P-001 **Comparative cytotoxicity study between spray-dried and cross-linked xylan/Eudragit® S100 microparticles with usnic acid**

Silva AE[1], Gomes MCS[1], Marcelino HR[1], Oliveira EE[2], Nagashima Jr T[3], Silva AE[4], Agnez-Lima FL[4], Magalhaes NSS[5] and Egito EST[1*#]
[1]LASID, UFRN, Natal-RN, Brazil, [2]UEPB, Joao Pessoa-PB, Brazil, [3]UFCG, Cuite-PB, Brazil, [4]LBMG, UFRN, Natal-RN, Brazil, [5]LIKA, UFPE, Recife-PE, Brazil.
* Supervisor # socrates@ufrnet.br

INTRODUCTION AND OBJECTIVES

Ideal drug delivery systems are supposed to target and release the active molecule in the right body compartment at a desired rate for a specific disease (Anal 2005). One of the strategies for developing those systems is the microencapsulation, which is defined as a technology of packaging solids, liquids or gaseous materials into microcarriers that are capable of releasing their contents at controlled rates under specific conditions (Anal 2007).

Depending on the formulation and the application of the microparticulate drug carriers, several approaches have been used for their production, such as coacervation, emulsion solvent evaporation, spray-drying and interfacial cross-linking polymerization (Ye 2010). Briefly, the spray-drying method consists of the atomization of a liquid feed into a spray under hot air contact followed by the drying stage initiated by heat transfer. Afterwards, the dried particles are collected (Sancin 1999). On the other hand, interfacial cross-linking polymerization is a usual and long-established technique that requires the use of organic solvents and cross-linking agents, which may include toxic and harmful reagents (Li 2009). In fact, not only efficacy but also the safety is a key feature of drug delivery systems.

In this work, usnic acid-loaded microparticles prepared by means of spray-drying and interfacial cross-linking polymerization were compared regarding their toxicity when in contact with human cells.

MATERIAL AND METHODS

Preparation of usnic acid-loaded xylan/Eudragit® S100 microparticles

Spray-dried xylan/Eudragit® microparticles containing usnic acid (SDM) were produced by the solubilization of xylan, Eudragit® S100 and usnic acid in the weight ratio of 10:30:1, respectively, in 50 mL of 0.6N NaOH solution. Afterwards, this solution was spray-dried at the feed rate of 1.2 mL/min (inlet temperature of 120ºC) using a Büchi Model 191 laboratory spray-dryer with a 0.7 mm nozzle.

Preparation of usnic acid-loaded cross-linked xylan/Eudragit® S100 microparticles

Cross-linked xylan microparticles containing usnic acid (CLM) were prepared by the following three steps: a) emulsification of an alkaline solution of xylan and usnic acid in 1:4 (v/v) chloroform:cyclohexane containing sorbitan triesterate; b) cross-linking reaction by adding a 5% (w/v) terephthaloyl chloride solution; and c) separation of the microcapsules by centrifugation and several washing steps with polysorbate (HLB=18.85) in ethanol, after with ethanol, and finally with water. Eudragit® S100 was added to coat the microparticles by spray-drying in the same weight ratio previously mentioned.

Cytotoxicity assay

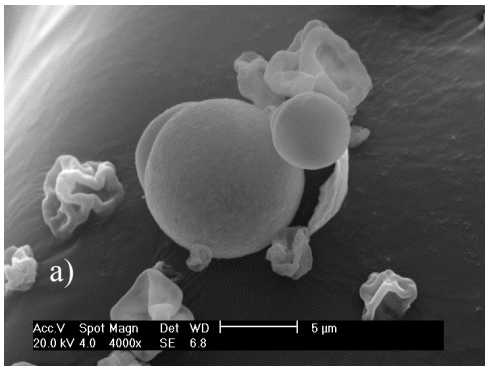
In order to assess the effect of the microparticles on the cell viability and proliferation rate, human embryonic lung fibroblasts (MRC-5 cells) were used. Cells (5×10^3/well) were seeded into a 96-well culture plate and incubated overnight. Afterwards, they were incubated with SDM and CLM dispersions at concentrations of 50, 125, 250 and 500 µg/ml for 24 h. Cell viability was determined by the MTT assay.

RESULTS AND DISCUSSION

Both spray-drying and interfacial cross-linking polymerization produced yellowish powders with yields of 82.15% and 88.08%, respectively.

According to the scanning electronic microscopy analysis (SEM), SDM and CLM were presented to be concave to spherical in shape (Figure 1a and 1b).

The mean particle sizes were presented to be 11.2 ± 1.9 µm and 10.1 ± 3.2 µm for SDM and CLM, respectively.



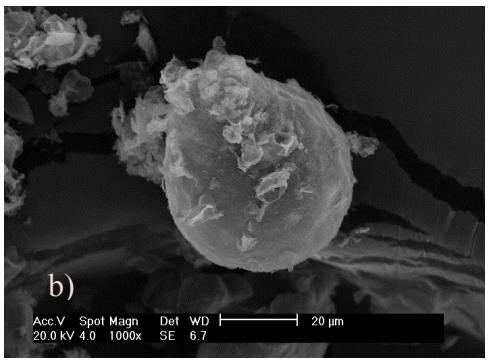


Figure 1. SEM images of a) SDM and b) CLM, at 4000 x and 1000 x magnification, respectively.

The mean particle sizes were presented to be 11.2 ± 1.9 µm and 10.1 ± 3.2 µm for SDM and CLM, respectively.

According to the MTT assay results, the cells treated with CLM presented an initial decrease in the cell viability of 56% at the lowest tested concentration (50 µg/mL) while the cell viability rate reached only 12.6% at the highest concentration (500 µg/mL) (Figure 2).

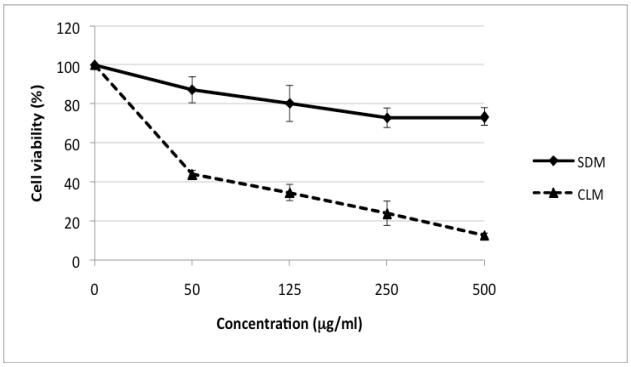


Figure 2. Effects of SDM and CLM in the survival rate of MRC-5 cells.

Nevertheless, SDM showed a maximum decrease in the cell survival rate of approximately 12% and 27% at the lowest and highest concentrations of microparticles, respectively (Figure 2).

The massive cytotoxicity induced by CLM may be explained by the presence of remaining molecules of terephthaloyl chloride, which plays the role of cross-linking agent during the formation of CLM and it is well-known as a toxic substance.

In contrast, the MTT assay for SDM did not show high cytotoxicity. This fact confirms the advantage of using spray-drying in order to avoid toxic and hazardous reagents such as terephthaloyl chloride and other cross-linking agents.

Additionally, such results indicate a relevant biocompatibility of spray-dried xylan/Eudragit® S100 microparticles containing usnic acid.

CONCLUSION

Both spray-drying technique and interfacial cross-linking polymerization presented to be feasible microencapsulation methods for producing usnic acid-loaded xylan/Eudragit® S100 microparticles. However, the latter demonstrated to produce highly cytotoxic systems.

Additionally, the spray-drying technique presents other advantages, such as the lack of organic and toxic solvents, the use of low-cost reagents, a single step process and ease of operation, which make it an eligible methodology for producing a controlled release system for usnic acid by using xylan and Eudragit® S100.

REFERENCES

- Anal A. K., et al. (2005). *Chitosan-alginate multilayer beads for controlled release of ampicillin.* International Journal of Pharmaceutics. (1-2). 45-54.
- Anal A. K., et al. (2007). *Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery.* Trends in Food Science & Technology. (5). 240-251.
- Ye M., et al. (2010). *Issues in long-term protein delivery using biodegradable microparticles.* Journal of Controlled Release. (2). 241-260.
- Sancin P., et al. (1999). *Effects of ultrasound-assisted compaction on Ketoprofen/Eudragit® S100 Mixtures.* European Journal of Pharmaceutical Sciences. (3). 207-213.
- Li B.-z., et al. (2009). *Physical properties and loading capacity of starch-based microparticles crosslinked with trisodium trimetaphosphate.* Journal of Food Engineering. (3). 255-260.