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In vitro evaluation of leuprolide-containing solid lipid-based nanosuspensions: ability to encapsulate, release, protect and permeate in the gastrointestinal tract

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PURPOSE

Peptides are interesting therapeutic molecules with specific and potent action over a wide variety of diseases. Unfortunately, oral bioavailability of peptides is strongly limited by **their proteolytic degradation** in the duodenal lumen as well as their **poor permeability across the intestinal epithelium** [1].

In this study, we considered encapsulation of peptides in biodegradable and biocompatible **Nanostructured Lipid Carriers (NLC)** and **Solid Lipid Nanoparticles (SLN)** to overcome both oral bioavailability limiting aspects.

OBJECTIVES

- To encapsulate Leuprolide (LEU), a hydrophilic model peptide in NLC and SLN
- To evaluate nanoparticle capacity to protect LEU from proteolytic degradation
- To evaluate nanoparticle ability to increase LEU intestinal permeability

METHODS

Formation of Hydrophobic Ion Pair (HIP)

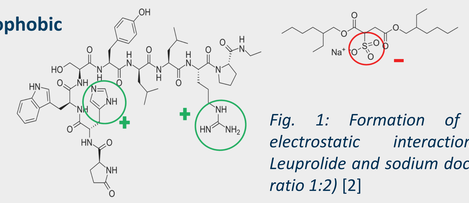


Fig. 1: Formation of a HIP by electrostatic interaction between Leuprolide and sodium docusate (molar ratio 1:2) [2]

Precipitation efficiency (**PE**, %) $= 100 - \left(100 * \frac{\text{Leuprolide concentration before HIP}}{\text{Leuprolide concentration after HIP}}\right)$ total amount of drug

Nanoparticles formulation:

SLN composed of Precirol®ATO5 (Glyceryl distearate) and Kolliphor®RH40 (PEG-40 hydrogenated castor oil) were obtained by hot High-Pressure Homogenization (HPH). Capryol®90 (propylene glycol monocaprylate) was added in the case of NLC.

Encapsulation Efficiency (**EE**, %) $= \frac{\text{Total amount of drug} - \text{unencapsulated amount of drug}}{\text{total amount of drug}} * 100$

Physico-chemical characterizations:

Particle size was determined by Diffraction Light Scattering (DLS) and confirmed by Cryo-TEM measurements. PE and EE were measured by HPLC.

Enzymatic degradation by trypsin:

Trypsin solution (0.3 mg/mL) was added to samples ([LEU]=100 µg/mL) at 37°C. Enzymatic activity was stopped at predetermined time points. Remaining LEU was analyzed by HPLC.

Permeability evaluation across intestinal cell models:

SLN and NLC loaded with HIP and DiD (lipophilic dye) were used. Interactions with Caco-2 and Caco-2:HT29-MTX (3:1) monolayers were observed by confocal microscopy. Internalization by Caco-2 cells was evaluated by flow cytometry. Transport studies across Caco-2 and Caco-2/HT29-MTX monolayers were conducted in HBSS. Transported LEU was quantified via ELISA and the apparent permeability (P_{app}) was calculated : P_{app} (cm.s^{-1}) $= \text{Transport rate} * \frac{1}{\text{Surface area x initial LEU concentration}}$

LEU release from SLN and NLC was studied in HBSS over 6 hours. LEU was quantified by HPLC.

RESULTS

Nanoparticles characterization

Table 1: Size distribution of NLC and SLN systems

	Blank NLC	NLC-LEU	NLC-HIP	Blank SLN	SLN-LEU	SLN-HIP
Z-average (nm)	114 ± 11	113 ± 1	125 ± 2	119 ± 4	124 ± 1	127 ± 1
PDI	0.2	0.2	0.2	0.2	0.2	0.2

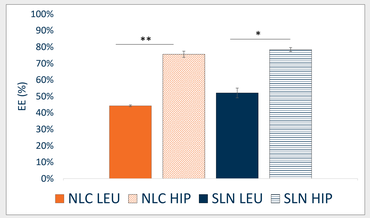


***Fig. 2**: HIP and LEU EE in NLC and SLN (* p<0.05, ** p<0.01) (mean ± SEM, n=3)*

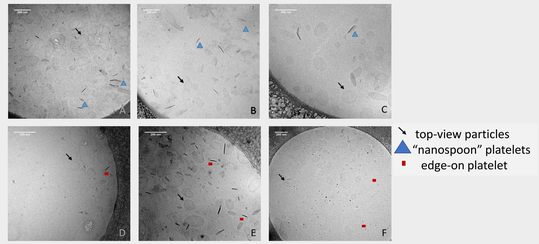


***Fig. 3:** Cryo-TEM images of blank NLC (A), NLC-LEU (B), NLC-HIP (C), blank SLN (D), SLN-LEU (E) and SLN-HIP (F).*

PE was 99.9% (n=110).
HIP formation enabled significant increase in EE. Reproducible NLC and SLN were obtained by HPH. The nanoparticles were platelet-shaped with a Z-average around 115 nm and a PDI of 0.2.

Protection from Trypsin degradation

Trypsin is not soluble in Capryol®90 [3]. Consequently, the addition of a C90 in NLC led to significant protection of LEU over trypsin degradation.

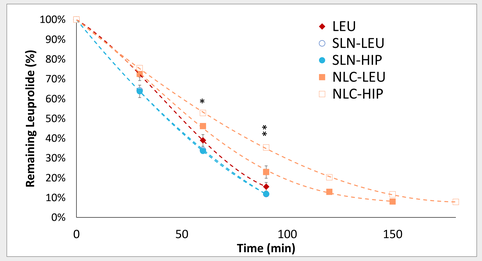
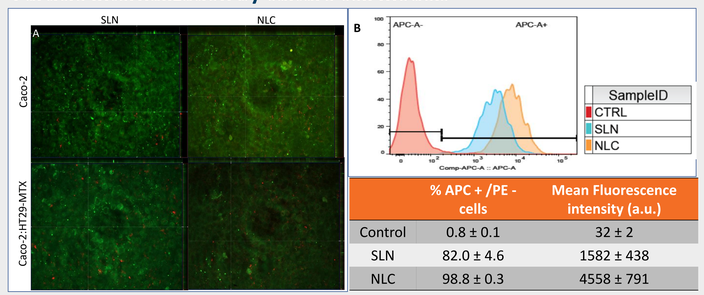


***Fig 4:** Degradation profile of encapsulated LEU compared to free LEU in presence of trypsin. (*p<0.05 and **p<0.01 when compared to suspensions dispersed in water) (mean ± SEM (n=3))*

Particle internalization by culture cell models



	% APC + /PE - cells	Mean Fluorescence intensity (a.u.)
Control	0.8 ± 0.1	32 ± 2
SLN	82.0 ± 4.6	1582 ± 438
NLC	98.8 ± 0.3	4558 ± 791

***Fig 5**: (A) confocal microscopy images of SLN and NLC interactions with Caco-2 and Caco-2:HT29-MTX, x25 (B) Flow cytometry quantitative interaction study of Caco-2 cells (n=10, N=3, mean ± SEM)*

Confocal microscopy images show SLN and NLC internalization by Caco-2 and Caco-2/HT29-MTX co-cultured cell monolayers, enlighting the ability of nanoparticles to cross mucus. Flow cytometry confirmed high particle uptake by Caco-2.

Permeability evaluation

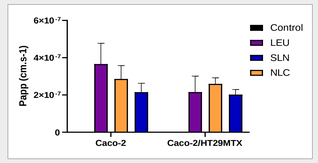


***Fig 6**: P_{app} of neat and encapsulated LEU on Caco-2 and Caco-2/HT29-MTX cell monolayers (n=3, N=3, data expressed as mean± SEM)*

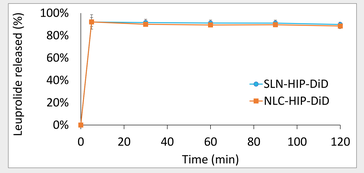


***Fig 7**: In-vitro release profile of LEU in HBSS at 37°C (data expressed as mean ± SEM, n=3)*

No improvement of LEU permeability was observed.
Indeed, the platelet-like structure of the particles, implying a large surface of exchange with the medium induced a high burst released of LEU from the nanoparticles [4]. Moreover, the HIP is not stable in this high ionic strength environment (150 mM) [5].

CONCLUSION(S)

- **Reproducible and monodisperse SLN and NLC were obtained by High Pressure Homogenization, a scalable and solvent-free method**
- **Leuprolide was successfully encapsulated in the nanoparticles**
- **Increasing peptide lipophilicity by Hydrophobic Ion Pair formation improved leuprolide encapsulation efficiency**
- **NLC provided a significant protection to trypsin-induced proteolytic degradation**
- **SLN and NLC were internalized by Caco-2 and Caco-2/HT29-MTX cell monolayers**
- **Important drug release in HBSS impeded permeability enhancement by encapsulation**
- **Stability of Leuprolide HIP needs to be improved to withstand biorelevant conditions**

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