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Method of investigation of non-stationary biological fluids

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Introduction

Biological fluids are specific objects for acoustical investigation due to the wide spectra of relaxation processes and as a consequence ultrasound velocity dispersion. Especially complicated media are non-stationary fluids as blood during its coagulation process.

Earlier the authors used ultrasonic spectrometry for the investigation of biological media, applying a broad band acoustic pulse transmission and Fourier analysis technique to determine media response over a frequency range such obtaining its attenuation characteristics [1]. The method is sensitive to the blood structure changes in clot formation as well [2], as the higher frequency components in the wide band informative pulse tend to be attenuated more than the low frequency components. But the quantitative parameters of the process are problematic to be determined.

The aim of the project was the development of the method of the investigation of biological fluids, especially non-stationary, based on the precise measurements of ultrasound velocity in a small sample and suitable to be applied in a clinical situation to distinguish between normal and pathological state, investigate the structural variations of the fluids.

Method

The measurement principle of ultrasound velocity is based upon the determination of the propagation time of acoustic pulses that are repeatedly transmitted to the sample. The method employs advantages of differential measurements. When the acoustic base is known with a necessary accuracy, it is easy to determine exactly the ultrasound velocity from the propagation time in the sample. It is the difference of total time and the propagation time in the buffer rod. Ultrasound velocity is an objective parameter of the fluid while the pulse propagation time is dependent on the base length of the cell and it is not possible to compare the results of various authors. The method is accurate (better than 0.1 m/s) and needs a small quantity of fluid (0.2 ml) as the acoustic base is short (5 mm).

Experimental technique

The implementation of the method depends on the way of acquisition of the information about the propagation time of the pulse. In the conventional case zero-crossing method is used, and only the specific instants of the transducer and received signals are determined. If the full signals were registered in the digital form more effects can be investigated.

The essential part of the equipment is the measuring cell. It is made of synthetic and biocompatible material, as well as the buffer rod, and is filled by means of the syringe. The cell can be equipped with changeable ultrasonic transducers of various characteristics. The temperature of the sample is monitored continuously by a small Pt thermistor probe, and the flat ceramic base of it serves as the reflector of ultrasonic waves. The cell was placed in a large water bath of a constant temperature. The measurement of the temperature of the object in principle enables to correct ultrasonic data and plays a decisive role at the first instants of the experiment, especially if the non-stationary fluid is investigated, e.g. in blood coagulation studies. The precision of the Pt thermistor is 0.01 K or better.

The block diagram of the universal instrument is depicted in Fig. 1.

It realises the two mentioned above different principles of ultrasound velocity measurements. In the case of wide-band one period signal, obtained from highly damped transducer, usually the analog zero-crossing technique is used, and the modified time interval averaging technique is applied. Narrow-band signals were obtained in two ways: weakly damped transducer was shock excited or highly damped transducer excited with a tone-burst with the rectangular envelope (e.g. 10 cycles), Fig. 2. These signals were digitised with a sampling rate 200 MHz to provide the best temporal resolution of the waveforms, and each zero-crossing of individual waves in the pulse was determined. The zero-crossing technique for the chosen wave may be used as well for such signals. Typically, highly damped transducers excited by a short duration rectangular pulse had a band-with 1-10 MHz with a central frequency 5 MHz. Narrow band signals contained only the fundamental frequency with a low harmonic content; this frequency may be increased up to 20 MHz. Due to the significant harmonic content the measurements with damped transducers didn′t represent single frequency values.

The counter clock period is τ =20 ns and averaging is n=256 in the time averaging method used with short ultrasonic signals. Due to the original scanning of clock signals according to the time intervals, the error in the overall measurements is several times less than is given by the known expression $\Delta t = \tau / \sqrt{6} \times 1/\sqrt{n}$. The measurement rate depends on a pulse repetition rate of the exciting generator; it is set at the fastest possible value consistent with allowed reverberations within the cell, rod and reflector to die away. Thus, the system allows to get about 4 results per second what corresponds to real time measurements. Measurement repetition rates in the vicinity of submultiples of the clock frequency were avoided.

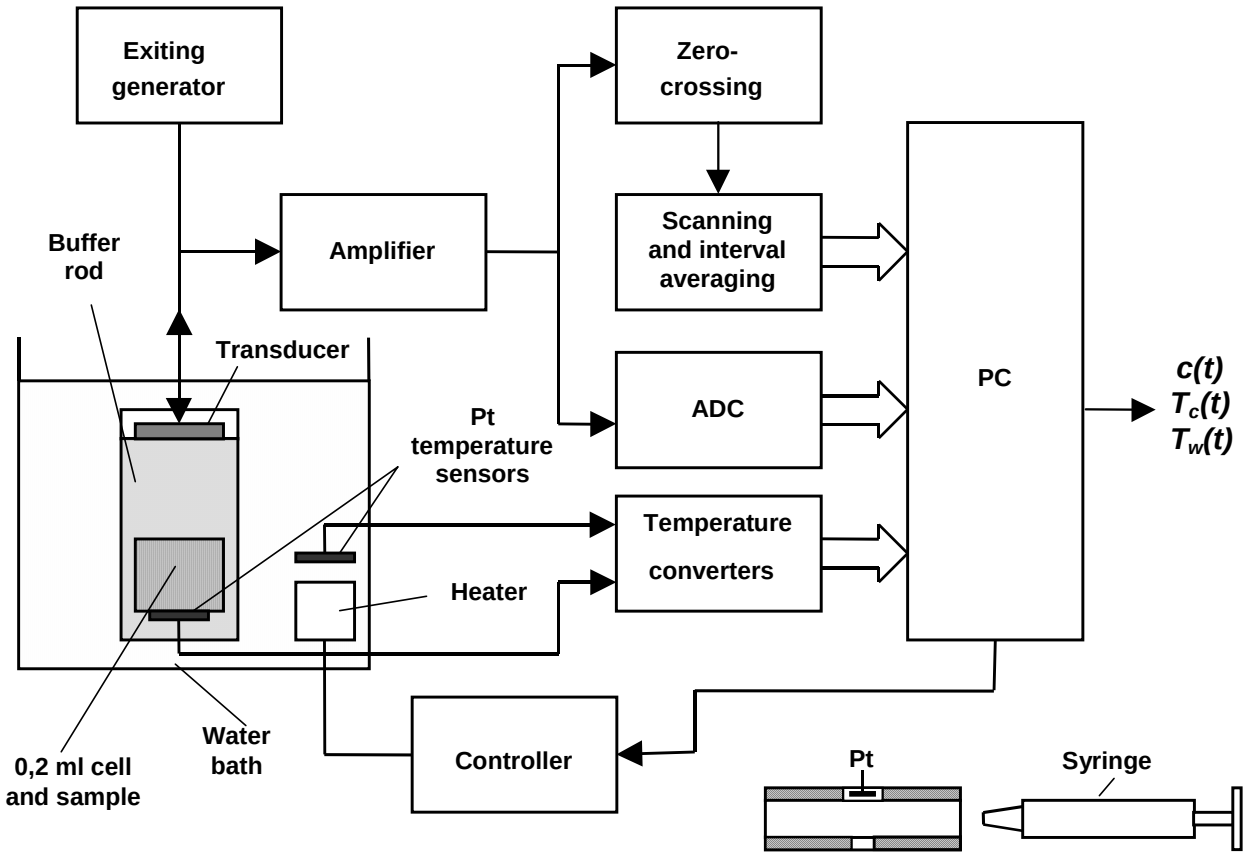

Fig. 1**. The block diagram of the instrument**

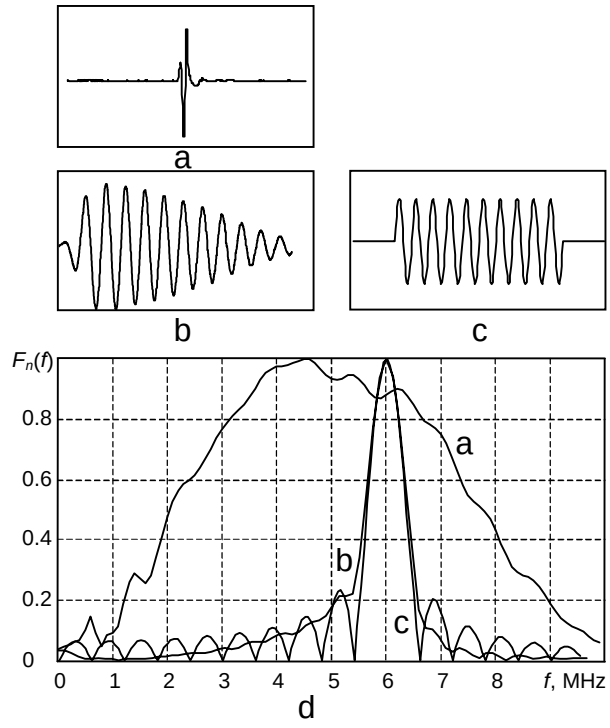

Fig. 2**. The signals used in the experiment and their spectra**

Narrow band multiperiod ultrasonic signals were converted into a digital form for easy and flexible signal processing made after the experiment.

Exciting generator produces nearly rectangular short pulses with variable width or a train of bursts (e.g. 5 MHz, etc.). Pt thermistor in a water bath has sensitivity 0.01 K, and in the cell 0.002 K. In the case of blood coagulation studies the temperature of the syringe is kept constant at 37°C before the blood is taken from the vein.

Experimental information is stored and processed by the Pentium type PC. Ultrasound velocity in a sample c as well as the temperature in cell T_c and in water T_w are displayed as they changes in the experiment. The sensitivity of ultrasound velocity measurements is estimated ±3 cm/s, by order of magnitude, and applying filtration of the results the coagulation curve may be smoothed. The accuracy is somewhat worse.

Results and discussion

In order to determine the effective cell length the first experiments must be carried out with non-dispersive reference liquid. For this purpose distilled water was used, and the length is defined from the measured pulse propagation time and known ultrasound velocity [3]. The effective length L=4.943 mm is a cell constant and is used in all successive measurements. Due to such a procedure diffraction effects are nearly eliminated, as ultrasound velocity in biological media is in principle close to that of water, and the buffer rod is of such a length that the cell volume is beyond the near field of the transducer.

Measurements in the reference liquid showed a long-term stability of the instrumentation. Native human blood is a particular object in biological investigations as it is

non-stationary, during the coagulation process changes its structure, and ultrasound pulse waves must respond to it. The effect was confirmed by a detailed investigation of the process with continuous standing ultrasonic waves, applying constant base interferometric method, but it is conservative and not flexible [4].

Alternative known methods based on electrical conductivity or optical absorption changes of the blood during the clot formation are not sensitive enough. Various attempts to employ ultrasound backscattering effects, changes of ultrasonic wave phase, interaction of the motion of spherical glass particles in ultrasonic field with the fluid undergoing coagulation doesn′t solve the problem as well [5].

The pulse method enables to vary ultrasound signals and investigate how they reflect the non-stationary process. The first experiments were provided with wide band signals. Ultrasound velocity changes in native human coagulating blood taken from the vein are presented in Fig.3.

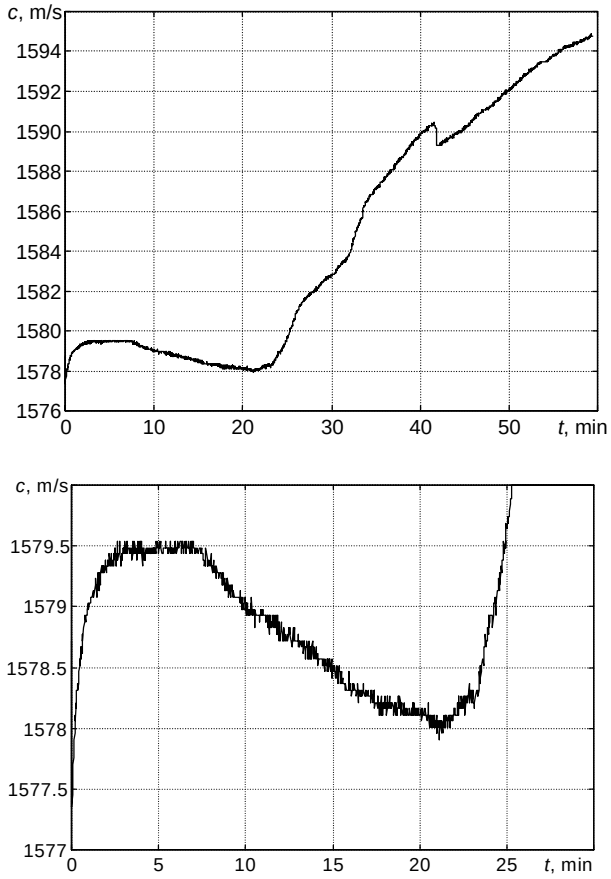

Fig.3. **Ultrasound velocity changes in coagulating blood registered with wide band signals without the temperature compensation; a-the full process, b-the initial most informative stage**

Short pulses being used, the method has a high resolution. The coagulation curve reflects the four different stages of the process [4]:

1-beginning of the clotting,
2-beginning of the latent retraction,
3-beginning of the main retraction,
4-the end of the retraction (up to 1 h).

Blood and its coagulation process is characterised by the starting times of different stages and their intensities (ultrasound velocity differences). The first two minutes after the cell is filled with blood, but no more, ultrasound velocity reflects not only the structural changes of the blood but the temperature stabilisation process of the sample as well (till T). Thus, the temperature effects can disturb the registration of the beginning of the clotting. The compensation of the temperature – dependent variations of ultrasound velocity is not so simple as it may seem. As a result of detailed investigation of the problem with a reference liquid it was noticed that there is a small systematic difference between the temperature as it was registered by propagating acoustic wave and the temperature registration with the thermistor probe. Acoustic waves are sensitive to the temperature of the media in the whole volume of the cell while thermistor probe only to the thin layer to it surface.

In this experiment the results are shown without the temperature compensation, as it stabilises before the beginning of the clotting. The peak in ultrasonic velocity curve approximately at 42-th minute is due to the separation of the clot from the cell walls. The same effect as well as the clot thickness is observed in A-scan of informative pulses (Fig. 4.).

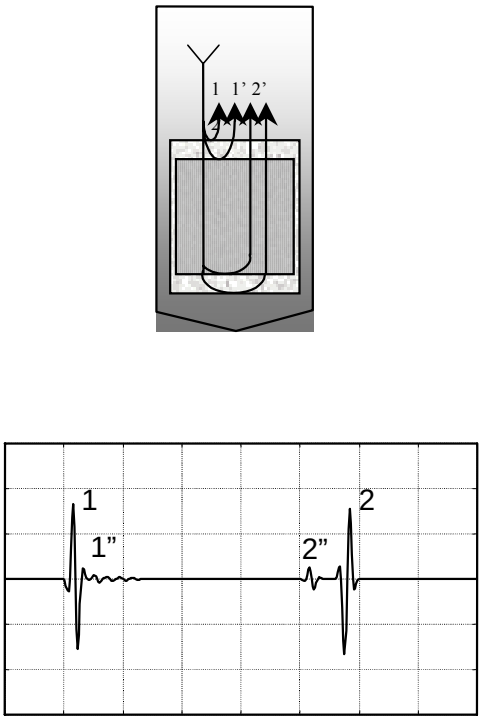

Fig. 4. **Additional reflections of short ultrasonic pulse from the clot separated from the cell walls**

As it is known a great care must be taken in velocity measurements using pulsed sine waves. Usually in such experiments phase velocity must be determined and because of a distortion that pulsed signals undergo in transmission through dispersive and atenuative media there is often a question as a what velocity a given measurement determines. Measurements of transit times of certain individual waves of the pulse at two positions give a phase velocity if they are close enough together and the

attenuation is low. Such a situation is in our case, as the cell length is only 5 mm and the attenuation loss is negligible; typical value for blood is 0.07 Np at 15 MHz for a propagation path of 10 mm [6].

Coagulation of blood is a complicated process and has a very important physiologic role. The coagulation process as well as blood acoustic properties are specific for every person and are affected by drugs that are used in the management of diseases and by some hereditary and acquired pathologies. Numerous experiments with different patients revealed the existence of common regularities of the process (4 stages), but in each separate case individual peculiarities were observed. Besides that the two successive coagulation curves of the same person never have been identical, they had certain differences reflecting different state of health. The most problematic was revealing of the beginning of the clotting, as it is weakly expressed.

The above-mentioned experiments were provided using modified time averaging technique.

Narrow band signals, shock excited or a train of bursts, were digitised and processed by PC. If the zero-crossing moments of the first individual waves of the informative pulses were taken, the similar dependencies of ultrasound velocity on time in coagulating blood were observed. Precise comparison of the results is difficult because there are no reference coagulating fluids, as in common ultrasound velocity measurements the reference liquid being degassed distilled water. Various individual waves of the shock excited pulse reflect the coagulation process differently, and the family of coagulation curves determined from successive zero-crossings is represented in Fig.5.

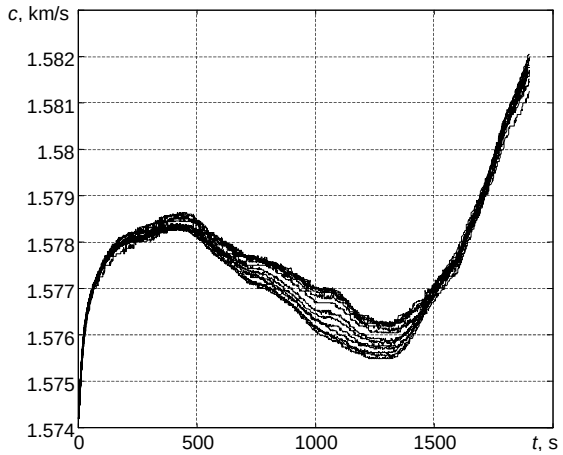

Fig. 5. **Family of coagulation curves obtained from successive zero-crossings of shock excited narrow band signals**

The character of separation of the curves obtained with a given signal may give an additional information about the process. This effect is observed due to the ultrasound velocity dispersion in coagulating blood. As the same pulse is propagating through the water sample of decreasing temperature simulating the velocity variations all individual waves of the pulse reflect the process identical, and there is no separation of the curves (Fig. 6).

The observed effect is due to the variation of the pulse length as it propagates in a dispersive media. The character

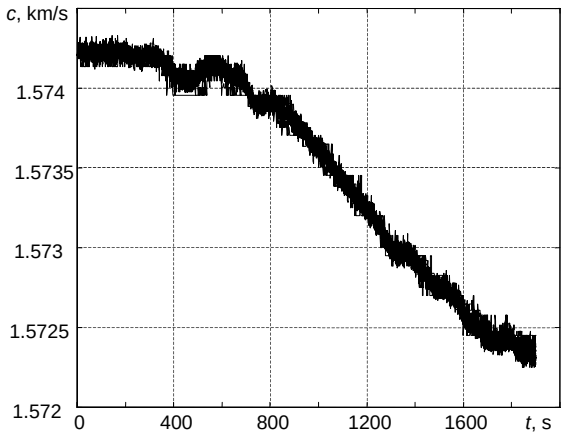

Fig. 6 **All individual waves of shock excited pulse reflect the varying ultrasound velocity in non dispersive media identical**

of this variation is identical with the ultrasound velocity variations and in principle reflects the coagulating process as well (Fig. 7).

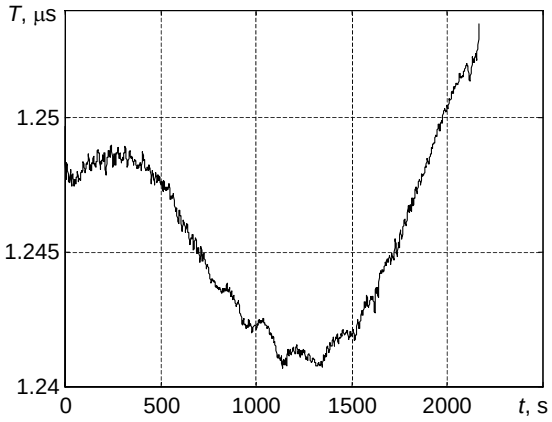

Fig.7. **Ultrasonic pulse length variations as it is propagating in coagulating blood (1-16 zero-crossings)**

If a narrow band pulse is a tone burst with the rectangular envelope it propagates in a dispersive media without changing its length, and all coagulation curves are identical. They are shown in Fig. 8. The lowest curve in the family is related to the velocity axis and the rest are moved away for a clarity.

It is feeled that a certain influence on the results may have the cell inclination about horizontal as the red blood cells tend to settle due to the gravity and they will experience a radiation force in ultrasonic field. The cell must be of a closed volume otherwise there is a problem of getting repeatable results. Such a case was observed when an open cell was situated in a chemical tube with coagulating blood.

Conclusions

The method proposed is sensitive and suitable for non-stationary biological fluids, e.g. for blood coagulation studies in the laboratory as it reveals the main stages of the clot formation not depending of the pulse type. It can be used to assess the coagulation characteristics in a clinical situation as well.

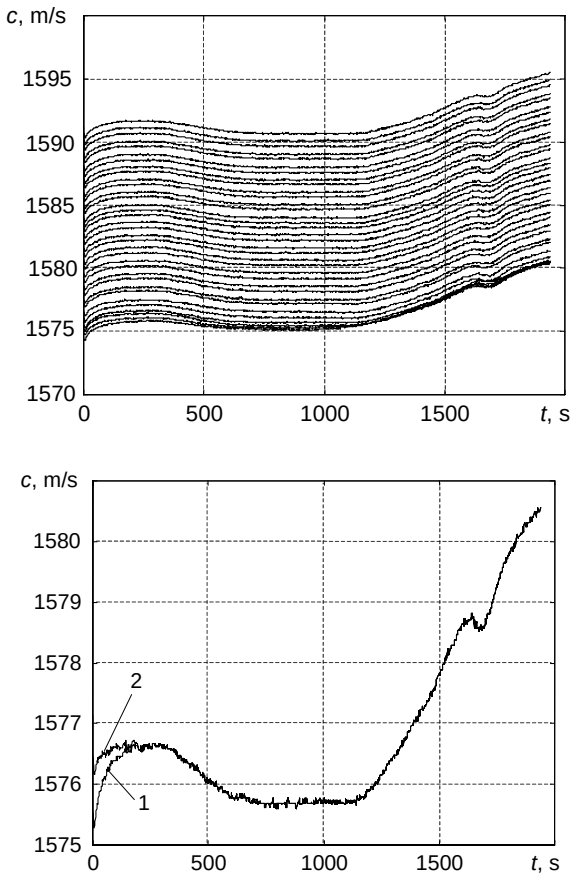

Fig. 8. **Coagulation curves as they are got with a tone burst signal; a-the family of the curves, b-the lowest curve without (1) and with (2) the temperature compensation**

Acknowledgements

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Nestacionarių biologinių terpių tyrimo metodas

Reziumė

Pasiūlytas ir ištirtas ultragarsinis impulsinis nestacionarių biologinių terpių tyrimo metodas. Metodo specifika – maži terpės tūriai (0.2 ml), geras skiriamumas (±3 cm/s). Nagrinėjama, kaip terpės parametrų dinamika veikia įvairių spektrų ultragarsinius signalus. Parodoma, kad metodas tinkamas kraujo krešėjimo proceso analizei ir gali būti taikomas klinikinėje praktikoje.