

# Dual-drugs delivery in solid lipid nanoparticles for the treatment of *Candida albicans* mycosis

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## ABSTRACT

Nowadays, a combinatorial drug delivery system that simultaneously transports two or more drugs to the targeted site in a human body, also recognized as a dual-drugs delivery system, represents a promising strategy to overcome drug resistance. Solid lipid nanoparticles loaded with clotrimazole (CLZ) and aliphatic lipophilic acid (ALA), considered as an effective agent in the reduction of reactive oxygen species, can enhance anti-infective immunity being proposed as a non-toxic and mainly non-allergic dual-drugs delivery system. In this study, uncoated and cationic CLZ-ALA-loaded SLN were prepared and compared. Suspensions with a narrow size distribution of particles of mean size below 150 nm were obtained, having slight negative or highly positive zeta potential values, due to the presence of the cationic lipid, which also increased nanoparticles stability, as confirmed by Turbiscan® results. Calorimetric studies confirmed the rationale of separately delivering the two drugs in a dual-delivery system. Furthermore, they confirmed the formation of SLN, without significant variation in presence of the cationic lipid. In vitro release studies showed a prolonged drug release without the occurrence of any burst effect. In vitro studies performed on 25 strains of *Candida albicans* showed the antimicrobial drug activity was not altered when it was loaded into lipid nanoparticles. The study has proved the successful encapsulation of CLZ and ALA in solid lipid nanoparticles that may represent a promising strategy to combine ALA protective effect in the treatment with CLZ.

## 1. Introduction

Fungal infections, most of all candidiasis, represent an increasing challenge affecting global health, that in many cases produces morbidity and mortality in immunocompromised and intensive care unit patients [1,2]. Among different types of fungi, *Candida albicans* represents the main cause of fungal diseases, responsible of both orally and vaginally yeast infections, overall when a decrease in *Lactobacillus* concentration appears [3]. Usually, polyenes, echinocandins and azoles are the most used drugs for the treatment of fungal infections [4], even if the requirement of early treatment and the occurrence of side effects still limit the efficiency of the therapy. Among azoles, Clotrimazole (CLZ) is one of the most broad-spectrum antimycotic drug used for the treatment of vulvovaginal and oropharyngeal candidiasis [5]. Moreover, CLZ has been claimed to show a selective antibacterial activity [6,7] and ability to inhibit *in vitro* *Streptococcus mutans* biofilm and

virulence [8]. CLZ antifungal activity is related to its capability to interfere with the biosynthesis of ergosterol, the major component of the fungal cytoplasmic membrane, with the consequent depletion of ergosterol and its replacement with the aberrant sterol species, 14- $\alpha$ -methylsterol, thus disturbing the membrane permeability and fluidity [9]. However, the increasing antifungal resistance to azoles, mainly among immunocompromised patients, strongly limits the effective treatment of mycoses [10].

Combinatorial drug delivery is a promising strategy to prevent potential drug resistance [11]. We have recently demonstrated the possibility to improve CLZ effectiveness exploiting its nanoencapsulation in lipid nanoparticles prepared by additionally using of essential oils from Mediterranean area, highlighting their synergistic effect [12]. Recently, the combination of two or more drugs in properly developed drug delivery systems (DDS) is attracting increasing efforts by the researchers as a potential strategy to improve the effectiveness also by reducing

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drug toxicity [13–18], since nanoparticles “4S” (size, surface, shape, stability) properties offer the possibility to overcome drug drawbacks such as instability, restricted efficacy, poor distribution, side effects [19]. In particular, different studies have been reported in literature concerning the possibility to enhance antifungal drugs bioavailability using lipid vesicles (liposomes) and lipid nanoparticles [20–28]. Among all, solid lipid nanoparticles (SLN) represent a versatile system for different routes of administration and they are able to the successfully enhancement of the incorporated drug bioavailability [29–31]. SLN have been developed for the combinatorial delivery of anticancer drug molecules and RNA material (siRNA or miRNA), increasing the cancer cells response to the anticancer treatment due to the synergic effect of the two agents [32,33]. Even if the co-delivery of antibiotic and linoleic acid has been recently reported to enhance the effectiveness of the treatment of infections due to *Staphylococcus aureus* [34], yet drugs dual-delivery has not been enough investigated.

In order to improve the effectiveness of CLZ treatment, alpha-lipoic acid (ALA) could be successfully used in association with the antifungal conventional drug, due to its ability in reducing the generation of reactive oxygen species [35,36], thus representing an innovative strategy to the advantage in the treatment of fungal infections [22,37]. Thereupon, the aim of this work was to develop SLN for the dual-delivery of ALA and CLZ, as promising strategy for the potential synergistic treatment of topical infection due to *C. albicans*. Since the coating with positively charged materials has been reported as an effective strategy for improving nanoparticles behavior [38,39], we compared slight negative and highly cationic SLN, obtained with the cationic lipid dimethyldioctadecylammonium bromide (DDAB). SLN were characterized by chemical-physical, technological and biological techniques to evaluate their properties, such as mean particles size, homogeneity, stability, drugs incorporation, *in vitro* release and antifungal activity against *C. albicans*.

## 2. Materials and methods

### 2.1. Materials

Glycerol Oleate (Tegin® O) was bought from ACEF (Piacenza, Italy). Polysorbate 80 (Tween® 80), didecyldimethylammonium bromide (DDAB), alpha-lipoic acid (ALA), RPMI 1640 (also known as RPMI medium) and Clotrimazole (CLZ) were purchased from Sigma Aldrich Co (St. Louis, MO, USA). Hydrogenated Coco-Glycerides (Softisan 100) was purchased from IOI Oleo GmbH (Oleochemicals, IOI group). All solvents were of HPLC grade and were bought from VWR International (Milan, Italy). Regenerated cellulose membranes (Spectra/Por CE; Mol. Weight Cut-off 3.5 Kda) were supplied by Spectrum (Los Angeles, CA, USA).

### 2.2. Nanoparticles preparation

SLN were prepared exploiting the low energy organic solvent-free phase inversion procedure (PIT method) as previously reported [19]. Both aqueous phase and lipid phase consisting of 9 wt. % of surfactant mixture of Tween 80/Glycerololeate (ratio 2:1) and 8 wt. % of solid lipid were separately heated until 80 °C. Then, the aqueous phase was added drop by drop to the lipid phase and the obtained formulation was stirred for 1 h. Briefly, DDAB (0.5 wt. % w/v) was added to the lipid phase during the nanoparticles preparation in order to obtain positively charged nanoparticles. CLZ (0.05 wt. % w/v) and ALA (0.25 wt. % w/v) were separately melted into the lipid phase before the addition of the aqueous phase.

### 2.3. Dynamic light scattering

The scattering intensity from Dynamic Light Scattering (DLS) measured by using Zetasizer Nano S90 (Malvern Instruments, Malvern, UK)

was exploited to determine the mean particle size (Z-Ave) and the polydispersity index (PDI), which provides the width of the particle sizes distribution in the colloidal suspension. DLS measurements were performed at a detection angle of 90°, at 25 °C, with a 4 mW He–Ne laser operating at 633 nm. Each value was measured at least in triplicate. Results are shown as mean  $\pm$  standard deviation (SD). The zeta potential values (ZP), which reflects the electric charge on the particle surface, was determined using the same equipment described previously at 25 °C. All samples were analyzed 24 h after the preparation, in order to allow the proper organization of lipids and surfactants into nanoparticles. Samples (50  $\mu$ L) were diluted in 1 mL of ultrapurified water, before DLS measurements.

### 2.4. Nanodispersions stability assessment using Turbiscan® AG Station

Stability studies were carried out using an optical analyzer Turbiscan® Ageing Station (TAGS, Formulation, L'Union, France), which is the TLAB equipped with an ageing station. Turbiscan® technology is based on static multiple light scattering for the analysis of concentrated dispersions. TAGS consists of a robot with three thermostated blocks for the storage of 54 samples. 10 mL of empty uncoated and DDAB-coated SLN were placed in a cylindrical glass cell and positioned in the Turbiscan® at three different storage temperatures: 25, 40 or 60 °C. The detection head was composed of a pulsed near-infrared light source ( $\lambda = 880$  nm) and two synchronous transmission (T) and back-scattering (BS) detectors. The T detector receives the light, which crosses the sample (at 180° from the incident beam). The detection head scanned the entire height of the sample cell (65 mm longitude), acquiring T each 40  $\mu$ m (1625 acquisitions in each scan).

### 2.5. Encapsulation efficiency and *in vitro* drug release estimation

Uncoated and DDAB-coated CLZ-ALA-SLN, were analyzed spectrophotometrically to evaluate the amount of the encapsulated drug. For each sample, the amount of the encapsulated drugs was determined after ultracentrifugation and dilution of the pellet in acetonitrile. The encapsulation efficiency (EE%) was calculated by the ratio between the amount entrapped inside the nanoparticles and the total amount of drug used for their preparation (Eq. (1)):

$$EE\% = (\text{amount of entrapped drug} / \text{total amount of drug used}) \times 100 \quad (1)$$

CLZ and ALA release from SLN was evaluated using Franz-type diffusion cells at 35.5 °C and stirring at 600 rpm. Before the experiment, the regenerated cellulose membrane was kept overnight in the receiving solution consisting in a mixture of physiological solution and ethanol (50:50 v/v). Each sample (500  $\mu$ L) was placed in the donor compartment. At different time intervals (2, 3, 4, 5, 6, 24, 48 h), 1 mL of the receptor phase was withdrawn and replaced with the same volume of fresh fluid. Each sample was analysed by UV spectrophotometer Shimadzu UV-1601 (Shimadzu Italy, Cornaredo, Italy) at two different absorption maxima ( $\lambda_{\text{max}}$ ), corresponding to 263 nm for CLZ and 325 nm for ALA. Drug quantification for drug release experiment was calculated as % of the amount of drug released at each time intervals in respect to the amount of entrapped drug. The procedure was performed at least in triplicate for each sample.

### 2.6. DSC analysis

A Mettler Toledo DSC 1 STARe system equipped with a PolyScience temperature controller (PolyScience Illinois, USA) was used to perform calorimetric analysis. The detection system was a HSS8 high sensitivity sensor (120 gold-gold/palladium-palladium thermocouples) and the ceramic sensor (Mettler Full Range; FR55) with 56 thermocouples. The signal time constant was 18 s and the digital resolution of the

measurement signal was less than 0.04  $\mu$ W. Resolution and sensitivity calorimetric determined by TAWN test were 0.12 and 11.9, respectively. The sampling rate was 50 values/second. The sensitivity was automatically chosen as the maximum possible by the calorimetric system, and the reference was an empty pan. The calorimetric system was calibrated, in temperature and enthalpy changes, by using indium by following the procedure of the DSC 1 Mettler TA STARE instrument. Raw materials, unloaded SLN, CLZ-loaded SLN, CLZ-ALA-loaded SLN and cationic cSLN were analysed. Samples were placed in a small aluminium pan and submitted to DSC analysis. The raw ingredients, empty and drug-loaded SLN were scanned in the temperature range of 25–220 °C with a speed of 5 °C/min. The results were evaluated by the heating scan. Transition temperature was calculated from peak areas with Mettler STARE Evaluation software system (version 13.00) installed on Optiplex 3020 DELL. Each experiment was performed in triplicate.

## 2.7. Determination of the antifungal activity

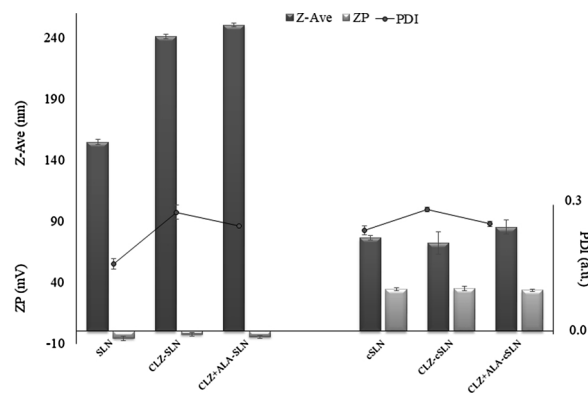
The antifungal activity of negative or cationic unloaded, CLZ-loaded and CLZ-ALA co-loaded SLN, was evaluated on 25 clinical strains of *C. albicans* previously isolated from vaginal swabs (collection of the Laboratory of Applied Microbiology, Department of Biomedical and Biotechnological Sciences, Università degli Studi di Catania, Italy), in comparison with an aqueous solution of CLZ. Minimum inhibitory concentration (MIC) was determined through the broth microdilution technique according to the recommendations stated in Clinical and Laboratory Standards Institute (CLSI) document [40]. Drug-loaded-SLN were tested at a concentration of drug equivalent to the free drug solution, thus making direct comparisons of the possible outcomes to evaluate the effectiveness of nanocarriers. Briefly, the inoculum was prepared by suspending colonies obtained from an overnight culture on non-selective nutritive agar medium in sterile distilled water. Suspensions were adjusted to  $1.0 \times 10^6$  CFU/mL and then diluted to obtain a concentration of  $1.0 \times 10^4$  CFU/mL in RPMI 1640 (also known as RPMI medium company). A mixture of 100  $\mu$ L of *C. albicans* suspensions in RPMI broth (final concentration  $0.5 \times 10^3$  CFU/mL) and 100  $\mu$ L of fresh medium were added to each well of a sterile 96-well microplate (Thermo Scientific™ Sterilin™, Waltham, MA, USA). The test range for free drug and drugs-loaded SLN was from 16 to 0.03  $\mu$ g/mL. Plates were incubated at 35 °C for 24 and 48 h, two readings at 530 nm were recorded, respectively. This assay was repeated six times for each formulation in order to ensure the reproducibility of the results.

## 2.8. Statistical analysis

All data are reported as mean values  $\pm$  sd. Differences, analyzed by Student's *t*-test using the Origin Software (version 8.5.1), were considered statistically significant when  $p < 0.05$ .

## 3. Results and discussion

The idea of delivering CLZ and ALA, separately incorporated into SLN suspensions (so-called dual-drug delivery system), finds its rationale in the possible interaction between drugs, that could limit their activity. In order to support the rationale of the work, firstly, DSC analysis of the two selected drugs (CLZ, ALA) and their physical mixture were performed, using the same proportion used in the study (Supplementary Fig. 1). As a result, the melting points of CLZ and ALA at 145.01 °C and 63.13 °C were noticed, respectively, confirming literature data. Furthermore, the thermogram of CLZ + ALA physical mixture revealed the absence of the endothermic peak of CLZ and a shift of the endothermic peak of ALA. These findings suggest the occurrence of interaction between the two drugs [41]. Thus, the potential separately dual-delivery would offer a promising strategy in order to take profit of the drugs combination avoiding the chemical interaction



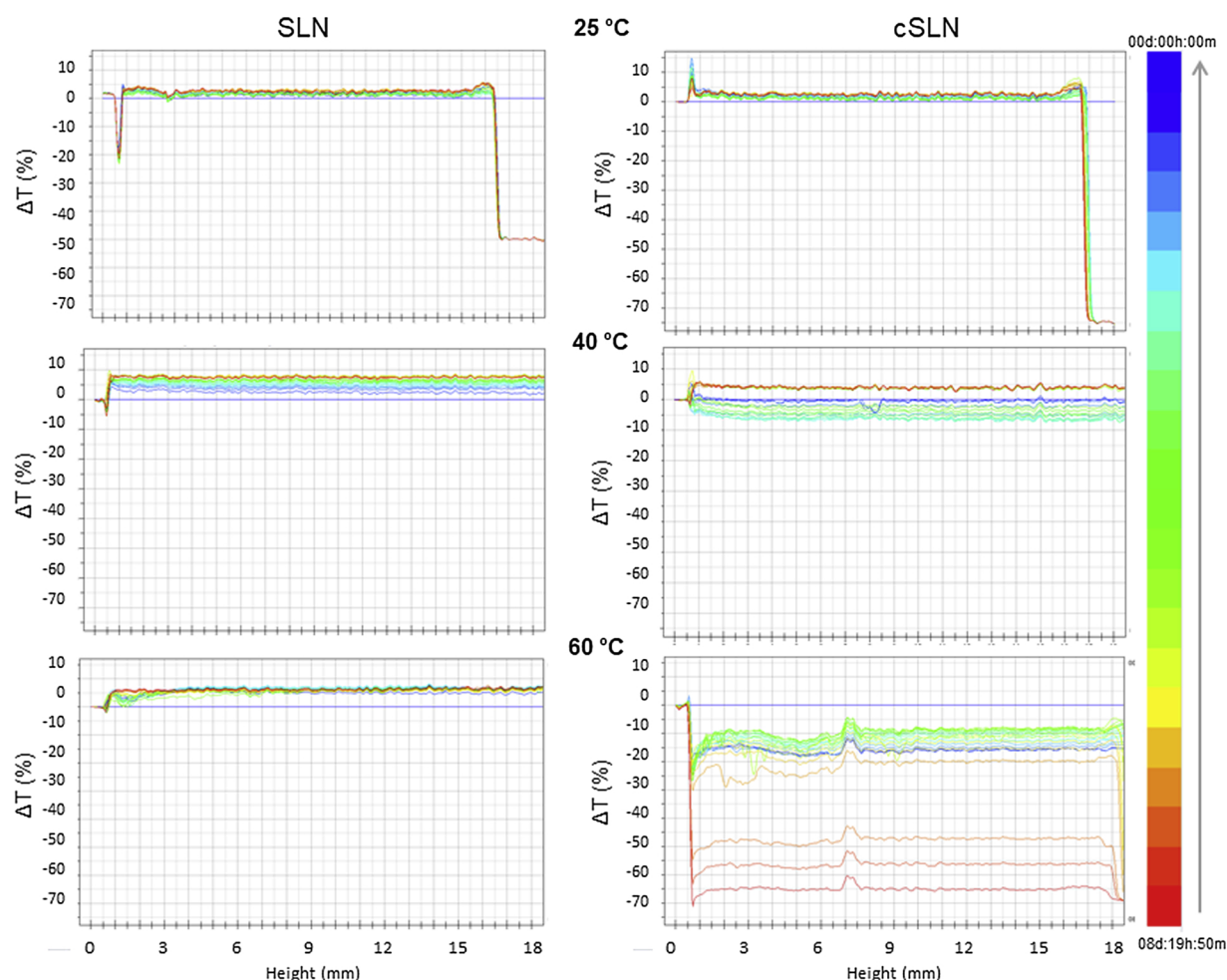
**Fig. 1.** Mean particles size (Z-Ave, [nm]), polydispersity index (PDI, [a.u.]) and zeta potential (ZP, [mV]) of neutral and cationic SLN: unloaded (SLN and cSLN), Clotrimazole-loaded (CLZ-SLN and CLZ-cSLN) and Clotrimazole-lipoic acid loaded (CLZ-ALA-SLN and CLZ-ALA-cSLN). Each measurement represents the mean value  $\pm$  standard deviation (SD),  $n = 6$ .

and enhancing their potential synergistic effects. Due to the high drug lipophilicity (log *P* values 0.5 and 2.1 for CLZ and ALA, respectively), SLN were selected as the most desirable nanocarriers for obtaining CLZ + ALA dual-delivery system.

The mean particles size, PDI and ZP values of unloaded and drugs-loaded SLN, unloaded and cationic SLN (cSLN), has been shown in Fig. 1. Based on the obtained results, it has been noticed that unloaded SLN have a narrow size distribution (PDI < 0.25) of particles of mean size below 150 nm, with slight negative (c.a. - 5 mV) and highly positive (c.a. + 35 mV) ZP values for SLN and cSLN, respectively. On the other hand, based on previous findings related to the effect of DDAB on lipid nanoparticles [39], it has been proved that the addition of the cationic lipid coating may significantly reduce the mean particle size, from 150 nm for SLN to about 75 nm in the case of cSLN (Fig. 1). This can be explained by a different ability of the cationic lipid to interact at the interface of nanoparticles depending on the qualitative composition of surfactants and lipids. Furthermore, as we previously demonstrated, the presence of DDAB can significantly improve nanoparticles uptake, thus enhancing drug effectiveness in the intracellular compartment [39].

Both drugs were loaded into SLN with similar EE% values, 77.86 and 80.63 % for CLZ and ALA, respectively. The addition of the drugs resulted in SLN with a significant increase of Zave and PDI values ( $p > 0.05$ ). In particular, the addition of CLZ alone or in combination with ALA led to the formation of particles whose size values increased from 150 to c.a. 250 nm (Fig. 1). In addition, PDI values showed a slight increase even if the value was found to be < 0.3, thus confirming the homogeneity of the nanoparticles. Interestingly, positively charged cSLN did not modify their size, maintaining values lower than 100 nm, even when CLZ or CLZ + ALA were loaded (Fig. 1). Drug incorporation into the SLN showed no influence on ZP values ( $p < 0.05$ ), indicating a homogeneous distribution of the drug with the lipid matrix, with EE% values of 96.66 % and 60.66 % for CLZ and ALA, respectively, whose difference could be attributed to the greater insolubility of the last one.

cSLN had higher ZP values compared to the uncoated system, which provides an adequate electric repulsion for assuring a high stability of the dispersions. In order to verify this hypothesis, Turbiscan® technology was exploited to evaluate the stability of unloaded uncoated (SLN) and cationic (cSLN) nanoparticles at three different temperatures (25, 40 and 60 °C) for 15 days. Fig. 2 clearly shows the PIT method allowed producing highly stable dispersions both in the presence or not of DDAB since no variations in the transmission profile were observed for both samples. A slight instability related to nanoparticles aggregation was revealed for samples stored at 40 °C (Fig. 2), even if the phenomenon was not significant due to the  $\Delta T$  values lower than 5 % in



**Fig. 2.** Turbiscan® transmission profile ( $\Delta T$ ) of empty neutral (SLN) and cationic (cSLN) nanosuspensions stored at 25, 40 and 60 °C. Data are represented as a function of time (0–8 days) of sample height (0–20 mm). The sense of analysis time is indicated by the arrow.

both samples. Increasing storage temperature to higher value (60 °C) caused a different behaviour, since important instability phenomena occurred in uncoated SLN (coalescence and flocculation), while cSLN showed a higher stability probably due to the presence of the cationic coating layer onto nanoparticles surface responsible of higher electric repulsion forces.

Thermal analysis was exploited to investigate the thermal behaviour of the prepared SLN. The thermograms of empty SLN, CLZ-loaded SLN and CLZ-ALA-loaded SLN as well as cSLN are reported in Supplementary Fig. 2. In the Supplementary Fig. 2A, the thermogram of uncoated SLN revealed a single endothermic peak related to the solid lipid. The presence of the drug, surfactant and co-surfactant incorporated into SLN did not modify the fusion temperature compared to empty SLN. These results proved that the drug was completely solubilised inside SLN lipid matrix and it did not crystallize. The calorimetric curve of Softisan was characterized by the presence of a peak at 40.63 °C due to the melting of the solid lipid. Table 1 shows the melting temperatures of raw materials and either empty or drug-loaded SLNs. Empty SLN presented well-defined peaks for lipid fusion, with a slight shift to lower temperatures and slightly broader compared to the bulk material's signals [26]. Therefore, based on the results reported by Laserra S. et al. [42], we can infer that SLN were obtained, since the shift of the melting temperature at lower temperature demonstrated that the solidification process occurred. CLZ addition affects the solidification process of the lipid matrix during SLN formation. Thereupon, the melting transition could also be broadened due to the fractions of different particle sizes, which melt at different temperatures. Indeed, a

**Table 1**

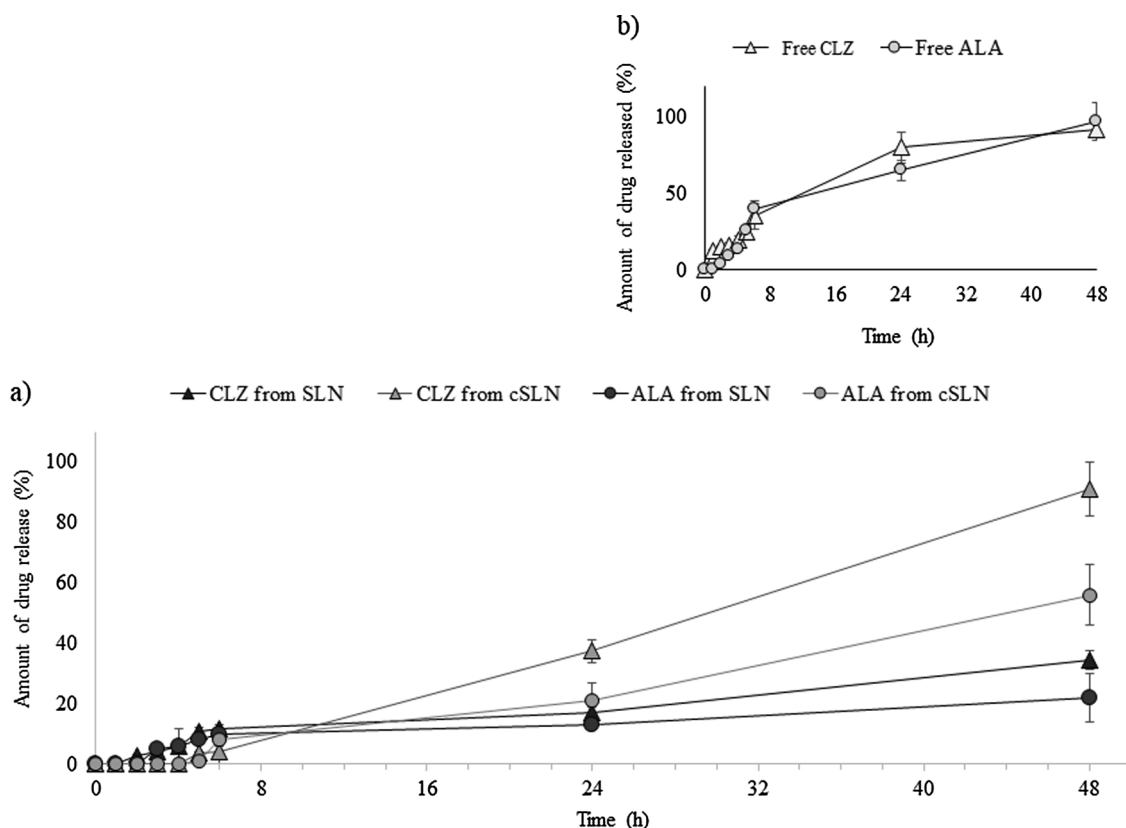
DSC melting temperature ( $T_m$ ) of raw materials, CLZ-loaded and CLZ-ALA loaded neutral SLN and cationic cSLN.

Samples	$T_m$ of the first principles (°C)	$T_m$ of the first peak (°C)	$T_m$ of the second peak (°C)
Softisan	41.18	–	–
ALA	63.13	–	–
CTZ	145.01	–	–
Glycerol oleate	35.32	–	–
SLN	–	–	40.63
CLZ-SLN	–	32.79	40.62
CLZ-ALA-SLN	–	32.83	41.37
cSLN	–	31.89	40.29
CLZ-cSLN	–	32.92	40.68
CLZ-ALA-cSLN	–	34.44	42.71

slight increase of PDI of loaded SLN compared to unloaded systems were evidenced. The addition of the cationic layer did not significantly affect the thermal properties of empty SLN, as well as CLZ or CLZ-ALA-loaded-SLN. Similar findings were observed for cationic SLN, both unloaded or drug loaded, which showed a similar behaviour compared to uncoated systems (Supplementary Fig. 2B).

The *in vitro* release profile of CLZ and ALA from the co-loaded SLN have been depicted in Fig. 3a. As shown in Fig. 3b, CLZ + ALA as free drugs showed a fast diffusion, while their nanoencapsulation provided a controlled release rate (Fig. 3a). As widely reported in literature, the burst effect is related to the occurrence of adsorption phenomena due to





**Fig. 3.** Percentage (wt.%) of Clotrimazole (CLZ) and alpha-lipoic acid (ALA) released in co-administration from neutral SLN and cationic cSLN (a) at different time intervals until 48 h. In (b) the free drugs release is reported.

the presence of drug molecules on the nanoparticles surface [43–45]. In our study, both drugs were well accommodated into the lipid inner matrix of SLN, as suggested by DSC results. The avoidance of drug adsorption phenomena was confirmed by the slow and constant release observed for both uncoated and cationic SLN, without the occurrence of burst effects in the early stage of the study. In particular, a slower release was observed for ALA from both systems, in respect to neat CLZ, thus suggesting that drug release from SLN was not affected by drug affinity for the lipidic phase. It is possible that the lower molecular weight and steric hindrance of ALA molecules allows a stronger interaction of the drug molecule with the other components of the SLN, thus providing a promising prolonged release for the topical administration, with the advantage to protect the reduction of reactive oxygen species during the treatment with the antifungal drug.

Antifungal activity defined as MIC values, i.e. the prominent decrease in turbidity corresponding to approximately 50 % inhibition of cell growth, was determined spectrophotometrically. Results reported in Table 2 showed that the effects of all samples did not change after 24

or 48 h of treatment. As expected, empty SLN and cSLN did not exhibit any antimicrobial effect. Concerning CLZ-loaded SLN, the uncoated system showed a similar behaviour compared to the free drug, thus confirming that the drug did not modify its antimicrobial activity when loaded into lipid nanoparticles. It is worth to highlight that the cationic CLZ-cSLN proved halved MIC values in respect to other samples in all tested strains of *C. albicans*. Comparing the obtained results with literature findings, it can be inferred that SLN prepared in our study represent a promising nanocarrier on the basis of MIC evaluation [12] as well as of gain by using DDS [46]. Furthermore, the antimicrobial activity of CLZ was not altered in presence of the co-delivered ALA (data not reported), thus confirming the possibility to exploit the potential beneficial protective effect of ALA, which will be further investigated.

#### 4. Conclusions

According to the findings presented in this study, lipid nanoparticles were successfully developed as dual drug delivery system for the treatment of topical infections related to *C. albicans*. In particular, uncoated and cationic CLZ-ALA-loaded SLN were obtained with high homogeneity, reduced mean particles size and high physical stability, as confirmed by Turbiscan® results of samples stored at 25 °C. CLZ and ALA were effectively incorporated into the lipid inner matrix of SLN, from which they released slowly and constantly, without the occurrence of burst release in the early stage of analysis. Results of the antimicrobial activity study proved that CLZ, even when loaded in combination with ALA, did not alter its antimicrobial effectiveness. DSC results showed the occurrence of interactions between the two drugs when combined in mixture, thus giving the rationale of the dual-delivery strategy exploited. Furthermore, calorimetry studies confirmed that the solidification process of SLN occurred. Therefore, the dual-delivery SLN herein developed would offer a promising strategy in order to take profit of the potential beneficial protective effect of ALA in the treatment of candidiasis.

**Table 2**

*In vitro* antimycotic activity of SLN (neutral and cationic) unloaded or loaded with CLZ evaluated at 24 and 48 h against 25 clinical strains of *Candida albicans*.

Samples	(μg/ml) 24 h		(μg/ml) 48h	
	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>
Free CLZ	≤ 0.03	≤ 0.03	≤ 0.03	≤ 0.03
SLN	No inhibition	No inhibition	No inhibition	No inhibition
cSLN	No inhibition	No inhibition	No inhibition	No inhibition
CLZ-SLN	0.03	0.03	0.03	0.03
CLZ-cSLN	0.015	0.015	0.015	0.015

MIC - Minimum Inhibitory Concentration.

## Author contributions

Carbone Claudia conceived and designed the experiments, performed and optimized the formulations, performed the chemico-physical and technological characterization and financed the project. Virginia Fuochi, Giulio Petronio Petronio and Pio Maria Furneri performed the *in vitro* experiments on *Candida* species. Teresa Musumeci performed DSC studies. The manuscript was drafted by Claudia Carbone, Aleksandra Zielińska and Teresa Musumeci. The manuscript appears in its final form by the contributions from Angela Bonaccorso, Carmelo Puglia and Eliana Maria Barbosa Souto.

## Declaration of Competing Interest

The authors declare no conflict of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.colsurfb.2019.110705>.

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