Hindawi Publishing Corporation
Journal of Nanomaterials
Volume 2011, Article ID 928728, 6 pages
doi:10.1155/2011/928728

*Research Article*

# Preparation and Characterization of Zein and Zein-Chitosan Microspheres with Great Prospective of Application in Controlled Drug Release

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Received 2 June 2010; Revised 26 July 2010; Accepted 6 September 2010

Academic Editor: Libo Wu



Biomaterials applied as carriers for controlled drug delivery offer many advantages over the conventional systems. Among them, the increase of treatment effectiveness and also a significant reduction of toxicity, due to their biodegradability property, are some special features. In this work, microspheres based on the protein Zein (ZN) and ZN associated to the natural polymer Chitosan (CHI) were prepared and characterized. The microspheres of ZN and ZN/CHI were characterized by FT-IR spectroscopy and thermal analysis, and the morphology was analyzed by SEM images. The results confirmed the incorporation of CHI within the ZN-based microspheres. The morphological analysis showed that the CHI added increased the microspheres porosity when compared to the ZN microspheres. The chemical and physical characterization and the morphological analysis allow inferring that ZN/CHI microspheres are good candidates to act as a carrier for controlled drug release.

## 1. Introduction

Carriers for drug delivery based on polymeric systems have been widely used not only for providing slow and gradual release of active components but also for targeting to specific organs of the body where the medicines may heal inflammation, tumor, and other diseases [1, 2]. One of most important concerns on drug administration is the need of maintenance of its characteristics during the delivery up to the target without changes in its molecular structure which could alter the drug-action capability [3]. The drug-loading on a polymer matrix, which one could act as an efficient carrier, is an interesting mean to ensure the preservation of drug molecular stability [4]. Polymer matrixes obtained from colloidal systems are good examples often employed for such purpose [5]. Among them, liposome, micelles, emulsions, and particles with micro, or nanodimensions can be mentioned [2].

The employment of natural polymers on obtaining colloidal systems enables the achievement of materials with many interesting features (low toxicity, biocompatibility, and biodegradability), thus allowing their use as carriers. Several works have been published related to the use of natural polymers such as collagen, cellulose, zein, alginate, and chitosan in those systems [6–8]. Zein (ZN), a protein that belongs to the prolamine class, is the major protein from corn [9, 10]. Due to its ability for acting as a water barrier, ZN has been widely used as a coating for candies, nuts, fruits as well as food packaging [9]. There are also studies concerning medical application of ZN such as carriers for drug release at specific sites in the human body [11]. Although ZN is a water-insoluble protein it remains soluble in aqueous solutions containing at least 70% alcohol [12]. On the other hand, ZN has the ability to form films, suitable for coating, in the presence of water through intermolecular interactions which are responsible for joining the molecules together [9]. This property is also important for studies of edible films and coatings, both in the food industry, as well as gastroresistant film in the pharmaceutical and biomedical research.

Chitosan (CHI) [or poly($\beta$-(1-4)-2-amine-2-deoxy-D-glucopyranose)] is a natural polysaccharide obtained from partial or total deacetylation of the biopolymer chitin, which is the major constituent of the invertebrates exoskeleton [13]. CHI shows well-known physical and biological features [14, 15]. It has been widely used in biomedical and pharmaceutical applications, such as carriers for controlled drugs and DNA release, in the manufacture of contact lenses, artificial membranes and skin, and periodontal and orthopedic applications [16–18].

The association of zein (ZN) with chitosan (CHI), forming microparticles, was investigated in this paper aiming the application of such biomaterial as carrier for controlled drug release.

## 2. Materials and Methods

*2.1. Materials.* Chitosan (CHI) (Golden Shell Biochemical Co., China), with deacetylation degree equal to 15% and $M_V$ equal to $90 \times 10^3$ g mol$^{-1}$, was determined according to Mao et al. [19]; Corn zein protein was supplied in powder form by Química Brasil Ltda. (CAS number 9010-66-6), with a $M_W$ of $22 \times 10^3$ g mol$^{-1}$; Hydrochloric Acid (Nuclear, Brazil); Acetic Acid (F. Maia, Brazil); Ethanol (TEDIA, Brazil); and Sodium hydroxide (Nuclear, Brazil).

*2.2. Preparation of Microspheres.* The microspheres based only on ZN were prepared by the solubilization of 2.0 g of ZN in 100 mL of ethanol-water (rate 4 : 1 ethanol/water) under magnetic stirring. The ZN alcoholic solution was transferred to a flask coupled to a high-speed laboratory mixer (Quimis, model Extratur). After this, the solution was vigorously mixed (12,000 rpm) for 15 min, and 100 mL of distilled water was slowly dropped to the system during the stirring (rate flow: 4 mL min$^{-1}$). The water addition changes the ratio ethanol-water to 2 : 3 v/v, inducing the loss of solubility of ZN and allowing the formation of microspheres droplets. So, the insoluble microspheres were collected through filtration under vacuum, after that were frozen under liquid $N_2$ and then lyophilized during 24 h. The preparation of ZN/CHI microspheres followed the same procedures described above unless by the addiction of CHI solution instead of distilled water. The 1.0 wt/v-% CHI solution was prepared in 0.2 mol L$^{-1}$ acetic acid solution at 65°C under magnetic stirring. After the lyophilizing step, the ZN/CHI microspheres were washed with 0.2 mol L$^{-1}$ HCl solution to remove the free CHI. So, these microspheres were dried again under reduced pressure at room temperature. The two types of microspheres (ZN and ZN/CHI) were characterized by scanning electronic microscopy (SEM) images, Fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetric (DSC), thermogravimetric analysis (TGA), and differentiate weight loss (DTG) analysis.

*2.3. SEM Images.* The morphologies of ZN and ZN/CHI microspheres were investigated by SEM images (Shimadzu, model SS 550). The microspheres surfaces were sputter-coated with a thin layer of gold for allowing the SEM visualization. The images were taken by applying an electron accelerating voltage of 8 kV. The microspheres average diameters were calculated by means of the software Size Meter®, version 1.1, with differentiation threshold set according to the image scale.

*2.4. FTIR Spectroscopy.* The lyophilized ZN particles or the after-dried ZN/CHI particles were mixed with KBr to form thin discs that were characterized by FTIR (Shimadzu Scientific Instruments, Model 8300, Japan), operating in the region from 4000 to 400 cm$^{-1}$, resolution of 4 cm$^{-1}$. Also, for control FTIR, spectrum of CHI was obtained in the same conditions.

*2.5. DSC Analyses.* DSC analyses were performed on a calorimeter (Netzsch, model STA 409 PG/4/G Luxx, USA) operating in the following conditions: heating rate of 10°C min$^{-1}$, nitrogen atmosphere with flow rate of 20 mL min$^{-1}$, and temperature range from 22 to 400°C. For all analyses it was obtained firstly the respective baselines.

*2.6. TGA Analyses.* TGA analyses were carried out on a thermogravimetric analyzer (Netzsch, model STA 409 PG/4/G Luxx, USA) at a heating rate of 10°C min$^{-1}$ under nitrogen atmosphere with flow rate of 20 mL min$^{-1}$ in a temperature range from 22 to 400°C.

## 3. Results and Discussion

Figure 1 shows the SEM images obtained from particles of ZN (Figures 1(a)–1(b)) and of ZN/CHI (Figures 1(c)–1(d)). The SEM images show that the particles are microspheres; indeed, however the size distribution is widely scattered. The average diameter of microspheres was calculated, in each case, from the simple average of 50 microspheres diameters randomly chosen, using the software Size Meter®. The calculated values were equal to $1.23 \pm 0.47\,\mu$m for the ZN microspheres and $4.30 \pm 1.93\,\mu$m for the ZN/CHI ones. From these values, it was possible verifying that the CHI incorporation into ZN microspheres increases in 3.5-fold the average diameter compared to neat ZN particles. This is totally expected considering the fact that CHI chains are larger than ZN chains (c.a. 4 times higher); therefore, the incorporation of CHI chains should promote an increase in mobility of polymer chains inside the particles reflecting in an increase of the size of ZN/CHI microspheres, which agrees with the calculated values. Another fact that might contribute to the average size of ZN/CHI particles to be higher than ZN particles is the higher miscibility of CHI chains in the parent solution in which the particles were obtained. Thus, the presence of CHI should affect not only the size but also the porosity of particles as can be observed in the micrographs on Figure 1.

According to the SEM images of Figure 1, it can be inferred also that both types of microparticles have the predominance of spherical shape. However, it could be observed that the ZN microspheres have smoother surfaces while ZN/CHI microspheres have irregular surfaces with spongy

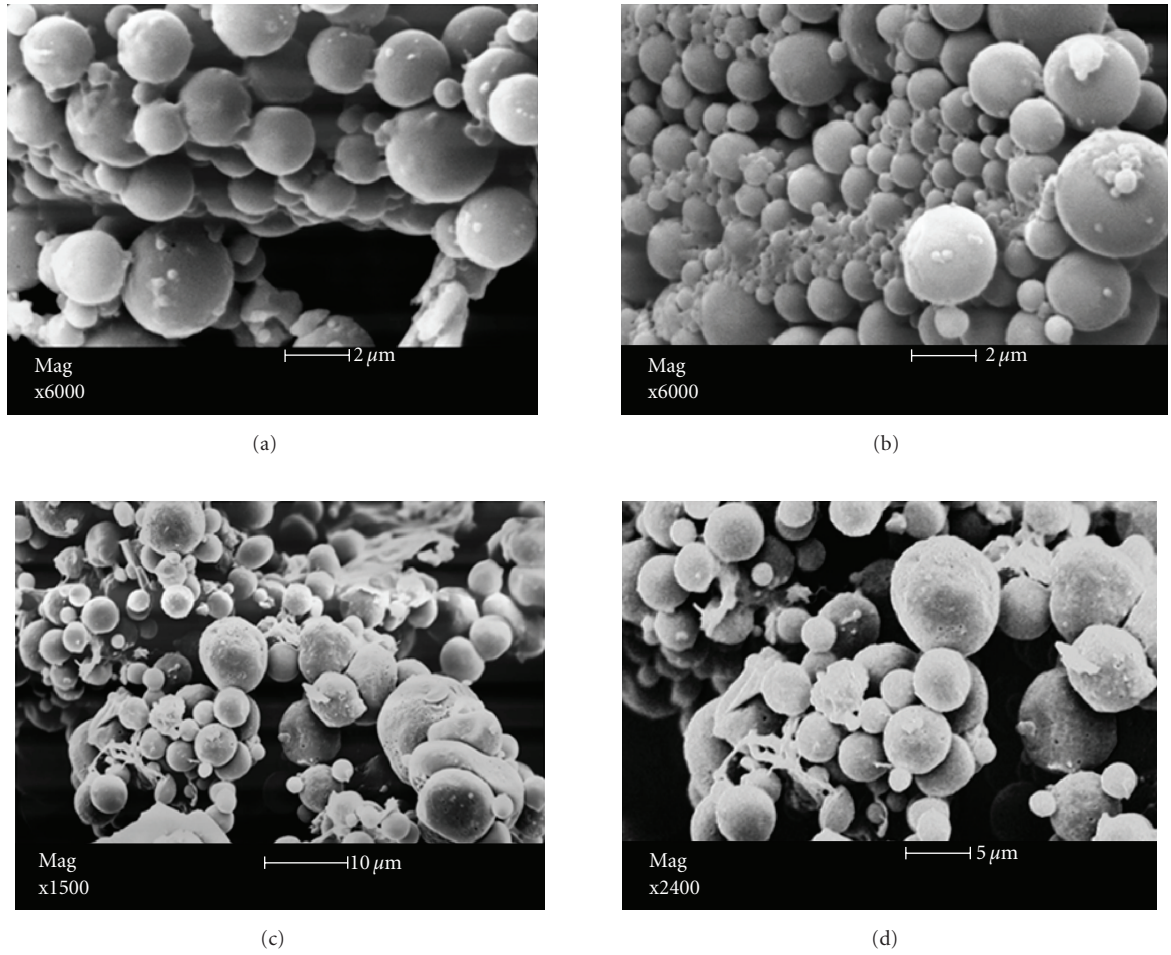


Figure 1: SEM images of ZN (a-b) and ZN/CHI (c-d) microspheres.

characteristics. This clear difference was interpreted having in the mind the fact that the two types of microparticles were prepared under different conditions. The microspheres constituted only by ZN were formed by the addition of distilled water in the ethanol-water solution, precipitating when the ethanol concentration lowered below the threshold value of 70%–30% (v/v) ethanol-water. Since the ZN/CHI microspheres were prepared by slow addition of aqueous CHI (0.2 mol $L^{-1}$ acetic acid) solution into alcoholic solution of ZN, with vigorous mixing, the two procedures are quite different. In this sense, the precipitation of microspheres occurred due to the fact that zein became insoluble in alcoholic medium of less than 70% (ZN particles) and CHI became insoluble in this condition and/or in medium with pH > 5 (CHI/ZN particles) [20].

Another synthesis factor that may have influenced the surfaces of microspheres particles is the difference in the forming solutions viscosity. Comparing the alcoholic solution of ZN with the solution obtained after the CHI addition, there is an increase in its viscosity. The alcoholic ZN solution exhibits a low intrinsic viscosity ($[\eta]$) at 25°C equal to 19.85 mL $g^{-1}$. The addition of CHI solution, which exhibits higher $[\eta]$ (equal to 950.10 mL $g^{-1}$), into the system, may have contributed to increasing the $[\eta]$ of ZN solution ($[\eta]$ equal to 308.50 mL $g^{-1}$). Thus, despite the vigorous mixing, the smooth surface observed on microspheres constituted by neat ZN was not achieved in ZN/CHI microspheres. Moreover, the higher surface roughness of ZN/CHI microspheres could contribute to increasing the drug adsorption on the microspheres and improving the encapsulation and releasing rates compared to neat ZN particles.

*3.1. FTIR Spectroscopy.* The ZN and ZN/CHI microspheres and pure CHI (powder) were characterized by FTIR spectroscopy technique (Figure 2). The FTIR spectrum of CHI (Figure 2(c)) exhibited a broad intense band at 3437 $cm^{-1}$ assigned to O–H vibrational stretching. Close to this wavelength, a N–H vibrational stretching is also commonly verified, but due to windening of OH band it was hindered. The band at 1633 $cm^{-1}$ was assigned to C=O from amide groups and to -$NH_2$ deformation [21], and the intense band at 1082 $cm^{-1}$ was assigned to the C–O vibrational stretching, characteristics of primary alcoholic groups on CHI structure. Figure 2(a) shows the FTIR spectrum for ZN microspheres, where a clear OH band at 3387 $cm^{-1}$, two intense bands at 1656 and 1545 $cm^{-1}$, assigned to the amide

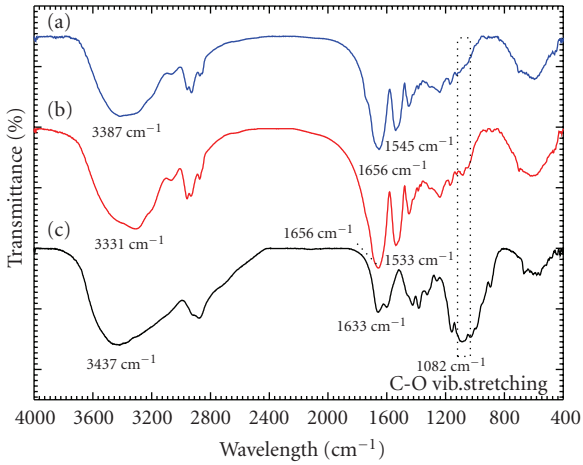


Figure 2: FTIR spectra of (a) ZN microspheres, (b) ZN/CHI microspheres, and (c) pure CHI.

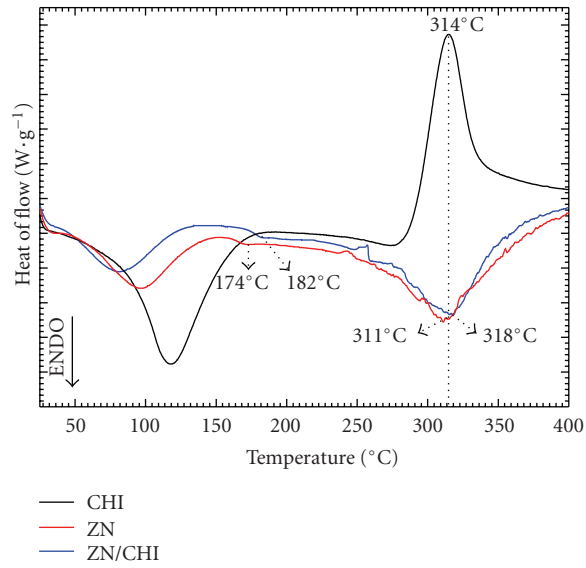


Figure 3: DSC curves of pure CHI, ZN and ZN/CHI microspheres.

bands, are observed. In one of amide band the predominance of C=O vibrational stretching occurs, and for the other amide band the C–N stretching predominates [22]. The FTIR spectrum from ZN/CHI microspheres (Figure 2(b)) presents a similar profile as compared to FTIR from ZN microspheres.

However, the band observed at 1545 $cm^{-1}$ in the ZN microsphere FTIR spectrum, which can be assigned to N-H bond, was shifted to 1533 $cm^{-1}$ in the ZN/CHI microsphere FTIR spectrum. This fact may indicate a possible interaction among the ZN and CHI chains, probably through hydrogen bonding among the amino groups present on both CHI and ZN chains. Furthermore, the formation of ZN/CHI microspheres was also confirmed by the arising of a band at 1082 $cm^{-1}$ in their spectrum of low intensity, which was not observed in the ZN microsphere spectrum, confirming the presence of CHI.

Different from work by Torres-Giner et al. [23], in which a common solvent for both compounds to form ZN/CHI blends was used; in this work, a nonsolvent for both components was used for precipitating the microspheres. Thus, simultaneous precipitation of ZN and CHI during the microspheres formation was expected and confirmed by FTIR spectroscopy. The almost complete precipitation should occur due to the interaction among the ZN protein and CHI chains by hydrogen bonding. Some other works addresses the interaction of the polysaccharide chitosan with proteins (i.e. gelatin, collagen, etc.) through hydrogen bonds [24, 25]. The hydrogen bonds are formed due to the interaction among the amino groups present in the protein (ZN) structure and the amino groups on the CHI chains. By adding water to the system, which acts as nonsolvent for the ZN, the precipitation of microspheres occurs by decreasing the relative amount of alcohol and increasing the pH. So, microspheres are formed and are composed mainly by ZN with CHI incorporated into their structures.

*3.2. Thermal Analyses.* The thermal profiles of ZN and ZN/CHI microspheres were evaluated by DSC, TGA, and DTG analysis. Figure 3 shows the DSC curves of neat CHI, ZN, and ZN/CHI microspheres. All the DSC curves show endothermic peaks in the range of temperature of 50 to 150°C. The presence of such peaks was attributed to the loss of volatile components or the possibility of chain relaxation [26]. Furthermore, in this temperature range is also verified the breakdown of hydrogen bonds which are present in zein structure and other molecular associations [27]. The DSC curve of pure CHI exhibits a strong exothermic peak at 307°C, which is attributed to the degradation of that polysaccharide [28].

Proteins have some features associated to their different tridimensional structure, such as the denaturation process. Some works show that the temperature of unfolding protein can be evaluated through thermal analysis [29, 30]. Mothe et al. [30] attributed the presence of an endothermic peak in proteins thermograms to the destabilization of their physical interactions, as hydrogen bonding, electrostatic interaction, and dipole-dipole interaction, thus causing the loss of three dimensional protein structures. DSC curves of ZN and ZN/CHI microspheres exhibited endothermic peaks at 311° and 318°C, which can be interpreted as the protein unfolding [31].

It was also verified through DSC curve, close to 174°C, an alteration on the linear profile of ZN curve. Such alteration was associated to the ZN glass transition temperature ($T_g$), and above this temperature the protein chains of ZN undergo to a flexible stage. This inference is supported by other studies that also determined the $T_g$ of ZN in a temperature range of 160–180°C [31, 32]. Moreover, the $T_g$ for the ZN/CHI microspheres was observed at temperature higher than that observed for the pure ZN microspheres. The $T_g$ of ZN/CHI microspheres increased c.a. 8°C from that without CHI. Thus, it can be evidenced that the incorporation of the polysaccharide CHI within the ZN microspheres makes the CHI/ZN structure get some flexibility in just a higher temperature. This fact was explained by the interaction among

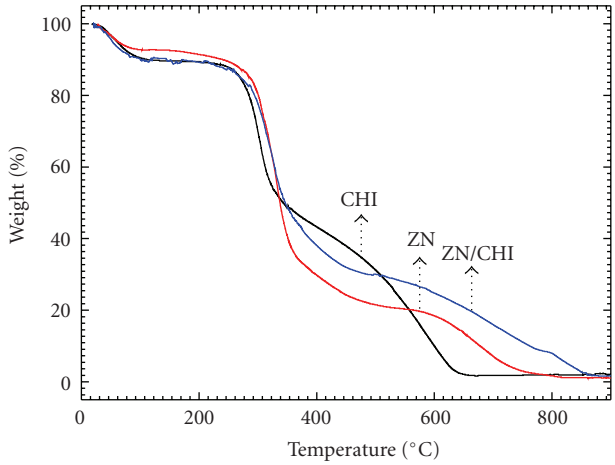


Figure 4: TGA curves of pure CHI, ZN, and ZN/CHI microspheres.

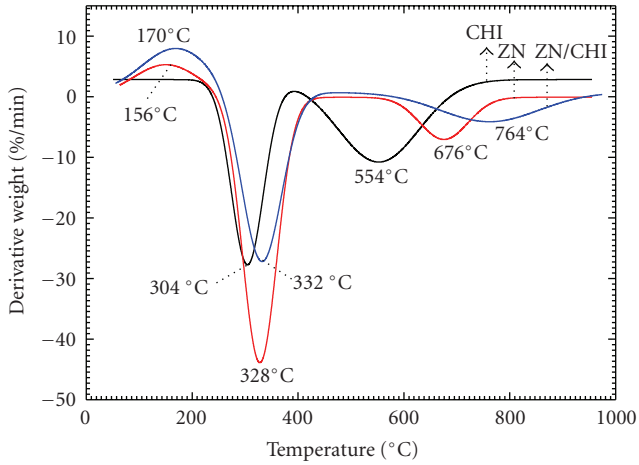


Figure 5: DTG curves of pure CHI, ZN and ZN/CHI microspheres.

the ZN and CHI chains after the microsphere formation, as observed through the FTIR spectra (see Figure 1).

Figure 4 shows the TGA curves of CHI powder and microspheres of ZN and ZN/CHI. The decrease of about 10% on mass between 50° and 150°C was due to the vaporization of water and volatile components from the material.

In the same temperature range, the decrease of mass observed for ZN is lower than that for CHI or ZN/CHI. This happens due to the lower hydrophilicity of ZN, while the pure CHI and ZN/CHI microspheres exhibit higher hydrophilicity. Thus, the amount of water volatilized is greater for these latter two. From 200 to 400°C, the TGA curves for CHI, ZN, and ZN/CHI presented significant mass loss being in this event more intense for ZN, followed by ZN/CHI and CHI. The mass loss in this range (200–400°C) for ZN particles is about 60% while for the ZN/CHI the decrease is 50%. The DTG curve (first derivative of TGA curves versus temperature), presented in Figure 5, provides information of thermal stability of ZN and ZN/CHI particles compared to CHI.

The incorporation of CHI on ZN allows the formation of material pursuing thermal stability similar to ZN. This was inferred after analysis of DTG curves (Figure 5).

Furthermore, as observed by analysis of DSC curves, the incorporation of CHI shifted the $T_g$ of microspheres to a larger value when compared with $T_g$ exhibited by neat ZN microspheres. Also, the incorporation CHI on ZN particles promoted an increase at the temperature of the unfolding of zein. Note that for the pure ZN microspheres the unfolding temperature was observed to occur at 328°C whereas for the ZN/CHI microspheres this temperature appears at 332°C. These results reinforce the hypothesis that the incorporation of CHI into ZN-based microspheres allows advantages and amplifying some of their properties, for instance, the thermal stability.

## 4. Conclusions

In this work, microspheres based on the protein zein (ZN) and on ZN having the polysaccharide chitosan (CHI) incorporated were prepared through the technique of precipitation by the addition of a nonsolvent under high stirring. SEM images allowed observing that both types of obtained microspheres presented spherical shape. Detailed analyses of SEM showed that surface of ZN particles is smooth while the surface of ZN/CHI particles is rougher. This difference on the microspheres surfaces could be an advantage to their application as carrier to drug delivery. The microspheres average diameter was calculated through the use of specific software. The average diameter was equal to $1.23 \pm 0.47\,\mu$m for the ZN microspheres and $4.30 \pm 1.93\,\mu$m for the ZN/CHI ones. Analysis of FTIR spectroscopy, allowed infering that the incorporation of the CHI into ZN microspheres effectively occurred. Thermal profile of the two types of microspheres was evaluated through DSC and TGA. Comparing the results, it was verified that the ZN/CHI microspheres presented similar thermal profile to that of the ZN microspheres. Furthermore, the CHI incorporation provided an increase in the $T_g$ of ZN microspheres inducing increasing the temperature at which ZN chains get flexibility. So, in despite of both types of materials prepared, this work exhibited interesting features; such materials might be applied in studies to obtain viable carriers for controlled drug release. As discussed previously, the CHI incorporation provides ZN/CHI microspheres with some advantages over neat ZN microspheres.

## Acknowledgments

Vinícius Müller thanks Prati, Donaduzzi, and Co. Ltd. (Toledo, Paraná, Brazil) for the undergraduation fellowship. Juliana Francis Piai, André Ricardo Fajardo, and Silvia Luciana Fávaro thanks CNPq and Capes for the doctorate fellowships. Edvani Curti Muniz and Adley Forti Rubira thanks CNPq for the financial support (Grant no. 309005/2009-4).

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