THIN FILM MICROELECTRODES USED IN ELECTRICAL MONITORING OF BLOOD SEDIMENTATION

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Abstract: Planar microelectrode chips with thin film interdigitated arrays of electrodes (IDAE) have been developed. We investigated the use of IDAE for impedance measurements of human blood sedimentation which is an important factor of health status of a patient.

Introduction

Miniaturization of electrochemical analytical instrumentation has increased the importance of thin film technology as a part of microsystem technologies. Microfabrication of electrochemical thin film cells provide a powerful means of exploiting unique properties and new phenomena which take place in micro-nano-meter scale (high mass transport, and collection efficiency, low response time, high ratio of faradic to capacitive currents). Use of interdigitated arrays of microelectrodes for impedance measurements is favorable because of their exceptional attributes in the field of accuracy and response time. Various kinds of liquids and solutions have specific electrical properties, which are dependent on the amount of addition agents in the solutions, from impurity and outer influences (temperature and pressure), as well as chemical interface between electrode and solution. Any change of one of the parameters (concentration of addition agents, temperature of solution, its impurity, etc) will result in a change of the solution impedance. Measuring the conductivity of a small concentration (up to 0.1 mM) of electrolyte is difficult. Specific conductivity of these electrolytes is very low [1].

We simulated and consequently measured electrical parameters of IDAE chips and compared it with impedance measurements of KCl solution of various concentrations, as well as impedance of human blood.

Theory

If we apply any electrode in a solution, potential will evolve on interface, which depends on the material of the electrode and chemical composition of measured solution (Figure.1). The total interfacial capacitance (C_1) is the series combination of the capacity of a fixed sheet of charge at the interface (C_H) and the capacity of the diffuse ionic cloud (C_D).

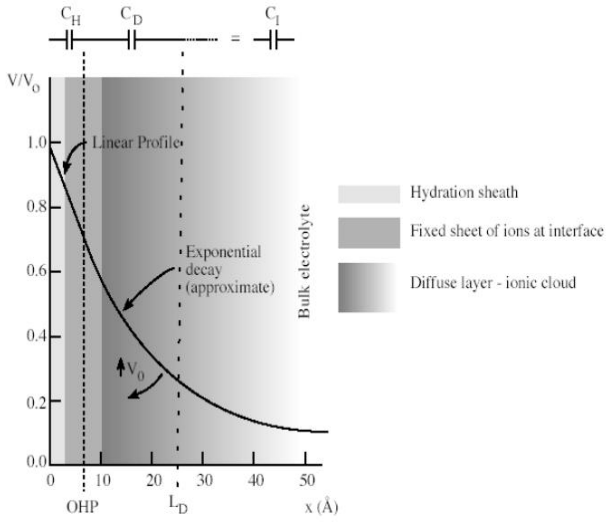

Figure 1: A schematic representation of an electrode - electrolyte interface. The hydration sheath, inner and outer Helmholtz planes (OHP) are shown as is the diffuse space charge layer (L_D) [2].

In the region between the electrode and the OHP, a linearly graded potential exists as the voltage is dropped across the oriented water dipoles. Beyond the OHP, the potential decays almost exponentially.

This decay is dependent on the voltage at the interface and becomes sharper as the potential (V_0) is increased (as shown by the arrow). The position of LD will shift to the left as the ionic concentration of the electrolyte is increased [2].

This model also consists of Warburg impedance Z_W which represents substance transport. Z_W isn't ideal element because it is frequency dependent.

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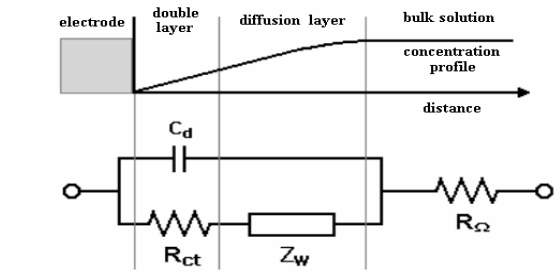
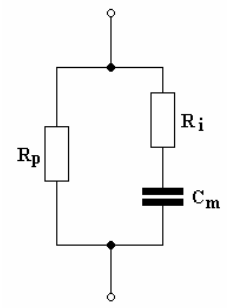


R_Ω - resistance of liquid
R_{CT} - resistance of electrical charge transmission
C_D – capacitance of double-layer
Z_W - Warburg impedance

Figure 2: Correlation of the different equivalent components of a faradaic process with electrochemical phenomena.

The sedimentation of red blood cells (Erythrocyte Sedimentation Rate – ESR) is one of the most commonly used clinical tests useful for diagnosis, prognosis and monitoring of certain contagious and cancer diseases. The measuring of ESR is based on the method of Westergren which is simple but takes a long time (0.5 to 2 hours) and requires a relatively large volume of blood.



R_p – plasma resistance (60-300 Ωcm)

R_i – cell interior resistance (300-1000 Ωcm)

C_m – cell membrane capacitance (300-1000 Ωcm)

Figure 3: Equivalent circuit model of blood impedance

An improvement in ESR measurements was achieved using a bioelectrical impedance technique [3]. In the sedimentation process of red blood cells we can distinguish three phases: aggregation, rapid setting and packing. During the aggregation period, red cells with a biconcave dics shape adhere together face to face into cluster – rouleaux. The forming speed and the final size of these macromolecules are critical for the outcome of the ESR. The purpose of our work was study ESR of blood in early phase of sedimentation (the agregation period) using the impedance method and thin-film microelectrode chips [4].

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Experimental techniques

The basis of measuring setup is impedance bridge from Hewlett Packard 4277A which was controlled by a computer. Bridge was a source of sinus signal with frequency range from 10 kHz to 1 MHz with output voltage of 20 mV and the magnitude of absolute value and phase of output signal from IDAE chip was measured (Figure 4).

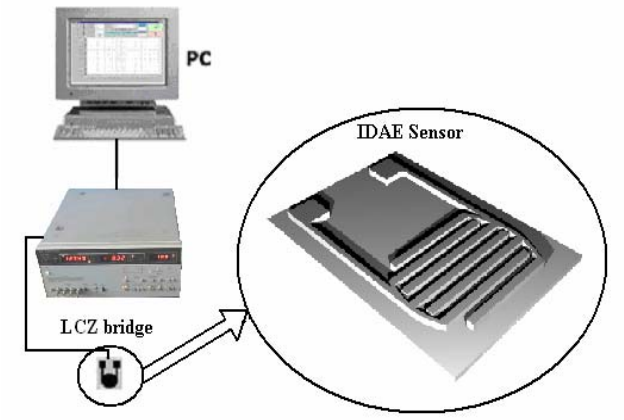

Figure 4: Measurement setup and detail of IDAE chip with dimensions 400x400 μm

Production of chips was realized by microelectronic technologies. Metal layers were deposited by diode RF radio high frequency sputtering on Al_2O_3 ceramic substrates. To remove the polarization effect we used Pt. For increase of adhesion between ceramics and platinum we used interlayer of Titan beneath functional platinum layer. Microelectrodes were patterned in metal film with help of photolithography and „lift-off" technology.

The dimensions of symmetric IDA microelectrode configurations were:

- 100 μm/100 μm (finger/gap width)
- 200 μm/200 μm
- 400 μm/400 μm

Small amount (drop of valvue 60 – 80 μl) of measured liquid (blood, KCl) was applied on active surface of the chip followed by automated measurement. Because material of electrodes is chemically inert against measured solutions, we were able to reuse chips after we clean down of it.

Measurements of human blood

Structure of human blood is more complicated compared to KCl. Blood it self consists of 15 parts (e.g. blood platelets, enzymes, minerals etc) and we are only interested in concentration of erythrocytes and lymphocytes in blood plasma. An improvement in Erythrocyte Sedimentation Rate (ESR) measurements was achieved using a bioelectrical impedance technique

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[3]. In the sedimentation process of red blood cells we can distinguish three phases: aggregation, rapid setting and packing. During the aggregation period, red cells with a biconcave disc shape adhere together face to face into cluster – rouleaux. The forming speed and the final size of these macromolecules are critical for the outcome of the ESR. Increased sedimentation has been helping us to find out various unknown illnesses like cancer or various forms of inflammations for decades. When blood chemical attributes change, impedance also changes and therefore we measure this change.

System of measurement for human blood is similar to that one of KCl. We not only focused on the dependence of absolute value of impedance on frequency but also its change during aggregation phase of sedimentation (period of time about 15 minutes). Because we only work with small amount of blood that coagulates, every measured sample had to be secured with anti-coagulate agent (citrate). On the Figure 5 is a comparison of absolute value impedance courses for 3 different sizes of chips and the same sample of human blood.

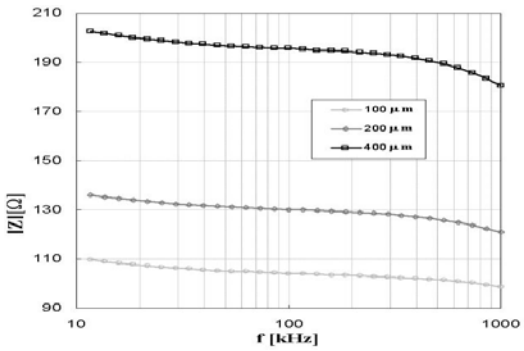

Figure 5: Influence of IDAE dimensions on the absolute value impedance course for identical blood sample in frequency range 10 kHz – 1 MHz

From gained results we can see that measuring of blood by IDAE with larger dimensions are more suitable. For high conductivity of electrolyte (like a blood) 400x400 µm IDAE with less amount of fingers are appropriate. On the other hand, solutions with lower conductivity require higher amount of IDEA fingers.

For measuring of blood it is important that the samples are as fresh as possible. In reality it means that fresh sample has to be measured after blood taking because its quality decreases despite of storing it in a cool and dry place for one months (Figure. 6).

At various pathological organism symptoms the impedance of blood changes, whereas healthy blood has the highest impedance. Change in blood impedance is sensitive to various illnesses and a little regression can identify as a no serious illness, e.g. simple cold. We measured dependences of absolute value of impedance and phase on frequency in the range of 10 kHz – 1 MHz. We found that the absolute value impedance for healthy blood was in the range between 245 Ω (for 10 kHz) and 195 Ω (1 MHz). For cancer blood was range of 205-155 Ω (10 kHz-1 MHz).

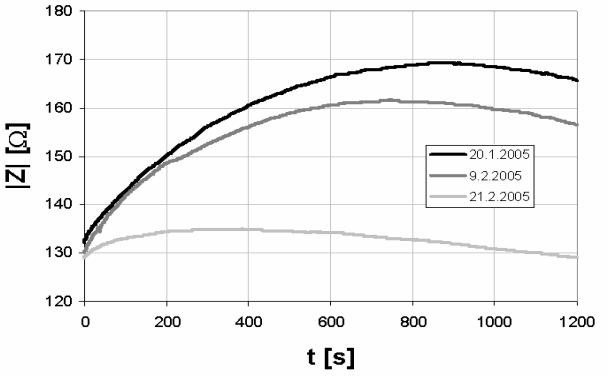

Figure 6: Decrease of human blood quality presented by change of impedance for long time by IDAE 200 µm

We observed the difference between healthy and cancer blood. From results given by IDAE with sizes of 100 µm and 200 µm was not possible to exactly specify range of healthy and cancer blood and therefore we used only 400 µm IDAE for next measurements.

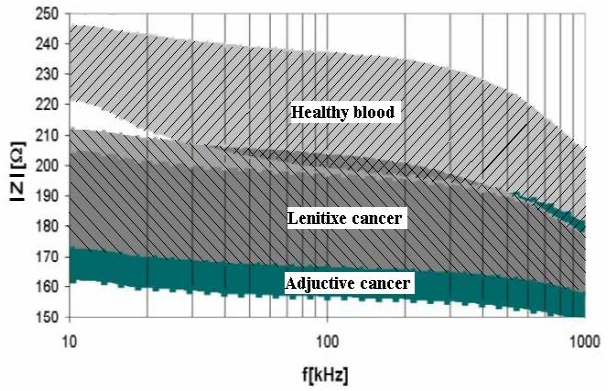

Figure 7: Zonal diagram distinguished between healthy and cancer (lenixite/adjuctive) blood obtained by IDAE 400 µm in frequency range 10 kHz –1 MHz.

Zonal diagram (Figure 7) was constructed from results of more than 70 blood samples of 45 patients. Frequency range up to 1 MHz has proven itself to be unnecessarily wide because the highest impedance differences occur at low frequencies and with increasing frequency the difference between healthy and cancer blood declines. This confirm also our preliminary results measured in the frequency range from 20 Hz to 10 kHz.

In Figure 8 we can see some selected measure samples of healthy and cancer blood samples, where using Cole-cole graph is shown approach of real and imaginary impedance part and this way even more accurate identification of healthy and cancer blood samples is possible.

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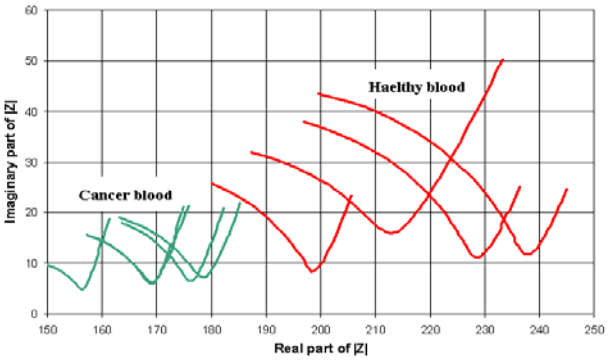

Figure 8: Cole-cole graph shows difference between healthy and cancer blood.

Absolut value of impedance is dependent on various types of external factors and approach of blood compounds. That is why we have to watch not only its absolute value but also phase of measured course, that can help us to identify real and imaginary part of impedance. Real part is dependent on sample resistivity and electrode, but imaginary part defines capacity changes in measured blood sample.

Conclusion

The preliminary results showed that we can define impedance properties of IDEA chips with the help of equivalent electric model.

We found out than for impedance measurement is better to use IDAE chip with large dimension because this chip is more sensitive for impedance changes. IDEA 100 µm and 200 µm chips were not able to differentiate healthy and cancer blood samples. We determined an optimal frequency range for impedance measurements of different solutions (KCL, blood) below 30 kHz. By means of IDAE chips 400 µm we can distinguish healthy and cancer blood samples with probability higher than 90 percent for lower frequencies than 30 kHz. Impedance measuring is not so complicated for patients, because we need only small quantity human blood and this method is easy combinable with other medical method. We predict that our impedance method could be used for early detection (screening) of critical human illnesses (cancer) and then these diagnoses will be checked in detail by classical medical laboratory methods. Another application is monitoring of guality of stored human blood. Their resistance and capacitance decrease as well as quality of blood. For this reason we must check impedance of human blood because it is important for good quality of blood.

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