Mixtures of the Biologically Active Substances as Model Systems for Animal Blood Diagnostics

S.Yu. Zaitsev[1,2*], *L.F.* Sotnikova[2], *M.V.* Stepanova[2], *Y.N.* Melikova[2], and *S.L.* Belopukhov[3]

[1]Federal Research Center for Animal Husbandry named after Academy Member L.K. Ernst, Dubrovitsy 60, Podolsk Municipal District, Moscow Region, 142132, Podolsk, Russia
[2]Research Laboratory of Ophthalmology, Oncology and Biochemistry of Animals, Federal State Budgetary Educational Institution of Higher Education "Russian Biotechnological University (ROSBIOTECH)" Volokolamsk Highway, 11, 1250806 Moscow, Russia
[3] Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, Timiryazevskay St. 49, 127434, Moscow, Russia

Abstract. There are numerous biochemical and physical methods for agrobio- and nanomedical sciences. The real-time interfacial tension (RTIT) of various liquids is a powerful analytical method. The main aims of the present work are the following: to study the parameters of the RTIT of the mixtures based on some proteins, lipids, salts as the model systems for animal blood diagnostics. The greatest differences in the RTIT values observed at different concentrations for the aqueous dispersions: albumin: lipid: salt "at the short time of existence of the surface". The high salt concentrations have some particular effects on RTIT values for these mixtures at all times. The changes in albumin concentration influence all RTIT values, but the changes in lipid concentration have insignificant influence and only "at the long time of existence of the surface". Such data have high importance both for fundamental studies and for possible applications in animal and human medicine.

1 Introduction

There are numerous biochemical and physical methods for "research and development" (R&D) in the fields of agrobio- and nanomedical sciences [1-4]. A one of the modern attempts is the further "development of the tensiometry methods" in order to measure a real-time interfacial tension (RTIT) of "blood serum or plasma samples" (BSPS) [5-7]. For the study of biological fluids are more suited the following RTIT methods: 1) the "maximum bubble pressure at the air-liquid interface" (MBP&ALI) and the "hanging drop at the air-liquid interface" (HD&ALI) [5, 6].

The MBP&ALI method allowed to obtain the RTIT curves (so-called tensiogram) in "the range of from 0.01 to 100 seconds with the reproducibility about 0.2%" [5-7]. Currently several types of devices are available which are operating according to the MBP&ALI method: PBS ("Electronetics Comp.", USA), Sensadyn ("Chem-Dyne Research Corp.", USA), BPA-1P ("Accurion GmbH", former "Sinterface Technologies GmbH", Germany),

* Corresponding author: s.y.zaitsev@mail.ru

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

MPT2 ("Lauda", Germany) [8-10]. These devices were adapted for the RTIT measurements of biological fluids at the 1-2 ml samples. For example, a significant advantage of the BPA-1P is a reasonable volume of the studied sample (about 1 ml), high rate of the analysis, "the complete automation of the measurement process, the computer processing of the information received" [8-10].

The major advantages of the HD&ALI method [5-7] are the following: a small volume of liquid to be analyzed (less than 0.5 ml); a simple and convenient temperature control of samples; a wide range of measurements of "the life-time existence" of a analyzed drop (from 10 to 10^4 seconds or more). There are two types of devices which are operating according to the HD&ALI method: "ADSA-Toronto" (Canada), PAT-1 ("Accurion GmbH", former "Sinterface Technologies GmbH", Germany).

These both methods (MBP&ALI and HD&ALI) can be used simultaneously that will give more information about biological systems in the "broad time interval from 10^{-3} to 10^4 seconds" [8-10].

It is important to highlight that a study of the complex systems of biologically active substances (BAS), modeling animal blood plasma or serum (such as proteins, lipids, etc.) is a modern and actual scientific fields [11-13]. There were some, but no pronounced, differences for time and sex of human and correlations with blood proteins, some enzymes, serum cholesterol levels, etc. [8-10], but only limited statistics for animals [5-7]. It is important to highlight that, in the field of veterinary science and practice, the RTIT investigations (especially, their dependences on a physiological condition of an animal and a biochemical compounds in biological liquids) can give valuable information for an early estimation of the physiological-biochemical status of the organism, for general inspections of animals before vaccination (immunization) or slaughter, for "quick separation" of healthy and ill animals in the case of infection, etc.

The main aims of the present work are the following: to study the parameters of the real-time interfacial tension of the mixtures based on some proteins, lipids, salts as the model systems for animal blood diagnostics.

2 Materials and Methods

The following chemicals were used in this study: albumin of the cattle serum (ACS) perched by "Sigma-Aldrich Chemicals" (USA), di-fatty-acids-phosphatidyl-choline from egg yolk (DFAPC) perched by "Fluka Analytical, Merck" (FRG), sodium chloride (NaCL) perched by "Chimmed" (Russia). So, ACS was used as a model protein, DFAPC - as a model lipid, Na^+ and CL^- – the major cation and anion in the human or animal blood. A bidistilled water was used for solution preparations. Solutions of the individual components, two- or three-component systems were prepared on the basis of ACS, DFAPC and NaCL.

The key MBP&ALI method for model solution measurements was applied by using BPA-1P tensiometer [5]. This method allows one to obtain a large row of RTIT values, which are visualized as tensiogram, i.e. the curve of the RTIT (σ) vs. time (t) dependence in the wide range of time intervals [5]. The following RTIT parameters were determined by BPA-1P tensiometer: σ_1 for a very short time period (0.1 s), σ_2 for an intermediate time period (1 s), σ_3 for a relatively long time period (100 s) and σ_4 for a very long time period (~1000 s), as well as the values of the tilt (slope) of the curve at the initial and terminal (final) regions at the tensiogram (λ_0 and λ_z) [4–7]. All manipulations were performed with the purified solutions prepared in bidistilled water. The statistical analysis of the obtained data was accomplished using common statistical programs for Windows with critical values of the reliability criterion on the basis of the Student distributions.

3 Results and Discussion

3.1 RTIT parameters of the individual components

It is well-known that the animal or human serum (or blood plasma) are very complicated mixtures of the numerous BAS. In order to understand their properties, one can prepare and study: first) the individual components, second) two-component systems, third) three-component systems on the basis of albumin of the cattle serum (ACS), di-fatty-acids-phosphatidyl-choline from egg yolk (DFAPC), sodium chloride (NaCL), etc. These BAS (ACS, DFAPC and NaCL) are the typical components of the animal or human serum (or blood plasma) which are presented there in the relatively high amount "as proteins, lipids and salts representatives" [5]. In order to understand the behavior of such complicated systems, it is essential to study them by some "integral complexed colloid" method such as MBP&ALI method and it is important to start the measurements coming from these individual components. Therefore, the RTIT-tensiogramms of the ACS solutions in concentrations (30-80 g/L) were obtained and studied (Table 1).

Table 1. Some RTIT parameters of the aqueous ACS solutions.

ACS, g/L	σ_1 mN/m	σ_2 mN/m	σ_3 mN/m	λ_0 $mN \cdot m^{-1} \cdot s^{1/2}$	λ_z $mN \cdot m^{-1} \cdot s^{1/2}$
30	71.6±0.2	69.1±0.2	58.4±0.2	6.51±0.12	13.30±0.28
40	71.9±0.3	67.2±0.2	56.7±0.2	6.10±0.20	13.82±0.12
50	70.9±0.3	67.1±0.2	57.1±0.3	7.32±0.12	11.51±0.12
60	70.1±0.3	67.5±0.3	55.9±0.3	5.43±0.12	15.42±0.13
70	71.9±0.2	66.3±0.3	56.6±0.3	5.60±0.19	12.70±0.20
80	73.9±0.2	68.3±0.3	56.3±0.2	7.12±0.13	15.92±0.12

It is important to highlight that these conditions are essentially close to the concentration of the total protein in the serum of the well-known domestic and productive animals [1-3]. The data in the Table 1 are in agreement with the data obtained earlier by another method – so-called "hanging drops" [3]. The RTIT parameters of the protein solutions at each ACS concentration were reduced by 4-6% for the middle times (σ_2), as compared to the short times (σ_1), respectively. At relatively long times (σ_3), the RTIT parameters reached the minimal values of about 55.9-58.4 mN/m that were about 25-30% lower as compared to the short times (σ_1). These last values (σ_3) corresponded to the equilibrium RTIT values [3] in the solutions with the lowest ACS concentration. In contrast, the tilts of inclination of the initial (λ_0) and final (λ_z) part of the tensiograms differed by 1.5-2 times that could be valuable RTIT parameters. The observed changes (Table 1) can be explained by the influence of uncompensated electric charge of the protein molecules adsorbed at the interface. So, all RTIT parameters differed slightly in solutions at ACS concentrations of 30-80 g/L in the particular time range. According to the authors [6] only when the concentration of globular proteins became less than 10 g/L (depending on the pH and electrolyte additives) they could be presented in such solutions as "separate" or "non-associated" molecules. Mainly such "non-associated" molecule was able to adsorb fast at the interface. At higher protein concentrations, the association of the protein molecules occurred in the volume of such solution. Therefore, the lower amounts of protein molecules were able to adsorb at the interface in the last case. It is important to underline that the concentration of such "non-associated" molecule in solutions was approximately constant, if there was a rather high concentration of total protein in solution. These effects can be useful to explains the "weak" influence of the ACS concentration on dynamic surface tension values of ACS solutions.

It was important to check an effect of phospholipids on the RTIT parameters of the model systems that can be considered for example as the aqueous DFAPC dispersions at concentrations close to those in the animal serum (Table 2).

Table 2. Some RTIT parameters of the aqueous DFAPC dispersions at concentrations (from 5×10^{-10} to 5×10^{-4} mol./L).

DFAPC dispersions, mol./L	σ_1 mN/m	σ_2 mN/m	σ_3 mN/m	σ_4 mN/m	λ_0	λ_z
					$mN\cdot m^{-1}\cdot s^{1/2}$	
5×10^{-10}	71.69± 0.13	71.66± 0.12	71.57± 0.14	70.66± 0.12	0.50± 0.20	2.61± 0.03
5×10^{-9}	71.70± 0.15	71.64± 0.14	71.54± 0.16	69.97± 0.13	0.60± 0.20	3.43± 0.09
5×10^{-8}	71.65± 0.13	71.61± 0.18	71.50± 0.16	69.41± 0.17	0.60± 0.20	2.52± 0.12
5×10^{-7}	71.59± 0.12	71.54± 0.14	71.42± 0.16	68.98± 0.14	0.65± 0.30	2.95± 0.07
5×10^{-6}	71.21± 0.12	71.20± 0.13	71.05± 0.17	68.44± 0.18	0.55± 0.20	3.24± 0.13
5×10^{-5}	71.85± 0.17	71.84± 0.13	71.72± 0.14	68.32± 0.18	0.50± 0.12	3.40± 0.04
5×10^{-4}	71.76± 0.12	71.28± 0.16	71.11± 0.15	49.44± 0.14	0.60± 0.20	3.19± 0.50

It is important to highlight that DFAPC amount has no significant effect on RTIT parameters of the aqueous lipid dispersions (very close to those of the distilled water), regardless of the lipid concentrations from 5×10^{-10} to 5×10^{-5} and "life-time range" (Table 3). But there are some significant changes of the RTIT parameters of the aqueous dispersions at 5×10^{-4} mol./L and "very long life-time range of about 1000 seconds" (σ_4). There were unexpected results because of the "high surface activity of any lipid molecules" [5]. But these effects can be explained by slow adsorption (kinetics) of such water-insoluble lipid molecules as DFAPC "at the air-water interface" [5].

Sodium chloride refers to surface inactive substances. That is why, the RTIT parameters of NaCl solutions (Table 3) were measured at relatively high concentrations (110-160 mM).

Table 3. Some RTIT parameters of the aqueous sodium chloride solutions.

NaCL, mM	σ_1	σ_2	σ_3	λ_0	λ_z
	mN/m			$mN\cdot m^{-1}\cdot s^{1/2}$	
110	72.93± 0.17	71.48± 0.18	70.80± 0.20	1.51± 0.11	1.12± 0.11
120	72.10± 0.20	71.60± 0.20	71.17± 0.19	0.72± 0.12	0.50± 0.30
130	72.90± 0.20	71.72± 0.11	71.20± 0.30	0.90± 0.20	0.70± 0.20
140	72.80± 0.30	71.77± 0.17	71.30± 0.30	0.90± 0.20	0.41± 0.12
150	73.90± 0.30	72.30± 0.20	71.9± 0.30	2.20± 0.20	0.50± 0.20
160	72.93± 0.17	71.48± 0.18	70.80± 0.20	1.51± 0.11	1.12± 0.11

So, NaCl did not adsorb at the interface and caused almost no changes in RTIT parameters of NaCl solutions at all concentrations (Table 3). Actually, the authors expected to find a small increase in the RTIT during the measurements, especially "at relatively long times (σ_3 and σ_4)" as compared to distilled water. Here, it was only small, even negligible, changes in RTIT parameters of NaCl solutions at "at relatively long times" (Table 3).

3.2 RTIT parameters of the two component systems

There are interesting to observe the changes in the RTIT parameters of the aqueous DFAPC dispersions in a mixture with an ACS solution (10^{-6} mol./L) (Table 4).

Table 4. Some RTIT parameters of the aqueous ACS solutions (10^{-6} mol./L) and DFAPC dispersions at concentrations (from 5×10^{-10} to 5×10^{-4} mol./L).

DFAPC dispersions, mol./L	σ_1 mN/m	σ_2 mN/m	σ_3 mN/m	σ_4 mN/m	λ_0	λ_z
					$mN\cdot m^{-1}\cdot s^{1/2}$	
5×10^{-10}	71.96± 0.15	71.94± 0.18	68.29± 0.17	69.60± 0.14	0.24± 0.05	2.37± 0.12
5×10^{-9}	71.84± 0.14	71.81± 0.17	68.21± 0.16	70.05± 0.18	0.18± 0.06	3.20± 0.20
5×10^{-8}	71.90± 0.17	71.93± 0.16	68.87± 0.14	69.49± 0.15	0.19± 0.08	2.40± 0.30
5×10^{-7}	71.82± 0.15	71.89± 0.14	69.44± 0.13	69.30± 0.12	0.36± 0.13	3.60± 0.20
5×10^{-6}	71.89± 0.16	71.92± 0.15	68.16± 0.14	70.21± 0.15	0.16± 0.05	2.60± 0.20
5×10^{-5}	71.84± 0.13	71.82± 0.14	68.20± 0.15	69.88± 0.14	0.18± 0.04	3.90± 0.20
5×10^{-4}	71.62± 0.15	68.51± 0.13	62.04± 0.17	53.17± 0.16	3.50± 0.30	26.70± 0.40

The RTIT parameters of the mixture of DFAPC dispersion with ACS solution (10^{-6} mol./L) decreased significantly only at a DFAPC concentration of about 5×10^{-4} mol./L by 6% at medium times and by 14% "at long times of surface existence" (Table 4). The equilibrium value of RTIT parameters was at 26% lower than the initial DFAPC dispersion and at 4% lower than that of an individual ACS solution (Tables 1, 2 and 4). Apparently, this is due to competition between the lipid and protein molecules "during adsorption at the interface and the predominance of the protein component of the mixture at the interface" [5].

There are pronounced RTIT parameters of the mixtures of aqueous ACS solutions (at concentrations 30-80 g/L) and NaCL (at concentrations 130-150 mM) (Table 5).

Table 5. Some RTIT parameters of the aqueous ACS solutions (at concentrations 30-80 g/L) and NaCL (at concentrations 130-150 mM)

Parameters ACS, g/L+ NaCl, mM	σ_1 mN/m	σ_2 mN/m	σ_3 mN/m	λ_0 $mNm^{-1}s^{1/2}$	λ_1 $mNm^{-1}s^{1/2}$
40 + 130	73.29±0.15	70.80±0.30	59.61±0.16	4.85±0.11	15.86±0.15
60 + 140	74.37±0.19	69.80±0.30	58.60±0.30	5.47±0.12	13.8±0.20
80 + 150	75.83±0.18	70.30±0.30	59.49±0.18	5.80±0.20	12.34±0.11

30 + 150	72.20±0.30	69.50±0.30	58.73±0.18	4.28±0.13	14.47±0.12
80 + 130	73.60±0.20	69.50±0.30	59.80±0.20	4.58±0.12	11.36±0.13

There are changes of the RTIT parameters by 4-5% at the ratio NaCL:ACS from 1:1.9 to 1:3.3 (g/g); by 2% at the ratio from 1:1.7 and 1:5 (g/g) of the mixtures of aqueous solutions of the ACS (at concentrations 30-80 g/L) and NaCL (at concentrations 130-150 mM) (Table 5). There is an increase in all RTIT values "at all life-times of the surface existence" by salt addition to the aqueous protein solution (Tables 1 and 5). In general, there is a reduction of the RTIT values (Table 5) that is due to the process of the "protein association by relatively high salt addition" [5]. This effect is less pronounced by lower content of salt in the solution of NaCL:ACS (Table 5).

3.3 RTIT parameters of the three component systems

There is an increase by 3% of the RTIT values of the aqueous dispersions DFAPC with ACS and NaCL solutions, i.e. DFAPC: ACS: NaCL of about 1:10:33; 1:10:50; 1:20:47 (g/g/g) at a short time, whereas some small decrease at medium and long times (Table 6).

Table 6. Some RTIT parameters of the aqueous DFAPC dispersions with ACS and NaCL solutions at various concentrations.

DFAPC, mM + ACS, g/L + NaCl, mM	**σ_1 mN/m**	**σ_2 mN/m**	**σ_3 mN/m**	**λ_0 mNm $^{-1}$s$^{1/2}$**	**λ_z mNm $^{-1}$s$^{1/2}$**
3 + 40 + 130	72.70±0.20	67.20±0.20	57.08±0.19	5.46±0.15	10.20±0.20
2 + 80 + 140	72.40±0.30	66.50±0.20	50.90±0.20	5.40±0.20	13.50±0.20
3 + 30 + 150	73.10±0.30	67.20±0.20	56.90±0.20	5.56±0.14	14.93±0.12
3 + 60 + 140	74.70±0.20	66.50±0.20	56.30±0.20	7.70±0.20	14.56±0.13
4 + 40 + 130	73.40±0.20	66.40±0.30	54.90±0.20	6.27±0.15	10.96±0.14
3 + 40 + 140	73.20±0.30	67.10±0.20	57.90±0.20	6.06±0.12	8.92±0.13

There is an decrease by 5-8% of the RTIT values for the aqueous dispersions DFAPC : ACS : NaCL =1:13:43; 1:13:47; 1:40:70 (g/g/g) "at all times of the surface existence" (Table 6). There is an increase by 2-3% of the RTIT values for increasing salt concentrations in the mixtures at short times (Table 6).

There are numerous RTIT data obtained for the aqueous dispersions DFAPC : ACS : NaCL at particular ACS : NaCL ratios and variations in the DFAPC amount "at all times of the surface existence" (Tables 7-11).

Table 7. Some RTIT parameters of the aqueous DFAPC dispersions (at various concentrations from 0.5 mM to 4 mM) with 30 g/L ACS and 150 mM NaCL solutions.

Concentration DFAPC, мМ	**0,5**	**1**	**2**	**3**	**4**
σ_0, mN/m	74.85±0.06	75.13±0.13	74.89±0.11	73.16±0.12	75.77±0.15
σ_1, mN/m	73.25±0.09	73.18±0.12	73.51±0.11	72.08±0.08	73.57±0.05
σ_2, mN/m	66.03±0.09	66.12±0.13	66.61±0.12	67.06±0.10	66.99±0.05
σ_3, mN/m	66.03±0.09	60.11±0.11	57.75±0.03	56.95±0.08	56.07±0.15
σ_4, mN/m	51.91±0.07	52.28±0.05	52.67±0.06	51.37±0.10	52.30±0.13
λ_0, mNm^{-1} s$^{1/2}$	10.3±0.2	10.1±0.1	9.3±0.1	5.5±0.3	10.9±0.2
λ_1, mNm^{-1} s$^{1/2}$	8.7±0.1	6.4±0.2	8.9±0.3	14.9±0.2	12.2±0.1

λ_2, mNm^{-1} s$^{1/2}$	31.1±0.1	24.7±0.2	21.4±0.2	22.7±0.1	31.8±0.2

Table 8. Some RTIT parameters of the aqueous DFAPC dispersions (at various concentrations from 0.5 mM to 4 mM) with 80 g/L ACS and 140 mM NaCL solutions.

Concentration DFAPC, мМ	**0,5**	**1**	**2**	**3**	**4**
σ_0, mN/m	77.96±0.01	75.11±0.04	72.89±0.13	75.24±0.17	74.31±0.06
σ_1, mN/m	77.35±0.15	73.35±0.15	71.51±0.16	72.61±0.15	75.87±0.13
σ_2, mN/m	70.19±0.16	65.99±0.17	66.29±0.17	64.49±0.18	65.39±0.12
σ_3, mN/m	65.93±0.11	56.09±0.12	49.80±0.13	56.94±0.15	57.99±0.15
σ_4, mN/m	49.95±0.13	50.8±0.11	50.15±0.09	49.53±0.05	49.35±0.14
λ_0, mNm^{-1} s$^{1/2}$	8.6±0.1	9.9±0.2	6.6±0.1	12.8±0.2	9.9±0.2
λ_1, mNm^{-1} s$^{1/2}$	4.2±0.3	11.6±0.3	16.4±0.2	9.4±0.1	8.1±0.2
λ_2, mNm^{-1} s$^{1/2}$	28.4±0.2	22.9±0.1	21.8±0.1	27.7±0.3	42.9±0.1

Table 9. Some RTIT parameters of the aqueous DFAPC dispersions (at various concentrations from 0.5 mM to 4 mM) with 40 g/L ACS and 130 mM NaCL solutions.

Concentration DFAPC, мМ	**0,5**	**1**	**2**	**3**	**4**
σ_0, mN/m	72.39±0.13	74.20±0.11	74.46±0.15	73.59±0.14	73.38±0.08
σ_1, mN/m	71.82±0.12	72.65±0.10	72.52±0.13	71.79±0.12	72.65±0.09
σ_2, mN/m	65.26±0.15	65.41±0.13	65.15±0.06	65.47±0.13	66.41±0.13
σ_3, mN/m	55.61±0.11	56.87±0.15	56.36±0.16	56.79±0.16	54.45±0.09
σ_4, mN/m	51.33±0.12	52.01±0.11	51.92±0.15	51.15±0.14	52.3±0.08
λ_0, mNm^{-1} s$^{1/2}$	8.3±0.3	10.4±0.2	12.8±0.1	8.9±0.2	6.2±0.2
λ_1, mNm^{-1} s$^{1/2}$	11.1±0.2	9.2±0.2	10.1±0.3	10.9±0.1	11.9±0.1
λ_2, mNm^{-1} s$^{1/2}$	25.1±0.1	29.1±0.2	30.9±0.1	26.6±0.13	31.8±0.11

Table 10. Some RTIT parameters of the aqueous DFAPC dispersions (at various concentrations from 0.5 mM to 4 mM) with 40 g/L ACS and 140 mM NaCL solutions.

Concentration DFAPC, мМ	**0,5**	**1**	**2**	**3**	**4**
σ_0, mN/m	74.68±0.11	73.55±0.09	75.08±0.08	76.1±0.16	74.79±0.05
σ_1, mN/m	73.36±0.13	72.74±0.15	73.77±0.12	73.35±0.20	72.03±0.07
σ_2, mN/m	65.94±0.11	65.56±0.07	65.6±0.08	65.74±0.09	65.26±0.13
σ_3, mN/m	57.71±0.17	56.59±0.15	57.89±0.16	55.39±0.12	58.38±0.13
σ_4, mN/m	52.16±0.15	52.6±0.14	51.92±0.12	51.12±0.11	52.18±0.14
λ_0, mNm^{-1} s$^{1/2}$	9.4±0.3	8.7±0.2	10.8±0.1	10.5±0.1	9.5±0.1
λ_1, mNm^{-1} s$^{1/2}$	8.8±0.1	11.1±0.2	8.3±0.3	13.3±0.1	7.2±0.2
λ_2, mNm^{-1} s$^{1/2}$	31.8±0.3	27.7±0.1	30.8±0.3	31.1±0.1	25.9±0.2

Table 11. Some RTIT parameters of the aqueous DFAPC dispersions (at various concentrations from 0.5 mM to 4 mM) with 60 g/L ACS and 140 mM NaCL solutions.

Concentration DFAPC, мМ	**0,5**	**1**	**2**	**3**	**4**
σ_0, mN/m	74.54±0.15	73.3±0.11	75.19±0.11	75.95±0.1	75.28±0.07
σ_1, mN/m	74.85±0.16	73.13±0.13	73.42±0.11	73.67±0.10	73.38±0.05

σ_2, mN/m	64.72±0.13	65.29±0.15	66.12±0.12	65.51±0.07	65.14±0.05
σ_3, mN/m	57.50±0.05	57.04±0.06	57.17±0.18	58.17±0.16	58.34±0.14
σ_4, mN/m	52.3±0.12	51.67±0.14	51.93±0.13	50.1±0.08	51.99±0.07
λ_0, mNm^{-1} s$^{1/2}$	10.8±0.2	7.9±0.3	10.7±0.1	11.3±0.2	10.3±0.1
λ_1, mNm^{-1} s$^{1/2}$	8.2±0.2	8.2±0.3	9.9±0.2	7.4±0.1	6.7±0.1
λ_2, mNm^{-1} s$^{1/2}$	27.7±0.1	27.4±0.1	25.9±0.1	22.2±0.1	27.5±0.2

There are some decrease in RTIT data at 9-15% observed for the aqueous dispersions DFAPC : ACS : NaCL at particular ACS : NaCL ratios and variations in the DFAPC amount "at all times of the surface existence" (Tables 7-11). In all cases, the equilibrium RTITvalues differ slightly. It is important to highlight that DFAPC influences RTIT "at long times existence surface", whereas the ACS concentration in the mixture affects the RTIT at medium times. Thus, for different ratios of the components in the mixture is different change values by varying the RTIT concentration of ACS, which is due to the mutual influence of the components of the mixture on top of each other. For the most RTIT values of these mixtures a pronounced decrease occurs with increasing protein concentration to 30-40 g/L. The only exceptions occur for the mixtures with high NaCL content (the ratio of DFAPC : NaCL as 1:70). In the last case RTIT values decrease only at a protein concentration of 80 g/L. This is due to "the salting out effect of salt on protein molecules, retarding their adsorption at the interface" [7-9].

4 Conclusions

Thus, the greatest differences in the RTIT values observed at different concentrations for the aqueous dispersions DFAPC : ACS : NaCL "at the short time of existence of the surface". The high salt concentrations have some particular effects on RTIT values for these mixtures at all times. The changes in albumin concentration influence all RTIT values, but the changes in lipid concentration have insignificant influence and only "at the long time of existence of the surface". Such data have high importance both for fundamental studies and for possible applications in animal and human medicine.

Acknowledgments

The part 1 of this article was fulfilled with support of the of the Ministry of Higher Education and Science of the Russian Federation in accordance with agreement No 075-15-2020-905 date November 16, 2020 on providing a grant in the form of subsides from the Federal budget of Russian Federation. The grant was provided for state support for the creation and development of the World-class Scientific Center "Agrotechnologies for the Future". The authors thank World-class Scientific Center "Agrotechnologies for the Future", which funded this research under grant No. 2744-r dated 10/24/2020.

The parts 2, 3 and 4 of this research were carried out within the project (code FSMF-2022-0003 of the Ministry of Higher Education and Science of the Russian Federation) of the Research Laboratory of Ophthalmology, Oncology and Biochemistry of Animals, Federal State Budgetary Educational Institution of Higher Education "Russian Biotechnological University (ROSBIOTECH)".

References

1. J.J. Kaneko, J.W. Harvey, M.L. Bruss, *Clinical Biochemistry of Domestic Animals*. (6th edn.) (San Diego: Academic Press, 2008) 916.

2. S.Yu. Zaitsev, N.V. Bogolyubova, G.V. Molyanova, *Biochemical blood analysis of a number of pig breeds and their hybrids* (Moscow: Agricultural Technologies, 2022) 256.
3. S.Yu. Zaitsev, Tensiometric and biochemical analysis of animal blood: fundamental and applied aspects (Moscow, Agricultural Technologies, 2016) 192.
4. V.N. Kazakov, O.V. Sinyachenko, G.A. Ignatenko, *Interfacial tensiometry of biological fluids in therapy* (Donetsk: Donechchina, 2003) 126.
5. S.Yu. Zaitsev, Advances in Colloid and Interface Science. **235**, 201–213 (2016).
6. S.Yu. Zaitsev, *Supramolekulyarnye nanorazmernye sistemy na granitse razdela faz: kontseptsii i perspektivy dlya bionanotekhnologii* (Supramolecular Nanosized Systems at the Interface: Concepts and Perspectives for Bionanotechnology), (Moscow, LENAND, 2010).
7. S.Yu. Zaitsev, Moscow Univ. Chem. Bull. **71**, 205-211 (2016).
8. V.N. Kazakov, O.V. Sinyachenko, V.B. Fainerman, U. Pison, R. Miller, in Studies in Interface Science, Amsterdam: Elsevier, **9,** 34 (2000).
9. V.B. Fainerman, R. Miller, in Proteins at Liquid Interfaces. Amsterdam: Elsevier, **7**. 51-102 (1998).
10. V.B. Fainerman, M. Zholob, K. Leser, J. Colloid Interface Sci. **274**, 496 (2004).
11. K. Holmberg, B. Jönsson, B. Kronberg, B. Lindman, *Surfactants and Polymers in Aqueous Solution*, (Chichester: Wiley, 2003).
12. M. Aguirre-Ramírez, H. Silva-Jiménez, I.M. Banat, M.A. Díaz De Rienzo, Biotechnol Lett. **43**, 523-535 (2021).
13. E.A. Vogler, Biomaterials, **33**, 1201 (2012).