Synthesizing Calcium Alginate Nanoparticles via a New Interfacial Cross-linking Technique

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Statement of purpose: The purpose of this project was to develop a new method to prepare calcium alginate nanoparticles [1]. Prospective and potential applications of these nanoparticles include targeted delivery of cancer chemotherapeutics in lung cancer therapy, delivery of anti-tuberculosis drugs to treat tuberculosis, delivering mucolytic agents to lungs in cystic fibrosis patients, mucosal vaccine delivery, and novel protein inhalation therapies. Our current research involves developing biomimetic methods to produce nanoscale materials [2].

Methods: Sodium alginate (low viscosity), Calcium Chloride, Cyclohexane and Ethanol were purchased from Fisher Scientific, Pittsburg, PA. Bovine Serum Albumin and Aerosol OT (AOT) were obtained from Sigma – Aldrich, St. Louis, MO. All chemicals were of certified ACS grade or better. The general method of calcium alginate nanoparticle (CANp) synthesis involved mixing two water-in-oil (w/o) microemulsions of AOT/cyclohexane/water, one containing sodium alginate and the other containing calcium chloride in the water phase. The particle size of microemulsions containing the reagents was determined by dynamic light scattering (DLS) prior to mixing. Samples were placed in cylindrical glass cuvettes and the particle size measured in a DLS instrument equipped with a He-Ne laser (100 mW, 658 nm). The scattered light was collected at 90° and analyzed using proprietary software supplied by the instrument manufacturer. The DLS instrument was used in the electrophoretic light scattering (ELS) mode to measure the zeta potential of prepared CANp's. The nanoparticles were directly visualized in a Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). SEM and TEM studies were performed to evaluate the morphology and structure of CANp's. The ability of CANp's to incorporate a biologically active agent was evaluated by using Bovine Serum Albumin (BSA). CANp's were synthesized using the method described above by adding BSA to the aqueous alginate dispersion prior to microemulsification. These nanoparticles were subjected to DLS, ELS, SEM, and TEM studies. The total amount of protein retained within the nanoparticles was determined spectrophotometrically via Lowry's method of total protein estimation. Further the integrity of protein molecules within CANp's was evaluated calorimetrically by differential scanning calorimetry (DSC) [3].

Results: DLS results of reactor microemulsions prior to mixing indicated presence of 10 nm droplets. When the microemulsions were mixed CANp's in the range of 100-300nm were produced. Zeta-potential measurements performed on CANp's demonstrated a negative surface charge (-20 mV) (Figure 1). The negative surface charge is consistent with the polyanionic nature of alginate polymer. This result indicates a potential route for coating the calcium alginate nanoparticle surface with an oppositely charged polymer. This can potentially impart tunable physico-chemical properties to the nanoparticles. SEM and TEM data (Figure 2) established that the particles were spherical in shape. Protein estimation results substantiated that 30% of the added protein was retained within the CANp's. The absence of thermal events characteristic to denatured protein fragments validated the retention of protein integrity in the final CANP's.

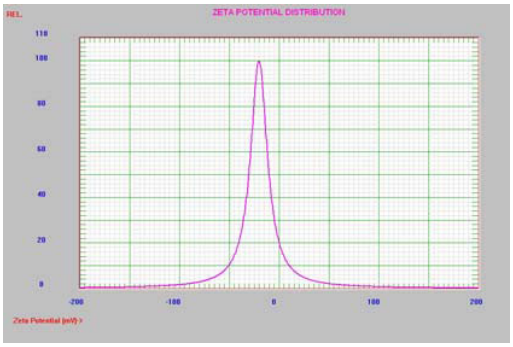

Figure 1: Zetapotential data of CANp's

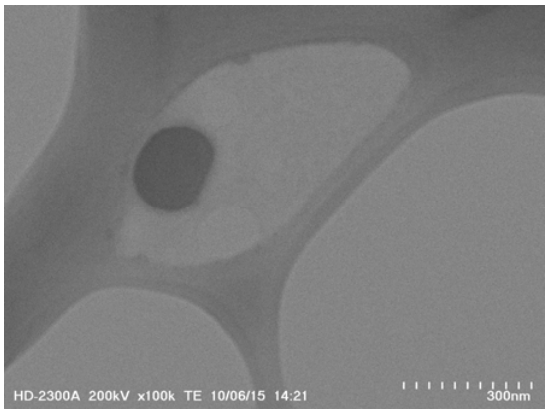

Figure 2: TEM of CANp's

Conclusion: Spherical calcium alginate nanoparticles of sizes between 100 nm and 300 nm were synthesized via a new interfacial cross-linking method developed in our research group. DLS, ELS, SEM, and TEM studies of the nanoparticles confirmed physico-chemical properties that are desirable in drug delivery applications. The use of BSA verified efficiency of these nanoparticles to incorporate biomacromolecules of therapeutic interest. Ongoing research will further authenticate the nanoparticle preparation process by delineating parameters influencing particle size and protein incorporation.

References:

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