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Zetasizer measurements of copolymer-drug carrier system: poly (maleic anhydrite-co-vinyl acetate) -acriflavine conjugate

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Copolymeric drug carrier system, poly (maleic anhydrite-*co*-vinyl acetate) copolymer (MAVA) carrying acriflavine drug (AF) has been examined by Zeta Potential Analyzer to determine the stability and controlled drug release tests in water with different pHs and simulated body fluids. Zetasizer measurements such as zeta potential, mobility, and particle size of prepared copolymer, drug and copolymer-drug conjugate (MAVA-AF) were done. The activity of copolymer-drug conjugate in different pHs and simulated body fluids was checked via UV-VIS spectrophotometer as a function of time.

Keywords: copolymer-drug conjugate, stability, particle size, zeta potential

1. INTRODUCTION

Recently, synthetic polymers are used for controlled drug delivery systems or drug carrier as biomaterials. There has been a growing interest in polymer conjugation with biologically active components. Such conjugates usually accumulate in tumors and can reduce toxicity in the body. Depending on the desired location, polymer conjugates can be synthesized to either have degradable or non-degradable chemical bonds with their associated drug. There is a strong desire to synthesize polymeric conjugates with bioactive components and other drugs. Polymer-drug conjugates are drug molecules held up in polymer molecules and have shown very promising results. The drugs remain attached to the polymer and are not activated until the enzymes associated with the diseased tissue are present. This process severely minimizes damage to healthy tissue [1-3]. Surface properties of drug carrier systems are responsible for their interactions with plasma proteins. Zetasizer measurements which are zeta potential, particle size and mobility provide valuable properties of particles or molecules in liquid medium. These characteristics directly affect bioavailability, dissolution and immunotoxicity [4-8].

In the present work, the particle size, mobility and zeta potential of novel copolymer-drug conjugate including nontoxic drug carrier MAVA [9], MAVA-AF [10] were measured as a function of pH in water and as a function of time in the simulated body fluids.

2. EXPERIMENTAL

2.1. Instrument and materials

The particle size and particle size distribution of MAVA, AF and MAVA-AF solutions in water were measured via Brookhaven 90 Plus/BI-MAS (Multi Angle Particle Sizing) and electrophoretic mobility and zeta potential measurements of all solutions were also determined by Brookhaven Zeta Potential Analyzer in water as a function of pH and in the different medias such as phosphate buffer saline (PBS; biotechnology grade, 137 mM NaCl, 2 mM KCl ve 10 mM phosphate buffer, pH 7.4 ± 0.1) tampon (Sigma) and Dextrose (5 %) at physiological temperature (37ºC) as a function of time. The Shimadzu UVmini-1240. UV/Visible spectrophotometer was used to check the activity of MAVA, AF and MAVA-AF.

2.2. Zetasizer Measurements

Electrophoretic mobility, μ_e referred as mobility is the average velocity of dispersed particles in an electiric field of 1V/m:

$$V_S = \mu_e E \quad (1)$$

Where E is electric field and V_S is an average drift velocity of particles. The sign of mobility shows the surface charge of particles. A positive mobility of a particle means the surface is positively charged, negative mobility means the surface is negatively charged. The zero mobility value shows the velocity is zero and electrostatic repulsion is small.

Zeta potential is the electrostatic potential at the electrical double layer surrounding a nanoparticle in solution. In other words, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle [4].

Zeta potential, ζ was determined ten times for each sample. Results were automatically calculated by the analyzer using the following Smoluchowski equation:

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$$\mu_e = \frac{\varepsilon\zeta}{\eta} \quad (2)$$

where μ_e is electrophoretic mobility, ε is the dielectric constant, ζ is the zeta potential and η is the viscosity of electrolyte [11]. Nanoparticles with a zeta potential between -10 and +10 mV are considered approximately neutral, while nanoparticles with zeta potentials of greater than +30 mV or less than -30 mV are considered strongly cationic and strongly anionic, respectively [12].

According to general colloid chemistry principles, a dispersed system typically loses stability when the magnitude (i.e. absolute value) of the zeta potential decreases to less than approximately 30 mV. As a result, there will be some region surrounding the condition of zero zeta potential (i.e. the isoelectric point, or IEP) for which the system is not particularly stable. Within this unstable region, the particles may agglomerate, thereby increasing the particle size [4].

3. RESULTS AND DISCUSSION

The particle size, mobility and zeta potential of MAVA, AF and MAVA-AF solutions in water were measured as a function of pH and different media such as PBS buffer (Sigma) and dextrose (5 %). The copolymer-drug activity in studied pHs and medias was monitored by UV-VIS spectrophotometer.

The pH effects on particle size, mobility and Zeta Potential of MAVA solution, AF and MAVA-AF were given in Table 1, Table 2 and Table 3, respectively.

MAVA in water is negatively charged and it is anionic. Particle size of MAVA increases via increasing pH and MAVA showed maximum zeta potential at pH=7. MAVA was stable in water all studied pH.

AF in water is negatively charged and it is anionic. Particle size of AF does not change with increasing pH and AF is stable at basic media. it is more stable via increasing pH.

MAVA-AF in water is negatively charged and it is anionic. Particle size of MAVA-AF does not change via increasing pH and it is nearly neutral at acidic media and it is more stable via increasing pH.

The activity of MAVA, AF and MAVA-AF in water at the different pHs were checked by UV-VIS and spectrums were given in Fig.1.

Table 1. pH effect on particle size, mobility and zeta potential of MAVA in water

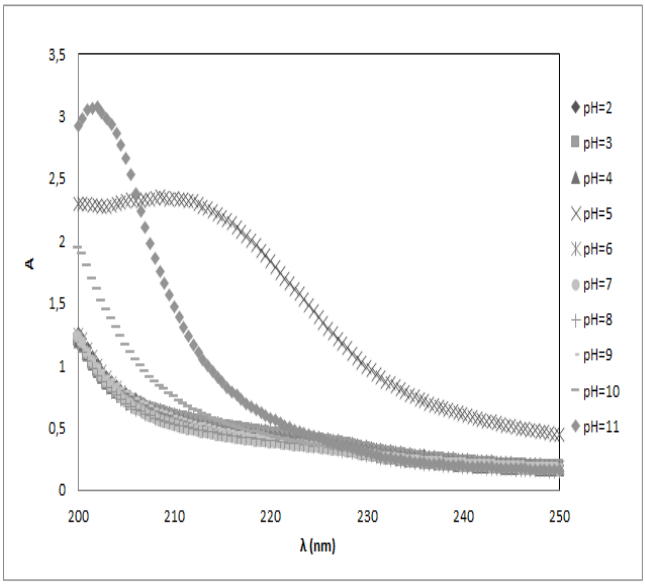
pH	Particle Size (nm)	Mobility	Zeta Potential (mV)
2	145	-2.8	-35.7
3	160	-3.7	-47.2
4	150	-3.4	-43.1
5	170	-4.5	-58.2
6	180	-2.2	-28.5
7	185	-5.5	-71.1
8	195	-5.3	-67.7
9	220	-5.0	-63.6
10	260	-4.6	-58.3
11	320	-3.5	-50.0

Table 2. pH effect on particle size, mobility and zeta potential of AF in water

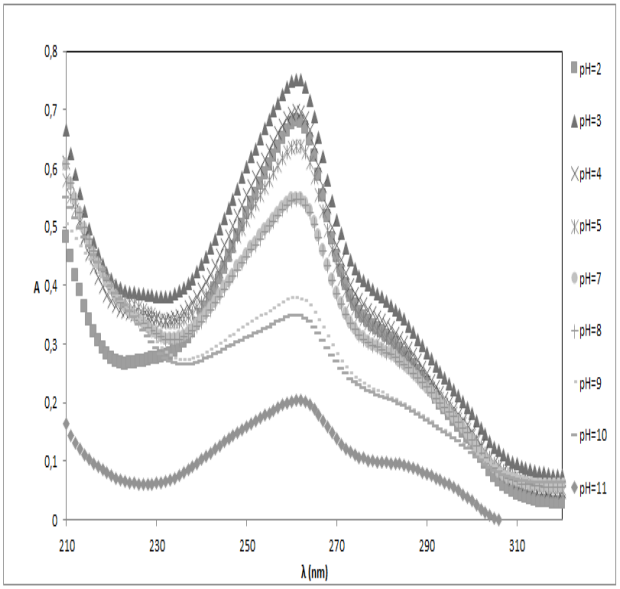
pH	Particle Size (nm)	Mobility	Zeta Potential (mV)
2	335	-0.7	-8.5
3	345	-0.8	-9.9
4	315	-1.1	-13.7
5	570	-1.2	-15.8
6	660	-2.3	-29.5
7	560	-2.5	-30.3
8	470	-2.8	-36.0
9	440	-2.9	-37.3
10	270	-2.6	-33.3
11	260	-2.8	-35.8

Table 3. pH effect on particle size, mobility and zeta potential of MAVA-AF in water

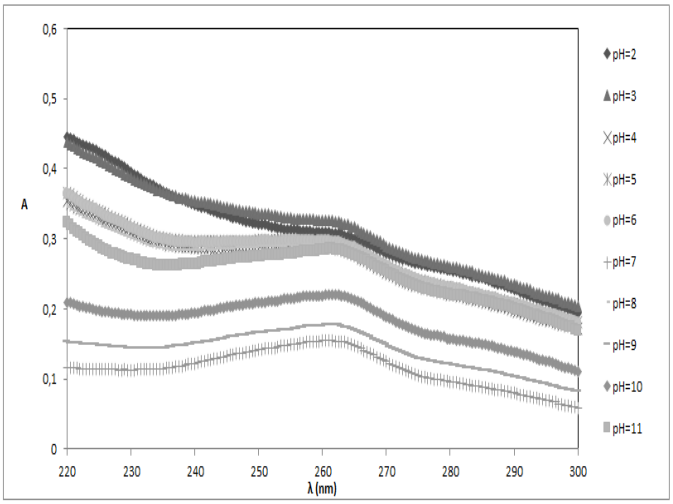
pH	Particle Size (nm)	Mobility	Zeta Potential (mV)
2	380	-0.1	-1.0
3	420	-0.2	-2.3
4	475	-0.3	-3.3
5	685	-0.8	-9.6
6	710	-1.2	-14.7
7	680	-2.1	-27.0
8	650	-1.9	-25.2
9	600	-1.6	-20.9
10	530	-1.7	-21.3
11	500	-1.8	-23.5



MAVA



AF



MAVA-AF

Figure 1. pH effect on activity of MAVA, AF and MAVA-AF in water as a function of pH

According to Figure 1, the activities of MAVA, AF and MAVA-AF were shown as pH=8 (the lowest), pH=11(the highest); pH=11 (the lowest), pH=3 (the highest) and pH=7 (the lowest), pH=3 (the highest), respectively.

The results of particle size, mobility and zeta potential measurements of MAVA, AF and MAVA-AF in 5 % dextrose as a function of time were given in Table 4, 5 and 6, respectively.

Table 4. Time effect on particle size, mobility and zeta potential of MAVA in 5 % dextrose

Time (h)	Particle Size (nm)	Mobility	Zeta Potential (mV)
0	120	-1.4	-17.6
0.5	119	-1.7	-21.8
1	119	-1.5	-19.4
2	120	-2.3	-28.9
3	120	-1.2	-15.0
96	120	-1.7	-21.3
168	100	-1.3	-16.5

MAVA in 5 % Dextrose is negatively charged and it is anionic. Particle size of MAVA does not change with time and it means it did not show any aggregation with time.

Table 5. Time effect on particle size, mobility and zeta potential of AF in 5 % dextrose

Time(h)	Particle Size (nm)	Mobility	Zeta Potential (mV)
0	960	-1.1	-13.6
0.5	620	-1.2	-15.4
1	270	-1.6	-20.1
2	170	-1.0	-12.8
3	270	-2.3	-28.9
96	590	-1.2	-15.3
168	480	-0.7	-9.0

AF in 5 % dextrose is negatively charged and it is anionic. Particle size and zeta potential of AF does not change with time and it means it did not show any aggregation with time.

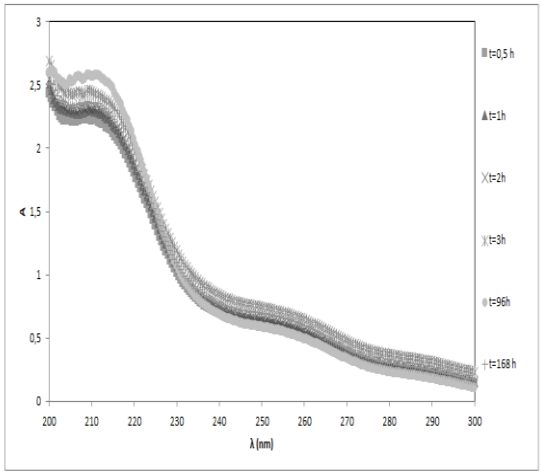
Table 6. Time effect on particle size, mobility and zeta potential of MAVA-AF in 5 % dextrose

Time(h)	Particle Size (nm)	Mobility	Zeta Potential (mV)
0	380	-1.3	-16.9
0.5	390	-1.4	-17.8
1	390	-1.3	-16.8
2	390	-1.5	-19.5
3	390	-1.1	-14.0
96	560	-1.1	-13.6
168	580	-1.4	-18.3

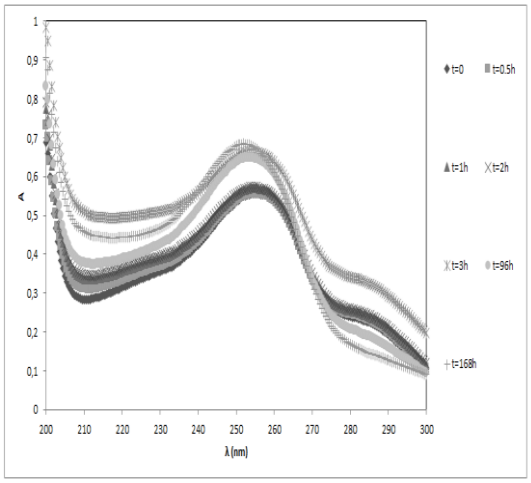
MAVA-AF in 5 % dextrose is negatively charged and it is anionic. Particle size and zeta potential of MAVA-AF did not change with time and it means it did not show any aggregation with time. According to the zeta potential results of MAVA, AF and MAVA-AF in 5 % dextrose, conjugation of MAVA and AF is good and no any size and stability change. The activity of MAVA, AF and MAVA-AF in 5 % Dextrose as a function of time were checked by UV-VIS and spectrums were given in Fig.2.

According to Fig.2, activity of MAVA, AF and MAVA-AF in 5 % dextrose is increasing as a function of time. It means they did not show any degradation in 5 % dextrose with time.

The results of particle size, mobility and zeta potential measurements of MAVA, AF and MAVA-AF in PBS as a function of time were given in Table 7, 8 and 9, respectively.



MAVA



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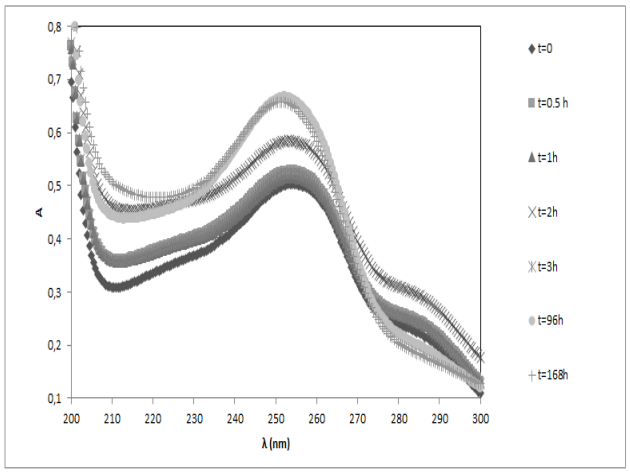


MAVA-AF

Figure 2. Time effect on the activity of MAVA, AF and MAVA-AF in 5 % dextrose.

Table 7. Time effect on particle size, mobility and zeta potential of MAVA in PBS

Time(h)	Particle Size (nm)	Mobility	Zeta Potential (mV)
0	180	-1.2	-15.1
0.5	190	-1.1	-15.0
1	230	-1.2	-15.1
2	240	-1.2	-15.1
3	250	-1.2	-15.0
96	270	-1.7	-21.3
168	290	-1.3	-16.5

MAVA in PBS is negatively charged and it is anionic. Particle size and zeta potential of MAVA in PBS did not change with time and it means it did not show any aggregation and coagulation with time.
Table 8 Time effect on Particle size, Mobility and Zeta potential of AF in PBS.

AF in PBS is negatively charged and it is anionic. particle size and zeta potential of AF in PBS did not change with time and it means it did not show any aggregation and coagulation with time.

Table 8. Time effect on particle size, mobility and zeta potential of AF in PBS.

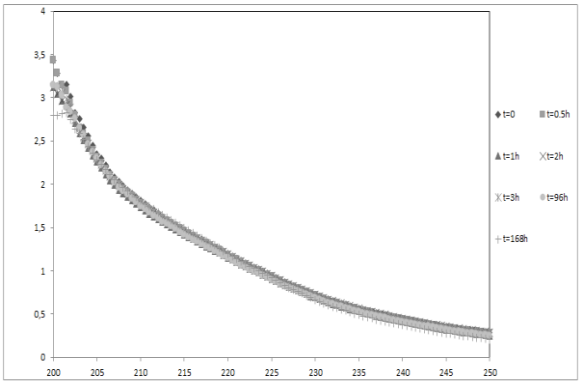
Time(h)	Particle Size (nm)	Mobility	Zeta Potential (mV)
0	460	-1.0	-12.9
0.5	360	-1.1	-13.6
1	280	-1.2	-15.7
2	340	-1.3	-17.5
3	310	-1.2	-15.7
96	270	-1.3	-17.5
168	230	-1.1	-13.6

Table 9. Time effect on particle size, mobility and zeta potential of MAVA-AF in PBS

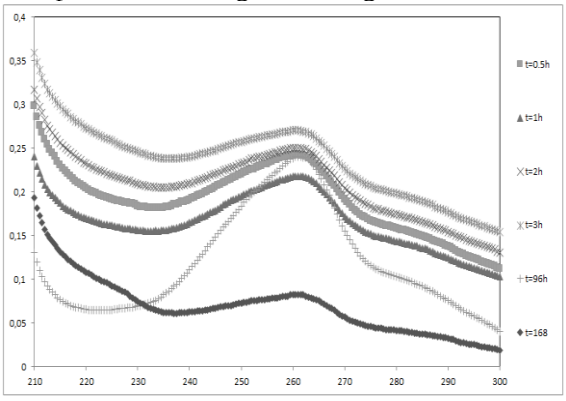
Time(h)	Particle Size (nm)	Mobility	Zeta Potential (mV)
0	810	-6.4	-8.1
0.5	870	-5.8	-10.3
1	880	-1.3	-16.4
2	810	-1.6	-20.2
3	850	-1.7	-21.4
96	810	-1.8	-22.1
168	850	-1.9	-24.8

MAVA-AF in PBS is negatively charged and it is anionic. Particle size and zeta potential of AF in PBS did not change with time and it means that it did not show any aggregation and coagulation with time. Particle size of MAVA-AF in PBS is bigger than MAVA and AF in PBS. It means conjugation of MAVA and AF is successful.

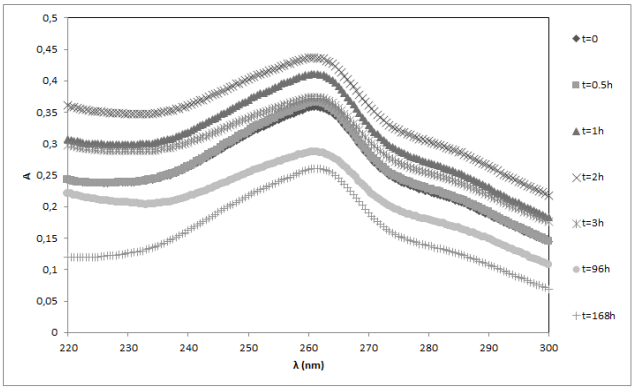
The activity of MAVA, AF and MAVA-AF in PBS as a function of time were checked by UV-VIS and spectrums were given in Fig.3.



MAVA



AF



MAVA-AF

Figure 3. Time effect on the activity of MAVA, AF and MAVA-AF in PBS.

According to Fig.3, activity of MAVA did not change with time in PBS, maximum activity of AF and MAVA-AF in PBS was shown at 2 h and their activities decreased with time.

4. CONCLUSIONS

Determination of stability and charge of novel copolymer-drug conjugate, MAVA-AF was carried out using zetasizer measurements in water as a function of pH and in simulated body fluids as a function of time. Activity of novel copolymer-drug conjugate was controlled via UV spectrophotometer in water as a function of pH and in simulated body fluids as a function of time. It was determined that the copolymer, drug and copolymer-drug conjugate are negatively charged. They show anionic character in water and simulated body fluids. The change of pH showed positive effect on the stability of copolymer-drug conjugate. Activation of copolymer-drug conjugate is increasing with time in dextrose solution and it was not shown any degradation or aggregation in this solution.

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