EVALUATION OF LACTOFERRIN LOADED LIPOSOMES IN GASTRIC AND INTESTINAL SIMULATED MEDIA

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1. Introduction

Freeze dried Lactoferrin liposomes is a lactoferrin loaded formulation of liposomes within the nanometric range. In this study, physicochemical parameters of liposomal formulations have been elucidated in order to confirm that gastric and intestinal media do not damage the formulations**.**

2. Materials and methods

2.1 Physicochemical characterization

For this test, a solution of the product *Liposome lyophilizate* with a concentration of 1mg/ml has been made.
This solution is transferred to a cuvette and the average size (Z_{av}), polydispersity index (PI) are analysed by dynamic light scattering on a Zeta Sizer (NanoZS, Malvern Instruments) (E Sánchez-López et al., 2018). Zeta potential (ZP) is analysed using electrophoresis laser-doppler in a Zeta Sizer (NanoZS, Malvern Instruments).

2.2. Preparation of *in vitro* intestinal drug release

Preparation of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) was carried out as reported elsewhere (Singh & Sarkar, 2011). In order to elucidate the effect of the enzymes, pepsin will be added to SGF and pancreatin will be added to SIF (concentrations of 0.032 mg/ml).
For this test the samples were incubated in a water bath at 37 ºC. The samples were diluted in the corresponding media of SGF and SIF with and without enzymes (pepsin or pancreatin, respectively) and incubated for 2 hours under magnetic stirring.

At different timepoints (0, 15, 30, 60, 120 min) 1 ml of samples will be extracted and Z_{av}, PI and ZP will be analysed (Liu, Ye, Liu, Liu, & Singh, 2013).

3. Results and discussion

3.1 Physicochemical characterization

Physicochemical characterization of liposomal formulations has been carried out and can be observed in Table 1. Liposomes show and average size below 200 nm and a PI below 0.2. In addition, the formulation show a negative surface charge which may indicate a suitable stability of the formulation (Elena Sánchez-López et al., 2020).

Table 1. Physicochemical characterization of *Liposoma liofilittzato*

Z_{av} (nm)	PI	ZP (mV)
192.9± 4.5	0.181 ± 0.02	– 40 ± 0.5

3.2 Physicochemical behaviour in in vitro intestinal and gastric fluids

Liposomal formulations were dispersed in SIF and SIG with and without enzymes and results can be observed in Table 2.

Table 2. Physicochemical characterization of Lactoferrin liposomes dispersed in SGF with Pepsin at different timepoints.

Release media	Time (min)	Z_{av} (nm)	PI	ZP (mV)
SGF with pepsin	0	260.5 ± 5.9	0.402 ± 0.021	17.1 ± 1.31
	15	311.3 ± 11.6	0.326 ± 0.066	11.2 ± 0.45
	30	266 ± 4.2	0.227 ± 0.017	11 ± 1.58
	60	278.4 ± 37.3	0.360 ± 0.031	11.3 ± 0.57
	120	236.8 ± 7.5	0.397 ± 0.025	10.9 ± 1.54
SGF	0	365.6 ± 60.0	0.412 ± 0.068	22.2 ± 0.27
	15	530.3 ± 26.9	0.531 ± 0.016	22.6 ± 3.36
	30	395.3 ± 96.2	0.519 ± 0.175	16.6 ± 0.49
	60	348.3 ± 18.6	0.371 ± 0.014	20.2 ± 0.75
	120	570.8 ± 26.5	0.508 ± 0.081	21.6 ± 1.31
SIF with pancreatin	0	327.5 ± 12.9	0.455 ± 0.081	-16.8 ± 0.55
	15	318.2 ± 63.0	0.366 ± 0.055	-17.7 ± 1.16
	30	227.1 ± 2.8	0.401 ± 0.051	-14.4 ± 4.45
	60	255.6 ± 14.4	0.352 ± 0.053	-12.3 ± 0.21
	120	262.0 ± 22.4	0.358 ± 0.021	-19.8 ± 1.86
SIF	0	964.0 ± 130.1	0.785 ± 0.120	-18.9 ±1.21
	15	550.5 ± 89.4	0.532 ± 0.081	-15.8 ± 0.89
	30	612.9 ± 194.0	0.580 ± 0.077	-20.9 ± 1.63
	60	317.0 ± 24.1	0.369 ± 0.038	-17.8 ± 1.40
	120	326.9 ± 24.4	0.383 ± 0.042	-17.3 ± 1.31

In all the cases, liposomes increase their size when dispersed in all the media as well as their PI. In addition, surface charge also seems to be shifted from negative to positive only in SGF. In SIF the surface charge is reduced to more neutral values but does not present a shift.

Regarding liposomal characteristics regarding time, in both cases the addition of enzymes to the media seems to stabilize the liposomes avoiding abrupt modifications. In the media prepared with enzymes, liposomal samples are below 400 nm.

4. Conclusions

Liposomal contact with intestinal and gastric delivery medium prepared *in vitro*, increases their size and PI. However, the addition of enzymes, which reproduces more accurately the in vivo behaviour, favours the preservation of physicochemical characteristics over time.

5. References

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