The results of the studies of PVEP–BSA complex solutions with different composition formed by polycations with a different molecular mass, Mw, are listed in Table 2

Table 2. Molecular characteristics of the particles of soluble Q-PVP-BSA polycomplexes with different composition at pH = 7, in 0.01 N NaBr.

$M_{w_{Q\text{-}PVP}}$	Mixture composition $\xi \cdot 10^{-2}$	$n_{BSA}/n_{Q\text{-}PVP}$	$(\partial n/\partial c)_{\mu}$ dl/g	$M_w \cdot 10^{-5}$	$A_2 \cdot 10^4$	Polycomplex composition $n_{BSA}/n_{Q\text{-}PVP}$
$1.2 \cdot 10^5$	3.3	3:1	0.188	3.6	3.25	3.4:1
	2.5	4:1	0.179	4.2	-	4.3:1
	2.0	5:1	0.181	5.1	3.33	5.5:1
	1.8	6:1	0.200	5.7	2.08	6.4:1
$2.5 \cdot 10^5$	3.3	7:1	0.188	7.6	2.16	7.3:1
	2.5	9:1	0.179	8.6	0.94	8.7:1
	2.0	11:1	0.181	10.5	0.72	10.3:1
	1.7	12:1	0.200	11.2	0.50	12.4:1
$3.2 \cdot 10^5$	3.3	8.1	0.188	8.9	0.82	8.1:1
	2.5	11:1	0.179	11.3	0.23	11.6:1
	2.0	14:1	0.181	13.2	0.17	14.3:1
	1.8	16:1	0.200	14.1	0.01	15.6:1

As it is seen an increase in the complex molecular mass with increasing protein content in the reaction mixture is characteristic of all the studied PVEP-BSA systems. If to know the molecular masses of the molecular masses of the complex particles and those of individual components it is possible to calculate the molecular composition of the complex particles. The results of such calculations are listed in Table 2. It is seen that at any content of protein in the mixture being in excess of the characteristic composition the soluble polyelectrolyte complex composition within the experimental error coincides with that of the reaction mixture. This fact is in agreement with the high speed sedimentation data and indicates that only one PVEP macromolecule enters each polycomplex particle over the studied range of concentrations and component ratios. Since an increase in the polycation DP results in an enhancement of the number of BSA globules in the polycomplex and the number of protein molecules increases proportionally to the polycation length, it is also convenient to express the polycomplex composition in terms of the ratio $\xi = \frac{DP}{N_i}$, where N_i is the number of BSA molecules sorbed by one chain of PVEP. This ratio does not depend on the polycation molecular mass. Note that the polycomplexes with the characteristic composition formed by BSA and the polycation chains of different length correspond to the composition $\xi=3.3 \times 10^2$ An increase in BSA content in the polycomplex results in a decrease in the ξ value.

As it seen in Table 2 PVEP-BSA polycomplex particles of the characteristic composition have the minimum molecular mass and the greatest affinity for the solvent. Addition of the protein to the reaction mixture in amounts exceeding the characteristic ones causes together with an increase in molecular mass of polycomplex particles a decrease in the values of the second viral coefficients of the solutions. This behaviour of the systems permits to assume that the addition of the protein to the solution in amounts greater than N_i causes a reaction in which the particles of the polycomplexes with the characteristic composition and free protein globules are interacting components. Since under the conditions of the reaction when pH=7 BSA globules are negatively charged (pI = 4.9) it is responsible to assume that ionogenic groups of the polycation included to the polycomplex and having not formed salt bonds with protein molecules

are responsible for BSA binding to polycomplexes with the composition $\xi=3.3 \times 10^2$. The existence of the charged polycation sequences in the soluble polycomplexes directly fallows from the viscometric data.

The further definition of the PVEP-BSA complex structure is obtained from the comparison of the intrinsic viscosity and the molecular weight for these complexes. Figure 26 shows the dependence of the intrinsic viscosity logarithm for solutions of the polycomplexes with the characteristic composition on the logarithm of their molecular weight.

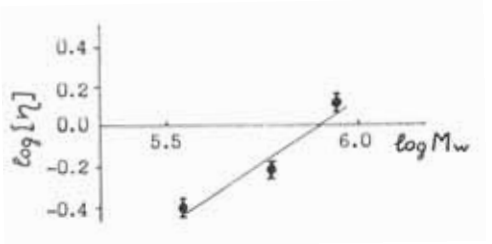


Figure 26. The dependence of log[η] on log M_w for the Q-PVP-BSA SPEC. $\xi = 3.3 \times 10^2$.

This dependence obeys Mark-Kuhn-Houwink equation:

$[\eta]=KM^a$ with the parameters $K=0{,}11.10^{-5}$ and $a=1.4 \pm a^3$

The latter value corresponds to rather rigid asymmetrical rods and suggests a rod-like model for the soluble polyelectrolyte complex particles. To determine the shape of the polycomplex particles with the composition $\xi=3.3x10^2$ the dependences of the root mean square radii of gyration on their molecular mass Mw were studied by light scattering. It is seen in Figure 27 that these dependences are linear for all studied compositions.

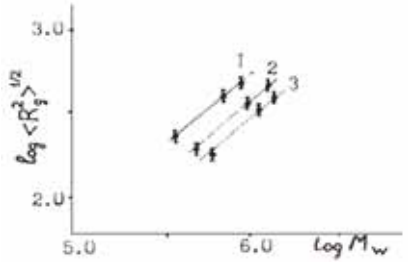


Figure 27. Dependence of log $<R^2>^{1/2}$ on $\log M_w$ for the particles of the Q-PVP-BSA complexes. $\xi = 3.3 \times 10^2$ (1); $\xi = 2.0 \times 10^2$ (2); $\xi=1.8 \times 10^2$ (3).

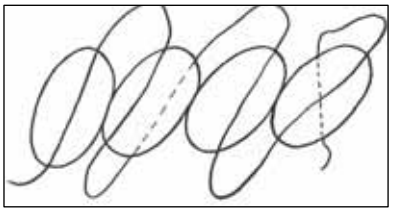


Figure 28. Proposed structure of Q-PVP-BSA soluble complex with characteristic composition.

It means that the polycomplexes with the similar composition may be considered as the representatives of one polymer homologous series. The dependence of the root mean square radius of gyration on the molecular mass of the polycomplex particles can be described by a known equation: $[R_g^2]^{1/2}=\text{const}.M^{\alpha}$ where $\alpha= 1 + 0.15$ that also corresponds to the asymmetrical rods.

Model of the Structure of Polycomplex particles with characteristic composition (N_i). Based on above-mentioned results Kabanov and Mustafaev proposed a rod-like model for the soluble polyelectrolyte complex particles with characteristic composition. This is shown in Figure 28.It should be accepted that protein globules join with each other in some way forming an asymmetric

stack. The latter may be approximated by a cylinder. The polycation chain encircles the butt-joined protein globules and the longer the polycation chain the longer the cylinder is.
It is remarkable that the described above scheme of the complex structure, obtained as a result of the analysis of the overall physical-chemical measurement data is confirmed by the data of electron microscopy. The complex micrograph obtained for the systems (BSA-PVEP and BGG-PVEP) in which $n_{BSA}/n_{PE}= N_i$ are given in Figure 30.

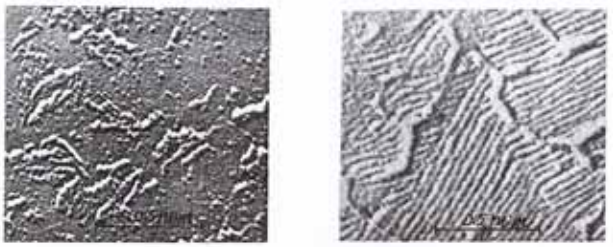


Figure 29. Micrographs of Q-PVP-BSA polycomplex when $n_{BSA}/n_{Q\text{-}PVP} = N_i$ DP=10^3. a-sample is prepared by putting a drop of the complex solution (0.01 g/dl) on the preparative grid with a substrate and removal of the solution bulk with filtering paper; b- sample is prepared by a slow evaporation of the solvent from the complex dialyzed solution (0.01 g/dl).

Extended linear formations consisting of globular particles coupled one with the other is distinctly seen in the micrograph. The thickness of each rod consisting of globules is about 100 A°. The solubility of the complex was attributed to the hydrophilic contribution from the loops of the bound polymer chain.

Figure 30. Electron micrographs of PVEP-BGG polycomplex when n_{BGG}/n_{PE}=2, DP=0,95.10^3. (Sample preparation as in the case of BSA-PVEP).

Model of the Structure of Polycomplex particles over the component ratios corresponding characteristic composition (at $N > N_i$). As shown in Figure 31an increase of the number of protein molecules in the mixture (the weight concentration of polycation is kept constant) leads to a decrease of the values of the reduced viscosity (η_{sp}/C) of PVEP-BSA mixtures When the ratio of components is $N=N_s$ the successive addition of BSA result in sharply decreases of the reduced viscosity and the (η_{sp}/C) characterized by very low values (0.03-0.05). The described situation is typical for all studied fractions of PVEP interacting with BSA. Comparison of the inherent viscosities and the sedimentation coefficients for these complexes formed by the polycations with different length when $n_{BSA}/n_{PE}=N_s$ shows that as the viscosity of the solutions remains practically unchanged with increasing degree of polymerization of the poycation the sedimentation coefficients for polycomplexes increases. As it fallows from light scattering data of the polycomplexes at any content of protein in the mixture being in excess of the characteristic

composition ($N > N_i$) an increase in the complex molecular mass (Mw) is characteristic of all the studied PVEP-BSA systems (Table 2) From obtained values of Mw of complexes and ratios of components it fallows that only one polycation chain is contained in the particles of BSA-PVEP complexes. Thus, the obtained relation of this kind may exist for sufficiently high compactness of particles of soluble polycomplexes at their elongation. These data allow to consider the structure of water-soluble PVEP-BSA complexes formed at $N > N_i$ (N_s) also as conglomerates of BSA globules assembled by one chain of PVEP. In this model the bound polycation chains are localized in a hydrophobic center wherein the positive charges from PVEP are neutralized by the negative BSA charge; the hydrophobic center is also surrounded by negative charged BSA, which provides a net complex surface charge and is thus responsible for the solubility of the complex (Figure 31).

The phase separation in the PVEP-BSA systems occurs only at some critical protein concentrations ($n_{BSA}/n_{PE} \geq N_s$) depending on the degree of polymerization of polycation (see Figure 31). As it follows from the curve of turbidimetric titration of a PVEP solution with a solution of proteins (BSA, OA, BGG) and vice versa titration of protein solutions (BSA, HSA, BGG) and mixtures of different proteins (BSA+BGG, BSA+β_1-G; β_1-G+BGG, BSA+β_1-G+BGG, whole serum and blood) with solutions of polycations one can distinguish three regions of reaction mixture composition (Figure 30).

In region I (for the systems when polymer solutions were titrated with protein) the solutions remains homogenous due to formation of water-soluble polyelectrolyte complexes. The successive addition of protein molecules at $n_{BSA}/n_{PE} > N$ leads to phase separation-region II. The maximum value of turbidity, as it was noted in numerous studied polyelectrolyte-protein systems, is reached at equivalent ratio of oppositely charged groups of proteins and polycations. The addition of the protein above this equivalent ratio is accompanied by a decrease in the turbidity of the solution, and at some critical ratios (region III) the system again becomes homogeneous. The soluble fractions of PVEP-BSA mixtures (supernatants) was studied by sedimentation analysis after removing of the precipitate. Figure 31 shows the dependence of the amount of precipitate (1), concentration (area of the peaks) (2) and coefficient sedimentation (3) of soluble complexes and concentration of free protein molecules in the supernatants (4) on the ratios of components.

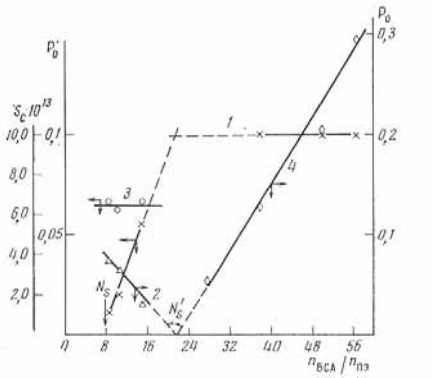


Figure 31. Dependence of the amount (P_o^1) of concentration (2) and sedimentation coefficient (3) of soluble complexes and concentration of free protein molecules in matrix solutions (4) on n_{BSA}/n_{PE}; CPE=0,15 g/dl; pH 7.0.

From the linear form of these dependences it follows that an increase protein concentration in mixture leads to a corresponding increase of the amount of precipitate and then attains a limiting value, while the concentration of soluble complexes decrease and their peak on sedimentograms disappears at N_s ratios (region II.). Sedimentation coefficients of the peaks remain the same; the composition of soluble complexes remains virtually unchanged. At the

ratios corresponding to maximum value of precipitate there are no reaction components and products (the protein, the polycation or water-soluble polycomplexes) in the solution. The addition of the protein above this ratio is accompanied by a decrease in the turbidity of the solution, and at some critical values of ratios (region III) the system again becomes homogeneous.

As it mentioned above about 55 negatively charged carboxylate groups of the native BSA molecules are able to form salt bonds with the polycation, though the total number of carboxylic acid groups of BSA is 125 (50 Asp +75 Glu) [97]. It means that only part of the carboxylic groups settled on the surface of BSA globule is accessible for the interaction. The composition (Q) of polycomplex [98,99] was expressed as the ratio of the total quantity of negative charges on the protein molecules (a) to the total number of positive charges on the polycations (b) in the mixture. The value of Q is related to the mass concentration of the components C (g/l) by the equation:

$$Q=a^-/b^+ = 55 \times (C_{BSA}/M_{BSA}) \times (M_{PEVP}/C_{PEVP})$$

Figure 32 shows the dependence of relative optical density A/A_{max} of a mixture of BSA and PVEP on the mixture composition Q. On sedimentograms of the solutions (supernatants) obtained at Q>1 two peaks are observed. The sedimentation coefficient 4.3 S of slowly sedimenting particles ("slow" peak) virtually coincided with that one of free BSA while quickly sedimenting particles were characterized by rather large value 25 S ("fast" peak). This means that at 2.5 > Q > 1 free protein coexists with newly formed water-soluble PVEP-BSA complex particles and insoluble PVEP-BSA complex particles. Addition of BSA till Q=2.5 is accompanied by a concurrent increase in the areas of both peak, and the area of "fast" peak is much greater than that one of "slow" peak. The successive addition of BSA after complete solution of the precipitate (Q > 2.5) does not lead to noticeable change of the "fast" peak area, while the rate of growth of "slow" peak area increases. Sedimentation coefficients of both peaks at Q > 1 (in both heterogeneous and homogeneous regions) remains the same, 4.3-4.5 S and 25-26 S respectively. The character of turbidimetric titrations virtually did not change under variation of PVEP degree of polymerization in the studied region P_{PEVP} = 200-1500.

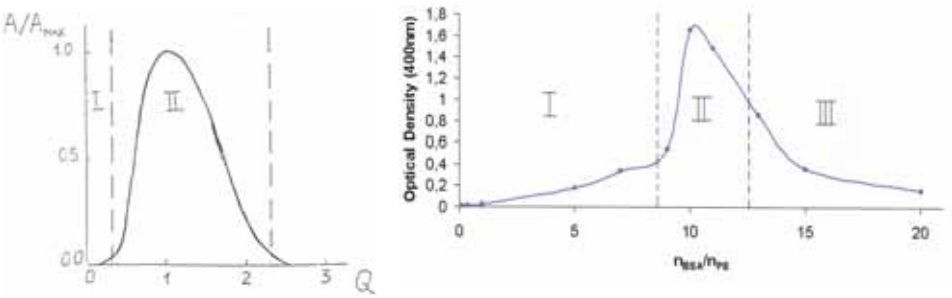


Figure 32A. Dependence of relative optical density A/A_{max} of a mixture of BSA and PEVP or the mixture composition Q. [PEVP] = 1 g/l, Pη=1,3.10^3

B. Dependence of optical density (OD_{400} nm*)* of a mixture of BSA and PEVP on the n_{BSA}/n_{PE} Pη=10^3; pH 7. (unpublished results of Dr. Zeynep Mustafaeva and Eray Dalgakıran).

From the areas of sedimentation peaks of soluble complexes BSA-PVEP and free BSA calculated the amount of BSA (C_{BSA}) including in the complexes and then a composition φ of the complexes, that is expressed as the ratio of total quantity of negative charges on the protein molecules (a^-) to the total number of positive charges on the polycations (b^+) in the complexes:

$$\varphi = a^-/ b^+ = 55 \times (C_{BSA}/M_{BSA}) \times (M_{PVEP}/C_{PVEP})$$

The composition of complexes provide to be not influenced by both P^{-}_{PVEP} and mixture composition Q in the whole studied region Q> 1. The value of this characteristic composition φ_c was equal to 2.0 + 0.3.

Figure 33 shows the dependence of the average molecular mass M_{sd} of the water-soluble BSA-PEVP complex on the degree of polymerization of polycation. The values of average molecular mass M_{sd} of the complexes were calculated from both the sedimentation data and QELS data using Svedberg's formula: $M_{sd} = \frac{RT}{(1-vp)} x(s/D)$, where R is the universal gas constant, T is the temperature (293K), p is solvent density, s and D are respectively the constants of sedimentation and diffusion of the complex particles. The specific partial volumes v of the BSA-PVEP complexes and free BSA virtually coincide, v = 0.75 + 0.03 [100]. From the linear form of this dependence it follows that an increase in the chain length leads to a corresponding increase in the number of protein molecules in the complex, while its composition φ_{sd} remains virtually unchanged. The average value of φ_{sd} calculated from the tangent of the slope of the line in Figure 33 is 1.9 + 0.3 that is in a good agreement with the value φ calculated from sedimentation peaks areas. The number of BSA molecules N_{BSA} and polycation molecules N_{PVEP} in the complex which were calculated from known values of M_{sd}, and the molecular mass of the components are given in Table 2.

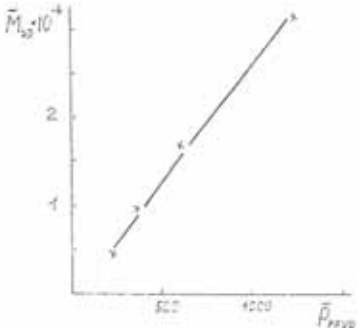


Figure 33. Dependence of the molecular mass $\overline{M}_{sd}$ of the BSA-PVEP complex on the degree of polymerization of the PVEP.

Table 3. Molecular characteristics BSA-PVEP complexes

$\bar{P}_{PEVP}$	$\bar{M}_{sd} \times 10^{-3}$, Da	N_{PEVP}	N_{BSA}
250	700	1	10
400	1100	1	15
650	1600	1	20
1300	3000	1	40

From these data is follows that only one polycation chain is contained in the particles of dissolved BSA-PVEP complex.

One can distinguish such three regions of reaction mixture composition also in the solutions of polycations containing the different proteins. Figure 34 shows the curve of turbidimetric titration of a BSA + BGG mixture solutions with a solution of PVEP. Curves of the titration are presented as the dependence of the amount of the precipitate (m). On the concentration of PE which were added to solution of BSA+BGG mixtures with constant concentrations. The dependence passes through a maximum. Starting with very low concentrations of polycation in protein mixtures (Cproteins = 0.5g/dl BSA+0.5g/dl BGG; C_{PE}=0.025 g/dl PVEP Cprot./C_{PE} = 40) phase separation took place, which indicates the formation of an insoluble triple polycomplexes.

On further increase in polycation concentration, the amount of the precipitate increases and then attains a limiting value. In this region as it fallows from sedimentation and electrophoretic datas of the mixtures, after removing of the precipitate, free protein fractions (BSA, sedimentation coefficient 4.5 S and electrophoretic mobility u = 6.0 sm.v.sec.; BGG, 7.2 S and u=1) coexists with soluble and insoluble polycomplexes containing both BSA and BGG. At the ratio of components, which correspond to situation of maximum amount precipitation in mixture, all components were included in composition of insoluble polycomplex particles. In matrix solution there are no polymeric compounds. On sedimentogram of the supernatant solution at C_{prot}/C_{PE}=13 only one peak (sedimentation coefficient 8S) which correspond to soluble polycomplex particles is observed (Figure.curve 4). One can assume that its correspond to mixed ternary PVEP-BSA-BGG complex This means that at C_{prot}/C_{PE}=13 ternary water-soluble complexes coexist with insoluble ternary complexes. After complete solution of the precipitate the soluble complexes protein-PE are formed by separate distribution of individual proteins at the matrix (it is remarkable that decrease of polycation molecules in the system results in the formation of a soluble complex of mixed composition). These polycomplexes (BSA-PVEP and BGG-PVEP) coexists with free polycation macromolecules. Such situation corresponds to those ratios where uneven distribution of protein molecules between polycation chains is observed [34].

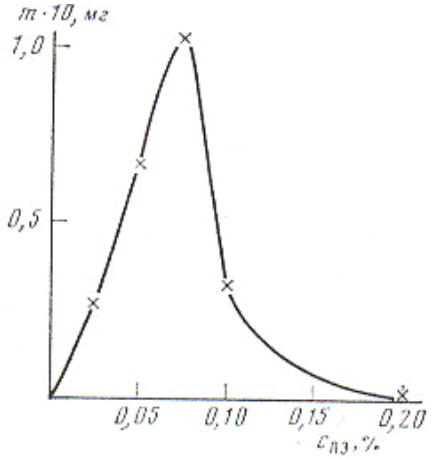


Figure 34. Dependence of the amount (m) of the insoluble complexes on the initial concentrations of adding PVEP (C_{PE}); C_{BSA}=0,5g/dl.

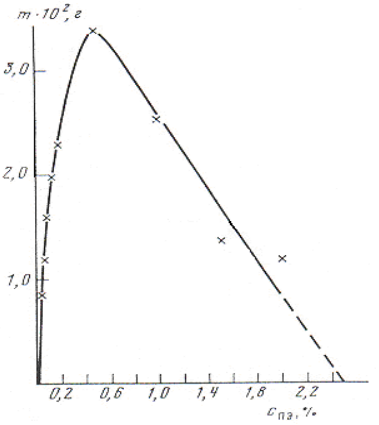


Figure 35. Dependence of the amount (m) of the insoluble complexes on the concentrations of adding PVEP (C_{PE}) in serum-PE mixtures. Initial serum is diluted 1:3 ratio. pH 7.5.

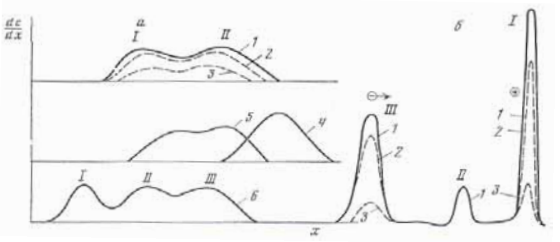


Figure 36. Sedimentograms (a) and electroforegrams (b) of the artificial mixtures BSA+BGG in the absence (1) and in the presence of PE at the different C_{PE}: 0.025 (2); 0.05 (3); 0.075 (4); 0,1 (5); 0.3 g/dl (6); $C_{Proteins}$= 0.5 g/dl BSA+ 0.5 g/dl BGG.

It has been shown that above mentioned dissolve character of insoluble polycomplexes and selectivity at binding protein fractions is observed in both artificially prepared systems (BSA+BGG, β_1-G + BGG, BSA + β_1-G + BGG) (Figure 36) and in serum and whole blood [38]. As it seen in Figure 37 one can observed the same peaks on sedimentograms of the supernatants of PVEP-serum and PVEP-whole blood, which are corresponds to soluble polycation-proteins complexes. Decrease of polycation concentration in the systems results in the formation of a soluble complex of mixed composition. When an insoluble complex is formed serum-PVEP selective sorption of β_1-globulin fractions is observed. These phenomenons were systematically studied by Mustafaev [33, 38].

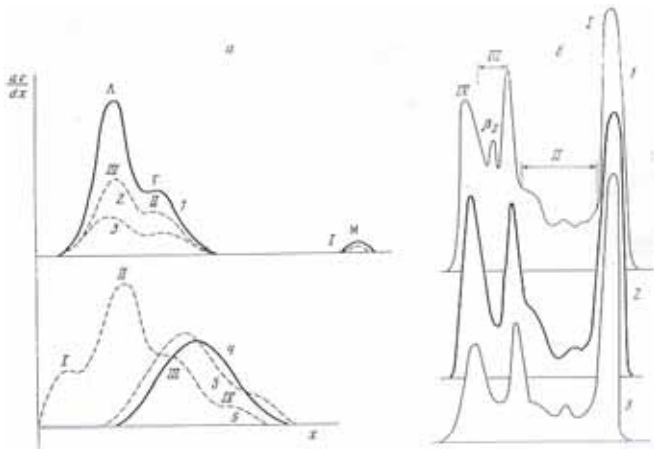


Figure 37. Sedimentograms (a) and electroforegrams (b) initial whole serum and matrix solutions of serum+PE, at different concentrations of PE (g/dl): 0.1 (2); 0.3 (3); 1.0 (4); 1,5 (5); 2.5 (6); pH 7.5; A-albumin, G-globulin, M-macro globulin, serum is diluated 1:3 ratio, with 0,025N NaCl solution.

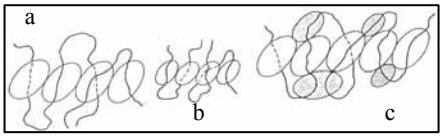


Figure 38. Proposed structures of complexes protein-PE: a-(BGG-PE) at $n_{BGG}/n_{PE} \leq N_i^1$, b-(BSA-PE), (α-globulin-PE), (β-globulin-PE) at $< N_i$; c-structure of polycomplex particles with different protein fraction which form in multicomponent mixtures at $n_{protein}/n_{PE} > N_i$

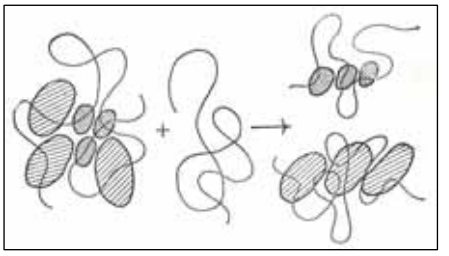


Figure 39. Redistribution of different protein globules between linear polycations

In [34] an important property of protein-polycation complexes was noted, that is the solubility in water when there is more than three-fold excess of positively charged PVEP units relative to negatively charged protein carboxylate groups. Segments of PVEP chains carrying excess charge act as the lyophilizing fragments of the complex particles. Region I in Figure 36

corresponds to formation of such positively charged water-soluble protein-polyelectrolyte complexes (see structure in Figure 38).

The maximum amount of precipitate is reached at equivalent ratio of oppositely charged groups of proteins and polycations (Q=1, n_{BSA}/n_{PE} = N's). The values of N_s and N_s are actually correspond to maximum number of protein globules which can join in composition of water soluble and insoluble polycomplexes at the given degree of polymerization of polycations, correspondingly. As it seen in Table 3 N_s and N_s as well as N_i is linear increased by increasing degree of polymerization, i.e. the length of counter of polycation. It means that in the average the site of the polycation chain of the approximately constant definite length is used for such globule binding, i.e. the number of additionally binding protein molecules per one protein in composition of water-soluble and insoluble polycomplex particles with characteristic composition is constant and equal 2 ($(N_s-N_i)/N_i$=2) and 3, correspondingly. Data obtained in [34] allow to consider the structure of such complexes in this region. Positively charged rod-like polycomplex particles at certain critical n_{BSA}/n_{PE} ratios aggregate with additional number of protein molecules and form soluble and insoluble cooperative complex particles with a more compact structures (Figure 39). In this strongly hydrophobic structure, which is on the limit of solubility, largely charged groups of polycation chain participate in formation of ionic bonds with protein globules.

Region III corresponds to formation of negatively charged water-soluble protein-polyelectrolyte complexes. The precipitate is dissolved by means of formation of the complex, which is almost doubly enriched in the protein. The characteristic composition φ =2 of the dissolved complex is determined by the minimum amount of protein necessary for attachment to the complex to provide the hydrophilicity of the particle as a whole. It is clear that with an increase in Q at Q > 1 ($n_{BSA}/n_{PE} = N_i$) the total number of salt bonds calculated per polycation remains unchanged: only their number taken over one protein globule decreases. Therefore the conclusion of an additional number of globules in the soluble complex of composition, does not occur, since this would lead to a decrease in the total number of particles that is unprofitable entropy wise, and is not compensated for by decrease in the enthalpy of the system. Data obtained in different systems [34,35,98,99] allow to consider the structure of negatively charged water-soluble protein-polyelectrolyte complexes formed at Q > 1 as a conglomerates of BSA globules assembled and reinforced by one chain of polycation. In this model the bound PVEP polycation chains are localized in a hydrophobic center wherein the almost all positive charges from polycation are neutralized by the negative protein charge; the hydrophobic center is also surrounded by negative charged proteins, which provides a net complex surface charge (negative) and is thus responsible for the solubility of the complex (Figure 40).

Figure 40. Proposed structure of the soluble BSA-PVEP complexes by Zaitsev. (from Ref. [98])

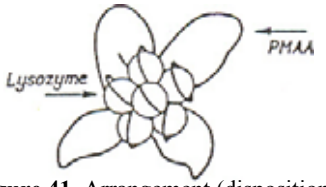


Figure 41. Arrangement (disposition) of structured parts of the lysozyme-PMMA polycomplex in the aqueous solution when the relative amount of protein in the solution is 1 and α_{PMAA} = 0.4) (from Ref. [68]).

For the lysozyme-PMAA complex, Anufrieva [68] recently suggested that some PMAA domains are filled with protein, while other domains remain unoccupied (Figure 41) for the excess protein case.

Soluble protein-polyelectrolyte complexes are usually formed at a pH close to the (protein) isoelectric point of the protein (IEP), and the soluble complexes aggregate to form coacervates toward phase separation by adjusting pH. It was suggested the existence of the primary intrapolymer complexes (in which a single polymer chain is bound to several protein molecules) for the excess protein case. These primary complexes could aggregate to form interpolymer complexes, in which several polymer chains are involved, as shown in Figure 42. The interpolymer complex could be soluble up to the point of the large-scale aggregation.

Kokufuta [87a] proposed a model for stoichiometric insoluble protein-polyelectrolyte complexes in salt-free system (see below).

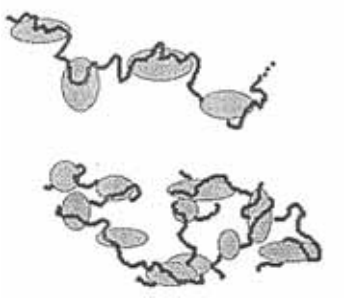


Figure 42. Intrapolymer and interpolymer structures of protein-polyelectrolyte complexes (from Ref. [79b]).

2.2. Water-soluble Complexes of Polyelectrolytes with Samely Charged Proteins

It is generally believed that the electrostatic interactions constitute the primary driving force for the formation of protein-polyelectrolyte complexes. Association of proteins with polyelectrolytes at pH > pI for polycations, and at pH < pI for polyanions has been attributed to salt linkage.

Complexes of Polyanions. It was found that some polyanions bind proteins at pH > pI [37, 57, 78]. It has been shown by high-velocity sedimentation that BSA and FDH form soluble complexes with polyacrylic acid (PAA), polymethacrylic acid (PMAA) and sodium polystyrene sulfonate (PSSNa) in neutral water solutions [37]. The typical sedimentograms of PSSNa and its mixtures with BSA at different ratios of their macromolecule concentrations in the solution as example are given in Figure 43. As it fallows from this figure in the general case the system is characterized by a unimodal distribution (by one peak) of sedimenting components. An increase of the number of protein molecules in the mixture leads to an increase both of the area of the peak and sedimentation coefficient of singular peak. These results unambiguously show protein and polyanion binding to a complex. Protein heterogeneous charge distribution (and strong binding capacity of $-SO_3$ anions [37]) and possible hydrophobic interaction of protein globules with hydrophobic polystyrene fragments can help to interpret this observation.

The analysis of the BSA-PAA, BSA-PSSNa and FDG-PSSNa systems by the sedimentation method showed that after adding NaCl salt, the soluble protein-polyanion complex particles lose some protein molecules in consequence with the compactization of complex particles.

Recently, the effects of protein charge heterogeneity in protein-polyelectrolyte complexation was described by Dubin [57]. The interaction between three monomeric globular proteins of substantially different isoelectric points (BSA, chicken egg lysozyme, and bovine pancreas ribonuclease) and synthetic polyelectrolytes with different chemical composition was investigated by turbidimetry and quasielastic light scattering (QELS) techniques in water-salt solutions. It was examine the association behaviour of two basic (RNAse and lysozyme) and one acidic (BSA) protein with polycations and polyanions of varying linear charge densities. As shown in the results of computer modeling for proteins RNAse can simultaneously have both

positive and negative potential regions in aqueous solution. This local non-uniform potential region or protein charge patch is believed to provide an attraction force that overcomes the repulsion between the global protein charge and the polyelectrolyte. Therefore, the complexes formed by polyanions with global negatively charged proteins are suggested to be a manifestation of local interaction between the protein charge patch and polyelectrolyte.

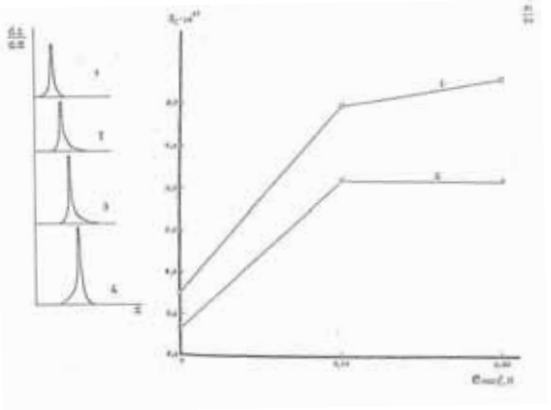


Figure 43. Sedimentograms (a) for the PSSNa-BSA mixtures at different n_{BSA}/n_{PE}: 0 (1); 1 (2); 10 (3); 20 (4); t=40 min; pH 7; b-dependence of sedimentation coefficients for the PSSNa-BSA mixtures (1) and pure PSSNa (2) on adding concentrations of NaCl. C_{PE}=0.15 g/dl; n_{BSA}/n_{PE}=10; pH 7.

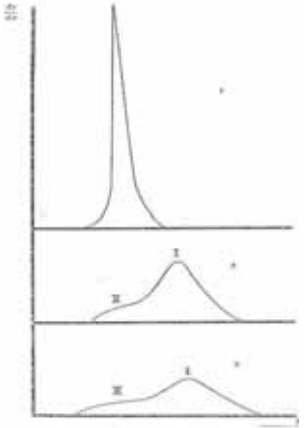


Figure 44. Sedimentograms for BSA-PSSNa mixtures at different concentrations of adding NaCl (C_{NaCl}): 0 (1); 0.15N (2); 0.3N (3); n_{BSA}/n_{PE}=10; pH 7.

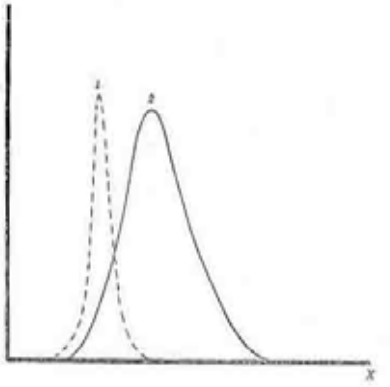


Figure 45. Sedimentograms for PSSNa (1) and its mixture with FDH (2): n_{FDH}/n_{PE} =10; pH 7.5; C_{PE}=0.15 g/dl.

On the bases of the results obtained by sedimentation analysis it was suggested that the complex formation between proteins and polyanions occurs by the random distribution of protein globules between polyion chains. Interaction between proteins and polyanions is not followed by any changes of protein α-helix structure (Figure 46). The data on Optical Rotary Dispersion and Circular Dichroism Spectroscopy of BSA and FDG were not affected by adding polyanion solutions to the protein solution. Strelzowa et al. [70] have observed CD spectral changes in the

range of 250-330 nm for α-chymotrypsin upon mixing the protein with dextran sulfate. However, the CD spectrum of α-chymotrypsin is not affected by adding dextran sulfate to the protein solution. The linear dimensions of the fractions of polyanions are considerably larger than those of the binding protein globules and every polyanion chain can bind some protein globules in succession. It may be deduced that this type complexes are formed as a result of the uniform all loading of protein globules between the polyelectrolyte chains, i.e., the protein molecules are randomly distributed between the adsorbing polyions. One polyelectrolyte molecule forms a complex with many of the protein molecules until the polyion fragments are populated with the protein globules. An outline of such a situation is given by the schematic illustration shown in Figure 46. In this case the structure of water-soluble complexes of proteins with samely (negative) charged polyelectrolytes being formed the protein molecules are randomly distributed along polyanion chains which retains the conformation of a statistical coil of the polyelectrolyte carrier. This scheme is conformed with the results of viscosity data of protein-polyanion mixtures. (The inherent viscosity of BSA-PSSNa mixtures is not essentially changing by the titration with protein solutions).

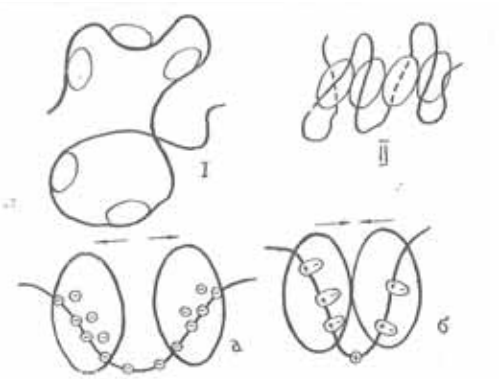


Figure 46. Proposed structures of complexes protein-PE: II-(PVEP-Protein); $n_{Protein}/n_{PE} \leq N_i$; I-(PAA)PSSNa)-Protein); "a" and "b" schematic illustration of the complex formation.

Thus, depending on the chemical nature of the polymeric carrier, protein composition and environmental conditions, two types of soluble polyelectrolyte-protein complexes may be constructed. Complexes of the first type (structure I) is on principle differ from the complexes of the second type (structure II). This difference between two types of the structures of polycomplexes by origin dictates by acting of the factors, which controls the interaction (by attraction or repulsion) between adsorbing protein globules on the polyelectrolyte chains. Initial protein globules are negatively charged in both cases, i.e. electrostatic factor prevent theirs draw together. At the same time, it is known that the non-polar interaction is the promoting factor for the association of protein molecules in water solutions. The formation of electrostatic (salt) bonds between negatively charged groups on the surface of protein globules and positively charged monomer units of polycations constitute the primary driving force at the sorption of the proteins by polycations. As a result the negative charges of protein molecules are neutralized (screening) and do not prevent to join of the bound globules, i.e. manifestation of non-polar interaction. At the same time it is achieved the partial neutralization and screening of the charge of polycations which promote draw together separate part of polycations at the self organization of the structure I. At the sorption of proteins by polyanions of polyacid (for example, PSSNa) the situation is quite different. Binding of negative charged protein globule with the polyelectrolytes takes place despite of electrostatic repulsion. Under this the efficient of negative charge of every sorbing protein globules may only increases at the expence of "stuck" units of polyanion. Then the

"docking "of globules in the polycomplex particles is not profitable (or less profitable) and they settle down separate each other by forming the structure II.

As it was mentioned above the excessive desalination of the system BSA-PVEP results in the electrostatic destabilization of particles of cooperative polycomplex (structure I) with the formation of rather bulky non-ordered aggregates being the products of the statistical interaction of albumin with the polycation (like structure II). Complexation of HSA, human or bovine hemoglobin (Hb), and bovine trypsin (BT) with poly (diallyldimethylammonium chloride) (PDDA) and potassium poly(vinyl alcohol sulfate) (KPVS), in a salt-free system were recently reported by Kokufuta [87a]. A model for stoichiometric complexes of proteins with polyelectrolytes is proposed on the bases of the results obtained (Figure 47).

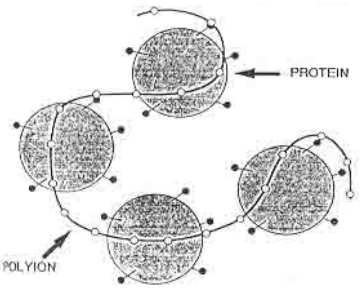
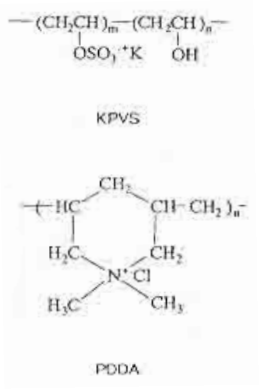


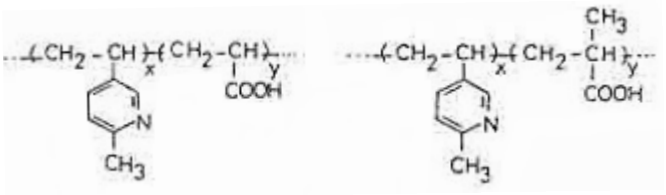
Figure 47. Schematic illustration of stoichiometric complex consisting of protein and polyion. Open and solid circles represent ionizable groups in the polyion and protein, respectively [87a].



Structures of polyelectrolytes studied. The physical data for KPVS and PDDA are as follows: DP_n for KPVS, 1500; [η] (in 1 N NaCl at 25°C) for PDDA, 1,67 $dl.g^{-1}$; equivalent weight referring to the molecular weight of polymer assigned to one mole of ionizable groups, 166 for KPVS and 158 for PDDA; degree of esterification of KPVS which is expressed as *m/(m+n),* 0.925. Both KPVS and PDDA maintain a completely dissociated state in the pH range 2 to 13.

In this model one polyelectrolyte molecule forms a complex with many of the protein molecules until all of the polyion charges are stoichiometrically neutralized with the opposite charges of the proteins. An outline of such a situation is given by the schematic illustration shown in Figure 47 in which the complex consists of a number of inflexible and global protein molecules bridged with loosely extended polyelectrolyte ions. The salt linkages maintaining the structure of the complex as an amorphous precipitate seem to be very "loose", because changes in pH or additions of other polyions sever some of the salt linkages, particularly the linkages between the protein imidazolyl and the KPVS sulfate groups. This looseness may make it possible for the protein and polyion molecules to undergo stoichiometric neutralization or 1:1 binding with their oppositely charged groups through thermal motion.

Complexes of Polyampholytes. Interaction of BSA, BGG and FDH with copolymers (CP) 2-methyl-5-vinylpyridine (MVP),acrylic (AA) and methacrylic acids (MAA) in different proportions in aqueous solutions was studied by Kabanov and Mustafaev [36].



The physico-chemical characteristics of CP are shown in Table 4

Table 4. The physico-chemical characteristics of CP

Copolimer	$[\eta_{sp}/C]$, dl/g	So, 10^{-13}	$M_{sd(\eta)}$	Isoelectric point (pI)
KP-I-66(x)	0,4	2	30000	6
KP-I-50	0,4	1,6	20000	5,5
KP-I-40	0,3	3,8	70000	5,2
KP-I-40	0,7	2,5	60000	4,5 + 6
KP-I-30	0,5	5,4	160000	4,5 + 6

These MVP and AA copolymers (CP-I) contained 66 (CP-I-66),50 (CP-I-50) or 34 (CP-I-34) mol% links of MVP. The MVP and MAA copolymers (CP-II) contained 30 (CP-II-30) and 40 (CP-II-40) mol% links of MVP. These copolymers are characterized with higher polydispersity [36]. According to this, the ratio of the components (n_p/Z) can be calculated as follows:

$$\overline{Z} = C_{cp}.10^{-2}.N_A/[\alpha.M_{AA} + (1-\alpha).M_{MVP}]$$

$$\eta_p/\overline{Z} = C_p.[\alpha.M_{AA} + (1-\alpha).M_{MVP}] \Big/ C_{cp}.M_P$$

Where C-concentration, g/100ml; N_A-Avogadro number; M_o-molecular weight of protein; $M_{AA(MAA)}$ and M_{vp} – molecular mass of AA(MAA) and MVP monomer units of CP correspondingly; α-part of AA(MAA) monomer units in composition of CP.

As it follows from the Table 4 both CP at pH 7 is the above of their isoelectric point and charged negatively. Under identical conditions the molecules of BSA, BGG and FDH also carry a summary of negative charges and preexisting electrostatic repulsive forces between CP and proteins will prevent the formation of stable polycomplexes. However, analysis of interactions revealed that the tightness of their binding to each other depended critically on the MVP monomer units in composition of copolymers.

The typical sedimentograms of CP-I and its mixtures with protein at different ratios of components (n_P/Z-the number of protein macromolecules per number of monomer units of CP) are given in Figure 48. In the general case the system is characterized by a bimodal distribution of sedimenting components. The value of Sc for a slowly sedimenting substance (peak I) is 2.0 saved, i.e. it corresponds to a free CP-I -66. One may assume that rapidly sedimenting substance (peak II) is a complex CP-BSA. Figure shows the dependence of the area of the free CP peak on the ratio of components for CP of a different composition.

A linear decrease of the free CP concentration in the solution during titration with protein in the case of CP-I-66 unambiguously shows its binding to a complex (Figure 48 curve 1). The CP-I-50 also forms the complex with protein molecules. As it follows from this figure, the area of the peak corresponding to free CP-I-50 is decreased by increasing protein concentration in the mixture (Figure 48 curve 2). However, the rate of decreasing of the area of free copolymer

fraction in mixture protein-CP-I-50 is lower than those of the copolymer containing 66 mol % MVP monomer unites. CP-I-40 show only relatively weak tendency to bind with BSA. Decreasing of the area of free CP-I-40 peak quickly end. The intersection points obtained at the extrapolation of the plots in Figure 50 to the zero area of the free CP peak correspond to $(n_{BSA}/Z)x10^3$ when all copolymer macromolecules are bound to a complex with BSA. One may consider that lim $(n_{BSA}/Z)x10^3=N_i$, when $P_0 \rightarrow 0$. This limit equals the number of protein molecules bound by a 1000 monomer units of copolymer in polycomplex particles. The corresponding characteristics for CP-I-66 and CP-I-50 are N_i=6 and 14, respectively. The value of N_i in the case of CP-I-50 is practically twice as much than those of CP-I-66.One can assume that this result may indicate on the more dence surrounding protein globules by chains of CP-I-66 (higher number of intermolecular contacts per protein globule). Another possible reason– increasing the number of intramolecular contacts between hydrophobic methylvinylpyridine monomer unites of CP with the formation of compact structure ("cluster" or "tack"), which are not participating in binding with protein.

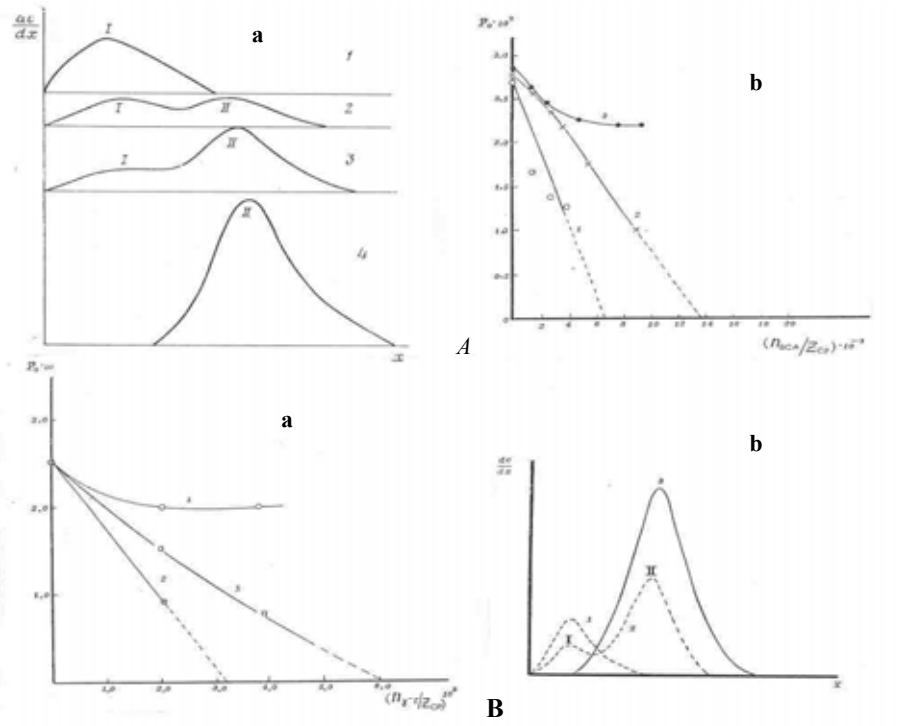


Figure 48 A. Sedimentograms (a) and dependence (b) of the sedimentation peak area (P_o) of free CP in the CP-BSA system on $(n_{BSA/z}).10^3$; a-correspond to CP-1-66; ratios: 1(2); 3 (3); 9 (4); b-3 (CP-1-40); 2(CP-1-50); 3 (CP-1-66); pH 7.0.
B. Dependence of the sedimentation peak area (P_o) of free CP on ratios $(n_{BSA/Z}).10^3$ for CP: (CP-1-66) (1), (CP-1-50) (2); (CP-1-40) (3) (a); Sedimentograms of free CP-1-66 (1) and its mixtures with FDH at different $(n_{FDH/Z}).10^3$: 1 (2); 10 (3); t=70 min. (b).

It is important to emphasize, that the free CP-I-66 and CP-I-50 still remains in the system over a sufficiently wide range of the molar ratios n_{BSA}/Z .It was suggest that these results indicate a non-random distribution of the protein molecules between the amphoteric polyions.

Therefore, it was shown, that in a wide range of ratio of the components soluble cooperative complexes were formed. Ability of amphoteric copolymers to form complexes with proteins depend both on the composition of the copolymers and the pH of the medium. Copolymers of methacrylic acid with MVP (CP-II-40) in contrary to CP-I-40 form a stable complex with proteins (Figure 49) and ability of CP-I-40 to form stable complexes increased with decreasing pH of the solution.

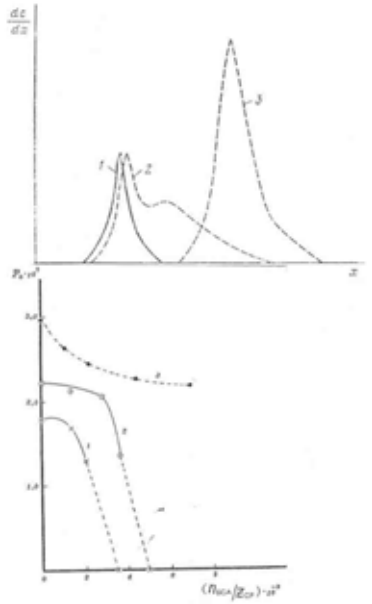


Figure 49. Sedimentograms for free CP-11-40 (1) and its mixtures with BSA at different $(n_{BSA/Z}).10^3$: 3 (2); 6 (3); Dependence of the sedimentation peak (P_0) of free CP in the CP-BSA system on ($n_{BSA/Z}$) ratios. (BSA- (CP-11-40) (1); (BSA-(CP-11-30) (2); (BSA-(CP-1-40)) (3); pH 7.0.

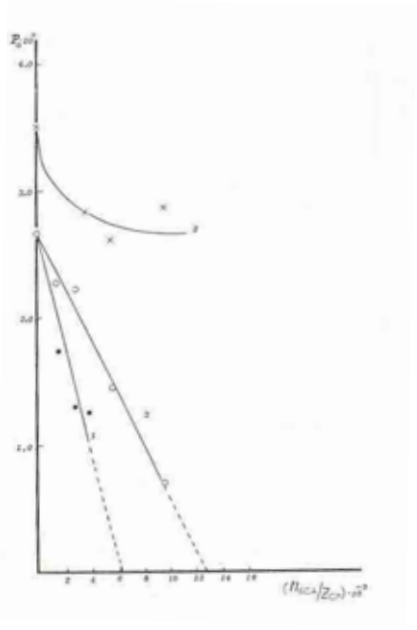


Figure 50. Dependence of the sedimentation peak (P_0) of free CP on $n_{BSA/Z}$ in the BSA-(CP-1-66) system at different pH 7 (1); 5 (2); 4 (3).

It was suggest that, under conditions where both CP and proteins have negative charges formation of hydrogen bonds and nonpolar interactions of MVP monomer units with protein globules promote the formation of stable water-soluble complex. The non-polar interactions of the protein globules with hydrocarbonic 2-methyl-5-vinylpyridine chain fragments of the polyampholyte play an important role in the association of similarly charged particles. It was shown that besides this effect of chain fragments of 2-methyl-5-vinylpyridine a significant contribution in the association is made by non-polar interactions, created by methyl groups in copolymer 2-methyl-5-vinylpyridine and methacrylic acid. It is confirmed with the fact that these complexes are not destroyed in the presence of sufficient amount of NaCl. At the some time the values of inherent viscosity of free copolymers and its mixtures with proteins decreased with increasing of the concentrations of NaCl (Figure 52).It means that in the structure of polycomplex particles exist the charged free polyion sequences, which was not involved in protein interaction.

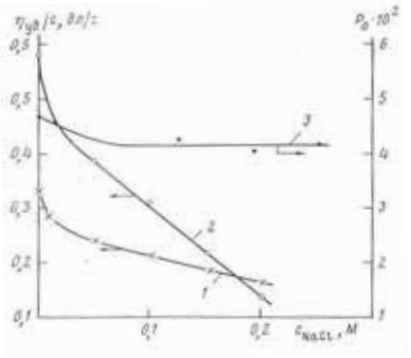


Figure 51. Dependence of the inherent viscosity ($\eta_{Sp/C}$) and sedimentation coefficient (Sc) for free BSA, CP (1-5) and complexes (1'-5') on $n_{BSA/Z}$: 1,1'-BSA+ (CP-1-66); 2, 2'-BSA+(CP-1-50), pH 7.0; 3, 3'-BSA+ (CP-1-66), pH 5.0; 4, 4'-BSA+(CP-11-40), 5, 5'-BSA+ (CP-11-30); pH 7.0.

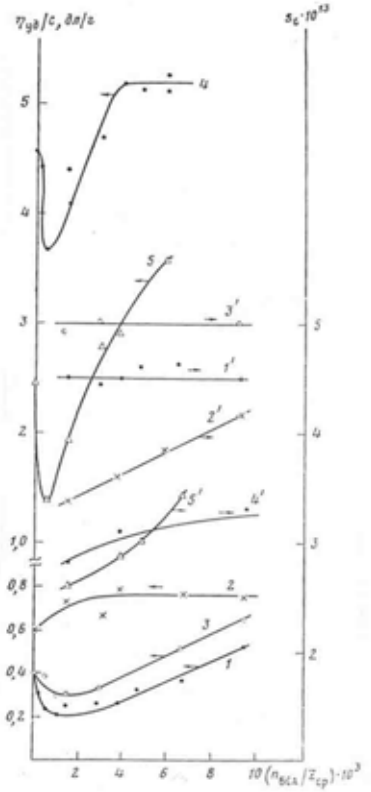


Figure 52. Dependence of the inherent viscosity ($\eta_{Sp/C}$) for CP-1-66 (1), complex (BSA+(CP-1-66) (2) and area of peak (P_0) of complexes (3) on the adding concentrations of NaCl. $(n_{BSA}/Z).10^3=6$; pH 7.0.

The association mechanism and structure of the formed complexes was studied. The dependence of the inherent viscosity of CP-BSA complex solution on the ratios is given in Figure 51.

It is seen that an increase of protein concentration in the mixture is followed by an increase of the inherent viscosity showing the formation of the polycomplex particles with more asymmetric structure than the coils of free copolymer polyions. At the same time the sedimentation coefficient changes insignificantly that conforms to the data of viscosity. Asymmetry of the structure of BSA-CP-I-66 complex is confirmed by the data of electron microscopy. The complex micrograph obtained for the system in which $n_{BSA}/Z=N_i$ is given in Figure 52. Extended linear formations are distinctly seen in the micrograph. The thickness of each rod consisting of globules is about 100 A^o. The rods, in all probability, form from contacting protein globules, which are "stick together" by the chains of linear polyampholytes.

Figure 53 shows the proposed structure of soluble protein-copolymer complex particles. In this model complex formation is accompanied by non-equal distribution of the chain copolymer between the protein globules and appearance of asymmetric particles of the complex. A correlation between the ability of polyampholytes to bind protein and their physiological action was found (see below).

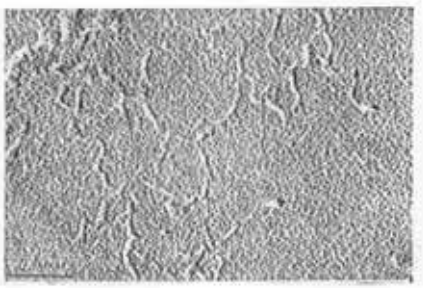


Figure 53 Electron micrographs of BSA+CP-1-66 complexes at $(n_{BSA/Z}).10^3$=6,4. (2x22000).

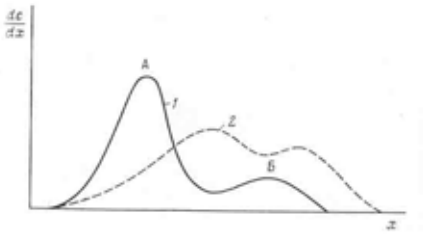


Figure 54. Sedimentograms of the initial serum (1) and its mixture with CP-11-40 (2). pH 7.5; C_{CP}=0,15 g/dl.

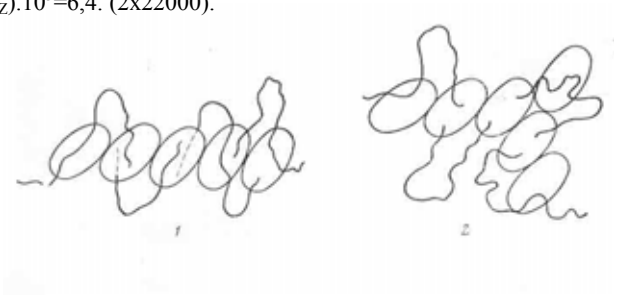
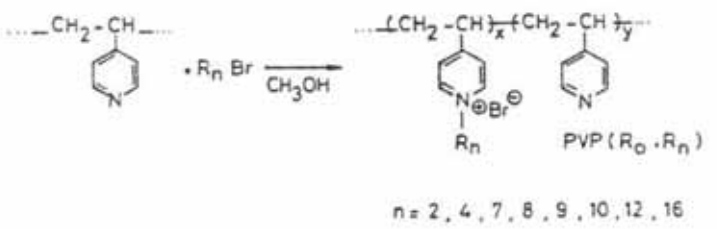


Figure 55. Proposed structures of BSA with copolymers at $n_{BSA/Z}=N_i$; 1-BSA+(CP-1-66), BSA+(CP-1-40); and BSA+ (CP-11-30), pH 7.0; 2-BSA+(CP-1-50); pH 7.0; and BSA+ (CP-1-66); pH 5.0.

Complexes of Polycations. A detailed analysis of physico-chemical properties of the mixtures of serum proteins (bovine serum albumin –BSA, human serum albumin-HSA) with poly-4-vinylpyridine (PVP) derivatives revealed that the chemical structure (charge density, hydrophobic-hydrophilic balance) of PE strongly affects their interaction with the proteins and, correspondingly, the stability of the polymer-protein complexes were formed thereby. It was found that in acidic media (pH 4.3) PVP acquires a weak positive charge and thus becomes unable to form complexes with positively charge BSA (isoelectric points of BSA pI=4.9) [97]. However after the loading of PVP with lateral hydrophobic radicals (PVP-Rn) the former acquire the ability to form complexes with BSA. The complex-forming capacity of PE molecules is different and depends both on the lengths and the amount of N-alkyl radicals.

These products whose general formula appears as:



where x/(x+y).100=7-8%, were obtained by quaternization of the PVP fraction (Pn=10^3) by corresponding alkyl bromides (from C_2 to C_{16}) as described previously [101-105]. For

convenience's sake these products will further be termed as PVP-Rn, where "n" is the number of carbon atoms in the N-alkyl fragment. It was obtained from the hydrodynamic analysis data that the viscosity of the solution of PVP-Rn whose hydrophobic radical length does not exceed eight carbon atoms is equal to the viscosity of the original PVP, i.e., in this sequence the size of the macromolecule does not change. The situation is quite different at n>8.In the range of R_{10},R_{16} the drastic decrease of viscosity is paralleled with an increase in the value of sedimentation coefficients, which facilitates the transition from the coil to the compact structure. (Figure 56)

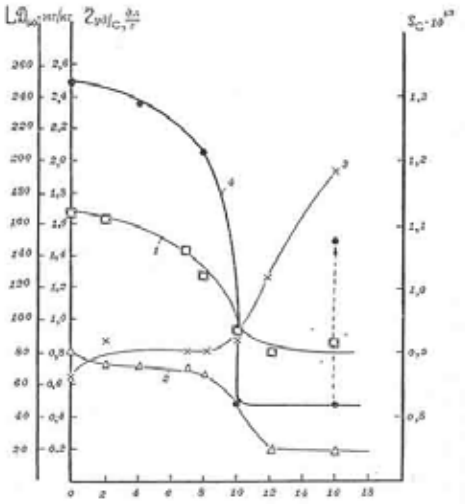


Figure 56. Dependence of the inherent viscosity ($\eta_{Sp/C}$) and sedimentation coefficient (Sc) on number of carbon atoms (n) in the N-alkyl fragment of PVP solution 0,5 % CH_3COOH (1,3) and 0,5 % CH_3COOH+0,2N NaCl (2); pH 4.3. Curve 4- correspond to toxicity (LD_{50}) of PVP(R_0, R_n).

In the next series of our experiments covalent conjugates of PVP(Ro, R_{16}) were obtained by quaternization of the PVP molecule with cetyl bromide ($C_{16}H_{33}$),a hydrophobic radical having a permanent length [101-105].In the case when the N-alkyl group represented rather a lengthy carbon chain and certain "critical" concentrations of N-alkylated bonds such PE underwent conformational transitions in dilute solutions, eventually resulting in the compactization of the macromolecular coils. It was suggested that the hydrophobic cooperative interactions between N-alkyl fragments play an important role in the transition of macromolecules from the coil to the compact structure.

PVP(Ro, Rn) conjugates thus obtained were further used for the study of complex formation with BSA. It was obtained that the complex-forming capacity of hydrophobically modified PVP in acidic media (pH 4.3) is different and depends on the length as well as on the number of alkyl radicals. Figure 57 shows plots of the area of the peaks corresponding to the free polyelectrolyte in the BSA-PVP(Ro, Rn) mixture is obtained from sedimentograms (ultracentrifugation experiments) of homogeneous systems.

An increase in the BSA concentration at a constant PVP-(Ro, Rn) concentration leads to a decrease in the area of the peak corresponding to the free PE, while the area of the presumed peak of the complex increases. At the peak of free PE disappears, and only one peak remains on the sedimentograms. It was also shown that the BSA/PE ratio at which the free PE disappears, at a fixed weight concentration of PE, depends on the length of alkyl radicals, i.e. the longer the radical length the less BSA are needed to make the free PE peak disappears. These results

indicate that, even at pHs < pI where net charges of PE and BSA are positive, electrostatic interaction is conjointly in effect with hydrophobic interaction of N-alkyl radicals for the binding (Figure 57). Hydrophobic interaction between N-alkyl fragments of PE, which are not including interaction with protein molecules, can help the stabilization of the complex structure as a whole.

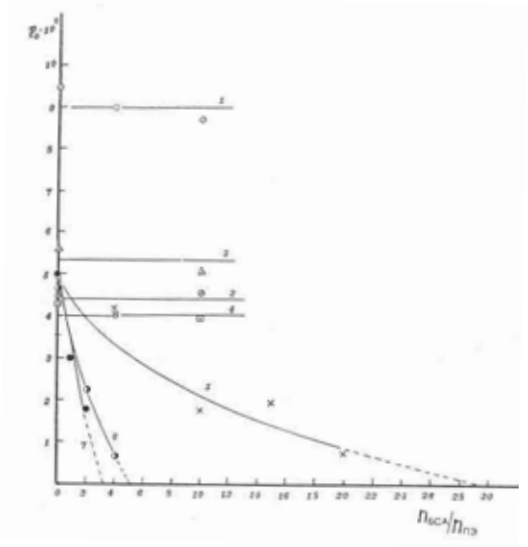


Figure 57. Dependence of the peak area (P_0) of free PVP(R_0, R_n) on n_{BSA}/n_{PE} in PVP(R_0, R_n)-BSA systems: 1-PVP(R_0); 2- PVP(R_0,R_2); 3- PVP(R_0, R_7); 4- PVP(R_0, R_9); 5- PVP(R_0, R_{10}); 6-PVP(R_0, R_{12}); PVP(R_0, R_{16}); pH 4.3 β=7-8%.

Noticeably, the binding of BSA to PVP(Ro, Rn) take place at a Rn, which corresponds to N-alkyl radicals realizing conformational transition of polymeric chains, i.e. exist the relationship between the complex-forming capacity of PE and the conformational transition in polyelectrolyte chains. Polycations of PVP(Ro, Rn) whose N-alkyl radicals are abundant enough to induce the formation of PE-BSA complexes but which are insoluble in neutral aqueous media can form stable electrostatic and hydrophobic complexes with BSA in acidic aqueous solutions. However, at physiological values of the ionic strength and pH such complexes lose, to a certain extent, their stability: some part of the protein molecules dissociate from the main complex to form an insoluble pellet, in which one polyionic chain corresponds to one protein molecule (so-called stoichiometric complexes). This phenomenon is very important also in view of the fact that electrostatic and hydrophobic cooperative interactions play an important role in many biological systems; hydrophobic effects on PEC formation of proteins should be an important problem to be investigated.

The interaction of BSA with various fractions of the copolymer of 4-vinyl-N-cetylpyridinium bromides has been studied depending on the length of macromolecules and the content of side hydrophobic cetyl fragments:

Introduction of hydrophobic cetyl radicals into PVP chains lead to compactization of macromolecular chains (Figure 57). The inherent viscosity decreased by increasing the amount of the cetyl radicals. As seen from Figure 58 curve this changing is acquire the character of conformation transition in salt containing water solution.

When BSA solutions are added to PVP(R_0, R_{16}) solutions at pH 4.3 the inherent viscosity of the mixtures was considerably increased (Figure 59).The degree of increasing of inherent viscosity so much the stronger the more compact of the conformation of initial PVP(R_0,

R_{16}) ,i.e. more the amount of cetyl radicals in the composition of polycation. So far as the viscosity of free BSA at corresponding concentrations is neglecting few this results direct testify on the binding of protein molecules with polycations. At pH 4.3 the globules of BSA acquires a positive charge (pI of BSA equal 4.9) and therefore one can assume that the interaction of hydrophobic N-cetyl radicals with hydrophobic section on the surface of protein globules is the driving force at the formation of protein-polycation complexes.

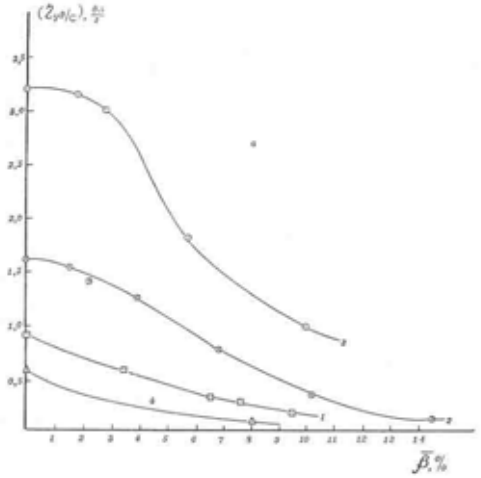


Figure 58. Dependence of inherent viscosity ($\eta_{Sp/}C$) of polycations on degree of quaternization (β) with ceytyl bromide at different P_w; 10^3: 1-0.6; 2-1.0; 3-2.15; 4-0.4; pH 4,3.

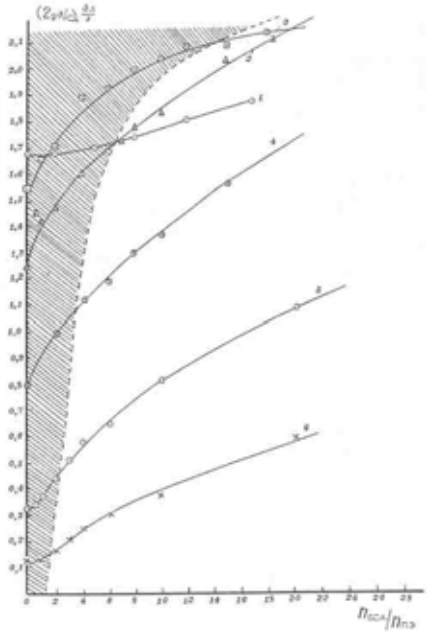


Figure 59. Dependence of ($\eta_{Sp/C}$) BSA-PVP(R_0, R_{16}) mixtures on n_{BSA}/n_{PE} at different β (Pη=const): 0 (1); 4 (2); 1,5 (3); 7 (4); 10 (5); 14,5 (6).

As it follows from sedimentograms of BSA-PVP(R_0, R_{16}) mixtures, in the general case the system is characterized by a bimodal distribution of sedimenting components (Figure 60). The free polycations coexist with the water-soluble protein-polymer complexes. An independent experiment has shown that the free protein is absent in the system when polycation chains contain some critical concentration of cetyl radicals ($\overline{\beta}$ ~7%) over the whole studied range of the ratios n_{BSA}/n_{PVP}. This results indicates that the non-uniform distribution of protein globules between polycations-sorbent is revealed.

P(R_0, R_{16}) sedimentation peaks plotted vs. the ratio of the number of protein molecules to that of polycation chains in the system.

As seen from this figure the function P_0= f(n_{BSA}/n_{PVP}) are not linear in contrary to above described protein-polycation mixtures. It means that there is polycation present in the system with different "capacity" as regards protein globules at given degree of polymerization and quaternization ($\overline{\beta}$). This phenomenon may be have to do with compositional heterogenecity of polycations as the degree of quaternization have strong influence on the binding capacity of

PVP(R_0, R_{16}). Therefore, the values N_i, which were obtained from Figure 61 are less exact than in the case of protein-PVP(R_2) and its characterizes the average number of protein molecules bound one polycation chain belonging to a given fraction of PVP(R_0, R_{16}). The dependence of N_i on degree of polymerization of polycations with different degree of quaternization ($\overline{P}_\eta$) is given in Figure 62.

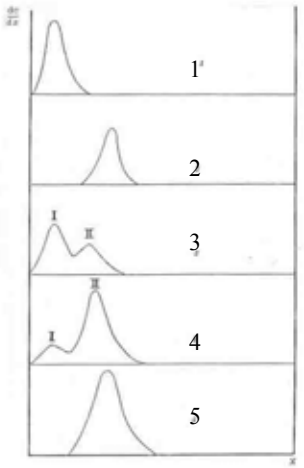


Figure 60. Sedimentograms of PVP(R_0,R_{16}) (1) and its mixtures with BSA (2) at different n_{BSA}/n_{PE}: 1 (3), 2 (4), 3 (5).

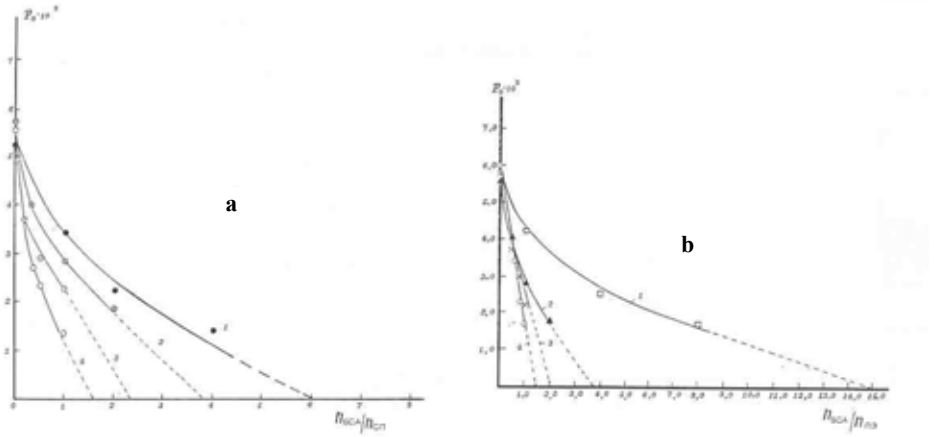


Figure 61. Dependence of the sedimentation peak area (P_0) of free PVP(R_0, R_{16}) in the BSA- PVP(R_0, R_{16}) system on n_{BSA}/n_{PE} for PVP(R_0, R_{16}) with different Pη, 10^3: 2,15 (1); 1,0 (2); 0,6 (3); a- β=4%; b- β=7%. (0,4 (4).

Within the experimental error these dependences are linear. The rate of this rise so much the higher than the lowest value of " $\overline{\beta}$ " in the system over the whole studied range of the " $\overline{\beta}$ ". From the data in Figure 62 one can approximately estimate the average number of