Chitosan nanogels for biomedical applications: Choosing a suitable sterilization method

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INTRODUCTION

BACKGROUND: The outbreak of nanotechnology in pharmaceutical and nanomedicine fields calls the need for developing strategies to guaranty the safety and effectiveness of nanostructured materials for treatment as well as for clinical trials progress.

Sterilization is a crucial step in the development of this type of materials[1]. Although often effective, the conventional sterilization methods may change the physico-chemical properties of the nanoparticles and their biocompatibility, compromising their functionality[1],[2].

AIM OF THE WORK: to evaluate the effect and effectiveness of different sterilization methods on a model chitosan-based nanogel.

MATERIALS AND METHODS

NANOGEL PRODUCTION (altakitin)

- Model chitosan-based hydrogel nanoparticles were prepared by **ionic gelation**, using sodium tripolyphosphate (**TPP**) as a crosslinking agent.

CHARACTERIZATION

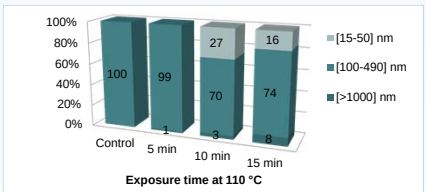
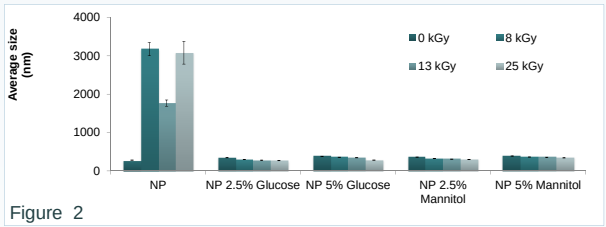
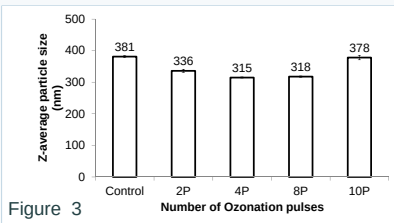
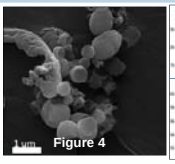
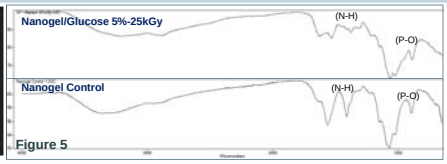
- **Zetasizer:** size, dispersion (PDI), zeta potential (ZP)
- **SEM:** size and surface morphology;
- **FTIR:** chemical composition;
- **Sterility tests**

STERILIZATION
With/Without protective sugars (Dextrose/Mannitol)

- **Autoclave** (110/120°C – 5-15min)
- **γ Irradiation** (8,13,25 kGy) + Glucose / mannitol
- **Ozone Gas** (2, 4, 8, 10 pulses)

Re-evaluation

RESULTS

	AUTOCLAVE	γ IRRADIATION	OZONE GAS
VISUAL	Loss of color and formations of large sediments	• **Without protective sugars:** aggregates and sediments • **With protective sugars:** no visual alterations	No visual alterations
EFFECT ON SIZE	 Figure 1 • **Degradation of the nanogel structure** • Very low particle count and several populations of different sizes (Fig 1).	 Figure 2 • Sterilization by **irradiation promotes aggregation** resulting in large particles agglomerates. • In the presence of both protective sugars (mannitol and glucose) the degradation effects decrease considerably (Fig 2)	 Figure 3 • No significant changes (Fig 3)
PDI/ZP	• PDI increases ↑ • ZP increases ↑	• **Without sugars:** PDI increases ↑ ZP decreases ↓ • **With sugars:** No significant alterations	• No significant changes
SEM / FTIR	• Not Tested	 Figure 4  Figure 5 • **SEM** did not reveal significant changes (Fig 4) • **FITR:** chemical alterations were observed in the presence of protective sugars. The amides and PO groups seem to be the most affected (Fig 5).	• No significant changes in SEM analysis • No significant changes in FTIR analysis
STERILITY	• Not Tested	• No bacterial growth in all tested conditions ✓	• ≤ 4 ozonation pulses: bacterial growth ✗ • 8 ozonation pulses: low bioburden ± • 10 ozonation pulses: no bacterial growth ✓

CONCLUSIONS

The nanogel severely degrades by autoclaving. Concerning gamma irradiation, the nanogel resistance increases considerably in the presence of protective sugars. Regarding ozone sterilization the nanogel seems to withstand the method without displaying significant physical adverse effects, although the method is not as effective as gamma irradiation.

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