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Interaction of Polymer Aggregates Based on Stearoyl-poly-N-vinylpyrrolidone with Blood Components

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Abstract—Stearoyl-poly-N-vinylpyrrolidone (PVP-stear) of various molecular weights (M_n = 1500-5500) self-assemble in aqueous medium. Particles prepared from PVP-stear were characterized in terms of shape and size distribution, and the mechanical stability of the particles was studied. The interaction of PVP-stear and its aggregates with blood components was investigated. Aggregates formed by the polymers with M_n = 1500-3500 in the presence of human serum are stable. The direct lytic action of PVP-stear preparations was studied using sheep and human erythrocytes. The influence of PVP-stear aggregates on the activation of complement system both on classical and alternative pathways was examined. The aggregates prepared from PVP-stear of various molecular weights had no effect on the activation of the complement system.

Key words: self-assembling systems, amphiphilic polymer, stearoyl-poly-N-vinylpyrrolidone, colloidal particles, blood serum, erythrocytes, complement system

The search for new medical drugs and production of more advanced forms of already known biologically active substances as well as the development of delivery systems for these substances into an organism is an important task of bio- and pharmaceutical technology. From this point of view, synthetic and natural polymers provide a unique possibility for the development of new and efficient drug formulations, where the most promising are various kinds of self-assembling polymer systems.

Amphiphilic polymers contain sufficiently large hydrophilic and hydrophobic moieties to make their aggregation into supramolecular associates (micelles, vesicles, liposomes) thermodynamically advantageous [1]. In this case, in the aqueous phase polar parts of amphiphilic polymer molecules are directed into the polar aquatic medium, while the non-polar parts of the molecules form the inner sphere of the particles. Because the whole system becomes dispersible in water, it is possible to homogenize low polarity medical drugs in an aqueous phase [2, 3].

Self-assembling systems based on amphiphilic polymers with gradual or controlled release of active substance are a key group of biologically active polymer systems. For instance, application of such systems decreases the role of side effects and allows introducing a higher dose of a drug into the body [4, 5].

To avoid the accumulation of amphiphilic polymer carrier in an organism after the function of the formulation is complete, hydrolyzable groups can be introduced into the main chain of the polymer, providing for its degradation in the organism into easily excreted fragments as well as different kinds of biocompatible polymers. Well studied polymers are most often used as biocompatible carriers in these drug formulations, those which are employed as blood substitutes: dextran, poly-N-vinylpyrrolidone, poly-N-(2-hydroxypropyl), methacrylamide, and other substances [6].

The biocompatible polymer used in this work is an amphiphilic polymer based on poly-N-vinylpyrrolidone (PVP). This polymer is a widespread component of injection medical drugs and also a blood substitute component [7]. Amphiphilic derivative of poly-N-vinylpyrrolidone containing a terminal hydrophobic group, which is a residue of long-chain aliphatic acid (stearic acid), was recently synthesized via radical polymerization of N-

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vinylpyrrolidone in the presence of functional mercaptans followed by an interaction with acid [8].

It is commonly known that an important factor for the distribution of polymer particles in the body is their binding by blood serum proteins. The question about the nature of plasma protein action on blood cells in the presence of different biologically active substances and delivery systems is virtually uninvestigated and demands solutions in both theoretical and practical aspects [9]. Elucidation of protein regulation of different types of erythrocyte lysis may facilitate the solution to cell protection under harsh conditions. Another interesting task is to study the activation of the complement system, which is a part of the immune system and provides nonspecific protection of the organism [10].

Hence, the investigation of the action of blood serum components on the polymer of the drug delivery system along with the action of these systems on the blood components is extremely important for the understanding of their interaction with a living organism. The goal of this work was to study PVP-stear self-organization processes in aqueous medium as well as to investigate the interaction of disperse systems (which are based on the studied polymer) with blood components.

MATERIALS AND METHODS

Materials. PVP-stear was synthesized by radical polymerization of N-vinylpyrrolidone in the presence of functional mercaptans (2-mercaptoethylamine, mercaptoacetic acid) with subsequent interaction with acid or stearoyl amine. By variation of reaction conditions amphiphilic polymers with different molecular weight (M_n = 1500-5500) were synthesized. The obtained polymers contain one terminal functional group, which is confirmed by the matching of molecular weights of the polymers determined by the content of terminal groups in them, and alternatively, by embullioscopy [8].

Materials used included the fluorescent label pyrene (98%, Sigma-Aldrich Chemie GmbH, Germany); support film for transmission microscopy, 0.2% polyvinylformal (Sigma, USA); dimethylsulfoxide (DMSO, 99%, ICN Biomedicals Inc., Germany). Other reagents were of chemically pure grade.

Isotonic veronal buffer (VBS_{conc}) was prepared according to [11]: 1 liter of solution contains 5.095 g of veronal, 41.5 g of NaCl, and the pH is adjusted to 7.4 with 0.1 M NaOH solution. Isotonic veronal buffer which contains Ca^{2+} and Mg^{2+} (VBS^{2+}) ions was prepared from VBS_{conc} as described above; however, before the volume was adjusted to 1 liter, 2.5 ml of 1 M $MgCl_2$ and 0.75 ml of 1 M $CaCl_2$ were added. Before use, the buffer was diluted 5 times with distilled water. EGTA buffer is a veronal buffer containing 5 mM $MgCl_2$ and 10 mM ethyleneglycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA).

Alsever's solution was prepared as described in work [11]. Eight grams of sodium citrate (dehydrate of sodium salt), 4.2 g of NaCl, and 20.0 g of glucose were dissolved in 750 ml of distilled water; ~5.5 ml of 10% citric acid was added to achieve pH 6.1, and then the volume was adjusted to 1 liter. Before taking a blood sample, Alsever's solution was sterilized. Sheep blood was placed in Alsever's solution, where it was stored for up to 4 months at 4°C (Gabrichevsky Institute, Moscow). Rabbit blood was placed in isotonic solution of sodium citrate and used on the day of experiment. The source for the complement system activity was blood serum from the donors and patients of different clinics in Moscow and St. Petersburg.

Preparation of PVP-stear aggregates. Two techniques for preparing polymer aggregates were used in this work: direct dilution and the dialysis technique. When using the direct dilution technique, the corresponding amount of PVP-stear was dissolved in distilled water or in physiological solution (0.15 M NaCl, pH ~7.4). The solution was stirred on a KS 500 shaker (Labortechnik, Germany) at 100 rpm for 20 min; then the samples were collected and analyzed. When preparing polymer aggregates via the dialysis technique, DMSO was used as the solvent. Solution of polymer in DMSO was dialyzed against 4 liters of water or physiological solution for 24 h using dialysis bags of MW 12,000-14,000 (Sigma) [12].

Determination of critical micelle concentration (CMC). CMC values for different PVP derivatives were determined based on solubilization of water-insoluble fluorescent dye (pyrene) by the colloid particles of amphiphilic polymers. Aliquots containing 10 μl of pyrene solution in acetone (10 mg/ml) in vials were dried under vacuum. To each dried pyrene vial 2 ml of PVP-stear solutions (10^{-4}-10^{-10} M) with different molecular weights in 0.15 M NaCl solution, pH 7.4, were added. Then the solutions were stirred for 24 h at room temperature [12].

The samples were filtered to remove non-solubilized pyrene residues. The fluorescence intensity of solubilized pyrene was measured at excitation wavelength 339 nm and emission wavelength 385 nm a luminescence spectrometer (Perkin Elmer LS-50B, USA).

Dynamic light scattering. The size of particles was determined by the dynamic light scattering technique. For this purpose, a monochromatic irradiation from a 2 mW helium-neon laser (632.8 nm wavelength) was passed through the thermostatted cuvette (t = 20°C). Light dissipated at 90° angle was registered by a photodetector, including a photomultiplier and a UNICOR-SP multichannel correlator (Sweden). Data processing was performed using UNICOR-SP software.

Transmission electron microscopy. Transmission electron microscopy was performed using a JEOL JEM-1000 apparatus (Germany) at 20 kV. To prepare the sample a drop of solution was placed on a 0.2% polyvinylformal surface grafted over a copper grid.

Study of mechanical stability of the aggregates. The dispersion of PVP-stear aggregates in physiological solution was filtered through 0.22 μm Millipore filters (Austria). The materials retained on the filters were subjected to 10, 15, and 30 sec ultrasonication using a UZO-5-01-MEDEL apparatus (Russia) at maximum power. The size of the particles obtained after filtration and ultrasonication was evaluated using dynamic light scattering.

Preparation of sheep erythrocyte standard suspension [13]. Sheep blood from the jugular vein was taken under aseptic conditions into equal volume of a sterile Alsever's solution with glass beads, stirred, and poured into vials, then stored for 3-7 days at 4°C for cell stabilization. The erythrocytes can be stored for approximately 2 months at 4°C. For the preparation of a standard suspension of sheep erythrocytes the erythrocyte pellet (E) was rinsed three times with 10-fold volume of VBS^{2+} followed by centrifugation (1000*g*) for 10 min. Rinsed E were resuspended in VBS^{2+} in such manner that after 15-fold dilution with water and centrifugation the supernatant would have an absorption equal to 0.7 optical unit (o.u.) at 541 nm, which corresponds to the cell concentration of $1 \cdot 10^9$ cells per ml of suspension. The number of lysed erythrocytes was determined based on the absorption of hemoglobin in the solution at 412 nm: $1 \cdot 10^7$ lysed cells in 1 ml have absorption equal to 1 o.u. The standardized erythrocytes were stored at 4°C and used in work for several days.

Preparation of sensitized sheep erythrocytes (EA). To the suspension of sheep erythrocytes (standardized to $1 \cdot 10^9$ cells/ml) an equal volume of a hemolytic serum, diluted 1 : 400 by VBS^{2+}, was added; the mixture was thoroughly stirred and incubated for 30 min at 37°C with occasional shaking. When the incubation was completed, the concentration of the E suspension was adjusted to $1.5 \cdot 10^8$ cells/ml by adding VBS^{2+} in a corresponding quantity so that the optical density of 200 μl of EA suspension lysed in 2.8 ml of water is equal to 1 o.u. at 412 nm. The standard EA mixture was stored at 4°C and used in the experiments for one day. For the determination of activation parameters of the complement via the classic pathway (CP), the employed standard suspension of sensitized sheep erythrocytes resulted in absorption of 0.560 ± 0.010 o.u. at 800 nm after dilution 8 times by the physiological solution.

Preparation of standard suspension of rabbit erythrocytes. Rabbit erythrocytes were rinsed three times with 10-fold volume of physiological saline followed by centrifugation at 1000*g* for 10 min. The rinsed E were suspended in VBS such that after 8-fold dilution with physiological saline the suspension would have an absorption equal to 0.560 ± 0.010 at 800 nm.

Study of stability of PVP-stear aggregates in the presence of blood serum. PVP-stear aggregates containing 10-30 μg/ml of solubilized pyrene were incubated in the presence of 50% human blood serum. The fluorescence intensity due to release of pyrene at different incubation times was measured at 339 nm excitation wavelength and 385 nm emission wavelength in the presence and in the absence of blood serum. Human serum and pyrene in physiological solution were used as a reference.

Investigation of lytic action of PVP-stear preparation on sensitized sheep erythrocytes. PVP-stear preparation solution (10-40 μl, $5 \cdot 10^{-3}$ M) in 0.01 M VBS^{2+} were mixed with 200 μl of EA ($1.5 \cdot 10^8$ cells/ml) and total volume was adjusted to 250 μl by adding 0.01 M VBS^{2+}. The mixture was incubated for 30 min at 37°C with periodic stirring. After the incubation, 2.5 ml of cold (4°C) 0.15 M NaCl solution was added to each sample; the mixture was centrifuged and lysis grade determined based on absorbance of the supernatant at 412 nm. The control sample did not contain the preparation.

Determination of influence of human albumin on lytic action of the preparations. For the determination of the influence of human albumin on the lytic action of PVP-stear preparations on sheep erythrocytes, 200 μl of EA was incubated with albumin solutions (7.7-30.8 μM) at constant lytic PVP-stear concentration in the total volume of 0.5 ml, adjusted with VBS^{2+} buffer. After 30 min of incubation, 2.5 ml of 0.15 M NaCl solution was added to the samples, and it was then centrifuged for 5 min at 1500 rpm. The lysis grade was measured based on absorbance of the supernatant at 412 nm. One lot of control samples did not contain albumin; another did not contain PVP-stear.

Study of the influence of PVP-aggregate on activation of the complement system. PVP-stear preparations were incubated for 1 h with human blood serum. For the determination of parameters of complement activation via the classic pathway (CP), 0.1 ml of control (without PVP-stear sample) and studied (containing PVP-stear) serum was places into a cuvette. Into a cuvette thermostatted at 37°C (K-5), 0.6 ml of VBS^{2+} buffer was added, and then (after heating for 3 min) 0.1 ml of sensitized sheep erythrocyte standard suspension were also added. Optical density was registered every 5 sec at 800 nm using an SF-2000 spectrophotometer (Russia). When the lysis of one portion of erythrocytes was completed, the same portions of standard suspension was added into the cuvette and the process of their lysis was registered until the exhaustion of the complement system was achieved, which was exhibited as a termination of hemolysis. The time between addition of erythrocytes and occurrence of hemolysis (lag-period or lag-t) value was determined; the rate of complement-dependent erythrocyte lysis (V of lysis by CP) was determined by the decrease in absorbance [14]; hemolytic capacity of the complement (HCC) [15] was determined by the number of standard portions of erythrocyte suspension that were completely lysed by the blood serum sample.

For the determination of parameters of the complement activation via the alternative pathway of comple-

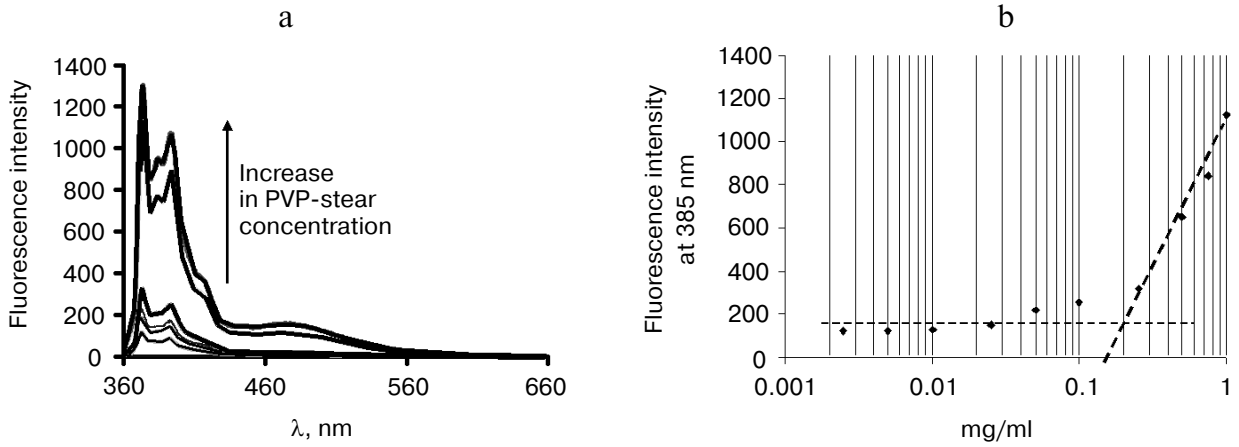

Fig. 1. Fluorescence spectra of pyrene ($6 \cdot 10^{-7}$ M) at different concentrations of PVP-stear (M_n = 1500) varying from 0.0025 to 1 mg/ml (a); dependence of fluorescence intensity at λ = 385 nm on the polymer concentration (b).

ment system activation (AP), 0.4 ml of EGTA-buffer and 0.2 ml of VBS^{2+} buffer were added to 0.1 ml of control or studied (containing PVP-stear) blood serum. After incubation for 3 min, 0.1 ml of standard suspension of rabbit erythrocytes was added to the incubation mixture. Time between introduction of erythrocytes and occurrence of hemolysis (lag-period or lag-t) and rate of complement-dependent hemolysis by AP was determined. The results are expressed as percentage of the control level [16].

RESULTS AND DISCUSSION

Preparations and characterizations of particles from PVP-stear. As a rule, the amphiphilic polymers are capable of self-assembling when the polymer concentration exceeds a certain threshold concentration, a so-called critical micelle concentration (CMC). In this case, micelle structures of different morphology can be observed in the solution [1].

All of the techniques for preparation of particles from amphiphilic polymers can be divided in two large groups: direct dissolution and dialysis [17]. In each case, the choice of technique for obtaining micelles is determined by the solubility of the micelle-forming block copolymers in the aqueous medium. In the case of direct dissolution techniques, the copolymer is directly added to an aqueous medium at room temperature or on the heating of the solution at temperatures much higher than the CMC. This technique is applicable for polymers, which are well solublized in water, such as pluronics and other copolymers of similar structure. For obtaining micelles from micelle forming copolymers with very low solubility in water, dialysis techniques are usually applied. Polymers are dissolved in a mixture of water and organic solvents such as DMSO, DMFA, acetonitile, or tetrahydrofuran and then this mixture is dialyzed against water. PVP-stear particles were obtained in this work using both techniques.

Critical micelle concentration for PVP-stear was determined by the technique based on solubilization of water-insoluble pyrene label into the obtained particles.

Figure 1a presents pyrene fluorescence spectra as the polymer concentration increases in the solution. CMC values were determined from the dependence of fluorescence intensity at 385 nm on the concentration of PVP-stear for different molecular weights (Fig. 1b, Table 1).

As seen from Table 1, CMC values fall into the micromolar range. Since the greatest contribution to the formation of these polymer aggregates is provided by the interaction between stearic acid residues, the increase in the hydrophilic part of the molecule leads to the weakening of these interactions. Therefore, in this case, for a polymer with higher molecular weight a higher concentration is required for aggregate formation.

The morphology of supramolecular aggregates of amphiphilic polymers is rather varied. It is usually assumed that amphiphilic polymers form particles whose

Table 1. Values of critical micelle concentration for PVP-stear of different molecular weights

M_n, daltons	1500	2000	2600	2800	4000	5500
CMC, μM	7.3	10.0	10.0	11.4	7.3	5.8

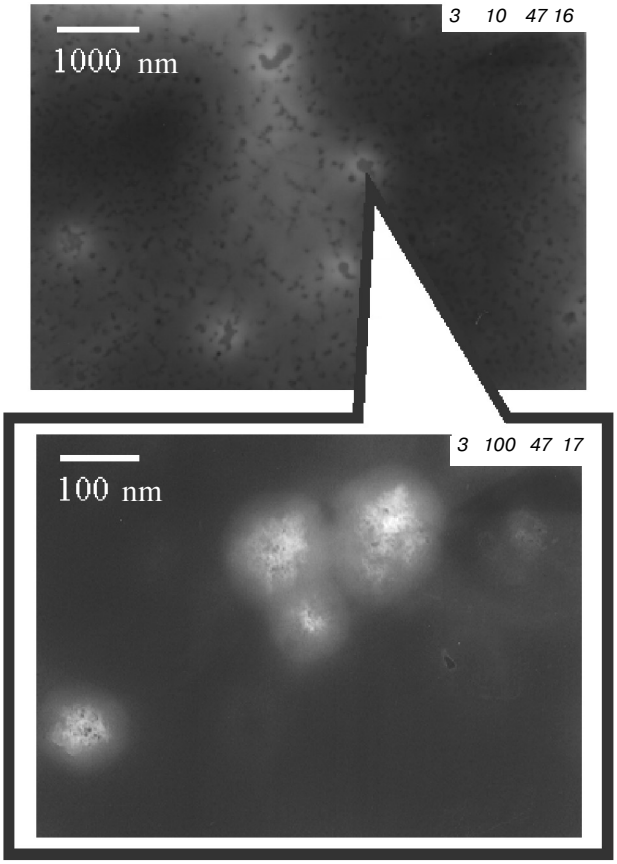

Fig. 2. Micrographs of particles formed by PVP-stear with M_n = 2600 (5.0 mg/ml) obtained by transmission electron microscopy.

shapes are close to spherical. However, a large number of publications reveal that there are many non-spherical micelle forms (rod-shaped, ring-shaped, lamellar sheets, worm-like structures, tubular structures, and hexagonal packages). Usually, these types of structures are formed from copolymers with asymmetric design; however, there is always a balance between different shapes in solution [18].

Particles formed from PVP-stear were investigated by transmission electron microscopy (Fig. 2). The electron microscopy data provide evidence for the formation of spherical particles with a diameter (for PVP-stear with M_n = 2600) of 100-500 nm.

Dimensions of the particles were studied by using the light scattering technique. Particle distribution by size (presented for PVP-stear with M_n = 2600 in Fig. 3) revealed that there are both small (80 nm) and large (440 nm) particles present in the dispersion.

Based on an assumption about the linear structure of the polymer (M_n = 2600), the maximal radius of its macromolecule at rough approximation will be 10 nm. In theory, the size of micelle particles should not exceed 20 nm. When correlating this value with the data obtained using dynamic light scattering, the conclusion is that

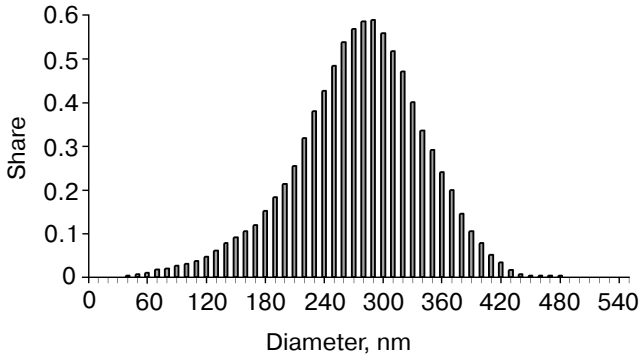

Fig. 3. Size distribution of PVP-stear with M_n = 2600 (5 mg/ml) particles in physiological solution.

there are particles with larger size than micelles. Therefore, in this case polymer aggregates are formed, and the more appropriate term would be the "aggregation concentration" C_{aggr} instead of CMC.

Table 2 summarizes average diameters of PVP-stear aggregates of different molecular weights. The average diameter of the particles in the physiological solution is 1.8 times smaller than in water. Such decrement in particle size can be explained by the increased hydrophobic interaction in the solution with higher ionic strength, which results in the formation of a more compact polymer structure.

Hence, it has been established that amphiphilic polymers based on PVP form large associates of spherical macromolecules.

An important characteristic of polymer particles is their mechanical stability. Using the dynamic light scattering technique, the sizes of polymeric aggregates were determined after filtration on a 0.22-μm Millipore filter, and it was also revealed that under applied pressure the size of the particles after filtration becomes approximately 200 nm, i.e., comparable to the size of the filter pores. Thereafter, particles obtained after filtration were subjected to 15 sec of ultrasonication. It would be reasonable to assume that polymer particles would be destroyed; however, the opposite trend was observed, where the particle

Table 2. Sizes of PVP-stear aggregates of different molecular weight determined using dynamic light scattering

M_n, daltons	Average diameter, nm	
	water	physiological solution
2000	540 ± 20	288 ± 10
2600	560 ± 20	298 ± 10
3000	560 ± 20	300 ± 10

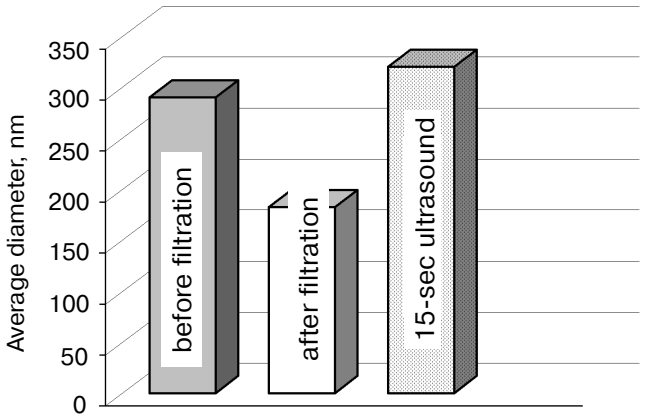

Fig. 4. Mechanical stability of PVP-stear (M_n = 3500) polymer aggregates.

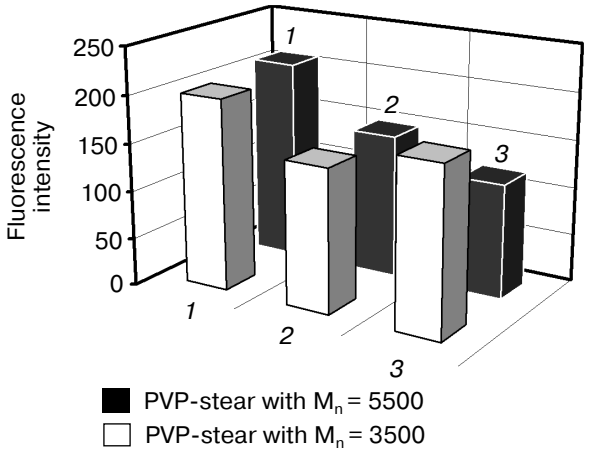

Fig. 5. Change in fluorescence intensity of pyrene label upon inclusion in PVP-stear aggregates (during the incubation with human blood serum for 30 min): *1*) PVP-stear aggregates containing solubilized pyrene; *2*) pyrene in the presence of human blood serum; *3*) PVP-stear aggregates containing solubilized pyrene in the presence of human blood serum.

size became equal to the original one. Apparently, this size of the obtained particles is the most thermodynamically advantageous (Fig. 4).

Interaction of PVP-stear aggregates with blood components. Adsorption of blood serum proteins after the intravenous application of polymeric medical drug formulations significantly affects their distribution in living tissues and organs. When studying the interaction of different formulations with blood components, the model systems most often used are human blood serum or guinea pig blood serum as well as sheep erythrocytes sensitized by rabbit antibodies towards them [13].

Since the properties of medical drug delivery systems are mostly determined by their interaction with different blood components, our first task was to analyze the stability of polymeric aggregates in the presence of human blood serum. As we presented earlier, PVP-stear aggregates are able to solubilize pyrene. By the change in fluorescence intensity of the included pyrene label, the degree of destabilizing action of human blood serum on polymer particles was determined.

Figure 5 illustrates that when the aggregates (formed by PVP-stear with low molecular weight) are incubated with blood serum, a certain decrease in fluorescence intensity of the included pyrene label occurs. This reveals either the destruction of PVP-stear polymer aggregates or the quenching of fluorescence by blood components. For polymers with the higher molecular weight, this effect is more noticeable.

The next step in the research included the test of direct lytic action of PVP-stear preparations on the sheep erythrocytes sensitized by rabbit antibodies (EA). Table 3 illustrates that PVP-stear 4500 and 5500 perform a powerful direct lytic action on sensitized sheep erythrocytes. Aggregates based on PVP-stear 3300 are less aggressive in action towards EA, while those consisting of PVP-stear with molecular weight of 1500 do not lyse sheep erythrocytes.

Table 3. Direct lytic action of PVP-stear aggregates with different molecular weight on sensitized sheep erythrocytes

[PVP-stear], mM	Lysis, %			
	M_n = 1500 daltons	M_n = 3300 daltons	M_n = 4500 daltons	M_n = 5500 daltons
0	3	3	3	3
0.2	2	3	8	8
0.4	0	5	93	73
0.6	0	10	94	85
0.8	2	13	96	92

Table 4. Influence of human albumin on the lytic action of PVP-stear preparation on sensitized sheep erythrocytes

PVP-stear 1500 (0.2 mM)	Human albumin, μM	Lysis, %
—	0	0
+	0	0
+	7.7	0
+	15.4	0
+	23.1	0
+	30.8	0
PVP-stear 3300 (0.3 mM)	**Human albumin, μM**	**Lysis, %**
—	0	0
+	0	2
+	7.7	5
+	15.4	3
+	30.8	3
PVP-stear 4000 (0.2 mM)	**Human albumin, μM**	**Lysis, %**
—	0	0
+	0	4
+	7.7	5
+	15.4	3
+	23.1	4
+	30.8	1
PVP-stear 5500 (0.3 mM)	**Human albumin, μM**	**Lysis, %**
—	0	0
+	0	8
+	7.7	4
+	15.4	6
+	30.8	4

The next aspect in the investigation of PVP-stear preparations was the study of the influence of human albumin on the cytolytic action of the polymer particles. In the presence of albumin, PVP-stear aggregates 1500 and 3300 still provide virtually no lytic action on sensitized sheep erythrocytes (Table 4).

Studies on the influence of albumin on the cytolytic action of PVP-stear preparations on EA revealed that lytic concentration of PVP-stear (4000 and 5500) in the presence of albumin at concentrations higher than 7.7 μM stops functioning after some time. The decrease in EA lysis indicated interactions between albumin and PVP-stear 4000 and 5500 preparations.

Hence, the investigation has demonstrated that PVP-stear preparations do not unambiguously interact with human albumin.

The following step of the work was a study of complement system activation in the presence of the polymer particles. The complement system is a part of the immune system and provides nonspecific protection against microorganisms and tumor- and virus-infected cells. In this work the influence of PVP-stear aggregates on the activation of the complement system was investigated. First, the activation of complement system by CP and AP was evaluated according to lag-t, i.e., time from the moment of erythrocyte introduction into the investigated serum until the initiation of lysis. This value characterizes the rate of limited proteolysis reaction cascade and formation of a membrane-attack complex. Second, the value of rate of lysis (V) was investigated. This is measured according to the decrease in opacity of erythrocyte suspension and reflects the "work" of the membrane attack complex in cells lysis. As seen from the data in Table 5, none of the investigated preparations had a significant influence on the activation of complement by CP and AP.

The conclusion from the data in Table 3 is that PVP-stear 4500 and 5500 exhibit a profound hemolytic action towards the sensitized sheep erythrocytes already at the concentration of 0.4 mM. Because of the fact that rabbit erythrocytes are even more sensitive to the lytic action of detergents [19], it can be assumed that they can also be subjected to lysis under the action from high-molecular-weight PVP-stear. At the same time, the sum of comple-

Table 5. Influence of PVP-stear on the activation parameters of the complement system via alternative (AP) and classical (CP) pathways. The results are presented in % of control ($n = 3$)

PVP-stear (5 mM)	lag-t (AP)	V (AP)	lag-t (CP)	V (CP)	HCC
PVP-stear 1500	87 ± 9	85 ± 22	98 ± 5	99 ± 5	100 ± 5
PVP-stear 3300	95 ± 19	94 ± 7	98 ± 5	97 ± 5	100 ± 5
PVP-stear 5500	91 ± 5	102 ± 13	98 ± 5	90 ± 7	100 ± 5

ment-dependent and detergent hemolysis is not seen in the presence of hemolytic concentrations of PVP-stear as well as there is no observed acceleration of erythrocytes lysis. This indicated that certain blood serum proteins, which are not affiliated to the complement system, bind the samples and prevent their action both on erythrocyte membrane and complement system.

The absence of an influence of PVP-stear on HCC value revealed by us once again proves that the investigated preparations do not initiate the complement cascades and do not decrease the lytic potential of the system. This fact can be seen as evidence for the biocompatibility of the investigated preparations. The absence of an influence of PVP-stear on both blood serum complement and on sheep erythrocyte membrane in the presence of albumin proves sorption of albumin and, perhaps, of other blood components.

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