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PREPARATION OF SODIUM ALGINATE NANOPARTICLES CONTAINING BOVIN SERUM ALBOMIN (BSA)

MEHRASA RAHIMI BORUMAND[a], ABBAS ZARE MIRAK ABADI[b], NASER MOHAMMADPOUR DUONIGHI[c], MONA DAMAVANDI[d], HOSSEIN RAHNAMAFARD[e]

[a]Department of chemistry Islamic Azad University, Shahre rey Branch, Tehran, Iran

[b]Razi Vaccine and Serum Research Institute, Karaj, Iran

[c]Razi Vaccine and Serum Research Institute, Karaj, Iran

*(email:rahimi.borumand@gmail.com)

[d]Department of chemistry Islamic Azad University, Tehran North branch, Tehran, Iran

[e] Department of Electrical and Computer Engineering, Islamic Azad University ,Ghazvin branch, Ghazvin, Iran

ABSTRACT

Alginate, a natural biopolymer extracted from brown algae (kelp), has several advantages made it to be used as a matrix for excavation and delivery of a variety of biological agents. The present study was carried out systematically to prepare a new kind of biocompatible and biodegradable nano-particles for loading Bovin Serum Albumin (BSA) and to evaluate their potential as protein delivery system. A series of alginate nano-particles were prepared in this study. Calcium chloride (CaCl2) was used as a cross-linking agent, because of its cationic property, and poly-l-lysine was also used as polycationic agent to prepare matrix under ionic-gelation process. The effect of some factors (BSA, polymer, CaCl2 concentration, homogenization rate and pH) on particles size, morphology, encapsulation efficiency (EE) and loading capacity (LC) were studied. FT-IR was used to confirm cross-linking processes of polymer with cationic agents and Brad Ford assay was also used to measure LC and EE. TEM and Z.SIZER were used for morphological and dimensional studies. In optimum condition, the results were as follows: %EE and %LC were 89.25% and 89.23%, respectively.

Keywords: Nanoparticles; Sodium Alginate; Cross-linking; Poly-L-Lysine; Encapsulation

INTRODUCTION

There are many natural and synthetic polymers that have been found for the controlled release system of proteins, but many of them can be harsh and sometimes they can cause denaturation for active agents. Alginate,a natural biopolymer extracted from brown algae (kelp), has several advantages made it to be used as a matrix for excavation and delivery of a variety of biological agents (Figure 1).

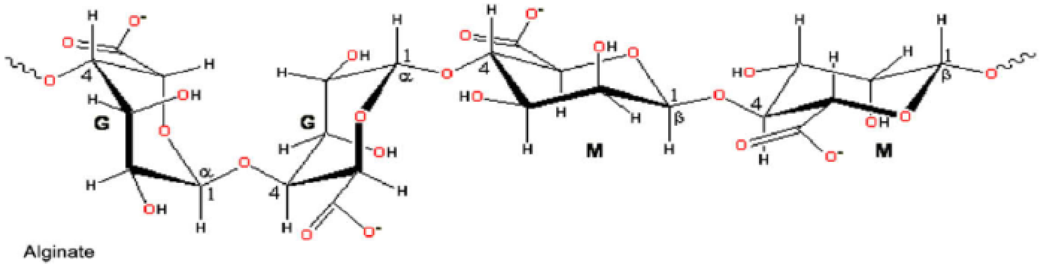

Fig.1: Alginate chemical structures

Alginates are also anionic polysaccharides consisting of a chain of(1-4)-linked B-D manuronicacid and a-l guluronic acid indifferent arrangements and quantities of the uronic acid residues.

Alginates, random, anionic, linear polymers, areconsisted of varying ratios of guluronic and man nuronic acid units. Salts of alginate are formed as the reaction of metal ions to the guluronic or mannuronicacid residues. The wide use of Alginate is in numerous biomedical applications such as drug delivery systems, as they are biodegradable, biocompatible, and mucoadhesive. Due to the addition of divalent ions such as, calcium, strontium, and barium, monovalent, water-soluble alginate salts undergo an aqueous sol–gel transformation to water-insoluble salts and as a result, these delivery systems are formed (Figure 2).

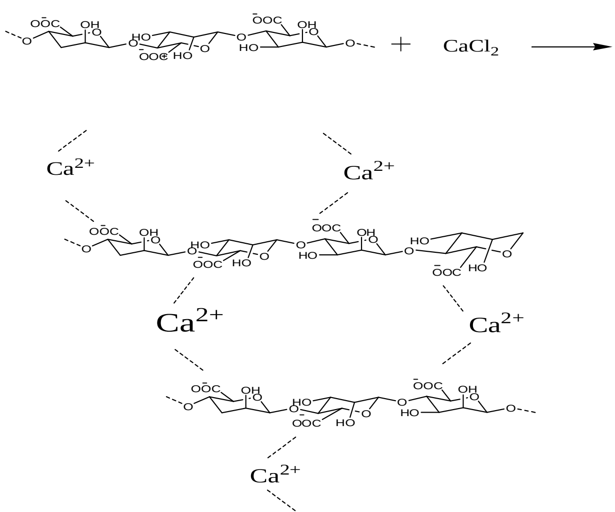

Fig 2: Reaction junction between Sodium Alginate &Calcium Chloride

By adding divalent cations to an aqueous solution, alginate can be ionic cross linked [1][2].

Due to their capacity for delivering different drugs, nanoparticles (NPs) have attracted the attention in the field of biomedicine [3]. Lots of studies have focused on the preparation of nanoparticles with the use of biodegradable hydrophilic polymers such as alginate and chitosan [4] [5]. It has been found that Alginate increases biotechnological and biomedical applications because of its several advantages, such as high biocompatibility, biodegradability, non-toxicity, non-immunogenicity, chelating ability, and the possibility of chemical modification [6], [7], [8], [9]. In addition, alginate has been widely used for encapsulation of cells [10], [11], proteins [12], DNA [13], venoms [14] and vaccines [15]. For the oral delivery of insulin alginate has been recently developed as a nanoparticle [16]. On the other hand, alginate has already acclaimed permission from US FDA [4]. Nanoparticles are prepared through various methods [17], such as ionic gelation in which nanoparticles are prepared based on polysaccharides [18]. Several advantages of this method are that it has a simple and mild preparation process without the use of organic solvent or high shear force [19], [20], [21].[22]

MATERIALS AND METHOD

Different series of Na-Alg Nanoparticles(0.25,0.3mg/ml) were prepared under Ionic-gelation process with $CaCl_2$ cross-linking agent .

In all cases, several concentration of BSA (43,64,150,500 ,700 µg/ml) were loaded on Na-Algnanoparticles. %LC and % AE were studied with Bradford assay. Morphological properties were studied with TEM and the exact size of NPs was determined with DLS (Figure 3).

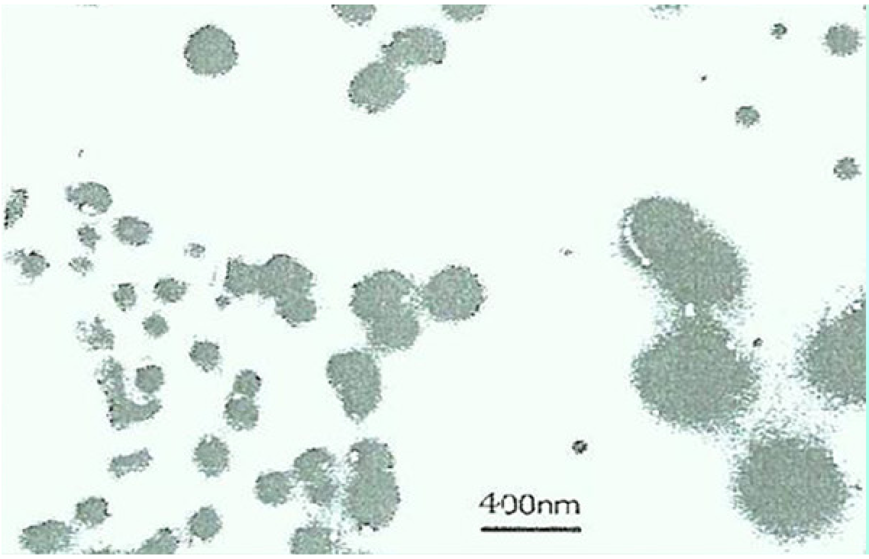

Fig 3: Nanoparticles of sodium alginate & calcium chloride 0.3 mg/ml containing 500 µg/ml

PREPARATION OF SODIUM ALGINATE NPs: Adapting the method of Rajaonarivony in the preparation of alginate–poly-L-lysine nanoparticles, the preparation of sodium alginate NPs has been done through ionic gelation method [18]. In order to impel gellification, 1 ml of calcium chloride was first added to 19 ml of sodium alginate solution. For liquefactionof nanoparticles, 8 ml of poly-L-lysine was then added to the solution. The obtained suspended nanoparticles were stirred for 2 h and were kept during the night for stabilization. Centrifugation (Sigma, USA) at 13000 rpm was used for 30 minutes to separate nanoparticles and then they were freeze-dried and stored at 4°C and their weights were also measured.

RESULTS AND DISCUSSION

In our study, a series of alginate nanoparticles were prepared and the factors such as NA-Alginate, $CaCl_2$ and BSA concentration, stirring rate that may be influenced in the preparation process were studied.

FTIR was used to confirm cross-linking processes of polymer with cationic agent.

Brad Ford assay was also used to demonstrate loading capacity and encapsulation efficiency (Figure 4, 5).

FTIR Result: As it can be determined from FTR spectrum, because of increasing condition, carboxyl peaks shift to higher frequencies as a result of adding $CaCl_2$ and BSA (Figure 6, 7).

TEM and Z.SIZER were used for morphological and dimensional studies, respectively.

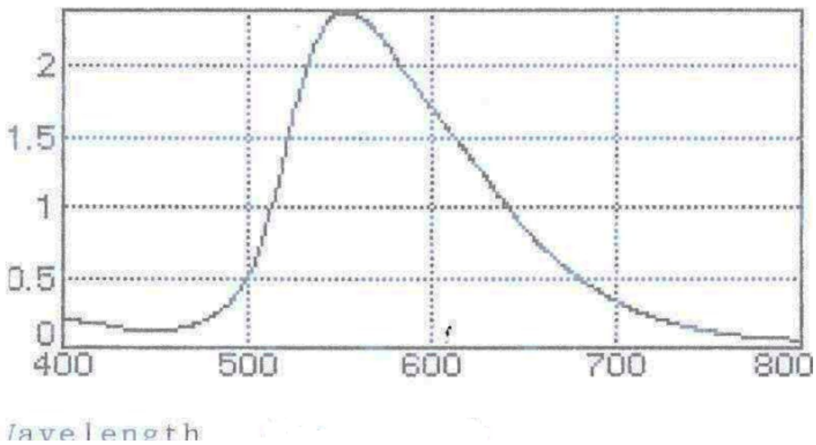

Fig4: Spectrum λmax solution sodium alginate within uv

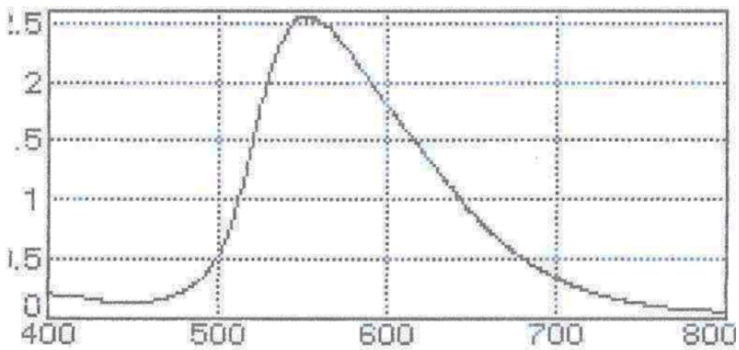

Fig.5: Absorption of sodium alginate solution 0.3mg/ml within 595nm - Spectrum FTIR related sodium alginate

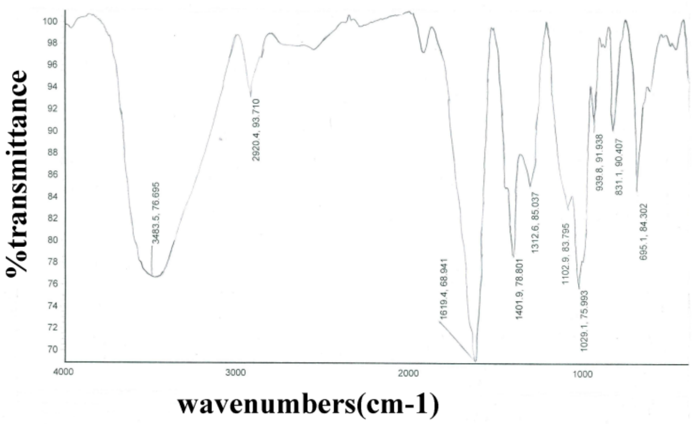

Fig.6: Spectrum FTIR related Sodium Alginate &Calcium Chloride

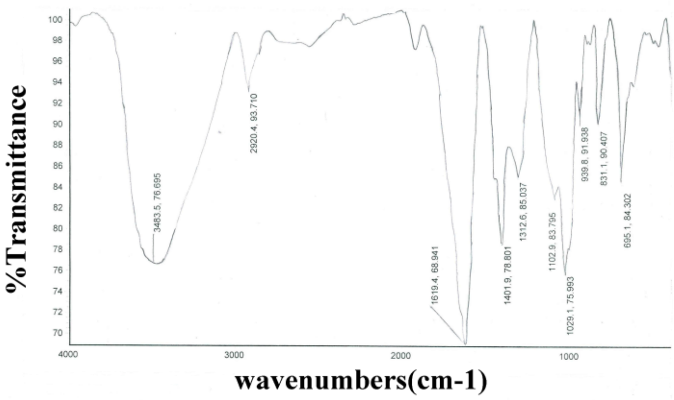

Fig 7: Spectrum FTIR sodium alginate &Calcium Chloride & bovine serum albumin

Finally the optimum condition for polymer and cross-linking agent concentration, protein concentration and stirring rate was evaluated. TEM of the nanoparticles and their surface morphology, as it is shown in the figure 3, are about 400 nm in size and spherical in shape (Figure 3). Quantitative absorption of alginate polymer in different concentrations of bovine serum albumine & Polymer is as follows (Table1).

Quantitative weight of nanoparticles alginate polymer containing bovine serum albumin is as below (Table2). Quantitative encapsulation efficiency of %EE &loading capacity %LC bovine serum albumin in nanoparticles alginate is also as follows (Table 3).

Table 1: Quantities Absorption alginate polymer

Absorption of nanoparticle alginate in 595nm	Concentration alginate polymer (mg/ml)	Concentration of bovine serum albumin under loading(µg/ml)
0.0007	0.25	43
0.004	0.3	
0.021	0.25	64
0.014	0.3	
0.044	0.25	150
0.024	0.3	
0.044	0.25	500
0.035	0.3	
0.066	0.25	750
0.036	03	

Table 2: Quantities of nanoparticles alginate polymer

weight of nanoparticles alginate µg	Concentration alginate polymer (mg/ml)	Concentration of bovine serum albumin under loading(µg/ml)
1700	0.25	43
1400	0.3	
1800	0.25	64
1700	0.3	
4500	0.25	150
1400	0.3	
4500	0.25	500
4700	0.3	
6699.70	0.25	750
7300	0.3	

Table 3: Quantities encapsulation efficiency

Encapsulation Efficiency %EE	Loading capacity (%LC)	Concentration alginate polymer (mg/ml)	Concentration of bovine serum albumin under loading(µg/ml)
%56.4	%13.79	0.25	43
%65.1	%19.54	0.3	
%42.71	%17.86	0.25	64
%57.3	%20.43	0.3	
%75.53	%59.89	0.25	150
%73.3	%61.43	0.3	
%87	%89.44	0.25	500
%89.25	%89.23	0.3	
%87.67	%87.78	0.25	750
%89.25	%89.33	0.3	

CONCLUSIONS

Lots of studies have recently been done on polysaccharides and their derivatives to be potentially used as nanoparticle drug delivery systems [23], [24], [25], [26]. It has been reported that modern advances of polymeric nanoformulations for the treatment of cancer are used to deliver anti-cancer agents in a sustained and controlled way [27], [28], [29], [30].

The important value for colloids and suspended nanoparticles is zeta potential. It's closely correlated with the value of suspension stability and morphology of particle surface [31], [32].

Zeta potential of aliginate and alginate-bovine serum albumin are shown with sharp peak within 127 nm particle size (Figure 3) According to figure 8, it was shown that particles' sizes are monotonous (Figure 8, 9).

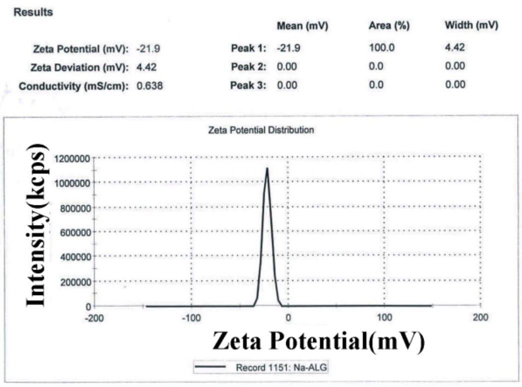

Fig 8: Diagram z.potential nanoparticles sodium alginate

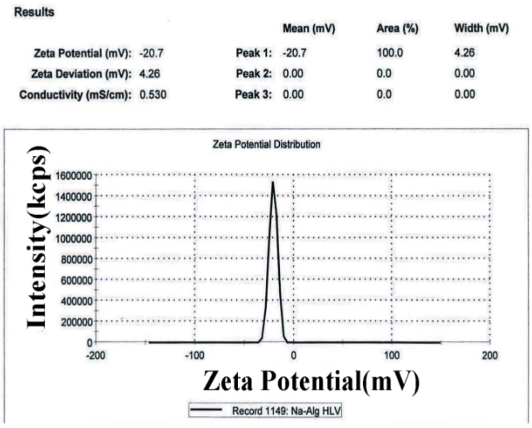

Fig.9: Diagram z.potential nanoparticles sodium alginate in concentration 500µg/ml of bovine serum albumin

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