Colloid Polym Sci 274:418–427 (1996)

ORIGINAL CONTRIBUTION

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The complex formation between polyacrylamide containing glycine end groups and bovine serium albumin in the presence of copper (II) in neutral aqueous media

Received: 15 February 1995
Accepted: 13 November 1995

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Abstract Complex formation between end group containing polyacrylamide and BSA has been studied in neutral water. Water soluble and insoluble complexes are formed when divalent copper ions are added to the solution. The contacts between protein and polyelectrolyte are achieved via chelate unit formation in which the copper ion is attached at the center. The solubility of the polycomplexes depends on protein/polymer ratio. Starting with very low concentration of protein in the system, phase separation takes place. Above the critical ratio of the protein/polymer, the mixture again exhibits water soluble character. The velocity of the formation of insoluble ternary complexes has been investigated by spectrophotometric method at different reaction conditions (preparation of mixture, ratio of components, low molecular salts, temperature and stirring, molecular weight of polyelectrolyte and Cu^{2+} concentration). A hypothetical structural scheme for the formation of soluble and insoluble ternary polycomplexes is proposed.

Key words Polyacrylamide – protein – copper – complex

Introduction

Intermolecular complexes and, in particular, protein containing polycomplexes (PEC) can be considered as a special class of polymeric compounds. Studies of mechanisms of cooperative binding of protein by synthetic polyelectrolytes (PE) is of interest for the construction of artificial immunomodulators and immunogens, for immobilization of enzymes, elucidation of the mechanism of PE physiological activity of polyelectrolytes [1–3] etc. In addition, such reactions may simulate the processes of assembling of viruses, chromatine, ribosome strands and other cell components in complicated biological systems.

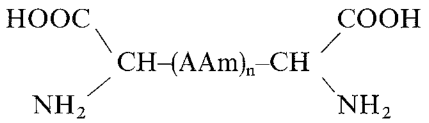
Recently a new technological approach has been developed for obtaining highly immunogenic complexes of protein antigens which can serve as a basis for the construction of artificial polymer-subunit antigens and synthetic vaccines [1, 4–6]. This method consists of the use of small concentrations of transient metal ions which promote the PE binding to the protein without causing any appreciable changes in the chemical structure of PE and, correspondingly, its biological activity. These studies revealed that in the presence of metal ions many nitrogen- and carboxyl containing PE, irrespective of their molecular mass, can form ternary PEC with proteins, both soluble and insoluble in water. The contacts between the protein and the PE are achieved via the formation of chelate units in which the metal ion is central. In these systems, depending on the ratio of components of mixtures, time to time partial or complete prevention of phase separation takes place. Time dependence of transformations of soluble ternary PEC to insoluble state demands the investigations of the kinetics of the formation of these PEC.

In this study, the rate of the interaction of water soluble polymers with bovine serum albumin in the presence of divalent copper ions was studied at different conditions.

Polymer used in this study was water soluble polyacrylamides (PAAm) which contain amino acid end groups [8]. Such polymers have potential for the construction of high molecular weight polymers containing weakly bounded structure which can be destroyed in physiological conditions.

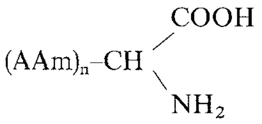
Experimental

The polymerization of the acrylamide monomer is initiated by a glycine radical which is formed by the reaction of Ce(IV) -Ce(III). The PAAm synthesized, using this procedure, have the following chemical structures.



$$\mathrm{(HOOC)(NH_2)CH{-}(AAm)_n{-}CH(COOH)(NH_2)}$$

or



$$\mathrm{(AAm)_n{-}CH(COOH)(NH_2)}$$

where $n = 280$ ($M_v = 20\,000$), 630 ($M_v = 45\,000$) and 3200 ($M_v = 230\,000$).

FT-IR results of PAAm indicate (1390–1410 cm^{-1}) the presence of carboxylic groups unlike the homopolyacrylamide. The titration of end carboxylic groups in PAAm was performed by using NaOH, and molecular weight of polymer (M_n) was calculated as following [8].

$$\text{Equiv. of carboxyl end groups}/10^6\ \text{g} = \frac{V \cdot N \cdot 10^3}{m}$$

$$M_n = 2 \times 10^6/\text{equiv. of carboxyl ends}/10^6\ \text{g}\ ,$$

where V, N, m are the volume, normality of titrant, and weight of polymer respectively.

The average molecular weight (M_v) was obtained by measuring viscosity of solution at 30 °C by using the following equation [8–11].

$$[\eta] = 6.8 \times 10^{-4} M_v^{0.66}\ .$$

Specific conductivity of PAAm at definite weight was measured by using E 518 Metrohm Harisau Conductometer. The results are shown in Table 1.

For the synthesis of polymer metal complexes (PMC), the polymer was dissolved in neutral water at pH = 7, and $CuSO_4$. $5H_2O$ was dissolved in water in acidic medium (pH = 4). The solution of $CuSO_4$ was then added to the polymer solution. The solution was stirred at 20 °C then pH was adjusted to pH = 7 by adding 0.1 N NaOH solution. The viscosity, the absorbance (at 254 nm) and the surface tension of solution were then measured.

The synthesis of ternary complexes of PAAm–metal–protein was performed as follows: protein (bovine serum albumin, BSA "Fluka", $M_w = 70\,000$) was dissolved in water at pH = 7, and then added to the polymer metal complex solution. The pH of solution was also adjusted to 7. The mole ratio of $CuSO_4$ to the polymer was kept constant at 0.1, i.e., $n_{CuSO_4}/n_{AAm} = 0.1$ where n_{CuSO_4} and n_{AAm} are the number of the molecules in one ml volume of solution.

For spectrophotometric measurement, the UV visible measurements (200–1000 nm) were performed using a Shimadzu UV-160A spectrophotometer equipped with a temperature control attachment. The FT-IR spectrum of the products was obtained with a Jasko 5300 spectrophotometer. The surface tension of solutions was measured by using Tansiometric method. The electrophoretic study was carried out by Sartorius.

The correlation coefficients (r) or straight lines have been calculated by the least-squares method including almost all the data points.

Table 1 Molecular weight and conductivities of polymers

PAAm	$[\eta]$	M_v	M_n	M_v/M_n	$n = M_v/M_{AAm}$	Specific[a] conductivity ($ohm^{-1} cm^{-1}$)$\times 10^6$
PAAm1	0.44	20 000	19 000	1.05	280	380
PAAm2	0.80	45 000	22 200	2.02	630	296
PAAm3	2.34	230 000	–	–	3200	168

[a] The specific conductance of 0.3 g polymer dissolved in 50 ml water.

[b] Since polymers have high molecular weight, carboxylic end groups were difficult to determine.

Results and discussion

The synthesis of the polymer-metal complex

For neutral water (pH = 7) the addition of metal ions to polymer (PAAm) starts to give homogeneous solution at a given value of $n_{Cu}/n_{AAm} = 0.1$ (concentration of PAAm $C_{PAAm} = 0.15$ g/dl, various concentration of Cu^{2+}) at pH = 7 and since $CuSO_4$ is not soluble in this system, suggests the formation of a soluble polymer metal complex. This was confirmed by UV-visible spectrophotometric measurements. The dependence of optical density at 254 nm on the different ratio of metal ions to acrylamide monomers is illustrated in Fig. 1. The absorbance increases linearly with an increase in copper ion concentration. The relation suggests that the copper ion is bounded to the PAAm molecules, which in turn generates a water soluble PMC.

The infrared spectra of PAAm and PMC have been given (Fig. 2a). Peaks were observed at 425, 592, 610, 800 and 1100 cm^{-1} in PMC spectra by the comparison of PAAm spectra. In a previous study of the glycino complexes with Cu(II), the bands at 439 (M–N stretching), 592 (ring deformation), 644 (NH_2 roching), 736 (C=O bending), 1058 (NH_2 waging) and 1151 cm^{-1} (NH_2 twisting) were observed [12]. These results suggest that the peaks observed at 425, 592, 610, 800 and 1100 cm^{-1} indicate M–N stretching, ring deformation, NH_2 roching, C=O bending and NH_2 waging or twisting respectively.

It was observed that the specific viscosity of the PAAm solution decreases in parallel with an increase in the copper ion concentration and passes through a minimum value (Fig. 2b). This effect can be readily explained by invoking two types of regimes. At low concentration of copper ions, the interaction can be considered to be intramolecular only, i.e., the Cu^{2+} ion forms the complex with only one polymer chain. This in turn leads to a more compact polymer coil. At high concentration one can speculate that the copper ion acts more effectively as a crosslinking agent between polymer coils. This intermolecular coil interaction leads to the increase in intrinsic viscosity.

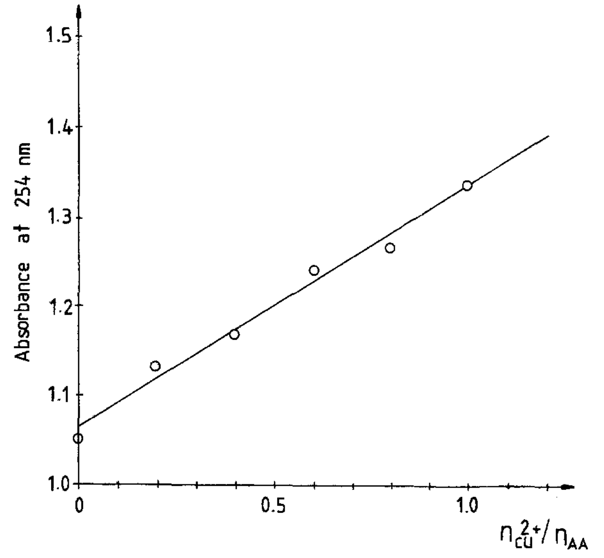


Fig. 1 The dependence of optical density of 254 nm (A_{254}) on the ratio of metal ions to acrylamide monomers ($n_{Cu^{2+}}/n_{AAm}$) (correlation coefficient, $r = 0.9931$)

The polymer metal complexes of BSA

Ternary complex formation

The solutions of the polymer metal complex were used to prepare the ternary complex. The physico-chemical parameters for the PMC are summarized in Table 2.

First, the neutral water medium, the solution of PAAm was mixed with the solution of BSA at about pH = 7 without copper ion. In a wide range of ratio of components, ($n_{BSA}/n_{PAAm} < 3.0$, $C_{PAAm} = 0.15$ g/dl, change BSA concentrations) the system remains homogeneous. This system was investigated by spectrophotometric measurements. Figure 3 shows the dependence of the optical density (A_{280}) for solutions of protein, protein–PAAm and protein–Cu^{2+}–PAAm.

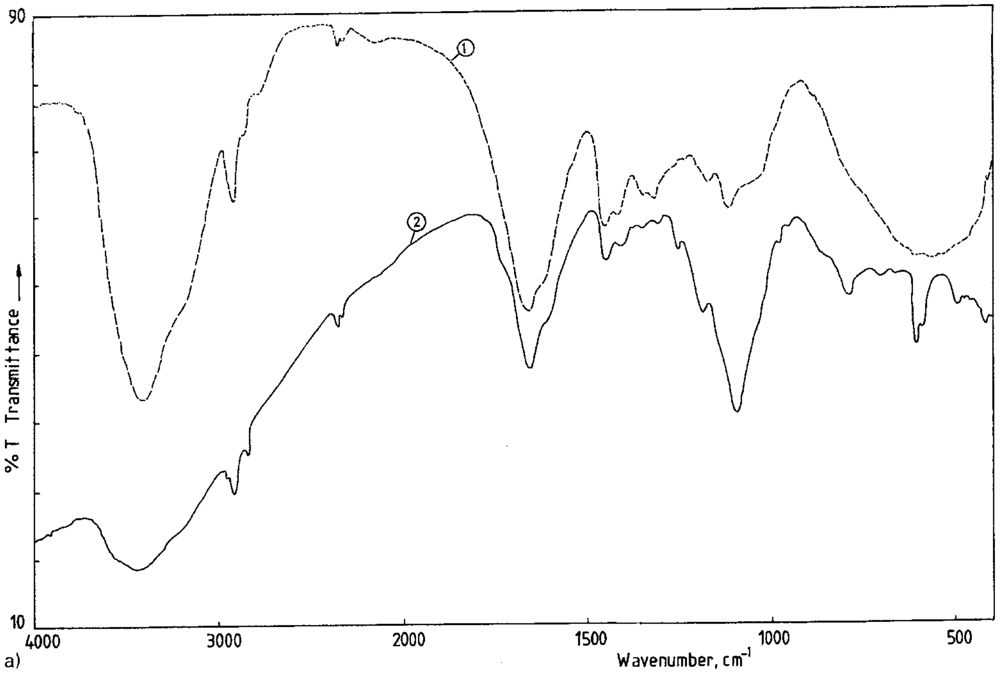
As shown in Fig. 3, curve 2 the value of A_{280} increases with increasing the protein concentration in the system. But in all cases A_{280} is practically identical to that obtained for the free protein solutions (Fig. 3, curve 1). These results suggest that there is no interaction between PAAm

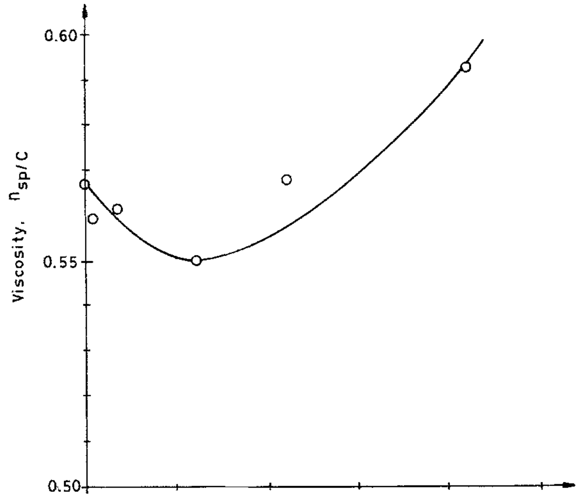
Table 2 The physico-chemical parameters of PMC

PMC	M_v(PAAm)	$n_{Cu^{2+}}/n_{AAm}$ [a]	η_{sp}/C(dl/g)	Specific conductivity (ohm^{-1} cm^{-1}) × 10^6
PMC 1	20000	0.1	0.552	986
PMC 2	45000	0.1	0.823	981
PMC 3	230000	0.1	2.558	931

[a] The interaction of BSA was studied at this concentration ratio.

Fig. 2 a) FT-IR spectra of PAAm ($M_v = 20000$) (1) and PMC1 (2). b) The dependence of η_{sp}/C on n_{Cu}/n_{PAAm}. $C_{PAAm} = 0.3$ g/dl; pH = 7; 20 °C





and BSA. But when we add the protein solution to PMC solution under the same condition, the absorbance value at A_{280} changed with increasing concentration of BSA (Fig. 3, curve 3). Comparison of these results and copper-binding properties of BSA [13] suggest copper ions lead to the formation of the complexes between polymer chains and protein globules.

The homogeneous mixture of the ternary system (PAAm–Cu^{2+}BSA) shows different behavior depending on the order of addition of BSA to PMC ($n_{BSA}/n_{PMC} \leq 3.0$; $C_{PMC} = 0.15$ g/dl or PMC to BSA ($n_{PMC}/n_{BSA} \leq 25$; $C_{BSA} = 0.5$ g/dl). For mixture prepared by the addition of various protein concentrations to PMC solutions in the early stage, all systems were water soluble and homogeneous. However, depending on time and on the ratio of these mixtures, the homogeneity was lost ($n_{BSA}/n_{PMC} \leq 2.0$) and insoluble particles appeared in the system. After 24 h, the absorbance of mixtures was measured. The dependence of the optical density (A_{400}) on the molecules of protein globules per polymer molecules (n_{BSA}/n_{PMC}) is shown in Fig. 4a. As seen, the dependence passes through a maximum. Starting with very low values of n_{BSA}/n_{PMC} in the system, phase separation take place which indicates the formation of an insoluble complex. On further increase in n_{BSA}/n_{PMC}, A_{400} increases and gives a limiting value. Depending on protein concentration in the mixture, partial or complete phase separation occurs. After the critical ratio of the protein/polymer ($n_{BSA}/n_{PMC} \geq 2$) is reached, the mixture shows water soluble character, i.e., the system behaves homogeneously. Similarly, the dependence of A_{400} on the ratio of n_{PMC}/n_{BSA} passes through a maximum (Fig. 4b) upon addition of the PMC solution at different polymer concentration to the solution of BSA, at constant concentration ($C_{BSA} = 0.5$ g/dl). In both cases, independent of the method of preparation, insoluble ternary polymer–metal–protein complex are observed.

The formation of insoluble ternary protein–metal–polymer complexes was investigated from FT-IR spectrophotometric measurements. The FT-IR spectrophotometric results for the BSA, PAAm–Cu^{2+} and

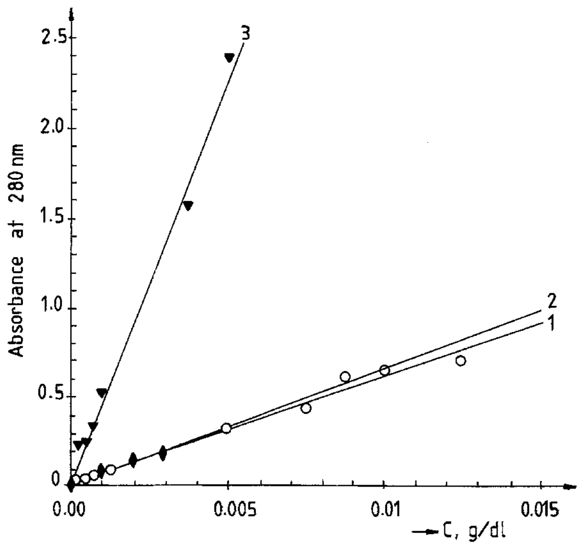


Fig. 3 The dependence of A_{280} of the mixtures on concentration of BSA. The correlation coefficients (r) of straight lines are 0.9901 (1, BSA–PAAm), 0.9972 (2, BSA) and 0.9948 (3, BSA–Cu^{2+}–PAAm), $M_v = 20000$; $C_{PAAm} = 0.15$ g/dl; $C_{CuSO_4} = 0.05$ g/dl

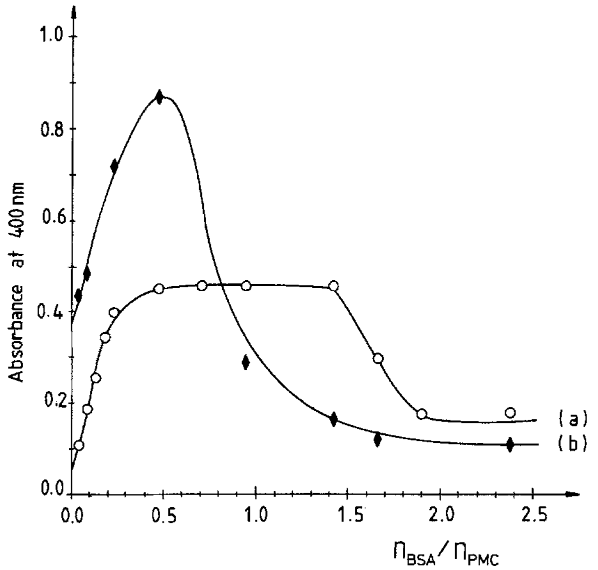


Fig. 4 The dependence of A_{400} on the ratio of components for the mixture PAAm–Cu^{2+}–BSA. a) The BSA solution at different concentrations added to the solution of PMC at constant concentrations of PMC (n_{BSA}/n_{PMC}). M_v(PAAm) = 20000; 20 °C; pH = 7; $C_{PAAm} = 0.15$ g/dl; $C_{CuSO_4} = 0.05$ g/dl. b) The PMC solution at different polymer concentrations added to the solution of BSA at constant concentrations of BSA (n_{PMC}/n_{BSA})· $C_{BSA} = 0.5$ g/dl; $n_{Cu}/n_{AAm} = 0.1$; ($C_{PAAm} \leq 3$ g/dl; $C_{CuSO_4} \leq 1.05$ g/dl)

insoluble products of the ternary mixtures of PAAm–Cu^{2+}–BSA are shown in Fig. 5. As seen from these results in ternary mixture spectra, we have observed that the peaks at 592, 610 and 800 cm^{-1} corresponding to polymer–metal complexes are invisible. Also, the peak at 1100 cm^{-1} which is characteristic for the PAAm–Cu^{2+} strongly decreases. These results showed that the metal ions bound with PAAm after adding protein molecules partially bound with proteins.

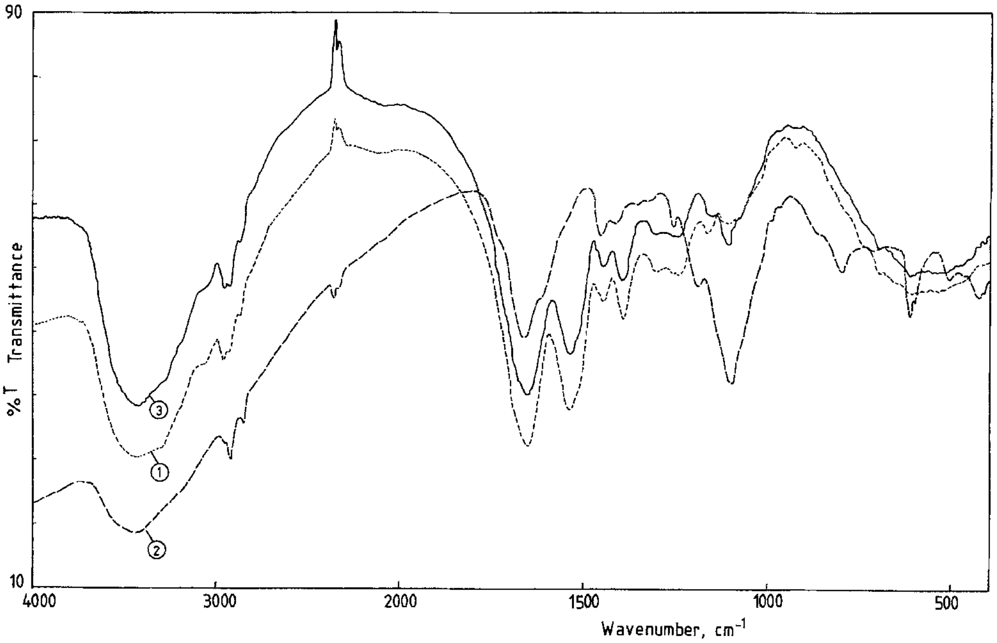
The formation of the insoluble products in the mixture has influence on the surface tension of polymer solutions (Table 3). Adding protein molecules to the PAAm–Cu^{2+} solution initially ($n_{BSA}/n_{PAAm} < 1$) the surface tension of polymer solution decreases and at the $n_{BSA}/n_{PAAm} > 1$ the surface tension takes a limiting value. These results indicate the formation of a new type colloid particle in systems, i.e., PAAm–Cu^{2+}–BSA complex.

The composition of the ternary polycomplexes was investigated by electrophoretic study of the mother liquors of the mixtures PAAm–Cu^{2+}–BSA. For this reason, the mixture containing different protein concentration separated from insoluble complexes and matrix solution was studied by electrophoretic method. Figure 6 presents the results of the study on the matrix solution with electrophoretic method. An electrophoretic study of this system showed that the proteins in the solutions are absent at the $n_{BSA}/n_{PAAm} < 1$. At the $n_{BSA}/n_{PAAm} > 1$ of BSA–Cu^{2+}–PAAm, the water soluble ternary complexes have been detected in the electrophoretic diagrams (at the same concentration, free BSA and ternary mixtures were shown to have different electrophoretic mobility and optical density). The results plotted in this figure could be interpreted in terms of water soluble and insoluble ternary complexes formed simultaneously, at the $n_{BSA}/n_{PAAm} > 1$. Further increasing the ratio n_{BSA}/n_{PAAm} leads to the amount of the insoluble ternary complexes decreasing and soluble ternary complexes increasing.

On the basis of the results shown in Fig. 5 as well as previous studies [2, 4, 6, 7], a critical ratio of $n_{BSA}/n_{PMC} \geq 2$ will favor the formation of a water soluble ternary complex, i.e., PAAm–Cu^{2+}–BSA. The metal Cu^{2+} acts as a crosslinking agent (chemical cross link) between the PAAm chains and the BSA globules. This interaction leads to the formation of insoluble or soluble ternary complexes, which was found to be greatly dependent on the quality of effective crosslinks in the system.

It is already known from literature that Cu^{2+} might bind to α-amino nitrogen, two intervening peptide nitrogens and the imidazole nitrogen of histidine residue [13] and carboxyl side chain groups of proteins [14–16]. According to both findings and analogous results, we propose the structure of the chelate of the ternary complex which may involve $-NH_2$ (side and end groups) and $-COOH$ groups of PAAm (other nitrogen ligands of BSA of the structure are not shown for the sake of clarity).

Fig. 5 FT-IR spectra of BSA (M_v = 70000) (1), PMC1 (2) and BSA-Cu^{2+}-PAAm (3)



The velocity of the formation of ternary complexes

It is critical that insoluble complexes, unlike soluble polycomplexes which form instantaneously, take place depending on time. These results permit investigation of the velocity of the formation of the ternary insoluble complex by the spectrophotometric method at different reaction conditions (the effects of the preparing mixture, ratio of the components, low molecular salts, temperature and stirring, molecular weight of polyelectrolyte and Cu^{2+} concentration).

The effect of the method of preparation: Figure 7a shows the variation of the absorbance at 400 nm vs. time for the two earlier mentioned methods of preparation, i.e., the BSA solutions were added to the PMC solutions (1), and the PMC solutions were added to the BSA solutions (2).

As is shown in Fig. 7, in both cases the A_{400} values increase with time, and absorbance values level off at about 150 min. The rate of the reaction (dA/dt) was obtained from the tangent to the curves in the early stages of the reaction, i.e., 0 to 60 min. The values of the $(dA/dt)_1$ and $(dA/dt)_2$ were found to be 1.93×10^{-2} and 1.22×10^{-2}, respectively. Evidently, the values indicate that reaction 1 is faster than reaction 2. Obtained results could be described as follows. When BSA solution was added to PMC solution, because the concentration of BSA was less

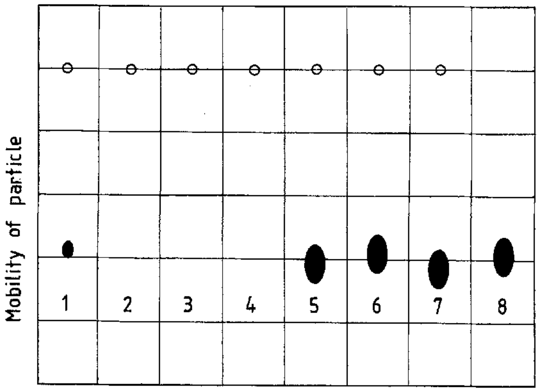


Fig. 6 Electrophoresis of BSA and the mixtures of BSA-Cu^{2+}-PAAm (t = 60 mins., V = 300 volt). C_{BSA} = 0.5 g/dl (1); The concentrations of BSA in the mixtures of BSA-Cu^{2+}-PAAm are 0.075 g/dl (2), 0.1 g/dl (3), 0.125 g/dl (4), 0.375 g/dl (5), 0.5 g/dl (6), 0.875 g/dl (7) and 1.0 g/dl (8); C_{PAAm} = 0.15 g/dl; C_{CuSO_4} = 0.05 g/dl

Table 3 The dependence of surface tension to n_{BSA}/n_{PAAm}

n_{BSA}/n_{PAAm}	0.055	0.143	0.476	0.952	1.667	1.904
Surface tension (dyn/cm)	72.17	67.19	63.65	61.88	63.15	62.89

M_v(PAAm) = 20000; 20 °C; pH = 7; C_{PAAm} = 0.15 g/dl; C_{CuSO_4} = 0.05 g/dl.

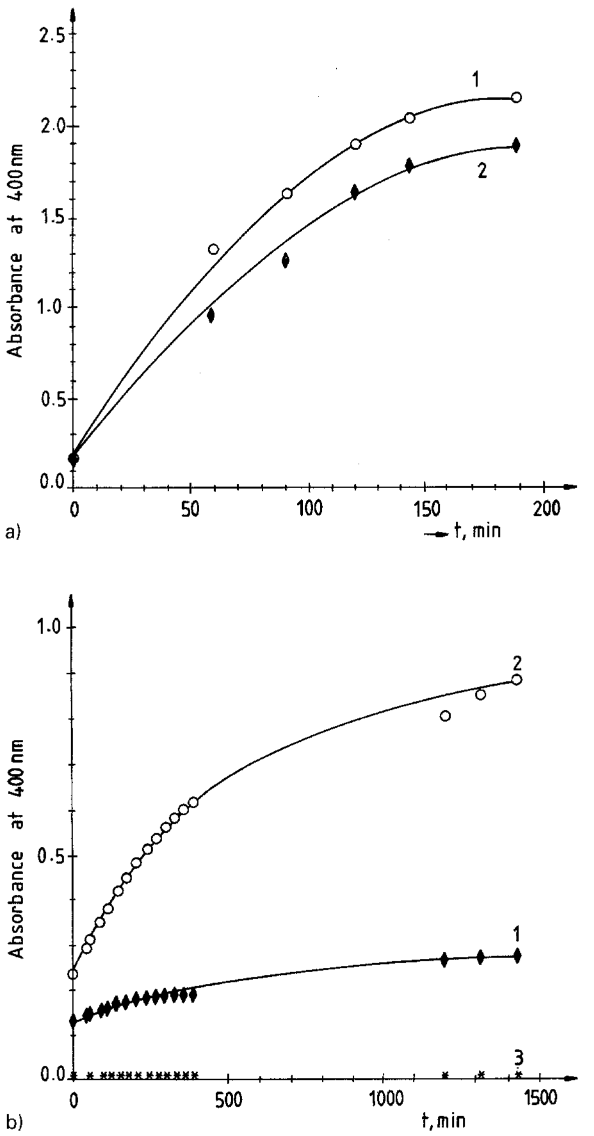


Fig. 7 a) The dependence of the A_{400} of two mixtures BSA–Cu^{2+}–PAAm on time: 1) BSA was added to the solution of PMC; 2) PMC was added to the solution of BSA; b) The dependence of the A_{400} of PAAm–Cu^{2+}–BSA mixture on the time at three different $(n_{BSA}/n_{PMC}) = 0.15$ (1, $dA/dt = 0.15 \times 10^{-3}$); 1.0 (2, $dA/dt = 1 \times 10^{-3}$); 2.0 (3, $dA/dt = 0$). (BSA was added to the solution of PMC) M_v (PAAm) = 20 000

than the concentration of PMC at the initial moment, water soluble complex occurred. When PMC solution was added to BSA solution, because the concentration of PMC was less than the concentration of BSA at the beginning of titration, insoluble ternary complex formed, therefore the reaction rate increased slowly, i.e., the diffusion process decreased.

The effect of the ratio of components: The influence of the protein polymer ratio on the absorbance at 400 nm is shown in Fig. 7b. The mixtures were prepared by adding BSA to the PMC solution. As suggested by Fig. 4, the n_{BSA}/n_{PMC} ratios are chosen as follow: initial state ($n_{BSA}/n_{PMC} = 0.15$), maximum precipitation ($n_{BSA}/n_{PMC} = 1.0$) and soluble complex ($n_{BSA}/n_{PMC} = 2$). It is clear that the A_{400} values greatly depend on the ratio used for these experiments. Moreover, the rate of reaction is also very sensible to the value of ratio.

As can be seen with increasing concentration of proteins, the rate of reaction increases to reach a maximum value and then decreases. For a ratio $n_{BSA}/n_{PMC} = 2$ which corresponds to soluble complexes, the value of $dA/dt = 0$. This behavior was also observed when the solutions were added in reverse order, i.e., the PMC solution is used to titrate the BSA solution. With variation in rate of reaction as well as the behavior illustrated in Fig. 4, in both cases equilibrium is reached at different speed (the reaction was faster in the case of forming a soluble system) i.e., different processes for addition methods suggest that different species (complexes) are formed depending on the method of preparation.

The effect of the dilution of systems: The ternary systems obtained at the ratio corresponding to maximum precipitation ($n_{BSA}/n_{PMC} = 1$) were dissolved with neutral water and investigated by the spectrophotometric method at different reaction time and constant temperature. The mixture was prepared by adding protein solution to PMC solution. The result of spectrophotometric measurements and the dependence of the obtained value of the dA/dt on the degree of dilution of the ternary mixtures are shown in Fig. 8. The dA/dt as a function of the degree of dilution increases and then attains a maximum value, at very high dilution of the ternary mixture, the value of dA/dt begins rapidly to decrease and attains a minimum value. It should be accepted that by the dilution, according to decrease of concentration, the velocity of diffusion and the process of exchange with the ternary particles in the system increase. This phenomena acts on association equilibrium and molecular aggregation of the system. Very high dilution leads to decrease of the interaction between the colloid particles. For this reason the velocity of the formation of the insoluble colloid particles decreases.

The effect of the low molecular salts: The mixture PMC–BSA was prepared at the different concentration of the sodium chloride to investigate the effect of the low molecular weight salts. The ratio of the n_{BSA}/n_{PMC} is constant and equal to 1.0. The analysis of the systems by the spectrophotometric method showed that the concentration of NaCl affect not only a solubility of the systems but also the velocity of the formation of the colloid particles. The dependence of A_{400} on time for PAAm–Cu^{2+}–BSA

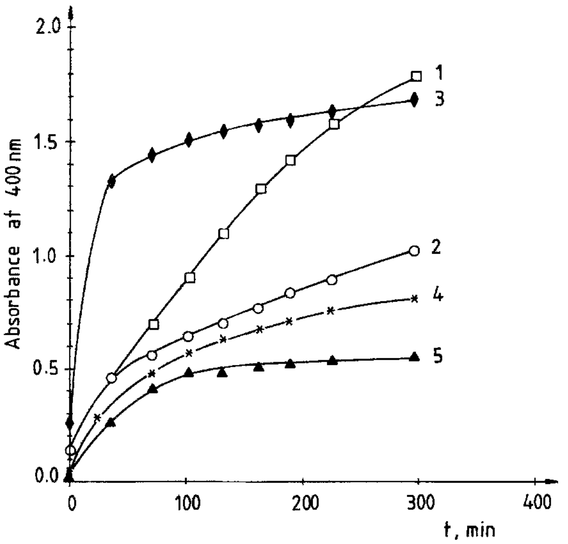


Fig. 8 The dilution effect on the formation of the insoluble ternary mixture. The initial concentration of the mixture of BSA–Cu^{2+}–PAAm: $C_{\mathrm{BSA}} = 0.5$ g/dl; $C_{\mathrm{CuSO_4}} = 0.05$ g/dl; $C_{\mathrm{PAAm}} = 0.15$ g/dl; pH = 7; 20 °C. C (1, $dA/dt = 0.67 \times 10^{-3}$); C/3 (2, $dA/dt = 0.89 \times 10^{-3}$); C/5 (3, $dA/dt = 2.98 \times 10^{-3}$); C/9(4, $dA/dt = 1.02 \times 10^{-3}$); C/17 (5, $dA/dt = 0.68 \times 10^{-3}$)

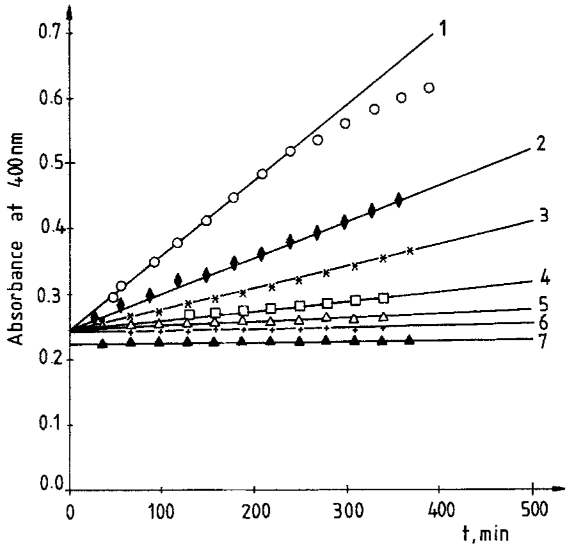


Fig. 9 The dependence of A_{400} for the NaCl concentration in the mixture of BSA–Cu^{2+}–PAAm. pH = 7; 20 °C; $C_{\mathrm{PAAm}} = 0.15$ g/dl; $C_{\mathrm{BSA}} = 0.5$ g/dl; $C_{\mathrm{CuSO_4}} = 0.05$ g/dl 0 M NaCl(1, $dA/dt = 117.7 \times 10^{-5}$); 0.01 M NaCl (2, $dA/dt = 56 \times 10^{-5}$); 0.025 M NaCl (3, $dA/dt = 34.2 \times 10^{-5}$); 0.05 M NaCl (4, $dA/dt = 15.4 \times 10^{-5}$); 0.1 M NaCl (5, $dA/dt = 6.25 \times 10^{-5}$); 0.125 M NaCl (6, $dA/dt = 3.37 \times 10^{-5}$); 0.15 M NaCl (7, $dA/dt = 1.78 \times 10^{-5}$)

mixtures at the different NaCl concentrations is shown in Fig. 9. With increasing NaCl concentration, the slopes of curves decrease and at higher concentration of the NaCl, the ternary system becomes homogenous and the absorbance A_{400} against time practically does not change. The relations of dA/dt and its logarithm on the concentration of NaCl fit the equation $(dA/dt) = 7e^{-26[\mathrm{NaCl}]}$.

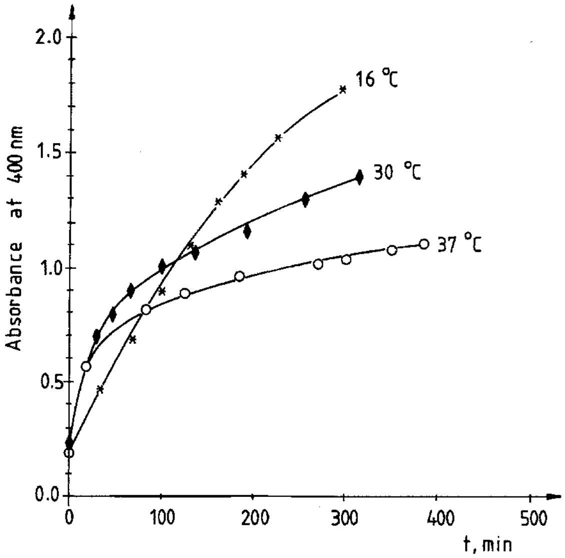


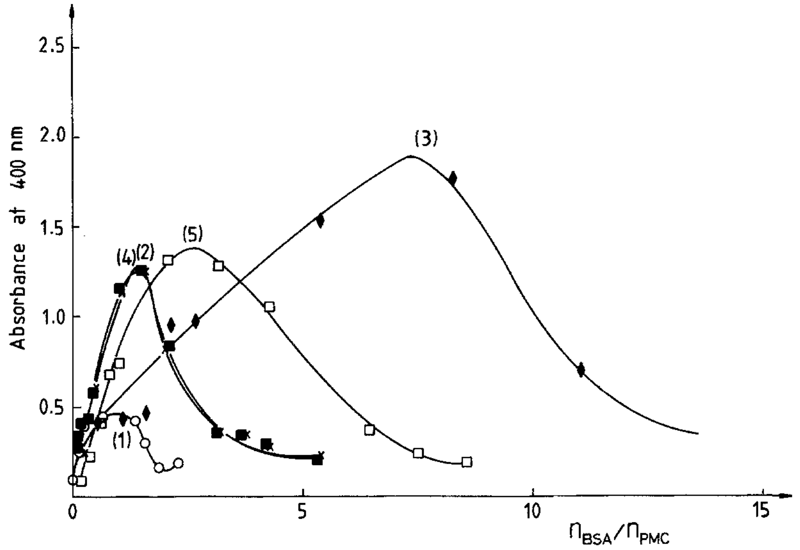
Fig. 10 The effect of temperature on the formation of the insoluble ternary mixture. pH = 7; $C_{\mathrm{PAAm}} = 0.15$ g/dl; $C_{\mathrm{BSA}} = 0.5$ g/dl; $C_{\mathrm{CuSO_4}} = 0.05$ g/dl

The effect of temperature and stirring: The effect of temperature was studied at 16–37 °C in absence of NaCl at the ratio of $n_{\mathrm{BSA}}/n_{\mathrm{PMC}} = 1.0$ by mixing the above mentioned. Figure 10 represents dependence of the optical density A_{400} of the ternary mixture on time at different temperatures. The dependence of the values of the $\ln(dA/dt)$ obtained from these curves at different temperatures is linear and fits the relation $(dA/dt) = 10e^{4500/T}$.

The formation of insoluble ternary complexes also depends on the effect of stirring of a complex mixture. The effect of stirring of a complex mixture increases the rate of formation of insoluble ternary complex.

The effect of the molecular weight of PAAm: The effect of the molecular weight of PAAm on the formation of soluble and insoluble ternary complexes has been studied at different molecular weights of PAAm: 20 000 (PMC1), 45 000 (PMC2) and 230 000 (PMC3). In all cases, the ratio of $n_{\mathrm{CuSO_4}}/n_{\mathrm{AAm}}$ was kept constant at 0.1. The dependence of A_{400} of the ternary system on the $n_{\mathrm{BSA}}/n_{\mathrm{PMC}}$ at different molecular weights of PAAm is shown in Fig. 11. The value of absorbance of the ternary systems depends on the molecular weight of PAAm. At each of the three state, this dependence passes through a maximum. However with increasing of molecular weight of PAAm (Fig. 11, curves 1–3), the maximum value of absorbance increases and soluble system takes place at higher values of $n_{\mathrm{BSA}}/n_{\mathrm{PMC}}$,

Fig. 11 The dependence of A_{400} on molecular weight of PAAm and Cu^{2+} concentration for the mixture of BSA-Cu^{2+}-PAAm. pH = 7; 20 °C; M_v = 20000 (1), 45000 (2) and 230000 (3); C_{PAAm} = 0.15 g/dl; C_{CuSO_4} = 0.05 g/dl (4); C_{CuSO_4} = 0.1 g/dl (5)



i.e., it is necessary to use more BSA molecules to obtain soluble ternary system for high molecular weight of PAAm in ternary mixture.

The effect of the Cu^{2+} concentration: The effect of the Cu^{2+} concentration on the formation of soluble and insoluble ternary system was investigated (Fig. 11). The ratios of $CuSO_4$ to the polymer are 0.1 and 0.2 and the molecular weight of PAAm was kept constant M_v = 45000. It was observed that the increase of the Cu^{2+} concentration in ternary system is proportional to the amount of BSA on the ratio of n_{BSA}/n_{PMC} to obtain soluble ternary system. Maximum precipitations were obtained for the ratios of $n_{BSA}/n_{PMC} = 1.5$ (Fig. 11, curve 4) and $n_{BSA}/n_{PMC} = 2.5$ (Fig. 12, curve 5) and for $n_{CuSO_4}/n_{AAm} = 0.1$ and 0.2 respectively. With increasing Cu^{2+} concentration in the system, the amount of Cu^{2+} concentration which is bound to polymer increased, i.e., the capacity of binding of PMC molecule to protein molecule increased. According to this idea, to obtain a soluble system (non stoichiometric binding of several protein molecules to one polymer molecule) in the case of greater Cu^{2+} concentration, it was necessary to add more BSA to system.

On the basis of the results represented here, as well as others from earlier investigations on analogous polymer–protein systems [2, 5, 6], one can propose a hypothetical structural scheme for the formation of the soluble and insoluble ternary polycomplexes. When PMC is titrated with BSA solution, an increase of BSA content to the component ratio of [BSA]/[PMC] = 1:1 leads to an increase of the system turbidity indicating the formation of stoichiometric (one polymer molecule binds to one protein molecule and positive and negative charges neutralize) [7] insoluble polycomplex in aqueous media. The further increase of BSA content in mixture ([BSA]/[PMC] > 1) leads to the formation of non-stoichiometric (the binding of several protein molecules to one polymer molecule) soluble polycomplexes in aqueous media followed by a decrease of turbidity. When the ratio of the components in solution is [BSA]/[PMC] ≅ 2, the system becomes soluble.

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