Bulgarian Chemical Communications, Volume 54, Issue 3 (pp. 271-276) 2022 *DOI: 10.34049/bcc.54.3.F010*

Stability investigation of PAA-BSA bioconjugate and PAA-Cu^{2+}-BSA ternary biocomplex at different pHs

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Accepted: July 14, 2022

Polycomplexes containing polyelectrolyte (PE), metal and protein reveal strong immunogenecity and provide a high level of immunological protection. The solubility, composition, and stability of these polycomplexes depend on pH, metal/PE, and protein/PE ratios. Zetasizer parameters such as particle size, polydispersity, mobility and zeta potential are used to control the stability of solutions. These parameters are also very important for polyelectrolyte–protein binary conjugate and polyelectrolyte–metal-protein ternary complexes because of bioavailability, dissolution and immune toxicity of these complexes. Polyacrylic acid (PAA) is a polyanion polyelectrolyte and has been used in vaccine adjuvants due to its safety and lower toxicity. In the current study, PAA-BSA bioconjugate and PAA-Cu^{2+}-BSA ternary biocomplex were freshly synthesized according to two different procedures in the presence of acrylic acid (AA), copper (II) ions and bovine serum albumin (BSA). The stability of PAA-BSA bioconjugate and PAA-Cu^{2+}-BSA ternary biocomplex at different pHs was investigated by zetasizer measurements and the pH effect on the stability of PAA-BSA bioconjugate and PAA-Cu^{2+}-BSA ternary biocomplex was determined.

Keywords: PAA-BSA bioconjugate, PAA-Cu^{2+}-BSA ternary biocomplex, stability, zeta potential

INTRODUCTION

Recently, synthetic polyelectrolytes (SPE) have been used quite frequently in biotechnological applications due to the enhancing immunoresponse to the immunizing antigen and provide an adjuvant effect. When SPEs are conjugated to microbial and viral antigens to obtain a stable complex, the immune response of the organism increases and provides immune protection. With this idea, artificial vaccines were started to be produced against infections that could not be controlled yet. SPEs can combine with antigen *via* metal (M) ion by means of activating the SPE surface. Thus, the synthetic vaccine model can be improved using SPE-M-protein ternary complexes. [1, 2] Polyanionic polymers have been used in vaccine adjuvants. Poly(acrylic acid) which is an effective adjuvant, is one of the linear polymers used as peptide carrier [3]. Poly(acrylic acid) is a polyanion in aquous solution and the structure of the PAA-M-protein ternary biocomplex is affected by pH and different salt concentrations [4]. Zetasizer measurements such as particle size, zeta potential give information about stability and charge of particles in solutions. Zeta potential parameter shows the degree of repulsion between the charged particles in the solution. High zeta potential values imply highly charged particles, which prevents aggregation of the particles due to electric repulsion. If the zeta potential is low, van der Waals attractive forces act upon particles and attraction between particles overcomes repulsion. Thus, the particles in solution combine and aggregate. The zeta potential values bigger than −30 mV and +30 mV are accepted as optimum condition for good physical stabilization of solutions due to supplying sufficient repulsive force [5-8].

PAA-BSA bioconjugate and PAA-Cu^{+2}-BSA ternary biocomplex were freshly synthesized according to the method in reference [4]. The effect of different concentrations of NaCl salt on the stability of the PAA-BSA bioconjugate and the PAA-Cu^{2+}-BSA ternary biocomplex has been published [9]. In this study, the pH effect on the particle size, conductance, stability and charge of PAA-BSA bioconjugate and PAA-Cu^{2+}-BSA ternary biocomplex was examined.

EXPERIMENTAL

Materıals

Polyacrylic acid (Aldrich 523925), bovine serum albumin (BSA) (Sigma A 7030), copper sulfate ($CuSO_4.5H_20$) (Merck 102788), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (Sigma) were used without any purification.

Preparation of BSA solution: BSA solution was prepared as 60 mg BSA/ 5 ml pure water. PAA- BSA bioconjugate was obtained by a conventional method.

Synthesis of PAA-BSA bioconjugate: Polyacrylic acid (1 mg) was dissolved in DMSO (0.1 ml) at room temperature and 1.9 mL of PBS solution was added to avoid micelle formation and to keep the organic

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phase in total solution max 5%. EDC (4 mg) was added to this solution and the pH was adjusted to 4.7 at which is the highest EDC reactivity. After 3 h mixing of the solution, BSA was added to the reaction mixture (n BSA /n PAA : 2.0). Reaction pH was adjusted to 7 with 1 N NaOH and reaction mixture was stirred for 17 h.

Synthesis of PAA-Cu^{2+}-BSA biocomplex (I method): PAA solution was prepared as 3mg/ml in PBS. $CuSO_4.5H_2O$ solution was prepared in ultra pure water. BSA solution was prepared in PBS. PAA-Cu^{2+} bioconjugate was prepared with 3333 µl (9.999 mg) of PAA solution and $CuSO_4.5H_2O$. This solution was stirred overnight and pH was adjusted to 7 with NaOH solution. BSA solution was added to this bioconjugate solution and the PAA-Cu^{2+}-BSA ternary biocomplex was obtained (blue colored solution, pH=7) ($n_{Cu^{2+}}/n_{PAA} = 0.4$; $n_{BSA} / n_{PAA} = 2$)

Synthesis of PAA-Cu^{2+}-BSA biocomplex (II method): The pH of 3333 µl (9.999 mg) of PAA was adjusted to 6 and stirred overnight. BSA solution was slowly added to this solution and stirred overnight. After that, $CuSO_4.5H_2O$ solution was added dropwise and the PAA-Cu^{2+}-BSA ternary biocomplex was obtained (blue colored solution, pH 7)($n_{Cu^{2+}}/n_{PAA} = 0.4$; $n_{BSA} / n_{PAA} = 2$) (Scheme 1).

Zetasizer measurements

Zetasizer measurements of particle size, conductance, electrophoretic mobility and zeta potentials of BSA, PAA-BSA and PAA-Cu^{2+}-BSA (I and II methods) at different pHs were performed *via* Brookhaven 90 Plus-Pals/BI-MAS (Multi Angle Particle Sizing) and zeta potential analyzer at 37ºC. The particle size and zeta potentials of BSA, PAA-BSA and PAA-Cu^{2+}-BSA were obtained by calculating the average of 10 measurements.

RESULTS AND DISCUSSION

PAA-BSA binary bioconjugate and PAA-Cu^{2+}-BSA ternary biocomplex were freshly prepared. The PAA-Cu^{2+}-BSA ternary biocomplex changed color from blue to purple with changing pH (Fig. 1).

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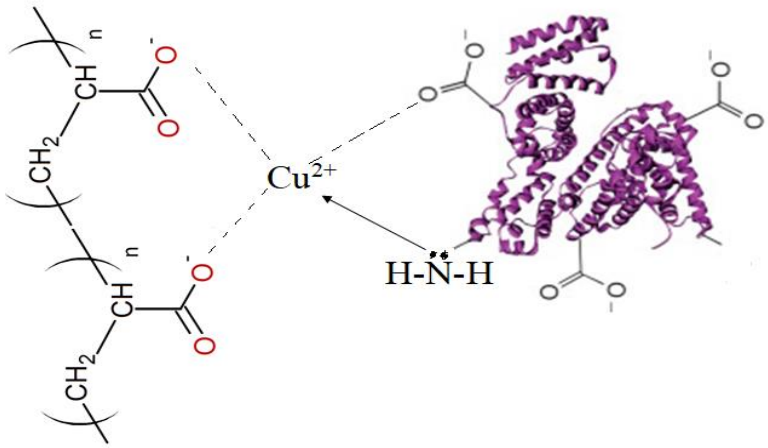

Scheme 1. Chemical structure of PAA-Cu^{2+}-BSA ternary biocomplex

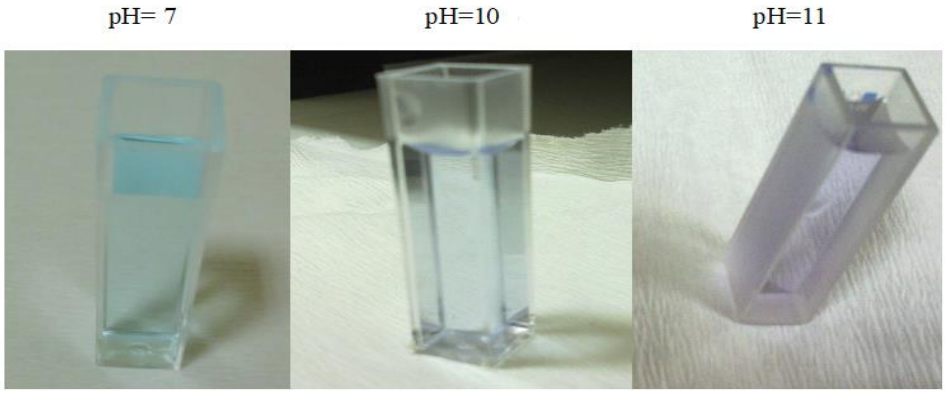

Fig. 1. Color change of PAA-Cu^{2+}-BSA ternary biocomplex at different pHs

This color change shows that copper reacts with the protein. The color of the PAA- Cu^{2+}-BSA ternary biocomplex solution is blue between pH 7 and 9, this blue color converts to purple with increasing pH to 10 due to the biuret reaction between copper and protein [10].

The particle sizes of BSA, PAA-BSA and PAA-Cu^{2+}-BSA between pH 7 and 11 were measured by dynamic light scattering technique *via* a zetasizer. The results are shown in Table 1 and Fig. 2.

Table 1. Particle sizes (nm) of BSA, PAA-BSA binary bioconjugate and PAA- Cu^{2+}-BSA ternary biocomplex at different pHs

pH	BSA	PAA-BSA	PAA-Cu^{2+}-BSA	
			I Method	II Method
7	315	475	480	495
8	420	560	520	450
9	630	620	380	445
10	650	360	480	565
11	930	280	275	360

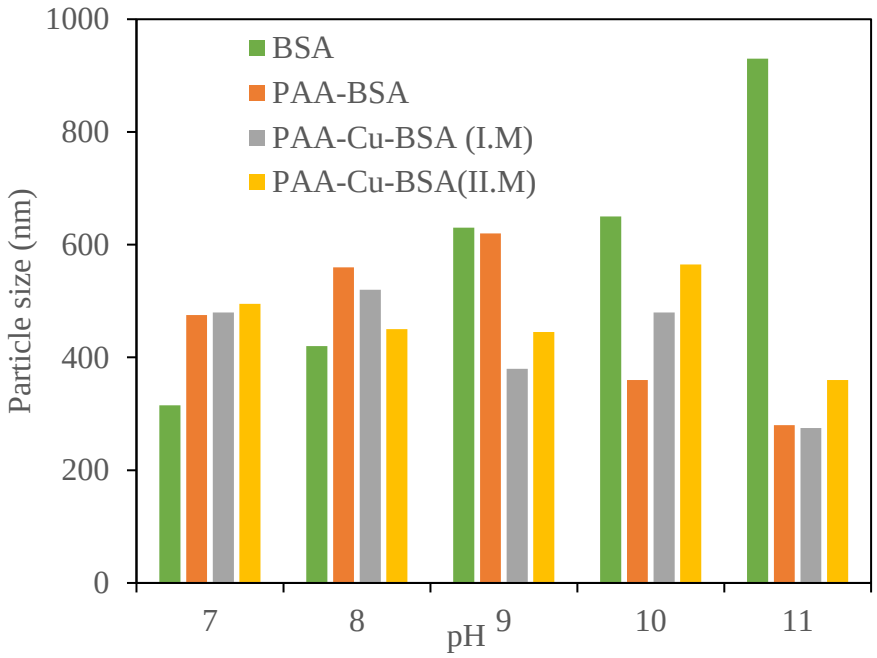

Fig. 2, Effect of pH on the particle size of BSA, PAA-BSA and PAA- Cu^{2+}-BSA (I and II M)

Table 2. Electrophoretic mobility (m^2/V.s) of BSA, PAA-BSA binary bioconjugate and PAA- Cu^{2+}-BSA ternary biocomplex at different pHs

pH	BSA	PAA-BSA	PAA-Cu^{2+}-BSA	
			I Method	II Method
7	-1.4	-2.1	-2.5	-2.9
8	-2.2	-2.6	-2.8	-2.9
9	-1.1	-2.0	-3.5	-2.1
10	-1.2	-2.6	-2.1	-2.8
11	-2.2	-2.7	-2.8	-2.7

Table 3. Zeta potentials (mV) of BSA, PAA-BSA binary bioconjugate and PAA-Cu^{2+}-BSA ternary biocomplex at different pHs

pH	BSA	PAA-BSA	PAA-Cu^{2+}-BSA	
			I Method	II Method
7	-21.7	-27.3	-31.7	-37.8
8	-28.6	-30.1	-36.4	-38.2
9	-14.8	-25.7	-30.6	-34.2
10	-18.3	-30.4	-34.8	-35.4
11	-28.0	-31.0	-35.8	-36.8

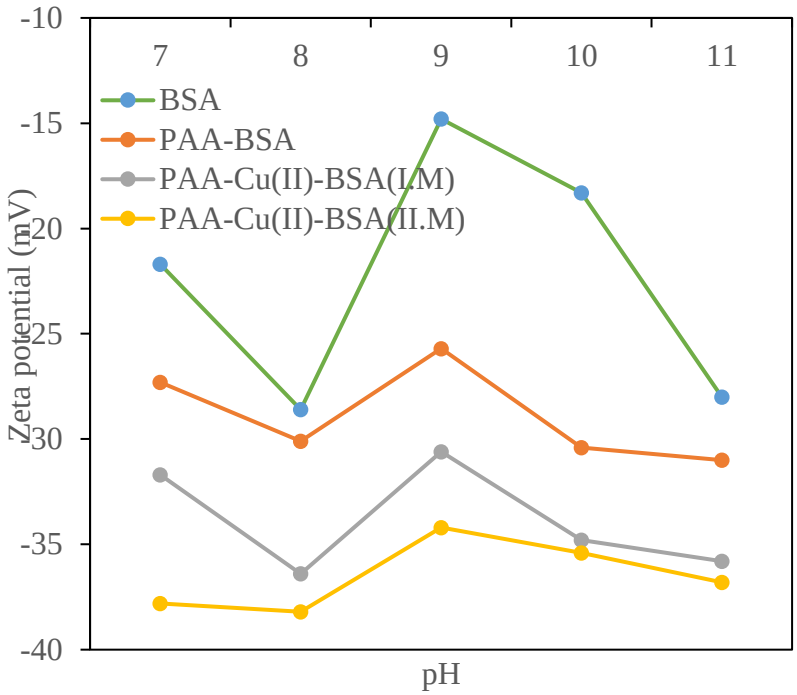

Fig. 3. Effect of pH on the zeta potentials (mV) of BSA, PAA-BSA and PAA- Cu^{2+}-BSA (I and II M)

Particle sizes of PAA-BSA and PAA- Cu^{2+}-BSA (I and II M), compared with BSA, increased at pH 7 and 8, but decreased in basic solutions. This trend suggests that the original closed structure of BSA is loosened after BSA binds with PAA and Cu ions at pH 7 and 8 while BSA structure shrinked between pH 9 and 11 [11].

The electrophoretic mobility and zeta potentials of BSA, PAA-BSA and PAA-Cu(II)-BSA between pH 7 and 11 were measured by phase analysis light scattering using a zetasizer. The results are given in Tables 2 and 3 and Fig. 3.

The electrophoretic mobility is proportional to zeta potential of solid particles in solution. Also, zeta potential indicates the surface charge property and stability of the solid particles in the solution. BSA protein contains many functionals such as amino group, carboxyl group and it can form complexes with polymers and metal ions and cause a change in surface charge.

It is noteworthy that the zeta potentials of BSA, PAA-BSA binary bioconjugate and PAA- Cu(II)-BSA ternary biocomplexes at different pHs were different. The zeta potential values of BSA were smaller than those of PAA-BSA and PAA-Cu^{2+}-BSA (I and II M) and all the samples had negative charge in the studied pH range. Comparing the stability of all samples, the most stable solution in the studied pH range was PAA-Cu^{2+}-BSA(II.M) The increase in negative zeta potential represents an electrostatic attraction between PAA-BSA and Cu(II) ions. The conductance of BSA, PAA-BSA and PAA-Cu^{2+}-BSA between pH 7 and 11 was measured by zetasizer. The results are given in Table 4 and Fig. 4.

Table 4. Conductance (μS) of BSA, PAA-BSA binary bioconjugate and PAA- Cu^{2+}-BSA ternary biocomplex at different pHs

pH	BSA	PAA-BSA	PAA-Cu^{2+}-BSA	
			I Method	II Method
7	440	6240	3330	2860
8	670	5830	4410	3300
9	1095	5580	3600	3065
10	2380	5210	3770	3450
11	9710	8400	7400	7090

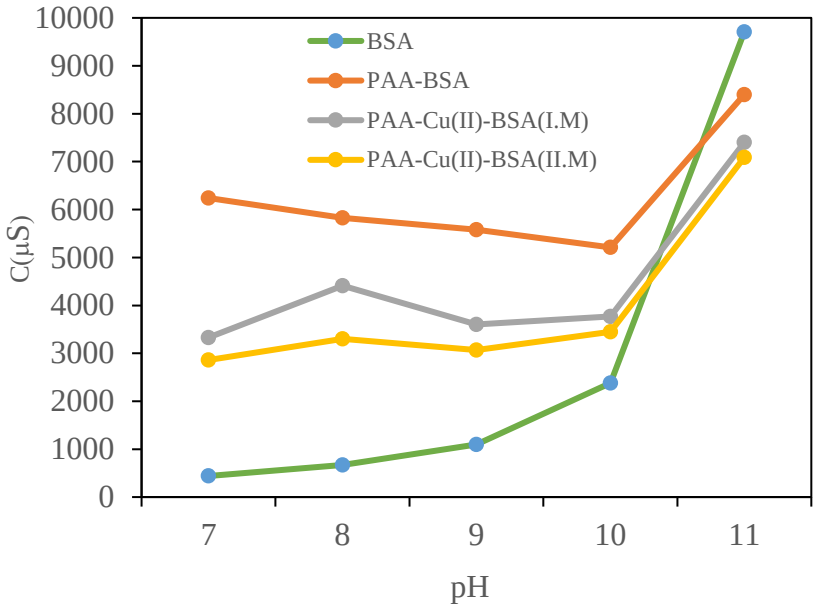

Fig. 4, Conductance change (μS) of BSA, PAA-BSA and PAA- Cu^{2+}-BSA (I and II M) as a function of pH

It was observed that the conductance of samples progressively increases with increasing pH. The conductance order of the samples was as follows: PAA-BSA> PAA-Cu^{2+}-BSA(I.M) > PAA- Cu^{2+}-BSA(II.M)>BSA. When the conductance of the samples was compared at increasing pH values, the highest conductance was registered for PAA-BSA. This increase in conductance of the PAA-BSA binary conjugate as a function of pH may be thought to be due to the exposure of more PAA ions with the solution.

CONCLUSION

In this study, PAA-BSA binary conjugate and PAA- Cu^{2+}-BSA ternary biocomplex were freshly synthesized according to two methods and their stability at different pHs was investigated *via* zetasizer measurements of particle size, zeta potential and conductance. The zeta potentials of BSA, PAA-BSA and PAA- Cu^{2+}-BSA (I and II M) surfaces between pH 7 and 11 were at negative values and they preserved the stability at the studied pHs (zeta potentials >-30). According to the results, it was observed that the Cu metal ion had a reducing effect on the conductance of the biocomplexes. This was thought to be because the ternary structure was more stacked and stable in the presence of Cu metal, and PAA was all bound to BSA.

Acknowledgement: *The current study was supported by the Scientific Research Project Coordination Center of Yıldız Technical University, Turkey (Project No: 2012-01-02-GEP04).*

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