Formulation and characterization of Chitosan coated PLA/PLGA encapsulated microparticles for vaccine development

B. Nayak, P.R. Routa, A. K. Panda, A. R. Ray

***Abstract*—** Thermoplastic aliphatic poly(esters) like poly(lactide) (PLA), poly(glycolide) (PGA), and especially the copolymer of lactide and glycolide, poly(lactide-co-glycolide) (PLGA) microparticles (MP) have generated immense interest due to their favorable properties such as good biocompatibility, biodegradability, and represent a promising and efficient delivery system for mucosal vaccination. The surface modification of these polymers and coating with polycationic polymers like chitosan can produce new formulations with unique properties. Chitosan coatings exhibit an important fraction of primary amino groups to the environment; these amino groups are freely accessible for a variety of mild coupling reactions for bioactive ligands. The positive charge of chitosan coating likely promotes the ionic binding of nucleic acids, either for gene vaccination purposes, e.g., plasmid DNA or mRNA or to deliver an immunoadjuvant to single-stranded RNA like in case of delivery of encapsulated rotavirus (RV). RV is one of the major causes of infantile gastroenteritis diarrhea. It is responsible for more than 150,000 deaths under the age of five in India every year. This emphasizes an urgent need for a vaccine to control such a large number of childhood mortality. Chitosan was used both as blend and coating to PLA and PLGA microparticle. The PLA-Chi, PLGA-Chi microparticles were prepared by solvent extraction evaporation method using a recently developed microextrusion-based technique along with the addition of various stabilizers. Along with the RV antigen, emulsifier mice serum albumin 2.5% W/V, lyoprotectant sucrose (10%, W/V) and 2% W/V Sodium bicarbonate were added to internal aqueous phases to stabilize the antigen. The particles were characterized with particle size determination, Zeta potential, while SEM and TEM studies along with their *in vitro* release studies are under progress. The details of the result obtained will be discussed during the presentation.

***Keywords*—**Biodegradable polymers, chitosan coated PLA/PLGA particles, Microparticles, Chitosan.

I. Introduction

Proteins and peptides have become the drugs of choice for the treatment of numerous diseases as a result of their incredible selectivity and their ability to provide effective and potent action and also due to their fewer side effects and higher potency to cure diseases [1]. Oral administration of drugs is so far the most widely used route of administration, although it is generally not feasible for peptide and protein drugs. The main reasons are pre-systemic enzymatic degradation and poor penetration into the intestinal membrane [8]. Biodegradable particles (0.1–1.5μm) prepared from poly(lactide-*co*-glycolide) (PLGA) and poly(lactic acid) (PLA) polymers have generated considerable interest in recent years for their use as a delivery vehicle for various pharmaceutical agents. Chitosan, a natural cationic polysaccharide has gained an increased attention in biomedical as well as pharmaceutical purposes due to its biocompatible properties such as non-toxicity, biodegradability [6,7, 9 and 10] and muco-adhesive properties. In this work, we aim to develop a novel oral drug delivery system to act as a carrier for therapeutic proteins with highest efficiency by using PLA/PLGA micro and nanoparticle and coating with chitosan polymer and as well as mixed blend.

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II. PREPARATIONS

A. Materials and Methods

PLA and PLGA polymer were used to encapsulate RV (strain SA11) to form nanoparticles and microparticles using solvent evaporation multiple emulsion method. Along with the RV antigen, mouse serum albumin (MSA); 2.5% W/V, sucrose; 10% W/V and Sodium bicarbonate; 2% W/V were added to internal aqueous phases (IAP) as stabilizers during primary emulsion step. Sucrose was also added to external aqueous phase (EAP) containing (1% W/V) PVA to form 10% W/V sucrose in PVA solution during secondary emulsion process. During the preparation process, chitosan solution dissolved in acetic acid was added drop wise to IAP under mild agitation at room temperature on a magnetic stirrer[11-15].

B. Loading and release study

The loading was performed by incubating different concentrations of Bovine serum albumin (BSA) and RV antigen and other protein antigens at concentrations (1.0, 2.0, 4.0, 8.0) mg/ml with chitosan under mild agitation at room temperature for 15 min. Loading efficiency (LE) of BSA and rotavirus antigen was calculated by indirect way by determining the free BSA and antigen remained in the supernatant after the performance of centrifuge[16-18].The best loading particles were taken for release studies.

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C. Morphological Characterization

The particle size distribution was detected by laser diffraction analyzer (Nano- ZS 90, Malvern instrument UK). The zeta potential of particle was examined by Malvern Zeta with ultrapure water as solvent (pH= 7, 25 °C). The morphological characterization like surface morphology, structure and size of the nanoparticles was carried out by SEM &TEM study[19-21].

III. RESULTS AND DISCUSSION

Biodegradable particles of size (0.1–1.5µm) were prepared from poly(lactide-*co*-glycolide) (PLGA) and poly(lactic acid) (PLA) -chitosan polymers. Then BSA (2.5%) (PI of 4.8) was used as a model protein and RV antigen was used for encapsulation. The loading efficiency was calculated using BCA standard curve for BSA protein by using spectrophotometer as shown in fig 1.

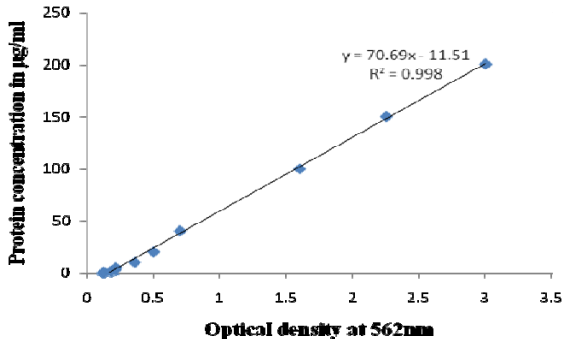
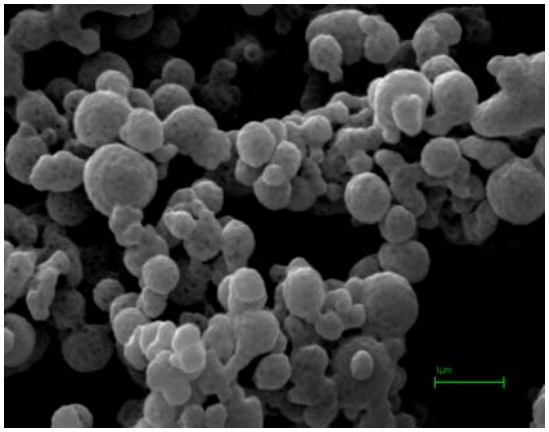


Fig. 1: BCA standard graph using BCA™ kit

The particle size was determined by Zeta sizer. Particles which were prepared by sonication for both primary and secondary emulsion were in nanometre range. The particle size was varied from 433 nm to 1073nm for PLA polymer and for PLGA polymer the particle size was 113.5 nm to 986 nm. When BSA was added to the particles (sample 102, 103 and 104), the size were as 258.3 nm, 196.8nm and 162.7nm. The loading efficiency was 58.7%, 69.6% and 82.4% for the samples 102, 103 and 104 respectively. For sample 104 the loading efficiency was high in this sample the OP: IAP ratio was 1:10. Similarly for RV antigen and plasmid DNA,it was ranged from 194.8nm to 1373.2 nm (sample 105, 106 and 107). Then the loading efficiency was calculated by spectrophotometrically. The loading or encapsulation efficiency for these samples were found as 42%, 37.6% and 31.12% respectively (Table 1). Fig.2 shows the SEM micrographs of PLA/PLGA-chitosan microparticles and Fig 3 shows in vitro release of RV antigen from PLA/PLGA-chitosan microparticles. Fig 4, Shows loading efficiency of BSA (A) and RV antigen (B) in PLA/PLGA-Chitosan microparticles.



(A)

(B)

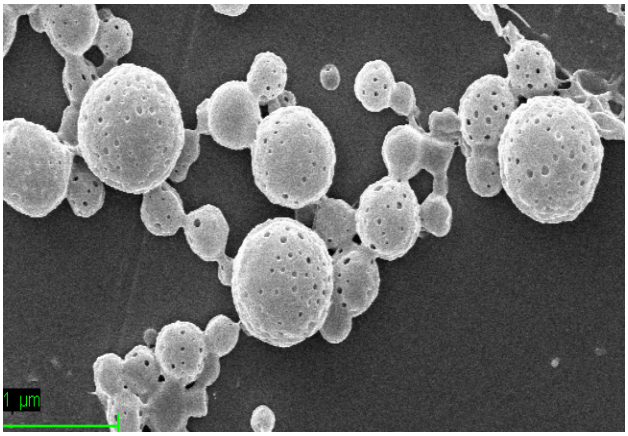


Fig. 2: (A) SEM structure of PLA-chitosan microoparticles, (B) SEM structure of PLGA-chitosan microparticles

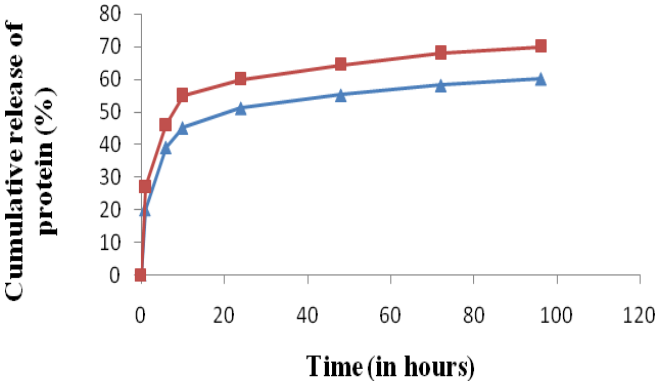


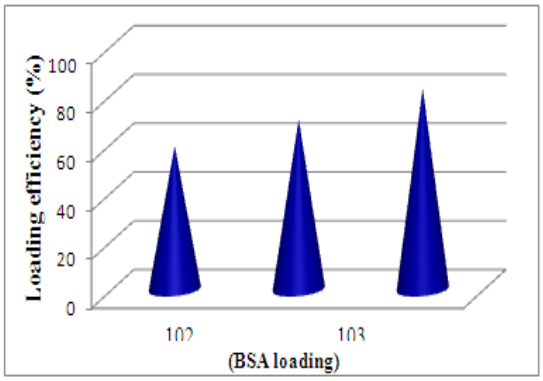
Fig. 3:*In vitro* release of RV antigen release from PLA-chitosan (blue) encapsulated and PLGA-chitosan (red) encapsulated microparticles

Table-1: Formulation of PLA/PLGA- chitosan microparticles with their mean particle size, PDI (poly dispersity index), zeta potential and loading efficiency**.**

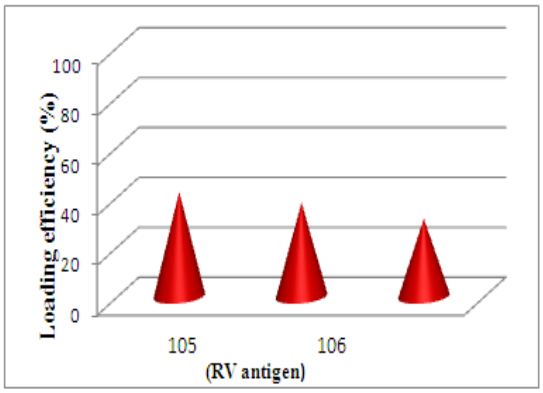
Sl.No	Sample Name	Poly dispersity index (PDI)	Zeta Potential	Loading Efficiency (%)
1	101	1.000	-24.8	No loading
2	102	0.337	-32.7	61.6
3	103	0.403	-20.5	69.6
4	104	0.465	-24.7	82.4
5	105	0.359	-19.5	41.95
6	106	0.987	-30.3	37.6
7	107	0.241	-19.7	31.12

IV. CONCLUSION

The formulation of PLA/PLGA-chitosan encapsulated protein (BSA) and RV antigen particles were obtained with desired characteristics of size, release profile. With the addition of stabilizer the size of the particles can increase and the release can be controlled. It can be concluded that the encapsulation efficiency of PLGA particles with chitosan is larger than PLA-chitosan particles as well as in case of release study encapsulated PLGA particles show faster release than encapsulated PLA particles and can be better proposed particulate system for vaccine development against rotavirus disease.



(A)



(B)

Fig. 4: Loading efficiency of BSA (A) and RV antigen (B) in PLA/PLGA-Chitosan microparticles.

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