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Alginate coated chitosan nanoparticles as potential oral vaccine carriers: Synthesis and *in-vitro* characterization

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ABSTRACT

One of the recent areas of research in the field of healthcare has been to explore stable carrier systems for sustained and controlled release of antigens to achieve enhanced immune response in an oral vaccine. We synthesized alginate coated chitosan nanoparticles (NPs) based on the principle of electrostatic interactions, exploiting the respective cationic and anionic properties of these biopolymers. The particles were characterized for their size, zeta potential and polydispersity index and later observed under SEM and TEM. Bovine serum albumin (BSA) was chosen as a model antigen for entrapment. Physiological conditions were simulated to carry out *in vitro* experiments in order to establish the efficacy of alginate coating on chitosan nanoparticles. Based on the entrapment efficiency of the NPs, the release study of BSA for a span of 48 hours was also noted. Nanoparticles stored at 4°C over six weeks were evaluated for their stability.

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KEYWORDS

Nanoparticles;
Chitosan;
Alginate;
Zeta potential;
Bovine serum albumin.

INTRODUCTION

Administering vaccines orally is the most preferred mode of vaccination to combat microbial infections that invade the human body and manifest through the gastrointestinal tract. Formulating effective oral vaccines has been a constant endeavour in the field of vaccine development against a wide array of bacterial and viral infections such as polio virus, rotavirus and cholera toxins^[1,2]. A significant immune response against these infections is elicited through the mucosal membranes with the generation of secretory IgA^[3]. It is desired that the antigens persist in the body long enough to allow their optimum interaction at these mucosal sites. Antigen deg-

radation caused by acid induced hydrolysis due to the low pH of stomach must be prevented for enhanced vaccine stability, hence perpetuating the need for suitable carrier systems^[7]. Liposomes, microparticles, and nanoparticles (NPs), have been identified as potential and emerging carriers for vaccines^[4]. In addition to imparting protection to the entrapped antigen, the reported features of nanoparticles also include slow and sustained release of the antigen^[5]. The biopolymers chitosan and sodium alginate have been studied for their properties of biodegradability, non toxicity and mucoadhesiveness^[6]. Hence in our study, we have synthesized nanoparticles with these and used them for entrapment of Bovine serum albumin as a model antigen. Prelimi-

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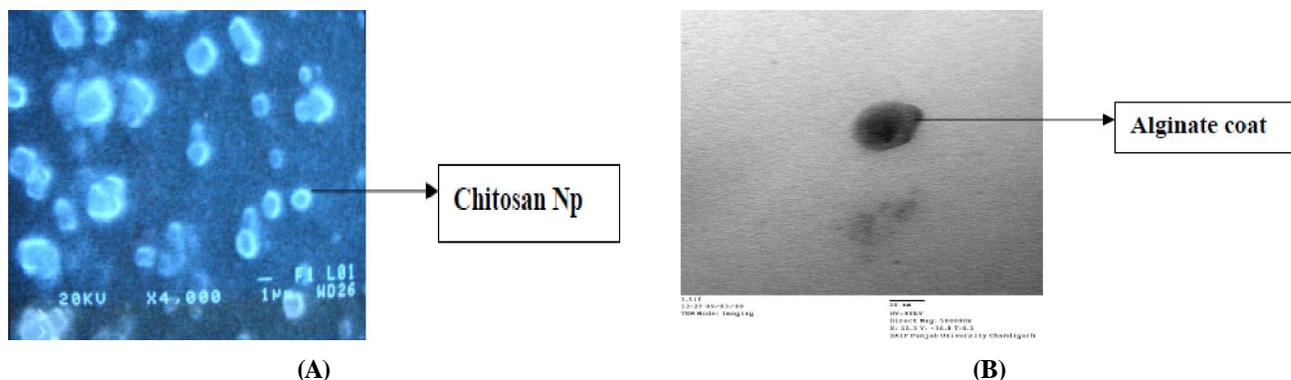


Figure 1 : [A] SEM of chitosan nanoparticles [B] TEM of alginate coated chitosan nanoparticles

nary studies for the characterization of these nanoparticles were conducted and an *in vitro* analysis of these nanoparticles as potential oral vaccine carriers was also carried out.

EXPERIMENTAL SECTION

Synthesis of chitosan/ BSA/alginate nanoparticles

Aqueous solution of Sodium Tripolyphosphate was added dropwise to the chitosan solution prepared in 1% (v/v) acetic acid containing BSA (1mg/ml) and polysorbate 80 as the surfactant^[8]. This was subjected to magnetic stirring at room temperature for 1.5 hours. Aqueous solution of sodium alginate was then added dropwise to enable coating of the NPs. The resultant sample was purified by repeated high speed centrifugation (Hitachi CT15E) and ultrasonicated (Sonics vibra cell). The nanoparticles were lyophilized (Delvac Lyodel) and stored for long term usage.

Particle size, PDI and zeta potential measurements

All the nanoparticle formulations were evaluated for their mean particle size, particle size distribution and zeta potential using a zeta sizer (Malvern instruments). The tests were conducted at National Institute of Pharmaceutical Education and Research (NIPER), Mohali, India.

SEM analysis of nanoparticles

The lyophilized sample of chitosan nanoparticles was analyzed under a scanning electron microscope (JSM 6100, JEOL) for its morphological characterization. The samples were sputtered with gold and ensured for complete dryness before observation.

TEM analysis of nanoparticles

Sample of the alginate coated chitosan nanoparticles in the liquid state was analyzed under a transmission electron microscope (Hitachi H-7500, 120 kV) to visually verify the presence of the alginate coating on the chitosan nanoparticles.

Acid degradation test

To compare the stability of alginate coated nanoparticles with non coated nanoparticles, an acid degradation test (0.01 M HCl, pH 2.0) was performed to note the extent of BSA degradation occurring in each case. The samples were incubated for two hours in the acidic medium followed by sustained release in PBS (pH 7.4) for 48 hours. The integrity of the bands was checked on 15% SDS-PAGE.

BSA release in PBS

The pellet obtained on centrifugation of the nanoparticle formulations was used to study the *in vitro* release of BSA upto 48 hours in Phosphate Buffer Saline (pH 7.4) at 37°C. Protein estimation was done using Bradford's reagent^[13] and the absorbance was measured at 595 nm (Jenway 6305 spectrophotometer).

Stability tests

Non lyophilized nanoparticles were stored at 4°C for a maximum of six weeks. These were evaluated at three weeks and six weeks for their mean particle size and polydispersity index, to check particle stability.

RESULTS AND DISCUSSION

Presence of alginate coat on the nanoparticles

Surface modification of the chitosan nanoparticles^[10]

with alginate due the electrostatic interaction between $-NH_3^+$ of chitosan and negatively charged $-COO^-$ of alginate resulted in decreased zeta potential of the nanoparticles (TABLE 1). BSA entrapment due to competitive electrostatic interactions^[11] was also responsible for inversion of charge from positive towards negative.

Agglomeration of particles

Higher positive or negative zeta potential signifies particle stability. Thus 6.36mV zeta potential and mean particle size of 4608 (TABLE 1) could be attributed to the phenomenon of agglomeration, often found in colloidal particle systems^[11,12].

Morphology of nanoparticles

SEM microphotographs revealed the unloaded chitosan nanoparticles to be spherical. Clusters of these nanoparticles gave them an irregular appearance. The spherical morphology was clearer in the TEM microphotograph, where the presence of alginate layer could also be well observed (Figure 1A, 1B).

Surface stability due to alginate coating

The presence of an additional coat of alginate could protect the entrapped BSA for at least two hours in an acidic environment of pH 2 (Figure 2, lane 2) whereas under similar conditions, chitosan alone failed to confer any protection to the entrapped protein leading to serious degradation (Figure 2, lane 3,). Acid denaturation of protein has also been seen in other studies^[14] using PLGA i.e. poly (lactic-*co*-glycolic acid) delivery system.

Sustained release from alginate-chitosan nanoparticles

A loading efficiency of 97.7% was obtained for chitosan nanoparticles. The loading efficiency (LE) was calculated according to the following equation:

$$LE (\%) = \frac{\text{Total amount of BSA} - \text{Free BSA}}{\text{Total amount of BSA}} \times 100$$

BSA release of 100% within two hours was observed in case of chitosan nanoparticles whereas alginate coated chitosan nanoparticles succeeded in showing a sustained release of nearly up to 80% BSA in eight hours (Figure 3). Our results are in accordance to the trends seen in similar studies done previously with

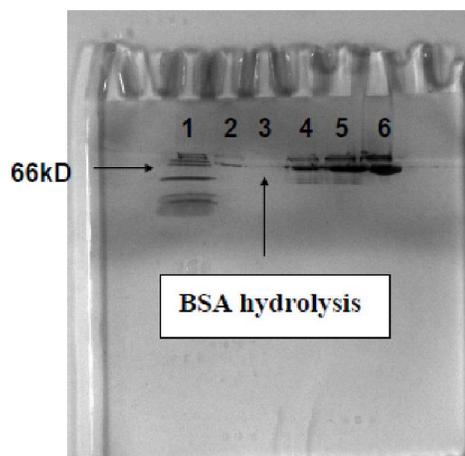


Figure 2 : SDS- PAGE: - Protein molecular weight marker (lane 1), alginate coated chitosan nanoparticles loaded with BSA pretreated with 0.01M HCl for 2 hrs and incubated in PBS for 24 hrs (lane 2), BSA loaded chitosan nanoparticles pretreated with 0.01M HCl incubated in PBS for 24 hrs (lane 3), BSA pretreated with 0.01M HCl for 2 hrs and incubated for 24 hrs (lane 4), BSA incubated with PBS for 24 hrs (lane 5), BSA (lane 6)

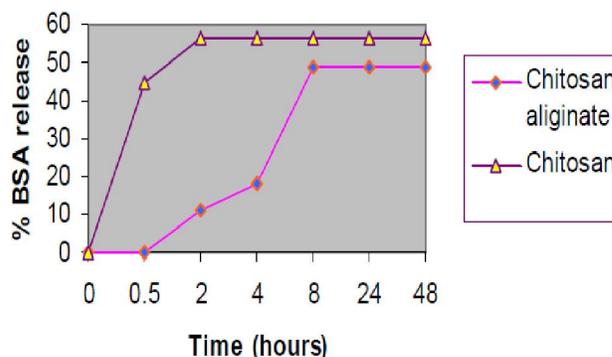


Figure 3 : Graph showing BSA release in PBS from chitosan nanoparticles and alginate coated chitosan nanoparticles

TABLE 1 : Size, PdI and Zeta potential of chitosan/ BSA/ alginate nanoparticles

Sample	Mean particle size (d.nm)	PdI	Zeta potential (mV)
Chitosan nanoparticles	342.3	0.483	23.3
Alginate coated chitosan nanoparticles (aggregates)	3583	0.310	-18.8
BSA loaded chitosan nanoparticles	154.3	0.370	21.2
BSA loaded alginate coated chitosan nanoparticles (aggregates)	4608	0.070	6.36

PLGA nanoparticles^[15].

Stability study tests

Alginate coated chitosan nanoparticles showed in-

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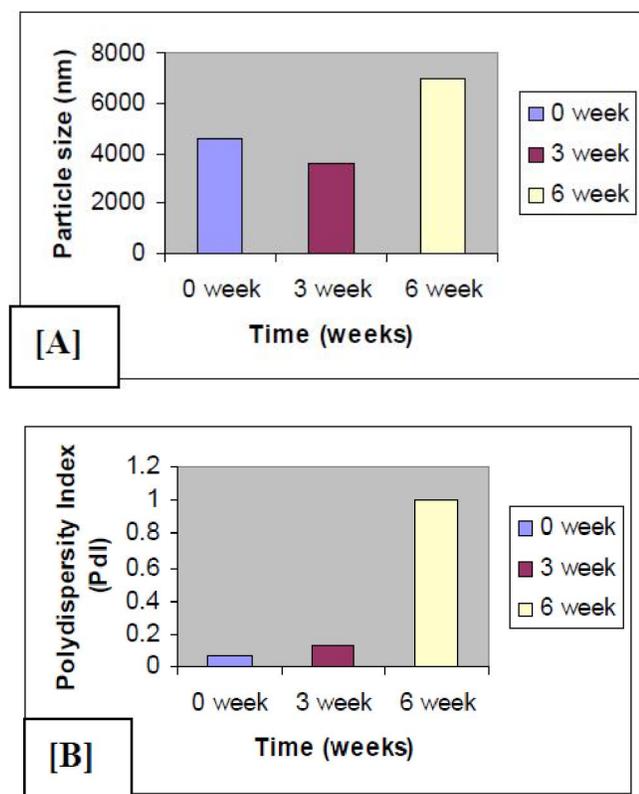


Figure 4 : Storage stability studies at 4°C [A] Effect on particle size [B] Effect on Polydispersity Index (PDI)

creased polydispersity index and average particle size. It can be inferred from figures 4 that the particles remained fairly stable upto three weeks but not till six weeks. These results could prove to be beneficial in formulating oral vaccines.

CONCLUSION

The data gathered from the preliminary studies encourage us to carry out a cohesive research in the field of oral vaccine delivery employing alginate coated chitosan nanoparticles. Substantiating the above results^[9] with more extensive *in vitro* and *in vivo* studies along with the usage of other proteins and peptides based antigens remains the future prospective of the given work.

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