Hemolysis Due to External Blood Circulation and its Effects on Conductivity

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***Abstract*—Many clinical applications require circulation of blood outside of the body. In these situations, the membrane of red blood cells may be ruptured due to shear stress, which is known as hemolysis. The contents within the hemolyzed cells are then released, most notably hemoglobin and its decompositional products. Hemoglobin and its byproducts are toxic and may cause damage within various bodily systems. Therefore, treatment must be administered to reduce the symptoms of hemolysis. In order to provide treatment, hemolysis must first be diagnosed. As the cell contents are electrically charged, when hemolysis occurs, the blood sample will decrease in resistivity. Therefore, detecting the change in resistivity of a circulating blood sample can be used to diagnose hemolysis. This in turn can signal a device to administer scavenging molecules to counteract hemolysis and reduce its symptoms. This device consists of a Wheatstone bridge with an instrumentation amplifier to determine percent hemolysis, and a comparator with a 555 timer to induce an infusion pump if hemolysis has occurred.**

Keywords*—hemolysis, hemoglobin, heme, iron ion, haptoglobin, hemopexin, ferritin, conductivity, resistivity, cardiopulmonary bypass, instrumental amplifier, Wheatstone bridge, anemia, hyperkalemia, spectrophotometry, blood

I. INTRODUCTION

A. External Blood Circulation

In treatments for various morbidities, the circulatory system must be run externally in order to bypass or supplement the organ of choice. External blood circulation is a medical technique in which blood is transported through an external device and then returned to the body. Some examples of this technique may be a cardiopulmonary bypass or dialysis. In a cardiopulmonary bypass, the device draws blood through an oxygenating system in order to bypass the heart and lungs. The device acts as the pulmonary system, diffusing oxygen into the blood while removing carbon dioxide. The oxygenated blood is then returned to the body with a pump [1]. In dialysis, the blood bypasses the kidneys due to their decreased function. This action cleans the waste from the blood, maintains safe mineral levels, and regulates blood pressure. At this point, the cleaned blood is returned to the body. In devices such as these, the erythrocytes are placed under high shear stress during artificial circulation [2]. This action may cause the red blood cells to rupture, resulting in hemolysis.

B. Hemolysis

Hemolysis occurs with the rupture of red blood cells (RBCs). In healthy RBCs, the inside contents of the cell – including hemoglobin – are protected by an outer lipid bilayer. [3] As the cell goes through stress, this outer layer can burst and release hemoglobin into the bloodstream. Levels of hemolysis exceeding 2% are considered above the healthy range [4]. Enzymes in the bloodstream break down the hemoglobin into heme and globin. The heme tetramer is then broken down into two heme dimers, composed of a heme alpha and beta complex. This dimer is then finally decomposed into bilirubin and biliverdin along with iron ions. Globin becomes amino acids [5]. Hemolysis occurs naturally in the spleen and liver [6]. However, other causes of hemolysis include external blood circulation machines [7], syringe sizes of less than 21 gauge [8], and kinked blood lines [9].

There are two classes of hemolysis: intravascular and extravascular. Intravascular hemolysis is characterized by cell destruction within the blood vessels, and the damaged cells then circulate through the body. Extravascular hemolysis occurs when the cell destruction is outside of the blood vessels, such as in the spleen [6]. Intravascular hemolysis occurs because the damaged cells are released into the bloodstream and begin to break down. Extravascular hemolysis is a naturally occurring process and gets resolved by the spleen and liver. Free hemoglobin, heme, and iron ions render the bloodstream toxic resulting in organ damage to the patient. Free heme offers severe toxic effects to the kidney, liver, central nervous system, and cardiac tissue [10].

Extracellular hemoglobin has been associated with adverse clinical outcomes in patients with hemolysis, such as acute and chronic vascular disease, inflammation, thrombosis, and renal impairment [11]. Some symptoms from this include hyperkalemia [12] and anemia [13]. Hyperkalemia is characterized by muscle weakness, numbness or tingling, nausea or vomiting, decreased reflexes, paralysis, respiratory failure, and arrhythmia. Anemia's symptoms include dyspnea, fatigue, muscle pain, angina and heart attack, chills or fever, yellowing skin, and dark urine. In order to diagnose hemolysis before symptoms worsen, there are very few developed techniques. A few of these techniques include a complete blood count (CBC) or other blood tests [14]. If there is hemolysis then the blood tests would show decreased haptoglobin, and increased reticulocyte and bilirubin. Another test would be a urine test, which would show urobilinogen and hemoglobin in the urine [14]. Other diagnostic tools include bone marrow aspiration or biopsy, and centrifugation and spectrophotometry [3]. These types of tests are invasive and are a separate process in addition to the external circulation system. In treatment, scavenging molecules like haptoglobin, hemopexin [15], and ferritin bind to hemoglobin, heme, and iron respectively, to neutralize their effects [16].

C. Goals of Design

Since hemolysis creates a difference in resistivity, the device can measure this change to detect the percentage of hemolysis in the blood. The goal of the device is to trigger a pump with scavenging molecules to neutralize the hemoglobin components. When the device senses a significant change in resistivity, it should output a voltage to start the pump.

II. RESEARCH

A. Hemolysis and Blood Resistivity

Hemoglobin and its components have a negative charge. When they are encased in the insulating cell outer lipid bilayer, their changes to conductivity are neutralized. When the cells rupture during hemolysis, the charged particles are released into the bloodstream, causing the resistivity to drop. This can be measured to determine the percentage of hemolysis. Each patient's blood resistivity is different, thus the change in resistivity should be specialized per patient. If the amount of hemolysis is measured, then treatment can be administered without further invasive procedures.

According to a study by Dr. Tyler Van Buren, as hemolysis percentage increases, the resistivity decreases [3]. Fig. 1, a graph from Dr. Van Buren's paper [3], demonstrates the inverse relationship of resistivity with percentage of hemolysis. This was measured with an LCR meter and spectrophotometer simultaneously. Inversely, with this resistivity change, the blood conductivity rises.

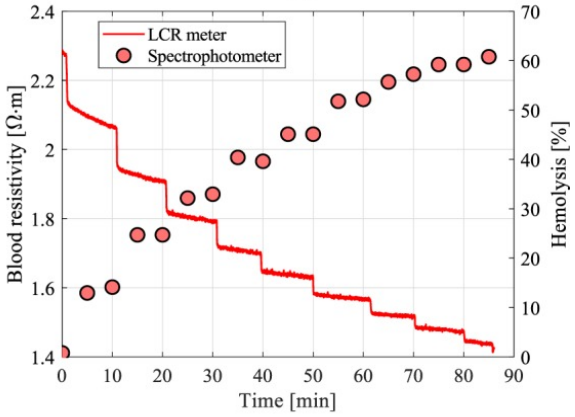

Fig. 1. Resistivity over time measured with an LCR meter overlaid over percent hemolysis over time measured with a spectrophotometer.

Fig. 2, also from Dr. Van Buren, shows the conductivity of blood based on percentage of hemolysis [3]. In order to find these values, two different samples of porcine blood were used. One sample was left untouched, while the other sample was mechanically damaged with an immersion blender. A series of samples were then prepared with varying levels of hemolysis.

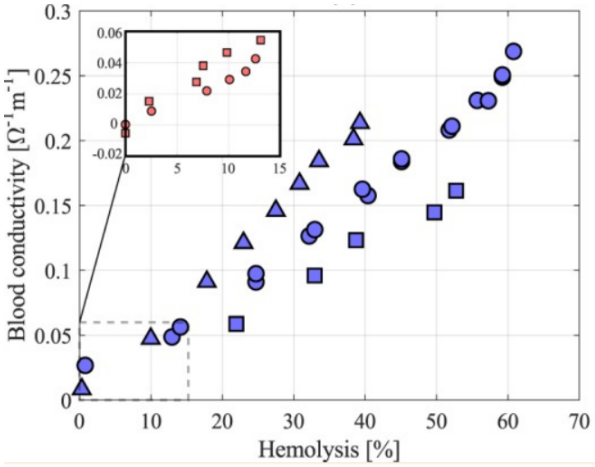

Fig. 2. Conductivity per percent hemolysis

III. METHODS

A. Device Design

To maintain a safe level of hemolysis, a negative feedback system is required. The mechanism for sensing resistivity in blood functioned by a Wheatstone bridge circuit of four resistors. Two of which are created by conducting current across a portion of the circulating blood sample. The resistance was calculated with the following formula obtained by performing a linear regression on Dr. Van Buren's models.

$$R_{Bl} = \frac{\rho(h\%)\cdot L}{A} = \frac{(2.28-0.0143\cdot h\%)\Omega m\cdot 0.01m}{(0.01m)^2} \quad (1)$$

If there was hemolysis, and therefore change in the resistance of these two samples, the potentials of the Wheatstone bridge would no longer be balanced and as a result, output a voltage.

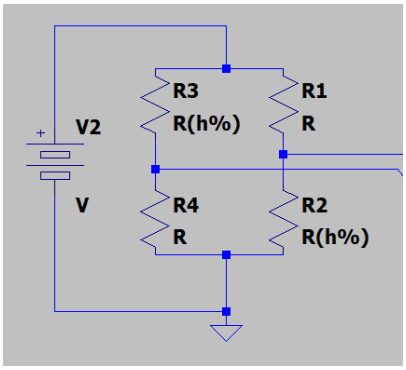

Fig. 3. Wheatstone Bridge Resistivity Sensor

This output was then connected to the AD8221 instrumentation amplifier (IA), which provided a circuit gain to the voltage obtained from the Wheatstone bridge. This resulted in a higher signal-to-noise ratio and an improved rejection of common mode signals (independent of resistance tolerances), leading to greater accuracy.

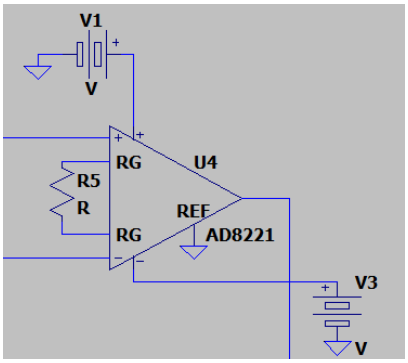

Fig. 4. AD8221 Instrumentation Amplifier

To determine if a treatment was necessary, a LT1017 comparator is then used to compare the output voltage of the IA to a reference voltage. This was then set equal to the voltage theoretically output by the IA at 2% hemolysis level. If the output exceeds reference voltage, the comparator, which was connected inversely, would send a trigger signal to the consequent NE555 timer IC.

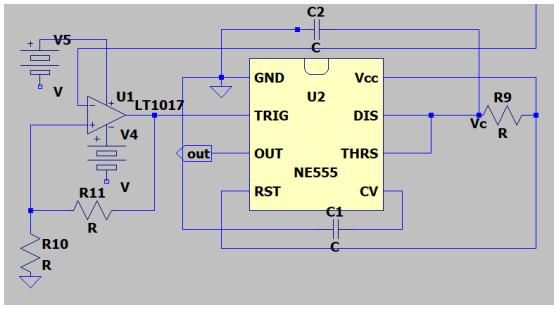

Fig. 5. LT1017 Comparator and NE555 Timer

This prompted the 555 timer to output a signal to the infusion pump for a designated amount of time (set with RC values) to inject scavenger molecules into the circulation. The pump would then also take an input directly from the IA to receive data on percentage hemolysis. The injection of scavenger molecules would then decrease conductivity of circulating blood, therefore providing negative feedback to the system.

B. Safety Implementation

The usage of an instrumentation amplifier instead of a single operational amplifier reduces risk of shock and equipment damage. It also aids in delivering a more accurate and precise dosage to the patient. In order for the device to be safe to use with blood still in circulation, values were selected to maintain a current of less than 10mA through the blood.

IIII. RESULTS

Parameters for the circuit components chosen in Fig. 5. were selected based upon estimates for resistivity that would correlate to normal levels of hemolysis. The Wheatstone bridge arranged as a strain gauge is powered by a standard 9V power supply, and functions to sense changes in resistivity in the blood induced by increased levels of hemolysis. The voltage outputs of this Wheatstone circuit increase with increasing hemolysis levels and pass these voltages to the instrumentation amplifier. The instrumentation amplifier amplified the signal by a gain of 10, as biosignals are often too small upon their initial collection to be analyzed properly. The resistor R5 which controlled the gain of the instrumentation amplifier was chosen by Eq. (2).

$$R_G = \frac{49k\Omega}{G-1},\ R_G = 5444\Omega \qquad (2)$$

Where R_G is equal to the resistance of the resistor connected between the gain inputs, and G is the gain, 10. The output voltage is then passed to the comparator for the next stage of the device, signaling the pump to output molecules. For the purposes of LTSpice simulation, a signal generator replaced the Wheatstone bridge and instrumentation amplifier section of the circuit, as simulating changing resistance values from an active blood pump in real time was not realistic with the limitations of the simulation software. An input voltage from a signal generator began at 0 V and slowly rose to a value greater than the voltage expected when hemolysis has reached an unsafe level. This simulates a patient slowly reaching an unsafe level of hemolysis during a procedure. When this simulation was run, the circuit provided outputs as shown in Fig. 6.

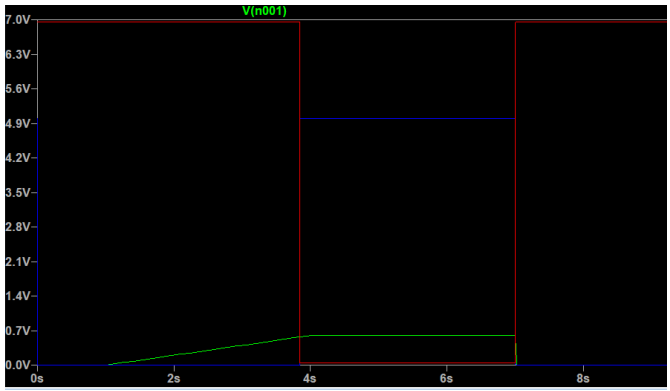

Fig. 6. Output voltages measured directly from signal generator (green) comparator (red) and 555 timer (blue)

In order to estimate the voltage output by the Wheatstone bridge and instrumentation amplifier with a gain of 10 using the resistivity of blood at 2% hemolysis, Eq. (3). was used.

$$G \cdot 9V \cdot \left(\frac{R_{Bl}(0\%)}{R_{Bl}(2\%)+R_{Bl}(0\%)} - \frac{R_{Bl}(2\%)}{R_{Bl}(2\%)+R_{Bl}(0\%)}\right) = 0.568V \quad (3)$$

Where R_{Bl} is the resistance of blood samples as functions of hemolysis percentage, and G is the gain, 10. Equation (3) calculates voltage output of the amplifier using voltage divider principles of the Wheatstone bridge.. After the threshold voltage of 0.568V was passed by the signal generator, the comparator had an inverting input that became higher than its non-inverting input. This triggered the comparator's output voltage to go low, shown by the red graph's sharply decreasing slope in Fig. 6. This voltage drop then triggered the 555 timer to discharge an output voltage, displayed by the blue graph's slope sharply increasing in Fig. 6. This voltage would then be passed to the pump to inject scavenging molecules to the bloodstream, combatting the hemolysis observed in the patient.

IV. CONCLUSION

This technique has applications in clinical environments during medical procedures where mechanical shear can result in the breakdown of the erythrocyte membrane. This design has the potential to relieve the risk of hemolysis-induced symptoms in the patients. Some future improvements to this project may be: adding a sensor module that measures the concentration of free scavenging molecules, designing our own pump to dispense scavenging molecules, researching the conductivity changes due to free scavenging molecules, and looking into other pathologies that might affect blood conductivity. However, there are a few weaknesses to this design. The differing concentrations of free hemoglobin, heme, and iron ions are not able to be measured by this design. Therefore, it is not possible to quantify the change in conductivity due to a particular molecule. Additionally, this design is only designed to measure changes in conductivity from hemolysis. In order to measure changes caused by any other molecule, the design would have to be revised. However, for the purposes of addressing hemolysis, this device works as expected.

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