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Novel Locust Bean Gum Nanoparticles for Protein Delivery

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INTRODUCTION AND OBJECTIVES

Effective oral delivery of many potential therapeutic macromolecules, such as proteins, is limited by the development of new carriers that enhance bioavailability and ensure stability against gastrointestinal degradation.

Polymeric nanoparticles have been presented as promising tools to successfully meet the challenge of delivering these biopharmaceutic drugs. These nanoparticles can be obtained by different methods, although the most accepted are those avoiding the toxicity of organic solvents and aggressive preparation conditions. In this context, methods involving ionic interaction offer great advantage. Moreover, nanoparticles based on natural and synthetic polymers have received great attention due to their stability and ease of surface modification, since they can be tailored to achieve both controlled drug release and cell targeting by tuning the polymer characteristics and surface chemistry. Nevertheless, natural polymers are usually preferred because they easily comply with requisites of biocompatibility, biodegradability and non-toxicity, required for biomedical applications.

Locust bean gum (LBG) is a neutral polysaccharide (galactomannan) very abundant in the Portuguese region of Algarve that has been reported as mucoadhesive (Sudhakar 2006). Its content in mannose units makes it a very attractive material to specifically target intestinal M-cells, located in the Peyer's patches, which over-express mannose receptors. In fact, the uptake of particles containing mannose residues bonded to their surface was demonstrated to be improved (Tomizawa 1993). Given the intimate contact between these cells and the underlying lymphoid tissues, LBG based nanoparticles can also act as a delivery system for oral immunization.

The objective of this work is to design a new nanometric drug delivery system, completely based on LBG. Nanoparticles are to be obtained by a simple ionic interaction method but, as LBG is devoid of charge, synthesis of positively and negatively charged LBG derivatives were carried out in order to produce pure LBG based nanoparticles.

MATERIALS AND METHODS

Synthesis and characterization of LBG derivatives

Three charged LBG derivatives, one cationic and two anionic, were synthesized by methods already used to modify other polymers, which were adapted for our purposes.

The sulphate derivative (LBGS) was obtained by reacting LBG with SO_3.DMF complex (Yuan 2005). The carboxylate derivative (LBGC) was obtained by oxidation with 2,2,6,6-tetramethylpiperidine-1-oxyl (Sierakowski 2000). The positively charged LBG derivative (LBGA), amine derivative, was obtained by alkylation with glycidyltrimethylammonium chloride (Simkovic 2009).

LBG derivatives were characterized by Fourier transform infrared spectroscopy (FTIR; Bruker, Tensor 27 FTIR spectrophotometer).

LBG nanoparticles preparation

LBG nanoparticles were assembled by ionic complexation. Briefly, 1.8 mL of various concentrations of the negatively charged LBG derivatives solutions were poured into 1.0 mL of a 0,2% solution of positively charged derivative, under magnetic stirring. Then, particles were isolated by centrifugation (16000 x g, 30 min, 15 ºC), supernatant was discarded and the pellet resuspended in water.

LBG nanoparticles characterization

Nanoparticles morphology was characterized by transmission electron microscopy (TEM; JEOL, JEM 1011). Nanoparticles' size was determined by photon correlation spectroscopy (25 ºC, detection angle of 173º) and zeta potential by laser Doppler anemometry, using a Zetasizer® (Nano ZS, Zen 3600). Measurements were performed on aqueous dilute nanoparticles suspension (n=3).

Stability assay

The stability of nanoparticles in aqueous medium was evaluated upon resuspension in water and storage at 4 ºC.

RESULTS AND DISCUSSION

Synthesis of LBG derivatives

LBG sulphate functionalization (LBGS) was confirmed by FTIR, through the appearance of a S=O asymmetric stretching band at 1255 cm^{-1}, as shown on Figure 1. Moreover, the band size suggests a high degree of substitution.

The carboxylate derivative (LBGC) was characterized by FTIR and the absorption bands at 1601 cm^{-1} and 1415

cm^{-1} are attributed to asymmetrical and symmetrical stretching vibration of $–COO^-$, respectively.

Since the quaternary ammonium groups do not display characteristic IR absorption bands, evidence for formation of the amino functionalized derivative (LBGA) comes from the broadening of the band at 1088 cm^{-1} (ether C-O symmetric stretching) and the new bands at 1480 and 914 cm^{-1} (C-H scissoring in methyl groups of the ammonium and ether C-O asymmetric stretching, respectively).

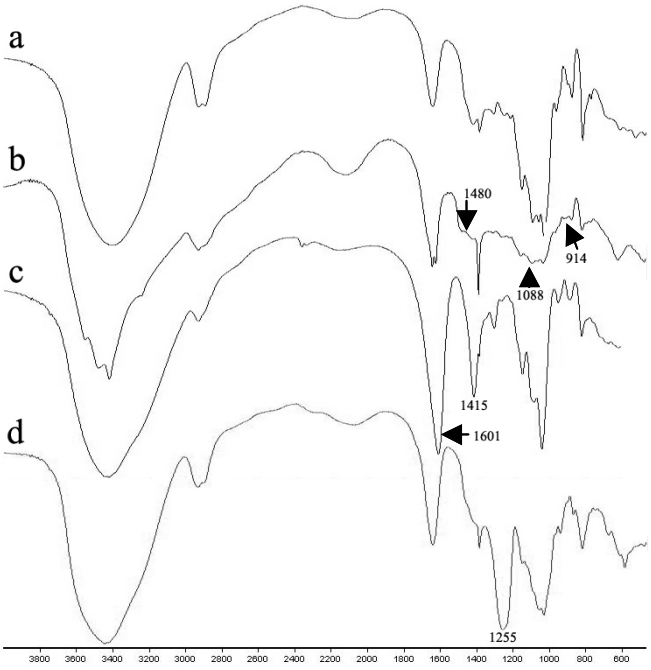

Figure 1: FTIR spectra of LBG (a), LBGA (b), LBGC (c) and LBGS (d).

LBG nanoparticles characterization

LBGA/LBGS particles were obtained with mass ratios of 1:1 and 1.5:1. TEM visualization evidenced spherical and compact structure. As displayed in Table 1, nanoparticles' sizes varied between 200 and 450 nm and they evidence negative charges around -30 mV. The negative charge of the formulation containing equal amounts of cationic and anionic derivative evidences the higher charge density of the negatively charged derivative.

Table 1: LBGA/LBGS nanoparticles characterization (mean±S.D.; n=3)

Mass ratio	Size (nm)	PI	ζ potential (mV)
1:1	215.2±17.1	0.39±0.15	-32.3±0.9
1.5:1	432.9±51.5	0.39±0,03	-26.0±3.5

As expected, when the amount of polymer composing the nanoparticles matrix increases, a corresponding increase in their size is observed. This incorporation of a higher amount of positive derivative in the formulation was accompanied by the corresponding increase of zeta potential from – 32 mV to – 26 mV.

Preliminary results demonstrate that LBGA/LBGS (1:1) nanoparticles remain stable when stored at 4 ºC for up to 48 h. Nevertheless, the monitorization is under course to allow more conclusive results.

Nanoparticles were also obtained with LBGA/LBGC derivatives and mass ratios of 1:1 and 1.5:1. Sizes varied within 110 and 480 nm and zeta potential was negative between -24 mV and -45 mV. Obtained results were very variable and therefore further characterization is needed.

CONCLUSIONS

Negatively and positively charged LBG derivatives were synthesized that allowed obtaining nanoparticles by a mild ionic interaction procedure. Developed LBG-based nanoparticles display adequate physicochemical properties for drug delivery purposes and their capacity to associate proteins is currently being performed.

REFERENCES

- Sierakowski MR. *et al*. (2000) *Specific modifications of galactomannans*. Carbohydrate Polymers 42 51-57.
- Simkovic I. *et al*. (2009) *Chemical modification of corn fiber with ion-exchanging groups*. Carbohydrate Polymers 76 250-254.
- Sudhakar Y. *et al.* (2006) *Buccal bioadhesive drug delivery - a promising option for orally less efficient drugs*. Journal of Controlled Release 114 15-40
- Tomizawa H. *et al.* (1993) *Uptake of phosphatidylserine lipossomes by rat peyer's patches following intraluminal administration*. Pharmaceutical Research 10 549-552.
- Yuan H. *et al*. (2005) *Preparation and in vitro antioxidant activity of k-carrageenan oligosaccharides and their oversulfated, acetylated, and phosphorylated derivatives*. Carbohydrate Research 340 685-692.

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