SYNTHESIS AND CHARACTERISATION OF ALGINATE MICRO- AND NANOSPHERES LOADED WITH BOVINE SERUM ALBUMIN

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[ENGINEERING OF BIOMATERIALS 143 (2017) 35]

Introduction

The development of new drug delivery systems (DDS) is improving the efficacy of controlled and targeted drug release systems. DDS are supposed to improve effectiveness of therapy and lessen the harmful side effects of drugs on organisms [1-3]. The preferred drug administration route is orally. Thanks to the composition of mucus and pH varied in different parts of the food track, scientists proposed biopolymers as potential materials for oral drug delivery. Among them, the most interesting one is sodium alginate (SA), a biocompatible and biodegradable polianionic polymer of non-animal origin, which shrinks at pH below 4 [2]. The aim of this study was to obtain alginate particles in two different size regimes with controlled particle-size distribution: nano (<100 nm) and micro (from 100 nm to couple of μm).

Materials and Methods

Sodium alginate spheres were obtained by double emulsion solvent evaporation technique with an external gelation mechanism. Double emulsion was formed using a sonicator probe. To prove the possible application of those particles as new DDS, bovine serum albumin (BSA) was selected as a model drug. The influence of two different types of surfactant was examined (anionic and non-ionic). After the optimisation, four different protocols of synthesis were created – two with sodium cholate (anionic surfactant) and two with Tween80 (non-ionic surfactant).

In brief, 3.5% (v/w) solution of SA, dichloromethane and a proper surfactant (1% Tween80 or 6% sodium cholate) were mixed together. Then, a second volume of surfactant was added to form a double emulsion. Finally, calcium chloride as a cross-linker was added dropwise with stirring. The double emulsion formed was stirred at 700 rpm for 6h in 30°C. Afterwards, the solution was centrifuged with addition of 0.01M $CaCl_2$. Efficiency of BSA encapsulation was calculated based on UV-vis spectroscopy according to BCA Protein Assay (Thermo Sccientific).

Results and Discussion

Nanospheres

Particles of spherical shape with an average size of 27.1 ± 8.2 nm were obtained using sodium cholate as an anionic surfactant (FIG. 1). Those particle-size distributions were confirmed by SEM and DLS analysis. Hydrodynamic radius of 192 ± 5 nm and ζ potential of -29.0 ± 1.6 mV were obtained. BSA was encapsulated in the polymeric matrix with an average efficiency of 48.6% ± 10.0%. Encapsulation was confirmed by FT-IR and confocal microscopy. Importantly, the average ζ potential changed from the initial -29.0 ± 1.6 mV to-12.6 ± 0.7 mV, which can be explained by charge of protein in pH = 5 (in which measurements were carried) – below isoelectric point charge of protein became positive (for BSA isoelectric point is 5.82), thus it could change ζ potential [4].

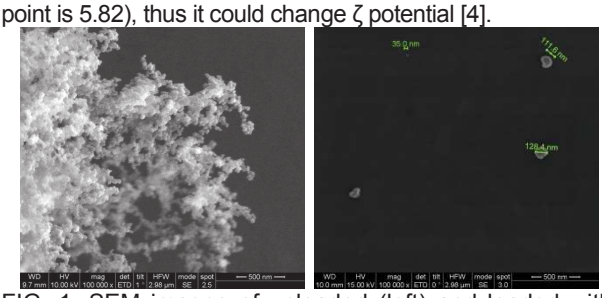

FIG. 1. SEM images of unloaded (left) and loaded with BSA (right) alginate nanospheres. Surfactant: sodium cholate.

Microspheres

Particles of spherical shapes with average size of 0.46 ± 0.11 μm and 0.58 ± 0.21 μm were obtained using Tween80 and lower (0.07% (w/v)) and higher (0.13% (w/v)) concentration of sodium alginate, respectively (FIG. 2). After encapsulation of BSA the increase of size from 0.46 ± 0.11 μm to 0.52 ± 0.13 μm was observed when using the lower concentration of alginate. In the second case (when a higher concentration of SA was used) two distributions were retrieved with sizes around 0.53 ± 0.12 μm and 1.03 ± 0.30 μm.

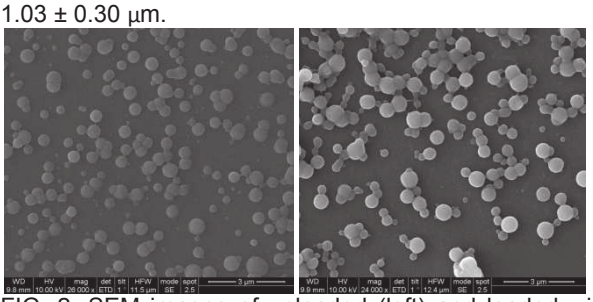

FIG. 2. SEM images of unloaded (left) and loaded with BSA (right) alginate microspheres. Surfactant: Tween80.

As well, it was possible to encapsulate the protein (BSA) in the alginate matrix with average efficiency of 65.71%. Release of protein was carried out in PBS (pH=7.4) at 37°C for 48h showing a release of 8 wt.%. The presence of protein was confirmed by confocal microscopy and FT-IR spectroscopy.

Conclusions

Protocols for the synthesis of alginate particles showing two different size-distribution regimes were successfully optimized. Particles made with Tween80 as a surfactant did not present tendency to aggregation and elevated monodispersity. In addition, particles obtained with the use of sodium cholate as surfactant showed also monodisperse morphologies. BSA was encapsulated in the polymeric matrix with efficiency up to 60%. Release tests revealed that up to 10% of the initial BSA was released after 48h.

Acknowledgments

This work was supported by Foundation for Polish Science within POMOST project "*Alginate/chitosan core-shell beads with bioactive functionalities*" (POMOST/2013-7/7).

References

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