The Study of the Coagulation Process in Droplet Samples of Bioliquids

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Statement of the problem and a description of the used method

The process of selection and training of astronauts involves performing a variety of medical tests. These tests include some coagulation tests. The study of blood clotting is of great scientific and practical importance, since any violations of this process are the result of various diseases and pathological conditions. Due to the fact that blood clotting is a multistage, multicomponent process, which involves a large number of factors, obtaining complete information about it has great complexity. Consequently, the chosen methods shall provide information about the process of clotting as a whole, and on the activity of the particular elements of the hemostatic system. Despite the large number of existing methods for the study of the hemostatic system, the search for the best and most informative methods, as well as improvement of the instrument base for coagulometric tests, have not lost their relevance.

We have conducted studies to assess the possibility of carrying out coagulation tests using the developed method of photometric study of samples formed as lying drops (Fig. 1). This scheme of analysis of liquid media has been tested on a number of medical laboratory techniques.

Figure 1 - Scheme of implementation of the method of photometric study of droplet sample

The optical measuring system includes a radiation source which is located below the test sample and the radiation detector, which is arranged coaxially source above the drop [1]. This scheme of study assumes the dependence of the current of photometric channel on the optical properties of the sample, that determined by the physical and physico-chemical processes in it.

These processes include the process of coagulation, i.e. formation of fibrin threads, resulting in a clot of insoluble stable fibrin and a general clouding of the medium. Consequently, the light transmittance value is changed and accordingly, the voltage at the output of the photoelectronic transducer (PET). The magnitude of this change is related to the concentration of the respective plasma clotting factors, as well as the presence in the blood of inhibitors of these factors and anticoagulants (heparin, hirudin, etc.). The article presents the results of testing this system for conducting the clotting tests used in the study of hemostasis.
Methodology of research

In experimental studies we used the test to determine the prothrombin time. Prothrombin time is the time of formation fibrin clot in plasma by the addition of calcium chloride and standardized tissue thromboplastin. The result of the study (prothrombin time) is expressed in seconds, which is normally is 14-20 seconds. To determine the prothrombin time we used Tehplastin-test reagents kit of firm “Technology-Standard” (Barnaul).

Methodology for conducting the experiments was as follows. According to the standard described in the instructions for the kit were carried out preparation of reagents and blood for analysis. Then the reagents and analyzed blood plasma was mixed directly on the photometric cuvette in the ratio 2:1 (two parts of tehplastin and one part of the plasma). The analysed samples were formed as a lying drops of 21 µl with a base diameter of 5 mm. After adding the tehplastin to the plasma, photometry during one or two minutes was conducted immediately.

Analysis of the shape of the photometric curves

Since the drop is plano-convex lens and the mixed components are sufficiently transparent, this system has the property to focus radiation passing through. It was experimentally established that the greatest changes of the optical signal associated with processes of the fibrin clot formation is recorded at the position of a receiver in an optical focus [2].

The shape of the curves, recorded in the focus area of the drop (Fig. 2), differs from the typical curves, obtained by optical coagulometer, where there is only an increase of optical density. We have analyzed the processes of changing optical properties of a droplet sample during formation of a fibrin clot, and their influence on the shape of photometric curve.

![Figure 2 - The shape of the optical signal recorded in the optical focus of drops during the formation of a fibrin clot (normal plasma)](image)

At this stage of our research we conduct a theoretical analysis of the shape of the curves. Explanation of their form requires learning a large amount of material on the subject, but we can assume that the decrease in light transmission almost immediately after mixing the reagents is due to the turbidity of the medium because of the formation of fibrin clots. The minimum value of transmittance corresponds to prothrombin time. Further enlightenment is due to the retraction of the clot.

The influence of clotting time of the sample on the dynamics of the curves

To evaluate the possibility of applying our proposed method to determine time parameters characterizing the coagulation process, we have conducted studies with plasma samples having different time of the formation of a fibrin clot. Since we have not had the opportunity to collect blood from people with different indices of hemostasis, to obtain samples with different time of clot
formation, we used the blood plasma with the normal clotting time of 13-15 seconds. The increase in clotting time was achieved by reducing the coagulation factors by the plasma dilution with physiological saline in various ratios. This technique is adopted in the calibration of coagulometers. Plasma was diluted with saline in the ratios: 4:1, 2:1, 1:1. The clotting time of the samples was checked on coagulometer "APG-02-P". Time of fibrin clot formation in normal blood plasma was 13-15 seconds, the "pathological" (diluted) plasma in the ratio of 4:1 - 18-20 seconds, the ratio of 2:1 - 26-30 seconds, the ratio of 1:1 - 41-50 seconds. According to the method described above, we conducted a photometry study of samples during the process of formation of fibrin clot after adding tehplastin to the plasma.

Fig.3 shows the optical curves for samples with different coagulation time. As can be seen from the graphs, the values of coagulation time of test samples, practically coincides with the time positions of the minima in the experimental optical curves.

![Optical curves of the samples with different time of clot formation](image)

Thus, on the basis of experimental studies of plasma samples with different clotting time it was shown that as an indicator on the coagulation time of blood, can be used a temporary position of the minimum, as well as the rate of decline and the rise in the curve of light transmission obtained at the position of a sensor in optical focus of droplet samples.

**Conclusions**

Our experimental studies have shown the possibility of using the method of photometric study of droplet samples to obtain information about the time of blood coagulation during prothrombin test.

Our further research will focus on improving the sensitivity and reproducibility of the method on the basis of experimental and theoretical (modeling) selection of optimal parameters of a droplet sample (volume, the diameter of the drops, the ratio of the reagents), improvement of the optical system and the design of the measuring chamber, as well as its application to other coagulations tests.

**References:**
