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Quantitative evaluation of blood glucose concentration using impedance sensing devices

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Abstract
This paper reports on the impedimetric investigation of glucose concentrations present in the human blood by using impedance sensing devices with different working electrode areas. It is evident from the experiment that, the impedance value increases with the increase of glucose concentration in blood and the trend follows in all the devices with various electrode areas. The measured complex admittance plot shows two semicircles which are due to double layer and coating capacitances respectively. Lower values of relative standard deviation in impedance data infer the reproducibility of these devices. A quantitative relationship, developed between the impedance and glucose concentration establishes a positive correlation between the blood glucose values and impedance.

Keywords: Blood glucose, impedance sensing devices, microelectrodes, microfabrication

Introduction

The enzymatic methods for glucose calculations using potentiometric and amperometric biosensors performed well and have been used in medical practices for decades. However, these methods are not suitable for online monitoring due to their invasive properties. Impedimetric glucose sensors are used less frequently as compared to enzymatic methods, but this technique has shown promising approaches in recent decades. Glucose does not affect the dielectric spectrum in the MHz frequency range and thus its concentration cannot be measured directly [1]. However, variations in blood glucose concentration induce electrolytic transportation through the membranes of blood cells. The altered electrolytic phenomena in blood can be measured by electrochemical impedance spectroscopy (EIS) that correlates with the concentration of glucose in blood [2]. Shervedani et al. developed a quantitative EIS method for the determination of glucose based on immobilizing glucose oxidase (GOx) on the modified gold electrode. The EIS measurements show that the charge transfer resistance decreases with the increase of the glucose concentration due to the increase of the diffusion current density by the hydroquinone oxidation [3]. Recently, several versatile biosensing materials such as semiconducting carbon nanotubes, conducting polymers [4], and nanotubes [5] have been used to measure glucose concentration in the blood by using impedance spectroscopy. However material properties such as active surface area, three-dimensional structure should be investigated to improve the performance and sensitivity of the EIS based glucose sensor [6]. This paper is the continuation work of the published paper of the authors [7] and in this paper the impedance of human blood with different concentrations of glucose is investigated by using different impedance sensing devices with various working electrode areas. The aim of the present experiment is to design and fabricate different electrode geometries to measure the blood glucose concentrations using impedance method. Also a quantitative approach has been carried out to understand the relations between impedance, glucose concentration, and various electrode areas of different electrode geometries.

Materials and methods

Design of microelectrodes
Three-electrode techniques with several dimensions of electrodes are used for this paper as described in authors' previous paper [7-8]. The principles for electrode design were extracted from previous literature. Mishra et al. [9] showed that the working electrode (WE) area should be smaller than the reference electrode (RE) area to enable detection of small changes in the sensing electrode. Brett and Brett [10] inferred that the surface area ratio of counter electrode (CE) and WE should be larger than 10 in order to support the current at the working electrode. In the present study, the ratio of WE and RE and WE and CE were fixed at 0.01. The CE and RE were placed at a distance of 100 μm from WE position in all the designs to avoid cross contamination [11-12]. The geometrical dimensions of WE, CE, and RE for Design 1, Design 2, Design 3, and Design 4 are described in Table 1.

Table 1. Dimensions of different designs of three-electrode devices.

Devices	Electrode dimensions (μm^2)	Distance between electrodes (μm)
Design 1	WE- 50×50, RE- 500×500, CE- 500×500	100
Design 2	WE- 100×100, RE- 1000×1000, CE- 1000×1000	100
Design 3	WE- 150×150, RE- 1500×1500, CE- 1500×1500	100
Design 4	WE- 200×200, RE- 2000×2000, CE- 2000×2000	100

Device fabrication

The impedance sensing devices were fabricated on 2 inch diameter Pyrex wafers using metal deposition and photolithography techniques. Initially, the wafers were cleaned and thin layers of chromium (Cr) and gold (Au) were thermally evaporated onto the wafers. Subsequently, the electrode traces and its contact pads were lithographically defined using positive photoresist and then the Cr and Au layers were selectively removed by wet etching process to define the metal patterns on the substrate. Finally, a second lithography step was performed to apply another photosensitive polymer (SU8) layer as passivation coating over the metal electrodes. In this step, only the electrode sensors and contact pads were exposed to make contact with the electrolyte as shown in Fig.1.

The photoresist was hard baked to impart stability and inertness to the polymer. The wafers were then diced into single devices for subsequent packaging and measurements. Finally the individual device was fixed in a printed circuit board and electrical connections were taken from the device to external equipment using thin metal wires and then cloning cylinders were aligned and attached to serve as electrolyte reservoir around the three-electrode system using PDMS as glue.

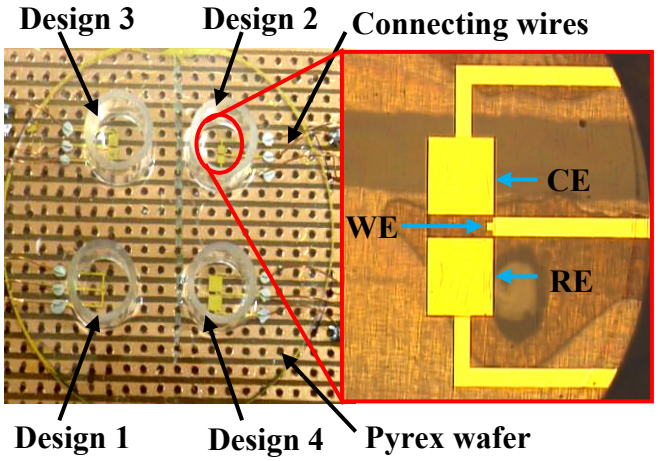

Fig.1: Microphotograph of different three-electrode devices.

Sample preparation

The blood samples, collected from BC Roy Technology Hospital, IIT Kharagpur were divided into five groups by considering the concentration of glucose of each sample. The first group contains the hypoglycemic blood (50±20 mg/dL of glucose), the second group possesses normal blood (100±20 mg/dL of glucose), and all other three groups possess 200±20 mg/dL, 300±20 mg/dL, and 400±20 mg/dL of glucose in blood samples respectively. Each group possesses 10 number of blood samples. As the present experiment was preliminary one and in the pilot scale the authors did not study the biochemical parameters of the blood.

Impedance measurement

The electrical impedance measurement of blood with different concentrations of glucose was carried out using computer controlled electrochemical work station SP 150 (Bio-Logic, France) by connecting the fabricated device directly to the system as described in Fig. 2. All the measurements were performed with actuation voltage of 10 mV and frequency range from 100Hz to 1 MHz with 51 sample points in between in a logarithmic scale. All the measurements were repeated ten times and then averaged to get the impedance value for each frequency. The present electrochemical workstation has an inbuilt facility to reduce the noise margin of the measured impedance data through software interpretation. The Nyquist data was obtained from the devices of four designs and subsequently the experimental data was exported to ZsimpWin software for further analysis with equivalent circuit simulation.

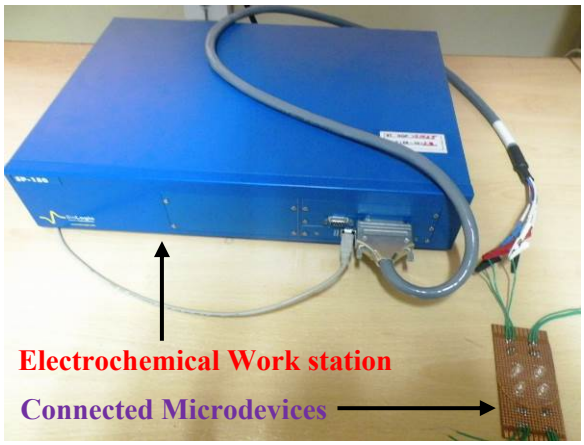

Fig. 2: Test setup for impedance measurement.

Equivalent circuit simulation

The Nyquist data obtained from the four designed devices was exported to ZSimpWin software for further analysis with equivalent circuit simulation. The equivalent circuit used in the present study was extracted from the authors' previous paper [7] and described in Fig. 3.

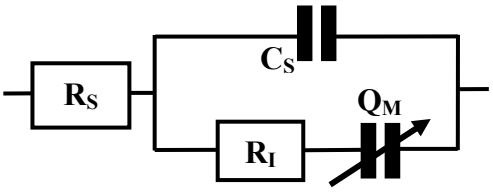

Fig. 3: Equivalent circuit of blood.

Quantitative analysis

From the above analysis, the qualitative approach of increased glucose concentration in blood is observed. However, a quantitative relationship is required to understand the relations between different variables. In the present paper four types of designs with different electrode areas are considered. Thus the blood glucose impedance not only depends on the glucose concentration but also upon the electrode area. Also the empirical equations obtained by

the correlation experiments are helpful to calculate the blood glucose concentration if other factors were known to the authors. Also these experiments help to design complex electrode geometries for further experiments [8]. In the first phase of the correlation experiment, the impedance data were fitted with the independent variables such as glucose concentration (100 to 400 mg/dL) and frequency (100 Hz to 1 MHz) for all the designs to establish the mathematical relationship between glucose concentration and impedance by using LAB Fit curve fitting software. In the second phase of experiment, the impedance data was fitted with glucose concentration and working electrode area (0.25 to 4 mm^2) by keeping frequency constant. In the same line of experiment, a direct empirical relationship between glucose concentration and impedance was established by keeping frequency and working electrode area constant. The equation of best fit was derived using the curve fitting procedure and later used to establish the mathematical relations.

Results and Discussion

Impedimetric evaluation

A typical Bode plot of different concentrations of glucose in blood for Design 1 is presented in Fig. 4. All other devices show the same trends. From the figures it is evident that with the increase of glucose concentration in blood, the impedance value increases and the trend follows in all the devices with various electrode areas. A single dispersion is found up to a frequency of 10 kHz in all the cases of hyoglycemic, normal, and diabetic blood for different designs.

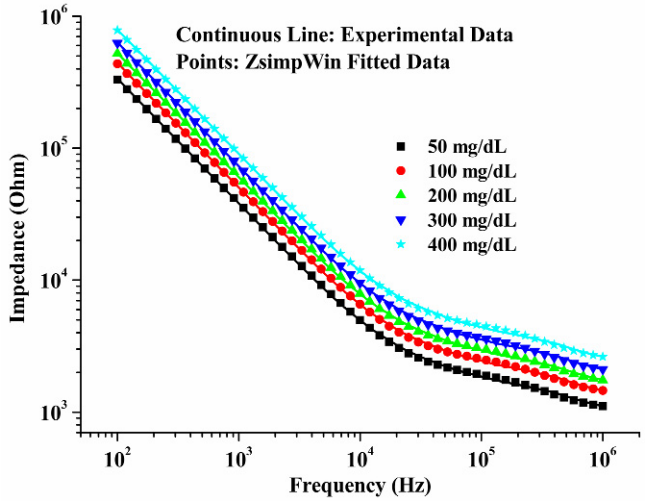

Fig. 4: A typical representation of Bode plot for different glucose concentrations.

The qualitative approach of bode plot of normal and diabetic blood is similar while the quantitative approach is different from each other. The values of impedance data increase uniformly as the glucose concentration increases in blood as evident from the graph for a particular design. This may be due to altered phenomenon in cell metabolism caused by increasing glucose levels. The high concentration of glucose modifies the properties of the cell membrane, which alters membrane capacitance. In the present study it has been observed that a significant variation in the impedance values is found for different glucose concentration of blood samples throughout the frequency range up to 1 MHz. The relative standard deviations of impedance for different devices remain below 10 % in the present experiment as shown in Fig. 5.

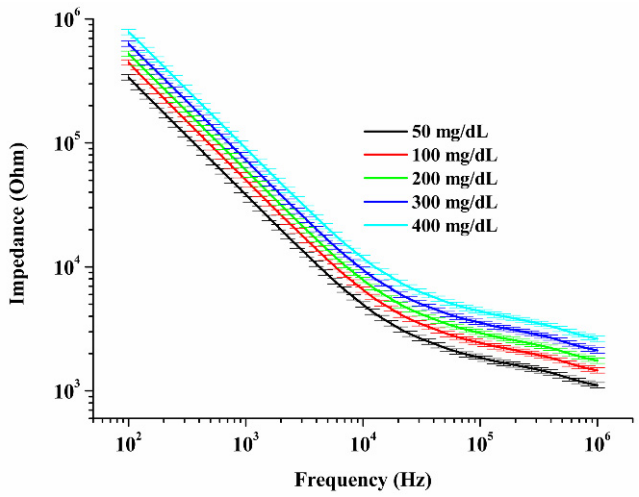

Fig. 5: A typical representation of Bode plot for different glucose concentrations with error bars.

It can be interpreted from the present experiment that variation in glucose concentration directly affects the dielectric properties of a solution and the results are similar and comparable to previously reported studies [13-14]. The well studied mechanism of sodium transportation through the cell membrane by Hillier *et al.* explored the decrease in sodium concentration in case of hyperglycemia [15].

As the measurements are phase sensitive, a typical complex admittance plot is required as described in Fig. 6 to understand the role of varying glucose concentration on impedance characteristics.

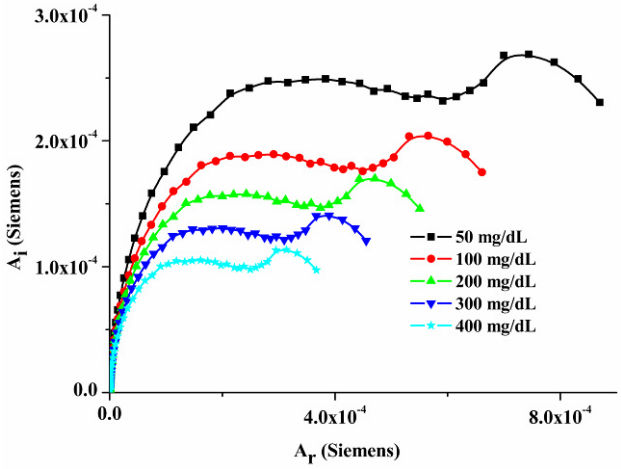

Fig. 6: A typical representation of complex Admittance plot of different glucose concentrations.

In the admittance plane the frequency decreases from right to left. It is evident from the figures that two semicircle are found. The first semicircle is due to the double layer capacitance and solution resistance of the Au/blood system. The resistance determines the radius of the complex plane plot, where as the capacitance determines the extent of the semicircle in the complex plane. The second semicircle present in the higher frequencies is smaller and it is due to the coating capacitance. Thus coating capacitance contributes in total impedance, however no polarization impedance is found in the present experiment.

Quantitative evaluation

The dependence curve for impedance and blood glucose concentration with respect to scanning frequency for impedance sensing devices is shown in Fig. 7.

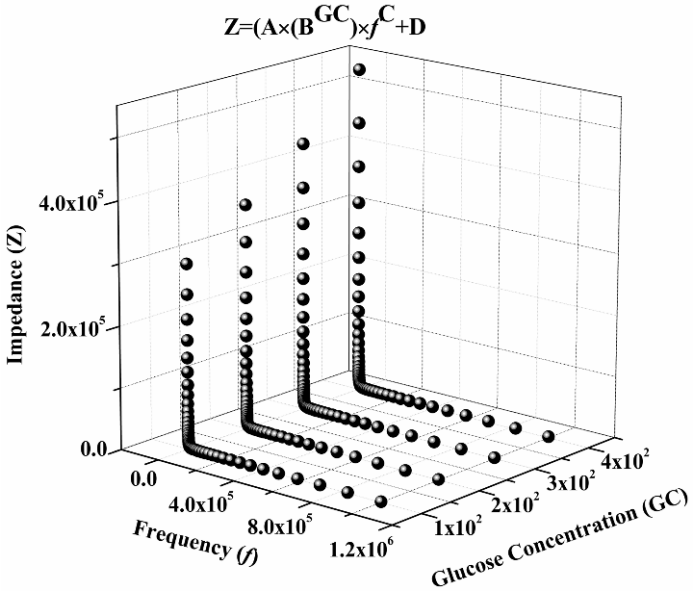

Fig. 7: Dependence plot of impedance with respect to scanning frequency and glucose concentration.

The empirical relation between impedance, glucose concentration, and frequency as obtained is expressed in Eq. (1).

$$Z = A \times (B^{GC}) \times f^{C} + D \quad (1)$$

Where, Z represents the impedance, GC is glucose concentration and f is the scanning frequency. A, B, C, and D are constant terms and their values are described in Table 2.

The constant values show a little variance between each other. However, the little difference in values along with variable blood glucose concentration is the main cause of decrease in impedance values with the increase of electrode dimensions.

Table 2: Constant values for different designs of three- electrode devices.

Devices	A	B	C	D
Design 1	0.2786×10^{8}	0.1002	-0.9475	0.1824×10^{4}
Design 2	0.2478×10^{8}	0.1002	-0.9958	0.1706×10^{4}
Design 3	0.2478×10^{8}	0.1002	-0.9581	0.1706×10^{4}
Design 4	0.1478×10^{8}	0.1002	-0.9581	0.1014×10^{4}

The dependence curve for impedance and blood glucose concentration is shown in Fig. 8. The empirical relation between impedance, glucose concentration, and working electrode area (EA) is expressed in Eq. (2).

$$Z = (P + GC) / (Q + R \times EA) \quad (2)$$

Here P, Q, and R are constants and their values are -0.2720×10^{3}, -0.6119×10^{-2}, -0.1685×10^{-6}, respectively.

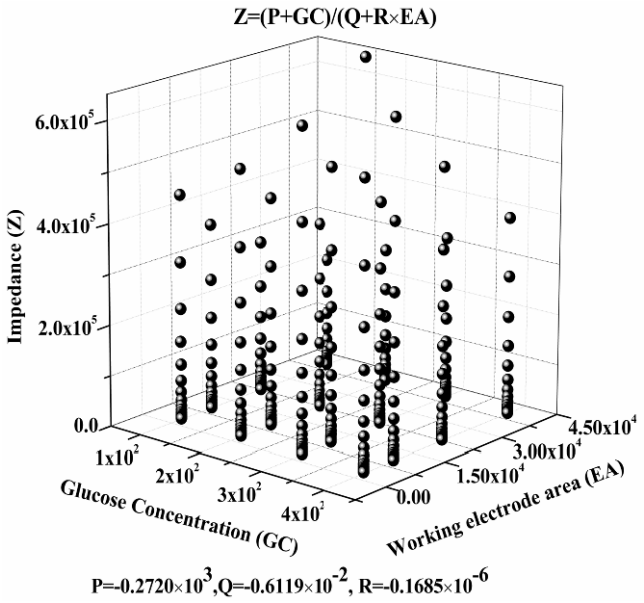

Fig. 8: Dependence plot of impedance with respect to working electrode area and glucose concentration.

However a direct correlation can be established between glucose concentration and impedance, keeping frequency and working electrode area as constants, which is described in Fig. 9. The empirical relation between impedance and glucose concentration is expressed in Eq. (3).

$$Z = S \times GC^{\,T \times GC} \quad (3)$$

where S and T are constant terms and their values are -0.3587×10^{5} and 0.2973×10^{-3}, respectively.

From the figure, it is evident that a positive slope is found during curve fitting of impedance data. Thus it can be said that with the increase of glucose concentration, impedance increases.

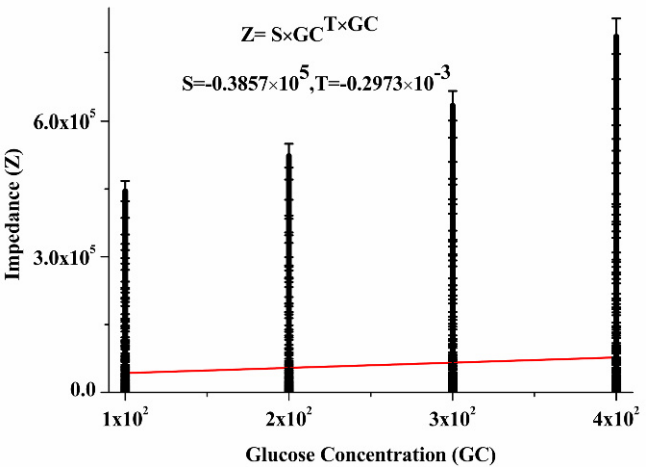

Fig. 9: Dependence plot of impedance with respect to glucose concentration.

Conclusion

The present study describes the design and fabrication of four microdevices with different dimensions by microfabrication technology and the measurement of blood glucose concentrations. From the present preliminary and pilot study it is evident that the variations in glucose concentrations in blood alter the impedance of the blood. With the increase of glucose concentrations in blood the impedance of the blood increases. Thus it can be said that the fabricated devices perform well for different blood glucose concentrations. Also the quantitative relation obtained by curve fitting also indicates a positive correlation between glucose concentration and impedance. However, the detailed mechanism of impedance alteration should be investigated further to establish the role of electrolytic factors present in blood.

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