

## Alternative way for tailoring acid stability, barrier and/or adsorbent properties of chitosan microspheres

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### INTRODUCTION

An important advantage of chitosan is the possibility of chemically modifying its structure with agents (cross-linkers) that are able to increase the time frame and controlled release property of active agents. Several chemical cross-linkers have been used such as glutaraldehyde or formaldehyde. However, the use of these cross-linking agents can lead to toxic side effects, which may impair the biocompatibility of cross-linked products. It is crucial to search for novel cross-linkers for chitosan microspheres.

To solve this problem, Ré and co-workers have long been studying the potential use of d,l-glyceraldehyde as a biocompatible cross-linking agent (Oliveira 2005, 2006). They are focusing now on a new alternative to control chitosan microsphere properties. This work was dedicated to explore the possibility of using nanosized hydrophobic silica to cross-link chitosan microspheres and to study the physicochemical properties of the microspheres.

### MATERIALS AND METHODS

Hydrophobic silica with a specific surface area of  $110 \pm 20 \text{ m}^2/\text{g}$  and a primary particle size of 16 nm (Aerosil® R972, Degussa-Huls, Brazil). Low molecular weight chitosan (75-85% deacetylated) and Bovine serum albumin-BSA (PI=4.8,  $M_w$  66kDa) from Sigma-Aldrich (St. Louis, MO, USA). Mannitol 99% (USP grade, Henri Farma Ltda, S. Paulo, SP, Brazil).

**Preparation of chitosan/nanoSiO<sub>2</sub> microspheres** The chitosan solution (1.0 %wt/wt) was prepared by dissolving the polymer in aqueous solution containing acetic acid (pH 5.5-6.0). Weighted portions of hydrophobic silica were dispersed in an ethanol-water mixture (20%:80% in mass proportion) at room temperature to yield a 2% wt/v suspension. The hydroalcoholic mixture containing the dispersed silica was added to the chitosan solution before being passed through a high-pressure APV-2000 homogenizer-HPH operated at  $800 \text{ kg}\cdot\text{cm}^{-2}$  for 5 homogenization cycles. The suspensions were then spray dried with a 0.5 mm two-fluid pressurized atomizer at a feed rate of 3 ml/min in a Büchi-B190 spray dryer. The inlet temperature was controlled at  $120 \pm 2^\circ\text{C}$  and the outlet temperature at  $89 \pm 3^\circ\text{C}$ .

**Characterisation of chitosan / nano SiO<sub>2</sub> microspheres** The particle size distribution was determined by laser diffraction (LS 230 COULTER®

granulometer). The surface area was measured (equipment ASAP 2010) by BET. The particles were observed by SEM (JEOL JSM-6360LV) and by TEM (Philips®CM120 80kV) microscopies. The acid stability of all the batches of microspheres was determined by measuring the transmission after the microspheres had been exposed to 0.1N HCl (Dhawan 2005). Dynamic vapour sorption (DVS) was used to assess the moisture sorption characteristics of the spray dried microspheres using an automated DVS-2 water sorption analyzer. In a separate series of experiments, we investigated the equilibrium and kinetics of adsorption of a model protein (BSA) on the chitosan/nanoSiO<sub>2</sub> microspheres. The adsorption experiments were performed using a static method. The best conditions for BSA adsorption were found after screening of pH (4, 5, 6, 7.4 and 8) and of initial BSA concentration in the adsorption media (0.2 to 10  $\text{mg}\cdot\text{ml}^{-1}$ ). Kinetic experiments were conducted at pH 6 and an initial concentration of BSA of 0.5  $\text{mg}\cdot\text{ml}^{-1}$ . Samples were taken and analyzed periodically. The zeta potential was also measured (Delsa TM Nano C). All experiments were triplicated.

### RESULTS AND DISCUSSION

Some characteristics of the chitosan/nanoSiO<sub>2</sub> microspheres are summarized in Tables 1 and 2.

**Table 1. Particle size distribution**

Chitosan: nanoSiO <sub>2</sub> (%wt)	D(4.3) ( $\mu\text{m}$ )	D(v.0.1) ( $\mu\text{m}$ )	D(v.0.9) ( $\mu\text{m}$ )
100 : 0	5.1 $\pm$ 0.1	1.9 $\pm$ 0.1	17.5 $\pm$ 2.1
76 : 17	6.6 $\pm$ 0.5	0.9 $\pm$ 0.0	15.3 $\pm$ 0.5
64 : 32	7.9 $\pm$ 0.1	2.6 $\pm$ 0.1	14.1 $\pm$ 0.6
50 : 50	5.9 $\pm$ 0.1	2.1 $\pm$ 0.1	10.5 $\pm$ 0.5

Although small variations in median diameter were observed for different samples (Table 1), there was no direct relationship between silica concentration and diameter. Representative SEM and TEM micrographs of the chitosan/nanoSiO<sub>2</sub> microspheres are shown in Fig. 1. The microspheres consist of spherical structures with visible colloidal silica layers on the surface. Table 2 shows that the residual water content ranged from 3.7 to 6.5 %wt, increasing with the chitosan mass proportion, and that the specific surface area of microspheres increased with the silica content from  $3.0 \pm 0.2 \text{ m}^2/\text{g}$  to  $21.7 \pm 0.7 \text{ m}^2/\text{g}$ , which could be

expected considering the high specific surface area of the silica ( $110 \pm 20 \text{ m}^2/\text{g}$ ).

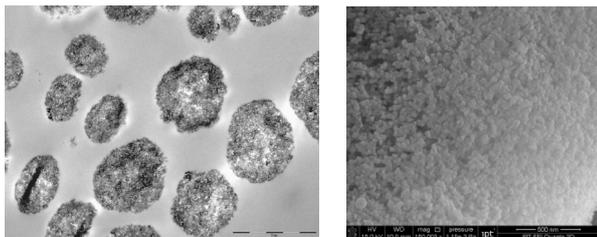


Figure 1. SEM and TEM micrographs

Table 2. Water content, superficial area and acid stability (% transmission)

Chitosan: nanoSiO <sub>2</sub> (%wt)	Water content (%)	Sup. Area BET (m <sup>2</sup> /g)	% transmission
100 : 0	-	3.0±0.2	92.1±0.3
76 : 17	6.5	4.1±0.3	72.9±0.4
64 : 32	4.3	8.8±0.3	30.0±0.8
50 : 50	3.7	21.7±0.7	10.6±2.8

The stability of all the batches of microspheres was determined by measuring the transmission after the microspheres had been exposed to 0.1N HCl. Since the decrease in turbidity is directly dependent on the disintegration of the microspheres, transmission is a measure of the concentration of non disintegrated microspheres. A low transmission indicates high stability, and a high transmission implies that the microspheres dissolved in HCl. Table 2 shows that chitosan/nanoSiO<sub>2</sub> microspheres with a silica content of 50%wt did not dissolve in 0.1N HCl. All other batches of microspheres were found to be unstable at pH 1.2.

The equilibrium moisture sorption isotherms (first sorption cycle) for the samples are plotted in Fig.1.

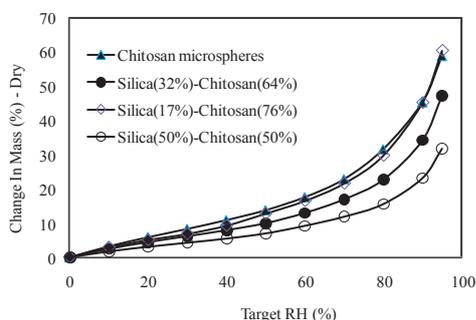


Figure 1. Sorption isotherms for chitosan/nanoSiO<sub>2</sub> microspheres with varying percentages of silica

The results suggest that for a silica content higher than 32% wt, the affinity for moisture sorption decreased, compared to the microspheres from chitosan only. From the data, a direct linear relationship was found between moisture sorption at 70% RH and silica concentration ( $R^2 = 0.9986$ ), with increased silica

content resulting in a decreased affinity for moisture sorption. The presence of hydrophobic silica resulted in less water affinity and probably acted as physical cross-linking points.

The kinetics results for experiments of BSA adsorption on chitosan/nanoSiO<sub>2</sub> are shown in Fig.3. The BSA adsorption equilibrium was reached at about 2h. It consists of 2 steps : the transport of BSA from the bulk solution to the surface of the microspheres and the attachment of proteins to the active adsorption sites on the microspheres. In the first step, the surfaces of microspheres were relatively free of protein and the protein that arrived at the microsphere surface could attach instantly to the surface sites. The adsorption rate may then be controlled by the number of BSA that diffused from the bulk solution to the microsphere surface. Electrostatic interactions can explain the high adsorption capacity at pH 6. The electrostatic interactions between the microspheres and BSA ( $PI = 4.8$ ) are repulsive at  $pH > 6.5$  (value of the pKa of the chitosan), but become attractive at  $pH < 6.5$ .

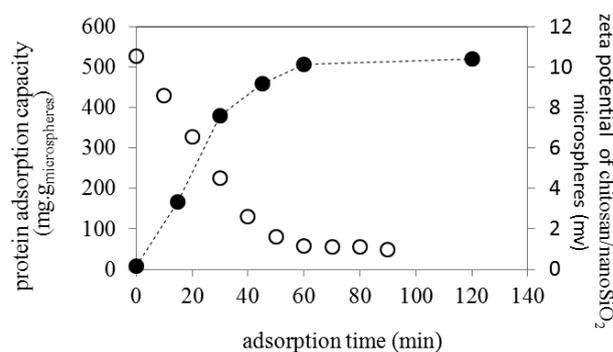


Figure 3. BSA adsorption kinetics

## CONCLUSIONS

The chitosan/nanoSiO<sub>2</sub> microspheres demonstrated a high stability in aqueous acidic environment, a decreased moisture sensitivity with increasing content of nanoSiO<sub>2</sub> and a high BSA adsorption capacity (520 mg/g<sub>microspheres</sub>). These results encourage the investigation of these microspheres as controlled delivery systems.

## REFERENCES

- Oliveira, B. F. et al (2005) *Spray-dried chitosan microspheres cross-linked with D, L-glyceraldehyde as a potential drug delivery system: Preparation and characterization*. Braz. J. of Chem. Eng. 22, 353-360.
- Oliveira, B. F. et al (2006) *Spray-dried chitosan microspheres as a pDNA Carrier*. Drying Technology, 24, 373-382.
- Dhawan, Set al (2005) *Application of poly (ethylene oxide) in drug delivery systems*. Pharm Technol.29, 72.