Ultrasonic multi-channel method for investigation of non-stationary biologic liquids

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Introduction

A typical non-stationary biologic liquid is a clotting blood, structure of which varies in time and in space. Blood coagulation serves as a protective mechanism against blood loss due to tissue damage. Inappropriate blood coagulation plays an important role in various diseases as well. There are a number of methods used for analysis of blood coagulation, including ultrasonic: spectroscopy, measurements of ultrasound velocity, attenuation, impedance, scattering and radiation force [1]. Measurements of ultrasound velocity variations reveal the main stages of clotting, however, due a random nature of this process it is still a problem to get a precise dynamic picture of the entire process.

It has been established experimentally that the ultrasound velocity in a blood sample is changing in a specific way during the whole blood clotting process [2]. In such experiments a small blood chamber (<1 ml) made of biocompatible materials, not initiating and not influencing the clotting process is used. In a differential pulse-echo method ultrasonic waves are transmitted into the medium through a buffer rod. Ultrasound velocity changes are measured in the blood sample during the entire blood clotting process, starting from the first minute structural changes. Immediately after the filling the medium in the chamber is homogeneous. At the same instant two main processes start: the blood clotting and sedimentation of erythrocytes. Gradually the medium becomes non-homogeneous. During the whole clotting process, which lasts approximately 1 hour, typical variations of the ultrasound velocity are observed. They reflect clotting, latent and main refraction and lysis. Most informative the clotting process is from the onset (~30 s), when a typical increase of ultrasound velocity is observed, till ~ 10÷15 min when a blood clot is formed. At a certain instant the clot separates from the chamber walls and a layered structure forms: serum-clot or serum-clot-serum with uncertain boundary surfaces. Ultrasound velocity in such layered structure must depend not only on velocities in each component, but on thicknesses and uniformity of the layers. That is why a phenomenon was observed that clotting curves in the blood of the same person are not strictly repeatable and measurement results depend on orientation of the chamber with respect to the horizon and the ultrasonic transducer position. Apparently, a clot shape slightly differs each time and that influences the ultrasound velocity. Additionally, it is not known if the clot forms simultaneously in the whole volume of the clot sample or it forms gradually in different zones.

In order to reveal regularities of a blood clotting both in the time and space domains a novel investigation method is necessary. In this paper we present a multi-channel ultrasonic method which enables to measure ultrasound velocity variations simultaneously in a few parallel channels thus enabling to monitor blood clotting process in different but closely located spatial domains.

Measurement system

The measurement system consists of a multi-channel ultrasonic measurement chamber, electronic units for excitation of ultrasonic transducers and amplification of received signals in each channel, a digital oscilloscope HP54645A for digitalization of the signals and a personal computer for acquisition, storage and processing of measurement data.

The primary measured parameter is the propagation time of the ultrasonic signal in each channel. After calibration in distilled water, ultrasound velocity variations in a non-stationary biologic liquid are determined. It is necessary to point out that only the ultrasonic velocity is an objective parameter of the medium, while the propagation time is an intermediate result, as it depends on a propagation distance, parasitic delays, etc.

The measurement chamber is immersed in a water bath of the temperature 37 ºC, controlled within ± 0.01 ºC. The chamber is filled by means of a syringe through a medical needle. The single channel measurement chamber used earlier in blood coagulation studies employs a plastic buffer rod with the diameter 10 mm and 15 mm length. For excitation and reception of ultrasonic signals 5 MHz the piezoelectric transducer with the diameter 6 mm is used, while the chamber length itself was 5 mm. In such a design an informative signal is observed between the first and the second reflected signals from the buffer rod end. A multi-channel version of the same dimensions is not optimal, because the transducers would be too small and the signals of the different channels would interfere due to divergence of the ultrasonic beam and a long buffer rod. Therefore, a measurement chamber of a different design is required. It is necessary to keep in mind that due to the required high accuracy of measurements diffraction effects play a significant role. Taking this into account two different designs of multi-channel measurement chambers, operating in through-transmission and pulse-echo modes were proposed and investigated: (Fig.1).

In both cases an axial symmetry is exploited, hence, the diffraction effects in all channels are almost the same. In a through-transmission mode the 5 MHz PZT

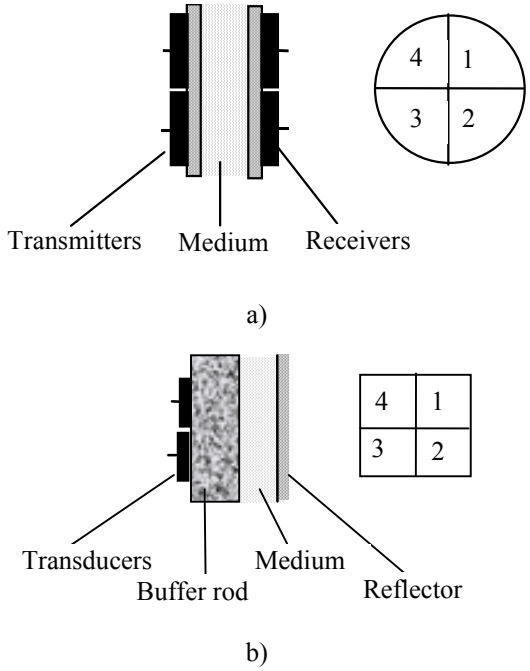


Fig.1. **Two designs of multi-channel chambers:a - through-transmission, b - with a buffer rod**

transducers of the diameter 12.7 mm (Pz27 Ferroperm) were glued to the λ/4 protectors made of the 10 MHz X-cut quartz plate and later cut precisely into the four 90º sectors by means of 200 μm diamond disc (DIA-TESSIN). In order to eliminate the delay time in the ultrasonic transducers and electronics during ultrasound velocity measurements, the first and the second received signals are used, i.e. the time of double propagation via investigated medium is measured.

In the chamber with a buffer rod four rectangular PZT 4×4 mm transducers symmetrically placed with respect to the axis of the chamber are used. The diameter of the buffer rod was 12 mm and the length was shortened up to 5 mm, so the ultrasonic signal propagating back from the reflector is situated between the second and the third signals reflected by the buffer rod end. Two different versions of acoustic chambers with a buffer rod were investigated- with transducers of the same 5 MHz frequency and with different frequencies: 5, 10, 15 and 20 MHz. In the last case diffraction effects are different and must be evaluated theoretically or determined experimentally for each channel.

In this paper experiments with the chamber operating in the through-transmission mode are presented. The frequency response of the 5 MHz transducer bonded to the λ/4 protector possesses two peaks at ~ 3 MHz and ~ 6 MHz. The peak of 5 MHz is absent as predicted theoretically in [3]. Thus the first and the second signals in each channel being almost identical are superposition of these two frequencies (Fig 2a). Signals of each frequency may be selected by an appropriate filtering procedure. It enables to perform measurements simultaneously at two different frequencies. Spectrum of the first directly transmitted signal in water is presented in Fig 2b. Spectra of the first and the second received signals are slightly different as the high frequency components are attenuated more at longer distances, especially in biological media.

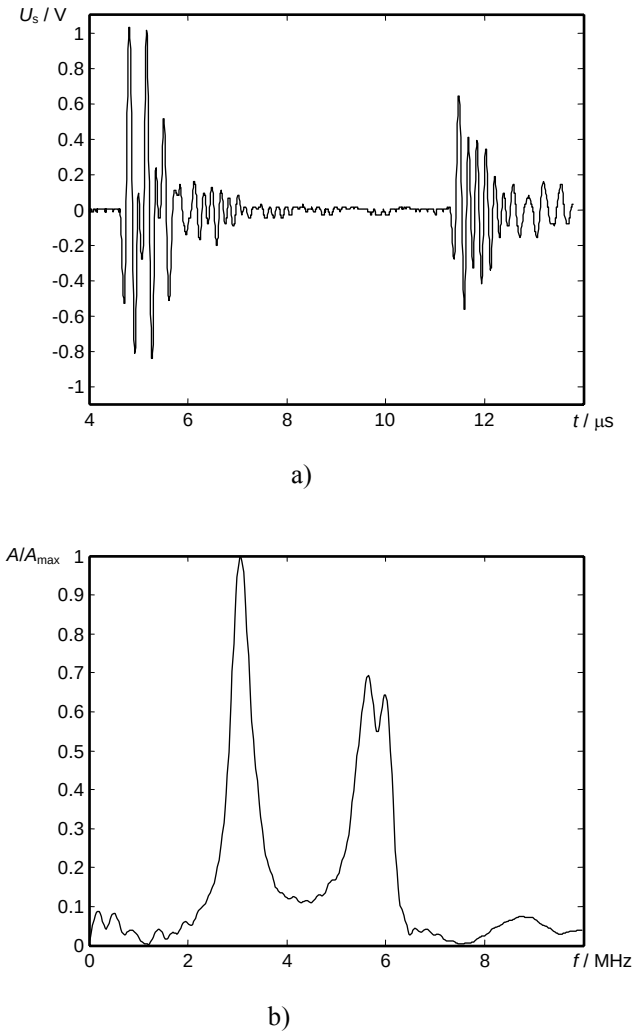
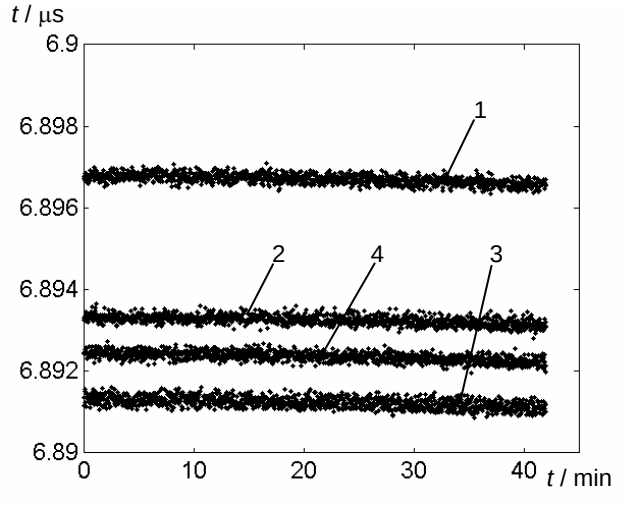


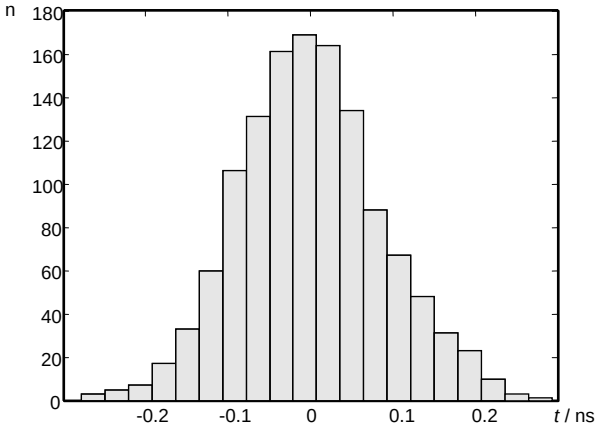
Fig.2. **The first and the second received signals in through-transmission multi-channel chamber (a) and a typical spectrum of the first signal (b)**

The identity of the measurement channels is of primary importance. The main problem is to get precisely the same distance between the ultrasonic transducer pairs in all four channels. First of all, the thickness of the spacing ring between the quarts protectors is 5.08mm, therefore the thickness difference only 1 μm corresponds to the difference in ultrasound velocities 0.3 m/s. In principle, the spacing ring may be machined up to this precision. However, the thickness of the quartz λ/2 protector is 297 μm and variations of it at different points may reach up to 1÷2 μm. Finally, it is difficult to assure a layer of epoxy glue of uniform thickness between the transducers and the protector in all channels. As a consequence, the propagation path of ultrasonic waves in different channels differs slightly and must be found by means of calibration in distilled water before the experiments. Fig 3a illustrates this situation and the necessity of calibration. The measured delay times in all four channels are the differences of arrival times of the second and the first received signal (Fig. 2a). The maximal difference between the channels is up to 5 ns, what

corresponds to the difference in ultrasound velocities 1.1 m/s.



a)



b)

Fig. 3. **Measured delay times in distilled water when the temperature slightly increases (a) and the distributions of delay time variations in the 1st channel (b)**

The delay time difference was calculated using cross-correlation function between the first and the second received signals. The exact position of the cross-correlation function maximum in the time domain was found using 5-order polynomial interpolation. The distribution of variations of the measured delay time is shown in Fig.3b. The standard deviation is σ_c=0.1ns what corresponds in the ultrasound velocity measurements to the uncertainty σ_c=0.02m/s.

The ultrasound velocity in the medium under investigation can be determined using the following expression

$$c_m(t) = \frac{2L_B}{[t_1(t) - t_0(t)] - \Delta t_{pr} - \Delta t_{gl} - \Delta t_{difr} - \Delta t_m},$$

where L_B is the measurement base of the chamber, $t_1(t) - t_0(t)$ is the measured delay time difference and Δt_{pr}=0.208μs is the delay time in the quartz protectors, Δt_{gl}=0.008μs is the delay time in the epoxy glue layers, Δt_{difr} is the diffraction error and Δt_m is the delay time measurement error caused by additional delays in electronic circuits and due to the measurement method. For determination of the measurement base L_B the measurements were performed in distilled water at two different temperatures 17.96°C and 36.91°C. The ultrasound velocity in distilled water at these temperatures according to Del Grosso [4] is 1475.90m/s and 1523.45m/s respectively. The calculated measurement base differences between the different channels are within 3.5μm. The determined measurement bases of individual channels were used for determination of ultrasound velocity values.

Experimental investigations

The objective of experimental investigations was to determine if the clotting process of the same sample in different spatial domains is going on in a similar way or some spatial non-uniformity exists. For this purpose measurements with the calibrated multi-channel ultrasonic measurement system first of all were carried out in the polymerizing acrylamide, which was proposed by us as the reference medium for a dynamic calibration of the ultrasonic system for investigation of whole blood clotting process [5]. A free-radical chain polymerization reaction of acrylamide is initiated by a certain ratio of persulphate and tiosulphate. This reaction is characterized by fast and slow variations of ultrasound velocity in the time domain in different reaction phases which are very close to the observed in clotting blood.

The composition of arcylamide polymerization compound mixture for experiments in this work is presented in Table 1.

Table 1. **Composition of acrylamide polymerization compound mixture**

Materials	Formula	Concentration	Quantity
Acrylamide	CH_2= $CHNH_2$	5 % on mixture weigh	5 ml
Potassium persulphate	$K_2S_2O_8$	0.04 moles/litre	2 ml
Sodium thiosulphate	$Na_2S_2O_3 \times 5$ H_2O	1×10^{-4} moles/litre	2 ml
Distilled water	H_2O	Laboratory grade	1 ml

Ultrasound velocity measurements in the polymerizing acrylamide were carried out at different spatial orientations of the measurement chamber. Orientation of the chamber schematically is shown in the upper or lower right corner of the corresponding figure. Two main orientations of the chamber were horizontal (Fig.4a) and vertical (Fig.4b).

From the results presented follows that in the horizontal orientation of the measurement chamber polymerization course differs significantly in various channels, especially in the upper and lower parts of the chamber. Moreover, experiments reveal that measured ultrasound velocities depend also on axial orientation of the chamber, i.e. which channel is on the top. In the upper

part of the chamber ultrasound velocities are lower, in the channels located at the bottom–higher. The observed phenomenon may be due to gravitation forces. Contrary to the expectations, in the vertical position of the chamber polymerization reaction courses are not identical in all channels. It may be explained by different polymerization course in the volume of the chamber and by existence of certain randomly located polymerization centers. Obviously, the same phenomenon exists in the first case also. It is interesting to note that the polymerization reaction begins after 5 min and terminates after approximately 20 min in both cases.

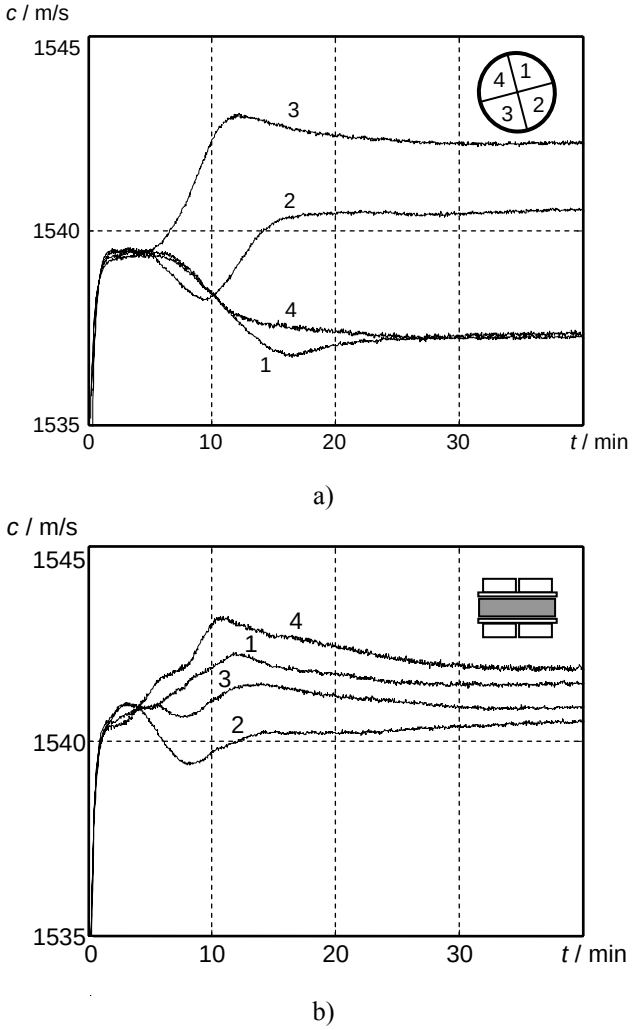


Fig. 4. **Acrylamide polymerization curves obtained with the multi-channel measurement chamber oriented horizontally (a) and vertically (b)**

The objectives of experiments in blood were the following: investigation of sedimentation of blood cells in a citrated blood and coagulation of blood, initiated by the addition of $CaCl_2$ solution.

As expected, the blood sample the bottom of the horizontal chamber (Fig. 5a, channels 2, 3) due to the sedimentation of the cells becomes more and more dense, and the velocity of ultrasound increases.

Contrarily, in the upper part (channels 1, 4) of the chamber the medium becomes more transparent, the ultrasound velocity slightly reduces and only after 30 min starts to increase. The beginning of the sedimentation, detected ultrasonically, starts approximately after 7 min. In this experiment the citrated blood was diluted by a physiological solution of 0.9 % NaCl by a ratio 3:1. Due to this reason the ultrasound velocity is lower than in whole blood. Similar dependencies were found earlier by the authors during investigation of sedimentation phenomenon up to 50 h after the cessation of stirring in red luminophor used in production of colored TV tubes.

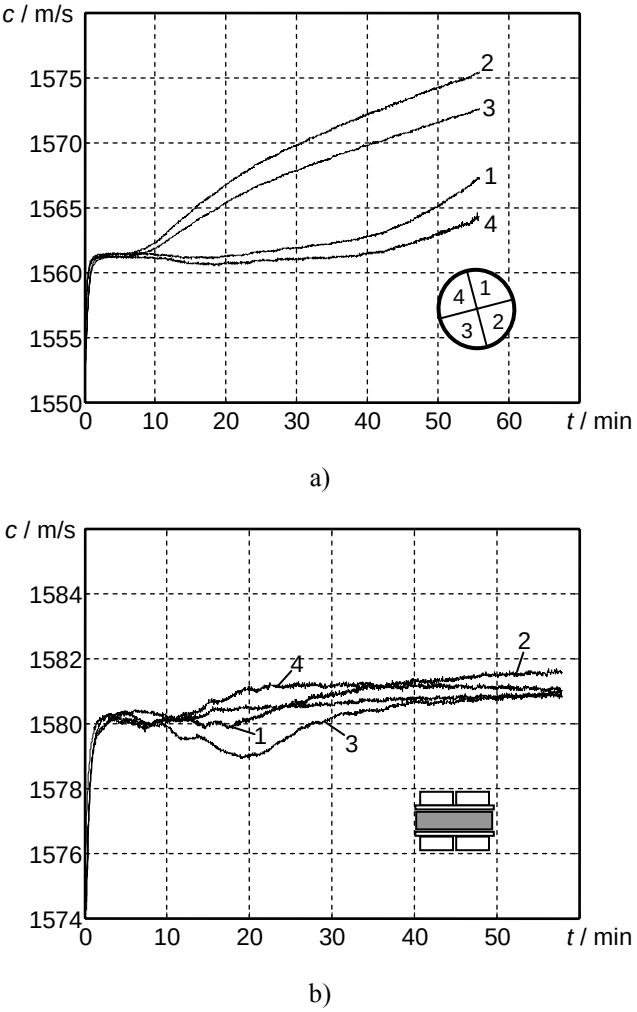


Fig.5. **Ultrasound velocity changes caused by sedimentation of red blood cells in a citrated blood at horizontal (a), and vertical (b) positions of the measurement chamber**

Experiments in a vertical chamber (Fig. 5b) were carried out with a non-diluted conserved blood, so the initial ultrasound velocity is typical for a blood and is approximately 20 m/s higher than in the previous case. It is obvious that at such orientation of the chamber no evident ultrasound velocity dependence on the channel number can be observed. Ultrasound velocity differences in all channels are due to random distribution of sediments.

Clotting experiments were performed using citrated blood: 0.06 ml of 10 % $CaCl_2$ solution was added to 2.5 ml blood in order to induce the coagulation process. The part of this blood was injected into the chamber kept at a constant temperature using a syringe. Native blood was not used due to a limited biocompatibility of the used experimental chamber.

Fig. 6a shows coagulation curves obtained with a horizontal chamber. As in the polymerization and the sedimentation experiments, the upper and the lower channels demonstrate different courses of the process. Differences may be due to a non-axially symmetric clot formation, obviously due to gravitation forces. At the 15 min. we can notice the separation of a clot, which causes the irregularity on coagulation curves.

In the vertical position of the measurement chamber (Fig. 6b) almost identical curves are obtained. After disassembling of the chamber it was found an axially symmetric clot, stuck to one of the quartz protectors. It was approximately of the same diameter as the chamber, while its thickness was about a half of the chamber length, the rest volume was filled with a blood serum. Clot separation is observed at 25-26 min after beginning of the measurement.

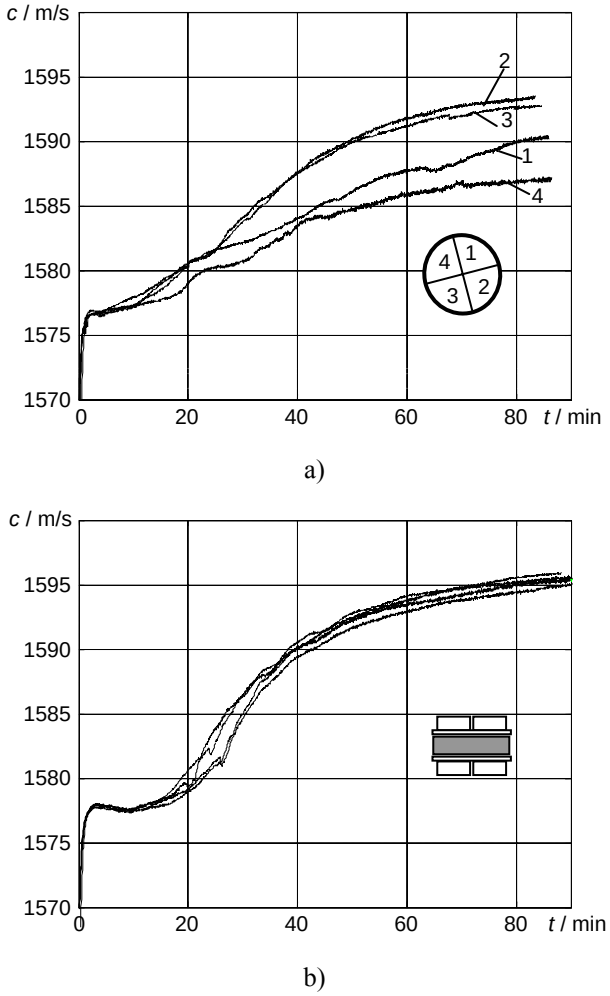


Fig.6. **Ultrasound velocities measured during coagulation of a citrated blood induced by addition of $CaCl_2$ solution in a horizontal (a) and vertical (b) positions of the chamber**

It is necessary to note that character of the curves differs in both chamber orientations. In the last case from the second to the tenth minute ultrasound velocity decrease is observed, which is associated with the so called latent clot retraction [2]. This decrease is absent in a horizontal chamber orientation.

Conclusions

Experiments reveal that in non-stationary liquid medium structure of which varies in volume and in the time domain (sedimentation, polymerization, blood clotting, etc.) the results of ultrasonic measurements depend on orientation of the ultrasonic wave propagation direction with respect to the horizon. In such cases the measurement results can be compared only at same experimental conditions. The proposed multi-channel measurement method enables to investigate spatial non-uniformities and variations in the time domain occurring in biologic liquids.

Acknowledgement

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Daugiakanalis ultragarsinis nestacionariųjų biologinių skysčių tyrimo metodas

Reziumė

Pasiūlytas ir ištirtas ultragarsinis metodas nestacionariųjų biologinių skysčių savybėms tirti realiu laiku. Metodas pagrįstas vienu metu lygiagrečiuose kanaluose atliekamais ultragarso greičio matavimais. Sukurtos keturių kanalų <1 ml tūrio ultragarsinės kameros, skirtos nestacionariems erdvėje ir laike procesams, pvz., kraujo krešėjimui, cheminėms reakcijoms ir kt., analizuoti. Matavimai atliekami siuntimo ir priėmimo arba atspindžio režimu, kai panaudotas akustinis bangolaidis. Ultragarso bangų dažniai kanaluose yra arba vienodi (5 MHz), arba skirtingi (5, 10, 15, 20 MHz). Pirmame etape atlikti elektroakustinių traktų tyrimai etaloniniame skystyje (distiliuotajame vandenyje). Tirta, kaip laike ir erdvėje vyksta akrilamido polimerizacija, kuri yra dinaminio kalibravimo terpė ultragarsiniam koagulometrui, skirtam kraujo krešėjimo proceso analizei. Atlikti eritrocitų nusėdimo bei citruoto kraujo krešėjimo tyrimai, esant įvairiai kanalų orientacijai.

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