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In-Vitro study on Nanopolymer encapsulated Protein isolated from *Emblica officinalis*

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Abstract: Nanoparticles are solid sub-micronic drug carriers made up of natural, semisynthetic, or synthetic polymers in the nanometer size range. Nanoparticles consisting of synthetic biodegradable polymers, natural biopolymers, lipids and polysaccharides such as Alginate/Chitosan,PACA,PIBCA,PIHCA etc have been developed and tested over the past decades. Nanoencapsulated drugs such as Alginate/Chitosan nanoparticle loaded Insulin, PIHCA-Cyclosporin encapsulates have been developed which had notably increased the bioavailability of the drug compared with that of the commercial formulation. Cyclosporine was encapsulated in PIHCA by interfacial and emulsion polymerization. Poly(FA:PLGA)-encapsulated insulin prepared by inversion phase method showed a better ability to regulate glucose, suggesting that the insulin crossed the intestinal barrier and was released from the microspheres in a biologically active form. Morphology and structural characterization of nanoencapsulated protein can be investigated by Transmission electron microscope (TEM),Scaning electronmicroscope(SEM) and Fourier transform infrared spectra (FTIR). The main aim of the study is to perform *in-vitro study* on Alginate/Chitosan nanopolymer encapsulated protein isolated from *Emblica officinalis.* The usage of nano polymers for encapsulation of proteins ensures biocompatibility and non-toxicity of such nanosystems.
Keywords : Alginate/Chitosan, Biopolymers, Emulsion polymerization

Introduction:

The colloidal carriers based on biodegradable and biocompatible polymeric systems have largely influenced the controlled and targeted drug delivery concept. Nanoparticles are subnanosized colloidal structures composed of synthetic or semisynthetic polymers[1]. Due to their small particle size, colloidal preparations lend themselves to parenteral administration and may be useful as sustained release injections for the delivery to a specific organ or target site. Nanoparticles useful for sustained drug release can be also obtained by electrostatic interaction between alginate and chitosan[2]. Chitosan is a natural cationic polysaccharide derived by deacetylation of chitin, a copolymer consisting of combined units of glucosamine and N-acetyl glucosamine[3,4]. In the pharmaceutical field chitosan is used as carrier of both drugs[5,6] and protiens[7,8]. Moreover, alginate has been widely used for encapsulation of cells, proteins, DNA, venoms and vaccines[9]. Drug loaded nanoparticles made of polyelectrolytes complexation have shown potential for use as drug delivery sytems. Polyelectrolyte complexes are formed by interactions between macromolecules that carry

oppositely charged ionizable groups. CS coated nanocapsules have shown enhanced penetration of some drugs. Several natural[10,11] and synthetic[12,13,14] polymeric nanoparticulate systems are used for insulin entrapment. Different types of forces are responsible for insulin and general protein physicochemical stability, such as hydrophobic and electrostatic interactions, covalent bonding, hydrogen bonding and van der Waals forces. The complex interaction between these forces places hydrophobic residues in the interior of the protein while directing hydrophilic residues to the outside of the protein, where they interact with the aqueous solvent. Manipulation conditions of proteins can lead to conformational changes, thus exposing hydrophobic areas, resulting in reduced solubility and an increased tendency to aggregation[15]. Because of their versatility for formulation, sustained-release properties, subcellular size, and biocompatibility with tissues and cells, nanoparticles seem to be a promising solution for peptide and protein administration[16].However, the use of peptides and proteins in medicine has been limited by low bioavailability, which results from their poor stability to proteolytic and hydrolytic degradation, low permeability across barriers, and short biologic half-life in the circulatory system[17-22].

The aim of this study was to determine the structural integrity of protein derived from *Emblica officinalis* upon entrapment in alginate/chitosan nanoparticles produced by ionotropic polyelectrolyte pregelation. Fourier transform infrared (FTIR) analysis, scanning electron microscopy (SEM) have been used in this study to examine the protein structure and interactions between the peptide and polymeric matrices after entrapment.

Experimental:

Extraction of Protein from *Emblica officinalis*

Seeds of *E.officinalis* were washed with distilled water and shade dried. The dried seeds were ground to fine powder and the proteins were extracted with extraction buffer[23] consisting of 10 mM Na_2 HPO_4, 15 mM NaH_2PO_4, 10 mM KCl, and 2 mM EDTA (pH 5.4) by constant stirring overnight at 4◦C. The crude protein extract was filtered using the muslin cloth and centrifuged at 10,000 rpm for 10 minutes. Proteins were precipitated from the crude supernatant using ammonium sulphate up to 80%saturation and lyophilized for further use. The lyophilized sample was dissolved in 10ml water and the purified protein was obtained using Ultrafiltration.(10MWCO ultrafilterate membrane from PALL Corporations.)

Preparation of blank Alginate/Chitosan nanoparticles

Alginate/chitosan particles were prepared in a two-step procedure based on the ionotropic pre-gelation of polyanion with calcium chloride followed by polycationic crosslinking according to Rajaonarivony's method of preparing alginate–poly-L-lysine nanoparticles[24] . 7.5 ml of 18 mM calcium chloride solution was added drop wise for 60 min under gentle stirring (800 rpm) into a beaker containing 117.5 ml of a 0.063% alginate solution to provide an alginate pre-gel. Then, 25 ml of different concentration(0.01% to 0.05%) chitosan solution was added drop wise into the pre-gel over 90 min. The pH of alginate and chitosan solutions was initially set to 4.9 and 4.6, respectively. A colloidal dispersion at pH 4.7 formed upon polycationic chitosan addition, visible as the Tyndall effect. Nanoparticles were stirred for 30 min to improve curing and subsequently collected by centrifugation (20,000g/45 min) at 4˚C.

Preparation of protein loaded alginate/chitosan nanoparticles

About 6mg of ultrafiltered protein sample was added to alginate solution before the addition of calcium chloride[25].The concentration of sodium alginate and calcium chloride was kept fixed with the concentration of chitosan changing from 0.01% to 0.05%.

Structural Studies

Protein loaded alginate/chitosan nanoparticles were stirred for 30 min and subsequently collected by centrifugation (20,000g/45 min) at 4˚C. The supernatant was discarded and the pellet which is in the form of a translucent gel was lyophilized. The structural morphology of ultra-filtered protein sample and protein loaded nanoparticles was studied using SEM results (FESEM FEI Quanta200G Field Emission Scanning Electron Microscope with EDS, EBL, STEM and Nanomanipulators, Agilent Technologies)

Fourier Transform Infrared Spectroscopy

The lyophilized sample was mixed with Kbr powder and compressed into Kbr pellets. The range for the analysis was set to 200cm^{-1} to 2000cm^{-1}. The FT-IR spectra was recorded for Chitosan(0.01%,0.05%), Sodium alginate, blank Alginate\Chitosan nanoparticle and ultrafiltered protein using FT-IR spectroscopy. (Resolution Pro FTIR Software, Agilent technologies)

Results & discussion

Chitosan and alginate are polycationic and polyanionic polyelectrolytes that have been used to form a polyelectrolyte complex to deliver various drugs. When the two polysaccharides are mixed, the carboxy residues of alginate and the amino groups of chitosan ionically interact to form the PEC complexation which has been found to reduce the porosity of alginate beads and decrease the leakage of encapsulated drug.[26,27] These polyelectrolyte nanoparticles have increased potential as drug carrier systems for proteins and peptides.[28,29]

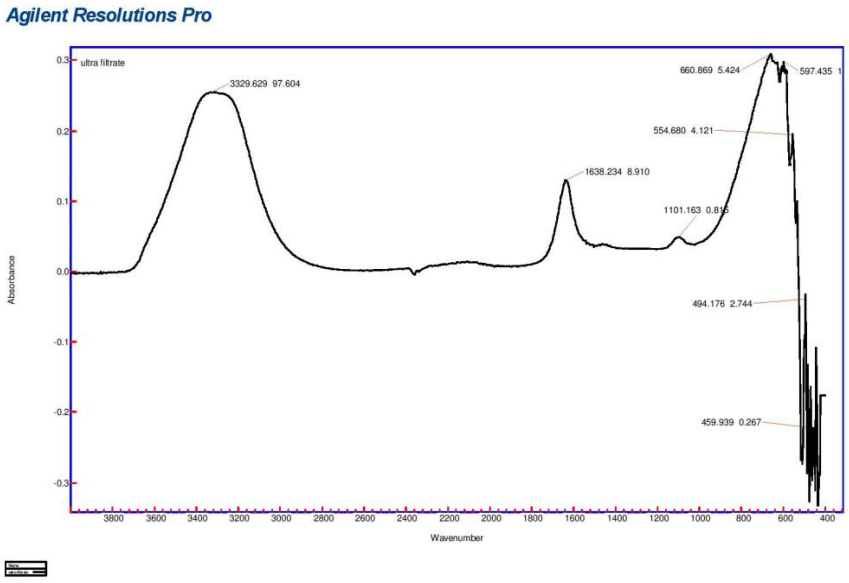

Figure 1 – FTIR spectroscopy of ultra-filtered protein sample

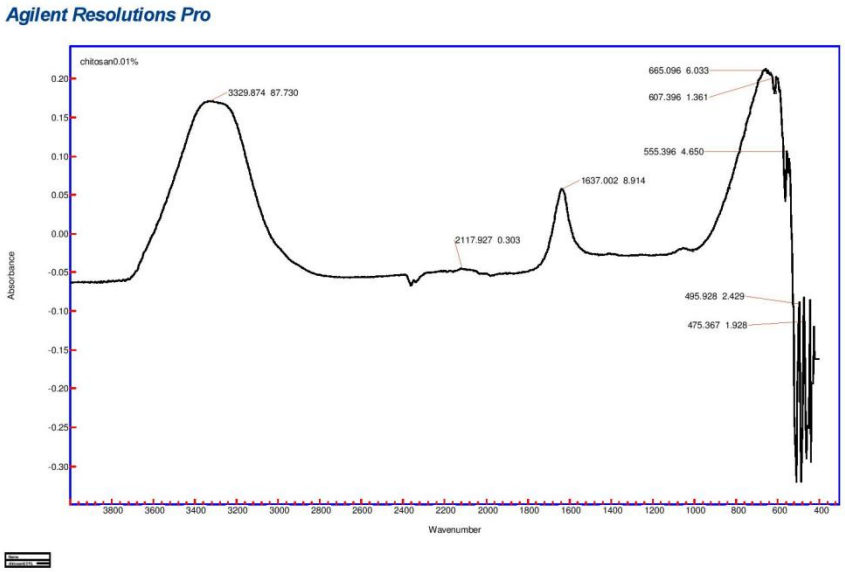

Figure:2 FTIR spectroscopy of 0.01% Alginate-Chitosan nanoparticle complex

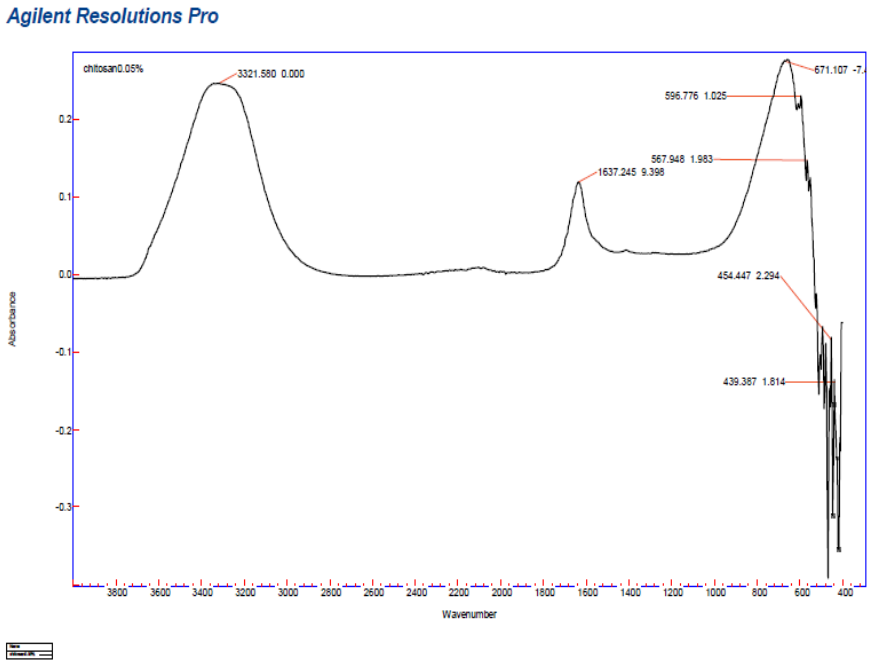

Figure 3 – FTIR spectroscopy of 0.05% Alginate-Chitosan nanoparticle complex

Fourier transform infrared (FTIR) analysis has also been proposed to examine interactions between polyelectrolyte complexes and the purified protein sample[30,31]. Figure 1 represents the FTIR spectra of ultra-filtered protein sample showing the presence of absorption bands at 3329.629 cm^{-1} which is characteristic of protein spectra. FTIR spectra of alginate-chitosan nanoparticles show broad bands at 3331.709 cm^{-1} (0.01%, Figure 2) and 3321.580 cm^{-1} (0.05%, Figure 3) which corresponds to the amine (N-H) and hydroxyl (OH) groups respectively.

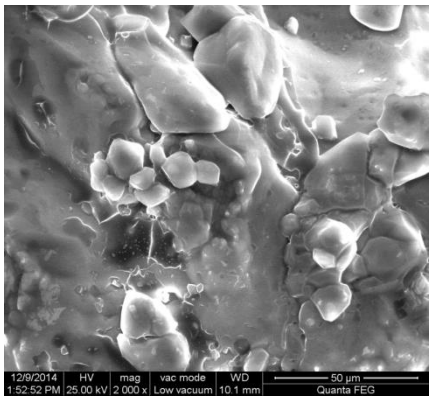

Figure 5 – SEM micrographs of ultra-filtered protein sample

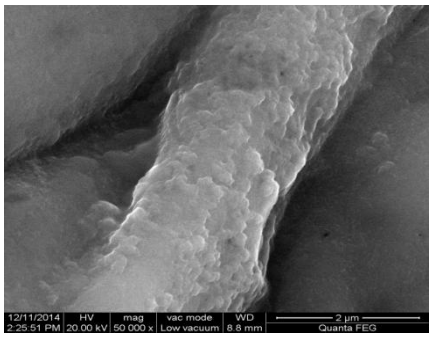

Figure 6 – SEM micrographs of 0.01% Alginate/Chitosan nanoparticles- showing nanosheet structures of 2 µm size

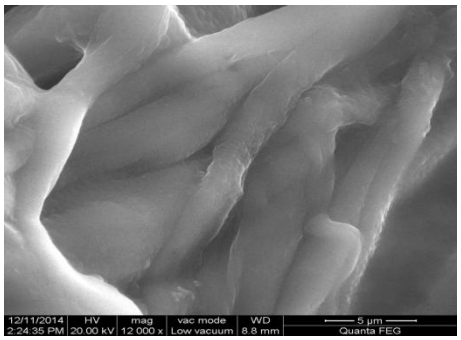

Figure 7 – SEM micrographs of 0.01% Alginate/Chitosan nanoparticles- showing nanosheet structures of 5 µm size

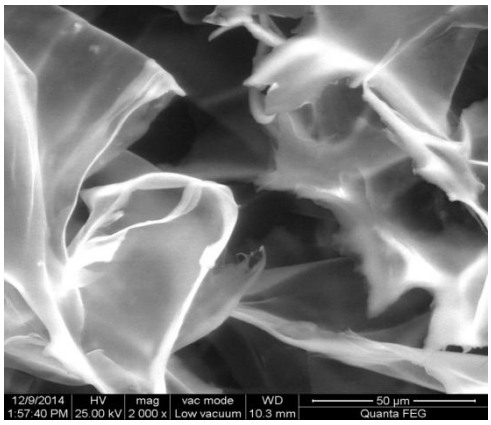

Figure 8 – SEM micrographs of 0.05% Alginate/Chitosan nanoparticles- showing nanosheet structures of 50µm size

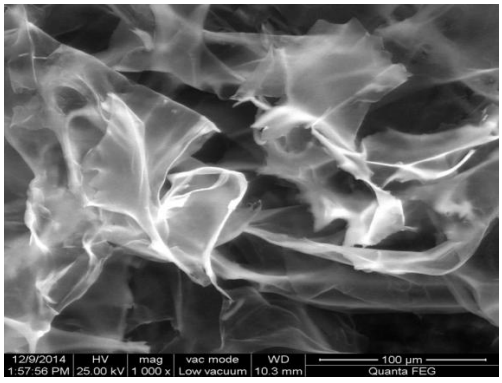

Figure 9 – SEM micrographs of 0.05% Alginate/Chitosan nanoparticles- showing nanosheet structures at 100µm size.

SEM micrographs of Ultra – filtered protein sample are presented in the Figure 5.Alginate – chitosan nanoparticles synthesized at concentrations of 0.01% and 0.05% show overlapping, thick sheets with the average sizes of 2µm, 5µm, 50µm and 100µm respectively as depicted in the SEM images (Figures 6-9). However, it is possible that these thicker sheets may consist of several thinner sheets aggregated to form a nanosheet network.Sheet-shaped carriers have a larger surface area and are more flexible than spherical carriers. These multi-layered nanosheets have alternate layers of chitosan, which has a positive electric charge, and alginate, which has a negative electric charge, in an aqueous solution. These nanosheets can overlap, internally and on their surfaces with substances such as drugs that carry electric charges similar to those carried by chitosan and alginate. These results are in accordance with the synthesis and characterization of new biodegradable polymeric poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles loaded with bovine serum albumin. Several studies have been reported for protein encapsulation in biodegradable polymeric nanoparticles.

Conclusion:

The results obtained in this study clearly indicate that there is a possibility to entrap the protein isolated from *Emblica officinalis* within CS/ALG nanoparticles using ionotropic pre-gelation technique and strong electrostatic interactions which exist in the nanoparticles. Ionotropic pregelation method can produce Alginate/Chitosan nanoparticles with optimum particle size and maximum entrapment of drug contents.

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