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Non-destructive method of blood serum composition analysis in forensic science

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Abstract. The article is devoted to consideration of non-destructive methods of blood serum composition analysis in forensics, problem solving and their improvement. At the current stage of creating materials and devices of a new generation, the task is not only to manage their properties, but also to effectively use them in various fields, in particular, in forensics. Analysis of the structural evolution of a heteroatomic liquid (in particular, blood) from the standpoint of theoretical and experimental approaches allows us to open new horizons in the practical plane, to determine the phenomenology of the process based on modern research methods. The authors of this work conducted a qualitative and quantitative analysis of experimental data related to the kinetics and mechanisms of structure formation of a multicomponent substance in the liquid state. It should be emphasized that the structural kinetics of a liquid such as blood serum, or blood itself, is a complex multistage process.

Keywords. Microscopic studies, kinetics, blood serum, physics, chemistry, properties.

Statement of the problem. At the present stage of creation of new generation materials and devices, the task is not only to control their properties, but also to use them effectively in various fields, in particular, in forensic science. The purpose of the article is to develop and use in the practice of forensic analysis an effective non-destructive technique that allows analyzing the structure of liquids, in particular, blood serum. The goal was realized by solving interrelated problems, namely: to improve the experimental technique based on the quartz weighing method described in works [1-3]; to identify the structure of a blood drop, to find out its topology; to analyze the physicochemical properties and morphology of the substance. Based on this, we defined the subject and object of the study. The object of the study is the process of studying the properties of a liquid (blood) by quartz weighing. The subject of the study is a multicomponent liquid (blood serum, patient's blood).

Structural evolution of a multicomponent liquid. The analysis of the structural evolution of heteroatomic liquids (in particular, blood) from the standpoint of theoretical and experimental approaches allows us to open new horizons in the practical plane, to determine the phenomenology of

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the process on the basis of modern research methods [3]. The authors of this paper have conducted a qualitative and quantitative analysis of experimental data concerning the kinetics and mechanisms of structure formation of a multicomponent substance in the liquid state. It should be emphasized that the structural kinetics of a liquid, such as blood serum or blood itself, is a complex multistage process. The latter can be divided into two stages: events occurring during the evaporation of free water, and structure formation itself. The last stage is the structure formation stage associated with water evaporation. As our experiments (2004-2015) show, a liquid that dries on a solid wettable substrate (under room ambient conditions) acquires a specific appearance (Fig. 1). Similar results were obtained in other laboratories [4], one of which is shown in Fig. 2. The reason for this is a complex of complex physical, chemical, and mechanical processes, and is defined as dehydration self-organization [5].

The kinetic characteristics of a liquid that dries on a substrate reflect the morphological state of the object to which it belongs. For example, if we analyze the biological fluid of the human body, the interpretation of the results allows us to use the phenomenon as an additional criterion not only in functional electronics [3], but also in biomedical diagnostics [6] and, most importantly, in forensics.

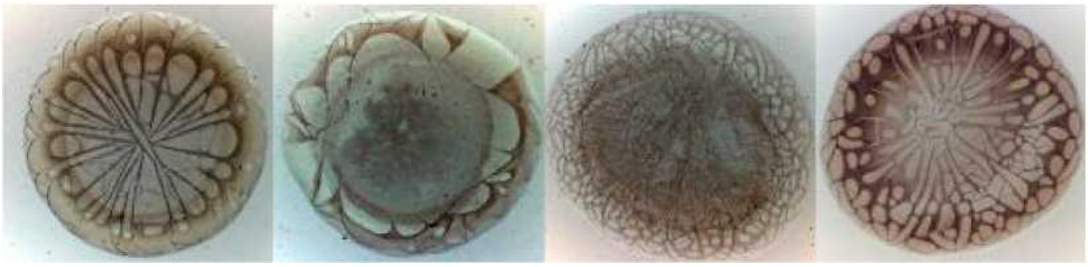

Figure 1. Dried serum drops [4]; 28 X magnification: a) practically healthy person; b), c), d) people with different types of diseases

Figure 2. Dried serum drops from patients with various diseases. Different structures [4]: a) tongues; b) foci and nuclei; c) plaques and wrinkles; d) spiral cracks

Based on the results of the study of the stages of nonlinear processes occurring in such systems using materials science methods [3,7], it is important to identify and consider the mechanisms that determine and have features of nonlinear processes at each stage of spatial and temporal structure formation in a multicomponent liquid such as blood serum. Our results allow us to note the following. The multistage

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process of drying a drop of blood serum on a rigid substrate occurs in two stages: 1) evaporation of free water, which lasts 20-35 minutes (for drops with a volume of 3-5 ml); 2) further evaporation of water, which lasts 2-3 days; 3) evaporation of water is accompanied by an increase in deformation and the formation of additional cracks, which form the final morphological appearance of the drying film suitable for microscopic studies.

Among the mechanisms responsible for the formation of morphological features of droplets at both the first (liquid) and second (solid) stages of drying, the following can be distinguished: a) interaction of the droplet with the substrate; b) distribution of components in the droplet with different surface activities; c) phase transitions in the droplet. At the beginning of the drying of a liquid droplet on a solid substrate, the colloidal phase is carried to the periphery, and the ratio of components in the liquid (inner) part of the droplet changes. As a result of water evaporation, the ionic strength of the solution increases, and the volume fraction of the colloidal phase decreases due to its removal to the periphery. The forces of attraction between particles increase due to a decrease in the Debye radius and an increase in the density of surface charges. The radius of interaction between particles decreases. The colloidal phase gradually loses its hydrate shells, the charge of the molecules approaches the isoelectric point, and the solution turns into a metastable state, followed by coagulation, as illustrated in Fig. 3. Colloidal particles can form different structures - from colloidal glass, with a very high volume fraction of colloid and a weak force of interaction between particles, to colloidal gels, with a very small volume fraction of colloid and a large force of attraction between particles.

Before a gel is formed, colloidal particles form fractal clusters, which subsequently combine into a spatial lattice - a gel. Any deviation from the fractal growth of nanoclusters (a detailed analysis of such systems was carried out by one of the authors, see monograph [3]) leads to a violation of gel formation. Given the existing relationship between salt concentration and pH value in protein solutions, it is expected that the shift in the isoelectric point of albumin when it is loaded causes a change in the coagulation kinetics.

Methods for analyzing the kinetic self-organization of a liquid. The process of self-organization of a drying liquid makes it technically possible to record the dynamics of this process. These processes can be coupled. Based on this, we propose a technology that opens up new prospects for the study of liquid media, in particular blood serum, as well as for the development of a number of practical applications based on it in forensics. Let us consider a method of analyzing multicomponent liquids based on a sensor device. The main feature of this method is to obtain electronic signatures of liquids (blood serum) suitable for their identification, certification and certification. The informational basis of the method is the dynamics of complex processes of self-organization of drying droplets, which is critical to the composition and structure of the liquid. The registration of these dynamics in the form of acoustic-mechanical impedance (AMI) allows to obtain quantitative differences between the compared liquids, which can be used to control their quality by comparing them with a standard.

To analyze the self-organization of liquid droplets, a resonator in the form of a quartz plate of xys/1030′ cut with a size of 48.0-4.5-1.2 (mm) was proposed. The device scheme and the resonator vibration mode are shown in Fig. 3. The resonator performs longitudinal oscillations of the compression-expansion type.

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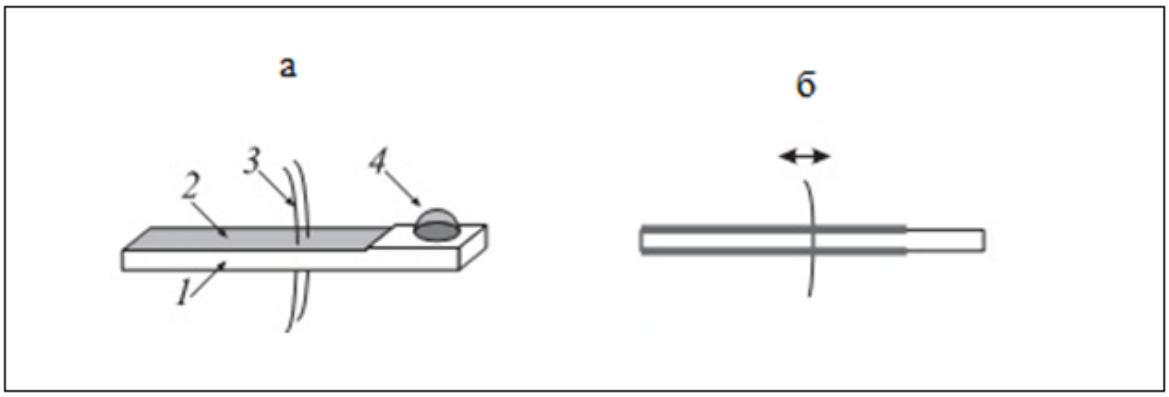

Figure 3.
Oscillation device of a quartz resonator. a - resonator with a drop of the investigated liquid, 1 - quartz plate, 2 - metal spraying, 3 - supporting conductors, 4 - drop of the investigated liquid, b - Distribution of the amplitude of longitudinal vibrations.

In the process of measuring the operating vibration frequency of the quartz plate, it is forced to maintain constant and equal to the resonant frequency of the unloaded resonator - 60 kHz. The vibration amplitude of the unloaded resonator is sinusoidally distributed along the length of the plate. A drop of the liquid under study is placed near the end of the plate. Estimates have shown that the distribution of the vibration amplitude along the length of the plate practically does not change when the resonator surface interacts with the object (drop). In other words, the drying droplet does not introduce errors into the distribution of the oscillatory velocity along the length of the resonator. The measured value is the complex electrical conductivity of the resonator loaded with the drop, while the resonator's own capacitance and the capacitance of the connecting cable are subtracted by the bridge circuit of the device. The AMI value of a drop of liquid (blood serum) is calculated from the measured electrical conductivity and displayed on the screen in real time. At the stage of droplet evolution, the AMI modulus value is measured, displayed and recorded.

AMI is the value of the acoustic or mechanical impedance of an object that loads quartz in the irrigation oscillation mode. In our case, we study such a specific object as a drop of liquid (blood serum) drying on the surface of quartz (substrate). Therefore, it can be assumed that AMI (A) integrally includes such physical characteristics of the object as viscosity, elasticity, friction, and mass with varying degrees of adhesion to the substrate using a ratio:

$$A = C_1(1+i)S + 2\pi f\eta\rho + C_2 \cdot 2\pi f m + C_3 \cdot \frac{k}{i \cdot 2\pi f} + C_4 k_T \qquad (1)$$

where
i is an imaginary unit;
S is the area of contact between the fluid and quartz;
f is the frequency of quartz oscillations;
η, ρ are the viscosity and density of the fluid at the initial stage, respectively;
m, k are the mass and elasticity of the fluid, respectively;
k_T is the coefficient of friction of the fluid on the surface of the substrate.

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The first term in formula (1) describes the value of the acoustic impedance of a viscous fluid tangent to the substrate over an area S; the second term is the value of the mechanical impedance of mass m oscillating with the substrate; the third term is the value of the acoustic or mechanical impedance of the load in the form of an elastic element; the fourth term is the value of the acoustic or mechanical impedance of dissipative losses (friction).

Each term has its own weighting factor, denoted by the letter C. These coefficients initially have different values for different liquids and change in different ways during the drying process, depending on the properties of the liquid, its composition and structure. This is what introduces the specific physical and chemical characteristics of the liquid, which contributes to the dynamics of AMI. At the beginning of the process, as long as the percentage of water in the liquid is high, the AMI is proportional to the characteristic viscous wave resistance, which allows us to write the equation:

$$A_0 = C_1(1+i)S + 2 \cdot \pi f \eta \rho \tag{2}$$

Where
A_0 is the AMI value at the beginning of the liquid drying process.

The values of η ~ 10- 2 Pa and ρ ~ 103 kg/m3 were chosen for the studied liquids. Under these conditions, the height of the liquid on the substrate did not affect the AMI value, since at the selected frequency the penetration depth of the viscous wave was of the order of 10μ. Therefore, at the beginning of the drying process, it can be assumed that the coefficient C1= 1 in formula (1), and the other weighting coefficients are equal to zero: C2= C3= C4= 0. At the end of the liquid drying process, a gel-like or even solid residue remains on the surface of the resonator. The resonator is loaded by the impedance of the mass of the mend residue. This can be analytically described by the following relation:

$$A_{end} = H_2 f \cdot 2\pi f m_{end} \tag{3}$$

where
A_{end} is the value of AMI at the end of the drying process; mend is the mass of dry residue.
Thus, the end of the drying process allows us to assume that the weighting factor $C_2 = 1$, and the other weighting factors are equal to zero: $C_1 = C_3 = C_4 = 0$.

The correspondence of the AMI to expressions (2) and (3) at the beginning and at the end of the drying process was experimentally verified by measuring the real and imaginary parts of the resonator electrical conductivity (or by the results of measurements of the real and imaginary parts of the AMI).
The results of the measurements proved that the initial and final values of the AMI can usually be described by expressions (2) and (3). However, such simple situations as "liquid sample at the beginning" and "solid residue at the end" do not always occur. For example, at the beginning of drying, some liquids have an AMI with unequal real and imaginary parts (in contrast to the situation described by (2)). Or, at the end of drying, some liquids have AMI with a non-zero real part (as opposed to the analytical relation (3)). Therefore, we believe that the decisive factor when using the AMI measurement in the shear vibration mode is the extreme sensitivity of its value to the emergence and growth of a new phase at the liquid-quartz interface, as well as to the acoustic and mechanical properties (e.g., elasticity) of the structures (solid or helium residues) that are formed.

Structural analysis of a liquid on a sensor. The kinetics of a liquid drying on a solid wettable substrate is a natural model of a self-organizing system with an infinite variety of process variants, depending on the composition and structure of the liquid. The drying process is determined by the initial parameters of the solution: surface tension, wetting, viscosity, internal structure, dispersion, thermal conductivity,

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ionic strength, pH. These factors affect processes such as aggregation, precipitation, sedimentation, gelation, and crystallization that accompany the drying process of a multicomponent liquid [4]. As a result, the physical properties of the liquid change, the dynamics of which is reflected in the form of a curve in the AMI-time coordinates. Our research has shown that the shape of the AMI curve is a passport characteristic of the liquid under investigation, such an important component in forensics as blood serum. An important, clearly controlled parameter of the liquid in our technology is its volume. It is this parameter that must be the same for the liquids being compared.

The liquid is deposited by means of a microscopic dispenser and its guide arms at a specific location on the quartz plate from a low height, which prevents splashing. The spreading area is not limited, as it reflects the degree of wetting of the substrate and is one of the important characteristics of the liquid. Therefore, a uniform surface quality of the sensor is critical for this technology. Before and after the measurement, the sensor surface is successively treated with water and isopropyl alcohol, and then thoroughly dried. This procedure for treating the quartz surface allows for highly reproducible results when repeatedly measuring the same liquid. Thus, the parameter that ensures the informativeness of the proposed technology is the wetting of the sensor surface. With the same volume of samples of the compared liquids and different degrees of wetting, the droplets of these liquids will have different shapes: one will be flatter, with a larger base area, and the other will be more convex, i.e., with a smaller base area. This will certainly provide a difference in both the initial value of the signal (2) and the dynamics of liquid structuring during the drying process.

Another important parameter affecting the dynamics of AMI is the physical properties of the adsorption layers. It has been experimentally proven that in a multicomponent liquid there is a redistribution of liquid phase components in accordance with their surface activity. The concentration and qualitative composition of surfactants that form the adsorption layer at the air interface determine the mechanical properties of the liquid, such as elasticity and density, limiting water evaporation to a different extent, and thus its structuring. The surfactant also reduces the surface tension at the liquid-substrate interface and reduces the rate of gel formation in the colloidal phase due to the formation of a structural and mechanical barrier. This is what ensures the occurrence of pressure during the formation of blood nanoclusters. These processes contribute to the dynamics of the AMI signal, since the size of nanoclusters significantly affects the evolution of structure formation in the drying liquid. The self-organization processes in the drying droplets of multicomponent liquids reflected by the AMI signal are completely reversible and well reproducible.

Below are the results of the practical application of the described methodology. However, it should be noted that the model description of the processes occurring in a liquid during drying is still an extremely technologically challenging task.

Results of quantitative analysis that can be used in forensic science. Fig. 4 shows the stages of drying of blood serum from a practically healthy donor and the corresponding sections of the AMI curve obtained by the authors of [4]. The section of the curve on the right reaches saturation and retains it due to the constant mass and sufficient elasticity of the formed gel with loosely bound water. It is this curve that should be considered the reference curve for quantitative analysis of the liquid structure. For example, Fig. 6 shows typical AMI curves of blood serum from individuals who died of drug and alcohol overdose compared to the serum of a healthy donor. The high content of drug metabolites in the blood inhibits gel formation in the drying serum droplets, making them extremely fragile. Even small compressive and tensile stresses on the quartz lead to intense cracking of the remaining droplets and detachment of their fragments from the sensor surface. This chaotically changing mechanical stress is shown on the display in the form of unsettled zigzag lines. The AMI curves of people who died from alcohol intoxication differ from the curves shown in Fig. 5.

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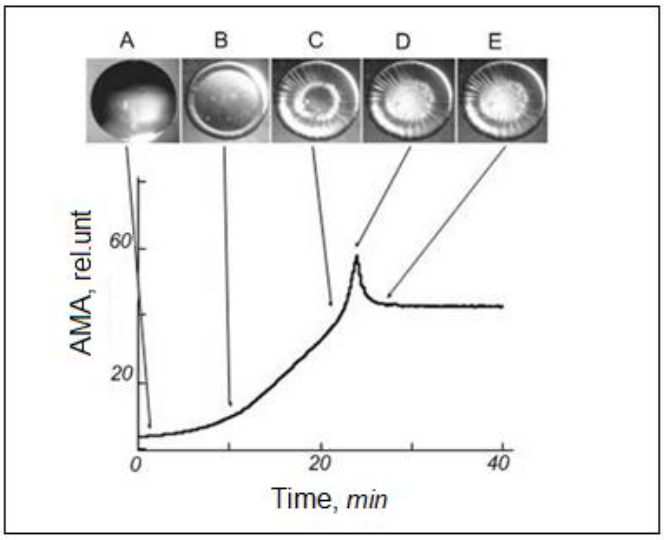

Figure 4.
The process of self-organization of a blood serum drop [4].
A-E - stages of drying of the donor's blood serum droplet (5 µl) and the corresponding parts of the AMI curve (bottom); A-B - formation of a protein roller along the periphery and flattening of the drop dome; B-C - gel formation; C-D - the process of salt crystallization in the gel matrix;
D-E - evaporation of the remaining free water and achievement of saturation of the AMI signal. This level reflects the relationship between the droplet mass and loosely bound water and the adhesion strength of the precipitate to the quartz surface.

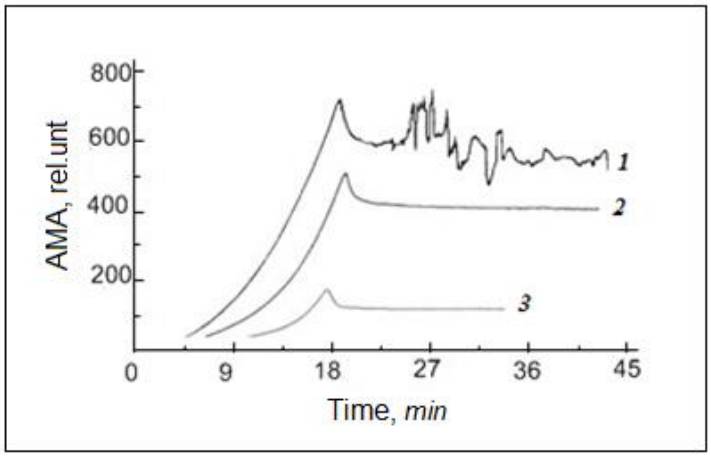

Figure 5.
Dynamics of AMI of drying droplets in the blood serum of a person who died from a drug overdose (1); a practically healthy donor (2); a person who died from alcohol intoxication (3).

Thus, the study of the kinetics of a liquid drying on the surface of an oscillating quartz resonator, due to controlled physicochemical parameters that integrally affect the shape of the AMI curve, provides information about the liquid that is sufficient for its identification.

Thus, our proposed method for recording the effect of a number of physical factors (magnetic field of UV radiation, odors, X-rays) on a liquid opens up the possibility of controlling the quality of wines, juices, milk, and other liquids.

Among other things, the identification technology we describe can be a convenient tool for detecting counterfeit medicines using an express method, as well as for one-step determination of the Ratio, the ratio of the concentration of soluble solids to acidity, the main indicator of juice quality used in expert laboratories.

Conclusions. The results of the research described in this article are systematized as follows: analyzed the method of quartz weighing; the structure of blood serum was identified; the topology of the multicomponent fluid was determined; analyzed the physical and chemical properties of the liquid.

The tasks we solved made it possible to determine the morphology of the liquid material, and thus the multicomponent substance. The method used to analyze the morphology of multicomponent liquids is based on the use of a sensor device, a characteristic feature of which is the acquisition of electronic signatures of liquids suitable for their identification and certification. The informational basis of our proposed method is the dynamics of complex processes of self-organization of drying droplets, which is critical to the composition and structure of the liquid. Recording this dynamics and expressing it in the form of AMI curves allows us to obtain quantitative differences between the compared liquids, which can be used to control their quality by comparing them with a standard.

It should be noted that the structural evolution of drying droplets of biological liquids is a complex multistage process in which two stages can be conditionally distinguished: events occurring during the evaporation of free water and structure formation associated with water evaporation. The stages of structural formation of the first stage are: interaction of the liquid with the substrate - under the condition of wetting, formation of an attachment line to the substrate and development of centrifugal flow of a capillary nature; formation of a glassy layer on the periphery of the drop; distribution of dissolved components in accordance with their surface properties and formation of adsorption layers along the interface; a cascade of phase transitions; crystallization of salt in the gel matrix.

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