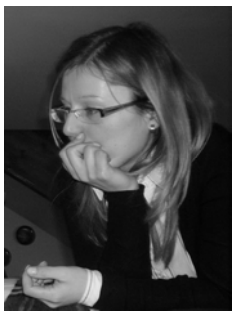
Feasibility studies of lipid-based carriers for dermal applications



E.B. Souto[1], A.J. Almeida[2], and R.H. Müller[1*]
[1] Department of Pharmaceutics, Biopharmaceutics and Biotechnology
Free University of Berlin, Kelchstrasse 31 12169 Berlin, Germany; [2] Faculty of Pharmacy, Lisbon University, Av. Prof. Gama Pinto, 1649-008 Lisbon, Portugal; Corresponding author: elianasouto@yahoo.com

Introduction

The aim of this study has been the physicochemical characterization of improved lipid-based formulations for topical, dermal and cosmetic purposes. Several substances usually applied onto the skin interact with its outermost layer, i.e. stratum corneum, which is the limiting step that controls the concentration and distribution of the drugs/actives acting on the skin [1]. The stratum corneum is a buildup of dead cells (corneocytes) surrounded by extremely hydrophobic epidermal lipids, mainly consisting of ceramides, cholesterol, long-chain free fatty acids, and also cholesteryl sulfate. These epidermal lipids form lamellae of orthorhombic packing but also a liquid phase and build up an efficient barrier against excessive water loss and harmful environmental substances. Additionally, drugs/actives penetrate the skin via the intercellular pathway between the corneocytes and through the air follicles. To improve and/or control the uptake of such molecules throughout the skin pharmaceutical technology is focused on their encapsulation into nanoparticulate systems based on lipid materials, such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC). These systems are composed of lipid materials characterized with a high melting temperature (above 50ºC) such as triacylglycerols (C_{14}, C_{16}, C_{18}), glyceryl behenate (C_{22}), glycerol monostearate, and waxes (cetyl palmitate) [2]. To prepare SLN only solid lipids are used, while for NLC a certain amount of solid lipid is replaced by a liquid lipid (oil) in order to nanostructure the lipid matrix allowing therefore a higher drug/active loading and a better control of its release rate [2-4].

Materials and methods

Aqueous dispersions of SLN composed of 10% (m/m) cetyl alcohol (Sigma-Aldrich, Deisenhofen, Germany) and optimized concentrations of Tween 80 (ICI Surfactants, Essen, Germany) as surfactant were produced. For the production of NLC 30% of the solid lipid has been replaced by medium chain triacylglycerols (Caelo, Hilden, Germany). SLN and NLC were produced by hot high pressure homogenization technique [5, 6]. The lipid materials were heated at 85ºC and further dispersed using an Ultra-Turrax T25 (Staufen, Germany) at 8000 rpm for 1 min in a hot surfactant solution at the same temperature obtaining a pre-emulsion. This pre-emulsion was then passed through an APV Micron Lab 40 high pressure homogenizer (APV Systems, Unna, Germany), at 85ºC and applying a pressure of 500 bar. The aqueous SLN or NLC dispersions were cooled at room temperature crystallizing the lipid phase and obtaining solid particles.
The particle size analysis was performed by laser diffractometry (LD) using a Coulter®LS 230 (Coulter Electronics, Germany). The LD data were characterized in terms of diameters d50%, d90% and d95% of the volume distribution in order to exclude the presence of particles above the nanometer range. To obtain the mean diameter of the bulk population and the polydispersity index (PI) photon correlation spectroscopy (PCS) has been performed in a Zetasizer 4 (Malvern Instruments, UK).
The degree of crystallinity of lipid nanoparticles was obtained by differential scanning calorimetry analysis (DSC) using a Mettler DSC 821^e (Mettler Toledo, Gießen, Germany). The samples were heated up to 80°C, kept for 15 min. at 80°C, cooled down to 20°C, kept at 20°C for 16 min.,

reheated again up to 80°C and cooled down again to 20°C. The scans were recorded at a heating and cooling rate of 5 K/min.
To assess the suitability of aqueous dispersions for dermal applications, rheological analysis of SLN and NLC was performed on a rheometer Rheo Stress RS 100 (Haake Instruments, Karlsruhe, Germany) equipped with a cone-and-plate test geometry (plate diameter 20 mm, cone angle 4º) at a temperature of 20±0.1ºC. An oscillation frequency sweep test was performed over a frequency range from 0 to 10 Hz at constant stress amplitude of 5 Pa.
To perform scanning electron microscopy (SEM) analysis aqueous dispersions of lipid nanoparticles were spread on a sample holder with double sided tape and coated under an argon atmosphere with gold to a thickness of 6.5 nm (SCD 040, Balt-Tec GmbH, Witten, Germany). The samples have been observed with a scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany) using secondary electron imaging at 10 kV in order to examine the surface morphology and to assess the particle size of lipid nanoparticles.

Results and discussion

The particle size and three-dimensional structure of SLN and NLC have been characterized by means of static and dynamic light scattering techniques, and by scanning electron microscopy (SEM) analysis. Spherical particles within the nanosize range (< 0.6±0.012 µm, *n = 3*) were measured by LD, showing a mean particle size of 310±1.5 nm (*n = 10*) assessed by PCS.
Structural parameters such as the dynamic storage modulus (G') and the loss modulus (G") are used to characterize mechanically and rheologically the pharmaceutical dosage forms intended for topical/dermatological administration. Both moduli are nearly independent of the angular frequency, and the values of G' (elastic component) should be at least one order of magnitude higher than the values G" (viscous component) [7]. Figure 1 shows the storage modulus (G'), loss modulus (G") and complex viscosity (Eta) of aqueous SLN dispersions as a function of frequency, recorded immediately after production.

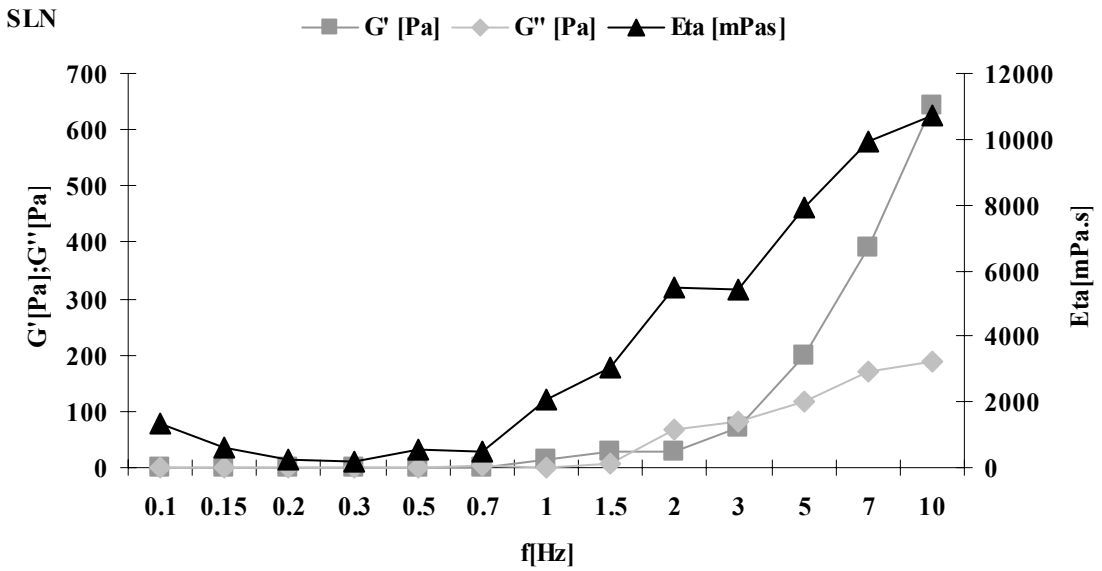


Figure 1: Storage modulus (G'), loss modulus (G") and complex viscosity (Eta) of aqueous SLN dispersions as a function of frequency, recorded immediately after production under a constant stress amplitude of 5 Pa.

Previously to oscillatory testing, the linear viscoelastic region for the aqueous lipid nanoparticles has been determined by a strain sweep. The complex modulus (G*) has been measured as a function of strain at a constant frequency [8]. All measurements have been carried out in the regime of linear viscoelasticity at stress amplitude of 5 Pa, i.e. where the material parameters are independent of the applied stress.
In Figure 1 one can see that there is a small linear viscoelastic region, G' and G" moduli are frequency dependent and at the maximum, the G' values are higher by one order of magnitude compared to the G" values [9]. Figure 2 shows the results obtained for NLC dispersions.

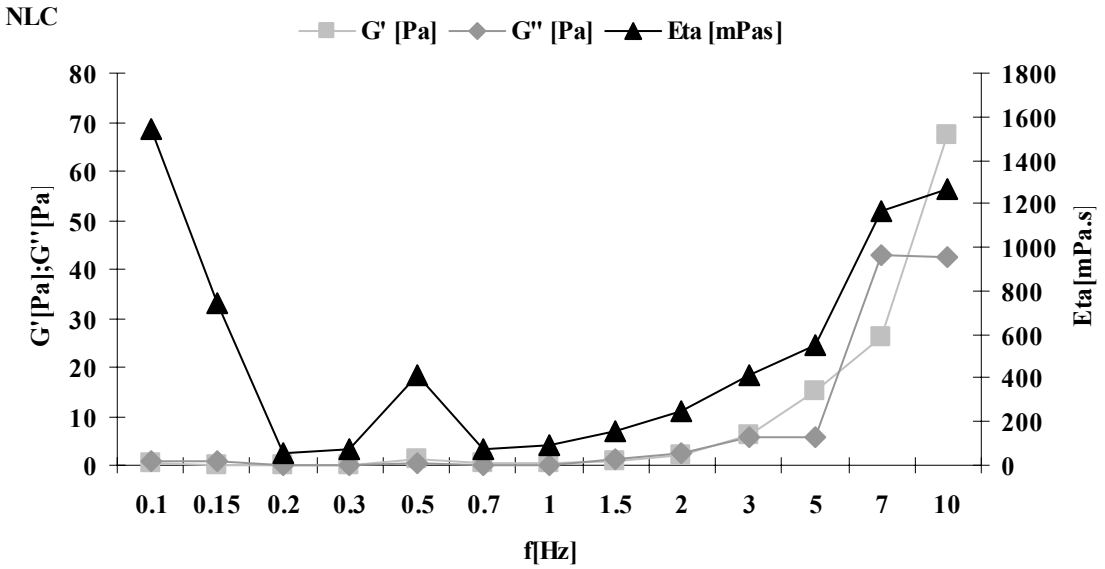


Figure 2: Storage modulus (G'), loss modulus (G") and complex viscosity (Eta) of aqueous NLC dispersions as a function of frequency, recorded immediately after production under a constant stress amplitude of 5 Pa.

From the results shown above, the effect of the presence of oil is clearly visible. Without oil within the lipid matrix the aqueous dispersions depicted a G' higher by about one order of magnitude than the G", which means that the system is more elastic than viscous in the investigated frequency range. Both parameters show a strong dependence on the applied frequency. The complex viscosity, depends also very much on the frequency, i.e. increases with increasing the frequency.
Looking at the values of G' and G" recorded for NLC dispersions (Figure 2) they are much smaller than the ones registered for SLN dispersions, which is due to the presence of Miglyol®812 inside the particles. Dynamic rheological analysis showed that NLC is a weaker structure in comparison to SLN where the storage modulus and the loss modulus are almost parallel and frequency dependent. These results can be analyzed in terms of tan φ, the loss tangent (the tangent of the phase angle), which is a measure of the ratio of energy lost to energy stored in a cyclic deformation. Because we can determine them so readily, spectra of log tan φ versus log frequency (consistency spectra) may be useful in quality control, in storage tests, or in formulation experiments. Table 1 depicts the values of the loss tangent of SLN and NLC dispersions. For each formulation, during the analysis tan φ is most of the time lower than 1, i.e. G' is greater than G", indicating that elastic properties dominate viscous behavior. In case of NLC its rheological behavior is not as uniform as in case of SLN.

Table 1: Loss tan φ of SLN and NLC dispersions, recorded immediately after production under a constant stress amplitude of 5 Pa.

Om [rad/s]	tan φ of SLN	tan φ of NLC
1	2.890	3.560
2	0.546	8.733
3	0.172	0.259
4	0.602	2.160
6	0.037	0.069
9	1.370	0.203
14	1.307	2.297
20	0.968	1.138
29	0.392	0.596
43	1.645	0.438
63	0.634	0.291

Conclusions

This paper shows that aqueous SLN and NLC dispersions behave as semi-solids, which are systems that possess the particular property that they readily deform when applied onto the skin yet they cling to the body, generally until washed or wiped off. Due to their lipid nature, they are valuable therapeutic aids and drug delivery systems in dermatology, being biocompatible, chemically similar to the lipids that figure on the skin. However, they are also the most difficult of materials to characterize rheologically because they combine solid behavior and liquid properties within the same material. It has been observed that lipid nanoparticles are more elastic than viscous. Of course, formulations need to be optimized regarding their suitable viscosity for topical administration.

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