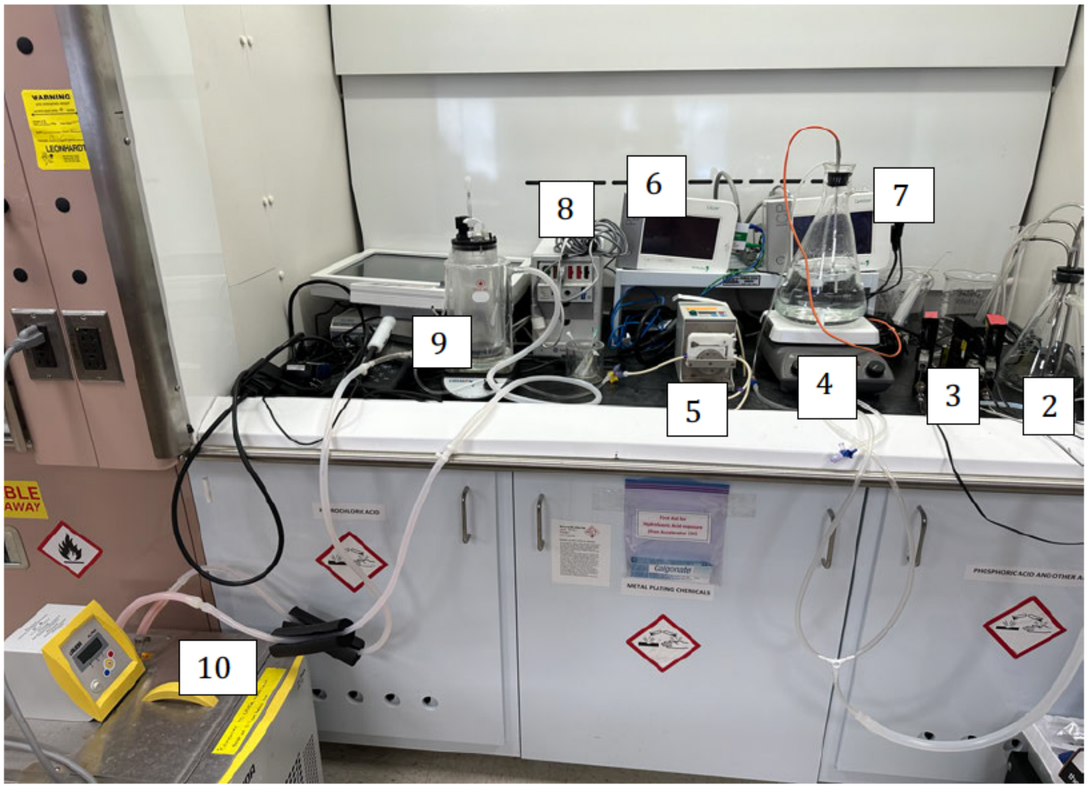

Figure 2: The experimental set-up

The experimental set-up for in vitro testing of Licox monitoring system showing the gas tanks (1), the gas mixing and collecting chamber (2), the mass flow meters (3), the saline and gas bubbling chamber (4), the peristaltic pump (5), and the experimental chamber inside the water jacket (9). The experimental chamber was heated using the water bath (10). Licox monitor (6) collected the pO_2 data, ICP Integra monitor (7) collected the pressure data within the chamber, and the GE solar (8) was a secondary monitor to collect pressure data.

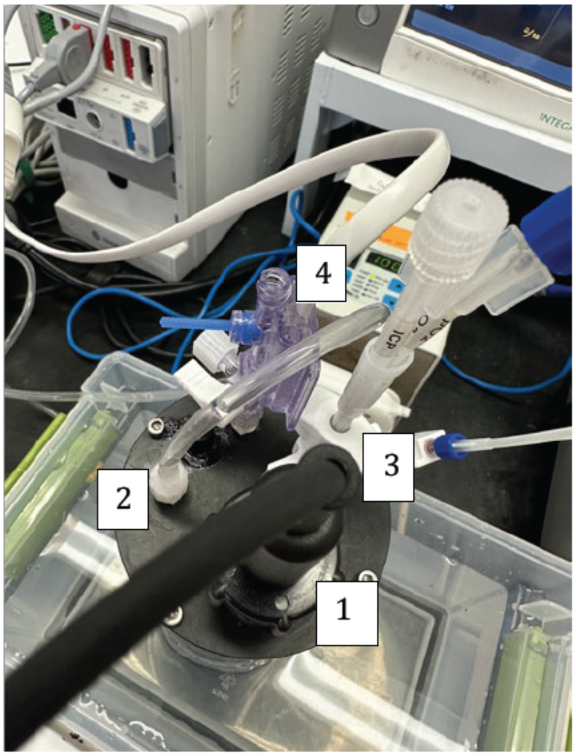

Figure 3: Experimental Chamber

This figure shows the ports in the experimental chamber with the Thermofisher DO Probe (1), the peristaltic pump connection (2), Licox probe (3), and GE solar catether (4)

RESULTS

Pressure Experiments

The performance of the Licox system was tested against changes in hydrostatic pressure to observe any changes in pO_2 correlated with fluctuations in pressure. Saline buffer solutions of three different pO_2 values, 20mmHg, 35mmHg, and 50mmHg, were tested against hydrostatic pressure. Three trials were performed for each pO_2value. For the solution set at pO_2 value of 20mmHg, as hydrostatic pressure was raised from 0mmHg to 70mmHg, the Licox pO2 value remained at 18.33mmHg, 95% CI [17.68mmHg,18.98mmHg]. For the solution set at pO_2 value of 35mmHg, as hydrostatic pressure was raised from 0mmHg to 70mmHg, the Licox pO_2 value remained at 34.33mmHg, 95% CI [33.68, 34.99]. Lastly, the solution with pO_2 value set at 50mmHg, as hydrostatic pressure was raised from 0mmHg to 70mmHg, the Licox pO2 value remained at 48.0mmHg, 95% CI [46.87, 49.13]. As shown in Figure 4, the results show no significant observable changes ($p>0.05$) in the Licox pO_2 in all three solutions during increases in hydrostatic pressure.

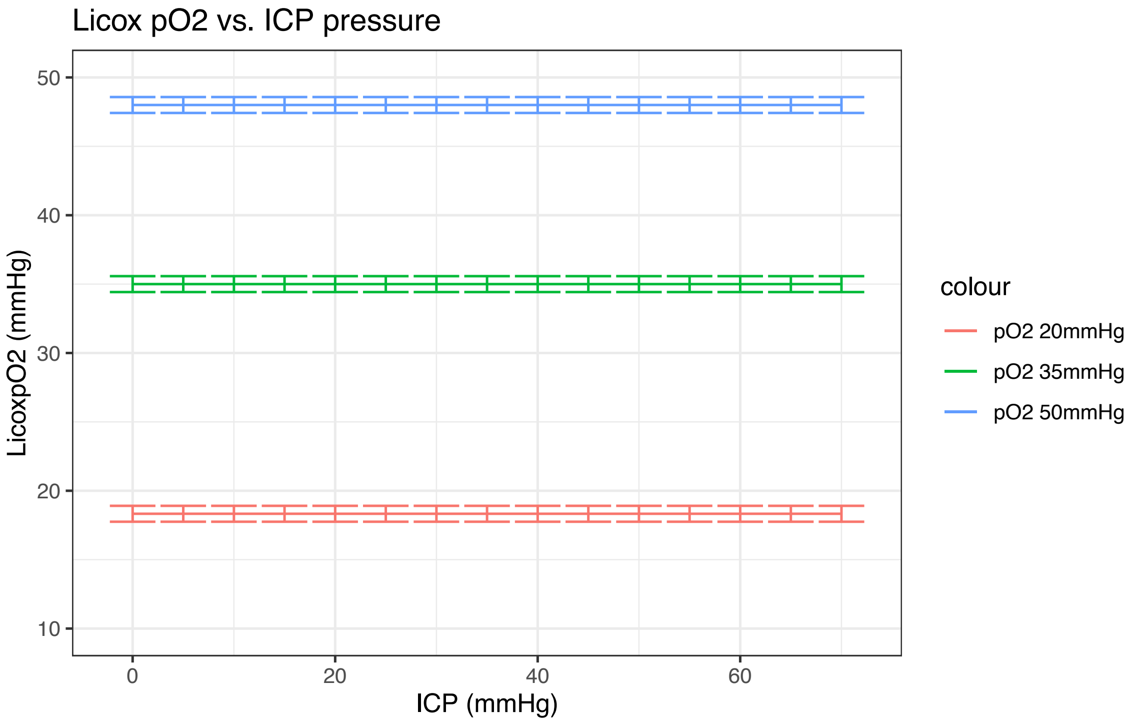

Figure 4: Graph of Licox pO_2 vs. ICP

This graph depicts changes in Licox pO_2 values, in solutions set at a oxygen partial pressure of 20mmHg, 35mmHg, and 50mmHg, as the hydrostatic pressure was increased from 0mmHg to 70mmHg by increments of 1mmHg. For the three different solutions the Licox pO_2 values remained at 18.33mmHg, 95% CI [17.68mmHg,18.98mmHg]; 34.33mmHg, 95% CI [33.68, 34.99; and 48.0mmHg, 95% CI [46.87, 49.13]. The graph indicates no significant observable changes in the Licox pO_2 in all three solutions during increases in hydrostatic pressure.

Temperature Experiments: Heating

The performance of the Licox system was tested against changes in temperature to observe any pO_2 changes correlated with changes in temperature. Two different solutions prepared at pO_2 values of 40mmHg and 20mmHg were tested. Two trials were performed for each different pO_2 solution. The results for both pO_2 solutions showed an increase in Licox pO_2 values with an increase in temperature, as shown in Figure 5 and Figure 6 respectively. In the 40mmHg pO2 solution the rise in Licox pO_2 value was found to be statistically significant ($p<0.001$). The Licox pO_2 rose by every degree with 95% CI [0.780, 0.977]. The Licox In the 20mmHg pO_2 solution, the rise in Licox pO_2 value was also found to be statistically significant ($p=0.0003$). The Licox pO_2 rose by every degree with 95% CI [0.864, 0.998]. The results indicate a correlation between the Licox pO_2 and a increase in temperature.

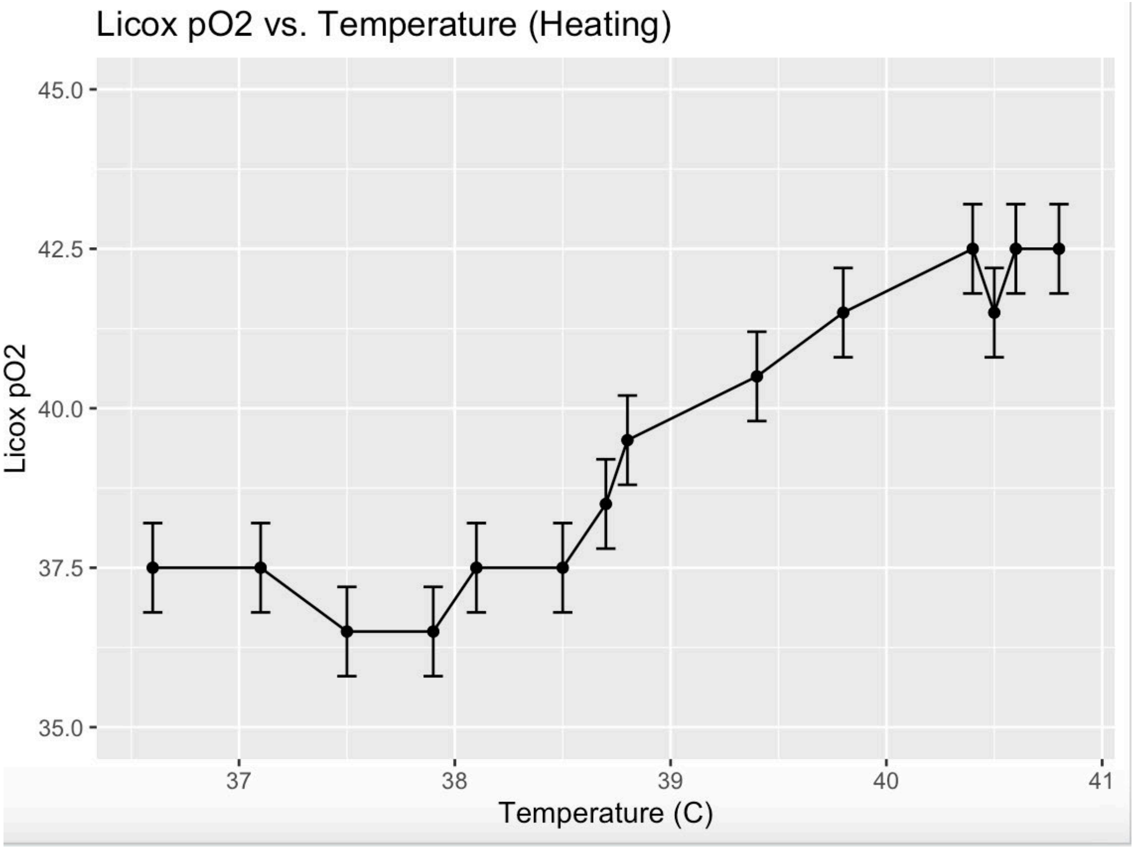

Figure 5 : Licox pO_2 vs. Temperature (Heating) at pO_2 of 40mmHg

This graph represents changes in Licox pO_2 values, in the solution set at pO_2 of 40mmHg, as temperature was increased from 37 degrees Celsius to 41 degrees Celsius. The trend indicates a statistically significant increase in Licox pO_2 with an increase in temperature ($p<0.001$) with a correlation coefficient of $r=0.98$.

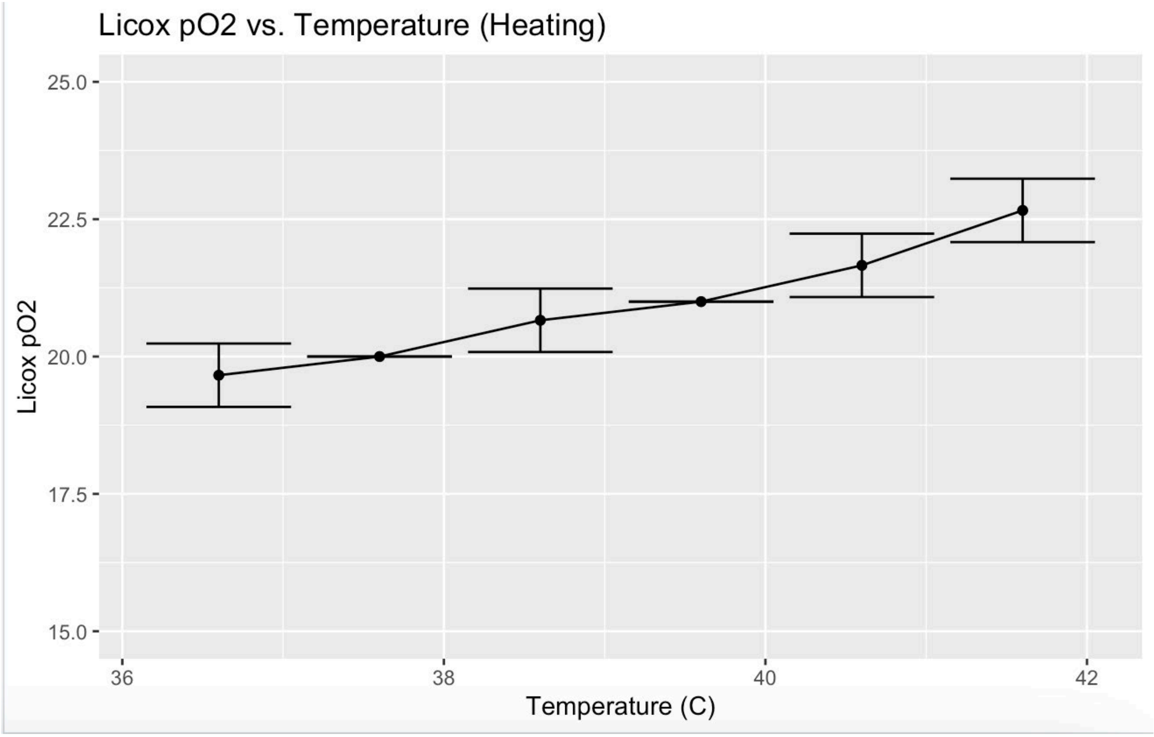

Figure 6: Licox pO2 vs. Temperature (Heating) at pO2 of 20mmHg

This graph represents changes in Licox pO_2 values, in the solution set at 20mmHg, as temperature was increased from 37 degrees Celsius to 41 degrees Celsius. The trend indicates an statistically significant increase in Licox pO_2 with an increase in temperature ($p=0.0003$) with a correlation coefficient of $r=0.98$.

Temperature Experiments: Cooling

Once each solution was heated up to 41 degrees Celsius. The solution was then cooled back down to 37 degrees Celsius. In regards to cooling, the results for both pO_2 solutions showed a decrease in Licox pO_2 value as shown in Figure 7 and 8. In the 40mmHg solution, the decrease in Licox pO_2 was found to not be statistically significant ($p=0.080$) with a correlation coefficient of $r=0.5$. In the 20mmHg solution, the decrease in Licox pO_2 was found to be statistically significant ($p=0.0180$) with a correlation coefficient of $r=0.8$. Both pO_2 solutions returned to close to baseline starting Licox pO_2 values when cooled back to 37 degrees Celsius. The results indicate a correlation between Licox pO_2 and decreases in temperature at the 20mmHg value.

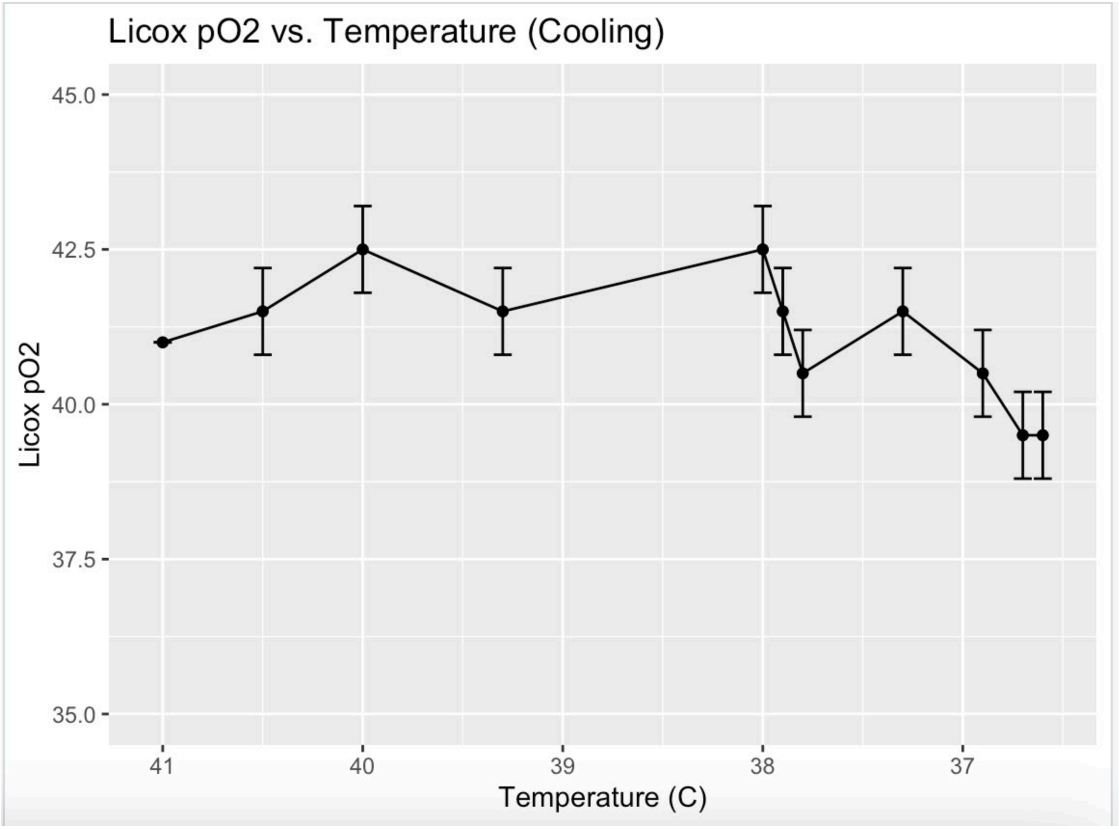

Figure 7: Licox pO2 vs. Temperature (Cooling) at pO_2 of 40mmHg

This graph represents changes in Licox pO_2 values, the solution set at pO_2 of 40mmHg, as temperature was decreased from 41 degrees Celsius to 37 degrees Celsius. The decrease in Licox pO_2 was not found to be statistically significant (p=0.080) with a correlation coefficient of r=0.5.

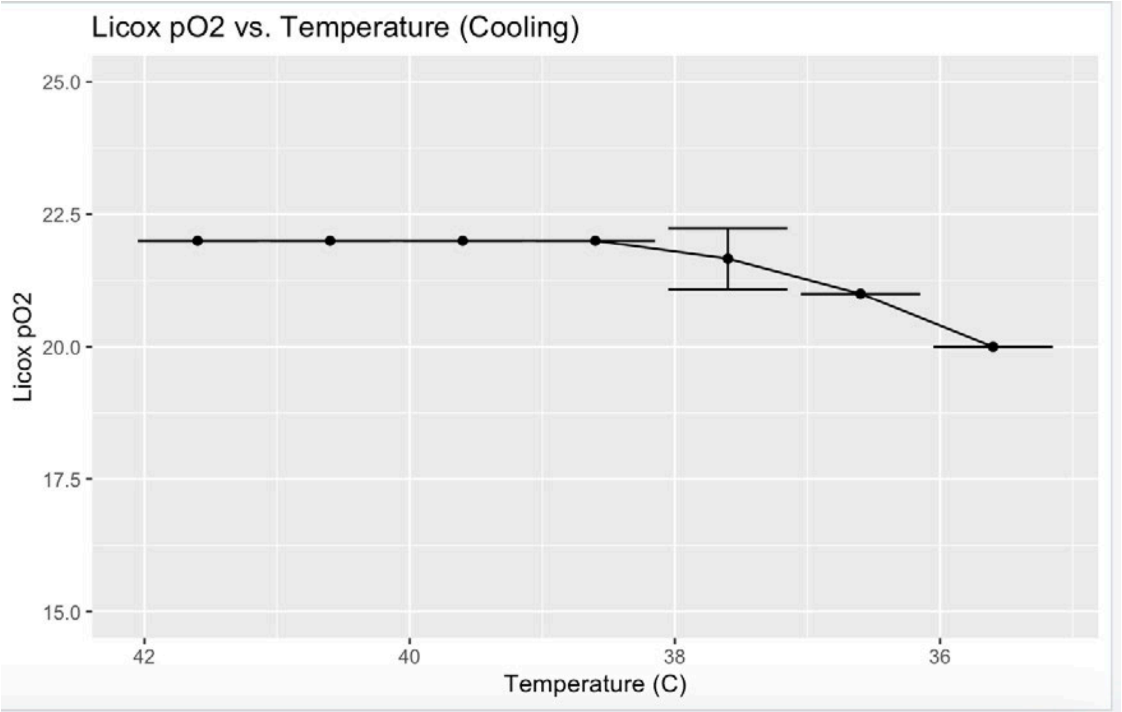

Figure 8: Licox pO2 vs. Temperature (Cooling) at pO2 of 20mmHg

This graph represents changes in Licox pO_2 values, the solution set at pO_2 of 20mmHg, as temperature was decreased from 41 degrees Celsius to 37 degrees Celsius. The trend indicates a statistically significant decrease in Licox pO_2 at the end of the cooling period, (p=0.0180).

Salinity Experiments.

The performance of the Licox system was tested against changes in salinity. Three separate buffer solutions with salinities of 150meq/L, 500meq/L, and 1000meq/L were created at the same pO_2 value of 145mmHg. The results indicate the Licox system did not read any differing values of pO_2 in the various saline solutions and the pO_2 value remained at 143.66mmHg, 95% CI [143.01, 144.32], as shown in Figure 9 respectively.

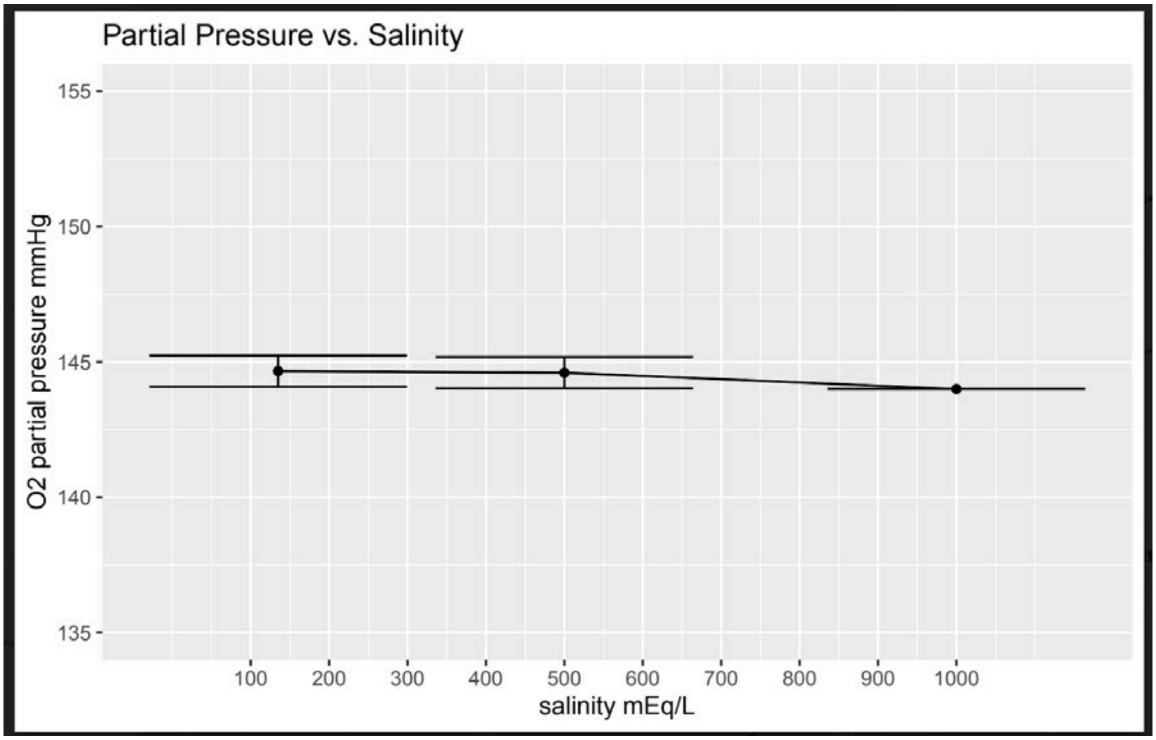

Figure 9: Licox pO2 vs. Salinity

This graph represents changes in Licox pO_2 values in solutions with differing salinities. The pO_2 value remained at 143.66mmHg, 95% CI [143.01, 144.32]. The trend indicates no significant changes in Licox pO_2 values against changes in salinity.

pH Experiments

The performance of the Licox system was tested against changes in pH. Three separate buffer solutions with pH's of 7.25, 7.35, 7.45 were created at the same pO_2 value of 35 mmHg. The results indicate the Licox system did not read any differing values of pO_2 in the various pH solutions and the pO_2 value remained at 34.33mmHg 95% CI [34.0, 35.3] as shown in Figure 10 respectively.

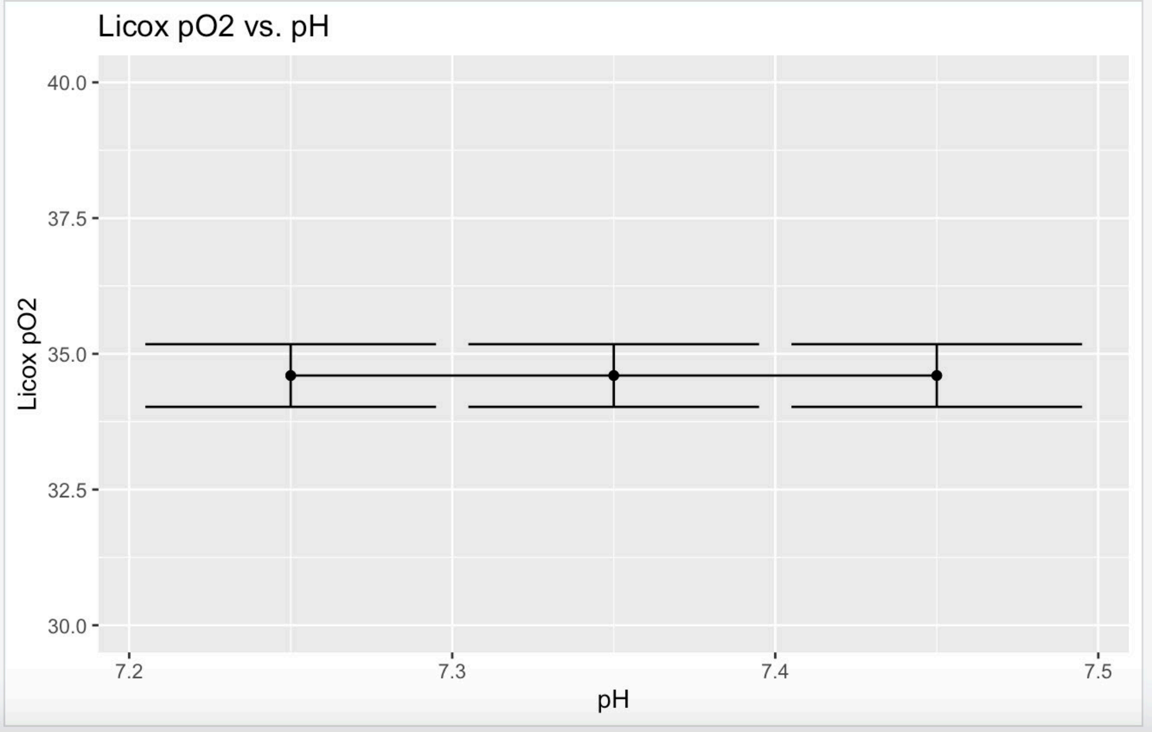

Figure 10: Licox pO_2 vs. pH

This graph shows the changes in Licox pO_2 against changes in pH. The results indicate no significant changes in Licox pO_2 associated with changes in pH. The pO_2 value remained at 34.33mmHg 95% CI [34.0, 35.3] across the different pH values.

Response Times

The Licox probe was tested for the probes response times to detect changes of pO_2 values in the solution. For the first experiment the Licox probe was submerged into a saline buffer solution with a pO_2 of 10mmHg and a new solution of higher pO_2 value was pumped through. The results show the Licox response times of 102 +/- 10s when going from a low pO_2 to high a pO_2, as shown in Figure 12 respectively.

For the second set of experiments, the Licox probe was submerged into a saline buffer solution with a pO_2 value of 40mmHg and a the lower pO_2 solution at 10mmHg was pumped through. The Licox response times for transitioning from a high to low pO_2 value were 240 +/- 16s, as shown in Figure 13 respectively. The results show that the Licox response times were slower when transitioning from a high to low pO_2 value.

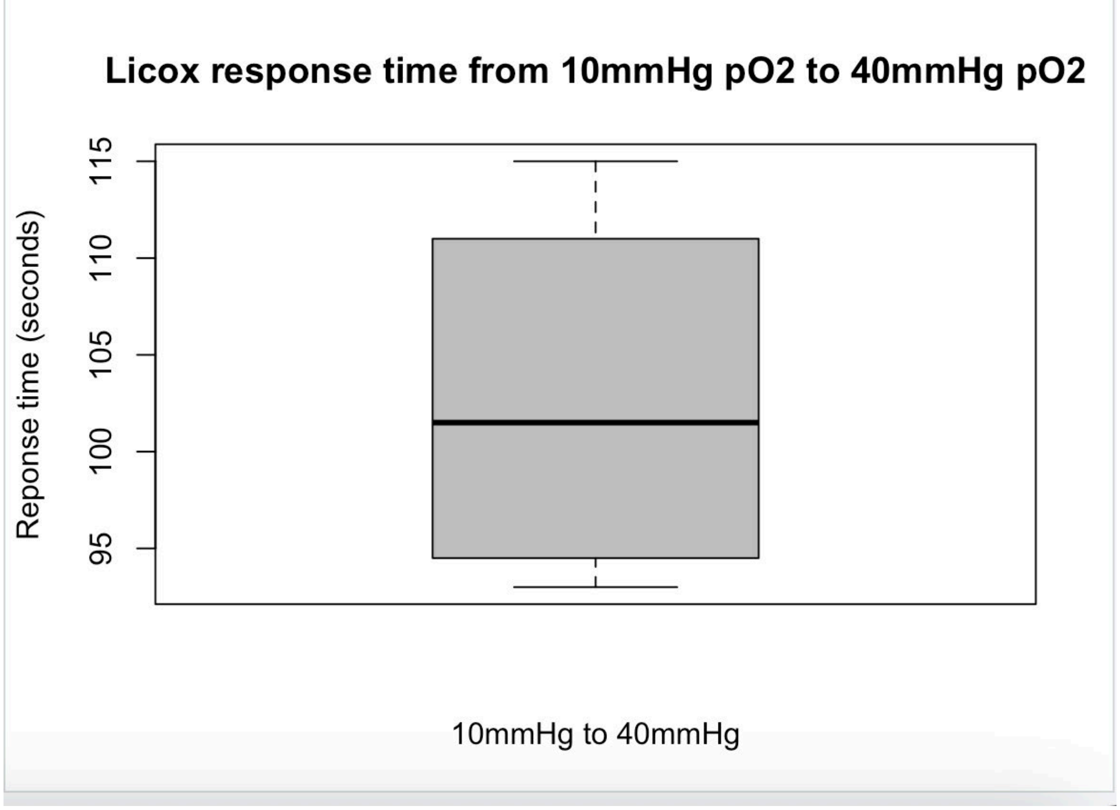

Figure 11. Response time from Low to High pO_2

This figure shows the Licox response times when going from a solution with a low pO_2 value to a high pO_2. The results show the Licox response times of 102 +/- 10s when going from a low pO_2 to high a pO_2.

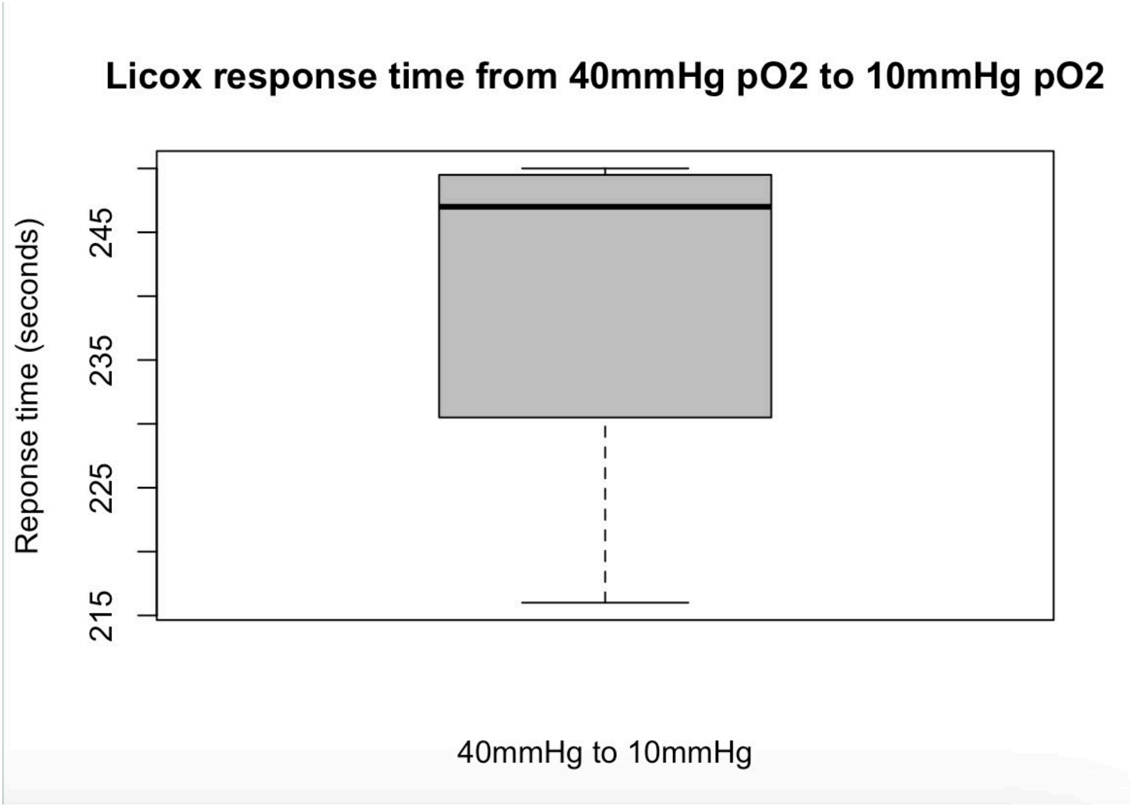

Figure 12. Response time from High to Low pO_2

This figure shows the Licox response times when going from a solution with a high pO_2 value to a low pO_2. . The Licox response times for transitioning from a high to low pO_2 value were 240 +/- 16s.

DISCUSSION

The purpose of this study was to test the performance of the Integra Licox brain tissue oxygen monitor under varying physiologic conditions such as temperature, hydrostatic pressure, pH, and salinity.

Hydrostatic Pressure

Looking at the hydrostatic pressure studies, saline buffer solutions of three different pO_2values, 20mmHg, 35mmHg, and 50mmHg, were tested against pressure. Our study indicates that the Licox system is not sensitive to changes in hydrostatic pressure in saline buffer solutions between pO_2 values of 20-40mmHg. For each separate buffer solution, the Licox pO_2 value remained near baseline starting value as shown in Figure 4 respectively.

Normal ICP values in adult brains are less than 20mmHg (Pinto et.al.,2022), however many TBI patients experience increased ICP and ICP fluctuations (Pinto et.al.,2022). In a healthy adult in the supine position, ICP values fall between 7-15mmHg (Haider et.al.,2018). While in a healthy standing adult, the ICP values measure at around 10mmHg (Haider et.al.,2018). ICP values >22mmHg are considered high and critical and usually are the threshold for starting treatment (Anania et.al.2020).

In our study, hydrostatic pressures up to 70mmHg were tested to observe the Licox sensitivity to changes in hydrostatic pressure. These results allow us to assume that if TBI patients are undergoing ICP fluctuations any concurrent changes in pO_2 indicated by the Licox monitor could be attributed towards changes in brain tissue oxygenation rather than pO_2 changes due to ICP fluctuations or Licox monitor calibration.

Temperature

In regards to temperature studies, the experiments were created to mimic the fast onset of fever and the slow stabalization back to baseline after intervention in TBI patients. TBI patients who experience bouts of higher fever that are (>39.0°C) within the first 72 hours of injury are associated with worse prognosis (Hinson et.al.,2018).

In our study, the results for both pO_2 solutions showed an increase in Licox pO_2 values with an increase in temperature, as shown in Figure 5 and Figure 6 respectively. In the 40mmHg pO_2 solution the rise in Licox pO_2 value was found to be statistically significant ($p<0.001$). In the 20mmHg pO_2 solution, the rise in Licox pO_2 value was also found to be statistically significant ($p=0.0003$).

The results indicate that the Licox is sensitive to changes in temperature and the Licox pO_2 values tend to change according to rise and fall in temperatures, while the salinity and pressures are held constant. The heating and cooling curves demonstrate that although the pO_2 value tends to rise during an increase in temperature from 37 degrees to 41 degrees, when cooled back down the pO_2 value tends to fall back to down to his original value +/- 1mmHg. Clinically, this is important as clinicians may be able to attribute these pO_2 changes to monitor calibrated changes due to temperature rather than a reflection of the changes in brain tissue oxygenation. These changes are especially of concern at the pO_2 value of 20mmHg as many clinical intervention decisions are made at this critical threshold value.

A previous study also explored the effect of temperature increases in $PbtO_2$ in TBI patients. The study results indicated that the $PbtO_2$ tends to slightly increase $\Delta P_{bt}O_2 = 0.9 \pm 6.1$ mmHg, $p = 0.022$) with rises in temperature (Rass et.al.,2021). Overall, the trends showed mostly stable or slightly increased $PbtO_2$ in the TBI patients during increases in temperature (Rass et.al.,2021). Our study cannot be compared to this study as our study was tested in in vitro conditions, while the Rass study was a clinical study conducted in patients with TBI injuries.

pH

The brain tissue acid-base homeostasis is found to be disturbed in TBI patients (Clausen et.al,2005). The increased brain tissue PCO_2 and lactate concentration during the time of injury is associated with brain tissue acidosis (Clausen et.al,2005). Previous studies have found that during the 1st day of trauma, the pH of the brain tissue was significantly lower (Clausen et.al,2005).

Due to frequent pH fluctuations found in TBI patients, it was important in our study to observe the changes in Licox pO_2 against changes in pH while temperature, salinity, and pressure were held constant. Our study results indicate that the Licox system did not show sensitivity to changes in pH. The pH in the physiological range of 7.25 to 7.45 was explored while pressure, temperature, and pO_2 were held constant. The pO_2 value remained at 34.33mmHg 95% CI [34.0, 35.3] across the different pH values.

This data is clinically relevant as any changes of pO_2 reflected on the Licox are not correlated with changes in pH. This is an important marker of analysis for clinicians, as TBI patients may experience acidodic expisodes where the pH can drop significantly, however, the Licox pO_2 values are not affected by these changes. The clinicians can attribute the Licox pO_2 changes to other variables other than pH changes.

Salinity

One common treatment for TBI patients is admisnitration of hypertonic saline solution to decrease the elevated ICP pressure. In standard practice, the hypertonic saline concentrations used are 3.0% to 23.4%, the dose of hypertonic saline can range from 1.0 to 4.0mL/kg (Shi et.al.,2020).

Thus, saline solutions of high concentrations were tested against the Licox to monitor an pO_2 changes. The trends in salinity versus Licox pO_2 system suggest that the Licox is not sensitive to changes in salinity. The results indicate the Licox system did not read any differing values of pO_2 in the various saline solutions and the pO_2 value remained at 143.66mmHg, 95% CI [143.01, 144.32]. The data suggests that if hypertonic saline solutions are administered to patients any concurrent changes in pO_2 are not correlated with salinity changes with CSF fluid.

Reponse Times

The Licox probes were tested for their response times during changes of pO_2 values in saline solutions. The results showed on average the Licox probes took 102 +/- 10s when going from a low pO_2 to high a pO_2, as shown in Figure 11 respectively. The Licox response times for transitioning from a high to low pO_2 value were 240 +/- 16s, as shown in Figure 12 respectively. The results show that the Licox response times were slower when transitioning from a high to low pO_2 value. Previous studies that have examined the response times of the Licox also reported values in this range. The Karlis et. al. study reported the Licox probe response time to be 78+/- 21s from a pO_2 of low to high and a response time of 215+/-63s from a pO_2 high to low (Karlis et.al.,2009). This is an important relevance for clinicians monitoring fluctuations of pO_2 in TBI patients, as rapid fluctuations or changes in pO_2 may not be reflected by the Licox due to its longer response time. Clinicians would have to wait for the equilibration time of the Licox to read the true value of the pO_2.

Study Limitations

One of the study limitations is the possibility of Licox pO_2 changes due to value drift over time. Previous studies have found the Licox pO_2 values tend to drift over time (Karlis et.al,2009). When the Licox probes are used in clinical practice for TBI patients, the probes are usually placed in the patient for a number of days at a time. The Licox pO_2 trends observed in our study may slightly differ if the Licox probes are kept in experimental chamber for longer periods of time.

Another study limitation is the Licox probe is studied in vitro experimental chamber of about 650mL of saline solution which is far greater than the volume of CSF fluid the Licox is submerged in when placed in the brain. These trends may be more or less pronounced in saline volumes that are far less than the ones studied in vitro in our study.

Lastly, the response times for the Licox were studied using saline solutions where as the brain fluid has a higher lipid content. This issue was addressed in the Stewart et. al. study where the study found the Licox probes were much slower in oil based solutions over water solutions. The Licox electrode rate limiting step for reading pO_2 is the rate of oxygen diffusion across the membrane. The rate of diffusion of oxygen can be influenced by the composition of the fluid it travels in. This brings an interesting concern for clinicians over the realibility of the Licox probe to detect instantaneous changes in pO_2 for TBI patients and should be further researched.

Future Studies

Our study was conducted in a controlled in vitro environment. For future experiments, the Licox probes should be tested in clinical animal or TBI patient models as well to observe if the trends are similar. Though the Rass et. al clinical study also found similar trends of an increase in Licox pO_2 values with an increase in temperature in TBI patients, our study is not comparable as the experiments were done in an controlled in vitro chamber.

For future studies it is important to study the relationship between dissolved oxygen and pO_2. The fluctuations of pO_2 may be explained with changes of dissolved oxygen content due to changing parameters of temperature, pressure, salinity, and pH. Based on literature, dissolved oxygen tends to increase with decreasing temperatures and dissolved oxygen tends to decrease with increasing temperatures. Secondly, the dissolved oxygen content decreases with increasing salinity in aqueous solution, and the dissolved oxygen concentration increases with decreasing salinity in aqeous solutions. Dissolved oxygen concentration provides the truest measurement of the brain tissue oxygen perfusion, so it is important find the relationship between dissolved oxygen concentration (mg/L) and the pO_2. There may be changes occurring in pO_2 values due to the changing parameters, however the Licox system have not have the precision to detect those small changes.

The Wrinkler Titration Method is an established method that precisely measures dissolved oxygen concentration in solutions. This titration can be used to monitor the dissolved oxygen changes that are occurring simultaneous to the changes in pO_2 values at the varying physiologic conditions that were tested in our study. The Wrinkler Titratin Method can also allow to us to test the accuracy of the Licox probes for measurement of oxygen concentration in a given solution.

CONCLUSION

The purpose of this study was to test the performance of the Licox probe and the Integra Licox brain tissue oxygen monitor under varying physiologic conditions such as temperature, hydrostatic pressure, pH, and salinity. There is plenty of literature that describes the accuracy of Licox probe in reading partial pressures of oxygen, however there is very little literature on testing Licox probe performance against varying physiological variables in a controlled closed in vitro environment. Our study is unique as the experiments have been conducted in a closed experimental chamber where we are able to eliminate confounding variables from outside sources.

Our study results indicate that the Licox probe is not sensitive to changes in pH, salinity, and pressure as there were no significant observable changes in pO_2 when these variables were changed. Our study does indicate that the Licox probe is sensitive to changes in temperature and has significant fluctutations in pO_2 values over the physiologic range of temperatures. In a clinical setting these results are important as they can help clinicans decide if the observed changes in pO_2 values can be attributed to actual changes in brain tissue oxygenation rather than fluctuations caused by Licox probe calibrations or changes in dissolved oxygen content.

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