Comparative study between the viscoelastic behaviors of different lipid nanoparticle formulations

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Synopsis

Application of drug substances to the skin for systemic absorption or action in a particular layer of the skin
is a rather old approach. However, over the last years it has received much more attention, as a consequence
of the development of new membrane-moderated and matrix reservoir devices. As new reservoir systems,
solid lipid nanoparticles (SLN™) and nanostructured lipid carriers (NLC™) have been successfully tested
for dermal application of different physicochemical substances. The knowledge obtained from rheological
investigations of these systems may be highly useful for the characterization of the newly developed topical
formulation. In the present study, an oscillation frequency sweep test was used for the evaluation of storage
modulus (G′), loss modulus (G″), and complex viscosity (η*) of twelve different SLN and NLC formulations,
over a frequency range from 0 to 10 Hz. The lipidic aqueous dispersions were prepared using three different
solid lipids (Softisan®138, Compritol®888, and stearyl alcohol) as matrix material. Miglyol®812, tocoph-
erol, sunflower oil, and long-chain triacylglycerols were the chosen liquid lipids for NLC preparation. The
objective of the present work was to investigate the effect of these different liquid lipids on the rheological
properties of aqueous dispersions of NLC as model systems. It was found that the liquid oil component of
the formulation has a strong influence on the viscoelastic parameters, which are dependent on the particle
size, zeta potential, and crystallinity of the lipid particles, as well as on the solid lipid used.

INTRODUCTION

The skin is the largest organ of the body, considered as a natural protective barrier
against either the penetration of dangerous exogenous compounds or the loss of excessive
amounts of water and other essential compounds from the body. At the same time, it can
also be a promising portal of entry of active substances to the systemic circulation. A
drug penetrates the stratum corneum and eventually it diffuses across the viable under-
lying tissues, according to its physicochemical properties (1).

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A wide spectrum of formulations is available for use in topical therapy, including simple solutions, fluid emulsions and suspensions, sprays and aerosols, gels, and creams and ointments (2). These most common topical formulations are essentially able to control the release of a drug substance but not its penetration rate or residence time in the different layers. The development of the new membrane-moderated and matrix reservoir devices improve the penetration of some drugs, by targeting either to the stratum corneum or to the follicles, and control their release profiles. The use of particulate carriers for this purpose presents an interesting challenge concerning the targeting of topically applied drugs to the different skin layers and appendages.

Solid lipid nanoparticles (SLN™) and nanostructured lipid carriers (NLC™) are novel particulate lipidic carriers that have been extensively studied for different administration routes, such as the gastrointestinal, ocular, and topical routes (3–5). Regarding topical administration, these systems show adhesive properties and, therefore, an occlusive effect, which is dependent on the size, crystalline status, and lipid composition of the particles (6,7). SLN and NLC can also protect chemically labile active ingredients in water-containing formulations against chemical degradation (4).

The aim of the present study was to characterize the rheological behavior of different SLN and NLC aqueous dispersions in order to elucidate the viscoelastic improving effects of these new drug vehicles for skin application.

MATERIALS

Softisan®138 was purchased from Condea (Witten, Germany), Miglyol®812 from Caelo (Hilden, Germany), and Compritol®888 and Tego Care®450 from Gattefossé (Weil a.R., Germany). Lutrol®F68 was a gift from BASF AG (Ludwigshafen, Germany), and sunflower oil and deoxycholic acid sodium salt were obtained from Fluka Chemie AG (Steinheim, Switzerland). Long-chain triacylglycerols (LCT) were purchased from Braun (Melsungen, Germany) and tocopherol from Sigma Aldrich (Deisenhofen, Germany). All samples developed for this study were prepared using ultra-pure Millipore water (Schwalbach, Germany) of specific resistance greater than 18 MΩ-cm^{-1}.

METHODS

PRODUCTION OF LIPID NANOPARTICLES

According to Müller *et al.* (4), SLN and NLC aqueous dispersions were prepared by the hot high-pressure homogenization technique using the high-pressure homogenizer APV Micron Lab 40 (APV Systems, Lübeck, Germany). Briefly, the lipid components were admixed and melted at 90°C, and this liquid lipid solution was dispersed in an aqueous surfactant solution heated to the same temperature, using an Ultra-Turrax T25 (Jankle & Kunkel GmbH and Co KG, Staufen, Germany) at 8000 rpm for one minute. The obtained pre-emulsion was then homogenized at 90°C, applying three homogenization cycles at 500 bar. The produced O/W nanoemulsion was cooled down, the recrystallized lipid providing SLN or NLC aqueous dispersions.

PARTICLE SIZE AND ZETA POTENTIAL ANALYSIS

Particle size analysis of the SLN and NLC aqueous dispersions was performed by laser diffraction using an LS230 (Coulter Electronics, Krefeld, Germany). The mean particle size and the polydispersity index (PI) were determined by photon correlation spectroscopy (PCS) (Malvern Zetasizer IV, Malvern Instruments, UK) (n = 5, standard deviation <2%).

Zeta potential (ζ) measurements were performed in distilled water (n = 3, standard deviation <5%) adjusted to a conductivity of 50 µS/cm by addition of 0.9% (m/V) NaCl, using a Zetasizer IV (Malvern Instruments, UK) (8). The electrophoretic mobility was converted to a ζ by the Helmholtz-Smoluchowski equation.

DSC ANALYSIS

The degree of crystallinity was determined by differential scanning calorimetry (DSC) measurements on a Mettler DSC 821e (Mettler Toledo, Giessen, Germany). Samples containing 15 mg of SLN or NLC aqueous dispersions, i.e., 1–3 mg of solid lipid, were accurately weighed in 40-µl aluminium pans, heated from 25°C to 85°C, and cooled from 85°C to 25°C under liquid nitrogen. DSC scans were recorded at a heating and cooling rate of 5 K/min. The melting points and crystallization points corresponded, respectively, to the maximum and minimum of the DSC curves. The recrystallization index was calculated using the following equation (9):

$$RI(\%) = \frac{\Delta H_{SLN\ or\ NLC\ aqueous\ dispersion}}{\Delta H_{bulk\ material} \times Concentration_{lipid\ phase}} \times 100$$

RHEOLOGICAL ANALYSIS

Rheological analysis of the SLN and NLC aqueous dispersions was carried out in order to compare the different viscoelastic behaviors of these carriers. Therefore, an oscillation frequency sweep test was performed with a RheoStress RS 100 rheometer (Haake, Karlsruhe, Germany), equipped with a cone-and-plate test geometry (plate diameter 20 mm, cone angle 4°). All measurements were carried out at a temperature of 20° ± 0.1°C, recording the variation of the storage (G′) and loss (G″) moduli, as well as the complex viscosity (η^*), over a frequency range from 0 to 10 Hz at a constant stress amplitude of 5 Pa. The complex modulus (G*) was measured as a function of shear stress (Pa) at a constant frequency of 1 Hz.

RESULTS AND DISCUSSION

SLN and NLC aqueous dispersions containing 10% (m/m) or 15% (m/m) of lipid phase were developed for the present investigation. Table I shows the composition of these formulations.

PARTICLE SIZE AND ZETA POTENTIAL ANALYSIS

Aqueous dispersions prepared with Softisan®138 and Compritol®888 showed physical stability and. a narrow size distribution (<0.25) one week after preparation when stored

Table I

Composition of the Developed SLN and NLC Formulations

Formulation	Solid lipid % (m/m)	Liquid lipid % (m/m)	Surfactant % (m/m)	Co-surfactant % (m/m)	Water ad % (m/m)
A	10% Softisan®138	—	1.5% TegoCare®450	—	100
A₁	7% Softisan®138	3% Miglyol®812	1.5% TegoCare®450	—	100
B	15% Compritol®888	—	2.5% Poloxamer 188	—	100
B₁	10.5% Compritol®888	4.5% Miglyol®812	2.5% Poloxamer 188	0.125% Sodium deoxicolate	100
B₂	10.5% Compritol®888	4.5% Tocopherol	2.5% Poloxamer 188	0.125% Sodium deoxicolate	100
B₃	10.5% Compritol®888	4.5% Sunflower oil	2.5% Poloxamer 188	0.125% Sodium deoxicolate	100
B₄	10.5% Compritol®888	4.5% LCT	2.5% Poloxamer 188	0.125% Sodium deoxicolate	100
C	15% Stearyl alcohol	—	3% Tween 80	—	100
C₁	10.5% Stearyl alcohol	4.5% Miglyol®812	3% Tween 80	—	100
C₂	10.5% Stearyl alcohol	4.5% Tocopherol	3% Tween 80	—	100
C₃	10.5% Stearyl alcohol	4.5% Sunflower oil	3% Tween 80	—	100
C₄	10.5% Stearyl alcohol	4.5% LCT	3% Tween 80	—	100

Table II

PCS, PI, and ζ of the Developed SLN and NLC Formulations
Measured One Week After Production, Stored at 20°C

Formulation	PCS diameter (nm)	PI	ζ (mV)
A	178 ± 4	0.16 ± 0.04	−15
A_1	192 ± 1	0.14 ± 0.05	−19
B	209 ± 3	0.25 ± 0.07	−23
B_1	220 ± 1	0.22 ± 0.08	−25
B_2	233 ± 1	0.23 ± 0.09	−26
B_3	231 ± 0	0.19 ± 0.10	−24
B_4	243 ± 4	0.18 ± 0.11	−28
C	397 ± 3	0.56 ± 0.13	−6
C_1	259 ± 2	0.35 ± 0.14	−10
C_2	267 ± 3	0.36 ± 0.15	−11
C_3	268 ± 1	0.33 ± 0.16	−9
C_4	254 ± 1	0.34 ± 0.17	−12

at room temperature (20°C) (Table II). SLN formulations (A and B) exhibited lower PCS diameters than the respective NLC formulations. Conversely, samples composed of stearyl alcohol revealed higher mean particle sizes (>250 nm) and PI values between 0.3 and 0.6, and for this lipid NLC exhibited lower PCS diameters than SLN. However, LD analysis revealed that all developed formulations had a maximum particle size lower than 700 nm, which is in the nanometer range (data not shown). These results confirm that suitable production parameters have been employed for the preparation of nanosized lipid particles composed of chemically different solid lipids and using Miglyol®812, tocopherol, sunflower oil, or LCT as liquid lipid for NLC formulations.

Concerning ζ measurements, it is clearly visible that stearyl alcohol formulations showed lower absolute ζ values than the Softisan®138 and Compritol®888 formulations, and for all tested solid lipids NLC exhibited higher electrostatic stability (i.e., higher |ζ|) than the respective SLN.

DSC ANALYSIS

For Softisan®138, no melting peak was detected, revealing a still liquid status or a supercooled melt seven days after sample production (Figure 1). SLN and NLC based on Compritol®888 showed a degree of crystallinity between 60% and 70% (seven days after production), calculated in comparison to the melting enthalpy of the raw material. SLN and NLC based on stearyl alcohol showed the highest crystallinity (75–80%) in comparison to the other developed formulations. Concerning NLC formulations, crystallinity decreased according to: tocopherol > Miglyol®812 > sunflower oil > LCT, for both Compritol®888- and stearyl alcohol-based NLC formulations (data not shown).

RHEOLOGICAL ANALYSIS

Semi-solid systems such as SLN and NLC aqueous dispersions are the most difficult of the materials to characterize rheologically because they combine solid behavior and liquid properties within the same material (10–12). In order to obtain information about
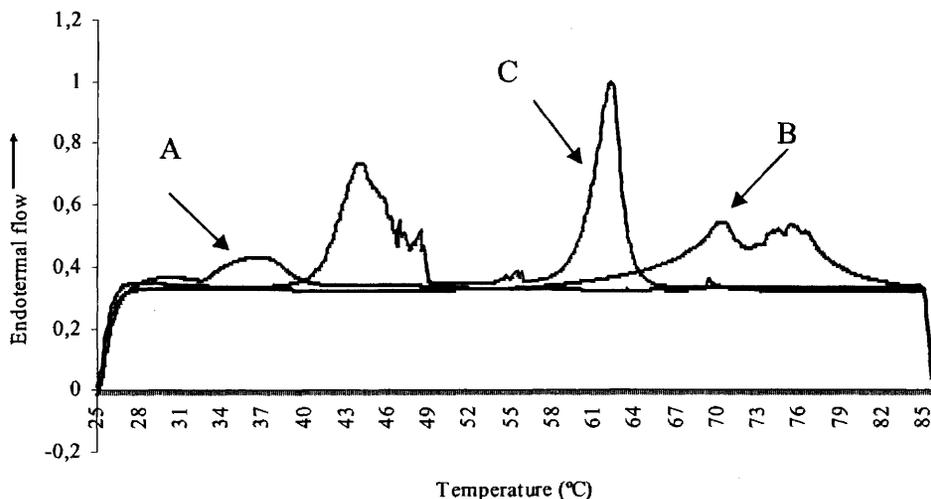
Figure 1. DSC curves of the developed SLN formulations (A, B, and C) recorded seven days after sample production.

the viscous and elastic behavior of the investigated systems and the network structure formed by particle–particle interactions, an oscillation frequency sweep test has to be performed (13).

When performing oscillation measurements, first the linear viscoelastic region has to be determined by a stress sweep at a constant frequency. The linear viscoelastic region is the range of stress over which G^* is independent of the applied stress. Over this linear region the structure of the dispersion remains intact. The stress sweep test is a dynamic test in which the complex modulus G^* is measured as a function of stress at a constant frequency. Being in the viscoelastic region for all tested formulations, 5 Pa was chosen as the stress amplitude in the following studies. The shape of the material function curves reveals structural characteristics of the system. Therefore, in the present work this test was performed over a frequency range from 0 to 10 Hz.

Figure 2 displays the storage modulus (G'), loss modulus (G''), and complex viscosity (η^*) of the developed SLN formulations (A, B, and C), after application of a frequency range between 0 and 10 Hz. With regard to formulation A (SLN based on Softisan®138), it can be seen that G' is lower that G'', which means that the system is more viscous than elastic. For both B (SLN based on Compritol®888) and C (SLN based on stearyl alcohol) formulations, G' is higher by about one order of magnitude than G'', and so the system is more elastic than viscous in the investigated frequency range and both parameters show weak dependence on the applied frequency. Note that formulation A is a supercooled melt; it has a lower η^* and shows less suitability for topical administration because it might readily flow out of the container. Conversely, formulations B and C are highly crystalline and show a prominent elastic component. Another reason for the obtained differences is the percentage of lipid phase in each formulation. In more concentrated dispersions (B and C), relatively large amounts of lipid may significantly increase the effective volume fraction of the dispersion, and hence the viscosity, due to the quantity of immobilized liquid between the lipid phase and the relatively high surface area of the nanoparticles. According to these results, formulations B and C might
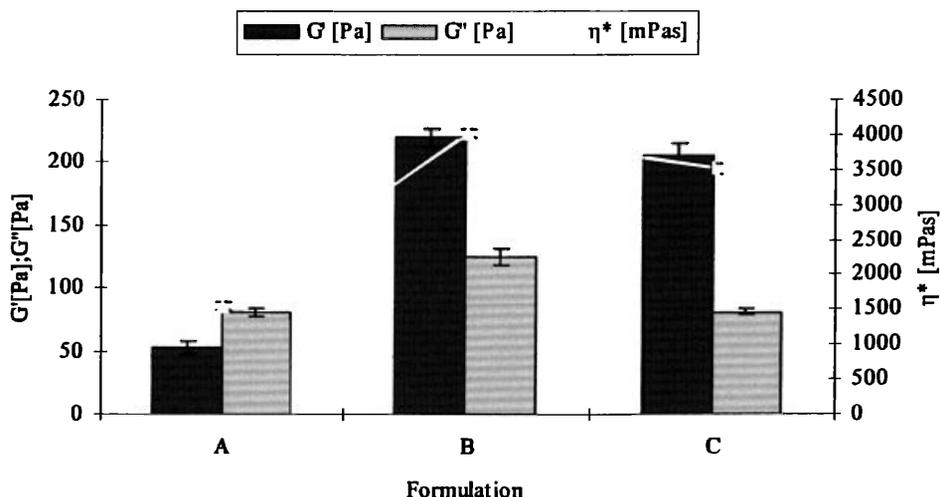
Figure 2. Storage modulus (G'), loss modulus (G''), and complex viscosity (η^*) of the developed SLN formulations, after application of a frequency range between 0 and 10 Hz.

be suitable for topical administration because they exhibit a more uniform resistance against flow.

Both G' and G'' moduli, as well as η^*, are also affected by the type and concentration of the surfactant in the formulation (14). Formulation B is sterically stabilized with poloxamer and, to provide a sufficient steric hindrance, a relatively large amount of this polymer is used, which might increase the effective volume fraction, resulting in higher viscosity. Therefore, the influence of the oil used for NLC preparation in its viscoelastic parameters has been evaluated in comparison to the respective SLN system. Regarding the obtained G' and G'' values for NLC formulations, a completely different rheological behavior can be observed as a function of the liquid lipids of the formulations. The results are shown in Figures 3 and 4.

Using Compritol®888 as solid lipid, Miglyol®812 (B_1) revealed a G'' value four times higher than G', and therefore this oil is responsible for a more viscous system. In contrast, when using stearyl alcohol (C_1), both moduli lie in the same range of magnitude. Tocopherol showed a greater influence on elasticity when formulated with stearyl alcohol as a solid lipid in NLC (C_2). Sunflower oil also displayed significant differences with regard to the solid lipid. In fact, when using Compritol®888 (B_3), the G' was twice as high as G'', showing that the system is more elastic than viscous, but the same was not detected when using stearyl alcohol (C_3). Concerning LCT, this oil did not reveal any significant difference with regard to the solid lipid used for NLC preparation (B_4 and C_4).

Regarding the obtained η^* values, formulations B_2 and B_4 indicated a significant decrease in viscosity in comparison to the SLN system (Figure 3). Stearyl alcohol-based formulations (Figure 4) showed higher values, although the influence of the liquid oils was more pronounced in Compritol®888-based formulations (Figure 3). The lower zeta potential values of the stearyl alcohol-based nanoparticles might be responsible for the higher η^* values recorded for these systems. In fact, the decrease in electrostatic repulsion between the suspended nanoparticles might be responsible for the develop-
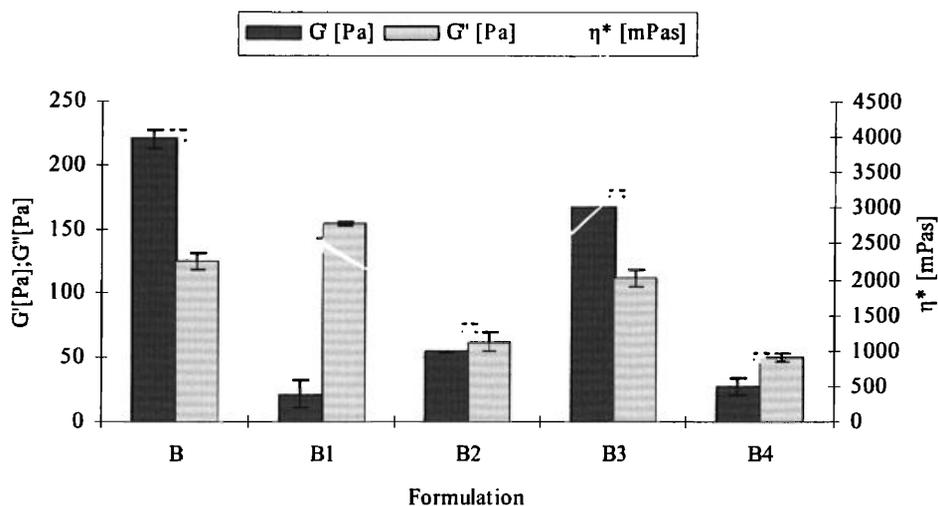
Figure 3. Storage modulus (G'), loss modulus (G''), and complex viscosity (η*) of the developed SLN and NLC formulations based on Compritol®888, after application of a frequency range between 0 and 10 Hz.
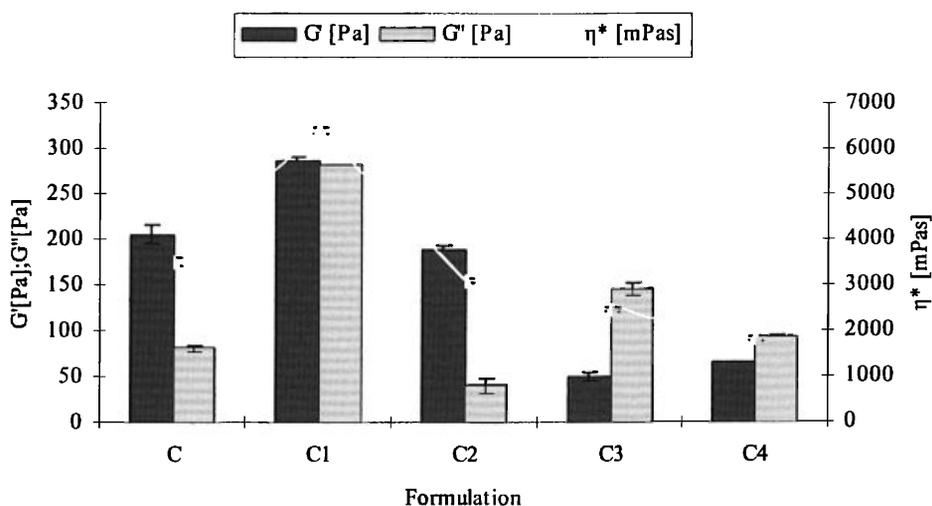

Figure 4. Storage modulus (G'), loss modulus (G''), and complex viscosity (η*) of the developed SLN and NLC formulations based on stearyl alcohol, after application of a frequency range between 0 and 10 Hz.

ment of a three-dimensional structure more difficult to disperse due to the possible formation of aggregates. Another possible explanation is the higher mean particle size of the stearyl alcohol-based nanoparticles in comparison to the other developed formulations.

CONCLUSIONS

Although much of the qualitative information about a material can be obtained by simply observing how a sample behaves when handled, the determination of the rheo-

logical properties of a dermatological formulation is extremely important. These studies allow (i) the evaluation of a the capability of a vehicle to suspend solids or immiscible liquids; (ii) the assessment of a topical formulation with respect to patient usage, e.g., the ease of removing the preparation from a container or spreadability and adherence to the skin; and (iii) the monitoring of the effect of the vehicle's consistency on the release of a drug from the preparation and its subsequent percutaneous absorption (bioavailability of the drug substance).

The determination of G', G'', and η^* as a function of the applied frequency, using an oscillation frequency sweep test, gives important information concerning topical administration. According to the obtained results, for Compritol®888 as a solid lipid for NLC preparation, sunflower oil seems to be the oil that presents the best attributes for a topical formulation, i.e., more elastic behavior and a higher viscosity. With regard to stearyl alcohol, tocopherol shows the best results. The presence of a more compact and organized system can have a significant beneficial effect on the stability and on the viscoelactic properties of particulate aqueous dispersions and, therefore, on the topical administration of drug substances.

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