Biodegradable Nanoparticles for Receptor-Mediated Oral Protein Delivery

Erik Brewer, Anthony Lowman.
Drexel University

Statement of Purpose: Biodegradable nanoparticles are a promising vehicle for orally-delivered therapeutic proteins. The nanoparticles, which are capable of encapsulating drugs, provide a protective outer shell that resist enzyme and acid degradation in the stomach and deliver them safely to the small intestine. Also, the shells of the nanoparticles are capable of covalently bonding to compounds that enhance transport across the GI tract. This eliminates the need to bond the drug itself to these compounds, which can be tedious and has a possibility of interfering with the therapeutic ability of the drug [1]. One attractive group of transport enhancing compounds contains those involved in receptor-mediated pathways. These pathways are active and do not rely on diffusion or other passive mechanisms and can be easily targeted for site-specific delivery.

While it is generally agreed that cells can more easily transport nanoparticles with smaller diameters, the nature of this correlation is not fully understood (for example, if size thresholds exist or there is an optimal nanoparticle diameter) [2]. Also, the nanoparticles size has important implications on its drug load: Larger particles have the ability to encapsulate larger amounts of drugs. Thus, even if smaller particles are taken up more easily by the cells, they carry a smaller amount of drugs.

The purpose of this study is to determine what factors influence the size and polydispersity of nanoparticles intended for receptor-mediated endocytosis. These particles will be designed for future *in vitro* work to determine the relationship between size and nanoparticle uptake.

Methods: Nanoparticles were made up of poly(lactic-co-glycolic) acid (PLGA), due to extensive use as a biomedical polymer. Bovine insulin was chosen as a model protein due to its resiliency and the detection methods available. Particles were made using two methods: the nanoprecipitation method and the double emulsion/solvent evaporation method. Nanoprecipitation involved dissolving PLGA and protein in acetone and then adding the solution drop-wise to a surfactant solution. The miscibility of the acetone and water forces the PLGA to precipitate into small particles. The double emulsion method involved fist mixing two immiscible solutions of PLGA in a solvent and insulin in water. The first emulsion was then transferred to a surfactant solution in water. This double emulsion was allowed to stir overnight, during which the solvent evaporated, leaving behind dried nanoparticles encapsulated with insulin. Two mixing apparatuses were used for the double emulsion procedure: a homogenizer, where ethyl acetate was used as a solvent, and a sonicator, where dichloromethane was used as a solvent. In all methods, polyvinyl alcohol (88% hydrolyzed) was used as a surfactant.

All particles were centrifuged and washed with water multiples times before freeze-drying. Dynamic light scattering was then used to determine average diameter and polydispersity. Chromatography techniques were used to determine insulin loading and encapsulation efficiency.

Results: Multiple variables in the nanoparticle fabrication process were investigated. These included the concentration of PLGA in the solvent, the concentration surfactant concentration in the water phase, and the speed of the homogenizer blade. PLGA concentration proved to be a factor in all methods, created larger diameter nanoparticles when a higher concentration was used. The double emulsion method produced nanoparticles with larger diameters compared to the nanoprecipitation method, ranging from 381-430nm with the homogenizer and 196-256nm when using the sonicator. The nanoprecipitation method provided the smallest nanoparticles obtained in the experiment, as low as 151nm when using a PLGA concentration of 10mg/ml. Conflicting results were observed in the double emulsion method when increasing the PVA concentration in the water phase from 2% to 10%. When using the homogenizer, the average diameter decreased from 536nm to 355nm, though when using the sonicator, it increased from 232nm to 415nm. PVA concentration had no effect in the nanoprecipitation method. However, size dispersity decreased with increasing PVA concentration for both methods. When using the double emulsion method, the homogenizer speed also proved to influence the nanoparticle diameter. When increasing the speed from 15krpm to 30krpm, the diameter decreased from 536nm to 386nm. Polydispersity also decreased from 0.141 to 0.071 over this range. In general, the use of the homogenizer in the double emulsion method produced the largest particles and polydispersity. The sonicator was capable of producing slightly larger particles than the nanoprecipitation method, though both methods produced similar size polydispersities.

Insulin encapsulation efficiency averaged 74% and 81% in the double emulsion method when using the homogenizer and sonicator, respectively. The nanoprecipitation method had the lowest efficiency of 26%. There was determined to be no correlation between insulin loading and all variables discussed

Conclusions: Nanoparticles containing insulin were successfully created using all methods. All studies combined produced nanoparticles with diameters ranging from 151nm to 536nm, with varying polydispersities. This will allow for transport studies to determine more statistically accurate correlations between nanoparticle size and cellular uptake.

References:

1. Lindsay DG. Biochem J. 1971:121:737-45.
2. Florence AT. Drug Discov. Today. Technol. 2005:2:75–81.