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Acoustical method and device for determination of lipid and protein spectra of blood serum

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New acoustic methods of determining total protein, protein fractions and lipid components of the human blood serum are presented. Acoustic methods are based on high-precision measurements of velocity temperature dependences and frequency and temperature dependences of ultrasound absorption. Acoustic characteristics of the blood serum were measured using the method of a fixed length interferometer in acoustic cells ~ 80 mcl in volume in the temperature range from 15 to 40 °C and the 4 – 9 MHz frequency range with the acoustic analyzer developed by BIOM company.

1 Introduction

Functional and pathological changes of tissues and liquids of the organism are accompanied by changes in their biochemical composition. These changes can be detected by means of ultrasound velocity and absorption measurements. Development of the new techniques providing precise measurements of ultrasound velocity and absorption in small volumes (0.1 cc) of liquids [1, 2] allowed to make systematic investigations of the solution of proteins, amino acids and other biological substances [3]. Such investigations provide necessary data to study dependences of acoustic properties of important biological liquids such as, for example, blood serum, gastric juice, on their composition.

The paper presents the results of systematic studies of acoustic characteristics of the human blood serum (in norm and in case of several diseases) which underlie a novel method of determining blood serum composition. At that, contrary to biochemical methods acoustic studies allow determining blood serum composition without expensive reagents which, what is more, are often hazardous for the health of the laboratory assistant. The results of comparative tests for conventional and acoustic methods at leading Russian medical centers are given. Based on these tests acoustic methods are recommended for application at clinical-diagnostic treatment-and-prophylactic establishments.

2 The principles of the functioning of the acoustic analyzer

To measure acoustic characteristics of the blood serum , the method of the interferometer of constant length or the resonator method was used. General view of acoustic cell is shown in Fig. 1.

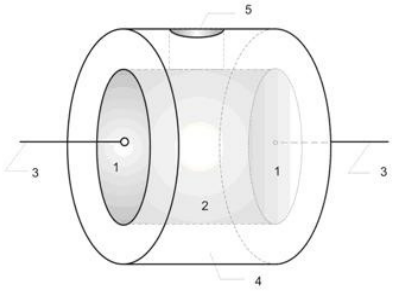

Figure 1:. General view of acoustic cell. 1-piezoelectric transducers, 2- the chamber for the sample,3-conductors, 4 – case of acoustic cell, 5 – aperture for filling up of the sample

The volume of the acoustic cells is 80 microliters. The cells are placed in a specially designed ultrathermostat providing +/- 0.005^0C temperature stability. The precision of measurement of the velocity of the ultrasound in the blood serum is $3x10^{-5}$ (3 thousandth of percent) and the coefficient of ultrasound absorption is determined with the precision of 2 percent.

Fig. 2 presents a block-diagram of differential acoustic analyzer comprising two independent channels of measurement. Let us note that every channel includes a block of acoustic thermostatted cells 6, (7), phase-sensitive circuits 4 (5), which are generators, controlled by the voltage, with a chain of phase auto-adjustment of the frequency. The alteration of the frequency of the generators is performed by the module of control 9 through the digit-analogue transformer 3. The outlets of the phase-sensitive circuits 4 and 5 through the switch 2 are alternately connected to the inlet of the frequency meter 1. The power block 8 provides voltage to the nodes of the analyzer and contains in itself circuits of control of the thermostats of acoustic cells. Control module 9 contains the unit of connection with PC.

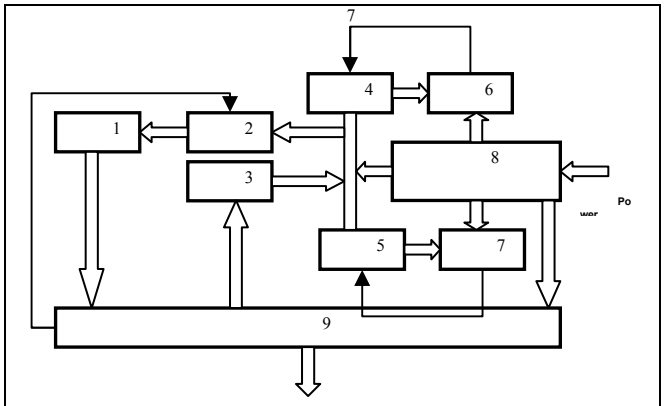

Figure 2: Simplified structural chart of the acoustic analyzer “BIOM”

Measurements start with a frequency sweep in the range from 5.0 to 10.0 MHz to assess all resonance peaks in the cells filled initially with distilled water and then with the tested fluid. Recorded data is used for calculating relative differences of resonance frequencies and the quality factor of the resonances in

the tested fluid relative to water. Measurements are conducted at two temperatures.

Calculated acoustic parameters include ultrasound velocity and absorption increments in the tested fluid relative to water, temperature and frequency slopes of the ultrasound velocity and absorption increments.

3 Ultrasonic assessment of the blood serum

Blood serum is a complicated biological liquid that contains a multitude of components, indispensable for the life of the human organism. The most important components of the blood serum are the globular proteins. Their contribution into the acoustic characteristics is the most significant (up to 90%) due to their high concentration in the blood serum (75 – 85 g/l) in the norm.

Various diseases are often accompanied by changes in the relative content of certain proteins in blood. Quantitative assessment of such changes may provide important diagnostic data.

The acoustic method of determining protein fractions in blood serum for diagnostic purposes is based on the measurement of the velocity of the ultrasound in the blood serum and in two modified sera, one of which does not contain γ-globulin, and the other – β- and γ-globulins. To determine the pro rata parts in the blood serum of C_{al} – albumin, $C_{\alpha 1}$ – α_1-globulin, $C_{\alpha 2}$ – α_2-globulin, C_β – β-globulin. C_γ – γ-globulin, the following system of linear equations is applied (1). This equations are derived under an assumption that the low-molecular components of the blood serum contribute insignificantly to the acoustic characteristics of serum.

$$\begin{aligned}
&Cal \times A_{al}^{T_1} + C_{\alpha_1} \times A_{\alpha_1}^{T_1} + C_{\alpha_2} \times A_{\alpha_2}^{T_1} + C_\beta \times A_\beta^{T_1} + C_\gamma \times A_\gamma^{T_1} = \varphi_1 \\
&Cal \times A_{al}^{T_2} + C_{\alpha_1} \times A_{\alpha_1}^{T_2} + C_{\alpha_2} \times A_{\alpha_2}^{T_2} + C_\beta \times A_\beta^{T_2} + C_\gamma \times A_\gamma^{T_2} = \varphi_2 \\
&Cal \times K_{al}^{T_1} + C_{\alpha_1} \times K_{\alpha_1}^{T_1} + C_{\alpha_2} \times K_{\alpha_2}^{T_1} + C_\beta \times K_\beta^{T_1} = \varphi_3 \\
&Cal \times K_{al}^{T_2} + C_{\alpha_1} \times K_{\alpha_1}^{T_2} + C_{\alpha_2} \times K_{\alpha_2}^{T_2} = \varphi_4 \\
&C_\gamma = 100\% - Cal - C_{\alpha_1} - C_{\alpha_2} - C_\beta
\end{aligned} \qquad (1)$$

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The relative changes of ultrasound velocity in blood serum at T_1 and T_2

$$\varphi_{1,2} = \frac{V_{1,2}^{(S)} - V_{1,2}^{(H_2O)}}{V_{1,2}^{(H_2O)}}$$

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The relative changes of ultrasound velocity in modified blood serum at T_1 and T_2

$$\varphi_{3,4} = \frac{V_{3,4}^{(mS)} - V_{1,2}^{(H_2O)}}{V_{1,2}^{(H_2O)}}$$

$$V^{(S)} = \frac{2lf_j^{(S)}}{j}$$

The methodology of obtaining the concentration coefficients of the velocity of the ultrasound to determine the protein fractions according to the system of equations shown above is based the results of acoustic measurements in solutions of both pure proteins (say, albumin, or γ-globulin), and proteins solutions, prepared out of blood serum through selective sedimentation of certain protein fractions.

Lipids do not dissolve in aqueous solutions, therefore they are incorporated in the lipoproteins in the blood serum. The structure and composition of lipoprotein is presented in Fig 3.

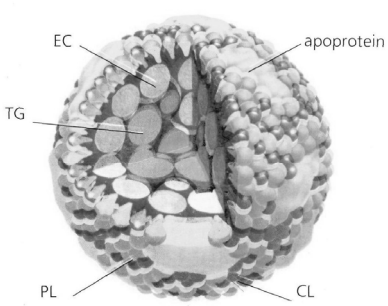

Figure 3: The composition of the lipoprotein. EC+CL – cholesterol, TG – triglycerides, PL – phospholipids.

The development of the reagent free acoustic method of determining lipid components of the human blood serum is based upon the measurement of the frequency dependences of the absorption of ultrasound (Fig. 4) and the temperature dependences of the velocity of ultrasound in the protein solutions and in the blood serum, containing a different amount of proteins and lipid components (Fig. 5).

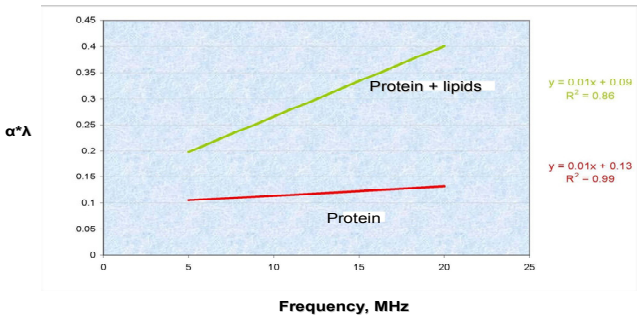

Figure 4: Frequency dependences of absorption coefficient.

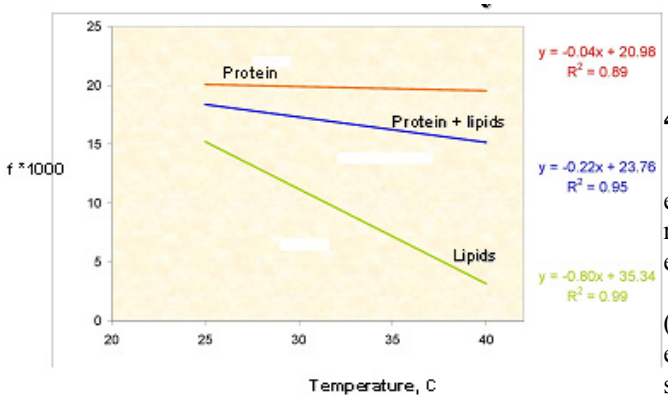

Figure.5: Temperature dependences of relative changes of ultrasound velocity.

Analysis of the results of measurement of blood serum with different concentration of lipid components allowed us to find out the exact correlations of acoustic characteristics (relative increments of ultrasound velocity and absorption) and the lipid composition of the serum. Under the assumption of additivity of the contribution of the separate lipid components into the temperature dependences of the ultrasound velocity in blood serum and in the frequency dependences of the absorption of the ultrasound, and also in the temperature dependences of the absorption of the ultrasound, one can determine the concentration of lipid components of the blood serum: total cholesterol, high density cholesterol, and triglycerides through the following system of equations (2).

$$\begin{aligned} C_{tot-Ch}(A_{tot-Ch}) + C_{HDL-Ch}(A_{HDL-Ch}) + C_{Tr}(A_{Tr}) &= \delta\varphi_T \\ C_{tot-Ch}(B_{tot-Ch}) + C_{HDL-Ch}(B_{HDL-Ch}) + C_{Tr}(B_{Tr}) &= \delta\xi_T \\ C_{tot-Ch}(D_{tot-Ch}) + C_{HDL-Ch}(D_{HDL-Ch}) + C_{Tr}(D_{Tr}) &= \delta\xi_f \end{aligned} \quad (2)$$

$$C_{LDL-Ch} = C_{tot-ch} - \left(C_{HDL-Ch} + {C_{tr}}/{2.2} \right)$$

$$\delta\xi_f = {(\xi_{f1} - \xi_{f2})}/{\Delta F} \qquad \delta\xi_T = {(\xi_{T1} - \xi_{T2})}/{\Delta T}$$

$$\delta\varphi_T = {(\varphi_1 - \varphi_2)}/{\Delta T}$$

The values of the concentration coefficients in the system of equations for determining lipid components of the blood serum are obtained by making acoustic measurements in test samples with known lipid content: blood serum of the company Human (Germany); the Serodos – the serum with reference values for the normal content of lipid components, and the Serodos plus – the serum with reference values of lipid components for various pathologies.

4 Results and Discussion

A comparison of the results of acoustic experiments with the protein spectrum was made in reference to the data obtained using the electrophoresis system “Paragon” (Beckman, USA).

When testing the analyzed serum samples (Human, Germany) in the acoustic device and by the electrophoresis method on the unit “Paragon” it was shown that differences for the albumin, and α_1-, α_2-, β- and γ-globulins were within the range indicated in the specifications for reference sera. While making comparative examinations of the blood serum for different groups of patients by the method of electrophoresis and the acoustic method, a high degree of correlation for content of albumin, α_1-, α_2- β-and γ-globulins was shown (r = 0.95, 0.75, 0.82, 0.7 and 0.92 respectively).

For the assessment of the accuracy of determination of lipid components, the serum samples “Serodos” of the company Human (Germany) were used. Comparative measurements of the lipid components in the blood serum by the acoustic method and the conventional biochemical methods: the biochemical analyzers Hitachi and Konelab, were conducted. In total, blood sera of more then 2000 patients were examined. The results of the comparative examinations showed that the correlation coefficients have the value from 0.77 to 0.89, which is sufficient for correct diagnostics of the failure of lipid exchange.

5 Conclusion

Performed systematic study of the acoustic characteristics of the blood serum enabled to develop new acoustic methods of determining protein fractions and the lipid components of the human blood serum. These methods do not require costly equipment or biochemical reagents, but at the same time they have good correlation with the conventional methods. The duration of the acoustic analysis of protein fractions and the lipid components of the blood serum is reduced to 5 minutes, while using the traditional methods, all those components can be determined in no less than 1 hour with the use of sophisticated biochemical analyzers and a device for the electrophoresis of proteins.

Acknowledgments

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References

[1] F. Eggers, Th. Funck "Ultrasonic measurements with milliliter liquids samples in the 0.5–100 MHz range", *Rev. Sei. Instr.* .44, 969–978 (1973)

[2] A.V. Klemina, I.Yu. Demin, V.A. Klemin. "The investigation of the very small volume acoustic resonator for medical and biological applications" *Vestnik NNGU*, 4, 59-66 (2006). (In Russian)

[3] A. Sarvasyan " Ultrasonic velocimetry of biological compounds", *Annu. Rev. Biophys. Biophys. Chem*. 20, 321–342(1991)

[4] E.L. Carstensen, K. Li , H.P. Schwan "Determination of acoustic properties of blood and its components", *J. Acoust. Soc. Am.* 25, 286–289 (1953)