Evaluation of in vivo anti-inflammatory and analgesic effects of N-palmitoylethanolamide (PEA) loaded nanostructured lipid carriers (NLC)

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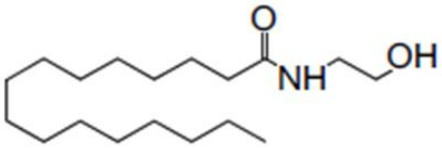
N-palmitoylethanolamide (PEA): what is it?

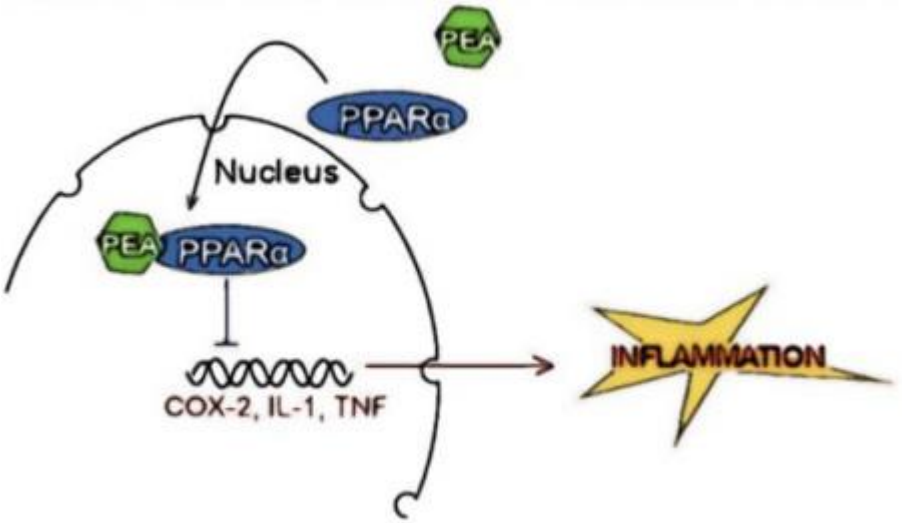
- Chemically it is the amide of palmitic acid and ethanolamine;



- It is one of the most investigated molecule belonging to the fatty acid esters (FAEs) family;
- It is a natural molecule presents in soy seeds and other vegetables

N-palmitoylethanolamide (PEA): therapeutic activi

- widely studied for its analgesic and anti-inflammatory effects, that seem mainly related to peroxisome proliferator-activated receptor α (PPAR-α) modulation[1]



[1]*From PAIN 153 (2012) 3–4*

N-palmitoylethanolamide (PEA): therapeutic activi

- When administered as a topical formulation to inflamed mouse skin, PEA inhibits inflammation and induces the expression of PPAR-α mRNA;

- Many skin diseases, such as acne, seborrhea, allergic dermatitis, itch and pain, psoriasis and hair growth disorders, might benefit from topical treatments with PEA and/or other FAEs[1].

[1]*From Petrosino S. et al. Allergy 65 (2010) 698–711.*

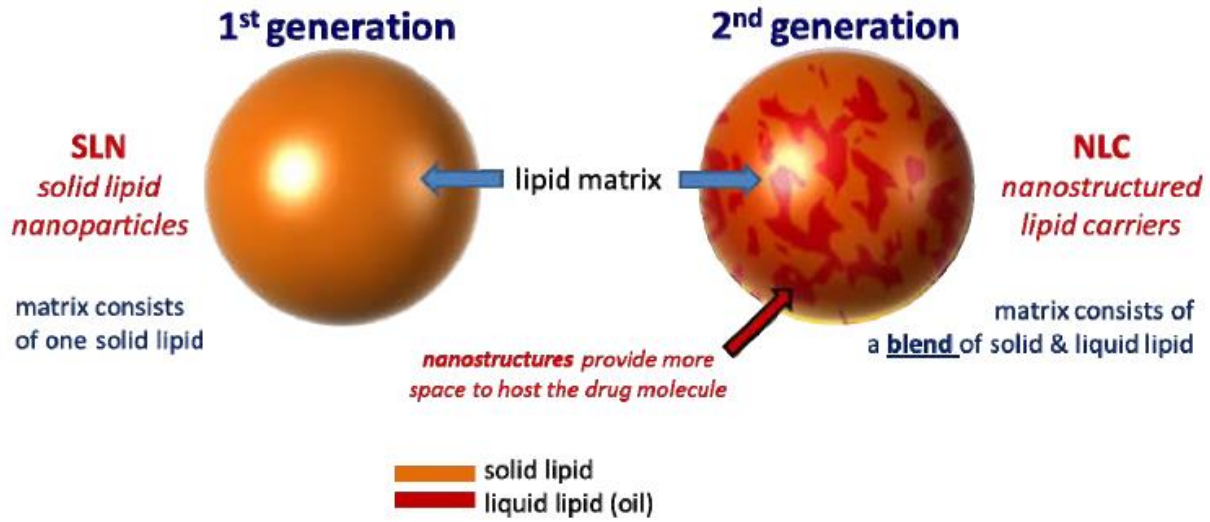
N-palmitoylethanolamide (PEA): drawbacks

- It belongs to the class of «poorly water soluble drugs» (PWSD)
- Limited penetration through the stratum corneum
- Difficulty in controlling and/or prolonging the absorption

Strategies to optimize PEA percutaneous absorpt

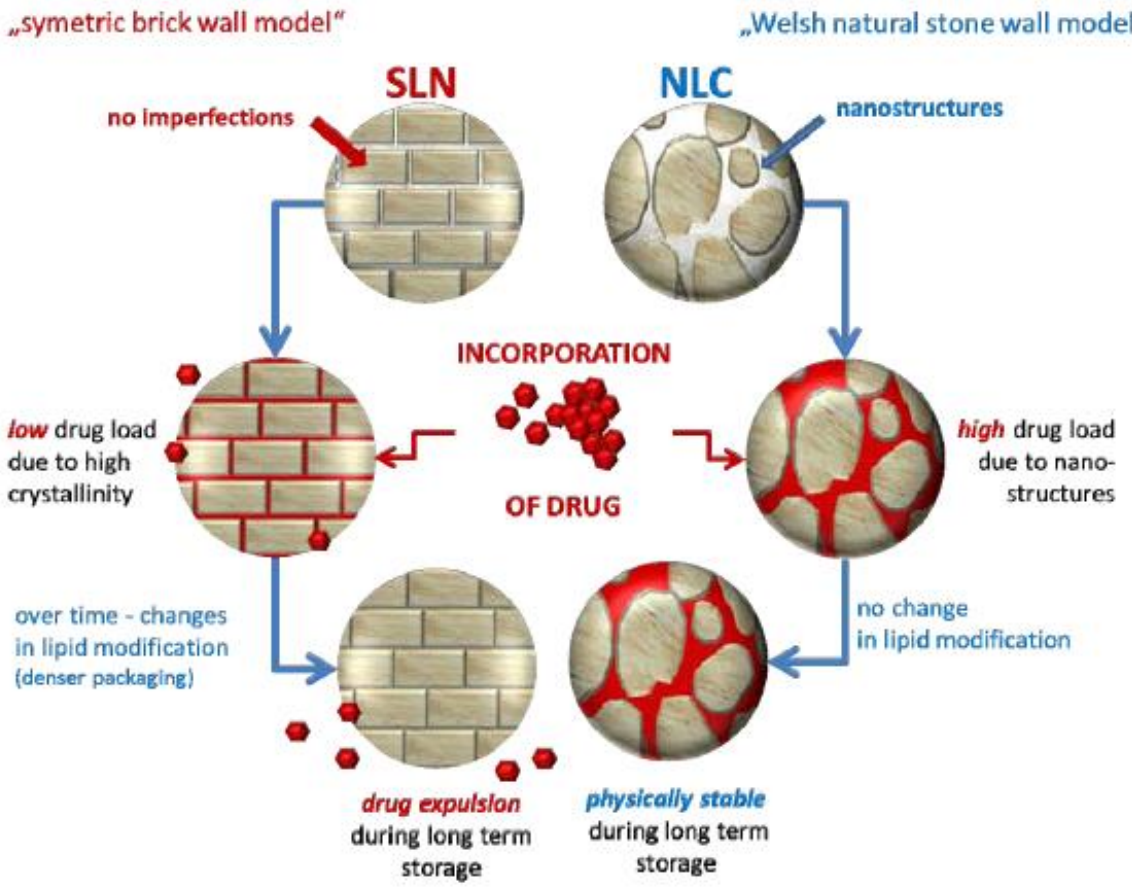
- Prodrug approach and derivatization
- Formulation of nanocarriers

Nanostructured lipid carriers (NLC): the second generation of lipid nanoparticles



From Müller RH et al., Curr Drug Discov Technol. 2011; 8(3):207-227.

Nanostructured lipid carriers (NLC): the second generation of lipid nanoparticles



From Müller RH et al., Curr Drug Discov Technol. 2011; 8(3):207-227.

Nanostructured lipid carriers (NLC): main features for topical application

- high biocompatibility
- good physical stability
- high occlusive effect on the skin
- improve re-epithelialization
- forming a reservoir on the skin surface, control and/or prolong the release of the active compound

From Puglia C, Bonina F. Expert Opin Drug Deliv. 2012 ;9(4):429-41.

Aim of the work

- Formulation of PEA loaded NLC
- Characterization for mean size, zeta-potential, morphology and internal structure
- In vitro percutaneous absorption study
- In vivo evaluation of anti-inflammatory an analgesic effects in a murine model

Experimental procedure: NLC formulation

PEA

Miglyol 812
(**oil phase**: Caprylic/Capric Triglyceride)

85°C
under stirring

Compritol 888 ATO
(**solid lipid**: Glyceryl dibehenate and glyceryl behenate)

85°C
under stirring

Water solution + Lutrol F68
(**surfactant**: Poloxamer 188)

85°C
under stirring

Ultraturrax:
10 min
13500 rpm

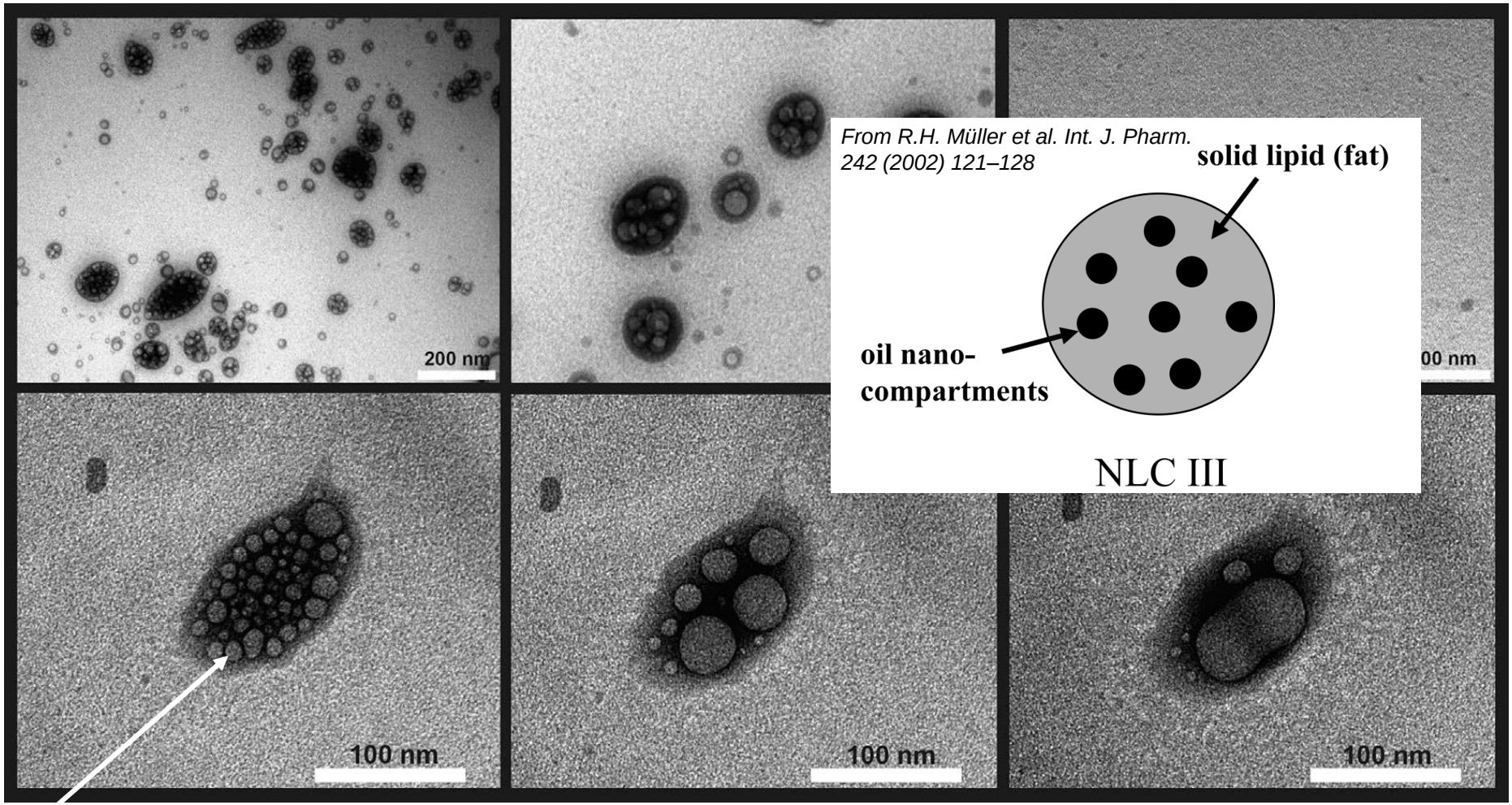
Cooling
(4°C)

NLC

Results: NLC characterization

Sample	Mean dimension (nm)	PDI	Zeta Potential (mV)
Empty NLC	155,7 ± 9,2	0,234	- 42,1
PEA-NLC	149,5 ± 7,1	0,175	- 39,1

Results: NLC characterization



Oil nanocompartments typical of type 3 NLC

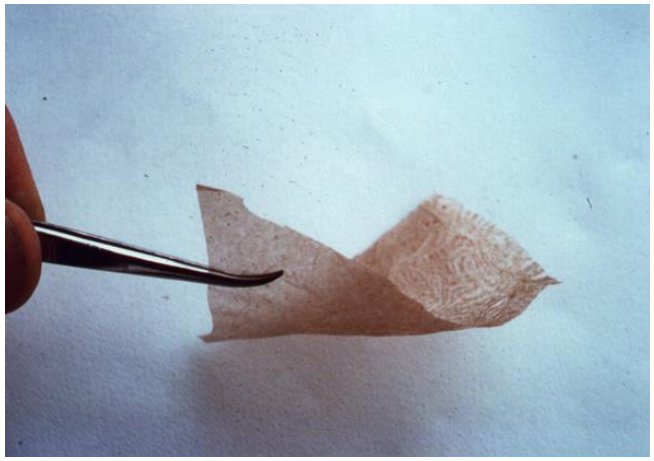
Experimental Procedure: Percutaneous absorption studies

batch	excipients		
NLC PEA NLC PEA OUT	xantan gum	glycerol	
	1%	10%	
PEA hydrogel	xantan gum	glycerol	water
	1%	10%	89 ml
PEA hydro-alcoholic gel	carbopol	ethanol	water
	1%	20 ml	80 ml

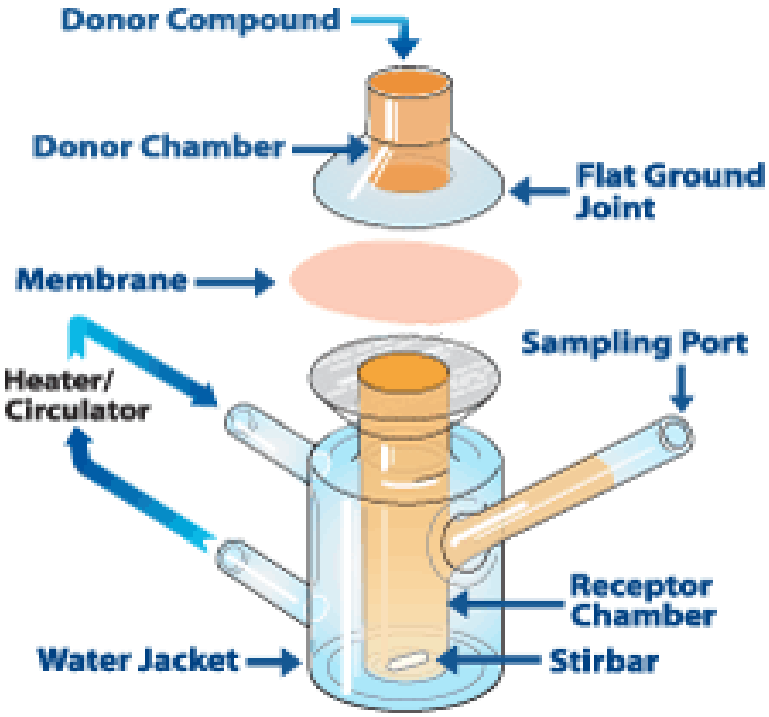
In all the formulations, PEA final concentration was 0.5% (w/v)

Experimental Procedure: Percutaneous absorption studies

SCE membrane

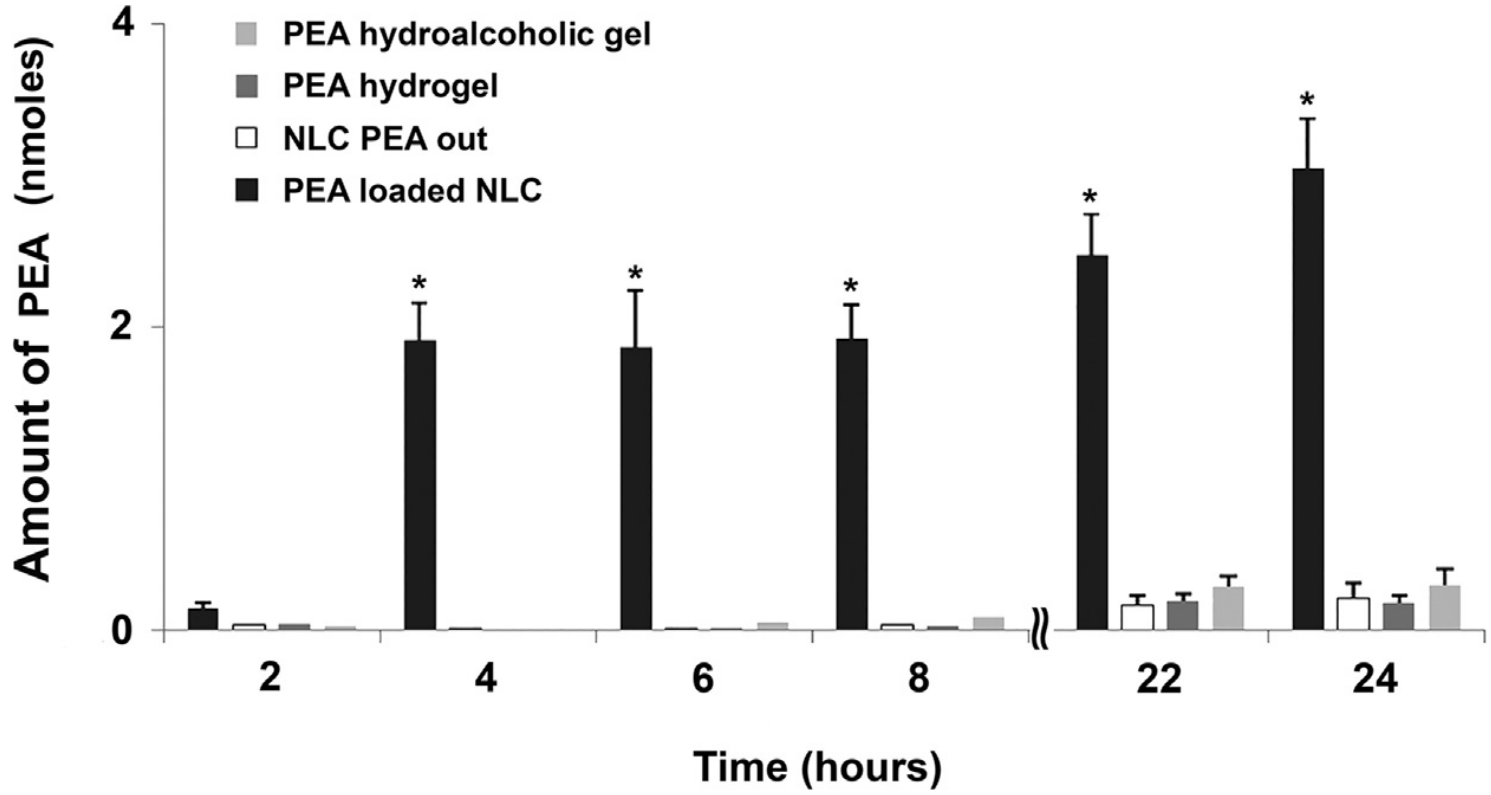


Franz Cell



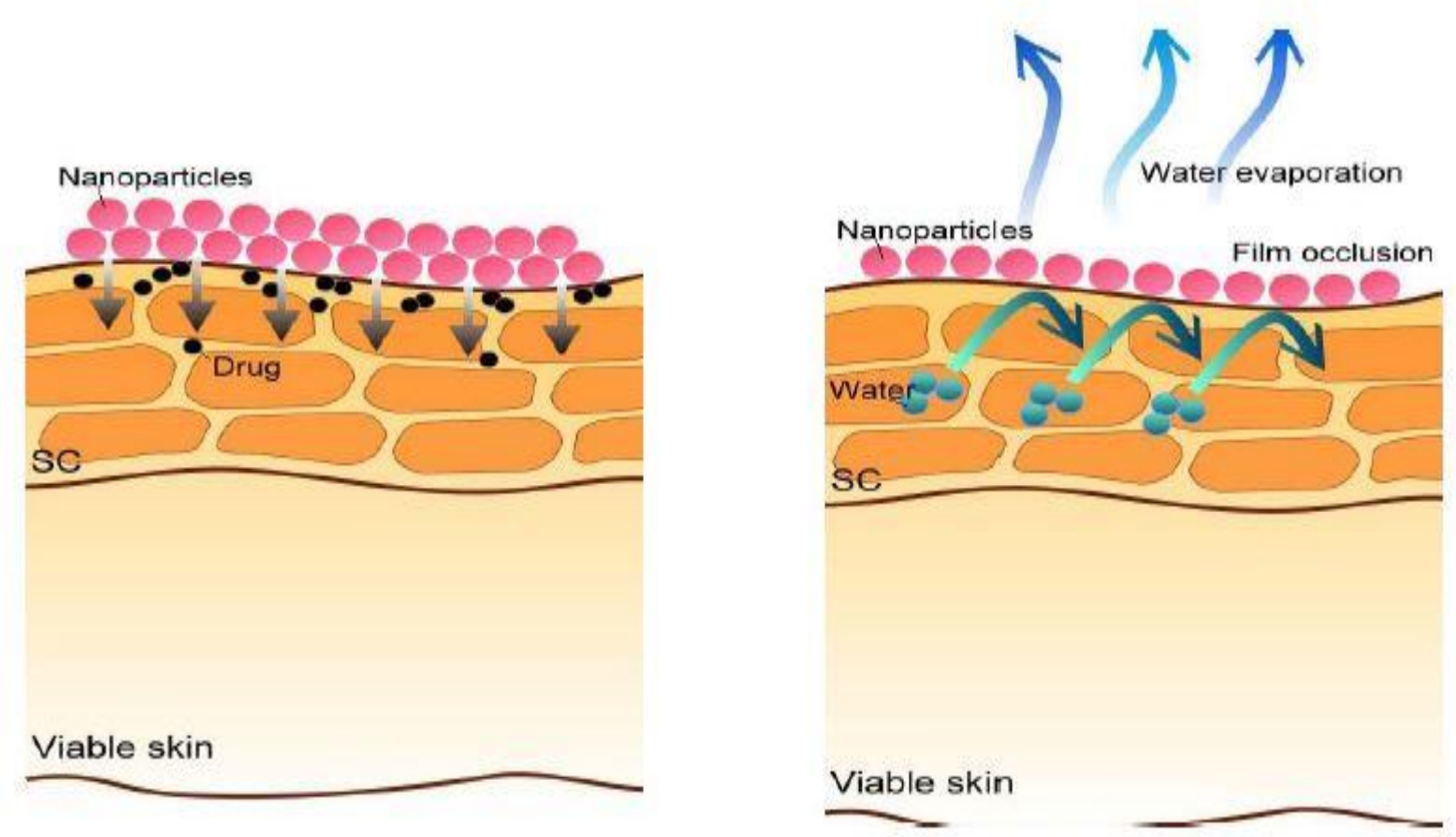
- SCE: stratum corneum/epidermis membrane
- Donor: 300 mg of each formulation
- Amount withdrawn during 24 hours: 200 µl
- Receiving compartment was filled with ethanol/water (50/50 v/v)

Results: Percutaneous absorption studies



Data from PEA in vitro percutaneous absorption through SCE membrane

Results: Percutaneous absorption studies



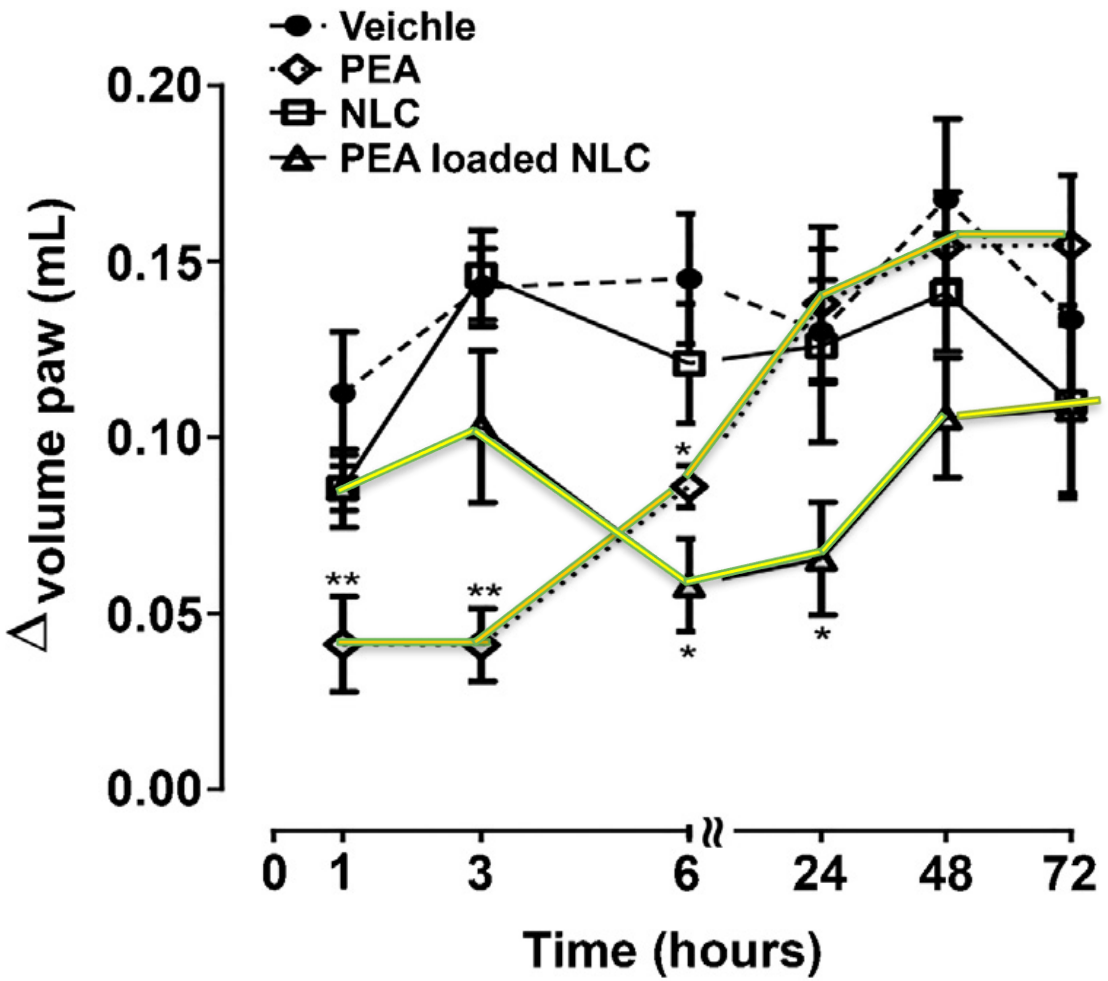
The occlusion effect due to the film formation after NLC application on skin surface lead to a skin hydration. This will facilitate the percutaneous absorption and drug penetration.

Experimental procedure: in vivo studies

- Mice received, by sub plantar injection, 0.5 µL of sterile saline containing 1% (w/v) of ʎ carrageenan into the left paws.
- After 30 min, mice received (in the same paw) saline, PEA (0.5% w/v) in saline, blank NLC, and PEA loaded NLC (0.5% w/v).
- At 1, 3, 6, 24, 48, and 72 h following the treatment, paw oedema and mechanical hyperalgesia were evaluated.

Results: in vivo studies

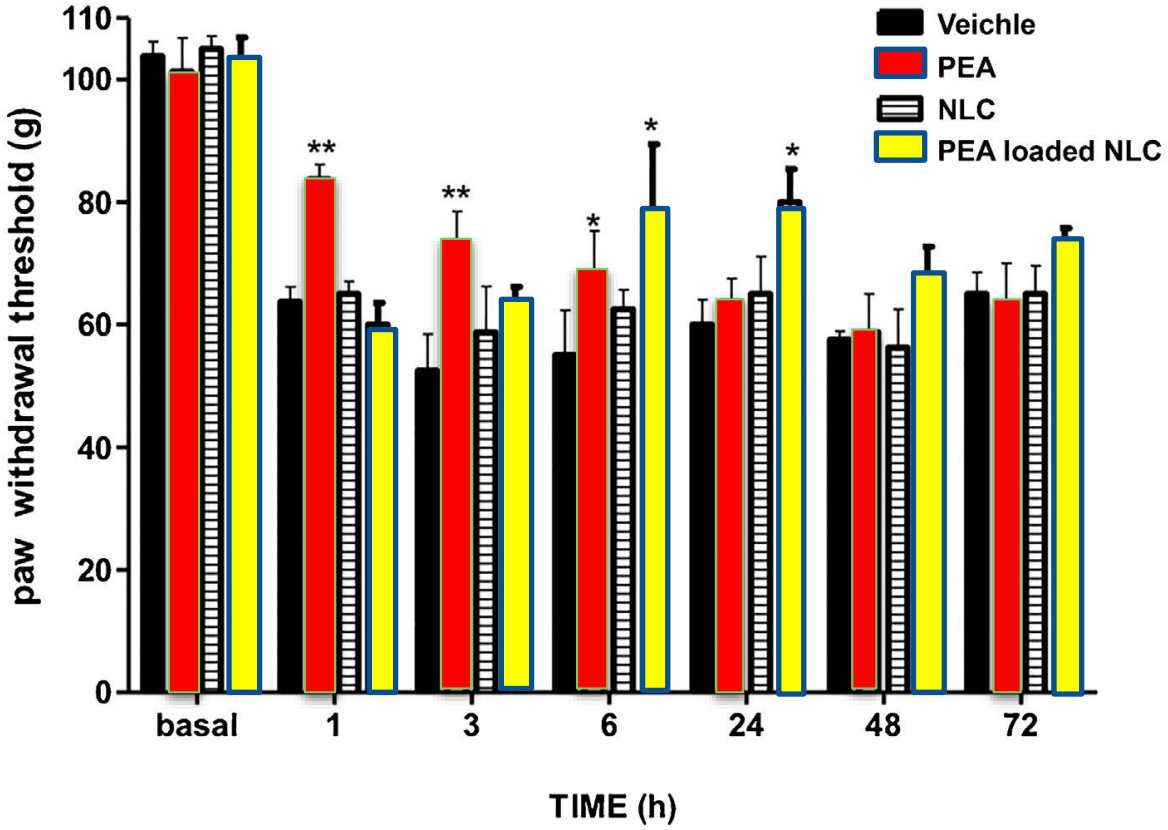
Effect on paw edema



*Data represents mean ± SEM of 6 mice. *p < 0.05 and **p < 0.01 vs vehicle.*

Results: in vivo studies

Effect on mechanical hyperalgesia



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CONCLUSIONS

- The HSH method was suitable to produce particles in nanometric range and characterized by a good homogeneity;
- Particle characterization has evidenced a peculiar internal structure (it has been hypothesized a type III NLC structure);
- NLC increase PEA percutaneous diffusion in comparison to control formulations;
- In vivo results prove that NLC are able to prolong PEA anti-inflammatory and analgesic effects

THANKS TO:

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THANK YOU
FOR YOUR ATTENTION