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MASTER'S THESIS

MODELING OF HEMOCOAGULATION PROCESSES AND DEVELOPMENT OF A METHOD OF OPERATIVE DIAGNOSIS OF THE HEMOSTASIS STATE

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ABSTRACT

Blood coagulation is a series of biochemical reactions that are needed to form a blood clot. Circulatory disorders can lead to blood flow, cardiac arrest, damage to vital organs, or death.

Thus, a quantitative understanding of how a clinical solution to problems associated with deficiencies and disorders works. The definition of blood coagulation is possible through mathematical modeling.

At present, the device of the system for regulating the aggregative state of the blood has been studied in detail, but this does not require a complete understanding of the processes and results of hemostasis, which makes it possible to correct certain violations of the RASB system. Developed mathematical models of processes based on simplified assumptions, except that the results were included in such models. It should also be noted that such models have not been tested in experiments on the assessment of hemostasis. In in vitro, in vivo and ex vivo experiments, a huge amount of experimental work is observed, which makes it possible to study various models of blood clotting in order to understand how they function and how it can affect the process of hemocoagulation.

In this regard, the purpose of this work is to develop a complex physical and mathematical model of whole blood coagulation processes for the assessment of hemostatic potential. In accordance with the goal, the following tasks were set:

1. Determine the viscosity-elastic characteristics of whole blood.

2. Determine the amplitude-phase and frequency characteristics of the oscillations of a pendulum in a viscoelastic medium.

3. To determine the dependence of the viscous-elastic characteristics of blood viscosity on the oscillation frequency of the pendulum in a viscoelastic medium.

4. To develop a comprehensive physical and mathematical model of the process of hemocoagulation.

5. Conduct quantitative and laboratory experiments, depending on the coefficients of blood viscosity.

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LIST OF ABBEVIATIONS

ARP-01M Mednord – diagnostic hardware and software for assessing the hemostatic potential of blood, acting on the basis of the registration of changes in the resistance of the test medium to the resonant oscillations of the resonator needle

APTT – activated partial thromboplastin time

BP – blood pressure

GP – hemostatic potential

DMA – dynamic mechanical analysis method

ICC – the intensity of contact coagulation

IPS – intensity polymerization clot

IRLS – intensity of retraction and clot lysis

ICD – the intensity of coagulation drive

ITS – the intensity of the total coagulation

KSPA – coefficient of total anticoagulant activity

CTA – thrombin activity constant

MA – maximum bunch amplitude

NPTEG – low-frequency piezotromboelastography

PC – personal computer

PV – prothrombin time

RASB – regulation of the aggregative state of blood

SSC – suspension stability of blood

TJ – gelling point

TV – thrombin Time

η – dynamic viscosity

τ – voltage

$\dot{\gamma}$ – shear strain rate

ε – strain tensor Koshi – Grina

σ – voltage

$\vec{f}$ – deforming force

ρ – density of the medium

ν – frequency

ω – requency set by the piezoelectric generator-generator of mechanical vibrations

ω_0' – natural frequency of a resonator needle in a fluid, which is determined by the shear modulus G

β – attenuation coefficient depending on η'

INTRODUCTION

The hemostasis system is one of the components of a single structurally and functionally defined polysystem, which plays a key role in maintaining the homeostasis of the body. It distinguishes three structural groups – blood vessel intima, blood cells (platelets, erythrocytes, leukocytes), plasma blood enzyme systems – and there are four functional units – coagulation, anticoagulant, fibrinolytic and antifibrinolytic. Their interaction allows the hemostasis system to be kept within the limits of physiological fluctuations between hypo- and hypercoagulation. At the same time, providing, on the one hand, the aggregative state of circulating blood, and on the other – prevention and relief of bleeding, the hemostasis system is one of the most labile systems of the body [1]. Adequate functioning of all components of the hemostasis system is an indicator of its usefulness.

Operational and objective assessment of the functional state of the hemostasis system plays an extremely important role because the late diagnosis of hemostasiological disorders carries the potential threat of both thromboembolic and thrombohemorrhagic complications, often fatal in nature (heart attacks, strokes, bleeding). The currently existing considerable arsenal of methods for laboratory control of the effectiveness of antithrombotic agents (amidolytic, clotting, immunofermental, “global” methods) studies of the vascular-platelet and plasma units of the hemostasis system provides only fragmentary information that does not allow to judge the state of the hemostatic system as a single system that functions complex and inextricably within their links [2].

The mentioned methods of laboratory diagnostics of the system for regulating the aggregative state of the blood (RASB) have a number of disadvantages: low sensitivity, lack of standardization, sample preparation time, research on the model of citrate plasma.

The emergence of new domestic technology – low-frequency piezotromboelastography – allows in real-time to receive information about all

phases of the clotting process when working with whole blood at the patient's "bed". It turns out that the use of a new instrument for assessing and monitoring the process of coagulation makes it possible to optimally select the dose and discreteness of prescribing antithrombotic drugs, thereby solving the problem of controlling thrombohemorrhagic complications in clinical practice [3].

In this regard, particular importance is attached to the development of complex physical and mathematical models describing the state of hemostasis, based on knowledge of the physical properties of blood, a detailed description of the physico-chemical processes of hemocoagulation and subsequent testing of these models in comparison with the experiment to determine the viscoelastic properties of whole blood .

Whole blood has two main rheological properties – viscosity and elasticity and belongs to the class of non-Newtonian fluids. For whole blood, the so-called apparent viscosity is a non-linear function of the shear strain rate depending on a number of factors: the concentration of blood cells and their aggregation parameters, the plasma composition and its spatial distribution, the kinetic characteristics of blood flow, the external factors; and various factors can have a mutual influence on their value. Thus, blood is a multiphase and heterogeneous disperse system refers to nonlinear viscous-plastic media [4, 5].

The proposed study is aimed at solving a particular issue within the framework of the designated problem, namely the development of a complex physical and mathematical model of whole blood coagulation processes for the assessment of hemostatic potential.

The aim of this work is to develop the physical principles and the physico-mathematical model of ultrasonic vibrations in a viscous medium as applied to the method of low-frequency piezotromboelastography. In accordance with the goal, the following tasks were set:

1. Determine the viscosity-elastic characteristics of whole blood.

2. Determine the amplitude-phase and frequency characteristics of the oscillations of a physical pendulum in a viscoelastic medium.

3. Determine the dependence of the viscosity-elastic characteristics of whole blood on the oscillation frequency of the physical pendulum in a viscoelastic medium.

4. To develop a comprehensive physical and mathematical model of the process of hemocoagulation.

5. Conduct numerical and laboratory experiments of the dependence of the coefficient of blood viscosity on time in the process of its coagulation under the conditions of the physiological norm.

1 Physical processes and mechanisms of blood coagulation

1.1 The system of regulation of the aggregative state of the blood: components, functions, diagnosis of disorders

The hemostasis system is a system of the body, the functional feature of which is, on the one hand, preventing and stopping bleeding by maintaining the structural integrity of the vessel walls and rapid local thrombosis of the latter during injuries, and on the other hand, maintaining blood in a liquid state and its volume in the bloodstream with a constant transcapillary transition of tissue fluid and plasma [6].

The system of regulation of the aggregative state of the blood maintains the aggregate state of the body at a level that is necessary for normal functioning. The blood coagulation system is part of the RASB.

The RASB system provides:

1) maintaining the liquid state of the blood;

2) restoration of the properties of vessel walls;

3) maintaining at the optimal level of coagulation factors in case of injury to organs, tissues, vessels.

The process of blood coagulation is an enzymatic chain (cascade) process of the conversion of soluble fibrinogen protein into insoluble fibrin. It is called cascading because in the process of hemocoagulation there is a sequential chain activation of blood coagulation factors. The process of blood coagulation is carried out in three phases.

1st phase – the formation of prothrombinase. In the blood, in the zone of the damaged vessel, an active prothrombinase is formed, which converts inactive prothrombin to thrombin. This is the longest period in the whole blood coagulation process.

Phase 2 – the formation of thrombin. Prothrombinase in the presence of Ca ++ ions converts the inactive plasma enzyme prothrombin into its active form, thrombin.

Phase 3 – fibrin formation. The transformation of soluble fibrinogen into an insoluble form of fibrin. Thrombin is a peptidase that causes partial proteolysis of a fibrinogen molecule, converting it to fibrin. Fibrin is the end product of the enzymatic coagulation cascade (Figure 1). The formation of the polymer network of fibrin in vivo, along with the adhesion and aggregation of platelets, are key events in stopping bleeding at the site of injury, as well as in pathological vascular occlusion (thrombosis).

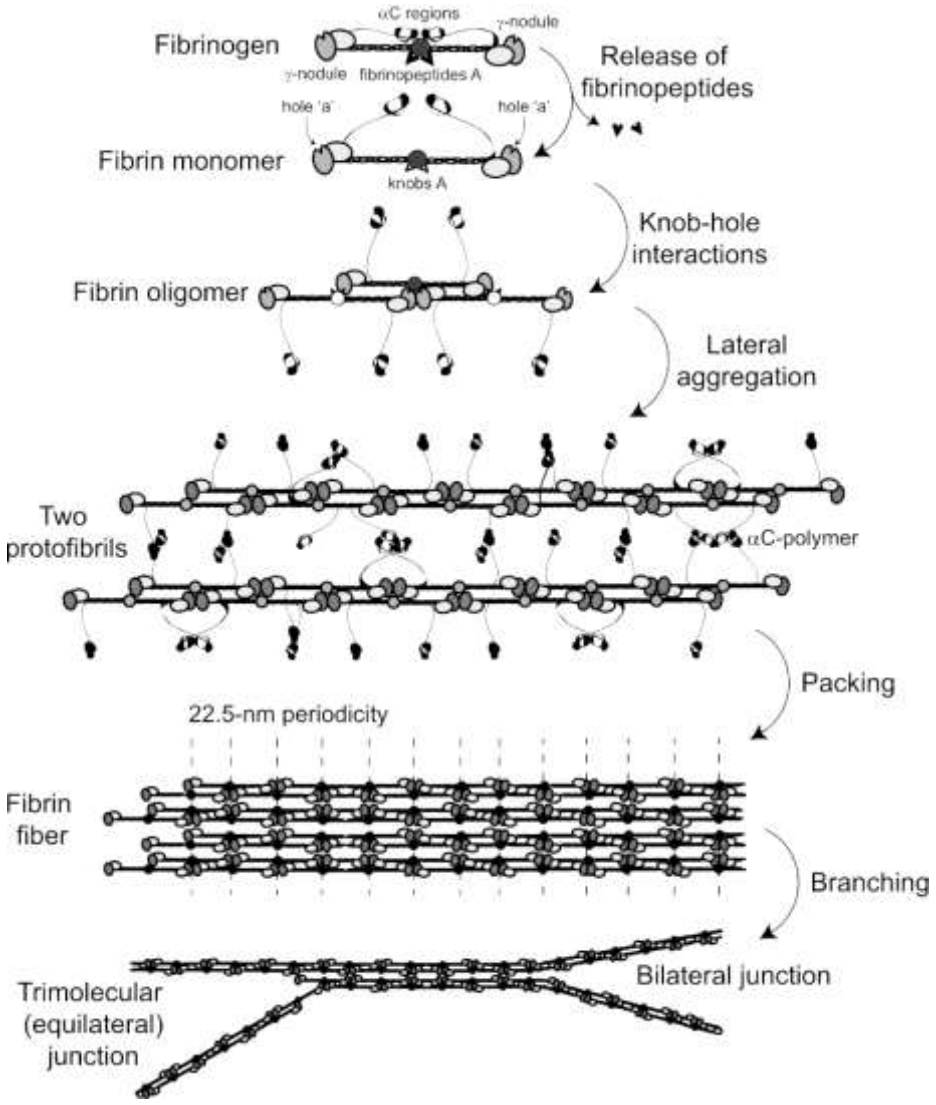


Figure 1 – Schematic representation of the sequential stages of fibrin polymerization

Fibrin polymerization involves a series of consecutive reactions, each of which affects the final structure and properties of the fibrin skeleton. These

properties determine the development and outcomes of various diseases, such as heart attack, ischemic stroke, cancer, trauma, surgical and obstetric complications, hereditary and acquired coagulopathy and thrombocytopathy. In addition, knowledge of the molecular mechanisms of fibrin formation provides the basis for new diagnostic tools and therapeutic approaches. Currently, there are many mathematical and kinetic models that describe fibrin polymerization during blood coagulation reactions [7, 8, 9].

Laboratory diagnosis of disorders of the blood coagulation system is difficult and expensive in laboratory practice. To date, there are a sufficient number of methods for evaluating the RASK system. Essential to the quality of laboratory research is the pre-analytical stage, which includes the selection and purpose of the test, patient preparation, receipt, storage and transportation of biological material, direct preparation of material for research. Material for coagulation studies is plasma, containing all components of the coagulation system. Strict adherence to the rules of the preanalytical phase is very important for the accuracy of hemostatic tests.

Diagnosing the causes of bleeding, determining the intensity of intravascular microcirculation and disseminated blood clotting syndrome, as well as monitoring of antithrombotic and hemostatic therapy are impossible without special laboratory tests.

Diagnostics can be carried out for each of the three phases of hemostasis: coagulation, the formation of blockage of platelets and fibrinolysis. The following test groups of methods can be distinguished:

1. Clotting methods allow to determine the biological activity of the studied factors of hemocoagulation. The unit used in these methods is the formation time of the fibrin clot.

2. Amidolytic methods using chromogenic substrates are used to analyze the time of hydrolysis of a peptide substrate.

3. Immunological methods allow to determine the concentration of the studied factor using monoclonal antibodies.

Separately, it is necessary to identify genetic methods that allow identifying the presence of mutations in the genes that determine the formation of individual coagulation factors and other participants in fibrinolysis and the process of hemocoagulation.

In any case, the laboratory test must have an established diagnostic value. It is necessary to take into account its sensitivity and specificity, as well as the method of calibration and standardization. In addition, there should be a procedure for monitoring the quality of research with an assessment of the correctness of the results. Properly monitored hemostasis system allows to predict hemorrhages during surgical interventions, to identify specific defects in patients with bleeding disorders. Based on the information received, appropriate therapy may be prescribed, for example, transfusion of fresh frozen plasma, intravenous control of blood clotting factors in case of their deficiency, or antifibrinolytic agents for systemic fibrinolysis.

When interpreting the results of laboratory tests for the diagnosis of disorders of the hemocoagulation system, it is necessary to proceed from modern ideas about the mechanisms of blood coagulation and individual information about the patient.

Currently, the number of laboratory tests, with the help of which different blood coagulation units are studied, exceeds several hundred. However, more and more attention is paid to the “global” tests for the operative / integrative assessment of plasma and cellular components of whole blood that are involved in the implementation of fibrinogenesis. Such methods involve two extremely important points:

– work with whole blood (start of analysis immediately after collection) with a sample containing aliquots of everything that is present in the vascular bed at the time of its receipt (endothelium producers, coagulation factors, inhibitors / activators of hemostasis and fibrinolysis, drugs, etc.) and affects the process of coagulation;

– registration of the process of coagulation in the conditions of standardized

contact activation and a graphic reflection of its characteristics of changes in the viscous and viscous-elastic properties of blood when its aggregative state changes.

In this context, the study of the diagnostic value of domestic development, the technology of low-frequency piezotromboelastography (NPTEG), acquires special significance. This technology allows you to visualize the process of blood coagulation, provides an opportunity in real time to evaluate all phases of blood coagulation and quantify the intensity of the pro- and anticoagulant potential. In the NPTEG method, the change in the aggregative state of whole blood is determined and recorded as the dependence of the amplitude of oscillations of the piezoelectric sensor ARP – 01M “Mednord” piezoelectric transducer on time during the period of “placing the sample in the measuring cell – achieving the maximum clot density during polymerization and retraction” [10].

Thus, blood coagulation is a very complex system. There are various biological models for describing this system. Coagulation disorders can lead to serious health complications in humans. Detection of such anomalies is sometimes difficult, so mathematical models can help. Mathematical modeling allows us to investigate and quantitatively describe some nuances in blood coagulation, which may not be obvious from some experiments. Such models can provide a safe way to explore different treatments before prescribing them to patients.

1.2 Viscous-elastic characteristics of whole blood

Viscosity is an important property of liquids, which describes the resistance of a liquid to spreading; it is associated with internal friction in the fluid. The most common type of fluidity is shear flow, in which the fluid layers move relative to each other under the action of shear stress, which is defined as the force acting per unit area of the fluid, and allows you to obtain a velocity gradient across the sample thickness, called the shear rate. The shear viscosity or dynamic viscosity associated with this process is determined by the ratio of shear stress to shear rate.

Simple unstructured fluids are classified as Newtonian, which means that

their viscosity does not depend on the magnitude of the applied shear. Examples include water and simple hydrocarbons. As the complexity of a fluid increases, liquids may exhibit more complex behavior and exhibit a non-Newtonian response, in which the viscosity depends on the magnitude of the applied shear. These types of fluids are commonly referred to as structured or complex fluids. Such non-Newtonian behavior is characteristic of many liquids, including blood, which are usually shear-thinning liquids, where the viscosity decreases with increasing shear rate.

Blood is a concentrated suspension of several formed cellular elements, erythrocytes, leukocytes and platelets in an aqueous polymer and ionic solution, plasma consisting of 93% water and 3% of particles, namely electrolytes, organic molecules, numerous proteins (albumin, globulins and fibrinogen). The physiological function of plasma is to transport these solutes, nutrients, wastes, and formed cellular elements of the entire circulatory system. Common red blood cells are biconvex discs with an average diameter of 6 to 8 microns and a maximum thickness of 1.9 microns. Their amount per cubic millimeter of blood is approximately from 5 to 6 $\times$ 10^6, and they represent approximately 40–45% by volume of normal human blood and more than 99% of all blood cells [7].

The first percent is called hematocrit. Leukocytes are roughly spherical and much larger than red blood cells, but they exist in smaller amounts in the blood: their diameter ranges from 6 to 17 microns, and their number is about 7 to 11 $\times$ 10^3 per cubic millimeter in an ordinary adult. Platelets have an even smaller size of approximately 2 to 3 microns in volume. Platelets are a vital component of the blood coagulation mechanism. The total volume concentration of leukocytes and platelets is only about 1%.

Blood plasma, which consists mainly of water, is a Newtonian fluid. However, whole blood has complex mechanical properties that become especially significant when the particle size is much larger or at least comparable to the size of the lumen. In this case, what happens at the level of microcirculation (in small arterioles and capillaries), the blood cannot be represented as a homogeneous fluid,

but as a suspension of blood cells (especially red blood cells) in the plasma. The presence of cellular elements of the blood and their interaction leads to significant changes in the rheological properties of blood, and it is necessary to carry out reliable measurements to obtain the appropriate microstructural models.

Numerous researchers have found that blood viscosity gradually decreases as the velocity gradient increases. This dependence manifests itself at relatively low velocity gradients up to 60–70 s^{-1}. With a velocity gradient of 60–70 s^{-1} and higher, the decrease in viscosity practically stops, and it becomes “constant” or, as it is often called, asymptotic. The characteristic viscosity curve for blood is concave toward the axis of strain rate. Consequently, judging by the flow curve, blood is inherent in pseudoplasticity. Considering that blood has a yield point, it (using the terminology used in rheology) can be attributed to nonlinear-viscous-plastic media.

Whole blood has two main rheological properties - viscosity and elasticity and belongs to the class of non-Newtonian fluids. For whole blood, the so – called apparent viscosity $\eta(\dot{\gamma})$ is a nonlinear function of the shear strain rate $\dot{\gamma}$ depends on a number of factors: the concentration of blood corpuscles and their aggregation parameters, the plasma composition and its spatial distribution, the kinetic characteristics of the blood flow, the rate of elastic deformations shear, external factors; and various factors can have a mutual influence on their value. Thus, blood is a multiphase and heterogeneous disperse system refers to nonlinear viscous-plastic media [4, 5].

Phase transformation of blood from a liquid state in the processes of fibrin polymerization and the formation of transverse intermolecular bonds, its retraction and subsequent lysis. Thus, by changing the viscosity coefficients, it is possible to judge the dynamic phase transformations of blood in the process of coagulation.

The main rheological equation is $\tau = \eta\dot{\gamma}$, where τ is the tensor of tangential stresses; $\dot{\gamma}$ is shear rate; η – viscosity. The so-called apparent viscosity $\eta(\dot{\gamma})$ of whole blood is a non-linear function of $\dot{\gamma}$. Thus, blood, which is a multiphase and heterogeneous dispersed system, belongs to nonlinear-viscous-plastic media [4, 5].

The main contribution to the elastic properties of blood is made by the aggregation ability of erythrocytes. At low shear rates $\dot{\gamma}$ the spatial structure created by erythrocytes does not collapse and forms the threshold behavior of shear deformation, at which the elastic properties of blood appear. At high speeds $\dot{\gamma} > 200\ s^{-1}$ the erythrocyte spatial structure is destroyed, which causes shear thinning of blood, leading to a decrease in viscosity, and in this state, the blood behaves like Newtonian fluid [15, 16].

In classical viscometry, the determination of the viscoelastic properties of blood is based on measuring the dependence $\tau(\dot{\gamma})$, as well as the threshold values τ and $\dot{\gamma}$. To account for the elastic properties of blood, the method of complex representation of the shear modulus is used $G = G' + iG''$, where G', G'' – storage modulus and loss modulus, respectively [17]. Similarly, for the coefficient of viscosity, taking into account the elastic properties of blood can be written $\eta^* = \eta' + i\eta''$, where η', η'' – coefficient of viscosity and coefficient of elasticity, respectively. To determine the real and imaginary parts of the complex viscosity, the oscillatory viscometry method or the method of dynamic mechanical analysis is used, when the dynamics of a viscoelastic medium is considered under the action of a force changing in time according to the harmonic law: $\tau = \tau_0 \sin\omega t$, where ω – frequency of driving force [17]. In this case, time and frequency dependencies are investigated $\tau^*, \dot{\gamma}$ and η^*.

2 Method of low-frequency piezotromboelastography

2.1 Elastography

Elastography is one of the important applications of physical knowledge in diagnosing the properties and pathologies of biological tissues and fluids. The term elastography is used to differentiate tissues and fluids by their viscous-elastic characteristics by mechanical action on them and analysis of deformations obtained using ultrasound diagnostic scanners or magnetic resonance tomographs.

The introduction of ultrasound elastography into medical practice has a

recent history and, despite the fact that the arsenal of diagnostic equipment already contains powerful tools and technologies, ultrasound elastography has far from exhausted its potential. Therefore, it is necessary to conduct further fundamental studies of the physical processes and mechanisms of interaction of ultrasonic waves with biological tissues and fluids that underlie the methods of ultrasonic elastography [21].

One of the important areas of such research is ultrasound elastography of the coagulation process of whole blood. Blood coagulation is an extremely complex biochemical process that starts when the vascular wall or blood cells are damaged and leads to the polymerization of fibrin with the formation of a clot, stopping the bleeding. Violations of this process are extremely dangerous and can lead to bleeding, thrombosis or other pathologies, which makes the study of blood clotting one of the priority applied tasks of the physics of biological systems. Existing approaches to the diagnosis and treatment of thrombosis and hemorrhagic complications are based on the results of studies of the functional viability of hemostatic potential using a set of “global” tests, clotting, amidolytic and enzyme immunoassay methods for evaluating hemostasis [22-24].

Unfortunately, the “limited” set of tests used in clinical practice predetermines obtaining only fragmentary information about the system of regulation of the aggregative state of the blood. In our opinion, only an assessment of the hemostatic potential in whole blood containing the entire totality of hemostasis factors, and performed in the “point-of-care test” mode, can determine the entire totality of the interaction of the elements of the RASK system [25]. This kind of test can be a method of low-frequency piezotromboelastography, which allows an integrative assessment of the state of the hemostatic potential based on the measurement of the dynamics of the viscosity-elastic characteristics of whole blood [11].

This method is based on the dependence of the viscosity-elastic characteristics of blood on the nature of the flow of the cascade of enzymatic fibrin polymerization reactions at all stages of the blood coagulation process. Thus, this

method registers the change in the resistance of whole blood to the forced oscillations of the resonator needle, which reflects the change in the state of aggregation of the blood over time. The result of measuring the dynamics of the aggregative state of blood is the curve of the integrative state of the full cycle of hemocoagulation – the time dependence of the amplitude-frequency characteristic of the oscillations of the resonator needle recorded by the piezo sensor [11]. Analysis of the measurement results at specific time points in the process of fibrin polymerization allows the rapid assessment of the hemostatic potential of the blood and the detection of irregularities in the blood coagulation system.

The limitation of this method is the problem of compliance of the integrative state of the hemocoagulation cycle as a result of measurement to a certain stage in the cascade of enzymatic reactions determining the aggregative state of blood, and, consequently, its viscosity-elastic properties. This is due to the fact that the device of the blood coagulation system has been studied, but does not reflect a sufficient understanding of the principles of operation of this device and the mechanisms of regulation of the system [19].

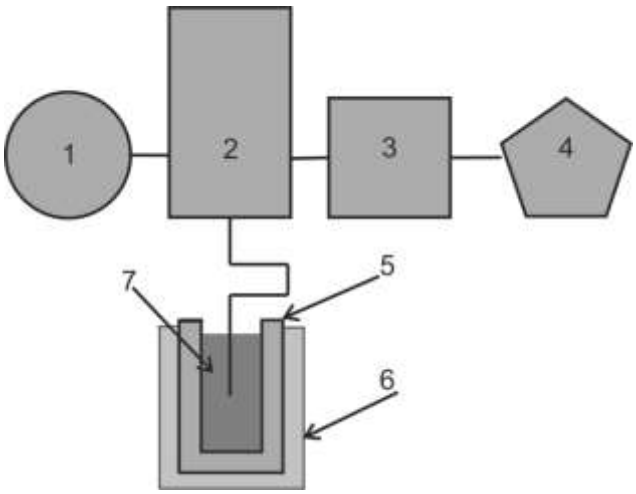
2.2 Equipment and algorithm for the diagnosis of blood hemostasis using the method of low-frequency piezotromboelastography

A description of the ARP-01M Mednord diagnostic hardware-software complex for evaluating the hemostatic potential of blood, which acts on the basis of recording the change in the resistance of the medium under study to resonant oscillations of the resonator needle, is given in [20]. The hardware-software complex is designed to study the process of hemocoagulation of whole blood, assess changes in the viscoelastic properties of a clot during the polymerization of fibrin and the formation of transverse intermolecular bonds, its retraction and subsequent lysis. The appearance of the device is shown in Figure 2.

Figure 2 – Apparatus for studying the rheological properties of blood ARP-01M Mednord

The principle of operation of the device is based on registering the change in resistance of the test blood to resonant oscillations of a resonator needle mounted on a piezoelectric element, which is a brass base on which a layer of piezoceramics is applied, divided into two circular segments, and lowered by the second end into the cuvette with the patient's blood (Figure 3). The resonator needle in its middle part is made with a bend in the form of a loop.

The frequency of oscillation of the needle-resonator in the air and in the liquid is maintained the same. A useful signal is the difference in the amplitudes of the needle oscillations in the air and in the liquid under study. The electromechanical tract is controlled by the measuring circuit of the apparatus, and all calculations, the output of graphs and research parameters, and the complex's operation are controlled by a personal computer (PC) that uses the specialized computer program "ICS HEMO-3".

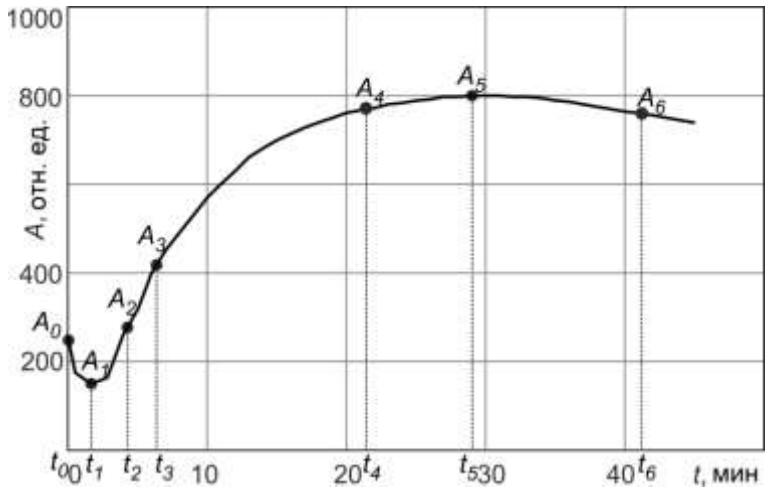


1 – pulse generator; 2 – piezoelectric sensor; 3 – output operational amplifier; 4 – information and computer system "ICS GEMO-3"; 5 – measuring cuvette; 6 – thermostat, 7 – blood.

Figure 3 – Structural and measuring circuit of ARP – 01M Mednord low-frequency piezotromboelastograph

The analysis of the graphical image of NPTEG is based on changes in the relative values of the viscoelastic properties of blood (*Ai*), occurring during coagulation, during the period “damage to the vascular wall of the vein for taking a blood sample – achieving the maximum density of the clot during its polymerization and retraction”. The dynamics of the process under study – the transfer of blood during coagulation from a liquid to a solid-elastic state – is determined by changes in the aggregative state of the blood and is recorded as an integrated NPTEG curve, each point of which (*Ai*) determined by the state of the system at a particular point in time of the study (*ti*).

Figure 4 shows a graph of changes in the aggregative state of blood (by the method of NPTEG) of a healthy volunteer, in which the amplitude A of the process under investigation is estimated on the ordinate axis in relative units, and on the abscissa is the study time t in minutes.



(*A0* – *A5*) – NPTEG amplitude at the stages of cross-linked fibrin formation; *A6* – amplitude at the 10th minute of clot lysis; (*t1* – *t5*) – time intervals of stages of fibrinogenesis; (*t3*) – gelling point (clotting time); MA – the maximum density of the clot.

Figure 4 – NPTEG indicators of whole blood of a healthy volunteer

During the measurement, the following parameters are recorded and determined: *A0* is the initial amplitude value at time *t0*, in relative units; *t1* is the reaction period (time from the beginning of the study to the maximum amplitude reduction *A1*); *t2* – time to reach amplitude *A2*; *A2* – increase in amplitude by 100 rel. unit; *t3* – blood clotting time (gelling point), is automatically determined by measuring tg of the slope of the curve by 50%; *A3* – amplitude value at the gelling point; *A4* – amplitude value 10 minutes after reaching the gelling point; *t5* – time to reach the maximum amplitude (*A5*) (time of formation of the fibrin-platelet structure of the clot); *A6* – amplitude value 10 minutes after reaching the maximum amplitude. According to the formulas shown in Figure 5, the following parameters are calculated in rel. unit: the initial stage of coagulation – the intensity of contact coagulation; thrombin activity constant; coagulation drive intensity; the intensity of the polymerization of the clot; coefficient of total anticoagulant activity; intensity of retraction and clot lysis; maximum amplitude of the clot.

The procedure for assessing the hemostatic potential provides for conducting a study in the “point-of-care test” mode, which is determined by the research

technique: the interval between blood sampling and placement into the measuring cell should not exceed 15-25 s. The coagulation process is recorded in a sample of whole blood, in a sample containing identical circulating blood concentrations of endothelial products, pro- and anticoagulant substrates, and even drugs entering the systemic circulation. Therefore, the study of the hemostatic potential of this method is as close as possible to the *in vivo* study.

Based on the fact that the NPTEG method is able to characterize the dynamics of the entire hemocoagulation process – from the initial stages of clotting (the initiation / amplification phase) to its lysis, each interval of the NPTEG curve demonstrates the result of biochemical blood coagulation reactions occurring in the cuvette where there is an aliquot of blood (Figure 5).

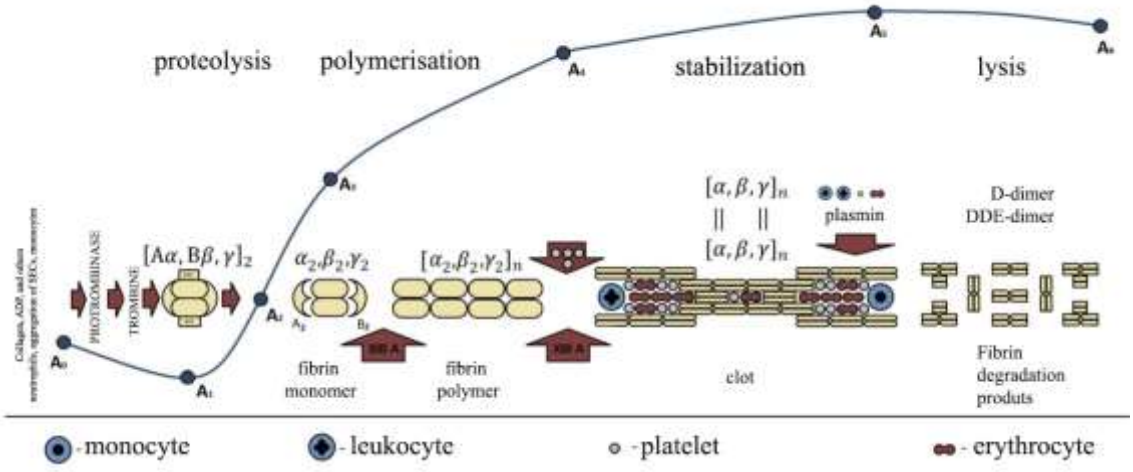


Figure 5 – Displays of the stages of fibrinogenesis relative to the NPTEG curve

NPTEG is a standardized test, the basis of which is the fixation of changes observed in the aliquot of whole unstabilized venous blood under alteration during transformation from the liquid stateto the solid-elastic state. The kinetics of the hemocoagulation process is determined by the change in the state of aggregation of the aliquot under study and is displayed by an integrated curve, on which each of the points (*Ai*) characterizes the state of the system at a fixed point in time (*ti*). A resonator needle, fixed on the piezoelectric element, registers the change in resistance occurring in the cell with an aliquot. The piezoelectric sensor converts

the input voltage of the low-frequency signal into self-oscillations of the resonator needle and, at the same time, converts the self-oscillations of the resonator needle into the voltage of the outgoing signal. Further signal processing is carried out by the information system “ICS HEMO-3”. Fixing of dynamic indicators and their transmission to a PC monitor is carried out in real time. Indicators and their interpretation are presented in tables 1 and 2.

Table 1 – Recorded NPTEG indicators

Indicator	Explanation of the indicator value
t1	The reaction period (time per minute from the beginning of the study to the minimum amplitude of NPTEG - A1), an indicator characterizing blood suspension stability (SSC)
t2	Gelling point (TJ) in min, is determined automatically when tg (tangent) of the slope of the curve is changed by 60% relative to the x-axis
t3	The time to reach the maximum amplitude of NPTEG (A5)

Table 2 – Estimated NPTEG indicators

Indicator	Explanation of the indicator value
IKK	$IKK = \frac{A1-A0}{t1}$ IKK, o.e. – the intensity of contact coagulation; A1, o.e. – the maximum decrease in the amplitude of the curve during the reaction period "t1"; A0, o.e. – the initial value of the amplitude of the curve at time t0; t1, min. – time from the beginning of the study to the minimum amplitude of the NPTEG curve – A1. This indicator reflects mainly the aggregation activity of blood corpuscles, phase I and II coagulation, or its suspension stability (SSC)

Continuation of table 2 – Estimated NPTEG indicators

ICD	$ICD = \frac{A3-A1}{t3}$ ICD, o.e. – intensity of coagulation drive; A3, o.e. – the magnitude of the amplitude of the curve at the “gelling point”; A1, o.e. – the maximum decrease in the amplitude of the curve during the reaction period "t1"; t3, min – blood clotting time - “gelling point”, fixed automatically when tg angle of curve changes by ~ 60%. This indicator mainly characterizes the proteolytic stage of the 3rd phase of hemocoagulation. A - part of the NPTEG curve near the gelling point (changing the tg angle of the curve by ~ 60%) reflects the beginning of the polymerization process, which at the gelling point (VSC) leads to the formation of a fibrin gel - the main structural framework of the hemostatic clot
CTA	$CTA = \frac{A2}{t2-t1}$ CTA, o.e. – thrombin activity constant; A2, o.e. – increase of the amplitude of the curve by 100 o.e. t2, min is the time to reach the amplitude of the A2 curve; t1, min is the time from the beginning of the study to the minimum amplitude of the NPTEG curve – A1. The use of this indicator in the analysis of NPTEG is due to the need to determine a universal criterion for assessing the intensity of the proteolytic stage of fibrin formation

Continuation of table 2 – Estimated NPTEG indicators

IPS	$IPS = \frac{A4-A3}{10\ min}$ IPS, o.e. – the intensity of the polymerization of the clot; A4, o.e. – amplitude value after 10 min from the “gelling point”; A3, o.e. – the amplitude value at the “gelling point”. Display the intensity of the polymerization stage. The use of a time interval of 10 min is due to the need to unify the method, since the formation of cross-covalent bonds is a fairly long stage of post-gel formation
MA	MA = A5-A1 MA, o.e. – the maximum amplitude of the bunch; A5, o.e. – the maximum amplitude of NPTEG recorded within 10 minutes; A1, o.e. – the maximum decrease in the amplitude of the curve during the reaction period "t1". Characteristics of the maximum density of the bunch, due to the activity of the FEC and the qualitative and quantitative characteristics of PSF, after the completion of the polymerization and retraction process
ITS	$ITS = \frac{MA}{t5}$ ITS o.e. – the intensity of the total coagulation; MA, o.e. – the maximum amplitude of the bunch, calculated by the formula MA = A5-A1; t5, min – the time of formation of the fibrin-platelet structure of the clot. The indicator characterizes the polymerization stage of the 3rd phase of hemocoagulation. Since the process of changing the viscoelastic properties of a clot during polymerization of fibrin and the formation of transverse intermolecular (covalent) bonds is long, and the moment of transition to the stabilization stage is quite arbitrary, a uniform time

Continuation of table 2 – Estimated NPTEG indicators

	interval equal to 10 minutes from the time of registration of the WSC point is used to unify the analysis of the PNTEG the initial stage of the clot polymerization is the formation of a viscoelastic gel (post-gel)
IRLS	$IRLS = \frac{A5-A6}{A5} \times 100\%$ IRLS,% – intensity of retraction and clot lysis. A5 –the maximum value of the amplitude of the NPTEG curve; A6 – the amplitude value of the NPTEG curve 10 minutes from A5. This indicator in the integrative characterizes the combination of plasmin, leukocyte proteases (granulocyte elastase, cathepsin G, monocyte cathepsin D, complement), erythrocyte kinases in a given blood volume (0.5 ml)
KSPA	$KSPA = \frac{ICD}{IPS}$ KSPA, o.e. – coefficient of total anticoagulant activity; ICD, o.e. – intensity of coagulation drive, calculated by the formula (A3 – A1) / t3; IPS, UE - intensity of bunch polymerization, calculated by the formula (A4 – A3) / 10 min. Anticoagulant blood activity is a key element in the regulation of the coagulation process and is due to the functioning of several groups of inhibitors: antiplatelet agents, specific and non-specific inhibitors of serine proteases, inhibitors of active coagulation factor complexes (TFPI), inhibitors of coenzymes (proteins C and S, thrombomodulin) and fibrin degradation products. This indicator is proposed due to the fact that the peak values of the functioning of the anticoagulant system are manifested mainly in the I and II phases of coagulation, as well as at the stage of proteolysis of the III phase prior to the beginning of the process of active polymerization of the clot (TJ)

3 Development of a complex physical and mathematical model of the process of hemocoagulation

3.1 Determination of the coefficient of viscosity of whole blood in the process of coagulation

To date, significant progress has been made in the development of theoretical methods and experimental tools for studying hemorheology and a deep understanding of the interaction of blood components that determine its viscoelastic properties. However, the problem of determining changes in the viscoelastic properties of whole blood in the process of coagulation remains open due to the lack of diagnostic tools. In existing devices for determining the rheology of whole blood, studies are carried out on its stabilized samples (capillary and rotational rheometers), and when evaluating the elastic properties, thromboelastographs are used to evaluate changes in this characteristic after the formation of a clot [10]. Measurement of the viscoelastic properties of whole blood is very limited in time due to the natural process of coagulation. In these cases, when measuring to prevent coagulation, stabilizing drugs are used (heparin, EDTA, etc.) [27, 28], which can lead to changes in hemorheological properties and to certain errors in measuring the absolute values of the real and imaginary components of the complex viscosity coefficient.

In this paper, to determine the complex viscosity index of whole blood, a resonant acoustic method was developed based on measuring the amplitude-phase and frequency characteristics of the oscillations of a physical pendulum in a viscoelastic medium. The amplitude-phase characteristics are measured on the ARP-01M “Mednord” low-frequency piezotromboelastograph [11], based on the dependence of the viscous-elastic characteristics of blood on the nature of the fibrin polymerization reactions at all stages of blood coagulation [18, 19]. This method belongs to the class of acoustic resonant methods for determining low-frequency viscoelastic characteristics of liquids by changing their resistance to forced oscillations of the piezoelectric resonator [30].

When the end of the resonator needle is immersed in a liquid, the amplitude and phase characteristics of the voltage on the recording piezoelectric element will change. This is due to the influence of the viscoelastic properties of the fluid on the amplitude and phase characteristics of the mechanical oscillations of the resonator needle. Thus, by changing the amplitude-phase characteristics of the voltage on the recording piezoelectric element, it is possible to obtain information about the magnitude of the viscoelastic characteristics of the fluid, which determine the complex viscosity index

$$\eta^* = \eta' + i\eta''$$

and its dynamics in the process of blood coagulation.

To solve this problem, an approach based on a mathematical model of forced oscillations of a cylinder in a viscoelastic fluid is applied. Denote the forcing periodic force acting on the resonator needle from the piezoelectric element,

$$f = f_0 e^{i\omega t} \ ,$$

where ω – frequency set by the piezoelectric generator-generator of mechanical vibrations. Under the action of this force, the lower end of the resonator needle performs harmonic oscillations in an elastic medium (air)

$$x = x_0 e^{i\omega t} \ ,$$

which are determined from the solution of equations (1). When the lower end of the resonator needle is immersed in a viscoelastic fluid, the amplitude-frequency characteristics of its oscillations can be determined from the equation

$$\ddot{x} + 2\beta\dot{x} + (\omega_0')^2 x = \varepsilon_0 e^{i\omega t} \ , \qquad (1)$$

where ω_0' – the natural frequency of the resonator needle in the fluid, which is determined by the shear modulus G; β – damping factor η' ; $\varepsilon_0 = \frac{f_0}{m}$, m – mass of the lower end of the resonator needle immersed in the liquid.

The solution to equation (1) is

$$x = \tilde{x}_0 e^{i(\omega t - \varphi)},$$

where

$$\tilde{x}_0 = \frac{\varepsilon_0}{\sqrt{[(\omega_0')^2 - \omega^2]^2 + 4(\beta\omega)^2}} \quad (2)$$

$$tg\varphi = \frac{2\beta\omega}{(\omega_0')^2 - \omega^2} \quad (3)$$

To calculate the coefficient β we use the model of oscillations of a cylinder with height h and radius R in a fluid with a viscosity coefficient η. Taking into account the amplitude value of the speed of the end of the rod Vx ~0.25 m/s Reynolds numbers are: for water Re~220; for glycerin Re ~0.2; of blood Re ~60, which allows us to conclude about the laminar flow around the lower part of the moving rod in these fluids. Therefore, in the calculations, you can use the model of viscous friction force proportional to the speed of movement of the lower portion of the rod relative to the liquid medium.

Figure 6 shows a section of a cylinder of radius R and height h, moving in a viscous fluid with a velocity υ. The element dS is acted upon by a viscous friction force equal to

$$dF_v = \eta \frac{dv_\tau}{dy} dS,$$

where $v_\tau = v \sin\alpha$ – tangential velocity component of the cylinder.

Then $dF_v = 2\eta \frac{dv}{dy} hR\sin\alpha d\alpha$.

Integrating this expression, we get $F_v = 4\eta \frac{dv}{dy} hR$.

To calculate the velocity gradient, we use the expression for the propagation velocity of shear waves in a viscous medium

$c = \sqrt{\frac{2\eta\omega}{\rho}}$, where ρ – fluid density.

Then you can put $\frac{dv}{dy} = \frac{\upsilon}{l^*}$, where $l^* = \frac{2\pi c}{\omega}$ – the thickness of the fluid involved in the movement.

As a result, for the coefficient β the formula is obtained:

$$2\beta = 4\eta/(\pi l^* \rho 0 R), \quad (4)$$

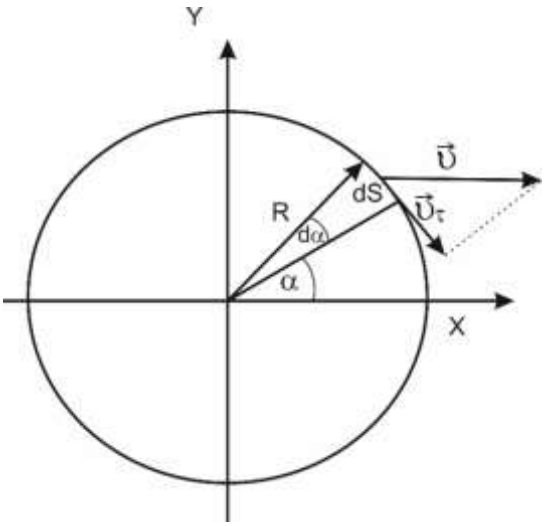


Figure 6 – Section of a cylinder oscillating in a viscous fluid

In the formula (4), ρ0 is the density of the material of the resonator needle. From formulas (3) and (4) you can determine the real part of the viscosity coefficient $\eta' = \eta$.

To determine the real part of the shear modulus characterizing the elastic properties of a fluid, we use the formula for the elastic force

$$dF_{el} = G\frac{x}{l^*}dS,$$

where $x -$ shear strain along the x axis.

Similar to the derivation of formula (4) and taking into account the relation for the tangential component of the shift $x_\tau = x\sin\alpha$, after integration over the entire lateral surface of the cylinder for the natural frequency of the resonator needle in the fluid ω_0' get the formula:

$$\omega_0' = \sqrt{\frac{4G}{\pi l^* \rho_0 R}} \quad . \qquad (5)$$

Formula (5) can be used to calculate the shear modulus $G' = G$ according to the experimentally determined value ω_0' . As a result, the modulus of the complex coefficient of viscosity η^* taking into account the ratios $\eta' = \frac{G''}{\omega}$; $\eta'' = \frac{G'}{\omega}$, can be

calculated by the formula:

$$\eta^* = \sqrt{\eta^2 + \left(\frac{G}{\omega}\right)^2}, \quad (6)$$

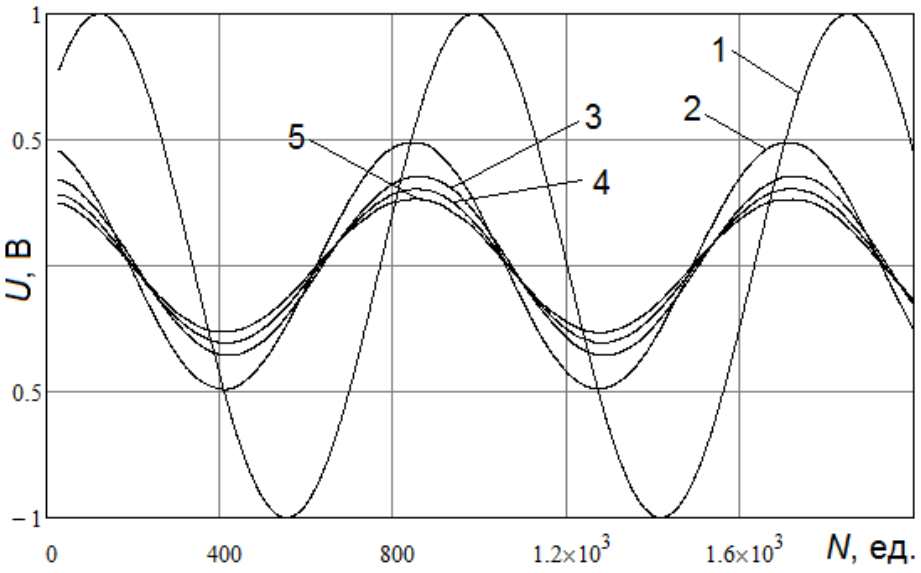
Thus, the experimental data on the change in the amplitude-phase characteristics of whole blood in the coagulation process (formulas 2, 3) allow using its formulas (4) and (5) to determine its viscoelastic characteristics (G, η).

3.2 Numerical experiment to determine the correlation function

As follows from the theory of mechanical oscillations in a liquid medium, the influence of the elastic-viscous properties of the medium on the motion of the resonator needle leads to a change in the amplitude-phase characteristics of its harmonic oscillations. A change in the viscous properties of the medium leads to a change in the attenuation coefficient β, and a change in the elastic properties of the medium leads to a change in the frequency of the natural oscillations of the resonator needle by an amount δω.

Using the developed numerical model of the acoustic oscillator in the ARP-01M Mednord installation, the correlation between the elastic-viscous properties of the medium and the amplitude-phase characteristics of the motion of the resonator needle in it was investigated. Numerical calculations were carried out at a signal frequency applied to a piezoelectric, 2950 Hz. The amplitude of the harmonic voltage applied was 1 V. The numerical calculations were carried out under the condition that the end of a 1 cm long cavity needle moves under the action of a viscous friction force with a viscosity coefficient η = 10-3, 5 × 10-2, 5 × 10-1, 1.5 Pa × s and elastic force with a coefficient G / m = 20, 25, 30, 40 s-1, where G is the coefficient of elasticity and m is the mass of the end of the needle-resonator.

Figure 7 presents the results of the conducted numerical calculation, where the dependence of the amplitude U of the supplied (1) and received (2-5) signals on a piezoelectric on the number of the time interval N is shown.



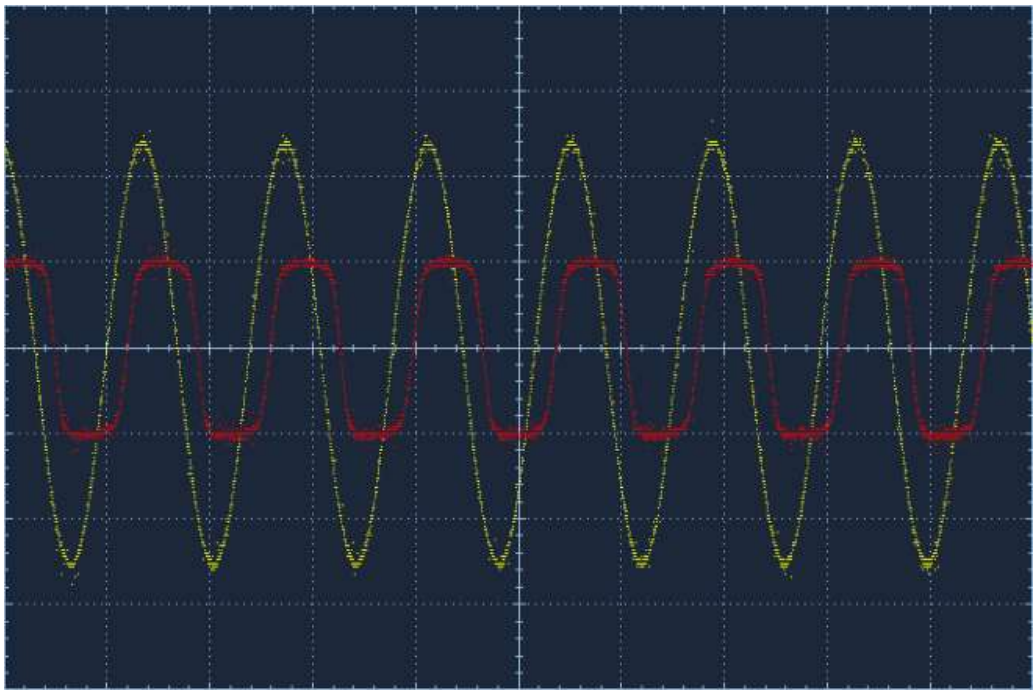
1 – signal applied; 2 – received signal for $\eta = 10^{-3}$ Pa × s and G / m = 20 s^{-1}; 3 – received signal for $\eta = 5 \times 10^{-2}$ Pa × s and G / m = 25 s^{-1}; 4 – received signal for $\eta = 5 \times 10^{-1}$ Pa × s and G / m = 30 s^{-1}; 5 – received signal for η = 1.5 Pa × s and G / m = 40 s^{-1}.

Figure 7 – Dependence of the amplitude of the signal U on the number of the time interval N

As follows from the results of the calculations, a change in the magnitude of the visco-elastic force leads to a change in the amplitude-phase characteristics of the received signal, which indicates their correlation. Using these data, we obtained the amplitudes Ap of the received signal and its phase shift относительнор relative to the input signal, the numerical values of which are presented in Table 3.

To compare the obtained results with the experimental data, the ARP-01M Mednord instrument measured the amplitude-phase characteristics of the received signal for an aqueous solution of glycerin with its percentage – 0, 80, 95, 100%, the viscosity coefficients of which correspond to the viscosity coefficients used in the calculations. The measurements were carried out at a resonant frequency in air using a digital oscilloscope. As an example, figure 8 shows the results of measurements for water. In this figure, yellow is the received signal, red is the input signal.

Using the results of the measurements, the amplitudes Ae of the received signal and its phase shift were obtained φэ relative to the signal, the numerical values of which are presented in Table 3.



Red – signal given; yellow – received signal.

Figure 8 – Waveform of signals on the ARP-01M Mednord device for water at a resonant frequency of 2881 Hz in air

Table 3 – Values of amplitude Ap of received signal and phase shift p relative to the supplied signal

Aqueous solution of glycerol,%	Ap, V	φp, deg.	Ae, мV	φe, deg.
0 (water)	0.49	51,2	124.5	50.9
80	0.36	46,8	88.9	45.5
95	0.31	49.3	76.5	48.2
100	0.26	50.5	66.5	49.1

As can be seen in Table 3, the calculation results and measurement results correlate well with each other. They show that with an increase in the visco-elastic properties of a liquid medium, the amplitude of the received signal decreases. The phase shift with increasing elastic-viscous properties of a liquid medium first decreases and then grows. The non-monotonic behavior of the phase shift is caused by the change in the attenuation coefficient β, natural frequency ω0 oscillations of a system in an elastic-viscous medium.

Attenuation coefficient β and own frequency ω0 can be calculated by the formulas:

$$\beta = \beta_0 \times \sin(\varphi) \times \frac{A_v}{A}, \ \omega_0 = \sqrt{\omega_v^2 - \frac{2 \times \beta \times \omega_v}{\tan(\varphi)}}, \quad (7)$$

where A_V is the oscillation amplitude of the resonator needle in the air, ω_v is the resonant frequency in the air. The results of the calculations for the measured values of Ae and φe are presented in Table 4. From the table it can be seen that a change in the viscosity coefficient within three orders of magnitude leads to a change in the attenuation coefficient β within ~ 20 s^{-1}. According to the data presented in Table 4, and the formulas, it is possible to calculate the viscoelastic properties of the media in which the resonator needle was immersed.

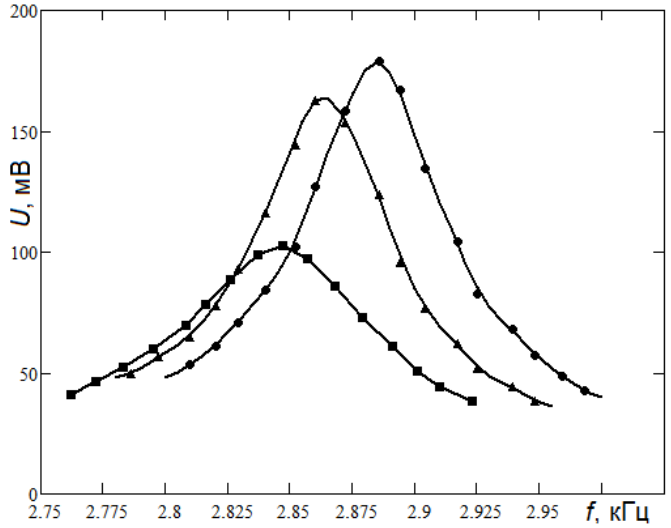
Table 4 – Values of the amplitude of oscillations Ae and phase shift φe

Aqueous solution of glycerol, %	β, s^{-1}	ω0, s^{-1}
0 (water)	22.2	2863
80	28.5	2853
95	34.7	2850
100	40.7	2846

Figure 9 shows the measured dependences of the amplitude-phase characteristics of oscillations of the resonator needle on the frequency for air, water, and glycerol at 100% concentration. As can be seen from the figure, the resonance frequency decreases with increasing fluid viscosity, which follows from the theory

$$\omega_{res} = \sqrt{(\omega_0')^2 - 2\beta^2}.$$

The frequency shift with increasing viscosity is much less than the resonant frequency.



● - air; ▲ - water; ■ - glycerol (100%).

Figure 9 – Dependence of the amplitude of the resonator needle in a viscous medium on the frequency

To calculate the viscosity coefficient and the elastic modulus of the fluid and its dynamics in the coagulation process, we use formulas 2, 5. As a medium for calibrating the amplitude of the piezoelectric recorder signal, we take the resonant amplitude of the resonator needle in air at the resonant frequency $\omega_{res} = 2884$ Hz, which we take for the frequency of forced oscillations of the needle of the resonator in a viscous fluid.

Then from formulas 2, 5 follows:

$$\delta A = \frac{\tilde{x}_{res}}{\tilde{x}_0} = \frac{\sqrt{\left[\delta\omega_{res} + \frac{\beta^2}{\omega}\right]^2 + \beta^2}}{\beta_0} \quad , \tag{8}$$

$$tg\varphi = \frac{\beta}{\delta\omega_{res} + \frac{\beta^2}{\omega}} \quad , \tag{9}$$

where δA – the ratio of the amplitudes of the oscillations of the needle of the resonator in the air and in the liquid under study

$$\omega = \omega_{res};$$

$\delta\omega_{res}$ – resonance frequency difference in liquid and air.

From equations (8) and (9) can be determined β and $\delta\omega$, and then from formulas (5) and (6) to calculate the shear modulus and viscosity of the test liquid.

Table 5 shows the results of calculations of the viscoelastic characteristics of water and glycerin by the formulas (5), (6), (8) and (9) according to the measured amplitude-phase characteristics of oscillations of the resonator needle. The measurements were carried out at a temperature of 37° C. The oscillation frequency of the resonator needle in air and in the liquid was maintained during the experiment with a constant and equal to the resonant frequency of oscillations of the resonator needle in air $\omega = 2884$ Hz. Measurements of the amplitude and phase characteristics for glycerol were carried out for several concentrations, this allows us to estimate the accuracy of the method in a wide range of changes in the viscoelastic properties of the liquid. Useful signals are the differences in the amplitudes and phase shifts of the resonator needle in air and in the liquid under study. For comparison, the first column gives the reference values of the viscosity coefficient. $\eta_{exp.}$, determined by direct rheometric measurements [31].

A comparison of the calculated values of the viscosity coefficient with the experimental data shows their good agreement, which indicates the high sensitivity of the method to changes in the viscoelastic characteristics of the fluid.

Table 5 – Viscoelastic characteristics of water and glycerin

Test liquid	ηexp, Pa×s	Calculated values		
		η, Pa×s	G, Pa	η*, Pa×s
Water	6.94·10-4	7.123·10-4	19.9	7.0·10-3
Glycerol (10 %)	8.79·10-4	8.999·10-4	23.1	8.13·10-3
Glycerol (25 %)	1.37·10-3	1.087·10-3	28.6	0.01
Glycerol (80 %)	0.043	0.046	223.2	0.091
Glycerol (95 %)	0.139	0.138	387.5	0.194
Glycerol (100 %)	0.357	0.350	765.3	0.441

To estimate the role of the elastic properties of a fluid during shear deformations, the shear modulus G is calculated, the results of calculations are given in Table 5. It is well known that the elastic properties of a fluid manifest themselves at high frequencies [31, 33, 34]. At these values of frequency, as can be seen from table 5, the shear modulus has an insignificant value, but its relative contribution to η* is essential. This is confirmed by the results of [34].

Taking into account the results of calculations in reference liquids, the viscoelastic characteristics of whole blood were computed during its coagulation.

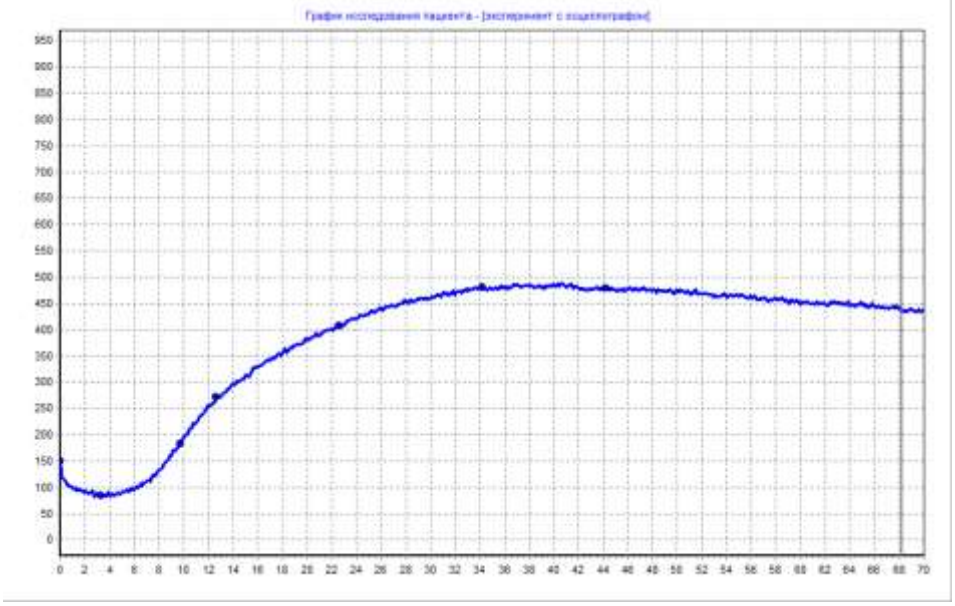
3.3 Results of a computational experiment to determine the viscoelastic properties of whole blood

In the NPTEG method, to register an electrical signal with a piezoelectric sensor that reflects the viscoelastic properties of an unknown fluid with a varying viscosity coefficient, the instrument is calibrated using reference liquids with known viscosity over a wide range of its magnitude. Thus, by calculating changes in the amplitude-phase characteristics of the displacement of the end of the needle-

resonators in the reference fluids relative to their calculated values in the air and comparing them with the amplitude-phase characteristics of the voltage on the recording piezoelectric element, you can determine the viscoelastic characteristics (η', η'') these fluids, which can then be used to determine the dynamics (η', η'') whole blood in the process of coagulation.

To compare the obtained results with the amplitude-phase characteristics of whole blood, measurements of these values were carried out as a function of time for whole blood in six healthy volunteers.

For the study, whole unstabilized blood was used, taken without the use of a harness with a disposable three-component siliconized syringe (the volume of collected blood was 1 ml) by an employee of the Scientific Research Institute of FIRM. E.D. Goldberg. Further, the obtained sample was placed in a single-use 0.45 ml cuvette (Mednord, Russia), located in the thermostat of the ARP-01M Mednord hardware-software complex (Mednord, Russia. FSF 2010/09767 of December 30, 2010). Figure 9 shows the NPTEG curve with dynamic indicators of one of the volunteers.



	Нормы	Показател
А0	*142...236*	**150**
А1	*85...105*	**84**
Т1	*0,8...1,6*	**3,3 мин**
ИКК	*-37...-14*	**-20,00**
А2	*183...203*	**183**
Т2	*3,5...4,8*	**9,7 мин**
КТА	*22,2...38,*	**15,63**
А3	*705...725*	**272**
Т3	*5,7...9,8*	**12,6 мин**
ИКД	*31,9...42,*	**14,92**
А4	*846...866*	**408**
Т4	*15,7...19,*	**22,6 мин**
ИПС	*15,6...22*	**13,60**
А5	*881...901*	**481**
Т5	*41...47*	**34,2 мин**
МА	*472...655*	**397,00**
ИТС	*14,68...20*	**11,61**
А6	*872...892*	**478**
Т6	*51...57*	**44,2 мин**
ИРЛ	-	**0,76 %**

Figure 9 – NPTEG curve of a healthy volunteer

Figure 10 shows a graph of changes in the aggregative state of the blood (by the method of NPTEG) in six healthy volunteers, on which the ordinate axis A is the amplitude A of the process under investigation in relative units, and the abscissa is the time t in minutes. Based on the presented results, the average values of the amplitude-frequency hemostatic potential of six healthy volunteers were calculated (Figure 11).

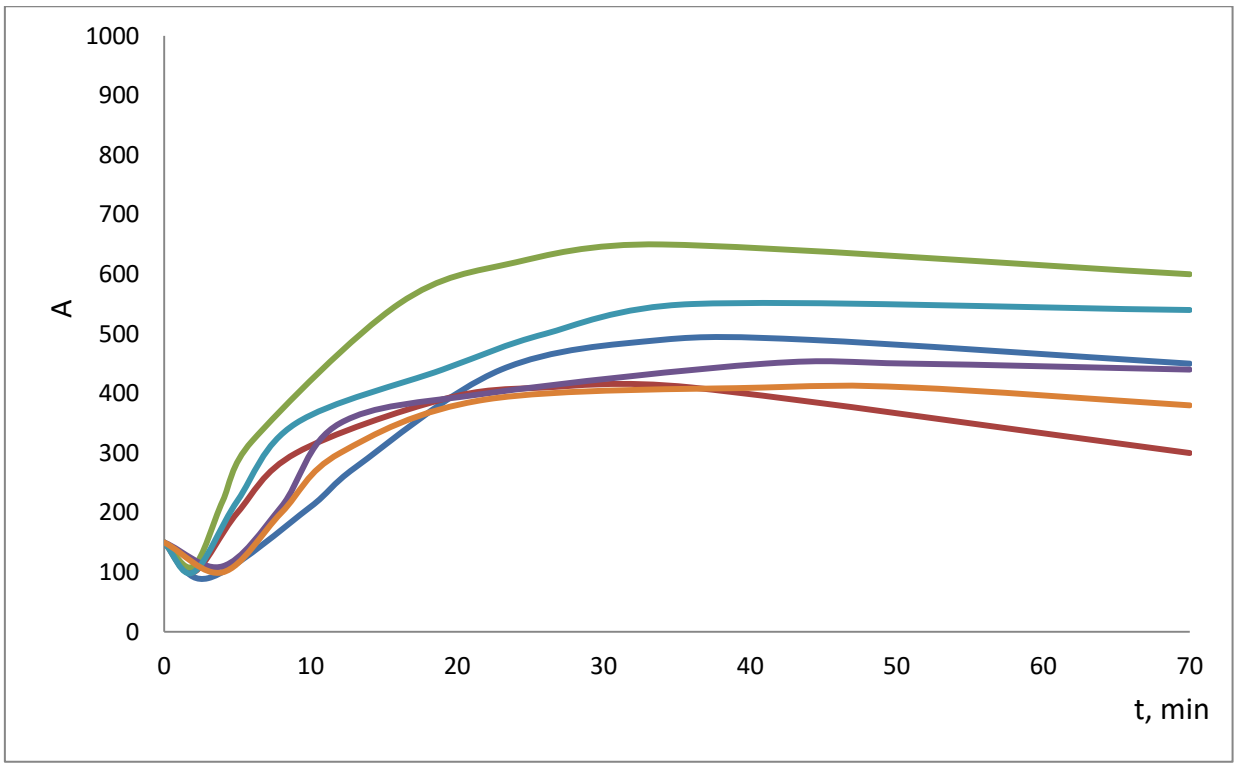


Figure 10 – NPTEG indicators of whole blood in six healthy volunteers

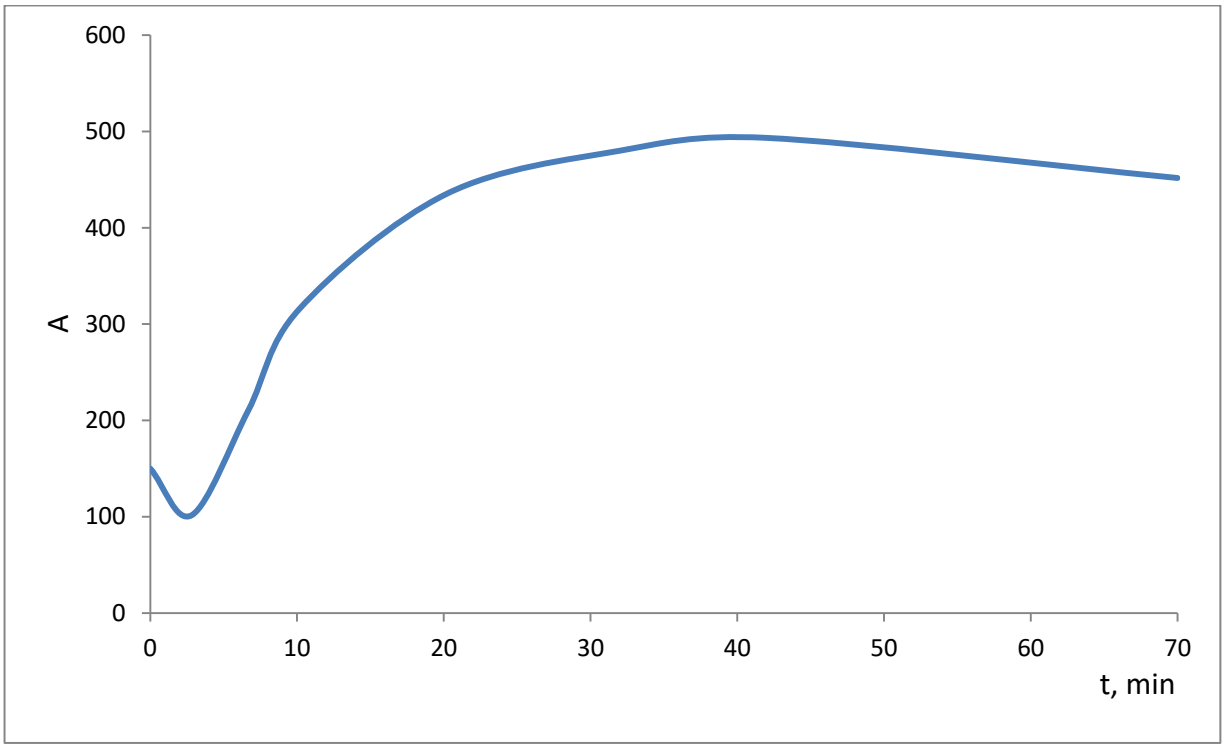


Figure 11 – Average values of the state of the hemostatic potential of six healthy volunteers

The measurements were carried out using a digital oscilloscope. Using the results of measurements, the amplitudes A of the received signal and its phase shift

φ relative to the supplied signal for each healthy volunteer were obtained, the numerical values of which are presented in Table 6.

Table 6 – Values of amplitude A of the received signal and phase shift φ relative to the supplied signal

Healthy volunteers	A, мV	φ, deg.
1	132.3	69.4
2	132.3	69.4
3	91.9	47.8
4	102	55.3
5	106.1	59.5
6	91.9	47.8

Figure 12 shows the dependence of the amplitude of the received signal on time, and Figure 13 shows the dependence of the phase shift of the received signal relative to the input signal from time.

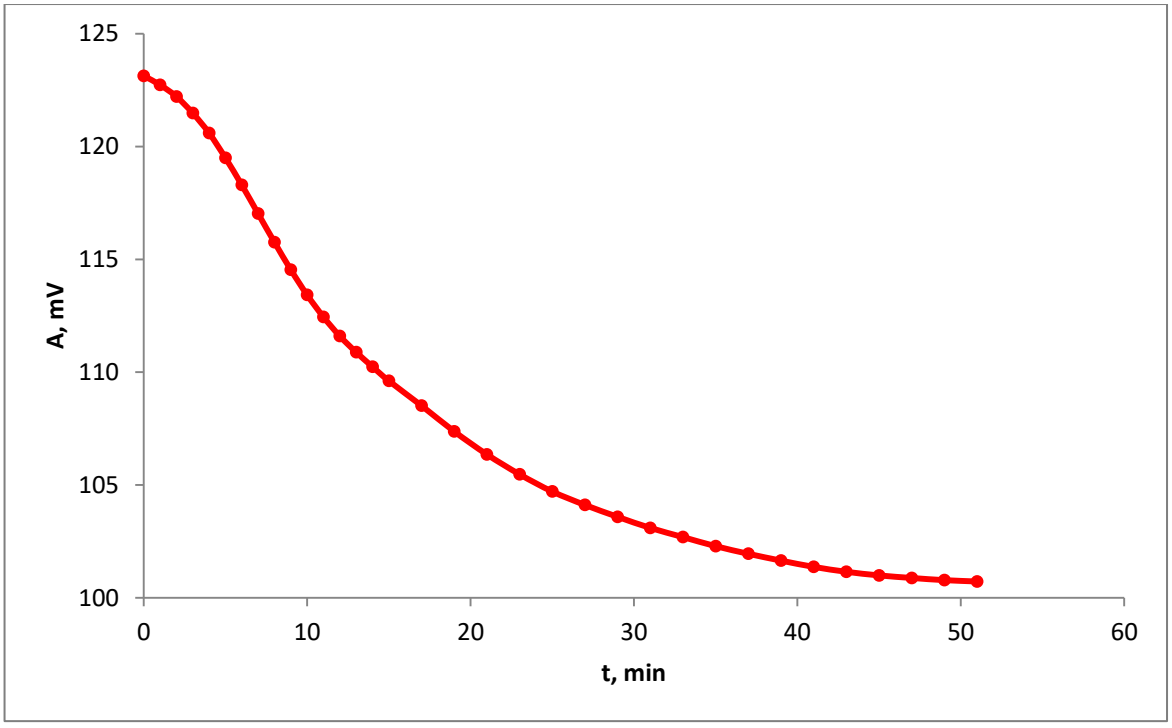


Figure 12 – Dependence of amplitude A of the received signal on time t

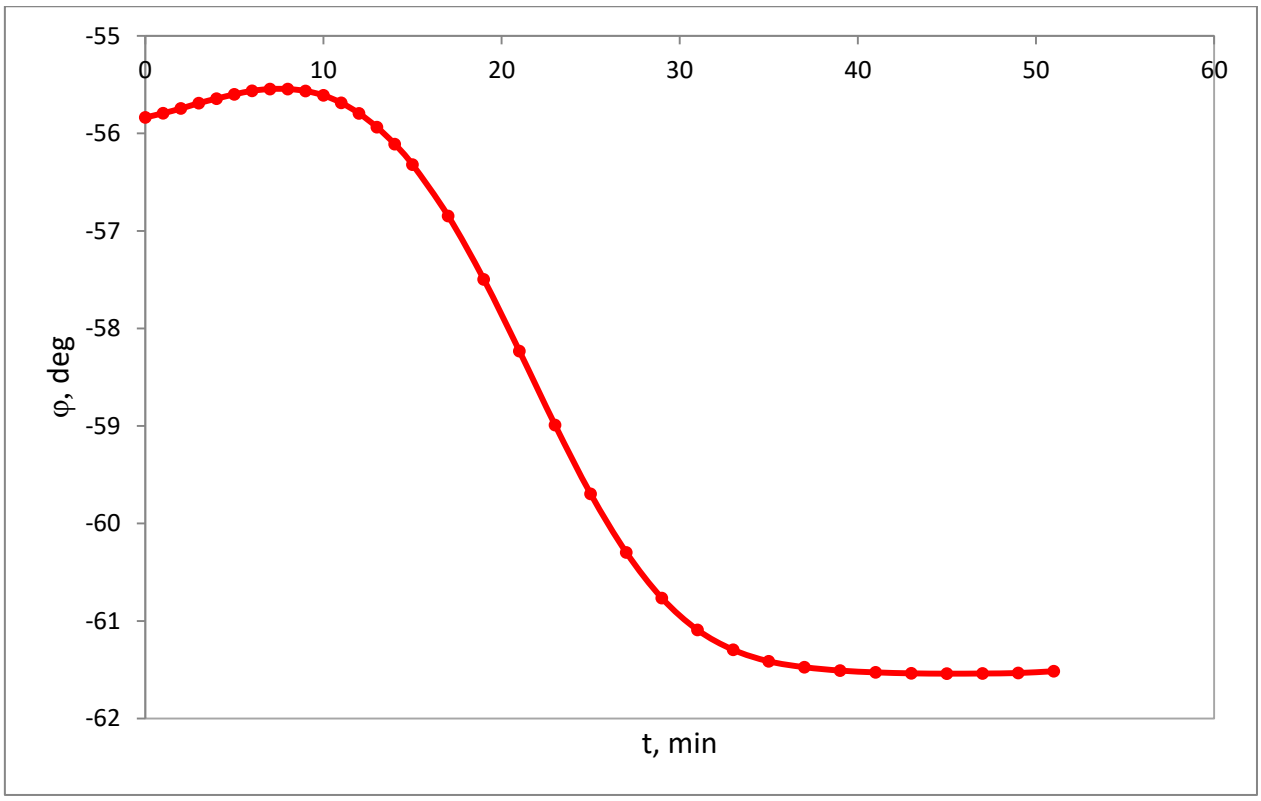


Figure 13 – Dependence of the phase shift φ of the received signal relative to the input signal from time t

The dependence of the amplitude-phase characteristics on the oscillation time of the resonator needle of the ARP – 01M Mednord piezoelectric transducer of the piezotromboelastograph, characterizing the change in the aggregative state of whole blood in the coagulation process, is non-monotonous, reflecting the nonlinear nature of the enzymatic coagulation reactions.

To analyze the coefficients of elastic-viscous properties of whole blood in six healthy volunteers during the coagulation process, according to the amplitude of oscillations of the resonator needle and the phase shift of the received signal relative to the input signal, the average attenuation coefficient β and the average natural frequency ω0 harmonic oscillations of the resonator needle in whole blood in the process of its coagulation. The calculations were carried out according to the formulas 7. The results of the calculations are presented in Figures 14 and 15.

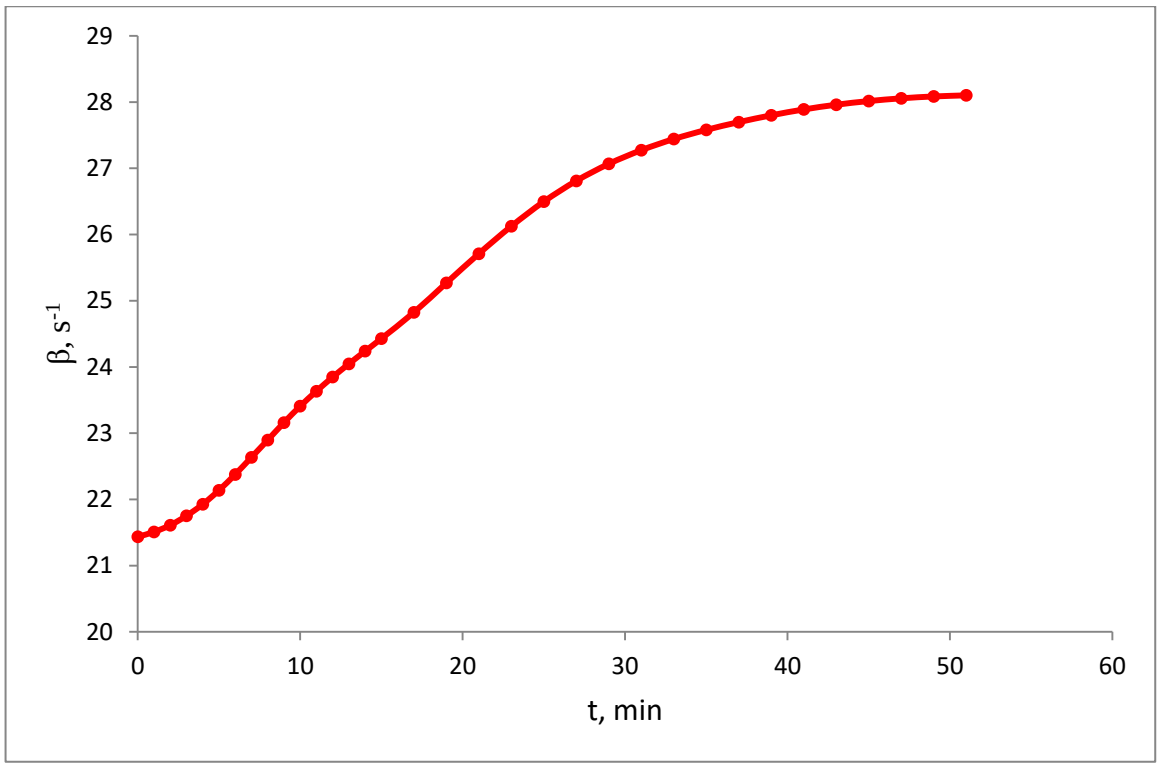


Figure 14 – Dependence of attenuation coefficient β of harmonic oscillations of a resonator needle in whole blood on time t

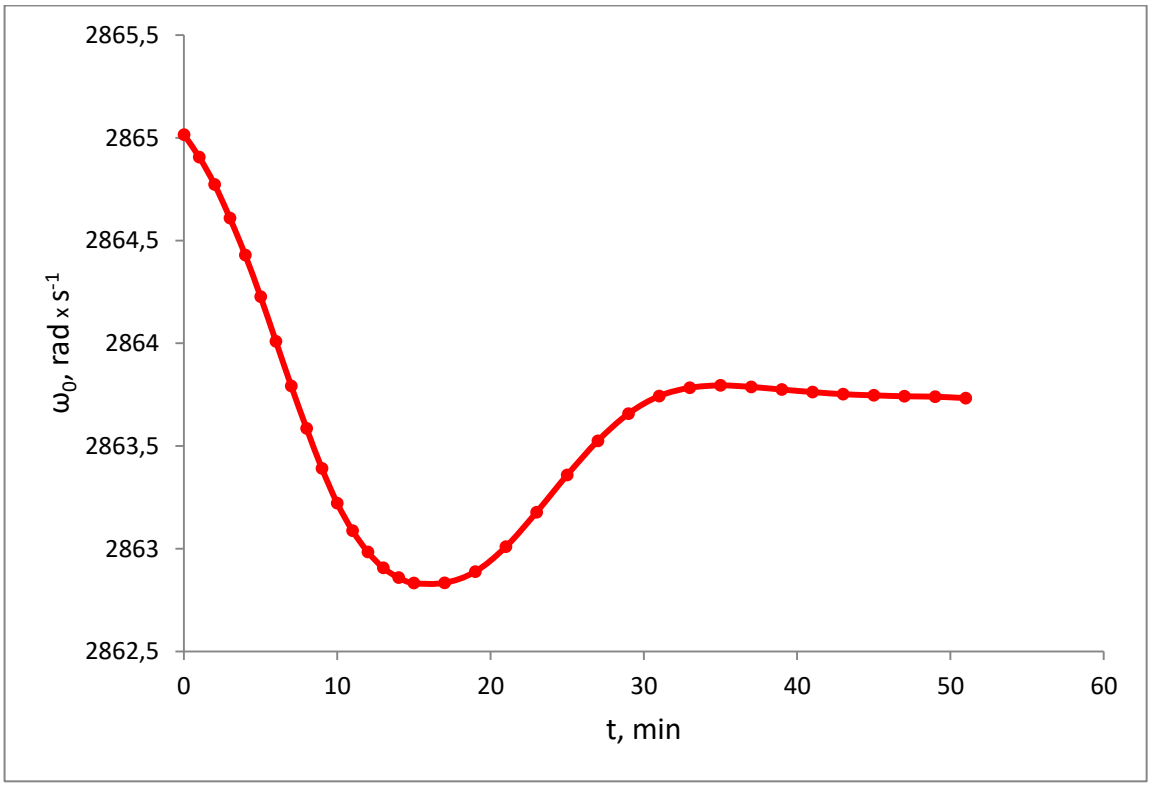


Figure 15 – Dependence of the natural frequency ω_0 harmonic oscillations of the resonator needle in whole blood on time t

As can be seen from the graphs, the dynamics of β and ω_0 have significant differences. At the initial time moment, β decreases, and ω_0 does not change. In the time interval Δt = 2-10 min, both values change significantly. Further, after a significant decrease, ω_0 ceases to change, and then a slight increase is observed. At β, an increase in its values is observed up to 40 minutes. This indicates the difference between the processes leading to the changes in β and ω_0.

Based on the expression:

$$2 \times \beta \times \upsilon = \eta \times \frac{d\upsilon}{dx} \times \Delta S\,, \qquad (10)$$

where υ is the speed of movement of the end of the needle of the resonator, ΔS is the area of the submerged part of the needle of the resonator in the blood, according to the values of the attenuation coefficient β shown in Figure 14, the coefficient of viscosity of the blood η was calculated during its coagulation. The results of the calculations are presented in figure 16.

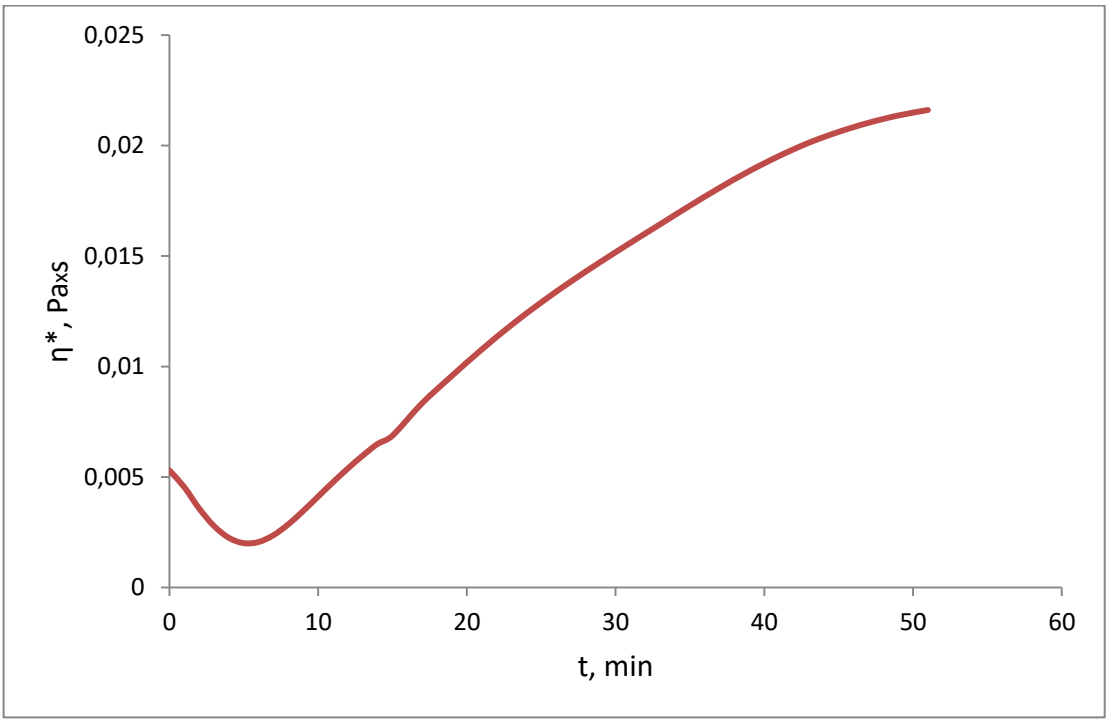


Figure 16 – Dependence of the coefficient of viscosity η blood from time t in the process of its coagulation

Table 7 shows the results of calculations of the viscoelastic characteristics of whole blood by the formulas (5), (6), (8) and (9) according to the measured amplitude-phase characteristics of the oscillations of the resonator needle.

Table 7 – Viscoelastic blood characteristics of healthy volunteers

Healthy volunteer	Calculated values		
	η, Pa×s	G, Pa	η*, Pa×s
1	0.005	25.8	0.010
2	0.005	25.8	0.010
3	0.038	163.7	0.065
4	0.311	134.0	0.056
5	0.038	118.2	0.057
6	0.030	163.7	0.065

Figure 17 shows the average time-dependent complex viscosity coefficient of whole blood $\eta^* = \eta + i\, G/\omega$ in the process of coagulation at a fixed temperature of 37°C based on the measured amplitude and phase characteristics. A comparison of calculations of the coefficient of blood viscosity at the initial moment of time with the available data of rheometric measurements shows their good agreement.

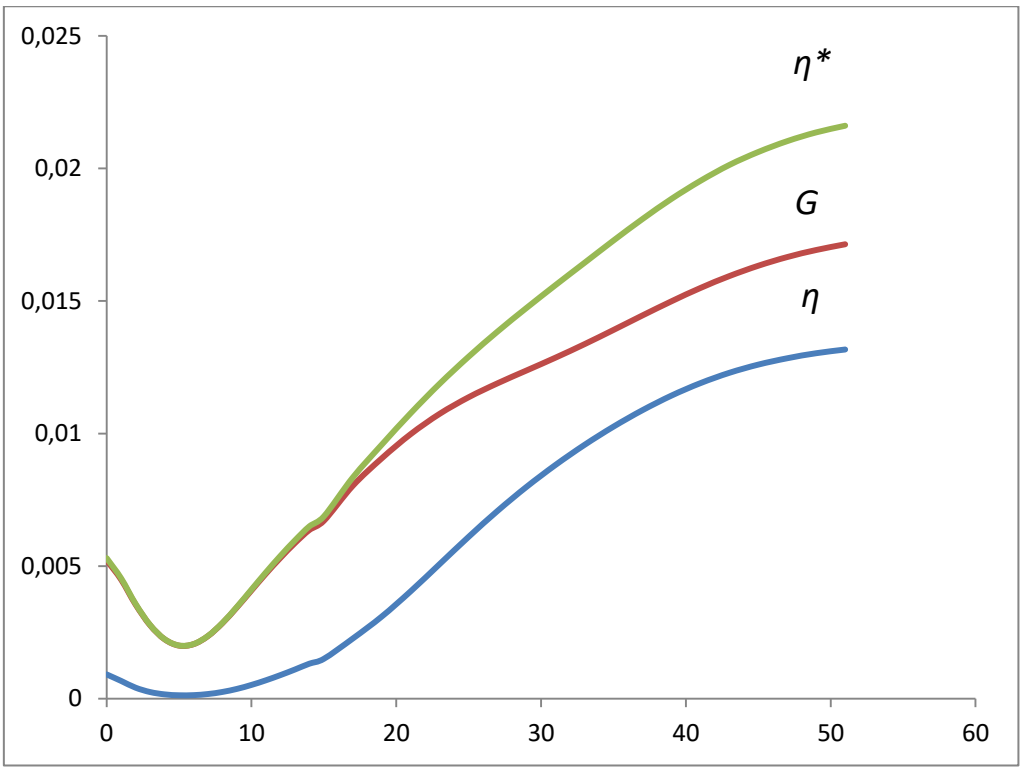


Figure 17 – Average time dependence of the complex viscosity coefficient of whole blood $\eta^* = \eta + i\,G/\omega$ in the process of its coagulation at 37°C

From figure 18 it follows that c in the process of blood coagulation, the real and imaginary parts of the coefficient η^* increase by orders of magnitude, reaching a maximum value during the formation of the fibrin-platelet structure of the clot. The shear modulus G, which reflects the elastic properties of the fluid in the time range under consideration, makes a significant contribution to the complex viscosity coefficient.

Thus, on the basis of the obtained results, using the developed complex physical and mathematical model of the hemocoagulation process, the time dependence of the viscosity coefficients of whole blood of six healthy volunteers was calculated. Figure 18 shows the calculated values of the coefficient of blood viscosity depending on the time obtained on the basis of data. Curve 1 was obtained on the basis of the experimental dependence of the viscosity coefficient on the instrument readings, and curve 2 was obtained on the basis of the

theoretically calculated dependence of the viscosity coefficient on the instrument readings using formulas (5) and (6).

Comparison of the obtained results gives good agreement between theoretical calculations and experimental measurements.

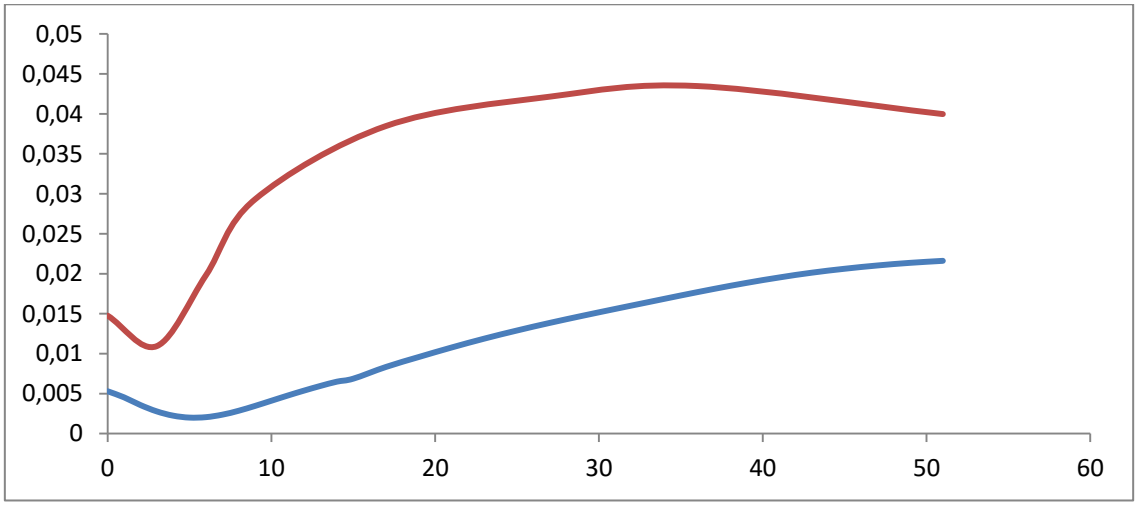


Figure 18 – Results of numerical (blue) and laboratory (red) experiments under the physiological norm

The value of the calculated viscosity coefficient η of blood in figure 18 at the initial moment of time was 5.53×10^{-3} Pa × s, which agrees well with the experimental results obtained by other methods.

A comparative analysis of the results obtained for different points in time shows that in the process of coagulation of whole blood, its viscosity coefficient η varies from $\sim 5 \times 10^{-3}$ to $\sim 2 \times 10^{-1}$ Pa × s, which indicates a significant change in its component composition. The study of patterns in the dynamics of the coefficient of viscosity of the blood in the process of its coagulation will allow to diagnose various deviations caused by those or other violations of this process.

CONCLUSION

Blood coagulation is a complex physiological process that is crucial to human health. It includes a balance of various factors, including biochemical reactions, clotting factors, platelets, binding sites, geometric, spatial limitations of the blood vessels and flow dynamics. In case of imbalance, people's lives may be in danger, for example, significant blood loss, pulmonary embolism, thrombosis, stroke, cardiac arrest or even death. In this regard, the study of blood coagulation is an important topic in medical research.

Given the complexity of coagulation, it is possible to use mathematical models to quantify the coagulation process. Mathematical models allow researchers to gain insight into the nuances of blood coagulation, as well as provide an opportunity to test potential treatment methods and drug therapy when a physical experiment may be too dangerous.

The obtained physical and mathematical model for calculating the complex coefficient of viscosity of whole blood is based on experimental data on the amplitude-phase characteristics measured by the method of low-frequency piezotromboelastography.

To conduct this study, the method of low-frequency piezotromboelastography (NPTEG) was chosen. The choice of this method was based on its ability to provide complete information about the process of fibrinogenesis, starting from the early stages of clot nucleation (initiation, amplification) and ending with the determination of the lytic activity of the blood aliquot under study. In addition, the use of this method involves the study of whole unstabilized blood, which helps to minimize the pre-analytical stage and, as a consequence, reduce errors associated with the selection, storage and sample preparation for the study.

In this work, we calculated the complex index of viscosity of whole blood and its real and imaginary parts, reflecting the viscoelastic characteristics of blood-based on measurements of the amplitude-phase characteristics of oscillations of the

resonator needle in whole blood during its coagulation. Based on the obtained results, using the developed complex physical and mathematical model of the process of hemocoagulation, the calculation of the time dependence of the viscosity of whole blood of six healthy volunteers was carried out. A comparison of calculations of the coefficient of blood viscosity at the initial moment of time with the available data of rheometric measurements shows their good agreement.

The obtained results confirm the possibility of using this approach to the determination of the viscoelastic properties of whole blood and analysis of their dynamics in the process of coagulation in the mode as close as possible to the in vivo study. Analysis of the results of measuring the amplitude and phase characteristics of the piezoelectric sensor at characteristic time points in the process of fibrin polymerization allows rapid assessment of the hemostatic potential of the blood and the detection of violations in the blood coagulation system.

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