



## INFLUENCE OF SLN MATRIX MODIFICATION ON "IN VITRO" AND "IN VIVO" NANOPARTICLE PERFORMANCES

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### ABSTRACT

This study focuses on the production of solid lipid nanoparticles (SLN) loaded with the hydrophilic carbonyl quencher N-acetylcarnosine (NAC) in order to develop topical formulations useful in several skin diseases and in the photoaging prevention and treatment. The addition of amphiphilic substances into lipid matrix, such as phosphatidylcholine (PC), polyglyceryl-3-diostearate and sorbitan monooleate, was evaluated. The influence of additives on "in vitro" SLN performances was investigated by Differential Scanning Calorimetry, size and zeta potential, active content analyses, release and occlusion tests. Preliminary "in vivo" investigations of cutaneous tolerability and short-term effect of SLN application were also carried out using non-invasive skin bioengineering techniques. Results obtained from this work allowed to conclude that the incorporation of these additives, in concentration of 15% with respect to lipid mass, strongly affects colloidal systems physical properties leading to a very different in "in vitro" and "in vivo" performances. In particular, encapsulation efficiency can be very good reaching the 65.75% by addition of PC into lipid matrix. In vivo evaluation evidenced good cutaneous tolerability for all batches produced. The presence of PC can significantly modify the short-term effect of SLN application on the skin with respect to both vehicle alone and unmodified SLN.

**Keywords:** SLN, Carbonyl-quencher agent, Phosphatidylcholine, Antioxidant topical delivery, Non-invasive skin bioengineering techniques.

### INTRODUCTION

Oxidative stress resulting from formation of reactive radical species, caused by chemical and physical compounds, temperature and food, is involved in the damage of cellular constituents, such as DNA, cell membrane lipids or proteins leading to skin diseases, autoimmune disease and cancer<sup>1</sup>. The same factors are responsible for the structural and functional alterations of photoaged skin as depolymerization and cross-linking with component of extracellular matrix (collagen fibers, elastine and hyaluronic acid)<sup>2</sup>. The skin contains a well-organised system of both chemical and enzymatic antioxidants that work in a synergistic manner to protect itself against the over-load of oxidant species. Skin antioxidant network protects cells against oxidative injury and prevent the production of oxidation products, such as 4-hydroxy-2-nonenal or malonaldehyde, which can induce protein damage, apoptosis or release of pro-inflammatory mediators, such as cytokines. When oxidative stress overwhelms the skin antioxidant capacity, the subsequent modification of cellular redox apparatus leads to an alteration of cell homeostasis and a generation of degenerative processes. Topical application or oral administration of antioxidants has been recently suggested as preventive therapy for skin diseases, photoaging and UV-induced cancer.

Therapeutical approach is based on the use of molecules with radical scavenging activity, able to deactivate radical species involved in oxidative process induction and propagation through electron transfer, and carbonyl quenching activity, able to deactivate secondary reactive carbonyl product, without radicalic structure. The molecules with these two activities are second generation antioxidants which act as functional hybrids thanks to the presence of a histidinic residual<sup>3</sup>.

N-acetyl-L-carnosine (NAC) is numbered among this class of compounds; it is a dipeptide composed by an acetylated alanine residual and a histidine one. It is metabolically correlated to L-carnosine, that is particularly beneficial in diabetes because of its ability to protect cellular chromosomes from oxidative damage and inhibits the process of cross-linking. Carnosine helps wound healing and prevents cataract formation because of its ability to rejuvenate connective tissue cells<sup>4</sup>.

Differently from carnosine, NAC is not a substrate of carnosinase or other dipeptidase because of its acetylated residual. In this way NAC can prolong and improve physiological response to antioxidant

treatment. The advantage of NAC to act as an in vivo universal antioxidant with physiological and therapeutic relevance deals with the ability of giving efficient protection against oxidative stress in the lipid phase of biological membranes and in aqueous environment due to the turnover into L-carnosine.

N-acetyl-L-carnosine is already used in ophthalmic treatment and prolongs of thirty minutes the effect of L-carnosine in aqueous humor of the treated eye<sup>5-6</sup>. Due to its relative hydrophobicity compared to L-carnosine, NAC may pass through the lipid membranes of skin cells easier than carnosine and may thereby gain faster access to cell's interior, which is primarily aqueous. N-acetylcarnosine can be gradually broken down to carnosine, which then exerts its beneficial effects. Solid lipid nanoparticles (SLN) represent a recent powerful carrier system for various pharmaceutical drugs and cosmetic active ingredients. They are able to encapsulate a great amount of lipophilic compounds such as steroids, retinol, sunscreens but for most drugs, especially hydrophilic ones, the payload is very low<sup>7-11</sup>.

This effect can be due to the crystalline structure of the lipid matrix or to the low solubility of hydrophilic substances into lipidic phase.

Two approaches have been evaluated to improve the payload of hydrophilic compounds. The first approach was the development of oil loaded SLN, also described as nanostructured lipid carriers (NLC). The alternative strategy is to modify the lipid matrix by incorporation of amphiphilic substances<sup>12,13</sup>. The aim of this work was to produce solid lipid nanoparticles loaded with the hydrophilic carbonyl quencher compound N-acetylcarnosine. For this purpose different amphiphilic substances were employed in order to modify the lipid matrix of SLN made of cetyl palmitate. SLN are used as topical vehicle because of their intrinsic occlusive properties that form a film on skin surface, reducing transepidermal water loss (TEWL). The increasing of the water content improves the appearance of healthy human skin and enhances the penetration of active through the skin; moreover the reduced particle size improves surface area and facilitates contact of encapsulated drugs with the stratum corneum<sup>14</sup>. Up to now, only few studies regarding in vivo evaluation of SLN topically applied are reported<sup>15,16</sup>. The cutaneous tolerability, in term of absence of adverse sensations and erythema appearances, is required for the safety assessment of new topical products. For this reason, in vivo evaluation of cutaneous tolerability of topical formulations containing SLN was carried out in

non-occlusive conditions using non-invasive skin bioengineering techniques. A "in vivo" short-term hydration effect of vehicle, SLN placebo, SLN loaded with NAC was also evaluated.

## MATERIALS AND METHODS

### Materials

Cetylpalmitate (CP) was provided by Gattefossé (France), Tego® Care 450 (polyglyceryl methylglucose distearate) by Goldschmidt GmbH (Germany), Lipoid E80 (PC) (egg lecithin, phosphatidylcholine content 83,3%) by Lipoid GmbH (Germany), Plurol diisostearique (P) (polyglycerol-3-diisostearate) by Gattefossé (France), Span 80 (S) (sorbitan monoleate) by Sigma (Germany), N-acetyl-L-carnosine (NAC) by Flamma (Italy).

### SLN preparation

Solid lipid nanoparticles were produced by the hot homogenization technique, suitably modified<sup>17</sup>.

Briefly, cetylpalmitate was melted at 85°C and NAC was dispersed into the melt in concentrations of 5, 10 and 20% with respect to lipid mass (batches SLN-5, SLN-10, SLN-20). When present, additives such as phosphatidylcholine (SLN-20-PC), polyglyceryl-3-diisostearate (SLN-20-P) and sorbitan monoleate (SLN-20-S) were used as dispersing agents for the active and were added to the melt lipid phase at concentration of 15% with respect to lipid mass. In these batches NAC was maintained in a concentration of 20%.

Surfactant aqueous phase, composed of 0.18% Tego Care 450 solution, was preheated at 85°C and added to the lipophilic phase and an O/W emulsion was obtained by stirring at 9500 rpm for one minute using an UltraTurrax® T25 basic (Ika, Staufen, D).

The emulsion was homogenized three times at 85°C, 500 bar with a high pressure homogenizer Panda 2K, Niro Soavi (Parma, Italy). SLN dispersion was cooled down into an ice bath for one hour after the preparation. All batches were purified with Amicon ultrafiltration cell (Millipore, Italy) equipped with a polyethersulfone disc, 300 kDa NMWL; SLN concentrated suspension was stored at 4°C in small glass vials with NaN<sub>3</sub> 10<sup>-4</sup> M solution as preservative. All batches were prepared in triplicate. Placebo SLN batches were prepared as control (batches SLN, SLN-PC, SLN-P, SLN-S)

### Morphology

Atomic force microscopy studies were used to image the surface structure of solid lipid nanoparticles and to show the shape of these particles. SLN morphology was investigated by using an AutoProbe CP Research scanning probe microscope (Thermo Microscope, Sunnyvale, CA, USA). The analyses were performed in air and under constant applied force condition (non-contact mode). SLN suspensions were fixed on a silicon support, and images were processed using the Image Processing Data Analysis 2.0 software provided by ThermoMicroscope.

### Particle size and zeta potential analyses

Particle size analyses were performed by laser diffraction technique using a Malvern Mastersizer 2000 (Malvern Ltd, Malvern, UK); this instruments works in a size range between 0.02 and 2000 µm. Samples of SLN suspensions were diluted in water and analysed under continuous stirring at 2500 rpm. Results are expressed as d<sub>10</sub>, d<sub>50</sub>, d<sub>90</sub>, that represent particle size of 10%, 50% and 90% of SLN populations, respectively.

Zeta potential measurements give informations about the particle surface charge; this value is very important in order to predict the long-term stability of nanoparticle suspension<sup>18</sup>. Zeta potential analyses were performed by diluting samples in water and the measurements were made using a Nicomp zetameter 380 ZLS (PSS, Holland).

### NAC content

The N-acetylcarnosine was quantified using a high performance liquid chromatography method, as provided by compound supplier. Briefly, an instrument model HP1100 was used, equipped with

Ultrasphere ODS column (250 x 4.6 mm, 5µm) and UV detector was fixed at 215 nm. Analyses were carried out isocratically with 1.7 ml/min as flow rate; the mobile phase was composed by 0.12% sodium hexansulfonate solution in phosphate buffer pH 2.5 and acetonitrile HPLC grade, 90/10 v/v. Retention time of NAC was 1.8 min, the limit of quantification (signal to noise ratio 10:1) 0.8 µg/ml. A calibration curve of NAC in a mixture water:acetonitrile (77:23 v:v) ranging between 16 and 320 µg/ml was used.

NAC content was determined by putting an amount of SLN in water at 85°C, under stirring at 1400 rpm for 10' (Thermomixer, Eppendorf AG, D); samples were centrifuged at 4°C for 10' at 16400 rpm (centrifuge model 5417 Eppendorf AG, D) and then filtered, diluted with water/acetonitrile (77/23 v/v), and analysed by HPLC.

Each batch was measured in triplicate and results are expressed as a percentage ratio between the active recovered and the total NAC used in the SLN preparation (encapsulation efficiency).

### Differential scanning calorimetry (DSC)

The crystalline status and lipophilic modification on raw materials, placebo SLN and NAC loaded SLN were investigated using a 2910 DSC (TA instruments, USA). Samples were weighted and put into aluminium pans, heated from -50°C to 150°C at a rate of 5°C/min under nitrogen purge.

### "In vitro" occlusion test

In order to evaluate the occlusive effect of SLN, in vitro test was carried out following the procedure explained by Wissing et al<sup>19</sup>. The occlusive property can be very important to improve skin hydration and active penetration through the skin.

SLN dispersion was spread in a concentration of 16.5 mg/cm<sup>2</sup> on a paper filter covering a beaker filled with water and stored at 37°C for 48 hours. Three beakers without sample represented the control. The evaporation of water through a membrane is measured by weighting the samples at 6, 24 and 48 hours and the occlusion factor F is calculated according to the following formula

$$F = 100 * [(A - B) / A]$$

where A is the water loss of the beaker without samples and B is the water loss of the beaker with samples.

### "In vitro" NAC release test

"In vitro" release test was performed by putting amounts of all batches of SLN prepared into test tubes filled with water. The filled test tubes were kept in an incubator at 37°C for 1 week. At prefixed times three tubes for each SLN batch were filtered through 0.22 µm membrane and analysed by HPLC method as previously reported for NAC content (section 2.5). Results are expressed as average cumulative amount of NAC released at each time ± standard deviations.

### In vivo instrumental study

#### Study population

Ten healthy female subjects 20-30 years old, with skin Fitzpatrick types II and III, participated in this study. Written informed consent was obtained from all volunteers and the study was approved by the local ethics committee.

Exclusion criteria were allergies, any skin diseases, consuming diseases, infections, pregnancy and lactation, systemic and topical medications, previous treatment of forearms with cosmetic formulation such as moisturizers or antiageing products and participation in another study in the last 2 months.

Six test square fields (4 cm<sup>2</sup>) were marked with a permanent marker on volar forearms of each volunteer: one for control and five for formulations. To avoid an influence of the anatomical localization on the results, a systematic rotation of the test areas was performed.

Before measurements subjects remained in the room for at least 20 minute to allow full skin adaptation to room temperature (20 ± 2°C) and humidity (45-60%). The temperature and humidity in the

investigation room were kept constant during the measurements. Prior to application basal values were recorded in all test area. Subsequently the test areas were treated with formulations except control site that remained untreated.

### Formulations

The formulation used as vehicle (V) was an emulsion-gel with the following composition: isopropylmyristate 10%, a mixture of polyacrylamide/ C13-14 isoparaffin/ laureth-7 (Sepigel 305, Seppic, Italy) 3%, preservative 0.2%, water 86.8%.

Two placebo formulations were prepared by dispersing the batches SLN and SLN-PC into vehicle in concentration of 20% w/w (named FP-SLN and FP-SLN-PC, respectively).

Two formulations containing NAC loaded SLN and NAC loaded SLN-PC into vehicle were also prepared (named FP-SLN-20 and FP-SLN-20-PC, respectively).

### Evaluation of SLN cutaneous tolerability

In order to evaluate SLN cutaneous tolerability a Single Open Application Test was performed. Physiological skin parameters were obtained by measurement of the skin erythema values using a Cutometer MPA 580 equipped with a Mexameter M18 probe (Courage&Khazaka, Germany).

Irritation was quantitatively evaluated by comparing basal erythema degree with values detected 30 minutes or 24h after formulations treatment.

### Short Term Hydration Test

In order to evaluate the effect of SLN application on skin hydration a Short Term Hydration Test was carried out. Instrumental evaluation was performed by using a Cutometer MPA 580 equipped with a Corneometer CM825 probe (Courage&Khazaka, Germany) able to quantify the stratum corneum water content.

Noninvasive biophysical measurement were repeated after 30, 60, 180, 360 minutes and 24 hours after skin treatment. All measurements were carried out according to the relevant guidelines<sup>20</sup>.

### Statistical analysis

The data are expressed as a ratio between erythema and water content values measured at each time in the application sites and in the untreated site, used as control. GraphPad Prism for windows software was used to perform statistical analysis on the data. Non-parametric Wilcoxon Signed Ranks Test was used to test for significance. A  $P < 0.05$  was considered statistically significant.

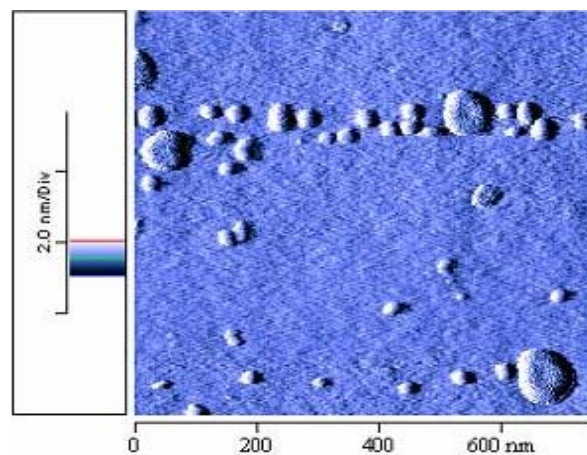
## RESULTS AND DISCUSSION

### SLN preparation and characterization

The problems related to a limited hydrophilic compound payload into SLN and the potential drug expulsion from the crystal lattice upon polymorphic transition, led researchers to develop new approaches in the SLN production. Our approach was to produce SLN with matrix modification by incorporating additives such as phosphatidylcholine, polyglyceryl-3-diisostearate and sorbitan.

Hot homogenization is a well known technique to produce SLN. This method, suitably modified, was successfully employed in this work to prepare NAC loaded SLN.

Atomic force microscopy represents a useful technique to visualize SLN morphology especially by operating in the non-contact mode<sup>21</sup>. In fact, AFM presents some advantages for the nanoparticles visualization such as the simplicity of sample preparation as no vacuum is needed during operation. The Figure 1 shows the AFM image of batch SLN-20. The AFM investigation revealed the round shape of nanoparticle made of cetyl palmitate and loaded with N-acetylcarnosine.



**Fig. 1: Atomic force micrograph of nanoparticles (batch SLN-20)**

The Table 1 reports size distribution and zeta potential values of all NAC loaded SLN batches.

**Table 1: Light diffraction diameters and zeta potential values of NAC loaded SLN**

Batch	d <sub>10</sub> ( $\mu\text{m}$ )	d <sub>50</sub> ( $\mu\text{m}$ )	d <sub>90</sub> ( $\mu\text{m}$ )	Zeta potential (mV)
SLN-20	0.073	0.168	0.928	-17.89
SLN-10	0.078	0.178	0.433	-26.97
SLN-5	0.074	0.171	0.785	-19.69
SLN-20-PC	0.066	0.131	0.269	-23.75
SLN-20-P	0.066	0.130	0.277	-28.59
SLN-20-S	0.069	0.144	0.404	-28.64

SLN particle size is always lower than 1 micrometer; additives permitted to obtain smaller diameter and size distribution.

Zeta potential is about -17.89 for the batch SLN-20; the addition of PC, P and S allowed to decrease this value to about -28.6, showing a stabilizer effect on SLN dispersion. As we known from literature that high zeta potential values corresponds to charged particles in which particle aggregation occur less likely. Otherwise, SLN with potential value of about  $\pm 10$  mV presents physical instability<sup>17,18</sup>.

The Table 2 reports the yields of production and the encapsulation efficiencies of all NAC loaded SLN batches prepared. The yields of production are always satisfactory, ranging between 42 and 67%. The addition of additive permitted to obtain a better dispersion of NAC into lipidic matrix during SLN preparation obtaining very good NAC content, especially for SLN-20-PC, reaching the 65.75% of encapsulation efficiency.

**Table 2: Theoretical drug contents, actual encapsulation efficiencies and yields of production of NAC loaded SLN**

Batch	Theoretical drug content (%)	Encapsulation Efficiency (%)	Yield of production (%)
SLN-20	20	46.84	42.92
SLN-10	10	23.33	44.70
SLN-5	5	26.67	61.64
SLN-20-PC	20	65.75	53.43
SLN-20-P	20	53.17	67.44
SLN-20-S	20	49.17	46.70

### Differential Scanning Calorimetry (DSC)

The thermal behaviour of the examined samples were investigated by means of Differential Scanning Calorimetry. In fact, DSC represents a useful tool to estimate the crystalline degree of nanoparticles since it provides information about crystal melting, polymorphic transitions and formation of eutectic mixtures.

The physical state of the particles is very important in relationship of technological aspect as well as the biopharmaceutical one.

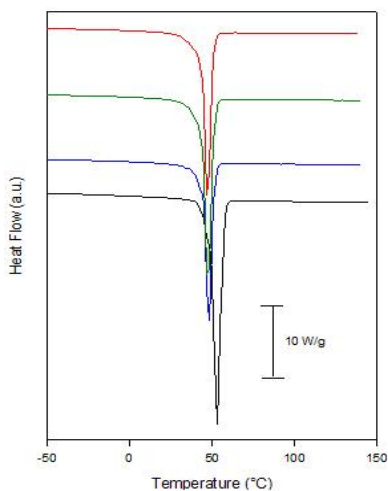
The DSC runs performed on pure cetylpalmitate (CP100) evidence only one sharp endothermic peak at 53°C with a peak  $\Delta H$  value of 218.5 J/g, as reported in the Table 3. All traces exhibit a shoulder just before the main peak; such a feature is expected for SLN's, on the other hand what concerns CP it can be due either to polymorphic forms or to impurities since its technical sheet reports a purity value significantly lower than that of CP100. It is also worth noting that preliminary DSC runs performed on all other raw materials used for SLN preparation did not evidence any thermal feature in the same temperature range (data not reported).

**Table 3: Shoulder onset temperature (1<sup>st</sup> T<sub>onset</sub>), main peak onset temperature, (Peak T<sub>onset</sub>) and the overall Enthalpy value (J/g) of CP, SLN placebo and NAC loaded SLN**

Sample	1 <sup>st</sup> T <sub>onset</sub> (°C)	Peak T <sub>onset</sub> (°C)	$\Delta H$ (J/g)
CP100	-	53.0 ± 2	218.5 ± 4.4
CP	41.3 ± 3	51.0 ± 2	212.5 ± 4.2
SLN placebo	43.2 ± 3	50.9 ± 2	180.9 ± 3.6
SLN-20	41.9 ± 3	49.4 ± 2	189.1 ± 3.7
SLN-20-S	34.3 ± 3	48.7 ± 2	158.3 ± 3.2
SLN-20-PC	34.0 ± 3	50.1 ± 2	165.4 ± 3.3
SLN-20-P	35.1 ± 3	48.7 ± 2	161.0 ± 3.2

The Table 3 reports the shoulder onset temperature (1<sup>st</sup> T<sub>onset</sub>), the main peak onset temperature, (Peak T<sub>onset</sub>) and the overall  $\Delta H$  value (J/g). Despite the difference in purity, the  $\Delta H$  value for CP raw material is pretty the same for CP100. In order to have a rough estimation of SLN's crystallinity degree, it is possible to assume the  $\Delta H$  of CP100 as a reference value. It corresponds to 100% of crystalline status to compare the enthalpic values for both raw material and preparations. By a comparison among  $\Delta H$  values, it is evident that non modified placebo and loaded SLNs present a crystallinity degree of about 83% and 86.5%, respectively. This result is in agreement with previously reported investigation on crystallographic behaviour of cetylpalmitate solid lipid nanoparticles in which CP is arranged into SLN in a lamellar lattice structure<sup>22</sup>. The addition of additives allowed to decrease the crystalline degree of SLN with respect to placebo SLN or SLN-20 down to 72%. The change in crystallinity can change the "in vitro" and "in vivo" performances of the systems.

DSC traces of NAC loaded SLN show a decrease of melting temperature for batches produced with the addition of phosphatidylcholine, polyglyceryl-3-diisostearate and sorbitan with respect to SLN-20; such a phenomenon is probably due to interactions between acetyl carnosine and additives.

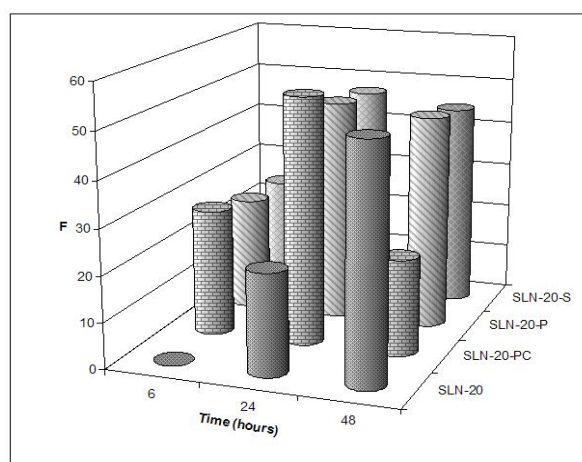


**Fig. 2: DSC traces of : red line SLN-20-S; green line SLN-20-P; blue line SLN-20-PC; black line: SLN-20**

The presence of additives results in a significant increase of NAC content, as reported in the NAC content analyses, and in a significant reduction of the melting area of the lipidic matrix. According to the collected data, the presence of acetylcarnosine seems to promote a further decrease of the 1<sup>st</sup> T<sub>onset</sub>, at least for what concerns the modified specimens, clearly indicating an appreciable interaction between active and additives.

### In vitro occlusion test

In vitro occlusion test results are reported in the Figure 3.



**Fig. 3: "In vitro" occlusion test results of NAC loaded nanoparticles**

SLN-20 showed an increasing F value in 48 hours while all modified batches highlighted the higher F value at 24 hours that remains constant for SLN-20-S and SLN-20-P batches. SLN-20-PC batch shows a very different behaviour. In all cases, additive addition causes a modification in SLN matrix. According to literature an increased skin hydration is based on an occlusive effect which occurs during film formation of the nanoparticles on the skin. Highly crystalline SLN show the most pronounced occlusive effect compared to NLC and nanoemulsions<sup>10</sup>. In our work it is evident that not modified SLN-20 presents an increasing of "in vitro" occlusive factor during the tested time period. These results are similar to those reported in literature about the occlusion factor of SLN crystalline state<sup>17</sup>. The only preparation that does not present this behaviour is the SLN-20-PC batch, prepared with phosphatidylcholine.

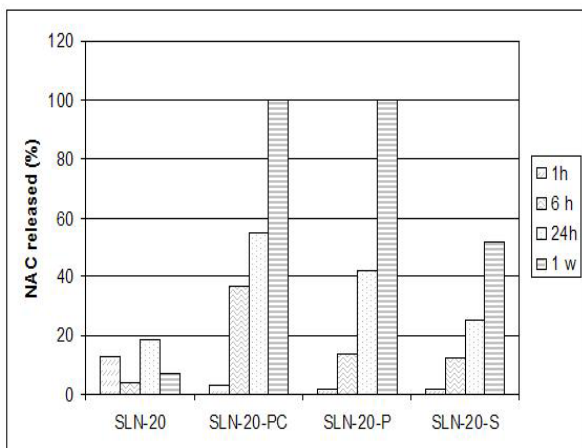
### In vitro release test

Drug release from lipidic nanoparticles depends on several factors concerning preparation method parameters (hot or cold homogenization, temperature of homogenization and cooling phase), drug characteristics (lipophilic or hydrophilic), particle size, viscosity and matrix physical modifications. The "in vitro" method used for the release test is also very important for its results. In fact, Franz diffusion cells technique have the advantage to be the most suitable method to predict percutaneous absorption but for a very short period of time only; in fact, this method can have the drawback to turn the fluid SLN dispersion into a semisolid gel through water evaporation inducing polymorphic transitions<sup>8</sup>. An other choice can be the paddle method USP; in this case the very large volume of acceptor led to a too diluted SLN dispersion that can increase the burst release and, at the same time, cannot be useful to predict a topical application.

The "in vitro" release test used in this work can be successfully employed for hydrophilic molecules because a very small amount of acceptor is needed in a close tubes that prevent water evaporation.

Figure 4 reports NAC "in vitro" release profiles from SLN batches.





**Fig. 4: "In vitro" release profiles of NAC from SLN batches produced**

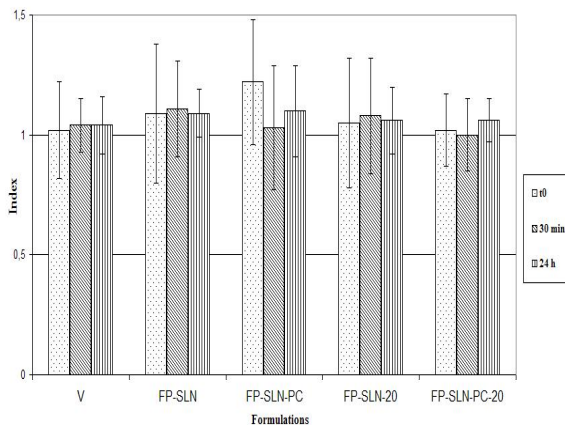
Results show that the addition of additives can strongly affect NAC release from nanoparticles. In fact, NAC release is completed undergone in a week only for SLN-20-PC and SLN-20-P batches. The modification of NAC release can be attributed to the capability of additives to disperse the active homogeneously inside the lipidic matrix instead of, without additives, the homogenization technique used in this study led to a drug incorporation in a drug-enriched core model, according to literature<sup>7</sup>.

#### In vivo evaluation

##### Evaluation of SLN cutaneous tolerability

SLN cutaneous tolerability was evaluated by a Single Open Application Test.

Figure 5 shows results obtained by Single Open Application Test as index between formulation site and control at each time evaluated.



**Fig. 5: "In vivo" cutaneous tolerability results of vehicle and of formulations containing placebo SLN and NAC loaded SLN**

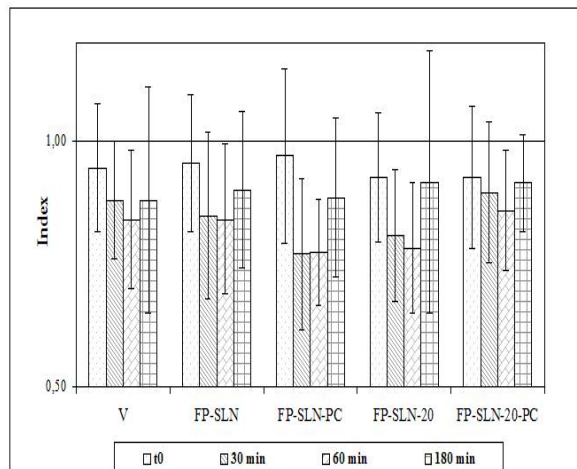
No significant differences are present between basal values, 30 minutes and 24 hours after application.

Irritation assessment highlights that the vehicle (V) is well tolerated and the presence of SLN and PC-SLN placebo (FP-SLN and FP-SLN-PC) or containing NAC in a percentage of 20% w/w (FP-SLN-20 and FP-SLN-PC-20) does not modify cutaneous tolerability of the formulation.

##### Short term hydration test

The effect of skin hydration is an important requirement in the development of new topical products. Skin hydration can be

influenced by a suitable vehicle or by active ingredients incorporated in the formulation<sup>16</sup>. In this study we evaluated the effect of solid lipid nanoparticle application on skin hydration. Non invasive biophysical measurements allowed us to study the influence of SLN composition and the effect of N-acetylcarnosine presence on skin hydration. Corneometer data are expressed as ratio between formulation site and control site values at each time considered, as shown in the Figure 6.



**Fig. 6: "In vivo" Short Term Hydration Test results carried out on vehicle and on formulations containing placebo SLN and NAC loaded SLN**

By comparing hydration indexes, an important reduction of stratum corneum hydration is noticeable for formulation containing placebo PC-SLN (FP-SLN-PC) in the first 180 minutes after application. These results could be probably due to a capability of PC of binding water by forming micelles. In this way water can reach deeper epidermis layers and it is not more detectable by corneometer, that measures only water present in the stratum corneum. The influence of PC is confirmed by FP-SLN results in which no differences are evidenced respect to vehicle values.

NAC presence strongly modifies skin hydration indexes. In fact, FP-SLN-20 shows an evident reduction of the stratum corneum water content indicating that the dipeptide is able to increase hydration of deeper skin layer or bind water and remove it from the stratum corneum.

This effect disappears when both NAC and PC are present (FP-SLN-20-PC). Some interaction between NAC and phosphatidylcholine might modify the affinity of the ingredients for the water and the ability to bind it.

#### CONCLUSION

The aim of this work was to produce solid lipid nanoparticles loaded with the hydrophilic carbonyl quencher compound N-acetylcarnosine. Our approach, in order to promote hydrophilic compound payload into SLN, was to modify SLN matrix by incorporating additives such as phosphatidylcholine, polyglyceryl-3-diisostearate and sorbitan.

Results obtained from this work allowed to conclude that the incorporation of these substances, in concentration of 15% with respect to lipid mass, led to a modification of the lipidic matrix. The lipid modification of SLN matrix strongly affects physical properties of the colloidal systems produced leading to a very different in "in vitro" and "in vivo" performances. In particular, encapsulation efficiency can be very good reaching the 65.75% by addition of PC into lipid matrix.

As reported in the DSC analyses, the modified SLNs present the lowering of the melting process onset, of about 34°C, that is very near to skin temperature (of about 32°C) and clearly indicates a possible appreciable interaction between matrix and skin after "in

vivo" application. The modification of the crystalline state of SLNs is evident also from occlusion test and "in vitro" release test results.

Preliminary "in vivo" experiments permitted to conclude that SLN and PC-SLN placebo or containing NAC in a percentage of 20% w/w are well tolerated after topical application. Furthermore, the presence of PC or NAC strongly affects stratum corneum hydration in the first 180 minutes after application.

Further studies are in progress in order to evaluate the effect of PC in a "in vivo" long-term application study and in the evaluation of active efficacy after topical application.

In conclusion, our approach can be successfully employed to develop topical formulations containing NAC loaded SLN for the prevention of skin diseases and photoaging.

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