Glucose detection of ringer-lactate solution using electrical bioimpedance: preliminary results

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Abstract. Continuous glucose monitoring is essential to reduce the damages caused by diabetes and for choosing the right treatment approach. In most cases, non-invasive glucose measurement devices generate their results through statistical tools (e.g., artificial neural networks) with an error that increases the further away from the training sample the measurement is. An analytical model would contain only propagated errors. Impedance measurements of lactate ringer’s solutions with egg albumin containing different concentrations of sugar were performed to validate the model proposed for measuring glycemia in human blood using the electrical bioimpedance meter AD5933. The curve fitting showed errors lower than 1.5%. Chemical phenomena, such as reduced sugar, fructosamine and solvation, might explain the behaviors observed in the experiments. The results suggest that the relaxation coefficient has significant changes with the increase of sugar in the solutions. The findings encourage future research with bovine blood for a more realistic analytical model.

1. Introduction
At SUS (Brazilian National Health Service), the costs with hypertension, diabetes and obesity have reached BRL $3.45 billion in 2018. From that costs, 30% was directed to the treatment of diabetes [1]. Diabetes Mellitus (DM) is characterized by hyperglycemia due to insulin secretion and/or insulin action defects. These defects can lead to dysfunction and failure of different organs, such as eyes, kidneys, nerves, heart and blood vessels. [2].

The blood sugar control is essential to avoid diabetes but also to reduce the side effects of it. That control is a quite difficult task as each person holds its own reference. There are many methods for assessing the blood sugar level in a long-term basis, such as glycated hemoglobin, self-monitoring of capillary glycemia and continuous glucose monitoring [3].

The use of continuous glucose monitoring (CGM) has grown rapidly in the recent years as a result of improved sensor accuracy, greater convenience and the ease of use [4]. Pedro et al (2020) presented an analytical model correlating the electrical impedance spectra to the blood glucose levels variations. The mathematical model is based on Bruggeman’s equations and the Theory of the Effective Environment (EMT) [5]. The EMT equations can be extended to high volume fraction by gradually adding particles of interest. EMT can be used to describe the behavior of percolation systems, unlike other approaches [6]. The application of adequate electrical percolation models in experimental and theoretical researches on composites is common in several fields of science [6][7][8].

The model proposed by [5] assumes that blood is a composite formed by non-conductive particles (hematocrit and glucose particles) immersed in a conductive matrix (plasma).
That model describes the blood glucose levels by using electrical impedance spectroscopy. Adjustments are needed for this type of model, which requires practical experiments in a phantom system simpler than human blood.

In order to validate the mathematical model, a simple composite of ringer’s lactate solution with albumin is used to calculate glucose by BIA measurements. It is expected to analyze the results by fitting the curves of the proposed model to the experimental data, and then to ascertain whether the model satisfy the objective of this work.

2. Methodology

This a similar composite to human blood for measuring its electrical impedance spectra by means of a commercial impedance meter, AD5933. It is then verified the functionality of the mathematical model developed by [5].

2.1. Composite

The composite chosen to represent a simpler system than human blood has its conductive phase represented by the ringer’s lactate solution. The composition of this solution is similar to that of extracellular liquids. Among the commercial options for electrolytic solutions in Brazil, RLS would be the only solution capable of promoting the simultaneous correction of dehydration, electrolyte imbalances and metabolic acidosis [9]. Furthermore, the non-conductive phase is found to be albumin, which is the most abundant protein in both plasma and extracellular fluids (60% of the total protein concentrations in plasma [10]). It has a long half-life (between 18 and 20 days), responsible for binding and transporting substances, for maintaining the plasma’s colloidosmotic pressure and for preserving the distribution of water in the body compartments. There are several types of albumin: Seroalbumin which is the blood serum protein, egg albumin which is the egg white albumin and lactoalbumin which is the milk albumin [11].

Recent research has shown that artificial derivatives of heme associated with human serum albumin provide a significant capacity for binding to oxygen, representing a promising line in the development of artificial blood substitutes. One of the most important studies for creating artificial blood compounds based on the mutation of the molecular micro-neighborhood of serum albumin [12]. Therefore, in order to make a blood-mimicking fluid, a mixture of egg albumin with ringer’s lactate was used. This is composed of calcium chloride $2\text{H}_2\text{O}$ a 0.02%, 0.03% potassium chloride, 0.1% sodium chloride and 0.3% sodium lactate.

2.2. Impedance meter AD5933

This device is a complete solution for measuring an impedance spectra (modulus an phase) in a frequency range from 100 Hz to 500 kHz. It is used together with an impedance probe, which is immersed into the blood samples, for example. The AD5933 can be tuned into a specific frequency required for each test. Both real (R) and imaginary (I) part of the impedance at each discrete frequency is recorded, processed and transmitted via its I2C serial interface. The magnitude and phase of the impedance are calculated according to the technical notes of the AD5933 [13].

2.3. Test bench

According to [14], an electrolyte solution with proteins and large molecules when crossed by a continuous electric current generates a so called double layer capacitance. This capacitance may have a high value, although it is not a perfect capacitor because of a leakage current. High capacitance values are a big issue in impedance measurements of the analysed sample. On of the solution is to discard the use of continuous voltage, since the alternating voltage

\[1 \text{ Calcium, zinc, magnesium, copper, long-chain fatty acids, steroids, drugs, etc.}\]
Figure 1. Schematic diagram of the experiment using the 2-electrode method from AD5933, where RFB is the feedback resistor.

shaking this fluid will prevent it from forming a layer of proteins at the electrode surface and, therefore, prevents the appearance of this double layer capacitance. Most solutions used the 4-point electrode technique for reducing the electrode-electrolyte interface impedance.

The AD5933 is designed to have a DC voltage of 2.5 V at its output, then the alternate signal has a mean value of 2.5 V. Therefore, the DC level contributes to the appearance of the double layer capacitance effect. The use of a DC blocking filter with a 0.1 Hz cutoff frequency is required at each electrode site. Here, it was used two capacitors of 470 nF between the electrodes and both input and output pins of the AD5933 (see figure 1).

The solution was put inside a beaker of 100 ml and 8 cm of diameter. The electrodes were placed 8 cm apart from each other. The electrodes are made of silver with an area of 0.4 cm$^2$ in contact with fluid.

2.4. Analytical model
Pedro et al. (2020) suggested that the electrical conductivity of blood can be modelled as a function of glucose concentration by using the EMT theory. The equation 18 in [5] represents the module of electrical impedance upon frequency and the concentration of blood glucose, which can be rewritten as:

$$|Z| = a \sqrt{1 + \left(\frac{f^2 + b}{f^2(cf^2 + d)}\right)^2} \quad (1)$$

where $a = \frac{L}{A\sigma_h(1-k+k')^{3/2}}$, $b = \frac{\varepsilon_\infty \Delta \varepsilon}{4\pi^2}$, $c = \frac{2\pi (\varepsilon_\infty + \Delta \varepsilon)^2}{\sigma_h(1-k+k')^{3/2}}$, $d = \frac{\varepsilon_\infty \Delta \varepsilon^2}{2\pi\sigma_h(1-k+k')^{3/2}}$, $k'$ is the volumetric fraction of glucose and $k$ is the volumetric fraction of red blood cells.

The blood vessel is modeled as a cylindrical conductor of constant volume ($L$=length of the conductor, $A$=area of the conductor section). $\sigma_h$ is the conductivity of the composite’s high electrical conductivity phase, which is here represented by the ringer’s lactate. Particularly at this experiment, $k'$ is the volume fraction of sugar, $f$ is the frequency of the electrical signal applied to measure the sample using the BIA technique, $\varepsilon_\infty$ is the electrical permittivity at high frequency and $\tau$ is the characteristic relaxation time.

2.5. Measurements
Three solutions with different concentrations of albumin were used. The 5g and 10g-solution were added sugar from 0 g to 15 g in a step of 5 g. The third solution used 15 g of albumin and 5 g of sugar in order to investigate the behavior of the impedance change due to albumin variations. All mixtures were carried out on a magnetic stirrer to reach homogeneity and to prevent any decant of solids.
The AD5933 was calibrated with a 2.2 kΩ resistor before solution measurements. The AD5933 was set to generates 15 signal wave cycles for averaging purpose in a frequency ranging from 10 to 60 kHz.

3. Results

The impedance measurements for the ringer’s lactate solutions with different concentrations of albumin are shown in figure 2. Figure 2(a) indicates the impedance modulus for a solution of 100 ml of ringer’s lactate and 5 g of egg albumin. Figure 2(b) shows the impedance modulus of the 100 ml solution of ringer’s lactate and 10 g of egg albumin.

The influence of albumin in the solution can also be observed in figure 3. The impedance modulus decreases as increasing the albumin concentration for 5 g of sugar.

Figure 4 shows the results from curve fitting by using equation 1 and concentrations of 100 ml of ringer’s lactate with 5 g of albumin. The 5 g albumin solution was chosen for the curve fitting due to its similarity to the experimental data shown in [15] and [5]. However, the 5 g albumin solution with 15 g sugar was discarded from our analysis because the curve does not indicate a similar behavior compared to other curves. In other words, the impedance was not expected to
increase as increasing glucose, whose phenomenon is explained further in the following.

The calculated impedance values were compared to the experimental ones collected with AD5933 (see figure 2). According to the curve fitting process shown in the figure 4, a minimum error of 0.03% and a maximum of 0.64% was obtained.

The percentage errors calculated for the curve fitting in figure 4(b) have a minimum and a maximum value of 0.02% and 0.71%, respectively. Finally, the errors calculated for the curve fitting in figure 4(c) have a minimum and a maximum value of 0.02% and 1.35%, respectively.

4. Discussions
It can be observed in figure 2 that the impedance modulus decreases as increasing sugar value. This might be explained by the dependence of the electrical conductivity on the number of free ions in the solution [16]. This dependency might be related to a sugar reduction process which yields negative ions to the solution, contributing to the increase in electrical conductivity which, in turns, to the decrease of the impedance modulus.

On the other hand, according to figure 2(a), the 15g-sugar-curve does not match the predictive

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**Figure 4.** Curve fit for a 100 ml ringer’s lactate solution, 5g of albumin and (a) 0 g, (b) 5 g and (c) 10 g of sugar.
curves. This might be explained by a process called fructosamine\textsuperscript{2}, which can cause such mismatch and also non-linearity in the results due to sugar saturation. Therefore, the amount of proteins in the solution decreases as decreasing the amount of free ions which, in turns, increases its impedance modulus.

It can be observed in figure 2(b) and figure 2(a) that the 0 g sugar curve is the lowest one. The explanation might be laid in the fact that the addition of 10 g of albumin saturates the solution, instead of adding sugar, in a process called solvation. Solvation is the phenomenon that occurs when an ionic or polar compound dissolves into a polar substance\textsuperscript{3}, without forming a new substance and generating bonds between the molecules of the solute and the molecules of the solvent. Proteins are macromolecules composed of amino acids, according to\textsuperscript{[16]}, then most of them are polar and are immersed in an ionic solvent. This supports the existence of the solvation phenomenon during the measurements.

Therefore, the explanation for the results of figure 2(b) is that when the concentration of albumin is increased to 10 g in the solution with ringer’s lactate, the fluid saturates because there are more proteins that the solvent is able to solvate. This results in an excess of proteins which triggers the fructosamine process for diluting the albumin which, in turns, increases the impedance modulus for the 5 g and 10 g sugar cases. Finally, since the fructosamine process is irreversible, when sugar is increased to 15 g the impedance decreases, acting similarly to the sugar reduction effect described in the previous case.

It was also observed that occurs an impedance drop with increasing the albumin at fixed values of sugar, as shown in figure 3(a). Solvation process is main cause of this result, as the increase of proteins contributes to increase the conductivity, then the impedance decreases.

It can be observed in figure 4 that the maximum percentage error was 1.35\%, when comparing experimental and numerical data. This may indicate that the coefficients are satisfactory and that the proposed analytical model by\textsuperscript{[5]} is feasible for modelling the materials analyzed in this work. Table 1 shows the coefficient values obtained for all curve adjustments.

Table 1. Comparison of the coefficient values obtained in the curve adjustments presented in the figure 4 for the equation 1.

| fit  | a       | b       | c       | d       |
|------|---------|---------|---------|---------|
| 0g   | 1.23 x 10\textsuperscript{-1} | -1.12 x 10\textsuperscript{6} | 9.30 x 10\textsuperscript{-2} | -1.55 x 10\textsuperscript{5} |
| 5g   | 1.17 x 10\textsuperscript{-1} | -3.90 x 10\textsuperscript{4} | 1.00 x 10\textsuperscript{-1} | -5.73 x 10\textsuperscript{3} |
| 10g  | 1.08 x 10\textsuperscript{-1} | -1.08 x 10\textsuperscript{2} | 1.49 x 10\textsuperscript{-1} | -1.61 x 10\textsuperscript{1} |

It can be noted that the coefficients a and c have almost the same order of magnitude, however b and d decrease by approximately two orders of magnitude as 5 g of sugar is added into the solution. According to the definitions given by equation 1, it can be noted that a, c and d depends on the glucose concentration (k’\prime) whereas d is significantly changed by the addition of sugar. However, only b and d depend on \(\tau^2\), leading to the conclusion that the sample’s characteristic relaxation time is responsible for this lag in the coefficients. As a result, it can be concluded that the addition of sugar in the solution affects the relaxation coefficient.

The relaxation phenomena also occurs in biological tissue, which has been investigated by electrical bioimpedance spectroscopy over the last 50 years. This may also be the case of

\textsuperscript{2} Fructosamine is a ketoamine derived from an irreversible non-enzymatic reaction of a sugar with a protein and is the general term to describe total glycated proteins. It is derived from the non-enzymatic binding of glucose to proteins present in blood plasma, such as albumin, and is an irreversible process.

\textsuperscript{3} There is a difference in electronegativity between the atoms, presenting a positive pole and a negative pole.
non-invasive glucose measurement by the same technique, where accuracy of the measuring instrument is high and then allowing the use mathematical modeling instead of artificial neural networks (ANNs).

The order of magnitude of $b$ and $d$ coefficients, with respect to the sugar concentration, might be an advantage for using impedance spectroscopy technique due to its sensitivity upon frequency for small changes in glucose.

5. Conclusion
The analytical model described by [5] is able to represent the proposed experiment and, probably, other composites with similar configurations. However, it cannot be used as a phantom for human blood due to its simplicity and saturation issues. Nevertheless, the results shown in figure 2(a), excepting the case of 15 g of sugar, presented a similar behavior found by [15]. The fitting process presented better results than those found by [5].

The findings here will promote future researches with bovine blood glucose at different frequency and fluid constituents by means of electrical impedance spectroscopy.

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