The Effects of Carboxymethylated Chitosan-Oxidized Sodium Alginate Hydrogel Microspheres on the Coating of Insulin in Low pH Conditions

Hypothesis: The carboxymethylated chitosan-oxidized sodium alginate hydrogel microspheres are able to coat inulin proteins while maintaining the integrity of its structure even in low pH conditions.
Purpose: To create an alternative mode of insulin delivery through oral administration. Oral insulin would be more convenient, more accessible to poorer areas, and decrease the use of used insulin needles in illicit drug use.

Abstract

The current experiment was designed to test the heat insulating factors of different types of glass used in windows. Different types of glass were tested, using the old types of glasses that are no longer allowed for construction as a baseline. The key aspect of our project is to find a balance between aesthetics and efficiency of glass that complies with Title 24 as dictated by the Building Energy Efficiency Program. We tested the heat conduction by shining a 125 watt lamp onto the glass and monitoring the temperature every minute for ten minutes. We predict that thinner glass will insulate heat worse than thicker glass, but double layered glass will be better than the thicker glass. Glass tempered with metallic properties and coated glass will also decrease thermal conduction. Shaded glass will block out the heat, but will decrease the amount of light let through.

Introduction

Even though there have been many advances in insulin therapy since the discovery of the pharmaceutical formulation of insulin in 1922, the form of delivery is still far from ideal (Brange and Langkjaer, 1997). The most common form of delivery is subcutaneous shots (CITATION). In order to make the mode of insulin delivery more psychologically and socially acceptable, oral administration would be the solution. The problem with the development of an oral insulin is the degradation of proteins by proteolytic enzymes through the acidic environment of the stomach (Woodley, 1994). Coating insulin with a protective covering may be able to maintain the integrity of the protein. The entrapment of insulin in microparticulate drug delivery systems using various polymers to create a hydrogels has been studied (Agarwal, 2001). Hydrogels are microporous structures that can have varying levels of porosities that allow it to become one of the most versatile and viable platforms for sustained protein release, targeted drug delivery, and tissue engineering (cite). Carboxymethyl chitosan (CMCS) has low toxicity, biocompatibility, and a good ability to form films, fibers, and hydrogels. Therefore, it has been used in biomedical fields that include moisture retention agents, bactericides, blood anticoagulants, and components in drug delivery matrices (Chen, Du, Tan, et al, 2005). An additional factor that may need to be considered is that the pH of the gastrointestinal tract increases from the stomach to the small intestines. The use of environmentally sensitive hydrogels are proving to be the most promising (Kim & Peppas, 2002).

Materials

- Sodium Alginate
- chitosan
- Ultrafine calcium carbonate
- Parraffin oil
- Insulin
- Streptozocin

Methods

Carboxymethylation of Chitosan
The chloroacetic acid method was used with the two reagents, glyoxylic acid and chloroacetic acid. Chitosan was suspended in a solution of NaOH kept at -20 degrees Celsius overnight. Isopropyl alcohol was then added to the frozen medium, which was followed by the addition of chloroacetic acid. The solution was stirred at 30 degrees Celsius for six hours and were washed with ethanol then vacuum dried.

Oxidation of Sodium Alginate
Sodium alginate was dispersed in 100 mL of ethanol. To get oxidized alginates, an aqueous solution of sodium periodate was added. The reaction was stopped with the addition of ethylene glycol and NaCl. The product was washed in ethanol and vacuum dried at 40 degrees Celsius.

Preparation of Nanosilver
$AgNO_3$ was dissolved in water then heated to 100 degrees Celsius. Then, 2 mL of a 1% sodium citrate was added to the solution to obtain a nanosilver dispersion.

Preparation of the Carboxymethylated Chitosan-Oxidized Sodium Alginate (CMCS-OSA) hydrogel
CMCS was mixed with OSA and was let to stand to create a hydrogel. There were different concentration of each substance within each hydrogel. Then varying amounts of nanosilver solution were added to the CMCS-OSA solution.

Preparation of microspheres
Microspheres were prepared using emulsification and internal gelation protocols. The previously prepared hydrogel solution was added to an insulin solution . A suspension of $CaCO_3$ was added to the chitosan-alginate solution. After homogenization, the mixture was dispersed into parraffin oil and was stirred at varying rotations per minute through the use of a mixer. After 15 minutes of emulsification, paraffin oil containing glacial acetic acid was added to the

Methods Cont.

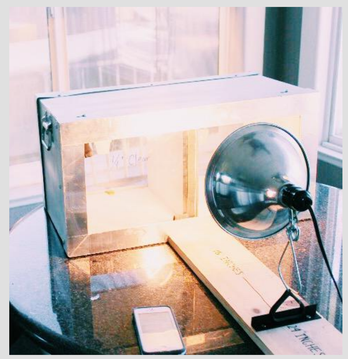
Morphological and particle size analysis
Morphology of the hydrated microspheres was observed with an optical microscope and circular dichroism spectroscopy. The size distribution of the microspheres was determined in a washing media using laser diffraction with a particle analyzer.

Encapsulation efficiency
To determine the encapsulation efficiency of the microspheres, 10 mg of the loaded microspheres were incubated in a 10 ml hydrochloric acid buffer at pH 1.2 under magnetic stirring. The sample was then transferred by centrifugation into a 10 ml phosphate buffer at pH 6.8 in order to obtain a complete dissolution. After withdrawing the sample from the acid and phosphate solution, the sample was analyzed by high performance liquid chromatography. The encapsulation efficiency was determined by calculating the difference between the theoretical initial amount of protein and the total insulin recovered from the microspheres.

In Vitro release studies
Multiple stirring point plates were used for in vitro

Predicted Results

The nanoemulsion was able to coat the insulin while maintaining original insulin structure. It was also able to retain secondary structure in the acid when analyzed by CD spectroscopy. [the size of the hydrogels must be small enough to be absorbed into the body, look at the different methods of creating the hydrogels (concentrations of alginate, insulin, rpm, ca ion concentration) look at the insulin release in the acidic environment.



Discussions

The results are significant because if alginate is able to stabilize insulin for oral digestion, then it could improve the lifestyle of diabetic patients. More people would be more likely to agree to insulin therapy. Once the insulin travels to the intestines, it can be absorbed directly into the bloodstream. Hi Dr. Malhotra

Conclusions

Orally administered insulin is still far from a reality, but it is closer than it seems. Since the CMCS-OSA was able to coat the insulin, it shows that hydrogels can maintain insulin structure and possibly survive the acidic conditions of the stomach.

Further Research

- Additional tests to be made include whether the emulsified insulin is able to function as normal insulin the the bloodstream. Also, seeing if the insulin can be absorbed by the small intestine/its stability in the intestines. Search to see if there are more consistent ways/ test the consistency of how much insulin can stay functional after going through the digestive system.

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