Research of Ionic, Molecular and Cellular Mechanisms for Formation of Electric Impedance in Biological Fluids and Tissues

A I Zuev\textsuperscript{1,2,*}, N N Shakirov\textsuperscript{1}, A I Sudakov\textsuperscript{1} and V Ju Mishlanov\textsuperscript{3}

\textsuperscript{1} Institute of Continuous Media Mechanics, Perm Federal Research Center of Ural Branch of Russian Academy of Sciences, Perm, Russia
\textsuperscript{2} Perm National Research Polytechnic University, Perm, Russia
\textsuperscript{3} Acad. Wagner Perm State Medical University, Perm, Russia

E-mail: *zal@icmm.ru

Abstract. Contemporary medicine applies the analysis of molecular composition and physicochemical properties of biological fluids as one of the main tools for diagnostics and control over body state and behaviour of pathological processes. However, most laboratory diagnostics methods (such as biochemical, enzyme-linked immunosorbent assay, etc.) are too expensive and time-consuming. They demand the availability of well-equipped laboratories, complex equipment, scarce and expensive reactants and materials and the highly skilled medical staff. Compared to them, bioimpedansometry is an incomparably simpler and cheaper method of laboratory research, becoming increasingly popular for diagnosing internal diseases. In the current research the main physical factors responsible for bioelectrical impedance variations in some conductive intracellular and extracellular biological fluids with different protein molecules, cell components and microbes were studied experimentally. The original laboratory impedance meter was developed for investigation of blood or interstitial biological fluids as well as condensates composition in order to determine the concentration of organic substances in them and the rate of biochemical and immune reactions.

1. Introduction

In modern medicine, the analysis of the molecular composition and physicochemical properties of intracellular and extracellular (interstitial and vascular) biological fluids is one of the essential tools for diagnosing and monitoring the body's state and its pathological course processes. Currently, various methods are widely used for this purpose, such as biochemical [1-2], enzyme immunoassay [3-4], immunofluorescent [5-6], radioimmune [7], etc. However, most of these instrumental and laboratory diagnostics methods are too costly and time-consuming. They require well-equipped laboratories, highly qualified medical staff, scarce and expensive reactants and materials. Therefore, the development of new promising methods for studying the content of various constituent components in biological fluids with the ability to monitor the dynamics of changes in their concentration during the development of various diseases can be of great practical importance for diagnosing these diseases. A rheographic method based on the registration of bioelectric impedance (electrical resistance of biological tissues and media to alternating current) may well be a promising method for conductometric diagnostics of the cellular composition of blood. In recent years, impedance-metric methods for non-invasive diagnostics of the human body's internal organs have have become popular, not least due to their simplicity and low cost. The areas of the modern application of these methods in clinical practice are the study of the state of the cardiovascular system (rheocardiography and similar techniques) [8-9]; differential diagnosis of diseases of parenchymal organs (liver, etc.); analysis of the water sectors of the human body; determination of fat and musculoskeletal mass of the human body [10-11], etc.
As is well known, electrical impedance is the total (complex) resistance of the medium to alternating current, which consists of active (ohmic) and reactive (capacitive) summands. Biological tissues are rather complex in chemical composition multicomponent media with significantly different physical characteristics, including electrical conductive and dielectric properties. Electrical conductivity is typical for all biological extra-cellular fluids based on electrolyte solutions (blood plasma, interstitial fluid, bile). The formation of free ions providing electrical currents in such fluids is due to the dissociation of inorganic salts and protein molecules. The main cations are salts of sodium, potassium, calcium and other monovalent and divalent metals; anions – chlorides and bicarbonates, lower concentration. Dielectric properties of electrolyte solutions are mainly characterized by relaxation times of charges and polarization phenomena – dipolar orientational polarization of dielectric-solvent (water) molecules and electrolytic concentration polarization of the solution near the electrodes.

In natural biological fluids, such as blood, containing various structural dielectric components (for example, organic lipid or protein molecules, cell complexes, etc.), additional macrostructural polarization with positive and negative movement ions appears opposite directions due to an externally applied electrostatic field. They reach the surface of these impenetrable to them objects and accumulate there, creating different dipole moments. Since macrostructural polarisation’s relaxation times are not very long (10^{-3}–10^{-8} sec), a significant reactive (capacitive) resistance, contributing to bioimpedance, appears in such biological media at enough low frequencies. As a result, the specific resistance of blood containing, in addition to the corpuscular elements, also solutions of proteins, fats and polysaccharides, is significantly higher than the resistance of electrolytic physiological solutions. Besides, inflammation of biological tissues is accompanied by cell damage and hydration, leading to a change in the cell’s capacity and intracellular membranes. The degree of inflammatory processes activity correlates with leukocytes and protein molecules’ content with characteristic electrical conductivity in blood. Therefore, the analysis of changes in the conducting and dielectric properties of biological fluids (such as blood, bile, interstitial fluid, etc.) is very promising in medicine for assessing the structure, condition and viability of tissues, as well as determining the intensity of biological processes occurring in them.

This study aimed to create an adequate biophysical model of the processes associated with the registration of electrical impedance in conducting model and biological fluids containing charged or dielectric protein and cellular inclusions and complexes; to experimentally study the main physical dependences of bioimpedance parameters on the properties and composition of organic liquids in a wide frequency range of probing alternating current; to develop an original laboratory impedance meter, measuring cuvettes and laboratory analysis techniques for studying the biochemical composition of blood and tissue fluids; to create software algorithms for calculating the concentration of organic elements in biological fluids and the rate of sedimentation processes, biochemical and immune reactions; to increase the accuracy and reliability of determining the leading indicators of the vital activity of biological tissues according to impedance measurement data.

2. Materials and methods

2.1. Electrical impedance analyzer

A multichannel semi-automatic device is designed to measure the complex electrical resistance (impedance) and phase angle between current and voltage in biological fluids in a wide range of changes in the probing frequency and measurement time [12]. The device is based on the ADC-DAC Zet-210 with other electronic boards of amplifiers, generator, phase analyzer and multiplexer of analogue signals. The device is connected via the "USB-2" port to a personal laptop computer (figure 1). The device will require no additional power supply.
Figure 1. Photo of a laboratory electrical impedance analyzer (laptop, measuring unit, immunological tablet).

For more accurate measurements, the device warms up for at least 10 minutes, disconnects the laptop power supply, and works on the battery since the power supply can create significant measurement noise.

2.2. Main technical characteristics of the device
The ADC digit capacity – 16 bits, DAC capacity – 14 bits.
Power supply +5 V with a consumption current <500 mA from the USB-2 port.
The number of channels (cells) for recording impedance and phase angle is 8.
The range of impedance measurement is from 10 Ohm to 100 kOhm (divided into two subranges).
The measurement range for the phase shift angle runs from 0 to 90 degrees.
The range of probing frequencies runs from 10 to 30 000 Hz.
The magnitude of the probing current is from 0.05 to 5 mA.
The measurement time for each cell runs from 1 to 60 sec.
The total measurement time in each experiment – up to 480 sec.
The maximum sampling rate of the recorded data in the experiment – 33 points per second (in this case, bad points are rejected and averaged over 167,000 measured points).
Instrument error – from 0.01% to 2% depending on the range of impedance measurement and angle.
The setting of measurement parameters: frequency, current, cell measurement time, total measurement time, registration frequency is performed programmatically using a control file. The output of the measurement results is in the form of graphs and text tables indicating the cell number, sounding frequency and the number of averaging points of this cell for the entire measurement time in the experiment.

2.3. Measuring cells and electrodes
Measurements of the electrolytic conduction of the liquids under consideration were carried out in model measuring cells (microchambers) in the form of a plastic tube 18 mm long and 1.2 mm in inner diameter. Thus, the microchamber volume amount to 20.4 μL. Special attention was paid to the thoroughness of filling the microchambers with the tested liquid, for which cells with both two and three outlets were used. In the first case, one of the holes was used to fill the chamber, and the other to adjust its uniform filling. In the second case, the chamber had an additional central opening, congruent to the pipette-dispenser's nozzle, for filling it with biological fluid and two lateral ones intended for adjusting its uniform filling.

At the ends of the microchamber, metal (steel or nichrome) electrodes of complex cylindrical and conical shape were fixed, the outer ends of which were funnel-shaped for a snug fit of the micropipette tip, and the inner ends were tapered to prevent liquid from flowing out during the measurement procedure. Before measurements, the chambers were tested on conductive liquids with a well-studied
and known resistivity (such as NaCl solutions). The measured impedance values were compared with those calculated for the given geometry of the working cells.

In clinical conditions, the studied biological fluids’ impedance parameters are measured in the cells of a standard immunological plate, widely used in various clinical medicine fields. The plate consists of 96 flat-bottomed plastic wells with a volume of 300 μl, interconnected by eight pieces in separate 12 immunological strips, fixed in a particular fixing frame. The measuring sensors are located in the cover of the immunological plate. The cover of the plate is made in the form of a textolite board 125×90 mm size, with wire vertical paired electrodes protruding from it on one side for eight plastic wells of the immunological plate. All 16 electrodes, made from rigid nichrome wires with a length of 5 mm and a diameter of 1 mm, are arranged in one row (the first cell of the tablet is located in the upper left vertical row). The distance between two paired electrodes in one well is 3 mm. The electrodes’ location allows them to be placed from above into the wells of the immunological plate without force or touching the electrodes to the plate walls well. The measuring sensors are connected to the measuring unit using a cable with a connector.

2.4. Tested liquids
In the experiments, the solutions of conducting liquids with various concentrations were investigated: sodium chloride NaCl (9, 20, 40, 70, 100 g/l); potassium chloride KCl (4.1, 5.1 mmol/l); calcium chloride CaCl₂ (2.0, 2.5, 3.0 mmol/l); magnesium sulfate MgSO₄ (0.5, 0.8, 1.0 mmol/l); glucose solutions in 0.9% NaCl (4, 5, 14, 26; 56 mmol/l); solutions of albumin in 0.9% NaCl (63, 67, 70, 71, 80, 90, 100 g/l); solutions of immunoglobulin in 0.9% NaCl (6.5, 10, 16, 40, 60, 100 g/l); solutions of total cholesterol in 0.9% NaCl (2.6, 3.1, 3.6, 4.1, 4.6 mmol/l); solutions of alpha-cholesterol in 0.9% NaCl (0.42, 0.56, 0.70, 0.85, 0.98, 1.13, 1.27, 1.41 mmol/l) and triglycerides (neutral fats) in 0.9% NaCl (0.23, 0.68, 0.91, 1.14, 1.60, 1.82, 2.05 mmol/l). Cholesterol at a concentration of 200 mg/deciliter (5.17 mmol/l), cholesterol-alpha at a concentration of 54.6 mg/deciliter (1.41 mmol/l), and the “Triglycerides” calibrator at a concentration of 200 mg/deciliter (2.28 mmol/l) were provided in a test kit for calculating the agent concentrations produced by Human.

The blood serum of 22 patients with ischemic heart disease in the form of unstable (progressive) stenocardia was also examined during their inpatient treatment at №4 City Clinical Hospital (Perm) and 20 patients with multiple myeloma treated in the cardiology department of the Perm Regional Clinical Hospital. Patients with ischemic heart disease were from 53 to 82 years, including 12 women and ten men. The age of multiple myeloma patients was 40-75 years (7 women, 13 men). At that six patients with multiple myeloma had a non-secreting variant, whereas 14 – variant G. The study was approved by the Ethic Committee of Perm State Medical University (Protocol No.12 from 23.12.2011). For comparison with the impedance analysis results, the concentration of total protein in the blood serum was determined by the biuret method which, at an ADC sampling rate (digitizing) of 192 kHz, gave almost 600,000 points. The scatter of measurements for 3 samples varied from 2 to max 5%. The dispersion dependences of the modulus and phase angle on the frequency and relationships of the impedance parameters on organic matter concentration were constructed.

The software written in the "Delphi" language for the operating systems "Windows-XP" and "Windows-7" has a convenient user interface of modern design, which ensures the registration of the examined patients with the creation of an appropriate database according to the parameters: name, age,
diagnosis, date survey, survey results. The volume of the database is at least 250,000 patients. The software allows sequential measurement of electrical impedance in each of the eight wells of the immunological plastic plate for eight patients at once, recording the measurement results, calculating the corresponding electrical parameters using unique mathematical formulas, and automatically entering the results database. The software also makes it possible to display the results of measurements and calculations on the screen and/or print with complete information from the database, differentially by the study's date and the patient's specific name.

3. Measurement results
Measurements have shown that, for purely conducting solutions of sodium chloride, potassium chloride, calcium chloride and magnesium sulfate, the modular value of the electrical impedance of biological solutions decreases depending on the increase in the frequency of the probing alternating electric current. When the frequency reaches 5 kHz, the impedance modulus's values cease to change and remain approximately constant. Simultaneously, a decrease in the phase angle is observed, which at high frequencies (more than 5 kHz) is only ~1-2 degrees. This fact indicates that at high frequencies, the liquid's electrical admittance is determined only by the active (ohmic) resistance, and the role of the capacitive component of the impedance becomes negligible. Its electrical properties determine the dependence of the modular value of the impedance on the biological component concentration. It turned out that two different types of biological fluids can be distinguished, differing in resistivity behaviour.

The first group includes solutions of the substances with molecules that have no electric charge and are dielectrics: for example, glucose or lipids (i.e. esters of glycerol and monobasic fatty acids), in particular, such neutral fats as triglycerides. Triglycerides can enter the body with food or be synthesized in adipose tissue, liver and intestines. They are the primary source of energy for tissue cells. In these solutions (fats + aqueous solution of sodium chloride NaCl), the increase in triglycerides' concentration while maintaining the total volume of the solution means a simultaneous decrease in sodium chloride concentration ions. This decrease leads to a significant (up to several times) increase in the active (ohmic) resistance of the solution (figure 2). As a result, it becomes possible to reliably (with relatively high accuracy) determine the triglycerides' concentration in such solutions from their electrical impedance values.

The second group includes mixtures of the substances with molecules that have an electric charge and can contribute to the solvent's ionic conductivity (saline NaCl solution): solutions of total cholesterol, alpha-cholesterol, albumin, immunoglobulin. In such compositions, with an increase in the protein concentration, the growth of active resistance due to the decrease in sodium chloride ions concentration is compensated by the additional ionic conductivity created by the protein molecules themselves. As a result, the total resistance (impedance modulus) of these solutions as a whole either does not change or slightly increases or decreases, depending on the specific substance features (figure 3). Therefore, the unambiguous determination of the concentration of proteins in such solutions by their active resistance values turns out to be impossible.

However, at low frequencies from 20 to 10,000 Hz (in the so-called region of alpha dispersion of electrical impedance), the contribution of the capacitive component of the resistance is, nevertheless, not small (the phase angle values reach 20-30 degrees). At the same time, it was found that the characteristic type of dependencies of the phase angle on frequency turned out to be very specific for each biological fluid (figures 4-7).
Figure 2. Dependences of impedance modulus on concentration of triglycerides at frequency 20, 100 Hz, 1, 5, 10, 20 kHz (lines 1-6).

Figure 3. Dependences of impedance modulus on concentration of cholesterol at frequency 20, 100 Hz, 1, 5, 10, 20 kHz (lines 1-6).

Figure 4. Phase angle of impedance versus frequency for albumin concentrations 63, 67, 70, 71, 80, 90, 100 g/l (lines 1-7).

Figure 5. Phase angle of impedance versus frequency for immunoglobulin concentrations 40, 60, 100 g/l (lines 1-3).

Figure 6. Phase angle of impedance versus frequency for cholesterol concentrations 2.6, 3.1, 3.6, 4.1, 4.6 mmol/l (lines 1-5).

Figure 7. Phase angle of impedance versus frequency for alpha-cholesterol concentrations 0.42, 0.56, 0.70, 0.85, 0.98, 1.13, 1.27, 1.41 mmol/l (lines 1-8).
Consequently, the form of observed dispersion dependences of phase angle can be used to identify and calculate the concentration of conductive substances in mono-solutions and mixed solutions of biological compounds and human blood serum in various diseases. However, to realize this possibility and determine in clinical practice the concentrations of biological substances by the parameters of their capacitive resistance, individually for each biological substance, the construction of mathematical models and equivalent electrical equivalent circuits of calculation the active and capacitive conductivity is required.

4. Discussion
The experimental and clinical studies' obtained results were processed by statistical methods using the Statistica 8.0 software package. Thus, the electrical impedance values of glucose solutions turned out to be quite large, although experimental studies were carried out with glucose solutions prepared not in distilled water but physiological sodium chloride solution. The electrical resistivity properties become apparent in glucose solutions with a concentration higher than physiological (more than 27 mmol/l, significantly more than 56 mmol/l), which is typical for the concentration of glucose in patients' blood serum complicated forms of diabetes mellitus. Cholesterol solutions are mixtures of a protein-coupled with complex cholesterol alcohol. Nevertheless, cholesterol solutions differ in electrical impedance characteristics from immunoglobulin solutions in that they have a more significant increase in the phase angle depending on the frequency of the probing alternating electric current. This fact indicates the increase in the capacitive resistance of tissues, blood and other biological fluids in patients with high total cholesterol levels.

Triglycerides are a class of lipoproteins with many fatty acids concerning the number of cholesterol and apo-protein molecules. Probably, it is just the chemical structure of the triglyceride molecule that causes the appearance of capacitive properties and increases the reactive component of the electrical impedance. A significant change in the phase angle's value at various frequencies of the probing alternating electric current was revealed. On the contrary, solutions of alpha-cholesterol, the molecule of which contains apo-proteins, are characterized by a decrease in electrical impedance concerning triglyceride solutions and even immunoglobulin solutions. In terms of electrical conductivity, alpha cholesterol is the exact opposite of triglycerides; with an increase in alpha-cholesterol concentration, the solutions' electrical impedance decreases, which is typical for protein solutions with low dielectric properties.

The study of the blood serum composition by traditional unified biochemical tests showed that the blood serum of patients with ischemic heart disease is characterized by a significant increase in the concentration of total cholesterol and triglycerides, has a slight increase in glucose concentration to the accepted norms and average values in the group of healthy individuals. Serum of patients with multiple myeloma revealed an increase in total protein concentration due to immunoglobulins' presence. Blood serum is a multicomponent solution based on electrolytes and proteins; lipids and carbohydrates are present in lower concentrations. The high electrical conductivity properties of ionic molecules and protein solutions are in antiphase with the characteristics of lipoproteins and glucose solutions with specific dielectric properties. Mathematical models of the biochemical parameters' relationships on changes in experimental biological fluids' electrical impedance characteristics and studied blood serum samples were proposed [13]. The obtained approximating formulas were used to calculate the concentrations of biological substances in the blood serum. Measurement of the electrical impedance of the blood serum of patients with coronary artery disease and multiple myeloma and the appropriate calculation of the concentrations of biological substances according to the correspondence formulas obtained based on the impedance analysis of their monocomponent solutions in the experiment made it possible to obtain values comparable to the results of traditional biochemical tests. The accuracy of the electrical impedance method of determination of the concentration of glucose, total cholesterol, triglycerides, alpha-cholesterol, albumin, immunoglobulin G and electrolytes Na+, K+, Ca++ was determined by indicating the average measurement error, 25% and 75% of the error range.

5. Conclusion
An experimental technique and a portable laboratory multi-frequency 8-channel electrical impedance analyzer have been developed to measure electrical impedance in conducting biological fluids containing charged (ionic) or dielectric (protein and cellular) inclusions complexes. The characteristic
frequency dependences of the modulus and phase shift angle of the impedance have been determined for solutions of model and biological fluids of various compositions and concentrations; the specific changes in biological solutions' electrical properties, determined by the concentration of various chemical components, were revealed. The accuracy limits of the new method for analyzing the concentration of glucose, total protein, immunoglobulins, albumin, lipid spectrum and electrolytes in the blood of healthy people and patients with coronary heart disease and multiple myeloma have been determined. Based on the relationships between the electrical impedance parameters and the concentration of biological substances in the study's solutions, a new original express method for laboratory diagnostics of the biochemical composition of blood serum is proposed. The obtained data were compared with the results of other clinical methods and demonstrated satisfactory agreement.

The developed equipment and research methods serve as the basis for designing a new type of medical impedance-meter biochemical analyzers intended for differential diagnosis of internal organs' diseases in clinical practice. The introduction of impedance-metric analysis methods into clinical practice will make it possible in the future to develop and recommend an optimal system for assessing the patient's condition in various socially significant diseases, such as heart failure, coronary heart disease, arterial hypertension, respiratory diseases, disorders immune system, oncology, etc.

Acknowledgments
The work was carried out under the Ministry of Education and Science of Russia program (topic No. 121031700169-1) and with the financial support of the RFBR grant No. 20-415-596008.

References
[1] Nazarenko G I and Kishkun A A 2005 Clinical assessment of the results of laboratory tests (Moscow: Medicine) (in Russian)
[2] Menshikov V V. 2002 Fundamentals of clinical laboratory analysis (Moscow: Agat-Med) (in Russian)
[3] Egorov A M, Osipov A P, Dzantiev B B and Gavrilova E M. 1991 Theory and practice of enzyme immunoassay analysis (Moscow: Vyshshaya shkola) (in Russian)
[4] Dolgov V V 2015 Immunochemical analysis in laboratory medicine (Moscow-Tver: Triada Publishing House) (in Russian)
[5] Coons A H, Jones R N, Berliner E and Creech H J. 1942 The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody J. Immunol. 45 159-170
[6] Goldin R B, Beletskaya L V, Kryukova I N and Shakhanina K L. 1977 Immunoluminescence in medicine (Moscow: Medicine) (in Russian)
[7] Yalow R S. 1980 Radioimmunoassay Annu. Rev. Biophys. Bioeng. 9 327
[8] Kubarev A M and Borisov V I. 2008 Pulsation of blood in arterial system and its influence on the body electric resistance Nizhegorodsky Medical J. 4 35-41 (in Russian)
[9] Tsverkov A A 2010 Bioimpedance methods for monitoring systemic hemodynamics (Moscow: Slovo) (in Russian)
[10] Nikolaev D V, Smirnov A V, Bobrinskaya I G and Rudnev S G. 2009 Bioimpedance body composition analysis(Moscow: Nauka) (in Russian)
[11] Jaffrin M Y. 2009 Body composition determination by bioimpedance: an update Curr. Opin. Clin. Nutr. Metab. Care. 12 482-486.
[12] Zuev A L, Mishlanov V Ju, Sudakov A I and Shakirov N V. 2012 Device for measuring the impedance of biological media Patent RF for invention № 2462185 from 27.09.2012
[13] Zuev A L, Mishlanov V Ju, Sudakov A I, Shakirov N V and Frolov A V. 2012 Equivalent electrical models of biological objects Russian J. of Biomechanics 16(1) 110-120.