IECP 2020
The 1st International Electronic Conference on Pharmaceutics
01–15 DECEMBER 2020 | ONLINE

Key Features of Solid Lipid Nanoparticles Prepared With Nanoclay and Spring Water Ingredients with Demonstrated Wound Healing Activity: a Pilot Study

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pharmaceutics
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ABSTRACT

In this pilot study we propose the formulation of a semisolid system formed by SLN (Solid Lipid Nanoparticles) embedded in an inorganic hydrogel with demonstrated wound healing activity.

The hot emulsification method was used to prepare the SLN. The SLN were embedded in a wound healing hydrogel composed of a clay mineral (PS9) and a natural spring water (ALI). Granulometry, pH, rheology and TEM microscopy were used to characterize the formulations.

Results showed that the use of natural spring water does not affect the SLN's particle size unlike PS9, which increased them. TEM microphotographs revealed that this increase in particle size was due to SLN coalescence in presence of PS9. The pH of all samples was stable for 3 months. Rheology was significantly influenced by the aqueous medium, better results obtained with ALI.

In conclusion, despite of the necessity of some improvements, the proposed SLN formulation would be very versatile for wound healing due to the possibility to load different actives inside the SLN together with the wound healing activity of the PS9/ALI hydrogel.

Keywords:

Solid Lipid Nanoparticles (SLN); sepiolite; spring water; hot emulsification.

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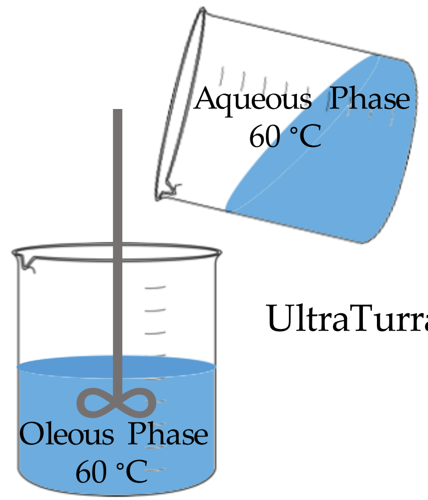
Materials and Methods

Aqueous Phase
10% w/w Kolliphor®   80% w/w of milliQ® or ALI water

Oleous Phase
10% w/w Crodamol™



UltraTurrax® 1500 rpm/3 min.

A/O emulsion

1) ultrasonication was performed for 15 minutes working under a constant 20% duty cycle

2) rapidly cooled in an ice bath to induce the crystallization of cetyl palmitate goticles

SLN@milliQ/SLN@ALI

Addition of 5% w/w of **PS9 (Sepiolite from Tolsa)** Homogeneization (UltraTurrax® 13000 rpm/5 min.)

FORMULATIONS

- **SLN@ALI@PS9**
- **SLN@milliQ@PS9**

Results and Discussion

➢ *Rheology studies*

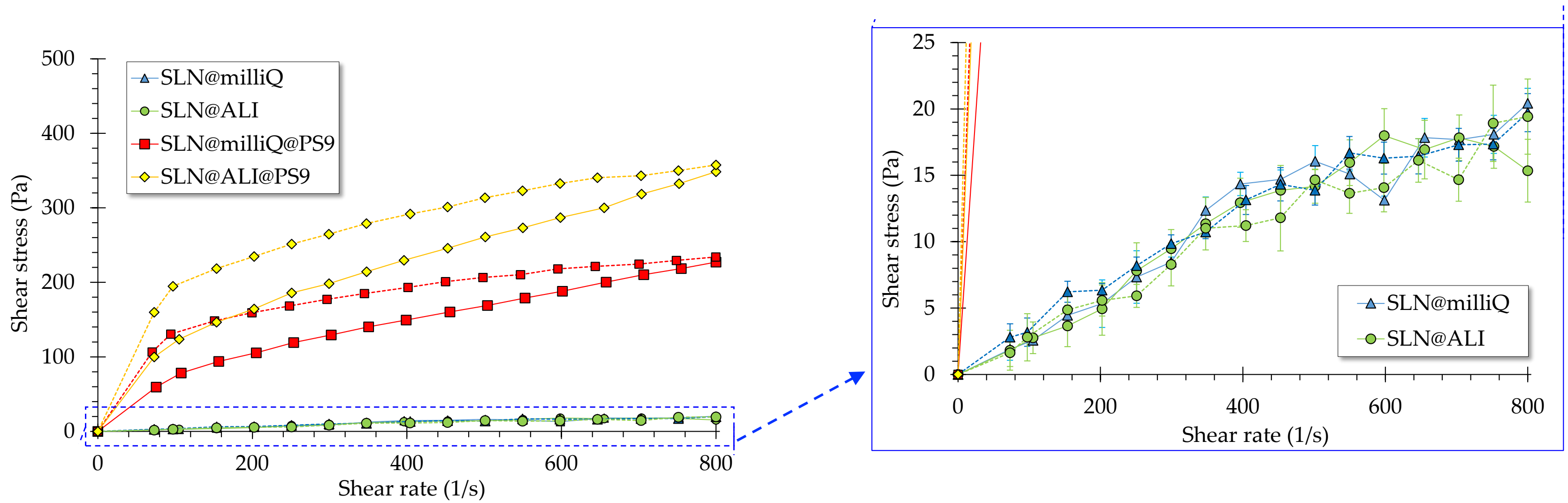


Figure 1. Flow curves of SLN@milliQ, SLN@milliQ@PS9, SLN@ALI and SLN@ALI@PS9. Uphill curves (70-800 s^{-1}) are represented with a continuous line, while dashed lines were used for the return flow curves (800-70s^{-1}).

- ✓ SLN@ALI and SLN@milliQ showed a Newtonian-like profile.
- ✓ SLN@ALI@PS9 and SLN@milliQ@PS9 displayed a non-Newtonian pseudoplastic behavior.
- ✓ Influence of SLN can be observed due to the rheopeptic profile of SLN@milliQ@PS9 and SLN@ALI@PS9.
- ✓ Type of water in SLN@ALI@PS9 and SLN@milliQ@PS9 influenced the final viscosity of the system, ALI water increasing this value with respect to the milliQ® system.

Results and Discussion

➢ ***pH measurements***: 1 week (1w), 15 days (15d) and 2 months (2m).

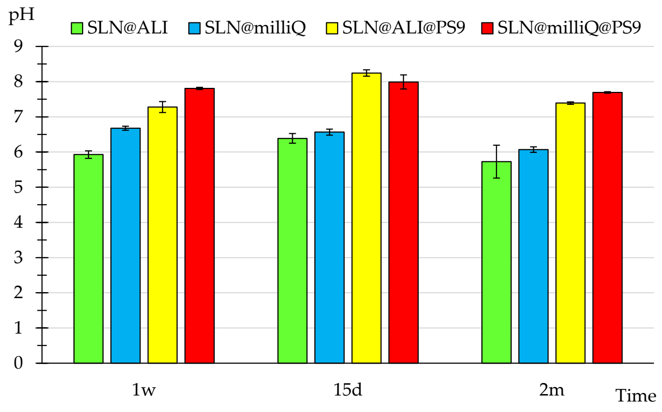


Figure 2. pH of the formulations through time (mean values ± s.d.; n= 8).

- ✓ SLN@milliQ and SLN@ALI showed a lower pH, around 6.
- ✓ SLN@milliQ@PS9 and SLN@ALI@PS9 possessed a pH between 7 and 8.

The presence of PS9 increased the pH value towards its natural value due to the intrinsic buffer activity of phyllosilicates.

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Results and Discussion

➢ ***Particle size*** : 1 week (1w) and 2 months (2m).

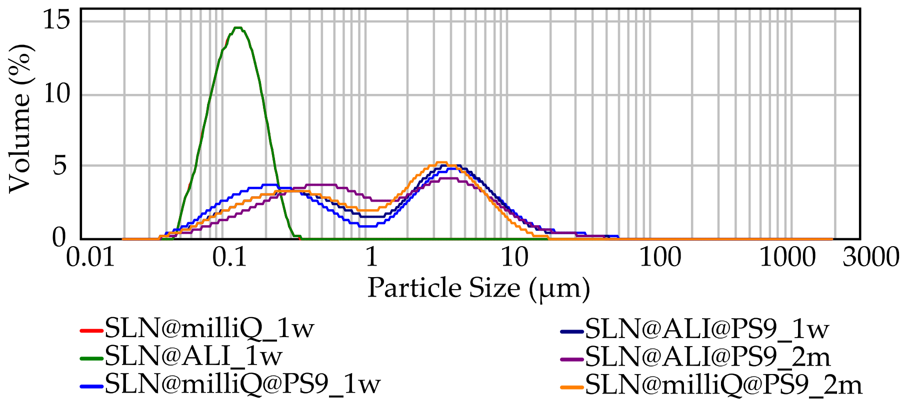

Figure 3. Particle size analysis of formulations. For simplicity, the most relevant results of 1w and 2m samples are shown (mean sizes; n=3).

- ✓ SLN@milliQ and SLN@ALI had a particle size between 0.3 and 0.04 μm, the main mode being 0.15 μm.
- ✓ The type of water did not influence the resultant particle size of the SLNs, thus demonstrating that ALI water is completely valid for the formulation of SLNs.

Table 1. Statistical particle size analysis.

Sample	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	SPAN factor
SLN@milliQ_1w	0.073	0.124	0.202	1.041
SLN@ALI_1w	0.074	0.124	0.203	1.042
SLN@milliQ@PS9_1w	0.116	1.595	8.277	5.116
SLN@ALI@PS9_1w	0.141	1.776	7.747	4.283
SLN@ALI@PS9_2m	0.180	1.235	7.650	6.046
SLN@milliQ@PS9_2m	0.142	1.435	6.143	4.181

- ✓ The presence of PS9 clearly influenced the final SLN particle sizes.
- ✓ The size changes of SLN caused by PS9 could be due to the adsorption of the tensioactive to the clay mineral surface.

Results and Discussion

➢ ***Transmission Electron Microscopy (TEM)***: 15 days (15d).

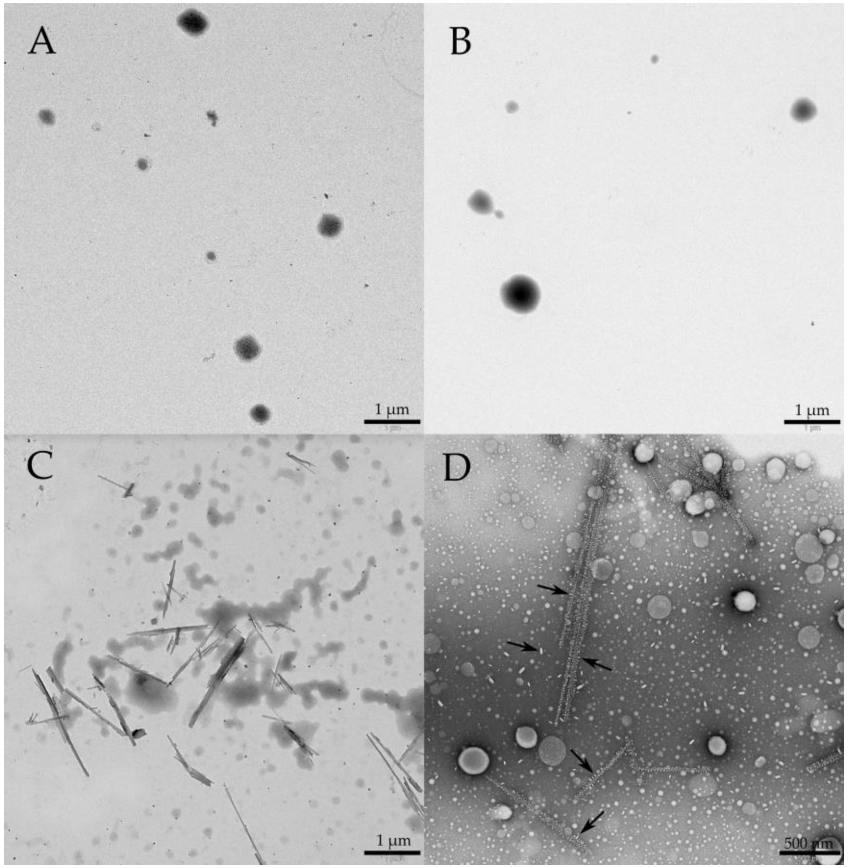

Figure 4. Microphotographs obtained during TEM studies of the formulations. A) SLN@ALI; B) SLN@milliQ; C) SLN@ALI@PS9; D) SLN@milliQ@PS9. Arrows mark tiny micelles surrounding sepiolite fibers.

- ✓ Coalescence of SLN nanoparticles is visible in both SLN@ALI@PS9 and SLN@milliQ@PS9, thus explaining the increase in particle size results.

- ✓ We hypothesize that the presence of the clay particles could alter the dimensions of the SLN due to adsorption of the surfactant molecules by the clay fibers. In fact, a concentrated zone was found in sample SLN@milliQ@PS9. In this area, sepiolite fibers are surrounded by tiny particles of around 30 nm.

- ✓ In view of the sizes and the aspect of these "particles", they can be identified as micelles formed by the concentration of surfactant molecules over the sepiolite particles. This result is in agreement with the hypothesis explaining the coalescence of the SLNs.

Conclusions

- This pilot study explores the main characteristics of a hybrid semisolid system formed by SLN embedded in an inorganic hydrogel made of spring water and a fibrous clay mineral.

- This kind of formulation would be very versatile for the treatment of skin diseases, specifically for wound healing, due to the possibility to load different actives inside the SLN together with the already demonstrated wound healing activity of the inorganic hydrogel.

- The results demonstrated that final particle size of SLN was not influenced by the use of Alicún de las Torres spring source. Nonetheless, the adsorption of surfactant molecules by the clay mineral particles induced coalescence of SLN, thus altering the final features of the formulation. Despite this inconvenience and the necessity of further studies, the formulations reported promising stability and rheological properties.

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Acknowledgments

Organizing of Congress

Institutions

Department of Pharmacy and Pharmaceutical Technology, University of Granada

Instituto Andaluz de Ciencias de la Tierra (CSIC-UGR)

Funding

Ministerio de Ciencia e Innovación, CGL2016–80833-R; Consejería de Economía, Innovación, Ciencia y Empleo, Junta de Andalucía, P18-RT-3786 and Ministerio de Educación, Cultura y Deporte, FPU15/01577.