P-059

Cross-linked chitosan/carrageenan nanoparticles as protein carriers

Rodrigues S. [#] **and Grenha A.** [*]
CBME/IBB, University of Algarve - Faro, Portugal
* Supervisor # susananasus@gmail.com

INTRODUCTION AND OBJECTIVES

Many biomolecules evidence therapeutic potential but their systemic administration is challenging and, thus, parenteral delivery is usually the unique option. Administration through alternative mucosal routes is promising but demands the development of adequate drug delivery systems that can improve the mucosal contact, while protecting sensitive bioactive materials from enzymatic and chemical degradation *in vivo*. In addition, carriers should enable a reduction of side effects, delivering the drug effectively and specifically to the site of action, thereby achieving greater efficacy compared to conventional drugs (Reis 2006).

The incorporation of bioactive agents into small polymeric particles such as nanoparticles, has been gaining popularity, because they are frequently associated to a more controlled drug release, allowing an increased therapeutic effect (Reis 2006). Many polymers have been applied to this end. Natural-origin polymers are usually preferred, since they easily comply with requisites always compulsory in drug delivery as biocompatibility, biodegradability and absence of toxicity (Malafaya 2007).

A previous study demonstrated the ability of two natural marine-derived polymers, chitosan and κ-carrageenan, to assemble into nanoparticles of 400-600 nm (Grenha 2010).

Chitosan (CS) is a cationic polysaccharide resulting from the deacetylation of chitin, the main component of the exoskeleton of crustaceans. It is composed of repeating units of N-acetilglucosamine and D-glucosamine and presents well-documented favourable biological properties such as biocompatibility, biodegradability and low toxicity, and it also displays mucoadhesive properties, rendering this molecule very attractive for drug delivery applications (Saboktakin 2010)

Carrageenan (CRG) is another polysaccharide, which is extracted from red seaweed, being composed of galactose and anhydrogalactose units, linked by glycosidic unions. Due to the strong ionic nature, carrageenan exhibits a high degree of protein reactivity becoming in this way a potential material to be used in the field of drug delivery (Malafaya 2007).

CS nanoparticles can be easily prepared by ionic complexation using a negatively charged molecule as precipitating agent. The very mild conditions that avoid using harmful organic solvents and the capability of retaining macromolecules bioactivity (protein, DNA etc) during the encapsulation are the principal advantages of this method (Saboktakin 2010).

The objective of this work was to produce nanoparticles based on the referred polysaccharides, including in the formulation pentasodium tripolyphosphate as cross-linking agent, and evaluating whether its presence contributed to the reduction of nanoparticles size. This would increase the surface area, improving the nanoparticles' contact with epithelial surfaces. The nanocarriers' ability to associate proteins is also assessed.

MATERIALS AND METHODS

Preparation of CS/CRG and CS/CRG/TPP nanoparticles

Nanoparticles were prepared according to the previously described polyelectrolyte complexation (CS/CRG) (Grenha 2010) with further gelation of chitosan (CS/CRG/TPP). Briefly, CS was dissolved in acetic acid 1% (w/w), while κ- CRG and pentasodium tripolyphosphate (TPP) were dissolved in purified water. CS/CRG nanoparticles were spontaneously formed at room temperature upon incorporation of CRG solution into CS solution (CS/CRG mass ratios from 3/1 to 6/1). CS/CRG/TPP nanoparticles were prepared by dropping into CS solution a previously prepared mixture of CRG/TPP (TPP ratio respect to CS ranged from 0,5 to 1). Nanoparticles were isolated by centrifugation (at 16000 x g during 30 minutes) and resuspended in 100 μL of milli-Q water. Bovine serum albumin (BSA) was dissolved in water and added to the CRG/TPP phase before the addition to CS solution.

Nanoparticles characterization

Nanoparticles production yield was calculated by gravimetry, comparing the real weight of nanoparticles with the initial amount of solids used for their production (n=3). Carriers' morphology was viewed by transmission electron microscopy (TEM) (CM 12 Philips) and their-size and zeta potential were measured by photon correlation spectroscopy and laser doppler anemometry, respectively (Zetasizer® Nano ZS, Zen 3600) (n=3). BSA encapsulation efficiency (E.E.) was calculated comparing the non-associated protein with the total amount used by the equation:

$$E.E.(\%) = \frac{Total\ BSA\,amount - Free\,BSA\,amount}{Total\ BSA\,amount} \times 100$$

Submitting the supernatant to the MicroBCA protein assay the free BSA amount was determinated measuring the absorbances by spectrophotometry at 562 nm. A calibration curve was made using the supernatant of blank nanoparticles.

Evaluation of nanoparticles stability

A study of the nanoparticles stability in water was performed at 4 ºC. Unloaded nanoparticles were isolated by centrifugation and resuspended in water afterwards. Size were monitored, using the above mentioned techniques (n=3).

RESULTS AND DISCUSSION

Nanoparticles characterisation

The incorporation of TPP in the formulation led to two remarkable effects: an increase in the nanoparticles production yield from 20% to 30-50% was observed, together with a size reduction of about 200 nm. In fact, CS/CRG/TPP nanoparticles (Figure 1) presented a diameter of 170 – 280 nm and a zeta potential around + 50 mV, while the CS/CRG nanoparticles showed sizes above 400 nm and zeta potential around + 70 mV. These results confirm the ability of TPP to cross-link chitosan as well as its great affinity for the chitosan amino groups, thus improving the process yield.

Preliminary results show that CS/CRG/TPP nanoparticles present the ability to encapsulate BSA (E.E. of 40-50%). Release assays are presently being performed.

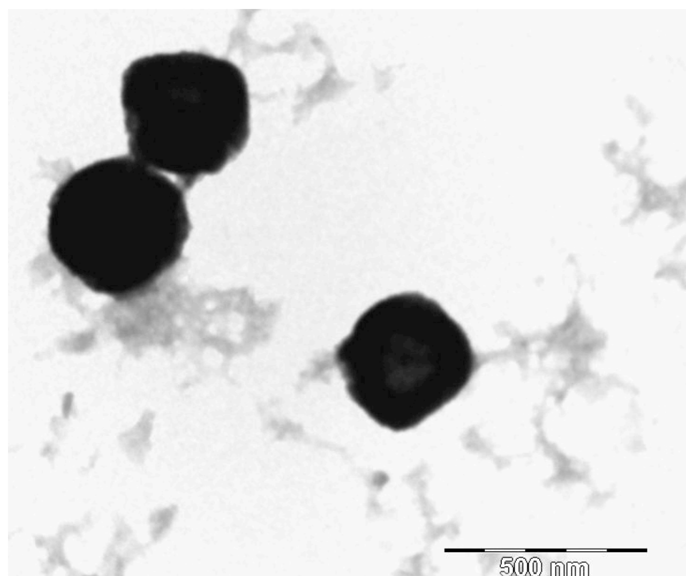

Figure 1: TEM microphotograph of representative CS/CRG/TPP nanoparticles.

Evaluation of nanoparticles stability

The stability assay revealed that, when stored at 4ºC, those nanoparticles containing TPP did not show significant size alterations for up to 6 days (Figure 2). The formulation without TPP showed a little increase until day 6 (15% against 5% only in TPP-containing formulations), although not statistically significant. Therefore, nanoparticles are considered physicochemically stable for the studied period. The monitorisation continues to be performed.

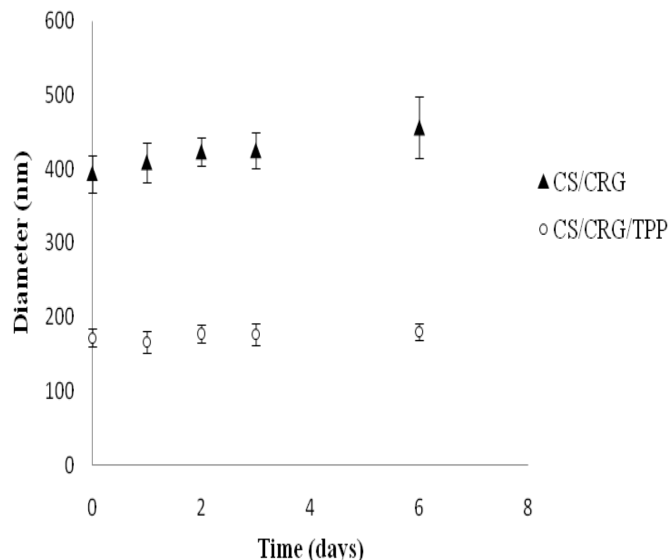

Figure 2: Monitorisation of CS/CRG (5/1) and CS/CRG/TPP (5/1/1) nanoparticles size over time

CONCLUSIONS

TPP was efficient at decreasing the nanoparticles size and increasing their production yield, thus proving its ability to cross-link the formulation. Considering the physicochemical properties, the ability to associate the protein and the demonstrated stability, CS/CRG/TPP nanoparticles are good candidates for drug delivery purposes.

REFERENCES

• Grenha *et al*. (2010) *Development of new chitosan/carrageenan nanoparticles for drug delivery applycations*. Journal of Biomedical Materials Research Part A 92A (4), 1265-1272

• Malafaya *et al*. (2007) *Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications.* Advanced Drug Delivery Reviews 59 207-233

• Reis *et al*. (2006) *Nanoencapsolation II. Biomedical applications and current status of peptide and protein nanoparticulate delivery systems*. Nanomedicine: Nanotechnology, Biology and Medicine 2 53-65

• Saboktakin *et al*. (2010) *Synthesis and characterization of superparamagnetic chitosan–dextran sulphate hydrogels as nano carriers for colon-specific drug delivery*. Carbohydrate Polymers 81(2) 372-376

ACKNOWLEDGEMENTS:

Funding from Fundação para a Ciência e Tecnologia is acknowledged (project PTDC/SAU-FCF/100291/2008).