Freie Universität Berlin, Department of Pharmaceutics, Biopharmaceutics & NutriCosmetics, Berlin, Germany

Preservative system development for argan oil-loaded nanostructured lipid carriers

A. HOMMOSS

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Aiman Hommoss, Arab International University (AIU), College of Pharmacy, Ghabaghib, Daraa-Damascus Highway, Damascus, Syria
a.hommoss@gmail.com

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Nanostructured lipid carriers (NLC) are used in many dermal cosmetic formulations. To prevent microbiological spoilage of NLC suspensions preservative systems must be used. Preservatives can impair the physical stability of NLC suspensions. Therefore, a systematic screening of preservative systems should be performed and the compatibility of these preservative systems with each NLC formulation has to be investigated. In this study three Argan oil-loaded NLC formulations were developed. Ethanol, propylene glycol and pentylene glycol were admixed to these formulations as preservative systems. The physical stability of the non-preserved and preserved formulations has been investigated. Upon admixing 20% w/w ethanol to the selected formulations, immediate particle aggregation could be detected using laser diffractometry and after 24 hours gelling occurred. This was accompanied with a lowering of Zeta potential value. Samples preserved with 10% w/w propylene glycol did not show any change in particle size or in Zeta potential, in comparison to the non-preserved formulation, when measured after one day and 120 days. Samples preserved with 5% pentylene glycol proved also to be stable after 120 days and did not show any change in particle size or Zeta potential.

1. Introduction

Nanostructured lipid carriers (NLC) are a well known delivery system for cosmetics and pharmaceuticals. They are the second generation of lipid nanoparticles, where the matrix of particles consists of a blend of solid and liquid lipids. This matrix is solid at body temperature like the matrix of the first generation of lipid nanoparticles, the solid lipid nanoparticles (SLN) (Müller et al. 2007a; Pardeike et al. 2009). The advantages of the NLC compared to the SLN are the higher loading capacity with actives and firmer inclusion of the active inside the particle matrix over a longer period of time (Müller et al. 2007b, 2002a, 2002b). Also the positive effects of NLC on the skin like increasing skin hydration and enhancing the penetration of actives into the skin as well as the protective activity against UV light. All of these properties made the way for this delivery system easier to enter the market as a successful delivery system (Müller et al. 2007a).

During product development, the physical and chemical stabilities as well as the microbiological stability of product should be guaranteed during its shelf life. The NLC, and due to the method they are prepared by (high pressure homogenization at high temperatures), are produced with a reduced microbiological contamination (Pereda et al. 2007; Lucks and Müller 1991, 1995, 2007c; Diels and Michiels 2006). On the other hand, NLC are usually neither produced under aseptic conditions, nor filled in sterile single-use containers. Therefore, and due to the relatively high water content in the NLC, a suitable preservative system should be added to the NLC suspension.

Moreover, the NLC suspension can be produced so that it is a concentrate of active compounds, which can be added as a constituent in final product formulations. In cosmetic industry NLC suspensions are usually admixed to the other constituents of the cream to obtain the desired concentration of the enclosed material in the NLC (Petersen et al. 2006; Müller et al. 2007b). Surely, the final product is stabilized with a preservative to guarantee that there will be no spoilage of the product during storage and use. Hence, the NLC suspension will be in direct contact with the preservative system in the final product.

It is known that preservatives could destabilize dispersed systems like emulsions, suspensions and liposomes (Gallardo et al. 1991; Sznitowska et al. 2002). It is also expected that instability could happen when the preservative system comes into contact with the NLC dispersion. Therefore, it is very important to insure that the NLC and the preservative system are compatible with each other and no destabilization will occur to the NLC, regarding their integrity or particle size, due to the preservative presence.

Some studies were already performed to understand the possible interactions between the NLC particles and the different preservatives used (Obeidat et al. 2010). This work is a continuation of what was started. In this study Argan oil-loaded NLC formulations were developed and suitable preservative systems for these formulations were chosen after studying the effect of different preservative systems.

2. Investigations, results and discussion

2.1. Production of Argan oil-loaded NLC

Twelve formulations were prepared. The lipid phase consisted of 12% w/w solid lipid, either Cutina CP or Cutina HR, and

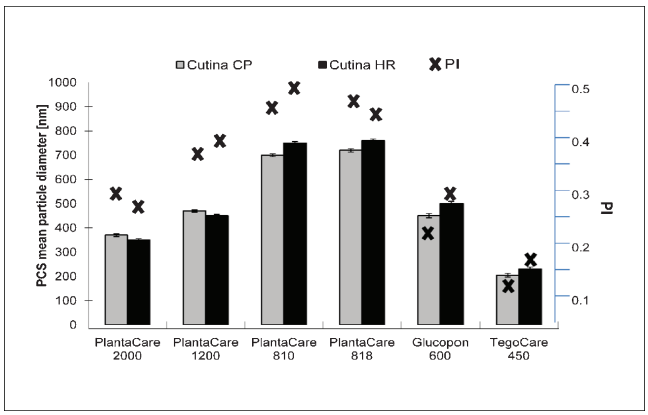

Fig. 1: The PCS mean particle size and the polydispersity index (PI) of the Cutina CP and Cutina HR NLC formulations stabilized with different surfactants

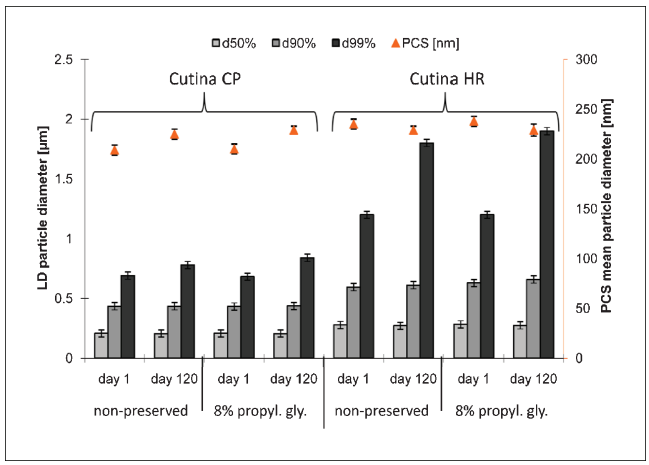

Fig. 2: The PCS and LD particle size measurements of the NLC formulations consisting of 8% w/w Argan oil and 12% w/w Cutina CP or Cutina HR, non-preserved and preserved with 8% w/w propylene glycol

8% w/w Argan oil and stabilized with one of the six surfactants mentioned in 3.1.

Not all the surfactants resulted in liquid suspensions or particles of small particle size. The most suitable surfactant was TegoCare 450 with a PCS mean particle size of 205 nm and 230 nm for particles made of Cutina CP and Cutina HR respectively. The polydispersity index was below 0.2 for both formulations indicating a narrow particle size distribution (Fig. 1). Moreover, all NLC stabilized with surfactants other than TegoCare 450 gelled on the next day giving a semisolid mass. This is because the surfactants used are suboptimal for the stabilization of the NLC hence, aggregation and gelling occur (Lippacher et al. 2002, 2004; Westesen and Siekmann 1997). Moreover, these surfactants are designed to stabilize creams and have thickening agent properties. Therefore, they increase the viscosity of the emulsion formed.

Another formulation was produced and added to these two formulations. This was a formulation containing 8% Argan oil and 12% w/w solid lipid mixture of the two lipids Cutina CP and Cutina HR at a ratio 1:1. The PCS mean particle size of this formulation was about 220 nm. The particle size of the NLC composed of these two lipids together is between the particle sizes of the NLC suspensions composed of each of the two lipids separately. By maintaining the homogenization pressure, the number of homogenization cycles and the concentration of the stabilizer the same, the only variable that influences the particle size is the viscosity of the lipid melt. When the viscosity decreases the particle size of the resulted homogenized emulsion will be smaller. Because the viscosity of the lipids mixture melt is between the viscosity of the melts of the two lipids separately, the resulted particle size of the new NLC was between the particle size of the Cutina CP NLC and Cutina HR NLC (Hommoss 2009).

The Zeta potentials in water for the three formulations stabilized with TegoCare 450 were all about -60 mV (Table 1). According to Riddick, a stable dispersion should have a Zeta potential larger than |30| mV (Müller 1996). The Zeta potential values in water indicate high Stern potentials and thus a good electrostatic repulsion of the NLC particles (Müller 1996).

2.2. Admixing preservatives to the Argan oil-loaded NLC formulations

The formerly mentioned selected formulations were admixed to different preservative systems. At the beginning ethanol 96% was admixed to the three formulations prepared (Cutina CP NLC, Cutina HR NLC and Cutina CP/Cutina HR NLC) at a concentration of 20% w/w to the total NLC formulation. Directly after admixing the ethanol to the three NLC formulations a change in the viscosity was noticed. After 24 h the formulations became semisolid and could not be poured out of the container. Upon measuring the Zeta potential of these formulations in water with a conductivity adjusted to 50 µS/cm, it was noticed that the absolute value of the Zeta potential was lower than the according Zeta potential of the non-preserved samples (Table 1).

Measuring the Zeta potential in the original dispersion medium is a measure of the thickness of the diffusion layer (Müller 1996). The lower the Zeta potential, the thinner the diffusion layer and the less stable the dispersion. By measuring the value of the Zeta potential for the three NLC formulations in a 20% ethanol solution it was noticed that the Zeta potential values are about -20 mV (Table 2). Theoretically, the Zeta potentials of the ethanol preserved dispersions are below the value of Zeta potential for stable dispersions (|30| mV). Moreover, Zeta potential is not the only stability determining parameter. Ethanol is a lipophilic preservative and has the ability to adsorb onto the surface of the NLC and to dissolve the surfactant from the surface of the lipid particles. It is also known that ethanol can compete with the stabilizing surfactant for the "hydration water". At a relatively high concentration of ethanol (20% w/w) this can result in a partial dehydration of the surfactant. All of this can lead to the destabilization of the particles and hence the aggregation and gelling occur (Obeidat et al. 2010).

Propylene glycol was admixed to the three formulations stabilized with TegoCare 450 at a concentration of 8% w/w to the total NLC formulation. After 120 days a growth in particle size of the non-preserved Cutina HR NLC was detected. The d99% grew from about 1.2 µm on day one to about 2 µm on day 120 (Fig. 2). This might be due to the recrystallization of the solid lipid to the more stable crystal form, which usually leads to an increase in particle size (Freitas and Müller 1999).

On day one the Zeta potential values, in water with a conductivity adjusted to 50 µS/cm, of the Cutina CP and Cutina HR NLC formulations admixed to 8% w/w propylene glycol were the same as the Zeta potential values of the according non-preserved formulations. Also after 120 days no change was detected in these formulations regarding particle size or Zeta potential in compare to the measured values on day one (Fig. 2 and Table 1). This is because propylene glycol is a hydrophilic preservative and has no tendency to adsorb to the surface of the particles and impair their stability. On the contrary, propylene glycol will dissolve within the water phase. Therefore, the NLC dispersions are not impaired by this preservative. Moreover, the Zeta potentials measured in the original dispersion solution (containing 8% w/w propylene glycol) of these two formulations were slightly

Table 1: Zeta potential, measured in 50 µS/cm conductivity water, of the preserved and non-preserved NLC at day 1 and after 120 days

Formulation	Preservative	Preservative %	Day 1	Day 120
Cutina CP	–	–	–63±5	–65±7
	Ethyl alcohol	20%	–30±5	–
	Propylene glycol	8%	–64±5	–63±6
Cutina HR	–	–	–61±3	–60±4
	Ethyl alcohol	20%	–32±6	–
	Propylene glycol	8%	–61±5	–63±7
Cutina CP/Cutina HR	–	–	–58±4	–60±6
	Ethyl alcohol	20%	–29±5	–
	Propylene glycol	6%	–59±6	–58±8
		8%	–59±5	–57±6
		10%	–61±5	–62±5
	Pentylene glycol	1.5%	–62±3	–60±6
		3%	–57±5	–59±4
		5%	–58±8	–59±7

Table 2: Zeta potential, measured in original solution, of the preserved and non-preserved NLC at day one and after 120 days (non-preserved samples were measured in 50 µS/cm conductivity water)

Formulation	Preservative	Preservative %	Day 1	Day 120
Cutina CP	–	–	–63±5	–65±7
	Ethyl alcohol	20%	–20±5	–
	Propylene glycol	8%	–31±4	–34±4
Cutina HR	–	–	–61±3	–60±4
	Ethyl alcohol	20%	–23±4	–
	Propylene glycol	8%	–32±4	–33±4
Cutina CP/Cutina HR	Propylene glycol	–	–58±4	–60±6
	Ethyl alcohol	20%	–17±5	–
		6%	–33±4	–31±7
		8%	–32±5	–31±6
		10%	–33±4	–36±6
	Pentylene glycol	1.5%	–29±5	–31±4
		3%	–32±3	–34±5
		5%	–33±5	–35±4

bigger than |30| mV (Table 2), indicating thick diffusion layers and stable dispersions according to Riddick (Müller 1996).

The formulation containing the mixture of the two lipids Cutina CP/Cutina HR was expected to be more stable, knowing that the re-crystallization of the solid lipids can be hindered due to the presence of two different lipids (Üner et al. 2005; Bunjes and Koch 2005; Müller et al. 2002b). During the study period Cutina CP/Cutina HR NLC formulation did not show any sign of physical instability. The particle size on day 120 was the same as on day one (Fig. 3). Propylene glycol was added to this formulation in three different concentrations (6%, 8% and 10% w/w to the total NLC formulation) and the particle sizes of these preserved preparations were assessed at defined time points. After 120 days all preserved preparations with propylene glycol did not show a change in the particle size nor in the Zeta potential. The Zeta potential was measured in both water with a conductivity adjusted to 50 µS/cm and in original preservative solution (Table 1 and Table 2). This indicates good physical stability also after adding propylene glycol at high concentrations such as 10% w/w to the total NLC formulation.

The preservative pentylene glycol was also added to the Cutina CP/Cutina HR formulation in three different concentrations (1.5%, 3% and 5% w/w to the total NLC formulation) and the

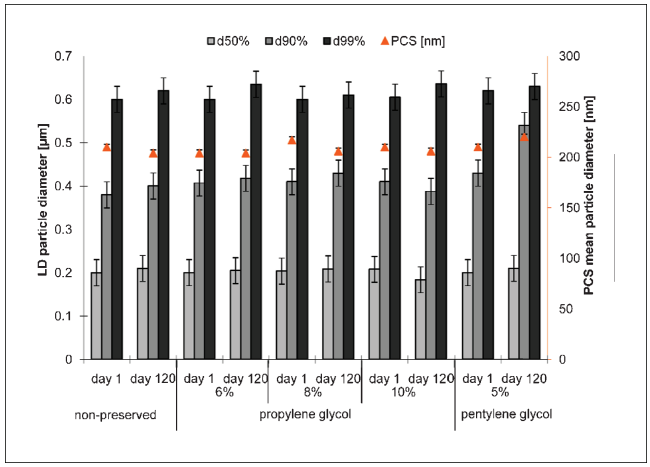

Fig. 3: The PCS and LD particle size measurements of the NLC formulations consisting of 8% w/w Argan oil and 12% w/w Cutina CP/Cutina HR non-preserved, preserved with 6%, 8% and 10% w/w propylene glycol and 5% w/w pentylene glycol

particle size of these preserved NLC preparations was assessed. After 120 days all the preparations with the three concentrations of the pentylene glycol did not show a change in the particle size nor in the Zeta potential indicating good physical stability. Figure 3 shows the PCS and LD particle size measurements results for the formulation containing 5% w/w pentylene glycol on day 1 and after 120 days. The Zeta potential measurements are listed in Tables 1 and 2. Pentylene glycol is a hydrophilic preservative like propylene glycol. Therefore, it has no tendency to adsorb to the surfaces of the particles and destabilize them; instead it will dissolve within the water phase without impairing the lipid particles stability.

2.3. Conclusion

Argan oil-loaded NLC formulations were produced by hot high pressure homogenization. The most suitable surfactant with the solid lipids used was TegoCare 450. The formulation containing a mixture of the two lipids Cutina CP and CUtina HR was the most physical stable formulation. Adding ethanol 96% at a concentration of 20% w/w impaired the physical stability of the original NLC dispersions. This impairment was by affecting the Zeta potential of the particles and by adsorbing on the particles surface. Propylene glycol and pentylene glycol, having high hydrophilicity and no tendency to adsorb on the NLC surfaces or to change their Zeta potential, were suitable preservative systems which did not interfere with the physical stability of the NLC dispersions.

3. Experimental

3.1. Materials

Argan oil was provided by Cognis GmbH, Germany. The solid lipids Cutina CP and Cutina HR as well as the surfactants PlantaCare 2000, PlantaCare 1200, PlantaCare 810, PlantaCare 818, Glucopon 600 and TegoCare 450 were all provided by Cognis GmbH, Germany. Ethanol 96% and propylene glycol were purchased from CG Chemikalien GmbH, Germany. Pentylene glycol was purchased from Cosnaderm Chemische Rohstoffe GmbH, Germany. As a dispersion medium, freshly prepared double distilled and ultra purified water Milli-Q water, Millipore GmbH, Germany was used.

3.2. Methods

3.2.1. Production of Nanostructured lipid carriers

The non-preserved formulations composed of 8% w/w Argan oil, 12% w/w solid lipid (Cutina CP, Cutina HR or a 1:1 mixture of the two lipids), 2.5% w/w of one of the surfactants mentioned above and 85.6% w/w Milli-Q water.

The surfactant was dispersed in Milli-Q water and the Argan oil was added to the solid lipid in a separate beaker. Both the lipid and aqueous phases were heated up to 90 °C. The lipid phase was dispersed in the aqueous phase using a high speed mixer, Ultraturrax T25 (Janke and Kunkel GmbH, Germany), for 30 s at 8000 rpm. The obtained emulsion was passed through a temperature-controlled high pressure homogenizer, Micro LAB 40 (APV Deutschland GmbH, Germany) having a capacity of 40 ml in discontinuous mode applying 2 cycles at 800 bar. The obtained hot o/w nanoemulsion was filled in silanized glass vials, which were immediately sealed. A water bath adjusted to 15 °C was used to control the cooling rate of the nanoemulsion and hence the solidification of the NLC (Pardeike et al. 2009; Petersen et al. 2006). The samples were stored at room temperature in the dark for a period of 120 days.

The effective concentrations of the preservatives used were not the same. Therefore, the Argan oil-loaded NLC were produced as a concentrate with reduced water content equal to the highest amount of the preservative to be added. The preservative was added to the NLC concentrate after cooling down the NLC to room temperature. The composition was completed to 100% w/w with Milli-Q water if the preservative concentration was less than the reduced water content at production. Gentle stirring was performed while adding the preservative successively.

3.2.2. Characterization of Nanostructured lipid carriers

Photon correlation spectroscopy (PCS) was performed using a Zetasizer Nano ZS (Malvern Instruments, UK). The analysis yields the z-average of the sample, which is the intensity weighted mean diameter of the bulk population. The polydispersity index (PI) is a measure of the width of the size distribution. The NLC samples were diluted in distilled water and measurements were performed at 25 °C temperature (Müller and Schuhmann 1996).

Laser diffraction was performed using the Mastersizer 2000 (Malvern Instruments, UK) in deionized water as dispersion medium. The instrument was operated with the Hydro S sample dispersion unit. LD yields volume weighted diameters d50%, d90% and d99% as characterization parameters. The results were analyzed using Mie theory with optical parameters 1.456 real refractive index and 0.01 imaginary refractive index of the lipid nanoparticles.

The surface charge of the particles was assessed by Zeta potential measurements using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK) applying a field strength of 20 V/cm at 25 °C. The Helmholtz–Smoluchowski equation was used for the Zeta potential calculation built into the Malvern Zetasizer software. The zeta potential of particles depends on the dispersion medium. Therefore, the surface charge has been measured in Milli-Q water adjusted to a conductivity of 50 μS/cm using 0.9% NaCl w/v solution and in the original dispersion medium of each sample (the corresponding preservative solution).

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