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"CHITOSAN-CYCLODEXTRIN NANOPARTICLES FOR HEPARIN ADMINISTRATION: DEVELOPMENT AND *EX-VIVO* EVALUATION FOR ASTHMA TREATMENT"

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Heparin is a macromolecule that has shown important effects in asthma, like the capacity for preventing mastocytes-degranulation and for inhibits smooth-muscle cell proliferation. Interestingly, these effects are independent of the anticoagulant activity and dependent of the molecular weight.
Chitosan (CS) is a cationic, natural, non-toxic, and biodegradable polymer that has shown to prolong the residence time of nanosystems in target sites due to their interactions with cell-membranes [1]. Carboxymethyl-β-cyclodextrin (CMβCD) is an anionic, non-toxic and biodegradable oligosaccharide that has shown to interact with CS to form nanoparticles with high capacity for associating drugs [2].
CS and different ratios of CMβCD-tripolyphosphate (TPP) including unfractioned or low molecular weight heparin (UFH and LMWH, respectively) were mixed to form nanoparticles following the ionotropic gelation method [3]. Resulting nanoparticles were between 221-729 nm and showed a positive zeta potential ranged in 32-55 mV. In selected formulations, heparin was loaded with an efficiency of ≈75%. The nanosystems maintained stable in HBSS pH 6.4 at 37℃ for 24 at least and release the loaded heparin in a slow-constant manner. TEM pictures showed the systems to be spherical (fig. 1).

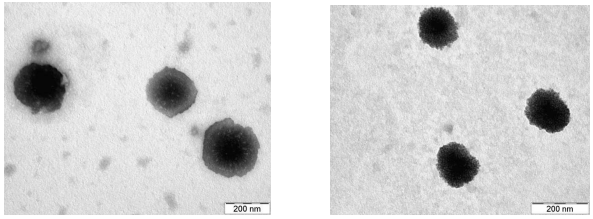

Fig. 1 TEM pictures of the systems with UFH (left) and LMWH (right).

Confocal microscopy experiments indicate that nanoparticles were homogeneously internalized in mastocytes extracted from rats.
Experiments for evaluating the capacity of heparin- loaded nanosystems in preventing degranulation in rat mast cells indicate a dose dependent effect similar to those achieved with heparin solutions (fig. 2). Importantly, a significant improve in the effect was achieved with the nanoparticles at the highest tested dose of heparin (0.2 mg/mL).

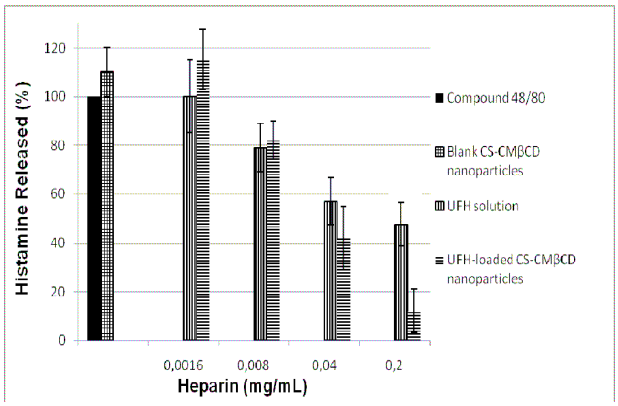

Fig. 2: Effect of heparin solutions and heparin-loaded CS-CMβCD NPs on histamine release induced by compound 48/80 in mast cells: UFH solution (vertical line bars), UFH-loaded CS-CMβCD NPs (horizontal-line bars). Compound 48/80 (black bar) and blank CS-CMβCD NPs (squared bars) (n=3, $p<0.05$).

Interestingly, the cellular viability of mastocytes after the contact with the nanoparticles was higher than 90% as confirmed by trypan-blue staning.

Our next task consists in evaluating the nanosystems in animal models of asthma.

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REFERENCES
[1] Soane, R., Frier, M., Perkins, A,. Jones, N., Davis, S., Ilum, L., *Int. J. Pharm.* **178** (1999) 55.
[2] Krauland, A., Alonso MJ., *Int. J. Pharm.* 340 (2007) 134.
[3] Calvo, P., Remuñan-Lopez, C., Vila-Jato, J., Alonso, MJ., *J. Appl. Pol. Sci.* 63 (1997) 125.