



## Investigation of different pHs and salt concentration effects on the stability of poly(methyl vinyl ether-co-maleic anhydride)-Bovine Serum Albumin bioconjugate and poly(methyl vinyl ether-co-maleic anhydride)-Cu<sup>2+</sup>-Bovine Serum Albumin biocomplex in aqueous solutions

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In this study, it is aimed to obtain preliminary information for the development of more effective and more stable biocomplexes as vaccine model. It is carried out synthesis and determine the stability at different pH and salt concentrations of synthesized poly(methyl vinyl ether-co-maleic anhydride)-bovine serum albumin (PMVEMA-BSA) bioconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex. For this purpose; PMVEMA-BSA conjugate was synthesized with poly(methyl vinyl ether-co-maleic anhydride) (PMVEMA) and bovine serum albumin (BSA), a water soluble synthetic polyelectrolyte, and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex in the presence of copper ions (Cu<sup>2+</sup>). The stability and surface charge of the prepared bioconjugate and biocomplex at varying pHs and salt concentrations were measured with Brookhaven 90 Zeta PULS Zetasizer device. It was observed that different pH and salt concentrations affect the stability of binary and ternary complexes.

Keywords: PMVEMA, BSA, binary conjugate, ternary complex, zeta sizer measurements.

### Introduction

Recently, new generation vaccine technology has been among the topics of interest for researchers. Synthetic peptide vaccines are new generation technological vaccines and they are low-risk, cheap, safe and low-cost vaccine technology because they contain microbial antigens<sup>1-5</sup>. Synthetic peptides with short or long chain of amino acids used in synthetic vaccine systems do not have sufficient size in terms of immunogenic activity<sup>6,7</sup>. In order to develop the immune response, their molecular weight and diameter should be increased<sup>8</sup>. For this reason, biopolymer systems are used in order to gain immunity-stimulating properties and to stimulate antibody production against peptides. Especially, water soluble polyelectrolyte polymers (PEP) and proteins such as microbial antigen conjugates (PEP-P) and biocomplexes of PEP-P with metal ions such as Cu<sup>2+</sup> are role model for vaccine<sup>9,10</sup>. Maleic anhydride copolymers are water soluble polyelectrolyte and the most used copolymer in biotechnological applications. One of the most used maleic anhydride copoly-

mer is poly(methyl vinyl ether-co-maleic anhydride) which is a biodegradable polyanhydride and non toxic<sup>11-13</sup>.

A stable complex is important for both developing an immune response and providing effective immunity. Zetasizer measurements provide an explanation of the properties of molecules or particles in the liquid medium such as the size of the particles and their mobility values. These properties of molecules directly affect the bioavailability of molecules, the effect of ions on blood coagulation, dissolution or immunotoxicity. Zetasizer measurements are used to explain the stability and the effect of ions on blood of the synthesized complexes<sup>14</sup>.

Zetasizer measurements are particle size, polydispersity, mobility and zeta potential. They give the valuable information about particle in solution. The particle size of biological molecules are factors affecting their properties such as immunogenic activity, toxicity, and intracellular uptake. Polydispersity index is a parameter that indicates how homoge-

neous the particle size is. It is the mobility capacity of ions in solution. The increase in mobility is an indication of increased stability. Zeta potential refers to the net surface charge. It also indicates the presence of functional groups on the surface. Zeta potential represents the surface charge of nanoparticles and reflects their long-term stability. The data obtained directly affect the bioavailability, dissolution and immunotoxicity of the molecules<sup>15,16</sup>. In the current study, it was investigated particle size and zeta potentials of BSA, PMVEMA-BSA bioconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex to determine stability of them at the different pHs and salt concentrations.

### Experimental

BSA (Sigma A 7030) solution was prepared in pure water at a ratio of 60 mg/5 ml.

#### Synthesis of PMVEMA-BSA bioconjugate:

PMVEMA-BSA bioconjugate was synthesized by conventional method. 1 mg PMVEMA (Gantiez AN-139 BF) solved in, 0.1 ml DMSO added in 1.9 ml PBS. pH 4.7' adjusted and added 4 mg (1-ethyl-3-(3-dimethylaminopropyl)carbodiimid hydrochloride(EDC) as crosslinking agent and BSA added in reaction media (nBSA/nPMVEMA : 2.0). The synthesis of PMVEMA-BSA is given Fig. 1.

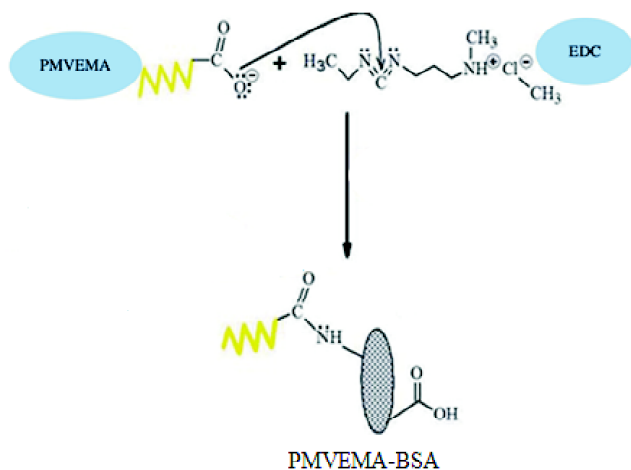


Fig. 1. The synthesis of PMVEMA-BSA bioconjugate.

#### Synthesis of PMVEMA-Cu<sup>2+</sup>-BSA biocomplex:

PMVEMA-Cu<sup>2+</sup>-BSA biocomplex was synthesized in the presence of metal. CuSO<sub>4</sub>·5H<sub>2</sub>O (Merck) added in 3333 μL PMVEMA solution and binary solution obtained (nCu<sup>2+</sup>/

nPMVEMA = 0.5) BSA solution slowly added in PMVEMA-Cu<sup>2+</sup> binary complex solution and mixed and obtained PMVEMA-Cu<sup>2+</sup>-BSA (nBSA/nPMVEMA= 2.0)<sup>17</sup>. The synthesis of PMVEMA-Cu<sup>2+</sup>-BSA is given Fig. 2.

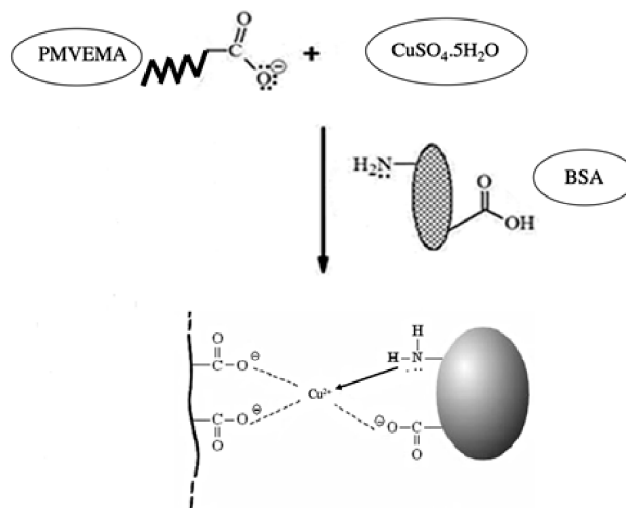


Fig. 2. The synthesis of PMVEMA-Cu<sup>2+</sup>-BSA biocomplex.

#### Zetasizer measurements:

Aqueous solutions of the BSA, bioconjugate and biocomplex were prepared and their Zetasizer measurements in different pH and salt concentrations were measured dynamic light scattering and electrophoretic light scattering technologies using with Brookhaven 90 Zeta PLUS Zetasizer device. Particle size was presented by intensity distribution, and size distribution was evaluated by polydispersity index (PDI).

### Results and discussion

The polydispersity of BSA, PMVEMA-BSA bioconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex were determined as 0.2 and 0.5. This value showed that BSA, PMVEMA-BSA bioconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex particles were monodispers in aqueous solution such as studied pHs and salt solutions. The particle size of BSA, PMVEMA-BSA bioconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex in different pHs and different salt concentrations was given in Table 1 and Table 2, respectively. The particle size values of PMVEMA-Cu<sup>2+</sup>-BSA is between BSA and PMVEMA-BSA particle size values. The zeta potentials of BSA, PMVEMA-BSA bioconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex in dif-

**Table 1.** Particle size of BSA, PMVEMA-BSA biconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex in different pHs

pH	BSA	PMVEMA-BSA	PMVEMA-Cu <sup>2+</sup> -BSA
6	645	480	626
7	700	395	520
8	745	350	540
9	755	375	530
10	766	415	626
11	760	540	555
12	755	530	450

**Table 2.** Particle size of BSA, PMVEMA-BSA biconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex in different salt concentrations

[NaCl]	BSA	PMVEMA-BSA	PMVEMA-Cu <sup>2+</sup> -BSA
0	360	415	425
0.001	365	425	415
0.002	370	428	405
0.005	370	435	355
0.008	380	370	320
0.01	385	365	310
0.02	390	370	370
0.05	425	450	335

**Table 3.** Zeta potentials of BSA, PMVEMA-BSA biconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex in different pHs

pH	BSA	PMVEMA-BSA	PMVEMA-Cu <sup>2+</sup> -BSA
6	-9.97	-21.18	-16.63
7	-10.02	-21.68	-14.62
8	-10.25	-19.67	-11.20
9	-10.23	-27.48	-12.90
10	-10.83	-26.36	-19.13
11	-10.45	-8.29	-15.23
12	-10.93	-6.62	-11.77

**Table 4.** Zeta potentials of BSA, PMVEMA-BSA biconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex in different salt concentrations

[NaCl]	BSA	PMVEMA-BSA	PMVEMA-Cu <sup>2+</sup> -BSA
0	-24.93	-18.01	-13.15
0.001	-25.93	-24.25	-12.35
0.002	-29.28	-24.96	-18.74
0.005	-29.77	-25.22	-23.68
0.008	-29.19	-25.45	-22.62
0.01	-28.95	-25.09	-22.67
0.02	-26.55	-22.76	-22.24
0.05	-24.89	-21.17	-20.25

ferent pHs and different salt concentrations was given in Table 3 and Table 4, respectively. They have negative zeta poten-

tial values. It means that the surface charge of them is negative at the studied pHs and salt concentrations. It was not seen any aggregation studied pH values and different salt concentrations for BSA, PMVEMA-BSA biconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex solutions. According to results, they protect the stability at the studied pHs and salt concentrations.

## Conclusions

It was investigated stability conditions of the freshly synthesized PMVEMA-BSA bioconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex according to Zetasizer measurements. The zeta potential measurement showed that the absolute value of zeta potential of the PMVEMA-BSA bioconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex particles was larger than 20 mV, which indicates that the PMVEMA-BSA bioconjugate and PMVEMA-Cu<sup>2+</sup>-BSA biocomplex can offer good stability in aqueous suspension. The stability of biocomplexes under organism conditions is a very important condition in terms of the emergence of their activities in the immune system or the activation of existing features. Zetasizer measurements provide information about the stability of the biocomplex and are an important indicator in terms of immunogenicity. For this reason, it is expected that the structure and stability in changing pH and salt conditions will be revealed, the data obtained will contribute to the studies to be carried out on the biocomplex, especially synthetic polymeric vaccine systems, and help the development of more effective and more stable biocomplexes.

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