

Preparation and characterization of microspheres constituted of Zein and Chitosan, as polymeric biomaterial

Edvani C. Muniz, Vinícius Müller, Juliana F. Piai, André R. Fajardo, Silvia L. Fávaro, Adley F. Rubira.

Grupo de Materiais Poliméricos e Compósitos, GMPC - Chemistry Department

Universidade Estadual de Maringá - Av. Colombo 5790 - 87020-900, Maringá, Paraná, Brazil.

Statement of Purpose: The drug delivery systems offer several advantages when compared to conventional dosage systems, for instance, better therapeutic efficacy. The motivation for the use of biopolymeric materials for preparation of biomaterial is their low toxicity, biodegradability, availability and relatively low price^[1]. Due to their good properties, mainly the degradability and biocompatibility, chitosan (CHI)^[2] and zein (ZN)^[3] were used in this work for preparing and characterizing microspheres aiming the potential application as biomaterial for controlled release of drugs.

Methods: For the preparation of ZN / CHI particles, two solutions were firstly prepared. CHI was solubilized in 100 mL of acid aqueous solution at 65 °C and stirred to obtain concentration equal to 1 wt-%. The ZN solution was prepared by addition of 2 g in 100 mL of ethanol-water mixture 80-20% (v/v) under stirring, obtaining a solution with concentration equal to 2 wt-%. The aqueous-alcoholic solution of ZN was poured into a flask coupled to dispersor Extratur (Quimis, Brazil). After this, the CHI solution was slowly (ca. 4 mL min⁻¹) added to the flask with ZN at stirring of 12,000 rpm. Furthermore, particles consisting only of ZN were prepared by addition of pure water instead of the acidic aqueous solution containing CHI. After all content has been added, a new mixture of ethanol-water 40-60% (v /v) was obtained in which the polymers became insoluble and precipitated forming particles. The system was kept under stirring for further 3 min. Thus, the precipitate formed was frozen and lyophilized. Following, the dried particles were washed with aqueous HCl solution (0.2 mol L⁻¹) to remove not-encapsulated CHI chains. The washed material was filtered and dried under reduced pressure at room temperature (25 °C). The after-dried particles were characterized by SEM images and FTIR.

Results: The formation of particles with spread out sizes can be observed from the images of Figure 1, obtained by SEM. The diameters of the particles were measured through an image analysis software (Size Meter 1.1©), and the average size of ZN / CHI microspheres was 4.30 ± 1.93 µm with the predominance of spherical shape. These microspheres show spongy characteristics. The size of microspheres prepared by only ZN averaged 1.23 ± 0.47 µm and the surface smoother compared to ZN/CHI ones. The ZN / CHI microspheres were formed from the slow addition of CHI aqueous solution to the alcoholic solution of ZN, by stirring. Thus, the particles were formed inside the new solution due ZN being insoluble in aqueous-alcoholic media smaller than 70% ethanol in the same way that the CHI precipitates in alcoholic solutions with pH > 5. The microspheres of only ZN were formed by the addition of pure water in the aqueous-ethanol, precipitating when the ethanol concentration lowered below the threshold value of 70% (v/v).

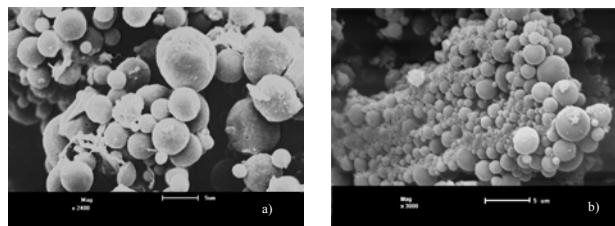


Figure 1. SEM images of microspheres constituted by: a) zein / chitosan (ZN/CHI); and b) zein (ZN).

Figure 2 shows the FTIR spectra of pure CHI, pure ZN and ZN / CHI particles. It can be seen that the spectra of ZN / CHI particles and of pure zein are very similar. However, it was noticed the presence of low intensity band at 1082 cm⁻¹ on ZN/CHI particles FTIR spectrum, characteristic of C-O bonds. According to Giner et al. (2009), the presence of this band corroborates that CHI is present on ZN / CHI blends^[4]. Thus, the band at 1082 cm⁻¹ confirms the occurrence of simultaneous precipitation of CHI and ZN during in the formation of microspheres. In such precipitation, the polymers can interact through H-bonding due to the presence of amide groups of protein (ZN) and the amino groups of the CHI, and become insoluble in the presence of non-solvent (ethanol and water for CHI and ZN, respectively). Thus, it can be inferred that the microspheres are composed mainly of ZN and small amount of CHI.

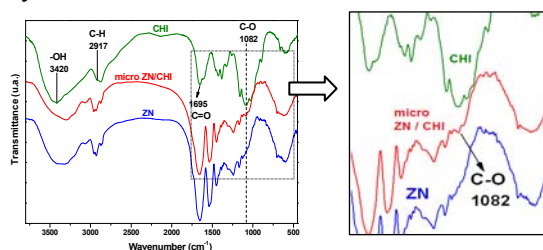


Figure 2. FTIR spectra of pure chitosan, zein and ZN / CHI particles.

Conclusions: Microspheres of ZN / CHI of different sizes were obtained by the above-described methodology. The ZN / CHI particles showed higher porosity and larger average diameters compared to ZN microspheres that was attributed to the presence of CHI in microspheres. FTIR spectra of ZN / CHI and ZN microspheres are not quite similar, but barely differed by the C-O stretching at 1082 cm⁻¹, which was clearly present on FTIR spectrum of CHI and not observed on ZN spectrum. Thus, it was concluded that ZN predominates in ZN / CHI microspheres. However, further studies are needed to better characterization and application of this material.

References:

- [1] Nascimento A. J Microencap 2001;18:679 – 684.
- [2] Kumar MNVR. Chem Rev 2004;104:6017 – 6084.
- [3] Lawton JW. Am Assoc Cer Chem 2002;79:1 – 18.
- [4] Giner ST. Carbohydr Polym 2009;77:261–266.