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Rheology of nanostructured lipid carriers (NLC®) suspended in a viscoelastic medium

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Colloidal lipid nanoparticle dispersions have been characterized by rheological measurements using two different nanostructured lipid carrier (NLC)-based formulations intended for cosmetic application of substances like sunflower oil and α-tocopherol. This study has shown that rheological and viscoelastic properties of aqueous NLC dispersions are quantitatively very different depending on the composition of the oil phase and the temperature of storage despite similar or even identical particle size. NLC were loaded with 30% active ingredient relative to the particle mass. Stearyl alcohol was used as lipid matrix and the particle sizes determined by photon correlation spectroscopy were in the range 210–270 nm. In general, sun flower oil-loaded NLC dispersions showed distinctly higher storage modulus (G′), loss modulus (G″) and complex viscosity (η^*). Storage at lower temperature (4 °C versus 20 °C) delay the build up of a microstructure affected not only by size and stabilizer but also loaded ingredient and storage history after preparation, i.e. storage at room temperature accelerates the build up of a final suspension structure.

1. Introduction

During the last ten years the interest in lipid nanoparticle technology has been growing rapidly. Nevertheless, the fundamental understanding of their flow behaviour is still rather limited. This is due to the fact that aqueous dispersions of lipid nanoparticles are much more complicated than characterization of macroscopic systems, such as powders. Systematic and reliable data are limited so far, although this is the kind of information needed for the development and assessment of a new pharmaceutical/cosmetic dermal product.

Formulations intended for topical application are usually developed to prolong the residence time on the skin and to undergo a wide variety of stresses during removal from the container and application to the desired area. Therefore, it is important to consider the effects of such stress on the microstructural properties of these products. Once lipid materials are regarded as safe substances for topical administration, the purpose of this study was to examine the viscoelastic properties of aqueous dispersions of lipid nanoparticles and to establish the suitability of these formulations for topical administration of cosmetics.

Lipid nanoparticles are colloidal carriers developed at the end of the last century as an alternative to systems like polymeric nanoparticles, liposomes and fat emulsions (Müller and Lucks 1996). Solid lipid nanoparticles (SLN®) were the first generation, while nanostructured lipid carriers (NLC®) are the second generation (Müller et al. 1998). The main difference between those particles is related to the morphology of their lipid matrix (Müller et al. 2002). In fact, SLN are composed only of solid lipids which create a relatively perfect crystal, and NLC are formed with a blend of solid and liquid lipids creating a more imperfect matrix which allows the assessment of a higher loading capacity of actives.

The advantages of lipid nanoparticles for cosmetic purposes are related to the fact that they exhibit an occlusive effect, which improves skin hydration, i.e. avoid water loss at the surface of the skin (Wissing and Müller 2002). In addition, it has been shown that lipid nanoparticles are also UV blockers themselves having a photoprotection effect avoiding therefore skin aging due to sun exposure. The clinical signs of aging are, for instance, dyspigmentation and wrinkles (Wulf et al. 2004).

Rheological experiments with topical creams, pharmaceutical or cosmetic products are generally performed in order to evaluate the effects of temperature, aging during storage, excipients and processing parameters. Moreover, these studies also allow the quantification of the flow of a material for quality control purposes. The liquid oils sunflower oil and α-tocopherol were selected for the present work due to their anti-acne and protective antioxidant properties, respectively. SLN dispersions were prepared and rheologically characterized.

2. Investigations, results and discussion

2.1. Particle size characterization of aqueous dispersions

Particle size parameters of lipid nanoparticles were determined in samples stored for 1 day after production. After

Table 1: Particle size parameters determined one day after preparation of NLC formulations

Evaluated parameters	Sunflower oil-based NLC		α-tocopherol-based NLC	
	25 °C	4 °C	25 °C	4 °C
PCS (nm)	268.5 ± 11.5	251.6 ± 9.2	267.2 ± 4.3	211.3 ± 3.6
PI	0.500 ± 0.031	0.321 ± 0.012	0.362 ± 0.050	0.150 ± 0.010
LD 50% (μm)	0.193 ± 0.018	0.165 ± 0.001	0.255 ± 0.022	0.203 ± 0.003
LD 95% (μm)	0.535 ± 0.001	0.478 ± 0.004	0.572 ± 0.013	0.423 ± 0.002
LD 99% (μm)	0.674 ± 0.002	0.591 ± 0.001	0.698 ± 0.006	0.531 ± 0.005

preparation, the obtained batches were divided in two equal parts, one stored at room temperature (25 °C) and the other stored at 4 °C. Table 1 shows the obtained results (mean ± SD) representative of three independent experiments.

Stearyl alcohol based NLC show mean particle sizes between 210 and 270 nm. The higher PI was registered for sunflower oil-based NLC stored at 25 °C (0.500 ± 0.031), while the lowest was obtained for α-tocopherol-based NLC stored at 4 °C (0.150 ± 0.010). SLN stored at 4 °C showed slightly lower PCS diameters, indicating some limited aggregation at least for the α-tocopherol-loaded SLN (from 211 to 267 nm). It was previously shown that incorporation of lipid particles into higher viscous gels or o/w creams physically stabilizes them. Therefore, if necessary, these formulations could be incorporated into gels/creams directly after production to avoid any relevant particle growth. By LD analysis the presence of lipid microparticles could be excluded.

2.2. Evaluation of the rheological properties

The rheology of complex materials, such as those presented here, should not be characterized by single-point measurements. Instead, a variety of conditions should be explored and a match between colloidal parameters and experimental rheological measurements should be attempted to obtain conclusive results. To do so, two different experiments were performed. Firstly, an oscillation frequency sweep test was applied over a frequency range from 0 to 10 Hz. The storage modulus (G'), loss modulus (G'') and the complex viscosity (η^*) of aqueous NLC dispersions were determined as a function of the frequency at constant stress amplitude of 5 Pa (linear viscoelastic region). Secondly, by continuous shear investigations the shear rate was evaluated over a shear stress range from 0 up to 50 Pa. Table 2 presents the results obtained after the first rheological experiments.

Table 2: Storage modulus (G'), loss modulus (G'') and the complex viscosity (η^*) of aqueous NLC dispersions as a function of the frequency (1–10 Hz) at constant stress amplitude of 5 Pa

Formulation	Storage temperature	Frequency	G' (Pa)	G'' (Pa)	η^* (m · Pas)
Sunflower oil based NLC	25 °C	1 Hz	1840	679	312000
		10 Hz	2660	669	43600
	4 °C	1 Hz	2.34	3.05	612
		10 Hz	49.9	145	2440
α-tocopherol based NLC	25 °C	1 Hz	76.5	81.8	17800
		10 Hz	670	264	11500
	4 °C	1 Hz	0.55	1.08	193
		10 Hz	189	39.9	3080

Viscoelasticity is defined as the simultaneous existence of viscous and elastic properties (Barnes et al. 1989). The dominant properties and the values for rheological parameters depend on the stress and the duration of stress application. Analysis of the presented viscoelastic NLC dispersions was designed in order not to destroy the structure, so that measurements can provide information on the intermolecular and interparticle forces in the material (Martin 1993). For a topical formulation which is tested in the linear viscoelastic region, the amplitudes of stress and strain are proportional and the stress waves alternate sinusoidally at the same frequency but are out of phase. This phase angle varies between 0° for a Hookean solid and 90° for a Newtonian liquid. In dynamic experiments such as oscillation tests the stress and the strain are sine or cosine functions and they may be treated as rotating vectors. As shown in Table 2 for each formulation, during the oscillation test G' became always greater than G'', indicating that elastic properties dominate viscous behaviour. Plots of G' and G'' as a function of frequency illustrate that the moduli for all samples are strongly dependent on the frequency, indicating short characteristic relaxation times for the micro-structures in the studied frequency range (1–10 Hz). This behaviour is typical for viscoelastic liquids in which the microstructure is able to store energy imparted by the oscillation and relax sufficiently to dissipate part of the energy by micro-structural rearrangement.

However, significant differences were registered for complex viscosity according to the storage temperature. As it can be observed, samples stored at room temperature exhibit higher levels of viscosity and higher moduli values than the samples stored at 4 °C. The differences between the rheological behaviour under the two storage conditions may be explained by the difference in particle size parameters of those formulations. In fact, the mean particle size of those preparations stored at 25 °C is approx. 270 nm, while storage at 4 °C yielded particles with approx. 210 nm. Also the former showed higher PI values (0.5 to 0.3) than the latter (0.4 to 0.2). The reduced particle size is believed to provide an enhanced physical stability to coalescence. It was also observed that during the applied frequency from 1 to 10 Hz the samples stored at 25 °C showed a decrease in the complex viscosity, while the samples stored at 4 °C showed an increase of this parameter. These results also reveal the effect of the storage temperature on the physical properties of colloidal dispersions.

Concerning the differences between the composition of the formulations (sunflower oil and α-tocopherol), Figs. 1 and 2 provide the plots of the shear rate (Gp) and the complex viscosity (η^*) as a function of the shear stress applied from 0 to 50 Pa, of the sunflower oil-based NLC and α-tocopherol-based NLC, respectively.

The flow curves provide additional information about the rheological behaviour of the aqueous NLC dispersions.

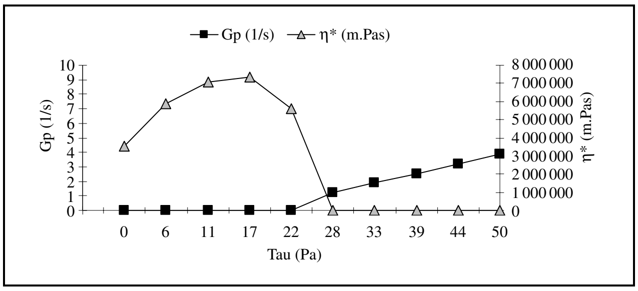

Fig. 1: Flow curves of sunflower oil-based NLC stored at 25 °C

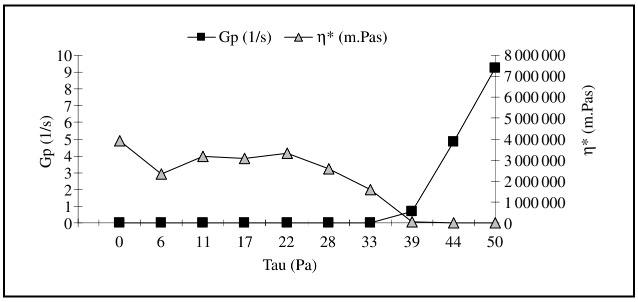

Fig. 2: Flow curves of α-tocopherol-based NLC stored at 25 °C

Although they are qualitatively similar, i.e. the increase of the shear stress yields a decrease of Gp and an increase of η*, non-Newtonian behaviour tends to disappear when the structure of both NLC dispersions is broken by shearing during test at different shear rate values. At 28 Pa the η* of sunflower oil-based NLC is approximately 0 Pas, while only at 39 Pa the same happens to the α-tocopherol-based NLC formulation. In addition, the former also exhibits a lower yield value (22 Pa) in comparison to α-tocopherol-based NLC (33 Pa).

2.3. Conclusions

The purpose of this study was to characterize aqueous NLC dispersions developed for cosmetic administration of active compounds, investigating the effect of particle size, oil phase composition and storage temperature on the rheological properties of the colloidal systems. Rheological data indicate that, at the maximum applied frequency (10 Hz), the magnitude of G″ is smaller than the magnitude of G′ and both moduli are strongly dependent on the applied frequency, indicating short characteristic relaxation times for the microstructures, which is a typical behaviour of viscoelastic liquids. Despite identical size, lipid particle concentration oil loading and storage temperature of 25 °C, the sunflower oil-loaded SLN showed much higher values for G′, G″ and η* proving the strong influence of the incorporated active on the rheological properties. The active is thought to affect surface properties of the particles and subsequently adsorption longer thickness/conformation of stabilizer and thus the microstructure via particle-particle interaction. Despite identical mean size, the sunflower oil-loaded SLN were slightly more polydisperse (PI 0.50 vs. 0.36) which might contribute to the observed differences in microstructure. Another interesting aspect was the observed effect of the storage temperature in the microstructure, i.e. rheological properties. A lower storage temperature (4 °C) obviously delays the build up of particle-particle interactions as indicated by the lower moduli and η*. These parameters need to be considered when preparing lipid nanoparticles-based topical formulations.

3. Experimental

3.1. Materials

For the present research two different NLC systems have been prepared using stearyl alcohol (Fluka, Neu-Ulm, Germany) as solid lipid. The liquid oils sunflower oil from *Helianthus annuus* and α-tocopherol were purchased from Fluka (Neu-Ulm, Germany) and Sigma-Aldrich (Deisenhofen, Germany), respectively. Tween®80 from Merck (Berlin, Germany) was used as stabilizer in all formulations. The materials were used as received.

3.2. Preparation of aqueous NLC dispersions

Mixtures of 70% (m/m) of stearyl alcohol and 30% (m/m) of liquid oil (sunflower oil or α-tocopherol) were melted at 85 °C. The melted lipid phase (15%) was dispersed in a hot aqueous solution of 3% (m/V) Tween®80 using an Ultra-Turrax G25 (IKA, Staufen, Germany) for 1 min at 8000 rpm. The obtained pre-emulsion was homogenized using the high pressure homogenizer Micron LAB 40 (APV Systems, Unna, Germany) heated at 85 °C, at 500 bar and applying 3 homogenization cycles. Aqueous NLC dispersions containing 15% of lipid phase were obtained after cooling the nanoemulsion at room temperature (Müller et al. 1995, 2000).

3.3. Particle size analysis

The mean particle size and the polydispersity index (PI) were determined by photon correlation spectroscopy (PCS) using the Malvern Zetasizer 4 (Malvern Instruments, UK). Laser diffractometry (LD) analysis was performed using the Coulter®LS 230 (Beckmann-Coulter Electronics, Krefeld, Germany) in order to detect the presence/absence of particles in the low micrometer range. The LD data were evaluated using the diameters LD50, LD95 and LD99, meaning the percentage of lipid particles (volume distribution) which are below the given size value.

3.4. Rheological experiments

Rheological experiments were performed on a rheometer Rheo Stress RS 100 (Haake Instruments, Karlsruhe, Germany). Cone and plate geometry was used in order to generate a homogeneous shear field throughout the tested samples, a pre-requisite for the analysis of viscoelastic behaviour. All experiments were performed at 293 K.

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