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Freeze-drying protocol for liposomes co-encapsulating fisetin and cisplatin

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In order to fight against glioblastoma, the antiangiogenic fisetin and the anticarcinogenic cisplatin were co-encapsulated in liposomes (75.3%mol of DOPC, 20.8%mol of cholesterol and 3.9%mol of DODA-Gly-PEG ; 8.5 mg of cisplatin/g of lipids and 15.5 mg of fistein/g of lipids). However, its stability needed to be improved. Indeed, 74.4 ± 0.7% of the fisetin and 35.2 ± 3.1% of cisplatin were released after 10 days. TEM highlighted a disruption of the liposomal bilayers (figure 1) explaining that the liposomal suspension was not stable, and that the release of the two drugs was higher when co- encapsulated than when alone in the liposomes. Freeze-drying is a process used to ensure the long-term stability of liposomes. The present study investigated the protective capacity of two lyoprotectants, sucrose and trehalose, at 3 concentrations, 5, 10 and 20%, during the freeze-drying of empty liposomes (75.3%mol of DOPC, 20.8%mol of cholesterol and 3.9%mol of DODA-Gly-PEG). Among these, only a 20% concentration of lyoprotectant could prevent the macroscopically aggregation of liposomes. Trehalose 20% could not prevent some aggregation and an increase in size and PDI. Sucrose 20% had the best protective effect concerning size (154.7 ± 1.3nm before freeze-drying and 155.5 ± 2.2nm after rehydration, NS) and PDI (0.094 ± 0.023 before freeze-drying and 0.121 ± 0.019 after rehydration, $p < 0.05$) of empty liposomes (figure 2). Sucrose 20% was chosen for further investigation: water content, freezing protocol and encapsulation of cisplatin and fisetin after freeze-drying and rehydration.

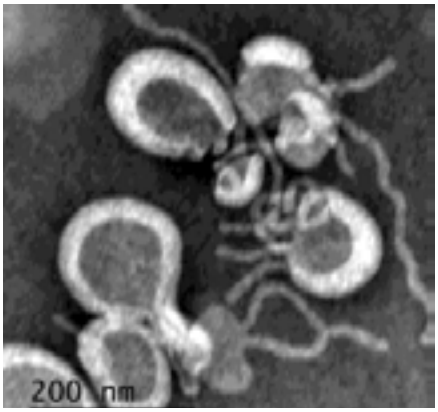

Figure 1 - Transmission electron microscopy photographs of fisetin and cisplatin-loaded liposomes at day 3

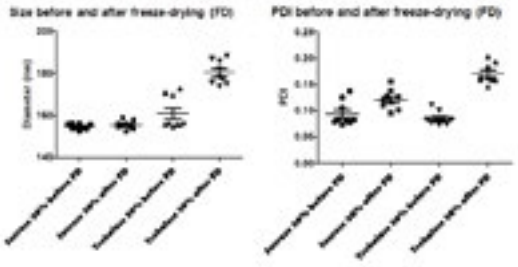

Figure 2 – Effect of sucrose 20% and trehalose 20% as lyoprotectants during freeze-drying of empty liposomes

Biography

Morgane Renault-Mahieux is a 26 years old PhD Scholar in pharmaceutical technologies at the Paris Descartes University, France.

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