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CHARACTERISTIC CHANGES IN THE DENSITY AND SHEAR VISCOSITY OF HUMAN BLOOD PLASMA WITH VARYING PROTEIN CONCENTRATION

The density and shear viscosity of human blood plasma and their dependence on the concentration of proteins (albumin, γ-globulin, fibrinogen, etc.) entering the natural blood composition have been studied. The biomaterial concentration is varied by diluting the blood plasma with the isotonic aqueous solution. It is shown that a decrease in the biomaterial concentration down to 0.91 of its initial value leads to a drastic change in the plasma density and to a change in the character of the concentration dependence of the shear viscosity of blood plasma. A hypothesis is put forward that the observed changes in the density and shear viscosity result from the structural transformations induced by oligomerization processes; first of all, by the albumin dimerization. A conclusion is drawn that the introduced blood substitutes should not exceed 10% of the blood mass; otherwise, structural transformations of a biomaterial in blood plasma can be provoked.

K e y w o r d s: blood plasma, density, shear viscosity, protein concentration, oligomerization of biomolecules.

1. Introduction

Blood plasma plays an important role in the formation of blood properties, first of all, its shear viscosity. This parameter either promotes or impedes the transfer of oxygen from lungs to tissues, as well as carbon dioxide in the opposite direction. A lot of works [1,2] were devoted to the study of the physical properties of blood plasma. The most important of them are the dynamic light scattering; the temperature, concentration, and pH dependences of the plasma shear viscosity [3,4]; and the low-angle thermal neutron and x-ray scattering [5].

In this work, we attract attention to the simultaneous study of the simplest parameters of blood plasma – its density and shear viscosity – under the same conditions. The study of the plasma density as a function of the temperature, bioprotein concentration, and acid-base balance (pH) makes it possible to determine the existence limits for certain structural features in the plasma biosolution. The parallel study of the plasma shear viscosity helps one to obtain the additional information about the features of structural transformations; in particular, the characteristic sizes of biocomplexes that can be formed in blood plasma.

In this regard, let us take into account that blood plasma can be considered, first of all, as an aqueous

solution of albumin, gamma globulin, and fibrinogen [6]. Perhaps, the former component may play a dominant role. Therefore, it is important to trace how the transformations in the structure of albumin molecules could affect the properties of the aqueous solutions of those proteins.

It is well known that an albumin molecule in the "dry" state is a "heart-like" complex about 80 Å in dimensions. This complex consists of three domains, and each of them has two subdomains. The subdomains, in turn, are formed from α-helices that are formed by the sequences of amino acids. When dissolving in water, the rigid spatial configuration of domains can be destroyed at certain temperature and pH values, and the albumin molecules transit to quasilinear complexes of three sequentially linked domains (the so-called F conformation).

The interaction between the domains of different macromolecules may result in the appearance of quasilinear complexes, i.e., dimers or, in the general case, higher-order albumin oligomers. Since the effective volume of a dimer exceeds the sum of the effective volumes of monomers, one should expect that the solution density will change in the area of the solution parameter values, where the development of the extended oligomerization of albumin macromolecules in blood plasma is favored. To a certain extent, a change in the course of the oligomerization will affect a change in the plasma shear viscosity.

But the interaction of domains with water molecules promotes the domain restructuring, especially if the solution pH changes. In particular, hydrogen atoms can partially compensate the negative charges of carbon groups, so that there emerge positively charged amino groups at the albumin "surface", which is accompanied by a change of the zeta-potential sign [7, 8]. This process should also, to some extent, affect the formation of complexes in the aqueous solution of albumin.

In this work, our main attention was focused on the experimental study of the dependence of the human blood plasma density on the bioprotein concentration, as well as on the behavior of the blood plasma shear viscosity.

2. Experimental Part

Changes in the density and shear viscosity of human blood plasma were invoked by adding an isotonic 0.9% solution of NaCl in water or a physiolog-

ical saline. This procedure is adequate to the infusion treatment or the replacement of some amount of blood in the circulatory system with blood substitutes. Modern medical protocols allow a physiological saline to be used as a blood substitute for the emergency management, because colloidal solutions and solutions providing the transport of oxygen and other gases have been developed nowadays. But the physiological saline remains to be a basis for the infusion of most medicines into the circulatory system. There is an empirical rule that the volume of saline added to blood should not exceed 10% of the total blood amount in a person. It is calculated according to the formula "7% of the body mass", and the due value of the latter in kilograms is equal to person's height in centimeters less 100.

Blood is a very complicated fluid system. Therefore, we carried out first experiments on the influence of water on the properties of its basic component, plasma. The latter was considered as an aqueous solution of macromolecular compounds. Native plasma was used.

The plasma density was determined by applying the pycnometric method, and the viscosity was measured making use of a Hess differential viscosimeter. Since the concentration of proteins in plasma and their spectrum are exclusively individual parameters and can vary in rather wide limits, we used the relative concentrations and viscosities, by normalizing their absolute values to their initial values obtained for native plasma. Further changes in the concentration were made by replacing some part of the sample volume with the physiological saline. Every time, the sample volume was reduced by one percent, and then it was brought back to the original value. The density and viscosity measurements might result in small losses of the sample material in a pycnometer and a viscosimeter. This circumstance was taken into account, when forming every new concentration; namely, the portions of the solution components were analytically weighed.

With the help of a 10-cm³ pycnometer and by applying the method of relative measurements (a comparison between the weights of water and a researched fluid with the same volume), the relative accuracy of 10^{-5} can be achieved for the density value. The masses of the sample, M_w, and the standard, M_l, were determined on an analytical balance with an accuracy of 0.2 mg. The density of the examined fluid

was determined by the formula

$$\rho = \frac{M_l}{M_w}.$$

Figure 1 demonstrates the measurement results for the density of human blood plasma diluted with an isotonic solution. Plasma was obtained from the clinical laboratory of therapeutic unit 411 of the Odessa Military Clinical Hospital. From Fig. 1, it follows that if the concentration of proteins in blood plasma reaches a characteristic value that is approximately equal to 0.9 of its initial magnitude, the plasma density drastically changes. This behavior can be explained by the fact that, at this concentration, plasma transits from a homogeneous jelly-like state into a inhomogeneous one generated by the formation of nuclei with a lower density of the protein material (see estimates in the discussion section). Quite unexpected was the fact that the value of this characteristic concentration was identical to that given by empirical recommendations and medical protocols, according to which blood substitutes should not exceed 10% of the blood volume.

A Hess viscosimeter was used to measure the concentration dependence of the plasma viscosity for the same samples. The viscosity was also taken in relative values, namely, normalized by its value for water. Figure 2 illustrates the measurement results obtained for the dependence of the human blood plasma viscosity on the relative protein concentration with respect to its initial value, $\tilde{C} = C/C_0$. This dependence was obtained by adding an isotonic NaCl solution immediately after plasma had been separated from blood.

It should be noted that the kinetic properties of blood plasma are more accurately characterized by the kinematic viscosity

$$\nu(T, \tilde{C}) = \frac{\eta(T, \tilde{C})}{\rho(T, \tilde{C})},$$

which, similarly to the density, also changes in a jump-like manner.

3. Behavior of Blood Plasma Density and Viscosity in 24 h after Preparation

The properties of blood plasma change in time, because its protein component gradually loses its ability of self-restoration [10]. Protein macromolecules lose their electric double layers, which prevent them from
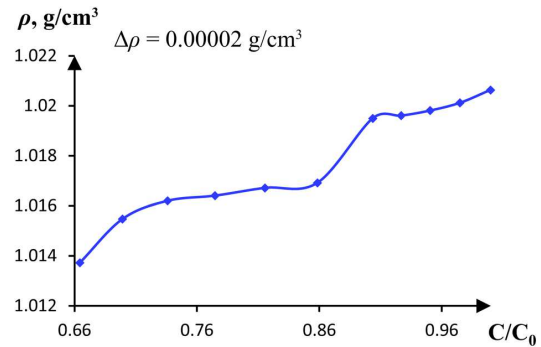

Fig. 1. Dependence of the human blood plasma density on the relative concentration of proteins in plasma diluted with an isotonic NaCl solution immediately after the plasma separation from blood. C_0 is the average protein concentration in human blood [9]
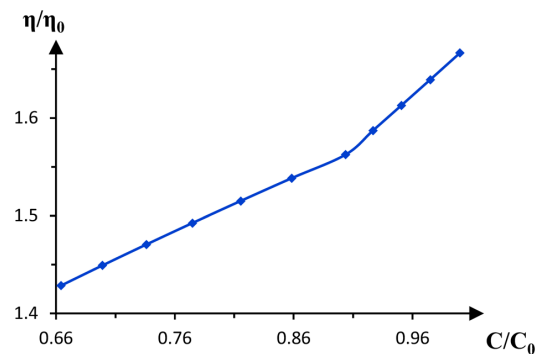

Fig. 2. Dependence of the relative viscosity of human blood plasma on the relative concentration of proteins in plasma diluted with an isotonic NaCl solution immediately after the plasma separation from blood, η_0 is the shear viscosity of water

sticking together in the living state, and begin to coagulate and precipitate. This effect was directly observed in a cuvette in which plasma was stored in a refrigerator at a temperature of 4 °C for 24 h. Due to the partial precipitation of proteins, their concentration in plasma decreases. Coagulation processes additionally reduce the volume fraction of proteins. Therefore, one may expect that the shear viscosity of plasma should decrease in time.

The concentration dependence of the plasma viscosity measured in 24 h after the plasma separation is shown in Fig. 3. As one can see, in accordance with our expectations, the shear viscosity of blood plasma decreases, but its dependence on the relative protein concentration in the intervals $0.6 < C/C_0 < 0.91$ and $0.91 < C/C_0 < 1.0$ becomes nonlin-
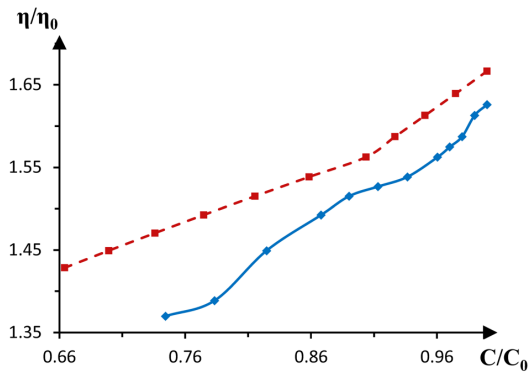
Fig. 3. Dependences of the human blood plasma viscosity on the relative concentration of proteins in plasma diluted with an isotonic NaCl solution immediately after the plasma separation from blood (dashed curve) and 24 h afterward (solid curve). In both cases, the experimental temperature was 16 °C

ear, in contrast to what was observed in as-prepared plasma. Such a variation testifies that the volume fraction of proteins in aging plasma decreases nonlinearly with the addition of an isotonic solution. This circumstance is extremely important and has to be studied thoroughly. The characteristic concentration $C/C_0 = 0.91$ becomes smeared, although the character of a nonlinear dependence of the plasma shear viscosity on C/C_0 changes appreciably in a vicinity of this point.

Furthermore, the execution of experiments with aging plasma is complicated owing to foaming effects. As a result, the magnitude of experimental measurement errors for the plasma density can approach the value of the density jump that is observed in as-prepared plasma. The foaming phenomenon reflects effects of the clustering of denatured protein molecules. Note that, for the measurement results to be adequately interpreted, they must be supplemented with results obtained at various temperatures, especially in the human temperature range between 34 °C and 42 °C, and at various acid-base pH values.

4. Discussion of Results

The character of the dependence of the blood plasma density on the relative concentration of main proteins in plasma, $x = C/C_0$, brought us to a hypothesis that plasma undergoes a smeared phase transition at $C/C_0 \approx 0.91$. This phase transition can be a physically justified factor responsible for the requirement in the empirical medical protocol on blood transfu-

sion that the restriction imposed on the amount of infused blood substitutes must be obeyed.

There are two main reasons for the abnormally rapid increase in the density of blood plasma diluted with an isotonic solution. First of all, this is a change in the character of the spatial ordering of proteins. Second, this is a change in the internal structure of proteins owing to their interaction with one another through the aqueous environment. Let us briefly consider some features of both factors.

We proceed from the behavior of plasma proteins in a dilute solution (note that the experiments were carried out in the opposite direction). The main protein components of plasma – these are albumin, gamma globulin, and fibrinogen – form a mixture of monomers. As the concentration increases, the interaction between the macromolecules begin to form protein oligomers; first of all, these are albumin dimers [11, 12].

Let us evaluate the volume fraction of proteins in blood plasma. According to the Khorolsky works [13–15], the effective radius of an isolated albumin macromolecule is approximately equal to

$$r_{\text{eff}}^{(\text{alb})} \approx 40 \text{ Å}.$$

According to work [6], the mass density of albumin molecules in human blood plasma is equal to

$$\rho_{\text{alb}} = 0.07 \text{ g/cm}^3.$$

With regard for the atomic mass of an albumin imacromolecule

$$M_a \approx 0.65 \times 10^5,$$

we obtain that the volume fraction of proteins in blood plasma equals

$$\varphi_{\text{alb}} = \frac{4\pi}{3} r_{\text{eff}}^3 \frac{\rho_{\text{alb}}}{m_{\text{alb}}},$$

where $m_{\text{alb}} = M_a \times 1.66 \times 10^{-24}$ g is the mass of an albumin molecule. Substituting the indicated values for the density and size of albumin macromolecules, we obtain the following estimate:

$$\varphi_{\text{alb}} \approx 0.2,$$

which is close to the percolation threshold value (see below). The total relative volume of proteins in blood plasma may probably be 1.5–2 times larger,

$$\varphi_{\text{prot}}^{(u)} \approx 0.30 \div 0.40,$$

which should substantially affect the behavior of both the density and the shear viscosity of blood plasma. At the maximum dilution of blood plasma with an isotonic solution, the relative volume of proteins equals

$$\varphi_{\text{prot}}^{(l)} \approx 0.17 \div 0.25.$$

When calculating the plasma shear viscosity, one can consider plasma biomolecules as particles in a suspension and use a formula obtained in work [19] (see also work [18]) in the framework of the cell approach. The dependence η/η_0 versus φ_{prot} calculated with the use of a formula obtained in work [19] is plotted in Fig. 4. In particular, the value $\varphi_{\text{prot}}^{(l)}$ corresponds to $\eta/\eta_0 \approx 1.5$, which almost exactly coincides with the result of experimental measurements shown in Fig. 2. From whence, it follows that, in the examined interval of relative protein concentrations $0.65 < C/C_0 < 1.0$, biomolecules form an ensemble that consists of monomers and oligomers of various orders. This is a direct consequence of the expansion of the $\eta(\varphi_{\text{prot}})/\eta_0$-dependence into an infinite power series in φ_{prot}. Every term in this series corresponds to a contribution from an oligomer of the appropriate order.

Already at the volume fraction of proteins in plasma $\varphi_P \approx 0.23$ [21], they form infinite percolation clusters, which dynamically transform into one another. Therefore, this is a bulk protein concentration at which some changes in system's density or its derivatives with respect to the concentration and temperature should be expected. At the same time, the formation of percolation clusters and their mutual transformations have to manifest themselves in the behavior of the plasma shear viscosity as well. We may assume that, in our experiments, the value of the percolation threshold actually corresponds to the relative protein concentration $C/C_0 \approx 0.91$. At $C/C_0 > 0.91$, the system may probably undergo a transition into the liquid jelly phase, "liquid kissel", the shear viscosity of which substantially depends on the temperature.

The abnormal density increase in the diluted plasma at $C/C_0 \approx 0.91$ may also occur due to the reorganization in the internal structure of protein macromolecules. Let us illustrate this mechanism on the example of albumin macromolecule. In the dry state, this molecule has a heart-like structure [22, 23] formed by three domains, and each of them
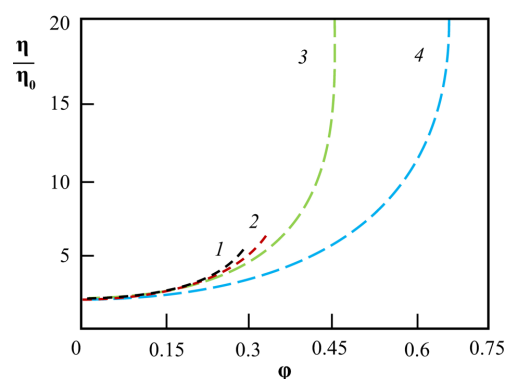

Fig. 4. Dependences of the relative blood viscosity (with respect to the plasma viscosity) on the specific volume φ: Einstein's formula for diluted suspensions [16] (*1*), Batchelor's formula [17] (*2*), formula from works [18, 19] (*3*), model formula for dense suspensions [20] (*4*)

is formed from two subdomains. The subdomains, in turn, consist of 4 or 6 α-helices that are fixed in certain configurations. In the aqueous medium, albumin macromolecules begin to "melt", i.e. their domains may fold or stretch, depending on the temperature and pH. The structure of subdomains also undergoes changes. To some extent, the process of macromolecule "melting" in water resembles structural transformations in water after the ice melting. Immediately after the melting, the local structure of water remains ice-like, and there emerge clusters in the water bulk. Their structure and the thermal motion of molecules in them remain close to those typical of ice. The lifetime of such clusters is finite, which is their feature the most distinct from the ice-like state. It is clear that the effect of the biomolecule decompaction should increase with the temperature.

The internal restructuring of protein macromolecules has the strongest influence on the behavior of the system heat capacity and dielectric permittivity. So, their further study may crucially change our understanding of structural transformations in dilute blood plasma.

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ХАРАКТЕРНІ ЗМІНИ ГУСТИНИ
ТА В'ЯЗКОСТІ ПЛАЗМИ ЛЮДСЬКОЇ КРОВІ
В ЗАЛЕЖНОСТІ ВІД КОНЦЕНТРАЦІЇ БІЛКІВ

Р е з ю м е

Досліджується залежність густини та в'язкості плазми людської крові від концентрації альбуміну, гама-глобуліну, фібриногену тощо, які утворюють природний склад плазми крові. Концентрація біоматеріалу змінюється шляхом розбавлення плазми крові ізотонічним водним розчином. Показано, що зменшення концентрації біоматеріалу до 0,91 від його середньої кількості приводить до різкої зміни густини плазми та зміни характеру концентраційної залежності зсувної в'язкості плазми крові. Висловлюється припущення, що спостережувані зміни густини та зсувної в'язкості плазми крові є пов'язаними зі структурними перетвореннями, викликаними процесами олігомерізації, в першу чергу, димеризації молекул альбуміну. Робиться висновок про те, що дозоване введення кровозамінників, яке не повинно перевищувати 0,1 від кількості крові, є тісно пов'язаним зі структурними перетвореннями біоматеріалу в плазмі крові.