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Minoxidil-loaded nanostructured lipid carriers (NLC): characterization and rheological behaviour of topical formulations

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Lipid nanoparticles are used as biocompatible carriers for several types of drugs intended for pharmaceutical, cosmetic, and biochemical purposes. The wide range of lipids and surfactants available for the production of such particles turns these carriers highly suitable for distinct applications (topical, dermal and transdermal, parenteral, pulmonary, and oral administration). This work describes the development of a special type of lipid particles, namely nanostructured lipid carriers (NLC), for minoxidil as an alternative to conventional topical alcoholic solutions. NLC were composed of stearic acid and oleic acid, being the matrix stabilized with poloxamer 188 in aqueous dispersion. To develop a suitable topical formulation, lipid dispersions were further mixed with freshly prepared Carbopol or perfluorocarbon based hydrogels. Minoxidil-loaded NLC were approximately 250 nm in size before the entrapment within the gel network and remained below 500 nm after mixing with both types of hydrogels. The occurrence of minoxidil crystallization in the aqueous phase of lipid dispersions was discarded under analysis by light microscopy and by scanning electron microscopy. Differential scanning calorimetry was used to assess the recrystallization index (i.e. measure of the percentage of lipid matrix that is crystallized) of the particles, which was shown to be 62% for minoxidil-free dispersions and 68% for minoxidil-loaded NLC dispersions. Rheological analysis of hydrogels containing NLC dispersions showed typical pseudoplastic behaviour which makes them suitable for topical purposes.

1. Introduction

Minoxidil was introduced in the 1970s as an oral antihypertensive agent (Limas 1973; Mehta 1975; Dargie et al. 1977; Swales 1982). However, its major clinical attraction has been related to its common side effect on the promotion of hair growth, i.e. hypertrichosis (Zappacosta 1980). In the last 20 years, minoxidil has been widely used for the topical treatment of androgenic alopecia in men and subsequently in women (Messenger and Rundegren 2004). The scientific literature describes several methods to increase drug permeation through the stratum corneum since its main function is related to barrier properties. The different pathways for a drug throughout the stratum corneum are: the intercellular routes existing between corneocytes, the intracellular routes through the corneocytes and the enveloping lipids, and the appendage routes, i.e. sweat ducts and hair follicles. To stimulate hair growth, topical minoxidil needs to act on the hair follicle. Nonetheless, a clear elucidation about its mechanism of action in humans is still rather limited. In animal studies, topical minoxidil has shown to short the resting phase (telogen), which causes the premature entry of resting hair follicles into the growing phase (anagen) (Messenger and Rundegren 2004).

Because of the lipophilic character of this drug, conventional topical formulations consist of propylene glycol-water-ethanol solutions (Tata 1995). Applications of such formulations may cause severe adverse reactions, such as scalp dryness, irritation, burning, redness and allergic contact dermatitis (Tosti 1985; Fiedler-Waiss 1987; Westesen and Wehler 1992; Whitmore 1992; Pavithran 1993; Scheman 2000). To minimise these side effects and to improve therapeutic efficiency of minoxidil, the development of new systems for topical delivery of such a drug is a demand. Minoxidil has already been formulated in colloidal systems to be delivered through the appendage routes, e.g. micelles, liposomes, niosomes, and polymeric nanoparticles (Ciotti and Weiner 2002; Shim et al. 2004; Mura et al. 2007). Other promising topical drug delivery vehicles are the aqueous dispersions of lipid nanoparticles (Souto 2004). These systems are derived from o/w emulsions by replacing the liquid lipid (oil) by a lipid being solid both at room and body temperature. The first nanoparticulate delivery system produced from a solid lipid was developed in the beginning of the nineties, the so-called solid lipid nanoparticles (SLN) (Müller 1996). Potential problems associated with SLN have been further minimised by a new generation of lipid systems developed at the turn of the millennium, the nanostructured lipid carriers (NLC), produced using a blend of a solid lipid with a liquid lipid (Müller 2002).

SLN and NLC are stable colloidal systems with notable advantages as drug delivery systems, i.e. physicochemical

stability, versatility, biocompatibility, biodegradability and controlled drug release. They are made from naturally occurring lipids from living systems, making them non-toxic, and are suitable for the incorporation of both lipophilic and hydrophilic drugs (Müller 2000; Mehnert and Mader 2001). The advantage of NLC over SLN results from the liquid lipid which is present in the solid matrix, avoiding the drug expulsion during storage that can occur when the lipid matrix undergoes polymorphic transformations from unstable to more stable configurations (Müller 2002). This means an improvement of the drug loading capacity and a possibility of modulation of the drug release profile from NLC comparing to SLN (Müller 2002).

Nowadays, aqueous dispersions of lipid nanoparticles are being investigated as drug delivery systems for different therapeutic purposes. One of their interesting features is the possibility of topical use, for which the systems have to be incorporated into commonly used topical vehicles, such as creams or hydrogels, in order to have a proper semisolid consistency (Souto 2004). In such cases, the lipid nanoparticle dispersions need to be stable only until they are incorporated into the vehicles, because they are stabilised by the semi-solid network (Müller 2005).

Topical solutions of minoxidil offer limited contact time with the scalp (Mura et al. 2007). To overcome this, topical gel formulations may be used, due to the increasing of the contact time, and therefore the local drug concentration. Nonetheless, the major obstacle of drug delivery across the skin is the low diffusion rate of drugs across the stratum corneum. One suitable approach to increase the permeation rate is the use of elastic colloidal carriers (Choi and Maibach 2005). The effectiveness of such carriers depends on their physicochemical properties as well as on composition of the final topical formulation. The aim of this work was the development and the characterization of a novel topical delivery system for minoxidil based on NLC, composed of stearic acid and oleic acid. These lipids have been successfully applied to prepare lipid-drug conjugate nanoparticles (LDC) by high pressure homogenization (Olbrich et al. 2004) and to prepare NLC by solvent diffusion method (Hu et al. 2005) and by melt-emulsification (Yuan et al. 2007).

In the present work, aqueous NLC dispersions have been produced (drug-free and drug-loaded) and further incorporated into Perfluorocarbon (PFC) or Carbopol 940 based hydrogels to achieve a proper semi-solid consistency. The developed formulations have been characterized regarding the mean particle size and the crystallinity of the colloidal carriers on the day of the production and during storage time, as well as regarding the rheological behaviour of semi-solid formulations before and after incorporation of NLC dispersion within the different gel networks.

2. Investigations, results and discussion

2.1. Identification and characterization of NLC dispersions

Based on the fact that many lipophilic drugs show high solubility in liquid lipids (oils) but poor solubility in solid lipids (Jenning 2000), several solid and liquid lipid combinations in different proportions %(w/w) have been tested for minoxidil during pre-formulation studies. A pre-requisite for a solid and liquid lipid combination to be selected as suitable is its melting point which must be higher that 40 °C. Furthermore, it should provide suitable encapsulation parameters (i.e. encapsulation efficiency and loading capacity) for the drug. The combination showing the most promising results (i.e. smaller particle size and higher loading of liquid lipid) was 70 : 30 stearic acid/oleic acid. The maximum lipid concentrations that allow a stable dispersion was selected and used for further analysis. The composition of the developed NLC formulations is shown in Table 1.

Table 1: Composition of NLC formulations [% (w/w)] (MF, minoxidil-free NLC dispersions; ML, minoxidil-loaded NLC dispersions)

Composition	Formulations [% (w/w)]	
	MF-NLC	ML-NLC
Stearic acid	13.3	13.3
Oleic acid	5.7	5.4
Poloxamer 188	1.0	1.0
Minoxidil	–	0.3
Water	79.0	79.0

For initial characterization lipid nanoparticle dispersions were examined by optical microscopy on the day of production, confirming the absence of aggregation phenomena between the particles (data not shown). SEM analysis was performed to evaluate the microscopic appearance and structure of NLC dispersions on the production day (Fig. 1).

The three-dimensional nature of the lipid nanoparticles was observed both before (Fig. 1, upper) and after (Fig. 1, lower) loading the nanoparticle matrix with minoxidil. The drug was successfully entrapped within the lipid matrix which remained below 1 μm in size. Fig. 1 depicts particles of approx. 250 nm. SEM analysis also revealed spherical NLC with a smooth surface. Such morphology is typical of an orthorhombic packing of lipid molecules characterized by the β′ polymorphic form in the matrix of these particles.

The effect of drug loading on the particle size during storage time at 25 °C was assessed by PCS analysis (Fig. 2). On the day of production, both placebo and minoxidil-loaded particles were of similar size (Z-ave of approx. 250 nm with pI < 0.45). Particles remained below 500 nm after 6 months of storage at 25 °C (pI < 0.45), revealing a good physical stability of minoxidil within NLC. Furthermore, formulations did not exhibit drug crystal growth during storage time (data not shown) which predicts that minoxidil remained solubilized in oleic acid nanocompartments inside the NLC matrix.

To confirm the presence of a solid lipid matrix produced by a modified oil-in-water emulsion procedure, DSC analysis was further performed. DSC analysis was used to characterize the state and the degree of crystallinity of lipid dispersions, because it allows the study of the melting and cristallization behaviour of crystalline material such as lipid nanoparticles. In the case of lipid particles, DSC experiments are useful to understand drug lipid interactions and the mixing behaviour of solid lipids with liquid lipids (Souto 2006).

To compare the crystallinity between minoxidil-free and minoxidil-loaded NLC dispersions the recrystallization index (RI) has been determined on the day of production. RI is defined as the percentage of the lipid matrix that has recrystallized during storage time and can be assessed applying the following equation (Freitas 1999):

$$\mathrm{RI}(\%) = \frac{\Delta \mathrm{H}_{\text{aqueous NLC dispersion}}}{\Delta \mathrm{H}_{\text{bulk material}} \times \text{Concentration}_{\text{lipid phase}}} \quad (1)$$

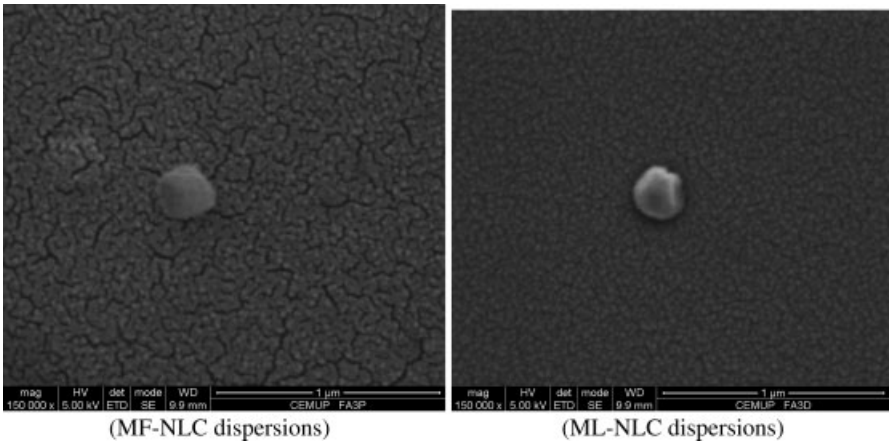

Fig. 1:
SEM pictures of aqueous NLC dispersions recorded on the day of production after 1 day of storage at room temperature (25 °C) (MF, minoxidil-free NLC dispersions; ML, minoxidil-loaded NLC dispersions)

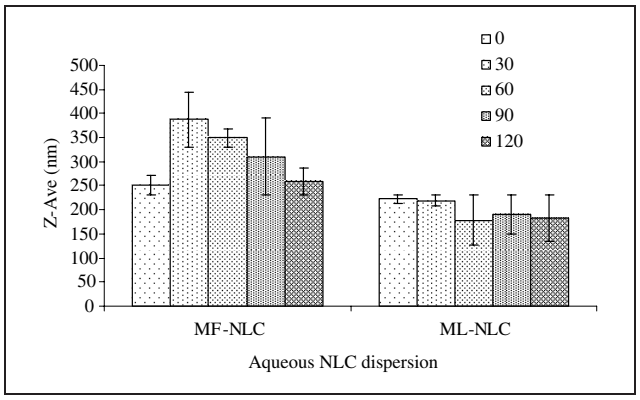

Fig. 2: Z-average (nm) of aqueous NLC dispersions measured on the day of production and after 30, 60, 90, and 120 days of storage at room temperature (25 °C) (MF, minoxidil-free NLC dispersions; ML, minoxidil-loaded NLC dispersions)

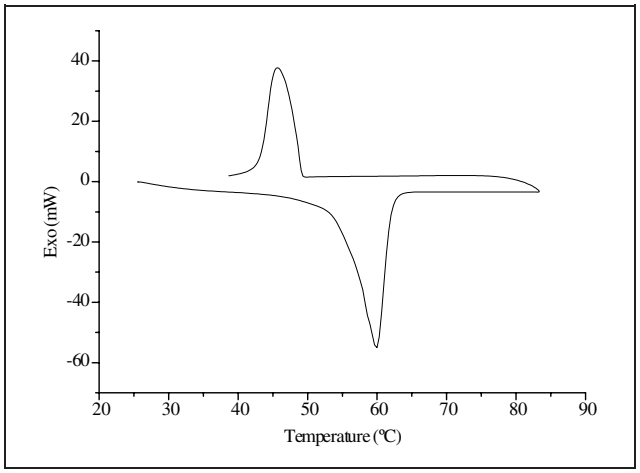

Fig. 3: DSC diffractogram of bulk lipid (stearic acid) recorded from 25–85–25 °C at a rate of 3 K min^{-1}

where ΔH is the molar melting enthalpy given by J/g and the concentration is given by the percentage of lipid phase in the dispersion. The method of preparation of the nanoparticles usually changes the polymorphism of the lipid matrix, which differs from the bulk lipid (Zimmermann 2005). Therefore, DSC analysis of the bulk solid lipid (stearic acid) was first performed (Fig. 3). Stearic acid showed a melting event at 59 °C with an onset temperature at 48 °C. Recrystallization peak was depicted at 46 °C. When analysing the diffractograms of lipid dispersions (Fig. 4) MF-NLC showed the melting peak also at 46 °C with a RI value of

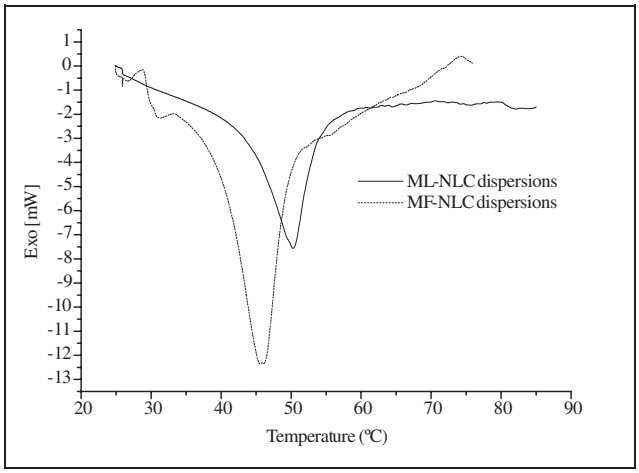

Fig. 4: DSC diffractograms of aqueous NLC dispersions recorded on the day of production after 1 day of storage at room temperature (25 °C) (MF, minoxidil-free NLC dispersions; ML, minoxidil-loaded NLC dispersions)

62% while ML-NLC revealed a RI of 68% with the melting peak occurring at 50 °C.

MF-NLC was shown to be less crystalline revealing as well a lower melting temperature in comparison to ML-NLC. The former has higher oleic acid content while in ML-NLC 5.3% of the oleic acid has been replaced by minoxidil. From the diffractograms it could also be observed that the drug was molecularly dispersed, i.e. solubilized in the NLC matrix. Minoxidil melts at 248 °C and has an aqueous solubility of 2200 mg/L (Huang et al. 2005), predicting the tendency of the drug to remain within the lipid matrix.

2.2. Preparation and characterization of NLC-based hydrogels

There are several approaches to develop a topical formulation of lipid nanoparticles, e.g. incorporation of these carriers into hydrophilic bases, hydrophobic bases, or the production of lipid nanoparticle gels or creams (Souto and Müller 2008). Lipid dispersions can be used as viscosity enhancers in topical hydrophilic formulations (Mei et al. 2005; Joshi and Patravale 2006; Souto et al. 2006). Hydrophilic bases can be single phase systems (hydrogels) or biphasic systems (o/w creams and lotions). Examples of the former are polyacrylates (Mei et al. 2005; Joshi and Patravale 2006) and hydroxyethylcellulose derivatives (Souto et al. 2004). Hydrogels differ from biphasic systems because they are not composed of a lipid phase but

Table 2: Composition of the developed NLC hydrogels [% (w/w)] (MF, minoxidil-free NLC dispersions; ML, minoxidil-loaded NLC dispersions; CP, Carbopol; PFC, perfluorocarbon)

Composition	Formulations [% (w/w)]			
	MF-NLC$_{CP}$	MF-NLC$_{PFC}$	ML-NLC$_{CP}$	ML-NLC$_{PFC}$
Stearic acid	5.32	5.32	5.32	5.32
Oleic acid	2.28	2.28	2.16	2.16
Poloxamer 188	0.40	0.40	0.40	0.40
Minoxidil	–	–	0.12	0.12
Propylenoglycol	3.00	3.00	3.00	3.00
Carbopol 940	–	0.60	–	0.60
PFC	0.60	–	0.60	–
Water	88.40	88.40	88.40	88.40

have higher water content. The incorporation of NLC in hydrogels can be achieved by admixture of the gel components, and a concentrated nanoparticle dispersion is added before starting the gelation process. To produce biphasic systems the NLC dispersions are mixed as a highly concentrated dispersion, i.e. with 50% solid content. This concentration is high enough to create an occlusive effect (Wissing et al. 2001) or to load a cream with chemically unstable compounds which are stabilized by incorporation in SLN/NLC (Jenning et al. 2000).

In the present case, NLC dispersions were first produced and further mixed with freshly prepared hydrogels. Two different hydrogel formulations were prepared, using an optimal stabilizer combination of water, gel-forming polymer and propyleneglycol. The freshly prepared aqueous NLC dispersions (40 g) were mixed with the hydrogels (60 g). Table 2 shows the final composition of the developed hydrogels.

As shown in Fig. 1, NLC dispersions did not reveal sedimentation of minoxidil nanocrystals six months after production. PCS was also used to evaluate the colloidal particle size of NLC dispersions after their incorporation in the hydrogels (CP or PFC). On the day of production, a mean particle size of 450 ± 3.3 nm and of 320 ± 4.5 nm was measured for NLC$_{CP}$ and NLC$_{PFC}$, respectively. The small difference in size between particles entrapped in different hydrogels suggests some effect of hydrogel type on the aggregation phenomenon. When producing polyacrylate gels (e.g. Carbopol hydrogels), these need to be neutralized in order to start the gelation process. Such neutralization can be achieved by means of strong electrolytes (e.g. sodium hydroxide) or by weak bases such as Tristan® (tromethamine) and Neutrol®TE (*N*,*N*,*N*,*N*-tetra(2-hydroxypropyl)ethylenediamine) (Jenning et al. 2000). Neutralization with sodium hydroxide, previously to addition of lipid nanoparticles can lead to aggregation because zeta potential can be strongly reduced. As a consequence of the decrease in zeta potential is the risk of particle aggregation due to the reduction of nanoparticle repulsion. In the present case, gelation process was performed using triethanolamine as neutralizing agent.

In order to clarify the effect of the type of hydrogel on the physicochemical properties of semi-solid formulations, rheological flow patterns were recorded for hydrogels containing NLC. Figs. 5 and 6 depict the plots of shear stress [Pa] as a function of shear rate [s^{-1}] of the different CP and PFC hydrogels, respectively, before and after admixture with NLC dispersions.

The effect of temperature on the rheological behaviour of the developed semi-solid formulations has been reduced

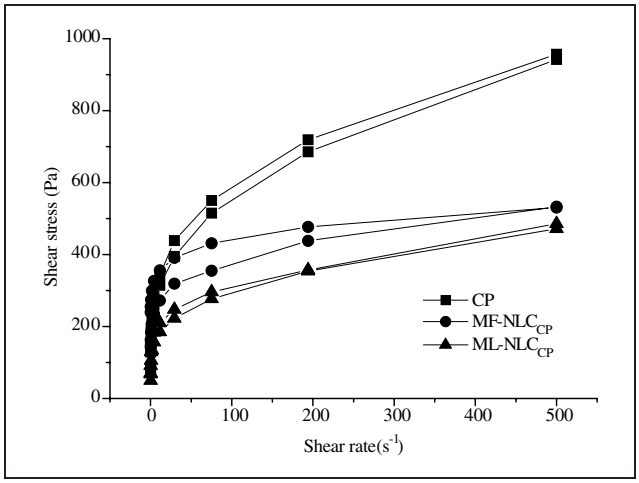

Fig. 5: Shear stress as a function of the shear rate, measured on the day of production, of Carbopol 940-based hydrogels without particles (CP) and with particles (MF-NLC$_{CP}$ and ML-NLC$_{CP}$)

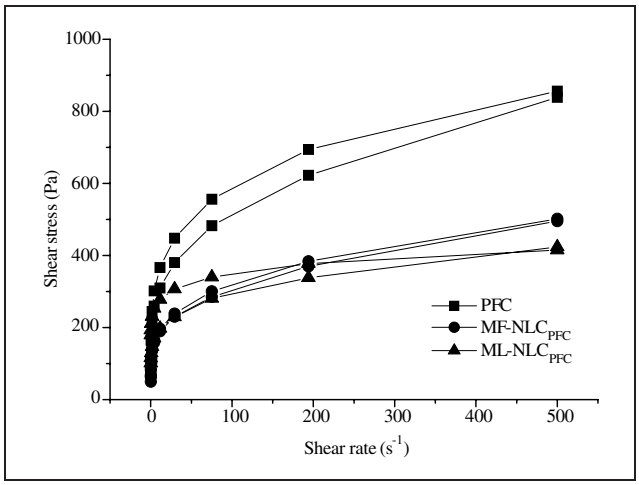

Fig. 6: Shear stress as a function of the shear rate, measured on the day of production, of perfluorocarbon-based hydrogels without particles (PFC) and with particles (MF-NLC$_{PFC}$ and ML-NLC$_{PFC}$)

because during all experiments, the temperature has been accurately maintained at 25 ± 1 °C, using a thermostatic water bath in order to avoid false positive results. From Figs. 5 and 6, the characteristic shape of the rheograms indicates that all developed formulations exhibited pseudoplastic flow. Thixotropy was also observed because the up and down curves did not overlap. The rheological behaviour of thixotropic fluids is usually a time-dependent pattern, i.e. the flow and viscosity of the systems are dependent on the time history of the sample. In the present case CP-hydrogels revealed higher thixotropy than PFC-hydrogels. Nonetheless, when mixing such gels with the NLC dispersions (60 : 40) similar patterns were recorded. Semisolids showing pseudoplastic flow reveal a rheological behaviour between Newtonian flow and plastic flow. While Newtonian materials flow at low shear rates, the liquid materials shows plastic flow at higher shear rates. In the present systems, viscosity effectively falls as shear rate is increased, nonetheless depending upon the shear applied. A thixotropy is similar to pseudoplastic flow once the viscosity decreases with increasing shear rate, but the time scale of the experiment also controls the viscosity, in that the longer the experiment takes place, the lower the viscosity. To develop a suitable semi-solid formulation for topical administration of drugs both properties should be

characteristic of the system. NLC are able to decrease the rheological differences between different types of hydrogels. The effect of minoxidil was hardly noticed between the rheograms recorded in both CP and PFC-based hydrogels.

In conclusions, with a modified oil-in-water emulsion procedure, NLC composed of stearic acid and oleic acid were prepared to incorporate minoxidil. NLC with a mean particle size of approximately 250 nm were obtained and remained physically stable at least during 6 months of storage at 25 °C. Minoxidil crystals in the aqueous phase were not detected by light microscopy and SEM. Furthermore, SEM revealed NLC with a smooth surface and a spherical-like shape. NLC dispersions were admixed with hydrogels to produce semi-solid systems intended for topical administration of minoxidil. The developed formulations were shown to be a promising alternative to the conventional alcoholic solutions for minoxidil once the former do not require ethanol to solubilize the drug, which has been previously solubilized in oleic acid prior too nanoparticle production. With this approach the risk of occurrence of adverse side effects, such as skin dryness and irritation, will be minimized/avoided. In addition, the drug is physically entrapped within the lipid matrix which can be useful to increase the bioavailability towards skin delivery.

3. Experimental

3.1. Materials

Stearic Acid, oleic Acid and minoxidil were purchased from Guinama (Spain). Poloxamer 188 (Lutrol®) was a gift from Gattefossé (France). For hydrogels preparation, perfluorocarbon (PFC), Carbopol 940 and triethanolamine were provided by Guinama (Spain). Propylene glycol and ethanol were obtained from Merck (Germany). The water used in all experiments was purified and lab supplied, obtained from a MilliQ Plus System.

3.2. Methods

3.2.1. Preparation of aqueous NLC dispersions

Three aqueous NLC dispersions were produced containing 20% (w/w) of lipid matrix (stearic acid and oleic acid) with different proportions (% (w/w)) of solid and liquid lipids (90 : 10, 80 : 20, 70 : 30), and stabilized with 1% (w/w) of surfactant (poloxamer 188). For the production of NLC a modified oil-in-water emulsion procedure has been applied. The mixture of solid and liquid lipids and surfactant was heated 5–10 °C above the melting point of the solid lipid (m.p. ≈ 69–72 °C), followed by the addition of purified water heated at the same temperature, and put into an Ultra-Turrax T25 (Janke & Kunkel GmbH, Staufen, Germany), at 8000 rpm for 20 min. The obtained emulsion was further diluted with 10 mL of purified hot water and cooled down under magnetic stirring, until 30 °C has been reached. To avoid nanoparticle aggregation under cooling, 4 mL of ethanol was added and stirred for more 25 min at room temperature.

For minoxidil-loaded NLC, the drug was dissolved in the liquid lipid (oleic acid) prior to emulsification, and it was used in a concentration of 5% (w/w) with regard to the liquid lipid. Minoxidil was shown to be soluble in oleic acid in a concentration higher than 5% (w/w) (data not shown).

3.2.2. Preparation of hydrogels

Two different hydrogels were prepared, one composed of perfluorocarbon (PFC) and other of Carbopol 940. The corresponding gel-forming polymer was dispersed in purified water and propylenoglycol was admixed to the aqueous solution. The mixture was stirred for 10 min at 1500 rpm in a Cito Unguator (Konietzko, Bamberg, Germany), and immediately neutralized with triethanolamine until pH 6.5. Hydrogels were further allowed to equilibrate for 24 h at room temperature.

3.2.3. Preparation of NLC-loaded hydrogels

Freshly prepared aqueous NLC dispersions (drug-free and drug-loaded) were incorporated in both hydrogels (PFC and Carbopol 940), using a high speed stirrer (Cito Unguator Konietzko, Bamberg, Germany) at approximately 1000 rpm for 3 min, in a concentration of 40% (w/w) of dispersion in the gel.

3.2.4. Scanning electron microscopy (SEM) analysis

Scanning electron micrographs were taken with a FEI Quanta FEG scanning electron microscope. Prior to the micrography, the samples were mounted on aluminium stubs covered with a glass lamella, air-dried, gold coated under vacuum and then examined.

3.2.5. Photon correlation spectroscopy (PCS) analysis

The mean particle size was assessed by PCS. All the samples were diluted with purified water to suitable concentration and measured with a Malvern Zetasizer 5000 (Malvern Instruments, UK). All measurements were performed in triplicate. To investigate the long-term stability as a function of storage conditions, the selected drug-free and drug-loaded NLC dispersions were stored at room temperature (25 °C) for 120 days and the Z-ave and PI of the nanoparticles were measured.

3.2.6. Differential scanning calorimetry (DSC) analysis

DSC analyses were performed on a Micro DSC III apparatus (Setaram Instrumentation, France). A sufficient amount of aqueous dispersion was accurately weighted in 40 μL aluminium pans. The DSC scans were recorded from 25 °C to 85 °C at a heating rate of 3 $Kmin^{-1}$, using an empty pan as reference. For the analysis of the bulk lipid, the heating and cooling runs were recorded from 25 °C 85 °C 25 °C at a constant rate of 3 $Kmin^{-1}$. Melting and recrystallization points correspond to the maximum and minimum of the DSC curves, respectively.

3.2.7. Rheological measurements

The rheological properties of hydrogel formulations were studied by continuous shear investigations, which were performed in order to evaluate the shear rate [s^{-1}] as a function of the shear stress [Pa]. This study started with a shear rate of 0.1 [s^{-1}] up to a maximum of 500 [s^{-1}] and the resulting shear stress was measured. Rheological measurements were carried out at 25 ± 1 °C on a rheometer Rheo Stress RS 100 (Haake, Karlsruhe, Germany) equipped with a cone and plate test geometry (a plate of radius 20 mm with a cone angle of 4°).

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