Hollow Hydroxyapatite Microspheres for Controlled Delivery of Proteins

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Introduction: Biodegradable polymer microspheres have been widely used as devices for controlled delivery of proteins. These polymer microspheres can be prepared with well-controlled physical and chemical properties, so they provide controlled release of the encapsulated proteins as they degrade. Day and Conzone1 invented a process for preparing porous or hollow hydroxyapatite (HA) microspheres by converting borate glass microspheres in an aqueous phosphate solution. The objective of this work was to evaluate hollow HA microspheres prepared by the borate glass conversion process as controlled delivery devices for a model protein, bovine serum albumin (BSA).

Methods: Glass microspheres (106–150 µm) with the composition (wt%): 15CaO, 10.63Li2O, 74.37B2O3, designated CaLB3-15, were prepared by conventional glass processing methods,1 and immersed for 48 h in 0.02M K2HPO4 solution (pH 9) at 37ºC to produce hollow HA microspheres. The as-prepared HA microspheres were characterized using scanning electron microscopy (SEM), X-ray diffraction (XRD), X-ray mapping in the SEM, the BET method, and gas adsorption. The ability to incorporate BSA into the hollow HA microspheres was studied by microscopy. A mass of 0.1 g HA microspheres was immersed in 5 ml of a solution consisting FITC-labeled BSA in phosphate-buffered saline (PBS) (BSA concentration = 5mg/ml). A low vacuum was applied to the system to assist the incorporation of the BSA into the hollow microspheres. After removal from the solution, the BSA-loaded microspheres were observed in an optical microscope. To study the release kinetics of BSA, HA microspheres loaded with a solution of BSA in PBS (5mg/ml), were placed in PBS, and the amount of BSA released into the PBS was measured using a micro-BCA reagent.

Results and Discussion: SEM images of the surface and cross sections showed that the as-prepared HA microspheres were hollow, with a nanoporous wall (Fig. 1a). The surface area and average pore size of the microspheres were 100.81 m²/g and 15.1 nm, respectively. X-ray mapping (Fig. 1b, 1c) showed that the shell microsphere wall was composed of Ca and P, which is consistent with the formation of HA as determined by XRD, and FTIR.

Optical images of the HA microspheres prior to loading with FITC-BSA (Fig. 2a) showed no fluorescence. On the other hand, the fluorescence of microspheres loaded with FITC-BSA (Fig. 2b) showed that the BSA was incorporated both in the nanoporous wall and in the hollow core of the HA microspheres.

The release of BSA from the hollow HA microspheres into the PBS solution (Fig. 3) was rapid (1.7µg/ml /h) during the first 10 h, then slowed during the next 14 h, and finally stopped after ~24 h.

Conclusions: Hollow HA microspheres with a nanoporous wall were prepared by a glass conversion process and loaded with a solution of BSA in PBS. The BSA was located both in the pores of the microsphere wall and in the hollow core of the microspheres. Release of BSA from the HA microspheres into a PBS medium was rapid initially, and stopped after 24 h, reaching a value equal to ~10% of the total amount incorporated into the microspheres. These hollow HA microspheres may have potential application as devices for controlled local delivery of proteins.

References:

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