

# SPECIFIC BEHAVIOUR OF THE BLOOD SEDIMENTATION PROCESSES EXAMINED BY THE ELECTROCHEMICAL IMPEDANCE MICROSENSOR

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## Abstract

*Electrochemical impedance microsensor for the fast monitoring of the blood sedimentation has been developed. Planar microsensor consisted of the interdigital array of electrodes (IDAE - finger/gap widths of different values from 5/5  $\mu\text{m}$  to 400/400  $\mu\text{m}$ ) based on Au or Pt thin films sputtered on Si/SiO<sub>2</sub> or ceramic alumina substrates. IDAE microsensor allows time measurements of electrical impedance changes - impedance rates - (at frequencies of order 0.1 kHz and 10 kHz) of small blood drop applied on it. The determination of the impedance rate during sedimentation and the impedance spectrometry at a low-frequency range of order of 1 kHz seems to be very helpful for a quick diagnostics of the health state. In the IDAE microsensor erythrocyte aggregation/rapid settling/packing periods associated with dry-period are overlapping due to the planar arrangement of dimensions in order of 1 -100  $\mu\text{m}$ . The time monitoring of the blood sedimentation (in the range of 10-900 seconds) by the impedance method can distinguish between healthy and cancer state of blood and could serve for the simple long-term diagnostics after the surgical operation or as a screening procedure for early diagnoses.*

## Keywords

*planar IDAE impedance microsensor, blood sedimentation*

## Introduction

Thin films are able to create "the bridge" between micro- and nano-technologies utilized in the fabrication of advanced biochemical microsensors. Thin films can serve as well-defined and reproducible supports and interfaces among sensing, recognition and transduction sites in electrochemical sensors, which may exhibit new and partly unique response characteristics due to their micrometer size. Thin-film interdigital array of electrodes (IDAE) is among the most used periodic electrode structure in sensors and actuators [1]. Planar IDAE permitted us to exploit also the new phenomena on micro-/nano- metric level and the miniaturization of biomedical sensors (e.g. amplification effect in cyclic

redox voltametry [2], monitoring of human stress - psychogalvanic skin effect - by impedance method [3] and a novel approach to ratio and relation measurements by the "smart" asymmetric combination and integration of IDAE structures [4]). 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 diseases. The standard measurements of ESR is based on the Westergren method which is simple but takes a long time (0.5 to 2 hours) and requires a relatively large volume of blood [5]. An improvement in ESR measurements by a triple-frequency impedance method has allowed faster analysis [6].

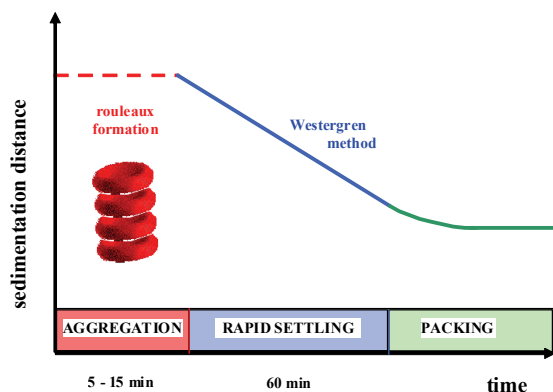


Fig. 1: Three phases of the standard red blood cell sedimentation.

We can distinguish three phases in the sedimentation process of red blood cells: aggregation, rapid setting and packing, Fig. 1. During the aggregation period, red cells - with a biconcave disc shape - adhere together face to face into clusters – rouleaux [7]. This process is reversible and takes place in the first minutes of sedimentation [8, 9]. The forming speed and the final size of these macromolecules are critical for the outcome of the ESR.

The aim of our work was to investigate specific features of red blood cell sedimentation by using impedance method and planar thin-film microsensor.

## Experimental

Thin film IDAE microsensors consisted of Si or ceramic chips with dimension of 3x10 mm, Fig. 2.

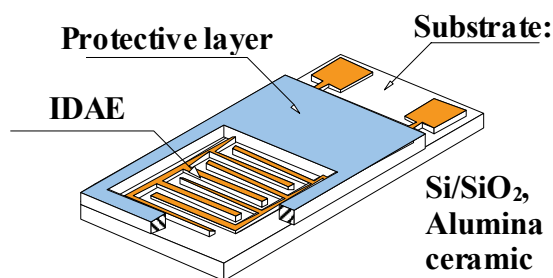


Fig. 2: Planar thin film IDAE microsensor.

We deposited Au and Pt thin films (thickness of 350 nm) with adhesion Ti/Pd layers (50 nm/50 nm) on Si/SiO<sub>2</sub> or alumina ceramic substrates in a planar RF sputtering diode system Perkin Elmer 2400/8L. IDAE of different finger/gap widths of values from 5/5 μm to 400/400 μm were patterned by photolithography and „lift-off“ technology. The protective Parylen thin film was deposited by evaporation and formed by plasmochemical etching.

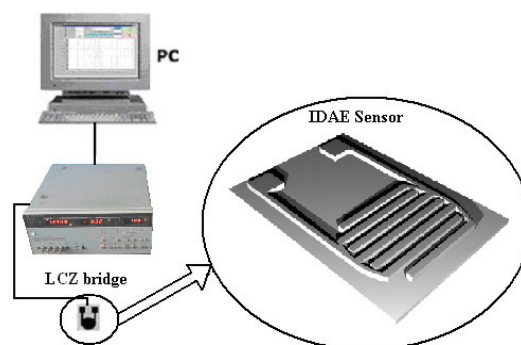


Fig. 3: Measurement setup by RLC bridge.

The red blood cell sedimentation rate was monitored by measurement of impedance  $|Z|$  after applying a drop of blood to planar IDAE microsensor. Impedance measurements were performed in two frequency ranges: (i) 10 kHz-100 kHz (RLC bridge, Fig. 3) and (ii) 10 Hz-10 kHz (measuring system with potentiostat PST-3 FM-99, Fig. 4).

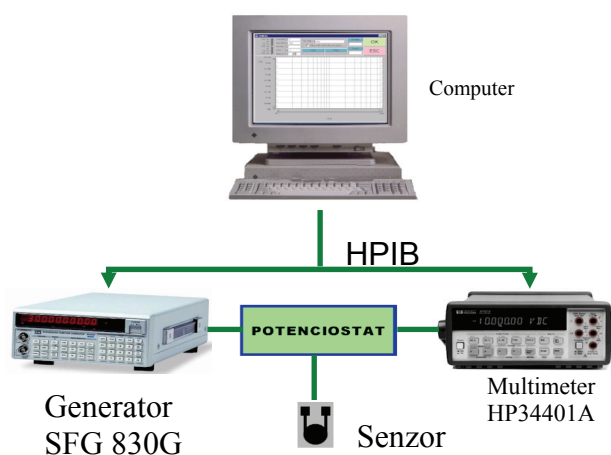


Fig. 4: Measurement setup based on potentiostat.

## Results and discussion

For evaluation of basic electrochemical impedance parameters of IDAE microsensors with different dimensions various concentrations of KCL solution were used (typical dependences of imaginary vs. real parts of impedance - Cole-Cole graphs - are shown in Fig. 5). An impedance of the electrochemical cell is given by the geometric capacitance of the cell electrodes, the resistance and the capacitance of the double-layer formed at the electrode/electrolyte interface, the resistance of an electrolyte, and by the Faraday impedance connected with the charge transfer [10]. The decrease of IDAE dimensions caused a shift of real/imaginary parts of impedance to lower values.

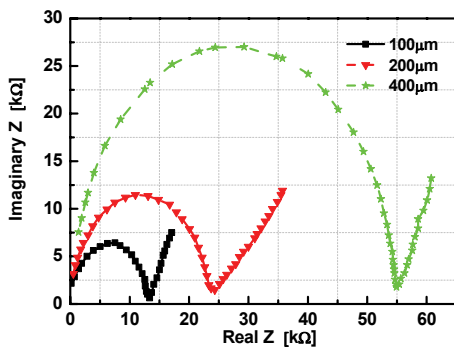


Fig. 5: Cole-Cole graphs for the different IDAE dimensions in 0.05M KCl solution.

The real part of impedance is getting smaller because it consists of double-layer resistance (it is of order of 1000 ohm in 0.05 M KCl solution) and solution resistance (of order of 100 ohm). The imaginary part decrease is caused by the IDEA/double-layer capacitance increase (from IDAE 400  $\mu\text{m}$ /20 pF to 100  $\mu\text{m}$ /70 pF [11]).

The time dependence of impedance was also influenced by the amount of the blood (the drop volume was in order of 10  $\mu\text{L}$ ), Fig. 6. These observations reveal the necessity of the exact dosing of blood on the IDAE microsensor. Also an effect of the blood drying took place after 1000 seconds (the second increase of the impedance is shown in Fig. 6).

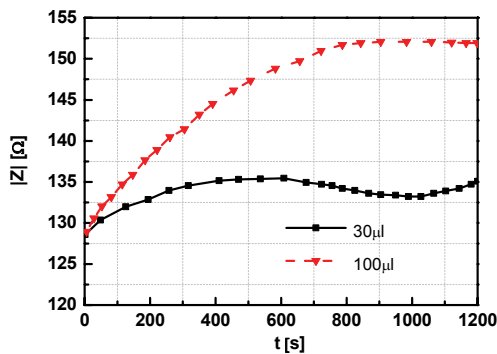


Fig. 6: Time development of the impedance for different sizes of blood drops.

During cold-store of the blood in vitro, its impedance (resistance and capacitance) showed downward trend with time throughout one month, Fig. 7. That phenomenon can be exploited for the monitoring of the blood quality when blood is kept for a transfusion.

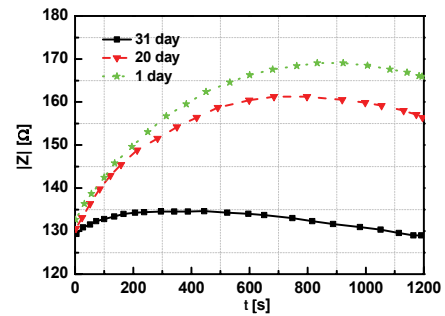


Fig. 7: Aging of the identical blood sample: the comparison of time dependencies of blood impedance in the period of 31 days.

At the beginning of our study we found that both the maximum of impedance  $|Z|$  and the time derivation of impedance – impedance rate  $d|Z|/dt$  – give very useful information in the early period of sedimentation (Fig. 8). The impedance rate eliminates an influence of non-desired static parameters (including absolute values and “offset” effects) and it allows relative comparison of different samples. The impedance rate is considerably higher for blood of cancer (malignant) donors in comparison with non-cancer (benignant) ones - Fig. 8 represents the examination of 25 patients.

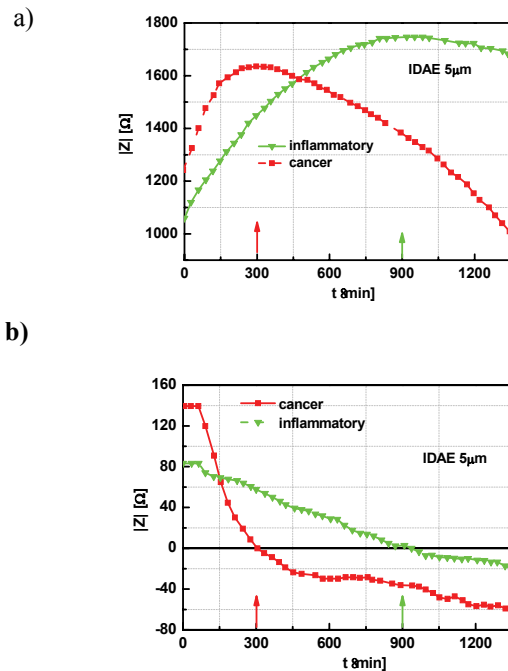


Fig. 8: Typical time dependencies of impedance a) and impedance rate of the benignant and malignant blood b).

An increase of impedance at the beginning of sedimentation is caused by the erythrocyte aggregation. They are negatively charged and repel each other under normal conditions. Many plasma proteins are positively charged and they can neutralize the surface charge of cells. Also change or damage of the blood cell structure can affect the aggregation process. Both mentioned effects shorten the aggregation period and cause high ESR. The aggregation period lasts tenth of seconds only as was confirmed by the kinetic model for erythrocyte aggregation [7] and optical analysis [8] of blood cell sedimentation. Therefore we can presume that the non-monotonous course of impedance (increase and decrease of the impedance rate) characterizes both aggregation and settling periods at the beginning – further on the settling period is going along with the packing period.

Spectral impedance measurements in the frequency range from 10 Hz to 10 kHz were performed by the setup based on potentiostat (Fig. 4). Impedances of the IDAE microsensor, blood and of the potentiometer input behaved as a low-pass filter with the maximum of several kHz [12]. The shift of the norm current maximum was 1.4 kHz for patients with the malignant disease in comparison with patients with benignant one, Fig. 9.

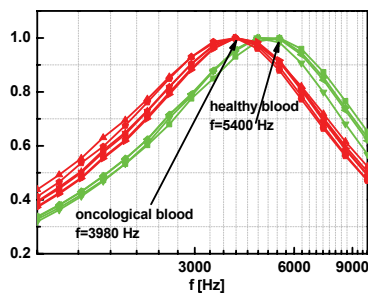


Fig. 9: The norm current output of the frequency spectral characteristic of healthy and oncological blood.

It is also interesting to monitor changes of the electrical activity of blood before and after the surgical treatment in the case of an oncology patient (Fig. 10).

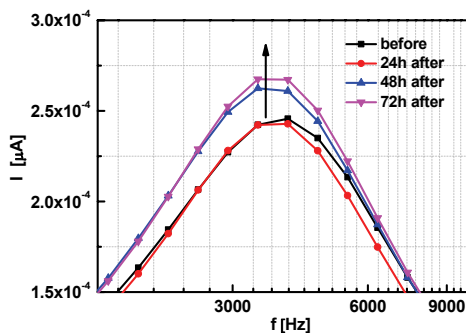


Fig. 10: Time development of electrical properties of blood before and after surgical operation of the malignant tumour.

After an operation of the malignant tumour, within 72 hours the value of impedance was increased a little, but the frequency corresponding to the current maximum did not change.

We can describe the specific behavior of blood sedimentation processes proceeding in the planar electrochemical impedance microsensor by the schematic diagram (Fig. 11). Every consecutive period is overlapping because of small volume of the blood drop and micrometer dimensions of the IDAE. The red cell aggregation caused the impedance rise resulting from the lowering of the charge concentration/transfer. The maximum of a curved impedance line in a graph (Fig. 11) is determined by both processes: rapid settling (an increase of the total impedance included also a lowering of the double-layer capacitance) and packing (a decrease of impedance by the shortening of the IDAE capacitance due to growth of the large red cell aggregates). In addition, there is a dry-period which starts at the edge of the blood drop. It reduces the active area of the IDAE structure, i.e. the total impedance is rising after 103 seconds approximately.

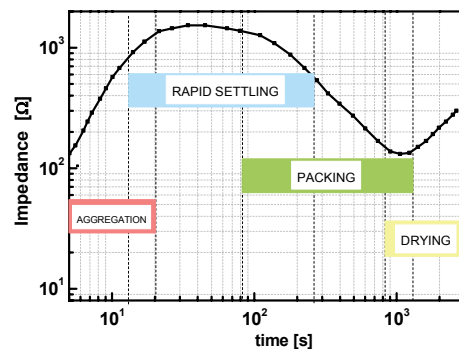


Fig. 11: Proposed scheme of red blood sedimentation processes characterized by the impedance in the planar IDAE arrangement on the microscopic level.

## Conclusion

Miniaturization of the planar microsensor using the interdigitated array of electrodes paves the way to the fast monitoring of the blood sedimentation by impedance method. The advantage of this technique is in the small amount of blood (~ 10 μL) necessary for diagnostics and quick sensor response. Blood sedimentation processes on the micrometric scale differ from the standard macroscopic one. In case of the IDAE microsensor erythrocyte aggregation/rapid settling/packing periods associated with dry-period are overlapping due to the planar arrangement of dimensions in order of 1-100 μm. The determination of the dynamic blood impedance parameter (impedance rate) during sedimentation seems to be very helpful for

a quick diagnostics of the health state. Also the impedance spectrometry of blood at a low-frequency range of order of 1 kHz provided useful information. The presented methods could be used for the simple monitoring of patient states after malignant or benignant operations. Another application seems to be an early detection (screening) of some human illnesses (incl. the fast differential diagnosis between cancer and inflammatory diseases) and in the monitoring of the quality of a stored human blood.

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